Programme

29th Annual Congress of the European Society for Biomaterials

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Scientific Programme Schedule Sunday

Room 0.4

YSF Workshop *Chairs: Arn Mignon and Nicola Contessi Negrini*

14.00 - 14.30 **Crossing the academia-industry bidirectional border: a route for innovation and competitiveness,** Zeinab Tahmasebi, Tony Feliciano, Rong Wang, Catarina Coelho and Gerard Cadafalch

14.30 - 15.00 **Academia & industrial research; the approaches and positions of young scientist,** Maarten Honing

15.00 – 15.30 **The Continuous Quest for Innovation,** Dirk Bontinck

15.30 - 16.00 **Coffee Break** Lobby

16.00 - 16.30 **How to publish journal articles,** Neil Hammond

16.30 - 17.00 **The adventure of starting a spin-off company: Ultroxa polymers,** Victor de la Rosa

17.00 – 17.30 **Round table discussion & QA**

17.30 - 17.45 **Closing**

Auditorium 1

18.00 - 18.30 **Opening of the Annual Meeting of the European Society for Biomaterials,** *Pamela Habibovic, Joelle Amedee & Joost van den Akker*

La Bonbonnière

19.00 - 21.00 **Welcome Reception**

Scientific Programme Schedule Monday

Auditorium 1 – Plenary

08.30-08.45 **Welcome,** *Rianne Letschert - Rector Maastricht University*

08.45-09.30 **Contrasting nanomedicine claims versus human clinical performance: why such disconnects?,** *David Grainger, Chair: Marc Bohmer, Sandra Hofmann*

09.30-10.15 **ESB George Winter Award 2018: Role of Functional Polymers in the development of Biomaterials for Application in the Biomedical Field,** *Julio San Roman, Chair: Liz Tanner, Matteo Santin*

10.15-10.45 **Coffee Break** Trajectum & Expo Foyer

Auditorium 1 - Parallel session

CREATIVE ENGINEERING IDEAS

Chair: Liam Grover, Niloofar Tahmasebi

10.45-11.15 **Bioinspired functionalisation of soft materials: materials and strategies,** *Katharina Maniura*

11.15-11.30 **Thermoresponsive nanofibers for engineering supramolecularly assembled tissue substitutes,** *Andrea De Pieri*

11.30-11.45 **Microfluidically generated single cell microgels as pericellular niches with temporally controlled biochemical and biophysical properties,** *Jeroen Leijten*

11.45-12.00 **Macromolecular crowding, mechanical stimulation and oxygen tension in tissue engineering - A step closer to cell-assembled biomaterials,** *Diana Gaspar*

12.00-12.15 **Thermo-responsive macrocarriers containing polycaprolactone grafted with Poly(N-isopropyl acrylamide) for cell culture,** *Linh Nguyen*

12.15-13.15 **Lunch Break** Trajectum & Expo Foyer

13.15-14.15 **Poster session A** Trajectum & Expo Foyer

14.15-15.00 **Jean Leray Award 2018: Meta-biomaterials,** *Amir Zadpoor, Chair: Gino Ambrosio, Aldo Boccaccini*

15.00-16.00 **YSF General Assembly,** *Sandra van Vlierberghe*

16.00-16.30 **Coffee Break** Trajectum & Expo Foyer

ELECTROSPINNING TECHNIQUES & APPLICATIONS

Chair: Elisabeth Engel, Marzia Brunelli

16.30-17.00 **Electrospinning techniques & possibilities,** *Fang Yang*

17.00-17.15 **Bioinspired topographically-tailored electrospun substrates for studying mesenchymal stromal cell behaviour,** *Beatriz Monteiro*

17.15-17.30 **Future trends in the electrospinning technique: The importance of using of benign solvents,** *Liliana Liverani*

17.30-17.45 **Experimental and Computational approach of cells' distribution during culture in a 3D porous electrospun scaffold,** *Foteini Kozaniti*

17.45-18.00 **Combining melt electrowriting of microfiber meshes with aggregated chondroprogenitor cells stimulated with GDF-2 to enhance cartilage tissue engineering,** *Florencia Abinzano*

Auditorium 2 - Parallel session

3D-PRINTED PERSONALIZED ORTHOPAEDIC IMPLANTS

Chair: Liesbet Geris, Holger Jahr

10.45-11.15 **Biodegradation Behavior, Infection Prevention, and Bone Regeneration,** *Amir Zadpoor*

11.15-11.45 **Towards a standardised and personalised in-vitro tool for 'functional' testing of 3D-printed (non)degradable orthopaedic implants,** *Jan Schrooten*

11.45-12.00 **3D additive manufactured scaffolds with antimicrobial activity for tissue regeneration,** *Maria Camara-Torres*

12.00-12.15 **Functionalization of poly(vinyl) alcohol fibers with polymeric brushes for reinforcement of calcium phosphate ceramics,** *Daniela Petre*

12.15-13.15 **Lunch Break** Trajectum & Expo Foyer

12.45-13.15 **Corporate rapid fire session**, Chair: Jan de Boer

13.45-14.15 **Poster session A** Trajectum & Expo Foyer

16.00-16.30 **Coffee Break** Trajectum & Expo Foyer

BIO-MOLECULAR SELF-ASSEMBLY FOR BIOMATERIALS DESIGN

Chair: Jacek Wychowaniec

16.30-17.00 **β-sheet forming peptide hydrogels: From self-assembly to functional biomaterials,** *Alberto Saiani*

17.00-17.30 **Biomimetic Polymer-based Self-Assembled Biomaterials,** *Sebastien Lecommandou*

17.30-17.45 **Biomimetic self-assembly of functional gold nanoparticles,** *Nonappa Nonappa*

17.45-18.00 **Biomimetic Nanostructured Polyurethane-based Patchy Colloidal Particles to Combat Biofilm and Planktonic Drug-resistant Bacteria,** *Patricia Varela*

18.00-18.30 **Rapid fire poster sessions 1** *Chair: Sabine van Rijt*

Room 0.4 - Parallel session

10.45-12.15 **Translational research symposium session 1: The industry vision,** *Chair: Patrick Boisseau and Yves Bayon.*

Introduction on ESTHER, *Patrick Boisseau*

A SME perspective, *Philip Procter*

Ultroxa®, *Richard Hoogenboom*

A multinational perspective, *Yves Bayon*

Brief introduction to Healthtech TAB, *Patrick Boisseau and Yves Bayon*

Panel discussion: the EU innovation ecosystem in healthcare?

12.15-13.15 **Lunch Break** Trajectum & Expo Foyer

13.45-14.15 **Poster session A** Trajectum & Expo Foyer

14.30-16.00 **Translational research symposium session 2: A patient centric perspective,** *Chair: Patrick Boisseau and Yves Bayon*

What do clinicians expect from industry and from technology in regenerative medicine? *Eelco de Koning*

From lab to OR in nanomedicine, *Didier Letourneur*

The European cooperation of clinical centers, *Jacques Demotes*

The patient's perspective, *Sofiane Naroun*

Panel discussion: the empowerment of patient in health technologies innovation

16.00-16.30 **Coffee Break** Trajectum & Expo Foyer

16.30-18.00 **Translational reserach symposium session 3: What is the vision of healthcare,** *Chair: Patrick Boisseau and Yves Bayon*

The ESB perspective on FP9, *Matteo Santin*

Healthtech 2030, *Klaus-Michael Weltring*

18.00-18.45 **Elevator pitches,** *Chair: Fabrizio Barberis, Yves Bayon and Peter Dubruel*

Room 0.5 - Parallel session

TOWARD APPLICATIONS OF ADDITIVE MANUFACTURING & BIOPRINTED SCAFFOLDS

Chair: Maria Grazia Raucci, Jessica Senior

10.45-11.00 **Preclinical evaluation of a composite implant made by stereolithography for orbital floor fracture repair,** *Olivier Guillaume*

11.00-11.15 **Towards bioprinting of vascularized bone tissue equivalents,** *Petra Kluger*

11.15-11.30 **Bioresin for printing of cell-laden hydrogels with 3D convoluted vessel-like microfluidic channels via digital light processing,** *Riccardo Levato*

11.30-11.45 **Platelet lysate fibrillar nanocomposite bioink for soft tissues bioprinting,** *Barbara Mendes*

11.45-12.00 **Bioprinted capillary beds as building block for organ-on-a-chip applications,** *Andreas Blaeser*

12.00-12.15 **Development of a biomimetic cell-laden hydrogel for bioprinting cellular tissues-like for bone regeneration,** *Ana Marina Ferreira*

12.15-13.15 **Lunch Break** Trajectum & Expo Foyer

13.15-14.15 **Poster session A** Trajectum & Expo Foyer

16.00-16.30 **Coffee Break** Trajectum & Expo Foyer

CONTROLLING BIOMATERIAL-BIOLOGIVAL SYSTEM INTERACTIONS

Chair: Julie Gough, Frederik Tengström

16.30-16.45 **Biomacs: Biomaterials advanced cell screening,** *Patrick van Rijn*

16.45-17.00 **Direct and stable immobilization of cell adhesive ligand on ePTFE through the oxidation of an anchor peptide containing Tyr residues,** *Sachiro Kakinoki*

17.00-17.15 **One step biocatalytic surface modification of viruses,** *Valentina Vignali*

17.15-17.30 **Metal-free alternating copolymer: - A novel nanomaterial synthesized by green chemistry approach for use in drug delivery/biomedical application,** *Piyush Kumar Gupta*

17.30-17.45 **BTA supramolecular hydrogelators as extracellular matrices for tissue engineering,** *Shahzad Hafeez*

17.45-18.00 **Influence of Dynamic Flow Conditions on Adsorbed Plasma Protein Corona and Surface-induced Thrombus Generation on Antifouling Brushes,** *Kai Yu*

Room 0.2/0.3 - Parallel session

SOFT TISSUE APPLICATIONS FOR BIOMATERIALS

Chair: Didier Letourneur, Denise de Bont

10.45-11.00 **Antithromboganic poly(ether-ether-ketone) by grafting of phospholipid polymer and immobilization of heparin,** *Kazuhiko Ishihara*

11.00-11.15 **Hydrogel based mesenchymal stromal cell delivery improves muscle regeneration via local modulation of CD4 and CD8 T Cell levels,** *Taimoor Hasan*

11.15-11.30 **A bioactive hyperbranched polyglycerol coating as an endothelial cell selective platform for vascular implants,** *Anouck Burzava*

11.30-11.45 **A multifactorial, multiplanar approach to inducing functional neural cell behaviour using electrospun scaffolds: Is fibre alignment the critical factor?,** *Kirstie Andrews*

11.45-12.00 **Microstructure and cyclic performance of composite hydrogel implants,** *Céline Wyss*

12.00-12.15 **Intraluminal guidance cues for nerve guide conduits,** *Jonathan Field*

12.15-13.15 **Lunch Break** Trajectum & Expo Foyer

13.15-14.15 **Poster session A** Trajectum & Expo Foyer

16.00-16.30 **Coffee Break** Trajectum & Expo Foyer

ADVANCES IN SOFT TISSUE ENGINEERING

Chair: Nicolas Blanchemain, Angela Carvalho

16.30-16.45 **Gelatin scaffolds for adipose tissue engineering with prevascular network by sacrificial template,** *Nicola Contessi Negrini*

16.45-17.00 **Optical fibers & MEMS for the micro-viscoelastic characterization of tissues and biomaterials in physiological conditions,** *Jakob Pyszkowski*

17.00-17.15 **Endothelial - Smooth Muscle - Fibroblastic co-culture systems prospecting the development of Tissue-Engineered Vascular Grafts,** *Tatiana Felizardo*

17.15-17.30 **3D Human skin equivalent (HSE) as a preclinical in vitro risk assessment platform,** *Ayesha Idrees*

17.30-17.45 **Bioactive, hydrophilic and stretchable polymer clay nanocomposites for soft tissue engineering,** *Sungkwon Yoon*

17.45-18.00 **Lactate-based strategy for cardiac tissue engineering,** *Jesús Ordoño*

Scientific Programme Schedule Tuesday

Auditorium 1 - Plenary

08.30-09.15 **Mechanobiology: a rapidly growing field with forceful implications,** *Viola Vogel Chair: Carlos Cabello, Debby Gawlitta*

09.15-10.00 **ESB International Award 2018: Biosynthetic Materials for Islet Encapsulation and Transplantation,** *Andres Garcia, Chair: Liz Tanner, Abhay Pandit*

10.00-10.30 **Coffee Break** Trajectum & Expo Foyer

Auditorium 1 - Parallel session

KSBM-ESB JOINT SESSION: HYDROGELS

Chair: Kidong Park and Peter Dubruel

10.30-11.00 **Control of properties of hyaluronate-based terpolymeric hydrogel for biomedical applications,** *In-Sup Noh*

11.00-11.30 **Towards angiogenic behavior of human mesenchymal stromal cells using 3D printed gelatin-based scaffolds,** *Sandra van Vlierberghe*

11.30-11.45 **Hydrogels that promote cell-cell interactions enhance therapeutic activity of mesenchymal stromal cells,** *Taimoor Hasan Qazi*

11.45-12.00 **Engineering hydrogels for cardiac 3D cell culture via hydrazone bioconjugation,** *Jenny Parraga*

12.00-13.00 **Lunch Break** Trajectum & Expo Foyer

13.00-14.00 **Poster session B** Trajectum & Expo Foyer

BIOMATERIALS SCIENCE SESSION

Chair: Neil Hammond, Agata Lapa

14.00-14.05 **Biomaterials Science Award,** *Neil Hammond*

14.05-14.30 **Leverage Physiology for Bioresponsive Drug Delivery,** *Zhen Gu*

14.30-14.45 **The use of chitosan porous 3D scaffolds embedded with resolvin D1 to improve bone healing,** *Daniela Pereira de Vasconcelos*

14.45-15.00 **The role of vascularization in the self-assembly of bioengineered islets,** *Frederrik Tengström*

15.00-15.15 **Engineering a functional muscle tissue using a fibrin-based gel for tissue modelling,** *Hironobu Takahashi*

15.15-15.30 **Graphene Oxide-containing Self-Assembling Peptide Hydrogels As 3D Platforms For Musculoskeletal Cell-based Therapies,** *Cosimo Ligorio*

15.30-16.00 **Coffee Break** Trajectum & Expo Foyer

CSB-ESB JOINT SESSION ON DRUG DELIVERY

Chair: Hasan Uludag, Ana Paula Pego

16.00-16.30 **siRNA and mRNA Delivery with Chitosans,** *Michael Buschmann*

16.30-17.00 **Gene Therapy for orthopaedic diseases,** *Laura Creemers*

17.00-17.15 **The increased therapeutic potential of pro-osteogenic MSCs delivered via an injectable thermosensitive hydrogel scaffold for the treatment of non-union fractures,** *Phil Chambers*

17.15-17.30 **Development of an injectable nanoparticle-loaded hydrogel system suitable for delivery of an angiogenic growth factor to the ischaemic myocardium,** *Joanne O'Dwyer*

DRUG-FREE ANTIBACTERIAL HYBRID BIOPOLYMERS FOR MEDICAL APPLICATIONS

Chair: Ipsita Roy, Xiang Yang

17.30-17.45 **Inorganic Antibacterial Materials,** *Aldo Boccaccini*

17.45-18.00 **Antibacterial Synthetic Polymers,** *Gianluca Ciardelli*

18.00-18.15 **Antibacterial Natural Polymers,** *Ipsita Roy*

18.15-18.30 **Clinical impact of HyMedPoly: Drug-free strategies and their future implementation,** *Jochen Salber*

18.30-18.45 **New concepts and goals of HyMedPoly - Drug free Antibacterial Strategies,** *Xiang Zhang*

18.45-19.00 **Scientific and Industrial Achievements in HyMedPoly,** *Ipsita Roy*

Auditorium 2 - Parallel session

ELECTRICALLY ACTIVE BIOMATERIALS: THE FUTURE OF TRANSLATABLE BIOELECTRONICS

Chair: Manus Biggs, Joseph Goding

10.30-11.00 **Materials for Biodegradable Electronics -> Soft, Skin-Interfaced Systems for Sweat Collection, Physiological Monitoring and Biochemical Sensing,** *Roozbeh Ghaffari*

11.00-11.30 **Design, Synthesis, and Characterization of Conjugated Polythiophenes for Interfacing Electronic Biomedical Devices with Living Tissue,** *David Martine*

11.30-11.45 **Optically active collagen-QD matrices for read-out and stimulation of neuronal activity,** *Stijn Jooken*

11.45-12.00 **PDMS/PVDFhfp core-sheath fibers with piezoelectric properties for stimulation of cells,** *Marzia Brunelli*

12.00-13.00 **Lunch Break** Trajectum & Expo Foyer

12.45-13.15 **Corporate rapid fire session**, *Chair: Lorenzo Moroni*

13.30-14.00 **Poster session B** Trajectum & Expo Foyer

RSC BIOMATERIALS CHEMISTRY GROUP AT ESB

Chair: Chris Sammon, Paul Roach

14.00-14.30 **Responsive polymers in diagnostics and therapeutics,** *Cameron Alexander*

14.30-15.00 **Highly branched poly(N-isopropyl acrylamide) responsive to fungi,** *Stephen Rimmer*

15.00-15.15 **Non-cytotoxic copper containing polyurethanes,** *Juan Valerio Cauich-Rodriguez*

15.15-15.30 **Degradable Poly(Ethylene Glycol) Hydrogels for Spinal Cord Injury Repair,** *Douglas Venters*

15.30-16.00 **Coffee Break** Trajectum & Expo Foyer

ADDITIVE MANUFACTURING OF POLYLACTIDES, FROM IDEA TO COMMERCIALIZATION

Chair: Dirk Grijpma, Bas van Leeuwen

16.00-16.30 **Aligning molecular and structural dynamics in fused deposition modelling: Novel routes to tailor product functionalities,** *Srinivas Vadrun*

16.30-17.00 **Thermoplastic materials in Additive Manufacturing assisted Tissue Engineering - A Review,** *Carlos Carvalho*

17.00-17.15 **Printability window for thermoplastic polymers in bioextrusion,** *Andrea Roberto Calore*

17.15-17.30 **Glaucoma-on-a-chip: an in vitro model for glaucoma drug discovery based on mimicking the mechanical stress of high eye pressure, Pascal Vroemen**

17.30-18.00 **Rapid fire poster session 2** *Chair: Aurelie Carlier*

Room 0.4 - Parallel session

TECHNOLOGICAL ADVANCES IN ADDITIVE MANUFACTURING & BIOPRINTING

Chair: Michael Gelinsky, Andrea Calore

10.30-10.45 **Rheology and processing of degradable polymers, considerations for additive manufacturing,** *Astrid Ahlinder*

10.45-11.00 **Real time measurement of spatial O2 distribution in 3D bioprinted constructs of clinically relevant sizes using sensor nanoparticles,** *Ashwini Rahul Akkineni*

11.00-11.15 **Using sound to pattern cells: 3D Sound Induced Morphogenesis (3D-SIM),** *Tiziano Serra*

11.15-11.30 **3D printing chimeric hydrogel scaffolds using suspended additive layer manufacturing (SALM),** *Jessica Senior*

11.30-11.45 **Validation of mid-infrared LIFT for absorber free laser assisted bioprinting,** *Richard Lensing*

11.45-12.00 **3D printing of porous scaffolds designed for biomedical implant development,** *Faezeh Shalchy*

12.00-13.00 **Lunch Break** Trajectum & Expo Foyer

13.00-14.00 **Poster session B** Trajectum & Expo Foyer

NANOMATERIALS FOR APPLIED FUNCTIONALITY

Chair: Sabine van Rijt, Diba Mani

14.00-14.15 **Surface functionalisation, nanoroughness and drug delivery by atmospheric plasma jet on scaffolds,** *Alessandro Patelli*

14.15-14.30 **A functional analysis of nanotopographically modified platinum iridium electrodes,** *Adriona Kelly*

14.30-14.45 **Attachment of functional molecules to the surface of ultrasmall gold nanoparticles by click chemistry for specific epitope targeting of proteins,** *Selina Beatrice van der Meer*

14.45-15.00 **Surface modification of tantalum via anodization for orthopedic applications,** *Batur Ercan*

15.00-15.15 **Effect of ion-doped hydroxyapatite nanoparticles for bone regeneration on bone cell viability and osteoclastogenesis,** *Carina Kampleitner*

15.15-15.30 **Modulating neural stem cells activity by nanoparticle light-activation,** *Catarina Rebelo*

15.30-16.00 **Coffee Break** Trajectum & Expo Foyer

3D SCAFFOLDS FOR MODELS & MANIPULATION

Chair: Anna Wistrand, Maria Camarra-Torres

16.00-16.15 **A cryogel toolbox for cell culture and ex vivo tissue manipulation,** *Ben Newland*

16.15-16.30 **Development of liver organoids in 3D porous polysaccharide**

scaffolds, *Marie-Noelle Labour*

16.30-16.45 **Regulatory Macrophages Control Fibroblast Behavior in a 3D in vitro Model of the Wound Resolution Phase,** *Franziska Ullm*

16.45-17.00 **Development of a three-dimensional collagen model for the in vitro evaluation of osteogenesis,** *Rebecca Zhiyu Yuan*

17.00-17.15 **Three-Dimensional (3D) Chitosan Scaffold to Mimic Breast Cancer Microenvironment,** *Sultan Gulce-Iz*

17.15-17.30 **Biofunctional Microparticles for Assembly of Tumor-Stem Cell Hybrid 3D In Vitro Models,** *Victor Gaspar*

Room 0.5 - Parallel session

COMBINING BIOMATERIALS WITH MICROFLUIDICS

Chair: Joao Mano, David Barata

10.30-10.45 **Development and Assessment of Microfluidic Platform for 3D Bone Cell Culture and Drug Evaluation,** *Hossein Bahmaee*

10.45-11.00 **Production of biomaterials for bone regeneration using droplet microfluidics,** *Victor Galván*

11.00-11.15 **From low- to high-throughput: Microfluidic fabrication of novel 3D biomaterial libraries for screening cell responses,** *Carlos Guimarães*

11.15-11.30 **Microengineering a curved alveoli-on-chip,** *Danielle Baptista*

11.30-11.45 **Tuneable collagen microgels for regenerative medicine,** *Jose Manuel Rey*

11.45-12.00 **Microfluidic manipulation of biomaterials mechanical properties by thermophoresis,** *Alexandros Kosmidis*

12.00-13.00 **Lunch Break** Trajectum & Expo Foyer

13.00-14.00 **Poster session B** Trajectum & Expo Foyer

DEVELOPMENTS IN APPLIED BIOMATERIALS

Chair: Karine Anselme, Irene Lodoso-Torrecilla

14.00-14.15 **Electrical stimulation on titanium for enhancing stem cell osteogenesis,** *Juan Shong Khaw*

14.15-14.30 **New biocompatible hydrogels with extreme mechanical properties,** *Andreia Pereira*

14.30-14.45 **Effects of Nrf2 signaling on cytotoxicity induced by HEMA from dental biomaterials,** *Bergitte Pearl Olderbø*

14.45-15.00 **Sterilization effects on the handling and degradation properties of calcium phosphate cements containing poly(lactic-co-glycolic-acid) porogens**

and carboxymethyl cellulose, *Nathan Kucko*

15.00-15.15 **Highly selective double atom activation of titanium alloys by directed irradiation synthesis: tailoring osteoblast behavior towards implants osseointegration,** *Ana Civantos*

15.15-15.30 **Hydroxyapatite coating of magnesium alloys for the tailored degradation of resorbable bone fixation products,** *Jonathan Acheson*

15.30-16.00 **Coffee Break** Trajectum & Expo Foyer

INSPIRED BY NATURE BIOMATERIALS FOR ENGINEERING AND REGENERATION

Chair: Jan Chlopek, Natalija Tatic

16.00-16.15 **Self-assembling silk hydrogels as a carrier matrix to treat stroke,** *Natalia Gorenkova*

16.15-16.30 **A Novel Biocomposite Based on Microporous Oxidized Bacterial Cellulose/Arginine and the Effect on the Behavior of Fibroblast/Endothelial Cell,** *Yudong Zheng*

16.30-16.45 **ClickECM – integration of click groups into cell-derived human extracellular matrix to create ECM-based biomaterials,** *Petra Kluger*

16.45-17.00 **Injectable hydrogels for single-surgery tracheal occlusion in congenital diaphragmatic hernia management,** *Chiara Emma Campiglio*

17.00-17.15 **Nano fracture behaviors of bone tissue,** *Fei Hang*

17.15-17.30 **Hyaluronic acid-based composite hydrogels with biofunctional tailored features,** *Ugo D'Amora*

Room 0.2/0.3 - Parallel session

UTILISATION OF BIOMATERIALS FOR WOUND TREATMENT

Chair: Jerome Sohier, Virginia Llopis-Hernandez

10.30-10.45 **A photo-triggerable NP library for skin cell targeting and efficient in-vivo small non-coding RNA delivery in wound healing,** *Josephine Blersch*

10.45-11.00 **A new 'spin"in chronic wound care: Delivering mir-31 via nanofibres,** *Eoghan Joseph Mulholland*

11.00-11.15 **Treatment of bacterial infection in a 3D skin model by transdermal application of curcumin,** *Berna Senturk*

11.15-11.30 **Development of an aerogel based on graphene oxide and polyvinyl alcohol with potential of transdermal use,** *Katherina Fernandez*

11.30-11.45 **Tiger 17 functionalized onto PVA and CA dressings accelerate clotting time and reduce microbial burden,** *Helena Felgueiras*

11.45-12.00 **Colloidal wound dressing with high tissue adhesiveness for digestive system cancer therapy,** *Akihiro Nishiguchi*

12.00-13.00 **Lunch Break** Trajectum & Expo Foyer

13.00-14.00 **Poster session B** Trajectum & Expo Foyer

BIOMATERIALS FOR ANTI-CANCER THERAPY

Chair: Du Chang, Ana Henriques Lourenço

14.00-14.15 **Development of biomaterials-based immunotherapy: chitosan and poly(gamma)-glutamic acid nanoparticles as immunomodulatory players at the tumor microenvironment,** *Flavia Castro*

14.15-14.30 **Conjugated linoleic acid functionalization enhances superparamagnetic iron oxide nanoparticles (SPIONs) cytotoxicity in 4T1 mouse breast cancer cells modulating PPARs,** *Marina Ricci*

14.30-14.45 **Development of a mRNA Vaccine in a Microneedle Patch for Castrate Resistant Prostate Cancer,** *Emma McErlean*

14.45-15.00 **Glucose-trigger hydrogen peroxide production for antitumor treatment combine with gene silencing therapy and photothermal therapy,** *Zi-Lin Zhou*

15.00-15.15 **Synergistic toxic effect of marine-origin polymeric nanoparticle loaded with gemcitabine over human breast cancer cells,** *Catarina Oliveira*

15.15-15.30 **Development of yttrium-containing magnetic microspheres for intra-arterial hyperthermoradiotherapy,** *Masakazu Kawashita*

15.30-16.00 **Coffee Break** Trajectum & Expo Foyer

IMAGING BIOMATERIALS & ENVIRONMENT

Chair: Dorothea Bruggemann, Julien Gautrot

16.00-16.15 **Structural details about the interface between biodegradable Mg alloys and cells or tissue,** *Regine Willumeit-Römer*

16.15-16.30 **Electrical impedance tomography with a lab-on-chip for imaging cells in culture,** *Marijn Lemmens*

16.30-16.45 **Multi-scale speciation imaging of metallic wear particles in peri-prosthetic tissues,** *Alexander Morrell*

16.45-17.00 **TEMPO-labeled hydrogels for magnetic resonance imaging,** *Emanuele Mauri*

17.00-17.15 **Optical projection tomography imaging of three-dimensional cell cultures as part of hydrogel scaffold development,** *Janne Koivisto*

17.15-17.30 **Thermal based sensor for real-time monitoring of cell growth,** *Seppe Bormans*

Room 0.8

12.15-13.00 **Lunch symposium: Beyond the pill: Smart drug delivery with Calcium Phosphate carriers – a scientific, regulatory and clinical perspective**

Room 2.9/2.10

16.00-17.30 **YSF workshop 'An interactive guide to good science writing,** *Neill Hammond*

Café Ipanema

20.00-00.30 **YSF's Night Out**

Scientific Programme Schedule Wednesday

Auditorium 1 – Plenary

08.30-09.15 **Nanoscale control of mesenchymal stem cells,** *Matthew Dalby Chair: Joelle Amede, Jeroen van den Beucken*

09.15-09.45 **Coffee Break** Trajectum & Expo Foyer

Auditorium 1 - Parallel session

CALCIUM PHOSPHATES

Chair: Matthias Schumacher, Selina Beatrice van der Meer

09.45-10.15 **Nature-inspired chemical process to retain highly bioactive chemistry and multi-scale hierarchic structure in 3-D bone scaffolds,** *Anna Tampieri*

10.15-10.30 **Biological events in bone formation instructed by submicron surface structured calcium phosphate ceramics,** *Huipin Yuan*

10.30-10.45 **Laser surface structuring of calcium phosphate bioceramics for cell behaviour assessment,** *Stéphane Hocquet*

10.45-11.00 **Influence of microporosity and macropores design upon cell colonization of calcium phosphate ceramic scaffolds for bone regeneration,** *Amandine Magnaudeix*

11.00-11.15 **A bioactive nano-calcium phosphate paste for in-situ transfection of BMP-7 and VEGF-A: Results of an in vivo study,** *Matthias Epple*

11.15-12.15 **Poster session C** Trajectum & Expo Foyer

12.15-13.15 **Lunch Break** Trajectum & Expo Foyer

13.15-14.30 **ESB General Assembly**

Chair: Pamela Habibovic

3D PRINTED SCAFFOLDS 7 HIERARCHY

Chair: David Eglin, Astrid Ahlinder

14.30-14.45 **3D printing of horizontal gradient scaffolds for bone regeneration,** *Luis Diaz-Gomez*

14.45-15.00 **Hierarchical biofabrication: Integrating molecular versatility and nano-to-macro scale structural control,** *Clara Hedegaard*

15.00-15.15 **Biphasic hydrogel with gradient drug release for articular cartilage regeneration,** *Xuetao Shi*

15.15-15.30 **Asymmetry in anisotropic ice-templated collagen scaffolds to mimic the structure of native tissue: from model to execution,** *Jamie Cyr*
15.30-15.45 **Morphological and mechanical evaluation of newly formed bone after spinal fusion treatment based on micro-CT scans,** *Bert van Rietbergen*
15.45-16.00 **An innovative sol-gel based hybrid biomaterial designed for bone tissue engineering,** *Henri Granel*

16.00-16.30 **Coffee Break** Trajectum & Expo Foyer

NATURAL BIOMATERIALS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE
Chair: Willeke Damen, Alicia Fernández-Colino
16.30-17.00 **Title T.B.C.,** *Manuela Gomez*
17.00-17.15 **Liquid platelet-rich fibrin (i-PRF) enhances the vascularization of a non-cross-linked collagen matrix in vivo,** *Shahram Ghanaati*
17.15-17.30 **Bone and spinal cord extracellular matrix hydrogels and their potential for utilisation in spinal cord injury,** *Natalija Tatic*
17.30-17.45 **Functionalized collagen scaffolds result in sustained postnatal regeneration after in utero closure of skin,** *Willeke Daamen*
17.45-18.00 **Antibacterial hydrogels based on Gellan Gum and Manuka honey for tissue engineering applications,** *Maria Addolorata Bonifacio*

Auditorium 2
NANOSTRUCTURED BIOMATERIALS FOR CELL REGULATION
Chair: Lino Ferreira, Nick Evans
09.45-10.15 **Light-activatable (nano)materials for cell regulation,** *Lino Ferreira*
10.15-10.45 **Emergent, collective behaviour of cell groups in long-range biomaterial mechanosensing ,** *Nick Evans*
10.45-11.00 **A High-throughput screening device to study 3D cell-material interactions,** *David Boaventura*
11.00-11.15 **Data-driven framework for rational design of nanotopographies on biomedical devices,** *Marie Cutiongco*
11.15-12.15 **Poster session C** Trajectum & Expo Foyer
12.15-13.15 **Lunch Break** Trajectum & Expo Foyer

COMPUTATIONAL TOOLS FOR BIOMATERIALS
Chair: Jan de Boer, Aurelie Carlier
14.30-15.00 **Application of computational methods to develop materials resistant**

to bacterial biofilm formation, *Andrew Hook*
15.00-15.30 **Computational tools for biomaterial design: from reverse engineering to mechanistic modeling,** *Lies Geris*
15.30-15.45 **Cell shape induced polarization: a computational modelling approach,** *Kerbaï Saïd Eroume*
15.45-16.00 **Analysis of sub-cellular structures in response to surface topographies,** *Linfeng Li*

16.00-16.30 **Coffee Break** Trajectum & Expo Foyer

BIOFABRICATION AND BIOMATERIALS FOR ADVANCED TISSUE GRAFTS AND MODELS
Chair: Carlos Mota, Riccardo Levato
16.30-17.00 **Designing and processing hydrogel bioinks for 3D printing applications,** *Jason Burdick*
17.00-17.30 **3D Printing and bioprinting with high spatial resolution, challenges and perspectives,** *Alexander Ovsianikov*
17.30-17.45 **Combining 3d-printing of a low-temperature setting calcium phosphate paste with melt electrowritten microfiber meshes for reinforcing interfaces in engineered osteochondral grafts,** *Paweena Diloksumpan*
17.45-18.00 **Magnetically responsive gelatin-based nanocomposite ink for remote control of 3D printed bio-inspired structures,** *Riccardo Tognato*

Room 0.4 - Parallel session
09.45-11.15 **The one billion euro gamble: are these the next four highest impact investment opportunities in biomaterials science,** *Andres Garcia, Serena Best, Abhay Pandit and Joachim Kohn*

11.15-12.15 **Poster session C** Trajectum & Expo Foyer
12.15-13.15 **Lunch Break** Trajectum & Expo Foyer

INTELLIGENT BIOMATERIAL PROPERTIES & APPLICATIONS
Chair: Hugo Fernandes, Urandelger Tuvshindorj
14.30-15.00 **Smart contact lens for biosensing and drug delivery applications,** *Sei Kwang Hahn*
15.00-15.15 **Spatiotemporal biomaterial modification via cytocompatible supramolecular complexation,** *Tom Kamperman*

15.15-15.30 **Couplings of macrophage phenotype, angiogenesis and bone formation in calcium phosphate ceramics,** *Rongquan Duan*
15.30-15.45 **High-throughput screening of topographically-patterned surfaces to identify novel bio-instructive & immunomodulatory materials,** *Mattew Vassey*
15.45-16.00 **Methylcellulose-based hydrogel for mechanobiology and cell sheet technology applications,** *Andrea Cochis*

16.00-16.30 **Coffee Break** Trajectum & Expo Foyer

NANOTECHNOLOGICAL TAILORING OF BIOMATERIALS
Chair: Mathhias Epple, Carina Kampleitner
16.30-16.45 **Optically Stable and Near-UV Activated Nanophosphors for in-vitro Dynamic Bioimaging,** *Georgios Sotiriou*
16.45-17.00 **Controllable synthesis of spherical calcium phosphate nanoparticles,** *Pichaporn Sutthavas*
17.00-17.15 **Nanopatterns to promote intercellular communication for cartilage regeneration,** *Ignasi Casanellas*
17.15-17.30 **Nanoceria-loaded nanostructured lipid carriers for the treatment of neurological diseases,** *Matteo Battaglini*
17.30-17.45 **Hyaluronan gradients for cells separation and identification,** *Ana Carvalho*
17.45-18.00 **Entrapment of autologous von Willebrand Factor on nanostructured substrates within a dynamic platelet assay,** *Joanna Ward*

Room 0.5 - Parallel session
ENGINEERING MICROENVIRONMENTS
Chair: Marlon Jetten, Aliaksei Vasilevich
09.45-10.00 **Engineering the tenocyte micro-environment by topographical architectures,** *Steven Vermeulen*
10.00-10.15 **Topography effects on macroscopic behavior and osteogenic differentiation of human bone marrow-derived mesenchymal stem cells: amplitude versus wavelength,** *Liangliang Yang*
10.15-10.30 **Biophyscial cues for modulation of tenogenic phenotype,** *Diana Gaspar*
10.30-10.45 **Studying cellular morphology using a high-throughput screening platform based on a library of arrayed cell-adhesive micro islands,** *Urandelger Tuvshindorj*
10.45-11.00 **Role of implant nanoroughness and bioactive coating on osseointegration and bacterial growth,** *Laila Damiati*

11.00-11.15 **Engineered Microenvironments for Efficient Regeneration of Bone Critical-Size Defects,** *Cristina Gonzalez-Garcia*

11.15-12.15 **Poster session C** Trajectum & Expo Foyer

12.15-13.15 **Lunch Break** Trajectum & Expo Foyer

DESIGN OF BIOMATERIALS FOR DRUG DELIVERY

Chair: Elzbieta Pamula, Jun Wu

14.30-14.45 **Multi-compartment collagen devices as modulators of skin fibrosis through controlled synergistic dual delivery of anti-fibrotics,** *João Coentro*

14.45-15.00 **Biodegradable microneedles for the delivery of proteins,** *Liliana Pires*

15.00-15.15 **pH-sensitive polymeric nanoparticles with antioxidant and antiinflammatory properties for the treatment of cisplatin-induced hearing loss,** *Maria Rosa Aguillar*

15.15-15.30 **Bioresorbable Silica Gel Fiber Systems - a Novel Platform Technology for Drug Release in Regenerative Therapies,** *Bastian Christ*

15.30-15.45 **Bio-resorbable polyesteramides: Aplatform for local sustained drug delivery concepts in management of pain,** *Jens Thies*

15.45-16.00 **Vancomycin and doxorubicin release from biodegradable ß-TCP-PLA nanocomposite scaffolds,** *Sanjaya Kumar Swain*

16.00-16.30 **Coffee Break** Trajectum & Expo Foyer

MODULAR ENGINEERING OF CELLS & TISSUES

Chair: Lorenzo Moroni, Naomi Lowry

16.30-16.45 **Computer Designed Topographical Surfaces for Instructing Cell Fate,** *Aliaksei Vasilevich*

16.45-17.00 **Synthesis of lithium carbonate nanoparticles with potential properties for bone tissue engineering,** *Covarrubias Cristian*

17.00-17.15 **Soft tissue biocompatibility of lithium, strontium and boron -doped bioactive silicate glasses,** *Inari Lyyra*

17.15-17.30 **Engineered bacterial biofilms to control stem cell differentiation,** *Aleixandre Rodrigo-Navarro*

17.30-17.45 **Enhancement of bone tissue regeneration via cross-talk between MSCs-derived osteogenic and angiogenic cells,** *Jidong Li*

17.45-18.00 **Immune response modulation by a Sr-releasing injectable biomaterial for bone regeneration,** *Ana Henriques Lourenço*

Room 0.2/0.3 - Parallel session

ANTIMICROBIAL ACTIVITY OF BIOMATERIALS

Chair: Wim de Jong, Jinlong Shao

09.45-10.00 **Lipid-based nanoparticles that counteract gastric infection burden,** *Christina Martins*

10.00-10.15 **The mechanism of action of surface-grafted caspofungin: definitive evidence from a series of Candida albicans mutants,** *Stephanie Lamont-Friedrich*

10.15-10.30 **Preparation of the antimicrobial surface with the antimicrobial peptide by click chemistry,** *Lin Wang*

10.30-10.45 **Photochemical internalization as a novel delivery strategy to enhance efficacy of antibiotic treatment of staphylococcal intracellular infection,** *Xiaolin Zhang*

10.45-11.00 **Graphene nanoplatelets coatings for antimicrobial silicone catheters,** *Iñes Concalves*

11.00-11.15 **Anti-biofilm coatings based on elastin-like recombinamers and antimicrobial peptides for preventing orthopedic implant infections,** *Sergio Acosta-Rodriguez*

11.15-12.15 **Poster session C** Trajectum & Expo Foyer

12.15-13.15 **Lunch Break** Trajectum & Expo Foyer

STEM CELL INTERACTIONS & BEHAVIOUR

Chair: Nicolas Dunne, Diana Lopes

14.30-14.45 **Material driven fibronectin assembly and growth factor presentation to investigate metabolic mechanisms for a bone marrow niche-like pericyte phenotype,** *Hannah Donnelly*

14.45-15.00 **Development of cold atmospheric plasma (CAP) functionalized membranes for the selective capture of adipose-derived mesenchymal stromal/stem cells,** *Tommaso Gallingani*

15.00-15.15 **Effect of ECM type and elasticity of iPS culture substrates on cardiac differentiation: cardiac marker expression and self-beating induction,** *Tetsuji Yamaoka*

15.15-15.30 **Differentiation of adipose tissue-derived stem cells into smooth muscle cells by chemical and mechanical stimuli for tissue engineering of heart valves,** *Elena Filova*

15.30-15.45 **Influence of microenvironmental cues on maintaining the phenotype of tenocytes,** *Dimitrios Tsiapalis*

15.45-16.00 **Zinc maintain ESC stemness through Zip7 activation via Akt pathway,** *Patricia Rico*

16.00-16.30 **Coffee Break** Trajectum & Expo Foyer

BIOINSPIRED DEVELOPMENTS IN BIOMATERIAL DESIGN
Chair: Maria Chatzinikolaidou, Daniel Pereira
16.30-16.45 **Effect of surface modification of nanofibers with glutamic acid and aspartic acid peptide on osteogenic differentiation of human mesenchymal stem cells,** *Ozan Karaman*
16.45-17.00 **Three-dimensional matrices of enamel like oriented calcium phosphate nanocrystals,** *Francesca Carella*
17.00-17.15 **Decellularized matrices as biomimetic platforms to unravel the role of CD44v6 in gastric cancer,** *Bianca n. Lourenço*
17.15-17.30 **Modulation of microstructural features of calcium phosphates for triggering specific osteoimmune response,** *Jordi Guillem-Marti*
17.30-17.45 **Material-driven fibronectin nanonetworks rescue collagen IV secretion in mutant cells,** *Marco Cantini*
17.45-18.00 **Collagen I based 3D networks as a tunable in vitro matrix for human mesenchymal stromal cells,** *Sarah Vogel*

Scientific Programme Schedule Thursday

Auditorium 1 - Plenary

08.45-09.30 **Can we grow valves inside the heart?,** *Carlijn Bouten,*
Chair: Silvia Fare, Chris Arts

09.30-10.15 **Klaas de Groot Award 2018: iomaterials - The Changing face of Bone Tissue Engineering,** *Lucy Di Silvio, Chair: Gino Ambrosio. Ana Paulo Pego*

10.15-10.45 **Coffee Break** Trajectum & Expo Foyer

Auditorium 1 - Parallel session

ENGINEERING ORTHOPEDIC TISSUES

Chair: Jan Schrooten, Francesca Giacomini

10.45-11.15 **Posterior spinal fusion with a microporous ceramic: a randomized, intra-patient controlled trial,** *Moyo Kruyt*

11.15-11.30 **Identification and in vitro screening of osteogenic metabolites through supplement-free nanovibration-driven mesenchymal stem cell differentiation,** *Tom Hodgkinson*

11.30-11.45 **A comparison of potential fibrillin-1 fragments for the functionalisation of a novel anterior cruciate ligament biomaterial scaffold,** *Zara Smith*

11.45-12.00 **Anisotropic direct current field stimulation significantly promotes the synthesis of cartilage extracellular matrix,** *Jun Hotta*

12.00-12.15 **Nanocomposite chemically modified hyaluronic acid hydrogel based on sol-gel methods for osteochondral regeneration,** *Alfredo Ronca*

Auditorium 2 - Parallel session

BIOMATERIALS BASED ON COLLOIDAL BLOCKS

Chair: Sander Leeuwenburgh, Jürgen Groll

10.45-11.15 **Bioactive glass nanoparticles with enhanced functionalities: progress and opportunities in biomedical applications**, *Aldo Boccaccini*

11.15-11.45 **Structuring hydrogels to enable suspended manufacture of cell-loaded constructs,** *Liam Grover*

11.45-12.00 **Composite colloidal gels with self-healing properties for regenerative medicine,** *Mani Diba*

12.00-12.15 **Protein self-assembly at oil-water interfaces controls nanoscale mechanics, cell adhesion and stem cell fate decision,** *Julien Gautrot*

Room 0.4 - Parallel session

INTERACTIONS IN AND WITH 3D SCAFFOLDS

Chair: Katarina Maniura, Faezeh Shalchy

10.45-11.00 **Platelet lysate based hydrogels with tunable mechanical properties as platforms for 3D cell culture,** *Sara Santos*

11.00-11.15 **Polyurethane foam scaffolds interpenetrated with crosslinked gelatin hydrogel for adipose tissue regeneration,** *Rita Sorrentino*

11.15-11.30 **Mechanobiology-enhanced tissue engineering: a collagen-scaffold-based delivery system for accelerating bone repair by activating JNK3 in stem cells,** *Arlyng Gonzalez-Vazquez*

11.30-11.45 **Designing peptide / graphene derivatives hybrid hydrogels through fine tuning of molecular interactions,** *Jacek Wychowaniec*

11.45-12.00 **Novel hierarchical silk fibroin-based scaffolds incorporating Sr- and Zn-ions for osteochondral tissue engineering,** *Viviana Ribeiro*

12.00-12.15 **Design of a perfusion bioreactor for the high-throughput study of combinations of extracellular matrix proteins on human mesenchymal stem cells performance in dynamic 3D conditions,** *Diana Lopes*

Room 0.5 - Parallel session

TOWARDS TISSUE-SPECIFIC APPLICATION OF BIOMATERIALS

Chair: Marco Lopez, Daniela Petre

10.45-11.00 **Importance of microenvironment, pH, in the design of biomaterials used for osteoporosis patients,** *Haobo Pan*

11.00-11.15 **A comparison of the linking arm effect on the biological performance of a CD31 agonist directly grafted on L605 CoCr alloy by a plasma based multi-step strategy,** *Sergio Diaz-Rodriguez*

11.15-11.30 **Multimodal Porogen Platforms for Calcium Phosphate Cements,** *Irene Lodoso-Torrecilla*

11.30-11.45 **Development of new methods for implant/tissue bonding and next generation dental/bioadhesives,** *Edward Cozens*

11.45-12.00 **The development of multi-substituted apatite for bone tissue repair,** *Naomi Lowry*

12.00-12.15 **Novel in vitro method for the evaluation of bioactive materials,** *Weitian Zhao*

Room 0.2/0.3 - Parallel session

INSTRUCTIVE SYNTHETIC BIOMATERIALS

Chair: Matt Baker, Helen Clough

10.45-11.00 **Towards corneal endothelium repair using synthetic polymer membranes,** *Jasper van Hoorick*
11.00-11.15 **Degradable Hydrogels Based on Star Shaped Copolypeptides: From Synthesis to 3D printing,** *Andreas Heise*
11.15-11.30 **Hyperbaric oxygen-generating hydrogels for facilitating wound healing process,** *Kyung Min Park*
11.30-11.45 **Oxygen-generating alginate hydrogels for enhanced wound healing process,** *Jeonil Kang*
11.45-12.00 **Synergistic effect of polymer molecular weight and Laponite concentration to tune the cell response of synthetic hydrogels,** *Arn Mignon*
12.00-12.15 **Synthetic Light-Curable Polymeric Materials Provide a Supportive Niche for Dental Pulp Stem Cells,** *Adam Celiz*

Auditorium 1 - Plenary
12.15-13.15 **Closing ceremony & awards,** *Pamela Habibovic, Michael Gelinsky*

13.15-13.30 **Take-away Lunch** Trajectum

Lunch symposia

Monday 10 September

Time: 12.30 - 13.15 hrs.

Room: 0.8

Registration is not required, however, the capacity of the room is limited and participation is based on a first come first serve base.

Programme:

Intorduction to BONE | Paul Wieringa

Melt ESP technology | Spraybase®

Electrospinning Technology to develop biomedical smart implants | ELECTROSPINNING COMPANY

Invited lecture | Jürgen Groll

Tuesday 11 September

cambioceramics

Beyond the pill: Smart drug delivery with Calcium Phosphate carriers – a scientific, regulatory and clinical perspective

Time: 12.15 - 13.00 hrs.

Room: 0.8

Registration is not required, however, the capacity of the room is limited and participation is based on a first come first serve base.

Programme:

Calcium phosphates for effective local drug delivery | Michael Gelinsky

Smart CaP based drug delivery products: clinical perspective | Ruben Osnabrugge

Regulatory strategies for drug eluting calcium phosphate carriers": "How to find your way in a jungle of rules | Herman Pieterse

Panel discussion

Wednesday 12 September

Materials driven Regeneration

Time: 12.15 - 13.00 hrs.

Room: 0.8

Registration is not required, however, the capacity of the room is limited and participation is based on a first come first serve base.

Programme:

Developing multiscale computational models for biological processes in tissue regeneration and biomaterial interactions | Aurelie Carlier

In situ cardiovascular tissue engineering | Anthal Smits

Imaging of the hearth at the interface of regenerative medicine and cardiology | Steven Chamuleau

Research Center for Materials-Driven Regeneration | Olga Goor

Satellite symposia

Wound care by design; biomaterials in action

Organizer: Interreg 2Seas DERMA Project

Date: Thursday September 13th

Room: Room 0.4

Price: Free of charge

The DERMA project (Design of Enabling Regenerative Materials) is designing and developing innovative technologies for the prevention and management of chronic dermal wounds with a special focus on elderly people and diabetics. Our work is expected to provide both social and economic benefits for the 2 Seas region (http://www.derma2seas.eu/).

Programme:

Time	Dissemination talks
13.40 hrs.	**Andrew Hook:** High throughput methods for the discovery of novel biomaterials: exploring the biological-material interface
14.00 hrs.	**Rachel Forss:** Wound Care & Ulceration: The Clinical Challenge.
14.20 hrs.	**Karel Claes:** Different wound dressings in the plastic surgery department and burn centre of Ghent University Hospital.
14.40 hrs.	**Gilles Brackman:** Advanced skin and wound care: Taking into account the complexity of the wound.
15.00 hrs.	**Matteo Santin:** To be announced shortly!
15.30 hrs.	**Stakeholder networking reception**
17.00 hrs.	End of programme

Young Scientist Forum (YSF)

Date: Sunday September 9th
Room: Room 0.4
Time: 14:00-18:00
Price: Free of charge

YSF Workshop: "How to publish journal articles''

Dr. Neil Hammond, Royal Society of Chemistry.

The YSF workshop aims at promoting biomaterials education and training in Europe, emphasizing the existing and emerging career, as well as research opportunities in the field. Discussion is fostered by creating an interactive environment amongst participants, thereby promoting an enriching discussion on topics that concern everyone active in Biomaterials. Furthermore, YSF encourages and provides opportunities for collaboration within the biomaterials European community.

Date: Tuesday September 11th
Room: Room 2.9/2.10
Time: 16:00-17:30
Price: Fully booked

YSF: "An interactive guide to good science writing"

Dr. Neil Hammond, Royal Society of Chemistry.

A special interactive session, providing insight into the guiding principles behind good scientific writing. The session will cover rules for the structuring of journal articles, along with practical advice on the most common errors to avoid, demonstrated through examples. Attendees are encouraged to bring along their current/latest work to receive tailored feedback. In order to facilitate direct feedback on an individual basis, attendance will be limited to 15 persons.

Scientific Programme abstracts Monday

Plenary lecture 08:45 - 09:30 Auditorium I 10/09/2018

Oral presentation

Contrasting nanomedicine claims versus human clinical performance: why such disconnects?

David W. Grainger
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Departments of Biomedical Engineering, and of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, USA

Thousands of publications to date claim evidence for nanomedicine anti-tumor drug delivery efficacy in cell and animal models of cancer. A few of these materials, particles or therapies are approved for human use. Sufficient global human clinical records are now published that allow meta-analyses of human effectiveness for limited nanomedicines. Few nanomedicines to date exhibit enhanced clinical anti-cancer effectiveness compared to free drug (clinical standard of care). Therefore, nanomedicines have yet to deliver much of their anticipated therapeutic value to patients, despite much hype and their increased costs. I will examine some case studies for nanomedicines now in clinical use and even commercialized. Scientific research credibility, lack of in vitro-in vivo correlations, and exaggeration of preclinical outcomes for nanomedicines are increasingly questioned by all stakeholders: political representatives, industrial and academic scientific peers, physicians patients, citizens and taxpayers. Erosion of this collective research support base must be stopped for research programs in advanced therapeutics and biomaterials to continue. Pressures in academic publishing as a common global academic performance indicator may be one important factor driving this inequality. Nevertheless, evidence supporting improved cancer treatments through applications of nanotechnology are largely unfounded by human experiences to date. Due to the costs to society and to industry for validating experimental nanomedicine designs that fail to produce human therapeutic progress, we must redouble efforts to (1) improve in vitro-in vivo validations, (2) examine differing biodistributions of nanomaterials in animal models versus humans, (3) better match drugs and their delivery systems to address disease sites, and (4) reduce the exaggeration in reporting preclinical model results that fail to translate to human use.

ESB George Winter Award 2018: 09:30 - 10:15 Auditorium I 10/09/2018

Oral presentation

Role of Functional Polymers in the development of Biomaterials for Application in the Biomedical Field

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The contribution of polymers to the development and application of biomaterials during the last 50 years has been well recognised and patented in very good products (prosthesis, devices, cements, bioadhesive membranes, drug delivery systems, etc.). In this presentation we will give information of the contributions of my research group and the most appreciate colaborations with very well recognised groups to the application of functional polymers to the development of advanced systems for the application of devices, drug delivery systems and “Polymer Drugs”.

The enormous possibilities offered by the macromolecular chemistry of polymer systems give opportunities for the development of very interesting formulations to build up the necessary components to reorganice the human body as well as the development of targeting and drug delivery systems. Essentially there is a clear relationship in the design applied by the nature in the formation of all the components and elements of the human body and the fabrication of biomimetic macromolecular systems for applications in the body for restoration of functions and the logical and controlled way of the application of bioactive molecules or macromoleculaes, like most of the drug delivery systems, growth factor or enzimatic compounds.

Examples of diferent systems developped and applied in the clinical practice by the group will be presented of different polymer families.

The development of biodegradable systems and composites based on polysaccharides for drug delivery systems has been an important cooperation with the 3B' group directed by Rui Reis and others, fruit of cooperation with Jan Feijen, Pedro Guillen, Antonio Lopez Bravo, and J. Antonio de Pedro. Bassically we interpret how to offer biodegradable systems or partially biodegradable systems to guaranttee the biomechanical estability in bone fractures. In this sense we developped partially acrylic – polylactide composites with a very good integration in the bone tissue without loss of the biomechanical estability during the remodelation process of the fracture.

One of the main activities of the group has been the development of bioactive polymer systems "polymer Therapeutics", based on self-assembling polymers in a biomimetic way.The design of copolymer systems with the adequated balance of hydrophilic and hydrophobic components has oppened excellent opportunities for the preparation of advanced bioactive systems with properties as antioxidant, antitumoral or antibacterial functions. Cooperative works with excellent groups directed by Sanjukta Deb, James Kirkpatrick, Andres García, Buddy Ratner, Claudio Migliaresi,Gino Ambrosio, Susi Borsachiello and many others has been well recognised.

Acknowledgements: The participation of all the components of my group, as well as the financial support from EU, CIBER-BBN, CSIC,and MAT programs is acknowledged.

Creative engineering ideas 10:45 - 12:15 Auditorium I 10/09/2018

Oral presentation

Bioinspired functionalisation of soft materials: materials and strategies

Katharina Maniura
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Biointerfaces, Empa, Swiss Laboratories for Materials Science and Technology, St. Gallen, Switzerland

Tissue engineering, disease modeling, and drug screening represent areas of research which benefit from advanced hydrogel systems that allow precise control of cells in their 3D microenvironments. A smart and highly cell-friendly hydrogel was developed that allows true 3D microfabrication of complex structures. Basis is a cell-compatible hydrogel resist that is sufficiently photosensitive, cell-degradable, and permissive to support 3D cell growth and multiphoton lithography is employed to paint functions in the 3D space.

The photosensitive cell responsive hydrogel is composed of peptide-crosslinked polyvinyl alcohol (PVA) and is designed to expand the biological applications of MPL. Hydrogels including cells are formed by use of ultraviolet light within 1 min. They provide fully synthetic matrices that are instructive for cell-matrix remodeling, multicellular morphogenesis, and protease-mediated cell invasion. Cell invasion can be precisely guided in 3D with micrometer-scale spatial resolution.

On the other hand there is need for new concepts for materials defending bacterial infection- e.g. for materials for wound healing. The occurrence of resistance to antibiotics has posed a high demand for novel strategies to fight bacterial infections. Antimicrobial peptides (AMPs) eliciting broad-spectrum activity and low likelihood for resistances, such as the model peptide “nisin” are among the most prominent candidates to achieve this goal. Hence, AMPs are a promising alternative to conventional antibiotics. However, their poor solubility in water and sensitivity to degradation has limited their application. Plant-derived nanocelluloses such as TEMPO-oxidized nanofibrillated cellulose (TONFC) are natural, non-toxic nanofibers with a high specific surface area that were previously shown to provide superior loading capacities for different active biomolecules. These attributes make TONFC promising substrates for the development of multi-purpose bioactive materials, for example as AMP-nanocarriers. The potential use of the polyelectrolyte TONFC fibrils as nanocarrier depends strongly on the capability to preserve their nanoscale dimensional properties. Hence, the understanding the three-dimensional nanostructures of TONFC fibrils at different conditions and upon immobilizion of nisin is of great interest for the design of advanced antimicrobial materials.

Creative engineering ideas 10:45 - 12:15 Auditorium I 10/09/2018

Oral presentation

17 Thermoresponsive nanofibers for engineering supramolecularly assembled tissue substitutes

Andrea De Pieri[1], Alexander Gorelov[2], Yuri Rochev[3], Dimitrios Zeugolis[4]
[1]Proxy Biomedical, Galway, Ireland
[2]4School of Chemistry, University College Dublin, Ireland
[3]Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), Ireland
[4]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL) National, Ireland

INTRODUCTION:

Tissue engineering by self-assembly is a technique that consists of growing cells on surfaces made of thermoresponsive polymers, allowing the production of contiguous cell sheets. In this approach cell-cell junctions and deposited extracellular matrix (ECM) remain intact, which provides a better cell localisation at the site of injury [1]. However, these systems lack the possibility to introduce topographical cues, that are fundamental for the organisation of many types of tissues. Moreover, the fabrication of ECM-rich cell sheets would be highly desirable. This limitation could be overcome by inducing macromolecular crowding (MMC) conditions during the culture period [2].

Herein we venture to fabricate aligned electrospun thermoresponsive nanofibres to sustain growth and detachment of ECM-rich cell sheets in the presence of a MMC microenvironment.

METHODS:

85% N-isopropylacrylamide/15% N-tert-butylacrylamide (pNIPAm/NTBA) copolymer was used. To create aligned nanofibers, the polymer were electrospun and collected on a mandrel rotating at 2000 rpm. Fibres diameter and orientation were assessed through scanning electron microscopy (SEM). Human adipose derived stem cells (hADSCs) were treated with media containing macromolecular crowders to enhance matrix deposition. Cell metabolic activity, proliferation, viability and morphology were assessed and immunocytochemistry was conducted in order to estimate matrix deposition and composition. Cell detachment was performed by decreasing the temperature of culture to 10°C for 20 minutes.

RESULTS AND DISCUSSION:

The electrospinning process resulted in the production of pNIPAm/NTBA fibres in a diameter range from 1 to 2 µm and an overall alignment of 80% (fig 1 A). Cell viability, proliferation and metabolic activity revealed that hADSCs were able to grow on the thermoresponsive pNIPAm/NTBA scaffold. The cells were able to align on the fibers after 3 days (fig 1 B) and they were able to detach as an intact cell sheet in presence of MMC (fig 1 C). Moreover, it was demonstrated that MMC, by a volume extrusion effect, enhances Collagen type I deposition, which is one of the main components of the ECM (fig 2). Collectively the pNIPAm/NTBA thermoresponsive fibres were able to sustain growth and detachment of ECM-rich cell sheets.

CONCLUSION:

By recapitulating the hierarchical organised structure of native tissues like tendons, we aim to improve the development of supramolecular assembled tissue substitute based on the principle of in vitro organogenesis

REFERENCES:

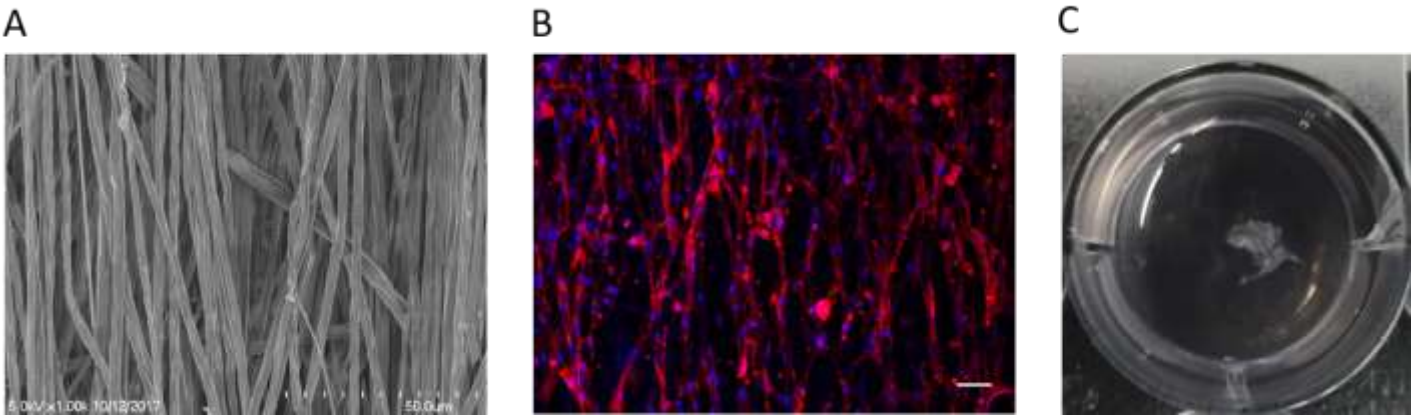
Yang et al., Biomaterials. ;28: 5033-5043, 2007

Satyam. et al., Adv Mater. 26: 3024-3034, 2014

ACKNOWLEDGMENTS:

Tendon Therapy Train-H2020-MSCA-ITN-2015-ETN (Grant no: 676338) and Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM) (Grant no: 13/RC/2073)

Picture 1: Caption 1: SEM image of the pNIPAm/NTBA fibres (A). hADSC aligned on the fibres after 3 days in culture (B) and detached as an intact cell sheet in the presence



Creative engineering ideas 10:45 - 12:15 Auditorium I 10/09/2018

Oral presentation

257 Microfluidically generated single cell microgels as pericellular niches with temporally controlled biochemical and biophysical properties

Jeroen Leijten, Tom Kamperman, Sieger Henke, Marcel Karperien
University Twente, Enschede, Netherlands

INTRODUCTION:

The modular design of tissues is of indispensable importance for proper organ function. A key example of this phenomenon is extracellular matrix, which is naturally modular. Specifically, cells are entrapped in a niche composed of a thin layer of pericellular matrix, which in turn is located in a bulk of territorial matrix. These two matrix types are highly distinct in their biochemical and biophysical properties: while the pericellular matrix provides a stimulating cellular microniche, the territorial matrix gives rise to organ level characteristics. Incorporating such a modular design into biomaterials is expected to allow engineered tissues to more accurately emulate native tissues. However, it has remained a grand challenge to engineer the functional counterpart of life's smallest living building block: a cell within its pericellular matrix.

METHODS:

A microfluidic droplet generation platform with delayed gelation was designed to produce enzymatically crosslinked single cell microgels that were mere micrometers larger than the single cell they encapsulated. The single cell microgels were engineered with on-demand tunable biophysical (temporal stiffening) and biochemical (dynamic macromolecular exchange) properties. The effects of temporal stiffening on the microgels were investigated using AFM and fluorescence quantitation and its effect on stem cell lineage commitment using histology, immunohistochemistry, label-free imaging (i.e., Hyperspectral Raman and CARS), qPCR, and RNAseq.

RESULTS AND DISCUSSION:

Single cell laden microgels with a diameter of 35 micrometer were produced in a monodisperse manner via on-chip enzymatic crosslinking of discrete prepolymer droplets. The single cell microgels remained metabolically active for at least month without any notable cell egression. The microgels' Young's modulus could be dynamically tuned from 2 to 50 kPa. Single cell analysis revealed that softer microgels stimulated adipogenesis, while stiffer microgels induced osteogenesis. Importantly, temporal stiffening of microgels revealed that the first three days of differentiation determined the stiffness-induced stem cell fate decision. Subsequently, we combined our single cell microgels with distinct biomaterials to create advanced bioinks. This modular approach effectively uncoupled the engineered tissues pericellular and territorial environments, which allowed for an unprecedented control over the design and behavior of living implants.

CONCLUSION:

We here present a novel microfluidic single cell microgel-based concept that advances the engineering of hierarchical tissues by incorporating pericellular microniches within biomaterials in a facile yet highly controllable manner.

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ACKNOWLEDGMENTS:

Dr. Leijten would like to thank the European Research Council (Starting Grant, #759425) for providing financial support to this project.

Picture 1:

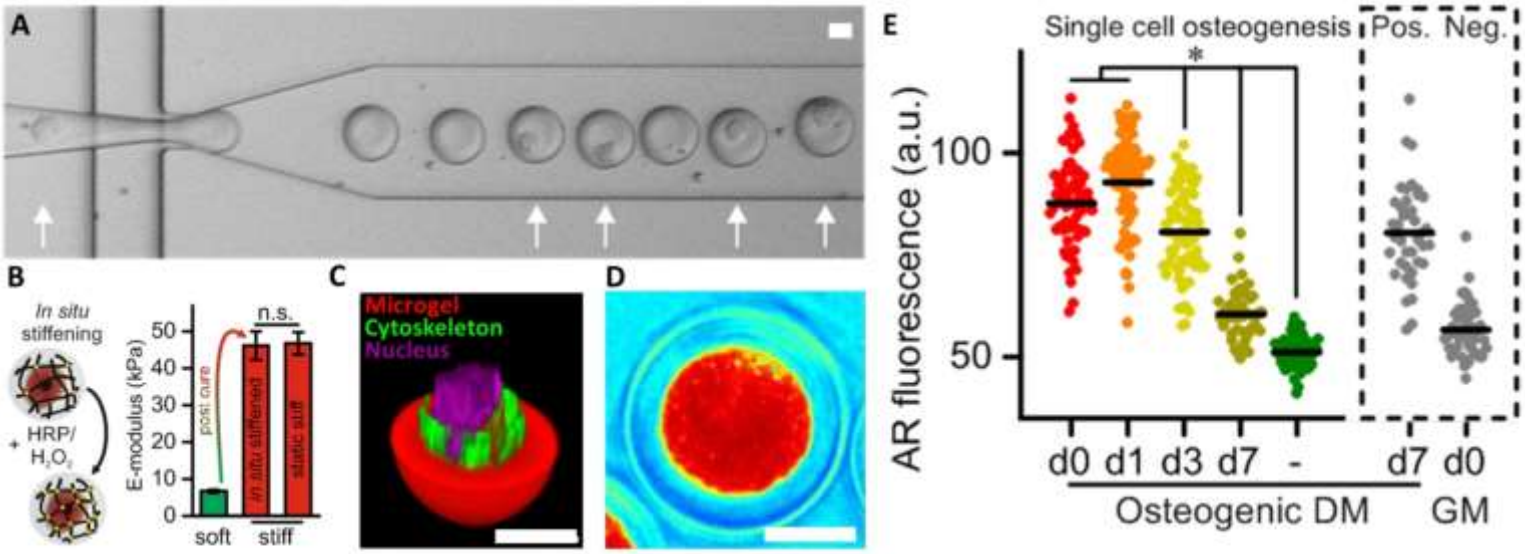

Figure 1: On-demand stiffening single cell microgels. A) Microfluidic formation of cell-in-prepolymer droplets (white arrows). B) Enzymatic post-crosslinking allows for on-demand microgel stiffening. C) 3D reconstruction of confocal fluorescent microscopy confirmed that each cell was located in the center of its microgel. D) Fluorescent investigation of biomarkers (calcification) allows for facile analysis of stem cell differentiation with single cell resolution. E) Microgel stiffness played a crucial role in the osteogenesis of mesenchymal stem cell during the first three days of differentiation, but not during the subsequent days.

Creative engineering ideas 10:45 - 12:15 Auditorium I 10/09/2018

Oral presentation

391 Macromolecular crowding, mechanical stimulation and oxygen tension in tissue engineering - A step closer to cell-assembled biomaterials

Dimitrios Zeugolis, D Gaspar, D Tsiapalis, V Graceffa, S Kearns, J Kelly
NUI Galway, Galway, Ireland

INTRODUCTION:

Macromolecular crowding is a biophysical phenomenon that governs intracellular and extracellular processes and increases thermodynamic activities by several orders of magnitude. *In vitro* data have demonstrated that macromolecular crowding dramatically accelerates extracellular matrix (ECM) deposition in skin, lung and corneal fibroblast and naïve mesenchymal stem cell cultures (1-4). Herein, we hypothesized that macromolecular crowding alone or in combination with other *in vitro* microenvironment modulators (e.g. oxygen tension, mechanical loading) will control cell function in vitro.

METHODS:

Human tendons were obtained from University Hospital Galway, after obtaining appropriate licenses, ethical approvals and patient consent. Subsequently, tenocytes were extracted using the migration method. Human chondrocytes were purchased from Lonza (Switzerland). Human osteoblasts were purchased from ATCC (UK). Human bone marrow was purchased from Lonza (USA). For variable oxygen tension cultures, a Coy Lab (USA) hypoxia chamber was used. For mechanical loading cultures, a Cell Scale (Canada) MCFX uniaxial stimulator bioreactor was used. all experiments were conducted at passage 3 to 5. All cultures were supplemented with ascorbic acid sodium salt (Sigma Aldrich, UK). Macromolecular crowding was carried out using 50 to 100 *m*g/ml

carrageenan (Sigma Aldrich, UK). ECM deposition was assessed using SDS-PAGE (BioRad, UK) and immunocytochemistry (ABCAM, UK) analysis. Gene analysis was conducted using a gene array (Roche, Ireland).

RESULTS AND DISCUSSION:

Macromolecular crowding (MMC) enhanced deposition of collagen types I and III in tenocytes and bone marrow stem cells. Gene analysis showed upregulation of scleraxis, thrombospondin-4 and cartilage oligomeric matrix protein in tenocytes after 7 days of mechanical stimulation. BMSCs exhibited upregulation of alkaline phosphatase under MMC and downregulation of collagen type I at day 3 under mechanical stimulation in the absence of MMC. Human tenocytes treated with MMC at 2% O_2 tension showed increased collagen type I synthesis and deposition after 7 days. In chondrocyte culture, MMC both in monolayer and alginate system, increased collagen type I deposition, whilst collagen type II was barely detectable.

CONCLUSION:

This study provides insight into modulation of cell behavior and phenotype using microenvironmental cues.

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ACKNOWLEDGMENTS:

The authors would like to acknowledge Science Foundation Ireland (15/CDA/3629 and 13/RC/2073) and the European Union (H2020, ITN, 676338) for financial support.

Creative engineering ideas 10:45 - 12:15 Auditorium I 10/09/2018

Oral presentation

21 Thermo-responsive macrocarriers containing polycaprolactone grafted with Poly(N-isopropyl acrylamide) for cell culture

Linh Nguyen, Akinlolu Odeleye, Chih-Yao Chui, Hua Ye, Zhanfeng Cui

University of Oxford, Oxford, United Kingdom

INTRODUCTION:

Thermos-responsive containing poly (N-isopropyl acrylamide) (PNIPAAm) have gained particular attention because of their ability to propagate and recover cells without physical damage[1]. This newly developed technique has been shown not to alter cell physiology, morphology and immunophenotype[2]. In the current study, we used polycaprolactone (PCL) beads coated its surface with PNIPAAm. The purpose of this study is to produce thermos-responsive macrobeads suitable for cell harvesting without the use of generally employed proteolytic enzymes.

METHODS:

PCL-PNIPAAm beads were prepared through amidation reaction in the presence of EDC and NHS and were characterized and confirmed by FTIR, XPS, SEM and EDS. Human dermal fibroblast cells (HDF) and Mesenchymal stem cells (MSC) viability and proliferation were determined by CCK-8 assay and Live-Dead assay. ECM protein

expression of HDF was analysed by immunofluorescence staining and western blot analysis. All quantitative data were expressed as mean ± SD. Statistical analysis was performed with two-way analysis of variance (ANOVA) with Tukey's honest significant difference post hoc test.

RESULTS AND DISCUSSION:

PCL-PNIPAAm allowed HDF and MSC to adhere, spread, and grow successfully. By reducing the temperature to below 30°C, more than 70% of HDF cells were detached from PCL-PNIPAAm with around 85% cell viability. The cell detachment ratio by trypsin treatment was higher than by reduced temperature, however, cell detachment from PCL-PNIPAAm by temperature change significantly reduced cell death and increased cell viability. The expression of some structural ECM proteins such as Laminin and Fibronectin was also found affected by trysinization process but not with reduced temperature process.

Unlike enzymatic treatments, this method of detachment may reduce the risk of damage to the physiology of the cell[3]. Thermo-responsive macrocarriers could provide the next generation of cell detachment technology for clinical applications such as tissue engineering, cell transplantation and biological production.

CONCLUSION:

In-vitro studies confirmed that the newly grafted beads were non-toxic, risk-free and biocompatible with HDF and MSC. By reducing the temperature from 37°C to 30°C, more than 70% of the cells were collected without the need for physical forces or enzyme treatment. The less damage to the cells and the ECM protein expression for cells detached from macrocarriers by temperature reduction compared to trypsin treatment confirmed the potential of thermos-responsive macrocarriers for future use in large-scale cell recovery.

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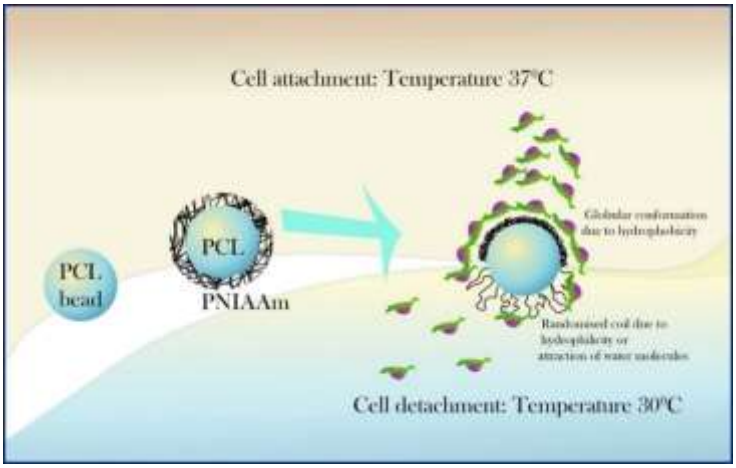
ACKNOWLEDGMENTS:

This work was supported by China Regenerative Medicine International (CRMI).



Picture 1: Caption 1: Figure 1. Schematic diagram of thermal response of cells on PNIPAAm grafted on PCL surfaces

Jean Leray Award 2018 14:15 – 15:00 Auditorium I 10/09/2018

Oral presentation

Meta-biomaterials

Amir A Zadpoor[1]
[1]Department of Biomechanical Engineering, Delft University of Technology

Metamaterials have emerged as promising candidates for creating advanced functionalities thorough adjustment of their mechanical, electromagnetic, or acoustic properties. The properties and, thus, functionalities of metamaterials are direct consequences of their small-scale architecture. Given the fact that design of arbitrarily complex micro-architectures is crucial for creating properties and functionalities, the advances made in additive manufacturing (3D printing) techniques are of particular relevance to the design and manufacturing of metamaterials. For biomedical applications, this creates a great opportunity to develop porous biomaterials with unprecedented combinations of mechanical, mass transport (permeability, diffusivity), and biological properties. Additively manufactured porous biomaterials are, however, very limited in terms of the surface access they allow, meaning that surface-related functionalities cannot be easily added to such biomaterials. Starting from a flat shape and folding the porous biomaterials up using origami techniques is a solution that allows for fabrication of porous biomaterials that combine properties originating from their 3D porous structure with those stemming from surface features (e.g. surface nanopatterns). In this talk, I will present an overview of the research carried out in my lab that has led to introducing the concept of meta-biomaterials and will discuss how meta-biomaterials could be used to improve bone tissue regeneration and prevent biomaterials-associated infections.

A part of the research for this paper was financially supported by the Prosperos project, funded by the Interreg VA Flanders – The Netherlands program, CCI grant no. 2014TC16RFCB046.

Electrospinning techniques & applications 16:30 - 18:00 Auditorium I 10/09/2018

Oral presentation

Electrospinning techniques & possibilities

Fang Yang
Department of Biomaterials, Radboud University Medical Center, The Netherlands

Electrospinning is a cost-efficient technique to prepare ultrafine polymeric fibers, which can be easily carried out in a laboratory and scaled up to an industrial process. It utilizes electrostatic forces to spin polymer solutions or melts into whipped jets, resulting in continuous fibers with diameters from a few nanometers to micrometers after solvent evaporation in the spinning process. Along with the booming research in the field of tissue engineering and regenerative medicine, electrospinning has gained exponentially increasing popularity as it can be used to generate a large variety of novel structured materials for biomedical applications, including tissue engineering scaffolds and drug carriers. Here, we summarized the methods of using electrospinning to produce one-dimensional fiber bundles/yarns, two-dimensional meshes, and three-dimensional scaffolds. Examples of applying such electrospun structures were given based on our research work.1-4 In addition, we underlined the techniques of using electrospinning for drug or growth factor delivery, including physical adsorption, blend electrospinning, coaxial electrospinning, and covalent immobilization. Moreover, we highlighted the use of a nano-in-nano system, i.e. enrichment of electrospun fibers with additional nanosized materials for the improvement of drug delivery.5 Existing challenges and opportunities of using electrospinning for biomedical use were identified based on our experiences and the literature survey.

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Electrospinning techniques & applications 16:30 - 18:00 Auditorium I 10/09/2018

Oral presentation

585 Bioinspired topographically-tailored electrospun substrates for studying mesenchymal stromal cell behaviour

Beatriz jc Monteiro[1], Thomas Patterson[1], Ilida Ortega[1], Paul Hatton[1], Simon Cool[2]
[1]The University of Sheffield, Sheffield, United Kingdom
[2]Agency for Science, Technology and Research (A*STAR), Singapore

INTRODUCTION:

Musculoskeletal disorders including trauma, bone-related disease and congenital defects have the potential to severely reduce human quality of life. Understanding the regeneration mechanisms associated to musculoskeletal tissues is a complex and challenging task; there is a pressing need for the development of new *in vitro* models that reproduce to a higher degree how cells with intrinsic regenerative capability are found within their *in vivo* native environments [1]. The aim of this research is to fabricate electrospun 3D biofunctional *in vitro* models using topographical cues and incorporation of specific biomolecules to ultimately influence mesenchymal stromal cell (MSC) behaviour towards osteogenic differentiation pathways.

METHODS:

Patterned stainless steel collectors were designed via CAD software and manufactured using the additive manufacturing technique selective laser melting (SLM). Polycaprolactone (PCL) was electrospun onto these templates to produce micropatterned scaffolds. Scaffolds were biofunctionalised with collagen and heparin and its content assessed. After scaffolds characterisation Rat MSCs were cultured onto coated and non-coated micropatterned and plain scaffolds in order to assess its effect on cell morphology (DAPI; FITC) and viability over time (Presto Blue®) (n=3). Statistical analyses were performed on GraphPad Prism software using one way analysis of variance (ANOVA) where p values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION:

Micropatterned membranes were produced by electrospinning, with the fibrous scaffolds mimicking extracellular matrix structure. SEM imaging found that sterilisation and functionalisation did not alter the scaffolds fibrous structure. Collagen and heparin were used to biofunctionalise the membranes. Optical imaging and staining showed successful biofunctionalization of collagen and heparin of the scaffolds through adsorption. Metabolic assays showed that the scaffolds could support MSC survival and viability over time.

CONCLUSION:

Combining topographical cues with biomolecules into electrospun scaffolds is a novel strategy towards the development of bioinspired cell responsive environments. Biofunctionalised micropatterned scaffolds supported cell

attachment. Future work will focus on the impact of surface topography and biomolecules on cells osteogenic potential. This innovative methodology will provide us with new tools to enable a better understanding and control of stem cell fate, opening the door to the development of the new generation of biomedical devices for musculoskeletal regeneration.

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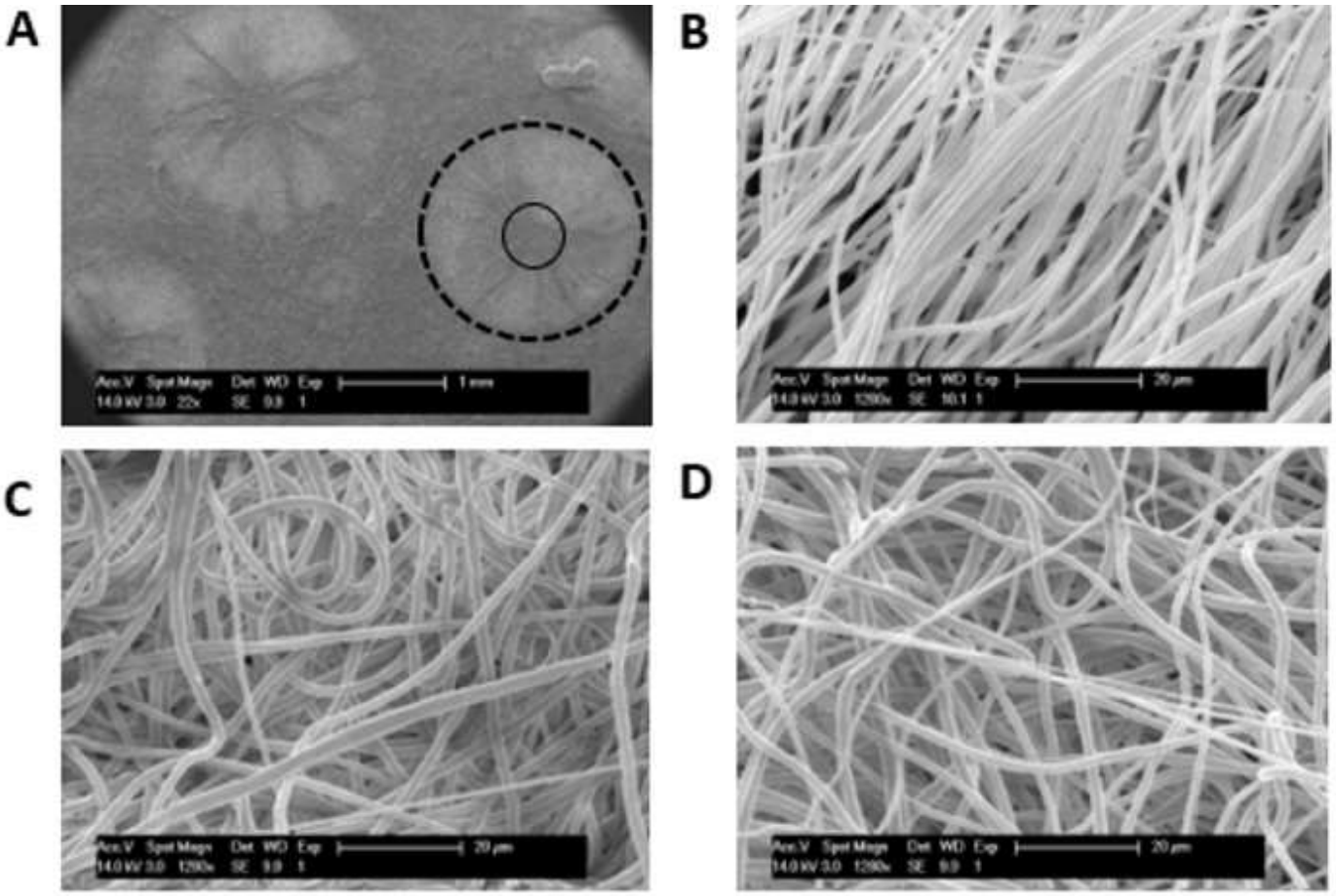
ACKNOWLEDGMENTS:

The authors would like to thank MeDe for providing financial support to this project.



Picture 1: Caption 1: SEM images of micropatterned PCL scaffolds and zoom in images of the different fibre distributions existing within one of our micropattern

Electrospinning techniques & applications 16:30 - 18:00 Auditorium I 10/09/2018

Oral presentation

344 Future trends in the electrospinning technique: the importance of using of benign solvents

Liliana Liverani[1], Aldo R. Boccaccini[2]
[1]University of Erlangen-Nuremberg, Erlangen, Germany
[2]University of Erlangen-Nuremberg / Institute of Biomaterials, Germany

INTRODUCTION:

The electrospinning is an established method for the obtainment of fibers for several applications[1]. Solvent systems commonly used for this process are toxic and the presence of their residuals in the obtained fibers could limit their biomedical applications or it forces the use of post-processing treatment for the neutralization of toxic remnants. The progressive elimination of their use in favour of benign (environmental friendly, non-toxic) solvents offers several benefits, like the prevention of proteins denaturation during the solution preparation and the process itself, improvements in laboratory workers safety and reduction of the environmental impact. Considering the novelty in the field, even if the use of benign solvents requires longer time for the process optimization, studies increasingly report their versatility for the fabrication of polymeric and composite fibers[2-3]. The aim of the present work is to report the successful use of benign solvents for electrospun scaffolds fabrication and to highlight the relevance of investigations on the possible degradation of polymers and inorganic particles during the contact with such benign solvents.

METHODS:

Synthetic polymers like poly(epsilon caprolactone) (PCL) and natural polymers (Sigma-Aldrich and Primex), like chitosan (CS), zein, silk and their blends were electrospun by using benign solvents, like acetic acid, formic acid and ethanol (VWR). For composite fibers, bioactive glass particles with different sizes and compositions were considered. Sample morphology was characterized by SEM and ATR-FTIR was used to investigate fibers degradation in Phosphate-Buffered Saline and Simulated Body Fluid.

RESULTS AND DISCUSSION:

Homogeneous beads-free fibers were obtained for all the investigated neat polymers and blends (Figure 1). Fiber average diameters changed in the blends respect to the neat polymers. The presence of inorganic particles affected the fiber morphology and the bioactivity of bioactive glass particles was preserved. In comparison to fibrous PCL scaffolds obtained from standard solvents[4] (e.g. chloroform/methanol), the obtained samples showed lower values of the fiber average diameter, both for nanofibers and microfibers, and higher values of the Young´s modulus.

CONCLUSION:

The focus of the scientific community is converging on the use of benign solvents and greener approaches for electrospun scaffolds fabrication. Examples discussed in this presentation contribute to the relevant amount of data being generated which supports the wider application of solvents such as replacing toxic solvents.

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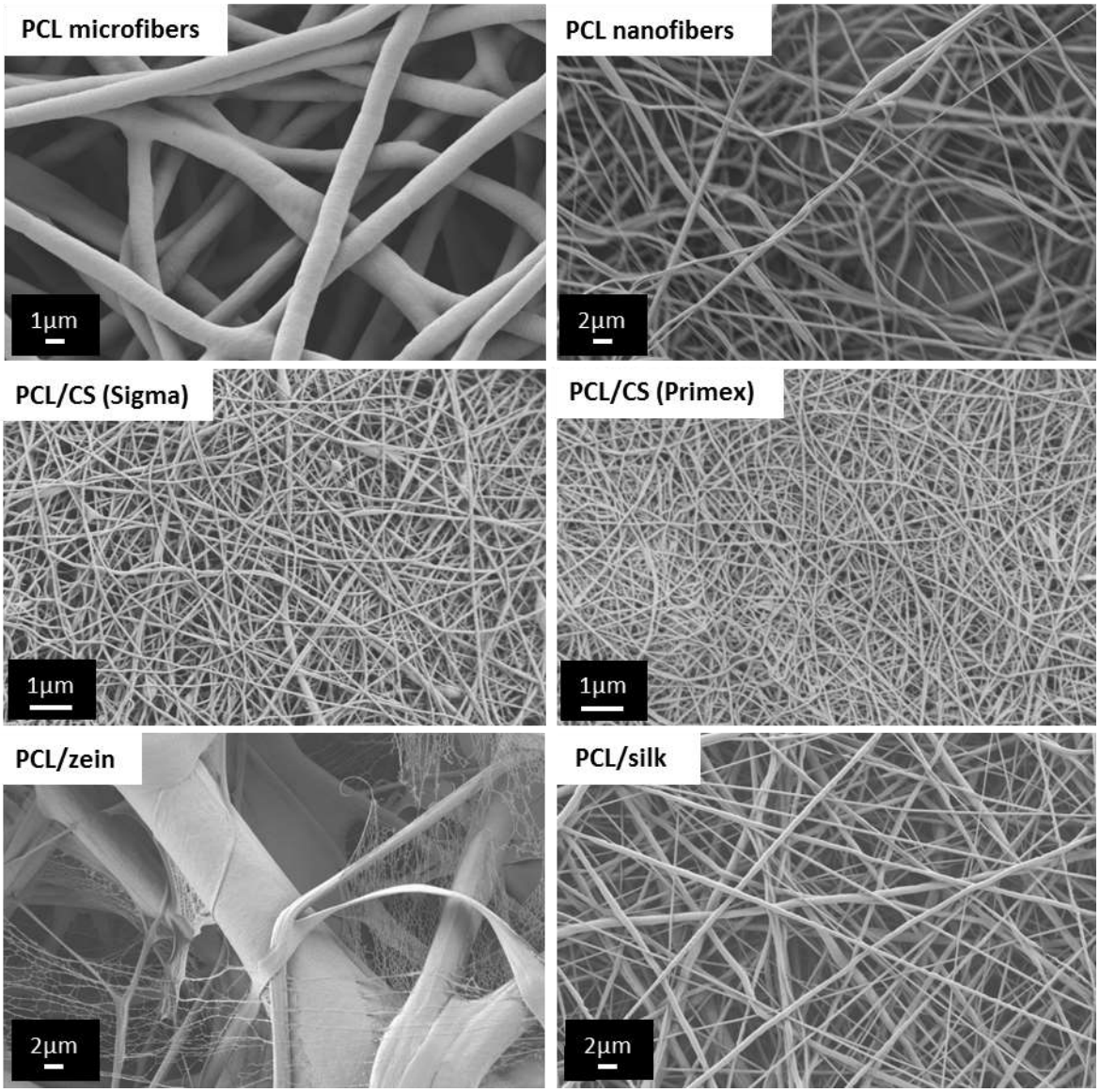

Picture 1: Caption 1: Figure 1 SEM micrographs of the different polymeric blends obtained by using benign solvents.

Electrospinning techniques & applications 16:30 - 18:00 Auditorium I 10/09/2018

Oral presentation

242 Experimental and Computational approach of cells' distribution during culture in a 3D porous electrospun scaffold

Foteini Kozaniti[1], Foteini Kozaniti[2], Margarita Georgiou[3], Paschalis Pantsios[2], Dimitrios Charisiadis[2], Petros Koutsoukos[3], Despina Deligianni[2]
[1]University of Patras, Rio/patras, Greece
[2]Department of Mechanical Engineering & Aeronautics, University of Patras, Greece
[3]Department of Chemical Engineering, University of Patras, Greece

INTRODUCTION:

3D porous scaffolds have gained increasing interest in tissue engineering due to their evident advantages in providing more accurately the actual microenvironment where cells reside in tissues. The construction of these scaffolds has many technical limitations when the electrospinning process is used. A stainless-steel mesh can be used as a collector to overcome these difficulties[1]. Furthermore, bioreactors have proved to be crucial tools to initiate, maintain and direct cell cultures and tissue development in a three-dimensional environment[2]. Computational methods can be used to determine cell density and spatial distribution in the 3D scaffold. This has proven to be important for cell differentiation and tissue development in vitro[3]. In this study, cell behavior was investigated both experimentally and computationally during culture in a 3D porous electrospun scaffold, using a bioreactor.

METHODS:

Poly-Caprolactone pellets were dissolved in glacial Acetic Acid by gently heating while on roller. The electrospinning method was performed to manufacture the porous scaffold. To fabricate a porous layer, a stainless-steel mesh was used as a receiver to replace the traditional collector. Repeating the above procedure, a porous scaffold was obtained by binding multiple layers.

MSCs were obtained from umbilical cord and were seeded in the scaffold. A bioreactor has been employed to perfuse culture medium directly through the pores of the seeded 3D scaffold.

The seeding procedure was modelled, using Computational Fluid Dynamics to evaluate the experimental results. The scaffold was designed, using Solidworks Dassault Systems. The seeding process was simulated in COMSOL Multiphysics 5.2a.

RESULTS AND DISCUSSION:

Macroscopically, the required multilayered scaffold seems to have the desired mesh textured morphology. The design of the scaffold, which was used in the modelling procedure, is presented below (Fig.1). The bioreactor experiments revealed that the cell seeding process has an important impact on cell behavior. The fluid shear stress applied on the construct's surface by the culture medium, computed by CFD, showed a close relationship with the distribution of cells in an in vitro experiment under the same seeding conditions.

CONCLUSION:

Combining the experimental and the computational results, a better insight of cell behavior was obtained.

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ACKNOWLEDGMENTS:

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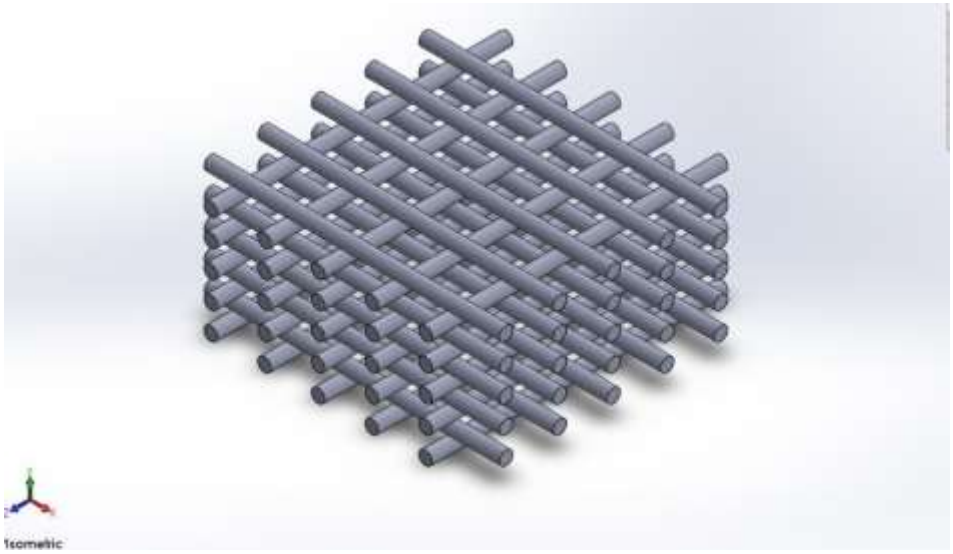

Picture 1: Caption 1: Fig 1 Design of the patterned electrospun meshed scaffold

Electrospinning techniques & applications 16:30 - 18:00 Auditorium I 10/09/2018

Oral presentation

557 Combining melt electrowriting of microfiber meshes with aggregated chondroprogenitor cells stimulated with GDF-2 to enhance cartilage tissue engineering

Florencia Abinzano[1], Mylène de Ruijter[1], Anneloes Mensinga[1], Miguel Castilho[1], Ilyas Khan[2], Riccardo Levato[1], Jos Malda[1]
[1]University Medical Center Utrecht, Utrecht, Netherlands
[2]Swansea University Medical School, United Kingdom

INTRODUCTION:

Articular cartilage-derived progenitor cells (ACPCs) are a promising cell source for cartilage regeneration, as they can be expanded in culture, achieving clinically relevant numbers without losing differentiation capacity[1]. However, culture protocols for ACPCs are not optimized and are based on media used for mesenchymal stromal cells. Also, little is known of their interaction with 3D scaffolding biomaterials. The aim of this study is to fabricate durable, organized cartilage constructs by i) maximizing cartilage production by ACPCs via optimized growth factor supplementation, and ii) incorporating ACPCs within 3D meshes of organized microfibers to guide the proliferation and eventual condensation of these cells into a cartilage-like tissue.

METHODS:

Adult ACPCs were cultured in pellets of 250.000 cells for 4 weeks, supplementing the media with 100 ng/ml of growth differentiation factor 2 (GDF-2). As control, pellets were grown with the standard dose of 10 ng/ml of TGF-beta1. To test if an initial boost of GDF-2 was sufficient for differentiation, a group was switched from GDF-2 to TGF-beta1 after the first week. Matrix production was evaluated with histology and GAG/DNA quantification. Next, GDF-2 grown chondrogenic pellets were combined with polycaprolactone meshes, fabricated via melt electrowriting. Microfibers (20µm) were printed to form a boxed structure (0-90° laydown, fiber spacing 800µm). Pellets were individually housed into each printed box.

RESULTS AND DISCUSSION:

GDF-2 has been reported as potent factor for chondrogenic differentiation of immature ACPCs[1]. In this experiment, GDF-2 supplementation significantly improved and hastened cartilage-like matrix deposition. After 1 week, these pellets were 40% larger in size compared to growing in TGF-beta1. Histology showed GAG and collagen II-rich matrix. After 4 weeks with GDF-2, the pellets presented 30x higher GAG production compared to pellets cultured with TGF-beta1, and 25% higher than the switched group (see figures A-B-C).

PCL microfiber meshes have been shown to have a reinforcing effect on gels[2]. Incorporating the pellet into the box structure (see figure D) provides structural stability, mechanical reinforcement, and can guide its growth and condensation into a larger tissue construct, enhanced through the addition of GDF-2.

CONCLUSION:

Overall, the combination of an optimized culture condition based on GDF-2 with the physical support given by the microfibrous scaffold, provides a promising platform for developing a long-term and durable solution for implantation of cartilage tissue.

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ACKNOWLEDGMENTS:

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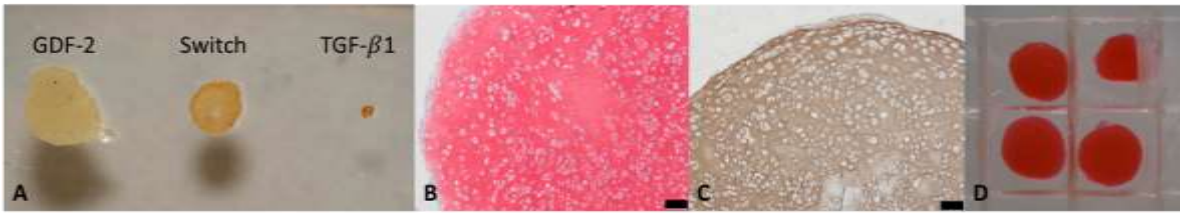

Picture 1: Caption 1: GDF-2 improves growth (fig. A) and GAGs/Col II production (fig. B-C) on ACPCs pellets. A microfiber mesh helps guide their maturation (fig. D).

3D-Printed personalized orthopaedic implants 10:45 - 12:15 Auditorium II 10/09/2018

Oral presentation

3D Printed Orthopaedic Implants: Biodegradation Behavior, Infection Prevention, and Bone Regeneration

Amir A Zadpoor[1]
[1]Department of Biomechanical Engineering, Delft University of Technology

3D printing otherwise known as additive manufacturing has enabled fabrication of volume-porous implants which may or may not be personalized in terms of their overall shape. The fully-interconnected, highly porous, and topologically ordered micro-architecture of such biomaterials has important implications for infection prevention and improved bone regeneration. First, the surface area of volume-porous implants is much larger than the corresponding solid implants. The much larger surface area amplifies the effects of coatings and surface treatments aimed at reducing the risk of infections and stimulating the growth of bone tissue. Second, there is a

very large pore space that could be used to accommodate drug delivery vehicles for local delivery of growth factors and/or antibacterial agents (e.g. antibiotics, inorganic antibacterial agents, or antimicrobial peptides). Finally, the biodegradation behavior of bone substitutes could be controlled using the topological design of volume-porous implants at the micro-scale to make sure bone regeneration continues unhindered to the maximum possible extent. Combined together, these three features of 3D printed volume-porous implants offer a host of opportunities to simultaneously reduce the risk of implant-associated infections and enhance bone regeneration performance. In this talk, the recent progress in this regard will be reviewed with a particular emphasis on metallic materials, their coatings and surface treatments, and new generations of 3D printed biodegradable metallic biomaterials.

A part of the research for this paper was financially supported by the Prosperos project, funded by the Interreg VA Flanders – The Netherlands program, CCI grant no. 2014TC16RFCB046.

3D-Printed personalized orthopaedic implants 10:45 - 12:15 Auditorium II 10/09/2018

Oral presentation

Towards a standardised and personalised in-vitro tool for 'functional' testing of 3D-printed (non)degradable orthopaedic implants

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For some time, researchers and companies are developing means to better mimic the 3D human tissue environment to make the implant development process more reliable, thereby (a) reducing in-vivo trial associated complications, (b) lowering costs resulting from late-stage failures, (c) ensuring quick abandonment of dead-ends, and (d) shortening the development process so that life changing implants reach the market sooner.
Development of 3D assays at the meso-scale have remained a challenge however, as the degree of precision required to emulate in-vivo cell-to-cell communication has proven elusive. Nowadays bioreactors enable a first step towards the in-vitro creation of more relevant cell and tissue-like environments for standardised and functional implant screening.
Currently, the ISO 10993-5:2009 guidelines describe the standard for biological in-vitro evaluation of novel materials for medical use. Non-toxic behaviour is indeed an essential material prerequisite for novel medical, implantable, applications. It, however, provides limited information on the actual device functionality. With the increasing number of geometrically more complex, customised and biologically active devices, also as part of the precision medicine revolution, including personalised and regenerative therapies, more functional in-vitro assays are needed.
Hence, there is a need to optimise decision making early on in the implant development process, supported by the growing insight that the relevance of preclinical models towards final patient outcome is limited. Establishing predictive correlations between implant bulk and surface properties and functional behaviour related to patient outcome will be essential to secure a sustainable clinical implementation of these personalised implants.
The development of a novel in-vitro standard can support the shift from assessing bio-tolerability and -compatibility towards bio-functionality. Using customised, closed, automated, stand-alone bioreactors, standardised functional screening of novel implants can become possible. Not only will these systems support the development of reproducible, operator-independent methodologies, they also enable testing of clinically relevant implant dimensions, geometries and other physicochemical surface and bulk features. Such systems also allow 'running' more complex, physiologically relevant and more patient-specific biological models on a lab scale.
The development of bioreactor-based standards for in-vitro 'functionality' testing of open porous, additive manufactured (non)degradable orthopaedic implants, using QbD-based 'functional' assessment of cell-medical device interaction in combination with in-silico predictive tools, will assist clinical translation of this next generation, customised and biologically active implants. Establishing R&D capacity for in-vitro screening of cell-medical device

interaction at this meso-scale, coupled to multi-parametric data assessment and processing will enable improved decision making early in (personalised) medical device development.

A part of the research for this paper was financially supported by the Prosperos project, funded by the Interreg VA Flanders – The Netherlands program, CCI grant no. 2014TC16RFCB046.

3D-Printed personalized orthopaedic implants 10:45 - 12:15 Auditorium II 10/09/2018

Oral presentation

348 3D additive manufactured scaffolds with antimicrobial activity for tissue regeneration

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INTRODUCTION:

Scaffolds with drug release properties to locally prevent infections are great candidates for regenerating tissues. Here, we report the additive manufacturing (AM) of polymeric scaffolds containing the inorganic lamellar fillers LDH (MgAl-based layered double hydroxides) and ZrP (zirconium phosphates), with intercalated antibiotics (ciprofloxacin (CFX) and gentamicin (GTM), respectively). The incorporation of these inorganic fillers gives double functionality to the fabricated scaffolds: i) it enables to achieve local controlled release of the antibiotics, unlike most current delivery systems, in which the drug is directly dispersed within the material, and ii) offers the potential to enhance tissue formation.

METHODS:

PEOT/PBT–LDH/CFX and PEOT/PBT–ZrP/GTM composites (5, 10, 20 wt% filler) were prepared by twin screw compounding. Initially, 2D films of each material were prepared at 190 °C, simulating the printing conditions. The influence of different fillers concentrations on the physico-chemical properties of the materials, in terms of antibiotic release kinetics, fillers distribution within the polymer matrix, and hydrophilicity, was assessed. Cells-antibiotics, cells-materials interactions, and the antimicrobial activity of each composite against Gram + and Gram – bacteria were evaluated. Ultimately, 3D scaffolds were manufactured with the materials by a melt extrusion based AM technique.

RESULTS AND DISCUSSION:

Release studies confirmed a filler concentration dependent release profile in both PEOT/PBT–LDH/CFX and PEOT/PBT–ZrP/GTM composites. An initial burst release was observed in the first 72h (concentrations lower than the toxic amounts for cells), followed by a nearly zero-order release stage, which extended until the end of the study (1 month). The fillers, exfoliated and homogeneously dispersed within the PEOT/PBT matrix (confirmed using XRD), increased the hydrophilicity of the material. This resulted in higher attachment of human mesenchymal stromal cells on composite films. Cell proliferation and morphology further confirmed the biocompatibility of the composites. Importantly, the activity of the antibiotics was preserved after the processing steps, as verified by the antimicrobial analysis. More specifically, PEOT/PBT–LDH/CFX and PEOT/PBT–ZrP/GTM composite films showed concentration dependent activity against *S. epidermidis* and *P. aeruginosa*, respectively. Each of the materials was melt extruded into 3D scaffolds (fiber diameter 250-350 μm) with printing parameters adapted to each filler concentration.

CONCLUSION:

New composites containing lamellar inorganic fillers were developed to fabricate 3D scaffolds. The unique controlled release of antibiotics, whose activity is preserved after thermal processing, is of great interest to

minimize infection risks during surgery and during tissue healing process. The potential influence of the fillers in tissue formation, in particular in osteogenesis, will be investigated.

ACKNOWLEDGMENTS:

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3D-printed personalized orthopaedic implants 10:45 - 12:15 Auditorium II 10/09/2018

Oral presentation

339 Functionalization of poly(vinyl) alcohol fibers with polymeric brushes for reinforcement of calcium phosphate ceramics

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INTRODUCTION:

Replacement and regeneration of damaged bone remains a challenge in the field of hard tissue engineering. Calcium phosphate ceramics(CPCs) are frequently used for bone grafting purposes due to their similarity to the mineral phase of bone. Nevertheless, these ceramics are brittle[1]. To improve their toughness, reinforcement of CPCs using fibers is a promising approach, where the adhesion between fibers and CPCs requires an interface of sufficient strength. A strong bond will lead to fiber rupture whereas a weak interface leads to fiber pull-out. Herein, we present a novel method to functionalize the surface of polyvinyl alcohol(PVA) fibers with thermoresponsive poly(N-isopropylacrylamide)(PNIPAm) brushes which shift from hydrophilic to hydrophobic behaviour above their lower critical solution temperature(LCST) of 32°C. We hypothesized that the hydrophilic nature of the fibers would facilitate their dispersion in the hydrophilic cement, while their hydrophobic behavior above the LCST would lead to toughening of the cement matrix through a frictional sliding mechanism.

METHODS:

The polymerization of PNIPAm brushes on the surface of commercially available PVA fibers is performed in a 3–step reaction[2]. The first step involves the introduction of an active site on the fiber surface(PVA-O_2) followed by the coupling reaction of the initiator(PVA-Br). The last step involves the growth of the PNIPAm brushes at different monomer:organometallic complex molar ratios(PVA-PNIPAm 25:1 and 50:1). To prepare fiber-reinforced CPCs, PVA fibers are mixed with the CPC powder and the liquid phase(Na_2HPO_4) at a 2.5wt% fiber weight fraction. The morphology of the fibers, before and after polymerization, is investigated by scanning electron microscopy(SEM), while the fiber-reinforced specimens(4x4x25mm^3) are tested at 21^oC and 37^oC in PBS to determine the work of fracture(WOF).

RESULTS AND DISCUSSION:

SEM reveals an increase in the fiber diameter and roughness of the modified PVA fiber. Following flexural testing of the composites, results show a 60-fold increase in the WOF values in all groups compared to pure CPC. Moreover, clear differences are shown between the WOF values for the two temperatures: cements reinforced with PNIPAm-modified fibers revealed significantly lower WOF values at 21^oC than at 37^oC. These results prove that the reinforcement efficacy of the PNIPAm-modified fibers was higher in their hydrophobic state.

CONCLUSION:

A new method is developed to functionalize PVA fibers with thermoresponsive polymeric brushes. These PNIPAm-modified fibers will be highly instrumental for the reinforcement of calcium phosphate ceramics, aiming at the development of bioceramics with load-bearing capacity.

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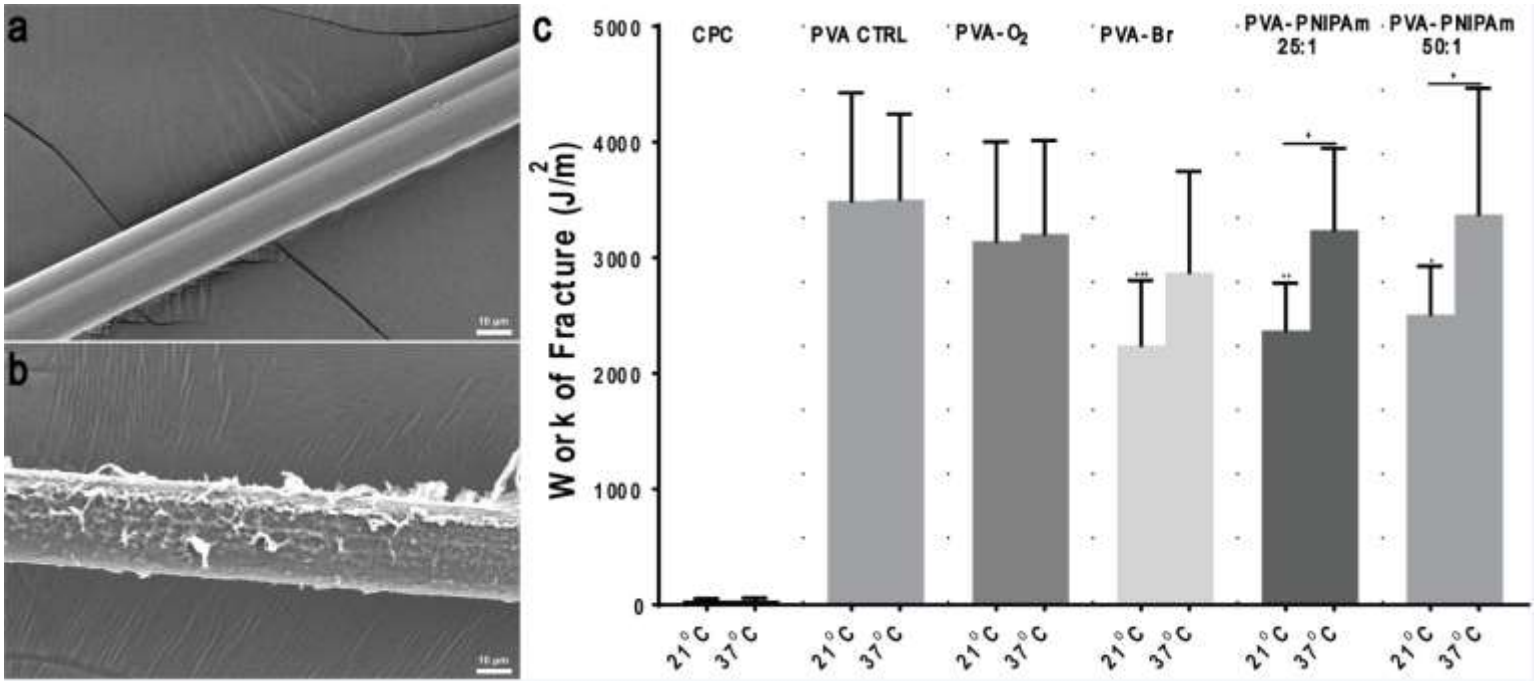

Picture 1: Caption 1: Fig. 1: SEM images of PVA fiber surface before a) and after b) PNIPAM surface modification (PVA-PNIPAM 25:1); c) Work of fracture (n=14);

Bio-molecular self-assembly for biomaterials design 16:30 - 18:30 Auditorium II 10/09/2018

Oral presentation

β-sheet forming peptide hydrogels: from self-assembly to functional biomaterials

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The use of non-covalent self-assembly to construct materials has become a prominent strategy in biomaterials science offering practical routes for the construction of increasingly functional materials for a variety of applications ranging from tissue engineering to in-vivo cell and drug delivery.1-2 A variety of molecular building blocks can be used for this purpose, one such block that has attracted considerable attention in the last 20 years is de-novo designed peptides.3 Peptides offer a number of advantages to the biomaterial scientists. The library of 20 natural amino acids offers the ability to play with the intrinsic properties of the peptide such as structure, hydrophobicity, charge and functionality allowing the design of a wide range of materials. Synthetic peptides are chemically fully defined and being build form natural amino acids they result usually in low toxicity and low

immune response when used in-vivo. Our group has focussed on the development of a technological platform for the design of novel biofunctional materials exploiting the self-assembly of β-sheet forming peptides.4-6 The β-sheet motif is of increasing interest as short peptides can be designed to form β-sheet rich fibres that entangle and consequently form stable hydrogels. These hydrogels can be easily functionalised using specific biological signals and or drugs. Through the fundamental understanding of the self-assembly and gelation of these peptides 5-7 we have been able to design hydrogels with tailored properties for a range of applications including cell culture8-11, cell and drug delivery12-13 and bio-printing14. We will present our group's journey from molecular self-assembly to bio-functional materials and their commercialisation.

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Bio-molecular self-assembly for biomaterials design 16:30 - 18:30 Auditorium II 10/09/2018

Oral presentation

Biomimetic Polymer-based Self-Assembled Biomaterials

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Polymers represent an important class of organic compounds that are used for many years in the development of biomaterials. Among them, amphiphilic block copolymers are among the most attractive systems for drug delivery applications. We report here an overview on the self-assembly in water of amphiphilic block copolymers into different nanomedicines, mainly focusing on polymer vesicles, also referred as polymersomes, and their applications in loading and controlled release of both hydrophilic and hydrophobic molecules and biomolecules.

We pay special attention to polysaccharide and polypeptide-based block copolymer vesicles and their development in nanomedicine.[1-5] Indeed, the field of synthetic polypeptides has seen many significant advances in recent years, including studies on block and hybrid copolypeptides that form vesicles, fibrils, and other structures with potential applications in medicine and materials chemistry. However, the development of glycosylated polypeptides has not kept pace, primarily due to the inability to readily synthesize glycopolypeptides

in a controlled manner. Glycosylation of natural proteins provides diverse functionality such as mediation of recognition events, modification of protein conformations, ect, that may find interest and application in biomedical field. In this context, we developed over the last years synthetic strategies for the design of glycosylated polypeptides and polysaccharide-polypeptide biohybrids with controlled placement of sugar functionality. We were especially interested in designing amphiphilic copolymers able to self-assemble into well- defined micelles and vesicles that can advantageously be loaded with drugs and present a surface with multivalent presentation of bioactive saccharides or oligosaccharides. The ability of these nanoparticles for different biomedical applications, from drug-delivery to inhibitor, will be presented. We especially evidenced the particular benefit of nanoparticles and their multivalency toward the interaction with biological receptors.[6-8]

Finally, our recent advances in using “biomimicry approaches” to design complex, compartmentalized and functional protocells will be proposed. Such a system constitutes a first step towards the challenge of structural cell mimicry and functionality, and may act in the future as an autonomous artificial cell that can sense and cure in situ any biological deregulation.[9-12]

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Bio-molecular self-assembly for biomaterials design 16:30 - 18:30 Auditorium II 10/09/2018

Oral presentation

725 Biomimetic self-assembly of functional gold nanoparticles

Nonappa Nonappa, Engelhardt Peter, Ikkala Olli
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INTRODUCTION:

In nature, biological particles, such as virus capsid self-assemblies are inspirational examples for hierarchical self-assembly with exceptional thermodynamic stability.[1] They follow the principle of genetic economy, efficiency, and error-free structure formation using the concept of subunit based self-assembly.[2] Related self-assembly concepts have been widely explored in supramolecular chemistry using molecular-level building blocks. However, a similar approach using colloidal level building blocks such as inorganic nanoparticles has certain limitations due to challenges in controlling their size, shape, interactions, and stability.[3-6] In this contribution, we show biomimetic self-

assembly of atomically precise gold nanoparticles into hollow capsids and their application potential in advanced drug-delivery systems (Figure 1).

METHODS:

In this work, atomically precise noble (Au, Ag) nanoparticles having an exact number of metal atoms and organic ligands) were used. The synthesis and assembly of the particles were achieved under aqueous environment.[4] The self-assembled structures were characterized using high resolution electron microscopy, Cryo-Transmission Electron Tomography, dynamic light scattering (DLS) and small angle X-ray scattering (SAXS).

RESULTS AND DISCUSSION:

The nanoparticles containing surface functional groups such as carboxylic acid groups (-COOH) offer directional hydrogen bonding under appropriate conditions. The dispersions of nanoparticles upon dialysis allow hydrogen bonding directed assembly into hollow superstructures with average size of 200 nm (Figure 1). Importantly, capsids contain monolayer thick shells allowing 90% of empty space. This allowed us to explore the possibilities to encapsulate poorly water soluble drugs.

CONCLUSION:

Colloidal self-assembly is a strongly growing area of research, where nanoparticle self-assembly is one of the key areas. In an analogy to supramolecular chemistry, which has matured to offer a rich toolbox to construct functional structures beyond the individual molecular scale, a challenge would be to develop rational methods for supracolloidal chemistry, i.e., structures beyond the colloidal scale using colloidal level building blocks for materials and biomedical applications.

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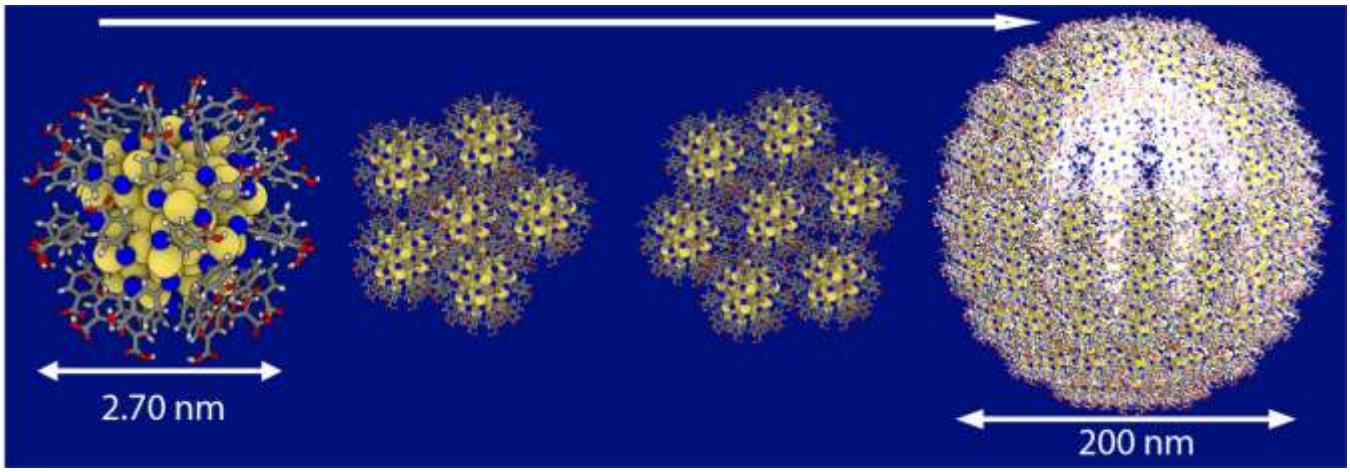

Picture 1: Caption 1: Structure of atomically precise gold nanoparticle and self-assembled capsid.

Bio-molecular self-assembly for biomaterials design 16:30 - 18:00 Auditorium II 10/09/2018

Oral presentation

377 Biomimetic Nanostructured Polyurethane-based Patchy Colloidal Particles to Combat Biofilm and Planktonic Drug-resistant Bacteria

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[3]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy
[4]Hospital of the Ruhr-University Bochum, Germany

INTRODUCTION:

Drug-resistant bacteria in planktonic or sessile form are the root cause of life-threating infections. Most of these infections are even proven fatal as currently used antibiotics are unable to treat them[1,2]. One of the main causes for this, is the fact that all biotic and abiotic surfaces have the risk of being colonized by biofilms which are often polymicrobial in nature[1,2]. To tackle this problem, we developed biomimetic polyurethane grafted polyionic liquid patchy colloidal particles. The novelty of these polymers is their ability to mimic the mesoscopic structural organization of antimicrobial peptides that is involved in its mode of antimicrobial activity[3].

METHODS:

A hydrophobic liquid monomer was grafted from the amphiphilic backbone of a custom-made polyurethane by redox initiated aqueous heterophase polymerization. Subsequently, the hydrophobic anion was exchanged with a hydrophilic one. Chemical structure was elucidated from analysis of ^{1}H, ^{13}C and ^{19}F NMR spectra. Structural organization was investigated from cryo-TEM images. Colloidal stability was determined by *ζ-potential* analysis. Most relevant bacterial strains in chronic wounds and medical device related infections were used to determine the Minimal Inhibitory Concentration(MIC) and Minimum Bactericidal Concentration(MBC) by the broth microdilution method. Safranin staining was used to quantify biofilm biomass. L929 murine fibroblasts were used for cytocompatibility evaluation.

RESULTS AND DISCUSSION:

Cryo-TEM images confirmed the formation of patchy colloidal particles consisting of self-organized mesophases. A strong bactericidal effect was observed mostly against susceptible and resistant strains of *Staphylococci* and *Enterococci*(table 1). After only 30 minutes in the presence of the colloidal dispersion at ~20µg/mL, there was a 99% reduction of *S.aureus* and MRSA CFU/mL. Interestingly, the particles had the capacity to disrupt pre-formed Gram-positive biofilms. The IC_{50} of the designed colloids on fibroblasts was higher than the MIC determined on susceptible and antibiotic-resistant *Staphylococci*, showing a selectivity index from 2.7 to 5.4.

CONCLUSION:

The developed patchy colloidal particles showed a very rapid bactericidal effect with very low concentrations against clinically relevant species of drug-resistant bacteria. Moreover, it inhibited biofilm formation and disrupted pre-formed biofilms of *S. aureus* and *E. faecalis*(non- and resistant) which makes it attractive to combat polymicrobial biofilms. Further studies are in progress to unravel the exact mechanism of antimicrobial action.

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Picture 1: https:\\www.eventure-online.com/parthen-uploads/40/18903/add_1_426897_853f0971-1702-4958-b171-095516033b0a. MIC and MBC values among non- and resistant Gram-positive bacteria.png
Caption 1: Table 1. MIC

Toward applications of additive manufacturing & bioprinted scaffolds 10:45 - 12:15 Room 0.5 10/09/2018

Oral presentation

61 Preclinical evaluation of a composite implant made by stereolithography for orbital floor fracture repair

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[2]Department of Biomaterials Science and Technology, University of Twente, Netherlands
[3]University and University Medical Center Groningen, Netherlands

INTRODUCTION:

Orbital floor (OF) fractures are commonly treated by implanting either bioinert titanium or polyethylene implants, or by autologous grafts. As alternative, we propose a personalized implant made of poly(trimethylene carbonate) loaded with hydroxyapatite (PTMC-HA). We previously characterized porous PTMC-HA scaffolds *in vitro* [1,2] and in small animal (calvarial defect) [3], revealing that the biomaterial was compatible and promoted bone formation. In this large-size animal study, we designed personalized PTMC-HA implants, manufactured them using stereolithography (SLA) and assessed their biomechanical and biological performance compared to standardized titanium implants in an OF defect sheep model.

METHODS:

Implants fabrication was done using SLA of photo-crosslinkable PTMC mixed with HA [1-3]. *Preclinical study:* (sheep n=12, ethic number 34_2016) was conducted by first scanning the OF bone of each sheep (**Fig. 1A**) in order to fabricate patient specific implants (PSI) made of PTMC-HA (**Fig. 1B and C**). The fabricated PSI was implanted after creating OF defect and bone formation and healing compared to preformed titanium mesh using time-laps X-ray analyses (**Fig. 1D**), histology (Giemsa-Eosin staining) and fluorochrome staining over 3-months. Additionally, the osteoinductive property of the biomaterials was assessed by intramuscular implantation (IM).

RESULTS AND DISCUSSION:

In this study, we showed that the composite PTMC-HA allowed for ectopic bone formation after IM implantation, without requiring any biotherapeutics (in 5 out of 11 samples). In addition, we could repair OF defect on sheep using SLA-fabricated PTMC-HA (**Fig. 1A-D**) with a good shape fidelity (compared to the virtual implant) and a better bone integration compared to the titanium mesh (**Fig. 1E**).

CONCLUSION:

This large-size animal study shows that a composite PTMC-HA personalized implant that exhibits osteoinductive activity could replace advantageously autologous bone and titanium implants in craniomaxillofacial surgeries.

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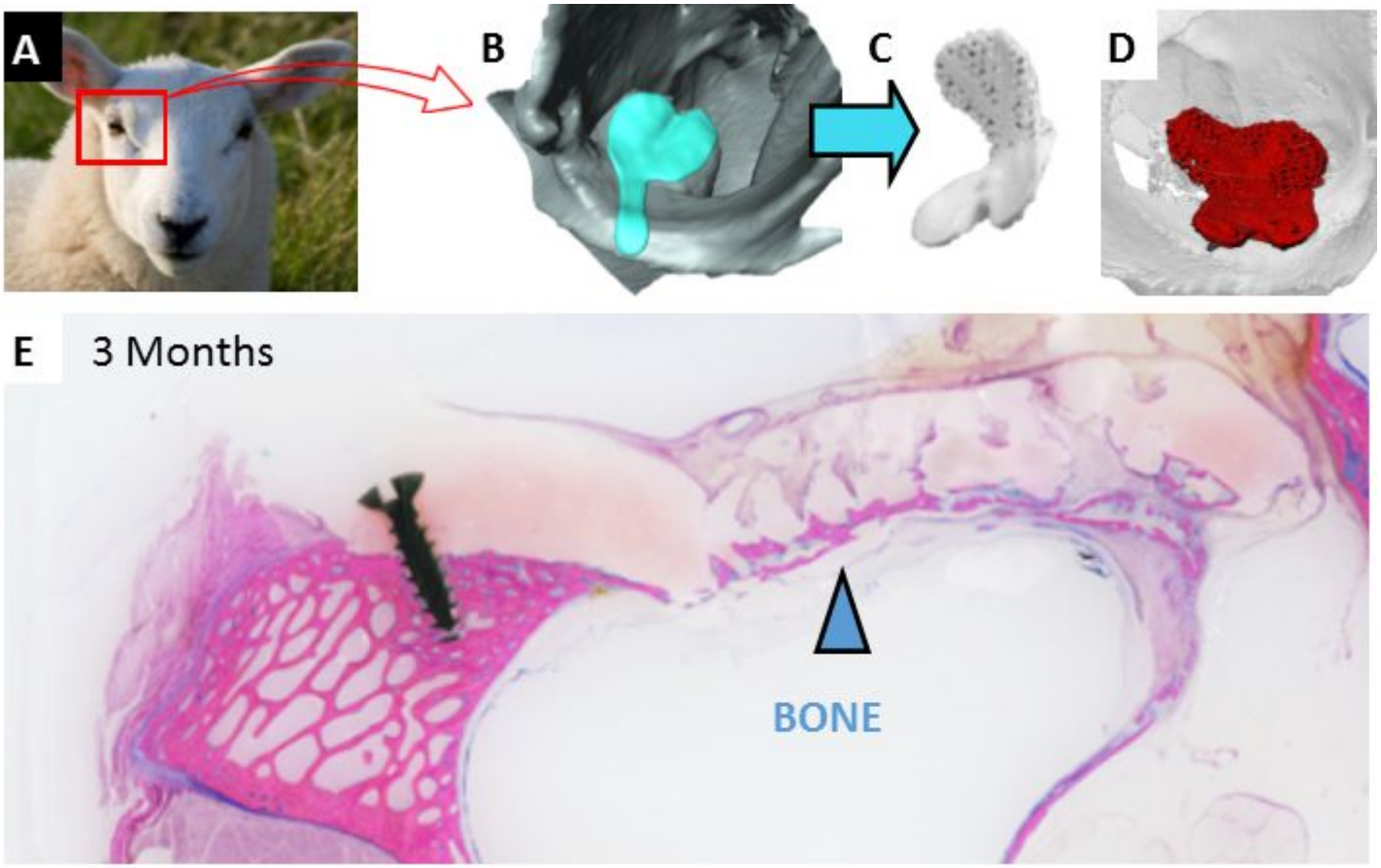

Picture 1: Caption 1: Figure 1: OF repair on sheep using novel PTMC-HA implant. Illustration of the work-flow (A-D), and histology analysis (E)

Toward applications of additive manufacturing & bioprinted scaffolds 10:45 - 12:15 Room 0.5 10/09/2018

Oral presentation

581 Towards bioprinting of vascularized bone tissue equivalents

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INTRODUCTION:

A crucial factor in tissue engineering is the vascularization of the constructed models to ensure supply of the included cells with nutrients and oxygen. For the fabrication of vascularized bone tissue equivalents, evolving manufacturing techniques like bioprinting can be used to construct geometrically defined three-dimensional structures.

METHODS:

Hydrogels based on methacrylated gelatin (GM)[1,2,3], hyaluronic acid and hydroxyapatite particles or methacrylated and acetylated gelatin were developed for the encapsulation of human adipose-derived stem cells (ASCs) or human microvascular endothelial cells (ECs), respectively. Hydrogel properties like rheological behaviour and swellability were assessed.

Encapsulated cells were cultured and bone matrix formation as well as the development of capillary-like structures was assessed by rheological measurements,staining of matrix components, EC markers and basal lamina. Combination hydrogels containing both ECs and ASCs were build up by microextrusion printing and matured. Again, formation of bone matrix and capillary-like structures was assessed. Last, bioprinted co-culture constructs were cultured in a perfusion bioreactor, and cell viability, bone formation and vascularization were evaluated as described before.

RESULTS AND DISCUSSION:

We developed bioinks on basis of GM that can - by further addition of hydroxyapatite - either support the osteogenic differentiation of ASCs and formation of a bone matrix, or the formation of vascular structures by ECs. The inks and resulting hydrogels, whose material properties like swellability, viscosity and elasticity can be adjusted to the requirements, were characterized. Additionally, the bioactivity of the hydrogels regarding the support of vessel-formation (Fig. 1A) and osteoconductivity (Fig. 1B) was verified. The bioinks were then used to build up various geometries (Fig. 1C) via a microextrusion-based printing system, which were afterwards cultured for up to four weeks under suitable co-culture conditions (Fig. 1D).

We detected the formation of a bone matrix and the generation of capillary-like hollow structures. Those processes were significantly increased in structures where bone and endothelial cells were co-cultured, in comparison to monocultured cells. Additionally, we could show the successful perfusion culture of printed constructs made of multiple bioinks and cell types, allowing the build-up of structures with higher dimensions (Fig. 1D).

CONCLUSION:

In conclusion, we were able to develop bioinks and a printing process which allow the successful build-up of bone tissue equivalents containing capillary-like structures, whose bioreactor culture enables the set-up of relevant geometries and sizes.

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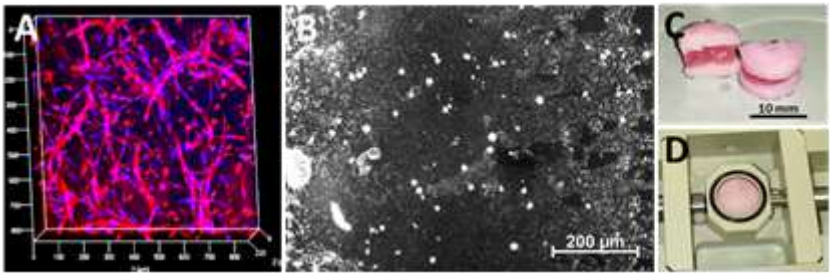

Picture 1: Caption 1: Figure 1: (A) Formation of extensive vascular networks in the developed hydrogel after 7 days culture (red: PECAM-1; blue: DNA). (B) Collagen-staining

Toward applications of additive manufacturing & bioprinted scaffolds 10:45 - 12:15 Room 0.5 10/09/2018

Oral presentation

411 Bioresin for printing of cell-laden hydrogels with 3D convoluted vessel-like microfluidic channels via digital light processing

Riccardo Levato[1], Khoon Lim[2], Debby Gawlitta[1], Tim Woodfield[2], Jos Malda[1]
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INTRODUCTION:

Hydrogels allow to culture cells in a native-like microenvironement, which is necessary in many tissue engineering and disease modelling applications. To further recapitulate the complex organization of living tissues, advanced biofabrication technologies are fundamental to accurately control the three-dimensional (3D) architecture of cell-laden hydrogels and to tackle major challenges in the field of tissue engineering, such as the incorporation of vessel-like structures.

The aim of this work is to develop a hydrogel-based strategy for digital light processing (DLP) bioprinting, to print high-resolution living constructs with embedded microfluidic, vessel-like channels. In DLP bioprinting, patterns of light are drawn layer-by-layer into a cell-laden hydrogel solution, termed bioresin, which gelates only where illuminated. With this approach free-form 3D biological structures with embedded microchannels are generated in a single-step process, without the need for sacrificial materials.

METHODS:

Photoresponsive gelatins, bearing methacryloyl and click chemistry-reactive norbornene groups, were investigated as bioresins for DLP printing (Perfactory 3 Mini, Envisiontec), using a recently described visible-light photoinitiator[1]. Working curve for the bioresins, optimal exposure settings and printing resolution were identified. Unconfined uniaxial compression and sol-gel analysis were conducted to assess mechanical and physico-chemical properties of the printed constructs as a function of polymer content. Cell response to the bioresin was assessed encapsulating and bioprinting bone marrow-derived Mesenchymal stromal cells (MSCs), and evaluating their potential for osteogenic differentiation.

RESULTS AND DISCUSSION:

Constructs displaying superior resolution compared to extrusion bioprinting (25-50µm), and embedding convoluted vessel-like networks, branching in the x, y and z axis (diameter down to 200µm) were successfully printed, and perfused through these microchannels, which also allow cell attachment for endothelialization. MSCs loaded into the bioresin and printed were homogenously distributed across the construct, high viability and proliferation up to 28

days. MSC synthesized bone-like mineralized matrix into the constructs, which provided a permissive environment for osteogenic differentiation.

CONCLUSION:

In conclusion, this DLP-bioprinting approach and the developed bioresin allow generating high-resolution, perfusable vessel-laden constructs with complex geometries with potential application in vascularized tissue engineering, and enable a new toolset for the design of the next generation of hydrogel-based organ-on-chip devices.

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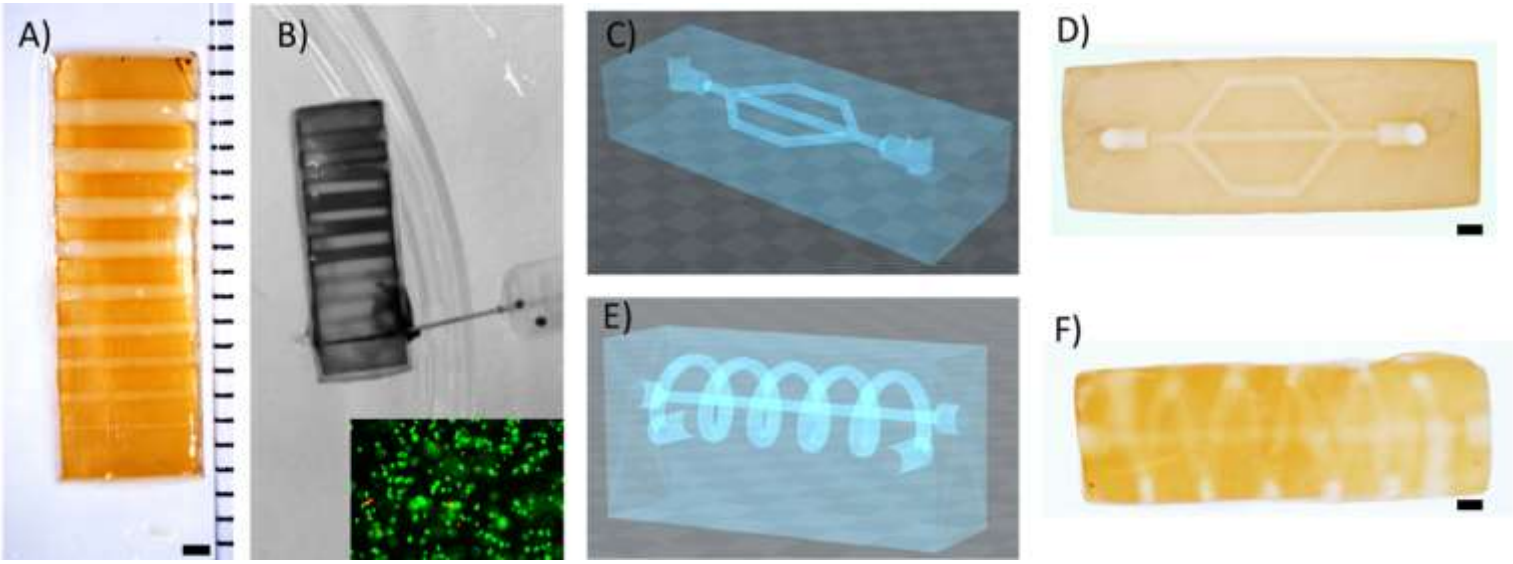

Picture 1: Caption 1: 3D printed bioresin structures via DLP. A) Hydrogel with channels of decreasing sizes, showing B) open and perfusable channels down to 200µm. Insert

Toward applications of additive manufacturing & bioprinted scaffolds 10:45 - 12:15 Room 0.5 10/09/2018

Oral presentation

628 Platelet lysate fibrillar nanocomposite bioink for soft tissues bioprinting

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INTRODUCTION:

Platelet lysate (PL) has shown outstanding biological properties as a scaffolding biomaterial, as it is composed of a myriad of biomolecules with relevant therapeutic function to promote tissue regeneration. However, these natural fibrillar scaffolds can be easily deformed or degraded[1]. Recently, we found that it is possible to reinforce the low strength PL matrix by crosslinking the produced fibril structure with aldehyde cellulose nanocrystals (a-CNC), through reversible Schiff base bonds. This strategy rendered PL as a stable injectable formulation with tuned mechanical and cellular behavior (unpublished results), which led us to explore PL-CNC as a potential nanocomposite bioink (nanoink) for additive manufacturing.

METHODS:

First, the protein content of PL was pre-optimized to maximize the network crosslinking and the effect of different CNC concentrations in rheological properties were studied. The extrusion of the PL-CNC nanoink was optimized using a double-barrel syringe (1:1) in Creatr 3D printer (Leapfrog). Barrel A was filled with PL and barrel B was composed of thrombin (2 U/ml), calcium (10 mM) and a-CNC (1.22 wt%). After the customization of the printer and its firmware to hold the syringe and print the fibrillar hydrogels in a gelatin supporting bath[2], 3D printing parameters (extrusion speed rate, infilling, noozle diameter and flow rate) were optimized to obtain self-supporting structures. Finally, we optimized the conditions to bioprinting human adipose tissue-derived stem cells (hASCs) in the developed bioink and assessed their biological performance.

RESULTS AND DISCUSSION:

Bioink components exhibited shear-thinning behaviour, which allowed extrusion at low printing pressures. Optimized bioink conditions allowed a fast hydrogel deposition with minimal diffusion, forming a solid structure. After removing gelatin support bath, 3D printed structures were mechanically robust and maintained a stable printed shape. Bioprinting with hASCs did not changed significantly the fidelity of the printed hydrogel and showed high viability immediately after extrusion that was maintained at the end of the cell culture period.

CONCLUSION:

In conclusion, the proposed nanoink showed cellular printing capacity of a biologically relevant extracellular matrix hydrogel. Moreover, it allowed to print shape-fidelity structures that has a great potential to fabricate soft structures.

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ACKNOWLEDGMENTS:

The authors would like to thank European Union's Horizon 2020 research and innovation programme for Marie Skłodowska-Curie grant agreement No 706996 (MGF) and TEAMING Grant agreement No 739572 - The Discoveries CTR, PD/59/2013 and PD/BD/113807/2015 (BBM), SFRH/BPD/112459/2015 (RD) and project Recognize (UTAP-ICDT/CTM-BIO/0023/2014) and NORTE-01-0145-FEDER-000021.

Toward applications of additive manufacturing & bioprinted scaffolds 10:45 - 12:15 Room 0.5 10/09/2018

Oral presentation

311 Bioprinted capillary beds as building block for organ-on-a-chip applications

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INTRODUCTION:

Capillary network formation is a fundamental step in the fabrication of living tissues and organs. *In-vitro* growth of capillary-like structures has been frequently reported in literature. However, without guidance capillary sprouting results in chaotic structures rather than the formation of an organized network. The aim of this work is to generate a macroscopically structured capillary network using 3D-bioprinting. The printed networks are sought to be applied as basic platform for the generation of different tissue-like *in-vitro* models.

METHODS:

Using a custom-built, drop-on-demand bioprinter fibrin networks loaded with human umbilical vein endothelial cells (HUVECs) and human mesenchymal stem cells (hMSCs) were printed (n > 3 donors). Mesh size and distance could precisely be controlled. The pores of the network could be filled with dots of agarose containing a third cell-type, e.g. hepatocytes. Post-printing cell viability, cell migration and spreading as well as capillary network formation were analyzed for up to two weeks using fluorescence microscopy (FDA/PI), immunofluorescence staining (CD31, DAPI) and two-photon imaging.

RESULTS AND DISCUSSION:

Fibrin networks with a mesh size of 500 µm and a mesh distance of 0.9, 1.25, 1.5, and 2.0 mm were readily fabricated. Printed cells maintained high cell viability (> 94 %) and proliferation potential following the printing process. After two weeks of culture pronounced capillary formation could be observed in all samples. Compared to the non-printed control, capillary network formation in the printed fibrin structures exhibited a significantly higher degree of organization. Capillaries predominantly aligned to the printed network fibers.

CONCLUSION:

Bioprinting technology was successfully applied to generate fibrin structures that enable the formation of capillary networks with a high degree of spatial orientation. A spatially oriented and *inter*-connected capillary network represents the basis for the fabrication of advanced tissue models. The possibility to readily integrate additional cell types in between the structured vasculature highlights the asset of the presented method for the generation of *organ-on-a-chip* models.

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Oral presentation

680 Development of a biomimetic cell-laden hydrogel for bioprinting cellular tissues-like for bone regeneration

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Newcastle University, Newcastle university, United Kingdom

INTRODUCTION:

Cell-laden hydrogels are highly demanded for tissue bioprinting, either for tissue repair or creating *in vivo* models as platforms for drug screening. The material to be printed is usually referred as bioink, and its formulation must allow printing of living cells. An optimal bioink should combine certain biological and materials specifications, including processability, mechanics, degradability and bio-functionality (including biocompatibility and/or bioactivity)[1]. This work aims to formulate a new cell-laden hydrogel based on collagen, fibrinogen and alginate biopolymers with tuneable properties for further bioprinting of human stromal stems cells for tissue mineralisation and bone formation

METHODS:

The cell-laden hydrogel is a mixture of collagen, fibrinogen and sodium alginate (CAF). Briefly, fibrinogen and alginate were dissolved in PBS at 37 °C and 60 °C for 3 hours, respectively. These were gently mixed, followed by collagen solution addition. As crosslinker, a mixture of cell medium with thrombin and $CaCl_2$ was used. The final concentrations were optimised for 3D printing. Stromal stem cells were gently mixed with the crosslinking solution before printing. The bioprinting was performed with an in-house reactive jet impingement (ReJI) printing system composed of two microvalves which jet both gel precursors simultaneously, with drops meeting and reacting in mid-

air before dropping onto the substrate (Fig 1B). Hydrogels were characterised in terms of physical-chemical, mechanical and biological properties, including cellular viability and mineralisation.

RESULTS AND DISCUSSION:

Physical-chemical characterisation of hydrogels demonstrated a quick crosslinking capacity with stiffness similar to native soft tissues. These exhibited a porous structure with pore sizes in the range of 40-120 μm (Fig 1A), and stability at cell culture conditions over 28 days of incubation. Cellular tissues were bioprinted into a cuboidal shape of 1x1 cm^2 (Fig 1B), with good cell viability and inter-cellular interaction (Fig 1D) and very high cell densities, up to 90 million cells/ml. Stromal stem cells showed sustained proliferation during the initial stages of incubation, followed by an increase of ALP activity

CONCLUSION:

A new cell-laden hydrogel based on collagen, alginate, and fibrinogen has been successfully formulated and characterised. The properties of CAF hydrogels can be tuned to ensure printability whilst supporting cell survival and function. Cellular constructs were 3D-printed achieving unprecedent cell densities, and exhibiting good cell viability, proliferation and differentiation during incubation

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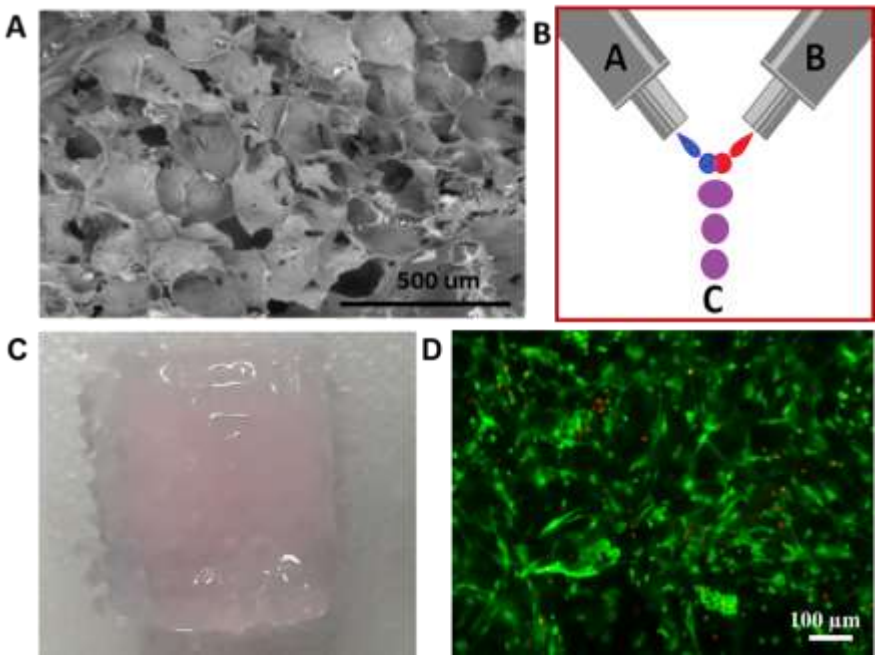
ACKNOWLEDGMENTS:

The research has been financially supported by the MRC-CiC at Newcastle University and the EPSRC Centre for Innovative Manufacture in Medical Devices (EP/K029592/1)



Picture 1: Caption 1: CAF hydrogel: A)morphology by SEM, B) ReJI printing system, C) bioprinted cell-laden hydrogels, D)Cell viability after 5 days of culture

Controlling biomaterial-biological system interactions 16:30 - 18:00 Room 0.5 10/09/2018

Oral presentation

246 biomacs: biomaterials advanced cell screening

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University Medical Center Groningen, Groningen, Netherlands

INTRODUCTION:

Biophysical and biochemical cues located on biomaterial surfaces profoundly affect (stem) cell response, which provides pivotal information for designing biomaterials.[1] However, most of the physicochemical properties were studied individually, which is not appropriate since cells always interact with multiple cues simultaneously.[2,3] We developed an orthogonal double gradient platform to identify the cell response towards thousands of these combined parameters to enable accelerate multi-scale design and optimization of material properties to enhance the function of biomaterials.

METHODS:

PDMS orthogonal double gradients are prepared by sequential shielded air plasma oxidation treatments and chemical modification using silanization reactions in accordance with previously published single linear gradients.[3,4]

RESULTS AND DISCUSSION:

Each position on the tested orthogonal double gradient surface has a unique combination of stiffness and wettability over a broad range (surface stiffness: 6-89 MPa; water contact angle: 29º-90º). We cultured hBM-MSCs on the developed gradient platforms for 24 h, imaged the cells via automated fluorescence microscopy, and quantified the cell response on 25 areas (division of the surface in a 5×5 grid)(Figure 1). We found higher cell surface coverage in WCA ranging from 29º-36º and 48º-90º on the soft part (6-19 MPa) of the substrate. However, there is similar cell surface coverage on the stiffer range from 19 to 89 MPa in WCA range from 29º to 90º. Also hBM-MSC adhesion, spreading, nucleus size and vinculin expression were affected and indicated that hBM-MSC behavior is non-linearly regulated by surface stiffness and wettability.

CONCLUSION:

We developed a novel approach to elucidate combined physical parameter influences on cellular behaviour using orthogonal double gradients and thereby gain insights in hBM-MSC responses. Most cell responses are non-linearly regulated by material stiffness and wettability. Our strategy allows for efficient analysis of multiple cue-response relationships to facilitate enhanced biomaterial development.

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ACKNOWLEDGMENTS:

Q.H.Z., L.G., and L.L.Y. are grateful for financial support from the China Scholarship Council (No. 201406630003; 201707720058; 201608310113).

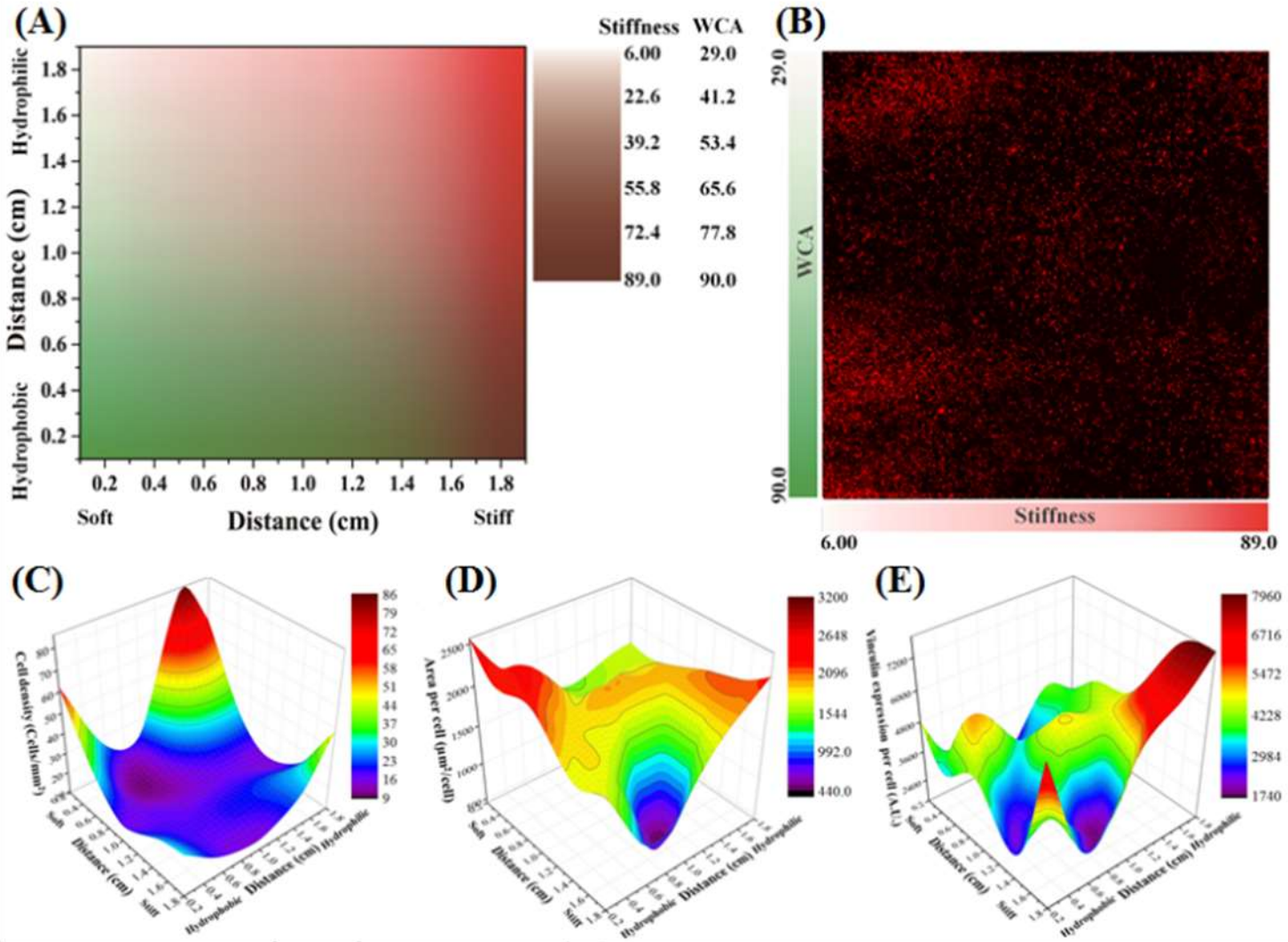

Figure 1. (A,B) hBM-MSCs cultured on the orthogonal double gradient for 24 hours. 3D heat maps for (C) cell density, (D) area per cell, and (E) vinculin expression per cell after 24 h culture (n = 3).

Picture 1: Caption 1: Figure 2. (A,B) hBM-MSCs cultured on the orthogonal double gradient for 24 hours. 3D heat maps for (C) cell density, (D) area/cell, and (E) vinculin.

Controlling biomaterial-biological system interactions 16:30 - 18:00 Room 0.5 10/09/2018

Oral presentation

541 Direct and stable immobilization of cell adhesive ligand on ePTFE through the oxidation of an anchor peptide containing Tyr residues

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[2]Osaka Medical College, Japan
[3]National Cerebral and Cardiovascular Center Research Institute, Japan

INTRODUCTION:

Expanded polytetrafluoroethylene (ePTFE) is widely used for cardiovascular prostheses due to its anti-platelet adhesion property. Meanwhile, the adhesion of endothelial cells is also inhibited resulting in the lack of endothelialization and stenosis. To solve this problem, the immobilization of cell adhesive ligands should be a powerful strategy. However, it is very difficult to modify ePTFE by chemical methods since ePTFE is a very stable material. In this study, we designed anchor sequences composed of tyrosine and charged residues to improve the immobilization efficiency of cell adhesive ligand onto ePTFE.

METHODS:

Peptides composed of an anchor sequence and fibronectin-derived ligand (LDV), Ac-YG_3LDV (Y-LDV), Ac-$(YK)_3G_3$LDV (YK3-LDV) and Ac-$(YE)_3G_3$LDV (YE3-LDV) were immobilized on ePTFE films through the oxidation with copper catalyst and hydrogen peroxide [1, 2]. Surface of modified ePTFE was analyzed using X-ray photoelectron spectroscopy. The adhesion behavior of endothelial cells was evaluated *in vitro*.

RESULTS AND DISCUSSION:

Nitrogen (N1s) was detected on all samples by XPS analysis, indicating that all peptides were successfully immobilized on the ePTFE surface through the Tyr oxidation. Interestingly, YK3-LDV immobilized on ePTFE was stable even after washing with the aqueous solution containing 1.0% SDS or 1.0 M NaCl. Because quinones produced by the oxidation of hydroxyphenyl trigger phenol coupling and Michael addition, YK3-LDV might form the stable layer on ePTFE through the adsorption and the polymerization of YK3 anchors. Adhesion of endothelial cells was improved on ePTFE surfaces immobilized with Y-LDV, YK3-LDV and YE3-LDV at the initial stage (3 hours). In the case of long-term culture, endothelial cells proliferated and form a single layer on YK3-LDV-immobilized ePTFE.

CONCLUSION:

The cell adhesive ligand, LDV was successfully immobilized through the oxidation of anchors having Tyr residues on ePTFE surfaces. Especially, YK3 anchor contributed to the stable immobilization and the improvement of endothelial cell adhesion.

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Controlling biomaterial-biological system interactions 16:30 - 18:00 Room 0.5 10/09/2018

Oral presentation

776 One step biocatalytic surface modification of viruses

Valentina Vignali[1], Barbara Santos Miranda[1], Mark Loznik[2], Frank Hollmann[3], Ulrich Commandeur[4], Patrick van Rijn[1]
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[3]Delft University of Technology, Netherlands
[4]RWTH Aachen University, Germany

INTRODUCTION:

Over the last years viruses proved to be versatile particles with a large variety of possible applications in nanomedicine and material science.[1,2] The grafting of polymers on their surface is highly desired to develop responsive therapeutic biomaterials as well as to improve their pharmacokinetics profile.[3]

In order to minimize the synthetic efforts which characterize the conjugation methods now available, an highly selective modification via a single biocatalytic approach is here proposed, providing a general platform for virus-acrylate hybrid particles.

The Tobacco Mosaic Virus (TMV) and the M13 bacteriophage have been successfully modified and new properties were introduced such as temperature responsiveness, high charge density, hydrophobicity and fluorescence making them versatile particles suitable for different type of applications. This new approach can also be applied to other biomolecules with tyrosine residues on the surface.

METHODS:

TMV and M13 have been modified via laccase induced free-radical oxidation of tyrosine residues as initiating step. The resulting radical formation triggers the functionalization with acrylates (Fig. 1A).

The reaction take place in phosphate buffer at pH 6.0. Temperature and concentration of acrylates and enzyme were optimized for the different types of virus. After purification, the virus-acrylate hybrids were characterized with Fluorescence Spectrometry, Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM), High Performance Liquid Cromatography (HPLC) and MALDI-ToF mass spectrometry.

RESULTS AND DISCUSSION:

TEM images of the TMV and M13 modified particles reveal that the viral structure is maintained, indicating the stability towards the reaction conditions (Fig. 1B). Hybrid particles are assembled due to hydrophobic interaction. The coat is too thin to be visible with TEM, but the altered behavior indicates successful modification as confirmed by mass spectrometry and HPLC analysis. With the use of a fluorescently labeled monomer, after purification, still the fluorescence was detected both for TMV and M13 modification.

CONCLUSION:

The development of a new route to modify virus particles with various functional monomers adds interesting properties to the TMV and the M13 bacteriophage. The same approach could be applied to other bionanoparticles with accessible tyrosine residues, allowing an easy access to protein-acrylate hybrid structures. Moreover, the possibility to obtain fluorescent hybrids is useful for tracking biomolecules.

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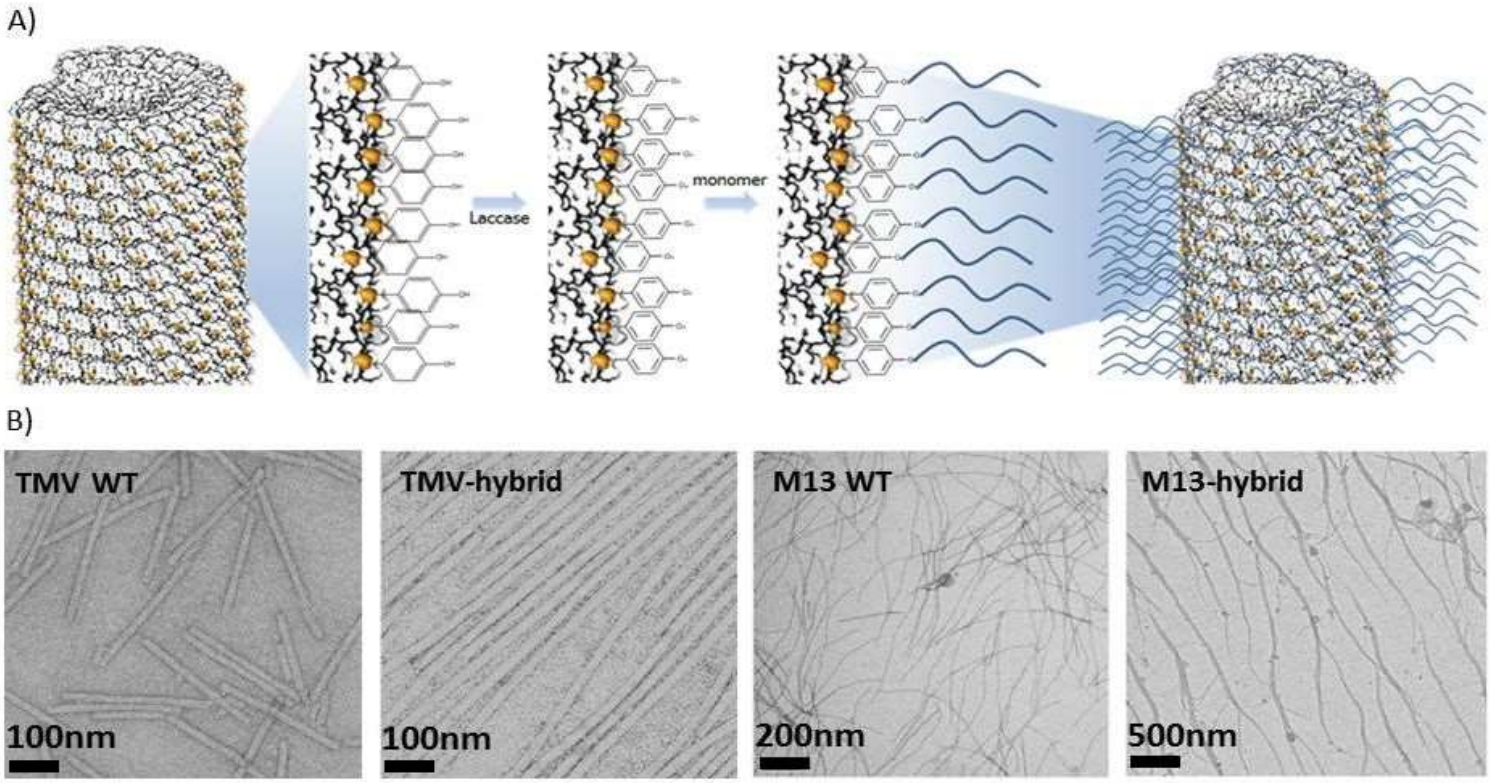

Picture 1: Caption 1: Fig. 1. A) Modification of TMV via free radical oxidation. B) TEM images of the wild-type and modified TMV and M13

Controlling biomaterial-biological system interactions 16:30 - 18:00 Room 0.5 10/09/2018

Oral presentation

736 Metal-free alternating copolymer: - A novel nanomaterial synthesized by green chemistry approach for use in drug delivery/biomedical application

Piyush Kumar mr. Gupta, Santosh mr. Gupta, Sreenath dr. Pappuru, Debashis prof. Chakraborty, Rama Shanker prof. Verma
IIT-Madras, Chennai, India

INTRODUCTION:

Polymeric nanoparticles are widely used nanomaterial, found to be toxic to healthy cells at higher concentration due to its polymer chemistry and its synthesis procedure**1**. These nanoparticles are made up of copolymers, synthesized by ROCOP using different monomers and organometallic catalyst. The metal component of such catalysts are toxic in nature**2**. Therefore, to eliminate such toxicity, there is need to develop a green synthesis approach for metal-free copolymer synthesis. In this proof of study, we have developed a green chemistry approach for the synthesis of metal-free alternating copolymer. Further, we have also developed and characterized different nanodrug formulations (NDF) for drug delivery application in cancer therapy.

METHODS:

The poly(*t*BGE-*alt*-PA) copolymer was chemically synthesized by ROCOP reaction**1** using non-metallic activators. Then, this copolymer was physiochemically characterized using NMR, GPC, and MALDI-TOF MS to confirm the molecular structure and calculate experimental molecular weight. Further, this copolymer was used to develop novel

NDF loaded with both doxorubicin and curcumin drugs. These NDF were characterized by XRD, DSC, TEM, Particle size analysis, Zeta potential and also used to calculate drug loading and encapsulation efficiency for both drugs. In addition, several *in vitro* biological studies were carried out for all NDF. All data were represented as mean ± SD from three independent experiments.

RESULTS AND DISCUSSION:

The poly (*t*-BGE-*alt*-PA) copolymer of 9.3 kDa size was molecularly characterized and found to be fully alternating in nature. The average diameter of these NDF ranged between 200 and 300 nm and presented higher drug encapsulation efficiency for both drugs i.e. more comparable to other similar NDF etc. These NDF further displayed a sustained drug release behavior for both drugs in a defined physiological environment. Moreover, the anti-tumor efficacy were examined on different cancer cell lines and showed higher toxicity on pancreatic cancer cells (MIA PaCa-2) with very low IC_{50} value. These NDF also inhibited the proliferation of MIA PaCa-2 cells due to arrest in G2/M phase that induced apoptosis with increased ROS production and significant changes in mitochondrial membrane potential.

CONCLUSION: In this study, poly (*t*-BGE-*alt*-PA) copolymer was used in preparation of novel NDF showing enhanced anti-cancerous activity against several cancers. Overall, there is need to open up several novel avenues for metal-free alternating copolymers in the field of biomedical science in near future.

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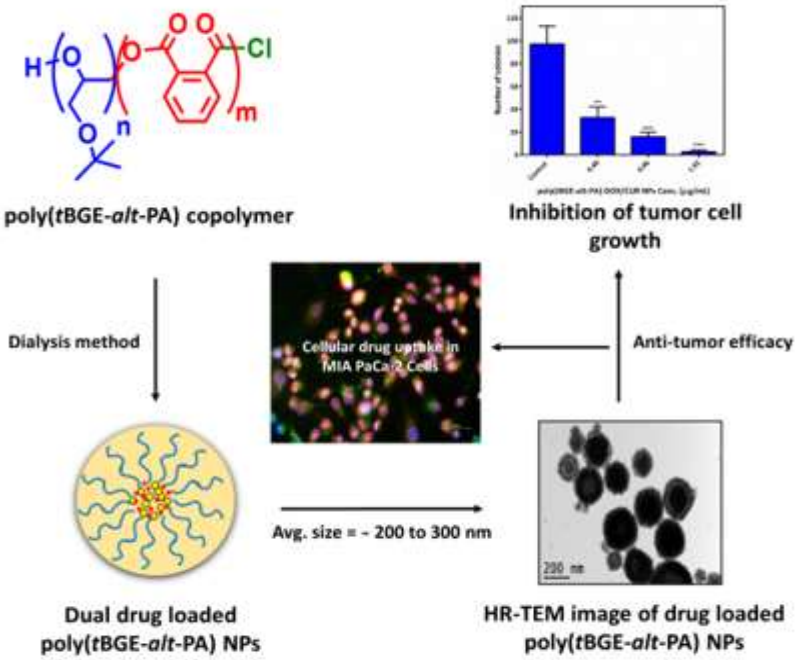

Picture 1: Caption 1: Graphical abstract

Controlling biomaterial-biological system interactions 16:30 - 18:00 Room 0.5 10/09/2018

Oral presentation

779 BTA supramolecular hydrogelators as extracellular matrices for tissue engineering

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[2]Institute of Complex Molecular Systems, Eindhoven University of Technology, Netherlands
[3]Department of Complex Tissue Regeneration, MERLN Institute, Maastricht Uni, Netherlands

INTRODUCTION:

The extracellular matrix (ECM) provides essential biological and mechanical cues required for tissue growth. Hydrogels are used as synthetic ECM as they better mimic mechanics of soft tissues, support cell adhesion, and proliferation. Traditional hydrogels do a poor job of mimicking ECM as they are static, built from covalent bonds, while ECM is made up of covalent and non-covalent bonds, allowing a more dynamic environment. High water content and dynamic interactions of noncovalent bonds among ECM components (proteins, glycoproteins etc.,) give rise to viscoelastic properties of ECM[1, 2]. To better mimic dynamicity and viscoelasticity of ECM synthetically, we are developing self-assembled supramolecular hydrogels based on 1,3,5-benzenetricarboxamides (BTAs) owing to their dynamic nature on cell-relevant timescales, protein-like fibrous structures and the ease of adjusting mechanical properties by controlling interactions at the molecular level. Previous work has shown that BTA architectures can be made to stack over each other through 3-fold hydrogen bonding and hydrophobic interactions forming long self-assembled fibers, resulting in hydrogels[3, 4].

METHODS:

Cell studies: BTA hydrogelators were tested for cytotoxicity with Fibroblasts, and chondrocytes. Cytotoxicity was evaluated by staining cells with calcein-AM and ethidium homodimer-1. Chondrocytes spreading/growth within hydrogels were evaluated using Alexa Fluor 488-Phalloidin and DAPI staining.

BTAs synthesis: To create a new BTA gelator library via a short route work was done on desymmetrization of BTAs; a penta-fluorophenol BTA synthon (BTE-P5Ph) has been synthesized and desymmetrized using hexylamine as a model reaction.

RESULTS AND DISCUSSION:

Cell studies: These hydrogels caused very little cytotoxicity (<10%) over 24 hrs. Further investigation with chondrocytes over 7 days showed ≈20% cytotoxicity. Furthermore, chondrocytes form cell aggregates when cells seeded within gels. We believe chondrocytes aggregate formation within gels is because of their dynamicity which is linked with non-covalent crosslinking of gels.

BTAs synthesis: BTE-P5Ph has been desymmetrized successfully and work is in progress on creating BTAs gelator library.

CONCLUSION:

Limited cytotoxicity was observed for chondrocytes even over 7 days and these non-covalent gels allowed chondrocytes aggregate formation within hydrogels. The comparison will be made with static hydrogels to evaluate if dynamicity of hydrogel is responsible for the aggregate formation and if it would enhance cartilage matrix production. In creating well defined ECM matrix, we aim to develop a library of hydrogels with varying dynamicity and viscoelasticity to understand better cell-ECM interactions and the role that materials time-scales play on tissue formation.

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ACKNOWLEDGMENTS:

This project/research has been made possible with the support of the Dutch Province of Limburg and European Research Council under H2020.

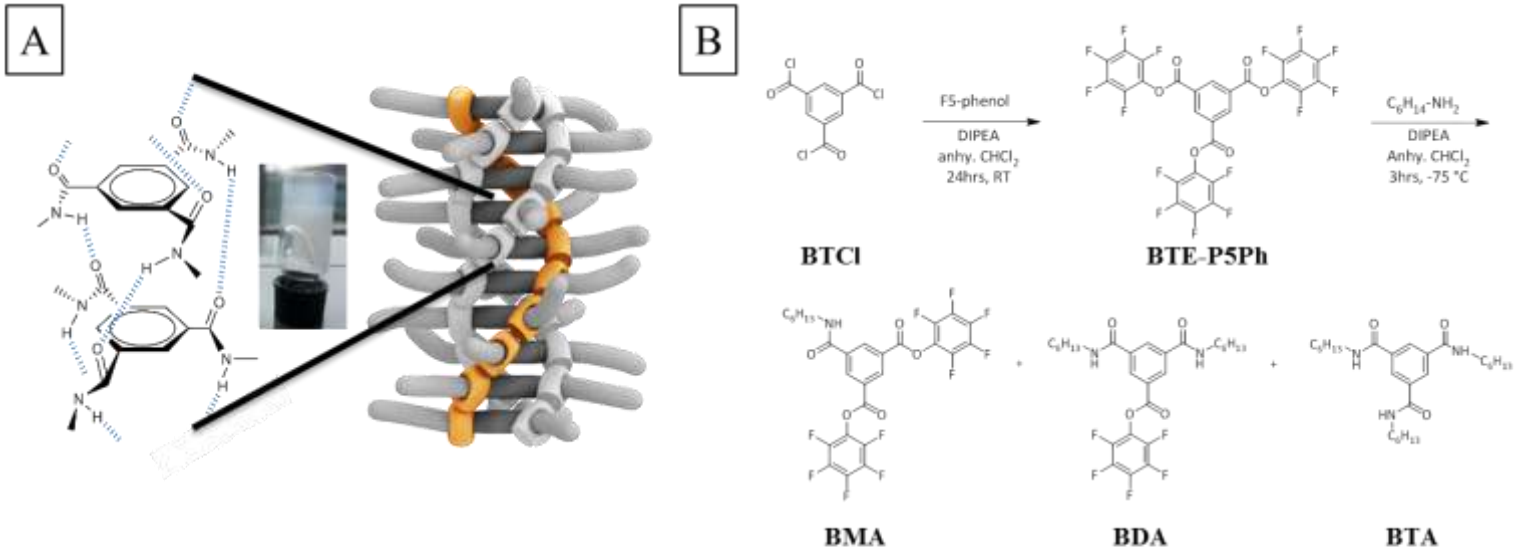

Figure: (A) BTA molecules stack over each other via hydrogen bonding to form long fibers resulting in hydrogels, (B) Penta-fluorophenol BTA synthon (BTE-P5Ph) was synthesized and desymmetrized using hexylamine as a model reacting molecule (RM). Three molecules were obtained as desymmetrization products; mono-substituted, di-substituted and tri-substituted are labeled as BMA, BDA, and BTA, respectively. These molecules were separated using flash column chromatography.

Picture 1: Caption 1: (A) Stacked BTA molecules form fibers, resutig in hydrogel, (B) desymmetrization of BTE-P5Ph synthon using hexylamine

Controlling biomaterial-biological system interactions 16:30 - 18:00 Room 0.5 10/09/2018

Oral presentation

6 Influence of Dynamic Flow Conditions on Adsorbed Plasma Protein Corona and Surface-induced Thrombus Generation on Antifouling Brushes

Kai Yu, Paula Andruschak, Han Hung Yeh, Dana Grecov, Jayachandran Kizhakkedathu
University of British Columbia, Vancouver, Canada

INTRODUCTION:

Synthetic biomaterials used for vascular applications promote surface-induced thrombus generation and inflammation to various extents.[1] Surface modification using polymer brushes can be utilized as a method to

increase the biocompatibility and tailor the biological response to biomaterials. Other than the surface characteristics, hydrodynamic shear force acting on the biomaterial surface also play an important role in surface-induced thrombus generation.[2] The information regarding the nature of protein corona and cell binding under dynamic flow conditions is critical to dissect the mechanism of surface-induced thrombosis. Thus in the present work, we investigated the proteins and blood cells binding on well-defined antifouling hydrophilic brushes with distinct chemistry in flow (arterial and venous) and static conditions using a blood loop. The data presented here will help in understanding the role of protein corona and its changes with flow and chemistry of the surfaces on surface-induced thrombus generation in a situation that closely mimic the *in vivo* conditions.

METHODS:

Three highly hydrophilic antifouling polymer brushes, (poly(*N*, *N*-dimethylacrylamide) (PDMA), poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) and poly[N-(2-hydroxypropyl) mehacrylamide] (PHPMA)) were prepared by surface-initiated atom transfer radical polymerization. A simplified computational fluid dynamics model based on COMSOL Multiphysics 5.2a to better understand the resulting flow patterns in a vertical channel containing flat substrates. The adsorbed protein corona from the blood was investigated by using ellipsometry, fluorescence microscopy and by mass spectrometry based proteomics analyses. The platelet adhesion from the whole blood/PRP was analyzed by scanning electron microscopy (SEM) and lactate dehydrogenase (LDH) assay.

RESULTS AND DISCUSSION:

Blood or PRP was circulated using a peristaltic roller pump (Fig. 1A) with two different flow rates to simulate the blood flow in the artery and vein. The fluid dynamics computational model (Fig. 1B) shows that the WSS profiles on each of the five substrates were very similar, although there is difference between the inlet and peak velocities. PDMAL and PDMAH brushes demonstrated good resistance to the deposits from blood in the static condition (Fig. 1C). The chemistry of the brush seems to influence the flow induced platelet adhesion. The platelet adhesion was greatly reduced on the PDMAH and PDMAL surface to almost complete in static condition to 99.7% ($P < 0.01$) and 99.9% ($P < 0.05$) in flow condition (Fig. 1D), respectively. As for the PMPC surface, the platelets adhered more in static condition (78.6 % compared to control) and decreased to 99.9 % ($P < 0.05$) in arterial flow condition. PHPMA brush showed the greatest number of adherent platelets either in static and flow conditions. Proteomics study revealed that the chemistry nature of polymer brushes and flow/static condition have influenced the species of proteins adsorbed onto the substrate. Adsorbed protein profiles on the PDMA samples (static) shows that serum album was nominally abundant. As to the PDMA at flow conditions, fibrinogen has the highest number of spectra count. The presence of vitronectin and fibronectin on the PDMA at flow conditions may be contributing to the slightly higher platelet adhesion. For the PMPC sample at both flow and static conditions, Apolipoprotein B-100 and apolipoprotein(a) were the most abundant of the plasma proteins, which could contribute to the RBC adhesion on this surface under static conditions.

CONCLUSION:

We determined the profiles of adsorbed plasma proteins and blood cell interaction on three different highly hydrophilic brushes in static and dynamic flow conditions. The flow/static condition influenced the protein corona on brushes modified substrates which is influencing the blood cell interaction especially platelets. The brush structure can not only alter the amount of proteins but also the composition of the adsorbed layer. The current study shows the importance of testing conditions for assessing the biocompatibility of the coated surface *in vitro*.

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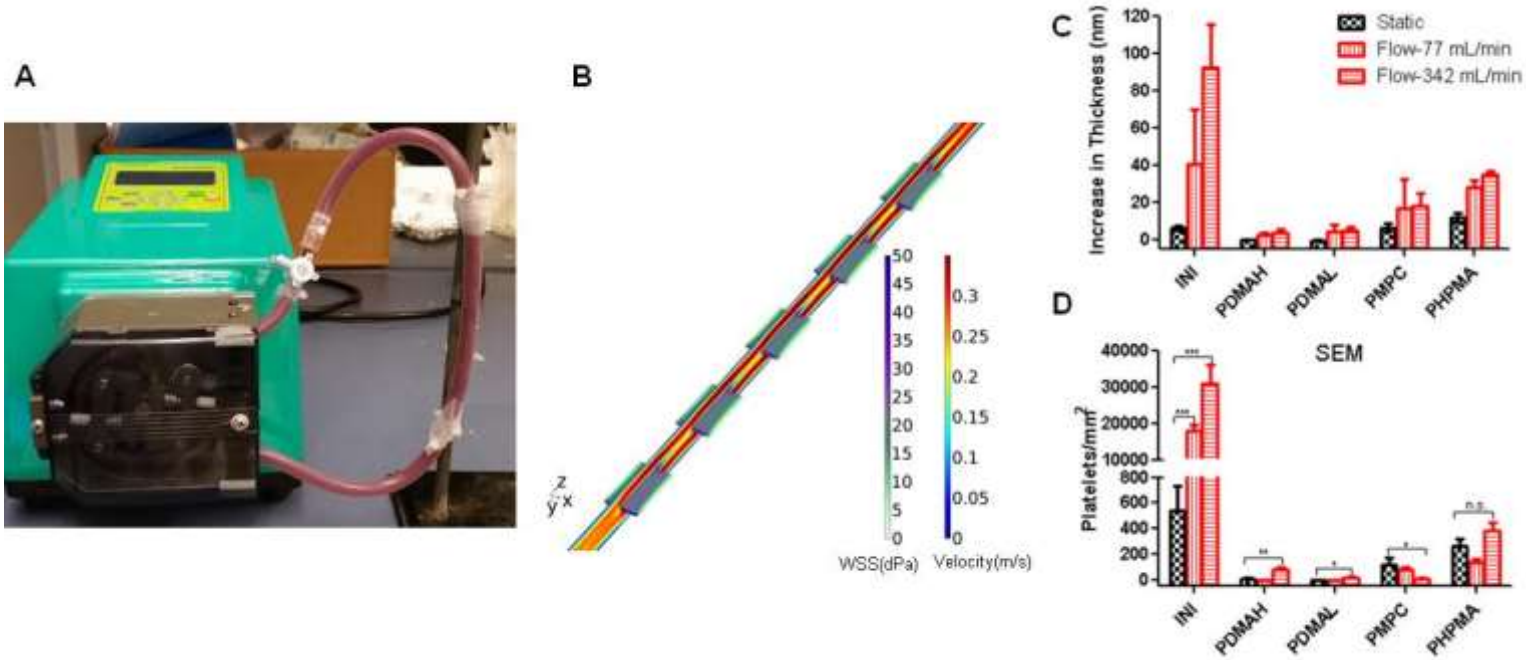


Picture 1: Caption 1: Set up of blood loop (A), fluid dynamic model (B), deposition (C) and platelet adhesion from whole blood

Soft tissue applications for biomaterials 10:45 - 12:15 Room 0.2/0.3 10/09/2018

Oral presentation

227 Antithromboganic poly(ether-ether-ketone) by grafting of phospholipid polymer and immobilization of heparin

Kazuhiko Ishihara, Satoshi Yanokuchi, Yuuki Inoue, Kyoko Fukazawa
The University of Tokyo, Tokyo, Japan

INTRODUCTION:

Since poly(ether-ether-ketone) (PEEK) have excellent mechanical strength and chemical stability, they are extremely effective for the design of these devices as next-generation medical materials in place of metals and ceramics. In particular, PEEK generates radicals on the surface by irradiation with UV light and forms polymer graft layer by radical polymerization. They control the interaction at the interface between the material and the living system. 2-Methacryloyloxyethyl phosphorylcholine (MPC) polymer and heparin (Hep) were modified on the surface of PEEK by the surface modification method. It is expected that progress of multiple blood coagulation processes due to the hybrid-effects of MPC polymer and Hep can be suppressed. The MPC and 2-(methacryloyloxy)ethyl trimethylammonium chloride (TMAEMA) were grafted onto the PEEK, and Hep was immobilized by ionic bonding with TMAEMA unit. Platelet adsorption and blood clotting activity were evaluated.

METHODS:

Poly (MPC-co-TMAEMA) (PMT) was grafted onto the PEEK surface by self-initiated photopolymerization. Immobilization of Hep into PMT-graft-PEEK was performed. To quantify Hep immobilized on each Hep/PMT-graft-PEEK surface, anti-factor Xa activity was measured. To evaluate the platelet adhesion on the Hep/PMT-graft-PEEK, platelet-rich plasma was contact with the substrate. After 1 h, the number of platelets adhered on the substrate was

counted from the SEM after normal treatment. To evaluate blood coagulation activity for each Hep/PMT-graft-PEEK, blood clotting time was measured by a whole blood coagulation test using the Lee-white method.

RESULTS AND DISCUSSION:

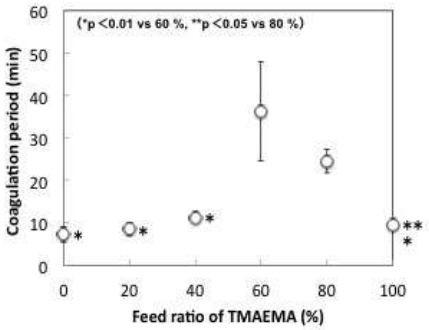
On the surface of the PEEK substrate with TMAEMA/MPC unit ratio of 0/100 to 40/60, the amount of immobilized Hep increased with the TMAEMA unit, and then it became constant about 40 ng/mm^2. Adhesion of platelets was suppressed on the surface of the sample with the TMAEMA/MPC unit ratio until 60/40, indicating an increase in platelet adsorption amount with increase in the TMAEMA/MPC unit ratio up to 80/20. The blood clotting time was about 10 min on the surface of TMAEMA/MPC unit ratio as 0/100. On the other hand, it extended on with the TMAEMA unit fraction and the longest clotting time was observed at Hep/PMT-graft-PEEK (60/40) as 40 min (Fig 1).

CONCLUSION:

When the substrate was contact with blood components, both platelet adhesion and clot formation were suppressed effectively by the hybrid effects of the MPC polymer and Hep. This surface modification technology will have applied for development of PEEK-based cardiovascular implants, such as an artificial valve.

ACKNOWLEDGMENTS:

This research was supported by S-innovation project of AMED, Japan.



Picture 1: Caption 1: Fig. 1. Coagulation time on the Hep/PMT-graft-PEEK surface

Soft tissue applications for biomaterials 10:45 - 12:15 Room 0.2/0.3 10/09/2018

Oral presentation

230 Hydrogel based mesenchymal stromal cell delivery improves muscle regeneration via local modulation of CD4+ and CD8+ T Cell levels

Taimoor Hasan Qazi, Michael Fuchs, Manuela Hoffmann, Agnes Ellinghaus, Norma Schulze, Georg Duda, Sven Geissler
Charité Berlin, Berlin, Germany

INTRODUCTION:

Skeletal muscle has significant regenerative potential that is lost in case of severe injury. Previously, we demonstrated that using hydrogels with distinct structural microenvironments to deliver autologous mesenchymal stromal cells (MSCs) can stimulate muscle regeneration [1]. It was shown that MSCs do not undergo differentiation, but stimulate regeneration via paracrine signaling [2]. Similar outcomes have been observed by our group in a clinical Phase I/II study [3]. However, the underlying mechanisms of regeneration remain unclear. Since our earlier study confirms a tight interaction between immune and musculoskeletal systems [4], we now investigated the relevance of the adaptive immunity for successful muscle repair and how it is altered by MSCs.

METHODS:

We analyzed muscle samples obtained 1, 3, 7, 14 and 21 days after trauma and MSC transplantation. Immune cells were monitored using the LSR II flow cytometer with specific antibodies against CD45, CD3, CD8a, CD4, CD25, CD44, CD62L. The depletion of specific immune cell subpopulations was achieved via the injection of neutralizations antibodies (300 µg per injection for 2 consecutive days, with the last day being the day of trauma).

RESULTS AND DISCUSSION:

The longitudinal comparison of the immune cell compositions in severely injured muscle with the corresponding levels in the peripheral blood or uninjured muscle tissue revealed that specifically T cells are important modulators of the healing process. In particular, we found that accumulation of conventional CD8+ and CD4+ T cells in the muscle trauma area correlates with the severity of the injury and limits the regeneration potential. Conversely, local transplantation of MSCs improves the functional healing outcome and correlates with reduced levels of conventional T-cells and enhanced levels of regulatory CD4+ T cells. Depletion of the whole CD8+ T cells (anti-CD8, OX-8) or more specifically of the CD4+ & CD8+ effector T cells (anti-CD45RC; OX-22) almost completely restores the function of severely damaged muscle, whereas the depletion of whole CD4+ T cell (anti-CD4; OX-38) has no beneficial effect.

CONCLUSION:

Our study refutes the scientific opinion that conventional CD4+ or CD8+ T cells are only found in persistently inflamed muscle. The local reduction of conventional T cell levels and concurrent enrichment of regulatory T cells is a promising therapeutic strategy to improve muscle repair.

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Soft tissue applications for biomaterials 10:45 - 12:15 Room 0.2/0.3 10/09/2018

Oral presentation

8 A bioactive hyperbranched polyglycerol coating as an endothelial cell selective platform for vascular implants

Anouck Burzava[1], Eli Moore[2], Marek Jasieniak[3], Michaelia Cockshell[2], Frances Harding[4], Nicolas Voelcker[5], Claudine Bonder[2], Hans Griesser[1]

[1]Future Industries Institute, University of South Australia, Mawson lakes, Australia
[2]Centre for Cancer Biology, University of South Australia and SA Pathology, Australia
[3]Cooperative Research Centre for Cell Therapy Manufacturing, Australia
[4]Cell Therapies Pty Ltd, Australia
[5]Monash Institute of Pharmaceutical Sciences, Monash University, Australia

INTRODUCTION:

Cardiovascular disease (CVD) is the leading cause of death worldwide, accounting for 31% of all deaths in 2012.[1] In coronary artery disease, the CVD of highest prevalence, reduced blood flow arises due to plaque formation on the arterial wall. To restore blood flow, vascular grafts or stents can be deployed into the occluded artery. However, due to the complexity of the environment, blood-contacting materials still face a number of complications, such as thrombosis or restenosis. Invariably, the implanting of these devices requires the use of long-term anticoagulant therapy.[2, 3]

Herein we present a novel 'low-fouling and endothelial cell selective' coating that will facilitate the formation of new vascular endothelium, while preventing the adhesion of unwanted blood components.

METHODS:

Hyperbranched polyglycerol (HPG) polymer[4] is grown from polystyrene-coated silicon wafers. Due to its functional end groups, HPG can be activated to enable the covalent conjugation of key biomolecules.[5] Surfaces are analyzed via x-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS). In parallel, human endothelial colony forming cells (ECFC), smooth muscle cells or mononuclear cells are left to adhere on the substrates and later stained, imaged and counted. Student t test is used, and experiments are performed in triplicate.

RESULTS AND DISCUSSION:

HPG is shown to be a very effective anti-fouling coating for both cells and proteins. Ex-vivo data demonstrate greatly reduced thrombus formation in coated stents, and no indication of activation of inflammation pathways such as complement and neutrophil activation. After mild oxidation to produce surface aldehyde groups, biomolecules can be covalently grafted onto HPG and are still functional. When decorated in this manner, the HPG is able to specifically capture endothelial cells, while inhibiting the adhesion of other blood constituents such as platelets or mononuclear cells.

CONCLUSION:

We show that after functionalization with specific biomolecules, our coating is able to capture endothelial cells that can assist with the rapid re-endothelialization of the vascular lining around the implant. Such coatings offer a promising alternative to improve the biocompatibility and performance of vascular implants.

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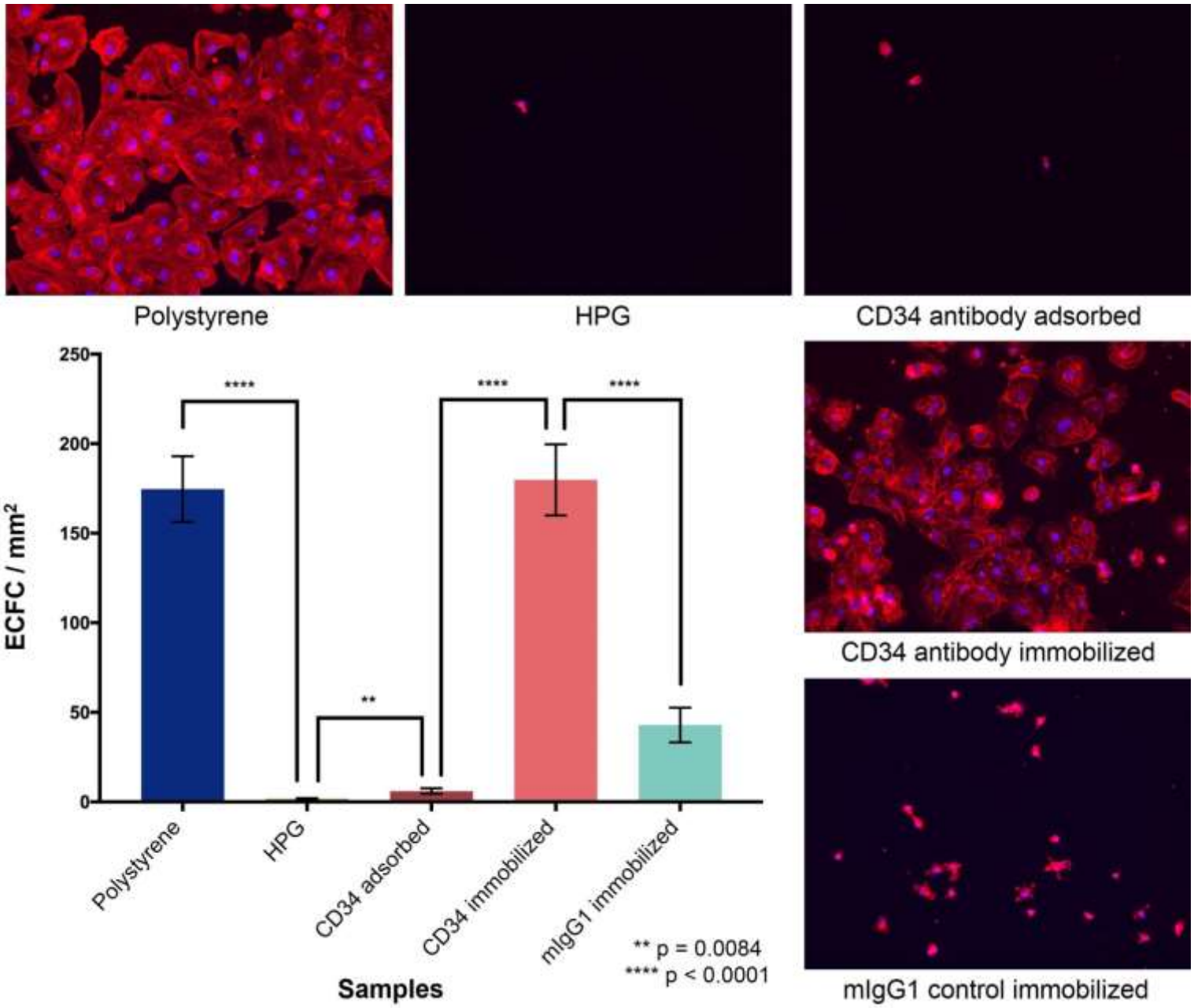
ACKNOWLEDGMENTS:

The authors would like to thank the Cooperative Research Center for Cell Therapy Manufacturing and the Playford Trust Foundation for providing financial support to this project.



Picture 1: Caption 1: HPG coating on polystyrene-coated material is low-fouling, and can be functionalized with biomolecules to specifically capture endothelial cells

Soft tissue applications for biomaterials 10:45 - 12:15 Room 0.2/0.3 10/09/2018

Oral presentation

708 A multifactorial, multiplanar approach to inducing functional neural cell behaviour using electrospun scaffolds: is fibre alignment the critical factor?

Kirstie Andrews
Manchester Metropolitan University, Manchester, United Kingdom

INTRODUCTION:

There is an increasing need to develop tissue engineered constructs for peripheral nerve regeneration applications. Research to date has targeted nano-scale, aligned fibrous electrospun scaffolds, to create structures mimicking natural nerves[1]. Recent studies have shifted to micro-scale fibres to improve mechanical properties; however, the focus remains the alignment, fibre diameter and choice of material/surface chemistry[2].

There has been little investigation into the potential to use other properties to induce optimum neural cell functionality, or to confirm the critical importance of fibre alignment.

Therefore, the aim was to investigate the effect and significance of multiple structural and topographical parameters of electrospun scaffolds upon inducing functional and biomimetic neural cell behaviour.

METHODS:

Ranging electrospun polyurethane scaffolds were UV-Ozone sterilised and cell-seeded with human neurons (HN) and Schwann cells (HSC): HN; HSC; HN + HSC. Seeding densities of $5x10^4$ cells/type/sample were used, for 4, 7, 14, 28 days.

Cell-scaffold behaviour was examined for cellular growth, process length/involvement/function and myelination potential, utilising a series of cell markers and neurotropic factor expression (n=4 per sample/condition).

Means, standard deviations and ANOVA statistical tests, with Tukey post hoc analysis, were performed. Assessed substrate parameters included: inter-fibre separation (IFS); fibre diameter (FD); surface roughness (SR); void fraction (VF); fibre orientation (FO).

RESULTS AND DISCUSSION:

Key aspects of cell behaviour and functionality were directly induced by separate scaffold properties showing noteworthy differences across samples (Figure 1). HN functionality, including axonal growth and alignment, was significantly affected by scaffold parameters in the horizontal plane (e.g. FD, $P<0.001$, and 1/SR, $P=0.003$); HSC behaviour, including myelination potential, correlated to the vertical plane (e.g. VF, $P=0.044$). Co-culture maintained these trends, with a lag in responses, particularly for HN. This suggested an ability to mimic natural neural regeneration processes, through manipulation of distinct scaffold properties, with a larger range of parameters than previously reported shown as significant.

CONCLUSION:

A multiplanar, multifactorial consideration of electrospun scaffolds was shown to be critical in the approach to developing substrates capable of producing functional, biomimetic neural regeneration behaviour.

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ACKNOWLEDGMENTS:

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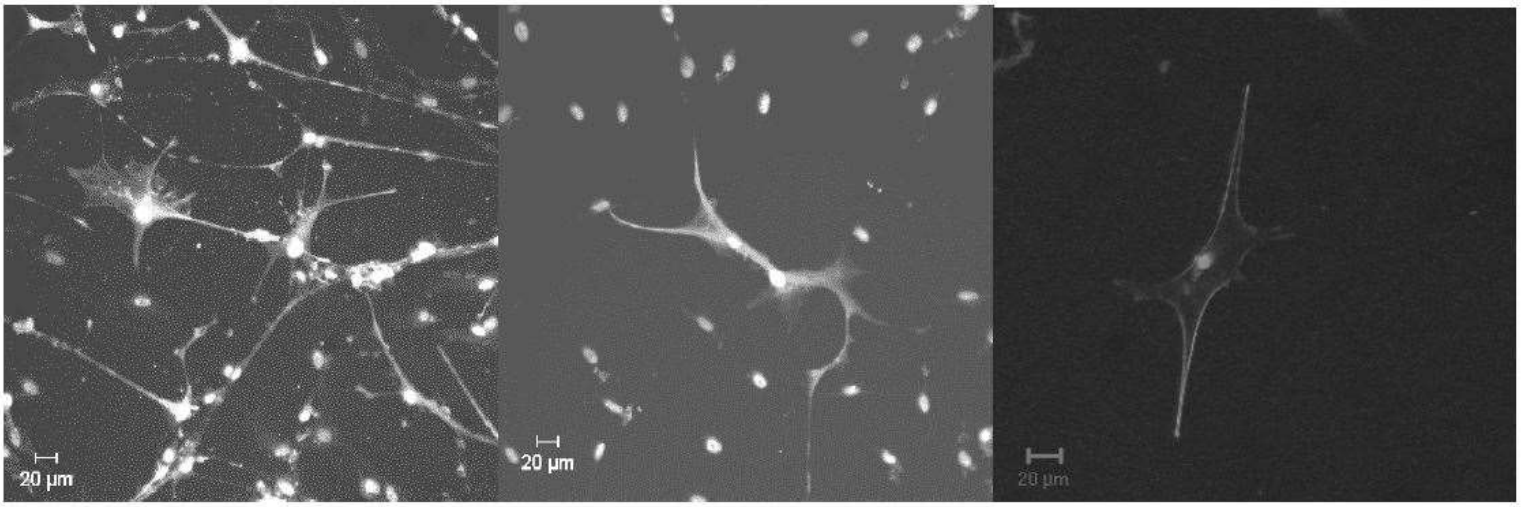

Picture 1: Caption 1: Figure 1. The effect of altering topography on neuronal cell responses.

Soft tissue applications for biomaterials 10:45 - 12:15 Room 0.2/0.3 10/09/2018

Oral presentation

120 Microstructure and cyclic performance of composite hydrogel implants

Céline Wyss[1], Azadeh Khoushabi[1], Peyman Karami[2], Dominique Pioletti[2], Pierre-Etienne Bourban[1]
[1]EPFL / Laboratory for Processing of Advanced Composite (LPAC), Lausanne, Switzerland
[2]EPFL / Laboratory of Biomechanical Orthopedics (LBO), Switzerland

INTRODUCTION:

Hydrogels are promising implantable biomaterials owing to some similarities to living tissues. To apply hydrogels in load bearing situations, a well-known long-term reliability is essential for clinical applications. Indeed, mechanical and/or physical properties could be changed under fatigue loading. For example, similar to the Mullins effect, conventional double network hydrogels become softer after the first loading cycles[1]. In this study, the evolution of composite hydrogels properties including swelling ratio, stiffness and fracture strength were evaluated before, under, and after cyclic loading.

METHODS:

Poly(Ethylene Glycol) Dimethacrylate (PEGDM) hydrogels reinforced with different Nano-Fibrillated Cellulose (NFC) fibers and representative hydrogels without fibers were synthesized as described previously[2]. The fatigue behavior of composite hydrogels was evaluated in tension and compression with an extensive parametric study incorporating the applied strain amplitude, the swelling ratio and the fiber concentration. Single edge crack tests were performed on untreated and treated hydrogels that were previously pre-conditioned under cyclic tensile loading. Finally, the 3D microstructure of composite hydrogels was observed with fluorescence confocal microscopy.

RESULTS AND DISCUSSION:

Fig. 1 shows different microstructures of the NFC fibers in a swollen composite hydrogel before and after high strain loading conditions. Bundle of NFCs and clouds of tightly entangled and randomly oriented fibrils are observed. The largest stiff bundles (arrows in Fig.1) start to break after the first loading cycles, before to offer a more homogeneous and stabilized structure. Microscopy results reveal the deformation and damage mechanisms responsible for the hydrogel properties, such as tensile and compression modulus, fracture strength or swelling ratio. For example, cyclic compression at 70% maximal applied strain shows that composite hydrogel becomes 44% softer after the first loading cycles and then maintains their properties up to 10 million cycles with a constant modulus 250% higher than the neat hydrogel.

CONCLUSION:

Composite hydrogels reinforced with cellulose fibers are envisaged for load bearing implants such as nucleus pulposus and cartilage replacement. This study investigates their performance in terms of modulus under cyclic compression and tensile loadings and fracture strength. The results illustrate how their long-term reliability is clearly linked to the loading history.

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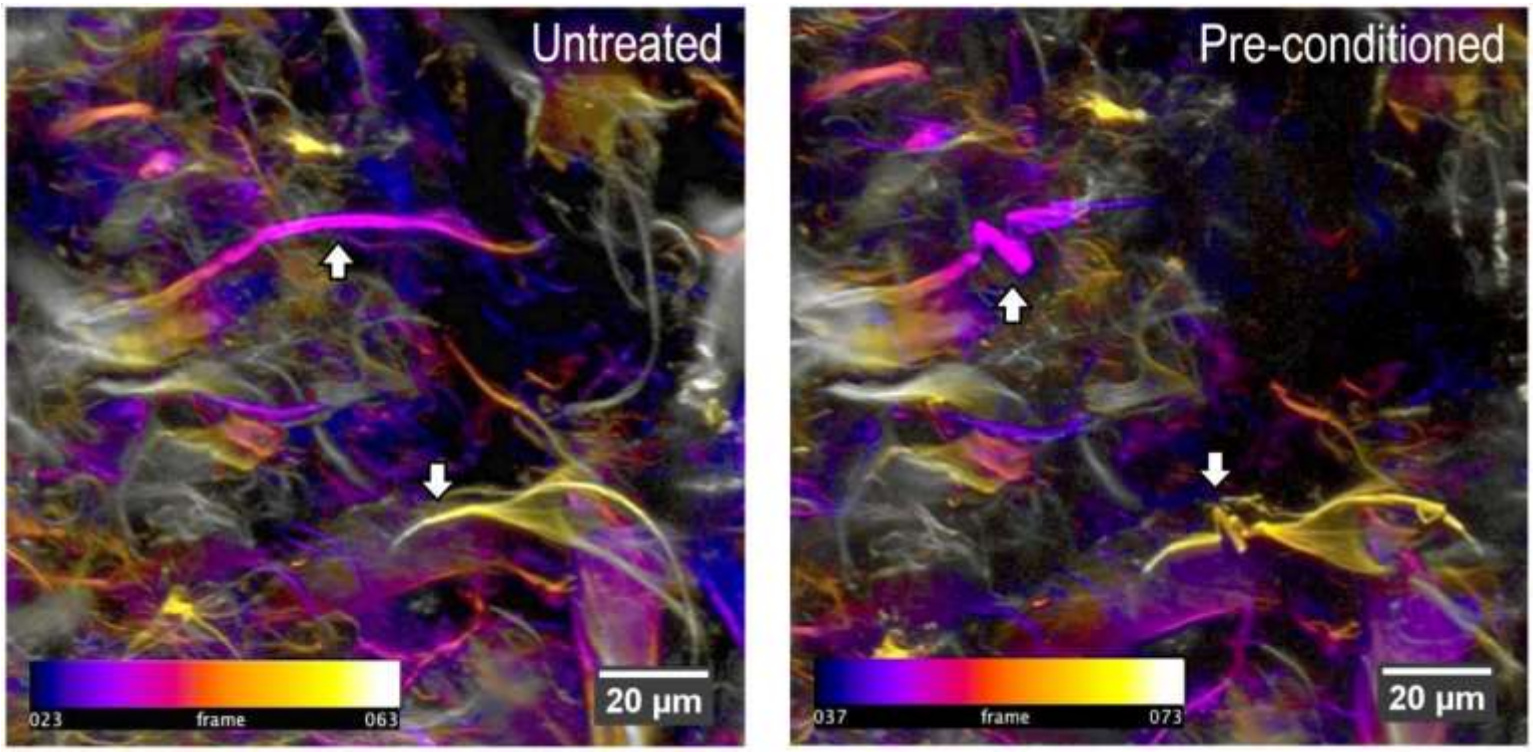
ACKNOWLEDGMENTS:

The authors thank the Swiss National Science Foundation, EPFL-BIOP, EPFL-LBO, EPFL-LAPD and EMPA Dübendorf.



Picture 1: Caption 1: Fig.1 - Hyperstack images of NFC fibers in composite hydrogels showing first damage of fibers after being compressed 10 times at 70% of deformation

Soft tissue applications for biomaterials 10:45 - 12:15 Room 0.2/0.3 10/09/2018

Oral presentation

221 Intraluminal guidance cues for nerve guide conduits

Jonathan Field[1], Adam Harding[1], John Haycock[2], Fred Claeyssens[2], Fiona Boissonade[1]
[1]School of Clinical Dentistry, University of Sheffield, Sheffield, United Kingdom
[2]Department of Materials Science and Engineering, University of Sheffield, United Kingdom

INTRODUCTION:

Nerve guidance conduits (NGCs) can be used as an alternative to the 'gold standard' autograft to overcome disadvantages such as donor site morbidity and the need for multiple operation sites. Commercial NGCs are available but all have simple designs and are only successful in short distance nerve gaps. Here, two types of conduits were produced containing intraluminal guidance cues to guide the migration and growth of regenerating cells. NGCs were separately fabricated containing either internal microgrooves or aligned, electrospun microfibres and were investigated *in vivo* to assess the influence of each conduit type on peripheral nerve regeneration.

METHODS:

Polycaprolactone-methacrylate (PCL-MA) was synthesised and used via microstereolithography to fabricate plain tubes and tubes containing internal microgrooves. Aligned PCL microfibers were electrospun and inserted into the plain tubes to act as intraluminal guidance structures. The resulting constructs were analysed via scanning electron microscopy (SEM), helium pycnometry and micro-computed tomography.

In vivo implantations were carried out in Thy-1-YFP mice to determine the effectiveness of each conduit type in promoting nerve regeneration in situ: NGCs were used to repair a 3 mm injury of the common fibular nerve and regeneration was assessed via confocal imaging.

RESULTS AND DISCUSSION:

PCL fibres with diameters ranging from 2–16 μm were electrospun to a high degree of alignment (figure 1A) and inserted into the plain PCL-MA tubes (figure 1B) to give a composite structure (figure 1C). Packing density was quantified with helium pycnometry to give a percentage fill between 10-50%. Control over this aspect of conduit design is important as it has been shown that a high volume of material within the lumen can impede regeneration. PCL-MA conduits were also produced with aligned grooves along the luminal wall, with no material occluding the luminal area (figure 1D).

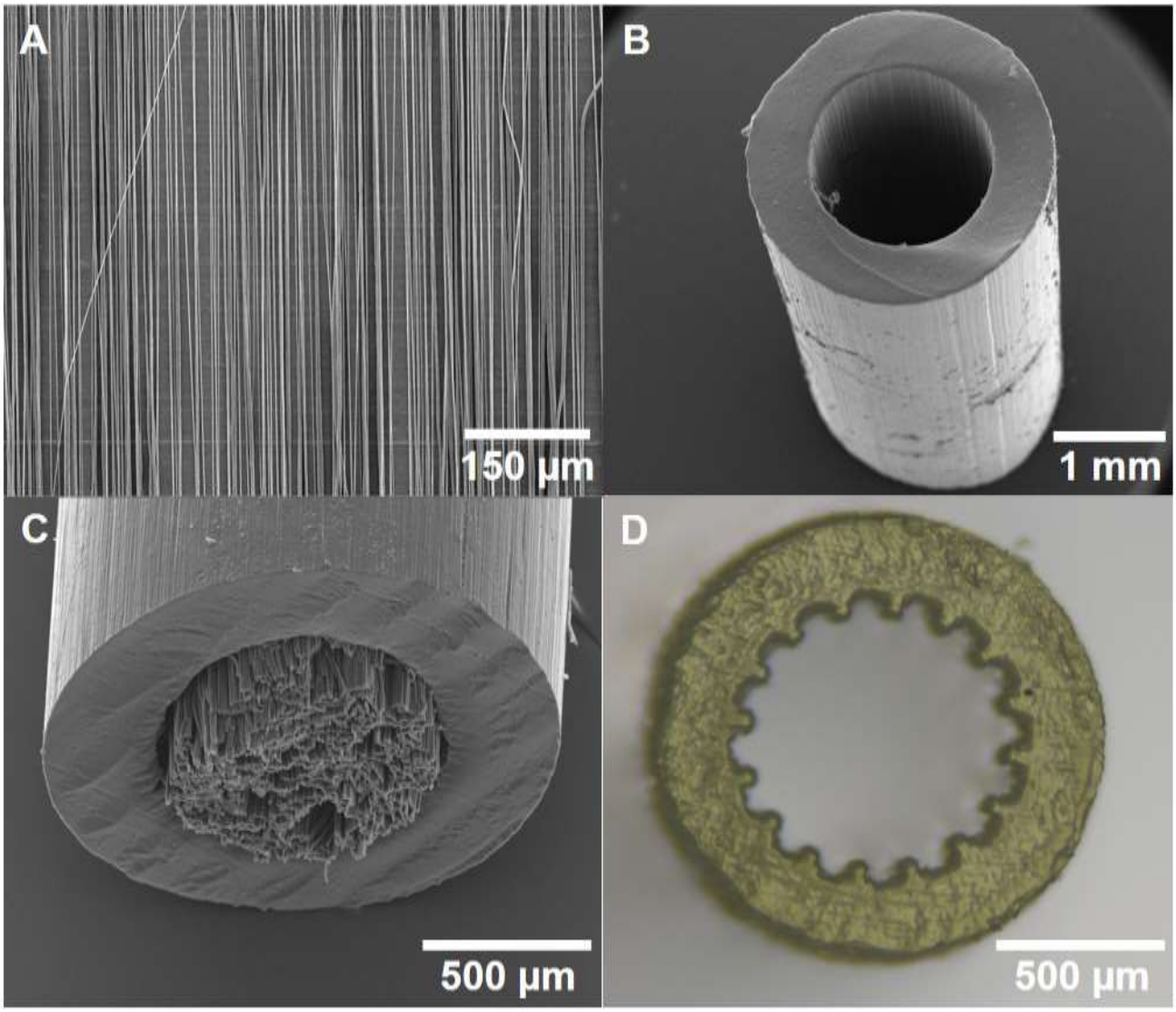
In vivo nerve regeneration through the microgrooved and fibre-filled NGCs was quantitively assessed via the tracing of fluorescent axons across the 3 mm nerve gap. The results were compared to empty conduit controls and autograft repairs. In preliminary in vivo trials, axons were seen to traverse the 3 mm gap in each conduit type after a 3 week recovery.

CONCLUSION:

Advanced NGCs containing intraluminal guidance structures have shown potential for promoting nerve regeneration *in vivo* and the Thy-1-YFP mouse model allows us to compare the effect of different guidance structures within NGCs.

ACKNOWLEDGMENTS:

The authors would like to thank the EPSRC and the University of Sheffield, Faculty of Medicine, Dentistry and Health for funding this research.



Picture 1: Caption 1: Figure 1. A-C: SEM images of electrospun fibres (A), plain tube (B) and fibre-filled conduit (C). D: optical micrograph of grooved conduit.

Advances in soft tissue engineering 16:30 - 18:00 Room 0.2/0.3 10/09/2018

Oral presentation

5 Gelatin scaffolds for adipose tissue engineering with prevascular network by sacrificial template

Nicola Contessi Negrini[1], Mathilde Bonnetier[2], Giorgio Giatsidis[3], Orgill Dennis Paul[4], Silvia Farè[5], Benedetto Marelli[2]
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[2]Massachusetts Institute of Technology, Cambridge, MA, United States of America
[3]Division of Plastic Surgery, Brigham and Women's Hospital, Boston, MA, United States of America
[4]Brigham and Women's Hospital, Boston, MA, United States of America
[5]Politecnico di Milano, Milan, Italy

INTRODUCTION:

Current challenges in designing scaffolds for adipose tissue (AT) engineering regard the obtainment of adequate morphological, structural and biological properties [1], together with a promoted vascularization, recognized as the main limitation to their clinical success [2]. We propose gelatin hydrogel scaffolds with adequate properties for AT regeneration and hollow channels with desired geometry, useful for a controlled vascularization, by simultaneously using alginate microbeads and 3D printed alginate hydrogels as sacrificial templates.

METHODS:

Gelatin hydrogels (GEL) were prepared by crosslinking via methylene-bis-acrylamide. Alginate microbeads and 3D printed strands were embedded in GEL during its synthesis. After GEL complete crosslinking, alginate templates were removed by immersion in an optimized salt solution. SEM was used to investigate the scaffold structure, DMA to measure its compressive properties, weight variation at 37 °C and collagenase enzymatic degradation to test its stability and degradability. A dyed fluid was injected in the hollow channels to check their morphology; human mesenchymal stem cells (hMSC) were seeded in the hollow channels and their viability checked by LIVE/DEAD staining. Finally, viability of hMSC seeded on GEL and cultured in adipogenic medium was investigated by LIVE/DEAD staining; lipid droplet deposition was observed by Oil Red O staining.

RESULTS AND DISCUSSION:

A successful strategy was here optimized to simultaneously obtain scaffolds with randomly distributed macropores and a controlled distribution of hollow channels. Macropores (Ø = 200-400 mm) were observed in GEL after alginate microbeads removal; compressive properties (E = 3 kPa) suitable for mimicking subcutaneous AT were achieved; stability in water at 37 °C was observed up to 21 days and possibility of enzymatic degradation, fundamental for the *in vivo* remodeling, was confirmed. Obtained hollow channels (Ø = 300 mm) reproduced with fidelity the designed channels geometry, after 3D printed alginate removal; viable hMSC colonized the hollow channels walls. GEL scaffolds supported hMSC adhesion and proliferation, with viable cells colonizing the pores of the scaffold; lipid droplets accumulation, evidence of adipogenic differentiation, was detected.

CONCLUSION:

A combination of sacrificial alginate microbeads and 3D printed alginate networks embedded in GEL hydrogels was used to obtain scaffolds with suitable physico-mechanical properties for AT engineering, with hollow channels that can be used, after *in vivo* anastomosis, to promote scaffold vascularization for a future successful clinical application.

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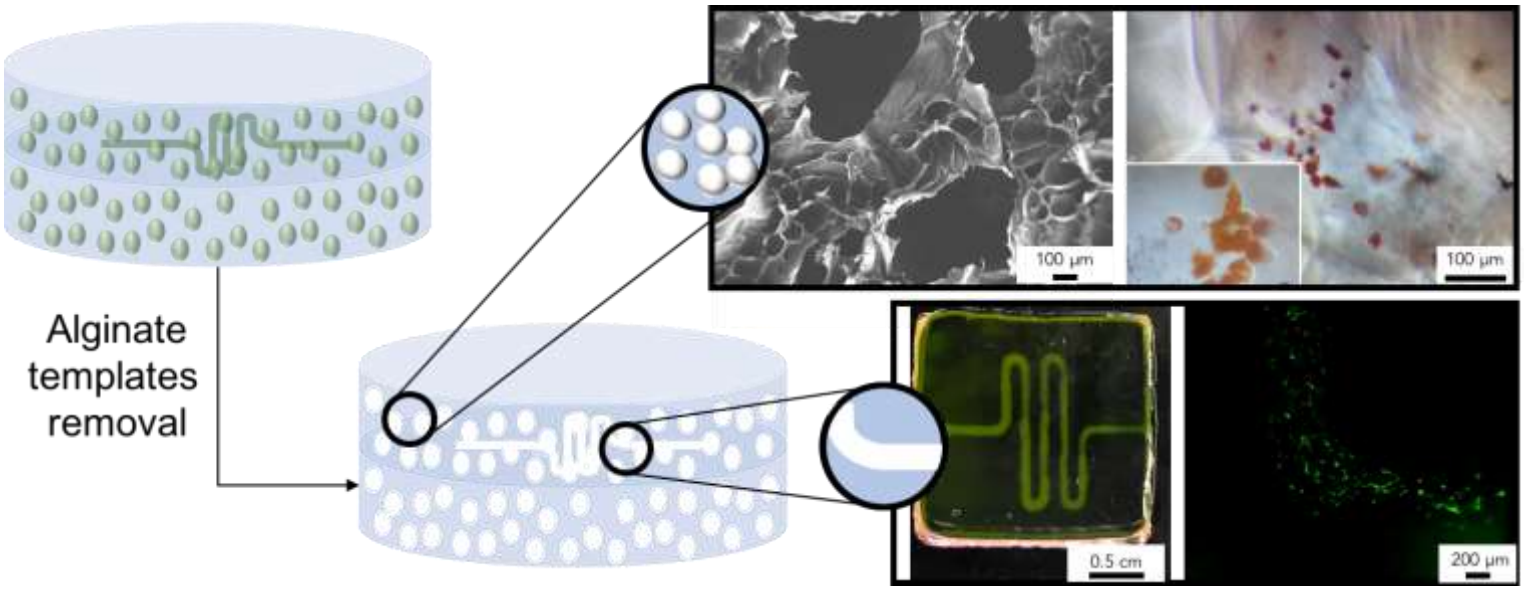
ACKNOWLEDGMENTS:

Authors acknowledge the financial support of Progetto Roberta Rocca.



Picture 1: Caption 1: Porous gelatin scaffold with prevascular network obtained by sacrificial removal of alginate microbeads and 3D printed alginate

Advances in soft tissue engineering 16:30 - 18:00 Room 0.2/0.3 10/09/2018

Oral presentation

Optical fibers & MEMS for the micro-viscoelastic characterization of tissues and biomaterials in physiological conditions.

Jakob Pyszkowski
[1]Optics11, The Netherlands

INTRODUCTION:

Mechanics of soft materials is recognized as an increasingly important in tissue engineering to both cell function as well as replicating the micromechanical architecture in human tissues. Nanoindentation is emerging as a popular technique to obtain information on the micromechanical architecture at cell length scales. Optics11 introduces a new technology based on optical fibers & MEMS to increase and optimize mechanical sensitivity and compatibility with biological samples and physiological environments, required for next-generation mechanobiology experiments.

METHOD:

The Optics11 Piuma and Chiaro instruments are featured with a fiber-optical ferrule-top micro-machined force transducer, providing inherent liquid compatibility combined with a low spring-constant force transducer. This combination offers unique advantages when performing measurements in liquids and on soft materials (Chavan et al, *Rev Sci Instrum* 83:115110, 2012). The instrument can be operated in three modes (displacement, load and indentation control) and in quasi-static and dynamic mode to allow practically any type of experiment to investigate visco-elastic sample behaviour including quasi-static experiments to derive *elastic* moduli, to step-response tests (e.g. creep, stress-relaxation) and dynamic mechanical analysis (DMA). Applications to hydrogels, tissues and single cells will be presented during this oral presentation.

Advances in soft tissue engineering 16:30 - 18:00 Room 0.2/0.3 10/09/2018

Oral presentation

580 Endothelial - Smooth Muscle - Fibroblastic co-culture systems prospecting the development of Tissue-Engineered Vascular Grafts

Tatiana Felizardo, Nuno M. Neves, Albino Martins, Rui L. Reis
3B´s Research Group, University of Minho, Barco - guimarães, Portugal

INTRODUCTION:

Cardiovascular diseases, including coronary artery diseases, are a major cause of mortality worldwide. Consequently, there is a pressing need to develop small-diameter vascular vessels for bypass surgery and other vascular reconstructive procedures. Tissue engineering offers the prospect of being able to meet this medical demand, as it allows the development of structurally complex blood vessels substitutes[1]. Accordingly, the ultimate aim of this work is to develop small diameter vascular substitutes based on layering multiple cell types (i.e. endothelial, smooth muscle and fibroblastic cells)[2].

METHODS:

Electrospun nanofibrous meshes, which restrict cell migration although enabling biochemical communication[3], were used as substrate. Co-culture systems of human endothelial-smooth muscle cells and fibroblastic-smooth muscle cells were established. These co-cultures were then assembled to develop a tri-culture system, which mimics the structural organization of a blood vessel.

RESULTS AND DISCUSSION:

In both co-culture conditions was possible to observe a viable and proliferative cell population through the 7 days of culture. From the histological (Figure 1) and immunofluorescence micrographs of the co-culture systems was possible to observe that the endothelial, smooth muscle and fibroblastic cells remained phenotypically stable, even in the presence of another cell type.

Concerning the tri-culture system, cell viability and proliferation presents a similar trend to the co-cultures. Interestingly, the tri-culture system presents values of protein synthesis much higher than the co-cultures, mostly of collagen. Quantification of lineage-specific growth factors, namely vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), on conditioned media of co- and tri-culture systems demonstrated a synergistic interplay between VEGF and bFGF. VEGF is mainly expressed by smooth muscle cells, which leads to increase levels in the co- and tri-culture systems. A similar trend is observed with the levels of bFGF, expectedly produced by the fibroblast cells. By its side, PDGF levels remain unaltered among conditions.

Altogether, these results demonstrate the potential inter-cellular communication mediated by these soluble proteins.

CONCLUSION:

This study demonstrated the fundamental importance of the intercellular crosstalk between endothelial, smooth muscle and fibroblastic cells, in the mechanism of vessels' regeneration. These experimental results reinforce the potential of a tri-culture system in the development of tissue engineered blood vessel substitutes.

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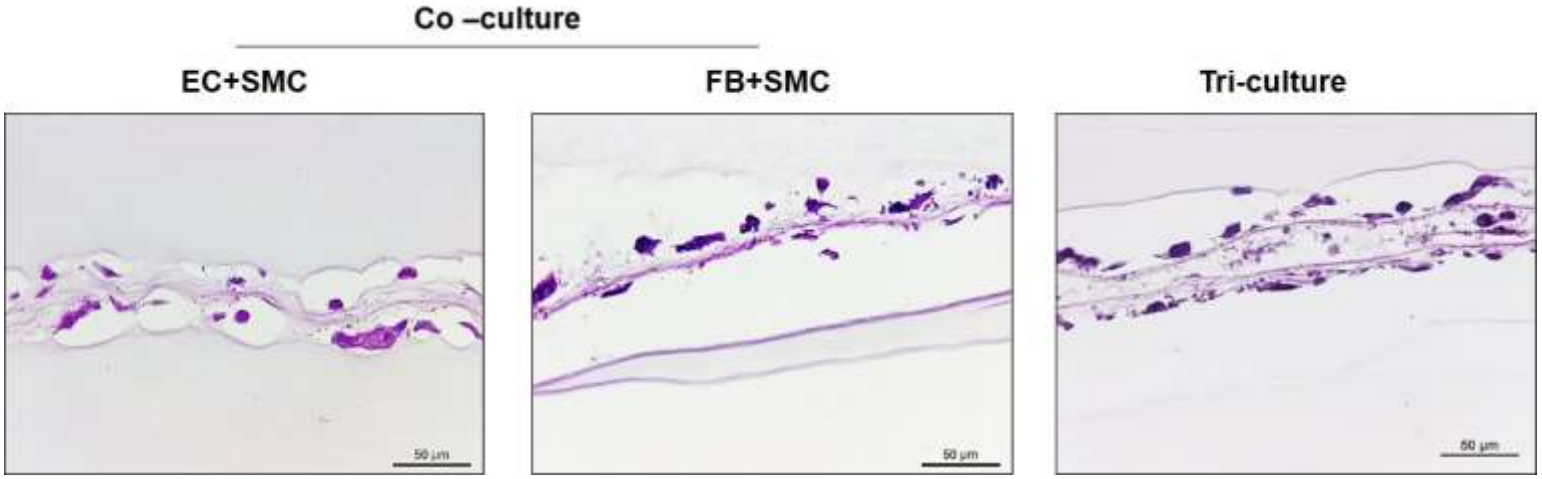
2 - Costa, *et al.*, WO/2013/085404.

3 - Martins, *et al.*, WO/2014/016816, EP2878314, US20160038645.

ACKNOWLEDGMENTS:

The Portuguese Foundation for Science and Technology for financing the project VESCells (PTDC/BBB-BMD/5468/2014) (POCI-01-0145-FEDER-016909) and the IF grant of A.M. (IF/00376/2014).



Picture 1: Caption 1: HE staining of endothelial - smooth muscle cells (EC+SMC) and fibroblasts - smooth muscle cells (FB+SMC) co-cultures and tri-culture systems.

Advances in soft tissue engineering 16:30 - 18:00 Room 0.2/0.3 10/09/2018

Oral presentation

126 3D Human skin equivalent (HSE) as a preclinical in vitro risk assessment platform

Ayesha Idrees[1], Valeria Chiono[2], Gianluca Ciardelli[2], Xiang Zhang[3], Richard Viebahn[4], Jochen Salber[5]
[1]1.Center for Clinical Research (ZKF),RUB,Bochum,Germany; 2.Politecnico di Torino, Turin, Italy
[2]Politecnico di Torino, Italy
[3]Lucideon Limited, United Kingdom
[4]UK Knappschaftskrankenhaus, Germany
[5]1.UK Knappschaftskrankenhaus 2.Center for Clinical Research (ZKF),RUB,Bochum, Germany

INTRODUCTION:

Infected wounds still represent a great challenge in public health. With an increasing need for novel strategies to combat wounds colonized with resistant microbes, more reliable preclinical data is needed to bioanalyse antimicrobial polymeric biomaterials (AMBs). A 3D human skin equivalent (HSE) as wound infection model is highly demanded to serve as a biomimetic system for the testing of AMBs.

METHODS:

The HSE was obtained having both a dermal and an epidermal compartment, by embedding human primary fibroblasts in rat tail tendon collagen type I hydrogel and then seeding human primary keratinocytes on it to generate the epidermis. The cultural conditions were optimized to obtain closely mimicking *in vivo* skin. Skin wound models were created by full thickness incision and colonized with relevant skin infectious bacteria e.g. *S. aureus* at wound site, to generate an *in vitro* skin infection model. The model was fully characterized by histology staining,

immunohistochemistry, confocal microscopy and TEM analysis. To validate the system, different antimicrobial wound dressings were tested for cytocompatibility and antimicrobial properties in our HSE infection model.

RESULTS AND DISCUSSION:

Confocal imaging revealed the filopodia like morphology and a uniform distribution of fibroblasts at different planes inside the dermal matrix. Histological results showed the characteristic multilayered epidermal structure (Fig. 1). Immunohistology showed that basal epidermal cells were in a proliferative state and expressing keratin 14, while supra-basal early differentiation marker (keratin 10) was restricted to supra-basal layers only. Terminal differentiation (filaggrin) was found in the subcorneal layers of the epidermis. The basement membrane protein (laminin 5) was present at dermal-epidermal junction. TEM revealed basement membrane with lamina lucida, lamina densa, regular hemidesmosomes and anchoring fibers. Our HSE infection model revealed that *S. aureus* invades through the wound to the whole dermal depth, disrupting epidermal layers while surrounding keratinocytes. Cytocompatibility and antimicrobial activity of different wound dressings showed marked differences when compared with common 2D cell culture systems. With an increasing need for skin substitutes as reliable *in vitro* test systems[1], we successfully created a full thickness infected skin equivalent, to serve as a risk assessment platform.

CONCLUSION:

Our HSE is a suitable tool for diverse topics of skin research, including cytocompatibility evaluation, drug testing, wound healing and skin infection.

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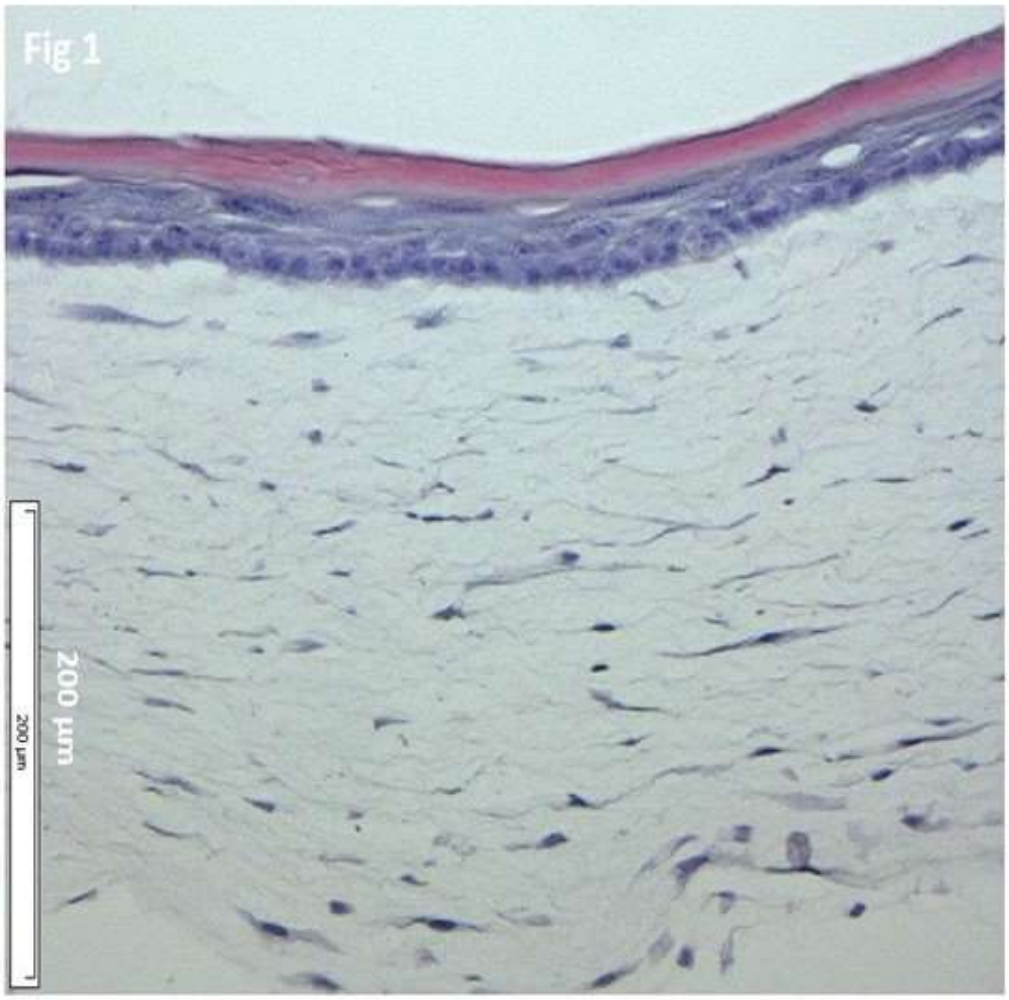
ACKNOWLEDGMENTS:

The authors like to thank European Union's Horizon 2020 research and innovation programme (643050) for providing financial support to HyMedPoly project.



Picture 1: Caption 1: Fig. 1: Histological cross section of H & E stained in vitro Human Skin Equivalent (HSE).

Advances in soft tissue engineering 16:30 - 18:00 Room 0.2/0.3 10/09/2018

Oral presentation

560 Bioactive, hydrophilic, and stretchable polymer clay nanocomposites for soft tissue engineering

Sungkwon Yoon, Biqiong Chen
Queen's University Belfast, Belfast, United Kingdom

INTRODUCTION:

Elastomeric biopolymers are an important class of biomaterials especially for soft tissue engineering applications. Among them, poly(glycerol sebacate) (PGS) has been widely studied due to its good biocompatibility and biodegradability, as well as biomimetic mechanical properties.[1] However, PGS is hydrophobic and holds no additional bioactive functionalities.[2] In this study, we report PGS-based clay nanocomposite gels with bioactivity such as induced angiogenesis and absorption of biogenic amines, as well as excellent hydrophilicity and stretchability.

METHODS:

To achieve nanocomposite gels, hydrophobic PGS and hydrophilic poly(ethylene glycol) (PEG) were copolymerised while clay dispersed in the polymer matrix. The samples were analysed by infrared and ultraviolet-visible spectroscopy, X-ray diffraction, electron microscopy, tensile tests, water swelling and vapour transmission studies, *in vitro* biocompatibility and biodegradability tests, as well as chick chorioallantoic membrane (CAM) assay. A freeze-drying approach was utilised for the *proof-of-concept* fabrication of foams.

RESULTS AND DISCUSSION:

The prepared nanocomposites were characterised by chemical crosslinking *via* ester bonds between PGS and PEG and well-dispersed clay within the polymer matrix. Hydrophilicity of the copolymer was improved due to the water swelling ability of clay. Stretchability and flexibility originated from PGS and hydrated PEG were further enhanced by the presence of clay, withstanding complex mechanical deformation such as stretching and knotting unlike many other conventional hydrogels. Non-cytotoxicity was confirmed by cell metabolic assay with L929 cells *in vitro*, with increasing metabolic responses. Biodegradation kinetics was controllable with the addition of clay. Induced angiogenesis by clay was demonstrated using CAM assay. The ability to absorb biogenic amine compounds, such as cadaverine and putrescine, was gifted by the large surface area and the intercalating capability of clay. Finally, an architecture of three-dimensional porous foam structure was also introduced to help illustrate its potential application in wound dressings and tissue scaffolds.

CONCLUSION:

The incorporation of clay into a PGS-based polyester resulted in novel bioactivities such as induced angiogenesis and absorption of biogenic amines, as well as improved hydrophilicity and excellent elastomeric mechanical behaviour. Together with its good biocompatibility and biodegradability as well as the ability to fabricate large three-dimensional porous foams, this novel elastomer-clay nanocomposite is an attractive biomaterial for multifunctional soft tissue engineering applications.

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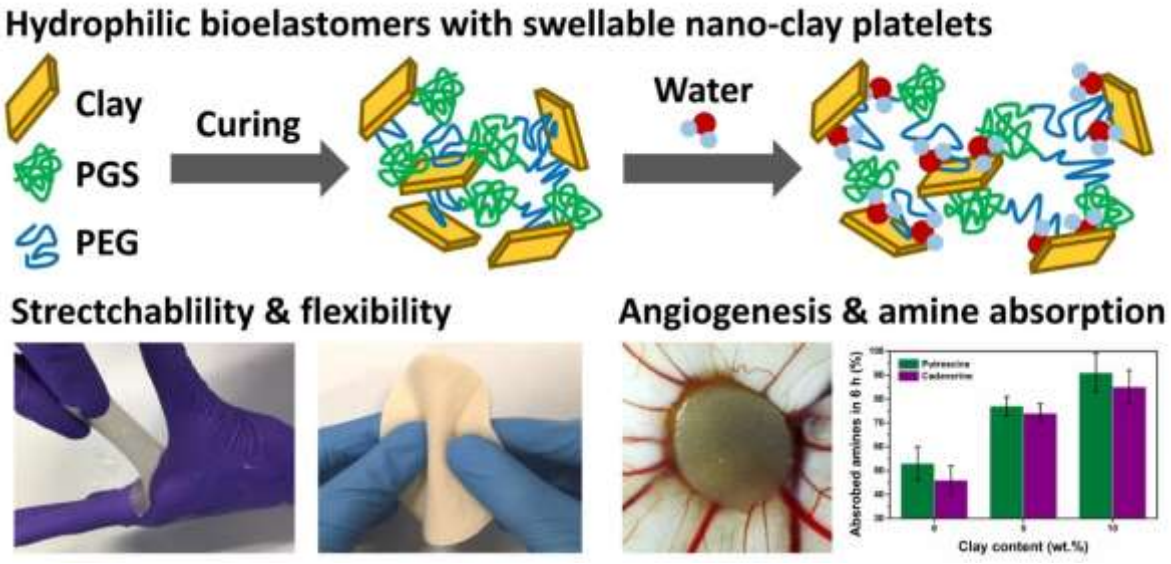
2. Patel A. et al., Biomaterials 34:3970-3983, 2013

ACKNOWLEDGMENTS:

The authors thank Sheila McNeil and Serkan Dikici (the University of Sheffield) for carrying out the CAM assay. S. Y. thanks the University of Sheffield for a University Prize Scholarship.

Picture 1:



Advances in soft tissue engineering 16:30 - 18:00 Room 0.2/0.3 10/09/2018

Oral presentation

410 Lactate-based strategy for cardiac tissue engineering

Jesús Ordoño, Soledad Pérez-Amodio, Lourdes Sánchez-Cid, Maria Valls-Margarit, Miguel Angel Mateos-Timoneda, Elena Martínez, Elisabeth Engel
Institute for Bioengineering of Catalonia (IBEC), BIST, Barcelona, Spain

INTRODUCTION:

Lactate is a typical metabolite of glycolysis, commonly produced by cells consuming glucose. Growing evidences suggest that lactate can also act as a signalling molecule and a key factor in many processes, influencing epigenetic programs and gene expression[1,2]. However, little is known on its effect on postnatal cardiac tissue and how can this molecule modulate cell behaviour to promote regeneration.

We explored the use of lactate as a novel *in situ* tissue regeneration strategy.

METHODS:

Cardiac cells were isolated from neonatal mouse. L-lactate solution was used for cellular assays. Proliferation was evaluated using ki67 immunostaining and the expression of c-kit and cell cycle proteins by RT-qPCR. An inflammation antibody array was used to detect 40 secreted proteins, and collagen production was assessed by the hydroxyproline method. Cardiac cells were cultured on a 3D scaffold of collagen and elastin in the presence of lactate. Square-wave pulses were applied to determine the electrical response. The expression of MCT1, MCT4, GPR81 and microstructural assessment on cardiomyocytes was evaluated using confocal microscopy.

All experiments were performed with at least three replicates and analysed by two sample T-student test with a $p<0.05$ as statistically significant criteria.

RESULTS AND DISCUSSION:

Lactate enhanced proliferation of cardiomyocytes (Figure) and the expression of c-kit, suggesting that this compound can reprogram cardiomyocytes towards a more immature stage. It also modified the expression of cell cycle proteins. Lactate modulated several factors related to cardiac regeneration and decreased collagen synthesis in cardiac fibroblasts. *Ex vivo* culture of mouse hearts revealed the ability of lactate to increase survival and beating capacity of the tissue. Histological analysis of the hearts confirmed the cellular *in vitro* results.

Cardiac cells cultured on 3D scaffolds in the presence of lactate showed an electrical response typical of a more proliferative and immature phenotype. Additionally, cardiac cells showed expression of specific lactate receptors and transporters, such as MCT1, MCT4 or GPR81. The development of sarcomeric structures was confirmed, as well as the coupling and presence of intercalated disks.

CONCLUSION:

The modulating activity of lactate on cardiac cells makes this compound an attracting molecule to be incorporated in suitable biomaterials as a novel tissue engineering strategy.

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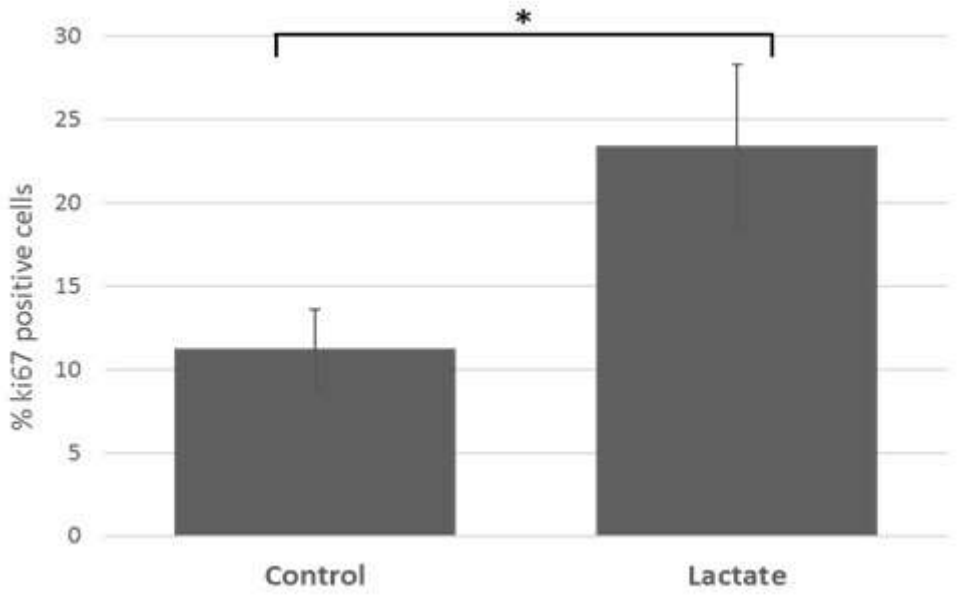
ACKNOWLEDGMENTS:

MINECO (MAT2015-62725-ERC and MAT2015-68906-R) and EUIN73 are acknowledged for funding.



Picture 1: Caption 1: Figure. Cardiomyocytes relative expression of ki67

Scientific Programme abstracts Tuesday

Plenary session 08.30 – 09.15 Auditorium I 11/09/2018

Oral presentation

Mechanobiology: a rapidly growing field with forceful implications

Viola Vogel
Laboratory of Applied Mechanobiology, Institute of Translational Medicine, Department for Health Sciences and Technology, ETH Zurich, CH-8093 Zürich, Switzerland

Mechanobiology is a rapidly growing field which explores the underpinning mechanisms by which mechanical forces and other physical factors can tune a large diversity of functions, from those of single molecules to the nuclear functions of cells, all the way to the tissue level. Forces are thereby emerging as an additional dimension of functional regulation, as they play central roles in tissue growth and regenerative processes, but also if misbalanced, in the onset and progression of various diseases. Macrophage biology has been at the centre stage of immunology and in clinical research, and more recently in regenerative medicine and bioengineering to learn how to improve on tissue healing processes. Far less is known though about the mechanobiology of macrophages and how to perhaps use physical factors to regulate their epigenetic profiles and functional outputs. Also major transformations of extracellular matrix (ECM) composition, architecture as well as of its mechanical properties accompany inflammatory diseases, including musculoskeletal diseases. Novel insights into the mechanobiology of pro-inflammatory macrophages and the ECM mechanobiology at the cell and tissue level will be discussed.

ESB International Award 2018 09.15 – 10.00 Auditorium I 11/09/2018

Oral presentation

Biosynthetic Materials for Islet Encapsulation and Transplantation

Andrés J. Garcia
Executive Director, Parker H. Petit Institute for Bioengineering and Bioscience
Regents' Professor, George W. Woodruff School of Mechanical Engineering
Georgia Institute of Technology, Atlanta, GA, U.S.A.

Hydrogels, highly hydrated cross-linked polymer networks, have emerged as powerful synthetic analogs of extracellular matrices for basic cell studies as well as promising biomaterials for regenerative medicine applications. A critical advantage of these synthetic matrices over natural networks is that the biophysical and biochemical properties of the material can be tuned with high control and precision. For example, bioactive functionalities, such as cell adhesive sequences and growth factors, can be incorporated in precise densities. We have engineered poly(ethylene glycol) [PEG]-maleimide hydrogels that support improved pancreatic islet engraftment, vascularization and function in diabetic models. Two biomaterial strategies will be discussed. We have developed proteolytically degradable synthetic hydrogels, functionalized with vasculogenic factors for localized delivery, engineered to deliver islet grafts to extrahepatic transplant sites via in situ gelation. These hydrogels induce differences in vascularization and innate immune responses among subcutaneous, small bowel mesentery, and epididymal fat pad transplant sites with improved vascularization and reduced inflammation at the epididymal fat pad site. This biomaterial-based strategy improves the survival, engraftment, and function of a single pancreatic donor islet mass graft compared to the current clinical intraportal delivery technique. In a second application, we have developed a localized immunomodulation strategy using hydrogels presenting an apoptotic form of Fas ligand (SA-FasL) that results in prolonged survival of allogeneic islet grafts in diabetic mice. A short course of rapamycin

treatment boosts the immunomodulatory efficacy of SA-FasL-hydrogels, resulting in acceptance and function of allografts over 200 days. Survivors generate normal systemic responses to donor antigens, implying immune privilege of the graft, and have increased T-regulatory cells in the graft. This localized immunomodulatory biomaterial-enabled approach may provide an alternative to chronic immunosuppression for clinical islet transplantation.

KSBM-ESB Joint session: Hydrogels 10.30 – 12.00 Auditorium I 11/09/2018

Oral presentation

Control of properties of hyaluronate-based terpolymeric hydrogel for biomedical applications

Dipankar Das,1, 2 Thi Thu Hien Pham,1, 2 Eunjae Chung3 and Insup Noh1, 2*

[1]Department of Chemical and Biomolecular Engineering, , Seoul National University of Science and Technology, Korea (Republic of)
[2]Convergence of Institute of Biomedical Engineering and Biomaterials, Seoul National University of Science and Technology, Korea (Republic of)
[3]Department of Otorhinolaryngology, Seoul National University College Hospital, Korea (Republic of)

INTRODUCTION

Hyaluronic acid(HA) is is a biopolymer composed by repeating units of D-glucoronic acid and N-acetyl-D-glucosamine and has advantageous biological properties1 in its applications to biomaterials in divers forms such as hydrogel and scaffolds for drug delivery and tissue engineering. Even though it has been fabricated as a hydrogel for long time, but its applications have been still limited due to its inherent physico-chemical properties.2,3 A new HA-based hydrogel with both higher mechanical and biological properties has been requested in medical society. We here report a synthesis of new hydrogel by graft-polymerization of 2-hydroxyethyl acrylate (2-HEA) and either poly(ethylene glycol) diacrylate (PEGDA) or bioactive polymers by the mechanism of radical polymerization for its potential applications to druge delivery and tissue engineering.4,5

METHODS

To obtain hydrophobicity and elasticity, 2-HEA was graft-polymerized onto hydroxyl groups of HA side chains, and then crosslinked using different amounts of PEG-DA or methacrylated polymers to obtain sufficient mechanical strength and porous morphologies of hydrogel (HA-g-p(2-HEA)-x-PEGDA). While chemical analyses were performed by FTIR, 1H HR-MAS-NMR, and TGA, physical analyses were done by TGA, swelling measurement and rheologies as well as drug delivery of tetracycline, bovine serum and dimethyloxalylglycine from hydrogel over acidic and basic medium at 37 °C. Diverse biological analyses of hydrogels were done in vitro and in vivo by implanting subcutaneously in rats for 3 weeks. Diverse histological analyses such as hematoxylin and eosin Y (H&E), and Masson's trichrome (MT) staining and others were done. All data were stated as mean ± standard deviation. Statistical significance was evaluated with one-way and multi-way ANOVA by using the SPSS 18.0 program. The comparisons between two groups were performed by t-test and significant difference has been reported when $p < 0.05$.

RESULTS AND DISCUSSION

The chemcial structure and compositions of the fabricated HA-g-p(2-HEA)-x-PEGDA polymer was verified by the methids of FTIR, 1H HR-MAS-NMR, and TGA analyses, indicating hydrogel formation. The SEM images indicate that the terpolymer contains interconnected porous network structures. The measurement of equilibrium swelling ratio showed physco-chemcial properties depending different pHs, and elastic modulus (G') value in rheology study confirmed the viscoelastic properties of HA-g-p(2-HEA)-x-PEGDA hydrogel in water at 37 °C. The polymeric gel demonstrated pH-dependent release behaviros of bioactive molecules such as tetracycline, bovine

serum albumin and dimethyloxalylglycine at 37 °C. The in vitro cell study indicated that the hydrogel supported outstanding adhesion, proliferation, and viability of osteoblastic MC3T3 cells as well as cell compatibility. The H&E and MT in vitro stains demonstrated that the native gel itself was excellent substrate for the bone tissue regeneration of extracellular matrix and collagen, respecitively, in absence of any extra growth factors after 3 weeks. In vivo evaluationa are underway of stainings after explanting the gel samples from rats. Currently we are under processing of its applications to the tissue regeneration scaffolds, which have been fabricated by 3D printing technology.

CONCLUSION

The results demonstrated that the synthesized HA-based hydrogel could be obtained by controlling a secondary polymer as biomateirals for both drug delivery and tissue engineering applications.

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ACKNLOWLEDGMENTS

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KSBM-ESB joint session: hydrogels 10:30 - 12:00 Auditorium I 11/09/2018

Oral presentation

213 Towards angiogenic behavior of human mesenchymal stromal cells using 3D printed gelatin-based scaffolds

Taimoor Qazi[1], Liesbeth Tytgat[2], Georg n Duda[1], Sven Geissler[1], Peter Dubruel[3], Sandra van Vlierberghe[3]
[1]Charité Universitätsmedizin Berlin, Berlin, Belgium
[2]Vrije Universiteit Brussel, Belgium
[3]Ghent University, Belgium

INTRODUCTION:

Mesenchymal stromal cells (MSCs) are known to secrete bioactive cytokines and growth factors, some of which exert potent angiogenic effects and stimulate angiogenesis of injured tissues. Identifying physical or mechanical properties that can enhance the angiogenic effects of MSCs is a promising approach to design new biomaterials to treat ischemic or injured tissues. Here, we utilized 3D printed gelatin scaffolds to elucidate the role of different pore sizes on the angiogenic effects of MSCs.

METHODS:

Gelatin-methacrylamide scaffolds were fabricated using an extrusion based bioprinter (Bioscaffolder 3.1, GeSIM), and subsequently crosslinked using UV light (320-500 nm). Scaffolds with three different pore sizes were created: 400, 500, and 600 mm. Primary human bone marrow MSCs were seeded onto the scaffolds and cultured for up to 7 days in static culture conditions. Cell growth was assessed with Presto Blue assay. Conditioned media were collected using Endothelial basal medium (Gibco) without addition of supplementary growth factors. Secreted factors were characterized using membrane based angiogenic cytokine arrays. 2D tube formation was assessed by

culturing HUVECs in conditioned media for 16 hours. Tubes were visualized by fluorescence microscopy after staining with DAPI (nuclei) and Phalloidin (F-Actin).

RESULTS AND DISCUSSION:

Due to swelling of scaffold struts, pore sizes during the culture period were slightly smaller than immediately after fabrication. Scaffolds with larger (>500 mm) pores promoted migration and 3D penetration of the seeded cells. No differences were observed on the metabolic activity and growth rate of cells on the different scaffolds over 7 days. MSCs cultured on 500 mm pore size scaffolds secreted significantly higher concentrations of bioactive growth factors including Angiogenin, PDGF, and VEGF. Moreover, conditioned media collected from these scaffolds stimulated robust 2D tube formation by HUVECs – leading to significantly higher mean tube lengths and junction points.

CONCLUSION:

3D printed scaffolds can act as a synthetic niche for MSCs, and the physical environment of this niche can define the cells' regenerative behavior. Our results show that scaffolds with an optimized pore size can potently enhance the angiogenic function of MSCs. These optimized scaffolds could find broad utility in tissue engineering applications

ACKNOWLEDGMENTS:

Liesbeth Tytgat would like to thank FWO for providing her a PhD fellowship.

KSBM-ESB joint session: hydrogels 10:30 - 12:00 Auditorium I 11/09/2018

Oral presentation

226 Hydrogels that promote cell-cell interactions enhance therapeutic activity of mesenchymal stromal cells

Taimoor Hasan Qazi[1], David Mooney[2], Georg Duda[1], Sven Geissler[1]
[1]Charité Berlin, Berlin, Germany
[2]Harvard University, United States of America

INTRODUCTION:

Mesenchymal stromal cells (MSCs) are attractive cell candidates for regenerative medicine applications due to their differentiation capacity and their ability to secrete bioactive cytokines and growth factors. Controlling their function has been a long-standing goal of designing biomaterials for cell therapies. While intensive efforts in the past decade have elucidated that biomaterials can effectively guide cell fate and differentiation, it remains unclear how material properties influence the paracrine activity of MSCs. Here, we used hydrogels (gels) to investigate the effect of structural cues (macro- and nano- porosity) on the paracrine activity of MSCs.

METHODS:

To create macroporous gels, RGD-alginate (2 % w/v) was crosslinked with Ca^{2+} ions, frozen at -80 °C, and lyophilized. MSCs were seeded onto the gels at a density of $2x10^5$ cells/gel. To create nanoporous gels, MSCs were encapsulated in RGD-alginate prior to crosslinking. Serum free conditioned media (CM) was harvested and used to study paracrine effects on myoblasts in *in vitro* assays. ELISAs and cytokine arrays were used to quantify concentration of secreted growth factors. Fluorescence microscopy was used to visualize cell morphology and N-

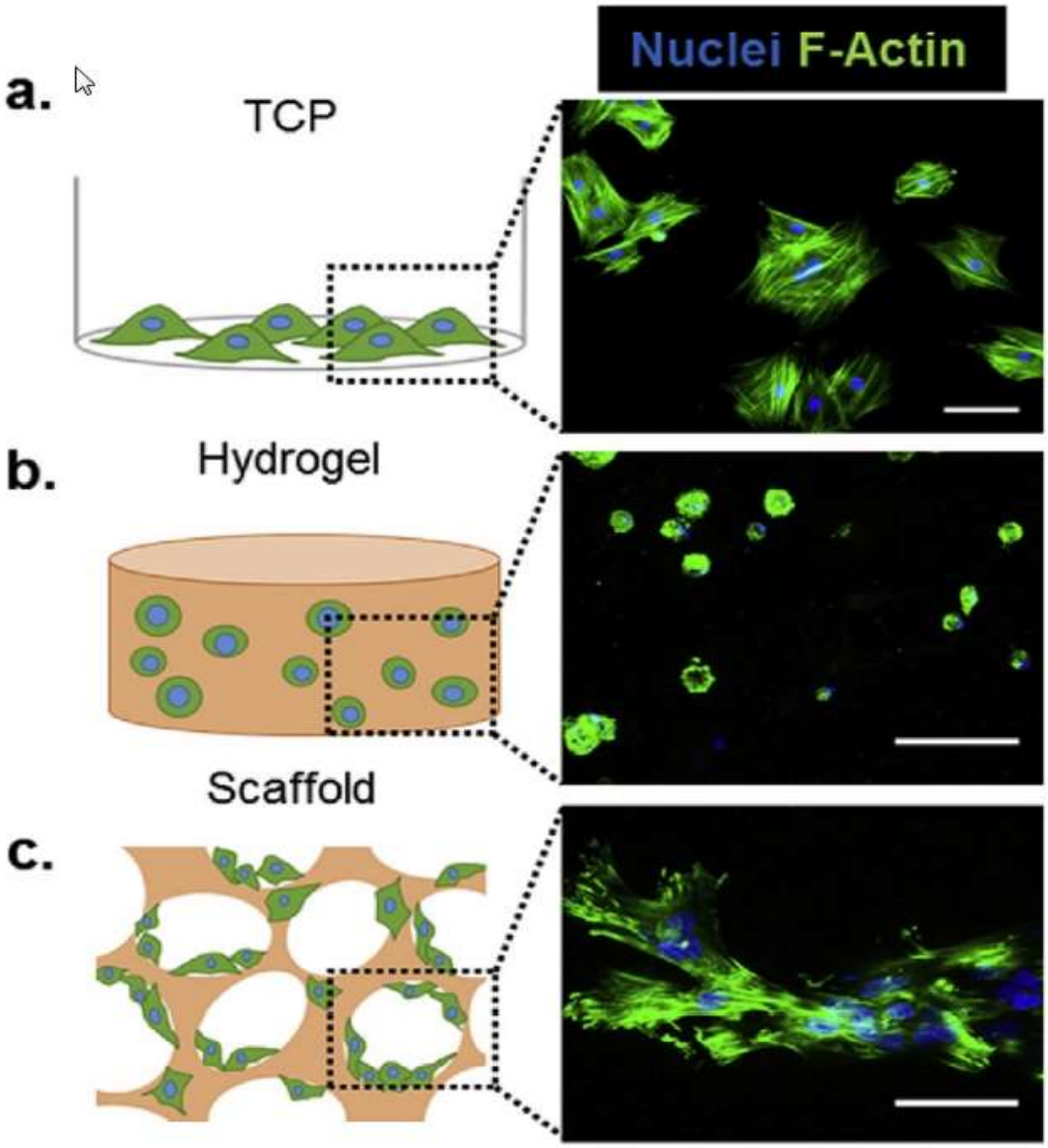
cadherin expression was assessed with qPCR. N-cadherin blocking experiments were performed by pre-incubating the cells with a neutralizing antibody prior to cell seeding or encapsulation.

RESULTS AND DISCUSSION:

MSCs in macroporous gels secreted significantly higher levels of various cytokines and growth factors (e.g. IGF, VEGF, HGF, LIF, and FGF) compared to those in nanoporous gels. Macroporous-CM also exerted more potent paracrine effects on myoblast migration, differentiation, viability, and proliferation. The difference between the two gel groups was attributed to the formation of N-cadherin based cell-cell contacts in macroporous – but not nanoporous – gels. Functionally blocking N-cadherin attenuated the paracrine effects of MSCs in macroporous gels to the same level as nanoporous gels.

CONCLUSION:

Changes in the physical microenvironment of MSCs - such as porosity and structure - can dramatically improve the therapeutic potential of MSCs, and can potentially lead to better outcomes of cell therapies in the clinic. Strategies to further harness cell-cell interactions could prove to be a promising approach to enhance the regenerative capacity of multipotent cells such as MSCs.



Picture 1: Caption 1: Schematic illustrating MSC culture in 2D (TCP) and 3D environments

KSBM-ESB joint session: hydrogels 10:30 - 12:00 Auditorium I 11/09/2018

Oral presentation

617 Engineering hydrogels for cardiac 3D cell culture via hydrazone bioconjugation

Jenny Párraga M[1], Janne Koivisto[2], Gerin Christine[2], Jennika Karvinen[2], Birhanu Belay[2], Hyttinen Jari[2], Katrina Aalto-Setälä[3], Minna Kellomäki[2]
[1]BioMediTech Institute and Tampere University of Technology, Tampere, Finland
[2]BioMediTech Institute, Tampere University of Technology, Finland
[3]BioMediTech Institute, University of Tampere, Finland

INTRODUCTION:

Successful 3D cell culture is determined by the biochemical and mechanical signals that regulate the cell behavior and fate[1]. Rationally designed hydrogels can recreate 3D environments that mimic the extracellular matrix. We hypothesized that soft chemical modifications of the biocompatible polymers gelatin and gellan gum (GG) keep the good features of both polymers while forming a gel. Gelatin provides cell responsive properties due to the presence of key peptides[2]. On the other hand, GG offers mechanical strength and stability for the hydrogel[3]. In this work, gelatin-gellan gum hydrogels (Gela-GG-Hyd) were obtained through hydrazone bioconjugation under cell culture conditions. Gela-GG-Hyd were tested with relevant cell lines, aiming towards cardiac 3D disease modelling.

METHODS:

Hydrazide gelatin was synthetized to form hydrogels through covalent interaction with oxidized GG. The hydrogels were characterized mechanically. Stability and degradability were studied in biologically relevant media. To verify the cell biocompatibility of hydrogel formation, human fibroblasts were grown with different formulations. We assessed cell viability and morphology of encapsulated fibroblasts by live/dead staining and confocal microscopy. The 3D cell characterization of macroscopic samples (750µL) was achieved by optical projection tomography. Finally, we analyzed 3D cultures of encapsulated cardiomyocyte aggregates by optical microscopy and *BeatView*® software.

RESULTS AND DISCUSSION:

The soft modification of the polymers facilitate spontaneous covalent bonding, leading to the gel formation. The gelation occurs within few minutes and mechanical properties and degradability can be controlled by varying the concentration of the components. Gela-GG-Hyd showed elastic behavior, similar to soft tissue. Crosslinking does not affect the polymers capacity to facilitate cell adhesion and matrix metalloproteinase degradation. Encapsulated human fibroblasts show elongated morphologies after 48h incubation. Human induced pluripotent stem cell derived cardiomyocytes show favorable cell response to the hydrogels since they are beating spontaneously after 24h in 3D in vitro studies.

CONCLUSION:

Gel-GG-Hyd with tunable properties were obtained by spontaneous crosslinking under cell culture conditions. The hydrogel creates a suitable 3D micro-environment for the cell spreading and proliferation. This work may provide an insight into the design of biomaterials for cardiac cell culture, specifically for 3D disease models.

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ACKNOWLEDGMENTS:

The authors thank to Human Spare Parts program of Tekes – Finnish Funding Agency for Innovation and Finnish Cultural Foundation Pirkanmaa Regional Fund, for support this project.

Biomaterials science session 14.00 - 15:30 Auditorium I 11/09/2018

Oral presentation

Leverage Physiology for Bioresponsive Drug Delivery

Zhen Gu
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Spurred by recent advances in materials chemistry, molecular pharmaceutics and nanobiotechnology, stimuli-responsive “smart” systems offer opportunities for precisely delivering drugs in dose-, spatial- and temporal-controlled manners. In this talk, I will discuss our ongoing efforts in developing physiological signal-triggered bio-responsive drug delivery systems, especially based on artificial cells or engineered cells. I will first present the glucose-responsive synthetic systems for biomimetic delivery of insulin for diabetes treatment. Bio-responsive microneedle patches and vesicle fusion-mediated synthetic beta cells will be emphasized. I will further discuss the local and targeted delivery of immunomodulatory therapeutics for enhanced cancer therapy. Our latest study utilizing platelets and injectable hydrogels for targeted/local delivery of immune checkpoint inhibitors will be specifically introduced.

Biomaterials Science session 14:00 - 15:30 Auditorium I 11/09/2018

Oral presentation

335 The use of chitosan porous 3D scaffolds embedded with resolvin D1 to improve bone healing

Daniela Pereira de Vasconcelos[1], Madalena Costa[2], Nuno Neves[3], José H. Teixeira[1], Susana G. Santos[4], Artur P. Águas[2], Mário a. Barbosa[1], Judite N. Barbosa[1]
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INTRODUCTION:

Bone tissue engineering, consists in the use of a scaffolding biomaterial that will induce formation of bone from surrounding tissues and act as a carrier of bioactive agents[1]. Several strategies have been proposed to improve the efficiency of bone regeneration. Among them, is the association between biomaterials and molecules capable of modulating inflammation[2]. The aim of this study was to investigate the effect chitosan (Ch) porous 3D scaffolds embedded with resolvin D1 (RvD1), an endogenous pro-resolving lipid mediator, on bone tissue healing. Previous studies of ours have demonstrated that with RvD1 incorporated in Ch scaffolds we were able to develop an

immunomodulatory strategy, that induces *in vivo* a shift in the macrophage phenotypic profile towards an M2 reparative phenotype thus creating an environment more prone to tissue repair[3].

METHODS:

Incorporation of RvD1 in the 3D scaffolds was performed by an embedding technique using a 1.66 ng/ml solution. We have used a rat femoral defect model created in the anterolateral wall of the lateral condyle. Femurs were retrieved two months after implantation for histological and micro-computed tomography (μCT) analysis. Three experimental groups were used: non-operated, and animals implanted with Ch scaffolds or with Ch+RvD1 scaffolds.

RESULTS AND DISCUSSION:

Bone histological analysis revealed a layer of newly formed bone delimiting the defect area, being thicker in the case of the animals implanted with Ch+RvD1 scaffolds. In addition, animals with Ch+RvD1 scaffolds exhibited a statistically significant increase in coll type I fibers when compared with animals with Ch scaffolds, being the ratio of Coll I/Coll III also increased for this group.

The μCT reconstruction of the defect area showed that the defects were still open in both experimental groups, being the defect smaller in the femurs of rats submitted to Ch+RvD1 scaffolds implantation. In addition, animals implanted with Ch+RvD1 scaffolds presented a statistically significant increase of the bone trabecular thickness (Tb.Th).

CONCLUSION:

We have used an immunomodulatory strategy, consisting of chitosan (Ch) scaffolds embedded with resolvin D1 (RvD1), to improve bone healing. The results obtained suggest that Ch scaffolds embedded with RvD1 favours the formation of new bone with improvement of trabecular thickness.

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ACKNOWLEDGMENTS:

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Biomaterials Science session 14:00 - 15:30 Auditorium I 11/09/2018

Oral presentation

554 The role of vascularization in the self-assembly of bioengineered islets

Fredrik C. Tengström, Clemens A. van Blitterswijk, Vanessa L.S. Lapointe
Maastricht University, MERLN, Maastricht, Netherlands

INTRODUCTION:

Vascularization is still one of the greatest challenges in regenerative medicine. Its importance is particularly evident in islets, which are especially sensitive to the delivery of oxygen and nutrients for their survival and function. Various strategies have been attempted both *in vitro* and *in vivo,* but reliable vascularization remains elusive. By using

bioengineered “pseudo islets” of Langerhans, which are three-dimensional (3D) spheroids of alpha and beta cells, we investigate the influence of endothelial cells on islet organization and function.

In order to do so, we will identify the molecules involved in the communication between endothelial and pancreatic cells and study how vascularization influences the self-organisation of the pancreatic cells. We intend to determine how endothelial cells and vascularization influences the function of the islet cells *in vitro*.

METHODS:

To establish a model for assessing the interactions and the spatial arrangement changes between islet and endothelial cells, we aggregate beta (INS1E), alpha (alpha TC-1 clone 6) and endothelial (HUVEC) cells in agarose microwells. In order to investigate the location and migration of single cells within the pseudo islet, we are currently studying solutions to follow single cells in a 3D environment.

RESULTS AND DISCUSSION:

We are able to identify each cell type by using an optimized non-destructive imaging setup. After fourteen days of culture, the beta cells have organized themselves into the core of the islet, and the alpha cells are situated in the periphery. This is also seen in native rodent islets. In our current experimental model, the endothelial are also in the periphery of the pseudo islets.

We also observe that endothelial cells might have an affinity for alpha cells, which is in agreement with what is known in the pancreas. As to why this also happens in *in vitro* self-organization and what the implications are for the islet integrity, function and vascularization remain to be seen.

CONCLUSION:

We aim to bring single cell techniques into regenerative medicine to study the spatial distribution of cells and how their distribution and organization affect function. This knowledge can contribute to a better understanding of the role of endothelial cells in the self-organization of bioengineered islets. Our research could result in new knowledge to improve the prospects of bioengineered islets in regenerative medicine.

ACKNOWLEDGMENTS:

The authors would like to thank the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant agreement No. 694801)

Biomaterials Science session 14:00 - 15:30 Auditorium I 11/09/2018

Oral presentation

347 Engineering a functional muscle tissue using a fibrin-based gel for tissue modelling

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INTRODUCTION:

In mature skeletal muscles, the muscle fibers are highly oriented and the bundle structure is important to produce its mechanical functions. Previously, our group have developed a micropatterned thermoresponsive substrate to regulate orientation of myoblasts and then harvest the aligned myoblasts as a single continuous cell sheet[1]. In this study, a new culture method for functionalizing this cell sheet construct was developed to produce an in-vitro muscle tissue model.

METHODS:

Human skeletal muscle myoblasts were seeded onto the patterned surface. After reached confluence, aligned myoblasts detached as a single cell sheet from the surface by lowering culture temperature and transferred onto a fibrin-based gel. After further cultured in a differentiation medium for 3 weeks, functionality of the muscle tissue construct was investigated by stimulating electrically using electrical pulse generator. Fluorescence imaging was also carried out to observe microstructures of these myotubes. In addition, ryanodine was added to a medium for investigating drug responsibility of the tissue construct.

RESULTS AND DISCUSSION:

Myoblasts aligned on the surface were successfully transferred onto a fibrin-based gel with maintaining their aligned orientation. In this study, it was confirmed that most myotubes spontaneously detached from a normal culture dish due to the contractile force of mature myotube. In contrast, the aligned myotubes were able to be cultured on the gel for 3 weeks. They formed sarcomere structures which are required for muscle contraction, and actually showed contraction by electrical pulse stimulation (EPS). According to the EPS frequency, twitch or tetanic contraction was observed. Importantly, the directionality of the muscle contraction was regulated by organizing the aligned tissue structure. Furthermore, this muscle contraction was significantly suppressed by adding ryanodine even under EPS. These results indicate that this tissue construct has a potential as a tissue model for studies of muscle physiology.

CONCLUSION:

In this study, we have developed a new method to produce a functional muscle tissue using a micropatterned substrate and a fibrin-based gel. Since it has the biomimetic structure and functions, this tissue construct is expected to be used as an in-vitro physiological tissue model.

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ACKNOWLEDGMENTS:

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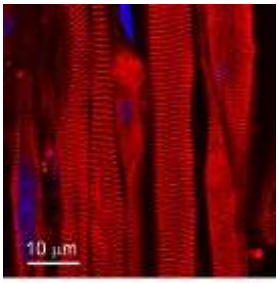

Picture 1: Caption 1: Engineered muscle tissue produced on a fibrin gel.

Biomaterials Science session 14:00 - 15:30 Auditorium I 11/09/2018

Oral presentation

530 Graphene Oxide-Containing Self-Assembling Peptide Hydrogels As 3D Platforms For Musculoskeletal Cell-based Therapies

Cosimo Ligorio[1], Mi Zhou[2], Aravind Vijayaraghavan[3], Judith Hoyland[2], Alberto Saiani[1]
[1]School of Materials, The University of Manchester, Manchester, United Kingdom
[2]School of Biological Sciences, The University of Manchester, United Kingdom
[3]National Graphene Institute, The University of Manchester, United Kingdom

INTRODUCTION:

Osteoarthritis and low back pain are two major contributors to global disability. Golden standard treatments are highly invasive, not targeted at the process of disease and poorly effective. For this reason, minimally invasive cell-based therapies are currently needed to achieve tissue repair of damaged tissues. Among synthetic biomaterials, self-assembling peptide hydrogels (SAPHs) represent potential candidates as three-dimensional (3D) cell carriers as they mimic the native tissue supporting cell viability and differentiation[1-3]. Moreover, the advent of graphene and its oxides as bioactive nanofillers has made appealing the fabrication of novel hydrogel-based graphene-containing nanocomposites[4] in which fillers can be further functionalised to promote stem cell differentiation. In this work, we hypothesised that graphene oxide (GO) could be incorporated within a SAPH to develop a novel biocompatible peptide-GO nanocomposite with potential as stem cell carrier and stem cell niche for musculoskeletal tissue engineering applications.

METHODS:

GO flakes (mean size <5μm) were dispersed in PGD-AlphaProA hydrogel at a concentration of 0.5mg/ml. GO dispersions and hydrogels were characterised by atomic force microscopy (AFM) and shear rheology. Patient bone-marrow derived mesenchymal stem cells (BM-MSCs) were encapsulated in the hybrid peptide/GO hydrogels ($4x10^6$ cells/ml) and cell viability assessed up to 7 days using a LIVE/DEAD® viability assay.

RESULTS AND DISCUSSION:

AFM images showed that GO was homogenously dispersed within the peptide matrix, creating a self-supporting system. Good dispersion of GO flakes anticipates a spatially-organised presentation of the nanomaterial to encapsulated cells, suggesting good suitability for future drug delivery applications. Moreover, 3D cell culture showed excellent cell viability and characteristic spindle-like morphology for BM-MSCs in peptide and peptide-GO hydrogels after 7 days (**Fig. 1**), indicating high biocompatibility as stem cell carriers.

CONCLUSION:

These preliminary results show that PGD-AlphaProA can be mixed with GO to fabricate homogenous and biocompatible nanocomposites with potential as stem cell carriers and niche. In future work, GO flakes will be functionalised to present relevant biomolecules for stem cell differentiation. In particular, the effect of GO and its payloads on stem cell differentiation within the peptide-GO nanocomposite will be investigated for cartilage and disc repair.

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ACKNOWLEDGMENTS:

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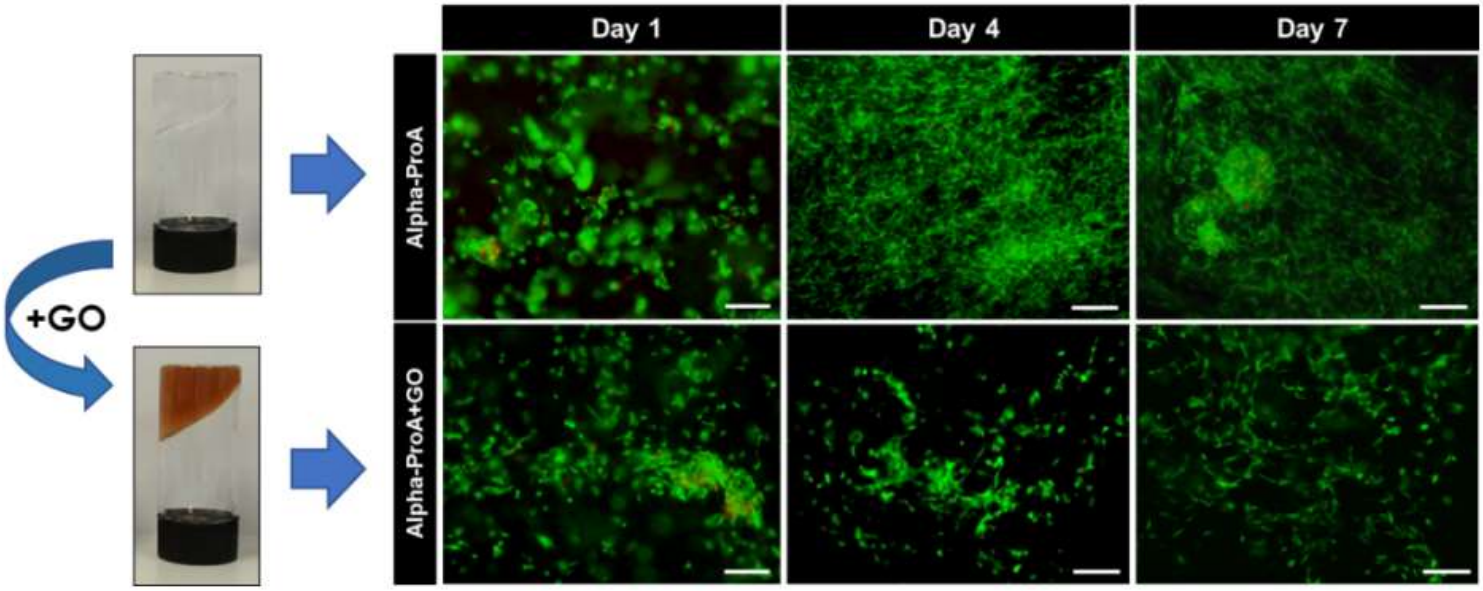

Picture 1: Caption 1: Figure 1: Cell viability of BM-MSCs encapsulated within GO-free (first row) and GO-containing SAPH (second row). Scale bar=100μm.

CSB-ESB Joint session on drug delivery 16:00 - 17:30 Auditorium I 11/09/2018

Oral presentation

siRNA and mRNA Delivery with Chitosans

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RNA delivery can be acheived by packaging in lipid or polymeric nanoparticles or by chemical modification and conjugation with targeting entities. The cationic natural-derived glucosamine-based polymer chitosan has been used for two decades, intially to deliver plasmid DNA, and subsequently siRNA and mRNA. Our previous studies of polyelectrolyte complexes of chitosan with these different nucleic acids has revealed significant differences in bioactivity that depend on the molecular weight (MW) of chitosan and its degree of deacetylation (DDA) which determines charge density and degradability. We found that particular combinations of low MW and high DDA were required for plasmid and were different from those where siRNA is most active that included high MW chitosans in the presence of high serum content. Physicochemical measurements of binding affinity and FRET-based imaging in cells showed that the binding affinity of chitosan to the nucleic acid was a main factor in controlling bioactivity through balanced protection against nuclease activity and effective release of payload intracellularly1. In vivo studies of siRNA knockdown in mice using tail-vein injections showed high targeting and knockdown of a reporter in proximal tubule epithelial cells in the kidney cortex, most likely through a process involving receptor mediated uptake of chitosan bound to siRNA by these cell types2. In the most recent study with mRNA we found that complexes of chitosan and an mRNA Luciferase reporter could express the mRNA in vitro most effectively when chitosan MW was lowest (5kDa) but only at slightly acidic medium pH which was not a requirement for siRNA activity. Coating these binary polyelectrolyte complexes with low molecular weight hyaluronic acid that was also sulfated increased expression and this expression was further increased when complexes were formed in the presence of trehalose, but again only at slightly acidic pH since expression at pH

7.3 was still very low for these systems. The main conclusion of this sequence of studies using chitosans to deliver RNA is that careful selection of chitosan molecular weight and DDA is needed for each type of nucleic acid and that siRNA/chitosan complexes can be directly bioactive in vivo while mRNA/chitosan activity needs to be acheived through further optimisation and modification of these systems.

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CSB-ESB Joint session on drug delivery 16:00 - 17:30 Auditorium I 11/09/2018

Oral presentation

Gene therapy for orthopaedic diseases; how to deliver?

Laura Creemers
Department of Orthopedics, University Medical Center Utrecht, Utrecht, the Netherlands

Gene therapy is a promising strategy in orthopaedic disease, especially nonintegrating and/or non-viral approaches; In most cases, temporary modulation will be sufficient for treatment, in contrast to oncological or genetic disease. Also widespread diseases such as osteoarthritis and chronic low back pain related to intervertebral disc (IVD) degeneration are in principle amenable to gene therapy. Several molecular targets involved in disease progresssion or symptom generation have been identified, which can be targeted much more specifically by antisense therapy than by –if available- small molecule inhibitors. Moreover, the growing body of evidence for the role of epigenetic modulation in health and disease suggests the possibility to use miRNA delivery to positively modulate cell phenotype in the joint or IVD. Also, plasmid DNA encoding regenerating genes can be delivered to this end.

As cartilage and the IVD are non vascularised tissues, however, systemic delivery of gene expression modulators is in most cases not feasible. Routes to take towards genetic modulation in the joint and IVD will therefore consist either of treating cells to be transplanted, before or during transplantation, or by local delivery to resident cells. Both anatomical structures are accessible for local injection and the associated diseases are in general local in nature. Even then, transport to resident cells of the cartilage and IVD may pose some challenges, given the tight extracellular matrix surrounding these cells. The use of smart delivery tools and gene modulating agents hold promise to overcome this barrier.

CSB-ESB joint session on drug delivery 16:00 - 17:30 Auditorium I 11/09/2018

Oral presentation

407 The increased therapeutic potential of pro-osteogenic MSCs delivered via an injectable thermosensitive hydrogel scaffold for the treatment of non-union fractures.

Phil Chambers[1], Monika Ziminska[1], Sreekanth Pentlavalli[1], Daniel Kelly[2], Nicholas Dunne[3], Helen Mccarthy[1]
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INTRODUCTION:

Limitations exist in the use of recombinant growth factors (GF) for the therapeutic treatment of bone regeneration defects (current gold-standard), such as ectopic bone formation and the requirement for supraphysiological dosage.[1] The goal of this study was to develop an alternative yet equally efficacious therapeutic strategy. More specifically, that encapsulation of MSCs pre-treated with nanoparticles (NPs) comprising of a novel amphipathic peptide (RALA)[2] and calcium phosphate (CaP), seeded within a thermo-responsive hydrogel (T-HG) scaffold possesses the required characteristics for enhanced osteogenesis and subsequent non-union repair.

METHODS:

NPs were prepared over a range of mass ratios, with NP size (d_h) and zeta potential (ζ) measured to determine the optimal NP characteristics (*n*=3). Human bone marrow-derived MSCs (hMSC) were treated with NPs and examined for intracellular delivery via confocal microscopy and biocompatibility via MTS assay (*n*=3). Expression of osteogenic marker genes were determined by RT-PCR to assess the osteogenic efficacy of the NPs (*n*=3). Alginate and poly(N-isopropylacrylamide) were polymerised to form a T-HG, characterised using ^{1}H-NMR, FTIR and rheological methods. Subsequently, NP-primed hMSCs encapsulated within a T-HG were injected subcutaneously into the rear dorsum of C57BL/6 mice (*n*=6) and analysed via µ-CT and histological staining.

RESULTS AND DISCUSSION:

The observed NP morphology and physiochemical characteristics were ideal for increased intracellular delivery[3] (ζ ~20mV, d_h <100 nm) (Fig.1A&B), exhibiting biocompatibility and an upregulation in key osteogenic genes (RUNX2, COL1, OCN). When NP-primed hMSCs were encapsulated within T-HGs, enhanced *in vivo* mineralisation occurred within an 8 week period, with µCT images exhibiting increased bone volume (Fig.1C).[4] Therapeutic efficacy was observed to be on par with a GF-only control group.

CONCLUSION:

In conclusion, we have successfully produced a thermo-responsive injectable polymer with the potential to behave as both a scaffold and delivery vehicle. RALA was capable of forming biocompatible NPs with the ideal physiochemical properties for the intracellular delivery of HA to hMSCs, resulting in the increased expression of osteogenic GFs and transcription factors, accelerating mineralisation and robust bone development *in vivo* at comparable levels to using the current gold-standard therapy options.

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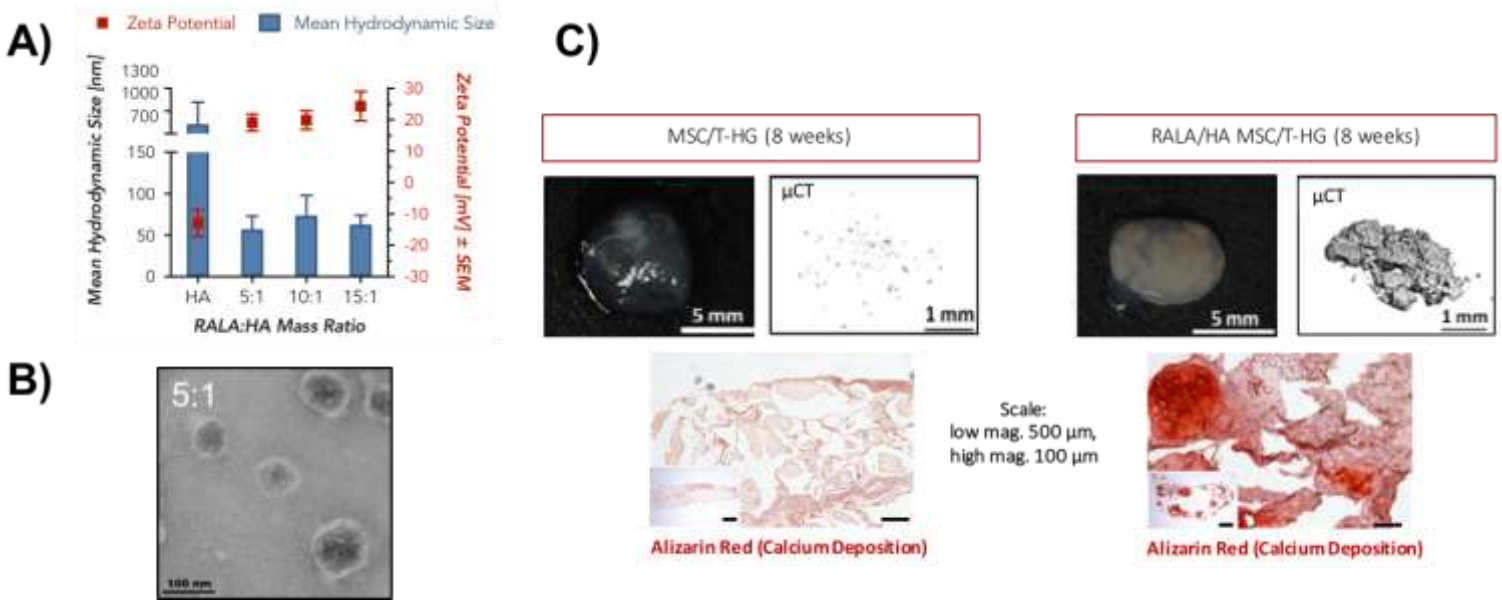

Picture 1: Caption 1: Figure 1: A) dh and ζ of RALA/HA-NPs, B) TEM image of 5:1 RALA/HA-NPs, C) T-HG scaffolds removed following 8 weeks in vivo.

CSB-ESB joint session on drug delivery 16:00 - 17:30 Auditorium I 11/09/2018

Oral presentation

526 Development of an injectable nanoparticle-loaded hydrogel system suitable for delivery of an angiogenic growth factor to the ischaemic myocardium

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INTRODUCTION:

The 5 year mortality rate for heart failure (HF) remains close to 50%[1]. The primary cause of HF is ischaemia related tissue damage. Increasing the vascularisation of the damaged area can prevent or slow the progression to HF. Previous efforts to achieve this have been unsuccessful due to rapid degradation or poor retention of angiogenic growth factors[2]. In this work we used a star-shaped Polyglutamic acid (PGA) polypepide and the angiogenic protein Vascular Endothelial Growth Factor (VEGF) to create self-assembling nanoparticles. Hyaluronic acid (HA) based hydrogels were screened to determine their ability to act as a delivery vehicle for the nanoparticles.

METHODS:

Dynamic Light scattering and Nanotracking Analysis were used to verify nanoparticle formation and size at various PGA:VEGF ratios. Nanoparticle biocompatibility testing was carried out on human Umbilical Vein Endothelial Cells (hUVECs) using Live/Dead and MTS assays. Bioactivity was ascertained by performing Matrigel® and Scratch assays on hUVEC's to give an indication of *in vivo* angiogenic potential. Rheology and disintegration testing were used as indicators of hydrogel robustness. Release of VEGF from the nanoparticle-loaded hydrogel was determined using a Float-A-Lyzer with detection via ELISA. The force required to inject the formulation through a 1.2m, clinically relevant catheter was measured using a Zwick mechanical testing machine.

RESULTS AND DISCUSSION:

PGA:VEGF ratios from 30:1 to 100:1 produced particles of 200nm (±25nm). No significant reduction in the metabolic activity of hUVECs was observed on exposure to the PGA:VEGF nanoparticles over 72 hours. Nanoparticles significantly improved microvessel formation on a Matrigel® assay ($p<0.05$) and cell migration on a Scratch assay ($p<0.01$) when compared to cells alone. The storage modulus of the HA hydrogels ranged from 0.8-2.2kPa depending on concentration and crosslinking density. These hydrogels were also able to remain intact for 7 days in the presence of surrounding phosphate buffered saline. Confocal microscopy confirmed that nanoparticles were distributed throughout the HA gel. Release of VEGF from the nanoparticle-loaded gel was detected for up to 35 days and the formulation could be easily pushed through the catheter at a force of 7.7N (± 0.17N).

CONCLUSION:

Optimal conditions for the fabrication of sustained release PGA:VEGF nanoparticles have been identified. The nanoparticles have successfully been incorporated into a HA hydrogel to produce an injectable, sustained release nanoparticle-loaded hydrogel with real translational potential.

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Drug-free antibacterial hybrid biopolymers for medical applications 17:30 - 19:00 Auditorium I 11/09/2018

Oral presentation

Inorganic Antibacterial Materials

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Antibacterial infections are one of the main complications after surgery. Conventional treatments (e.g. administration of antibiotics) are often characterized by lack of effectiveness, low delivery efficacy, high toxicity and other inconveniences for the patient. They can also lead to the development of drug-specific resistance by bacteria. Mesoporous bioactive glasses (MBGs) in the CaO–SiO2–P2O5 system were reported for the first time more than 10 years ago. The tunable and ordered pores, the high specific surface area and the high pore volume make MBGs optimal candidates for the local delivery of biologically active molecules.

In the past 10 years, different therapeutic ions, such as monovalent (Ag and Li), divalent (Sr, Zn, Cu, Co and Mg), trivalent (Ga, Ce and Fe) and tetravalent (Zr) ions, have been introduced in silicate matrices to develop bioactive glasses with biologically active ions release. The therapeutic ions can be easily incorporated in the framework and at the same time allow the material to maintain its well-ordered mesoporous structure, ideal for drug delivery. The release of ions can have various effects such as enhancing osteogenesis, promoting angiogenesis and cementogenesis or providing antibacterial activity, offering a multifunctional platform for applications in orthopedics and hard tissue regeneration.

In this work different MBG compositions were produced by sol-gel technique and specific concentrations of antibacterial ions were introduced during the synthesis process. Full characterization of the inner microstructure, specific surface area, pore size distribution and pore volume of the samples by TEM, BET and BHJ was performed. The samples were then characterized by soaking in simulated body fluid (SBF) in order to study their ability to form a hydroxycarbonate apatite layer (HCA) on their surface. SEM, FTIR and XRD were used to

characterize the glasses after the immersion in SBF. The control of MBG dissolution, ion and biomolecule release capability and the effect of HCA formation will be discussed.

Drug-free antibacterial hybrid biopolymers for medical applications 17:30 - 19:00 Auditorium I 11/09/2018

Oral presentation

Antibacterial synthetic polymers

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[3]Universitaetsklinikum Knappschaftskrankenhaus GmbH - Hospital of the Ruhr-University Bochum, Germany

The increasing occurrence of microbial infections has stimulated the development of new antibacterial materials for medical applications. The challenge is to develop novel medical polymers that have an intrinsic antibacterial functionality. In the design of innovative materials, scientists are increasing taking inspiration by Nature, developing biomimetic polymers, which contain building blocks that mimes part or whole natural antibacterial materials. To this aim, the development of different polymers, which mimic respectively honey and antimicrobial peptides have been explored in the Hymedpoly project.

A honey-like hydrogel was prepared via thiol-ene click chemistry of hyperbranched polyethylene glycol diacrylate (HB-PEGDA, 10 w/w%) and thiolated hyaluronic acid (HA-SH). The hydrogel produced antibacterial reactive oxygen species (ROS) in the form of hydrogen peroxide (H2O2), through the two components found in honey: glucose oxidase (GO) enzyme within HB PEGDA and glucose (G) in HA-SH. The hydrogel was able to produce 9.11 mmol H2O2 ROS after 24 hours with 250 U/L GO and 25 g/L G. This concentration demonstrated zone of inhibition as a measure of antibacterial activity against several bacterial strands. An electrospun polyurethane patch was prepared and surface modified with polydopamine for the immobilization of GO enzyme, to be used in combination with the above-described hydrogel.

Antimicrobial peptides (AMPs) are essential components of immune system forming the first line of defense against pathogenic bacteria1. AMPs consist of amphipathic structures in which clusters of hydrophobic and hydrophilic amino acids segregate, enables them in selective disruption of the bacterial cytoplasmic membrane1. In order to mimick the structural organization of AMPs, a novel polyurethane grafted polyionic liquid based patchy colloidal particles was developed. A hydrophobic liquid monomer was grafted from the polyurethane backbone by redox initiated aqueous heterophase polymerization. Subsequently, the hydrophobic anion was exchanged with a hydrophilic one. Chemical structure was elucidated by NMR analysis. Cryo-TEM images confirmed the formation of patchy colloidal particles consisting of the self-organized mesophases. The strong bactericidal effect is summarised in table 1.

Table 1. MIC and MBC values among non- and resistant Gram-positive bacteria.

Most relevant clinical Gram-positive bacterial strains in chronic wounds and medical device-related infections	Polyurethane-based Patchy Colloidal Particles (μg/mL)	
	MIC	MBC
Staphylococcus aureus ATCC 29213	10.4 ± 3.7	10.4 ± 3.7
Methicillin-Resistant Staphylococcus aureus (MRSA)	13 ± 3.7	13 ± 3.7
Staphylococcus epidermidis ATCC 12228	6.5 ± 1.8	6.5 ± 1.8
Macrolide-lincosamide-streptogramin B resistance in Methicillin Resistant *Staphylococcus epidermidis* (MRSE)	13 ± 3.7	18.2 ± 9.7
Enterococcus faecalis ATCC 29212	31.2 ± 0	46.85 ± 15.6
Vancomycin - resistant enterococci (VRE)	31.2 ± 0	62.5 ± 0

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Acknowledgment: HyMedPoly received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 643050.

Drug-free antibacterial hybrid biopolymers for medical applications 17:30 - 19:00 Auditorium I 11/09/2018

Oral presentation

Antibacterial Natural Polymers

Alexandra Paxinou[1, 2], Elena Marcello[1, 3],, Isabel Orlando[1, 3],, Sheila Piarali[1], [2]Pooja Basnett[1], Rinat Nigmatullin [1], Jochen Salber[2], Wenxin Wang[3,] Ipsita Roy[1]
[1]Applied Biotechnology Research Group, FST, University of Westminster, UK
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Natural Polymers have the potential to be used in a variety of medical applications due to their excellent biocompatibility, varied mechanical properties and sustainable resourcing. These include polymers such as alginate, chitosan, collagen, gellan gum, silk and chitin. This work has focused on two main types of natural polymers produced by bacteria including Polyhydroxyalkanoates (PHAs)1 and Bacterial Cellulose (BC)2. These are a distinct class of natural polymers produced by controlled bacterial fermentation.

PHAs are polymers of 3,4,5 and 6-hydroxyalkanoic acids produced by bacteria, mainly under nutrient limiting conditions. These are divided in to two main types, short chain length PHAs, with monomer chain length, C4-C5, which are normally hard and brittle in nature; and medium chain length PHAs, with monomer chain length, C6-C16, which are normally soft and elastomeric in nature. In this work we have modified PHAs with various natural antibacterial agents including antimicrobial peptides and active factors from garlic, among others. In addition, a naturally antibacterial class of PHAs, Thio-PHAs have also been produced3. All of these antibacterial polymers have been characterised with respect to their antibacterial activity against Staphylococcus aureus ATCC 6538 and Escherichia coli ATCC 8739 following ISO22916. The biocompatibility of these materials have been assessed using C2C12, L929, and NG108, a myoblast , fibroblast and neural cell line respectively. These antibacterial polymers have been used for the development of tissue engineering scaffolds and medical devices.

BC is produced by several bacteria including Gluconacetobacter xylinus and has an inherent hydrogel-like structure. This particular feature enables it to incorporate more than 90% of its weight by water, thus providing an optimal level of moisture and turning the material into a natural wound dressing. Moreover, BC is highly pure as it does not contain lignin and hemicellulose, ensuring a great degree of biocompatibility without needing harsh purification treatments. In this work the surface of cellulose was functionalized by wet chemistry to introduce

active groups. Antibacterial studies showed a decrease in the bacterial growth after 24 hours of contact with the samples. The cytotoxicity evaluation using HaCaT cells (keratinocytes) confirmed the cytocompatibility for both modified and unmodified BC.

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The authors would like to thank the European Union's Horizon 2020 research programme under the Marie-Sklodowska-Curie Grant Agreement No 643050.for providing financial support to this project.

Drug-free antibacterial hybrid biopolymers for medical applications 17:30 - 19:00 Auditorium I 11/09/2018

Oral presentation

Clinical impact of HyMedPoly: Drug-free strategies and their future implementation

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1Department of Mechanical and Aerospace Engineering (DIMEAS), Politecnico di Torino, Italy
2Medical Biomaterials, Center for Clinical Research of the Ruhr-University Bochum, Germany
3Universitaetsklinikum Knappschaftskrankenhaus GmbH - Hospital of the Ruhr-University Bochum, Germany

Antibiotic drugs performed a triumphal procession by saving countless patients life. But in the last decades a dramatically increasing antimicrobial resistance (AMR) poses a dangerous threat to the global public health. We need new antibiotics (drugs), but additionally alternative, complementary antimicrobial strategies (drug-free) to promote the success of major surgery and the application of implants. Thus, biomaterials play an indispensable role in modern medicine. In this context drug-free antimicrobial hybrid biopolymers are becoming important for clinical applications as implant coatings, wound dressings and tissue engineering approaches to kill bacteria, prevent bacterial adhesion and biofilm formation. This will open the critical question how to evalute their biocompatibility best. The authors' hypothesis is that 3D human tissue constructs are more suitable to analyse this than 2D culture systems according to the DIN EN ISO standards only. As one example an in vitro 3D wound infection model based on human cells will be demonstrated here as an in vitro tool giving enough valuable response for the analysis of drug-free antimicrobial hybrid biopolymers. The 3D skin equivalent was obtained having both a dermal and an epidermal compartment, by embedding human primary fibroblasts in collagen type I and then seeding human primary keratinocytes on it to generate well differentiated epidermal layers. This model was specifically injured and inoculated with clinically relevant bacteria (e.g Staph. aureus) at wound site, to generate a 3D wound infection model (Figure 1). The model was comprehensively characterised by histostaining, immunohistochemical analysis (e.g. Anti-Cytokeratin 10, Cytokeratin 14, Laminin 5, Filaggrin antibodies), SEM and TEM. The bacterial adherence and localization within epidermal tissue was observed through confocal microscopy. Results demonstrated the architectural features of dermal and fully differentiated epidermal layers. Immunohistology demonstrated the details of epidermal markers and remodelled intercellular connective soft tissue. The in vitro wound infection model better relates to the in vivo situation for the evaluation of biological properties (antimicrobial activity, cytocompatibility, etc.) of drug-free antimicrobial hybrid biopolymers. Next step will be the evaluation of gene expression and cytokine levels by keratinocytes to identify model skin response to bacteria that will also help exploring host-pathogen interaction and thus the antimicrobial strategies.

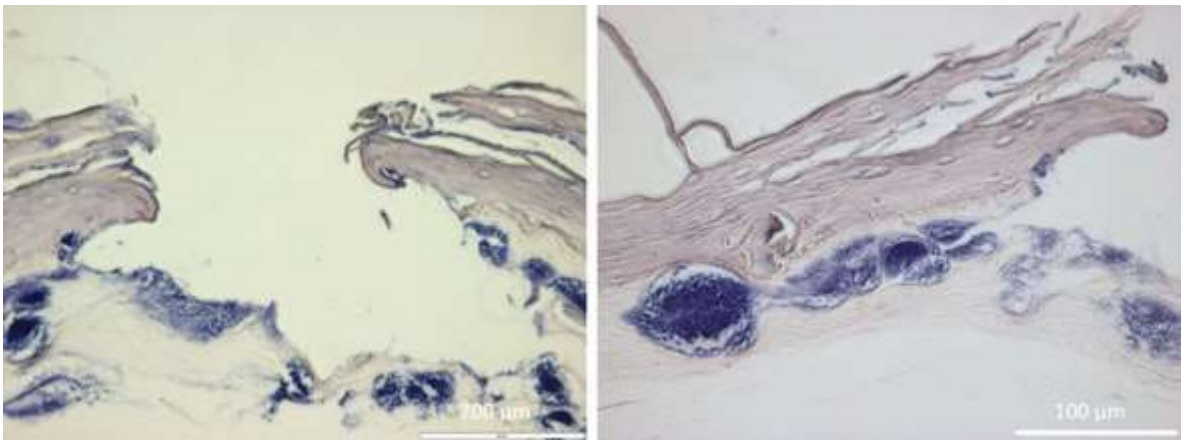

Figure 1: Cross-sections of the skin wound model inoculated with Staph. aureus. Bacteria are forming colonies in different compartments and separating the ECM and epidermal cell layers.

Acknowledgment**:** HyMedPoly received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 643050.

Drug-free antibacterial hybrid biopolymers for medical applications 17:30 - 19:00 Auditorium I 11/09/2018

Oral presentation

New Concepts and Goals of HyMedPoly - Drug free Antibacterial Strategies

Xiang Zhang
Principle Consultant at Lucideon,
Royal Society Industry Fellow at University of Cambridge

INTRODUCTION

The continuous advances in the treatment of disease/infection-related healthcare will lead to a strong demand for new therapeutic products. Medical device related infections account for substantial morbidity and cause a sharp increase in healthcare costs through prolonged hospitalisation and after-care. Particularly, implanted synthetic medical devices demonstrate a significant number of infections. The safety and efficiency of the medical devices need to be improved to drastically decrease infection rate from synthetic implants and medical devices that contact human tissue and/or body fluids. In Europe, at least 1.5% of the approximately 800,000 annually implanted orthopaedic devices are subjected to peri-prosthetic infections . In the United States alone at least 80,000 catheter related bloodstream infections occur annually in Intensive Care Units, leading to 24,000 patient deaths and increased healthcare costs ranging from approximately €7,000 to €45,000 per case. , . In this scenario, antibiotic resistance due to a lack of effective drugs to combat bacteria is one of the most serious health threats worldwide. Each year in the United States, at least 2 million people acquire serious infections from bacteria that are resistant to the antibiotics designed to treat them and at least 23,000 die as a direct result. The level of incidences is rising as some pathogens become resistant to multiple types or classes of antibiotics. The loss of effective antibiotics will undermine the ability to fight infectious diseases and manage the infectious complications. This presentation aims to introduce new concept and report outcomes of drug-free antibacterial therapeutic strategies vis a range of technologies that work in the best way that can potentially and efectively win the war on bacterias in most of applications.

METHODS

3 strategies - organic, inorganic and natural therapies, aiming to create antibacterial biomaterials with surface environments that make it difficult for bacteria to grow, including: (1) Honey Mimetic Polyurethane Based Wound Healing Scaffolds; (2) Therapeutic Polyurethane (PU): Amphipathic Antibacterial PU based Colloids; (3) Bioresorbable polyesters & Copper (II)-Chitosan Hybrids; (4) Functioned Biodegradable and Bioresorbable Polyesters; (5) Novel Natural Polymers with Antibacterial properties; (6) Hybrid polymers based antibacterial hydrogels for wound healing applications; (7) Bioactive Silica Glass with antibacterial functionality; (8) A Study of Substituted Hydroxyapatites for Antibacterial Applications; (9) Bioresorbable Phosphate Based glasses for antibacterial applications; (10) Development of natural polymers for Antibacterial Nerve Conduits; (11) Natural hybrid polymers equipped with antibacterial functionalities; (12) - New Antibacterial Study of Mechanobiology of Cell-Surface Interaction; (13) Mechanics of Porous and Structured Antibacterial Biomaterials; (14) Bio-analysis of Antibacterial Biomaterials - Prevention Microbial Wound Infections; (15) Study on Antibacterial Efficacy and Safety of Novel Amphipathic Polyurethanes

RESULTS AND DISCUSSION

The challenge is to develop new medical materials that have an intrinsic antibacterial functionality to achieve clinical effectiveness; to develop a new generation of industrial professionals is needed who will firstly understand new concept of innovation from concept to commercialisation, and can implement new strategies to combat bacteria; and the best way to achieve the goals of science and technology in HyMedPoly project: ORANIC - through synthetic pathway: develop new hybrid polymers synthetic and natural “equipped” with antibacterial functionality, INORGANIC – through design new molecular structure of inorganic nature to make them naturally processing antibacterial functionality LEARN FROM NATURE – build new materials with natural inhibitors that can permanently deactivate bacteriological proteases and In combination of (a), and/or (b), and/or (c)

CONCLUSION

Drug-free antibacterial strategies and prodcuts can be designed and developed with success

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ACKNOWLEDGMENTS

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Electrically active biomaterials : the future of translatable bioelectronics 10.30 – 12.00 Auditorium II 11/09/2018

Oral presentation

Soft, Skin-Interfaced Systems for Sweat Collection, Physiological Monitoring, and Biochemical Sensing

Roozbeh Ghaffari, PhD
Department of Biomedical Engineering,Center for Bio-Integrated Electronics, Northwestern University, USA

Unusual classes of electronics and microfluidics enabled by recent advances in materials science and mechanics can be designed with physical properties that approach the mechanical properties of human skin. These systems are referred to as epidermal electronics and epifluidics by virtue of their stretchable form factors and soft mechanics compared to conventional packaged electronics and sensors. In this talk, I present an overview of recent advances in novel materials, mechanics, and designs for emerging classes of fully-integrated epidermal electronics and soft microfluidic systems. These devices incorporate microfabricated arrays of sensors, microfluidic channels and biochemical assays, configured in ultrathin, stretchable formats for continuous monitoring of kinematics, cardiac, mechano-acoustic, neuromuscular, and electro-chemical signals. Quantitative analyses of strain distributions and circuit performances under mechanical stress highlight the utility of these wearable systems in the clinical and home environments. I will conclude with representative examples of these epidermal devices, which began as feasibility projects in research publications a few years ago, and have now entered the commercialization phase with leading industrial partners.

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Electrically active biomaterials : the future of translatable bioelectronics 10.30 – 12.00 Auditorium II 11/09/2018

Oral presentation

Design, Synthesis, and Characterization of Conjugated Polythiophenes for Interfacing Electronic Biomedical Devices with Living Tissue

David C. Martin
Materials Science and Engineering and Biomedical Engineering, The University of Delaware,

We have been exploring the use of chemical stable, electronically and ionically active polythiophenes for interfacing rigid, inorganic metallic and semiconducting biomedical devices with soft, organic, and wet living tissue1. Functionalized thiophene comonomers such as EDOT-acid and ProDOT-diene, shown in the schematic, make it possible to tailor the charge transport, mechanical properties, and biological activity of the resulting copolymers. Examples of targeted electronic devices include pacemakers, cochlear implants, retinal prostheses, cortical electrodes, and cardiac mapping devices. Recent efforts have focused on the use of carboxylic-acid and amine-functionalized monomers for improving interactions with adhesion to solid substrates, and multifunctional molecules for introducing covalent chemical crosslinking. The polymers are typically deposited from solution using oxidative electrochemical methods. The physical and chemical properties of the resulting films are characterized using a variety of methods including impedance spectroscopy, FTIR and Raman spectroscopy, optical microscopy, and electron microscopy.

This work was supported by the National Institutes of Health (NINDS-N01-NS-1-2338, 1R01EB010892), the Defense Advanced Research Projects Agency (N660011-11-C-4190), the Army Research Office (MURI W911NF-06-1-0218), the National Science Foundation (DMR-1103027), and the University of Delaware.

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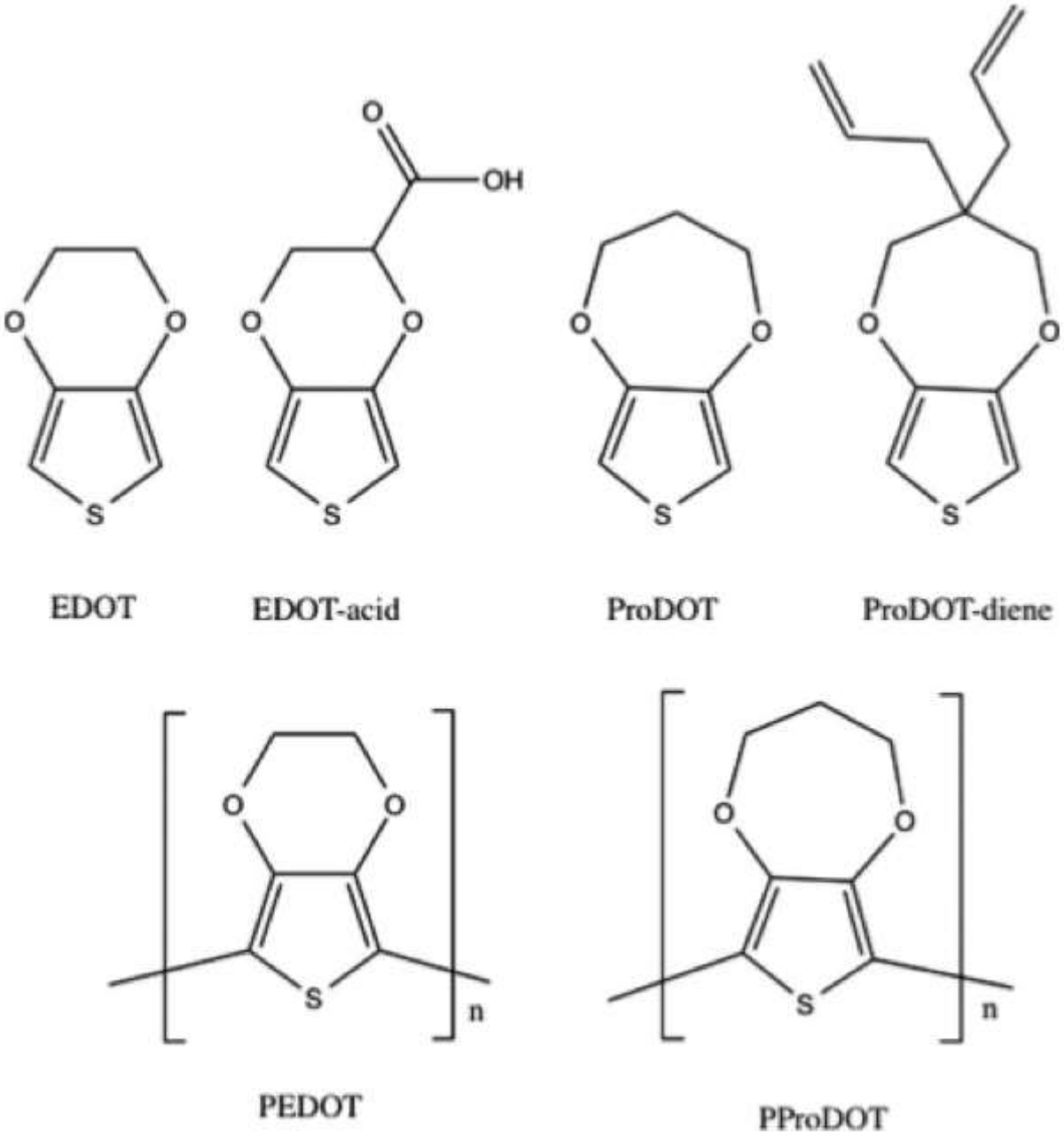

Electrically active biomaterials: the future of translatable bioelectronics 10:30 - 12:00 Auditorium II 11/09/2018

Oral presentation

286 Optically active collagen-QD matrices for read-out and stimulation of neuronal activity

Stijn Jooken, Olivier Deschaume, Yovan de Coene, Carmen Bartic

KU Leuven, Leuven, Belgium

INTRODUCTION:

The ability to stimulate neurons and record their activity in a non- or minimally invasive manner at (sub)cellular level is of enormous importance to neuroscience and medicine. In the class of nanoparticle probes, semiconductor quantum dots (QD) are promising tools. They possess voltage-sensitive optical properties and have been shown to be capable of stimulating electrogenic cells [1–2]. For efficient signal coupling, it is critical for the QDs to be in close proximity of the cell membrane as well as firmly tethered to a substrate to avoid QD internalization by the cell[1,3]. Our approach is to couple the QD probes to protein fibers that are constituents of natural extracellular matrices (ECM). Here, we report on the development of such a hybrid 2D biomaterial consisting of collagen tethered QDs.

METHODS:

2D fibrillar collagen matrices were prepared on glass coverslips silanized with a methyl ending group to increase hydrophobicity and promote fiber adhesion. Subsequently, the fibers were decorated with poly(styrene-co-maleic anhydride) (PSMA)-coated CdSe/ZnS core-shell QDs through EDC-NHS mediated covalent binding. The properties of hybrid collagen-QD films and their cellular compatibility were studied using atomic force microscopy (AFM), single and 2 photon fluorescence as well as second harmonics generation (SHG) imaging.

RESULTS AND DISCUSSION:

Different collagen-QD matrices have been constructed with varying coverage density and degree of orientation as shown in Figure 1. The quantum yield of the coupled dots has been optimized during the transfer from organic to water phase and amounts 30%. Using nonlinear optical techniques, the behaviour of cells on the hybrid scaffold can be easily studied as it allows to clearly distinguish between the different components of the matrices. The collagen fibers exhibit a high SHG output, while the photoluminescence of the QDs can be studied using 2P excitation. Moreover, cell viability studies on cultured HEK 293 show no cytotoxicity of the QDs. Collagen enhances the cell viability as compared to control substrates and this is maintained after the QD attachment.

CONCLUSION:

The constructed hybrid scaffolds combine the exceptional cellular affinity of collagen with the ability of QDs to excite/record action potentials. These materials provide a promising platform capable of overcoming current difficulties of creating an efficient and biocompatible interface between the cell and the nanoparticle film towards incorporating sensing/actuating nanoprobes in artificial extracellular matrices.

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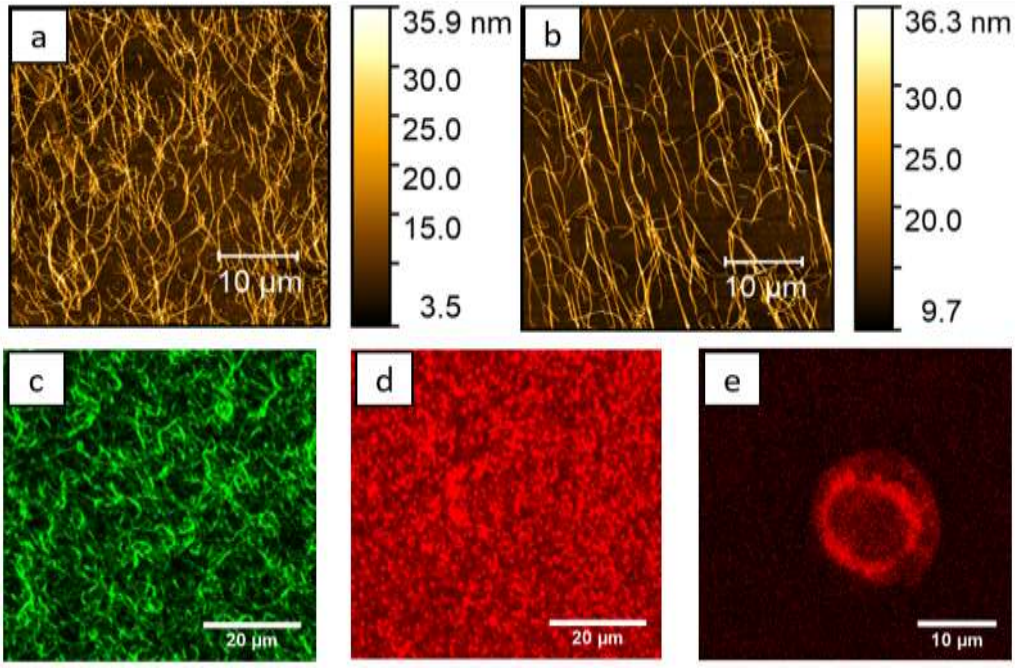

Picture 1: Caption 1: (a,b) AFM topography of collagen networks, (c) SHG imaging of collagen and 2P PL of (d) QDs and (e) a HEK 293 cell cultured on the hybrid scaffold.

Electrically active biomaterials: the future of translatable bioelectronics 10:30 - 12:00 Auditorium II 11/09/2018

Oral presentation

255 PDMS/PVDFhfp core-sheath fibers with piezoelectric properties for stimulation of cells.

Marzia Brunelli, Alexander Morel, M. René Rossi, Rolf Stämpfli, Giuseppino Fortunato
EMPA St. Gallen, St gallen, Switzerland

INTRODUCTION:

Electric stimuli influence orientation and differentiation of cells such as bone, cardiac, neural and stem cells [1,2]. When a mechanical strain is applied to a substrate, seeded cells sense an electrical stimulus driving their behavior. In this study, the elastomer PDMS was embedded in the core of PVDFhfp fibers to tailor for elastic properties, leading to enhanced piezoelectric response and output due to the β-phase within the PVDFhfp structure.

METHODS:

Solutions and coaxial electrospinning. Sylgard 184 (PDMS) was prepared mixing curing reagent and prepolymer (1:10 (PDMS1), 0.7:10 (PDMS0.7), 0.5:10 (PDMS0.5), 0:10 (PDMS0) (w/w)). 35% PVDFhfp (MW 400KDa) was diluted in DMF. The flow rates were 3.05 and 18.3 ul/min for PDMS and PVDFhfp. The voltage was +8/-1 KV while the distance was 13 cm. **Mechanical characterization**. Tensile tests (Zwick, Germany) were performed at 10 mm/min at 23°C and 50% RH. **X-ray scattering (WAXS-5 cm, SAXS-107 cm).** Scattering was measured using Nanostar instrument (Brucker) with an X-rays source (Incoatec GmbH). **Piezoelectric measurements.** A home-made apparatus was used to apply a strain and measure the resulting electrical signal. **Trasmission electron microscopy**. Fibers deposited on a copper grid were scanned by TEM (Hitachy).

RESULTS AND DISCUSSION:

TEM revealed the core-sheath structure (Fig.1a) denoting a clear embedding of the PDMS within the PVDFhfp sheath. The ratio between inner and outer diameter remains constant at 59±5% regardless the amount of crosslinker used, although an increase in the overall diameter is noticed. The elastic modulus measured by tensile tests amounted to 73.8±11.9 MPa, 11.3±1.3 MPa, 3.5±0.7 MPa,1.8±0.9 MPa,0.7±0.5 MPa for PVDFhfp, PDMS1, PDMS0.7, PDMS0.5, and PDMS0, denoting tunable mechanical properties. SAXS measurements revealed a ratio β/α phase of 96.8 % and 94.6 % for pure PVDFhfp and PDMS1 fibers, confirming the core-sheath structure does not affect the β-phase content in PVDFhfp. Preliminary piezoelectric measurements showed up to 3.5-fold increase in electric change for PDSM1 compared to mere PVDFhfp.

CONCLUSION:

By embedding PDMS into the core of PVDFhfp fibers, we proved the development of more deformable fibers compared to mere PVDFhfp fibers without affecting the amount of β-phase within the material. This substrate finds application for enhanced cell stimulation due to the higher electrical signal obtained at a certain value of strain.

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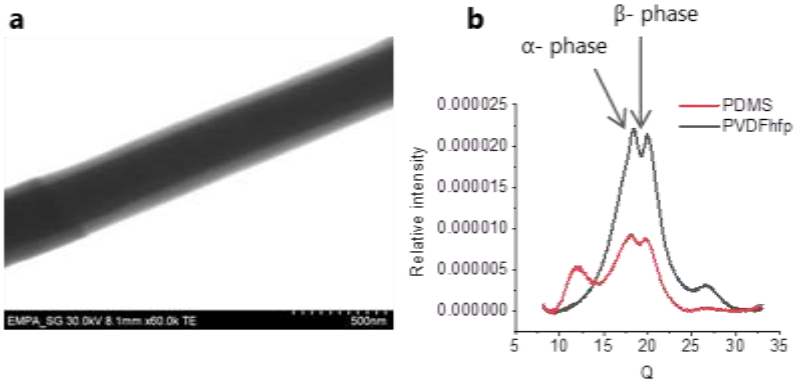


Picture 1: Caption 1: a) TEM image of PDMS1 fibers and b) SAXS graph of PVDFhfp and PDMS1 fibers.

RSC biomaterials chemistry group at ESB 14:00 - 15:30 Auditorium II 11/09/2018

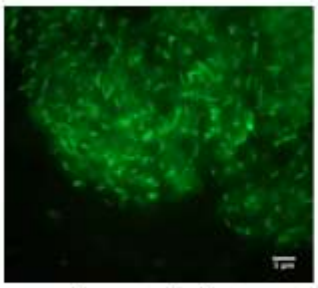
Oral presentation

Responsive polymers in diagnostics and therapeutics

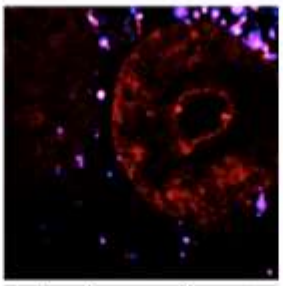
Cameron Alexander,1 Amanda Pearce,1 Patricia Monteiro, 1 Nishant Singh, 1 Vincenzo Taresco, 1 Arwyn T. Jones,2 Stefano Salmaso,3 Paolo Caliceti3 cameron.alexander@nottingham.ac.uk
1 School of Pharmacy, University of Nottingham, Nottingham, UK. 2 School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK 3 Dept. of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy

Responsive biomaterials have many applications ranging from anti-infectives, drug delivery and tissue engineering. However, the processes and mechanisms by which synthetic polymers and particles bind to

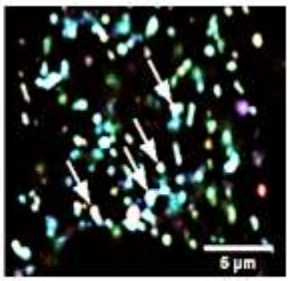
bacterial and mammalian cells, and how they transport within, are not always analogous to their natural analogues . We have been working on materials which can probe cell surface binding and intracellular transport phenomena through a range of (bio)responsive functionality. These include polymers which can reversibly change from a chain-extended to a chain-collapsed state in response to temperature, pH, ionic strength or redox potential. The responses of these polymers can lead to a hydrophilic/hydrophobic switch at a surface, the unveiling of a ligand to bind to a receptor, or a release of a therapeutic payload at a target site. Recent studies have shown that even relatively simple 'model' polymer systems can behave in unexpected ways in varying cell types and populations. The talk will accordingly focus on polymers that can (a) bind to cell surfaces and interfere with bacterial Quorum Sensing (QS)[1] and redox systems[2] (b) selectively enter cancer cells by a polymer-mediated ligand switch,[3] and (c) change pathways inside a range of cells.[4]



Polymer-bacteria interactions



Selective cell entry



Pathway switching

We will show that new synthetic polymers can exhibit a variety of intriguing properties in the presence of a range of cell types, and that the knowledge gained from these studies can give useful insights into disease processes and new therapies.

Acknowledgments: The UK Engineering and Physical Sciences Research Council (EPSRC) for grants EP/G042462/1, EP/H005625/1, EP/I01375X/1, EP/N03371X/1, EP/N006615/1 and the Royal Society (Wolfson Research Merit Award WM150086) for funding.

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RSC biomaterials chemistry group at ESB 14:00 - 15:30 Auditorium II 11/09/2018

Oral presentation

Highly branched poly(N-isopropyl acrylamide) responsive to fungi

Thomas Swift,1 Nagaveni Shivshetty,3 Abigal Pinnock,3 Ian Douglas,2 Prashant Garg,3 Sheila MacNeil,2 Stephen Rimmer1
1University of Bradford, Bradford, UK; University of Sheffield, Sheffield, UK;2

3 LV Prasad Eye Institute, Hyderabad, India

Fungal diseases are a growing problem in the tropics and can be fatal for patients with supressed immune systems. Treatment often requires the use of relatively toxic drugs such as amphotericin B. Therefore, increasing the efficacy of this and similar drugs addresses a significant need. On the otherhand detection of fungal infections and differentiation from bacteria is a key goal in the fight against antimicrobial resistance. Here we describe a branched polymer with amphotericin end groups. We show how the polymer binds to its usual target, ergosterol, and retains antifungal activity. The polymer responds to the binding of the target by desolvation of polymer chain segments and we report early indications of increased activity against some strains of fungi. The MIC against two strains of Candid albicans were 1.23 (SC5314) and 1.0 (ATCC90028) μmol of amphotericin mL-1 compared to MICs against the same strains of 0.48 amd 4.76 μmol of amphotericin mL-1 for amphotericin B not attached to the polymer. The action of the polymer against fungi is in contrast to our previously reported work on similar polymers, which respond to bacteria (by desolvation) but did not kill the organisms. We tentatively propose that the maintenance of the efficacy is associated with increasing local concentration of the amphotercin ligands and the potential for the desolvated globule to disrupt the cell membrane. Importantly the polymer showed no toxic effects to corneal epithelial cells even at concentrations as high as 5 mg ml-1. In contrast amphotericin B was toxic at and above 10 g ml-1.

Improved and faster diagnosis can inform treatment and negate strategies such as polypharmacy.

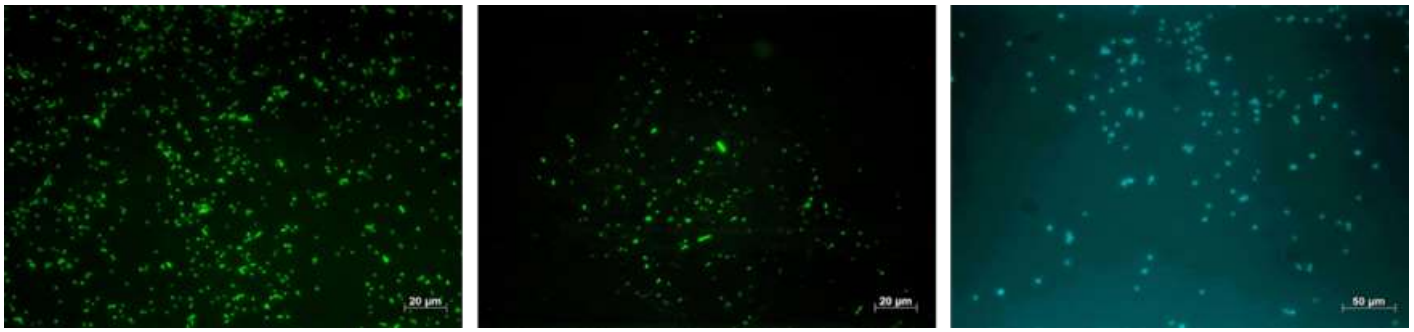

Figure 1 Bacteria and fungi attached to hydrogel functionalzed with H-PNIPAM carrying ligands each class of organism

We have, therefore, developed a diagnostic device that carries three HB-PNIPAM polymers functionalized with ligands for Gram-negative, Gram-positive or fungal infections. Each of these polymers is attached to a methacrylic hydrogel membrane. These three classes of organism can then be attached the membrane as shown in Figure 1. Importantly in this immobilized format the amphotericin HB-PNIPAM does not kill fungi. The device has been sown to be effective for sampling infections in in vivo.

RSC Biomaterials Chemistry Group at ESB 14:00 - 15:30 Auditorium II 11/09/2018

Oral presentation

200 Non-cytotoxic copper containing polyurethanes

Juan Valerio Cauich-Rodriguez, Rossana Faride Vargas-Coronado, Jose Manuel Cervantes-Uc, Teresa Hernandez-Sotomayor, Jorge Froylan Xool-Tamayo

CENTRO DE INVESTIGACION CIENTIFICA DE YUCATAN A.C., Merida, yucatan, Mexico

INTRODUCTION:

Copper is a trace metal involved in human biology and play a vital role in collagen, brain and nervous system formation in addition for maintaining healthy muscle tone and blood vessel elasticity[1]. Copper is incorporated into various metalloenzymes involved in hemoglobin formation, drug/xenobiotic metabolism, carbohydrate metabolism, catecholamine biosynthesis and the cross-linking of collagen, elastin, and hair keratin as well as in the antioxidant defense mechanism. However, copper in excess have important implications such as in the Wilson's disease or Menkes disease[2]. Therefore, the aim of this study was to obtain hybrid biomaterials based on segmented polyurethanes (SPU) and copper complexes with potential biomedical applications.

METHODS:

Copper complexes were prepared with $CuSO_4$ and either D-penicillamine (DP), L-penicillamine (LP), L-cysteine (LC) or glutathione (GR). Composites were prepared with the copper complex at 5 wt.% and a polyurethane matrix based on PCL, HMDI and as chain extenders the same thiol compound used for complex preparation. Copper complexes and hybrid polyurethanes were characterized by FTIR, Raman, SEM-EDX, XPS and TGA. Cytotoxicity of copper complexes and copper containing polyurethanes was studied by MTT using macrophages obtained from BALB/c mice. Composites of Tecoflex SG 80A and the Cu-complexes were used for comparison purposes.

RESULTS AND DISCUSSION:

FTIR and Raman spectra of copper complexes showed the disappearance of thiol related bands at 2550-2526 cm^{-1} during complex formation. EDX showed that more copper is chelated with DP, followed by LP and LC being GR the complex with less copper. However, their thermal stability followed the opposite trend i.e. penicillamine complexes were less stable (T_{d1}=160°C) than glutathione complexes (T_{d1}=205°C). Figure 1 shows macrophage viability on the different polyurethane hybrids. As seen in this figure, macrophage viability increased with time on DP and GR containing complexes while it tend to reduce with LP complexes. However, in all cases viability was higher than 70%. Composites prepared with Cu-complexes and Tecoflex SG 80A were of slightly lower toxicity compared with composites prepared with the synthesized SPU.

CONCLUSION:

Polyurethanes prepared with copper complexes were not cytotoxic at the studied concentration. However, it is clear that this depend on type of isomer (D or L) and the strength and amount of the chelated copper.

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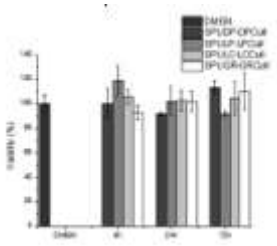
ACKNOWLEDGMENTS:

Authors wish to thank CONACYT 1360 Project



Picture 1: Caption 1: Figure 1 Macrophage cytotoxicity of copper containing polyurethanes

RSC Biomaterials Chemistry Group at ESB 14:00 - 15:30 Auditorium II 11/09/2018

Oral presentation

663 Degradable Poly(Ethylene Glycol) Hydrogels for Spinal Cord Injury Repair

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University of Glasgow, Glasgow, United Kingdom

INTRODUCTION:

Spinal cord injury (SCI) can profoundly affect mobility, independence and quality of life of those injured. Spontaneous regeneration of the damaged spinal cord is insufficient in the majority of cases resulting in chronic complications. Poly(Ethylene Glycol) (PEG) hydrogels hold potential as a suitable biomaterial therapy because of their biocompatibility, modifiability and tuneable elastic modulus in proximity to spinal cord tissue[1,2]. In this work PEG-based hydrogels are characterised and evaluated for their biological suitability in the context of SCI.

METHODS:

5% wt/vol 4-armed PEG-maleimide (PEG-4MAL) based gels were modified using integrin-recognisable peptide sequences RGD or IKVAV at 2mM concentration. Gels were cross-linked using a matrix-metalloprotease degradable peptide sequence mixed in variable ratios with non-degradable PEG-dithiol. These hydrogels were characterised for Young's modulus using atomic force microscopy (AFM) and for their degradation profile. The behaviour of astrocytes, neural stem cells and spinal cord neurons were also studied in 3D gel environments along with preliminary in vivo experiments in rat contusion models of injury, injecting the hydrogel into the contusion syrinx after onset of injury followed by immunohistochemistry analysis.

RESULTS AND DISCUSSION:

The fully degradable gels have Young's moduli of approximately 1kPa which fits well with published spinal cord data and degrade very rapidly within 1 day in concentrations of collagenase exceeding 10U/mL. Partially degradable gels persist for several weeks at the same concentration and had Young's moduli of approximately 2.5 kPa. *In vitro* studies showed RGD gels were effective in supporting cell attachment and process development with astrocytes and neural stem cells compared to other gel types. Spinal cord neurons were also capable of growing axons on RGD and IKVAV gels. Preliminary *in vivo* data indicates increased axonal regrowth through the spinal cord injury site with both RGD and IKVAV gels.

CONCLUSION:

The peptide-modified hydrogels show a capability for pervasive glial and neuronal support and growth in a 3D environment. Future work will aim at establishment of robust *in vivo* data to consolidate previously obtained information.

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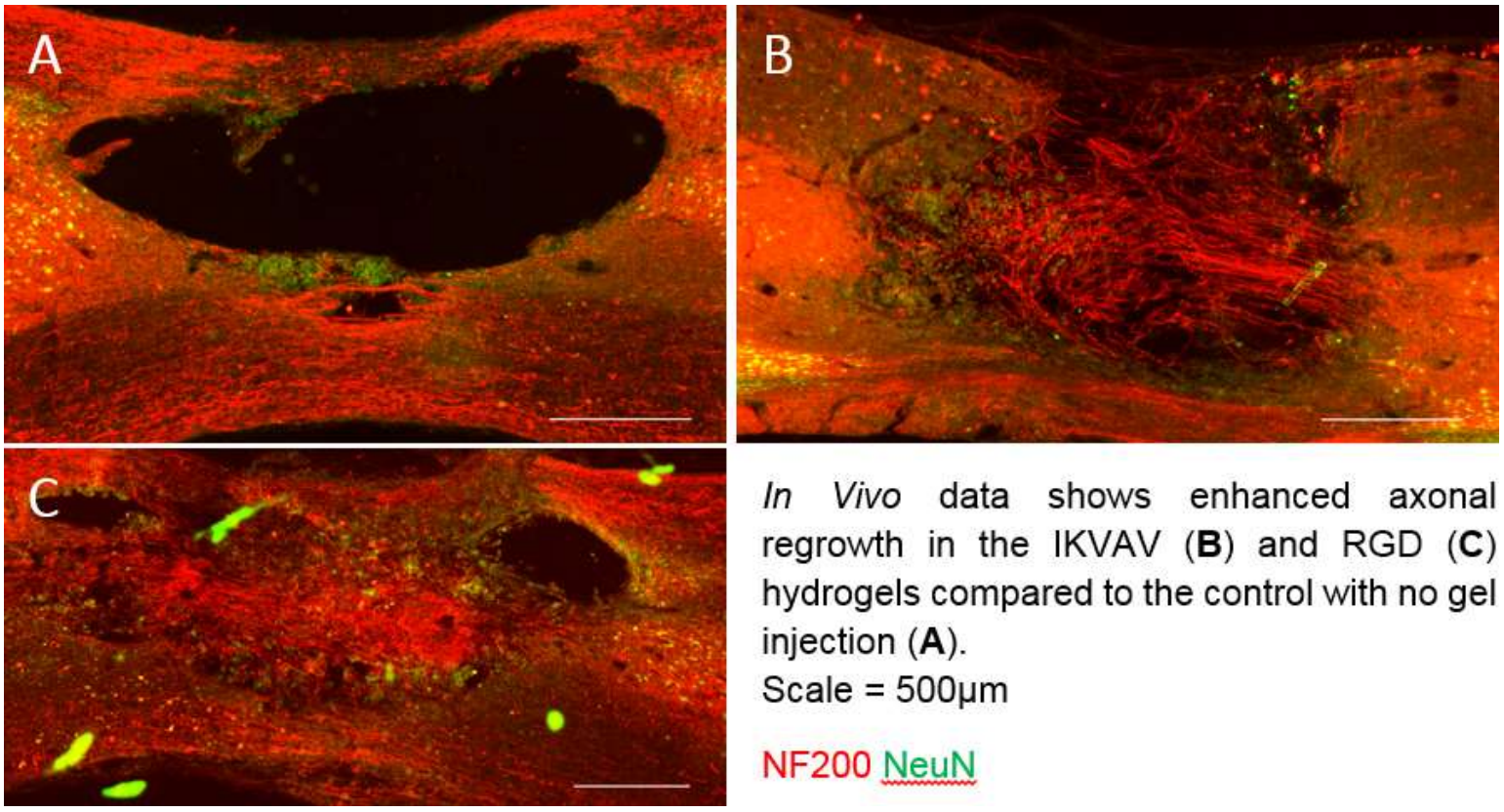
ACKNOWLEDGMENTS:

The authors thank the EPSRC for providing the project scholarship (EPSRC: 1653820) and the ERC for providing additional funding (ERC: 306990).

Picture 1:



Additive manufacturing of polylactides, from idea to commercialization 16:00 - 17:30 Auditorium II 11/09/2018

Oral presentation

Aligning molecular and structural dynamics in fused deposition modelling: Novel routes to tailor product functionalities

Varun Srinivas 1, 2; Tosca Van Hooy 2; Sanjay Rastogi 1; Jules Harings 1
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2 Zuyd University of Applied Sciences, The Netherlands.

Additive manufacturing, often referred to as 3D printing, is a rapid developing technology. Products are created layer by layer, rendering extraordinary dimensional flexibility and control in fabrication of complex geometries. Via a variety of 3D printing technologies, niche products of high added value are entering automotive, aerospace, defense, art and medical industries [1]. However, from economical perspective reservations rise due to mismatched expectations in production speed and functional product quality; the Achilles heel of 3D printing. Low production speed and disappointing product quality are particularly evident for materials of thermoplastic origin that are processed via fused deposition modeling (FDM).

FDM relies on the facile processability of thermoplastics, whereby material is extruded and shaped in liquid state, after which the new shape preserved upon solidification. However, thermoplastics differ from other materials classes such as ceramics and metals since covalent bonds and subsequent connectivity of elementary building blocks into large individual macromolecules can slow down the molecular mobility significantly [2]. In addition to the common time scales of layer build-up and temperature changes also those of conformational rearrangements

(like crystallization), intermolecular diffusion during shaping need to be taken into account and balanced in case of FDM with macromolecules, as depicted in Figure 1 (a).

In this study the local structural effects of (mis-)aligned timescales are mapped for polylactides via systematic variations in judiciously chosen molecular parameters. Polylactides varying in molar mass and enantiomeric purity, and consequently different fusion and crystallization dynamics, serve as model. Results indicate that complex heat management that deviates as function of build height and print speed lead to layer-specific inhomogeneity in degree of fusion, crystallization, crystallographic composition, thermodynamic and thus geometrical instability (Figure 1(a) and (b)). The molecular understanding of the dynamics of fusion, crystallization, print strategy, coupled with the use of additives (reinforcing phases, nucleating agents and blending strategies to promote fusion) provide opportunities to exploit, position and direct local structure induced functionalities.

Additive manufacturing of polylactides, from idea to commercialization 16:00 - 17:30 Auditorium II 11/09/2018

Oral presentation

Thermoplastic materials in Additive Manufacturing assisted Tissue Engineering - A Review

Carlos Carvalho
Process and Material Development, EnvisionTEC GmbH

Thermoplastic polymers, especially PLA, PLGA, PCL and their co-polymers, belong to the most widespread materials currently used in Additive Manufacturing assisted Tissue Engineering research. Besides their most well-known use in technical 3D printing technologies, like FDM, for prototyping and surgical planning, thermoplasts have been used with 3D bioprinters for Bone Regeneration as well as Cartilage Regeneration in combination with other materials to optimize their mechanical properties to the application's requirements. Additionally, researchers have increasingly used biodegradable polymers as reinforcement of hydrogel scaffolds, a type of hybrid scaffolds, to provide both flexibility as well as mechanical strength to cell laden implants. Lastly, thermoplastic polymers remain the gold standard for controlled drug releasing applications, which can be combined with scaffold fabrication processes.

This presentation will review the use of thermoplastic materials in Additive Manufacturing assisted Tissue Engineering using different manufacturing practices during the last 15 years.

Additive manufacturing of polylactides, from idea to commercialization 16:00 - 17:30 Auditorium II 11/09/2018

Oral presentation

256 Printability window for thermoplastic polymers in bioextrusion

Andrea Roberto Calore[1], Carlos Domingues Mota[1], Marnix van Gurp[2], Katrien V. Bernaerts[1], Jules Harings[1], Lorenzo Moroni[1]
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INTRODUCTION:

Despite the consolidated success of Additive Manufacturing (AM) in tissue engineering, the process of scaffold fabrication is still based on direct experience and on a trial-and-error approach when testing a new machine or

material. This highly limits the testing of research grades from small scale synthesis or expensive medical grades commercially available[1, 2].

Focusing on extrusion-based techniques, we show that it is possible to evaluate in advance the compatibility between a specific machine and a specific material and to choose the best set of deposition parameters to obtain the desired results in terms of morphological accuracy and mechanical properties. This can be done by knowing *a priori* the rheological and thermal properties of the polymer and the machine features.

METHODS:

The flow of material through the print head and the power requirement were modelled as in screw extrusion. The viscosity dependence on temperature and shear rate of a test polymer were measured by means of a plate-plate rheometer. The deposition speed at specific processing parameters could then be evaluated. The solidification kinetics were modelled via the lumped-capacity analysis and the polymer thermal properties were measured by means of DSC. The interlayer bond formation was modelled according to the reptation theory[3] and the related layer thickness, and mechanical properties were measured via microCT and torsional DMA.

RESULTS AND DISCUSSION:

The flow model correctly predicted the deposition speed at which the filament has a diameter equal to the nozzle. By evaluating the power requirement under specific conditions, it was possible to estimate the highest extrudable viscosity. The cooling kinetics model successfully identified the solidification window within which interlayer bonds are formed after deposition as shown in the figure below. Extruding at higher temperatures increased this time span, leading to enhanced bonding and mechanical properties. Additionally, the vertical displacement experienced by a fiber because of the increased degree of bonding could be successfully estimated. This allowed adjusting the layer thickness during fabrication accordingly.

CONCLUSION:

We developed a toolbox to evaluate in advance the compatibility between a specific bioextruder and a specific thermoplastic polymer, skipping the usual trial-and-error step. Thanks to it, it is possible to save time, material and therefore money by understanding if and how a polymer can be processed with a given machine, so that the focus can be set on creating a scaffold with the desired morphological and mechanical properties.

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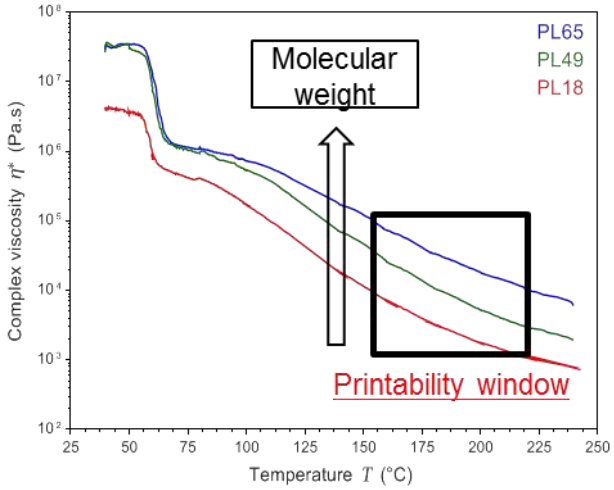
ACKNOWLEDGMENTS:

This work is supported by Province of Limburg, Brightlands Materials Center and Maastricht University.



Picture 1: Caption 1: Printability window for a set of thermoplastic grades with different molecular weights.

Additive manufacturing of polylactides, from idea to commercialization 16:00 - 17:30 Auditorium II 11/09/2018

Oral presentation

814 Glaucoma-on-a-chip: an in vitro model for glaucoma drug discovery based on mimicking the mechanical stress of high eye pressure

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[2]MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, Netherlands
[3]Department of Biomechanical Engineering, Enschede, Netherlands
[4]University Eye Clinic Maastricht UMC+, Maastricht, Netherlands

INTRODUCTION:

The pathology of glaucoma is characterized by optic neuropathy. At the cellular level, there is the loss of retinal ganglion cells (RGCs). Since the loss of these specialized neurons is irreversible, it is urgent to develop treatments that protect these cells. To achieve this, an *in vitro* glaucoma model would be very useful. The purpose of this study is to develop such a model by culturing RGCs and expose them to conditions that mimic increased ocular pressure (IOP), the main risk factor of glaucoma.

Normal IOP ranges from 12 to 22mmHg, but IOP levels can exceed 70mmHg in glaucoma. Elevated IOP leads to increased hydrostatic pressure in the eye, causing, amongst others, deformation and stretching of cells. To mimic this, we need a device that can apply both hydrostatic pressure and stretch simultaneously to cultured cells.

METHODS:

Stretching should be applied dynamically, in order to account for fluctuations in IOP that occur naturally in vivo. To realize this, we modified a recently developed device for stretch and shear stress(1). We use the immortalized neuronal PC12 cell line to establish the model; later RGCs will be used. Output parameters include cell density, survival and morphology.

RESULTS AND DISCUSSION:

A medium-throughput cell culture device has been constructed that can apply hydrostatic pressure via the culture medium (0-90 mmHg). In addition, simultaneously, cells can be dynamically stretched anisotropically and isotropically ranging from 0 to 20% (in 5 steps). All conditions are represented in fourfold on the device.

First experiments using PC12 cells indicate that cyclic stretch (1Hz, 10%) applied for 2 days, result in reduced cell density as compared to control (no stretch, T-Test, $P<0.05$). Variation in cell density was large, probably related to variation in seeding density. To cope with this variation, we now also record the initial cell density, before applying mechanical strain, and use this for normalization.

CONCLUSION:

We constructed the first cell culture device that can apply both pressure and stretch to cultured cells at the same time. This glaucoma-on-a-chip will help to identify the molecular mechanisms of mechanically-induced RGC death and help to design neuroprotective treatments. In the future, it can serve to characterize sensitivity of patient cells to mechanical stress.

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ACKNOWLEDGMENTS:

The authors would like to thank the University Eye Clinic Maastricht UMC+ and the Dutch province of Limburg ("Limburg INvesteert in haar Kenniseconomie" (LINK), Grant no: FCL67723) for providing financial support to this project.

Technological advances in additive manufacturing & bioprinting 10:30 - 12:00 Room 0.4 11/09/2018

Oral presentation

308 Rheology and processing of degradable polymers, considerations for additive manufacturing

Astrid Ahlinder, Tiziana Fuoco, Anna Finne-Wistrand
KTH Royal Institute of Technology, Stockholm, Sweden

INTRODUCTION:

The next generation of medical scaffolds will be produced through additive manufacturing. It promises numerous advantages over other manufacturing techniques, but common melt printing techniques often adversely affect the properties of the printed polymers compared to established salt-leaching techniques.[1] Aliphatic polyesters and their copolymers are of interest in the field of medical devices due to their degradability and biocompatibility.[2] Our aim is to produce a pliable scaffold which can provide mechanical support during the first part of the healing process and is subsequently replaced by the regenerated tissue. Knowledge of the polymeric melt behavior and stability improve the manufacturing process.[3,4]

METHODS:

Medical grade polylactide, polycarbonates, polyether(esters) and their copolymers have been evaluated using small amplitude oscillatory shear (SAOS) rheology to follow their melt behaviour and stability. The measurements have

been combined with thermal and chemical characterisation performed by differential scanning calorimetry and size exclusion chromatography.

RESULTS AND DISCUSSION:

A series of measurements, using SAOS rheology with varying temperature and frequency as a function of the melting points of the polymers have been used to map the elastic and viscous response to deformation. From the rheological analysis it emerged that all polymers showed a clear shear thinning behaviour. At temperature close to the melting point the frequency applied had a high impact on the elastic response, whereas at higher temperatures the elastic response decreased regardless of applied frequency. The rheological data have later been used to study the printability of the polymers for different additive manufacturing techniques.

CONCLUSION:

Rheology and chemical characterisation techniques gave us an insight into the melt behaviour of degradable polymers and the changes occurring in their microstructure and properties. The polymers had an overall similar melt behaviour in respect to their individual melting point, but care need to be taken to tailor the manufacturing process to the more fluidic and elastic responses of the different copolymers.

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ACKNOWLEDGMENTS:

The authors would like to thank Swedish Foundation for Strategic Research (RMA15-0010) for providing financial support to this project.

Technological advances in additive manufacturing & bioprinting 10:30 - 12:00 Room 0.4 11/09/2018

Oral presentation

477 Real time measurement of spatial O2 distribution in 3D bioprinted constructs of clinically relevant sizes using sensor nanoparticles

Ashwini Rahul Akkineni[1], Erik Trampe[2], Klaus Koren[2], Felix Krujatz[3], Anja Lode[1], Michael Kühl[2], Michael Kühl[4], Michael Gelinsky[1]
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[2]Marine Biology Section, Department of Biology, University of Copenhagen, Denmark
[3]Institute of Natural Materials Technology, TU Dresden, Germany
[4]Climate Change Cluster, University of Technology Sydney, Australia

INTRODUCTION:

Cell based therapies for tissue repair and regeneration have taken a quantum leap with introduction of 3D bioprinting in the fields of biomaterials and tissue engineering. However, lack of defined vasculature in bioprinted constructs

(especially in constructs having clinically relevant sizes) leads to low O_2 availability in deeper areas of the hydrogel matrix strongly limiting cell growth and development of functional tissue equivalents *in vitro*. Inclusion of angiogenic factors for developing vasculature or employing bioreactors offer a potential solution for construct maturation. However, these approaches are associated with problems such as increased culture time, non-uniform vasculature development or inefficient O_2 distribution within constructs. To understand and overcome these challenges, new tools are needed to monitor O_2 distribution dynamics within whole bioprinted constructs – which can lead to better printing designs and improved *in vitro* construct maturation.

METHODS:

Alginate/methylcellulose bioink[1] combined with O_2 sensitive nanoparticles[2] was loaded with O_2 consuming and/or producing cells, i.e., mammalian cells and photosynthetically active microalgae (*Chlorella sorokinania*), and used for extrusion-based bioprinting. 3D constructs of various complexity were fabricated to image the spatio-temporal dynamics of O_2 concentration. Cell laden constructs were cultivated in an adapted medium suitable for both, microalgae and mammalian cells, at 37°C, 5% CO_2 and defined O_2 concentrations (1-21%) with and without illumination (white light, 450 µmol photons $m^{-2}s^{-1}$). A simple SLR camera and custom-built 445nm LED (setup inside the incubator) was used to image O_2–dependent luminescence of the nanoparticles.

RESULTS AND DISCUSSION:

Addition of nanoparticles did not negatively influence the bioink rheological properties or 3D bioprinting process or the cell viability. Imaging of cell-laden constructs revealed that microalgae constructs showed significantly higher O_2 concentration dynamics compared to mammalian cell constructs, especially in response to light. Our data demonstrate the high potential of the method for non-destructive visualization of local metabolic activity, which now allows real time measurement of spatio-temporal dynamics of cell activity and the chemical microenvironment in constructs.

CONCLUSION:

Inclusion of O_2 sensitive nanoparticles in bioinks offers a simple and reliable method to measure spatio-temporal O_2 concentrations in 3D bioprinted constructs. The system can potentially be extended to other tissue engineering methods. As competitive O_2 consumption of different cell types in a construct can be studied, such measurements can lead to more efficient designs for clinically relevant constructs.

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Picture 1:

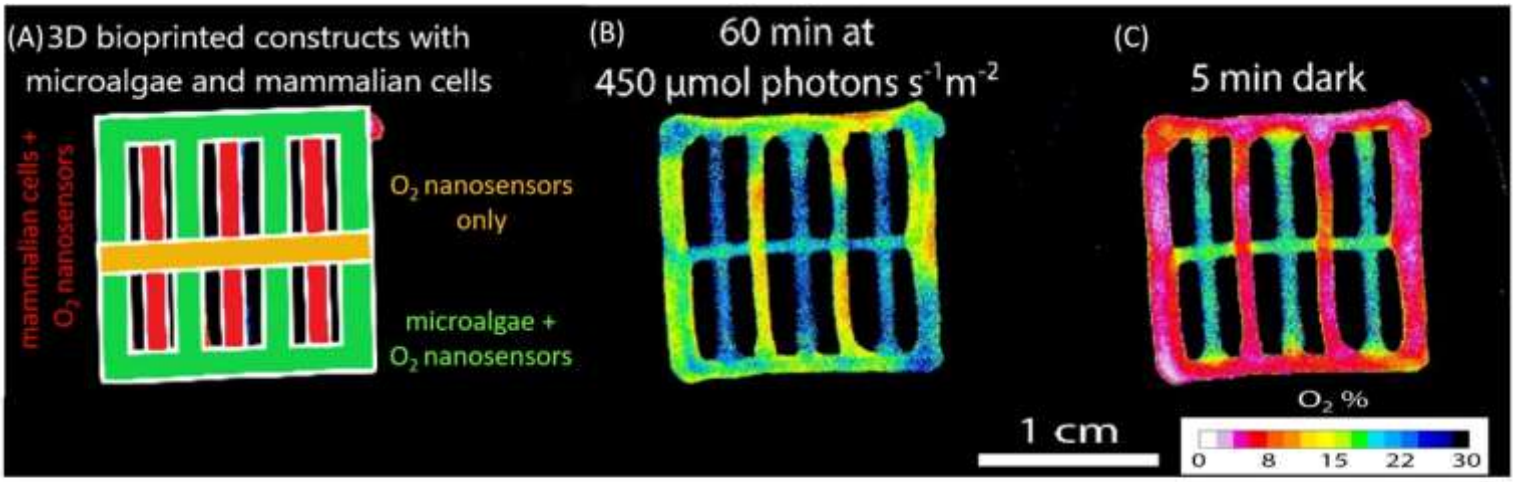

Figure 1: (A) Schematic diagram of 3D bioprinted construct with strands of microalgae + O_2 nanosensors (green), mammalian cells + O_2 nanosensors (red) and no cells + O_2 nanosensors (orange). (B & C) Visualization of O_2 concentrations in the scaffold after 60 min exposure to a photon irradiance of 450 µmol photons s^{-1} m^{-2} and 5 min of darkness, respectively.

Technological advances in additive manufacturing & bioprinting 10:30 - 12:00 Room 0.4 11/09/2018

Oral presentation

236 Using sound to pattern cells: 3D Sound Induced Morphogenesis (3D-SIM)

Tiziano Serra, Riccardo Tognato, Angela R. Armiento, Robert G. Richards, Mauro Alini, David Eglin
AO Research Institute Davos, Davos platz, Switzerland

INTRODUCTION:

3D cell technologies are revolutionizing drug discovery and precision medicine. They can better recapitulate native physiological milieu in comparison to standard cell cultures and preclinical models. A wide range of 3D cell technology products are on the market. These include products to make spheroids, gels embedding cells, synthetic scaffolds seeded with cells and the most advanced 3D-bioprinting technology to dispense cells and matrices. 3D-bioprinting tools allow precise dispensing of cell-loaded hydrogel and creation of complex 3D constructs. However, their main limitations are: a) lower cell viability, b) limited resolution, c) long processing time and d) high costs. Thus, an additive manufacturing process to create 3D models in a time-effective manner, with sufficient complexity, and retaining cell viability is crucially required. Here we report a 3D cell technology, named 3D Sound Induced Morphogenesis (3D-SIM), which allows producing hierarchically complex 3D cell constructs[1].

METHODS:

3D-SIM is based on an acoustic wave additive manufacturing technology. Acoustic waves move cells dispersed in a fluid. Depending on amplitude and frequency of the waves, multiple cell patterns are produced and stabilized through gelation of hydrogel precursors. Finite element analysis (FEA) was conducted to properly select cell pattern shapes. Morphological analysis via optical and confocal microscope has been carried out.

RESULTS AND DISCUSSION:

We show that acoustic waves can move cells dispersed in a fluid over an area of 28 cm^2 in less than 30s. The process is applicable to a wide range of off-the-shelf gelling biomaterial matrices (such as Matrigel®, Collagen and Fibrin). Layers composed by several combinations of hydrogel and cells/bioactive particles were generated and employed as matrices. Cell patterns morphology confirmed FEA investigation. 3D constructs were created by staking layers of patterned cells embedded in those matrices.

CONCLUSION:

We demonstrate that 3D-SIM is an affordable and user-friendly technology to create 3D cell models in a time-effective manner, with sufficient spatial complexity, retaining cell viability.

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ACKNOWLEDGMENTS:

The authors would like to thank BRIDGE programme (SNSF-Innosuisse) for providing financial support to this project (SNSF grant number: 20B1-1_178259).

Technological advances in additive manufacturing & bioprinting 10:30 - 12:00 Room 0.4 11/09/2018

Oral presentation

711 3D printing chimeric hydrogel scaffolds using suspended additive layer manufacturing (SALM)

Jessica Senior[1], Megan Cooke[2], Erik Hughes[2], Muhammad Ghori[1], Vasileios Kontogiorgos[1], Liam Grover[2], Alan Smith[1]
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[2]University of Birmingham, United Kingdom

INTRODUCTION:

Biopolymer hydrogels are known to have physicochemical similarities to extracellular matrix (ECM). Despite this, replicating the anisotropic nature of ECM is challenging. 3D-bioprinting offers a potential solution, however, using low viscosity biopolymers in 3D printing is particularly difficult. Firstly, pre-gelled solutions possess little structural integrity, flowing from the intended shape upon surface deposition. Another problem, is the inability to integrate multiple layers of material once gelled, preventing the production of composite gels with regional variations in mechanical behaviour. Using SALM recently pioneered within our laboratories, it is possible overcome these problems[1,2]. Here we report on how a range of different biopolymers can be 3D printed to create chimeric hydrogels that have regionally diverse mechanical behaviour and a distinct interfacial region all within a single construct.

METHODS:

Agarose fluid gels were prepared by shear cooling agarose solutions (0.5%w/v) and loading into deep petri dishes. Using a 3D bioprinter (INKREDIBLE®), an MC3T3 cell-loaded biopolymer material (gellan gum, ι-carrageenan, alginate and collagen) was deposited into the agarose fluid gel bed as a cylinder shape with dimensions 20x5mm. A secondary cell-loaded cylinder of a different biopolymer was then printed above the pre-deposited material forming an interfaced chimeric hydrogel. Once printed the constructs were crosslinked (thermally for collagen and ionically for the alginate, gellan and ι-carrageenan) prior to removal from the supporting bed. Alternatively, acellular hydrogels were printed, crosslinked and freeze dried for the fabrication of chimeric hydrogel sponges. The mechanical behaviour of the constructs was evaluated using rheology, dynamic mechanical analysis and texture analysis. Cell

viability was assessed using fluorescence microscopy and the microstructure evaluated using microCT and profilometry.

RESULTS AND DISCUSSION:

Rheological analysis of constructs exhibited large regional variations in mechanical properties. For example, the gellan-*i*-carrageenan constructs had G' values of 110, 750 and 9010 Pa for the i-carrageenan, interface and gellan regions respectively (figure 1). Freeze-dried constructs analysed using microCT and profilometry, revealed distinct localised variations in microstructure and porosity and cells were seen to remain viable over a 7-day culture with evidence of attachment to the regions of cell adhesive material (collagen).

CONCLUSION:

These results demonstrate that chimeric hydrogel scaffolds can be intelligently designed using a range of low viscosity bioinks in order to manufacture a tissue construct that contains chemical and mechanical gradients similar to the gradients present within native tissue.

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Picture 1:

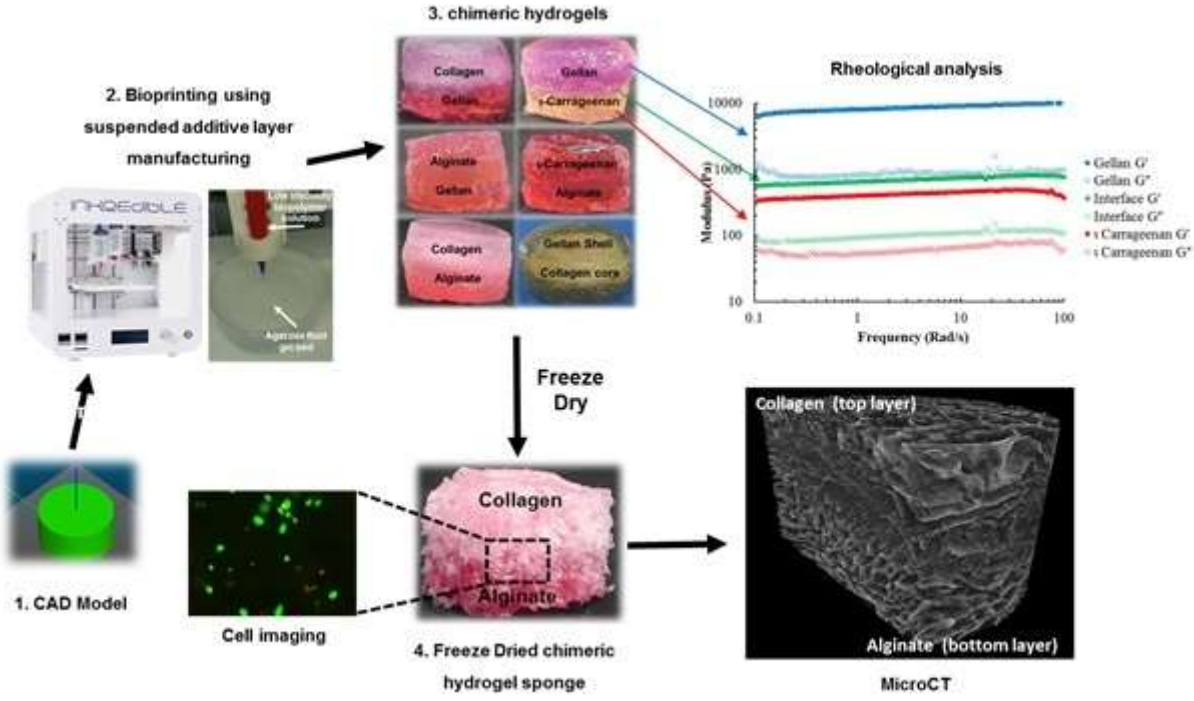

Figure 1| A schematic diagram for the production of 3D printed scaffolds and sponges for mechanical and optical analysis.

Technological advances in additive manufacturing & bioprinting 10:30 - 12:00 Room 0.4 11/09/2018

Oral presentation

713 Validation of mid-infrared LIFT for absorber free laser assisted bioprinting

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INTRODUCTION:

Laser Assisted Bioprinting (LAB) – using Laser Induced Forward Transfer (LIFT) – enables a wide range of applications in tissue engineering and stem cell research. LAB combines single-cell printing accuracy with a high transfer rate in a nozzle-free printing process. LIFT allows for transferring a wide variety of biological materials providing ideal conditions for the fabrication of complex tissue structures[1]. One drawback is the metallic absorber layer used in classical LIFT processes. Printed cell cultures demand a high cell viability and contamination-free cell transfer without nano-particles from the absorber layer. To omit the metallic layer, a mid-infrared (MIR) laser wavelength is used. At this wavelength the cell surrounding hydrogel matrix acts as an absorbing material for the underneath cells to stay unharmed. In this study we investigated a new LIFT process omitting the sacrificial metal layer and compared the results with classical LIFT experiments.

METHODS:

To validate the new absorber layer free LIFT process a glass coverslip was coated with a 50 µm thin gelatine hydrogel and cell culture medium containing CHOs cells. The jetting characteristics and cell viability have been investigated and compared to the classical LIFT process using the LIFTSYS®-Bioprinter[2]. The jetting characteristics are explored using high speed photography. For cell viability cells have been cultivated for up to eight days and stained with neutral red assay and live-dead staining.

RESULTS AND DISCUSSION:

With high speed photography a regime of stable jet formation was found leading to a controlled and reproducible jetting behaviour. The transfer of CHO cells with classical UV-LIFT and absorber layer shows that approximately 70% of transferred cells survived immediately after the LIFT. The number decreases within the following 24 hours to approx. 30% but stays stable afterwards. The new and preliminary results from the MIR-LIFT show survival rates between 50-60% which are stable for up to eight days. Proliferation of cells was observed for both processes after 24 hours in culture.

CONCLUSION:

The absorber free LIFT process seems to be promising alternative to the absorber based UV LIFT. Cells show a stable survival and proliferation rate for up to eight days.

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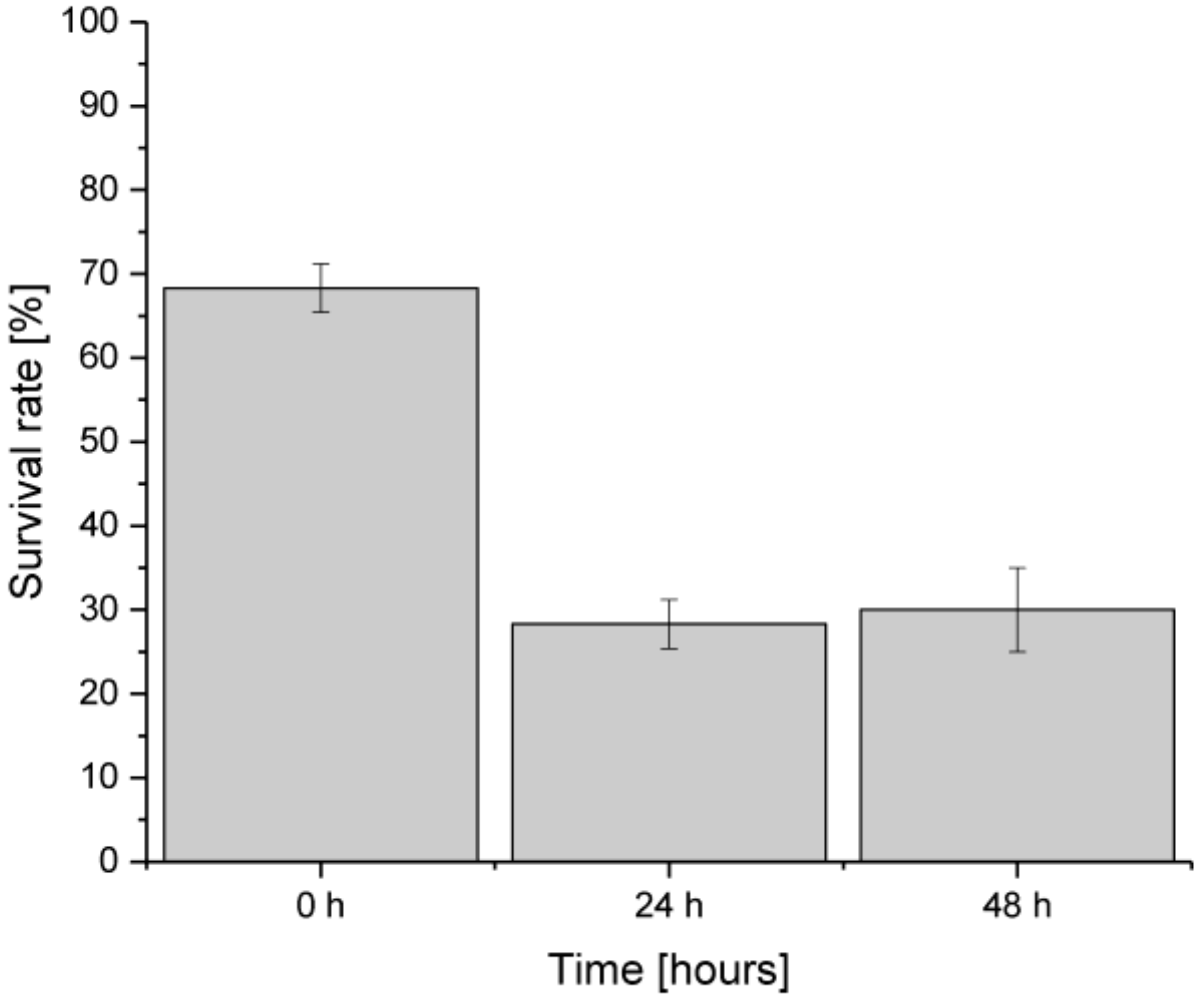
ACKNOWLEDGMENTS:

Authors thank the Fraunhofer Society for funding the absorber based LIFT experiments within the MAVO OptisCell and the Eurostars program for funding the absorber free experiments in the project MIR-LAB



Picture 1: Caption 1: Survival rate of CHO cells transfered with UV LIFT

Technological advances in additive manufacturing & bioprinting 10:30 - 12:00 Room 0.4 11/09/2018

Oral presentation

721 3D printing of porous scaffolds designed for biomedical implant development

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[2]Lucideon, United Kingdom

INTRODUCTION:

Development of scaffolds which facilitate the growth of healthy functioning tissues is a key challenge for advances in implant technologies and artificial organs. To enhance the engineering of such tissues it is beneficial to have a degree of porosity within the support structure [1]. As such, there are several fabrication processes to produce parts

with a controlled porosity [2] of which Additive Manufacturing is one example. In this study, we explore a novel idea to control the intrinsic porosity of the filaments used in the production of 3D printed scaffolds. Our ultimate aim is to develop methods to tailor the microscale topographies of scaffolds to influence cell responses which could benefit engineering of complex tissues.

METHODS:

Combinations of poly(lactide) (PLA), hydroxyapatite (HA) and sacrificial phases were blended using a Noztek filament extruder to produce composite filaments. A range of filaments were made varying the loading of inorganic phase and the blend ratio of PLA to the sacrificial phase. These filaments were then used to fabricate “wood-pile” scaffolds using an Ultimaker 2 3D printer. Two types of sacrificial phase were investigated, sodium chloride (NaCl) and poly(vinyl alcohol) (PVA), which were removed from the scaffolds through water immersion. Particle size analysis was used to quantify size distributions of the NaCl and HA fillers. PLA/PVA filaments were analysed using thermal analysis and SEM. Characterisation the composite scaffolds was performed using SEM, porosimetry and mechanical tests.

RESULTS AND DISCUSSION:

For salt, scaffold porosity depended on the particle size distribution and ratio of the soluble filler to matrix. Similarly, with PVA, the porosity of the filaments depended upon the blend with PLA. A transition from surface porosity to an open porous structure throughout the filaments was observed with increased loading of the sacrificial phases which impacted significantly on the mechanical strength of the composite scaffolds.

CONCLUSION:

We have demonstrated an effective method to 3D print scaffolds with controlled random porosity from PLA filaments prepared using both polymer and inorganic sacrificial phases.

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ACKNOWLEDGMENTS:

The authors would like to thank *Marie Skłodowska”Curie grant* (Grant no: 643050) for providing financial support to this project.

Nanomaterials for applied functionality 14:00 - 15:30 Room 0.4 11/09/2018

Oral presentation

222 Surface functionalisation, nanoroughness and drug delivery by atmospheric plasma jet on scaffolds

Alessandro Patelli[1], Federico Mussano[2], Paola Brun[3], Tullio Genova[2], Emanuele Verga Falzacappa[4], Emmanuele Ambrosi[4], Niccolo' Michieli[3], Marco Scatto[5], Giovanni Mattei[3], Paolo Scopece[5]
[1]Unversity of Padova, Padova, Italy
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[4]University of Venezia, Italy
[5]Nadir srl, Italy

INTRODUCTION:

Novel atmospheric plasma jets (APPJ) offer increasing possibilities for bio-applications. Here we present a new patented technology [1] that pushes forward the compromise between low temperature processing and efficiency. The novel jet potentialities are presented here as example in an innovative way to control nanoroughness, chemistry and drug delivery on scaffolds even on biogradable polymers.

METHODS:

The novel approach based on a APPJ is tested on titanium alloys also with commercial large grits and acid etched surface treatments for dental implants and on flat molded PCL films. Surface roughness is controlled by a 200nm nanoparticles aerosol spraying, to match the size for focal adhesion points. Two different type of particles are used: inert silica for roughness control and fluorescent PLA particles to simulate the drug delivery. Then particles are embedded on the surfaces by a 100-200 nm coating obtained by APPJ with ammine or carboxylic functional groups. The surfaces have been characterized by FT-IR, AFM and SEM. Titanium alloys are tested with osteoblasts while PCL with fibroblasts. Cells growth is evaluated by viability assay, protein absorption, proliferation and focal adhesion, SEM. The release of the fluorophore is checked in the growing media, simulating a drug release.

RESULTS AND DISCUSSION:

The APPJ patented technology is based on the coupling of RF and HF frequencies in a DBD discharge in argon and allows real room temperature plasma polymers deposition. In the case study presented here, the nanoparticles are easily fixed on the surface without substrate and particles damaging or melting. The nano-roughness increases cells adhesion independently by the chemistry, while the preferred surface chemistry depends on culture media and cells type. Osteoblasts cells adhesion increases of 20% relative to SLA titanium alloys. On the other side compared to smooth PCL substrate fibroblasts adhesion increases of a factor 10. The release of the fluorophore by the dissolution of the PLA nanoparticles is verified, confirming that the fluorophore encapsulated is not damaged.

CONCLUSION:

The atmospheric plasma offers an easy and scalable tool for surface treatments that widen the potentialities of bioscaffolds design. In this case study as example, the whole process is in open-air, fast and localized, it is compatible with 3D-printing, allows gradients designs and to face surface chemistry, roughness, drug release and mechanical properties singularly.

REFERENCES:

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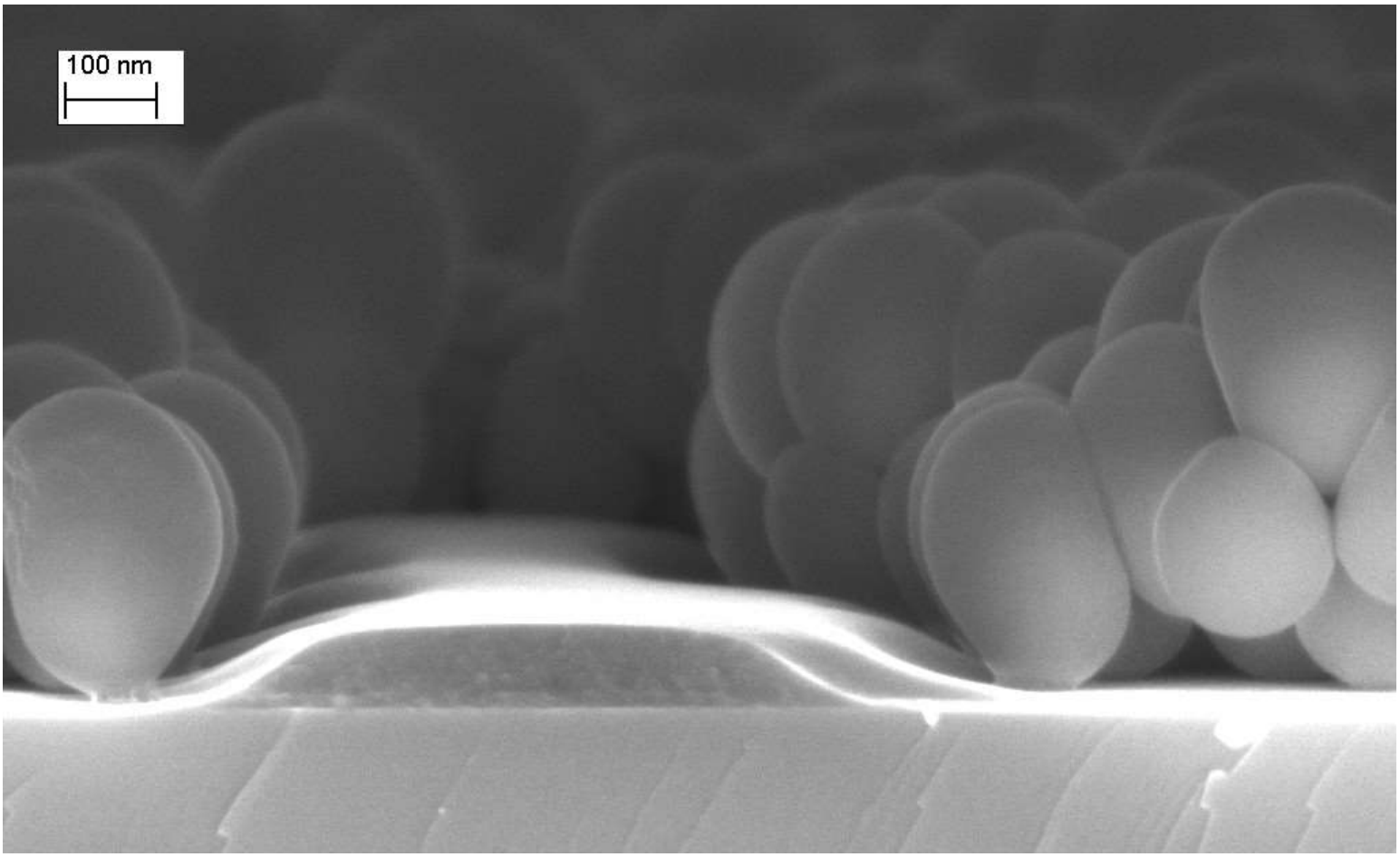
ACKNOWLEDGMENTS:

We are grateful to H2020-NMP-PILOTS-2015 (GA n. 685825) for financial support.



Picture 1: Caption 1: SEM cross section of 200 nm silica particles sprayed on silicon substrate and embedded by silane APTES coating deposited by atmospheric plasma.

Nanomaterials for applied functionality 14:00 - 15:30 Room 0.4 11/09/2018

Oral presentation

332 A functional analysis of nanotopographically modified platinum iridium electrodes

Adriona Kelly[1], Nazar Farid[2], Gerard O'Conner[2], Manus Biggs[3]
[1]Centre for Research in Medical Devices (CÚRAM), National University of Ireland, Galway, Ireland
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[3]Centre for Research in Medical Devices (CÚRAM), NUI Galway, Ireland

INTRODUCTION:

Brain Machine Interface (BMI) technologies have seen major advances in recent years but widespread clinical translation has been impeded due to a general deterioration in electrode charge transfer capabilities following implantation. This is attributed to an intrinsic host tissue response, namely glial scarring or gliosis[1]. This process prevents the injured neurons from regenerating, drives neurite processes away from the neuroelectrode and increases signal impedance by increasing the distance between the electrode and its target neurons[2]. Modification

of the nanoscale geometry of the implanted probe could enhance the electrode's electrical capabilities, and improve the physical coupling between the electrode and the surrounding neurons, whilst reducing gliosis.

METHODS:

Commercially available platinum Iridium (Pt/Ir) microelectrodes and planar Pt/Ir substrates were nanotopographically (NT) functionalised via femtosecond laser processing to generate Laser Induced Periodic Surface Structures (LIPSS), manifest as surface rippling. Four different topographies (generated through varied pulse laser number) were analysed for their physical properties using scanning electron microscopy (SEM) and atomic force microscopy. The electrochemical properties of these interfaces were then investigated using impedance spectroscopy and cyclic voltammetry. The in vitro response of mixed cortical cultures (embryonic rat E14), was subsequently assessed by confocal microscopy and ELISA. Statistical analysis was performed with Mann Whitney test.

RESULTS AND DISCUSSION:

Preliminary analysis showed that microelectrodes functionalised with LIPSS features possessed significantly reduced electrochemcial impedance profiles relative to unmodified Pt/Ir electrodes (due to increase in surface area) (**Fig.1 a&b**) and an increase in the electrode electroactive surface area ($n=3$, $p \leq 0.05$). Confocal microscopical analysis indicated that neurons were aligned along nanofeatures relative to control, implicating contact guidance of cells on NTs was achieved (**Fig.1 c&d**). Ongoing protein analysis via ELISA indicated that LIPSS functionalisation impacted on the synthesis of pro-inflammatory signalling in complex mixed neural cultures.

CONCLUSION:

The explored LIPSS features improved overall electrochemical properties of the electrodes. Cell orientation observed on LIPSS features is not only pivotal for neural regeneration but also enhanced neural recording[3]. These NT features could pave the way towards reducing tissue encapsulation in situ and improve overall neuroelectrode functionality.

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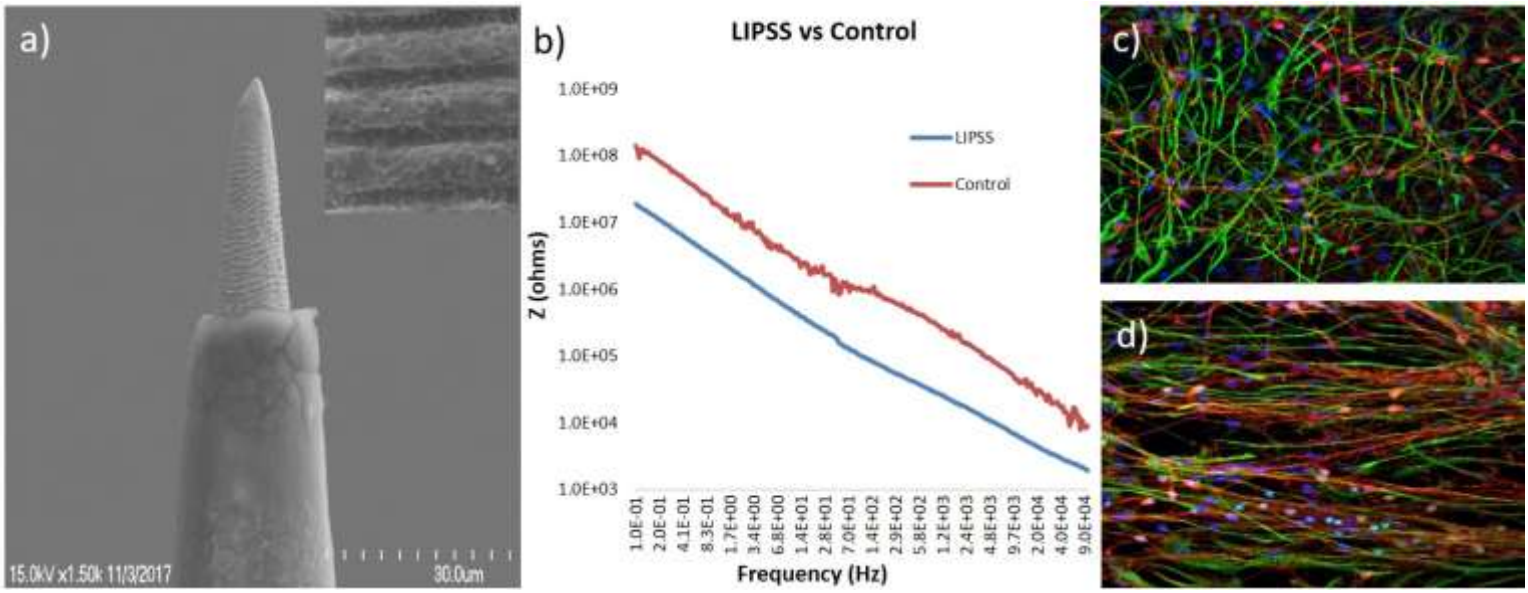
ACKNOWLEDGMENTS:

The authors would like to thank SFI CÚRAM (Grant agreement no. 13/RC/2073), Hardiman Scholarship, CMI & NCBES -NUI Galway



Picture 1: Caption 1: SEM image of LIPSS on microelectrodes a) Impedance of LIPSS b) Confocal analysis of cells on control c) compared to orientated cells on LIPSS

Nanomaterials for applied functionality 14:00 - 15:30 Room 0.4 11/09/2018

Oral presentation

27 Attachment of functional molecules to the surface of ultrasmall gold nanoparticles by click chemistry for specific epitope targeting of proteins

Selina Beatrice van der Meer[1], Matthias Epple[2]
[1]University of Duisburg-Essen, Essen, Germany
[2]University of Duisburg Essen, Germany

INTRODUCTION:

Due to the aurophilicity of sulphur, the thiolated amino acid cysteine is excellently suited as ligand for gold nanoparticles. Azide groups allow the orthogonal covalent bonding of receptor molecules to the nanoparticles by click chemistry. Surface-functionalized ultrasmall gold nanoparticles (diameter 2 nm or less) allow a specific targeting of epitopes on a protein surface as they are smaller than most proteins.[1] This is of special interest to influence the function or the conformation of a protein, e.g. to influence its function. Using click chemistry, molecules with an alkyne group were covalently bound to the particles under mild reaction conditions.

METHODS:

Ultrasmall nanoparticles with a diameter of 2 nm or smaller were prepared in a one-pot synthesis by reducing tetrachloroauric acid in the presence of functional ligands,[2] e.g. a modified tripeptide (Lys(N_3)-Cys-Asp). Cysteine binds to the gold nanoparticle via the thiol group. By click chemistry to the azide group, functional molecules with an alkyne function were covalently attached to the particles. To assess the molecular structure of the gold nanoparticle surface, nuclear magnetic resonance spectroscopy (NMR) was used. Disc centrifugal sedimentation (DCS) and transmission electron microscopy (TEM) were performed for size determination. FT-IR spectroscopy was used to

identify characteristic band of azide groups. Atomic absorption spectroscopy (AAS) was used to determine the gold concentration and thereby the absolute particle number concentration.

RESULTS AND DISCUSSION:

Ultrasmall gold nanoparticles with a diameter of 2 nm were synthesized. The FT-IR spectrum of the azide-functionalized gold nanoparticles showed a large band at 2100 cm^{-1}, due to the symmetric vibration of azide groups. ^{1}H-^{1}H-TOCSY (total correlated spectroscopy; NMR) showed the binding between gold and the cysteine of the tripeptide. Furthermore, the signals of the other amino acids were found and assigned. To demonstrate the successful clicking of the alkyne to the azide-functionalized ultrasmall gold nanoparticles, the fluorophore 6-fluorescein-alkyne was used.

CONCLUSION:

The functionalization of ultrasmall gold nanoparticles with cysteine-containing and azide-modified peptides was demonstrated by NMR spectroscopy. These particles can be biorthogonally bound to a variety of biomolecules and are available for a specific targeting of proteins, including therapeutic applications.

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ACKNOWLEDGMENTS:

We thank the Deutsche Forschungsgemeinschaft (DFG) for funding within the framework of CRC 1093: Supramolecular Chemistry on Proteins.

Nanomaterials for applied functionality 14:00 - 15:30 Room 0.4 11/09/2018

Oral presentation

18 Surface modification of tantalum via anodization for orthopedic applications

Batur Ercan, Ece Uslu
Middle East Technical University, Ankara, Turkey

INTRODUCTION:

Commonly used orthopedic implant materials experience premature failure at the implantation site due to corrosion, fatigue, wear debris formation and, most importantly, lack of sufficient osseointegration with the juxtaposed bone tissue[1]. To overcome these challenges, tantalum based implants have been investigated in several studies due to their excellent corrosion resistance and mechanical properties[2]. Despite having superior physical and chemical properties, implants made of tantalum are bioinert, and fail to promote desired osseointegration with bone.

Bioactivity of tantalum can be improved by different surface modification, *i.e.* PVD, CVD, plasma modifications, and etc. In this project, tantalum surfaces were modified by anodization, which is an electrochemical surface modification technique to fabricate nanostructured oxide on metallic components. By altering electrochemical parameters, four different surface morphologies having nanofeatures were obtained and the effect of these surfaces on bone cell functions was assessed.

METHODS:

1cmx1cm tantalum samples were anodized using a two electrode system with a platinum mesh as a cathode. Different dilutions of HF, H_2SO_4 and NH_4F were used as electrolytes for the anodization process. Potentials (5-50V) and different anodization times (90s to 3h) were chosen depending on electrolyte concentration and they were iterated to obtain uniform surface morphologies on tantalum. To investigate biocompatibility of the samples, osteoblast (ATCC CRL-11372) adhesion and proliferation were examined up to 5 days of culture.

RESULTS AND DISCUSSION:

When H_2SO_4:HF 9:1 (v/v) electrolyte was used, nanotube/nanodimple morphology were obtained (Fig.1a). Upon addition of water, a nanoporous surface morphology was formed (Fig.1b). By using a fluoride free electrolyte, *i.e.* 1M H_2SO_4 and 3.3 wt% NH_4F, nanocoral morphology was fabricated on the tantalum surfaces (Fig.1c). Finally, incorporation of 5% DMSO into concentrated H_2SO_4:HF electrolyte produced nanodimples on tantalum surfaces (Fig.1d). When bone cells were cultured on these surfaces having different nanophase topographies, enhanced cellular adhesion was observed, highlighting the biocompatible nature of the fabricated surfaces.

CONCLUSION:

Tantalum was modified via anodization to obtain four different surface morphologies. Results showed enhanced cellular adhesion compared to their non-anodized tantalum counterparts.

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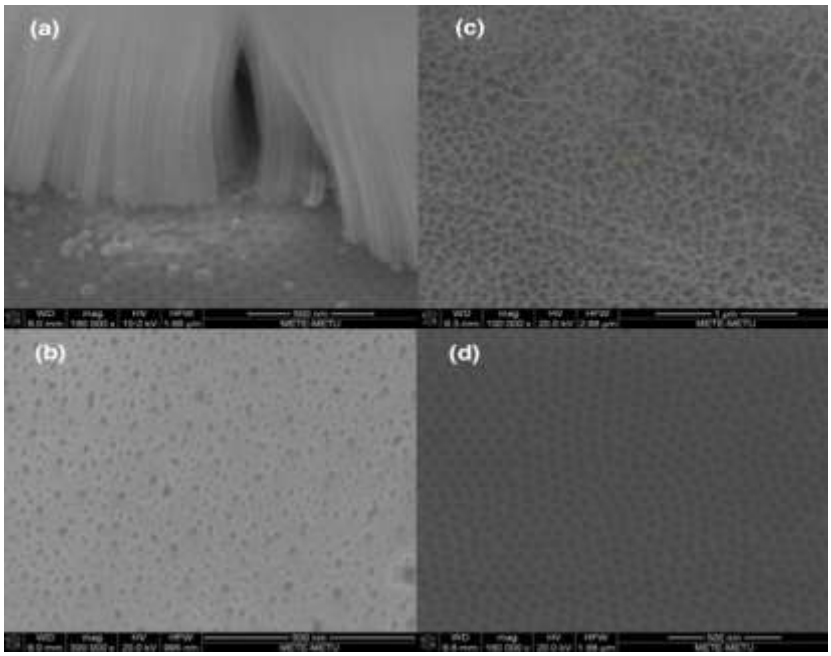
ACKNOWLEDGMENTS:

The authors thank Eyüp Can Demir for his help in microscopy and the Scientific and Technological Research Council of Turkey (Grant no: 117M187) for providing financial support.



Picture 1:Caption 1: Fig.1 Anodized tantalum samples having a) nanodimple/nanotube, b) nanopore, c) nanocoral and d) nanodimple surface morphologies.

Nanomaterials for applied functionality 14:00 - 15:30 Room 0.4 11/09/2018

Oral presentation

702 Effect of ion-doped hydroxyapatite nanoparticles for bone regeneration on bone cell viability and osteoclastogenesis

Carina Kampleitner[1], Oscar Gancitano[1], Montserrat Espanol[2], Maria-Pau Ginebra[2], Oskar Hoffmann[1]
[1]Department of Pharmacology and Toxicology / University of Vienna, Vienna, Austria
[2]Dept. Materials Science and Metallurgical Eng. / Univ. Politècnica de Catalunya, Spain

INTRODUCTION:

Nanosized hydroxyapatite (HA) is chemically similar to the mineral phase of hard tissue. Its good biocompatibility and bone integration ability have led to extensive research to use HA nanoparticles (NPs) for bone tissue engineering[1]. In recent years, ionic-substitution into HA nanocrystals became a promising strategy to improve osteoconductive and osteoinductive properties. While it is well known that HA-NPs interact with bone cells and modulate cell behaviour, HA-NP features such as size, composition, surface charge, morphology, and dose, can differently affect cellular response[2]. Thus, we aimed to determine bone cell viability and effects on bone-resorbing osteoclasts (OCs) for four recently developed HA-NPs types: non-doped HA (HA), carbonate-doped HA (10 wt%, cHA), strontium-doped HA (6 wt%, srHA) and silicon-doped HA (2 wt%, siHA).

METHODS:

We evaluated HA-, cHA-, srHA-, and siHA-NPs (250 µg/ml) for cytocompatibility on primary mouse osteoblasts (OBs) and co-cultured OBs with mouse bone marrow-derived OC-precursors. Cell viability was assessed by using WST-1 reagent after a 24-hour NP-incubation. To determine whether these NPs affect OCs, we used a mouse OB-OC co-culture to investigate OC differentiation and activity. NPs were tested at 50, 100 and 250 µg/ml, tartrate-resistant acid phosphatase positive OCs were counted and cathepsin K, a marker for OC activity, was measured.

RESULTS AND DISCUSSION:

We observed that non-doped and ion-doped HA-NPs evaluated in our cell viability assays were cytocompatible and non-toxic. Also, the different ion-dopings did not lead to adverse effects at 250 µg/ml when compared to HA-NPs and control. We found that all NPs dose-dependently reduced (250>100>50 µg/ml) OC differentiation and activity. This response was similar to all ion-doped NPs tested. Interestingly, the stimulation of NP-treated OB-OC co-cultures with RANK-ligand increased OC numbers compared to OB-OC co-cultures treated with NPs alone suggesting that OB-mediated osteoclastogenesis is susceptible to NPs. Taken together, these data show that NPs can modulate OC behaviour, which might affect bone metabolism, and indicate more in-depth investigations on the mechanisms involved.

CONCLUSION:

Although HA-NPs are potential candidates for bone regeneration, their effect on all cell types within the bone microenvironment should be considered.

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ACKNOWLEDGMENTS:

The authors would like to thank the European Seventh Framework Programme “InnovaBone” (Grant no: 263363) for providing financial support to this project.

Nanomaterials for applied functionality 14:00 - 15:30 Room 0.4 11/09/2018

Oral presentation

387 Modulating neural stem cells activity by nanoparticle light-activation

Catarina Rebelo[1], Sonia Pinho[2], João Peça[2], Liliana Bernardino[3], Lino Ferreira[2]
[1]University of Coimbra, Coimbra, Portugal
[2]Center for Neurosciences and Cell Biology, Coimbra, Portugal
[3]Health Sciences Research Centre (CICS-UBI), University of Beira Interior, Portugal

INTRODUCTION:

No current therapeutic strategy is yet sufficient to completely restore brain function upon demand. As neurogenesis persists throughout life within specific regions of the brain (namely the subventricular zone, SVZ), modulation of endogenous pools of neural stem cells (NSCs) present a straight forward solution for brain disorders. Gene editing technologies are one of the today’s most promising biological tools[1]. The delivery of gene editing enzymes like Cre-recombinase is a powerful tool that could potentially be used to manipulate NCSs behavior. Although intracellular delivery of proteins is extremely useful for the manipulation of cellular processes, it still presents great challenges. In the last years, reports of nanoparticle-formulations with the ability of intracellular delivery of proteins have emerged[2,3]. Nevertheless, light-triggered strategies reported for Cre-recombinase are still viral-based and relay on low penetrating UV/blue light[4].

METHODS:

Preparation of light-triggered UCNPs: We have designed a protein delivery system based on lanthanide-doped upconversion nanocrystals that respond to near infra-red (NIR) light to intracellularly release functional Cre-recombinase (CRE-UCNPs). A new photo-cleavable linker was synthesized and linked to the UCNPs through one end, while the other end was used to immobilized Cre-recombinase.

In vitro assays: A reporter cell line (mouse fibroblasts) for Cre-recombinase activity was used to access cytotoxicity, delivery and activation of the Cre-recombinase *in vitro* by a combination of microscopy and flow cytometry techniques.

Animal experiments: Deep-tissue light-activation was performed in mice to consolidate the hypothesis of high tissue penetration of NIR-light through the transplantation of reporter cells. A transgenic mice reporter for Cre-recombinase activity was used and recombination efficiency in the SVZ region was evaluated by fluorescence microscopy.

RESULTS AND DISCUSSION:

We show the potential of CRE-UCNPs in the internalization of Cre-recombinase and its escape from endocytic compartments. We further confirm the specificity of NIR-light-induced cargo release both *in vitro* and *in vivo*, with the key achievement of *in vivo* DNA recombination, within the SVZ region of transgenic mice.

CONCLUSION:

This gene editing platform with a spatio-temporal control over intracellular Cre-recombinase delivery represents a generalist tool for *in vivo* modulation of SVZ cells.

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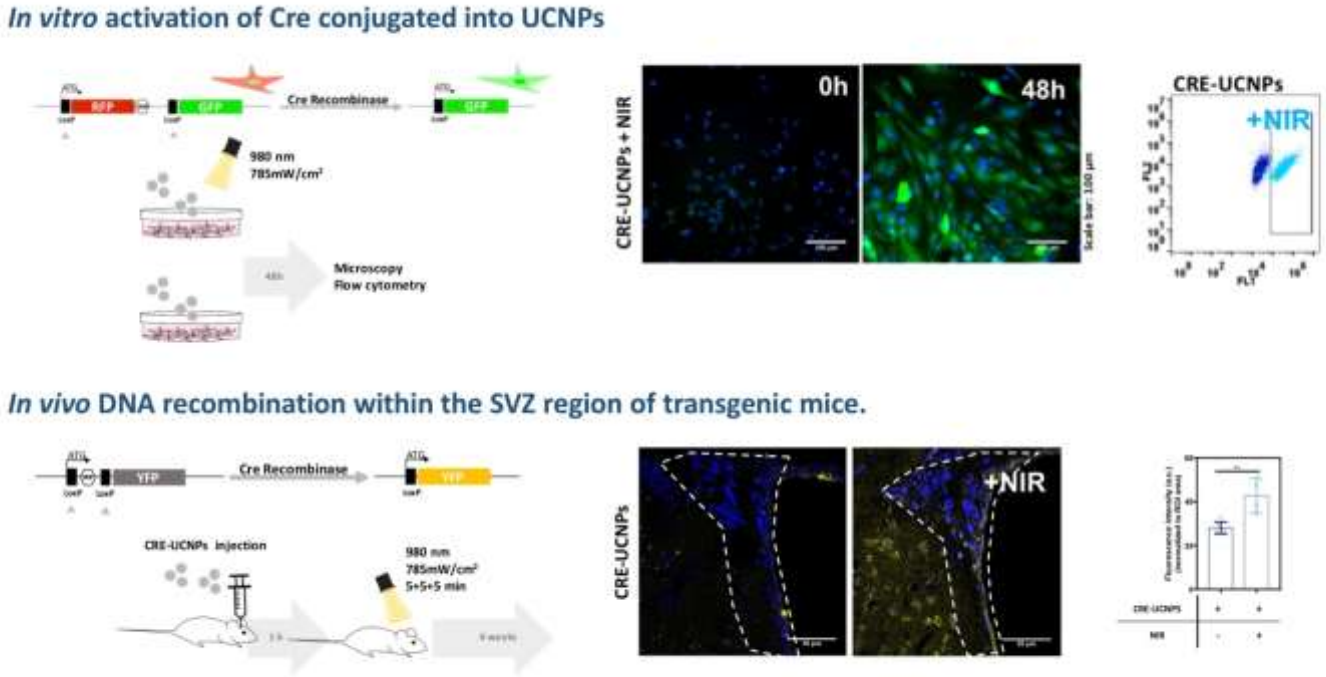
ACKNOWLEDGMENTS:

The authors would like to thank the financial support of Fundação para a Ciência e a Tecnologia (SFRH/BPD/9604/2013; SFRH/BD/52337/2013), and EC funding (ERC project nº 307384, “Nanotrigger”; European project ERAatUC ref.669088).

Picture 1:



3D scaffolds for models & manipulation 16:00 - 17:30 Room 0.4 11/09/2018

Oral presentation

261 A cryogel toolbox for cell culture and ex vivo tissue manipulation

Ben Newland[1], Eigel Dimitri[2], Welzel Petra[2], Werner Carsten[2]
[1]Cardiff University, Cardiff, United Kingdom
[2]Leibniz Institute for Polymer Research, Germany

INTRODUCTION:

Cryogels are highly porous hydrogel materials produced by freezing the precursor components either prior to, or during the gelation process.[1] The ice crystals that formed during freezing leave behind a macroporous network. The strut and pore structure of cryogels makes them tough and robust during handling, yet, as a bulk structure, be compressible and soft. The aim of this work is to tailor make a range of cryogel structures of various shapes and chemistries, to suit the end application. This work represents the progress that we have made, together with our project collaborators, towards this aim. There are three distinct cryogel scaffolds described herein (all unpublished), each of which is tailored to different specific uses. Each cryogel scaffold fits to one of the three objectives as follows:

Cryogel cylinders – Create a delivery device for focal demyelination on *ex vivo* mouse brain slices

Sulphated cryogel lines – Deliver extracellular matrix molecules to the developing human neocortex *ex vivo*

Heparin based cryogel microcarriers – Provide an adherent niche-like scaffold for neural precursor cell growth and differentiation *in vitro*.

METHODS:

A range of hydrogel precursor units (including poly(ethylene glycol) diacrylate, heparin, heparin mimics) were polymerized at sub-zero temperatures, either within templates (objectives 1 & 2), or in a water-in-oil emulsion (objective 3) in a similar manner as described previously. [2]

RESULTS AND DISCUSSION:

Figure 1 shows scanning electron microscopy analysis of the completed cryogel structures and a sample image of its use towards the specific objective. The shape of the cylinders and lines was directly determined by the polystyrene template used. The cryogel cylinders deliver lysophosphatidylcholine (LPC) to a limited area of the brain slice, creating focal demyelination. The sulphated cryogel lines produce a pattern of delivery to the developing fetal cortex which is currently being used to elucidate mechanisms of cortex folding. The heparin based microcarriers have been used to culture multipotent neural precursor cells for 28 days prior to successful differentiation into neurons.

CONCLUSION:

Cryogels offer a versatile platform technology from which to spatially restrict the delivery of molecules to tissue in culture. In addition, the macroporous structure makes them well suited for culturing cells in a three dimensional, extracellular matrix-mimicking environment.

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B.N. would like to thank the Wellcome Trust for funding.

Picture 1:

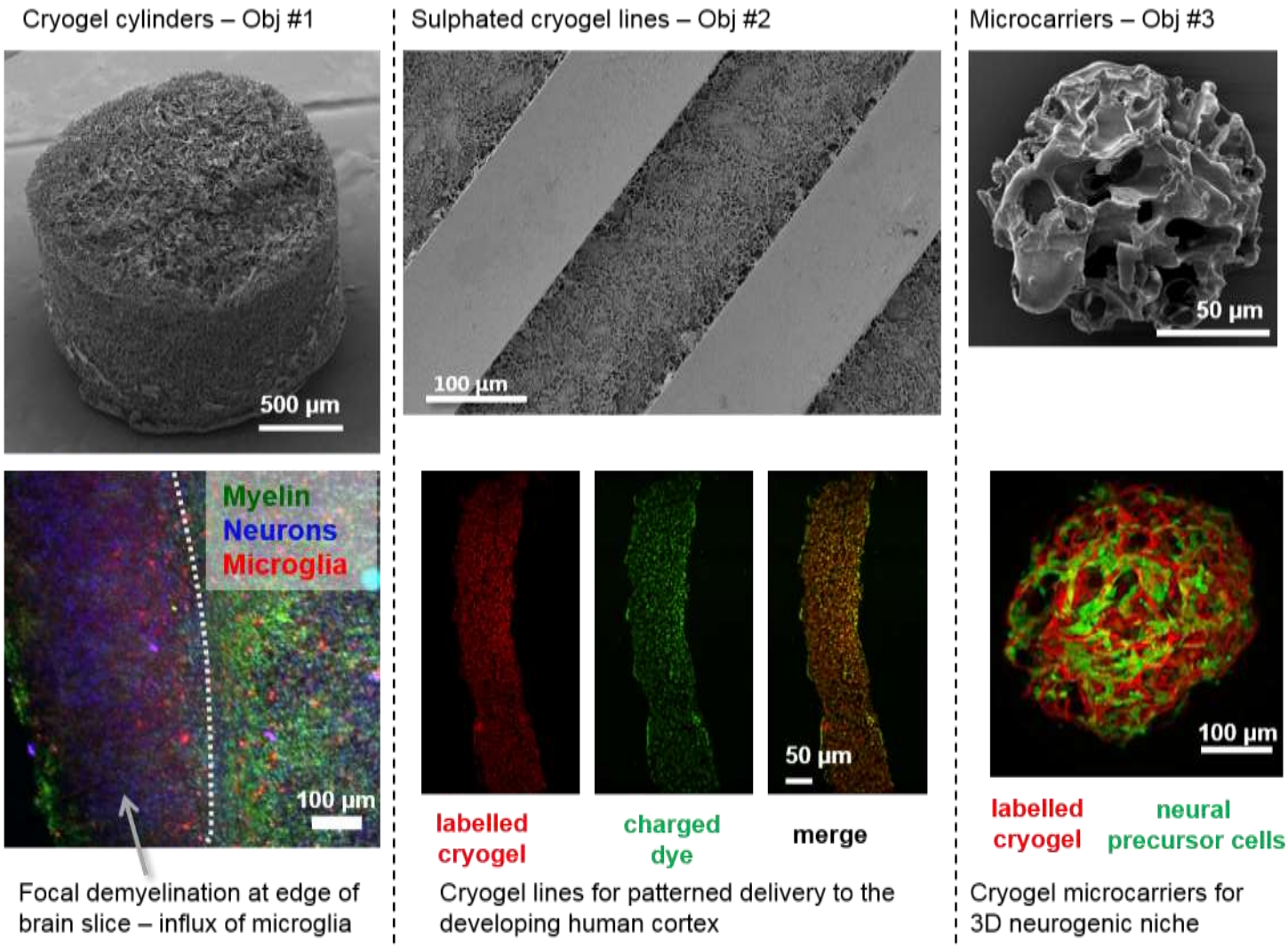

Figure 1 – The cryogel toolbox for applications in tissue engineering.

3D scaffolds for models & manipulation 16:00 - 17:30 Room 0.4 11/09/2018

Oral presentation

105 Development of liver organoids in 3D porous polysaccharide scaffolds

Marie-Noelle Labour, Teresa Simon-Yarza, Didier Letourneur
Inserm U1148, Institut Galilée, Université Paris 13, Paris, France

INTRODUCTION:

The liver is one of the biggest organ in the body and present vital functions. Many affections including hepatitis, cancer or genetic diseases induce liver fibrosis followed by cirrhosis which is irreversible and often lead to liver

failure associated with poor prognostic. Currently, allografts are the gold standard to treat acute liver failure. However, due to the lack of donors, tissue engineering strategies represent a promising therapeutic route for liver diseases[1-2]. Some attempts have been investigated but with limited success due to a lack of viability or functionality of the implants. The aim of this study is to produce a 3D porous scaffold to develop functional hepatic organoids that will then be associated with a bile and vascular network to form a functional artificial liver.

METHODS:

We used a combination of pullulan and dextran[3] with different porogen formulations to form matrices with various geometries and pore sizes. Structural properties were analysed by confocal microscopy and electron microscopy. Two cell lines were cultivated within the scaffold: the hepatocellular carcinoma cell line HepG2 and HepaRG which possess similar properties as primary hepatocytes. Viability and proliferation was analysed as well as the shape, sizes and distribution of cell organoids.

RESULTS AND DISCUSSION:

Polysaccharide scaffolds are rapid to prepare, easy to handle and allow confocal observations thanks to their transparency. The scaffolds porosities ranged from 30 µm to several hundreds of microns and pore geometries are characteristic of the porogen type and quantity. The seeding method allowed a rapid impregnation of the scaffolds with the cell suspension and a homogeneous distribution of the cells within the scaffolds. HepG2 cells formed characteristic aggregates within the pores of all types of scaffolds. The size and shape of cell aggregates reflects pore geometries and cell viability and proliferation was maintained over 7 days. HepaRG cells forms dense organoids that can be maintained over three weeks.

CONCLUSION:

Overall these results present the formulation and characterisation of 3D scaffold with controllable pore geometry and size. Hepatic cells incorporated within the pores form aggregates, remain viable and maintain their proliferative capacity. The *in vitro* functionality of hepatocytes in 3D is currently under investigation.

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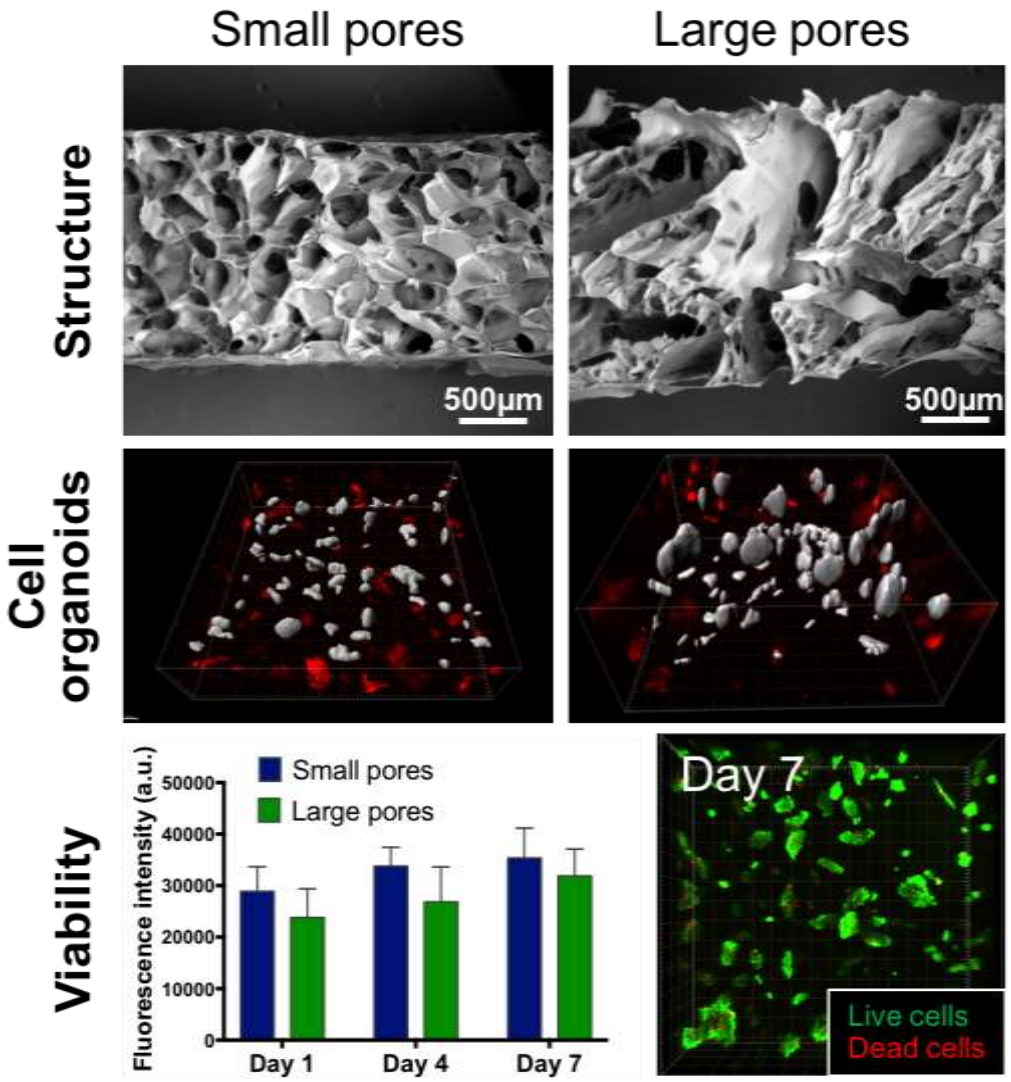
ACKNOWLEDGMENTS:

We acknowledge RHU lLite ANR-16-RHUS-0005 for financial support.



Picture 1: Caption 1: Effect of scaffold porosity on organoids size, shape and cell viability

3D scaffolds for models & manipulation 16:00 - 17:30 Room 0.4 11/09/2018

Oral presentation

114 Regulatory Macrophages Control Fibroblast Behavior in a 3D in vitro Model of the Wound Resolution Phase

Franziska Ullm, Jiranuwat Sapudom, Katja Franke, Tilo Pompe
Universität Leipzig, Leipzig, Germany

INTRODUCTION:

Impaired tissue repair can be attributed to a dysfunction in cell-cell or cell-matrix interactions during wound healing. The mechanism, which clears sustained inflammation to avoid overshooting scar formation and tissue fibrosis, is not well understood so far. *In vitro* models are highly appropriate to mimic physiologically relevant conditions like 3D microstructure, composition of the extracellular matrix (ECM) and mechanics of an *in vivo* tissue. In this study we

develop and test an *in vitro* co-culture model of the wound resolution phase using primary human fibroblasts (FB) and macrophages (Mϕ).

METHODS:

Our model system is based on fibrillary 3D matrices reconstituted from collagen I. Pore size and network stiffness are adjusted through reconstitution conditions and optional modifications by subsequent cross-linking or functionalization with other ECM molecules, e.g. glycosaminoglycans (GAGs) or fibronectin. Primary human dermal FB and human monocyte-derived Mϕ were studied in their response to these matrices in single cell type and co-culture studies *in vitro*. Differentiation of FB into myofibroblasts (MyoFB) was ascertained by gene expression analysis and staining of the TGF-β1 downstream target Smad2/3 and visualization of αSMA incorporation into the actin cytoskeleton. Identification of Mϕ in co-culture was achieved by visualization of the monocyte-specific cell surface marker CD14.

RESULTS AND DISCUSSION:

Under global TGF-β1 presentation FB differentiated into matrix-producing, contractile and less invasive MyoFB. Differentiated MyoFB were further stimulated with IL-10 to mimic the influence of IL-10 releasing immunomodulatory Mϕ in the wound resolution phase. While removal of TGF-β1 from differentiated MyoFB led to increased cell death, IL-10 treatment without TGF-β1 presence led to dedifferentiation of MyoFB into FB with marginal cell death nicely mimicking regulation of fibroblast function to homeostatic conditions at the end of wound repair[1].

Co-culture experiments with both cell types, FB and IL-10-releasing regulatory M2-Mϕ in these 3D matrices revealed a direct interaction of FB and Mϕ with a dose-dependent regulation of FB differentiation. Under systemic delivery of TGF-β1, proliferation of FB increased while TGF-β1 dependent differentiation into MyoFB decreased with the amount of co-cultivated Mϕ.

CONCLUSION:

We have nicely shown that our 3D collagen I based matrices resemble a biomimetic model of the wound resolution phase with a control of FB proliferation and differentiation by Mϕ in co-culture.

REFERENCES:

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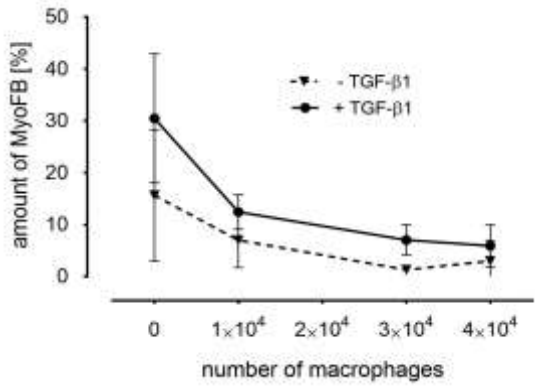
ACKNOWLEDGMENTS:

This study was supported by Deutsche Forschungsgemeinschaft grant (SFB/TRR67 project B10). The authors would like to thank Ronald Weiß and Sunna Hauschildt as well as Ulf Anderegg (Universitätsklinikum Leipzig) for support with cells.



Picture 1: Caption 1: Fig.1: Dose-dependent regulation of FB differentiation under systemic delivery of TGF-β1.

3D scaffolds for models & manipulation 16:00 - 17:30 Room 0.4 11/09/2018

Oral presentation

240 Development of a three-dimensional collagen model for the in vitro evaluation of osteogenesis

Rebecca Zhiyu Yuan[1], Robert Allaker[1], Kaveh Memarzadeh[2], Abish Stephen[1], Jie Huang[3]
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[2]Orthopedic research uk, United Kingdom
[3]Mechanical Engineering, University College London, United Kingdom

INTRODUCTION:

Magnetic stimulation can be applied to enhance bone regeneration and non-union fracture treatment; however the cellular and molecular mechanisms of repair require better understanding. 3D *in vitro* models have been developed to understand the process and can be used to investigate the effects of magnetic stimulation on osteogenesis[1]. Although collagen hydrogels have an established track record as potential 3D models for tissue regeneration applications, conventional collagen hydrogels are low in collagen density with large excesses of fluid. This results in poor mechanical properties as well as the lack of orientated architecture. Plastic compression (PC) was developed with dense, cellular and mechanically stronger native structures[2]. In this study, osteoblasts and magnetic iron oxide nanoparticles (IONPs) were incorporated into PC collagen gels to produce a range of cell-laden models. A magnetic bio-reactor to support cell growth under static magnetic fields (SMFs) was designed and fabricated by 3D printing. The influences of SMFs on cell proliferation, differentiation, mineralization and gene expression was then evaluated.

METHODS:

The plastic compression protocol was modified from a study of Brown et al [2] with IONPs embedded inside the collagen matrix at the point of self-assembly. MG-63 cells (Homo sapiens, osteosarcoma, ATCC) were seeded within the PC collagen gel at a density of 10,000 cells/ml. A magnetic bio-reactor was designed by ANSYS Maxwell and 3D printed. Alamar blue (AB) assay was used to measure cell proliferation, alkaline phosphatase (ALP) was used to investigate cell differentiation, alizarin red s (ARS) staining and extraction was employed to study mineralization and polymerase chain reaction (PCR) was conducted to evaluate gene expression of Runx2, BMP-2 and BMP-4. TEM and histology were used to investigate the microstructure of the 3D model. Statistical analysis was carried out by analysis of variance to determine the presence of any significant differences between groups.

RESULTS AND DISCUSSION:

Results (as shown in Figure 1) demonstrated that SMFs and IONPs can stimulate the expression of Runx-2, BMP-2 and BMP-4 genes in collagen matrix ($p<0.01$; day 14), hence accelerating cell proliferation ($p<0.0001$; day 14) and differentiation ($p<0.0001$; day 21). Histology and TEM images demonstrated the microstructure of the cell-laden 3D model, with fibrous, lamella collagen structure and healthy attached cells. Results from this study indicate that a combination SMFs and IONPs can enhance the osteogenesis process of MG-63 cells when embedded in 3D collagen matrix without unwanted inflammatory response.

CONCLUSION:

Plastic compressed collagen hydrogels were used as a 3D bio-mimetic model with dense, cellular and mechanically strong native structures to study the effects of magnetic stimulation on osteogenesis; which paves the way for further applications in tissue engineering and regenerative medicine.

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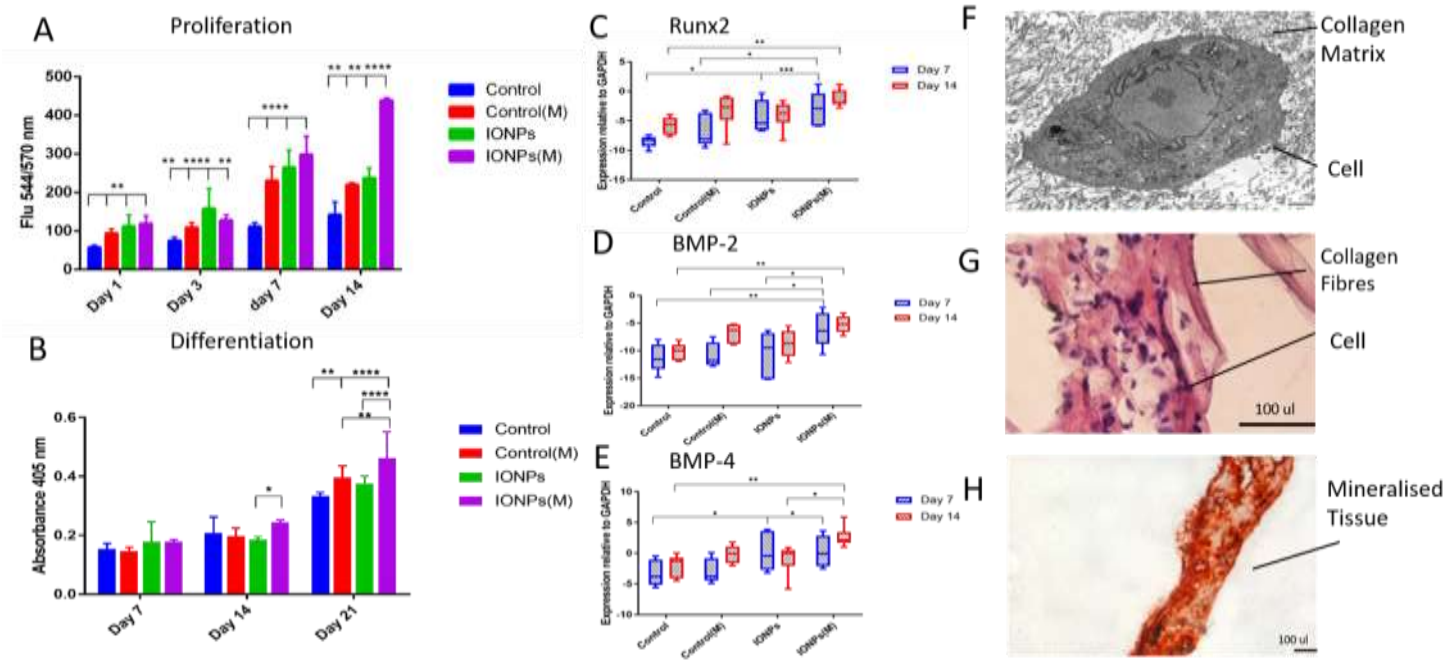

Figure 1. A comparison of the effect of SMFs and IONPs on (A) cell proliferation and (B) cell differentiation. SMFs and IONPs can stimulate the cell proliferation and differentiation after 14 and 21 days, respectively. (C) (D) and (E) demonstrate the expression of Runx-2, BMP-2 and BMP-4 with/without IONPs, and with/without magnetic fields, respectively. (F) Microstructure of cells incorporated in the 3D model. (G) Histology of MG-63 cells in collagen scaffold, with fibrous, lamella collagen structure and healthy attached cells shown. (H) ARS staining of cells embedded in the PC hydrogel, indicating the level of mineralisation. (n=3, *p< 0.05, **p< 0.01, ***p< 0.001, ****p< 0.0001.)

Picture 1: Caption 1: Figure 1. A comparison of the effects of SMFs and IONPs on osteogenesis of osteoblasts.

3D scaffolds for models & manipulation 16:00 - 17:30 Room 0.4 11/09/2018

Oral presentation

621 Three-Dimensional (3D) Chitosan Scaffold to Mimic Breast Cancer Microenvironment

Sultan Gulce-Iz, Muge Anil-Inevi, Pelin Saglam-Metiner, Evrim Ceren Kabak, Aylin Sendemir-Urkmez
Ege University, Izmir, Turkey

INTRODUCTION:

A subset of cancer cells called as “circulating tumor cells” (CTCs) having a key role in cancer metastasis and able to escape immune system, have become widespread issue in recent years. Previously, epithelial cell adhesion molecule (EpCAM)[1] and human epidermal growth factor receptor 2 (Her2)[2] have been reported as proper targets for isolation of CTCs in peripheral blood of metastatic breast cancer patients. However, there are several pitfalls for usage of these markers alone. Thus, it is important to study cancer cells that overexpress both of these markers to have an aggressive tumor microenvironment. Chitosan, previously reported to provide a better model for the evaluation of the drug cytotoxicity[3], was employed as a 3-dimensional (3D) scaffold for EpCAM(+)/Her2(+) CTC subsets to mimic *in vivo* tumor microenvironment.

METHODS:

EpCAM(+), Her2(+) and EpCAM(+)/Her2(+) cells were isolated from MDA-MB 453 breast cancer cell line by MACS®. All subsets and MDA-MB 453 cells as control were characterized with regard to the growth and attachment characteristics, ability of sphere forming. Chitosan scaffolds were prepared using the freezing and lyophilizing method and used for 3D cultures. 2D and 3D cultured cells were observed by SEM (Scanning Electron Microscopy) (Fig1) and mRNA expression of pluripotency genes (Sox2, Oct-3/4, Nanog and Nestin) was evaluated by qPCR. Finally, the chemotherapeutic agent Doxorubicin was tested both in 2D and 3D cultures.

RESULTS AND DISCUSSION:

Her2(+) and EpCAM(+)/Her2(+) cells had higher proliferation rate and low tendency to attach to the surface than MDA-MB 453 cells. 3D cultured CTCs hag higher expression of pluripotency genes than 2D cultures. Moreover, Sox2 and Oct-3/4 expression levels of CTCs were higher than MDA-MB 453 cells. The over expression of Sox2 and Oct-3/4 may indicate their contribution in metastasis and thus 3D models may provide more convenient microenvironment to study nature of these aggressive subsets. After doxorubicin treatment, 3D cultured EpCAM(+)/Her2(+) cells showed a higher viability than 2D.

CONCLUSION:

EpCAM(+)/Her2(+) CTC subsets might be used for an aggressive breast cancer tumor model *in vitro*. Using 3D chitosan scaffold mimics *in vitro* breast cancer microenvironment that is a promising model for investigation of new anti-cancer therapy strategies against CTCs.

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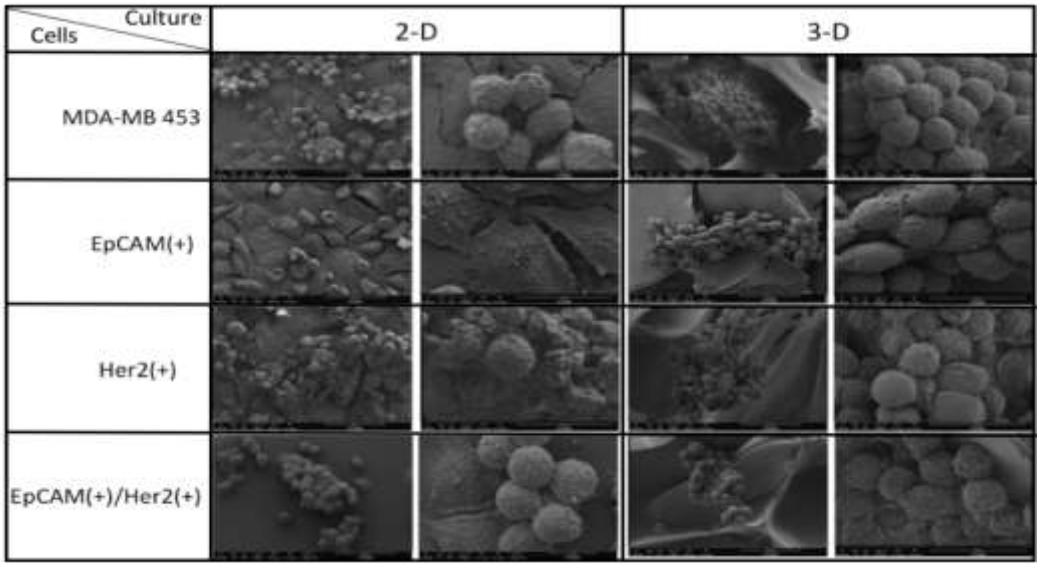
ACKNOWLEDGMENTS:

This study was funded by 'Ege University, Directorate of Administrative and Financial Affairs by a grant number 15-MUH-038 given to Sultan GULCE-IZ.



Picture 1: Caption 1: SEM analysis of 2D and 3D culture of breast cancer cells

3D scaffolds for models & manipulation 16:00 - 17:30 Room 0.4 11/09/2018

Oral presentation

796 Biofunctional Microparticles for Assembly of Tumor-Stem Cell Hybrid 3D In Vitro Models

Vítor Gaspar[1], Vítor Gaspar[2], João Mano[2]
[1]Universidade de Aveiro, Aveiro, Portugal
[2]University of Aveiro, CICECO - Aveiro Institute of Materials, Portugal

INTRODUCTION:

3D multicellular tumor spheroids (3D-MCTS) that mimic the tumor microenvironment in vitro are gaining increased interest as platforms for screening innovative anti-cancer therapeutics. Herein we engineered 3D-MCTS that mimic the existence of tumor stromal and ECM components by combining, for the first time, heterotypic Fibroblasts/MSCs/A549 cancer cells triple co-cultures with bioinstructive hyaluronan-functionalized microparticles.

METHODS:

Bioinstructive microparticles production via Layer-by-Layer

PCL microparticles where produced by using the oil-in-water (O1/W1) emulsion-solvent evaporation technique. Prior to surface functionalization microparticles were subjected to plasma treatment. For Layer-by-layer (LbL) surface functionalization plasma treated PCL MPs (LbL-MPs) w were immersed in Poly(L-lysine) and washed in distilled water. For the buildup of the negative layer, PLL-MPs were transferred into an Hyaluronan solution and re-washed. This process was repeated 3 times to allow the formation of 3 PLL-HyA bilayers.

3D ***In vitro*** **lung tumor model's assembly via Liquid-Overlay Technique**

Homotypic monoculture 3D-MCTS with A549 cells, heterotypic co-culture spheroids: A549-HF and A549-MSCs cells, or tricultures: A549-HF-MSCs cells, were self-aggregated at different cell ratios, by using the liquid-overlay technique in low-adhesion U-bottom 96-wells plates (Corning).

RESULTS AND DISCUSSION:

Preliminary optimization of spheroid cultures was performed using mono and dual coculture spheroids of A549 and HF, formed by 5, 10, 15, 30 and 45 thousand cells and with varying quantities of LbL-MPs. Analysis of size variations in mono, dual and triculture coculture 3D-MCTS revealed distinct patterns of growth and contraction resultant from the inclusion tumor microenviroment associated populations. Monoculture spheroids of A549 presented the largest areas and slowest contraction ratios of the four tested coculture conditions. Live/Dead analysis of more complex A549-HF-MSCs triculture 3D-MCTS, with and without LbL-MPs, revealed the establishment of well-defined necrotic regions at 7 days of culture (Figure 1). Anti-cancer drug screening assays revealed that The Inclusion of LbL-MPs into 3D-MCTS lead to the observation of significant differences regarding resistance profiles when compared to control 3D-MCTS.

CONCLUSION:

Overall Hybrid 3D-MCTS with LbL-MPs serve as effectively tool for tackling one of the main flaws of spheroids-based models, i.e, the integration of pre-existing ECM derived components and for drug screening.

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ACKNOWLEDGMENTS:

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Combining biomaterials with microfluidics 10:30 - 12:00 Room 0.5 11/09/2018

Oral presentation

491 Development and Assessment of Microfluidic Platform for 3D Bone Cell Culture and Drug Evaluation

Hossein Bahmaee, Gwendolen Reilly, Cecile Perrault, Frederik Claeyssens
University of Sheffield, Sheffield, United Kingdom

INTRODUCTION:

This paper presents a novel bone tissue engineering system that combines a microfluidic bioreactor with a microscale-porosity, polymerised High Internal Phase Emulsion (polyHIPE)-based scaffold. Bone regeneration is a well-organized but complex physiological process that involves synergistic chemical and mechanical stimulation [1]. Our system combines for the first time high precision manufacturing of a porous, biocompatible PolyHIPE scaffolds via laser photo-polymerisation witha microfluidic bioreactor that can control the mechanical and chemical stimulation. The system permits monitoring of cell viability and differentiation over long time periods, enables computational studies of the environment, and provides a platform to evaluate drugs used to treat bone disorders.

METHODS:

The mold for the scaffold and microfluidic chamber were created via laser photocuring of polyethylene glycol diacrylate (PEG-DA). Scaffolds were produced by casting 80% porosity 2-ethylhexyl acrylate/isobornyl acrylate blend polyHIPEs and curing under UV light. Scaffolds were sealed inside microfluidic chambers via air plasma binding. Human embryonic stem cell-derived mesenchymal progenitors (hES-MPs) were cultured on scaffolds for up to three weeks in both static and continuous and intermittent dynamic conditions. MTT, resazurin reduction and DNA assays assessed cell viability and number, and alkaline phosphatase activity (ALP), Alizarin Red S (ARS, calcium), and Direct red 80 (DR80 collagen)) staining measured osteogenic differentiation. Finally, effects anabolic bone drugs (Lactoferrin, Icariin) on bone cells were tested under intermittent flow and compared to the static condition to assess the chips suitability for *in vitro*.

RESULTS AND DISCUSSION:

Viability assays confirmed cell proliferation in the chip, and MTT displayed a confined growth of cells in the PolyHIPE channels in the dynamic condition. Normalized ALP activity and ARS and DR80 staining indicated the effect of different flow regimes on osteogenesis, with increased osteogenic differentiation and bone-matrix deposition was observed under intermittent flow. These results permit a better understanding of the mechanical and biochemical cues associated with osteogenesis and demonstrate the potential of a more advanced *in vitro* system.

CONCLUSION:

We successfully created a microfluidic bioreactor platform for bone tissue engineering. The precise geometry of the chamber and scaffold enables the computational study of fluid flow. Short and long-term studies of hES-MPs showed significant positive results for osteogenesis on the chip with intermittent flow. Therefore, long-term studies of hES-MP cell lines treated with therapeutic reagents will provide us more accurate, cheaper, and easier methods to investigate drug production process *in-vitro*.

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Combining biomaterials with microfluidics 10:30 - 12:00 Room 0.5 11/09/2018

Oral presentation

148 Production of biomaterials for bone regeneration using droplet microfluidics

Víctor P Galván, David Barata, Pamela Habibovic
MERLN Institute - Maastricht University, Maastricht, Netherlands

INTRODUCTION:

Affordable and therapeutically effective biomaterials are required for successful treatment of orthopaedic critical-size bone defects. Calcium phosphate (CaP) ceramics are widely used for bone repair and regeneration, but there is still much room for improvement when it comes to their properties and biological performance. To improve the existing CaP bone graft substitutes, novel engineering approaches are needed that provide a fine control of the mineralization process, high chemical yields and versatility in the delivery format. In our work, a microfluidic strategy for production of CaP microparticles derived from highly monodisperse droplets is proposed for the controlled synthesis of bioactive ceramic materials.

METHODS:

Based on computational fluids dynamics simulations, a PDMS microfluidic chip able to produce monodisperse droplet emulsions through flow focusing was fabricated. The CaP precursor solutions and the oil phase were introduced through independent inflows, resulting in a downstream droplet formation after flow crossing. The analysis of the synthesized materials was performed using optical and scanning electron microscopy (SEM), X-Ray Diffraction (XRD), Energy Dispersive Spectroscopy (EDS) and Fourier transform infrared spectroscopy (FTIR).

RESULTS AND DISCUSSION:

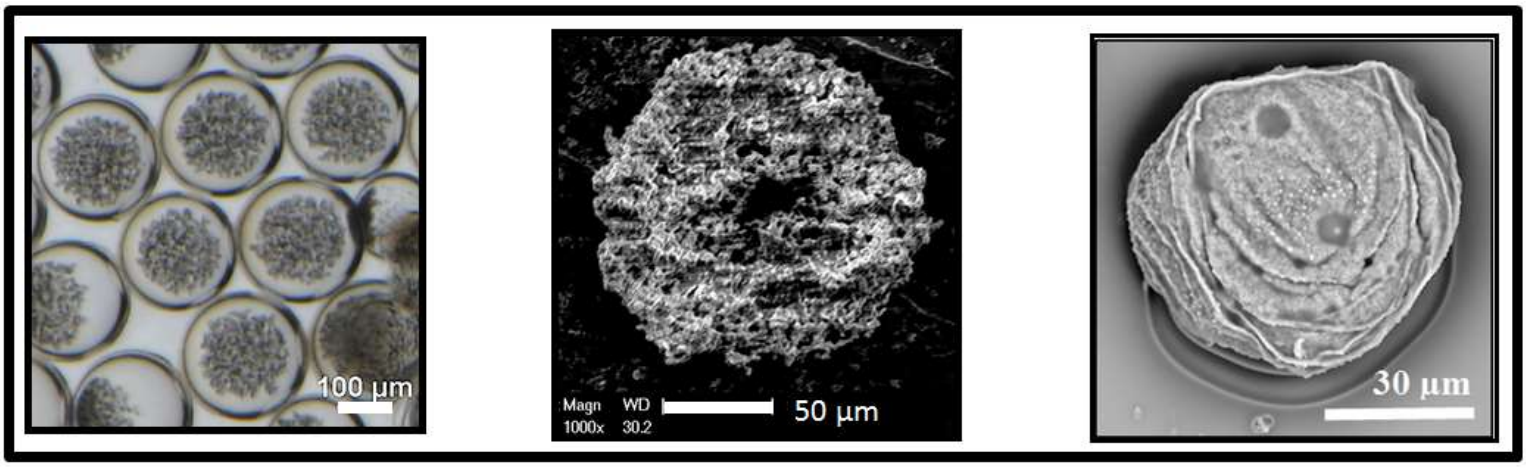
Highly monodisperse droplets containing CaP solution were produced using droplet microfluidics. After collection, ammonia solution was added to increase the pH and induce the mineralization reaction (Fig. 1 left). Eventually, the droplets collapsed, liberating the particles (Fig.1 center). After collection and sintering, the droplets were disrupted, exposing mineral particles which were homogenous in size (Fig. 1 right). The EDS analysis revealed the presence of Ca and P on the sintered ceramic particles. XRD patterns showed that the droplets were initially composed of brushite, that transformed into β-TCP after the sintering process. The FTIR spectra of the brushite and β-TCP particles showed the bands characteristic of phosphate groups.

CONCLUSION:

We successfully produced brushite and β-TCP microparticles using a droplets-based microfluidic device. This result shows that droplet microfluidics is a powerful tool for the production of ceramic microparticles with highly homogeneous properties. This method can potentially be used to produce new ceramic materials and gain deeper insight into their biological performance.

ACKNOWLEDGMENTS:

This research has been in part made possible with the support of the Dutch Province of Limburg. PH acknowledges financial support by Innovative Research Incentives Scheme Vidi (#15604) of the Netherlands Organization for Scientific Research (NWO).



Picture 1: Caption 1: Fig. 1: Left) Mineral precipitates inside the droplets. Center) SEM image of a brushite particle. Right) SEM image of a β-TCP particle.

Combining biomaterials with microfluidics 10:30 - 12:00 Room 0.5 11/09/2018

Oral presentation

637 From low- to high-throughput: Microfluidic fabrication of novel 3D biomaterial libraries for screening cell responses

Carlos Guimarães, Luca Gasperini, Raquel Ribeiro, Andreia Carvalho, Alexandra Marques, Rui Reis
3B's Research Group, Guimarães, Portugal

INTRODUCTION:

Platforms that allow high-throughput (HT) screening of cell responses to wide ranges of biomaterial modifications are a primal challenge within Tissue Engineering. To tackle this issue, we used a natural polymer to fabricate 3D hydrogel fibers built in a gradient fashion where encapsulated cells can sense their varying 3-dimensional surroundings. In the proposed platform, by imaging one single cell-laden gradient, it is possible to pinpoint the most appropriate condition for a desired cellular event.

METHODS:

As a low-throughput approach, 0.5 wt% and 1 wt% gellan gum (GG) solutions were developed and mixed in varying ratios and their post-gelation mechanical properties were evaluated. Simultaneously, fibers composed of a gradient-like transition from 0.5% to 1% solutions were developed through a custom microfluidic circuit (figA). Cell-laden fibers of 0.5% and 1% GG were fabricated as control. Adipose stem cells (ASCs) were encapsulated in the developed hydrogel systems and subjected to chondrogenic stimulus. In addition to cell viability, the effect of the composition and consequently of the mechanical properties of the hydrogels on triggering chondrogenic differentiation were assessed through fluorescent imaging and analysis (figE) [1].

RESULTS AND DISCUSSION:

The mechanical analysis on hydrogels revealed a significant increase on the storage modulus of the 0.5-1% GG hydrogels, from 5 to 20 kPa. These values were slightly lower upon the introduction of ASCs but still within the physiological range[2]. A higher percentage of viable cells was observed in the fibers, independently of the concentration of polymer, which reinforces the improved diffusion properties of the proposed HT platform in relation to the low-throughput approach. Additionally, the optical properties of fibers allowed clear microscopic analysis (figB-D) thus supporting the accuracy of the platform. The triggering of the chondrogenic differentiation, as detected by the

levels of Sox9 expression at early timepoints, varied among the different hydrogel systems evidencing the effect of the 3D mechanical stimuli without the need of direct cell-cell interactions. As such, the HT platform was validated by confirming the distinguished triggering of ASCs chondrogenic differentiation throughout the 0.5-1% polymer concentration spectrum.

CONCLUSION:

We have created a novel approach to develop simple-yet-integrative 3D biomaterial libraries through spatial variation of hydrogel composition, allowing HT screening complex cellular events. In the future, different cues and materials will be integrated in these platforms, generating increasing amounts of information as we move forward in library complexity.

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ACKNOWLEDGMENTS:

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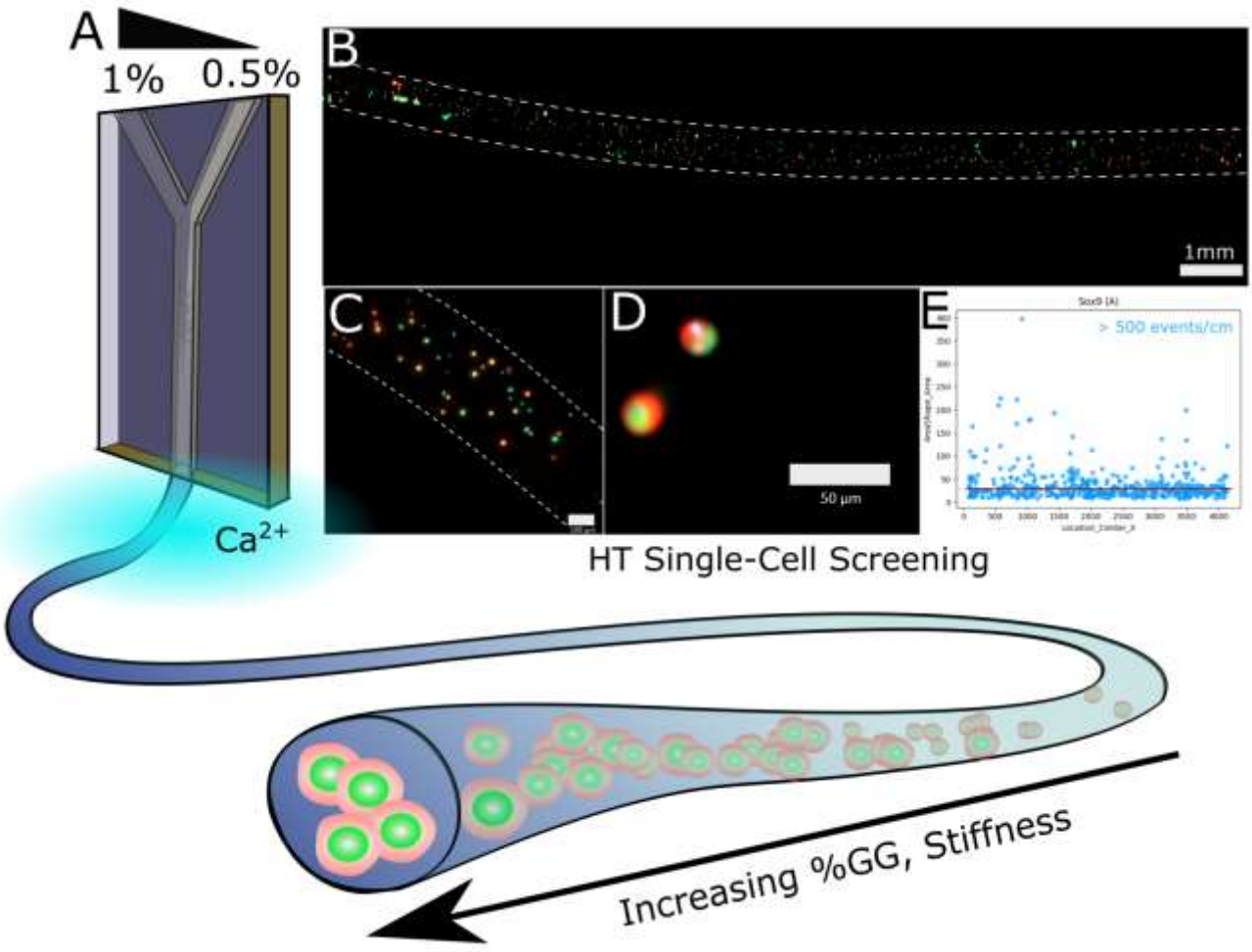

Picture 1: Caption 1: Microfluidic fabrication of a cell-biomaterial library (A), fluorescent imaging (B-D, red-Phall., green-Sox9), cell-by-cell marker analysis (E).

Combining biomaterials with microfluidics 10:30 - 12:00 Room 0.5 11/09/2018

Oral presentation

233 Microengineering a curved alveoli-on-chip

Danielle Baptista, Liliana Teixeira, David Barata, Pamela Habibovic, Stefan Giselbrecht, Roman Truckenmuller

MERLN Institute, Maastricht, Netherlands

INTRODUCTION:

The vast majority of *in vitro* lung models are still based on 2D cell culture, which does not reflect the native, curved microanatomy of the lung. Amongst the few processes that allow the controlled fabrication of curved cell culture substrates, microthermoforming [1] has become highly attractive as a simple and yet powerful technology which can fabricate features at the cell-scale level. We hypothesize that substrate curvature is essential in establishing and maintaining functional alveolar tissue *in vitro.* Here, we describe an innovative microfluidic model of the alveoli, also described as alveoli-on-chip, which integrates a microengineered biomimetically curved membrane.

METHODS:

Arrays of uniform hemispherical microwells were fabricated by a combination of microthermoforming and ion track-etching of polycarbonate films [2]. A two-part microfluidic chip from polydimethylsiloxane was fabricated with two perfusable compartments in a central chamber separated by the thermoformed porous film/membrane once bonded between the housing halves (Fig. 1). Lung epithelial cells were seeded on the concave side of the microwells and lung microvascular cells on the opposite side of the membranes. Cell behaviour was then characterized with regard to cell density, proliferation, apoptosis and barrier function, comparing dynamic (perfused) and static cell culture.

RESULTS AND DISCUSSION:

Cells were cultured for up to three weeks and were able to line the curved walls of the microwells, thereby creating and epithelial-endothelial interface. The behaviour of both cells types was found to be influenced by curvature. Tight junctions, as a pre-requisite for a functional barrier, could be observed between epithelial cells. The expression of CD31 indicated that microvascular cells preserved their phenotype over time. In general, cells on curved membranes were observed to arrange in higher densities compared to cells on flat membranes and showed differences in rates of proliferation and apoptosis.

CONCLUSION:

In this study, we successfully analysed the influence of substrate curvature on alveolar cell behaviour, more specifically regarding cell packing density, proliferation and apoptosis. Further studies will focus on analysing respective implications on alveolar barrier function and regeneration on-chip. The microengineered curved alveolar membrane-on-chip described above is expected to become a valuable tool as an advanced 3D lung model for recapitulation of regenerative strategies, drug screening and adjustment of personalized treatments.

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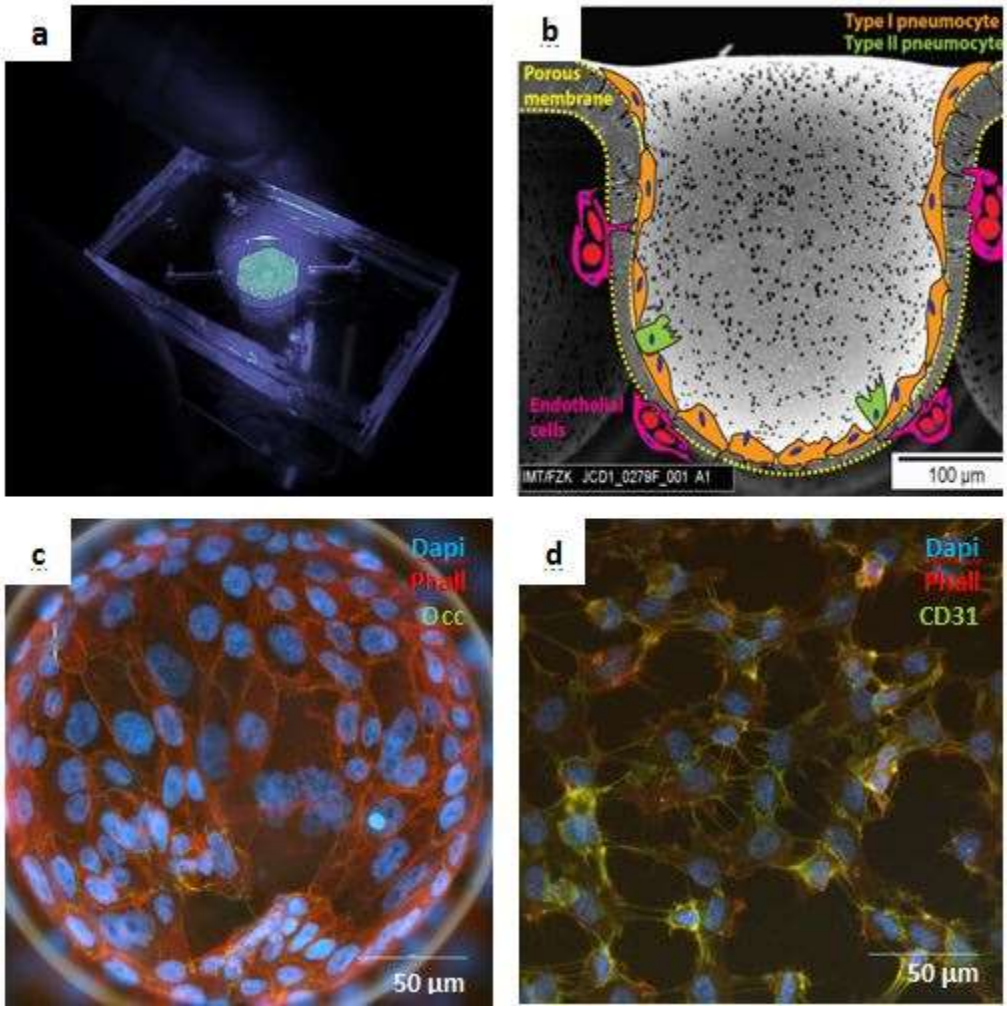

Picture 1: Caption 1: 1 (a) Alveoli-on-chip. (b) Biomimetically curved membrane where (c) epithelial and (d) endothelial cells line both sides forming the interface.

Combining biomaterials with microfluidics 10:30 - 12:00 Room 0.5 11/09/2018

Oral presentation

322 Tuneable collagen microgels for regenerative medicine

Jose Manuel Rey[1], Abhay Pandit[2], Grahame Busby[3], Manuel Salmerón-Sánchez[4], Cristina González-García[4]
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[2]Centre for Research in Medical Devices, National University of Ireland Galway, Ireland
[3]Collagen Solutions PLC., Nova Business Park, United Kingdom
[4]Division of Biomedical Engineering, School of Engineering, University of Glasgow, United Kingdom

INTRODUCTION:

During recent years, microgels have emerged as an effective type of drug delivery system (DDS), showing advantages such as tuneable size, increased surface area and injectability[1]. Collagen has been extensively studied as a scaffold for regenerative medicine and drug delivery due to its biocompatibility, non-immunogenicity and degradability[2]. The current study focuses on the use of microfluidic techniques for the automated generation of monodisperse type-I collagen (col-I) microgels crosslinked with PEG-4S, encapsulating hollow collagen spheres with

glial-derived neurotrophic factor (GDNF) and bone morphogenetic protein 2 (BMP-2) for regenerative therapies in Parkinson's Disease and bone repair, respectively.

METHODS:

Col-I (Collagen Solutions, UK) stock was characterized in a viscometer and UV/Vis spectrophotometer to determine the fibrillogenesis conditions. Collagen was crosslinked with PEG-4S succinimide glutarate (Jenkem, USA) to form hydrogels. Crosslinking occurred after merging col-I with PEG-4S crosslinker in the nozzle of a double-chamber capillary within an oil flow, and complete gelification took place in a coiled tube connected to the device. Microgel size and mechanical properties were controlled through flow rates, pH and concentration of collagen/crosslinker. Microgels were characterized with TNBS assay and their cytotoxicity with LIVE/DEAD® (Neu-7 astrocytes, n=3). Hollow spheres were prepared by covalently attaching collagen to silica templates, that are later dissolved with hydrofluoric acid. Morphochemical characterization included: scanning electron microscopy, dynamic light scattering and FTIR spectroscopy. Hollow spheres were loaded with GDNF and BMP-2 by diffusion, seeded with different cell-types and encapsulated in the microgels within the microfluidic device.

RESULTS AND DISCUSSION:

Microgels with different size and stiffness were successfully synthetized in a glass microfluidic device. The microgels are non-cytotoxic to cells (fig.1C) and foster cell growth at different crosslinker concentrations. In the synthesis of hollow spheres, no harmful by-products appeared, and their size ranged around 200 nm (fig.1D). Microfluidic-generated hydrogels encapsulating hollow spheres can therefore be used for sustained delivery of GDNF and BMP-2.

CONCLUSION:

We demonstrate that microfluidics is an adequate technique for automatically generating monodisperse collagen microgels and provides a simple and useful tool for the posterior encapsulation of nanospheres and cells. The combination of microgels encapsulating hollow spheres is expected to provide a controlled and sustained delivery system of different therapeutic factors.

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ACKNOWLEDGMENTS:

The authors would like to thank European Union H2020 Programme (H2020-MSCA-ITN-2015) and Grant No. 676408 for providing financial support to this project.

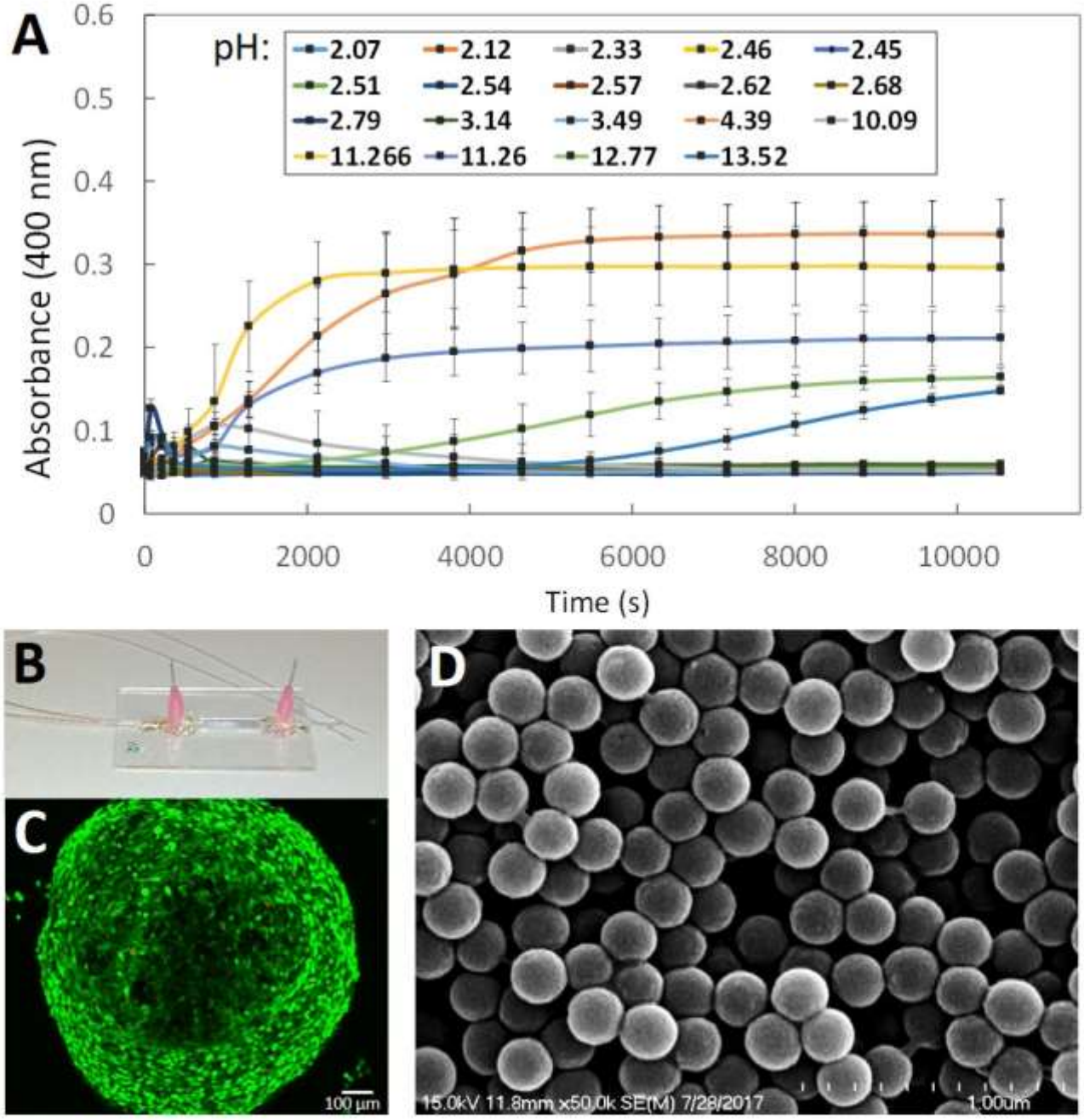

Figure 1. **A**. Effect of pH in collagen fibre formation as measured by UV-Vis absorption. **B.** Glass microfluidic device. **C.** LIVE-DEAD ® assay in collagen type-I microgel. **D.** SEM images of collagen hollow spheres.

Picture 1: Caption 1: A. Effect of pH in collagen fibre formation in UV-Vis absorption. B. Microfluidic device. C. LIVE-DEAD® in microgels. D. SEM image of microspheres

Combining biomaterials with microfluidics 10:30 - 12:00 Room 0.5 11/09/2018

Oral presentation

49 Microfluidic manipulation of biomaterials' mechanical properties by thermophoresis

Alexandros Kosmidis, Daniele Vigolo
University of Birmingham, Birmingham, United Kingdom

INTRODUCTION:

The optimisation of cell-material interactions is essential in the process of wound healing and tissue regeneration. Particularly, biomaterials exhibiting a gradient of mechanical properties can be used to control cell behaviour which is actually dependent upon the elasticity of the substrate [1,2]. However, the possibility to create these materials is still limited especially at the micron scale. By carefully imposing and controlling temperature gradients [3,4] across a microfluidic channel and exploiting the phenomenon of thermophoresis [5,6], we demonstrated the possibility to fabricate biocompatible hydrogels based on Gellan gum presenting a gradient of mechanical properties along their width. Furthermore, we monitored cell behaviour over time by seeding osteoblasts MC3T3 on the surface of the biomaterial. We studied the cells evolution and evaluated their migration and proliferation along the gradient, which provided us with insights for the design of future 3D scaffolds that could potentially act as biocompatible implants.

METHODS:

The PDMS based microfluidic device consists of a main microchannel and two larger side channels used to impose the temperature gradient. Particularly, a low melting point alloy is used to create a Joule heater within one of the side channels [4,6] while we flow cold water as coolant on the other side channel. A Peltier module, placed below the glass base, regulates the average temperature of the device. The flow of gellan gum is then halted on the main channel to apply the temperature gradient. By using Atomic Force Microscopy we characterised the biomaterial by evaluating locally the Young's modulus across the 600 μm obtaining values of about 100 Pa/μm. In the end, we seeded osteoblasts MC3T3 on the surface of the biomaterial and studied their activity and mineralisation degree over time.

RESULTS AND DISCUSSION:

We demonstrate the fabrication of a biomaterial with a stiffness gradient along its width by using thermophoresis in microfluidic devices. Additionally, cell migration and mineralisation are observed on different stiffness areas, giving us valuable information for future applications such as patterned cell culture, wound healing implants etc.

CONCLUSION:

We demonstrate the feasibility to fabricate and accurately control concentration gradients hydrogels by thermophoresis and thus, effectively, customise the local Young's modulus of the biomaterial at the single-cell scale. Finally, we demonstrated the excellent biocompatibility of the material via live-dead essays.

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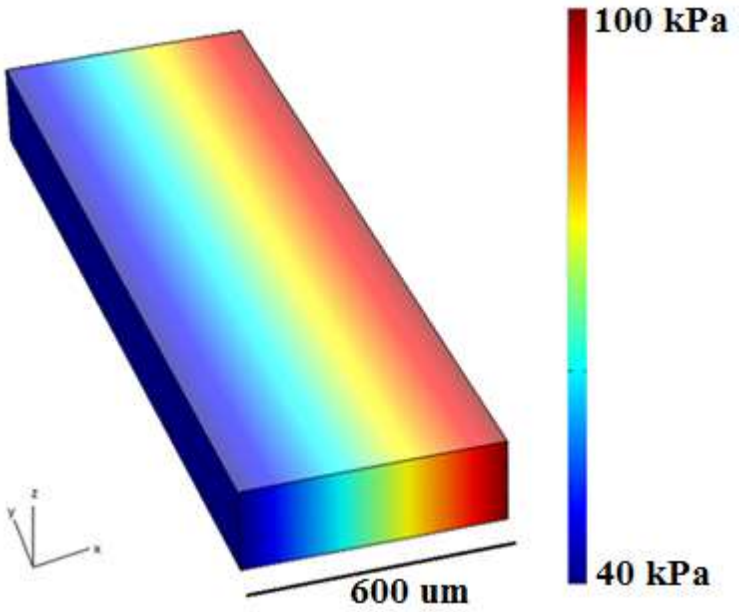

Picture 1: Caption 1: Figure 1: Sketch of the typical stiffness across the width of the biomaterial

Developments in applied biomaterials 14:00 - 15:30 Room 0.5 11/09/2018

Oral presentation

295 Electrical stimulation on titanium for enhancing stem cell osteogenesis

Juan Shong Khaw[1], Nigel Cassidy[2], Sarah Cartmell[1]
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[2]Department of Civil Engineering, University of Birmingham, United Kingdom

INTRODUCTION:

Electrical stimulation (ES) offers excellent control on cell growth, orientation, and intracellular calcium level. Electrical signals have the capability to induce intracellular calcium concentration which helps in bone remodelling and mineral deposition [1].

Capacitive coupling (CC) stimulation involves two parallel electrodes placed above and below, or left and right to the well plate, with no direct contact of electrodes to the culture medium, hence the effect of toxicity, pH changes and current fluctuation can be minimised. With appropriate electrical regime, homogenous electric field (EF) can be generated. This allows equal amount of stimulation for every cell regardless of the position in the culture vessel [2].

METHODS:

A customised CC stimulation bioreactor is designed and simulated using COMSOL Multiphysics. The EF in culture medium and titanium disc were also simulated to obtain an applied voltage for the actual stimulation.

Titanium discs (0.25 mm thick, 99.5% purity, Alfa Aesar) were sonicated in degreasing solution followed by sterilisation before conducting the experiments. The bioreactor was stimulated continuously with desired applied voltage for 14 days, under incubation at 37^{o}C & 5% CO_2, with medium change every 3 days. Human bone marrow derived mesenchymal stem cells lineage (passage 8) was employed in the experiments.

RESULTS AND DISCUSSION:

Homogenous EF was simulated across the culture medium and titanium disc. Simulated and actual experimental current were both zero. No visible effect on pH changes under this ES regime.

AlamarBlue assay suggested that the cell metabolism rate was higher under CC-ES as compared to non-stimulated discs. Live/Dead Imaging showed that hMSCs cultured on the discs were majority aligned in a direction and greatly stretched across the discs – either multiplying themselves, or differentiating into osteoblastic cells. Alkaline Phosphatase (ALP) assay revealed that the ALP activity was higher under CC-ES, denoting an early marker of bone mineralisation and osteogenic cell differentiation.

CONCLUSION:

Titanium itself has an excellent osseointegration property; while CC-ES offers long term stimulation with no harmful byproducts and pH changes to the media. The combination of titanium and CC-ES does not only help to improve proliferation and orientation, but also capable of enhancing differentiation and bone mineralisation.

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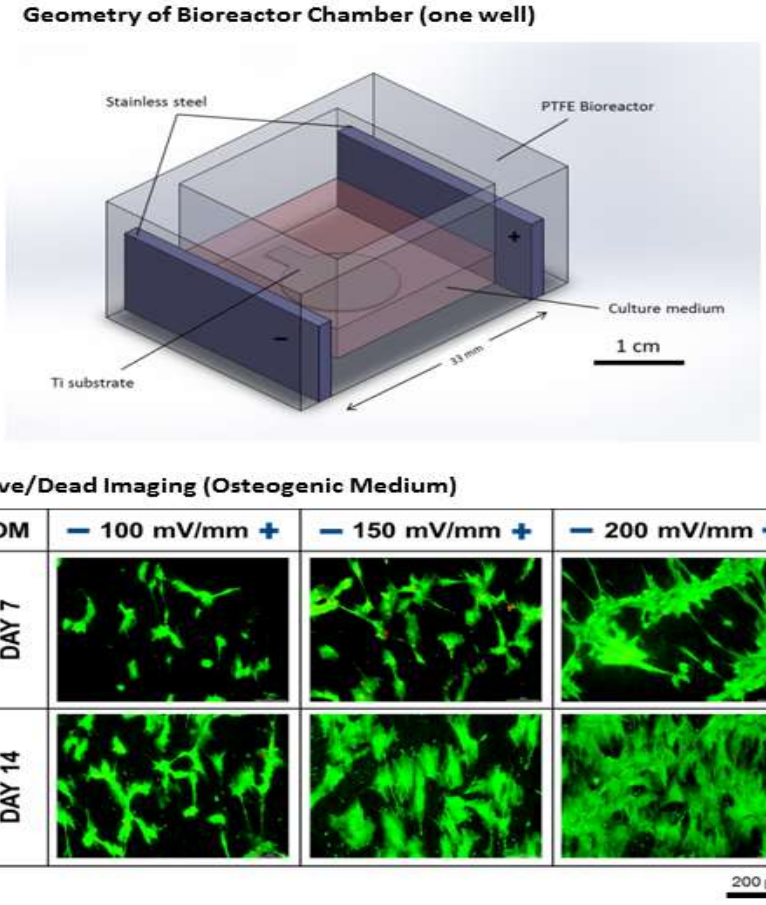

Picture 1: Caption 1: Capacitive Coupling Electrical Stimulation (CC-ES)

Developments in applied biomaterials 14:00 - 15:30 Room 0.5 11/09/2018

Oral presentation

709 New biocompatible hydrogels with extreme mechanical properties

Andreia T Pereira[1], Patrícia Henriques[2], M. Cristina L. Martins[2], Fernão D. Magalhães[3], Inês C. Gonçalves[4]
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[4]INEB - Instituo de Engenharia Biomédica / i3S, Portugal

INTRODUCTION:

Designing hydrogels with high-strength and stiffness remains a challenge, limiting their usage in load bearing applications.[1] Thus, the aim of this study was to develop hydrogels with suitable mechanical properties to suppress this limitation. For this, graphene based materials (GBMs) with differing lateral size, oxidation degree and thickness were incorporated in poly(2-hydroxyethyl methacrylate) (pHEMA) and evaluated regarding their bio/hemocompatibility.

METHODS:

Hydrogels were fabricated by *in situ* polymerization of 2-hydroxyethyl methacrylate and tetraethylene glycol dimethacrylate[2] in the presence of increasing concentrations (0-5% w/v) of five different GBMs, namely graphene oxide (GO) and graphene nanoplatelets with varying lateral size (GNP-M5, GNP-M15) and oxidized forms (GNP-M5ox, GNP-M15ox). pHEMA/GBMs composites were evaluated regarding polymerization degree (Infrared spectroscopy- FTIR-ATR), GBMs dispersion (optical microscopy and TEM), surface topography (SEM), wettability (contact angle measurements), swelling capacity (gravimetry) and mechanical properties (tensile and compression tests). Biocompatibility was evaluated using mouse fibroblasts (NIH3T3) and endothelial cells (HUVECs) regarding cell adhesion/proliferation by immunofluorescence and cytotoxicity of medium extracts by resazurin assay. Hemocompatibility was assessed regarding hemolytic potential by hemolysis assay and platelets adhesion/activation by SEM.

RESULTS AND DISCUSSION:

Incorporation of GBMs increases surface roughness of pHEMA while polymerization, swelling capacity (~50%) and surface wettability (~25º contact angle) are not affected. Regarding the mechanical properties, the presence of non-oxidized GNPs does not influence the performance of neat pHEMA. GO is the most efficient oxidized nanofiller, with incorporation of 5%GO in pHEMA increasing its compressive and tensile stiffness 4x and 8.5x (4MPa and 6.5MPa, respectively) and tensile resistance 7.8x (1.2MPa). This is an outstanding value for tensile resistance since for most of hydrogels described in the literature GO incorporation increases only 2-3x, reaching about 400 kPa.[3] The elasticity of the hydrogel can be improved in 50% using concentrations of up to 2% GO. Regarding the biological properties, GBMs incorporation in pHEMA does not promote cell and platelets adhesion at surface, neither hemolysis nor cytotoxicity.

CONCLUSION:

We propose a facile method for producing bio/hemocompatible non-fouling hydrogels with outstanding stiffness and tensile resistance and improved elasticity. This new material will pave the way for hydrogels application in several load-bearing application, namely in the designing of tissues where non-adhesive properties are an asset, such as cartilage, intervertebral disc and blood-contact devices.

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ACKNOWLEDGMENTS:

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Developments in applied biomaterials 14:00 - 15:30 Room 0.5 11/09/2018

Oral presentation

620 Effects of Nrf2 signaling on cytotoxicity induced by HEMA from dental biomaterials

Bergitte Pearl Olderbø, Else Morisbak, Solveig Uvsløkk, Jan Tore Samuelsen
Nordic Institute of Dental Materials - NIOM, Oslo, Norway

INTRODUCTION:

Resin-based biomaterials used in dentistry consist of methacrylate monomers that are polymerized *in situ*. The conversion to polymer is never complete and cause patient exposure to electrophilic monomers such as 2-hydroxyethyl methacrylate (HEMA). In addition, dental personnel are exposed through handling of uncured materials. *In vitro*, methacrylates are reported to be cytotoxic in a dose-dependent manner. The mechanisms involved are not fully elucidated, but increased oxidative stress is a suggested key event. Based on this presumption, activation of nuclear factor erythroid 2-related factor 2 (Nrf2) is assumed to play a central role in defending exposed cells against oxidant and electrophilic stresses[1]. In this study, we aim to elucidate the ability of Nrf2 regulated genes to counteract HEMA toxicity.

METHODS:

The effects of 24 hours HEMA exposure (0-8 mM) on the human bronchial epithelial cell line BEAS-2B and the human alveolar epithelial cancer cell line A549 were compared. The first cell line holds a normal regulation of Nrf2 activity, whereas the latter contain a mutation that results in continuously Nrf2-activity. Western blotting was used to explore altered levels of Nrf2-associated gene products known to be affected by HEMA exposure. Furthermore, viability after 24 hours HEMA exposure (0-8 mM) was measured using MTT assay. Additionally, viability in BEAS 2B cells after 24 hours preincubation with 1 mM HEMA followed by 24 hours of HEMA exposure (0-8 mM) was measured. At least three independent experiments were performed. Statistical analysis was calculated using one-way ANOVA with Dunnett multiple comparisons test by GraphPad prism.

RESULTS AND DISCUSSION:

Protein measurements using western blotting revealed that BEAS-2B cells exposed to HEMA significantly increased the investigated Nrf2-associated proteins, while no change was observed in A549 cells. The basal levels of these proteins in A549 cells, however, were comparable to HEMA-exposed BEAS-2B cells. Both BEAS-2B and A549 cells exposed to HEMA resulted in a similar dose-dependent decrease in viability. Although preincubation of BEAS-2B cells with 1 mM HEMA increased Nrf2-activity, the viability loss caused by HEMA was not affected.

CONCLUSION:

These results indicate that HEMA induce the Nrf2 activity in BEAS-2B, while the Nrf2 pathway is constantly activated and not further inducible by HEMA in A549 cells. Furthermore, the results argue that increased Nrf2 activation protects against HEMA induced toxicity.

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ACKNOWLEDGMENTS:

The authors would like to thank Aurora Lima, Ingelin Neeraas, Renate Ravndal and Siv Jensen for excellent technical assistance.

Developments in applied biomaterials 14:00 - 15:30 Room 0.5 11/09/2018

Oral presentation

307 Sterilization effects on the handling and degradation properties of calcium phosphate cements containing poly (lactic-co-glycolic acid) porogens and carboxymethyl cellulose

Nathan Kucko[1], Marcela Garcia Martinez[2], Ihtesham ur Rehman[2], Ann-Sissel Teialeret Ulset[3], Bjørn Christensen[3], Sander Leeuwenburgh[1], Ralf-Peter Herber[4]
[1]Radboud University Medical Center, Nijmegen, Netherlands
[2]Kroto Research Institute, University of Sheffield, United Kingdom
[3]Norwegian University of Science and Technology, Norway
[4]CAM Bioceramics B.V., Netherlands

INTRODUCTION:

Calcium phosphate cements (CPCs) exhibit excellent biocompatibility, osteoconductivity, and injectability[1]. Their clinical applicability, however, can be compromised due to insufficient cohesion upon injection into the body. Therefore, we incorporated carboxymethyl cellulose (CMC) into CPCs to increase their cohesion and injectability[1], and poly(lactic-co-glycolic acid) (PLGA) microparticles to enhance their degradation rate[2].

Gamma irradiation of CPCs containing polymeric additives can affect the properties of the cements by forming free radicals and reducing the molecular weight (Mw) of the polymers[3]. Therefore, the aim of this study was to investigate the influence of gamma irradiation doses on the handling and degradation of CPCs.

METHODS:

To prepare CPC specimens, polymeric and ceramic powders were first mixed and then irradiated at various doses. Subsequently, the powder phase was mixed with a 4 wt% aqueous solution of $NaH_2PO_4 \cdot 2H_20$, after which the handling properties were tested by determining their setting times, injectability, and cohesion. The degradation was tested by immersing hardened CPC specimens in PBS solution at 37°C and measuring the change in mass, pH, and calcium concentration in supernatants after 1, 2, 3, and 4 weeks. Finally, size exclusion chromatography was performed to measure the Mw of CMC as a function of irradiation dose.

RESULTS AND DISCUSSION:

Fig. 1 represents the relationship between the Mw of CMC and how this influences the cohesion of CPCs after irradiation at different doses. It can be seen that the Mw decreases hyperbolically as the irradiation dose increases.

Further, this reduction in Mw negatively affects the injectability and cohesion score of the CPC, where higher scores indicate worse cohesion. Further, the final setting time for both CPC formulations increased by nearly 7 min after irradiation at 80 kGy.

The decrease in CMC Mw is due to the cleavage of glycosidic bonds within the CMC chain which causes poorer CPC cohesion and injectability. Moreover, the formation of these smaller, more mobile anionic CMC chains adsorb onto the surface of the ceramic particles thereby hindering the setting reaction and increasing the setting times.

CONCLUSION:

Using CMC with a higher starting Mw and lower irradiation dose would be preferred as it is capable of retaining more of its viscosifying properties that translates into improved handling properties.

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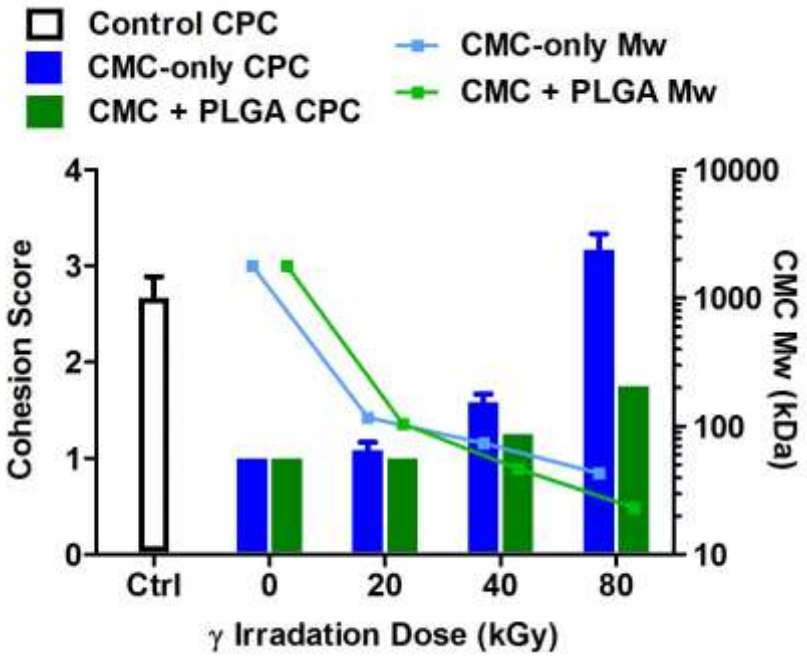

Picture 1: Caption 1: Fig. 1: Cohesion score of composite CPCs and CMC molecular weight as a function of gamma irradiation dose.

Developments in applied biomaterials 14:00 - 15:30 Room 0.5 11/09/2018

Oral presentation

808 Highly selective double atom activation of titanium alloys by directed irradiation synthesis: tailoring osteoblast behavior towards implants osseointegration

Ana Civantos, Alethia Barnwell, Camilo Jaramillo, Jean Paul Allain
University of Illinois, Urbana, United States of America

INTRODUCTION:

Despite favorable biocompatibility and mechanical properties of Titanium (Ti) and its alloys (Ti6Al4V), challenges remain with its intrinsic inertness, stress shielding, bacterial adhesion and ultimately aseptic loosening that drives implant rejection[1]. To address these challenges current surface modification technologies seek to design suitable surface activation properties on Ti6Al4V improving osteoblast growth while diminishing bacterial attachment[1,2]. Previous work by Allain et al reported enhanced in-vitro tendencies for osseointegration indicators with directed irradiation synthesis (DIS) by tailoring surface topography by modulation of energy density deposition using inert particle beams[3]. These surface changes at the nanoscale level demonstrated an increase in cell adhesion and differentiation compared to control surfaces (polished Ti). In this work, we use a multiple-beam approach with DIS to control Ti alloy surface activation by combining inert and reactive particle-beam interaction to control topography and surface chemistry independently resulting in a controlled surface activation to improve Ti6Al4V osseointegration

METHODS:

Titanium alloy (Ti6Al4V) samples were modified by DIS using different gas species (Argon, Nitrogen, and Oxygen) by normal and off-normal incidence angles. Other DIS parameters (energy and fluence) were kept constant. Topography, roughness, and hydrophilicity were evaluated by scanning electron microscopy (SEM), atomic force microscopy (AFM) and contact angle (CA) respectively. Surface charge density correlated to physical and biological properties was also completed. *In vitro* experiments were carried out using a preosteoblastic (MC3T3E1) and macrophages (J774A1) cell lines to evaluate cells adhesion, differentiation, morphology and early immune response by cytokine expression of TNF alpha and Il-10

RESULTS AND DISCUSSION:

Each ion species, Ar^+, N_2, and O_2, revealed different nanofeatures morphology ranging from nano sharp cones, nanoplatelets, and nano blunt pillars respectively using similar irradiation parameters. These nanofeatures and chemistry changes also showed a remarkable influence on cells behavior, inducing different cell metabolic activities as well cell differentiation. N_2 surfaces reached the highest ALP values and the lowest TNF alpha levels compared to O_2 and Ar^+ irradiated Ti surfaces showing an interesting ion surface modification.

CONCLUSION:

Using DIS we are able to control with high-fidelity surface topography and chemistry changes towards an improved cell adhesion and differentiation of osteoblast which will enhance implants osseointegration

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Developments in applied biomaterials 14:00 - 15:30 Room 0.5 11/09/2018

Oral presentation

437 Hydroxyapatite coating of magnesium alloys for the tailored degradation of resorbable bone fixation products

Jonathan Acheson, Stephen Mckillop, Patrick Lemoine, Adrian Boyd, Brian Meenan
Ulster University, Co. antrim, United Kingdom

INTRODUCTION:

The use of bio-resorbable bone fixation products offers the opportunity to eliminate the need for secondary removal operations and negate the associated complications such as infection and pain. Magnesium alloys display great potential as biodegradable metal implants; however, the corrosion rate of magnesium can be difficult to control *in vivo* thereby limiting its use[1]. In this study, sputter deposited hydroxyapatite (HA) coatings, acting as a barrier layer to tailor the degradation of magnesium alloys, have been investigated.

METHODS:

Magnesium alloy (AZ31, Goodfellow, UK) coupons (10 x 10 x 1 mm) were coated with HA from CAPTAL R (Plasma Biotal, UK) via RF magnetron sputtering, a well-established methodology[2]. A design of experiments approach was used to create a response surface plot that allows for a tailored degradation rate to be chosen based on the required *in vivo* response. HA coated, and uncoated control coupons were exposed to simulated body fluid (SBF) for up to 14 days. Samples were characterised via micro-computed tomography (microCT), x-ray photoelectron spectroscopy, optimal microscopy, scanning electron microscopy, atomic absorption spectroscopy, time of flight secondary ion mass spectroscopy (ToF-SIMS) and gravimetric analysis.

RESULTS AND DISCUSSION:

The presence of a 700 ± 350 nm HA coating, determined from ToF-SIMS depth profiling, significantly reduced magnesium alloy corrosion, with a mass loss of 3.92 ± 0.38 mg for HA coated coupons and 7.57 ± 4.98 mg for uncoated controls (Figure 1a). The nature and scale of the corrosion that occurred was clearly different with much less pitting observed in the presence of the HA coating (Figure 1b and c).

CONCLUSION:

Hydroxyapatite coatings offer potential as a barrier layer to reduce the corrosion rate of magnesium alloys in SBF by controlling the diffusion of ionic solution to the underlying metal. Variation of the coating thickness allows for tailored degradation rates to be achieved. The presence of the HA in the biological environment associated with magnesium alloy breakdown offers the additional advantage of enhanced bone cell response.

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ACKNOWLEDGMENTS:

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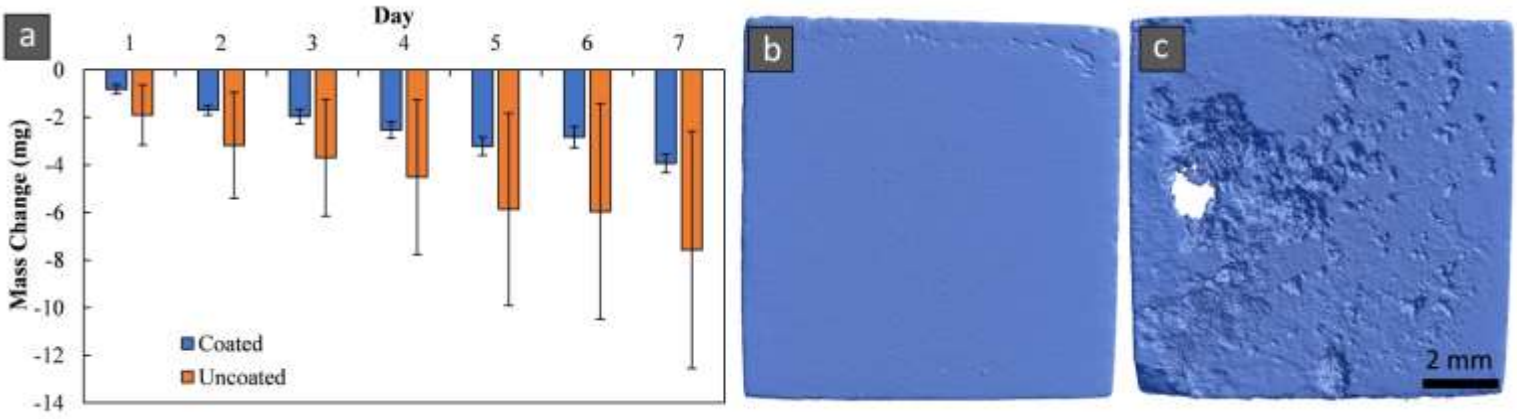

Picture 1: Caption 1: Figure 1 a) Mass change of coated and uncoated AZ31 alloy coupons. MicroCT reconstructions of AZ31 alloys after 7 days in SBF b) HA coated c) uncoated

Biomaterials for engineering and regeneration inspired by nature 16:00 - 17:30 Room 0.5 11/09/2018

Oral presentation

196 Self-assembling silk hydrogels as a carrier matrix to treat stroke

Natalia Gorenkova, Osama Ibrahim, Hilary V.O. Carswell, F. Philipp Seib
University of Strathclyde, Glasgow, United Kingdom

INTRODUCTION:

Advanced cell therapies require robust delivery strategies[1]; one promising contender is the biopolymer silk.[2] Silk already has a long clinical track record for load bearing applications and can be assembled into a range of material formats, including hydrogels.[3] The aim of this study was to (i) assess the biocompatibility of self-assembling silk hydrogels in the stroked brain and (ii) optimise self-assembling silk hydrogels as a mesenchymal stem cell (MSC)-support matrix.

METHODS:

B. mori silk was reverse engineered as described previously.[4] Sonication energy was used to control silk self-assembly. The resulting hydrogels were characterised using FTIR, CD, rheology and SEM. During the solution-gel transition MSCs were embedded and cytocompatibility was assessed; analogous studies were performed to examine the impact of injection on cell viability. Next, stroke studies were performed according to ARRIVE guidelines. Rats underwent transient middle cerebral artery occlusion 2 weeks before random assignment and blinding to treatment groups including stereotaxic injection of self-assembling silk hydrogels into the stroke cavity. Space conformity, GFAP glial scar manifestation, microglia/macrophage localisation were examined 1 and 7 weeks later.

RESULTS AND DISCUSSION:

We used sonication energy to programme the transition of the silk secondary structure from a random coil to a stable β-sheets configuration. It was thus possible to fine-tune self-assembling silk hydrogels to achieve space conformity in the absence of any silk hydrogel swelling and to support uniform cell distribution as well as cell viability. Embedded cells showed significant cell proliferation over 14 days but 2%w/v hydrogels supported proliferation best. When injected through a 30G needle, MSC viability was significantly better when gels were in the pre-gelled state

versus post- gelled state. Silk hydrogels with matched physical characteristics of brain tissue were able to show excellent space conformity and presented no overt microglial/macrophage response.

CONCLUSION:

Overall, this study demonstrates that self-assembling silk hydrogels are emerging as a promising cell delivery platform for CNS diseases, especially stroke.

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ACKNOWLEDGMENTS:

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Biomaterials for engineering and regeneration inspired by nature 16:00 - 17:30 Room 0.5 11/09/2018

Oral presentation

492 A Novel Biocomposite Based on Microporous Oxidized Bacterial Cellulose/Arginine and the Effect on the Behavior of Fibroblast/Endothelial Cell

Hui Qiao[1], Yudong Zheng[2], Liang Zhao[1], Yajie Xie[1], Yi Sun[1]
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[2]University of Science and Technoloty, Beijing, China

INTRODUCTION:

Bacterial cellulose (BC) has been studied widely. Although there are many methods to modify BC such as the oxidized BC, the nano-structure of BC makes it difficult to be oxidized, and high oxidation degree makes the content of aldehyde high, which make the cell biocompatibility poor. Therefore, many challenges still exist for modifying BC easyly, and improving its biocompatibility and biodegradation, or promoting the https:\\www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/cell-migration and proliferation.

METHODS:

In the study, a novel biocomposites based on oxidized BC with microporous structure and in-situ grafted with Arginine (Arg) were fabricated (Fig1). The composites were characterized by infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and roughness, contact angle and surface energy test. Oxidation degree, grafting percentage of Arg to MOBC and release percentage of Arg were also measured. Finally, effects of the composites on the cell proliferation, migration and expression of Collagen-I of normal human dermal fibroblasts (NHDF) and human umbilical vein endothelial cells (HUVEC) were evaluated by use of microfluidic chip.

RESULTS AND DISCUSSION:

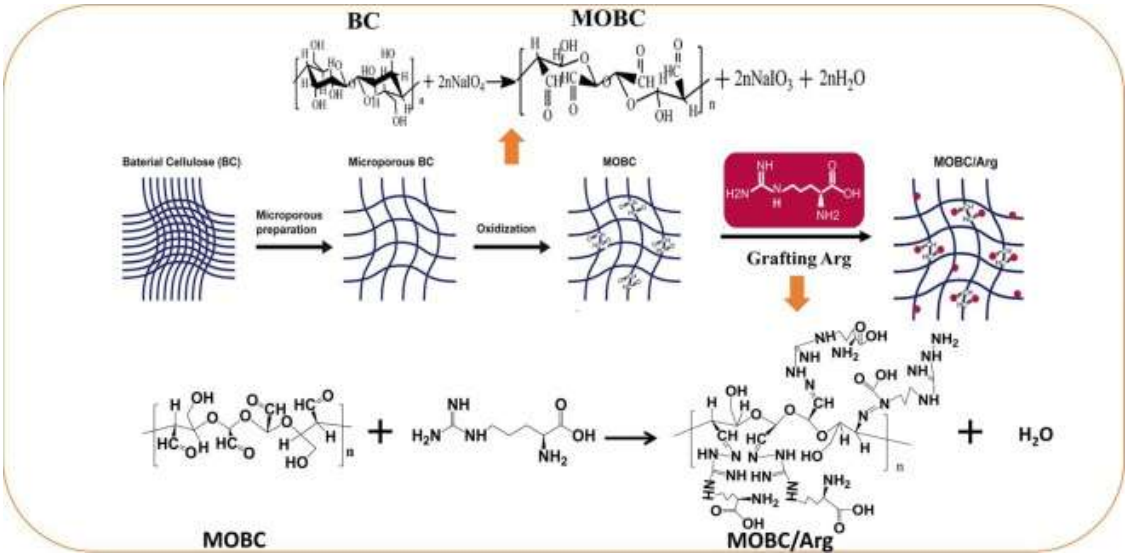
The in-situ grafted Arg, which is reacted with -CHO through the Schiff base reaction, forming strong C=N bond and enhances the biocompatibility. The roughness, surface energy for MOBC/68.68 % Arg was 1.5 and 1.16 times than that of BC respectively. In vitro studies indicated that the cells showed high viability and proliferation on MOBC/Arg, which can also promote the expression of Collagen-I. Moreover, by employing a microfluidic chip, we found that MOBC/Arg was more effective in promoting wound healing (both on NHDF and HUVEC) than BC and MOBC. Quantitative data from microfluidic analysis indicates that MOBC/Arg can significantly improve the migration of HUVEC than that of NHDF.

CONCLUSION:

The microporous structure of MOBC/Arg increased the contact area of MOBC in modified process. The in-situ grafted Arg with MOBC,forming strong CN bond and reducing the cytotoxicity of the aldehyde group. In vitro studies indicated that MOBC/Arg can improve the cells viability and proliferation , and also promote the expression of collagen-I. MOBC/Arg has high roughness and surface energy ,and can promote the migration of NHDF and HUVEC faster than that of BC. Our study highlights the importance of MOBC/Arg in cell motility and thus is potential to be used as wound dressing.

ACKNOWLEDGMENTS:

The authors would like to thank Beijing Municipal Science and Technology Plan Projects (No. Z161100000116003) and National Natural Science Foundation of China (No. 427 51473019, 21675011).



Picture 1: Caption 1: Fig1 Schematic illustration of the formation of MOBC/Arg composites

Biomaterials for engineering and regeneration inspired by nature 16:00 - 17:30 Room 0.5 11/09/2018

Oral presentation

587 clickECM - integration of click groups into cell-derived human extracellular matrix to create ECM-based biomaterials

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INTRODUCTION:

The extracellular matrix (ECM) with its various functions can be isolated after several days of cell culture[1, 2, 3, 4]. However, the use of ECM is limited due to the lack of functional groups, which are often required coatings or scaffolds[3]. Therefore, we performed Metabolic Glyco Engineering (MGE) to introduce azide groups into the glycan structures of the ECM to create a variously deployable “clickECM”.

METHODS:

Substrates were functionalized with activated alkynes to covalently immobilize the azide-modified *click*ECM on material surfaces via copper-free click reaction. The bioactive properties of these coatings were evaluated by quantifying the cell proliferation. Histochemical and immunofluorescence analysis were performed to characterize the biological composition of *click*ECM. We further increased the denseness of the *click*ECM-coating by using centrifugal filters. Moreover, we cross-linked the azide-modified biomolecules of the *click*ECM with a bi-functional alkyne crosslinking agent in order to further increase the coating thickness and denseness.

RESULTS AND DISCUSSION:

We could for the first time show that MGE can be used to introduce click groups into the ECM of human dermal fibroblasts (Fig. 1).This *click*ECM consists of glycans, collagens, and non-collagenous proteins. The *click*ECM was covalently immobilized on alkyne-modified surfaces resulting in a significant increase in coating stability compared to a conventional ECM coating via physisorption. Cell proliferation was significantly enhanced on the *click*ECM-coated surfaces what proved its exceptional biological activity. We were able to increase the denseness and the thickness of our *click*ECM-coating.

CONCLUSION:

These results demonstrate that our *click*ECM is a promising biomaterial e. g. for the generation of bioactive coatings with an increased stability and a high biological complexity. The covalent immobilization mediates an increased stability while preserving the high biological activity of the human dermal ECM.

We propose that the additional incorporation of selected alkyne-functional (bio)molecules into the *click*ECM-coatings could enable the preparation of stable, tissue-specific, and bioactive scaffolds for tissue engineering and regenerative medicine.

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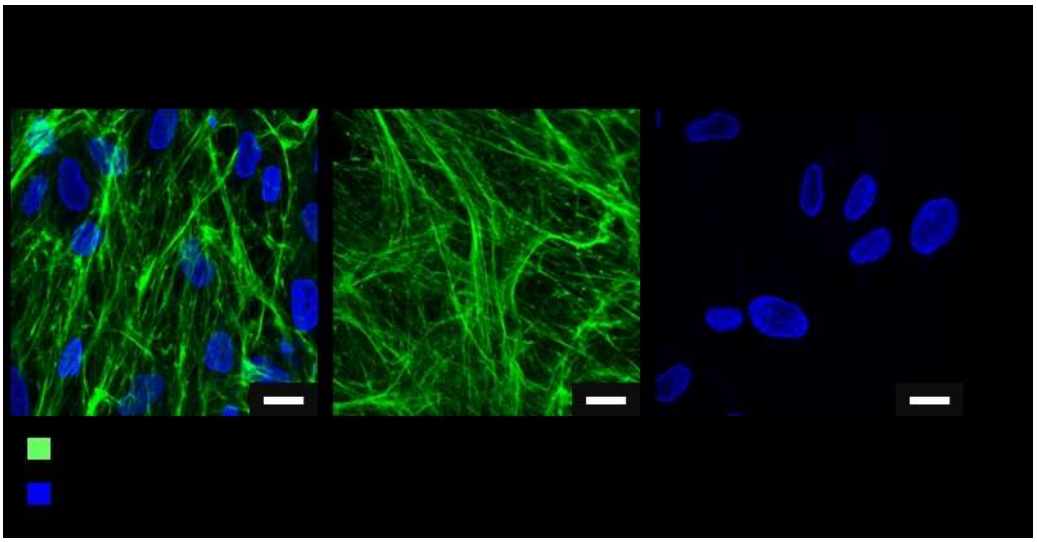
ACKNOWLEDGMENTS:

This work was supported by the Baden-Württemberg Stiftung (Glycobiology/Glycomics, Grant number: P-BWS-Glyko/09) and by the Fraunhofer Internal Programs under Grant No. Discover 828 335.



Picture 1: Caption 1: Fig. 1: MGE is a useful tool to incorporate click groups into the glycans of cell-derived ECM. Source: adapted from3.

Biomaterials for engineering and regeneration inspired by nature 16:00 - 17:30 Room 0.5 11/09/2018

Oral presentation

564 Injectable hydrogels for single-surgery tracheal occlusion in congenital diaphragmatic hernia management

Chiara Emma Campiglio[1], Martina Villonio[1], Raffaele Dellacà[2], Fabio Mosca[3], Lorenza Draghi[1]
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INTRODUCTION:

Congenital Diaphragmatic Hernia (CDH) is a fetal anomaly characterized by failed closure of diaphragm, herniation of abdominal organs in the thoracic cavity and impaired lung development[1]. In most severe cases, fetoscopic endoluminal tracheal occlusion (FETO) is performed through a catheter balloon to accumulate lung fluid and contain pulmonary hypoplasia[2]. However, the risk to benefit ratio of this technique, which requires a second fetoscopy for balloon removal, is still debated. To support the success rate of FETO and overcome some of the associated drawbacks, this work aimed at finding an injectable, degradable substitute for the balloon not requiring surgical prenatal removal.

METHODS:

Two different hydrogels were evaluated as tracheal plugs: calcium-alginate and hyaluronan-methylcellulose blend (HAMC). Different concentration, molecular weights, composition and ratios were rheologically screened. Anatomical tracheal models were fabricated and filled with simulating lung fluid to assess injectability, cohesiveness, sealing pressure and persistence. *In vitro* cytotoxicity and adhesion of mouse fibroblasts (L929) on hydrogels were also evaluated.

RESULTS AND DISCUSSION:

For both hydrogels, adjusting formulation enabled to obtain gelation time in the design specification range. When injected in the tracheal model, selected hydrogels showed good cohesion and capability to adapt to the anatomical shape. Under pressure, effective sealing up to 80 cm_{H2O} was observed for composition with superior mechanical properties. Occlusion was maintained for over 4 weeks under 10 cm_{H2O} while weight loss varied from few days to several weeks. Although no evidence of cytotoxicity was shown *in vitro*, cell-adhesion was favorably low.

CONCLUSION:

Biodegradable, injectable gel plugs have the potential to improve FETO patients' prognosis by removing the need for a second surgery. Moreover, for their anatomical adaptability, they can also prevent the large tissue deformation entailed in the current balloon procedure. An effective gel plug needs to withstand very strict gelation time, cohesiveness, swelling and degradation profiles design criteria. For both type of hydrogels evaluated here, alginates and HAMC blends, initial design specifications ranges were met by adjusting their composition. However, because of the favourable shear-thinning behavior, that does not require intraoperative control of gelation times, HAMC blends appeared as a particularly promising alternative for temporary tracheal occlusion.

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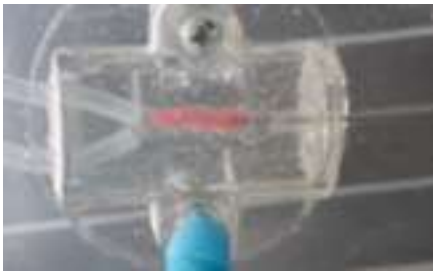

Picture 1: Caption 1: Injection of hydrogels in the silicon rubber tracheal model

Biomaterials for engineering and regeneration inspired by nature 16:00 - 17:30 Room 0.5 11/09/2018

Oral presentation

485 Nano fracture behaviors of bone tissue

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INTRODUCTION:

A comprehensive understanding in nano-mechanical behaviors of bone are important but yet to be determined [1-3]. A novel in-situ experimental technique using AFM and micro-electro-mechanical system (MEMS) is used to investigate the mechanical behaviors of bovine cortical bone at nano-scale during fracture. The separation of intra-fibrillar mineral and micro-bridging were recorded for the first time.

METHODS:

The bovine cortical bone close to femur bone marrow was cut into 20×3×3mm along axial direction.The natural surface close to bone marrow was kept. All samples were immersed in Hank buffered solution for sonication cleaning for 30 mins. While samples were tested under three-point bending using a custom-built MEMS, live images were continuously recorded on the nature surface by AFM (MFP3D,Asylum Research,Dutch) as shown in the figure.

RESULTS AND DISCUSSION:

When load increases, the layered mineral separation occurs shown as the blue arrow in figure BCD. In early stages of bone deformation, intrafibrillar mineral crystals started to separate, and the main deformation may occur in the mineral phase. A decreased diameter of collagen fibrils in Fig B may indicate the fibrils are under stretch due to mineral displacement. As the load increases, fibril slipping occurs between the collagen fibrils. Meanwhile, the interfibrillar mineral particles fall off from the mineralized fibers, and then the formed small mineral particles are extruded to expose on the nature surface, seen in red circle area in Fig C. The fibrils have probably also ruptured, which relaxes the state of the fibrils, and collagen fibrils are not limited by the mineral particles, causing the fibers to become thicker. Finally, the bone is fractured to failure, mineral phase stratified separation, fibril breakage and slipping, and mineral particle shattering were observed in Fig D.

CONCLUSION:

An in situ AFM mechanical testing technique was used to investigate nanoscale behaviors on nature surface of cortical bone during fracture. Experimental observation of nano-strain with detailed mechanical behaviors of bone under macroscopic loading was recorded high resolution AFM for the first time. These behaviors include mineral phase stratified separation, fibril breakage and slipping, and mineral particle shattering. Our investigation indicates that the mineral phase deformation may occur in the early stages of bone deformation with subsequent breaking and slipping of the collagen fibrils.

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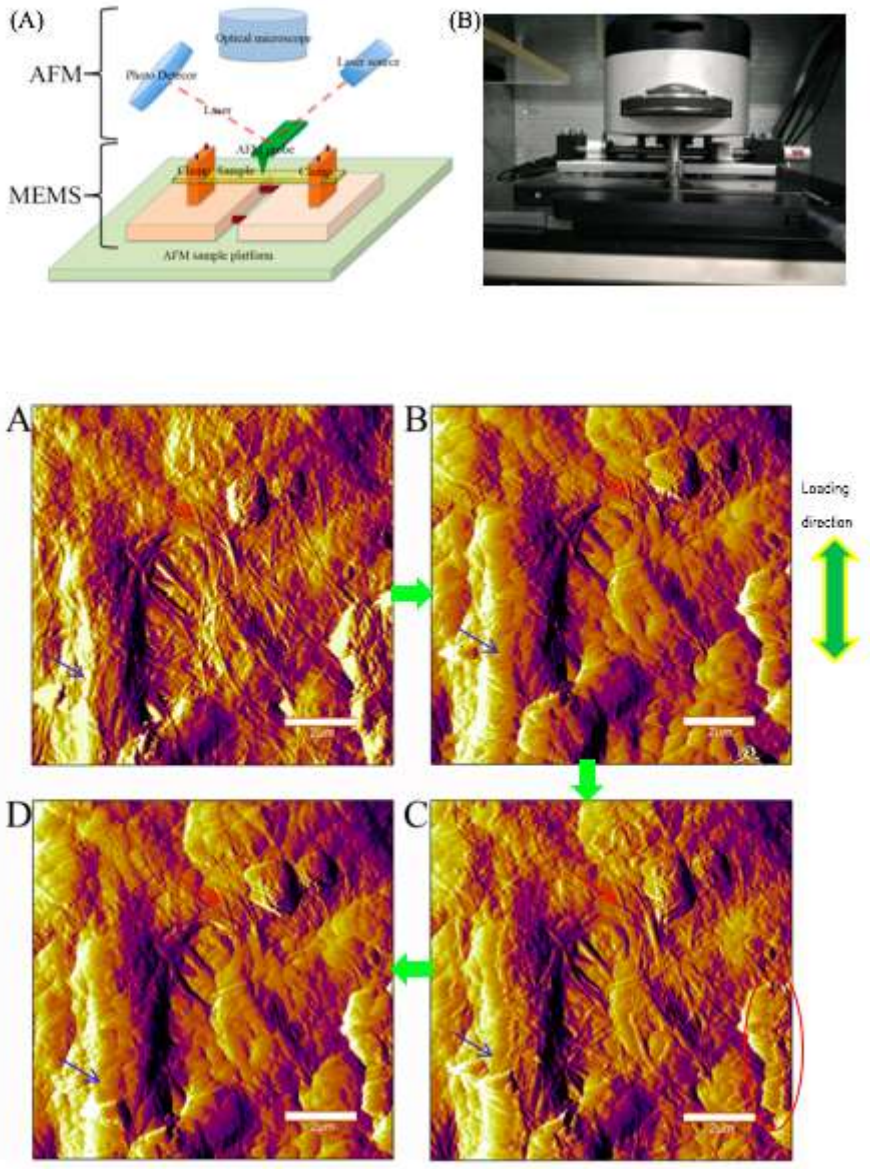

Picture 1: Caption 1: Figure (A) Schematic diagram of in-situ mechanical test device. (B) the mechanical testing device is located below AFM. A: un-loaded. B: 7N. C: 23N. D

Biomaterials for engineering and regeneration inspired by nature 16:00 - 17:30 Room 0.5 11/09/2018

Oral presentation

119 Hyaluronic acid-based composite hydrogels with biofunctional tailored features

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[1]Institute of Polymers, Composites and Biomaterials, Naples, Italy
[2]Institute of Science and Technology for Ceramics, Italy
[3]National Engineering Research Center for Biomaterials, China

INTRODUCTION:

Recently, great interest has been devoted to composite-based hydrogels synthesized by *in situ* sol-gel method for drug delivery and tissue engineering applications[1]. Traditionally, hard tissue-engineered nanocomposites were obtained involving physical mixing of hydroxyapatite nanoparticles. On the contrary, the versatile *in situ* sol-gel process ensures a controlled and finer distribution of nanofillers in the matrix and the possibility of incorporating biologically active compounds. The synthesis takes place at room temperature and it does not require the use of pharmaceutically unacceptable solvents. In this study, a modified hyaluronic acid was used as polymer matrix.

METHODS:

The polymer was methacrylated (MeHA) and maleated (MaHA) following adapted protocols[2-3]. Different degrees of substitution were also considered. Composite materials at 25 and 50 wt% of inorganic nanoparticles in the polymer matrix were produced by *in situ* sol-gel synthesis and characterized in terms of inorganic filler size and distribution through scanning and transmission electron microscopy. Furthermore, MeHA and MaHA-based composite materials were loaded with a non-steroidal anti-inflammatory drug, the Diclofenac Sodium (DS). At designated time points, DS release was evaluated. Biological assays were performed using murine fibroblast and macrophage cell lines for cytotoxicity evaluations and anti-inflammatory response of the DS-loaded materials. *In vitro* cell viability and the expression of pro, anti-inflammatory and chemotactic cytokines such as tumor necrosis factors and interleukins were measured at different time points of cell culture.

RESULTS AND DISCUSSION:

SEM and TEM results revealed a good dispersion of needle-like nanocrystals for MeHA-based composites, whilst nanoplates were observed in MaHA-based samples. Biological results proved the absence of any material's cytotoxic effect highlighting the best cell adhesion for composite hydrogels with the highest degree of substitution and lowest particle concentration. The viability of macrophages, seeded on DS-loaded and not, neat and composite hydrogels, was evaluated. The release kinetics study showed that the chemical modification has a strong effect on DS release, which can be ascribed to the different hydrogels' swelling behavior. The pro-inflammatory (IL-1β, TNF-α and IL-6) and anti-inflammatory (IL-10) cytokines release was evaluated. In particular, the presence of DS in composites reduced TNF-α release, stimulating the IL-10 production.

CONCLUSION:

The results obtained highlighted these materials as suitable candidates for drug delivery applications tailoring the inflammatory response.

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ACKNOWLEDGMENTS:

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Utilisation of biomaterials for wound treatment 10:30 - 12:00 Room 0.2/0.3 11/09/2018

Oral presentation

797 A photo-triggerable NP library for skin cell targeting and efficient in-vivo small non-coding RNA delivery in wound healing

Josephine Blersch[1], Adrian Jimenez-Balsa[2], Catarina Rebelo[2], Helena Henriques-Antunes[2], Vitor Francisco[2], Sandra Pinto[2], Malwina Kotowicz[2], Karsten Haupt[3], Klaus Liedl[4], Lino da silva Ferreira[5]
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[4]Institute for Inorganic and Theoretical Chemistry, University of Innsbruck, Austria
[5]Center for Neuroscience and Cell Biology Coimbra and University of Coimbra, Portugal

INTRODUCTION:

Impaired wound healing and its medical complications remain one of the most prevalent and economically burdensome healthcare issues in the world. RNA-based therapies have emerged recently as promising drugs for skin regeneration[1,2]. RNA-based therapies have distinct advantages over conventional drug therapies, such as specificity, potency, number of accessible targets, species crossreactivity, manufacturing, etc…[3] However, several obstacles need to be addressed before the clinical translation of RNA-based therapeutics. Particularly, formulations´ design that enable targeted delivery to the skin, reduction of off-targeting and simultaneously increase efficacy in the intracellular delivery. The hypothesis of the current work was that biocompatible light-activatable nanoparticles allowing precise control of the timing and spatial release of the RNA molecules could increase their efficiency and accelerate the translation of these therapies.

METHODS:

NP library: We have used nanoprecipitation for the formation of NP from polymers obtained by combinatorial polymer synthesis.

In vitro essays: siRNA against GFP was immobilized on NPs and gene silencing efficacy was measured using High-Content Imaging in HelaGFP cells relative to commercial standard before and after light activation. Skin cell targeting of lead formulations was analysed by confocal microscopy.

Wound healing: NP were combined with a skin regenerative microRNA and *in vitro* wound healing was performed in keratinocytes with(/out) light activation and relative to commercial standards. Finally, a full excisional wound healing model in C57BL/6 mice was used and wound closure was monitored daily.

RESULTS AND DISCUSSION:

We produced a library of light-activatable NPs (more than 300 NPs, size below 500nm and positive charge) dissociating at different rates once activated by UV. High-throughput screening for GFP silencing efficacy identified several formulations which are more effective as the commercial standard. Candidates were further characterized regarding their specificity to skin cells, endolysosomal escape and functional studies. Moreover, we have confirmed the advantages (kinetics and quality) of one of the candidate formulations *in vitro* and in a wound healing animal model, for the delivery of a skin regenerative miRNA identified recently by us.

CONCLUSION:

In conclusion, we have developed a powerful platform for the delivery of RNA-based therapeutics delivery both *in vitro* and *in vivo*.

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ACKNOWLEDGMENTS:

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Utilisation of biomaterials for wound treatment 10:30 - 12:00 Room 0.2/0.3 11/09/2018

Oral presentation

244 A NEW ‘SPIN' IN CHRONIC WOUND CARE: DELIVERING MIR-31 VIA NANOFIBRES

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[2]Dublin City University, Ireland

INTRODUCTION:

In the UK alone, some 200,000 patients present annually with chronic wounds.[1] MicroRNAs (miRs) act as post-transcriptional regulators of multiple proteins and therefore have great potential as genetic therapeutics. Herein, we aim to develop a new genetic nanomedicine for the efficacious treatment of chronic wounds. This treatment will consist of nanoparticles (NPs) designed to deliver DNA encoding miR-31, which regulates multiple proliferative and angiogenic target genes. A novel peptide system ‘*PEP’* has been designed to deliver the pmiR-31 cargo. The NPs will be delivered via electrospun PVA nanofibres (PVA-NF), designed to facilitate temporal controlled delivery, and provide a physiologically moist microenvironment at the wound site.

METHODS:

The NPs were synthesised by self-assembly and the size and zeta potential determined by Dynamic Light Scattering. NCTC-929 fibroblast, HMEC-1 endothelial and HaCat keratinocyte cells were transfected with PEP/pmiR-31 NPs, and this was quantified via RT-PCR. The functional effects of miR-31 upregulation on cell migration, proliferation and angiogenesis were subsequently assessed via the wound scratch, cell viability and tubule formation assays. The experimental design approach was used to optimise the electrospinning of PVA-NF. PVA-NFs were crosslinked prior to NP loading. The functionality of the NPs was determined upon release from the PVA-NFs.

RESULTS AND DISCUSSION:

PEP condensed pmiR-31 into NPs with sizes of <100 nm and a zeta potential of ~10 mV from N:P ratios ≥4. Furthermore, transfection analysis with each cell line revealed significant upregulation of miR-31. Expression of miR31 increased cell migration rates in the HaCat (**, P=0.0062), HMEC-1 (*, P=0.0286) and NCTC-929 (*, P=0.0454) cell lines. Treatment increased the proliferative capacity of the HaCat (**, P=0.0028) and the NCTC-929 (*, P=0.0311) cell lines, and resulted in a significant increase in the rate of angiogenesis (*, P=0.0202) with HMEC-1 cells. Complete release of all loaded NPs from the PVA-NFs was achieved within 48 h, and NPs were found to be of comparable size and zeta potential to fresh NPs. The released NPs also successfully transfected each cell line and induced significant functional outcomes

CONCLUSION:

Results indicate miR-31 upregulation increases cell migration and proliferation of fibroblast, keratinocyte and endothelial cells, as well as angiogenesis, which should provide an environment that promotes healing. The combination of PVA-NFs with NPs forms the basis of a functional chronic wound healing system. The next stage of this research will involve functional assessment of the device in vivo, in a diabetic murine wound model.

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Utilisation of biomaterials for wound treatment 10:30 - 12:00 Room 0.2/0.3 11/09/2018

Oral presentation

180 Treatment of bacterial infection in a 3D skin model by transdermal application of curcumin

Berna Senturk[1], Claudia Fessele[1], Brigitte von Rechenberg[2], Friedrich von Hahn[3], Markus Rottmar[1]

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INTRODUCTION:

Chronic wounds represent a significant burden to patients and health care systems. Current treatments such as wound dressings, hydrogels and drugs for chronic wounds fail to achieve effective therapeutic outcomes. Local delivery of active pharmaceuticals is an interesting, yet insufficiently explored therapeutic approach for treating chronic skin wounds. For this, ideal pharmaceuticals should have both anti-microbial and wound healing-promoting properties, as well as potential for easy and extensive topical application in clinical settings. Although wound healing properties of curcumin have been described previously, its activity is limited by poor solubility and difficult application to the wound area[1]. In this work, the anti-microbial and anti-inflammatory properties of curcumin, and its delivery via transdermal application to promote wound healing were investigated using different in vitro models.

METHODS:

Cell viability, migration and proliferation were investigated by alamarBlue assay, wound scratch assay and brdU proliferation assay, respectively. Anti-inflammatory effect of curcumin was examined by RT-PCR analysis of pro-inflammatory markers (CD197, CXCL10) on human THP-1 derived macrophages treated with LPS. The antibacterial and anti-biofilm activity of curcumin was tested on *S. aureus* and *P. aeruginosa*.

A 3D full thickness skin model, encompassing a dermal and an epidermal compartment consisting of primary human fibroblasts, and primary human keratinocytes differentiated with air-lift culture was established. We use the 3D skin model to study the effects of curcumin on skin infection. For this, curcumin is applied to the infected skin using TDA technology and the healing process is evaluated by histology.

RESULTS AND DISCUSSION:

Expression of pro-inflammatory markers CD197 and CXCL10 was significantly reduced in curcumin treated THP-1 cells compared to control (Figure1). Notably, with increasing curcumin concentration, cell viability decreased while the anti-inflammatory effect increased. Curcumin treatment had an anti-bacterial effect on S.aureus, but was not efficient on *P. aeruginosa*.

CONCLUSION:

Curcumin shows anti-inflammatory and anti-bacterial activity and is thus promising for the treatment of chronic wounds. Furthermore, these results strongly suggest that transdermal application of curcumin is a feasible approach to support the impaired healing process in chronic wounds.

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ACKNOWLEDGMENTS:

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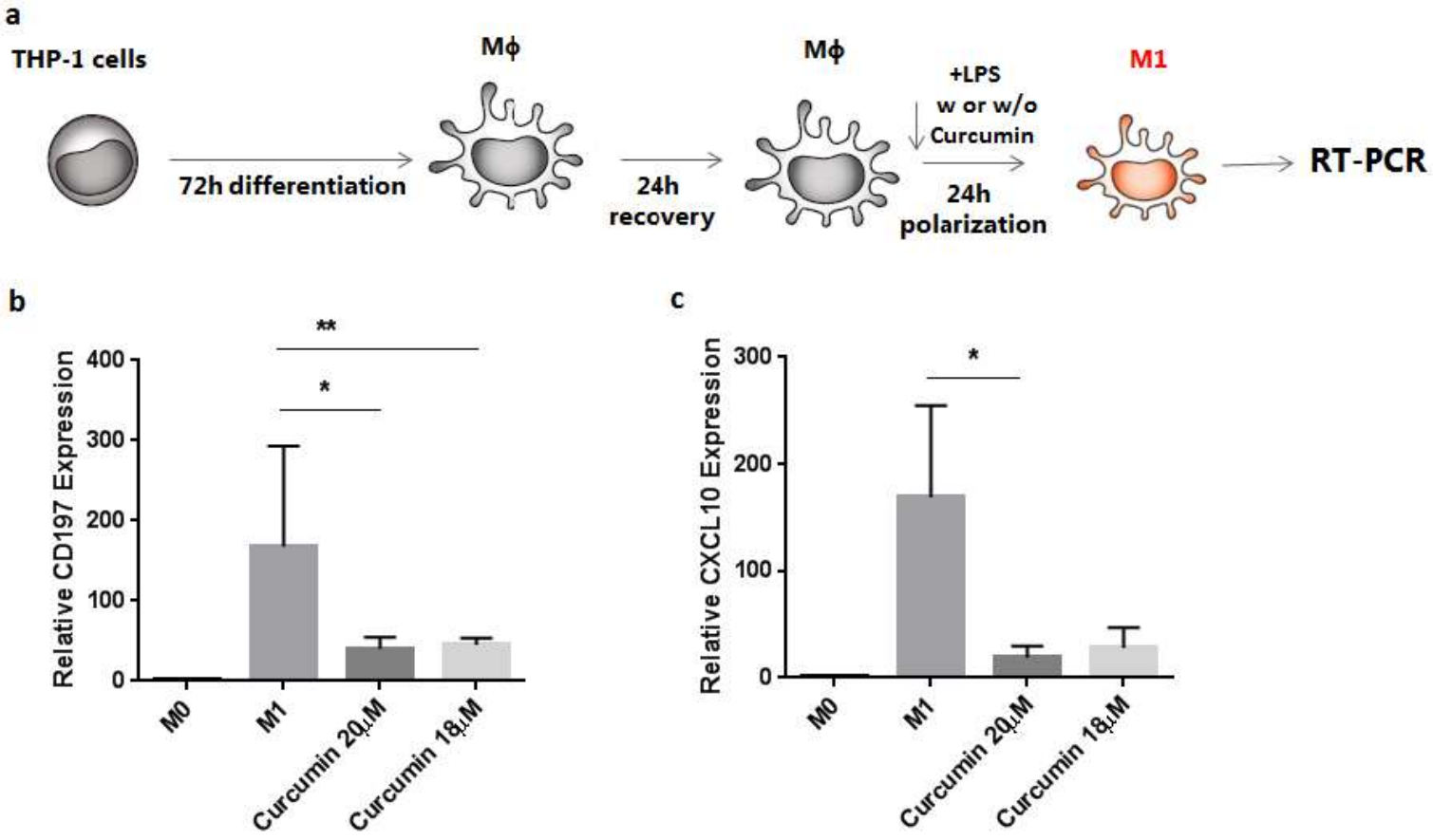

Picture 1: Caption 1: Figure 1. RT-PCR analysis of anti-inflammatory activity of curcumin treatment.

Utilisation of biomaterials for wound treatment 10:30 - 12:00 Room 0.2/0.3 11/09/2018

Oral presentation

282 DEVELOPMENT OF AN AEROGEL BASED ON GRAPHENE OXIDE AND POLYVINYL ALCOHOL WITH POTENTIAL OF TRANSDERMAL USE

Katherina Fernandez, Mellado Constanza, Toribio Aguilar, Manuel Melendrez, Berta Schulz
University of Concepcion, Concepcion, Chile

INTRODUCTION:

Effective hemostatic materials that can rapidly control bleeding are vital for wounds healing, as excessive hemorrhaging is the main cause of trauma death[1]. Graphene-based materials, as graphene oxide (GO) has been successfully explored as a nanomaterial for biological applications, one of which is a platform for load drugs[2], but not yet natural compounds such as Proanthocyanidins (PAs). The aim of this research is to develop a dry and stable composite aerogels based on graphene oxide (GO) and polyvinyl alcohol (PVA). Furthermore, we incorporated natural extract of País grape seed (SD) and skin (SK), rich in Proanthocyanidins (PAs or condensed tannins), into the aerogels and compared the effectiveness of each one, in order to generate a new material for use in dermal, hemostatic and/or healing applications.

METHODS:

An aerogel was elaborated based on GO functionalized with PVA and loaded with natural extracts from seed and skin grapes, high in PAs. The aerogels were characterized by SEM, AFM, ξ-potential, elemental composition, FT-IR, and XDR. The effect of the incorporation of the grape extracts was investigated in relation to the aerogels' structure, coagulation performance and the release of the extracts (kinetic of adsorption and desorption).

RESULTS AND DISCUSSION:

The results demonstrated that the aerogels have a porous structure and low density, capable of absorbing water and blood (73 times their own weight). The incorporation of PA extracts into the aerogel increased the coagulation of whole blood. The negative zeta potential of the material also rose by 33% (-18.3 ± 1.3 mV), and as a consequence the coagulation time was reduced by 37% and 28% during the first 30 and 60 seconds of contact between the aerogel and whole blood, respectively. The release of extracts from the GO-PVA-SD and GO-PVA-SK aerogels was prolonged to 3 h but reached only 20%, probably due to the existence of strong binding between PAs and GO-PVA, both characterized by the presence of aromatic and hydroxyl groups that can form non-covalent bonds but strong and stable enough to avoid a greater release into the medium.

CONCLUSION:

This work provides a new GO-based aerogel, which has a great potential use in the field of dermal delivery.

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ACKNOWLEDGMENTS:

The authors thank Project Fondecyt nº1170681 for the financial support for this investigation.

Picture 1:

DEVELOPMENT OF AN AEROGEL BASED ON GRAPHENE OXIDE AND POLYVINYL ALCOHOL
WITH POTENTIAL OF TRANSDERMAL USE

Utilisation of biomaterials for wound treatment 10:30 - 12:00 Room 0.2/0.3 11/09/2018

Oral presentation

42 Tiger 17 functionalized onto PVA/CA films accelerates clotting time and reduces microbial action

Helena P. Felgueiras, M. Teresa P. Amorim
Centre for Textile Science and Technology, University of Minho, Guimarães, Portugal

INTRODUCTION:

Typically, acute wound healing is a well-organized process that evolves/ends in a predictable amount of time. Chronic wounds result from gradual tissue degradation, and are characterized by defective cell matrix, high bacteria counts, prolonged inflammation and moisture imbalance[1]. Treatment of chronic wounds requires expensive and individualized therapies: mechanical debridement to remove necrotic tissue, topical administration of antibiotics/anti-inflammatory agents, dressings to provide moisture/manage exudates, and drugs to promote tissue regeneration[2]. Antimicrobial dressings, that combine dressing and antiseptics/antibiotics in one formulation, have been suggested as potential strategies to treat chronic wounds[3]. However, the rising of antibiotic-resistant infection agents has turned these systems obsolete, revealing antimicrobial-peptides (AMPs) as viable alternatives. AMPs aside from displaying a broad spectrum of activity against pathogens, act rapidly at multiple sites within microbial cells, reducing their propensity to develop resistance[4].

In this work, poly(vinyl alcohol) (PVA) and cellulose-acetate (CA), all well-established polymers in biomedicine, were prepared in the form of films and functionalized with Tiger17 (c[WCKPKPKPRCH-NH_2]). Tiger17 is a little explore AMP endowed with immunoregulatory abilities with great potential for wound healing. Its antimicrobial features are unknown.

METHODS:

PVA and CA films were produced by phase inversion. PVA was prepared at 10w/v% in dH_2O, autoclaved at 120ºC for 20min, and combined with glutaraldehyde. CA films were prepared at 10w/v% in acetone at RT. All traces of acetone were eliminated by periodic exchange of dH_2O bath. Commercial woven-swabs were used as control. Tiger17 was prepared at 10-40µg/mL in pure water and functionalized using dopamine as binding agent or combined with the polymer ("all-in-one" approach). AMP functionalization was confirmed using sulfo-SDTB. SEM, ATR-FTIR, DMA and contact-angle techniques were used for characterization purposes. Tiger17 physiological stability was evaluated in presence of proteolytic enzymes. Tiger17 antimicrobial performance was tested against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli*. Its hemocompatibility and clotting-time was determined using human platelets and by following the loss of movement of re-calcified plasma, respectively.

RESULTS AND DISCUSSION:

Tiger17 presence on PVA and CA films was confirmed. Films were very hydrophilic, possessed an interconnected-porous structure and were stable to enzymatic degradation. Preliminary testing revealed Tiger17 functionalized films to reduce bacterial presence compared to control, and to accelerate clotting time. Biological testing are still ongoing.

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Utilisation of biomaterials for wound treatment 10:30 - 12:00 Room 0.2/0.3 11/09/2018

Oral presentation

16 Colloidal wound dressing with high tissue adhesiveness for digestive system cancer therapy

Akihiro Nishiguchi, Tetsushi Taguchi
National Institute for Materials Science, Ibaraki, Japan

INTRODUCTION:

Minimally-invasive therapies using endoscopy such as endoscopic submucosal dissection has attracted increasing attention for the treatment of digestive system cancer. Although wound healing of tissues after the dissection is important to avoid scar contracture, bleeding, and inflammation, commercially available materials lack tissue adhesiveness, biodegradability, and easy handling for delivery. In this study, we aim to develop a colloidal wound dressing with strong adhesiveness to tissues after digestive system cancer treatment (Figure 1a).

METHODS:

Porcine gelatin (Mw=100 kDa) was modified with fatty aldehydes with various alkyl groups (C2~C12) though Schiff base. The gelatin particles were prepared by spray drying and thermally crosslinked at 150 ^{o}C for 3 hours. Adhesion strength was evaluated using porcine stomach according to ASTM (F-2258-05).

RESULTS AND DISCUSSION:

Hydrophobically-modified gelatin was synthesized through the reaction between fatty aldehydes with various alkyl groups and amine groups in gelatin. We previously reported that hydrophobically-modified gelatins show strong adhesiveness to soft tissues[1]. We prepared micro/nano-meter sized particles of hydrophobically-modified gelatin by spray drying method and thermal crosslinking method (Figure 1b). Hydrophobized gelatin particles strongly adhered to the tissue surface under wet conditions after dissection of mucosal tissue (Figure 1c). Adhesion strength depended on the degree of substitution and the length of alkyl groups in fatty aldehydes, indicating that hydrophobic groups increased tissue adhesiveness.

CONCLUSION:

This colloidal wound dressing would be promising for promoting wound healing after the dissection of digestive system cancer.

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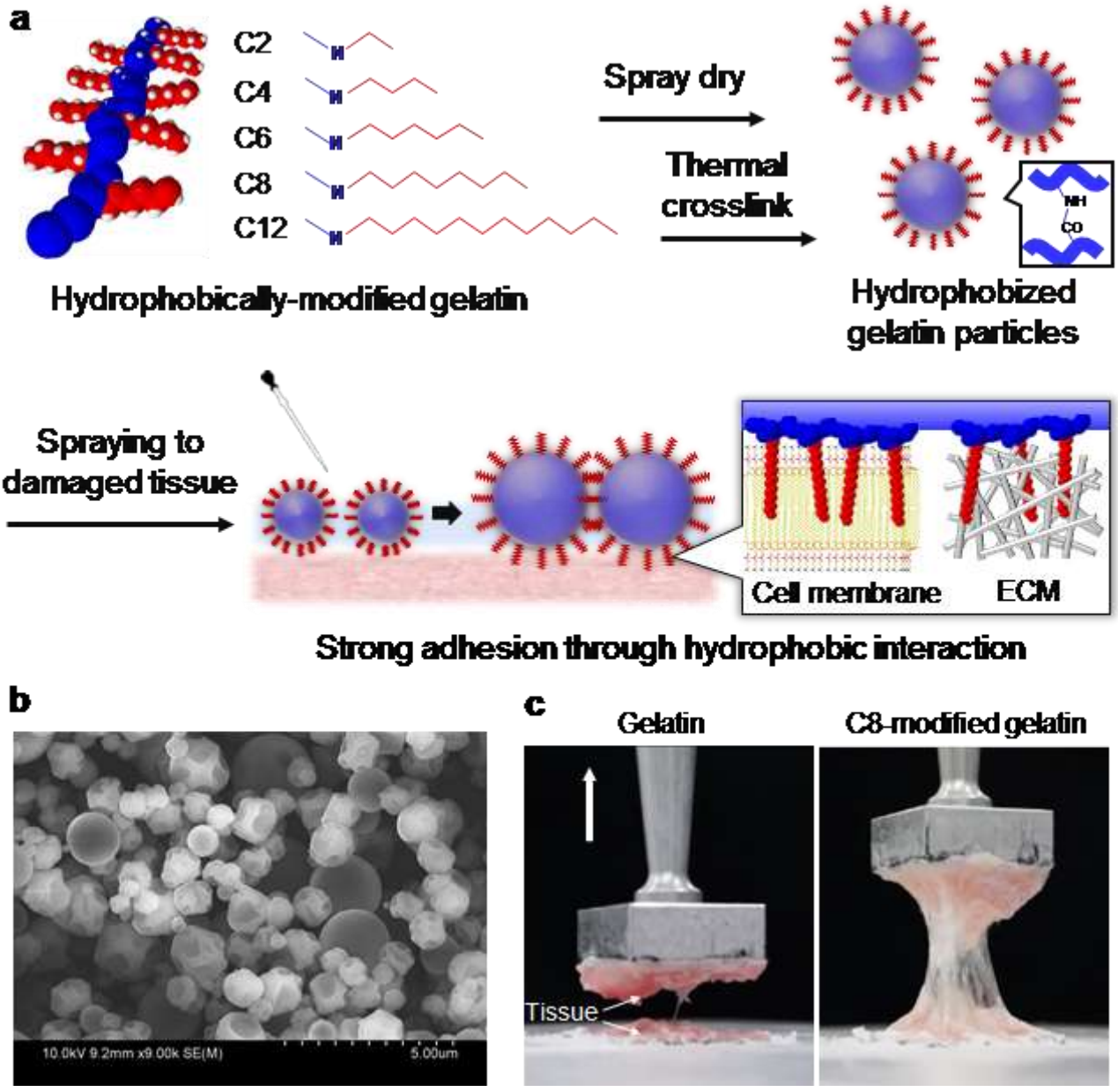

Picture 1: Caption 1: Figure 1. (a) Schematic illustration of colloidal wound dressing composed of hydrophobically-modified gelatin and the adhesion to living tissues. (b)

Biomaterials for anti-cancer therapy 14:00 - 15:30 Room 0.2/0.3 11/09/2018

Oral presentation

782 Development of biomaterials-based immunotherapy: chitosan and poly(gamma)-glutamic acid nanoparticles as immunomodulatory players at the tumor microenvironment

Flávia Castro[1], Marta L. Pinto[2], Andreia M. Silva[1], Catarina L. Pereira[1], Graciosa Q. Teixeira[1], José H. Teixeira[1], Maria G- Lázaro[1], Susana G. Santos[1], Mário A. Barbosa[1], Olivier de Wever[3], Karine Serre[4], Raquel M. Gonçalves[5], Maria J. Oliveira[5]

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[5]I3S-Instituto de Investigação e Inovação em Saúde, Portugal

INTRODUCTION:

The tumor microenvironment is a complex and dynamic niche that has a fundamental role in tumor cell proliferation, angiogenesis, invasion and metastasis. Macrophages (Mac) are described as having a central role in tumor progression and dendritic cells (DC) as having an immature/immunosuppressive profile, which limits T cells activity. Therapeutic strategies targeting these cells have been emerging as adjuvants to anticancer conventional treatments, aiming at their recruitment and repolarization towards an immunostimulatory phenotype favoring cancer cell elimination. Here, we focused on the potential of Chitosan/Poly(γ-glutamic acid) nanoparticles (NPs) to modulate cellular immunity and, consequently, to affect cancer-cell related activities.

METHODS:

NPs were prepared by co-acervation method. Primary human monocyte-derived M2 Mac (obtained by stimulation with IL-10) were incubated with NPs. Control experiments with unstimulated Mac and LPS-stimulated Mac (M1 phenotype) were performed. After 72h, cell metabolic activity, cell phenotype and cytokine production were evaluated. Mac ability to induce T cell proliferation/activation and tumor cell invasion were also assessed. The same studies were performed with human-monocyte derived DC. For *in vivo* models, $1x10^6$ E0771 cells were injected in the mammary fat pad of C57BL/6 mice. After 10 days of tumor implantation, animals were treated every two days with NPs. Tumor growth was assessed by caliper every two days.

RESULTS AND DISCUSSION:

NPs re-educated IL-10-stimulated Mac towards a pro-inflammatory profile, decreasing CD163 expression and promoting IL-12p40 and TNF-α secretion. NPs also induced an immunostimulatory phenotype on DC, enhancing the expression of the co-stimulatory molecules CD86, CD40 and HLA-DR, and secretion of the pro-inflammatory cytokines TNF-α, IL-12 and IL-6. Interestingly, these phenotypic alterations induced both $CD4^+$ and $CD8^+$ T cell activation/proliferation and counteracted cancer cell invasion*, in vitro*. Regarding the *in vivo* tumor model, we observed a decrease in tumor growth in NPs-treated animals comparing to the control, after 23 days of E0771 cell implantation. Additional experiments with other tumor models are currently being explored.

CONCLUSION:

Overall, our findings open new perspectives on the use of NPs as an immunomodulatory therapy for tumor microenvironment reprogramming, providing a new tool for anticancer therapies.

ACKNOWLEDGMENTS:

The authors would like to thanks to FEDER funds through the COMPETE 2020 Operacional Programme for Competitiveness and Internationalisation (POCI), and to FCT- Fundaçao para a Ciência e a Tecnologia in the framework of the project “Institute for Research and Innovation in Health Sciences” (POCI-01-0145-FEDER-007274) and through their FCT PhD Programmes and FCT Investigator Programme and by Human Capital Operational Programme (POCH), specifically by the BiotechHealth Programme.

Biomaterials for anti-cancer therapy 14:00 - 15:30 Room 0.2/0.3 11/09/2018

Oral presentation

523 Conjugated linoleic acid functionalization enhances superparamagnetic iron oxide nanoparticles (SPIONs) cytotoxicity in 4T1 mouse breast cancer cells modulating PPARs

Marina Ricci[1], Giuliana Muzio[1], Ester Borroni[2], Cristina Multari[3], Marta Miola[3], Sara Ferraris[3], Enrica Vernè[3], Rosa Angela Canuto[1], Antonia Follenzi[2]
[1]University of Torino, Torino, Italy
[2]University of Piemonte Orientale, Italy
[3]Polytechnic of Torino, Italy

INTRODUCTION:

Superparamagnetic Iron Oxide Nanoparticles (SPIONs) are promising cancer nanomedicine candidates for diagnostics and therapeutics, thanks to the improved tumor targeting and delivering chemotherapeutic drugs, nucleic acids, monoclonal antibodies or engineered viral vectors[1]. Conjugated linoleic acid (CLA) has proven anti-tumor properties[2], thus, SPIONs functionalized with CLA (SPIONs+CLA) were prepared to improve their cytotoxicity[3]. Since SPIONs+CLA are more effective than SPIONs alone in reducing mouse breast cancer cell viability, the mechanisms underlying the effects of SPIONs+CLA were investigated paying particular attention to the type of cell death induced, to the effect on inflammation process and to the possible involvement of PPARs (Peroxisome Proliferator-Activated Receptors). PPARs were investigated because CLA is a natural ligand of these nuclear hormone receptors that regulate the expression of genes involved in several intracellular signal transduction pathways, including proliferation and survival.

METHODS:

Cell viability and type of cell death were evaluated in 4T1 mouse breast cancer cells by flow cytometry; proliferation regulation *(PPARγ)* by western blotting; modulators of inflammation by western blotting (PPARα) and ELISA (TNFα, IL-1β). An in vivo biodistribution study was performed injecting SPIONs (10 mg/g of mouse) through the tail vein of NSG mice. After 2 weeks the presence of SPIONs in different organs was evidenced by iron staining.

RESULTS AND DISCUSSION:

Flow cytometry analyses showed that both free and CLA-conjugated SPIONs decreased cell survival while inducing necrotic cell death, being the stronger effect observed in the presence of SPIONs+CLA after 48 hours. After 72 hours, the cells treated with CLA-SPIONs, restarted growing. Decreased cell proliferation inversely correlated with *PPARγ protein expression, a negative modulator of cell proliferation.* The induction of cell death associated with the increase of pro-inflammatory TNFα and IL-1β and by the decrease of anti-inflammatory PPARα. The analysis of biodistribution evidenced SPIONs accumulation mainly in the spleen and, to a lesser extent, in the liver, being the higher uptake for CLA-SPIONs.

CONCLUSION:

The results show that CLA-functionalized SPIONs effectively impair breast cancer cell growth triggering necrotic cell death. The cytostatic/cytotoxic effect seems to be correlated with changes in PPARs and in pro-inflammatory mediator release.

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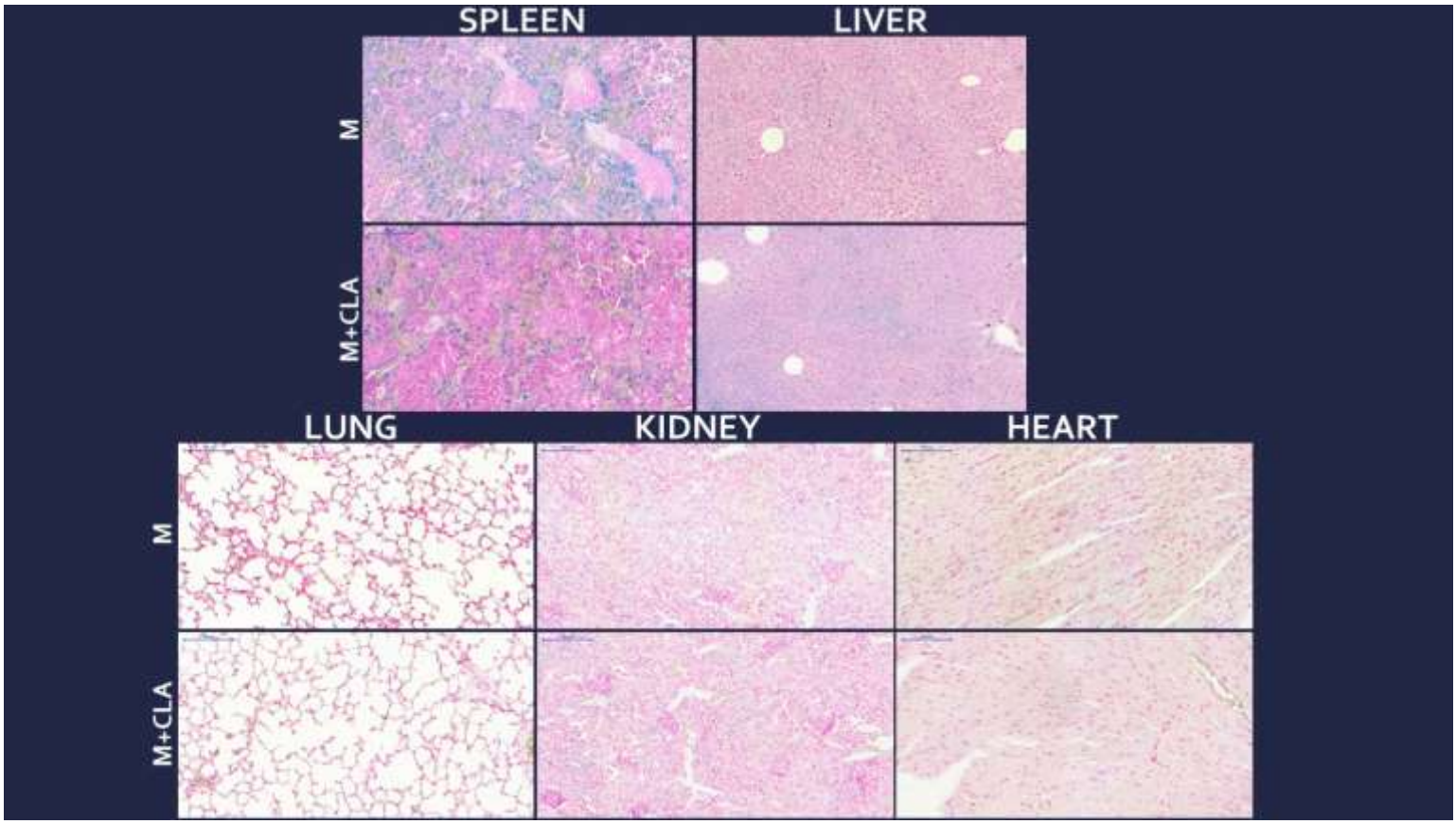
ACKNOWLEDGMENTS:

This work was supported by AIRC, Italy: IG n. 13166, to A.F., from Compagnia di San Paolo, Italy, and from the University of Torino, Italy.



Picture 1: Caption 1: SPIONs accumulation in different organs.

Biomaterials for anti-cancer therapy 14:00 - 15:30 Room 0.2/0.3 11/09/2018

Oral presentation

245 Development of a mRNA Vaccine in a Microneedle Patch for Castrate Resistant Prostate Cancer

Emma Mcerlean
Queen's University Belfast, Belfast, United Kingdom

INTRODUCTION:

RALA peptide has significantly improved the potency of a DNA vaccine where the E6/E7 antigens were delivered via polymeric microneedle (MN) patches; successfully inducing potent cytolytic T-cell responses, delaying tumour initiation in a prophylactic model, and slowing tumour growth in a therapeutic model of cervical cancer [1–3]. Messenger RNA (mRNA) vaccines are more potent than DNA, with smaller quantities stimulating greater immune responses [4,5]. In this study, mRNA has been designed to correlate with the stages of CRPC and formulated into nanoparticles (NPs) with RALA, lyophilised and loaded into MN patches for delivery to resident immune cells in the skin.

METHODS:

RALA complexed mRNA in NPs, lyophilised with 5% trehalose and characteristics of NPs were assessed by DLS. *In vitro* functionality was assessed by transfection in HaCaT keratinocyte and DC2.4 dendritic cells. Following loading of RALA/mRNA NPs into polyvinyl alcohol (PVA) MN patches, strength and penetration across the stratum corneum were analysed using optical coherence tomography, and NP integrity and functionality assessed. *In vivo* NP release from MN patches and gene expression were analysed in C57/BL6 mice.

RESULTS AND DISCUSSION:

RALA/mRNA formed stable NPs and transfected HaCaT and DC2.4 cells successfully, with negligible toxicity. NP loaded MN patches were resistant to compression with no fracturing observed; indicating MN/NP patches retain integrity under application pressure. Lyophilisation of NPs facilitated increased mRNA dose delivery, and MN/NP integrity and functionality confirmed that RALA protects genetic cargo in the MN polymeric matrix. Following *in vivo* application, NPs were released from the MN patch resulting in localised gene expression, demonstrating the functionality of the MN/RALA/mRNA.

CONCLUSION:

This technology consists of i) RALA peptide, to condense mRNA into NPs, protect from degradation, facilitate intracellular delivery of mRNA; and ii) a polymeric MN patch encapsulating NPs, dissolving upon intradermal insertion, releasing mRNA cargo to skin-resident dendritic cells. Future work will utilise the MN/RALA/mRNA platform to deliver clinically relevant tumour-associated antigens (TAAs); stimulating an immune response for both prophylactic and therapeutic vaccination against castrate resistant prostate cancer.

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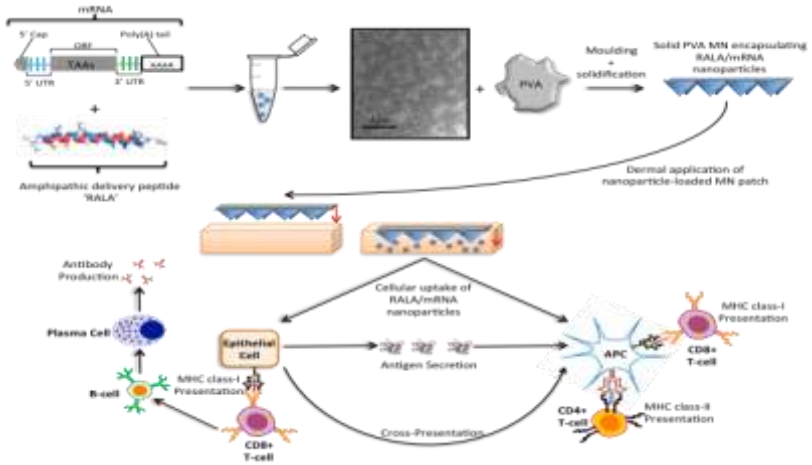

Picture 1: Caption 1: Figure 1: Schematic of RALA/mRNA nanoparticle encapsulation into, and subsequent release from, dissolvable PVA MN patches for intradermal immunisation

Biomaterials for anti-cancer therapy 14:00 - 15:30 Room 0.2/0.3 11/09/2018

Oral presentation

169 Glucose-trigger hydrogen peroxide production for antitumor treatment combine with gene silencing therapy and photothermal therapy

Zi-Lin Zhou, Hung-Wei Yang
National Sun Yat-sen University, Kaohsiung, Taiwan

INTRODUCTION:

Lung cancer (LC) is the second most common cancer. There're two major types of lung cancer: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), about 75% of NSCLC is diagnosed at an advanced stage which isn't amenable to surgery. Prohibitin 1 (PHB1) belongs to prohibitin family and encoded by nuclear genes PHB. PHB1 has been shown have an action to block cell cycle progression, and also found to be overexpressed in many tumors.[1-3] Therefore, we developed multifunctional core-shell nanoparticles (NPs) using polypyrrole (PPy) coating based on glucose oxidase (GOD)-adsorbed gold NPs (PPy@GOD/AuNPs) for glucose-trigger hydrogen peroxide (H_2O_2) production/photothermal therapy (PTT) with PHB1 gene silencing for the combination LC treatment.

METHODS:

The PPy@GOD/AuNPs were prepared by incubating the GOD/AuNPs with Py solution for 30min at 25°C then the $FeCl_3$ was further added in the solution for Py polymerization. The purified PPy@GOD/AuNPs, which can be used to adsorb the pSUPER-gfp-siPHB1 on the surface to form pSUPER@PPy@GOD/AuNPs. After that, poly(β-amino ester) (PAE) in DMSO (300 μg/μL) was diluted to 3 μg/μL in 25 mM acetate buffer (pH 5), and rapidly combined with pSUPER@PPy@GOD/AuNPs, the mixed solution was allowed to form PAE@pSUPER@PPy@GOD/AuNPs for 15 min (Figure 1A). The obtained PAE@pSUPER@PPy@GOD/AuNPs were then incubated with LC cells to investigate the cell uptake, photothermal efficiency, H_2O_2 production, and gene expression.

RESULTS AND DISCUSSION:

We have successfully formulated PAE@pSUPER@PPy@GOD/AuNPs with uniform size distribution of 45 nm (Figure 1B). What attracted us is that the synthesized PPy/AuNPs display strong NIR absorption with a broad peak at ~850 nm (Figure 1C). We next investigated the photothermal effect of PPy@GOD/AuNPs, the temperature increased to 49.3°C from 29°C after 5 min irradiation by 808 nm laser with a power density of 1 W/cm 2 (Figure 1D). After incubation with U87 cells, the PAE@pSUPER@PPy@GOD/AuNPs can be taken into the cytoplasm to express the GFP showing green fluorescence (Figure 1E).

CONCLUSION:

In this study, we have successfully formulated PAE@pSUPER@PPy@GOD/AuNPs and the results shown the PAE@pSUPER@PPy@GOD/AuNPs are highly biocompatible and potential to be used for combination therapy in LC.

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ACKNOWLEDGMENTS:

This work was supported by the Ministry of Science and Technology, Taiwan (R.O.C.), for the financial assistance provided (MOST106-2628-B-110-001-MY4, MOST106-2628-E-110-001-MY3).

Picture 1: Caption 1: Figure 1

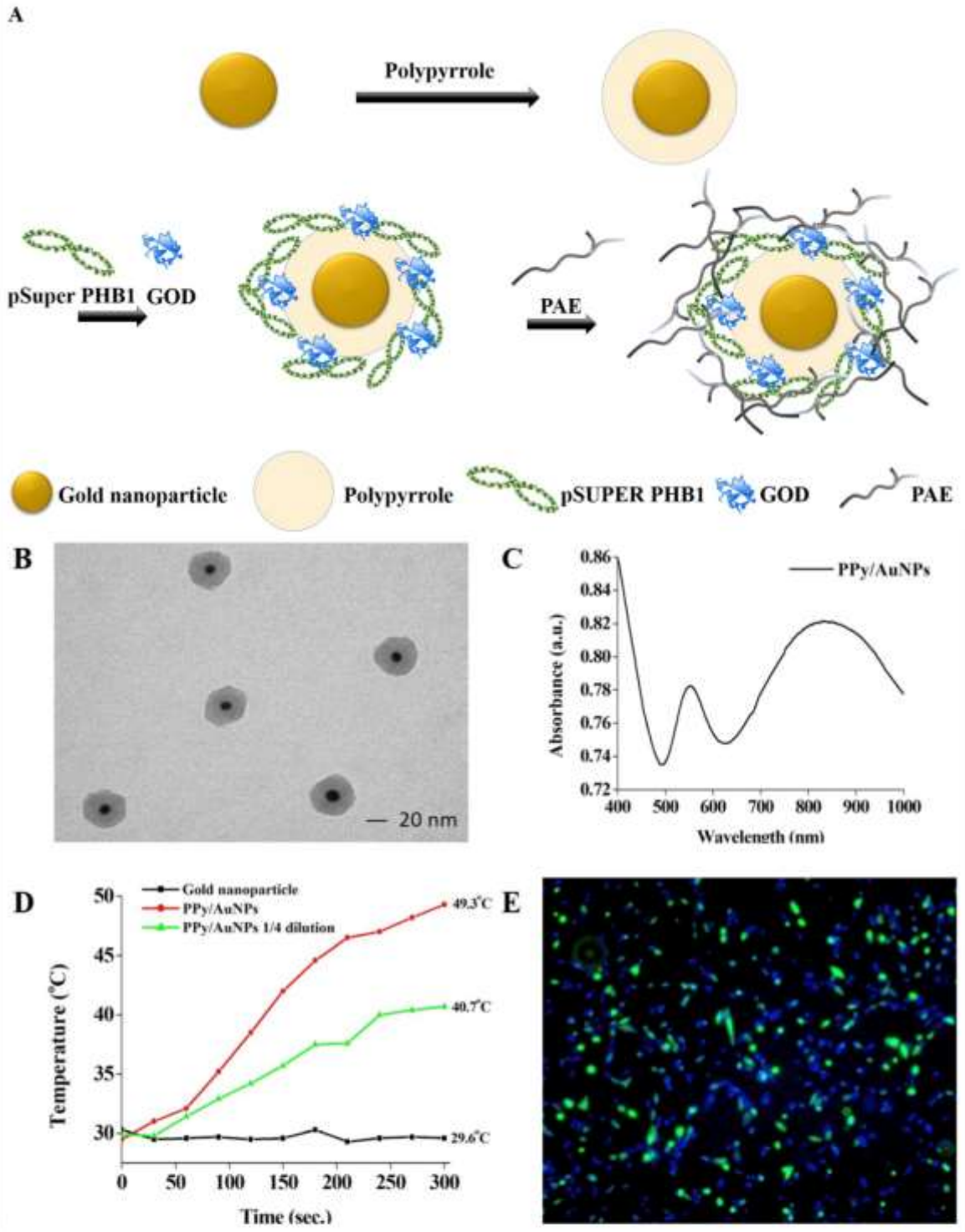

Figure 1. (A) Schematic diagram of the multifunction PAE@pSUPER@PPy@GOD/AuNPs.(B) TEM image of PPy coated AuNPs (PPy/AuNPs). (C) The UV-Vis-NIR spectra of Ppy/AuNPs. (D) Photothermal efficiency of PPy/AuNPs after irradiation by 808 nm laser with a power density of 1 W/cm^2 for 5 min. (E) Fluorescence image of U87 cells incubated with PAE@pSUPER@PPy@GOD/AuNPs for 48 h (green: GFP expression; blue: cell nuclei).

Biomaterials for anti-cancer therapy 14:00 - 15:30 Room 0.2/0.3 11/09/2018

Oral presentation

698 Synergistic toxic effect of marine-origin polymeric nanoparticle loaded with gemcitabine over human breast cancer cells

Catarina Oliveira, Nuno m Neves, Rui l. Reis, Albino Martins, Tiago h Silva
3B's Research Group, Guimarães, Portugal

INTRODUCTION:

Breast cancer is the most common cancer among women. To circumvent some drawbacks of current treatments, the use of natural polymers as biologically active compounds or in drug delivery devices may be a promising therapeutic alternative due to their similarities to native tissues. Fucoidan has been reported to present different biological activities, namely antitumor. However, from a previous study we observed that not all fucoidan extracts presented the desired antitumor behavior, which was related with different chemical features. In this work, a non-antitumor fucoidan extract was used to develop nanoparticles for the delivery of anticancer drugs.

METHODS:

Two marine origin polymers, fucoidan (negatively charged) and chitosan (positively charged), were chosen to produce nanoparticles by polyelectrolyte complexation. Gemcitabine, a hydrophilic drug commonly used to treat breast cancer, was encapsulated into the nanoparticles and the entrapment efficiency, release profile and the toxicity of the developed nanoparticles were evaluated over human endothelial and breast cancer cells.

RESULTS AND DISCUSSION:

Nanoparticles final formulation presented a size around 115-140 nm and a polydispersity index <0.2, demonstrating nanoparticles' size homogeneity. Crosslinked and non-crosslinked nanoparticles were stable up to 2 months in storage conditions, whereas crosslinked nanoparticles were more stable at physiological conditions, as expected. Nanoparticles cytotoxic effect was evaluated over human breast cancer and endothelial cells, being only toxic for the highest concentrations tested. Gemcitabine was used as antitumor drug model and encapsulated at a maximum entrapment efficiency of 35-42%. Drug release studies demonstrated that around 84% of gemcitabine is released within 4h. Cytotoxicity results of gemcitabine-loaded nanoparticles showed increased toxicity (around 25%) when compared with free gemcitabine, without increasing toxic effects over human endothelial cells.

CONCLUSION:

The devolved drug delivery system was able to increase the toxic effects over human breast cancer cells without affecting endothelial cells viability. This system may be a promising and interesting approach to be further explored on the development of more effective breast cancer therapies.

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Figure 1. A) Nanoparticles characterization B) Nanoparticles morphology observation by SEM and C) Cytotoxic effects of gemcitabine-loaded nanoparticles (NPs_GEM) and free gemcitabine (gem) over human breast cancer cell lines at day 3.

ACKNOWLEDGMENTS:

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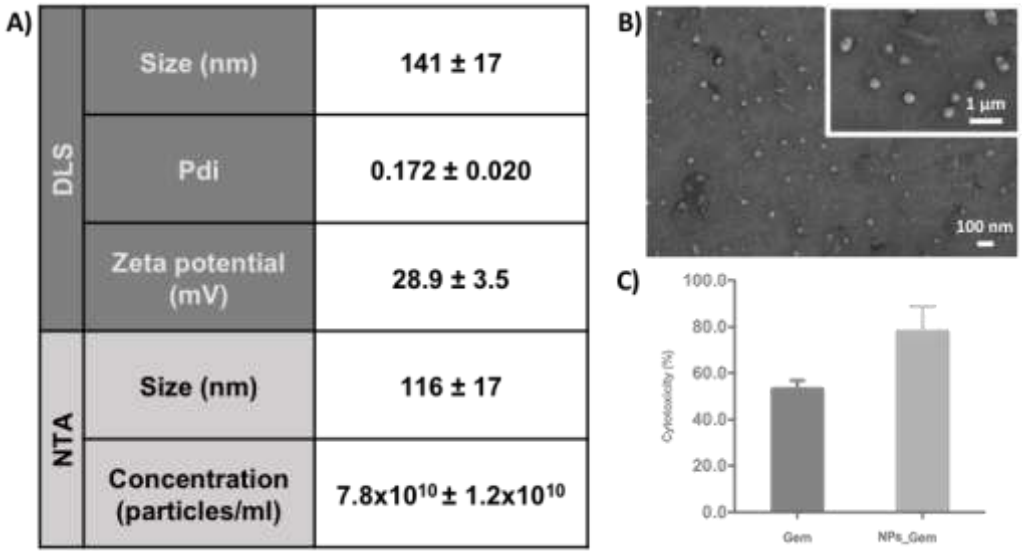


Picture 1: Caption 1: Please see references section for Figure 1 caption.

Biomaterials for anti-cancer therapy 14:00 - 15:30 Room 0.2/0.3 11/09/2018

Oral presentation

368 Development of yttrium-containing magnetic microspheres for intra-arterial hyperthermoradiotherapy

Masakazu Kawashita[1], Eisuke Nagabuchi[1], Tomoyuki Ogawa[1], Masahiro Hiraoka[2]
[1]Tohoku University, Miyagi, Japan
[2]Japanese Red Cross Wakayama Medical Center, Japan

INTRODUCTION:

Radiotherapy and hyperthermia are method of curing cancer without surgical resection, but the external irradiation or external heating have drawbacks such as unwanted damages to healthy tissues and low efficacy against deep-seated tumor. Yttrium (Y)-containing magnetic microspheres 20–30 μm in size might be useful for intra-arterial hyperthermoradiotherapy since they can deliver β-ray to tumor as well as heat the tumor under alternating current (AC) magnetic field when injected into capillary vessel of tumor after neutron bombardment. In this study, we investigated the structure, magnetic property and *in vitro* heat-generating ability of Y-containing magnetic ceramics and then attempted to prepare microspheres composed of the Y-containing magnetic ceramics.

METHODS:

Sodium hydroxide aqueous solution was added to yttrium nitrate – iron nitrate aqueous solution under stirring. The resultant precipitates were collected by centrifuge separation and heated at 700-1100ºC for 1 h to obtain Y-containing magnetic ceramics. On the other hand, gelatin droplets containing the precipitates were dispersed and solidified in corn oil, collected by filtration and finally heated at 1000ºC for 1 h to obtain microspheres composed of Y-containing magnetic ceramics. The structure and magnetic property of the samples were investigated by X-ray

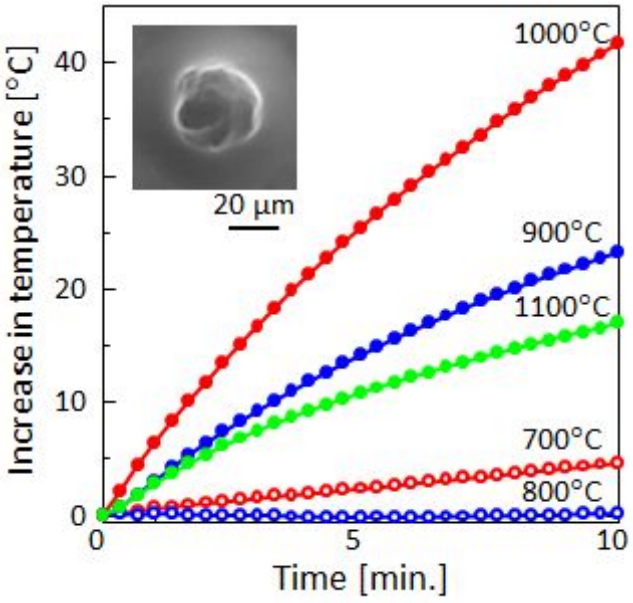
diffractometer and vibrating sample magnetometer, and the *in vitro* heat generation of the samples in agar phantom was measured under AC magnetic field of 300 Oe at 100 kHz.

RESULTS AND DISCUSSION:

α-Fe_2O_3, $YFeO_3$ and $Y_3Fe_5O_{12}$ started to be formed in the precipitates heated at 700, 800 and 900ºC, respectively. The precipitates heated at 800ºC or below hardly generated heat, but those heated at 900ºC or above generated heat under 300 Oe at 100 kHz (Figure 1). The precipitate heated at 1000ºC showed higher heat-generating ability than one heated at 1100ºC although a larger amount of antiferromagnetic α-Fe_2O_3 was formed in the precipitate heated at 1000ºC than one heated at 1100ºC. This is because the higher coercive force of precipitate heated at 1000ºC provides the hysteresis loop with larger area under 300 Oe. Finally, we obtained microspheres around 25 μm (inset in Fig. 1) providing the hysteresis loop with large area under 300 Oe, which suggests that the microspheres can show excellent heat-generating ability.

CONCLUSION:

Y-containing magnetic ceramics with excellent heat-generating ability were obtained by co-precipitation from yttrium nitrate – iron nitrate aqueous solution and subsequent heat treatment at 1000ºC. Further, we successfully prepare microspheres 20–30 μm in size, which were composed of the Y-containing magnetic ceramics. The microspheres would be useful for intra-arterial hyperthermoradiotherapy.



Picture 1: Caption 1: Time-dependent temperature curves of the agar phantoms in which the precipitates were dispersed under AC magnetic field of 300 Oe at 100 kHz.

Imaging biomaterials & environment 16:00 - 17:30 Room 0.2/0.3 11/09/2018

Oral presentation

251 Structural details about the interface between biodegradable Mg alloys and cells or tissue

Regine Willumeit-Römer[1], Jörg U. Hammel[1], Diana Krüger[1], Julian Moosmann[1], Florian Wieland[1], Silvia Galli[2], Berit Zeller-Plumhoff[1]

[1]Helmholtz Center Geesthacht, Geesthacht, Germany
[2]Faculty of Odontology, Malmö University, Sweden

INTRODUCTION:

Magnesium with its biodegradable properties can become an alternative to non-degradable metal implants. Still, it remains an open question how the material degrades in the body and how the material is finally remodelled into bone matrix. There are indications that bone-forming osteoblasts actively remodel the degradation layer[1] and that proteins can determine which precipitates are formed[2]. High-resolution small angle X-ray scattering (SAXS) offers a look into the ultrastructure of bone and material[3]. In combination with high-resolution computed tomography[4] a link between material degradation and bone structure can be established.

METHODS:

Mg-5Gd and Mg-10Gd screws were prepared at HZG (mold direct chill casting, solid solution heat treated (T4), extrusion and turning/milling). They were implanted in the femur of Sprague Dawley rats for 4, 8, and 12 weeks. Explants were measured at the imaging beamline P05, HZG at PETRA III / DESY, Hamburg for computed tomography (at 38.4 keV, 2.4 µm effective pixel size) and at P03 for SAXS experiments (at 13 keV, 1.7x1.7 μm^2 beam size). The P05 data was reconstructed using an in-house algorithm and the P03 data was analysed using Matlab. The data was manually registered and overlaid using Avizo 9.3.

RESULTS AND DISCUSSION:

Imaging revealed a very good contact between the degrading implant and the newly formed bone (Fig 1). Different degradation products are visible at the interface, due to the difference in greyscales. By registering the scanned SAXS region with the CT data (red-boxed subfigure) it is possible to overlay the degree of mineralization of the formed hydroxyapatite (yellow-boxed subfigure). The high attenuating regions (brighter greyscales) of the newly formed bone tissue coincide with regions of higher mineralisation (purple and yellow colours).

CONCLUSION:

With the combination of high-resolution small angle scattering and synchrotron tomography the quantification of mineralization and corrosion properties are possible. It thus becomes feasible to track the influence of Mg degradation on tissue growth and mineralisation.

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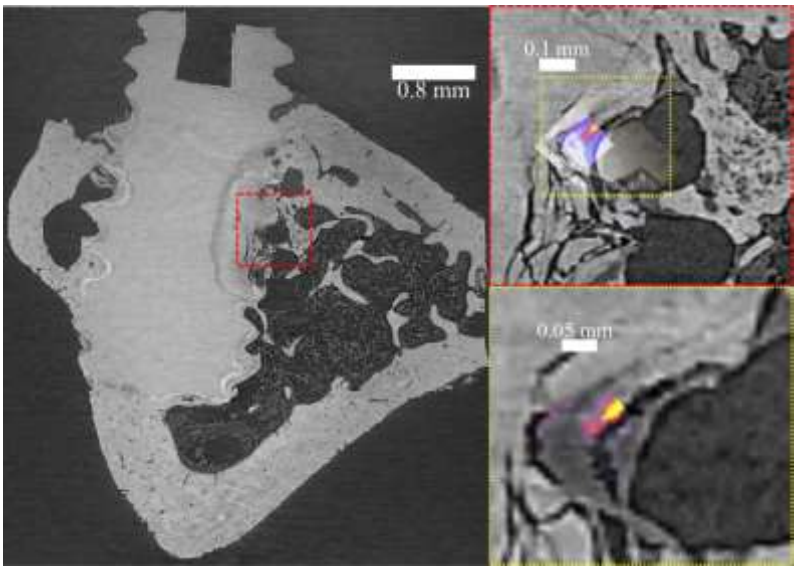
ACKNOWLEDGMENTS:

We wish to acknowledge funding by the BMBF (SynchroLoad project, 05K16CGA and MgBone project, 05K16CGB) and provision of beamtime (proposals I-20170009 and I-20170312, at beamline P05 and P03 at PETRA III at DESY, Hamburg). We are also very grateful for the support by Monika Luczak.



Picture 1: Caption 1: Fig 1: Overlay of synchrotron tomography and SAXS data: Mg-5Gd screw in bone after 8 weeks of healing.

Imaging biomaterials & environment 16:00 - 17:30 Room 0.2/0.3 11/09/2018

Oral presentation

525 electrical impedance tomography with a lab-on-chip for imaging cells in culture

Marijn Lemmens, Seppe Bormans, Fredrik Vreys, Thijs Vandenryt, Thijs Thoelen
Hasselt University, Diepenbeek, Belgium

INTRODUCTION:

In Regenerative Medicine (RM), imaging is imperative for the characterisation purposes as well as monitoring the progression of the regeneration. Many types of imaging techniques already exist in the translational research, these techniques often rely on synthetic or genetic fluorescent labels. To overcome this EIT can be used as a valid alternative.

METHODS:

A circular 8-electrode array inside a culture well was fabricated, doing this gives a controlled environment to do the first test based on EIT. A finite element model, based on EIDORS, is used to reconstruct the spatial characteristics of an object[2]. To monitor the growth of yeast, Saccharomyces cerevisiae was used in a concentration of 0.02 g/ml and cultured in a medium of 10 % glucose solution. Every 60 second, EIT measurements are taken and afterwards this data is post-processed into conductivity distribution images.

RESULTS AND DISCUSSION:

The results show a clear change in conductivity values in function of time, this can be explained by the changes in the membrane potential values. Due to the small volume of a cell, large surface-to-volume ratio and high transport capacity of proteins through the proton pump in the cell barrier the electrical properties of a cell chance over time[3]. These things all contribute to the ion homeostasis in a yeast cell.

CONCLUSION:

A feasibility study was conducted in this research to determine the viability of monitoring cell proliferation with the use of the EIT imaging technique. Based on the results, the applicability of EIT as an alternative way for analysing the cell-drug interaction is proven. The study resulted into a working miniaturised experimental setup were cellular growth within this region can be detected.

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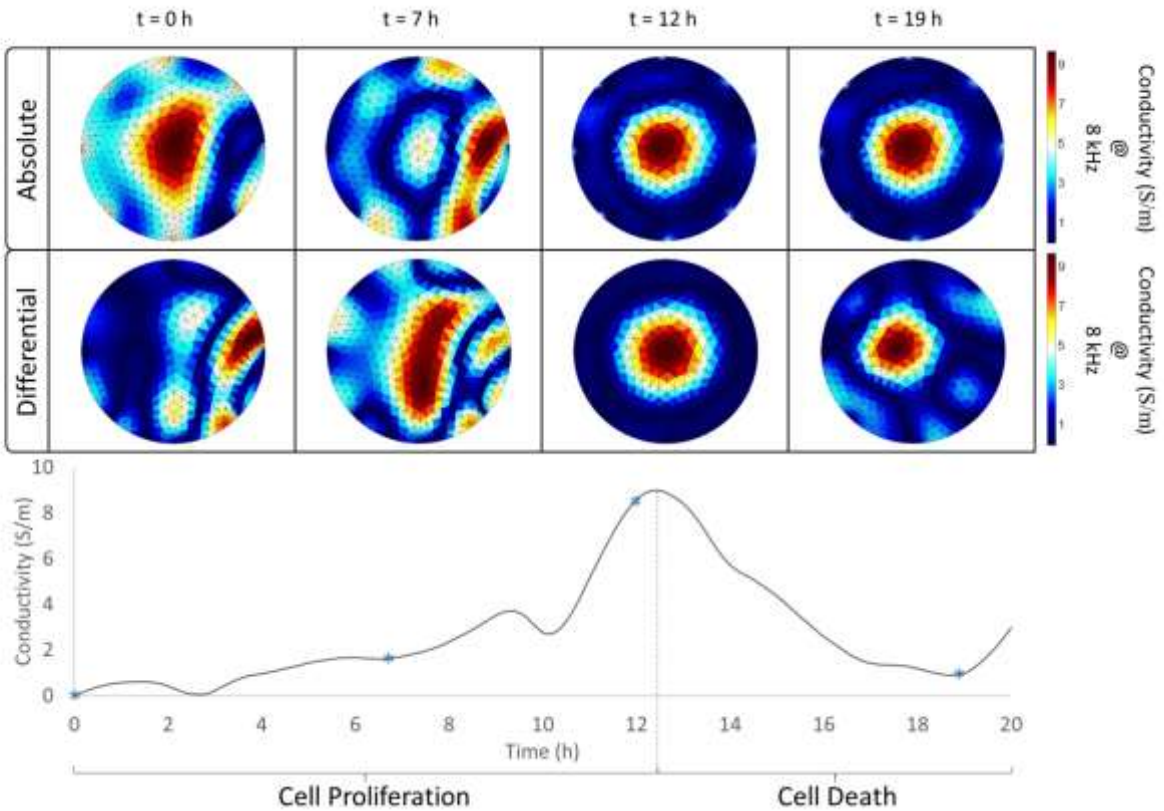
ACKNOWLEDGMENTS:

This work is funded by the BIOMAT project which is carried out under Interreg V-A grensregio Vlaanderen - Nederland and is supported by the European Union and The European Regional Development Fund and with financial support of province of Limburg - Belgium.



Picture 1: Caption 1: Figure 1 (top figure) The EIT images shown at several intervals: 0 h, 7 h, 12 h and 19 h. (bottom figure) Plotting the mean value of each frame.

Imaging biomaterials & environment 16:00 - 17:30 Room 0.2/0.3 11/09/2018

Oral presentation

141 Multi-scale speciation imaging of metallic wear particles in peri-prosthetic tissues.

Alexander P Morrell[1], Richard A Martin[1], J. Frederick Mosselmans[2], Hiram Castillo[3], Owen Addison[4]
[1]Aston University, Birmingham, United Kingdom
[2]Diamond Light Source, United Kingdom
[3]European Synchrotron Radiation Facility, France
[4]University of Birmingham, United Kingdom

INTRODUCTION:

There is interest in the biological reactions associated with implant debris. The underlining response is strongly dictated by local cellular interactions, which can be influenced by the speciation of metallic wear particles [1]. To date there has been limited speciation identification of this debris at sub-micron scales, generally it is the bulk composition that is reported. Here we show a multi-scale approach to identify, using a spectroscopic method, the spatial speciation variation in tissues that once surrounded metallic implants.

METHODS:

A section of synovial sheath was taken following scheduled revision surgery of failing hip arthroplasty. The section was stored at -80^{o}C at a Human Biomaterials Resource Centre approved site at the University of Birmingham (REC 09/H1010/75). Frozen tissue sections (4-8μm) were prepared using a cryotomed and mounted on silicon nitride membranes (*Silson, Ltd*). X-ray fluorescence (XRF) and X-ray absorption near edge structure mapping (XANES) was undertaken at Diamond Light Source with a beam resolution of 3x3μm. To further explore the state of wear particles in the tissues, they were taken to ID21 at the ESRF with a 600 x 600nm resolution.

RESULTS AND DISCUSSION:

From Figure 1a it is evident that there are significant deposits of the metallic debris. The ratio of Co: Cr present is not the same ratio as the bulk implant which is in agreement with previous literature [2]. There is a significant amount of Cr without a Co counterpart, which may be accounted for by Cr re-precipitating off the implant surface in-situ. Figure 1b displays a XANES map, with a 600nm resolution it is evident the chromium species changes from pixel to pixel. Chromium is observed in range of organic and inorganic Cr (III) complexes such as oxides, phosphates, hydroxides and malates some of which have been previously identified [3]. When interrogating with a 3μm beam this variation was not observed highlighting the importance of interrogating such samples with a high spatial resolution.

CONCLUSION:

It has been demonstrated that, within tissues associated with CoCrMo metal-on-metal hip replacements, chemical variability at the nanomeric scale is observed. The distinctiveness of this sub-micron XANES mapping approach is that it enables the entire chemical variability of an image field to be captured. The biggest impact of this work is understanding that a few point spectra with a broad beam cannot represent the full chemical variability present within a heterogeneous sample.

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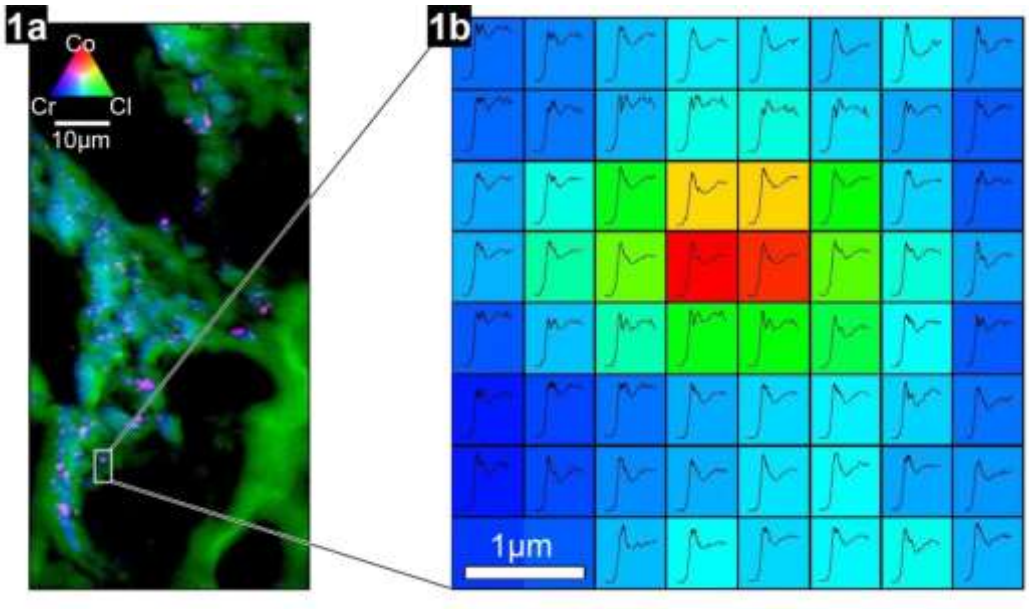

Picture 1: Caption 1: Figure 1a. A high resolution XRF map (600nm). 1b A XANES map shown graphically.

Imaging biomaterials & environment 16:00 - 17:30 Room 0.2/0.3 11/09/2018

Oral presentation

70 TEMPO-labeled hydrogels for magnetic resonance imaging

Emanuele Mauri[1], Alessandro Sacchetti[1], Lucio Melone[1], Carlo Punta[1], Filippo Rossi[2]
[1]Politecnico di Milano, Milan, Italy
[2]IRCCS Mario Negri Institute for Pharmacological Research, Italy

INTRODUCTION:

Magnetic resonance imaging (MRI) is the first-line imaging method suitable to brain, spinal cord, nerves and muscles disease detection, diagnosis and treatment monitoring[1]. In this context, the design of biomaterials is generally based on charge interactions or coordinate bonds between the contrast agent and the polymer-based material, obtaining a tool that is sensitive to the biological environment (pH, temperature or fluids)[2]. Moreover, the adverse effects and the toxicity of some contrast agents are questioned. To overcome these limits, we proposed a chemical functionalization strategy linking TEMPO to polymeric chains in a stable manner, preventing the diffusion of the heterocyclic compound in *in situ* application. The resulting product can be used in synthesis of MRI three-dimensional scaffolds[3].

METHODS:

4-Hydroxy-TEMPO was used as MRI molecule. Polyethylene glycol (PEG) was functionalized with azide preserving its hydroxyl groups, whereas TEMPO was modified with alkyne group. Click chemistry was used to permanently graft the contrast agent to PEG through the formation of a stable triazole linker. Then, the resulting PEG-TEMPO was used in microwave-assisted hydrogel synthesis designing an injectable PEG-based biomaterial with paramagnetic properties.

The chemical functionalizations were characterized by NMR, FT-IR, GPC, MRI analysis and *in vitro* and *in vivo* experiments were performed.

RESULTS AND DISCUSSION:

MRI studies assessed that the polymer functionalization route did not affect TEMPO paramagnetic properties: the longitudinal relaxivity of TEMPO-conjugated hydrogel (0.29 mM s^{-1}) was very similar to hydrogel sample where the contrast agent was only physically entrapped (0.31 mM s^{-1}). The ability of TEMPO to maintain its magnetic activity, even linked to PEG, was tested in *in vivo* mouse model: TEMPO-conjugated hydrogel was injected onto the spinal cord and analyzed with MRI. Its signal was clearly recognizable within the surrounding environment (Fig. 1), at least 1 month.

CONCLUSION:

We designed a MRI traceable hydrogel that can be imaged *in vivo* throughout the whole-time period of a longitudinal study, without any release of the contrast agent. In particular, the synthesis of a biocompatible polymer with a detectable magnetic moiety appears as a versatile material for three-dimensional scaffolds intended to non-invasive detecting techniques.

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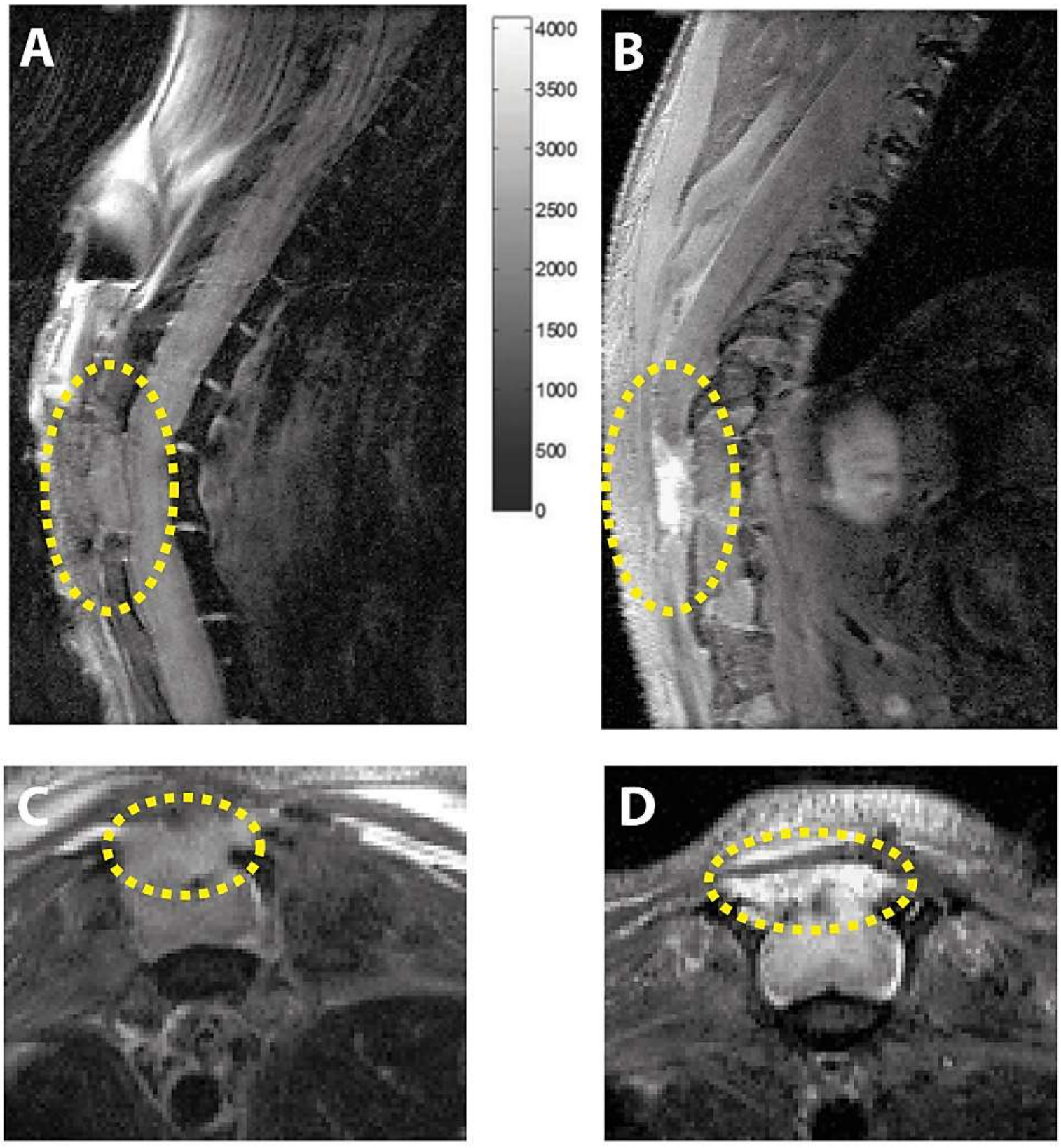

Picture 1: Caption 1: MR longitudinal and axial images of: hydrogel without TEMPO conjugation (A and C) and hydrogel grafting TEMPO (B and D), 6 h after injection.

Imaging biomaterials & environment 16:00 - 17:30 Room 0.2/0.3 11/09/2018

Oral presentation

239 Optical projection tomography imaging of three-dimensional cell cultures as part of hydrogel scaffold development

Janne Koivisto[1], Birhanu Belay[2], Jenny Parraga[2], Toni Montonen[2], Olli Koskela[2], Edite Figueiras[3], Minna Kellomäki[2], Jari Hyttinen[2]
[1]Tampere University of Technology and University of Tampere, Tampere, Finland
[2]BioMediTech, Faculty of Biomedical Sciences and Eng., Tampere University of Tech, Finland
[3]Nanophotonics, International Iberian Nanotechnology Laboratory, Portugal

INTRODUCTION:

Tissue engineering (TE) and cell biology have a constant need to characterize new hydrogel biomaterials. In fact, the clinical translation of TE requires establishing critical parameters in order to ensure rigorous screening and quality control. We demonstrate that optical projection tomography (OPT)[1] provides an efficient, non-destructive method for bright field and fluorescence imaging of transparent 3D hydrogel cell cultures in mesoscopic sample size[2].

METHODS:

To evaluate the capability of OPT in finding differences between hydrogel materials, two types of hydrogels were used for cell encapsulation: ionic bioamine crosslinked gellan gum (GG)[3] and gelatin functionalized GG. Human fibroblast cell line WI-38 was seeded in 3D hydrogel (750 µl, at least 2 parallel samples) and cultured for one week. The conventional Live/Dead assay was improved for 3D samples by increasing stain concentration and incubation time. In-house built OPT setup[1] was used in transmission and emission modes using 5x objective. The sample was rotated 360° while a total of 400 projection images were captured and the 3D images were reconstructed using standard filtered back-projection algorithm

RESULTS AND DISCUSSION:

The optimized Live/Dead assay shows that the fibroblasts were viable in all tested hydrogels. However, the functionalized GG showed more cell elongation compared to the bioamine crosslinked GG. The accompanying figure shows an example of transmission OPT projection and 3D reconstruction of cells in bioamine crosslinked GG hydrogel. The ability of OPT imaging to visualize cell-hydrogel interaction and morphology throughout mesoscopic scale samples provides broader 3D visual information of the sample compared to standard microscopy methods.

CONCLUSION:

We have shown that OPT is a suitable method for studying live 3D hydrogel cell cultures. It can be used for identifying essential aspects in two different approaches for formulating GG hydrogels. Imaging large volume cell-hydrogel samples is possible, enabling a more thorough analysis of cell response and enhancing understanding the sample as a whole.

REFERENCES:

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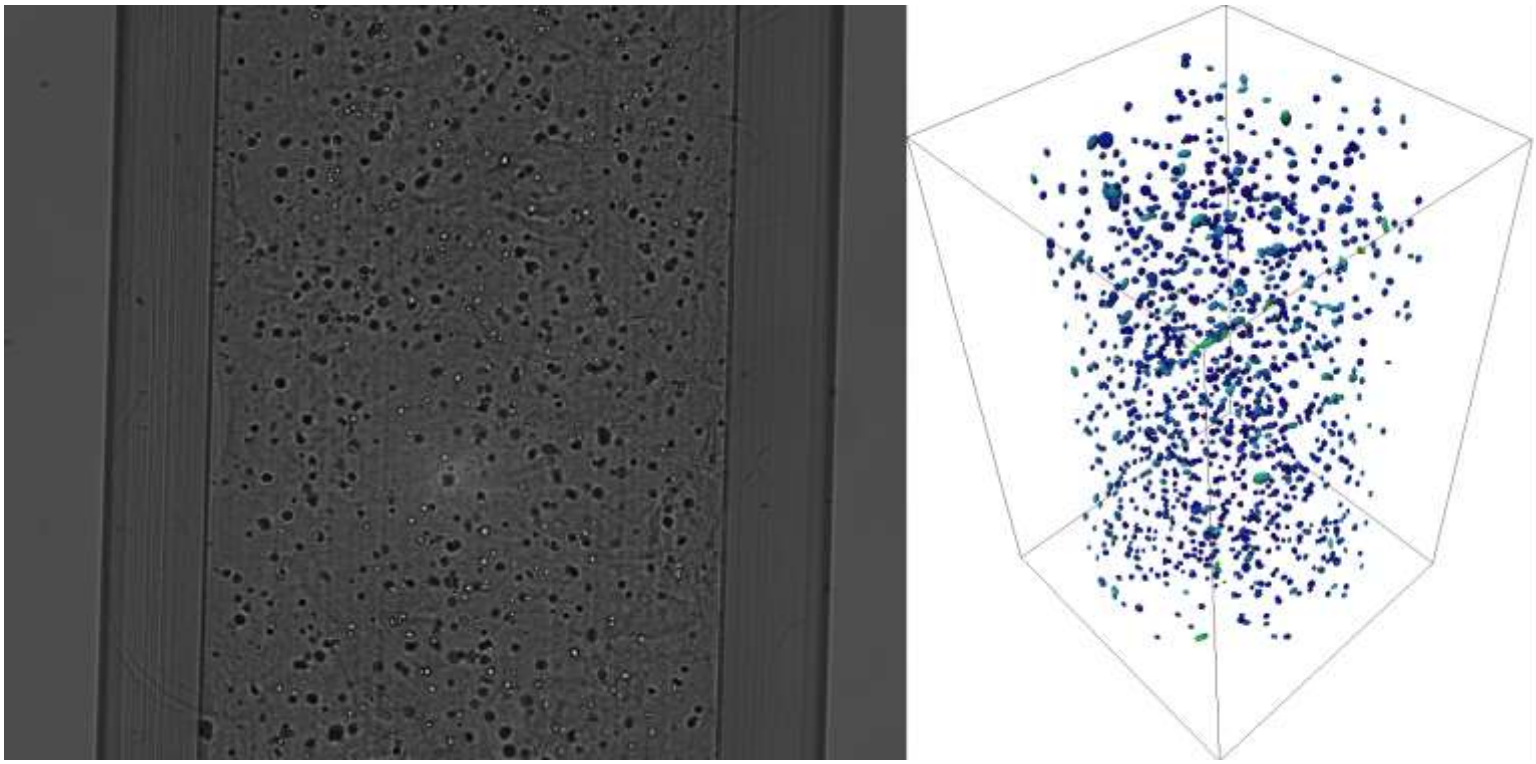

Picture 1: Caption 1: Transmission OPT image of fibroblasts in bioamine crosslinked GG hydrogel, (left) 2D projection image, (right) 3D reconstruction of cells.

Imaging biomaterials & environment 16:00 - 17:30 Room 0.2/0.3 11/09/2018

Oral presentation

660 Thermal based sensor for real-time monitoring of cell growth

Seppe Bormans, Anouk Haselaars, Jeroen Lambrichts, Gilles Oudebrouckx, Ronald Thoelen
Hasselt University, Diepenbeek, Belgium

INTRODUCTION:

Nowadays there are many techniques that can be used for continuous monitoring the cell growth process. Think of optical techniques such as Raman spectroscopy or optical waveguide light mode spectroscopy which use either absorption, transmission or reflection for target detection[1]. The Heat Transfer Method is based on the transport of heat through a functional layer or liquid, where changes at the interface of the layer or the composition of the sample fluid can lead to a change in thermal resistance[2]. We modified this technique and combined it with a sensor-heater in order to investigate if it is possible to detect and monitor the growth process of cells in real-time. This can be achieved by looking at the changes of the thermal characteristics of the interface of the heater of these cells.

RESULTS AND DISCUSSION:

The performed measurements where done using *Saccharomyces cerevisiae*. Measurements with varying factors like (starved) yeast and glucose concentrations where performed. In order to obtain the starved yeast, the yeast cells where first suspended overnight in MilliQ, so that all the energy still present in the cells would be gone. During the measurements, first growth medium was added to the heater to let is stabilize for 3 hours. After stabilization, yeast was added to the growth medium and the yeast started to grow.

Figure 1 shows the results of the growth process of the starved yeast cells. The four common stages of a growth process, lag phase, exponential growth phase, stationary phase and death phase, can be clearly observed.

CONCLUSION:

Combining heater-sensors with the HTM measurement setup makes it possible to monitor the growth process of cells in real-time. The four growth phases can be recognized in the data as well as the effect of the yeast amount on the growth speed.

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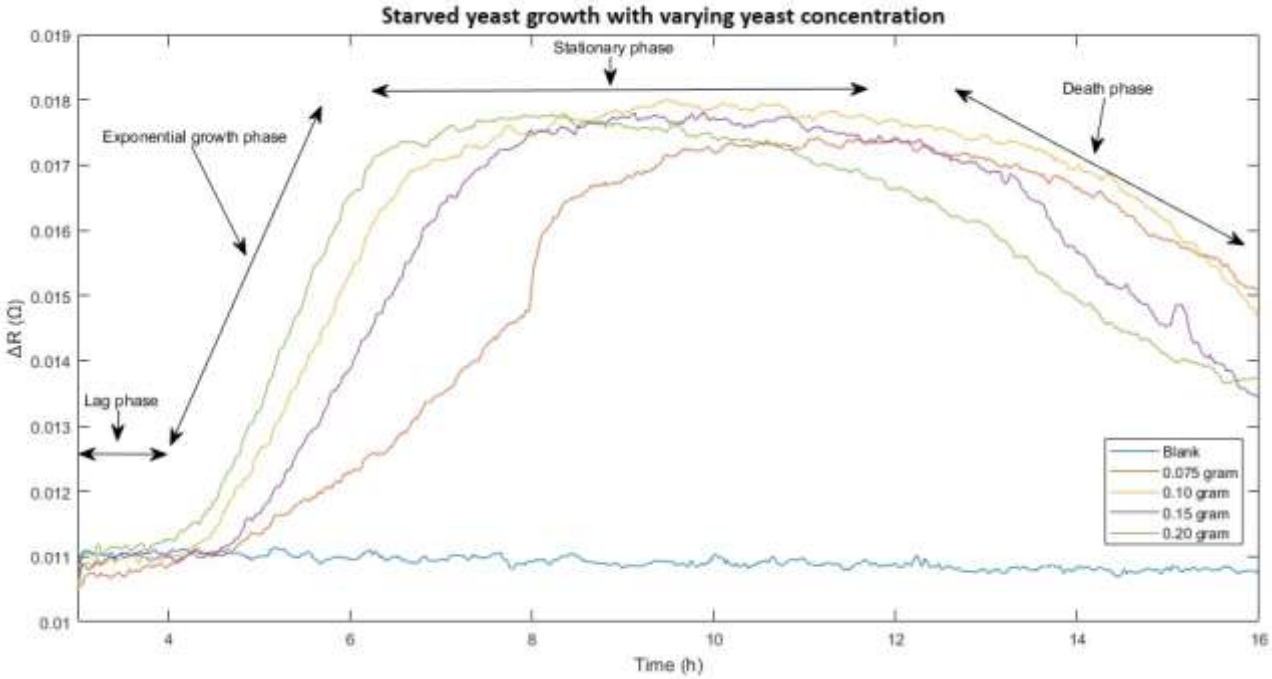

Picture 1: Caption 1: Figure 1: Starved yeast growth with varying yeast concentration: 0,075 g, 0,1 g, 0,15 g and 0,20 g.

Scientific Programme abstracts Wednesday

Plenary sesison 08.30 – 09.15 Auditorium I 12/09/2018

Oral presentation

Nanoscale control of mesenchymal stem cells

Matthew J Dalby
Centre for the Cellular Microenvironment, Joseph Black Building, University of Glasgow, Glasgow G12 8QQ.

Bone and the bone marrow niche are regenerative tissues comprising multiple cell types. This presentation will focus on using nanoscale approaches to drive bone regeneration and to bioengineer niche environments to allow enhanced self-renewal and retention of immune suppressive mesenchymal stem cell (MSC) phenotype.

For bone formation, we have used nanoscale topographies to show that physical cues alone can drive osteogenesis[1]. This data highlighted that integrin/growth factor receptor co-localisation is critical for efficient MSC osteogenesis[2]. Thus, we have developed a simply-engineered polymer (polyethylacrylate, PEA) system that facilitates integrin/BMP-2 co-localisation for the cells with the aim to further enhance osteogenesis[3]. With this bioengineered system, it is easy to upscale and move to 3D as the polymer can be applied via spin coating or plasma polymerisation. Recently, we successfully trialled bone graft coated with the PEA - BMP-2 in a compassionate veterinary case, a giant Münsterländer, Eva, who had suffered a major non-union fracture and was facing amputation; she now enjoys a normal quality of life with enhanced bone regeneration allowing her to retain her foreleg. Finally, from understanding that as cells adhere, they vibrate their focal adhesions, we have developed a nanovibrational bioreactor that uses 1000 Hz, 40 nm vibrations to drive osteogenesis in 3D hydrogels; the Nanokick[4]. This non-invasive and non-chemical differentiation protocol is allowing us to prepare lab-grown graft in readiness for a human trial in 2021.

For MSC self-renewal, we, again, used a nanotopography to show that MSCs could be grown with a retained MSC phenotype in the lab for prolonged periods[5]. This is important as out of their marrow niche, MSCs tend to quickly differentiate into e.g. fibroblasts. This makes it hard to grow large numbers of high quality stem cells in vitro. It is notable that MSCs are finding use in transplant treatments – not for their regenerative capacity per se, but for their immune-suppressive capacity. This capacity is also lost with time in vitro, but can be maintained using nanotopography. Using a metabolomics pipeline that we developed to understand MSC differentiation[6], we identify key glycolytic pathways that can be modulated with drugs in order to achieve prolonged immune suppressive effects and thus generate better MSCs for use with transplant protocols. Going forwards, we are using this information to develop bioengineered MSC niche environments.

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Calcium phosphates 09:45 - 11:15 Auditorium I 12/09/2018

Oral presentation

Nature-inspired chemical process to retain highly bioactive chemistry and multi-scale hierarchic structure in 3-D bone scaffolds

Anna Tampieri1, Monica Sandri1, Andrea Ruffini1, Silvia Panseri1, Monica Montesi1, Simone Sprio 1
1Institute of Science and Technology for Ceramics, National Research Council, Italy

INTRODUCTION

Tissue regeneration requires the use of scaffolds able to take part to the physiological metabolic processes and act as an instructing guide for cells. To this purpose, calcium phosphates, particularly metastable ion-doped apatites are reputed as best-in-class biomaterials for bone regeneration; however, the common processing techniques to consolidate 3-D ceramic bodies endowed with biomechanical performance degrades the bioactive phases into more inert scaffolds with scarce regenerative potential. The inability of the current fabrication methods to generate bone scaffolds retaining bioactive chemistry, nanocrystallinity and multi-scale structural hierarchy has so far prevented effective regenerative therapies, when human tissues with high compositional and structural complexity such as bone have to be treated. In this respect, nature-inspired synthesis approaches are rapidly gaining ground in material science, for the development of biomaterials with unique composition, structure and performance. Methods for assembling/nucleation in the 3-D state such as biomineralization1,2 and biomorphic transformation processes3 are relevant examples of this concept.

METHODS

I) Biomineralization process is carried out in aqueous environment where pH-controlled fibration of collagen fibres is induced concurrently with nucleation of bioactive calcium phosphates. The process is directed to generate bone-like and functionally-graded scaffolds with high mimicry of mineralized and non-mineralized regions present in osteochondral tissues.

II) Chemical processes based on heterogeneous chemistry in the 3-D state start from natural sources inspired by the hierarchical structure of compact bone, and imply a sequence of heterogeneous reactions at the interface with reactive gas mixtures. The original 3-D structure is maintained throughout the whole transformation process, leading to bioactive nano-structured apatite with bone-like hierarchical organization.

RESULTS AND DISCUSSION

Hybrid composites with composition and highly porous structure mimicking woven bones are the product of biomineralization process. In particular, the process yields heterogeneous nucleation of biologic-like apatitic phase occurred onto assembling collagen matrix, with multiple ion substitutions and crystal orientation inducing specific chemotactic and topotactic interaction with cells, all features at the basis of regenerative ability demonstrated in various pre-clinical and clinical tests carried out in the osteochondral district.

Heterogeneous gas-solid reactions generate hierarchically organized porous ceramics made of biomimetic, multi-doped nanocrystalline apatites, directly nucleated in the 3-D state to form a scaffold retaining original bone-mimicking structure with open and interconnected porosity and multiscale hierarchical organization. Such features determined very high mechanical strength and damage-tolerant mechanical behaviour. In vitro tests in static and dynamic conditions showed high biocompatibility and bioactivity, as well as overexpression of the most relevant genes involved in bone formation.

The above approaches represent a conceptual revolution in bioceramic science, where the application of assembling/nucleation processes in the 3-D state uniquely enables the formation of biological-like mineral phase as 3-D scaffolds. The obtained results confirm that, contrary to the apatitic phases obtained by conventional methods, such biomimetic phases are involved in the natural remodelling process leading to bone tissue regeneration.

CONCLUSION

The obtained results confirm that the use of innovative processes based on controlled assembling/nucleation in the 3-D state is an effective approach to obtain highly bioactive materials as 3-D scaffolds possessing innate cell-instruction ability. Therefore, these new approaches promise to be a breakthrough in the synthesis of bioceramics where the “metabolic crystals” organized in the 3-D state are able to boost bioactivity and potentially open to new applications in regenerative medicine.

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ACKNOWLEDGMENTS

N/A

Calcium phosphates 09:45 - 11:15 Auditorium I 12/09/2018

Oral presentation

324 Biological events in bone formation instructed by submicron surface structured calcium phosphate ceramics

Huipin Yuan[1], Davide Barbieri[2], Rongquan Duan[3], Florence de Groot[2], Joost d de Bruijn[2]
[1]MERLN Institute, Maastricht, Netherlands
[2]KUROS Bioscience BV, Netherlands
[3]BST Twente University, Netherlands

INTRODUCTION:

Having submicron surface structure, calcium phosphate ceramics gave rise to bone formation following non-osseous implantation [1,2]. Given the fact that bone formation induced by calcium phosphate ceramics starts directly with bone, it has long been suggested such bone formation resembles the intramembrane ossification in development [3]. However recent studies [1, 4, 5] have indicated that upon implantation, osteoinductive CaP ceramics may affect not only the osteogenesis but also other processes in wound healing (e.g. inflammation, angiogenesis and bone remodeling). It is thus necessary to clarify the biological processes involved in material-driven osteoinduction.

METHODS:

CaP ceramic (namely NCS-CaP) with submicron scaled needle-crystal surface (Figure 1) was fabricated with a thermal treatment, and implanted in granule form (1-2mm, 1cc per implant) in back muscle of canine for 6 weeks (n=3) and 3 weeks (n=5) together with non-osteoinductive micron surface structure calcium phosphate ceramic (namely TCP-B) [1,2]. The explants were subjected to biochemical assays regarding Alkaline phosphatase (ALP)/ Tartrate-resistant acid phosphatase (TRAP), histology and histomorphometry with respect to bone formation.

RESULTS AND DISCUSSION:

Bone was formed in all NCS-CaP implants 6 weeks (Figure 1D) after implantation (n=3); by percentage, 2.8±1.9% available space was occupied by bone. No bone was formed in any TCP-B implants (neither 3 weeks nor 6 weeks). No bone was formed in NCS-CaP 3 weeks after implantation either. As compared to the non-osteoinductive TCP-B, more TRAP was detected in NCS-CaP implants, and TRAP in NCS-CaP increased from 3 weeks to 6 weeks (Figure 2). Meanwhile ALP was detected at week 6 in MagentOs implants. Moreover prior to ALP, TRAP was presented in NCS-CaP implants at week 3.

CONCLUSION:

This study demonstrated that material factors greatly affected TRAP production in in vivo implants and confirmed a strong link between TRAP and ALP. The presence of TRAP prior to ALP (bone formation) indicated a more complicated cascade in material-driven osteoinduction other than osteogenesis alone. The earlier presence of TRAP than ALP supports partially the hypothesis that material-driven osteoinduction mimics the secondary bone formation during bone remodeling.

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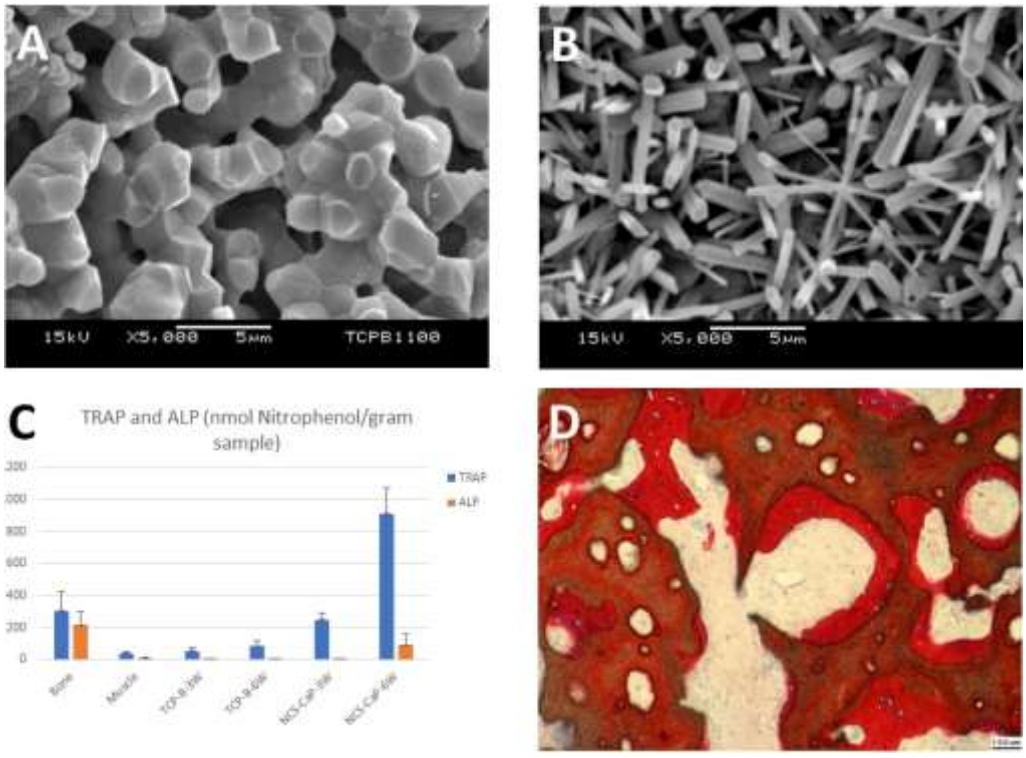


Picture 1: Caption 1: Surface structure of TCP-B (A) and NCS-CaP (B), TRAP and ALP production along time in implants (C) and bone formation in NCS-CaP at week 6.

Calcium phosphates 09:45 - 11:15 Auditorium I 12/09/2018

Oral presentation

574 Laser surface structuring of calcium phosphate bioceramics for cell behaviour assessment

Marie Lasgorceix[1], Cédric Ott[1], Laurent Boilet[1], Stéphane Hocquet[1], Anne Leriche[2], Maria-Helena Fernandes[3], Fernando Monteiro[4], Véronique Lardot[1], Francis Cambier[1]
[1]Belgian Ceramic Research Centre, Mons, Belgium
[2]LMCPA-UVHC, France
[3]Faculdade de Medicina Dentária, Porto, Portugal
[4]Laboratorio de Biomateriais, INEB, Porto, Portugal

INTRODUCTION:

The bioactivity of synthetic bone implants is highly impacted by their surface topography, especially by the presence of micro-patterns likely to influence cell behaviour.

METHODS:

In this study, laser machining technology was employed in order to produce controlled regular micro-patterns on dense calcium phosphate surfaces, without any contamination. The choice of the source was directed towards a femtosecond pulsed laser, with 1030 nm wavelength and 270 fs pulse width, in order to limit the thermal impact of such a process and thus to avoid the unwanted phase transformations potentially induced by the temperature elevation. The microstructural characteristics were investigated by microscopy (optical, confocal, scanning electron) and the phase identification was performed by X-Ray Diffraction and Raman spectroscopy.

RESULTS AND DISCUSSION:

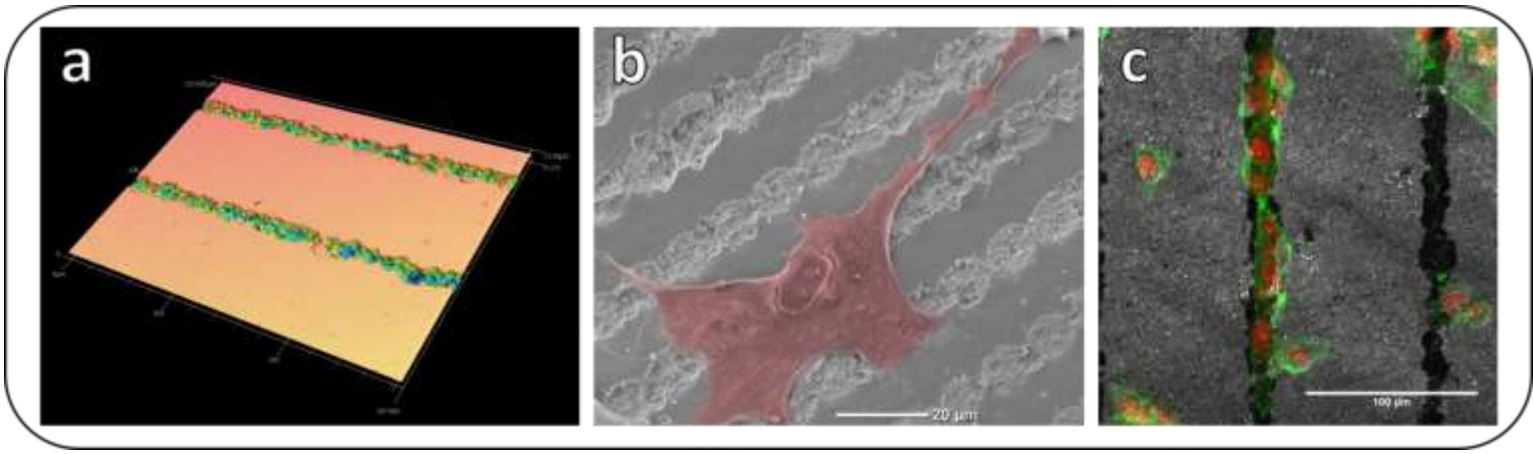
Beta tricalcium phosphate substrates with perfectly controlled micro-patterning and without any secondary phase were obtained by controlling the process parameters (laser power, scanning speed, pulse frequency). An accurate optimization of the process parameters allowed to obtain micropatterns with several complex designs. This work allowed to highlight the effects of the process parameters on the patterning. The influence of surface micro-patterning on osseous cells behaviour was highlighted *in vitro*. In particular, an elongation of the cells shape was observed along linear grooves made by laser machining, whereas cells appeared more spread on smooth surfaces with same chemical composition. These results show that linear patterning should promote cell migration. The effect of the micro-patterning design, and particularly the presence of angular shapes, on cell adhesion, is currently being evaluated *in vitro* with stem cells.

CONCLUSION:

To conclude, femtosecond laser machining technique seems to provide an interesting alternative to conventional ceramic surface treatments of calcium phosphates. This technology, which allows a minimization of the thermal impact, appears promising and can now be envisaged for the surface treatment of calcium phosphate ceramics or even calcium phosphate coatings used for bone tissue engineering.

ACKNOWLEDGMENTS:

The authors are grateful to the Walloon Region for financial support, within the “BEWARE” program (convention n°1510392) co-funded by Wallonia and European Union (FP7 – Marie Curie Actions). All the authors also thank the JECS Trust Action “Frontiers of Research” for the financial support facilitating the collaborative work between laboratories.



Picture 1: Caption 1: a) Laser micropatterned surface ; b) hMS cells attached on micropatterned substrate ; c) Alignment of MG63 cells along grooves

Calcium phosphates 09:45 - 11:15 Auditorium I 12/09/2018

Oral presentation

409 Influence of microporosity and macropores design upon cell colonization of calcium phosphate ceramic scaffolds for bone regeneration

Amandine Magnaudeix, Urda Rüdrich, Marie Lasgorceix, Eric Champion, Patricia Pascaud-Mathieu, Chantal Damia, Thierry Chartier, Joel Brie
IRCER, UMR CNRS 7315, Limoges, France

INTRODUCTION:

Tissue engineering for bone regeneration involves the use of osteoconductive scaffolds to guide the formation of new tissues into their porous network. The scaffold architecture is of great importance since it can influence cell behavior. At the microscopic scale, open micropores (< 10 µm) are beneficial [1]. At larger scales, macropore (> 100 µm) network and geometry influence the quality, velocity and rate of tissue growth and inner scaffold colonization.[2, 3, 4] The objective of this study was to evaluate how the macropore cell colonization was influenced by: (i) the macropore size and cross-sectional geometry, and (ii) the microporosity amount of the ceramic scaffold.

METHODS:

Silicated hydroxyapatite (SiHA – $Ca_{10}(PO_4)_{5.6}(SiO_4)_{0.4}(OH)_{1.6}$) scaffolds were manufactured by microstereolithography containing unidirectional macropores with 5 cross-sectional geometries and three open microporosity contents. MC3T3-E1 pre-osteoblastic cells were seeded onto these scaffolds for 7 and 14 days. Several parameters were quantified by computer-assisted image analysis of the macropore microphotographs. The data were statistically analyzed using principal component analysis.

RESULTS AND DISCUSSION:

Analysis of cell colonization inside the macropores shows that the cellular filling is proportional to the macropore size and strongly influenced by macropore shape. Straight edges and convex surfaces are detrimental. High aspect ratios, the absence of reentrant angles and the presence of acute angles, by creating concavities and minimizing flat surfaces, facilitate cell colonization[2,3]. Rhombus and triangle cross-sections are thus particularly favorable, while square and star geometries are the least colonized. Importantly, an increase in the microporosity content strongly impairs cell growth in the macropores. Microporosities modifed the local topography and likely the cell orientations

with regards to each others disturbing the cell tissue coherence. The intrinsic geometry of the cell tissue controls its growth and its progression,[5-6] which may explain these results.

CONCLUSION:

The results indicate the important cell sensing of topography at micro- and macrometric scales during the initial step of cell adhesion and proliferation. They evidence the need for considering together the micro- and the macroporosities to optimize scaffold design.

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ACKNOWLEDGMENTS:

This work was supported by institutional grants from the LabEX SigmaLim (ANR-10-LABX-0074-01).

Calcium phosphates 09:45 - 11:15 Auditorium I 12/09/2018

Oral presentation

415 A bioactive nano-calcium phosphate paste for in-situ transfection of BMP-7 and VEGF-A: Results of an in vivo study

Matthias Epple[1], Carsten Schlickewei[2], T.O. Klatte[2], Y. Wildermut[2], G. Laaff[2], Johannes M. Rueger[2], Johannes Ruesing[1], Svitlana Chernousova[1], Wolfgang Lehmann[3]
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[2]Trauma, Hand and Reconstructive Surgery, University Hospital Hamburg, Germany
[3]Trauma, Orthopaedics and Plastic Surgery, University Hospital Göttingen, Germany

INTRODUCTION:

The aim of this study was to create a new injectable DNA-functionalized calcium phosphate as bone graft substitute. For this we used the well-known potential of calcium phosphate as bone graft substitute and supplemented it with the corresponding DNA for the transfection of BMP-7 and VEGF-A at the implantation site.

METHODS:

24 New Zealand white rabbits were randomly divided into two groups: One group with BMP-7- and VEGF-A-encoding DNA on the calcium phosphate nanoparticles and a control group with calcium phosphate nanoparticles only. A bone defect was created at the proximal medial tibia and filled with the injectable calcium phosphate paste, either loaded with DNA or without DNA. To evaluate the potential as bone graft, the proximal tibia was harvested 2, 4 and 12 weeks after the operation. Histomorphological analysis was focused on the dynamic bone parameters using the Osteomeasure system.

RESULTS AND DISCUSSION:

The animals treated with the DNA-loaded calcium phosphate showed a statistically significantly increased bone volume per tissue volume after 4 weeks in comparison to the control group. Additionally, a statistically significant increase of the trabecular number and the number of osteoblasts per tissue area were observed. These results were confirmed by radiological analysis. The DNA-loaded bone paste led to a significantly faster healing of the critical size bone defect in the rabbit model after 4 weeks. After 12 weeks, all defects had equally healed in both groups. No difference in the quality of the new bone was detected.

CONCLUSION:

The injectable DNA-loaded calcium phosphate paste led to a faster and more sustained bone healing and induced an accelerated bone formation. The graft was not only incorporated into the bone, but new bone was also formed on its surface. The calcium phosphate paste without DNA generated a regular healing of the critical size bone defect but slower than the DNA-loaded paste. The in-situ transfection with BMP-7 and VEGF-A significantly improved the potential of calcium phosphate as bone graft substitute.

ACKNOWLEDGMENTS:

The authors thank the Deutsche Forschungsgemeinschaft (DFG) for funding this project within the framework of the CRC 1093: Supramolecular Chemistry on Proteins and to personal grants to ME and WL.

3D Printed scaffolds & hierarchy 14:30 - 16:00 Auditorium I 12/09/2018

Oral presentation

764 3D printing of horizontal gradient scaffolds for bone regeneration

Luis Diaz-Gomez, Sean Bittner, Brandon Smith, Anthony Melchiorri, Antonios G Mikos
Rice University, Houston, United States of America

INTRODUCTION:

Tissue scaffolds for bone regeneration must maintain adequate mechanical properties while balancing and directing biodegradation and tissue ingrowth (1). The use of graded porous composite scaffold with adequate pore size and interconnectivity has been investigated before. However, these scaffolds usually lack uniformity and delamination is often reported (2). 3D printing technology allows the preparation of porous scaffolds with precise topographies incorporating graded properties (3). Specifically, the printing of fibers with composition gradients to create horizontally-graded scaffolds has not been reported. The aim of this work was to develop a printing method based on the integration of additive manufacturing and computer-aided design to prepare radial gradients of hydroxyapatite in a highly controlled and reproducible manner.

METHODS:

PCL, PCL-HA15% and PCL-HA30% powdered admixtures were loaded in printing heads and heated at 160ºC. 3D template and printing parameters were tuned to fabricate cylindrical scaffolds composed of continuous fibers and creating a radial gradient of HA using a Bioplotter printer (Envisiontec, Germany). HA incorporation was varied lengthwise in printed fibers and was evaluated via micro-CT imaging, EDX and TGA. Morphological and structural analyses were carried out by micro-CT and SEM. Mechanical tests were performed using a compressive bench. Accelerated and standard degradation studies were also carried out.

RESULTS AND DISCUSSION:

The printing methodology developed allowed the fabrication of radial composition gradient of HA within the same fibers (Fig 1A). The resulting scaffolds presented pore sizes ca. 300 µm and with 100% interconnectivity.

Mechanical evaluation confirmed that the incorporation of 30% HA in the composition and the segment printing did not decrease the compressive and elastic modulus compared to single material controls. All values were in the range of trabecular bone. Degradation studies showed degradations rates were dependent on HA content.

CONCLUSION:

We developed a novel 3D printing technique to producing tissue scaffolds with precise topographies incorporating graded compositions and interconnected porous structure suitable for bone tissue regeneration. This printing methodology serves as a template for the 3D printing of complex tissues requiring gradients of mixed materials to modulate scaffold osseointegration and degradation.

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ACKNOWLEDGMENTS:

L.D-G. acknowledges Xunta de Galicia for a Postdoctoral Fellowship (EDB481B 2017/063). Authors thank the support of Rice University's Biomaterials Lab, and the Center for Engineering Complex Tissues.

Picture 1:

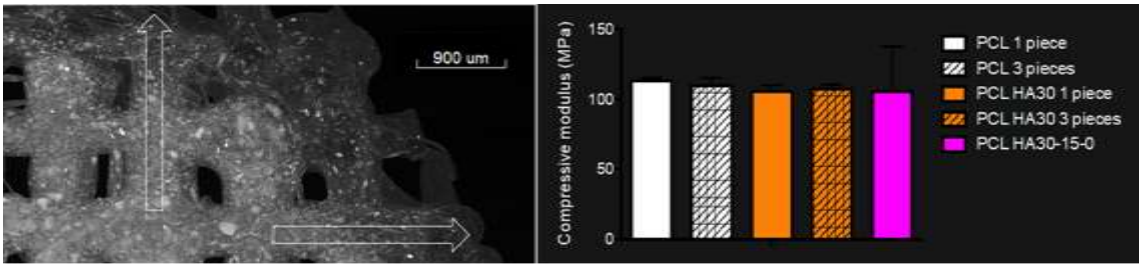

Figure 1: Micro-CT image showing the radially decrease in the HA content of the layer (left). Compressive modulus of the PCL-HA scaffolds printed as a continuous strand or in graded segments (right).

Caption 1: Micro-CT image showing the radial decrease in the HA content of the layer (left). Compressive modulus of the graded and control scaffolds (right)

3D Printed scaffolds & hierarchy 14:30 - 16:00 Auditorium I 12/09/2018

Oral presentation

198 Hierarchical biofabrication: Integrating molecular versatility and nano-to-macro scale structural control

Clara Hedegaard[1], Estelle Collin[1], Carlos Redondo-Gómez[1], Luong Nguyen[2], Kee Woei Ng[2], Alfonso Castrejón-Pita[3], Rafael Castrejón-Pita[1], Alvaro Mata[1]
[1]Queen Mary University of London, London, United Kingdom
[2]Nanyang Technological University, Singapore
[3]University of Oxford, United Kingdom

INTRODUCTION:

A major goal in tissue engineering is the capacity to recreate the extracellular matrix (ECM) with hierarchical control. Typical approaches include molecularly complex materials or advanced biofabrication techniques. Whilst biofabrication can build precise tissue-like architectures, many of the bioinks are constrained by stringent printing requirements[1]. Conversely, self-assembling biomaterials can be molecularly designed to better mimic the ECM[2], but lack macroscale precision. The objective of this study was to develop a biofabrication method that integrates the benefits of bioprinting with molecular self-assembly, enabling the fabrication of bioactive macroscopic constructs with molecular to macro scale control.

METHODS:

An in-house designed acoustic based print head was used to generate droplets-on-demand (DoD). Different peptide amphiphile (PA) sequences were co-assembled with a range of macromolecules (keratin, collagen, fibronectin and hyaluronan). All solutions were prepared using a cell friendly buffer at concentrations 5-20 $mg.mL^{-1}$. The co-assembly creates nanofibrous hydrogels. Scanning electron microscopy was used to characterise the gel structure. Cell viability was assessed using live/dead assay.

RESULTS AND DISCUSSION:

The droplet into liquid bioprinting method allowed interfacial liquid forces to be used to guide the self-assembling process into gels with specific shape and topography. We show that the gels can be individualised by changing the macromolecule, altering the PA, integrating biological signals, aligning the nanofibers and/or controlling the macroscopic structure. As such, one fabrication set-up can be tuned according to the target tissue without the need to redesign the print-head. We show that the individual gels can be assembled into larger structures or used individually. The printing method can be carried out in presence of cells (viability >80%). We demonstrate spatial control of cell positioning as well as co-culture, by encapsulating cells in the PA or protein solution. Versatility was demonstrated using different cell lines. The developed method enables for the first time the possibility to bioprint whilst controlling biomolecular and structural elements of the printed scaffold[3].

CONCLUSION:

By combining self-assembling materials with DoD based printing, we create a biofabrication method with both nano and microscale control. The merits of the ink and method allow the fabrication of complex bioactive scaffolds for applications such as tissue engineering, *in vitro* models, and drug screening.

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ACKNOWLEDGMENTS:

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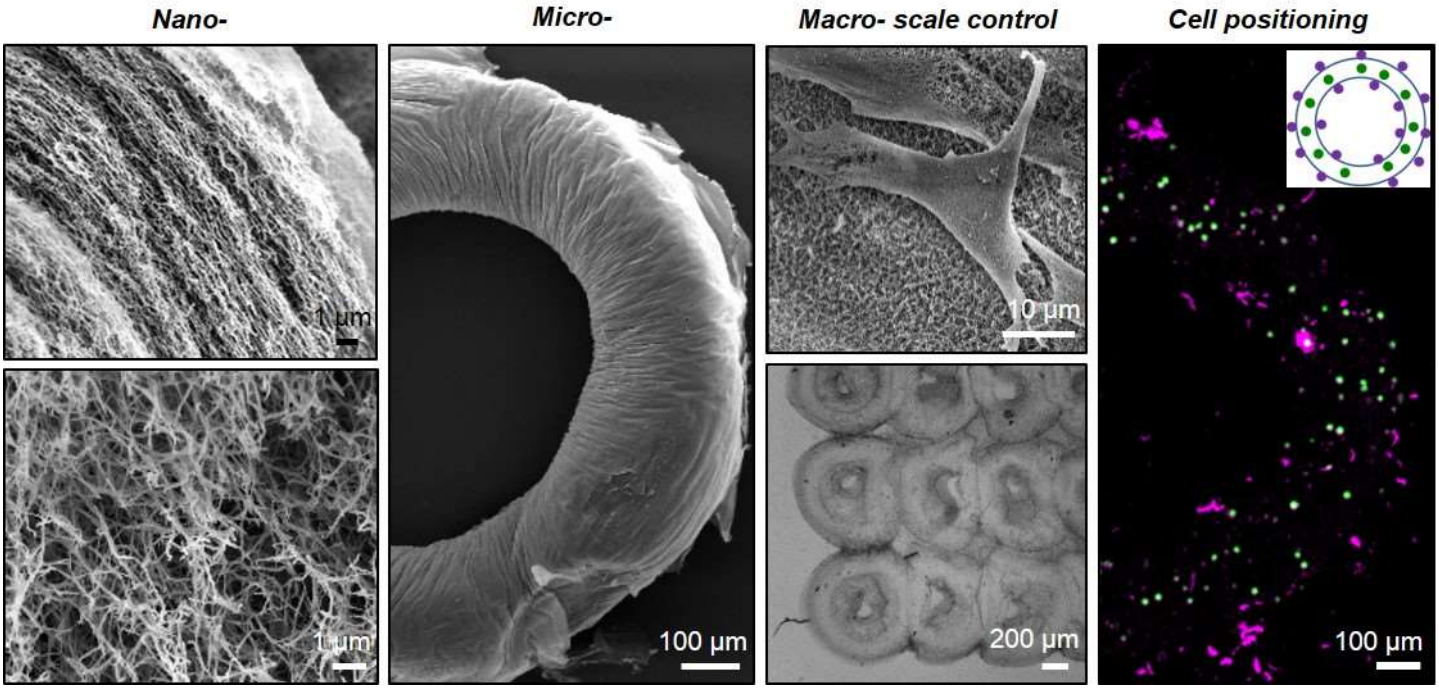

Picture 1: Caption 1: From Nano to Macro Scale Control | a) aligned and random fiber orientation, b) structure and topography, c) gel sheets, and d) cell positioning.

3D Printed scaffolds & hierarchy 14:30 - 16:00 Auditorium I 12/09/2018

Oral presentation

484 Biphasic hydrogel with gradient drug release for articular cartilage regeneration

Xuetao Shi
South China University of Technology, Guangzhou, China

INTRODUCTION:

The subchondral bone, covered by cartilage, cannot be underestimated in the pathogenesis of articular cartilage damage.[1] Three-dimensional tissue scaffolds, be able to support cell growth/proliferation, has been attached with significant advantages. [2] Cartilage was known to have different composition and cell type with subchondral bone region and exist a transition region between cartilage and bone. Therefore, it must take chondrogenic/osteogenic directional differentiation of stem cells into consideration for integrative repair of bone and cartilage. [3]

METHODS:

In this study, biphasic hydrogel consisting of gelatin methacryloyl (GelMA) and hyaluronic acid methacryloyl (HAMA) respectly crosslinked with modified beta-cyclodextrin (β-CD-AOI) were applied for the restoration of cartilage layer or subchondral region. Hydrophobic drugs melatonin for osteogenesis and kartogenin for chondrogenesis were encapsuled in β-CD beforehand in a host-guest way. Therefore, we could achieve controlled drug release in vivo environment. GelMA and HAMA based hydrogels for integrative restoration of subchondral bone and cartilage and expect to form a continuous transition from bone to cartilage. Live/Dead assay, stem cell differentiation experiments, and rabbit femoral joint repair experiments were performed to assess biocompatibility and ability of inducing directional differentiation of stem cells and repairing bone joints of the biphasic hydrogel.

RESULTS AND DISCUSSION:

The results suggested that drug-loaded β-cyclodextrin-based hydrogel had appropriate release performance and induced directional differentiation of stem cells. Cytotoxicity experimentph revealed a good viability of adipose tissue-derived stromal cells encapsuled in the hydrogel in a evenly distributed 3-dimensional way.

Immunohistochemistry staining proved chondrogenic and osteogenic differentiation of those cells. Rabbit femoral joint defect repairing experiment and micro-CT analysis results represented that gradient drug-loaded diphasic hydrogel repair the osteochondral interface and full-thickness articular cartilage defects more effective than control group.

CONCLUSION:

Taken together, this study showed the potential of biphasic drug-loaded hydrogel for integrative repair of bone and cartilage. Different materials can be used to obtain more biphase materials for the repair of transitional layers of tissue, and combine the advantages of those materials to provide biomaterials with optimum properties.

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ACKNOWLEDGMENTS:

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3D Printed scaffolds & hierarchy 14:30 - 16:00 Auditorium I 12/09/2018

Oral presentation

755 Asymmetry in anisotropic ice-templated collagen scaffolds to mimic the structure of native tissue: from model to execution

Jamie Cyr, Ruth Cameron, Serena Best, Anke Husmann
Cambridge University, Cb3 0fs, United Kingdom

INTRODUCTION:

Ice-templated collagen scaffolds may be used to structure cell migration and provide key mechanical cues in a range of regenerative medical applications [1]. However, there is a need for techniques to precisely control the direction of ice growth during scaffold production in order to create scaffolds which mimic the heterogeneous structures of complex tissues [2]. Here we use finite element modelling to design an apparatus to create complex temperature gradients within a freezing slurry. This enables the production of defined heterogeneous scaffold structures.

METHODS:

The novel freeze-casting protocol presented here was modeled via finite element modeling software, Abacus. Three-dimensional solutions to the heat diffusion partial differential equations were derived numerically for a variety of imposed boundary conditions to simulate the experimental set-up (Figure 1.A.). The physical set-up was comprised

of a programmable heat sink contacting the base of the collagen slurry-containing mould. Thermal gradients within the slurry were dictated by patch heaters placed at the periphery of the mould (Figure 1.A.). After freezing, the structures were dried via sublimation and imaged with X-ray micro-computed tomography and scanning electron microscopy. The resulting collagen structures were analysed for pore alignment, directionality and consistency with the simulation.

RESULTS AND DISCUSSION:

It was determined that in the presence of a sufficiently large temperature gradient, ice crystals grow in the direction of the gradient with the pores of the ice-templated collagen structures reflecting the simulated three-dimensional thermal profile of the freezing slurry (Figure 1). Thus by adjusting the thermal environment during freezing, pore directionality and alignment could be controlled. Additionally, model consistency allows for mould design to be tailored to produce structures that replicate specific tissue architectures within the body.

CONCLUSION:

By exploiting the relationship between the thermal profile of the freezing slurry and the final scaffold architecture and by simulating and constructing a freezing apparatus that allows full three-dimensional control of the thermal profile, increased architectural control of freeze-cast collagen structures has been achieved. The success of this approach has been demonstrated with a scaffold with pores which follow a defined and controlled curve. The approach has the power to be generalized to create chosen variable architectures for a host of heterogeneous target structures.

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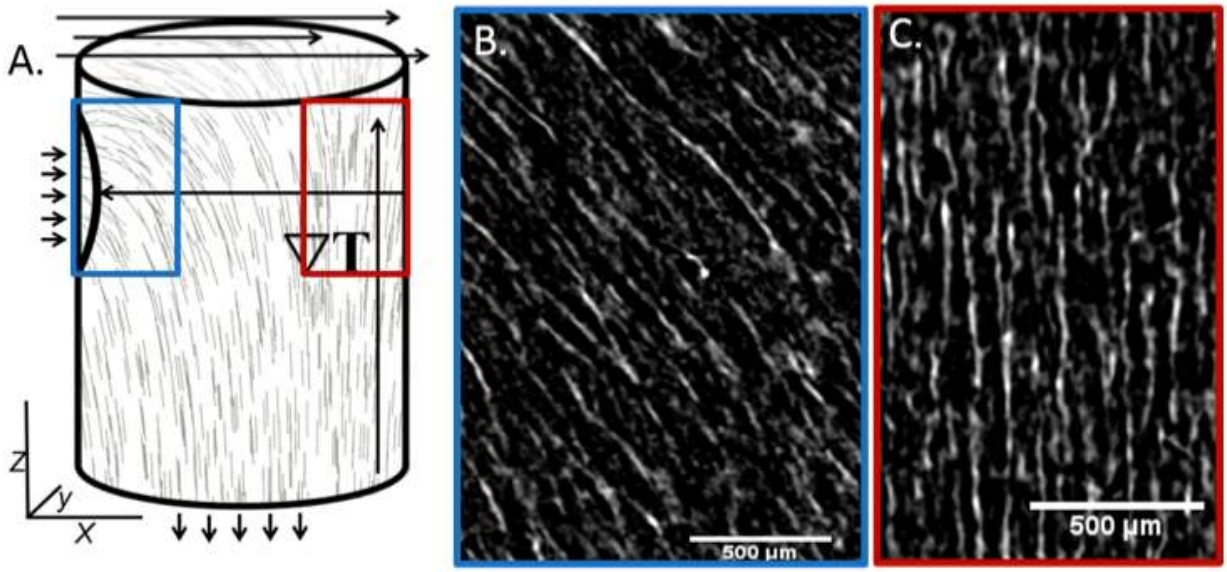

Figure 1 Techniques to control pore alignment and directionality. **A)** shows a schematic of thermal sources and sinks present around a cylindrical mold. The arrows at the base of the mould represent the heat sink, the arrows across the top of the cylinder represent ambient air temperature as a thermal source, and the inward arrows on the side of the cylinder represent a controlled heat source. The grey vectors inside the cylinder represent the modeled temperature gradients present within the mould under the given boundary conditions as heat sources and sinks. **B)** and **C)** display uCT images of the structure produced. **B)** displays pore alignment and directionality of the structure proximal to the heat source (blue), while **C)** displays the alignment and directionality of the structure opposite the heat source (red).

Picture 1: Caption 1: Figure 1 Techniques to control pore alignment and directionality. A) shows a schematic of thermal sources and sinks present around a cylindrical mold.

3D Printed scaffolds & hierarchy 14:30 - 16:00 Auditorium I 12/09/2018

Oral presentation

595 Morphological and mechanical evaluation of newly formed bone after spinal fusion treatment based on micro-CT scans

Bert van Rietbergen[1], Scott Johnson[2], Jerome Connor[2], Chris Arts[3]
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[2]Cerapedics Inc., United Kingdom
[3]Maastricht University Medical Centre, Netherlands

INTRODUCTION:

Spinal fusion aims to mechanically stabilise the motion segment using a combination of instrumentation and targeted bone regeneration. Iliac crest bone graft (ICBG) remains the 'gold standard' for fusion but numerous synthetic options are available. It is expected that substitute biomaterials progress towards near-normal trabecular organisation as fusion progresses; however, there is a lack of information regarding the morphological changes and development of subsequent mechanical stability during the progression to fusion. The goal of this study is to quantify the morphology and mechanical properties of bone formed after single level interbody spinal fusion treatment using a peptide enhanced bone graft (i-FACTOR P15L) in an animal model.

METHODS:

Mature female sheep (<1 year) underwent 1-level fusion surgery (L2-L3) using a PEEK interbody spacer. Animals were divided into two groups: one received a cage filled with P15L and the other a cage filled with ICBG. Animals were sacrificed at 30 (n=6), 90 (n=12), and 180 (n=12) days following surgery. A micro-CT (25micron voxel) was acquired at sacrifice.

A total of 30 micro-CT scans were evaluated from the three time-points. Morphological analysis was performed for the region of newly formed bone at the centre of the interbody cage. Parameters included metrics for the volume of new bone formed and trabecular organisation.

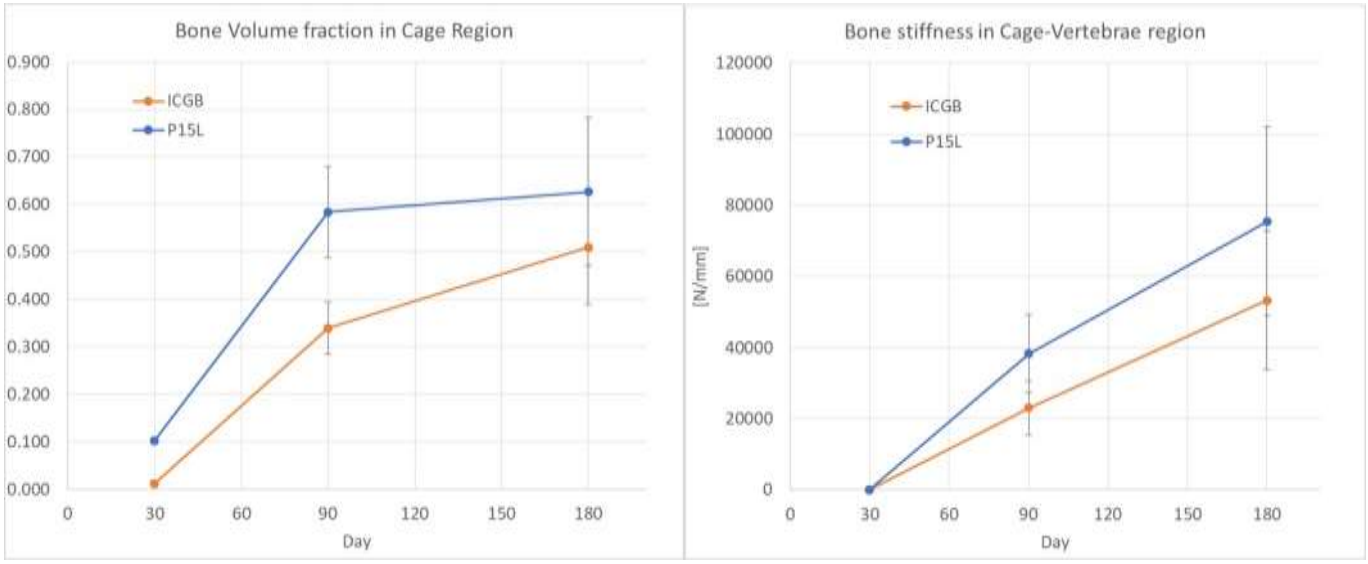
An analysis for mechanical stability was undertaken to assess the load-bearing capacity of the graft-vertebrae region using micro- finite element (FE) analysis. Micro-FE models were generated for a region of 4 mm in height above and below the marker-affected region and the stiffness of these bone regions was calculated and averaged. Statistical significance between treatment groups was assumed for p values < 0.05.

RESULTS AND DISCUSSION:

At D30 there was no significant bone formation in the ICBG group whereas significant bone formation was found in the P15L group (Figure, left). At D90 substantial amounts of bone were formed in both groups, but significantly more (72%) in the P15L group than in the ICBG group. At D180, both groups developed similar amounts of bone and differences were no longer significant. The calculated stiffness linearly increased over time for the P15L group, while for the ICBG group it started slower. Significant differences between the groups were found for D90.

CONCLUSION:

Although both groups reached similar amounts of bone volume and mechanical stiffness, the i-FACTOR peptide enhanced bone graft (P15L) group did so significantly faster than the 'gold standard' (ICBG).



Picture 1: Caption 1: Bone volume fraction and stiffness were greater in subjects treated with P15L compared to ICBG.

3D Printed scaffolds & hierarchy 14:30 - 16:00 Auditorium I 12/09/2018

Oral presentation

58 An innovative sol-gel based hybrid biomaterial designed for bone tissue engineering

Henri Granel[1], Cédric Bossard[2], Fabien Wauquier[1], Edouard Jallot[2], Jonathan Lao[2], Yohann Wittrant[1]
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[2]Laboratoire de Physique Corpusculaire, France

INTRODUCTION:

Glasses were among the first bioactive materials studied. Discovered in the early 70's, they have been described for their ability to strongly bind bone and stimulate its regeneration. However, their fragility restricts their field of application. To overcome these restrictions, synthesis processes of bioactive glass (BG) have evolved towards organic-inorganic hybrids associating the osteoinductive properties of BG and the mechanical strength of polymers. Members of our consortium have recently patented a method for synthesizing binary bioactive glass (SiO2-CaO) and polycaprolactone (BG-PCL) hybrid scaffolds using sol-gel at room temperature. *In vitro* and *in vivo* characterization of the biological properties of this material have been investigated.

METHODS:

Photonic and electron microscopy studies were performed to evaluate the adhesion capacity of rat primary osteoblastic cells (pOB) to the surface of the biomaterials. Western blots were performed to implement the data regarding the activities of transcription factors involved in bone formation. Differentiation of pOBs within the scaffold was assessed by enzymatic activities, RT-qPCR and mineralization assays. Finally, scaffolds were implanted *in vivo* in a model of craniotomy in mice. The BG-PCL hybrids were compared to partially deproteinized bovine bone (DBB).

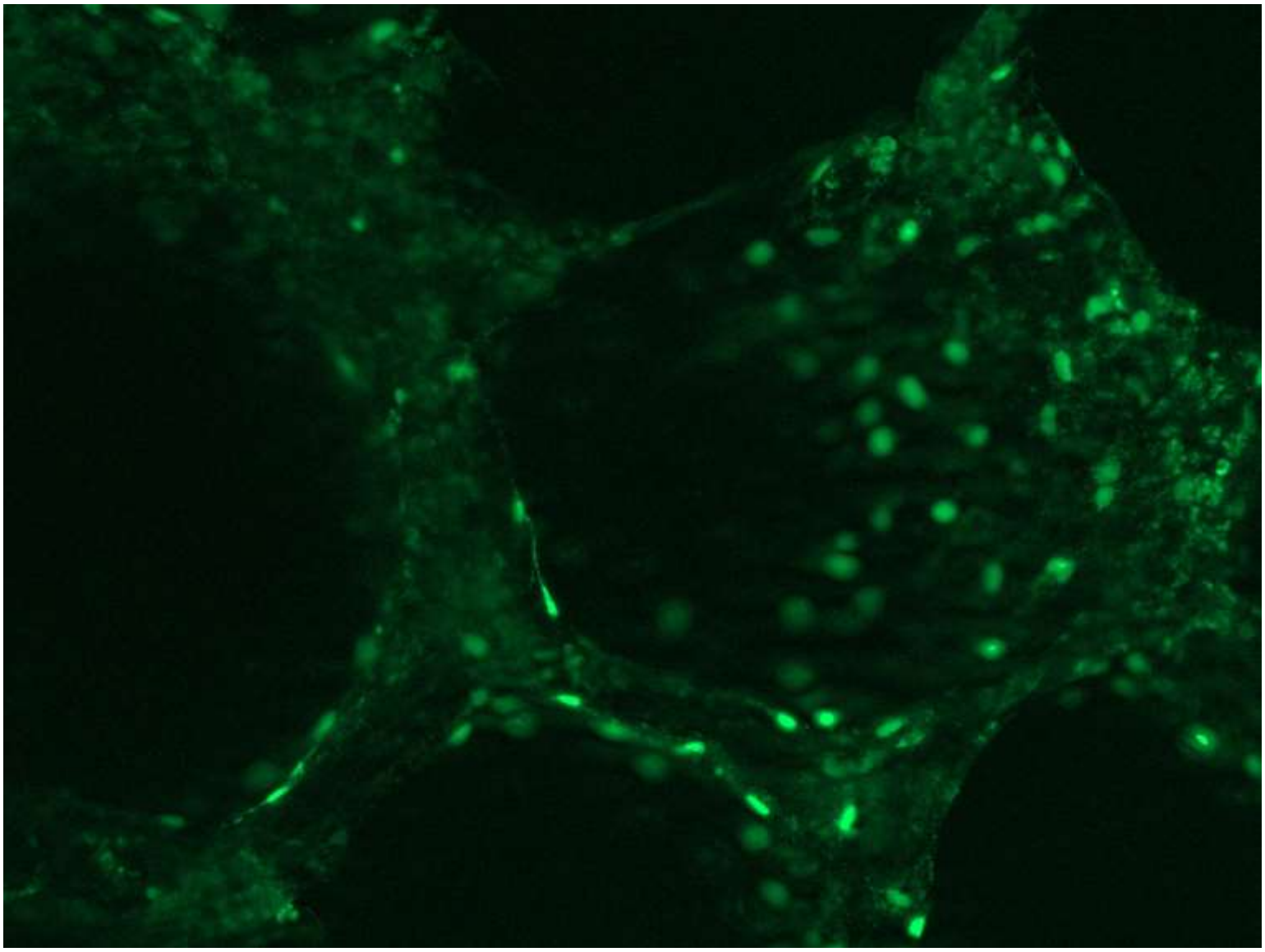
RESULTS AND DISCUSSION:

Electron and photon microscopy studies have shown that pOB are able to bind, spread and proliferate on the surface of the BG-PCL and in 3D scaffolds. These results are supported by the phosphorylation of Focal Adhesion

Kinase (FAK) on its tyrosine 397, observed in western blot. Consistently, the study of cell proliferation demonstrates the absence of cytotoxic effects by the dissolution products from BG-PCL. When grown in the presence of BG-PCL, pOB exhibit a greater stimulation of the cell differentiation markers compared to DBB (control). Western blot reveal an up-regulation of the proteins runx2 and osx, two transcription factors involved in osteoblastogenesis (≈200% BG-PCL vs DBB). Besides, alkaline phosphatase assays confirmed an enhanced activity (+ 2100% after 14 days) in the presence of BG-PCL, suggesting a greater stimulation of osteoblast mineralization processes by BG-PCL. This benefit is validated by the µCT data obtained after the implantation of BG-PCL in a robust craniotomy mouse model.

CONCLUSION:

In vitro and in vivo results support the development of BG-PCL biomaterials for clinical outcomes in regenerative medicine. In addition, the synthesis method at room temperature allows homogeneous organic doping of the BG-PCL. This breakthrough innovation was not feasible so far and is currently being evaluated in our laboratory.



Picture 1: Caption 1: Primary rat osteoblasts grown in a PCL-BG hybrid scaffold and observed by fluorescence microscopy

Natural biomaterials for tissue engineering and regenerative medicine 16:30 - 18:00 Auditorium I 12/09/2018

Oral presentation

T.B.C.

Manuela Gomez

Natural biomaterials for tissue engineering and regenerative medicine 16:30 - 18:00 Auditorium I 12/09/2018

Oral presentation

383 Liquid platelet-rich fibrin (i-PRF) enhances the vascularization of a non-cross-linked collagen matrix in vivo

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[2]Pain center Nis France, France

INTRODUCTION:

Vascularization is highly necessary for tissue regeneration[1]. Platelet-rich fibrin (PRF) is a blood based concentrate system obtained from the centrifugation of peripheral blood without additional anticoagulants[2]. This system is clinically highly relevant and was developed to support regeneration in damaged tissues. In vitro studies proved that reducing the relative centrifugation force (RCF) during the preparation of PRF leads to significant enrichment of the matrices with platelets and leukocytes in addition to the significant increase of growth factor release[2,3]. The aim of the study was to investigate the influence of liquid PRF on the in vivo vascularization and integration of a non-cross-linked collagen matrix in vivo.

METHODS:

Liquid PRF (i-PRF) was gained from human blood. The collected blood was immediately centrifuged at either high-RCF (966 g) or low-RCF (60 g) and applied on the collagen matrix. The resultant biomaterial PRF construct was subcutaneously implanted in severe combined immunodeficiency (SCID) mice (n=4 per group). For the control group, the biomaterial was rinsed in phosphate-buffered saline (PBS) and subcutaneously implanted. After 10 days, the implantation areas were analyzed histologically. Immunohistochemical staining and histomorphometrical analysis was performed for the vessel density using CD-31 for mice and the distribution of human inflammatory cells, expressing vimentin, CD-3, CD-20, or CD-68 in the implantation bed.

RESULTS AND DISCUSSION:

The vessel density within the biomaterial was significantly higher in the case of low-PRF compared to high-PRF (P<0,001) and control-group (P<0,0001). Vimentin expression as a pan marker for human cells was highly expressed in the group of low-PRF, whereas no cells were found in the group of high-PRF. Thereby, the expression of human cell markers was significantly higher in the group of PRF-low compared to PRF-high and control-group (CD-3 P< 0,001; CD-20 P<0,05 and CD-68 P<0,0001). Previous studies proved the angiogenesis effect of preculturing biomaterial using primary cells as a tool to enhance vascularization[1]. However, this concept is difficult to translate to clinical application.

The present findings highlight the use of PRF as an alternative to primary cells to enhance the biomaterial vascularization. Centrifugation using low-RCF led to significant increase of the cell-mediated vascularization in vivo. This concept is highly relevant for clinical application because of its simple preparation and high potential for translation to clinical application.

CONCLUSION:

The use of i-PRF as an autologous source of inflammatory cells and growth factors provides pro-angiogenic stimuli and supports biomaterial vascularization as a clinically applicable tissue engineering concept.

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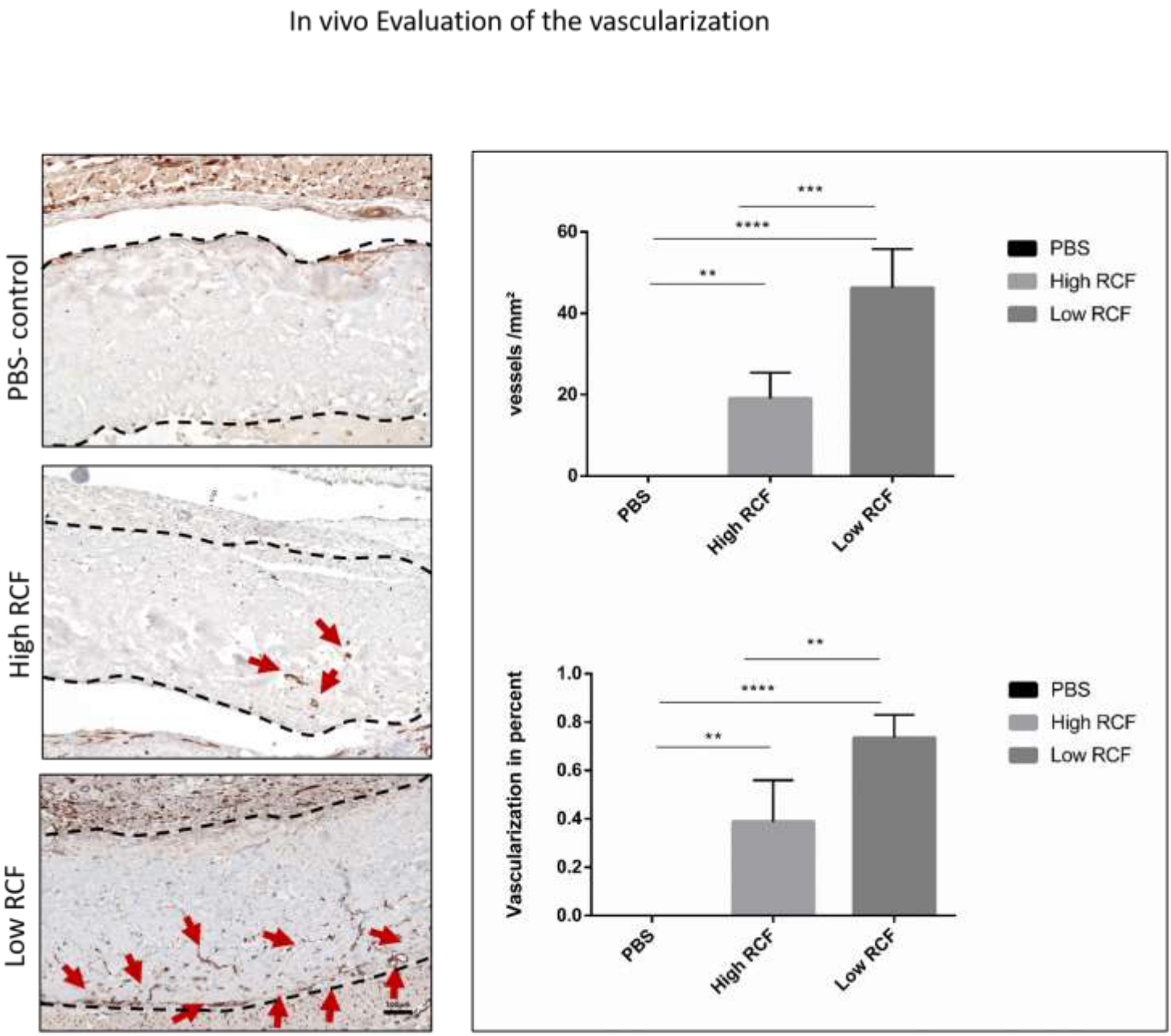

Picture 1: Caption 1: The vascularization patern in vivo after 10 days

Natural biomaterials for tissue engineering and regenerative medicine 16:30 - 18:00 Auditorium I 12/09/2018

Oral presentation

275 Bone and spinal cord extracellular matrix hydrogels and their potential for utilisation in spinal cord injury

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INTRODUCTION:

Spinal cord injury (SCI) induces chronic inflammation creating an unsuitable environment for regeneration. Injectable extracellular matrix (ECM) hydrogels have been shown to influence inflammation[1] and have been used as cell delivery vehicle[2]. This study determined whether ECM hydrogels from spinal cord (scECM) and bone (bECM) can be combined with mesenchymal stem cells from dental apical papilla (SCAP) and potentially delivered to spinal cord lesion as a strategy to mitigate inflammation.

METHODS:

Hydrogels were formed as previously described[3]. SCAP viability in hydrogels was determined using PrestoBlue (N=3, n=3). A time sweep test determined rheological properties (N=3). *In vivo* gelation was tested in rat brain biopsy model (N=3). Inflammation mitigation was assessed on LPS-stimulated murine microglia, BV2 cells (N=3, n=3). One-/two-way ANOVA and Tukey's post-test were performed in GraphPad Prism 7.02 ($p<0.05$).

RESULTS AND DISCUSSION:

SCAP viability in 8mg/mL scECM (S8) was above 75% over the 7 days. Viability in 8mg/mL bECM (B8) was above 55%. With an increased bECM concentration (10 mg/mL (B10)), viability decreased significantly. 10mg/mL scECM was heterogeneous and difficult to deliver reproducibly. Lower storage modulus and slower gel formation of S8, compared to B8, prompted the addition of B10 and fibrin. S8 and B10 combinations supported the viability similarly to S8. Fibrin (F) in S8 lowered the viability only on day 0 by 30%. S8/B10 75/25 and S8/F 75/25 had significantly higher storage moduli and quicker onset of gelation compared to S8. Mechanical properties of S8/B10 75/25, S8/F 75/25 and B8 allowed *in vivo* gelation whilst S8 could not gel. Anti-inflammatory properties of S8 and B8 were tested in two forms: solid gel formed on cell culture insert (indirect contact) and gel suspended in medium (direct contact). Neither S8 nor B8 in any of the forms had significant influence on TNFα secretion in unstimulated BV2, suggesting that they, as other ECM hydrogels[1], do not cause inflammation. No significant reduction in *Tnf* or *Nos2*/*Arg1* expression in LPS-stimulated BV2 (Figure 1) suggested the lack of anti-inflammatory activity of S8 and B8 on these cells.

CONCLUSION:

Mechanical properties of scECM appeared inadequate for SCAP delivery to SCI, unless improved with fibrin or B10. B8 characteristics implied wider utilisation potential. S8 and B8 showed no effect on inflamed microglia cells.

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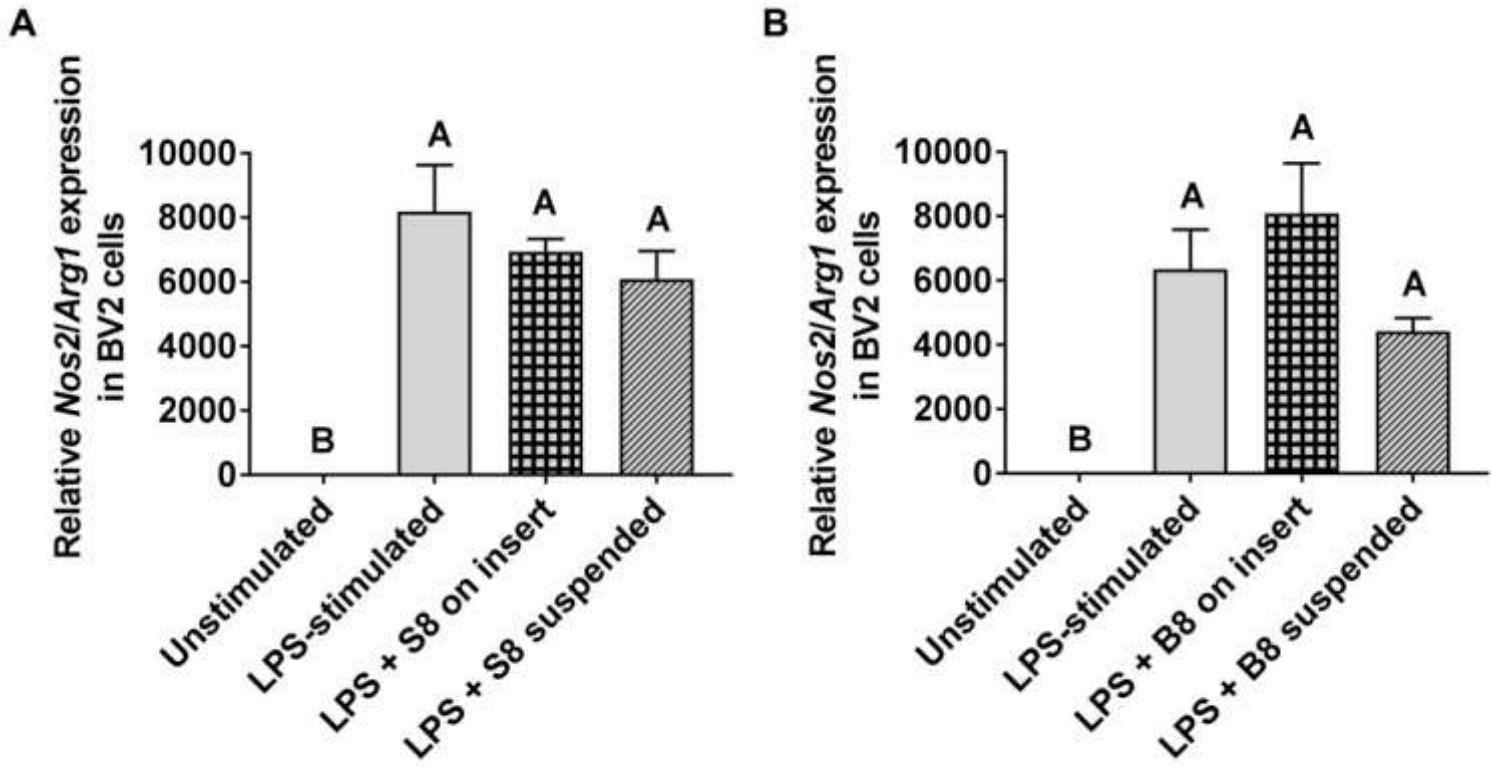
ACKNOWLEDGMENTS:

The project was funded by Erasmus Mundus NanoFar.



*** Conditions not connected by the same letter are significantly different (p<0.05).**

Picture 1: Caption 1: Influence of scECM 8mg/mL (S8) and bECM 8mg/mL (B8) hydrogels on LPS induced inflammation in two different settings: pre formed hydrogel on cell cultu

Natural biomaterials for tissue engineering and regenerative medicine 16:30 - 18:00 Auditorium I 12/09/2018

Oral presentation

50 Functionalized collagen scaffolds result in sustained postnatal regeneration after in utero closure of skin defects in sheep

Willeke Daamen[1], Corien Oostendorp[2], Paul Geutjes[3], Frank Smit[4], Dorien Tiemessen[5], Sjoerd Polman[2], Aya Abbawi[2], Katrien Brouwer[6], Alex Eggink[7], Wout Feitz[5], Toin van Kuppevelt[2]
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INTRODUCTION:

For a number of congenital anomalies, like spina bifida, it may be advantageous to close the defect in the prenatal period. In case of spina bifida, this would prevent *in utero* degeneration of the exposed spinal cord tissue and improve neurological outcome.

METHODS:

In this study, three full-thickness skin defects were created in sheep at 79 days post gestation of which one was treated with a collagen scaffold (COL), one with a collagen scaffold loaded with heparin, vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2) (COL-HEP/GF) and one was left untreated. Skin regeneration was evaluated 1 and 6 months postnatally using semi-quantitative histological analysis on excised skin specimens and comprehensive gene expression microarray analysis on laser-dissected dermis and epidermis.

RESULTS AND DISCUSSION:

Histology showed that the addition of heparin and growth factors to the collagen scaffold significantly increased the total surface area of regenerated skin with hair follicle generation. One month after birth epidermal thickness was increased, but at six months this was reduced to near normal thickness. In COL-HEP/GF, scaffold degradation was significantly decreased. Gene expression microarrays showed a shift in the total number of differentially expressed genes from the epidermis at one month to the dermis at six months. At one month in the epidermis, GO-terms “mitochondrial translational termination” and “mitochondrial translational elongation” were enriched in COL-HEP/GF compared to the untreated defect. At six months in the dermis, both collagen scaffold treatments led to enrichment of GO-terms related to extracellular matrix organization compared to native skin. Furthermore, fold change analysis revealed that the gene expression pattern of COL-HEP/GF more closely resembled normal skin than COL.

CONCLUSION:

Our data indicate that *in utero* intervention during skin wound healing with functionalized collagen scaffolds loaded with heparin, VEGF and FGF2 has long-term beneficial effects on skin regeneration. This approach offers new possibilities for the treatment of closure defects such as spina bifida.

ACKNOWLEDGMENTS:

This work was supported by the European Union by the projects EuroSkinGraft (grant agreement 279024) and EuroSTEC (LSHB-CT-2006-037409).

Natural biomaterials for tissue engineering and regenerative medicine 16:30 - 18:00 Auditorium I 12/09/2018

Oral presentation

549 Antibacterial hydrogels based on Gellan Gum and Manuka honey for tissue engineering applications

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INTRODUCTION:

One of the hardest challenges in biomaterials science consists in the repair of cartilage. Hydrogels represent very promising candidates even if their clinical application is still limited by unsuitable mechanical performances and

proneness to infection. Accordingly, here we present an innovative antibacterial hydrogel with superior mechanical properties. Gellan gum (GG) was used to prepare a composite hydrogel, adding Manuka honey (MH) as molecular spacer to enhance the gel viscosity and introduce antibacterial properties. Furthermore, halloysite nanotubes (HNT), natural biocompatible clays, were added to increase the gel compressive modulus up to ~140kPa. Compared with other natural hydrogels for cartilage regeneration described in literature, the developed gels showed superior mechanical performances. Moreover, the MH doping inhibited the formation of *Staphylococcus aureus* and *epidermidis* biofilm without influencing stem cells viability and chondrogenic differentiation.

METHODS:

To prepare the composite hydrogels, an aqueous solution of MH (2%w/v) was mixed 1:1 with GG, dissolved in water in presence of HNT (0.5w/v). The final mixture was poured into molds and ionically crosslinked as previously described[1]. Freeze-dried samples were exploited for characterization techniques, while the as prepared hydrogels underwent mechanical and biological studies.

RESULTS AND DISCUSSION:

An accurate spectroscopic characterization was performed by XPS and FT-IR/ATR. The elemental composition obtained by XPS analysis revealed, beyond the main elements due to organic materials, the presence of calcium or magnesium employed for crosslinking and, in the case of nanoclays reinforced hydrogels, the presence of silicon and aluminum in the characteristic 1:1 stoichiometry. From FT-IR/ATR analysis, the region typical of honey's major components was evident (1500-750cm^{-1}). The water uptake profile, as well as the degradation behavior of the hydrogels were studied. Mechanical compression and stress-relaxation test showed high compressive moduli (>100kPa) for all the prepared samples.

The antimicrobial effectiveness of the gels was demonstrated against clinical isolates of *S. aureus* and *S. epidermidis*, whose viability was noticeably decreased up to 72h. Furthermore, human mesenchymal stem cells (hMSCs) were successfully cultured on the gels surfaces and properly differentiated in chondrocytes after 21days.

CONCLUSION:

The prepared hydrogels showed superior mechanical properties and, at the same time, were effective against two multidrug resistant *staphylococci* strains without interfering with hMSCs proliferation and chondrogenesis. Therefore, the obtained results encourage a deeper investigation of the developed hydrogels, which have the potential to become, in future, smart cartilage substitutes.

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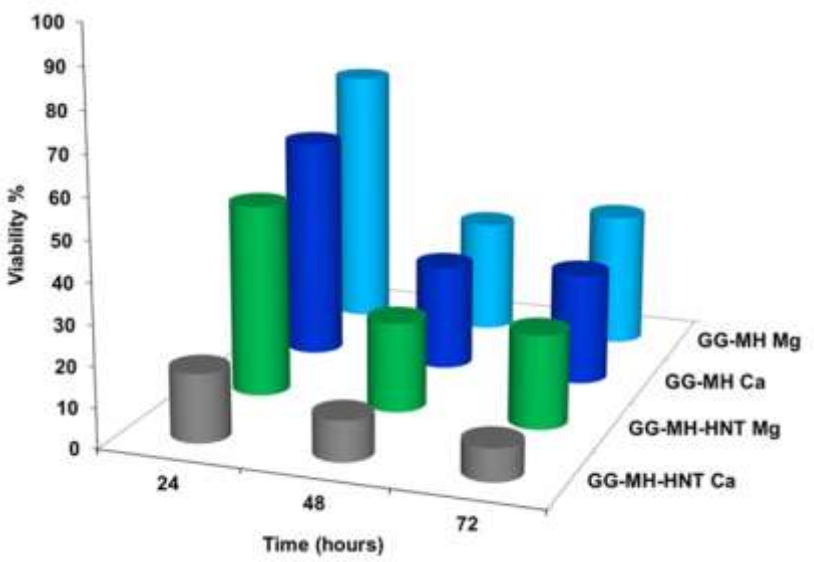

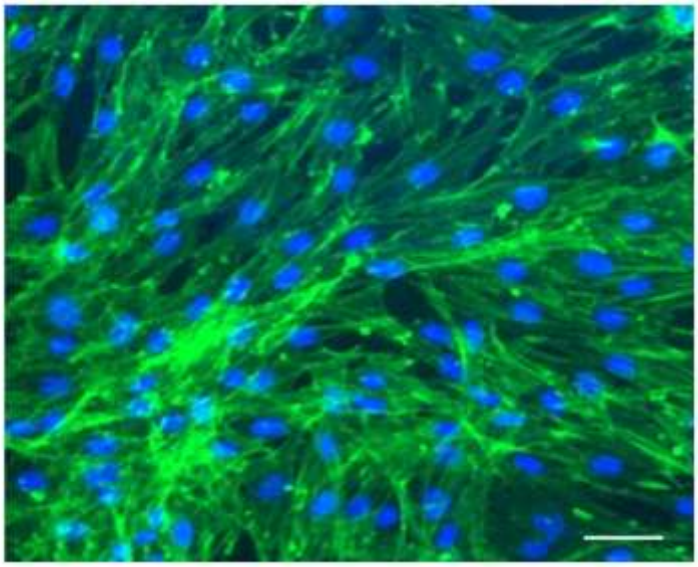

Picture 1: Caption 1: Fig.1 S. aureus biofilm viability evaluated by XTT assay (left). Fluorescence microscopy image of hMSCs seeded onto hydrogel (right). Scale bars:50µm.

Nanostructured biomaterials for cell regulation 09:45 - 11:15 Auditorium II 12/09/2018

Oral presentation

Light-activatable (nano)materials for cell regulation

Miguel Lino1,2, Carlos Boto2, Sonia Pinho2, Catarina Rebelo1,2, Akhilesh Rai2, Catarina Almeida1,2, Lino Ferreira1,2
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Nanomaterials are an emerging platform to control the activity of endogenous stem cells [1, 2]. These nanomaterials can be fabricated from a wide variety of components, including polymers, lipids and metals [3-5]. Because of their small size, surface chemistry for cell targeting, the possibility of remote activation, the chance of encapsulate both hydrophilic or hydrophobic molecules while protecting them during their circulation in the body, these nanomaterials are very promising for the control of (stem) cell activity. These nanomaterials may be used to replace viral vectors for gene edition and therapy, and thus preventing undesired side effects.

In case of stem cell niches spatially well defined, nanomaterials can be administered directly into the niche. This is the case of the subventricular zone niche located in the brain that hosts an important niche of neural stem cells. In this case, formulations may be administered by intracerebroventricular/intracerebral infusion [6]. This strategy increases the success of targeting, maximizing the amount of bioactive agent that reaches the stem cells. However, in most cases, the stem cell niche is not spatially well defined. For example, the hematopoietic stem cell niche is located in the bone marrow within the human body. In this case, the nanomaterials are administered systemically (alone or conjugated to stem cells [7, 8]) to access the stem cell niche. Alternatively, the nanomaterials should have components (e.g. ligands or antibodies) or properties (e.g. plasmonic activity) in their surface to overcome the multiple barriers in the human body and finally target the stem cell niche. After stem cell targeting, the nanomaterials need to overcome the endolysomal compartment and reach the cell cytoplasm.

An attractive possibility to facilitate the targeting of the formulations to the stem cell niche is by the use of external stimuli. In this case, nanomaterials respond to magnetic forces, light, or ultrasounds and release the cargo with spatio-temporal control. The use of external stimuli may improve significantly the therapeutic effect of the formulations in the stem cell niches. In addition, the nanomaterials may open biological barriers. For example, the

thermal energy generated by magnetic heating of magnetic nanoparticles may increase BBB permeability [9]. During my talk, I will present our latest results in the use of light-activatable nanomaterials to regulate cell activity and cross biological barriers.

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Nanostructured biomaterials for cell regulation 09:45 - 11:15 Auditorium II 12/09/2018

Oral presentation

Emergent, collective behaviour of cell groups in long-range biomaterial mechanosensing

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INTRODUCTION

Cells attach to and exert tensile and compressive forces on extracellular matrix (ECM), ‘measuring’ the resultant deformation that develops. The degree of resistance of an ECM to deformation is dependent on its stiffness. Cells use information obtained in this way to make fundamental decisions in how to move, divide and differentiate.

Stiffness is not only dependent of the elastic modulus of the ECM, however, but also on its dimensions. This has the corollary that single cells are able to sense underlying stiff substrata through soft ECMs at low (<10 μm) thicknesses[1]. Here, we hypothesised that groups of cells would be able to deform materials to a greater degree than individual cells, and therefore be able to act collectively to mechanosense underlying substrates or features at greater depths than individual cells.

METHODS

To test this, we fabricated polyacrylamide hydrogels in the range of 1 - 1000 μm in thickness and of 0.5 – 40kPa elastic modulus adhered to glass substrates. Hydrogel surfaces were then covalently modified with ECM proteins, and MG63 cells were plated on hydrogels either at low density or in compact colonies/islands. Cell density, cell aspect, and cell perimeter was measured by microscopy, and hydrogel displacement by time-lapse imaging of hydrogel-embedded fluorescence fiduciary beads.

RESULTS

The spreading of separated, single cells on soft (1kPa) hydrogels increased exponentially as function of decreasing hydrogel thickness, with a half maximal response at ~3.2 μm. Similarly, the spreading of cells within cell islands of defined area (4×10^4 - 4×10^5 μm^2). also increased exponentially as a function of decreasing hydrogel thickness, but with a much greater half-maximal response of 54 μm^2. Depth-sensing was dependent on Rho kinase activity. Hydrogel displacements were greater for colonies vs. single cells and for thick gels *vs.* thin gels.

CONCLUSIONS

These results support the notion that groups of cells act collectively to mechanosense rigid materials beneath elastic hydrogels at greater depths than individual cells. This raises the intriguing possibility that the collective action of cells in tissues such as epithelia may allow cells to sense structures of differing stiffness at comparatively large distances. This has implications in cell patterning and differentiation in development, in tissue healing, and in the design of implanted biomaterials.

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ACKNOWLEDGEMENTS

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Nanostructured biomaterials for cell regulation 09:45 - 11:15 Auditorium II 12/09/2018

Oral presentation

656 A High-throughput screening device to study 3D cell-material interactions

Gomes boaventura David, Mota Carlos, Espinosa-Camarero Sandra, Moroni Lorenzo
MERLN Institute for Technology-Inspired Regenerative Medicine, Masstricht, Netherlands

INTRODUCTION:

Culture of cells on 3D micro-environments is far more complex than 2D systems (1). When cells are cultured in 3D systems, the effect of soluble factors, cell morphology, spatial distribution, cell-cell and cell-matrix interactions play a crucial role on cell homeostasis (2). However, when attempting to deconstruct the role of each of these parameters and their interplay, an increasing number of experiments, volume of material, cells and time are necessary. To

increase the experimental conditions while reducing the complexity and the volumes of hydrogel, we used a high-throughput screening (HTS) system based on a miniaturized well plate that allows for the simultaneous screening of the response of human mesenchymal stromal cells (hMSCs) to multiple 3D conditions.

METHODS:

The HTS system was fabricated by hot press-moulding of poly(ethylene oxide terephthalate)-poly(butylene terephthalate) (PEOT/PBT, 300/55/45, Polyvation B.V.) copolymer. Five microliter of peptide-conjugated alginate (FMC polymers) with hMSCs (Prockop) at a density of 10 million cell/mL was dispensed in each well and crosslinked with 100 mM sodium chloride solution.

RESULTS AND DISCUSSION:

HTS system master moulds were successfully designed and manufacured. After 24 hours of culture (Figure 1), analysis of cell viability showed similar values to the conventional 10 uL droplets cultured in individual wells. Extended culture periods of up to 5 days revealed values of cell viability, proliferation and apoptosis similar to traditional 3D systems. Finally, we prepared alginate conjugated with several peptide sequences found in the extracellular matrix (ECM) and evaluated hMSCs response in a combinatorial fashion, showing that this system enables the high-throughput analysis of the biophysical and biochemical effect of hydrogels on hMSCs behaviour.

CONCLUSION:

We have successfully optimized a high-throughput culture system as a tool to study interactions between the alginate containing ECM peptides and hMSCs. Such HTS system will ultimately screen the behaviour of cells when exposed to different peptide combinations *in vitro* as well as *in vivo*.

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ACKNOWLEDGMENTS:

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Picture 1:

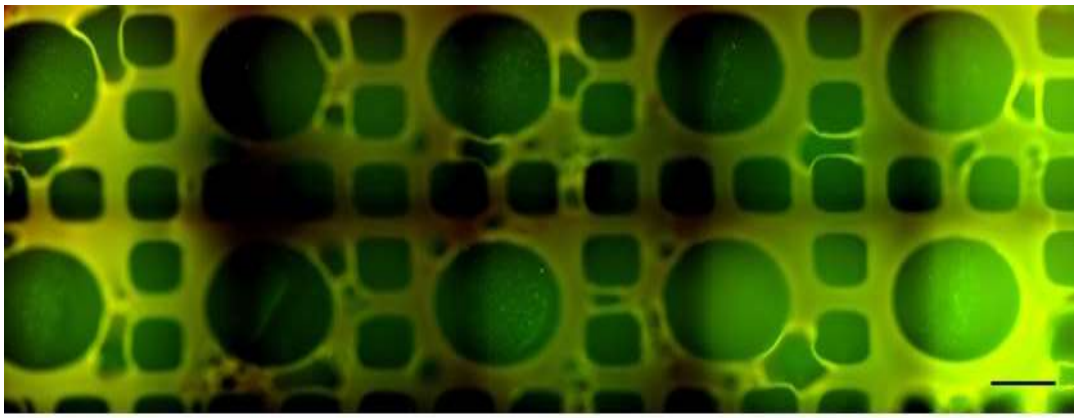

Figure 1 – Cell viability assay on hydrogels in the HTS device after 24 hours of culture (live as green and dead as red). Scale bar: 1 mm.

Nanostructured biomaterials for cell regulation 09:45 - 11:15 Auditorium II 12/09/2018

Oral presentation

96 Data-driven framework for rational design of nanotopographies on biomedical devices

Marie Cutiongco, Bjørn Jensen, Julie Russell, Paul Reynolds, Nikolaj Gadegaard
University of Glasgow, Glasgow, United Kingdom

INTRODUCTION:

Implantable biomedical devices are textured with nanotopographies to improve tissue regeneration in situ. However, surface texturing is mostly engineered for a specific cell function, while ignoring the regenerative challenges of the surrounding tissues. Thus, new strategies of biomedical implant design are needed to regenerate tissues holistically and effectively.

METHODS:

Here, a bioinformatics based platform is utilized for rational nanotopography selection exemplified for musculoskeletal regeneration. Nanotopographies with 100 nm pit depth, 300 nm centre-to-centre spacing and varying geometric arrangement, and a flat control were used. Image analysis[1,2] was performed on 5000 myoblasts, osteoblasts, chondroblasts and fibroblasts on nanotopographies at day 2. Meanwhile gene expression of the population was measured using quantitative polymerase chain reaction at day 7 across two independent experiments.

RESULTS AND DISCUSSION:

The cell morphome, consisting of multivariate measurements of focal adhesions, actin and chromatin intensity, texture[3,4]and geometry reflected nanotopography-induced responses. On nanopits with square arrangement[5], myoblasts upregulated myogenic gene expression and exhibited high actin and focal adhesion measurements. Osteoblasts showed highest osteogenic marker gene expression, high focal adhesion textures and radial distribution on nanopits with disordered square arrangement[5]. Nanopits with hexagonal arrangement[6] significantly upregulated chondrogenic gene expression and actin radial distribution in chondrocytes. On unpatterned substrates, fibroblasts minimized expression of pathogenic fibrotic genes and generally showed high actin, focal adhesion and chromatin measurements. Linear regression of the morphome allowed accurate prediction of myogenic, osteogenic, chondrogenic and fibrotic gene expression due to the inherent information in both substrate structure and cell. Furthermore, the morphome accurately (83%) distinguished combinations of cell type and nanotopography that promote formation of muscle, bone, cartilage and fibrotic tissue, as categorised by maximum gene expression. Measures of phosphorylated focal adhesion kinase intensity and texture were critical in predicting gene expression and discriminating functional class types.

CONCLUSION:

Our framework utilizes an information rich dataset containing focal adhesion measurements that encompass cell type and nanotopography. This framework identifies unique signatures of functional cell types for the rational implementation of nanotopographies on implantable biomedical devices.

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Computational tools for biomaterials 14:30 - 16:00 Auditorium II 12/09/2018

Oral presentation

Application of computational methods to develop materials resistant to bacterial biofilm formation

Andrew L. Hook1, David A. Winkler2, Paul Williams1, Morgan R. Alexander1
1University of Nottingham, UK

2Monash University, Australia

The prevention and eradication of bacterial biofilms remains a major unsolved global healthcare challenge. Bacterial biofilms are communities of bacteria that readily form on the surfaces of implanted medical devices such as urinary catheters and endotracheal tubes. Within a biofilm bacteria become up to 1000 times more tolerant to antibiotics and the host's immune defences1. To address this problem novel materials are required that are able to prevent biofilm formation. However, there are innumerable possible materials that could be screened and the interactions between bacteria and surfaces are poorly understood2.

To effectively search for novel materials resistant to bacterial attachment we have used a high throughput screening approach to assess the bacterial-material interaction for hundreds of different polymers in parallel3. We then applied computational modelling to develop structure-function relationships between the material properties and their biological performance4-6. Such models can be used to screen virtual libraries of materials to identify potential candidate polymers for further experimental screening. Two approaches were taken, in the first we modelled hundreds of diverse polymers that represented a large chemical space4,5. Although these models could be broadly applied they were difficult to interpret. Concurrently, we explored correlations between molecular descriptors and a subset of structurally related materials to create a model that more readily enabled the design of new monomers resistant to biofilm formation. Specifically, we have identified a correlation between the molecular rigidity and hydrophobicity of the pendant groups on polyacrylates with bacterial biofilm formation6.

The further development of computational modelling applied to bacterial-material interactions will lead to the identification of optimised materials with biofilm resistant properties by unravelling the complex structure-function relationships.

The authors acknowledge the Wellcome Trust (Senior Investigator Award refs: 103882 and 103884) for funding this research.

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Computational tools for biomaterials 14:30 - 16:00 Auditorium II 12/09/2018

Oral presentation

Computational tools for biomaterial design: from reverse engineering to mechanistic modeling

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Prometheus LRD division of skeletal tissue engineering, KU Leuven, Belgium, Biomechanics Section, KU Leuven, Belgium

One of the major challenges in tissue engineering and an essential step towards successful clinical applications is the translation of biological knowledge on complex cell and tissue behavior into predictive and robust engineering processes. Computational modelling can contribute to this, among others because it allows to study the biological complexity in a more quantitative way. Computational tools can help in quantifying and optimizing micro-environmental signals to which cells and tissues are exposed and in understanding and predicting the biological response under different conditions.

A wide variety of model systems has been presented in the context of tissue engineering ranging from empirical models (data-driven) over gene network models to mechanistic models (hypothesis-based), targeting processes at the intracellular over the cellular up to the tissue level. Each model system has its own benefits and limitations which delineate the context in which it can be used. Whereas mechanistic models are used as in silico tools to design new therapeutic strategies and experiments, empirical models are used to identify, in large data sets, those in vitro parameters (biological, biomaterial, environmental) that are critical for the in vivo outcome.

In this talk I will show a number of examples of these models, all related to the optimization of scaffold design in the context of skeletal tissue engineering. In order to optimize bioceramics-based biomaterials, we have developed models simulating the degradation of the biomaterials upon in vivo implantation, as well as the influence the degradation products have on the local biology. Extensive screening experiments have guided the model formation. In turn, the model is used to predict the bone formation capacity of bioceramics-based biomaterials in combination with a specific cell source. Other in silico models are able to predict the optimal scaffold geometry and quantify the created microenvironment for cells seeded onto the scaffold during perfusion bioreactor culture or try to identify the local mechanical forces put on cells inside hydrogel containing bioinks during 3D bioprinting

The talk will end with an outlook on the different actions that need to be taken when bringing in silico models from the bench to the bed side.

Computational tools for biomaterials 14:30 - 16:00 Auditorium II 12/09/2018

Oral presentation

231 Cell shape induced polarization: a computational modelling approach

Kerbaï Saïd Eroumé, Aurélie Carlier
MERLN, Maastricht, Netherlands

INTRODUCTION:

Topographical cues, coming from for example biomaterials, have been shown to influence cell shape and cell fate dramatically although the underlying mechanisms have not yet been elucidated [1,2]. Previously, Jiang et al. [2] have observed that cells migrate in different directions depending on the initial shape to which they are confined. Here we hypothesize that the initial cell shape influences the “front-to-back” cell polarization and in turn the migration direction.

METHODS:

To study the relation between cell shape and cell polarization, we implemented in Virtual Cell [4] the model developed by Marée et al. [3], for the shapes defined by Jiang et al. [2] (Figure 1A-B). Simulations were run at a mesh size of 70 by 70 elements with a fully implicit solver. Applying an initial linear gradient to the cdc42 activation rate as a function of the x-coordinate for the first 10s, ensured that the initial cell polarization was along the left-right direction (see Figure 1A).

RESULTS AND DISCUSSION:

Despite the initial polarization direction to the right (blue arrow-Figure 1A), the maximal cdc42 concentration, which is typically high at the “front” of the cell, shifted in the reverse direction (Figure 1B). The time at which the maximal cdc42 concentration started to shift and the maximal distance along the horizontal axis over which the front travelled, varied greatly with geometry (Figure 1C). Interestingly, the evolution of the cdc42 concentration was similar for similar cell shapes such as the teardrop, the triangle and the narrow teardrop (Figure 1C-D). The maximal cdc42 concentration travelled the furthest in case of the circle and square (Figure 1C). Note that these are symmetrical cell shapes, without an asymmetrical orientation with respect to the horizontal axis. Moreover, for these symmetrical shapes, the DC, which is the difference between the maximal concentration and the concentration at x=50 micron (the right side of the cell shape) is larger than for the asymmetric shapes (Figure 1D).

CONCLUSION:

The preliminary results of the calculations indicate that initial cell shape affects cell polarization. Future work will focus on model validation through various perturbation tests where the effect of particular pharmacological inhibitors (e.g. latrunculin) are explored both *in silico* and *in vitro*.

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ACKNOWLEDGMENTS:

Dutch province of Limburg LINK (FCL67723) project and Dutch Science Foundation (NWO) VENI grant (number 15057).

Picture 1:

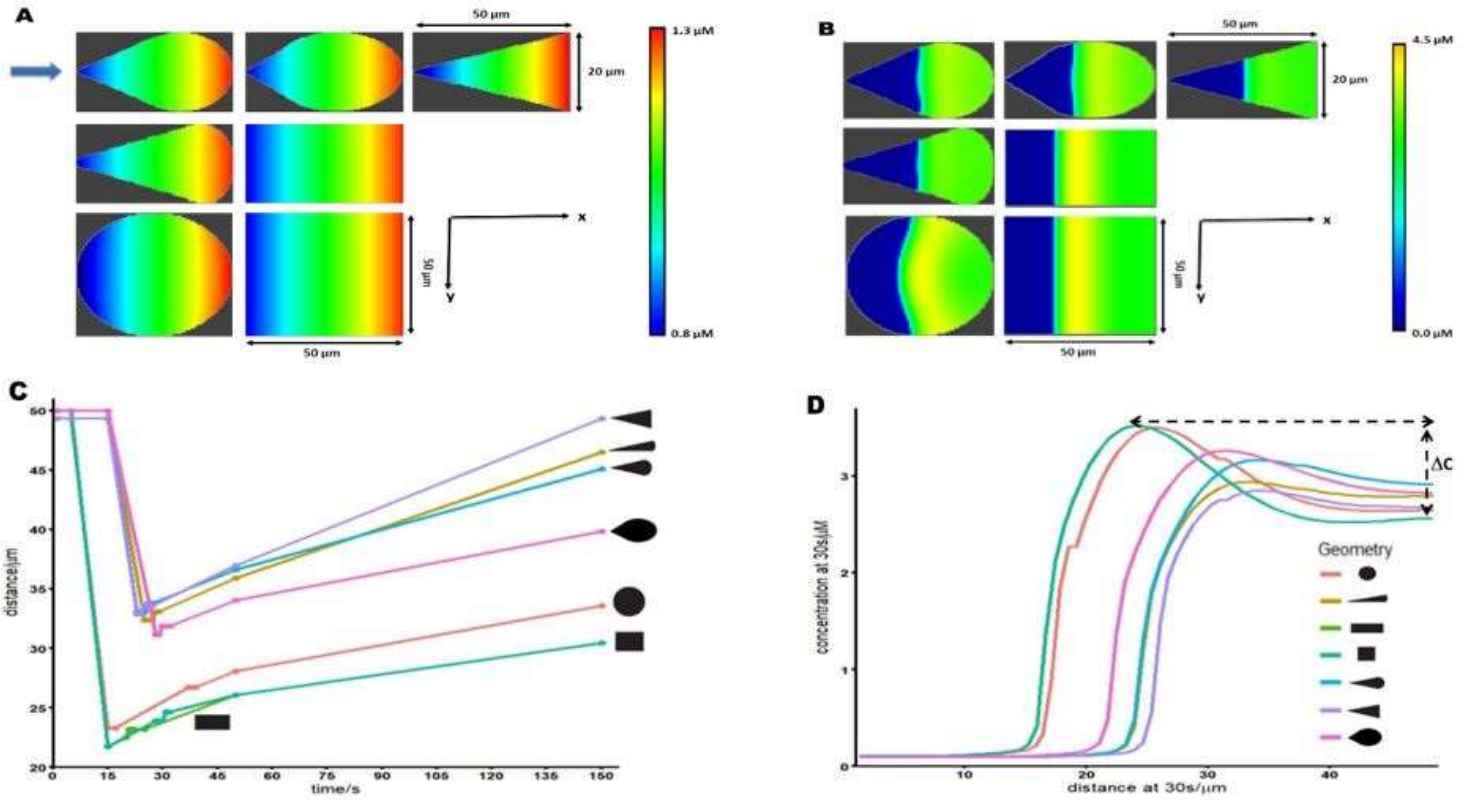

Figure 1: *(A) Spatial distribution of active cdc42 at 1ϟ for all geometries. The blue arrow indicates the direction of initial polarization* ***(B)*** *Spatial distribution of active cdc42 at 30s for all geometries. Note that the scale bars of Figures A and B are different.* ***(C)*** *Evolution of maximal cdc42 concentration as a function of horizontal distance and time.* ***(D)*** *Evolution of cdc42 concentration as a function of distance at time 30s. The rectangle and the square have fully overlapping profiles.*

Computational tools for biomaterials 14:30 - 16:00 Auditorium II 12/09/2018

Oral presentation

229 Analysis of sub-cellular structures in response to surface topographies

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INTRODUCTION:

Surface topography affects cells behaviour, including cells phenotype (e.g. size, shape) and gene expression. The influence of surface topography can be visualised through the morphology of the cytoskeleton, which shapes the cell architecture and gives mechanical resistance to deformation[1]. Here, we used a combination of a high-throughput screening approach and robust data analysis to study the effect of surface topographies on cytoskeleton morphology.

METHODS:

U2OS osteosarcoma cells were cultured on the Nano-Topochip[2], stained with DAPI for nuclei and phalloidin for F-actin after three days of culture and imaged using fluorescence microscopy. The image analysis pipeline is schematically shown in Fig.1A. Images were analysed in CellProfiler[3] to segment and quantify the F-actin. With specific parameters chosen to represent the characteristics of F-actin, we performed hierarchical clustering, and determined the number of optimal groups using the dispersion index criterion. The medoid cell, which had the smallest dissimilarity to all the other objects in the group, was calculated to be the representation of each group.

RESULTS AND DISCUSSION:

Based on the subcellular F-actin traits, surfaces with different features were clustered into 34 groups. From the medoid cells and data comparison, we found that F-actin features valued differently due to various surface topographies as examples shown in Fig.1B. The results show that sub-cellular traits like the cytoskeleton structure are affected and could be used as a proxy to measure the changes of cells on surface topographies.

CONCLUSION:

Using high-throughput screening platforms, we studied the variation sub-cellular trait changes. We observed that features of cell-morphology-related sub-cellular traits like F-actin change along with surface topographies. More specific factors like genes or proteins involved in the morphology change processes will be studied in the future upon upscaling of surfaces of interest. Using this approach, we hope to model cell reaction and behaviour in response to surface topographies.

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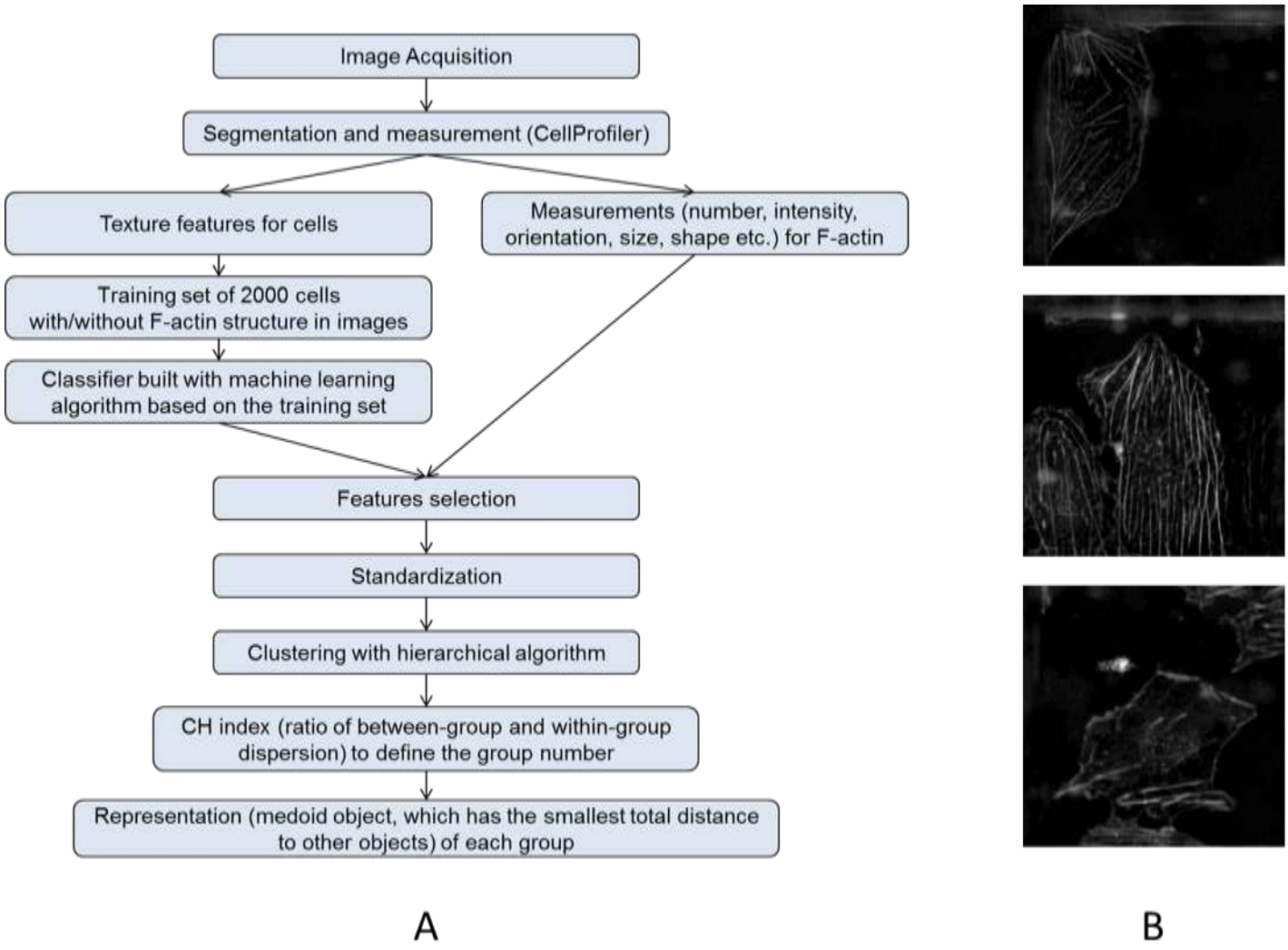
ACKNOWLEDGMENTS:

This work was supported by the Dutch province of Limburg in the LINK (FCL67723) (“Limburg INvesteert in haar Kenniseconomie”) knowledge economy project. AC gratefully acknowledges her VENI grant (number 15057) from the Dutch Science Foundation (NWO).



Picture 1: Caption 1: (A) The schematic overview of workflow from high-content imaging to robust data analysis; (B) Examples of medoid cells from Group 5, 7 and 13 which sh

Biofabrication and biomaterials for advanced tissue grafts and models 16:30 - 18:00 Auditorium II 12/09/2018

Oral presentation

Designing and processing hydrogel bioinks for 3D printing applications

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Department of Bioengineering, University of Pennsylvania, USA

Hydrogels represent a class of biomaterials that have great promise for the repair of tissues, particularly due to our ability to engineer their biophysical and biochemical properties1. 3D printing approaches are now being developed to process hydrogels into structures with the appropriate shapes and patterns for tissue repair2; however, printing processes are often not compatible with hydrogels optimized for a desired cell response. Thus, we have developed techniques to both screen hydrogels for a desired cell response and to process these materials into printable bioinks.
Towards MSC chondrogenesis, we have developed a screening platform using the patterning of photocrosslinkable norbornene-modified hyaluronic acid hydrogels with biochemical signals3. These include

peptides that mediate cell-matrix adhesion (i.e., RGD) or cell-cell adhesion (i.e., HAVDI). When cells are encapsulated within the hydrogels incorporating orthogonal gradients, optimal formulations can be identified through imaging of MSC differentiation markers (e.g., Sox9, aggrecan). As these are non-viscous precursor solutions, they are difficult to 3D print using traditional printing approaches, such as extrusion-based printing. With extrusion-based printing, a bioink must flow during extrusion, but then be rapidly stabilized post-extrusion to maintain the desired printed structure. To address this, we have developed two approaches to 3D print non-viscous bioinks: (i) curing the material with light through a transparent conduit immediately prior to extrusion4, and (ii) processing the materials into microgels using microfluidics that can be jammed and printed as solids. Both of these approaches have been successful in the processing of non-viscous hydrogel precursors into stable structures and have been used to encapsulate cells with high cell viability. Ultimately, the design of new bioinks and printing processes will lead to successful applications of 3D printing in the repair of tissues.

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Biofabrication and biomaterials for advanced tissue grafts and models 16:30 - 18:00 Auditorium II 12/09/2018

Oral presentation

3D Printing and bioprinting with high spatial resolution, challenges and perspectives

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Additive Manufacturing Technologies Group (amt.tuwien.ac.at/Ovsianikov)
Austrian Cluster for Tissue Regeneration (www.tissue-regeneration.at)

Additive manufacturing technologies, also referred to as 3D printing, are experiencing rapid development by providing disruptive solutions across different industrial sectors. They also play an increasingly important role in biomedical and tissue engineering applications. Bioprinting aims at additive manufacturing from materials containing living cells. These materials, often referred to as bioinks, are based on cytocompatible hydrogel precursor formulations, which gel in a manner compatible with different 3D bioprinting approaches [1]. Among the most widespread bioprinting technologies are methods based on extrusion and ink-jet material deposition. The achievable spatial resolution is therefore in the range of tens of micrometers, limited by intrinsic properties of these methods. In the context of biomedical and tissue engineering applications of 3D Printing and Bioprinting, multiphoton lithography (MPL) is an outstanding approach as it can produce features smaller than a single mammalian cell (down to around 100 nm). For example, it has recently enabled realization of highly porous microscaffolds capable of hosting individual cell spheroids [2]. MPL is also fundamentally different in that it does not rely on material deposition, instead the materials is locally modified using photochemistry induced by multiphoton absorption of ultra-short laser pulses [3]. Depending on the material MPL can produce high-resolution volumetric structures, induce photodegradation or spatially resolved covalent binding of specific molecules in the volume of the sample [4]. 3D printing of cell-containing hydrogel structures with high spatial resolution opens exciting perspectives for the engineering of 3D biomimetic cell culture matrices. Development of cell compatible and photopolymerizable hydrogels is an important step towards the latter goal. Current challenges include possible cell damage, resulting from generation of free radicals, and necessity for faster processing [5]. In this contribution, our recent progress on MPL development will be presented. Current state of the art, challenges and future perspectives will be discussed.

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Biofabrication and biomaterials for advanced tissue grafts and models 16:30 - 18:00 Auditorium II 12/09/2018

Oral presentation

686 Combining 3d-printing of a low-temperature setting calcium phosphate paste with melt electrowritten microfiber meshes for reinforcing interfaces in engineered osteochondral grafts

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[2]University Medical Center Utrecht (UMC), Netherlands

INTRODUCTION:

Calcium phosphate-based ceramics are promising biomimetic materials for bone regeneration, due to the similarity with the mineral composition of native bone. During manufacturing, some bioceramics need post-treatment with high temperature or acidic pH. These processes hinder several applications, for instance, direct incorporation of biological substances and low-melting, thermolabile biomaterials, which are promising in combination with bone substitutes to recapitulate biological interfaces, such as the osteochondral boundary.

In this study, we developed a 3D-printing approach to create an osteochondral graft with improved bone-to-cartilage interfacial integration by combining a low-temperature setting printable calcium phosphate paste (PCaP) with hydrogel-laden thermoplastic microfiber meshes obtained by melt electrospinning writing (MEW).

METHODS:

The PCaP consisted of α-tricalciumphosphate, nano-hydroxyapatite and biodegradable, crosslinkable poloxamer[1]. PCaP was printed into porous bone substitutes via an extrusion-based printer (3D discovery, regenHU). To form an osteochondral construct, PCaP was directly deposited on a polycaprolactone (PCL) mesh, fabricated via MEW (custom-made device). Finally, gelatin methacryloyl (gelMA) was infused to fill the space between PCL microfibers that were anchored into the PCaP and protruded to the chondral region. Compressive properties of PCaP scaffods and reinforced gelMA, including the strength of interconnection at the hydrogel-ceramic interface were investigated. The biological activity of mesenchymal stem cells (MSCs) on PCaP scaffolds was investigated. Results were analyzed by Wilcoxon rank sum and Kruskal-Wallis tests. Statistical significance was considered when $p > 0.05$.

RESULTS AND DISCUSSION:

The PCaP scaffolds were printed at ambient temperature through a 250µm-diameter nozzle and set under humidity at 37°C. MSCs seeded onto such bone substitute were able to proliferate and undergo osteogenic differentiation. PCL microfibers were efficiently embedded within PCaP, as showed by SEM micrographs. Microfiber incorporation improved 7-fold the interfacial shear stress compared to the structure without this embedded microfiber mesh. Tangent modulus and ultimate strength of bone region were in the range of cancellous bone. The compressive modulus of the reinforced hydrogel, used as chondral compartment, was also increased 3-fold compared to MEW-reinforced hydrogels and 19-fold compared to the hydrogel alone.

CONCLUSION:

3D-printing of a low-temperature setting PCaP allowed accurate integration of microfibrous MEW-printed meshes with controlled architecture, which acted as interconnection at the interface between the PCaP bone substitute, and a soft hydrogel optimized as chondral substitute. Both the strength of interconnection at hydrogel-to-ceramic interface and the modulus of the otherwise soft gelMA were greatly increased, providing important cues for the design and fabrication of complex multimaterial constructs.

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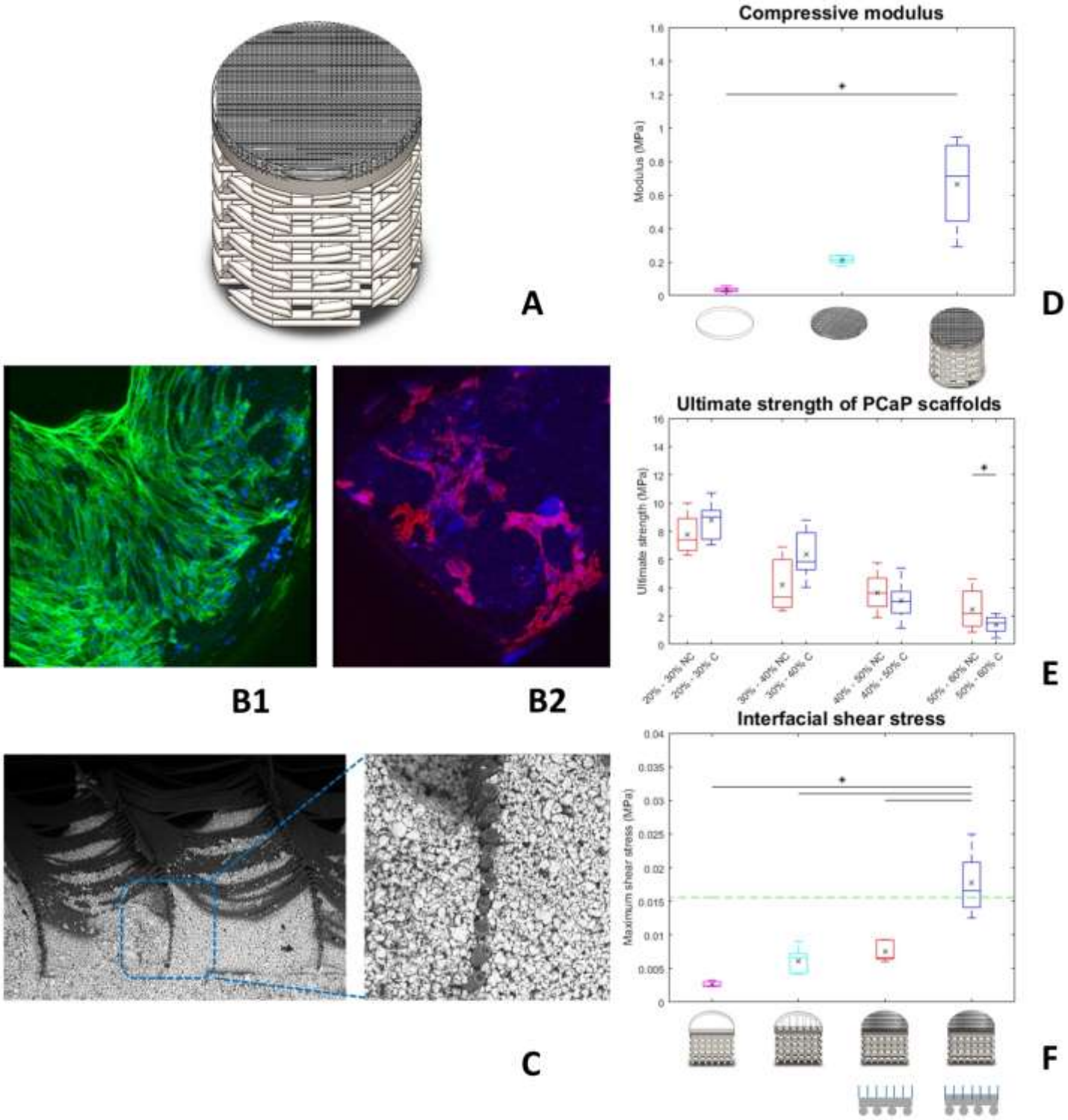

Fig 1 : A; An osteochondral structure, B1; MSC proliferation on PCaP scaffold at day 14 under proliferation media, B2; Osteonectin expression from MSCs on PCaP scaffold at day 21 (osteogenic induction media), C; SEM images of embedded microfibers in PCaP, D; compressive modulus of reinforced hydrogel at chondral region, E; Ultimate strength of PCaP scaffolds and F; Interfacial shear stress at the interface

Picture 1:

Biofabrication and biomaterials for advanced tissue grafts and models 16:30 - 18:00 Auditorium II 12/09/2018

Oral presentation

234 Magnetically responsive gelatin-based nanocomposite ink for remote control of 3D printed bio-inspired structures

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[1]AO Research Institute Davos, Davos platz, Switzerland
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INTRODUCTION:

Gelatin Methacryloyl (GelMA) is a promising photopolymerizable hydrogel for medical additive manufacturing applications thanks to its tunable physical properties and likeness to extracellular matrix (ECM)[1]. In addition, quick-responsivity and remote controllability through low external magnetic field can be achieved by incorporation of magnetic responsive elements in polymeric networks[2]. Thus, we report the development and the characterization of a GelMA-based magnetically responsive ink using PEG capped Iron Oxide Nanoparticles (IOPs).

METHODS:

IOPs, prepared following previous protocol[3], were homogeneously dispersed at several concentrations in GelMA (10 to 15% w/v) with a photo-initiator (IRGACURE2959, 0.03%w/v). The system was subjected to static external magnetic field and subsequently UV-crosslinked to obtaining 3D hierarchically patterned nanocomposites. Morphological analysis via optical and scanning electron microscopy (SEM) were performed. To understand the influence of the IOPs and temperature in the process, rheological properties were investigated. Metabolic activity, morphology and alignment of eukaryotic cells seeded on top (2D) or embedded (3D) in the nanocomposite hydrogels were evaluated. 3D GelMA/IOPs bio-inspired architectures were successfully printed by a nozzle-based bioprinter (3D Discovery, RegenHU Ltd) and their potential use for soft-robotic applications tested.

RESULTS AND DISCUSSION:

SEM images show homogeneous dispersion of IOPs within the hydrogel and formation of aligned IOPs rods in the GelMA exposed to magnetic field. Rheological characterization indicates shear thinning behavior of the ink, and no influence of the IOPs on the gelation time. No viscosity changes upon addition of IOPs were observed in GelMA 10% w/v and 15% w/v. Metabolic activity of cells seeded on top of GelMA and GelMA/IOPs nanocomposite showed no statistical differences. Interestingly, cells showed significantly higher metabolic activity when encapsulated within homogeneously dispersed IOPs. This suggests an UV-protection effect of IOPs. Cells align with the alignment of the IOPs rods. 3D printed constructs immersed in water and exposed to an alternating magnetic field respond with a controlled swimming motion.

CONCLUSION:

This work reports a simple approach to create a magnetic and cytocompatible gelatin-based nanocomposite ink. Here we demonstrate its potential use in the field of bio-inspired soft-robotic through the printing of 3D complex architectures responding to alternate magnetic field.

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Intelligent biomaterial properties & applications 14:30 - 16:00 Room 0.4 12/09/2018

Oral presentation

208 Smart contact lens for biosensing and drug delivery applications

Sei Kwang Hahn, Do Hee Keum
Pohang University of Science and Technology, Pohang, South-Korea

INTRODUCTION:

As an attractive alternative to the conventional healthcare systems, a wide variety of biosensors and drug delivery systems (DDS) have been developed for diagnostic and therapeutic applications. However, these devices require bulky external power supplies, which are not convenient and practical for theranostic applications in a human body. Accordingly, there have been strong medical unmet needs for implementable, wirelessly powered biomedical wearable devices as a platform for ubiquitous healthcare. Among these medical devices, wirelessly powered smart contact lens has been considered to be very promising for real-time healthcare applications because it can be used as an efficient interface between the human body and the electronic device.

METHODS:

A power receiver coil, biosensor and flexible DDS (f-DDS) were fabricated on the peripheral area of a contact lens. A microcontroller integrated circuit (IC) chip was implemented through standard 0.18 μm CMOS process. The Cu power receiver coil was attached on ultrathin polyethylene terephthalate (PET) film with f-DDS using adhesive PDMS. Afterwards, working electrode (WE), counter electrode (CE), and reference electrode (RE) of the biosensor were deposited. IC chip was attached between the biosensor and the f-DDS. Finally, the integrated devices were molded into silicone contact lens.

RESULTS AND DISCUSSION:

We developed a smart contact lens with novel functionality for continuous monitoring and treatment of diabetic retinopathy. The contact lens device, built on a standard biocompatible polymer, contains ultrathin, flexible electrical circuits and a microcontroller chip for real-time electrochemical biosensing, controlled drug delivery, remote power management and data communication. In animal tests using diabetic model rabbits, we could measure tear glucose levels that were consistent with those by the conventional invasive blood-glucose tests and allow drugs to be released from reservoirs for the treatment of diabetic retinopathy. Taken together, we confirmed the feasibility of smart contact lenses for non-invasive and improved management of diabetes, diabetic retinopathy and possibly other diseases.

CONCLUSION:

We developed a smart contact lens system for theranosis of diabetic retinopathy as a model for wirelessly powered healthcare systems. Connected with a commercially available wireless communication electronic device, tear glucose level could be measured as a non-invasive alternative to blood glucose tests. Furthermore, as a proof-of-concept, we demonstrated the controlled pulsatile drug delivery from gold coated reservoirs integrated in the remotely powered lens for various ocular theranostic applications.

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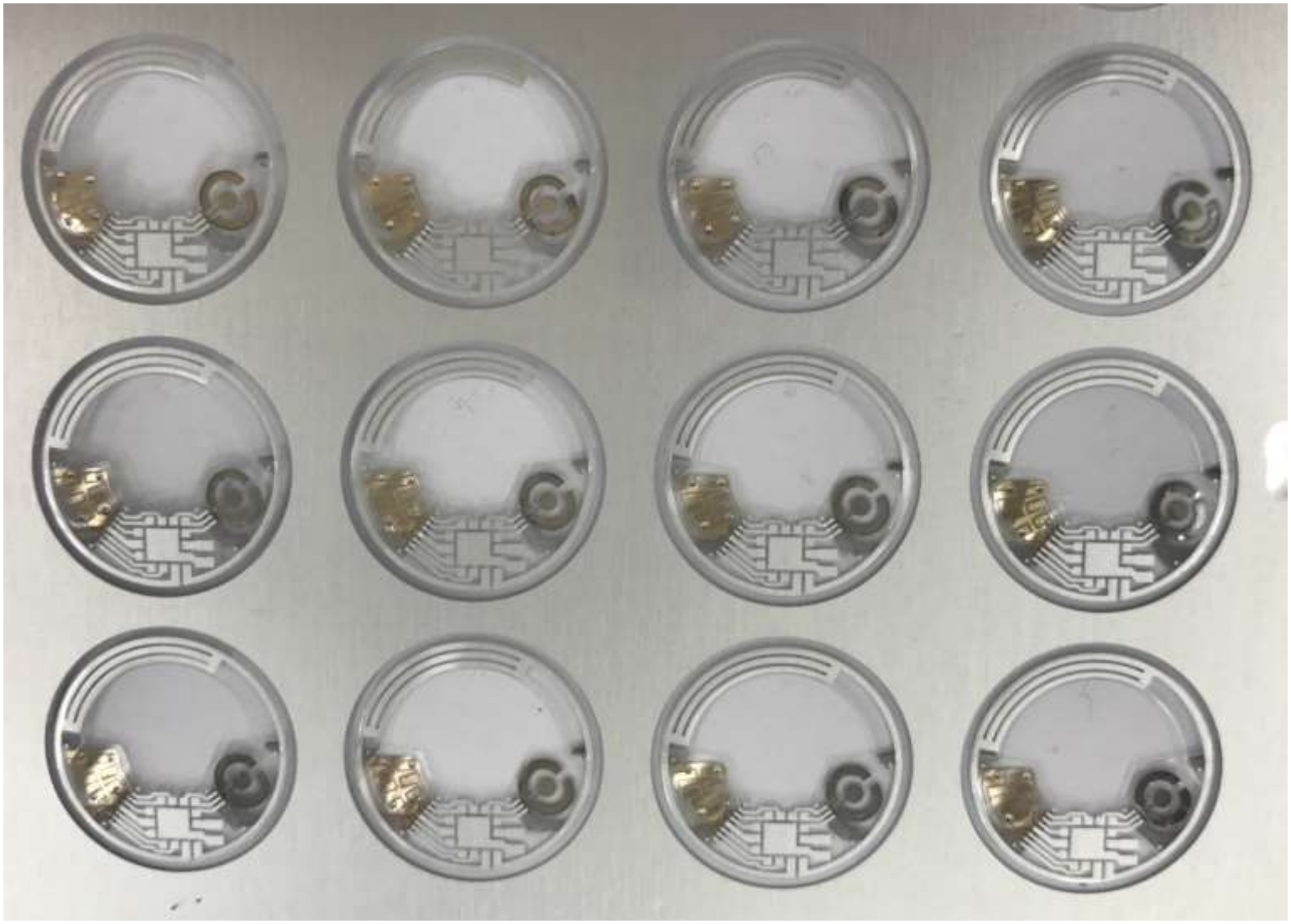
ACKNOWLEDGMENTS:

This research was supported by the Nano·Material Technology Development Program (No. 2017M3A7B8065278), Korea.

Picture 1:



Intelligent biomaterial properties & applications 14:30 - 16:00 Room 0.4 12/09/2018

Oral presentation

507 Spatiotemporal biomaterial modification via cytocompatible supramolecular complexation

Tom Kamperman, Michelle Koerselman, Cindy Kelder, Jan Hendriks, João Crispim, Xandra de Peuter, Piet Dijkstra, Marcel Karperien, Jeroen Leijten
University of Twente, Enschede, Netherlands

INTRODUCTION:

Native tissues are characterized by a dynamic nature. Recapitulating such dynamicity in engineered tissues requires the temporal control over their biochemical composition. Typically, spatiotemporal modification of biomaterials relies on photoresponsive strategies, which pose the inherent risk of cytotoxic UV-light and radical-based reactions[1]. Here, we pioneered supramolecular desthiobiotin/avidin complexation to enable the dynamic modification of

biomaterials. Desthiobiotin is a non-sulfur containing analog of biotin that also interacts with avidin, but with substantially lower binding affinity than biotin ($K_{d,biotin}$~10^{-15} M vs $K_{d,desthiobiotin}$~10^{-13} M)[2,3]. We hypothesized that a supramolecular desthiobiotin/biotin displacement strategy would grant spatiotemporal control over the biochemical composition of biomaterials in a novel, facile, and cytocompatible manner.

METHODS:

Dextran-tyramine-biotin (Dex-TA-biotin) was synthesized as previously described[4]. Hydrogels were prepared by mixing 5% Dex-TA-biotin, 3 U/ml horseradish peroxidase, and 0.05% H_2O_2. Hydrogels were further functionalized with 1 μM tetravalent neutravidin (i.e., avidin analog) and 1 μM desthiobiotin-FITC, biotin-atto565, and/or biotin-FITC, and subsequently analyzed using fluorescence recovery after photobleaching (FRAP) and fluorescence confocal microscopy.

RESULTS AND DISCUSSION:

Fluorescence confocal microscopy and FRAP confirmed that biotin-FITC was coupled to Dex-TA-biotin hydrogels via neutravidin, but not to non-functionalized (i.e. Dex-TA) hydrogels, which validated the successful generation and functionality of Dex-TA-biotin hydrogels. As shown in Figure 1, the reversible and sequential modification of hydrogels was demonstrated by displacing desthiobiotin-FITC (i.e. green) with biotin-atto565 (i.e. red). By tuning the concentration and incubation time of biotin-atto565, we could reproducibly control its penetration depth into the hydrogels. This strategy granted spatial control over the hydrogels' biochemical composition by determining the thickness of the biotin-displaced shell. Performing the supramolecular displacement strategy in the presence of cells did not reveal a cytotoxic effect, as assessed by live/dead cell staining. Moreover, the method enabled the spatiotemporal capturing and presentation of, for example, bioactive peptides (e.g., RGD) and endogenous growth factor, which was validated using surface plasmon resonance.

CONCLUSION:

In situ tuning of the biochemical composition of engineered tissues is key to mimic the dynamic nature of native tissues. We have successfully demonstrated a novel method for the spatiotemporal modification of biomaterials based on reversible and cytocompatible desthiobiotin/avidin complexation.

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ACKNOWLEDGMENTS:

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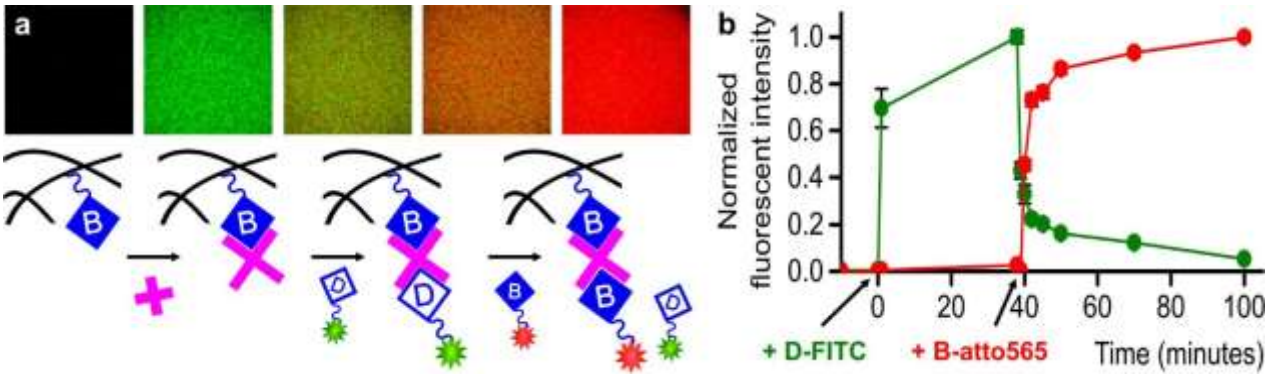

Picture 1: Caption 1: Figure 1: Dynamic biomaterial modification was demonstrated by desthiobiotin-FITC / biotin-atto565 displacement.

Intelligent biomaterial properties & applications 14:30 - 16:00 Room 0.4 12/09/2018

Oral presentation

652 Couplings of macrophage phenotype, angiogenesis and bone formation in calcium phosphate ceramics

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[4]Kuros Biosciences BV; Twente University, Netherlands
[5]Kuros Biosciences BV; Twente University;Queen Mary University of London, Netherlands
[6]Kuros Biosciences BV;Maastricht University, Netherlands

INTRODUCTION:

Calcium phosphate (CaP) ceramics are often considered as bone substitutes and their bone forming ability has been proven to vary with the physicochemical properties [1,2]. Given the fact that following surgical implantation, CaP ceramics initiate a series biological response of inflammation, angiogenesis, tissue formation and tissue remodeling [3], we addressed in the this study the biological cascades in submicron surface structured CaP ceramic-instructed bone formation.

METHODS:

Standard non-osteoinductive TCP-B and osteoinductive TCP-S [1,2] were used in this study. TPH-1 derived momocytes were cultured on materials with the presence of PMA for 2 days and 4 days. Culture media were subjected to ELISA assay of proinflammatory cytokines (TNF-a, IL-1β), anti-inflammatory cytokines (TGF-β and CCL18). Meanwhile the culture media harvested at day 4 were used to culture human umbilical vein endothelial cells (HUVECs) regarding angiogenesis and human bone marrow stromal cells (hBMSCs) with respect to osteogenic differentiation. TCP-B, TCP-S and TCP-S loaded with an angiogenesis-inhibiting chemical (KRN633) were further implanted in back muscle of canines for 3, 6 and 12 weeks to investigate blood vessels and bone formation. Ink was injected to at sample harvest to view blood vessels.

RESULTS AND DISCUSSION:

TCP-S facilitated more M2 macrophage polarization as shown by higher production of TGF-β and CCL8, while TCP-B displayed more M1 macrophage formation as indicated by the enhanced TNF- α and IL-1β secretion. The condition medium harvested from the THP-1 cultured on TCP-S enhanced tube formation from HUVECs, but none of the condition medium enhanced osteogenic differentiation of hBMSCs. Histology and hitomorphometry showed significantly more blood vessels were formed in TCP-S at all time points, and bone formation was formed in TCP-S starting from week 6 but no bone in TCP-B. Moreover, (KRN633) inhibited both angiogenesis and bone formation in TCP-S.

CONCLUSION:

The results show a strong link between macrophage phenotype, angiogenesis and bone formation in CaP ceramics. The submicron surface structure instructs the formation of M2 macrophages, which secret cytokines to enhance angiogenesis, facilitating bone formation.

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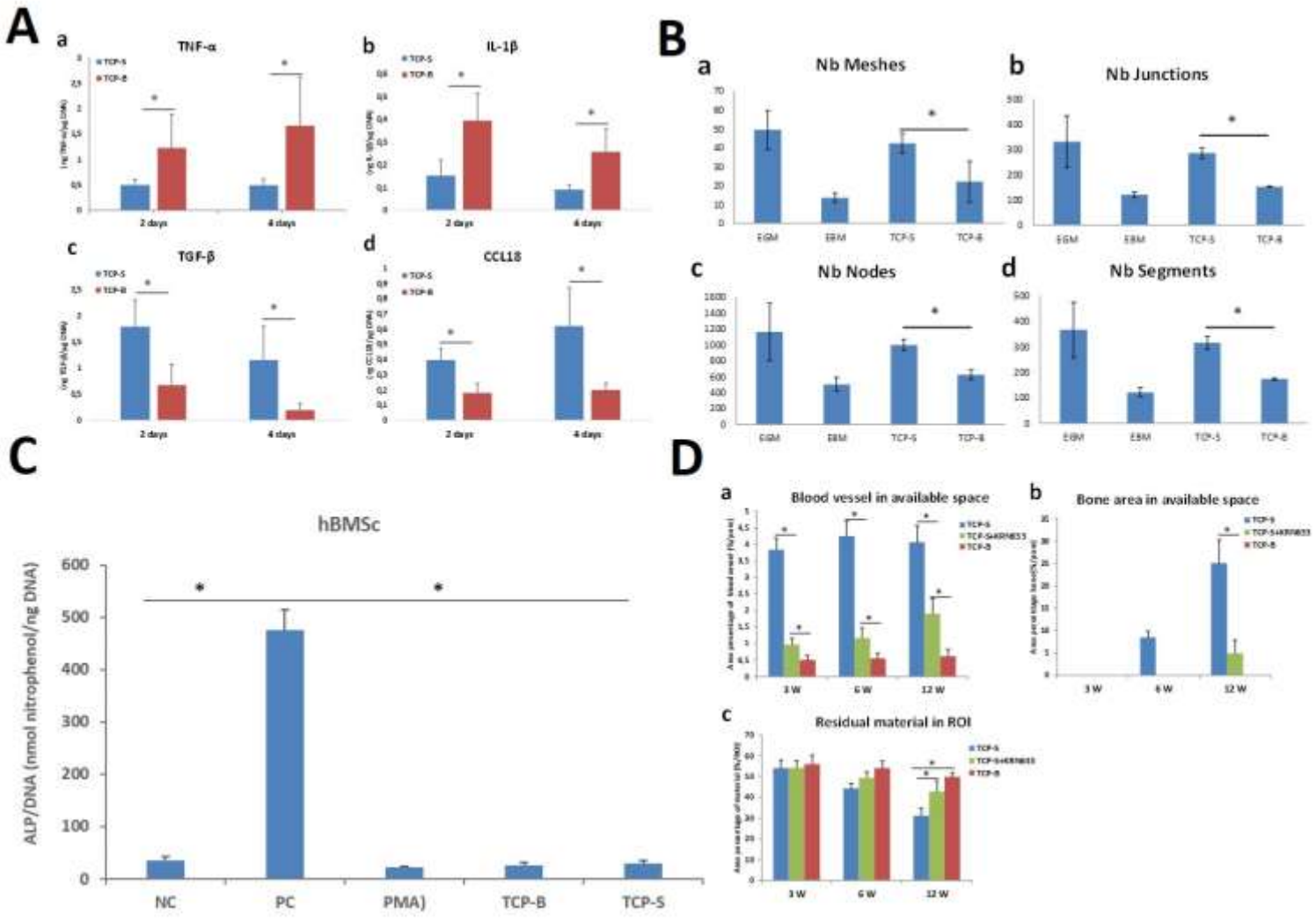
ACKNOWLEDGMENTS:

The authors thank the European Union's Horizon 2020 research and innovation program for the financial support to this project (grant agreement no. 674282).

Picture 1:



Intelligent biomaterial properties & applications 14:30 - 16:00 Room 0.4 12/09/2018

Oral presentation

337 High-throughput screening of topographically-patterned surfaces to identify zovel bio-instructive & immunomodulatory materials

Matthew dr Vassey[1], Blessing dr Mukonoweshuro[1], Steven dr Vermeulen[2], Nick dr Beijer[2], Jan prof. de Boer[2], Morgan R prof. Alexander[1], Amir prof. Ghaemmaghami[1]
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INTRODUCTION:

Macrophages play an integral role in the immune response to biomaterials and can determine the fate of implanted devices due to the plasticity of their phenotype; the M1 phenotype mediates pro-inflammatory responses that can lead to implant failure while the M2 phenotype promotes anti-inflammatory regenerative processes that may result in successful implant integration and function[1]. Biomaterial surface topography and chemistry, can influence macrophage response[2], providing the opportunity to rationally design biomaterials function by surface engineering. Successful control of these responses point the way to immune-instructive devices with tuneable biological responses. The aim of this study was to develop a high throughput screening strategy to identify surface topographies that modulate human macrophage polarisation *in vitro.*

METHODS:

TopoChips, representing about 2000 unique combinatorially assembled topographies, were designed and fabricated on a polystyrene substrate as described previously by Zhao *et al*.[3] Peripheral blood monocytes of healthy donors were isolated and cultured on TopoChips for 9 days to allow differentiation to macrophages (with no polarising cytokine stimulation). Cells were stained (intracellularly) for pro and anti -inflammatory cytokines TNFα (M1) and IL-10 (M2) respectively and imaged using automated wide-field microscopy. Image analysis was carried out on the individual micro-topographies using CellProfiler (Version 2.2.0) and multi-parametric profiles of cell responses were obtained. A total of 5 chips (each cultured with a different donor) each containing 2 duplicates were analysed.

RESULTS AND DISCUSSION:

We show that specific surface topographies modulate polarisation of human monocyte derived macrophages in the absence of exogenous polarising cytokines as evidenced by changes in the level of TNF and IL-10 expression compared to the cells differentiated on a flat surface. The TopoChip enabled the assessment of cell responses to 2176 unique topographies in a high-throughput screen and show a number of important topographical “hits” that polarise macrophage response to M1 (pro-inflammatory), M2 (anti-inflammatory).

CONCLUSION:

We developed a reproducible automated method to screen micropatterned surfaces for immune-modulatory properties on human macrophages and have identified a number of key “hit” topographies that influence macrophage polarisation status. Further research will focus on the mechanism of this interaction and subsequent cell signalling that drives this cell phenotype. Ultimately this will lead to greater understanding of immunomodulatory processes and the development of bio-instructive surfaces for use in medical device.

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ACKNOWLEDGMENTS:

Authors acknowledge EPSRC for grant funding (EP/N006615/1).

Intelligent biomaterial properties & applications 14:30 - 16:00 Room 0.4 12/09/2018

Oral presentation

641 Methylcellulose-based hydrogel for mechanobiology and cell sheet technology applications

Andrea Cochis[1], Nicola Contessi Negrini[2], Rita Sorrentino[1], Lina Altomare[2], Barbara Azzimonti[1], Elena Varoni[3], Silvia Faré[2], Lia Rimondini[1]
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[3]Università di Milano, Italy

INTRODUCTION:

Smart hydrogels represent an attractive category thanks to their ability to respond to an environmental stimulus. They are proposed as cells carrier or substrate to allow cells proliferation followed by cell sheet (CS) detachment. Here, we describe a methylcellulose (MC) based hydrogel: its chemical structure is characterized by the presence of hydrophobic (–CH_3O) and hydrophilic (–OH) groups, so that MC-based hydrogels exhibit a thermo-responsive behavior, showing a sol behavior at lower temperature (<20°C) and a gel behavior at higher temperature (>35°C). The MC-hydrogel was investigated as temporary substrate to biofabricate CS and in combination with 3D polyurethane (PU) foam to mechanically induce chondrogenesis of human mesenchymal stems cells (hMSC).

METHODS:

MC-hydrogel was obtained using a dispersion technique by mixing 8% MC powder in a 0.05M Na_2SO_4 solution[1]; thermo-reversibility was verified by rheological analysis. To obtain CS, NIH-3T3 mouse embryo fibroblasts expressing GFP were seeded onto hydrogel surface in the gel state (37°C) and detached as a continuous monolayer by cooling down at 4°C, allowing the gel-sol transition (Fig. 1, upper panel). To mechanically induce chondrogenesis, hMSC were loaded in the MC hydrogel that was used to fill the pores of a 3D PU foam. Compression and shear forces were applied to the hybrid scaffold by a custom-made bioreactor to induce chondrogenesis (Fig. 1, lower panel), thus avoiding the use of any biochemical factor[2].

RESULTS AND DISCUSSION:

The selected MC hydrogel composition showed a sol-gel phase transition close to 34°C, validating hydrogel suitability to support cell seeding and proliferation at 37°C. For CS, cells were seeded onto the MC-based hydrogel at 37°C, cultivated until 100% confluence and detached as a continuous monolayer of cells tightly interconnected to each other (Figure 1, upper panel). When the MC-based hydrogel was used as cells carrier within the pores of the porous PU scaffold, it was able to transfer the mechanical stress (i.e., compression + shear) to hMSC that were successfully induced towards chondrogenesis as demonstrated by safranin-O and PCR analysis (Fig. 1, lower panel).

CONCLUSION:

The here presented MC-based hydrogel represents a very promising tool for different applications in regenerative medicine. Sol-gel transition allows its use for CS formation and as injectable cell carrier.

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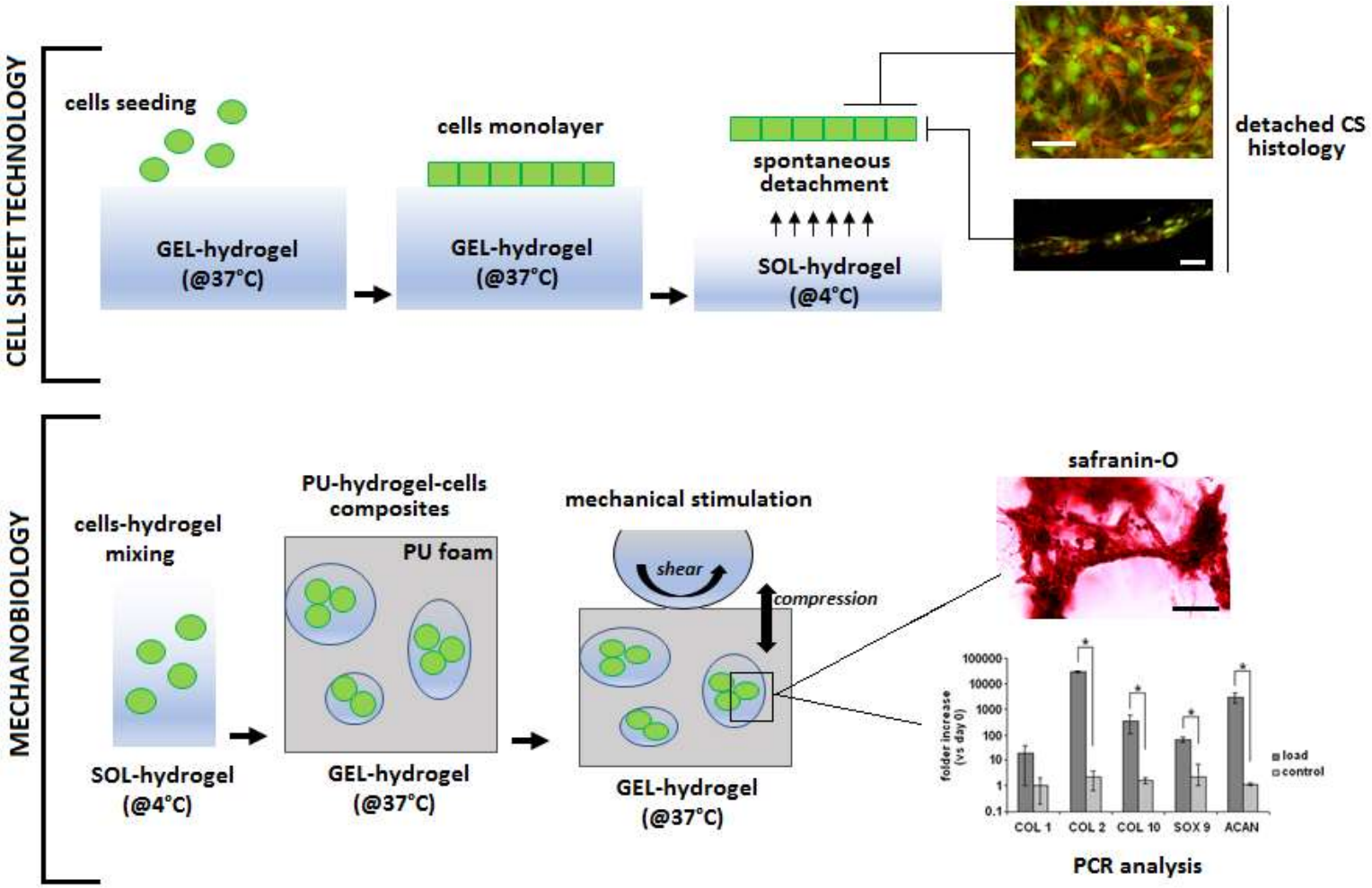

Picture 1: Caption 1: Figure 1. Schematic representation of CS detachment (upper panel) and mechanobiology for cartilage repair (lower panel).

Nanotechnological tailoring of biomaterials 16:30 - 18:00 Room 0.4 12/09/2018

Oral presentation

495 Optically Stable and Near-UV Activated Nanophosphors for in-vitro Dynamic Bioimaging

Georgios Sotiriou
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INTRODUCTION:

Luminescent rare-earth-based inorganic nanoparticles (nanophosphors) are promising bioimaging agents due to their high photostability, sharp emission bands and high biocompatibility overcoming toxicity-related concerns associated with the commonly-used heavy-metal containing quantum dots. Flame aerosol technology provides a scalable and highly reproducible process for production of such nanophosphors (e.g. Y2O3:Eu3+,Tb3+) with precise control of their composition and properties [1,2,3]. Nanophosphors that can be excited in the near- ultraviolet and visible region, such as YVO4:Eu3+,Bi3+, provide a useful tool for bioimaging and in vitro dosimetry studies using conventional fluorescence microscopes.

METHODS:

Here, YVO4:Eu3+,Bi3+ nanophosphors are made by flame spray pyrolysis. The optimal Bi content for maximum red- shift of their excitation band edge towards the visible region is identified through systematic experiments. The nanophosphors with the optimal composition are highly crystalline and appear bright red under a conventional fluorescence microscope.

RESULTS AND DISCUSSION:

Their photostability during dynamic imaging of cells in vitro is confirmed, contrary to commercial fluorescent (organic-dye labeled) SiO2 nanoparticles that exhibit 50% photobleaching within 3.5 h. Furthermore, the feasibility of the developed nanoparticles as superior bioimaging agents is demonstrated by studying the pathogen-host interactions of human lung endothelial cells with S. pneumoniae over time upon (i) the nanoparticle labelling of the cells incubated with GFP-expressing *S. pneumoniae* bacteria, and (ii) by the biofunctionalization of the luminescent nanoparticles with targeting antibodies. The nanobiointeractions are examined in both upright and inverted cell culture orientations [4], validating the antibody selectivity towards the targeted cells.

CONCLUSION:

These $YVO_4:Eu^{3+}/Bi^{3+}$ nanophosphors can provide a non-photobleaching tool for further dynamic nanoparticle-cell interaction studies with conventional fluorescent microscopes.

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ACKNOWLEDGMENTS:

Funding from the Karolinska Institutet Board of Research, the Swedish Research Council (2016-03471), the Jeansson Foundations (JS2016-0029) and the Åke Wiberg Foundation (M16-0098) is kindly acknowledged.

Nanotechnological tailoring of biomaterials 16:30 - 18:00 Room 0.4 12/09/2018

Oral presentation

171 Controllable synthesis of spherical calcium phosphate nanoparticles.

Pichaporn Sutthavas, Pamela Habibovic, Sabine van Rijt
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INTRODUCTION:

Biomaterials based on nanostructured calcium phosphate (nCaP) have received increased interest over the years especially for use in bone regeneration as they share chemical and crystallographic similarities to inorganic components of bone (1). However, synthesis of stable uniform nano-size ranged CaP particles in high quantities has been challenging because particles often rapidly aggregate during synthesis (2). In this study we developed synthesis methods which allowed control over particle size and crystallinity of spherical CaP nanoparticles. We

further investigated whether these particles can be surface functionalized with polyethylene glycol derivatives, with the final aim to develop novel nanostructured nCaP biomaterials for bone regeneration.

METHODS:

A method based on the precipitation of Ca+ and PO3-4 ions using a micelle template was developed to achieve spherical shaped nanoparticles. The effect of different organic molecules to stop layer growth was investigated, as well as the effect of ion concentrations and the rate of addition to obtain control over particle size. The possibility for further functionalization of the nanoparticles with hydrophilic polymers was also investigated. Characterization of particles was done using dynamic light scattering, X-ray diffraction, electron dispersive spectroscopy, Fourier transform infra-red spectroscopy, differential thermal and thermo-gravimetric analysis, and atomic force, scanning and transmission electron microscopy.

RESULTS AND DISCUSSION:

Spherical amorphous CaP particles with sizes ranging from 20 nm to 1 µm were successfully synthesized and characterized. The colloidal suspensions were homogeneous in size and could be stored long-term. Particularly starting concentrations of Ca^{+} and PO^{3-}_{4} solutions and the pH of the reaction chamber played important roles in the particles' growth rate.

CONCLUSION:

By altering ions' concentrations, precipitation time, and organic coating, the size and colloidal stability of CaP nanoparticles could be controlled. These synthesis methods open up new ways for developing nanostructerd biomaterials based on CaP with control over critical features such as size, shape and crystallinity.

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ACKNOWLEDGMENTS:

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Nanotechnological tailoring of biomaterials 16:30 - 18:00 Room 0.4 12/09/2018

Oral presentation

72 Nanopatterns to promote intercellular communication for cartilage regeneration

Ignasi Casanellas[1], Anna Lagunas[2], Iro Tsintzou[3], Yolanda Vida[4], Daniel Collado[4], Ezequiel Pérez-Inestrosa[4], Cristina Rodríguez Pereira[5], Joana Magalhaes[6], José A. Andrades[7], José Becerra[8], Josep Samitier[9]
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[7]Instituto de Investigación Biomédica de Málaga (IBIMA), UMA / CIBER, Spain
[8]IBIMA, UMA / CIBER / Andalusian Centre for Nanomedicine and Biotechnology, Spain
[9]Institute for Bioengineering of Catalonia (IBEC) / CIBER / UB, Spain

INTRODUCTION:

There is currently a need for improved stem cell therapies to treat widespread cartilage damage in conditions such as osteoarthritis. We have previously shown that arginine-glycine-aspartic acid (RGD) dendrimer nanopatterns with uneven densities at the nanoscale can be tuned to influence cell-substrate adhesion[1] and promote chondrogenesis of human mesenchymal stem cells (hMSCs)[2,3]. The establishment of a gap junction intercellular communication (GJIC) network during cartilage formation is crucial for the final tissue homeostasis. We here demonstrate that nanopatterns also promote GJIC in the developing cartilage.

METHODS:

Nanopatterns were produced by letting RGD dendrimer solutions at various concentrations adsorb on polymeric biocompatible surfaces, and characterized with Atomic Force Microscopy.

hMSCs were cultured on the nanopatterns in chondrogenesis-inducing conditions.

Expression of chondrogenesis markers and of genes related to cell-cell and cell-substrate interactions, including gap junctional connexin 43 (Cx43), was determined with RT-qPCR, as well as immunostaining and confocal fluorescence microscopy. Cx43 connectivity was evaluated through 3D mapping bioinformatics tools.

A neurobiotin-based assay was performed to further assess GJIC among differentiating cells.

RESULTS AND DISCUSSION:

Nanopatterns obtained from a medium concentration of RGD dendrimer, leading to intermediate cell-substrate adhesiveness, promoted cell condensation and differentiation, and Cx43 expression (Figure 1). Cx43 was also interconnected forming a tridimensional network across the condensates.

CONCLUSION:

Uneven nanopatterns of RGD dendrimers on biocompatible surfaces can be easily produced, permit tuning cell adhesion and sustain hMSC culture. Nanopatterned substrates of intermediate adhesiveness promote not only chondrogenesis, but also GJIC. This is an important milestone for the development of a carrier that allows stem cell predifferentiation before implantation into patients for cartilage regenerative therapies.

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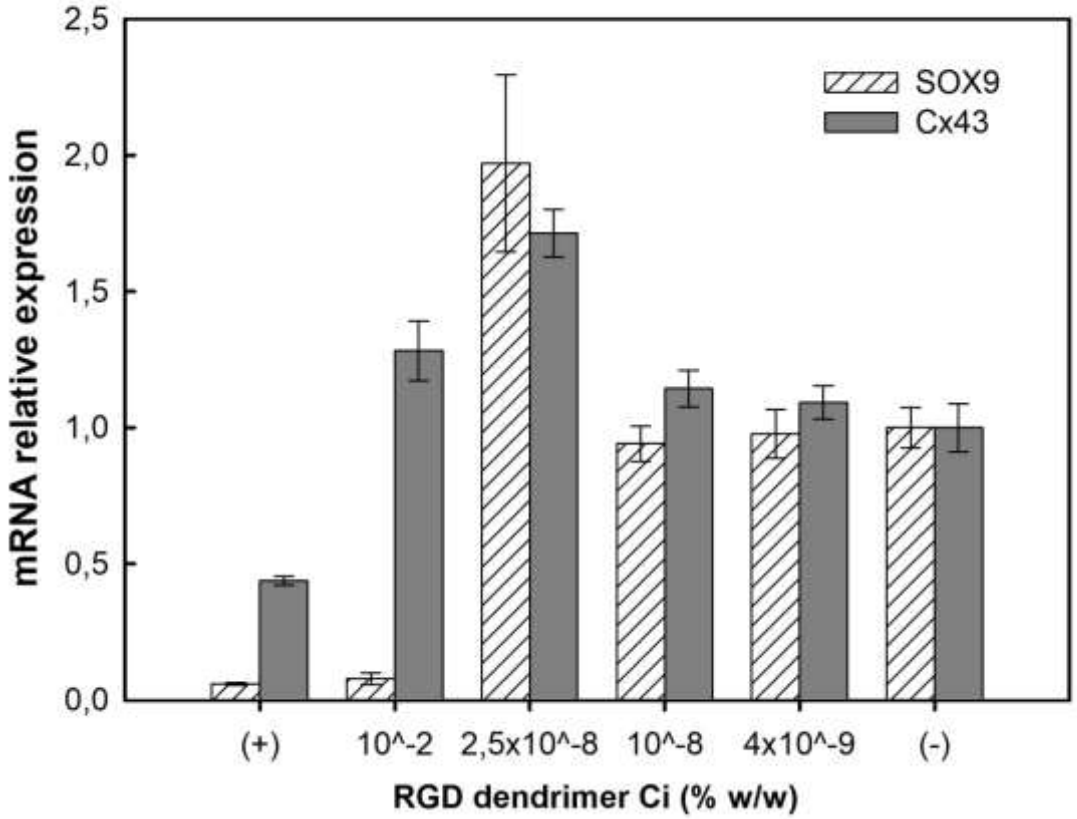
ACKNOWLEDGMENTS:

This work was supported by the Networking Biomedical Research Center (CIBER), Spain. CIBER is an initiative funded by the VI National R&D&i Plan 2008-2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions, and the Instituto de Salud Carlos III, with the support of the European Regional Development Fund. This work has been supported by the Commission for Universities and Research of the Department of Innovation, Universities, and Enterprise of the Generalitat de Catalunya (2014 SGR 1442). It was also funded by the projects OLIGOCODES (No. MAT2012-38573-C02) and CTQ2013-41339-P, awarded by the Spanish Ministry of Economy and Competitiveness (MINECO), in addition to INTERREG V-A Spain-Portugal 2014-2020 POCTEP (0245_IBEROS_1_E). I.C.M. and C.R.P. acknowledge support from MINECO (BES-2016-076682, IFI15/00151).



Picture 1: Caption 1: Figure 1: mRNA expression of chondrogenesis marker SOX9 and gap junctional Cx43, after 6 days of culture on nanopatterns.

Nanotechnological tailoring of biomaterials 16:30 - 18:00 Room 0.4 12/09/2018

Oral presentation

89 Nanoceria-loaded nanostructured lipid carriers for the treatment of neurological diseases

Matteo Battaglini[1], Christos Tapeinos[2], Ivana Cavaliere[3], Gianni Ciofani[3]
[1]Italian institute of technology/Scuola Superiore Sant'Anna, Pontedera, Italy
[2]Italian institute of technology, Italy
[3]Italian institute of technology/Politecnico di Torino, Italy

INTRODUCTION:

Currently only in Europe the 35% of all disease burden is attributed to neurological problems[1]. The treatment of neurological diseases is even more difficult due to the presence of the blood-brain Barrier (BBB), a continuous structure enveloping brain capillaries and preventing the passage of the most commonly used drugs[2]. Despite the extreme heterogeneity of brain disorders, a common hallmark is the presence of a high level of reactive oxygen species (ROS) that interferes with several normal functions of the cells, causing damage to cellular stuctures[2]. Therefore, the development of antioxidant compounds able to cross the BBB and target neurons could prove a useful tool against neurological diseases. In the scope of this, we have fabricated a "smart" nanovector able to cross

the BBB and to deliver cerium oxide nanoparticles (well-known inorganic antioxidants), which elicit both a neuroprotective and a neuro-regenerative effect.

METHODS:

Nanostructured lipid carriers loaded with cerium oxide nanoparticles (NLCs-Ce) were fabricated using a combination of hot ultrasonication and high-pressure homogenization (HPH). After synthesis, NLCs-Ce were characterized in terms of morphology (transmission electron microscopy, dynamic light scattering), cerium content (thermo-gravimetric analysis), and antioxidant properties. The ability of NLCs-Ce to cross the BBB was assessed through an *in vitro* triple culture model, using both confocal microscopy and flow cytometry. NLCs-Ce antioxidant abilities were assessed on neuronal derived cells (differentiated SH-SY5Y).

RESULTS AND DISCUSSION:

NLCs-Ce of a hydrodynamic diameter 95 nm and with high antioxidant capacity were successfully fabricated. The ability of NLCs-Ce to cross BBB and to be internalized by various cell lines was demonstrated using a triple culture system consisting of endothelial cells (bEnd.3), astrocytes (C8d1A) and neuronal cells (differentiated SH-SY5Y). Preliminary viability studies demonstrated the ability of the NLCs-Ce to act as neuroprotective agents against pro-apoptotic stimuli (1mM TBH treatment).

CONCLUSION:

NLCs-Ce were shown to be able to cross an *in vitro* BBB model and to be internalized by different cellular component of BBB (endothelial cells, astrocytes and Neuronal Cells). NLCs-Ce demonstrated also a high antioxidant capacity and a cellular-protective effect.

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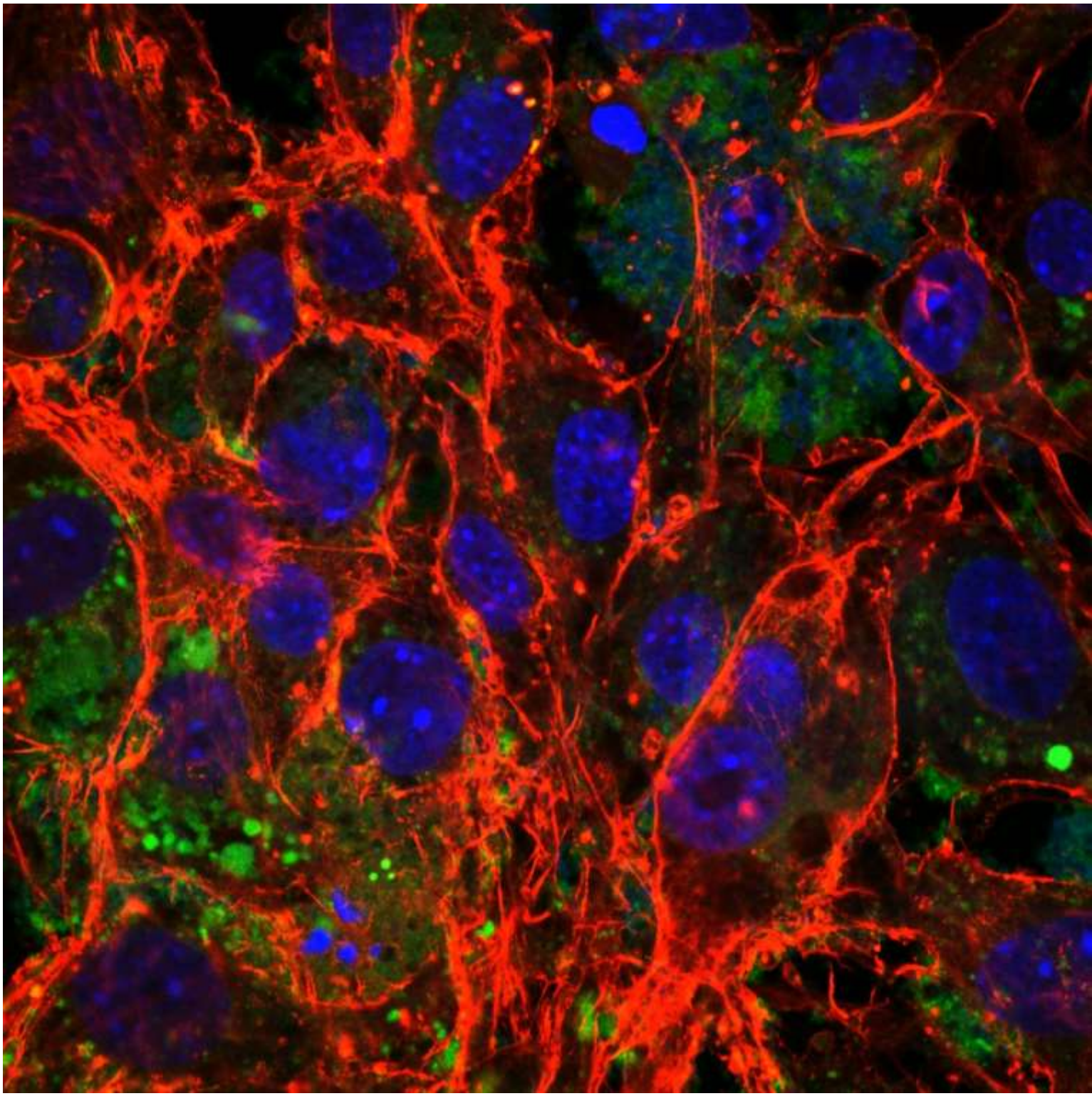
ACKNOWLEDGMENTS:

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement N°709613, SLaMM).



Picture 1: Caption 1: Confocal image showing the internalization of NLCs-Ce particles in bEnd.3 endothelial brain cells (in green particles, in red f-actin, in blue DAPI)

Nanotechnological tailoring of biomaterials 16:30 - 18:00 Room 0.4 12/09/2018

Oral presentation

456 Hyaluronan gradients for cells separation and identification

Ana M. Carvalho, Diana Soares Da Costa, Rui L. Reis, Iva Pashkuleva
3B's Research Group - Biomaterials, Biodegradable and Biomimetics, Guimarães, Portugal

INTRODUCTION:

Hyaluronan (HA) is a linear non-sulfated glycosaminoglycan present in the extracellular matrix and known to modulate cell-cell and cell-ECM interactions. For instance, HA main receptor (CD44) is overexpressed in several cancers and has been correlated with disease progression inducing cell proliferation, migration and chemoresistance.[1] CD44 is therefore considered a potential diagnostic tumor marker. Herein, we report on the development of a HA-based gradient for cancer cells separation and identification based on CD44 expression.

METHODS:

HA-gradient was achieved by colloidal gradient of gold nanoparticles (20 nm) immobilized on aminated glass; HA end-on functionalization with alkanethiol ($C_{11}SH$) and covalent linkage of HA to gold gradients. The gradient's formation was confirmed by XPS and SEM. HA stability and biofuctionality was confirmed by histochemistry with lectin wheat germ agglutinin. Breast cancer cell lines with different CD44 expression levels (Sk-Br-3($^{-}$), MDA-MB-231($^{+}$) and MDA-MB-468($^{++}$) were studied in contact with HA-gradients.

RESULTS AND DISCUSSION:

Different cells' response was observed through the gradient. At low density, small number of adherent cells were found for all studied lines. These cells had low expression of CD44, and round morphology. Cells adherent to the areas with high HA density presented higher expression of CD44 and a spindle-like shape. These differences were more pronounced for $CD44^{++}$ cells. MDA-MB-468 form long filopodia when adhered to areas with middle to high HA density. Of note, colocalization of CD44 and actin was observed at the filopodia edges. Cell motility was also affected by the gradient – at low densities cells presented higher motility and longer persistent length, which decreased with the increase of HA density. Besides this common trend, we observed differences among the studied cells. $CD44^{++}$ cells had shorter persistent length displacement than $CD44^{+}$ and $CD44^{-}$ cells. Upon CD44 blockage, $CD44^{++}$ adherent cells decreased dramatically, and cell motility increased, i.e. they behave similarly to $CD44^{-}$ cells. These results suggest that cells interact with HA-gradients through CD44 receptors and that the overexpression of these receptors leads to a stronger interaction between cells and surface.

CONCLUSION:

Gradients of bioactive HA were successfully developed. The results show that CD44 receptors recognized these gradients and cells interact differently with them. $CD44^{-}$ and $CD44^{+}$ cells behavior can be distinguished in HA-gradient, not only by CD44 expression, but also by morphology and motility.

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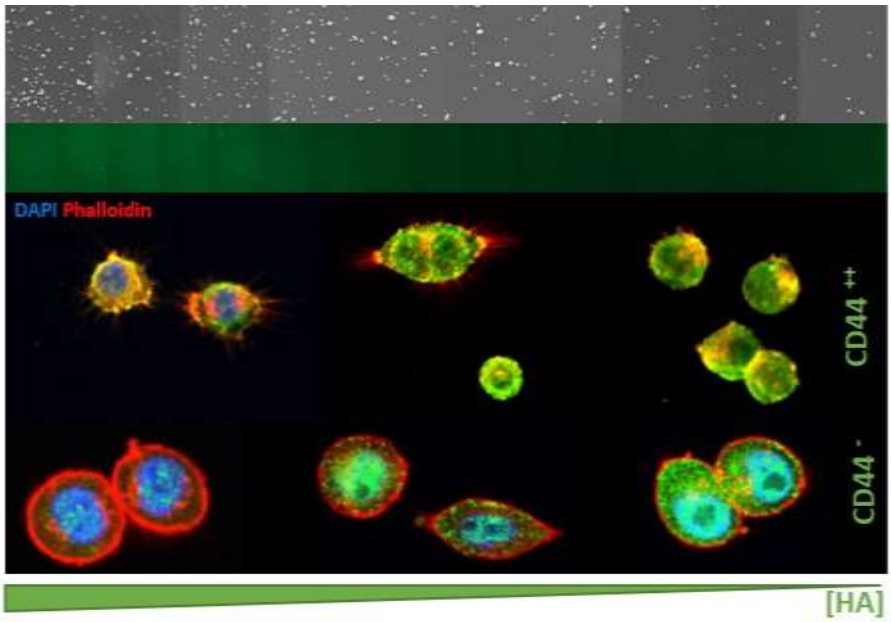
ACKNOWLEDGMENTS:

The authors thank the Portuguese FCT (Grants no: SFRH/BD/114847/2016, SFRH/BD/114847/2016, IF/00032/2013), and EU (ComplexiTE ERC-2012-ADG 20120216-321266 and H2020-TWIN-CHEM2NATURE-692333) for the provided financial support.

Picture 1:



Nanotechnological tailoring of biomaterials 16:30 - 18:00 Room 0.4 12/09/2018

Oral presentation

176 Entrapment of autologous von Willebrand Factor on nanostructured substrates within a dynamic platelet assay

Joanna Ward[1], Eimear Dunne[2], Adrian Boyd[1], Dermot Kenny[2], Brian J. Meenan[1]
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INTRODUCTION:

Blood platelets are concerned with haemostasis and thrombosis[1]. Upon damage to the blood vessel endothelium, the haemostatic mechanism acts to control bleeding via platelet adhesion and aggregation. This platelet interaction is dependent on von Willebrand Factor (vWF). Recent advances in dynamic platelet function testing uses an assay substrate in which small volumes of whole blood are brought into contact with immobilised endogenous vWF[2]. This work reports the development of a surface that can entrap the vWF directly from the same whole blood sample, to facilitate a patient-specific assay to determine platelet function.

METHODS:

PS and PMMA were used to create a 25PS/75PMMA w/w stock solution in chloroform which was then diluted to a 3% casting solution in the same solvent. Uniform 50µm PS microspheres, provided as 5k/mL (aqueous solution), were added to the casting solution. Thin films of the polymer demixed solution were then created on glass cover slips using a spin coater. A spin cast 100% PMMA solution was used as a control. All substrates were subject to physical and chemical characterisation, then incorporated within the DPFA device whereby labelled whole blood was perfused over the substrate at an arterial shear rate of 1500 s^{-1} and platelet function measured.

RESULTS AND DISCUSSION:

AFM analysis of 25/75 surfaces displayed features with FWHM values ranging from 2 to 4µm, overlaid with nanoscale features with FWHM 100nm to 1µm. Introduction of 750 PS microspheres per mL into casting solution, resulted in coatings with topographical features of 40-50nm in height, and FWHM of approximately 1µm (Fig. 1). Increased platelet adhesion and aggregation was observed for the latter compared to the 25/75 samples while the 100% PMMA control (R_a=0.33nm, R_q=0.567nm) showed no significant platelet interactions. Platelet receptor blocking experiments suggest that interactions observed are a direct result of vWF entrapment and uncoiling to expose the relevant attachment domains. This indicates that vWF is being captured by the substrate topography rather than the chemistry and that feature size and uniformity effects the nature and scale of the these interactions[3].

CONCLUSION:

Nanotopography is essential to successfully entrap autologous vWF from whole blood and can allow for its uncoiling to enable subsequent platelet interaction. Introduction of uniform PS microspheres offers a means to regulate the required topography, whilst the surface chemistry remains unchanged.

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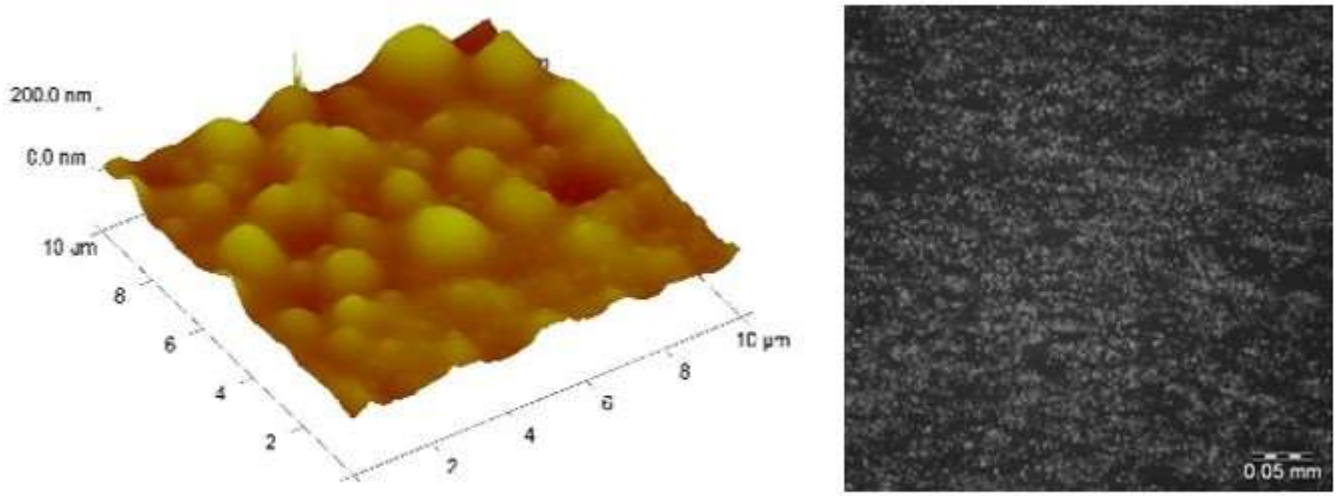

Picture 1: Caption 1: Figure 1 (a) AFM 3D plot and (b) fluorescence micrograph of DIOC6 labelled platelets adhered to 25/75+750 surface.

Engineering microenvironments 09:45 - 11:15 Room 0.5 12/09/2018

Oral presentation

189 Engineering the tenocyt micro-environment by topographical architectures

Steven Vermeulen[1], Aliaksei Vasilevich[2], Dimitris Tsiapalis[3], Aysegül Dede[2], Dimitrios Zeugolis[3], Jan de Boer[2]
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[2]MERLN, Netherlands
[3]University of Galway, Ireland

INTRODUCTION:

Due to its acellular and hypovascular nature, tendons have limited regenerative capabilities, often leading towards scarred and inferior tissue. Clinical interventions usually do not recover the original tendon architecture, whereby the best outcome is achieved through autografts. Tissue engineering a functional tendon however is hindered by the loss of tenogenic associated markers of tenocytes, the main cell type of the tendon. Since tenocytes require mechanical stimuli for their proper function, we reasoned that micro-topographical architectures can elicit biomechanical cues supporting the phenotype of tenocytes and can guide differentiation of mesenchymal stem cells towards this lineage.

METHODS:

Using a design algorithm, we have generated numerous different patterns, which can first be reproduced on a silicon mold and then imprinted onto polymers using microfabrication[1] (**Fig. 1a**). As such, we created the TopoChip platform with 2176 unique micro-topographical architectures in a 2x2 cm^2 design space[2]. After cell seeding on this platform, we used quantitative high content imaging and machine learning algorithms to characterize the response of the cells to the thousands of different surfaces and learn more about the relation between surface topography and cell response[3].

RESULTS AND DISCUSSION:

To investigate the beneficial influence of topographies, we seeded rat tenocytes on the TopoChip and screened for Scleraxis expression, an important tendon related transcription factor. We found micro-topographies inducing higher Scleraxis levels (**Fig. 1b-c**) and through machine learning algorithms, we associated morphological and topographical features with elevated Scleraxis expression. Upscaling the dimensions of these Scleraxis inducing topographies allowed in-depth tenogenic marker investigation. Starting from a tendon tissue, we migrated tenocytes on topographies and identified an upregulation of multiple essential tendon related genes, including COL-I, TNMD and MKX. These findings support the notion that micro-topographies can help maintain the tenogenic phenotype of tenocytes.

CONCLUSION:

We present micro-topographies as a xenofree stimulus in cell culture platforms that can support the phenotype of tenocytes.

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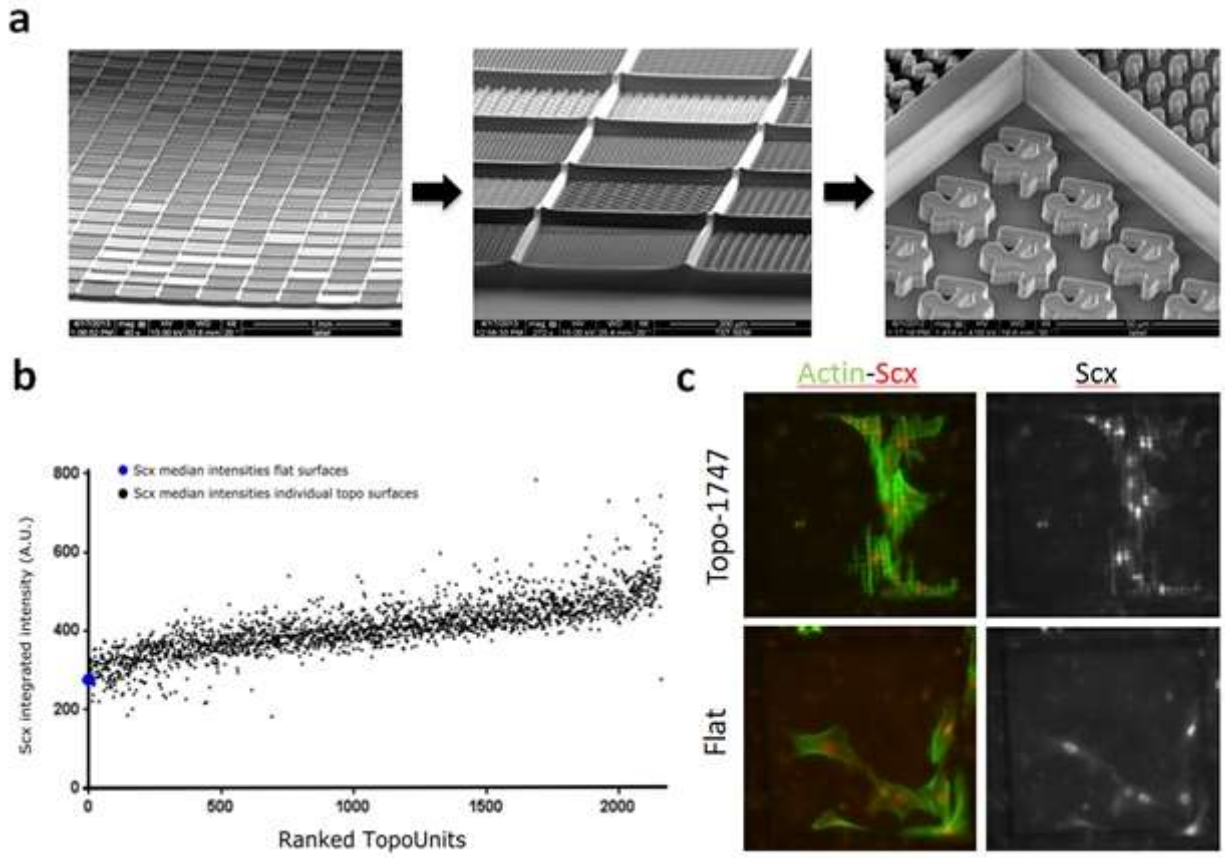
ACKNOWLEDGMENTS:

SV acknowledges the financial support of the Dutch province of Limburg and the European Union's Horizon 2020 Programme (H2020-MSCA- ITN-2015; Grant agreement 676338).

Picture 1:



Engineering microenvironments 09:45 - 11:15 Room 0.5 12/09/2018

Oral presentation

465 topography effects on macroscopic behavior and osteogenic differentiation of human bone marrow-derived mesenchymal stem cells: amplitude versus wavelength

Liangliang Yang, Zhou Qihui, Ge Lu, Rijn van Patrick
University of Groningen, Groningen, Netherlands

INTRODUCTION:

Self-renewal and differentiation of stem cells is influenced by chemical and physical features[1]. Cells can “sense” substrate elasticity and surface patterns ranging from 10 nm to 100 µm. Recent findings underscore that submicron and nanoscale topography can control cell behavior including proliferation, migration and differentiation. Tailored biomaterials with specific structures can mimic the topographic landscape of the niche. However, these structures are notoriously complex with multiple combinations of parameters and symmetry layout. For a better understanding of relevant topographic features, it is important to systematically vary one specific parameter at the time in order to determine its impact on cellular functions[2].

In this study we designed a nano and micro-wrinkled screening platform featuring a combination of 70 different structures with systematic variation of the wavelength and amplitude of wrinkles. The impact of these structures on cell behavior of hMSCs was subsequently analyzed.

METHODS:

2.1 Preparation of PDMS and Wrinkle Gradients

Wrinkled gradient PDMS elastomer samples were obtained via previously reported stretch-oxidation-release procedures[3]. Here a 10.5 × 9 cm wrinkle gradient substrate was used.

2.2 Creating the orthogonal wrinkle gradient and combine with bottomless 96-well plate

The oxidized wrinkle gradient was imprinted in fresh PDMS and divided into 7 equal areas. Plasma oxidation decreases the amplitude without affecting the wavelength, longer time of oxidation lowers the amplitude. All samples were re-imprinted to ensure that both surface chemistry and stiffness are equal and integrated the substrate with the bottomless 96-well plate.

RESULTS AND DISCUSSION:

The unidirectional gradients were obtained with amplitudes ranging from 144 to 3000 nm and wavelengths between 700 and 14000 nm. After different time of oxidation time, the amplitude value corresponding to 144 nm decreased to 6 nm and 3000 nm decreased to 800 nm.

With the decline of amplitude, the nucleus area per cell are slightly increased, and area per cell are increased dramatically, the minimum number is 1442 μm^2/cell, and the maximum is 3321μm^2/cell. However, aspect ratio are decreased with the reduction of amplitude, the biggest is 12.68 and the smallest is 4.16.

CONCLUSION:

We successfully prepared high-throughput screening platform to determine effects of different combinations of amplitude and wavelength of aligned topography on the morphological behavior and osteogenesis of hBMSCs.

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ACKNOWLEDGMENTS:

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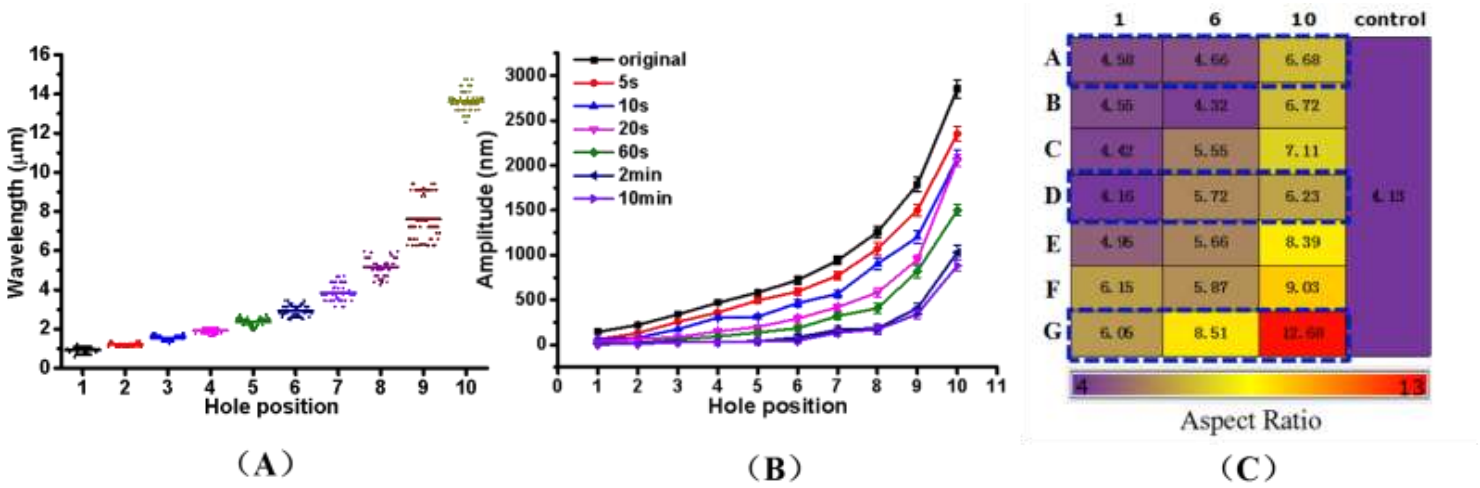

Figure 1. (A) wavelength change as a consequence of plasma oxidation treatment. (B) amplitude change curve on different hole position after plasma oxidation. (C) aspect ratio change with the decrease of amplitude.

Picture 1: Caption 1: (A) and (B) wavelength and amplitude change curve on different hole position, respectively. (C) aspect ratio change with the decrease of amplitude.

Engineering microenvironments 09:45 - 11:15 Room 0.5 12/09/2018

Oral presentation

570 Biophysical Cues for Modulation of Tenogenic Phenotype

Diana Gaspar, Dimitrios Zeugolis
Regenerative Modular & Developmental Engineering Laboratory (REMODEL), Galway, Ireland

INTRODUCTION:

Current cell-based tissue engineering strategies have limited clinical applicability due to the need for large cell numbers and prolonged culture periods that lead to phenotypic drift and cell senescence. *In vitro* microenvironmental modulators have been proposed to mimic the native tendon and induce tenogenic trans-differentiation / differentiation. Standard *in vitro* culture conditions result in delayed extracellular matrix (ECM) deposition, impairing the development of scaffold-free approaches. ECM deposition can be enhanced by macromolecular crowding (MMC), a biophysical phenomenon that governs the milieu of multicellular organisms. We assessed a multifactorial biophysical approach, using MMC and mechanical loading, on different cell sources to determine their suitability for *in vitro* fabrication of tendon-like tissue.

METHODS:

Human dermal fibroblasts (DFs), tenocytes (TCs) and bone marrow mesenchymal stem cells (BMSCs) were cultured with carrageenan (MMC) under static and dynamic culture conditions. Cyclic uniaxial strain was applied (MechanoCulture FX, CellScale) at 1 Hz and 10% strain. Cell morphology and ECM composition were assessed using fluorescence microscopy. Cell phenotype (tenogenic, chondrogenic, osteogenic lineages) was also assessed.

RESULTS AND DISCUSSION:

TCs and DFs exhibited alignment perpendicular to the load, whilst BMSCs did not show preferential alignment. When MMC was used, DFs and BMSCs showed increased deposition of collagen I, the main component in tendon ECM, while TCs only presented deposition when loading was combined with MMC. DFs presented ECM composition similar to TCs with collagen types III, V and VI present, while BMSCs also deposited collagen type IV. Gene expression analysis revealed upregulation of tenogenic markers by TCs and DFs, such as scleraxis and thrombospondin-4, under both loading and MMC, while BMSCs displayed upregulation of collagen type X.

CONCLUSION:

The combined use of MMC and mechanical stimulation is suitable for TCs phenotype maintenance and can modulate the phenotype of DFs and BMSCs differentially. It facilitated the fabrication of rich in ECM aligned cell sheets of TCs and DFs. This study provides insight into response of different cell sources to biophysical cues and contributes to further development of cell therapies for tendon repair and regeneration.

ACKNOWLEDGMENTS:

Irish Research Council (RS/2012/82), Health Research Board (HRA_POR/2011/84), Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM) (13/RC/2073).

Engineering microenvironments 09:45 - 11:15 Room 0.5 12/09/2018

Oral presentation

304 Studying cellular morphology using a high-throughput screening platform based on a library of arrayed cell-adhesive micro islands

Urandelger Tuvshindorj, Steven Vermeulen, Stefan Giselbrecht, Roman Truckenmüller, Jan de Boer
Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medi, Maastricht, Netherlands

INTRODUCTION:

Cell adhesion, spreading and shape are regulated by the local distribution of the extracellular matrix. It was found that micropatterned substrates can control the spatial distribution of cells and their adhesion to the engineered microenvironment and that different adhesion patterns regulate different cell functions such as cell polarity, migration, apoptosis, and differentiation[1]. In this study, we established a high-throughput micropatterned screening platform which contains 2176 different arrays of cell-adhesive micro-islands. We cultured human mesenchymal stromal cells (hMSCs) on this platform and studied the effect of pattern design on cell morphology using the high-content imaging technique “Cell Painting”[2].

METHODS:

The micro islands are designed by combining circles, squares and rectangles (3-10 µm in size) and arraying them in 290 µm x 290 µm units as previously published[3] (Figure 1a).The platform contains the duplicate of 2176 unique micro island units. The adhesive micropatterned platform was fabricated by photo-initiated thiol-ene click chemistry. Firstly, vinyl organosilane was vapor-deposited on the glass surface. Afterwards, the CGGG**RGD**S peptide was locally coupled to the substrate by shining UV light through a chromium mask, and finally, polyethylene glycol thiol was coupled to the rest of the substrate. Subsequently, hMSCs were cultured on the platform for 4 hours, fixed, and stained using Cell Painting. Cell-morphological features were quantified using the software CellProfiler and further analyzed with R.

RESULTS AND DISCUSSION:

On the platform, the hMSCs present different shapes and morphologies depending on the underlying micropattern (Figure 1b). Cells are aligned along the micropatterns and bridge the micro-islands. Cells on the smaller micro-islands (left) show small protrusions compared to cells on the bigger micropatterns (middle and right). As a next step, a quantitative analysis will be performed to understand the correlation between pattern design and cellular morphology.

CONCLUSION:

We created a high-throughput screening platform that can induce different cellular morphologies. In future experiments, we will select a subset of micropatterns that are able to induce specific cellular morphologies and study the corresponding cellular functions.

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ACKNOWLEDGMENTS:

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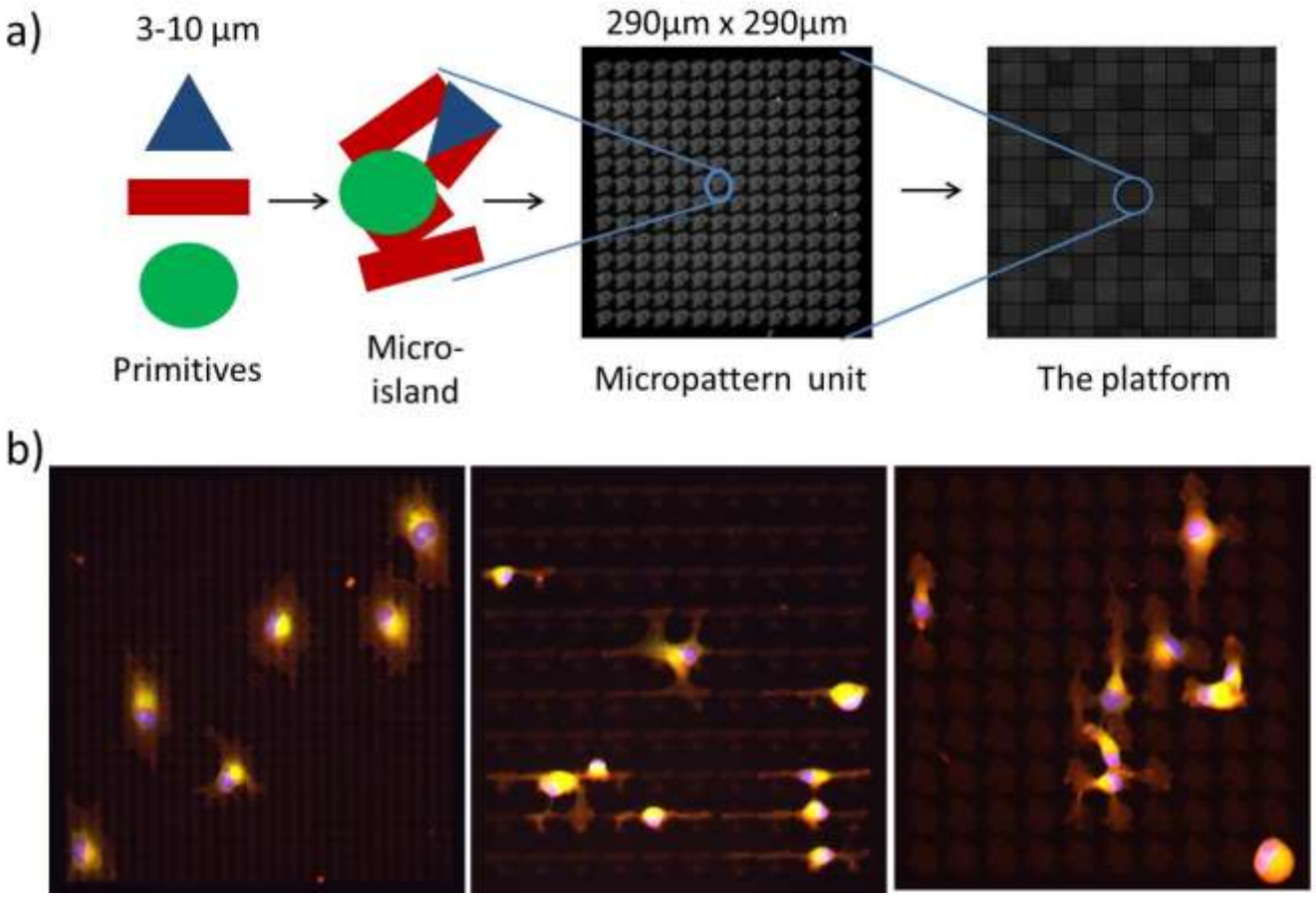

Picture 1: Caption 1: Fig.1 a) Design of micro islands and the high throughput screening platform. b) Varying morphology of hMSCs on 3 different adhesive micropatterns.

Engineering microenvironments 09:45 - 11:15 Room 0.5 12/09/2018

Oral presentation

65 Role of implant nanoroughness and bioactive coating on osseointegration and bacterial growth

Laila Damiati[1], Virginia Llopis-Hernández[1], Angela Nobbs[2], Bo Su[2], Richard Oreffo[3], Penelope Tsimbouri[1], Manuel Salmeron-Sanchez[1], Matthew Dalby[1]
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[2]University of Bristol, United Kingdom
[3]University of Southampton, United Kingdom

INTRODUCTION:

Titanium (Ti) and its alloys can be processed to tune their physical and chemical properties. In this project, we are interested in the ability of a material to induce osseointegration and subsequent mineralization dependent on the initial adhesion of mesenchymal stem cells (MSCs) onto the implant surface. Further, we are interested in developing topographies that can kill bacteria. Polymers are also important medical materials. An example is a poly(ethylacrylate) (PEA) as it causes a spontaneous unravelling of fibronectin (FN) upon contact which facilitates interaction with growth factors (GFs), allowing ultra-low dose GF presentation with high efficiency. We thus aim to investigate the effect of Ti surfaces with bactericidal nanotopographies coated with PEA/FN/BMP2 to see if the coating can improve MSC growth and differentiation while maintaining bacterial kill.

METHODS:

Two different Ti nanowire surfaces were produced through a thermal oxidation process under alkaline conditions (1h and 2h TiO_2, with average maximum height ~380- 550 nm respectively). Two different time points were used to coat the surfaces with PEA utilizing a plasma polymerization technique (90 seconds and 3 minutes). The biological coating was applied using FN/ BMP2 prior to Stro-1+ hBM-MSC culture. Physical and chemical characteristics were studied using SEM, AFM, WCA, and XPS. The availability of P5F3 (GF binding) and FN7.1 (cell adhesion) domains was tested using antibody-based ELISA assays.

RESULTS AND DISCUSSION:

AFM showed that the maximum average height of 1h and 2h TiO_2 are *Rt* ~350 and ~700 nm respectively after 90 secs PEA coating and *Rt* ~390 and ~740 nm after 3 mins PEA coating. Polymer coating increased the hydrophobicity of Ti, which resulted in increased protein adsorption. On the other hand, FN decreased the hydrophobicity, which improved cell adhesion. The number of cell- binding domains increased on the coated surfaces compared to coated/ uncoated flat surfaces, and the heparin-binding domain increased on coated surfaces compared with uncoated. XPS showed the PEA coating partially covered the surfaces with 90 sec PEA treatment and full coating with 3 min coating. The coating showed an improving in cell adhesion, growth, and osteogenic gene expression.

CONCLUSION:

An ideal bone implant should enhance the osteogenesis and reduce bacterial adhesion. However, increasing the implant surface area, e.g. a 3D format could improve the osteogenic and bactericidal effect we seek.

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ACKNOWLEDGMENTS:

This project is supported by a studentship to LD from University of Jeddah, Jeddah- Saudi Arabia and EPSRC grant EP/K034898/1.We are grateful for the technical support of Carol-Anne Smith.

Engineering microenvironments 09:45 - 11:15 Room 0.5 12/09/2018

Oral presentation

754 ENGINEERED MICROENVIRONMENTS FOR EFFICIENT REGENERATION OF BONE CRITICAL-SIZE DEFECTS

Cristina Gonzalez-Garcia[1], Andres Alba-Perez[2], David Shields[2], Matthew J. Dalby[3], Andres J. Garcia[4], Manuel Salmeron-Sanchez[2]
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[4]Woodruff School of Mechanical Engineering and Petit Institute for Bioengineering, United States of America

INTRODUCTION:

Material-based strategies seek to engineer synthetic microenvironments that recapitulate the characteristics of physiological extracellular matrices for applications in regenerative therapies, including bone repair and regeneration. In our group, we have developed a new technology using materials that promote fibronectin (FN) assembly to recruit and present growth factors (GF) in combination with the integrin binding domain of FN during bone tissue healing.

METHODS:

Synthetic biodegradable polymers or allogenic bone chips were functionalised with a thin layer of plasma-polymerised ethyl acrylate (EA). Then, FN was adsorbed on the implant surface, followed by the adsorption of the BMP-2 GF. A murine segmental defect and a fat pad models were used to evaluate the potential of this technology to induce bone regeneration and vascularisation *in vivo*, respectively.

RESULTS AND DISCUSSION:

Fibrillar conformation of FN adsorbed on polyethyl acrylate (PEA) favours the simultaneous availability of the GF binding domain (FNIII12-14) next to the integrin binding region (FNIII9-10), compared to standard polymers such as polymethyl acrylate (PMA), a material with similar chemistry to PEA, but where FN adopts a globular conformation. The crosstalk between integrins and GF receptors improves the osteogenic differentiation of mesenchymal stem cells (using BMP-2) and the vasculogenic response of human endothelial cells (using VEGF). The potential of this system as recruiter of GFs was investigated in a critical-size bone segmental defect in a mouse model. The synergistic integrin-GF signalling, induced by fibrillar FN, promoted bone formation and enhanced vascularisation *in vivo* with ultra-low doses of GFs compared to current advanced technologies[1,2]. Furthermore, we optimized the system for its potential use in translational research, seeking to address the clinical need of using biocompatible and biodegradable material implants. This allowed us to apply the technology to material systems with different geometries, including allogenic bone chips that were coated with a thin layer of plasma-polymerized PEA, which recruits and efficiently presents GF during healing of critical size defects.

CONCLUSION:

This technology, based on growth factor functionalised coatings, provides a new strategy to efficiently reduce the GF doses administrated in bone regenerative therapies and has been recently used to treat successfully a first veterinary patient.

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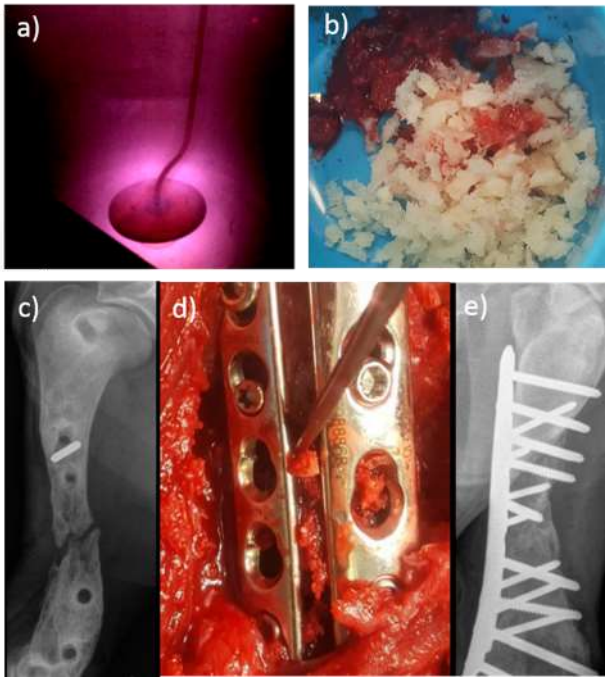

Picture 1: Caption 1: Humeral fracture healing after treatment with bone chips coated with pPEA, FN, and BMP-2. Plasma polymerisation was used to create a thin polymer coat

Design of biomaterials for drug delivery 14:30 - 16:00 Room 0.5 12/09/2018

Oral presentation

290 Multi-compartment collagen devices as modulators of skin fibrosis through controlled synergistic dual delivery of anti-fibrotics

João Coentro[1], Dimitrios Zeugolis[2]
[1]REMODEL/CÚRAM, National University of Ireland Galway, Galway, Ireland
[2]REMODEL/CÚRAM/National Unviersity of Ireland Galway, Ireland

INTRODUCTION:

Fibrosis is a phenomenon characterised by the formation of excessive fibrous connective tissue, which can compromise the skin's function and its mechanical properties. This can cause a huge global burden on healthcare, with millions of patients suffering from cosmetic or even functional tissue impairment, which considerably reduces their quality of life[1]. In order to treat this, Il-6 antagonists and decorin have been reported as potential anti-fibrotic therapeutics[2, 3]. Therefore, there is a need to develop multi-compartment systems, capable of delivering multiple bioactive agents in a controlled, safe and cost effective way[4], with single domain systems being a promising

alternative. In this study it was hypothesized that multi-compartment crosslinked collagen type I systems can modulate skin fibrosis through the controlled synergistic dual delivery of an Il-6 antagonist and recombinant decorin.

METHODS:

Multi-compartment hydrogel systems were prepared in a mould as follows: solutions of dialyzed type I collagen at a concentration of 5 and 8 mg/ml, were mixed with 10x PBS, neutralised and crosslinked with different densities, creating two separate compartments, that were loaded with decorin and an Il-6 antagonist. The systems were characterised through swelling assessment, collagenase degradation assay, rheological and compression tests. The release of encapsulated drugs from the hydrogels was studied by HPLC and the effect of the delivered bioactive agents was assessed through proteomic analysis and imaging for fibrotic markers in an *in vitro* keloid model.

RESULTS AND DISCUSSION:

Mechanical and biological resistance of the systems were found to be suitable for potential implantation in the skin *in vivo*. Release studies proved that the inner compartment was capable of promoting a sustained release of decorin over a long period of time (around 14 days), fitting the intended therapeutic release profile. Proteomic studies showed a decrease of endogenous collagen type I, TGF-β1 and α-smooth muscle actin expression indicating reduced fibrosis.

CONCLUSION:

It was observed that this system is mechanically robust and stable *in vitro*, besides being an attractive dual drug delivery system for skin fibrosis, ameliorating markers of fibrosis in an *in vitro* keloid model.

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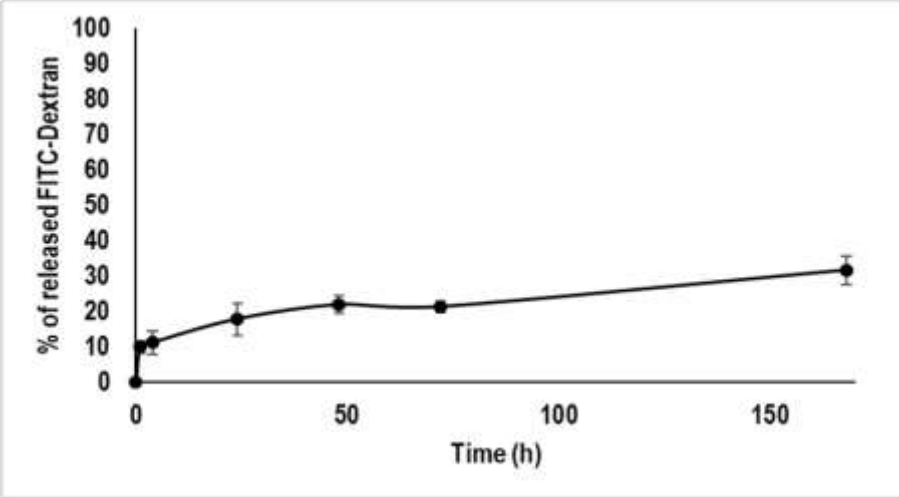
ACKNOWLEDGMENTS:

Science Foundation Ireland, co-funded under the European Regional Development Fund (Grant Number 13/RC/2073).



Picture 1: Caption 1: Release profile of FITC-dextran from the system's **inner** compartment over 7 days (n=3)

Design of biomaterials for drug delivery 14:30 - 16:00 Room 0.5 12/09/2018

Oral presentation

802 Biodegradable microneedles for the delivery of proteins

Liliana Pires, João Gaspar
INL-International iberian Nanotechnology Laboratory, Braga, Portugal

INTRODUCTION:

Microneedle (MN) arrays have been investigated as mean to deliver drugs, proteins or other molecules to the epidermal and/or intradermal space, overcoming the skin stratum corneum barrier. These devices allow painless, self-administration of therapeutic agents. Biodegradable MNs can dissolve in the skin, assuring safe disposal without biohazardous waste[1]. This project aims to prepare fully biodegradable polymeric MN arrays that promote the release of relevant amounts of biologically active proteins to the epi-/dermal space. Specifically, the design and microfabrication of silicon MN arrays is presented and the preparation of protein-loaded biodegradable structures.

METHODS:

To obtain sharp silicon MNs silicon wafer was patterned by lithography and MN shape was determined by sequential isotropic-anisotropic deep reactive ion etching (DRIE) process[2]. 33x33 arrays (2cm) with MNs of 200 or 125µm diameter, and 600µm height were obtained. These were replicated by poly(dimethylsiloxane) (PDMS) moldeling[3]. Polymeric MNs were prepared using chitosan, poly(vinyl acetate) (PVA) and poly(vinyl pyrrolidone) (PVP). Chitosan was firstly added in the molds and subsequently freeze-dried. Mixtures of PVA:PVP were then added onto molds. After drying (24hrs, RT), solid MN patches were peeled off. The mechanical properties were tested and different skin models. Protein-loaded MN arrays were produced using bovine serum albumin (BSA), and anti-tumor necrosis factor-α (anti-TNFα). Drug loading and release was quantified using fluorescence-based techniques.

RESULTS AND DISCUSSION:

Arrays of polymeric MN with different diameter and chitosan loading were prepared. Needle failure occurred for higher forces in 200 µm diameter MN comparing to 125 µm. MN with 125 µm tend to bend whereas 200 µm MN do not significantly change morphology after mechanical loading. Homogeneously fluorescent MN were obtained using fluorescein sodium salt and rhodamine. After 24hrs in physiological conditions (37ºC), the patches mostly dissolve. BSA was also successfully incorporated in the patches. More than 1mg of BSA could be loaded in the MN arrays. Remarkably, the presence of chitosan in the MN structure increased BSA loading. When MN with anti-TNFα were produced, improved loading was detected in MN arrays without chitosan.

CONCLUSION:

The work shows the preparation of sharp, biodegradable 600 µm MN that can accommodate and release therapeutic doses of biologically active proteins in the upper layers of the skin.

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ACKNOWLEDGMENTS:

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Design of biomaterials for drug delivery 14:30 - 16:00 Room 0.5 12/09/2018

Oral presentation

657 pH-sensitive polymeric nanoparticles with antioxidant and antiinflammatory properties for the treatment of cisplatin-induced hearing loss

Maria Rosa Aguilar[1], Sergio Martin-Saldaña[1], Raquel Palao-Suay[1], Luis Garcia-Fernandez[1], Humberto Arevalo[1], Almudena Trinidad[2], Rafael Ramirez-Camacho[2], Julio San Roman[1]
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INTRODUCTION:

Cisplatin (CDDP) is commonly used in the treatment of solid tumors, such as non-small cell lung cancer or testicular cancer due to DNA damage and effective induction of apoptosis of cancer cells. However, serious side effects, such as neurotoxicity, nephrotoxicity, and ototoxicity, are associated to the treatment with high doses of CDDP, due to its low selectivity against cancer cells and the massive accumulation of ROS in certain populations of cells (*e.g.* cochlear cells) that produces a severe inflammation in the affected tissues.

Dexamethasone (Dx) is used in the treatment of sensineural hearing loss by intra-tympanic administration. However, it is highly hydrophobic and its encapsulation and controlled release by polymeric nanoparticles (NPs) could improve its bioavailability, decrease its toxicity and avoid degradation processes. Moreover, the NPs could present pH-responsiveness in order to stimulate the drug release in an inflamed environment (pH between 5.5 and 6.8). Therefore, the aim of this work was the synthesis, characterization, *in vitro* and *in vivo* evaluation of new pH-sensitive NPs with antioxidant and anti-inflammatory properties that encapsulate and controlled release Dx for the treatment of CDDP-induced hearing loss.

METHODS:

Methacrylic derivatives of ibuprofen (HEI) and vitamin E (MVE and MTOS) were obtained and new copolymers (poly(1-vinylimidazole-*co*-HEI) were synthesized by free radical polymerization [1] and properly combined with poly(*N*-vinylpyrrolidone-*co*-MTOS) or poly(*N*-vinylpyrrolidone -*co*-MVE) [2]. The composition of the copolymers was adjusted to favour VI ionization in the slightly acidic environment of the inflamed tissue, and to induce the release of the encapsulated Dx. NPs were characterized by DLS, LDE, SEM and fluorescence spectroscopy.

In vitro biological activity was studied using HEI-OC1 cell line (from Dr. Federico Kalinec). An auditory steady-state responses (ASSR) test was performed in Wistar rats before and after the treatment to evaluate the biological effect *in vivo*.

RESULTS AND DISCUSSION:

The combination of copolymeric drugs formed surfactant-free NPs that were used to efficiently entrap Dx as antiinflammatory and antiapoptotic molecule. *In vitro* biological tests showed lower CDDP-induced cytotoxicity, down regulation of caspase 3/7 expression, and lower IL-1β release and intracelular ROS accumulation, while *in vivo* experiments demonstrated a reduced hearing loss when animals received the NPs.

CONCLUSION:

pH sensitive polymeric systems were succesfully used against cisplatin-induced hearing loss in an animal model.

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ACKNOWLEDGMENTS:

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Design of biomaterials for drug delivery 14:30 - 16:00 Room 0.5 12/09/2018

Oral presentation

271 Bioresorbable Silica Gel Fiber Systems - a Novel Platform Technology for Drug Release in Regenerative Therapies

Bastian Christ, Johannes Schneider, Sofia Dembski, Jörn Probst, Heike Walles
Fraunhofer ISC, Translational Center Regenerative Therapies TLZ-RT, Würzburg, Germany

INTRODUCTION:

Synthetic organic-inorganic hybrid biomaterials are in focus of biomaterials research since many years and already entered the marked as medical products.[1] These materials combine synergetically the advantages of organic and inorganic devices. Here the authors present sol-gel derived resorbable silica gel microfibers, whereof one fiber formulation is CE-approved for the regeneration of diabetic ulcer. The nanostructured surface of the fibers enhances the directed movement of cells[2] and the release of *ortho*-silicic acid while fiber degradation results in an anti-inflammatory wound healing[3], which opens up new perspectives in therapies as a smart bioactive implant. In a next step the silica gel fibers have been further developed into a carrier of therapeutically effective amounts of drugs. This study shows drug release profiles while fiber degradation and its optimization by adjusting fiber degradation properties.

METHODS:

Via sol-gel routes endless silica gel fibers were produced out of liquid precursors using a wet spinning process. Integration of polymer encapsulated drugs while sol synthesis results into drug-loaded fibers. Fiber degradation studies and drug release profiles are performed under defined flow rates of physiological buffer solutions. The materials are tested for biocompatibility according DIN ISO 10993-5.

RESULTS AND DISCUSSION:

Biocompatible sol-gel derived silica gel microfibers show high loading capacities of different drugs, e.g. antibiotics and cytostatic agents. Variation of sol composition as well as fiber treatment during spinning process result in different degradation behaviours. This parameters can be used to fine-tune the release of drugs in therapeutic amounts. Optimized fiber formulation show a drug release of at least 10 days.

In contrary to organic fibrous drug delivery systems, e.g. electrospun biopolymers and biomimetic hydrogels, hybrid silica gel fibers show no shrinkage effects under physiological conditions and also ensure a therapeutically effective drug release over a sufficient time period.

CONCLUSION:

In this work the authors present silica gel fibers as a novel hybrid drug delivery system, which allows a loading and release of drugs of different physicochemical nature. Fabricated to a fiber fleece with optimized mesh sizes and degradation behaviour, the fibrous hybrid material shows high potential as a smart drug eluting carrier for regenerative therapies.

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Design of biomaterials for drug delivery 14:30 - 16:00 Room 0.5 12/09/2018

Oral presentation

214 Bio-resorbable polyesteramides; a platform for local sustained drug delivery concepts in management of pain.

Jens Thies[1], Nina Woike[1], Julien Bérard[1], George Mihov[1], Imke Rudnik-Jansen[2], Anna Tellegen[3], Pieter Emans[4], Marianna Tryfonidou[3], Laura Creemers[2]
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INTRODUCTION:

An aging population and the increasing prevalence of chronic degenerative diseases such as Osteoarthritis and intervertebral disc degeneration (IVDD) means that many patients are living for long periods while suffering chronic pain. Current pain management in these patients is far from optimal and relies on prolonged use of systemic drugs such as non-steroidal anti-inflammatory (NSAIDs), steroids, or opioids. The side effects of these drugs are well known and devastating. Prolonged use of NSAIDs can lead to significant gastrointestinal and cardiovascular conditions, as well as musculoskeletal and renal side effects#_ftn1. The widespread prescription and related abuse of Opioids has led to an estimated 4% of the US population misusing opioids and was attribute to more than 33000 deaths in 2015#_ftn2. Therefore, a pressing need remains for effective pain management concepts where the appropriate drug is delivered locally to the area causing pain and for a prolonged duration, without the need for high systemic doses.

#_ftnref1 RESULTS AND DISCUSSION: In this paper we describe the use of bio-resorbable polyesteramide as a platform for local sustained release of drugs for the management of pain.

In-vitro and

in-vivo studies of PEA formulations for treatment of pain resulting from osteoarthritis#_ftn1 and IVDD are discussed. A main advantage of PEAs is that by design, they allow for tunable degradation profiles ranging from enzymatically driven surface erosion to slow hydrolytic degradation of the polymer matrix#_ftn2. Experimental results indicate that PEA polymers undergo degradation without lowering the pH in the test media or surrounding tissues. Additionally, in-vivo degradation studies demonstrated the capability to tailor degradation of different types of PEAs as well as superior biocompatibility in comparison to common biodegradable polymers. Furthermore, by choosing appropriate formulation and processing approaches drug release rates can be tuned to achieve desired release kinetics.

CONCLUSION:

Polyesteramides (PEA) are highly promising materials for drug delivery and their local application may have a significant impact of improving quality of life in large patient populations suffering from pain related to chronic degenerative diseases.

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Design of biomaterials for drug delivery 14:30 - 16:00 Room 0.5 12/09/2018

Oral presentation

369 Vancomycin and doxorubicin release from biodegradable β-TCP-PLA nanocomposite scaffolds

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[4]Institute of Pathology, University Medical Center of the Johannes Gutenberg Univ, Germany

INTRODUCTION:

Bioresorbable implants are gaining popularity in bone healing devices. Use of bioresorbable scaffolds can result in complete tissue regeneration, without the risk of scaffold-related infection. Bioresorbable Ca Phosphate ceramics are brittle and unsuitable for load-bearing sites[1]. High mechanical properties were reported for β-TCP-PLA ductile nanocomposites, prepared employing high energy attrition milling followed by cold sintering[2]. In[3] such nanocomposites were used for incorporation and release of vancomycin, resulting in strong antimicrobial activity. In present work, we report on processing of β-TCP-40vol.%PLA nanocomposite scaffolds and kinetics of vancomycin and anticancer drug doxorubicin release from these scaffolds. Effect of released drugs on viability of bacteria and of tumor cells was studied *in vitro.*

METHODS:

β-TCP nanopowder prepared as described in[2] was mixed with 40%PLA by high energy attrition milling resulting in nanocomposite[2]. β-TCP-40PLA scaffolds were produced from 300-400mm granules mixed with 50-70 vol.% sugar porogen. The blends were consolidated at 2.5 GPa and sugar was dissolved in water. The 10 mm diameter 3 mm thick scaffolds were loaded by vancomycin or doxorubicin water solutions in concentrations of 20-50 mg/ml under vacuum. Drug loaded scaffolds were immersed in 50 mM Tris buffer at 37°C. Vancomycin and doxorubicin concentrations were evaluated using a UV-spectrometer. Scaffolds were characterized in SEM with EDS. Mechanical properties were tested in compression and bending. Permeability was measured employing Darcy test.

RESULTS AND DISCUSSION:

The density of high pressure consolidated β-TCP-40PLA specimens was ³ 94 % TD. SEM revealed homogeneous distribution of macro pores. The scaffolds exhibit high strength in compression ~5MPa and high permeability value ~ 0.7×10^{-10} m^2. A gradual release of >95% vancomycin and doxorubicin was observed with after 2 weeks for 50% porosity scaffolds, with the mass loss not exceeding 2 %. Kinetics of vancomycin release is shown in Fig.1. Compressive strength decreases with immersion time still being >75% of initial strength after 2 weeks of immersion.

*In vitro*study showed significant antimicrobial efficacy of vancomycin and anti-tumor cells ability of doxorubicin loaded β-TCP-40PLA scaffolds.

CONCLUSION:

The developed load-bearing drug eluting bioresorbable β-TCP-PLA scaffolds are capable of providing temporary mechanical support, while preventing implant-related infection or suppressing activity of tumor cells.

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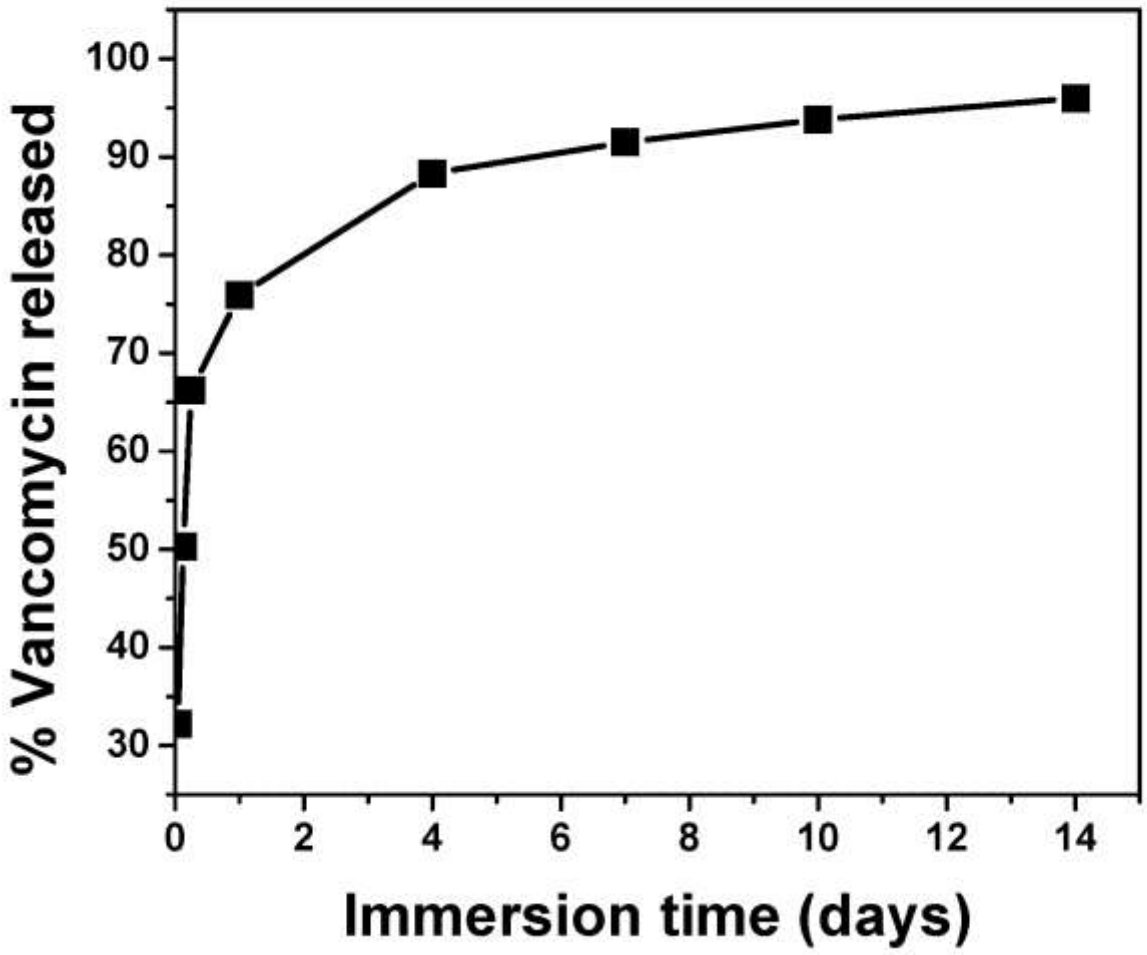

Picture 1: Caption 1: Cumulative vancomycin release from β-TCP-40PLA scaffold with 50% porosity at 37°C as a function of immersion time in Tris

Modular engineering of cells & tissues 16:30 - 18:00 Room 0.5 12/09/2018

Oral presentation

513 Computer Designed Topographical Surfaces for Instructing Cell Fate

Aliaksei Vasilevich[1], Steven Vermeulen[1], Marloes Kamphuis[1], Nick Beijer[1], Aurélie Carlier[1], Singh Shantanu[2], Jan de Boer[1]
[1]MERLN Institute, Maastricht University, Maastricht, Netherlands
[2]Broad Institute of MIT and Harvard, United States of America

INTRODUCTION:

Topographical cues have been repeatedly shown to influence cell fate dramatically. The exact mechanism of topographical control on cell behaviour is unknown. We have therefore developed a high-throughput technology in our laboratory to determine the optimal surface topography for a given biomedical application. The TopoChip enables assessment of cell response to 2176 unique topographies in a single assay [1]. In our previous studies, we have demonstrated that these surfaces exert a mitogenic effect [1], affect cell shape [2], modulate ALP expression on human mesenchymal stromal cells (hMSCs) [3], as well as pluripotency in human induced pluripotent stem cells [4].

METHODS:

hMSCs were incubated in aMEM media supplemented with 10% FBS at 37 °C in a humid atmosphere. Nuclei were stained with DAPI, Actin was stained with phalloidin. Cell morphology was captured by high-content imaging and we performed image analysis in CellProfiler. Microarray analysis was performed with Illumina microarrays.

RESULTS AND DISCUSSION:

We identified 28 clusters of distinct cell morphologies based on cell shape features. Corresponding topographies were further used to reveal how different cell shapes induced by topography can affect fundamental cell functions. We have performed various functional assays with hMSCs such as: differentiation, proliferation, migration, apoptosis and protein synthesis. By performing micro-array analysis on cells grown on these surfaces we revealed key genes involved in surface topography interaction.

CONCLUSION:

We identified 28 surfaces based on cell shape diversity with distinct designs. Using functional assays we identified topographies inducing the unique cell response. Analysis of gene expression on these surfaces allowed identifying key regulators of cells behaviour on investigated topographies.

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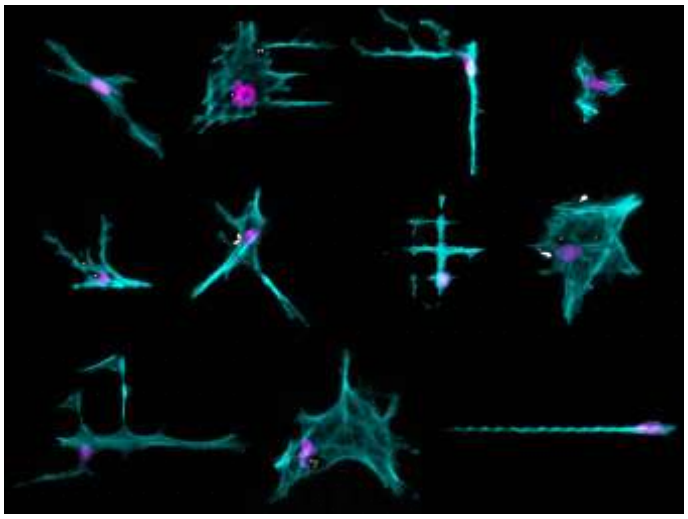
ACKNOWLEDGMENTS:

The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no 289720. AV, SV, MK, NB, AC and JdB acknowledge the financial contribution of the Province of Limburg. AC gratefully acknowledges her VENI grant (number 15057) from the Dutch Science Foundation (NWO).



Picture 1: Caption 1: Figure 1. hMSCs morphology on topographies with different designs.

Modular engineering of cells & tissues 16:30 - 18:00 Room 0.5 12/09/2018

Oral presentation

267 Synthesis of lithium carbonate nanoparticles with potential properties for bone tissue engineering.

Covarrubias Cristian, Juan Pablo Durán, Miguel Maureira, Margarita Torres
University of Chile, Santiago, Chile

INTRODUCTION:

Lithium is a therapeutic element with properties to stimulate bone tissue formation. Local application of lithium increases bone mineral density and accelerates the bone formation process[1]. Likewise, bioceramics have shown a superior osteogenic capacity when doped with lithium[2]. Until now, lithium bioactivity has been studied using only soluble Li^+ ions. The production of nanoparticles of lithium could offer technological advantages to design composite biomaterials, surfaces for biomedical devices or controlled drug delivery systems.

In this work, we reported the synthesis of Li_2CO_3 nanoparticles by facile chemical route and demonstrated for the first time their cytocompatibility and differentiation properties.

METHODS:

Nanoparticles were synthesized from a LiOH solution in the presence of polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), or cetyltrimethylammonium bromide (CTA) as capping agents. Capping agent was dissolved in water; LiOH was added and stirred for 1 h. The suspension was frozen at -80°C, lyophilized and calcined at 700 °C. Nanoparticles were analyzed by SEM/EDX microscopy, ATR-FTIR spectroscopy and XRD. The viability of dental pulp stem cells (DPSCs) cultured with nanoparticles was determined with the MTS assay. Alkaline phosphatase (ALP) activity was measured by dephosphorylation of p-nitrophenyl phosphate substrate. Lithium released in the cell culture media were measured by flame emission photometry.

RESULTS AND DISCUSSION:

Li_2CO_3 nanoparticles (nLi_2CO_3) of ~86 nm in size were produced preferably using PVA as a capping agent (Fig. 1a). FTIR-ATR analysis revealed that Li_2CO_3 phase is formed during calcination of the freeze-dried synthesis product.

Nanoparticles do not induce statistically significant changes in cell viability at concentrations up to 600 µg/mL. ALP activity is significantly increased ($p<0.001$) in DPSCs cultured with 600 µg/mL nLi_2CO_3 (Fig. 1b). The capacity of the nLi_2CO_3 to stimulate the cell differentiation is attributed to the Li^+ ions[3] generated by dissolution of the nanoparticles. Li^+ concentrations around 9.71 mM are more favorable to promote the osteogenic differentiation process.

CONCLUSION:

Li_2CO_3 nanoparticles can be synthesized by a facile chemical route and preferably using PVA. nLi_2CO_3 produces high cell viability and promote the osteogenic differentiation of stem cells. The biological properties exhibited by the nLi_2CO_3 are promising to be exploited in bone regeneration applications.

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ACKNOWLEDGMENTS:

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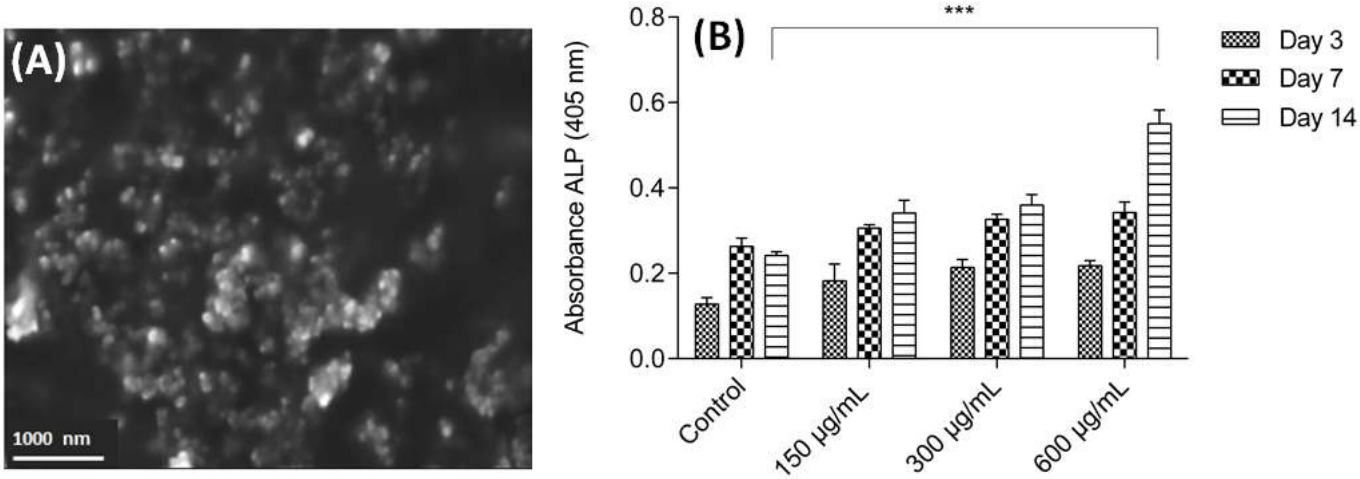

Picture 1: Caption 1: SEM image of lithium carbonate nanoparticles (A). ALP activity of DPSCs cultured for 14 days with different concentrations of nanoparticles.

Modular engineering of cells & tissues 16:30 - 18:00 Room 0.5 12/09/2018

Oral presentation

13 Soft tissue biocompatibility of lithium, strontium and boron -doped bioactive silicate glasses

Inari Lyyra[1], Reetta Sartoneva[2], Minna Kellomäki[1], Susanna Miettinen[2], Jonathan Massera[1]
[1]BioMediTech Institute, Tampere University of Technology, Tampere, Finland
[2]BioMediTech Institute, University of Tampere, Finland

INTRODUCTION:

Bioactive glasses (BAGs) are extensively studied for bone tissue engineering, but they also show potential for soft tissue regeneration[1]. One advantage of these materials is the ease of introducing therapeutic ions[2,3], and controlling their release[1]. However, BAGs are often tested with many different cell types in various conditions, leading to a lack of comparability between studies. Here, the objective is to understand and compare the effects of lithium, strontium and boron –doped BAGs on the viability, proliferation and morphology of human adipose stem cells (hASCs), fibroblasts and human uroepithelial cells (HUCs).

METHODS:

hASCs, human lung fibroblasts and HUCs were cultured for 14 days in cell culture medium based ionic extracts of 13-93 BAG doped with lithium, strontium or boron. The cell viability, proliferation (n=9) and phenotype were studied and the changes in ion concentrations in the extracts were quantified using ICP-OES (n=3). The glasses' dissolution properties were studied in a buffer solution and the ion concentrations were compared to the concentrations in the extracts.

RESULTS AND DISCUSSION:

The hASCs and the fibroblasts remained viable in the extracts and lithium and strontium seemed to promote their proliferation. These results are in line with an in vivo study, where Sr and Sr+Li -doped BAGs induced an abundant collagen network in rats[4]. Boron increased the cell size in hASCs and fibroblasts and seemed to inhibit the fibroblast proliferation, maybe because of the high boron content in the extracts. Lastly, all extracts changed the morphology of the HUCs, and did not promote their viability nor proliferation. This is attributed to the high calcium content, known to slow down HUC proliferation[5].

CONCLUSION:

Based on good viability and proliferation of hASCs and fibroblasts, the novel Li/Sr containing BAGs show potential for soft tissue applications. With these cells, mineralization effects should be studied carefully to avoid ectopic ossification. The therapeutic ion concentrations for HUCs will be confirmed. This screening study gives comparable information on the effect of BAGs on various cell types linking the ion concentrations to cell behaviour.

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ACKNOWLEDGMENTS:

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Modular engineering of cells & tissues 16:30 - 18:00 Room 0.5 12/09/2018

Oral presentation

674 Engineered bacterial biofilms to control stem cell differentiation

Aleixandre Rodrigo - Navarro[1], Aleixandre Rodrigo-Navarro[2], Jake Hay[2], Manuel Salmeron-Sanchez[2]

[2]University of Glasgow, Glasgow, United Kingdom

INTRODUCTION:

The use of materials for stem cell culture is attractive since it can be easily upscaled using techniques such as 3D printing or fibrous hydrogel scaffolds. These materials can be engineered to deliver specific and/or dynamic cues to the stem cells. However, these systems lack the ability to deliver the precise cues required for stem cell differentiation in a controlled manner, since stem cells usually reside in a highly complex niche and their fate is tightly regulated by a plethora of factors.

We present a novel approach using bacteria as a substrate to influence mesenchymal stem cells in a facile and temporal manner. *Lactococcus lactis* spontaneously develops biofilms on a variety of surfaces (e.g polymers, metals) and can be genetically modified to produce a variety of probiotic and efficacious proteins[1,2]. Here we show that controlled expression of fibronectin fragments supports growth and temporal regulation of secreted bone morphogenetic protein 2 drives osteogenesis in an on-demand manner.

METHODS:

Lactococus lactis NZ9020 has been genetically modified to express the III7-10 fragment of human fibronectin and human bone morphogenetic protein 2 (BMP-2) in a constitutive and inducible fashion. Protein expression was characterised with ELISA and Western blot.

Human MSCs were co-cultured with *L. lactis* biofilms for up to 28 days and assessed for osteogenic differentiation using von Kossa for mineralization, osteocalcin immunofluorescence and ALP expression.

RESULTS AND DISCUSSION:

L. lactis develops stable biofilms on a variety of substrates. These biofilms contain viable bacteria and are stable for up to 28 days, supporting hMSC adhesion, proliferation and differentiation.

L. lactis expresses biologically active III7-10 fibronectin and BMP-2. The expression levels of BMP-2 can be adjusted and induce osteogenic differentiation, as assessed with the techniques described in the previous section. The behaviour of hMSCs cultured on engineered *L. lactis* biofilms is equivalent to the use of a fibronectin coated substrate with the addition of 100 ng/mL of BMP-2, the standard for osteogenic differentiation of hMSCs.

CONCLUSION:

Engineered *L. lactis* biofilms expressing FN III7-10 and BMP-2 in an inducible manner can be as a functional, living biointerface between a wide range of substrates and hMSCs to drive its differentiation to the osteogenic lineage in a predictable and controlled way.

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ACKNOWLEDGMENTS:

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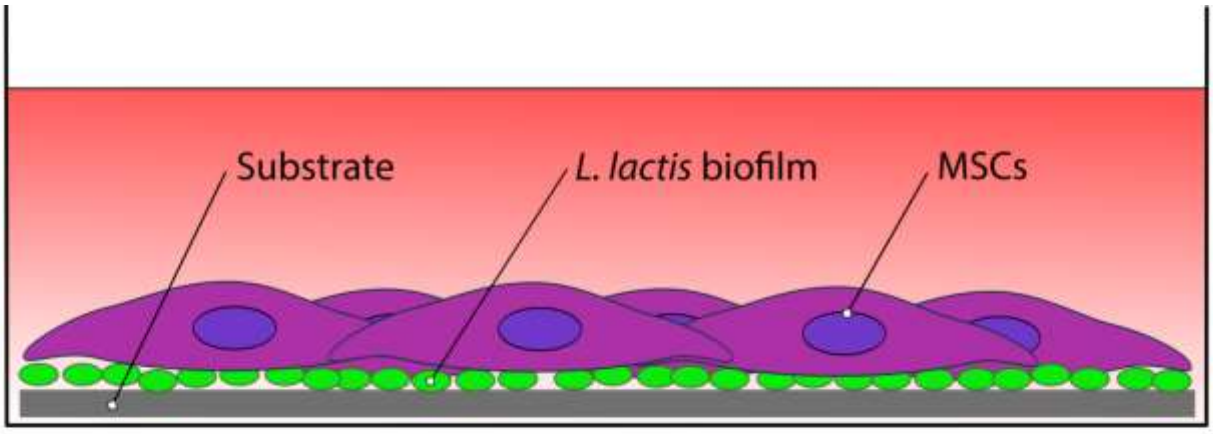

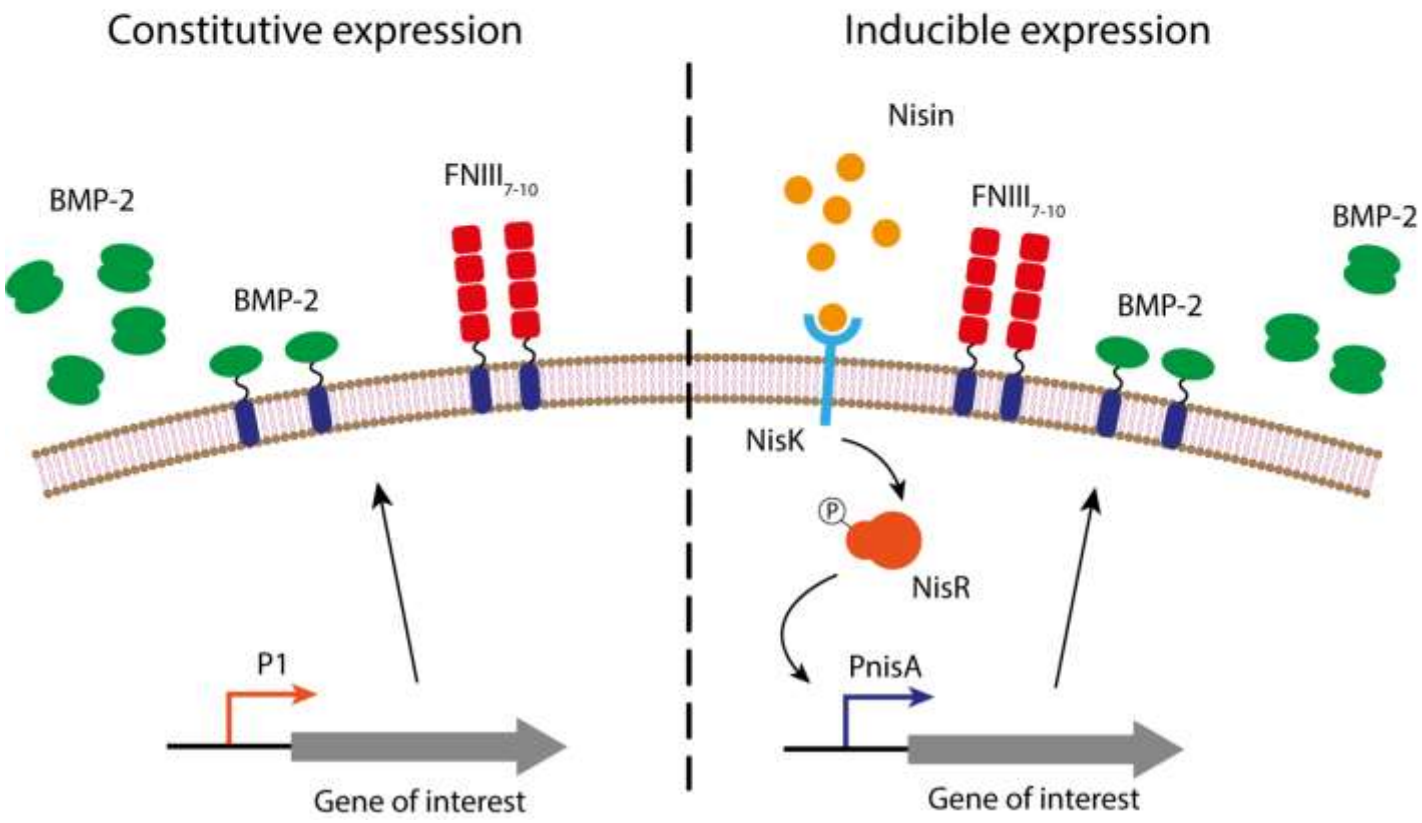

Picture 1: Caption 1: Engineered L. lactis biofilms act as a substrate for stem cell differentiation. Expressed FN III7-10 and BMP-2 are able to trigger osteogenesis.

Modular engineering of cells & tissues 16:30 - 18:00 Room 0.5 12/09/2018

Oral presentation

305 Enhancement of bone tissue regeneration via cross-talk between MSCs-derived osteogenic and angiogenic cells

Jidong Li, Limei Li, Qin Zou, Yi Zuo, Yubao Li
Sichuan University, Chengdu, China

INTRODUCTION:

Bone fracture healing is a complex process mediated by multiple factors; many cell types are involved in the formation, repair, and remodeling of bone[1,2]. Thus, we first induce the osteogenic and angiogenic differentiation of bone mesenchymal stem cell (MSCs) into osteogenic cells (OMSCs) and endothelial cells (ECs), respectively. After screening for the optimal ratio of OMSCs/ECs using *in vitro* experiments, the selected desired co-cultures were transplanted onto a scaffold to form a cellular bone graft to demonstrate the enhancement of new bone formation.

METHODS:

We dually induced MSCs to differentiate into OMSCs and ECs. Subsequently, the optimal ratio of OMSCs/ECs in the co-culture system was determined by exploring the level of cell cross-talk based on the functional markers of osteogenesis and angiogenesis expression. The selected desired co-cultures were transplanted onto a nano-hydroxyapatite/polyurethane (n-HA/PU) scaffold to form a cellular bone graft. A condylar femur defect model in rat was used to demonstrate the enhancement of new bone formation among tissue-engineered constructs.

RESULTS AND DISCUSSION:

ELISA and gene expression studies revealed that a 0.5/1.5 co-culture of OMSCs/ECs significantly elevated the transcription levels of osteogenic genes such as *ALP*, *Col- I,* and *OCN*, as well as transcription factors Msx2, Runx2, and Osterix via the BMP-2 pathway; it also upregulated angiogenic factors of vascular endothelial growth factor (VEGF) and CD31 when compared with cells cultured alone or in other ratios. As the cell numbers increased, the OMSCs/ECs group (ratio: 2.0/1.0) showed elevated levels of osteogenic and angiogenic factors, indicating the importance of cell numbers and ratios. The optimized OMSCs/ECs mixture seeded to the n-HA/PU scaffold had more abundant calcium phosphate crystal deposition, thus further facilitating their bone formation *in vivo* when compared to mono-cultures or MSCs/ECs co-cultures. The OMSC/EC-scaffold constructs at an optimal cell ratio (2.0/1.0) achieved enhanced osteogenic and angiogenic factor expression and biomineralization, thus resulting in more effective bone formation.

CONCLUSION:

This study demonstrated that osteogenesis and angiogenesis could be enhanced by augmenting the paracrine effects between OMSCs–ECs interactions at an optimal ratio (2.0/1.0) in co-culture treatment.

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ACKNOWLEDGMENTS:

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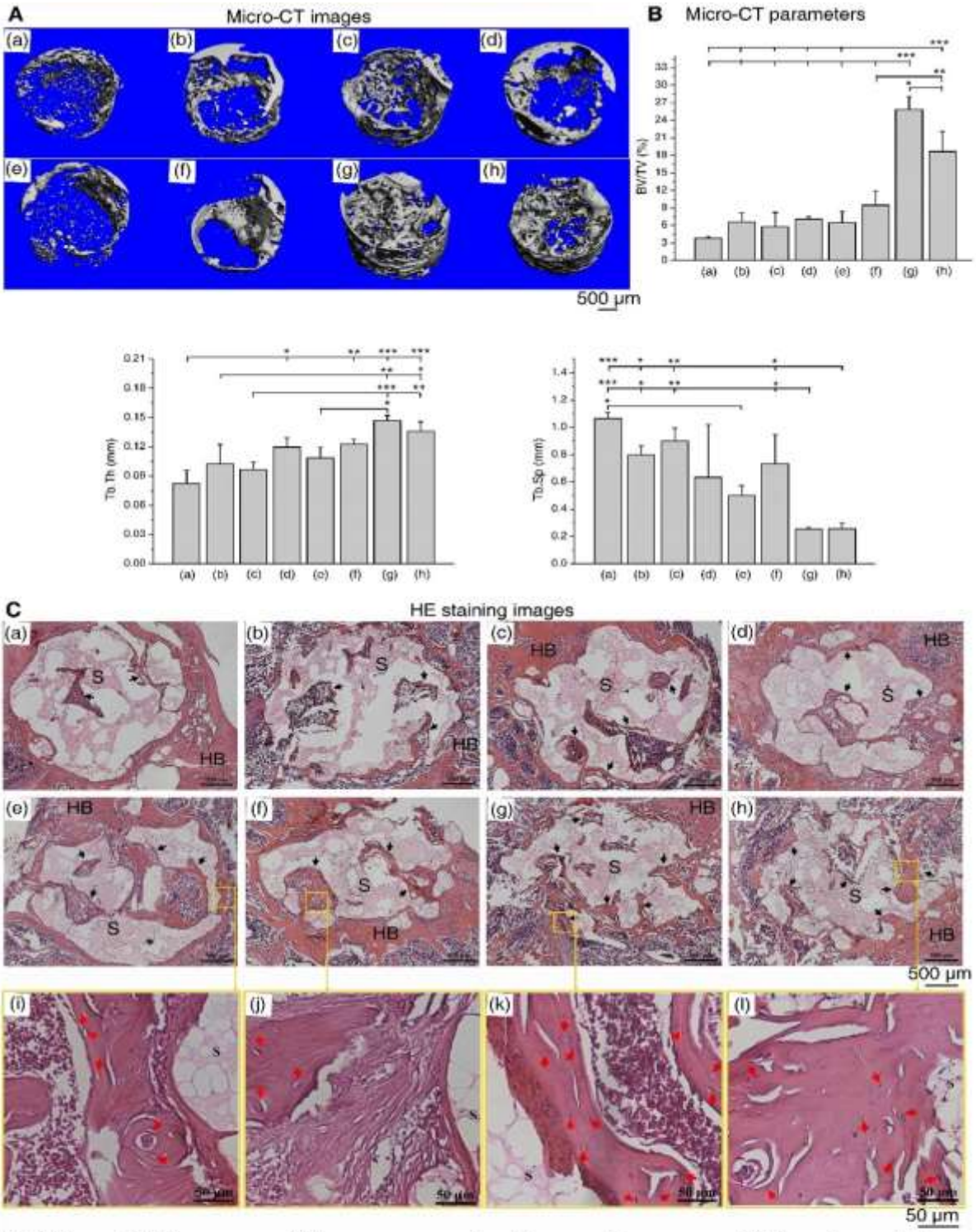

(A) Micro-CT images of the regenerated bone tissue and (B) relevant bone parameters of BV/TV (bone tissue volume/total volume), Tb.Th (trabecular thickness), and Tb.Sp (trabecular separation). (C) Histological evaluation (HE staining) of new bone formation within the pore for 8 weeks: (a) pure scaffold; (b) MSCs scaffold; (c) OMSCs scaffold; (d) ECs scaffold; (e) MSCs/ECs (2.0/0.5) scaffold; (f) MSCs/ECs (0.5/1.5) scaffold; (g) OMSCs/ECs (2.0/1.0) scaffold; (h) OMSCs/ECs (0.5/1.5) scaffold. HB – host bone; S – scaffold; black arrows – new bone; red arrows – vascular structure. **P*<0.05; ***P*<0.01; ****P*<0.001.

Picture 1: Caption 1: Bone regeneration of different research groups after 8 weeks implantation

Modular engineering of cells & tissues 16:30 - 18:00 Room 0.5 12/09/2018

Oral presentation

723 Immune response modulation by a Sr-releasing injectable biomaterial for bone regeneration

Ana Henriques Lourenço, Daniela P. Vasconcelos, Cláudia Ribeiro-Machado, Judite N. Barbosa, Cristina C. Barrias, Mário A. Barbosa, Cristina C. Ribeiro
I3s - Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Porto, Portugal

INTRODUCTION:

For bone tissue regeneration strategies, injectable bone substitutes are very attractive since they can be applied with minimally invasive surgical procedures and can perfectly fill irregular defects created in cases of trauma, infection or tumor resection. These materials must combine adequate mechanical properties with the ability to induce new bone formation. Incorporating strontium (Sr) in bone substitute biomaterials may be a strategy to achieve high Sr concentrations, not in a systemic but in a local environment, taking advantage of the osteoanabolic and anti-osteoclastic activity of this metal ion, for the enhancement of new bone formation [1,2]. However, the immune response upon implantation of a biomaterial is of major importance and ultimately may dictate the success of the implant. In this context, the aim of the present work was to evaluate a Sr-rich injectable hybrid material in an *in vivo* rodent model of inflammation.

METHODS:

Sr- and Ca-Hybrid materials, consisting of hydroxyapatite microspheres doped with Sr or Ca and an alginate vehicle crosslinked in situ with Sr or Ca (respectively), were produced. The air-pouch model of inflammation was used in male BALB/c mice with 7-9 weeks old [3]. Ca-Hybrid systems and empty defects were used as control. Inflammatory exudates were recovered after 3 days and skin and organs were processed for histology after 15 days.

RESULTS AND DISCUSSION:

An increase in F4/80+/CD206+ cells (M2 macrophages) in inflammatory exudates was observed upon Sr-hybrid implantation, comparing to the control, with a decrease in inflammatory cytokines such as TNF-RI, MIP-1gamma and RANTES, at day 3. Histological analysis of the materials and adjacent tissues at day 15 revealed a thin fibrous capsule (150 μm) with low cell infiltration and not statistically different from the control. Furthermore, histological analysis of the heart of animals did not detect collagen deposition, an important issue due to recent cardiovascular safety concerns regarding Sr ranelate administration.

CONCLUSION:

The Sr-releasing hybrid system promoted an M2 regenerative response, modulating therefore the inflammatory response towards a reparative phenotype.

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Antimicrobial activity of biomaterials 09:45 - 11:15 Room 0.2/0.3 12/09/2018

Oral presentation

763 Lipid-based nanoparticles that counteract gastric infection burden

Catarina leal Seabra[1], Cláudia Nunes[2], Paula Parreira[1], Patricia Henriques[1], Claudia Monteiro[1], Joana Gomes[3], Celso Reis[3], Inês Gonçalves[1], Salette Reis[2], Cristina Martins[1]
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[2]REQUIMTE, Portugal
[3]IPATIMUP/i3S, Portugal

INTRODUCTION:

Helicobacter pylori infection is one of the major risk factors for gastric cancer development[1]. Current treatment shows a rapid decline of *H. pylori* eradication rates, mostly due to bacterial resistance to available antibiotics[1].

Docosahexaenoic acid (DHA) is a polyunsaturated fatty acid with high bactericidal activity against *H. pylori*[2]. We demonstrated that DHA nanoencapsulation into lipid nanoparticles improve its bactericidal effect since DHA-NPs were able to inhibit *H. pylori* growth at lower concentrations (25 mM) than free DHA (>100mM)[3]. This work aims to evaluate the efficacy of DHA-NPs in the treatment of gastric infection, using an infection mice model, and to clarify if their bactericidal effect is specific to *H. pylori,* without antimicrobial activity against intestinal microbiota.

METHODS:

DHA-NPs were synthesized by hot homogenization and ultrasonication using a mixture of solid lipid/liquid lipid/stabilizer[5]. DHA-NPs were characterized in terms of morphology, size and charge, entrapment, stability in the simulated gastric fluid. *In vitro* efficiency were assessed using *H. pylori* J99 and *L. casei* and *E. coli* by colony forming units (CFU) counting for 24h. Bacteria morphology was evaluated by SEM and TEM. Cytocompatibility in MKN45 gastric cells was evaluated by metabolic activity (MTT assay) and cytotoxicity (LDH assay). *In vivo* efficacy was evaluated in SS1 *H.pylori*-infected C57BL/6 mice. After 4 weeks, DHA-NPs were administrated by oral gavage for 14 days. Stomachs were collected to assess *H. pylori* infection by CFU counting. Toxicity was evaluated by histological analysis of mice liver/stomach.

RESULTS AND DISCUSSION:

Homogenous DHA-NPs with a size ~300 nm, stable in the simulated gastric fluid, were able to release 40% of DHA after 3h in bacteria environment. DHA-NPs was bactericidal against *H. pylori* due to their ability to adhere and disrupt *H. pylori* membranes[3]. This effect was specific to *H. pylori* without affecting intestinal microbiota. At bactericidal concentration, DHA-NPs are cytocompatible to human gastric cells.

In vivo efficacy assays further confirmed that DHA-NPs was able to kill *H. pylori* reducing 91% of bacterial infection in mouse stomach. Toxicity assays demonstrated that DHA-NPs was biocompatible and well-tolerated by mouse.

CONCLUSION:

Our findings suggest that DHA-NPs should be envisaged as an antibacterial nanotherapeutic or an adjuvant agent in *H. pylori* infection treatment.

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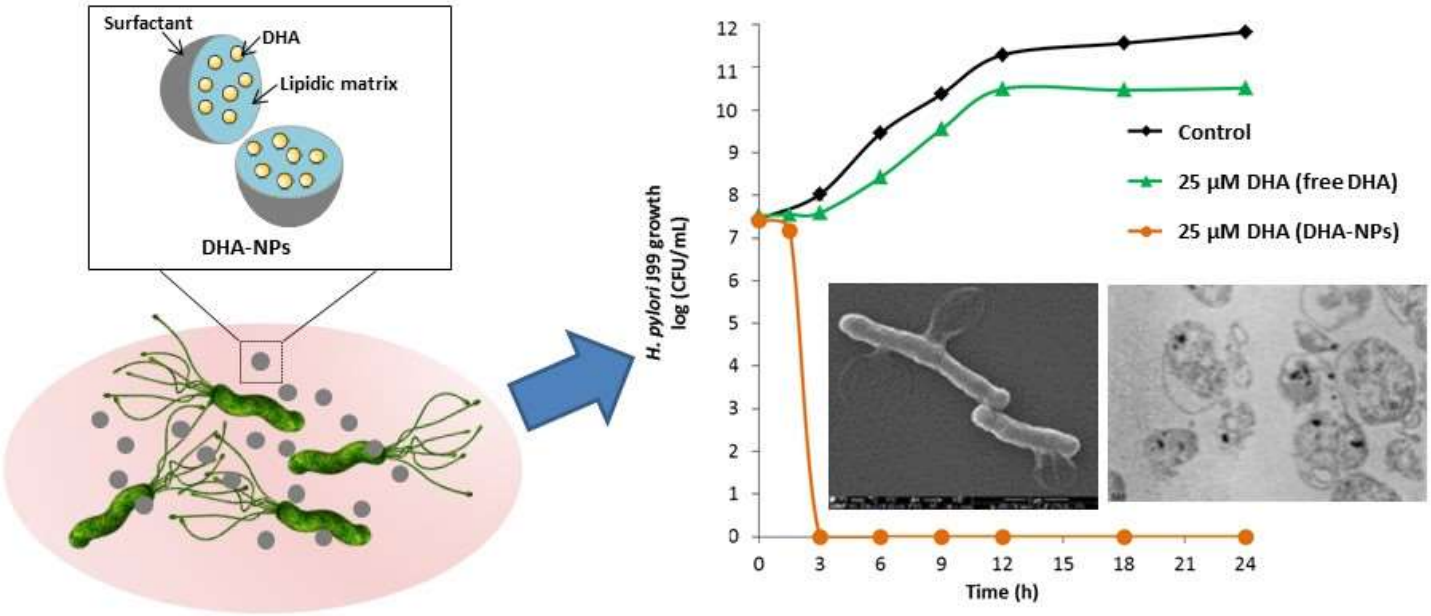

Figure 1. Anti-*H. pylori* efficacy *in vitro*: Antimicrobial activity of DHA-NPs against *H. pylori* J99 growth for 24 hours.

Picture 1: Caption 1: Anti-H.pylori efficacy

Antimicrobial activity of biomaterials 09:45 - 11:15 Room 0.2/0.3 12/09/2018

Oral presentation

540 The mechanism of action of surface-grafted caspofungin: definitive evidence from a series of Candida albicans mutants

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INTRODUCTION:

The most common fungal species in medical device infections is *Candida albicans*, with Candidaemia incurring a mortality rate of 30–40%[1]. There is currently no viable solution to combat fungal biofilms on biomaterials surfaces. Here we report an approach comprising the covalent-binding of antifungal drug caspofungin onto the surface of biomaterials[2]. We explore the mechanism of action of surface-grafted caspofungin molecules, using a series of *C. albicans* mutants with varying caspofungin sensitivities and resistance.

Caspofungin is an echinocandin-class antifungal drug that inhibits the enzyme (1,3)-β-D-glucan synthase, disrupting the integrity of the fungal cell wall[3]. The enzyme (1,3)-β-D-glucan synthase is encoded by several FKS genes, with mutations to FKS-1 shown to impact susceptibility of *C. albicans* to caspofungin[4].

We hypothesise that non-releasing coatings of surface-grafted casopfungin act by inhibiting the enzyme (1,3)-β-D-glucan synthase, disrupting the integrity of the fungal cell wall. Our key objective was testing caspofungin surface coatings against various FKS-1 mutants strains of *C. albicans*, either proving or disproving the hypothesis that the mechanism of action of caspofungin surface coatings is through inhibition of the (1,3)-β-D-glucan synthase fungal cell wall enzyme.

METHODS:

Six *C. albicans* FKS-1 mutant strains were tested for biofilm growth against caspofungin-coatings, using a previously established biofilm assay[2]. Two *C. albicans* mutants and one clinical isolate were caspofungin-resistant, and two mutants and one clinical isolate were caspofungin-sensitive. Each mutant strain was tested for biofilm growth against caspofungin-coatings. Each *C. albicans* mutant strain experiment was repeated to achieve statistical significance (n=9 minimum).

RESULTS AND DISCUSSION:

Two mutants and one clinical isolate were caspofungin-resistant, confirmed by biofilm growth on caspofungin-coatings. Two mutants and one clinical isolate were caspofungin-sensitive, confirmed with no colony growth on caspofungin-coatings. This confirmed that mutations to the FKS-1 gene in *C. albicans* directly influenced their sensitivity to caspofungin. These results confirm our hypothesis, that coatings of surface-grafted caspofungin act by inhibiting the enzyme (1,3)-β-D-glucan synthase, disrupting the integrity of the fungal cell wall.

CONCLUSION:

To conclude, we have shown the mechanism of action of surface-grafted caspofungin-coatings, shedding light on a potentially industrially-viable solution to fungal biofilms on materials surfaces.

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Antimicrobial activity of biomaterials 09:45 - 11:15 Room 0.2/0.3 12/09/2018

Oral presentation

584 Preparation of the antimicrobial surface with the antimicrobial peptide by click chemistry

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INTRODUCTION:

Biomaterial-associated infection is a serious problem in clinic. Surface modification of the biomaterial with silver nanoparticles or antibiotic could reduce the infection. However, the cytotoxicity of silver nanoparticles and bacterial drug-resistance caused by the antibiotic often limit their applications [1, 2].

Antimicrobial peptides (AMPs) are widely studied by researchers [3]. However, when applied to biomaterial surfaces, the bottleneck of using an antimicrobial peptide often lies in its stability. The AMPs only exhibit activity with proper orientation on the surface. The energy of the surface could disorder its orientation, and the enzymes in the environment could degrade the peptide, destroying its orientation and activity.

METHODS:

We employed the HHC36 peptide as the antimicrobial agent and the poly(zwitterion), poly[2-(Methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)-ammonium hydroxide (polySBMA), as the spacer molecule to prepare an antimicrobial surface by click chemistry. We characterized the antimicrobial activity of the surface against S. aureus, E. coli and P. aeruginosa, and the stability of the antimicrobial activity. We also employed mesenchymal stem cells (mBMSCs) to characterize its biocompatibility.

RESULTS AND DISCUSSION:

The integration of the antimicrobial peptide significantly increased the antimicrobial activity of the surface. Compared to the control group, the modified surface could kill 98.26% of E. coli, 83.72% of S.aureus and 81.59% of P. aeruginosa. Moreover, the peptide with spacer (Si-SBMA-PraAMP) has a much better enzymolysis-tolerance than the peptide without spacer (Si-PraAMP). After being treated with trypsin for 5 min, Si-PraAMP inhibited only 31.58% of E. coli on its surface, whereas Si-SBMA-PraAMP inhibited 84.80% of E. coli. These antimicrobial results indicate that the incorporation of polySBMA can markedly improve the peptide's enzymolysis-tolerance on the surface.

The biocompatibility results illustrated that both Si-PraAMP and Si-SBMA-PraAMP had negligible cytotoxicity towards the mBMSCs. After 24 h of the culture, 0.96- and 1.04-folds of cells were on Si-PraAMP and Si-SBMA-PraAMP, respectively, compared to pristine Si.

CONCLUSION:

We developed a method to modify the surface using the antimicrobial peptide with improved stability. The poly(zwitterion) spacer could increase the enzymolysis stability of the modified surface. The surface exhibited antimicrobial activity against E.coli, S.aureus, and P.aeruginosa and showed negligible cytotoxicity towards mBMSCs.

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Antimicrobial activity of biomaterials 09:45 - 11:15 Room 0.2/0.3 12/09/2018

Oral presentation

626 Photochemical internalization as a novel delivery strategy to enhance efficacy of antibiotic treatment of staphylococcal intracellular infection

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INTRODUCTION:

Staphylococci can survive intracellularly even in phagocytes, causing intracellular infections. Treatment of these intracellular infections often fails due to the low intracellular efficacy of most antibiotics. To address this problem, we used photochemical internalization (PCI) to improve treatment of staphylococcal infections using antibiotics with low intracellular activity such as gentamicin. In PCI, the membranes of endocytic vesicles containing drugs are disrupted by photosensitizers bound to these membranes, causing cytosolic release of the drugs from the vesicles allowing therapeutic action within cells.

METHODS:

Intracellular antimicrobial activity assay

RAW264.7 cells containing *S. epidermidis* were treated with gentamicin (1, 10, 30 µg/ml) with or without the photosensitizer $TPPS_{2a}$ (0.25 µg/ml) and illuminated for 10 or 15 minutes. After overnight incubation, cells were lysed and intracellular bacteria were quantitatively cultured. Data were analyzed by one-way ANOVA, and subsequently compared pairwise by Sidak's multiple tests.

Confocal microscopy

RAW264.7 cells were incubated overnight with 10 µg/ml Alexa Fluor 405-labeled gentamicin alone or combined with 1 µg/ml the photosensitizer $TPCS_{2a}$, then illuminated for 2 minutes and prepared for confocal microscopy.

Treatment of S. aureus-infected zebrafish embryos

Zebrafish embryos were injected with 3000 CFU of *S. aureus*, and randomly divided into groups (~30 embryos per group) for subsequent treatments of gentamicin alone (0.05, 0.1 or 0.4 µg/ml) or combined with 0.25 µg/ml $TPCS_{2a}$, and then illuminated for 10 minutes. Survival was monitored until 6 days post injection. Differences between pairs of survival curves were analyzed using log rank test.

RESULTS AND DISCUSSION:

PCI significantly enhanced the activity of gentamicin against intracellular *S. epidermidis* in RAW264.7 cells. Moreover, PCI-gentamicin treatment significantly improved survival of *S. aureus*-infected embryos. However, a

minimal dose of gentamicin is required to observe the enhancing effect of PCI both *in vitro* and *in vivo*. Confocal images implied PCI induced cytosolic release of gentamicin in RAW264.7 cells. Since PCI has no restrictions for compounds to be delivered into cells, it is expected to also improve the efficacy of other antibiotics with low intracellular activity (e.g. other aminoglycosides and glycopeptides).

CONCLUSION:

PCI can significantly enhance the efficacy of an antibiotic with limited activity (e.g. gentamicin) against intracellular staphylococcal infection, likely owing to the cytosolic release of the antibiotic.

ACKNOWLEDGMENTS:

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Antimicrobial activity of biomaterials 09:45 - 11:15 Room 0.2/0.3 12/09/2018

Oral presentation

704 Graphene nanoplatelets coatings for antimicrobial silicone catheters

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INTRODUCTION:

Silicone rubber (SR) catheters are susceptible to bacterial adhesion and biofilm formation[1]. Graphene-based materials (GBM) have been considered intrinsically antimicrobial[2], presenting higher antibacterial activity in coatings when GBM are exposed[3]. The aim of this work was to produce a stable antimicrobial coating for silicone based on graphene nanoplatelets (GNP). This innovative strategy explores two different coating techniques to evaluate the influence of GNP exposure and oxidation on its antimicrobial properties.

METHODS:

GNP with 5 µm lateral size (GNP-M5) and its oxidized form (GNP-M5ox) were dispersed in a THF dispersion containing SR and immobilized on the surface of silicone films by dip and spray coating. GNP distribution of the surface and physicochemical properties of SR/GNP coatings were evaluated by optical microscopy, SEM, XPS, contact angle and rubbing test. An antibacterial assay based on ISO 22196 was performed by incubating materials with *S. epidermidis* ($6x10^5$ CFU/ml) (37ºC; 24h). Adherent bacteria were quantified by LIVE/DEAD assay (fluorescence microscopy) and planktonic bacteria collected from supernatant evaluated regarding metabolic activity (resazurin assay) and viability (CFU counting).

RESULTS AND DISCUSSION:

GNP was successfully exposed using both techniques, and whereas dip coating provided better adhesion of the GNP to the surface and with no changes in surface wettability, spray coating induced higher amounts of GNP at the surface and more homogeneously. Antibacterial assays revealed that spray coating enhanced bacterial adhesion comparing to dip coating and uncoated silicone, most likely due to increased surface roughness. However, independently of the coating technique, SR/GNP-M5ox induced higher bacterial death than GNP-M5 or uncoated silicone, suggesting that oxidized GBMs induce bacterial death upon contact by oxidative stress or membrane disruption.

CONCLUSION:

SR/GNP-M5ox coatings performed by dip coating preserved bacterial adhesion levels of silicone while increasing bacterial death to 80%, demonstrating the potential use of coatings with oxidized graphene nanoplatelets in the development of an antimicrobial silicone catheter.

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Antimicrobial activity of biomaterials 09:45 - 11:15 Room 0.2/0.3 12/09/2018

Oral presentation

547 Anti-biofilm coatings based on elastin-like recombinamers and antimicrobial peptides for preventing orthopedic implant-associated infections

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INTRODUCTION:

Peri-implant inflammations are on the rise, challenging research across numerous fields towards coatings that aim to counter this pathology. One of the main threats for implants is bacteria colonization and biofilm formation[1]. In this regard, developing covalent coatings for titanium implants that prevent the bacteria attachment and the development of the infection, ensuring the biointegration is crucial. In this study, the antimicrobial and anti-biofilm activity of recombinant coatings was evaluated covalently attached onto Titatium discs. The coatings are based on a polycationic Elastin-Like Recombinamer (ELR) backbone and the antimicrobial peptide GL13K, combining the extracellular- matrix mimicking properties of the ELR and the antimicrobial properties of the GL13K[2]. In this work, we investigated the antimicrobial capacity of the chimeric recombinamer and the effectiveness of the coatings.

METHODS:

All the antimicrobial biopolymers were designed and constructed by recombinant DNA technology and produced by *E. coli* fermentation[3]. Titanium grade II discs were covalently functionalized via silanization with the ELRs and incubated in the presence of relevant pathogens in the implant colonization and biofilm formation (*S. gordonii* ML-5, *S. mutans* ATCC® 700610™, *S. epidermidis* ATCC® 35984™ and *S. aureus* ATCC® 25923™) and also against complex multi-species oral stocks that allow simulate oral microbiota[4]. Afterwards, ATP-activity, CFUs, SEM visualization and live-dead staining of the biofilms were assessed. *In vitro* cytocompatibility tests (Live-dead and AlamarBlue®) were performed after the incubation of human gingival fibroblast cells over the surfaces.

RESULTS AND DISCUSSION:

Bacterial analysis revealed that new coatings provides strong anti-fouling and anti-biofilm activity, enhancing cell adhesion and proliferation of oral cells. These results suggested that the chimeric recombinamers enable the formation of covalent coatings for medical materials with high resistance and antimicrobial activity.

CONCLUSION:

Biomimetic antimicrobial coatings for titanium implants were obtained with chimeric recombinant biopolymers. The covalent functionalization of Ti discs and the antimicrobial and anti-biofilm activity of the coatings were demonstrated. Thus, these coatings could lower the severity of bacterial infections, preventing biofilm formation and improving the implantation process due to extracellular-matrix mimicking properties of the ELR.

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Stem cell interactions & behaviour 14:30 - 16:00 Room 0.2/0.3 12/09/2018

Oral presentation

639 Material driven fibronectin assembly and growth factor presentation to investigate metabolic mechanisms for a bone marrow niche-like pericyte phenotype

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INTRODUCTION:

Pericytes are key bone marrow niche cells, a regulatory microenvironment that is hypoxic. They have immune modulatory and inflammatory functions, and act to support haematopoietic stem cells (HSCs). In culture however, both pericytes and HSCs lose their niche phenotype. Noting that soft gels can support nestin[1] expression, a key niche marker, we aim to create a system supporting a niche-like phenotype of pericytes. Here, poly(ethyl acrylate) (PEA) is used to control fibronectin (FN) fibrillogenesis, allowing growth factor (GF) tethering[2]. Then, a soft gel or hypoxia is used to investigate metabolic mechanisms required to support niche phenotypes in bone marrow-like microenvironments.

METHODS:

Material preparation and method

PEA was spin coated on 12 mm glass coverslips. FN from human plasma was adsorbed (20 ug/mL) and then GF adsorbed BMP-2 (50 ng/mL). Pericytes, isolated from human adipose tissue, seeded at 1 x 10^3/substrate and collagen gel (2.05 mg/mL, at stiffness to match bone marrow) or 1% hypoxia added after 72h.

Metabolomics and transcriptomics

Metabolites extracted 7 and 14 days after seeding and relative abundance measured using liquid chromatography-mass spectrometry (LC-MS). Whole transcriptome profiling after 7 days. (N= 4;3).

Immunocytochemistry

Immunofluorescence used to asses phenotypic markers, LDH levels (n=3, $p<0.05$), and HIF1a levels and activity (Hypoxyprobe).

RESULTS AND DISCUSSION:

Immunocytochemistry revealed a degree of hypoxic response with addition of soft gels, similar levels of activated HIF1a were observed compared to the system in hypoxia, but not in polymer controls supporting globular FN conformation. Correspondingly, levels of glycolytic enzyme lactate dehydrogenase (LDH) showed a trend towards increased levels with gel addition, suggesting a switch to an anaerobic metabolic profile. Metabolomic analysis revealed strong agreement in down-regulation of oxidative phosporylation-related metabolites with soft gels and hypoxia. Genome-wide transcriptomic analysis identified key genes similarly expressed, such as glycolytic enzymes, and identified genes differentially expressed, such as increased expression of nestin and HIF1a after 7 days with soft gels but not hypoxia. However, downstream analysis of HIF1a-driven VEGF production was not increased with gel addition, suggesting mechanistic differences.

CONCLUSION:

Using this material-based system, we have found soft gel addition drives a 'hypoxic-like' mechanistic response, that could be key in supporting niche-like phenotypes *in vitro.* This can have large implication for production of pericytes that can be used to support, for example, tissue engineered construct implantation via enhanced immune modulatory properties.

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Stem cell interactions & behaviour 14:30 - 16:00 Room 0.2/0.3 12/09/2018

Oral presentation

500 Development of cold atmospheric plasma (CAP) functionalized membranes for the selective capture of adipose-derived mesenchymal stromal/stem cells

Tommaso Gallingani[1], Giulia Boscaini[2], Vittorio Colombo[1], Giorgio de Santis[2], Roberto di Gesù[1], Massimo Dominici[3], Maria Letizia Focarete[1], Matteo Gherardi[1], Chiara Gualandi[1], Anna Liguori[1], Giorgio Mari[3], Maria Serena Piccinno[4], Valentina Strusi[3], Elena Veronesi[3]

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INTRODUCTION:

Polyesters have been widely employed as substrates for the covalent immobilization of bioactive compounds, due to their biocompatibility and bulk properties; however, many of them do not present significant amount of functional groups and, often, a surface treatment is needed prior to the conjugation. CAP is an effective technology for the introduction of functional groups[1]. This work reports on the use of CAP to introduce carboxyl groups on polybutylene terephthalate (PBT) fibrous membranes to enable the bioconjugation of anti-CD10 antibody, which can interact with the surface marker CD10+ expressed by Mesenchymal Stromal Cells (MSCs); in this way, MSCs can be captured and later be detached and employed in tissue engineering applications. This technology represents an innovative approach to capture MSCs from the stromal vascular fraction.

METHODS:

PBT membranes were subjected to CAP functionalization performed with an air Dielectric Barrier Discharge (DBD) driven by a function generator producing square voltage signals with microsecond rise time; peak voltage, frequency and treatment time were fixed at 13.5 kV, 500 Hz and 60 s, respectively. Carboxyl groups introduced by plasma were activated with 1-etil-3 (3-dimetilaminopropil) carbodiimide/n-hydroxysuccinimide and, then 1,4 diaminobutane (DAB) linker was bonded to the mats; the fluorescent antiCD10 antibody was finally grafted on the functionalized membranes. The membranes were characterized by water contact angle (WCA) measurements, Fourier Transform Infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM); a semi-quantitative determination of the antibody conjugation was performed by fluorescence microscopy. The potential of anti-CD10 functionalized membranes to capture MSCs was investigated by incubating the membranes with MSCs at 37°C using an orbital shaker.

RESULTS AND DISCUSSION:

WCA measurements revealed an increase of membranes' wettability after plasma exposure, due to the formation of polar groups. SEM analysis did not highlight any difference between plasma treated and pristine samples, underlining that plasma does not induce morphological damage. With respect to conjugated membranes, FTIR analysis confirmed the presence of the diaminic linker, while fluorescence microscopy revealed a homogenous distribution of the antibody. The biological assay demonstrated that anti-CD10 conjugated membranes doubled the MSCs capture with respect to the unconjugated membranes and did not result in any morphological alteration of the MSCs after their detachment.

CONCLUSION:

The work highlights the possibility to employ CAP assisted processes for the fabrication of anti-CD10 conjugated membranes suitable for the selective capture of MSCs from the stromal vascular fraction.

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Stem cell interactions & behaviour 14:30 - 16:00 Room 0.2/0.3 12/09/2018

Oral presentation

55 Effect of ECM type and elasticity of iPS culture substrates on cardiac differentiation: cardiac marker expression and self-beating induction

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INTRODUCTION:

Stem cell-based myocardial regeneration therapies have emerged as alternative strategies to heart transplantation for serious heart diseases. The effect of culture substrates on the cardiac differentiation of stem cells has been studying mainly with cardiac marker gene expression, but it does not correlate with the cell beating function.ツ Our challenge here is to divide the cardiac differentiation of induced pluripotent stem cells (iPSs) into three steps: (1) cardiac marker gene expression, (2) contractile gene expression, (3) self-beating, and (4) beating duration, and evaluate independently. However, each step is affected by the efficiency of previous steps, and it is difficult to evaluate them.ツ We therefore used neonatal rat cardiomyocytes (NCMs) which are already expressed cardiac phenotype to evaluated the latter steps, in addition to iPS cells.

METHODS:

These cells were cultured on substrates with different natures, hydrogel-based elastic substrate (Es) with the modulus of 9, 20, or 180 kPa, and hard tissue culture polystyrene dishes (TCPS) with 2GPa.ツ These substrates were immobilized with various proteins: collagen (Col), gelatin (Gel), fibronectin (FN), poly(L-lysine) (PLL) and used as cell culture substrates.

RESULTS AND DISCUSSION:

The effective niche for each step was very different. The cardiac marker gene (GATA4, Tbx5, MEF2C) level of iPSs was very high on the TCPS coated with FN or Gel, whereas FN-coated Es with 9 kPa modulus Nanog (undifferentiated marker gene) expression was maintained at a high level. In contrast, the contractile genes (ホア-MHC, TnC1, and TnT2) levels and the self-beating of the NCMs were very high on FN-coated TCPS and Col-coated Es. The beating duration of the NCMs was effective on Es substrates.ツ Overall, cardiac differentiation ‘preferred’ ECM-rigid culture substrates, and beating-behavior ‘preferred’ Col-soft culture substrates.

CONCLUSION:

These results clarified that single parameter of substrates cannot be the general determinant for effective stem cell differentiation, and their combination is very important.ツ In addition, we have to clarify the adequate stem cell niche for each differentiation steps independently.

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Stem cell interactions & behaviour 14:30 - 16:00 Room 0.2/0.3 12/09/2018

Oral presentation

433 Differentiation of adipose tissue-derived stem cells into smooth muscle cells by chemical and mechanical stimuli for tissue engineering of heart valves

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INTRODUCTION:

Recellularized porcine pericardium or vessels with autologous cells, e.g. adipose –tissue derived stem cells (ASCs) could improve function of bioartificial prostheses[1]. Pure fibrin, fibrin layers with bound FGF2 and/or VEGF coatings, and analysed *in vitro* with ASCs under static or dynamic culture conditions.

METHODS:

Pure fibrin and fibrin with attached FGF2 and/or VEGF was coated onto glass coverslips or on decellularized porcine pericardium and seeded with adipose tissue – derived stem cells (ASCs). The cells were seeded either onto fibrin assembly or embedded in fibrin gel and cultured in standard cell culture medium (DMEM supplemented with FCS and FGF2) or in a differentiation cell culture medium for smooth muscle cells (supplemented with TGFβ1 and BMP4) under static culture or in a bioreactor under pulse pressure loading of 110-130 Torr/70-90 Torr, 1 Hz for 7-21 days. Cell growth, differentiation or cell migration into pericardium was assessed by immunocytochemical (for alpha-actin, calponin, type I collagen), histological (hematoxylin eosin and elastica staining, AFOG) and immunohistological staining (for desmin, vimentin, and alpha-actin).

RESULTS AND DISCUSSION:

Differentiation of ASCs into smooth muscle cells was supported by both cell culture medium and dynamic loading conditions, the most effective is their simultaneous action. Fibrin with attached FGF2 stimulated predominantly migration of ASCs into pericardium tissue, on the other hand, VEGF seemed to hamper it. ASCs proliferation was strongly stimulated by dynamic loading, in addition, the cells produced calponin and type I collagen, which is necessary for improving mechanical properties of new tissue-engineered heart valve.

CONCLUSION:

Effective recellularization of decellularized pericardium with ASCs could be supported by their coating with bioactive fibrin layers or by dynamic pressure loading during cell culture. This allows pointed ASCs differentiation into smooth muscle cell and the production of new extracellular matrix by these cells. The newly-formed tissue will resemble physiological heart valve.

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Stem cell interactions & behaviour 14:30 - 16:00 Room 0.2/0.3 12/09/2018

Oral presentation

93 Influence of microenvironmental cues on maintaining the phenotype of tenocytes

Dimitrios Tsiapalis[1], Andrea De Pieri[2], Ignacio Sallent[1], Dimitrios Zeugolis[1]
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INTRODUCTION:

Cellular therapies play an important role in tendon tissue engineering with tenocytes being described as the most prominent cell population if available in large numbers. However, *in vitro* expansion of tenocytes in standard culture leads to phenotypic drift and cellular senescence[1]. Maintenance of tenogenic phenotype *in vitro* can be achieved by recapitulating different aspects of the native tendon microenvironment. One approach to modulate *in vitro* microenvironment and enhance extracellular matrix (ECM) deposition is macromolecular crowding (MMC). MMC is based on the addition of inert macromolecules to the culture media to mimic the dense ECM[2]. In addition, as tendon has been described to be a relatively avascular and hypoxic tissue and low oxygen tension can stimulate collagen synthesis and cross-linking through the activation of HIF1-α, we venture to assess the effect of MMC and low oxygen tension on human tenocyte phenotype maintenance by enhancing synthesis and deposition of tissue-specific ECM.

METHODS:

Human tendons were kindly provided from University Hospital Galway. Tenocytes were extracted using the migration method. Experiments were conducted at passage 3. Optimization of MMC conditions was assessed using 50 to 500 *m*g/ml carrageenan. For variable oxygen tension cultures, tenocytes were incubated in a Coy Lab hypoxia chamber. ECM synthesis and deposition were assessed using SDS-PAGE and immunocytochemistry analysis. Protein analysis for Scleraxis was performed using western blot. Gene analysis was conducted using a gene array. Experiments were performed at least in triplicate. MINITAB was used for statistical analysis.

RESULTS AND DISCUSSION:

SDS-PAGE and immunocytochemistry analysis demonstrated that human tenocytes treated with MMC at 2 % oxygen tension showed increased synthesis and deposition of collagen type I, which is the major component of tendon ECM. Moreover, immunocytochemistry for collagen type III, V, VI and fibronectin illustrated enhanced deposition when cells were treated with MMC at 2 % oxygen tension. In addition, western blot analysis revealed increased expression of tendon-specific protein Scleraxis, while a detailed gene analysis illustrated upregulation of tendon-specific genes and downregulation of transidifferentiation genes when cells cultured with MMC under hypoxic conditions. Finally, it was shown that that low oxygen tension and MMC metabolic activity, proliferation and viability of human tenocytes.

CONCLUSION:

Collectively, results suggest that the synergistic effect of MMC and low oxygen tension can accelerate the formation of ECM-rich substitutes, which stimulates tenogenic phenotype maintenance.

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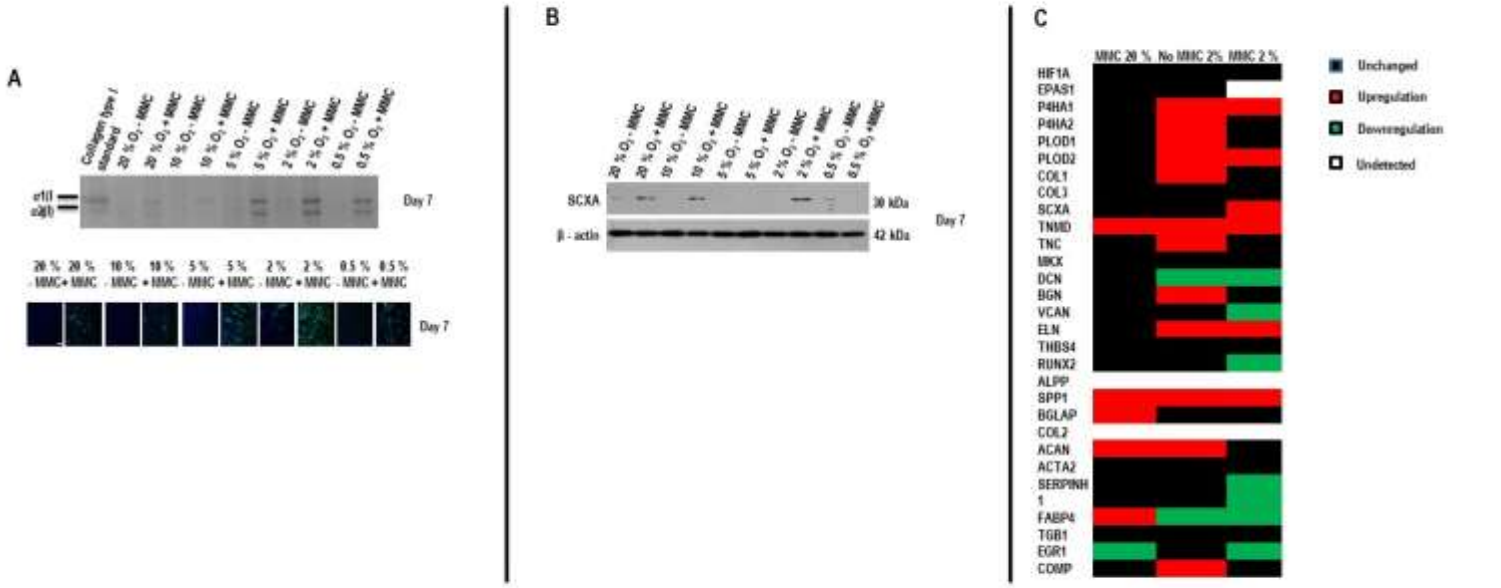
ACKNOWLEDGMENTS:

Tendon Therapy Train (H2020-MSCA-ITN-2015-ETN) and Science Foundation Ireland

Picture 1:



A. SDS-PAGE and subsequent immunostaining analysis for collagen type I of human tenocytes cultured under different oxygen tensions in the absence (- MMC) and presence of carrageenan (+ MMC) after 7 days.

B. Western blot for Scleraxis of human tenocytes cultured under different oxygen tensions in the absence (- MMC) and presence of carrageenan (+ MMC) after 7 days.

C. Gene analysis of human tenocytes cultured under different oxygen tensions in the absence (- MMC) and presence of carrageenan (+ MMC) after 7 days.

Stem cell interactions & behaviour 14:30 - 16:00 Room 0.2/0.3 12/09/2018

Oral presentation

517 Zinc maintain ESC stemness through Zip7 activation via Akt pathway

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[2]Division of Biomedical Engineering, School of Engineering, United Kingdom
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INTRODUCTION:

Embryonic stem cells (ESC) are a versatile tool for tissue engineering due to their capability for differentiation to all embryonic lineages. Nevertheless, approaches to prolong the *in vitro* lifespan of ESC while still maintaining their multipotency are a challenge that involve the use of multiple factors such as feeder layer cells, cytokines (LIF) and/or growth factors (Fgf2)[1], that exert its effects via PI3K/Akt pathway[2]. In this work, we propose Zinc (Zn) as an alternative of growth factors for promotion of ESC stemness via PI3K/Akt cascade through activation of Zn transporter Zip7[3].

METHODS:

$ZnCl_2$ was used as a supplement in media. ESC pluripotency was evaluated by immunofluorescence (Oct4, Sox2) and alkaline phosphatase staining (AP). Expression of pluripotency markers (Oct4, Nanog, Klf4) and differentiation lineage-specific markers (Foxa2, Brachyury/T, Sox1) were evaluated by qPCR. Embryoid body (EB) assays were

used for determination of ectoderm, mesoderm and endoderm formation after 15 days by histological analysis. Role of Zip7/PI3k/Akt in ESC stemness were evaluated by Zip7 silencing and PI3k/Akt inhibition with LY-29004.

RESULTS AND DISCUSSION:

Results show that addition of 40, 100 or 140 μM Zn induced ESC self-renewal capacity at similar levels to stemness-induced positive control condition (LIF-supplemented). Silencing of Zip7 transporter strongly reduced pluripotency marker levels only in ESC Zn-supplemented while no effect was observed in LIF or basal media conditions. Likewise, inhibition of Akt activity resulted in a strong reduction of pluripotency markers only in Zn and basal conditions while LIF condition remained unaltered. Our data suggest that Zn-induced stemness maintenance is acting through Zip7 activation via Akt, and LIF-induced stemness is acting via different pathway. We further evaluated ESC pluripotency culturing cells 30 days with Zn supplemented media and then stimulated to commitment during 15 days to ectoderm, mesoderm and endoderm from EB. Results showed that Zn-treated EB were capable of develop ectoderm, mesoderm and endoderm structures similar to those formed after LIF-treated ESCs.

CONCLUSION:

In this work, we report that zinc is able to direct stemness maintenance of ESC acting through Zip7 activation and via PI3k/Akt cascade. Moreover, ESCs cultured for 30 days in Zn presence maintain their pluripotency. We propose zinc as an alternative to the use of growth factors.

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ACKNOWLEDGMENTS:

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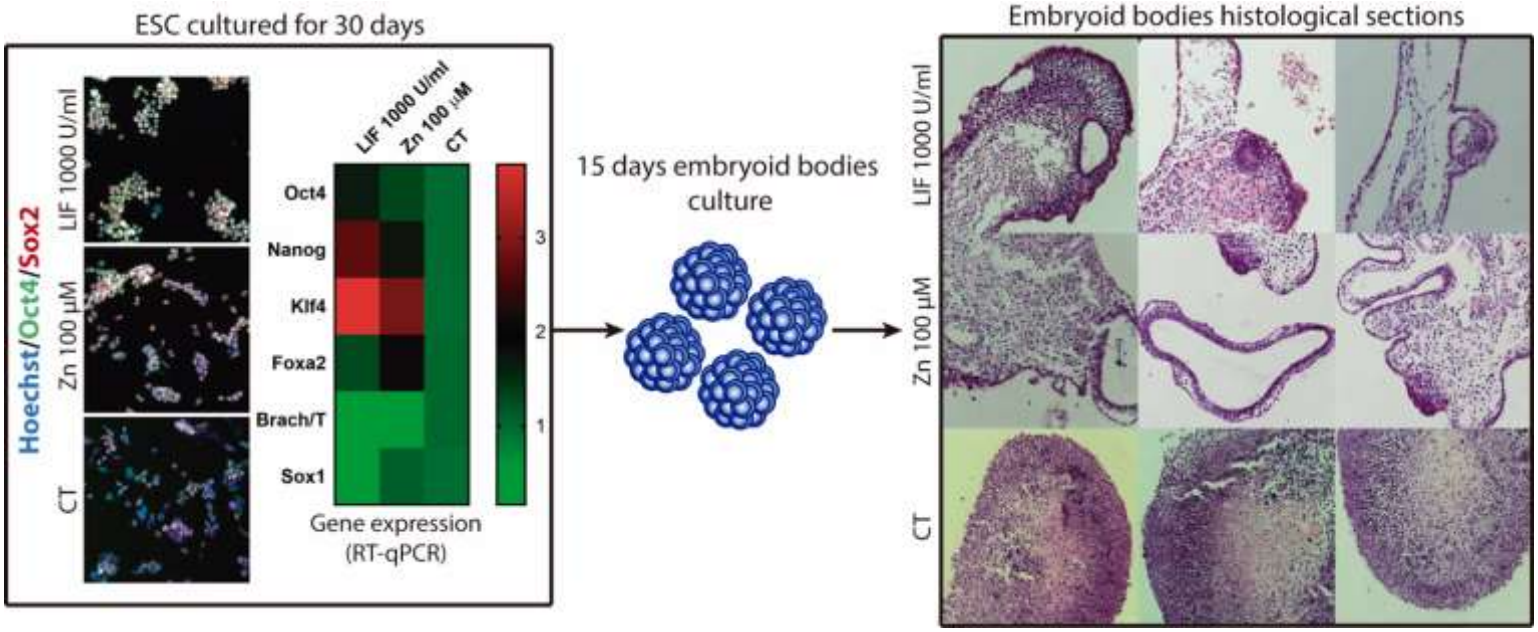

Picture 1: Caption 1: ESC cultured for 30 days with Zn express higher pluripotency markers than basal conditions and maintain pluripotency after embryoid body assay.

Bioinspired developments in biomaterial design 16:30 - 18:00 Room 0.2/0.3 12/09/2018

Oral presentation

529 Effect of surface modification of nanofibers with glutamic acid and aspartic acid peptide on osteogenic differentiation of human mesenchymal stem cells

Ozan Karaman[1], Günnur Onak[2], Mustafa Sen[2], Utku Kürsat Ercan[2], Nesrin Horzum[2], Bora Garipcan[3]
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INTRODUCTION:

Glutamic and aspartic acid templated peptides are known to have a more potent enhancer role for bone formation compared to other aa and highly expressed in non-collagenous proteins involved in bone mineralization such as bone sialoprotein (BSP). Although there have been studies that show the individual effect of GLU and ASP sequences on mineralization and osteogenic differentiation when those used to modify the surface of synthetic nanofibers (NF), to best of our knowledge comparison the effect of GLU and ASP on human mesenchymal stem cells (hMSCs) proliferation and osteogenic differentiation has not been studied. The objective of this study was to investigate the effect of nanofibres surface-modified with GLU and ASP on calcium phosphate nucleation and osteogenic differentiation of human mesenchymal stem cells.

METHODS:

Glutamic and Aspartic Acid sequences were synthesized manually on MBHA resin as previously described [1]. Cold atmospheric plasma was applied on nanofibers until maximum COOH groups are exposed by determining parameter roughly by water contact angle measurements. Peptide sequences were conjugated on nanofibers by using EDC/NHS chemistry. MSCs seeded on GLU-NF and ASP-NF microsheets and incubated in osteogenic medium. Ca Assay, ALP Assay and DNA quantification assay were evaluated on GLU-NF, ASP-NF and NF groups by 7d,14d, 21d and 28d to compare the effect on calcium phosphate nucleation and osteogenic differentiation of human mesenchymal stem cells.

RESULTS AND DISCUSSION:

The calcium and DNA content of all groups increased slightly with incubation time. The calcium and DNA amounts of ASP-NF groups at each time points were higher than other groups. However, the ALP activity of all other groups incubated in osteogenic medium peaked after 14 days and returned to baseline level at day 28. The ALP activity was maximum on GLU-NF groups compared to other groups. Calcium, DNA and ALP assay results indicated that glutamic acid sequence is more effective than aspartic acid sequences. For further studies, mRNA amount of MSCs on Peptide-NF microsheets will be analyzed by using real time-PCR amplification with appropriate gene-specific primers such as collagen type I, ALP, osteopontin and osteocalcin.

CONCLUSION:

The outcomes of this study would help to enhance biomaterial's function with most effective sequence on human MSCs on osteogenic differentiation for further modifications of the synthetic scaffolds for bone tissue engineering.

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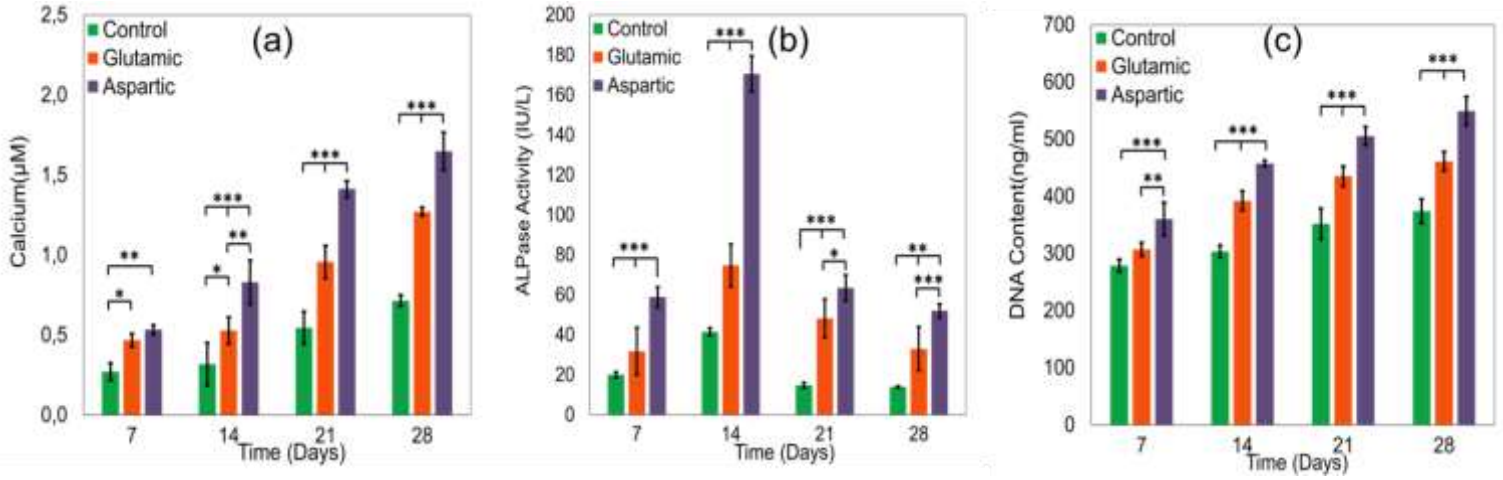
ACKNOWLEDGMENTS:

The study funded Scientific Research Projects Fund of Izmir Katip Çelebi University through the Research Projects 2016-ÖNP-MÜMF-0022.



Picture 1: Caption 1: Calcium content, ALPase activity and DNA content of Control (neat nanofibers), Glutamic acid and Aspartic acid templated peptide modified nano fibers

Bioinspired developments in biomaterial design 16:30 - 18:00 Room 0.2/0.3 12/09/2018

Oral presentation

624 Three-dimensional matrices of enamel like oriented calcium phosphate nanocrystals

Francesca Carella[1], Michele Iafisco[2], Alessio Adamiano[2], Lorenzo degli Esposti[2], Davide Barreca[3], Gianmario Martra[4]
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INTRODUCTION:

Understanding how living organisms form their extremely specialized mineralized structures, and identifying the processes controlling the final crystal size, shape and polymorphism, which in turn determine their unique mechanical and biological properties, is highly relevant. The hydroxyapatite (HA) biomineralization is a complicated process that arises on the interaction between ions and organic molecules.[1] The study of HA formation is crucial not only to understanding how living organisms form the mineralized structures of bones and teeth, but also to achieve synthetic advanced biomimetic materials for application in the fields of regenerative medicine and bone tissue engineering.[2] In particular, the preparation of three-dimensional materials mimicking the enamel tissue, which is formed by highly oriented nanocrystals arranged along the *c*-axis is a scientific challenge.

METHODS:

Amorphous calcium phosphate nano-powders were synthetized in presence and in absence of citrate. Powders were pressed to form discs with of 1 cm in diameter and then put in a growing solution containing calcium, phosphate and fluoride ions for different times. At scheduled times discs were removed from the solution, washed with ethanol and dried in air. Samples were characterized by SEM, XRD, EDS, FT-IR and XPS to evaluate their chemical-physical and morphological features.

RESULTS AND DISCUSSION:

Chemical-physical and morphological evaluations demonstrate the formation of highly oriented along the c-axis HA nano-needles (Figure 1) whose composition, size and morphology depend on the characteristics of substrate and growing solution. The analyses at different crystallization times, revealed that the crystallization pathway occurs through a formation of an intermediate layer consisting of nano-crystalline HA formed by transformation of the amorphous phase. After that, precipitation of highly oriented HA nano-needles on this intermediate layer takes place. It was also found that the ions concentration of the growing solution plays a fundamental role, in fact, different morphologies ranging from needles to lamellae were observed.

CONCLUSION:

Three-dimensional matrices of enamel like oriented HA nanocrystals were synthesized employing a simple and effective procedure involving the use of amorphous nanoparticles. Interestingly, these highly organized structures were obtained without the use of organic molecules that up to now are recognized as fundamental in controlling final crystal shape of mineralized tissue. These results provide new insights not only in the field of material science but also to better comprehend the enamel biomineralization process, where the role of amorphous phase might be wider than has been thought to date.

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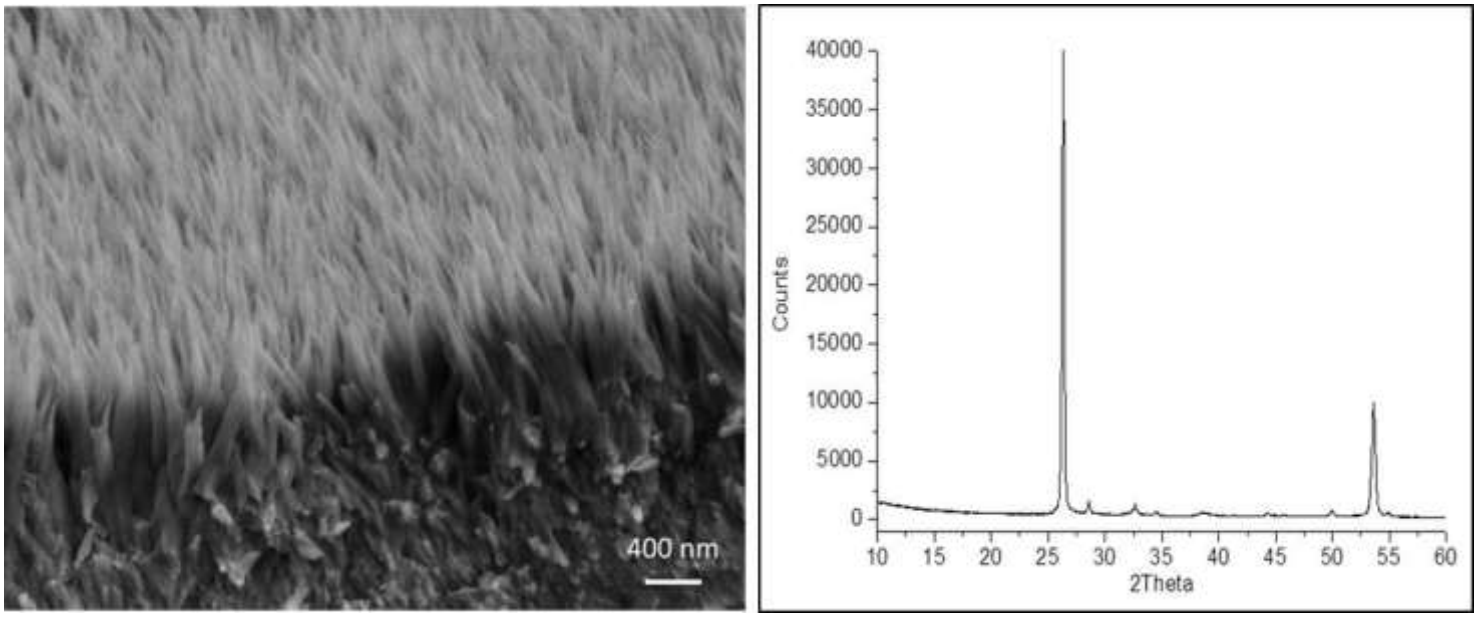

Picture 1: Caption 1: SEM micrograph (left panel) and XRD pattern (right panel) of HA nano-needles grew on amorphous calcium posphate substrate

Bioinspired developments in biomaterial design 16:30 - 18:00 Room 0.2/0.3 12/09/2018

Oral presentation

316 Decellularized matrices as biomimetic platforms to unravel the role of CD44v6 in gastric cancer

Bianca n. Lourenço[1], Nora Springer[2], Daniel Ferreira[1], Carla Oliveira[3], Pedro I. Granja[1], Claudia Fischbach[2], Mariana I Neves[4]
[1]I3S/ INEB, Porto, Portugal
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[4]I3S - Instituto de Investigação e Inovação em Saúde, Porto, Portugal

INTRODUCTION:

Cancer research has been driven by the need for biomimetic culture models that reliably recapitulate the extracellular matrix (ECM) of the tumor microenvironment. Decellularized matrices are increasingly utilized to elucidate the effect of cell-matrix interactions on fundamental aspects of tumor development, and progression[1]. We have utilized this approach to uncover the role of hyaluronic acid receptor CD44 variant 6 (CD44v6) in the context of gastric cancer (GC). More specifically, we have tested whether CD44v6, that is expressed *de novo* in 62.8% of GCs[2], promotes GC aggressiveness by modulating stromal cell behavior and whether the resulting effects on ECM remodeling play a role in this process.

METHODS:

To evaluate the effects of CD44v6 in GC, we developed an isogenic human GC cell line overexpressing CD44v6 and utilized its parental cells lacking endogenous CD44 expression as control. Conditioned media collected from these cell lines was applied to human adipose-derived stromal cells (ASCs) to test their differentiation into myofibroblasts, the primary cell type mediating ECM remodeling in the tumor microenvironment. GC cells were seeded on ASC-mediated decellularized ECMs to evaluate their behavior. *In vitro* results were validated by implanting the different GC cells into the subcutaneous space of SCID mice in the presence/absence of ASCs.

RESULTS AND DISCUSSION:

Our results indicate that soluble factors secreted by GC cells overexpressing CD44v6 increase proliferation and expression of myofibroblastic marker alpha-smooth muscle actin in ASCs relative to control conditions. This altered phenotype enhanced fibrotic/desmoplastic ECM deposition and remodeling as indicated by fibronectin immunofluorescence staining and Western Blotting of ASC-deposited ECMs. Importantly, decellularized CD44v6-associated ECMs stimulated the malignant behavior of GC cells, but this effect was decreased when GC cells were treated with a matrix-metalloproteinase inhibitor. Results confirmed that ASC-mediated changes in ECM remodeling contributed to altered tumor cell behavior. Finally, mouse studies supported the pathological relevance of our *in vitro* findings. CD44v6-derived tumors were larger and exhibited increased desmoplasia relative to control tumors.

CONCLUSION:

Our findings reveal that CD44v6 overexpression by GC cells is functionally linked to desmoplastic stroma remodeling and consequential changes in malignancy, opening new insights into potential CD44v6-targeted therapies.

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ACKNOWLEDGMENTS:

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Bioinspired developments in biomaterial design 16:30 - 18:00 Room 0.2/0.3 12/09/2018

Oral presentation

215 Modulation of microstructural features of calcium phosphates for triggering specific osteoimmune response

Joanna Maria Sadowska[1], Fei Wei[2], Jia Guo[3], Jordi Guillem-Marti[1], Maria-Pau Ginebra[1], Yin Xiao[2]
[1]Universitat Politècnica de Catalunya (UPC), Barcelona, Spain
[2]Queensland University of Technology (QUT), Australia
[3]Guanghua School of Stomatology, Hospital of Stomatology, China

INTRODUCTION:

The immune system actively participates in bone healing. Biomaterials interfere in this process, although the precise role of their specific properties is unknown. In the present work, we demonstrate that tuning some microstructural features of biomimetic CDHA allows altering the immune cell response, which subsequently affects the osteogenic differentiation.

METHODS:

CDHA discs with different microstructures and porosities were prepared varying L/P ratio (0.35 $mL \cdot g^{-1}$ or 0.65 $mL \cdot g^{-1}$) and particle size of starting α- tricalcium phosphate powder (coarse C or fine F). Detailed material characterisation was previously described[2]. Macrophages (RAW 264.7) were incubated for 12h with CDHA and evaluated in terms of immune response (RT-qPCR: pro-inflammatory cytokines IL-1β, IL-6, iNOS, TNFα and anti-inflammatory cytokines IL-10 and CD206; CLSM: iNOS visualisation), osteogenic activity (RT-qPCR: osteogenic factors OSM, TGFβ, VEGF) and osteoclastogenic activity (RT-qPCR: osteoclastogenic CTSK, CAR2, MMP9; SEM: visualisation of material- cell interphase, CLSM: visualisation of multinucleated cells; ICP-OES: Ca^{2+} and P_i of cell culture medium in presence/ absence of RAW). Osteoblastic cells (SaOS-2) were incubated with RAW- CDHA extracts to determine the effect of immune environment on osteogenic differentiation (RT-qPCR: Runx2, ALP, BSP, COLL I, OCN, BMP-2; Western blot: Runx2, ALP, COLL I; CLSM: ALP visualisation; ARS: Ca deposits).

RESULTS AND DISCUSSION:

Different immune response was observed depending of the topography of the substrate. Fine CDHA caused a more pronounced expression of pro-inflammatory cytokines compared to coarse CDHA (Figure 1). The RAW 264.7 showed osteoclastic activity through fusion to multinucleated cells and degradation of crystals of both C and F CDHA. Nonetheless, incubation with F CDHA led to downregulation of osteoclastic molecules (CTSK and CAR2) whereas the opposite behaviour was observed on C CDHA discs. Finally, the incubation of osteoblastic cells with RAW- CDHA extracts impacted to different extents the osteogenic differentiation, being more pronounced after stimulation with RAW- fine CDHA conditioned medium. Overall, coarse CDHA stimulated osteoclastogenesis whilst fine CDHA led to more favourable environment for osteogenesis.

CONCLUSION:

The immune response of macrophages, and its impact on osteoclastogenesis and osteogenesis, can be modulated through the microstructural features of CDHA.

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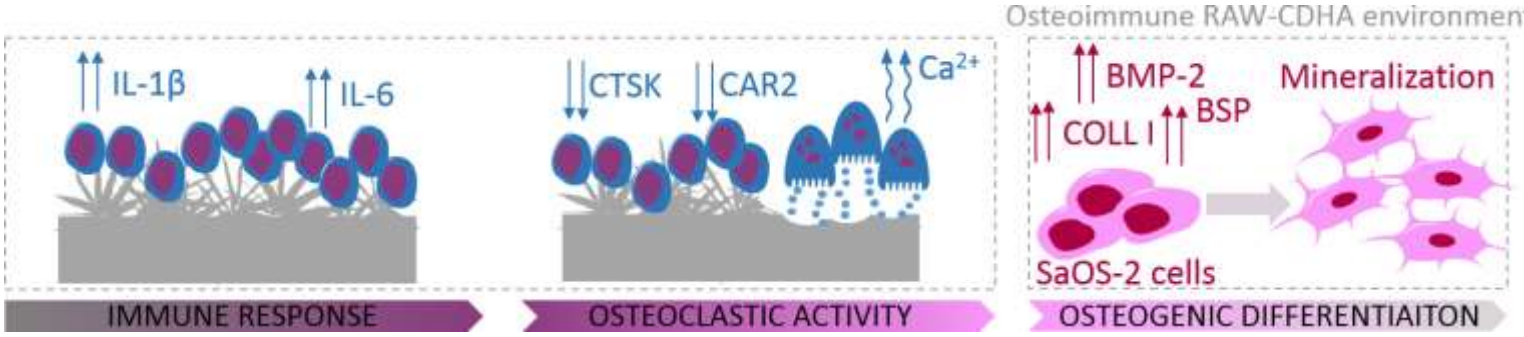
ACKNOWLEDGMENTS:

Spanish government (MAT2015-65601-R) co-funded by the EU through European Regional Development Funds.



Picture 1: Caption 1: Fig 1 Left: Scheme showing RAW 264.7 response to F CDHA substrates. Right: The effect of RAW- F CDHA environment on SaOS-2 osteogenic differentiation

Bioinspired developments in biomaterial design 16:30 - 18:00 Room 0.2/0.3 12/09/2018

Oral presentation

78 Material-driven fibronectin nanonetworks rescue collagen IV secretion in mutant cells

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[1]School of Engineering, University of Glasgow, Glasgow, United Kingdom
[2]Woodruff School of Mechanical Engineering, Georgia Institute of Technology, United States of America
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INTRODUCTION:

Mutations in the extracellular matrix (ECM) protein collagen IV (colIV) have been implicated in a broad spectrum of vascular diseases, including intracerebral haemorrhaging. Indeed, col IV is a major component of the specialised ECM structures that underlie endothelial and epithelial cells – the basement membrane (BM) – providing them with structural support and signalling cues. Mutations in the genes COL4A1/COL4A2 cause defects in the BM, via incorporation of mutant protein or its absence due to retention and accumulation in the endoplasmic reticulum (ER stress).

In this study, we aim to provide novel insights into the mechanisms of colIV diseases by investigating the behaviour of cells with a COL4A2 mutation, *COL4A2+/G702D* mutant fibroblasts,[1] using defined engineered microenvironments composed of synthetic polymers – poly(methyl acrylate), PMA, and poly(ethyl acrylate), PEA – which are able to organise the assembly of ECM proteins – laminin (LM), colIV and fibronectin (FN) – upon simple adsorption.[2]

METHODS:

Thin films of the polymers were prepared by spin coating. Distribution, conformation and amount of adsorbed ECM proteins were evaluated by AFM, BCA and immunofluorescence. Wild-type and mutant fibroblasts cultured onto the surfaces were analysed in terms of early adhesion and matrix secretion. The mechanical properties of secreted ECMs were measured using AFM.

RESULTS AND DISCUSSION:

On PEA, FN was adsorbed in the form of interconnected nanofibrils; these nanonetworks were found to induce increased secretion of colIV by mutant cells compared to PMA, where adsorbed FN maintained a globular conformation. Increased levels of molecular chaperones and reduced ER area suggested an increased colIV folding

capacity on PEA. Enhanced formation of focal adhesions was also seen on FN-coated polymers, where ligand density and actin-myosin contractility accounted for the observed increase in cell adhesion strength. Interestingly, the protein matrix deposited by mutant cells cultured on the FN nanonetworks showed a significantly higher Young modulus (~7.5 to 18 kPa) than on PMA (~4.3 to 11.5 kPa) (Figure).

CONCLUSION:

These findings provide a basis of concept that materials, through changes in the interfacial layer of adsorbed proteins, may be employed to modulate effects of collV mutations. Understanding the mechanisms through which these surfaces rescue collV secretion of mutant cells will prove valuable for the development of new therapeutic approaches to address pathologies due to these mutations.

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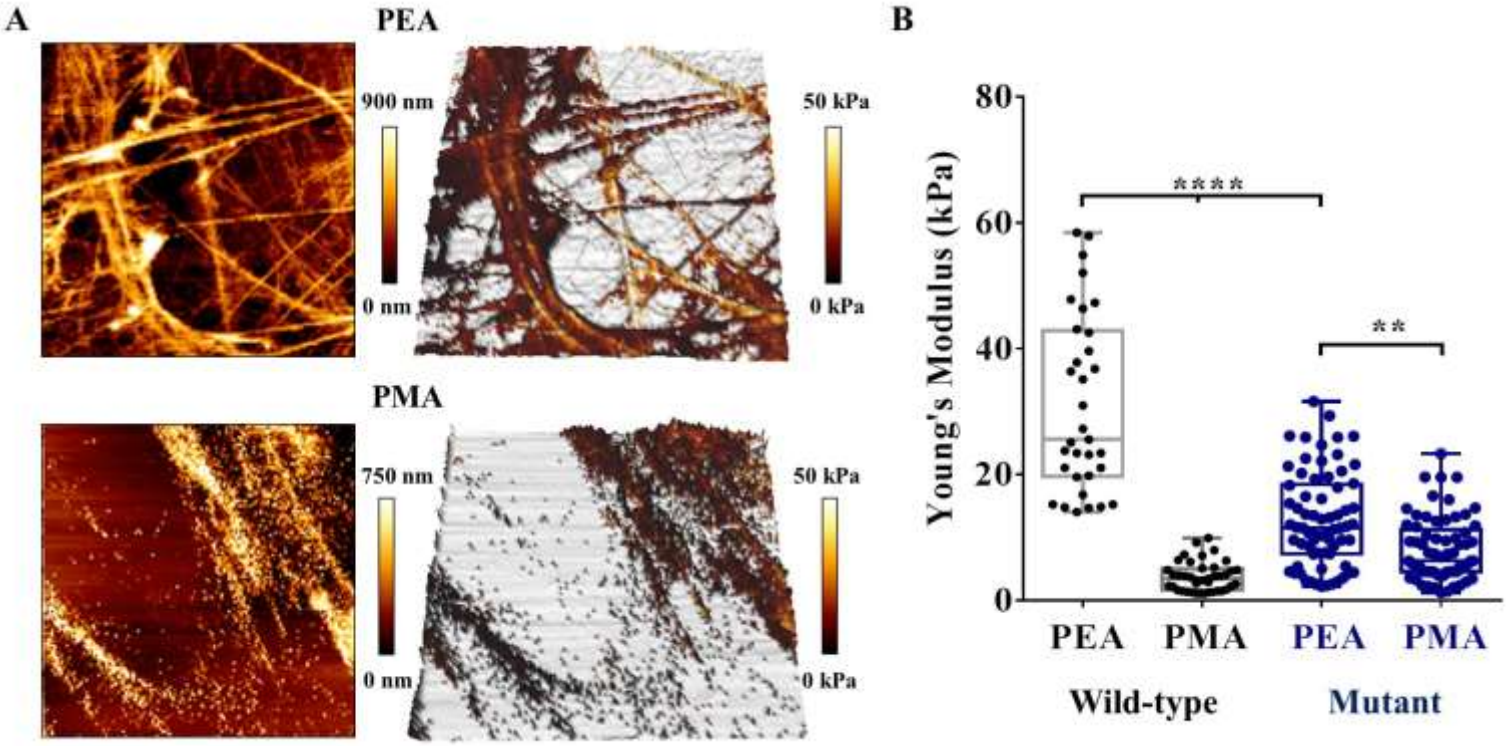

Picture 1: Caption 1: (A) Height and Young modulus images of ECM secreted by mutant fibroblasts, measured via AFM; (B) quantification of the Young moduli of the ECM fibres.

Bioinspired developments in biomaterial design 16:30 - 18:00 Room 0.2/0.3 12/09/2018

Oral presentation

417 Collagen I based 3D networks as a tunable in vitro matrix for human mesenchymal stromal cells

Sarah Vogel[1], Franziska Ullm[2], Tilo Pompe[2], Ute Hempel[1]
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[2]Institute of Biochemistry, Faculty of Life Science, Leipzig University, Germany

INTRODUCTION:

Mimicking the natural *e*xtracellular environment is a promising strategy to discover new osseointegrative biomaterials. 3D matrices [1] composed of fibrillar collagen I (Col) with varying stiffness adjusted by intrafibrillar crosslinking were used to study the behaviour of human mesenchymal stromal cells (hBMSC) during osteogenic differentiation. By that we questioned how the 3D environment affects cellular viability, morphology, invasion and osteogenesis as well as ECM remodelling.

METHODS:

Defined fibrillar Col matrices were engineered with covalent attachment on polymer-functionalized cover slips [1]. The resulting 3D matrix layers exhibit a thickness of 400-500 µm and an elastic modulus of ~120 Pa. Intrafibrillar crosslinking, performed after fibrillogenesis with 20 mM EDC, increased network stiffness to ~190 Pa, whereas network topology (pore size ~3.5 µm, fibril diameter ~0.7 µm) remained constant.

hBMSC were seeded onto the scaffolds (7.000 cells/cm²) and cultured up to 22 days in DMEM with osteogenic supplements [2,3]. Cell morphology and ECM remodelling parameters were detected by immunofluorescence, Western blotting and ELISA. Remaining thickness of matrix layers and cellular invasion were analysed using confocal laser scanning microscopy [1]. Tissue non-specific alkaline phosphatase (TNAP) activity and CaP-deposition were quantified from cell lysates to characterize osteogenic differentiation [2,3]. After 22 days, decellularized substrates were analysed for stiffness changes by colloidal probe force spectroscopy [1].

RESULTS AND DISCUSSION:

hBMSC adhered on and invaded in both matrices independent on crosslinking. A well spread morphology with elongated filopodia and prominent actin stress fibres could be observed (Fig. 1). Invasion depth and number of invasive cells increased over time.

ECM remodelling was observed including secretion and incorporation of ECM components (e.g. fibronectin) and proteolytic degradation (e.g. MMP-2) of non-crosslinked Col networks over time.

hBMSC committed osteogenic lineage independent on crosslinking and mineralized the scaffolds resulting in an increased substrate stiffness.

From these results it is concluded that important *in vivo* processes of hBMSC during bone repair could be modelled *in vitro* within engineered 3D Col matrices.

CONCLUSION:

3D Col matrices are a tunable biomaterials system that enables elucidating matrix remodelling during osteogenesis of hBMSC *in vitro*.

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ACKNOWLEDGMENTS:

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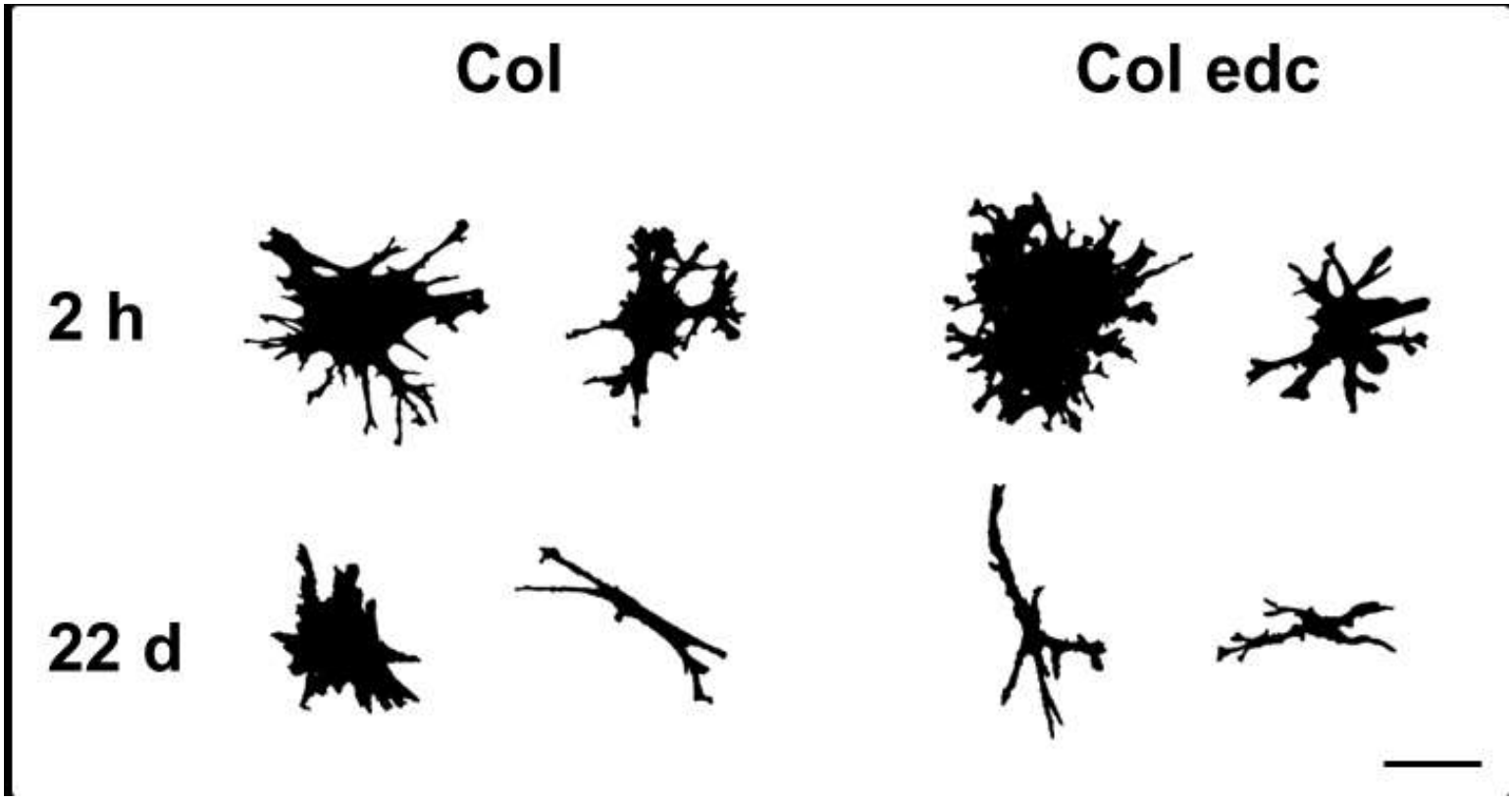

Picture 1: Caption 1: Fig. 1: Morphology of hBMSC in Col and Col edc after 2 h and 22 days. Representative phalloidin-stained cells were analysed using segmentation plugin

Scientific Programme abstracts Thursday

Plenary session 08.45 - 09.30 Auditorium I 13/09/2018

Oral presentation

Carlijn Bouten
Technical University of Eindhoven

Can we grow valves inside the heart?

Perspectives on material-based in situ heart valve tissue engineering.

We investigate and design *in situ* heart valve tissue engineering technologies using instructive, cell-free, biodegradable scaffolds as an approach to create living valves inside the human heart. This lecture addresses the challenges to develop scaffolds that i) function upon implantation and with time of tissue formation and scaffold degradation, ii) are capable of harnessing the natural host response, and iii) provide the necessary cues for a stable and organized load-bearing extracellular matrix in vivo. I will address how biomimetic *in vitro* models and computational analyses are used in direct comparison with preclinical studies to optimize scaffold biochemical, biophysical, and degradation properties. The resulting scaffolds have demonstrated sustained mechanical and biological functionality during long-term orthotopic (12 month FU) and transcatheter (6 month FU) implantations as pulmonary valve in large animals. These results offer new perspectives for endogenous heart valve replacement starting from readily-available synthetic grafts.

Klaas de Groot Award 2018 09.30 – 10.15 Auditorium I 13/09/2018

Oral presentation

Lucy Di Silvio

Engineering orthopedic tissues 10:45 - 12:15 Auditorium I 13/09/2018

Oral presentation

Posterior spinal fusion with a microporous ceramic: a randomized, intra-patient controlled trial

Moyo Kruyt

Engineering orthopedic tissues 10:45 - 12:15 Auditorium I 13/09/2018

Oral presentation

716 Identification and in vitro screening of osteogenic metabolites through supplement-free nanovibration-driven mesenchymal stem cell differentiation

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INTRODUCTION:

Bone is one of the most commonly transplanted tissues but obtaining suitable autologous grafts is difficult and associated with donor site morbidity and pain. There is a need for effective tissue engineered approaches to produce bone. In the laboratory, these approaches typically involve osteogenic differentiation of mesenchymal stem cells (MSCs) through media supplementation. We recently developed a supplement-free osteogenic differentiation protocol through nanovibrational-stimulation of MSCs[1]; the 'nanokick' bioreactor. Here, we hypothesised that nanovibrational differentiation of MSCs would allow metabolomic analysis of differentiation without confounding exogenous media supplements. We aimed to investigate MSC nanovibration-driven osteogenesis in 2D and 3D cultures, identify important osteogenic metabolites and investigate their osteogenic potential by supplementing these pathways *in vitro*.

METHODS:

Human MSCs were cultured in well plates (2D) or type I collagen gels (3D) over 28 days in three groups- nanovibrational stimulation, osteogenic media (dexamethasone) and MSC expansion media. Differentiation was tracked through gene expression (qPCR), protein expression (western blot and immunofluorescent staining) and extracellular matrix (ECM) production (histology). At key points, cell metabolites were extracted and metabolomic analysis performed (LC-MS; ZIC-pHILIC) to identify important metabolites. After selecting a promising metabolite, this was synthesised with several other structurally similar molecules. The osteogenic potential of these synthetic metabolites was investigated following supplementation to 2D and 3D MSC cultures.

RESULTS AND DISCUSSION:

Nanovibration upregulated key osteogenic genes and proteins in 2D and 3D cultures comparably to osteogenic media and consistent with progressive osteogenic differentiation, including early upregulation of RUNX2 (2D x14.5, $p<0.05$; 3D x11.5, $p<0.05$) followed by maturation marker osteopontin (2D x19, $p<0.05$; 3D x7.2, $p<0.05$). Interesting differences were observed in 2D and 3D processes, particularly in ECM associated-genes. Metabolomic analysis identified several key networks, with cholesterol sulphate (CS) identified as a promising metabolite target. CS and several derivatives were synthesised and supplemented at 1 mM to 2D and 3D cultures, inducing osteogenic differentiation. When compared to osteogenic media, selected metabolites induced greater gene and protein expression while having less effect on chondrogenic and adipogenic genes.

ONCLUSION:

Nanovibration is a novel tool for the supplement-free study of MSC osteogenic differentiation. Here we used the bioreactor to drive 2D and 3D osteogenesis comparing and contrasting 2D vs 3D differentiation. Further, we used the bioreactor as a tool to identify metabolites that in themselves can induce osteogenesis and that can be modified to tune effect; that supplements were not needed for osteogenesis is critical in producing an artefact free small molecule background.

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ACKNOWLEDGMENTS:

We would like to acknowledge the EPSRC for funding this project (EP/N013905/1) and Glasgow Polyomics.

Engineering orthopedic tissues 10:45 - 12:15 Auditorium I 13/09/2018

Oral presentation

264 A comparison of potential fibrillin-1 fragments for the functionalisation of a novel anterior cruciate ligament biomaterial scaffold.

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INTRODUCTION:

Anterior cruciate ligament (ACL) tears are the most common sports injuries, with over 200,000/year in the USA alone[1]. Current “gold standard” treatment involves autografting of either patellar tendon or hamstring muscle into the ACL integration sites[2]. Although autografting has a lower failure rate, autograft tissue differs in specific protein composition, resulting in inadequate mechanical properties[2]. Both grafting methods can result in surgical reintervention, causing increased trauma for the patient[3]. Alternatively, synthetic grafts are mechanically strong but lack the biological components required for local integration. To improve this, we propose a novel fibrillin-1 modified biomaterial scaffold, capable of directing correct extracellular matrix/ECM deposition, for regeneration of the native ACL.

METHODS:

Poly (e-caprolactone)/PCL (15% w/v) was electrospun into aligned fibres, sterilised (70% ethanol, 1hr) and plasma treated (P) (air, 1min). Re-sterilisation (UV, 1hr) was performed, before fibrillin-1 treatment (overnight, 4ºC) with commercial (AbF, Abcam, UK) or isolated (PF8, PF9, PF17.1) fragments. Canine ACL fibroblasts (cACLs, 60,000 cells/scaffold) were seeded. Protein adsorption, contact angle, live/dead and metabolic analyses were performed. Total neo ECM proteins were imaged using immunofluorescence and quantified (FIJI imageJ). Statistical analysis (two-way ANOVA, Tukey HSD post hoc) on a sample size of n=3, was used for all assays, with exception of live/dead (n=2).

RESULTS AND DISCUSSION:

All isolated fragments (PF8/PF9/PF17.1) adsorbed to PCL+P, with PCL and glass coverslips/GC being less successful. Live/dead staining showed good viability for all conditions. Although there appeared to be no inter fragment differences on GC, PF8 fragment encouraged cell integration on PCL[4], with slight elongation of the cells (day 7). Isolated fragments showed better integration of cells compared to AbF. Increased metabolic activity was observed across all isolated fragments (Figure 1B), which was not observed using AbF (no significances). Data from neo ECM immunofluorescence evidenced secretion of complex ECM proteins, organised in an aligned manner due to plasma treatment.

CONCLUSION:

PF8 appeared to be the ideal fibrillin-1 fragment to combine with PCL+P, suggesting that it could be used as an 'initiator' protein for guiding the formation and development of a mature ACL ECM.

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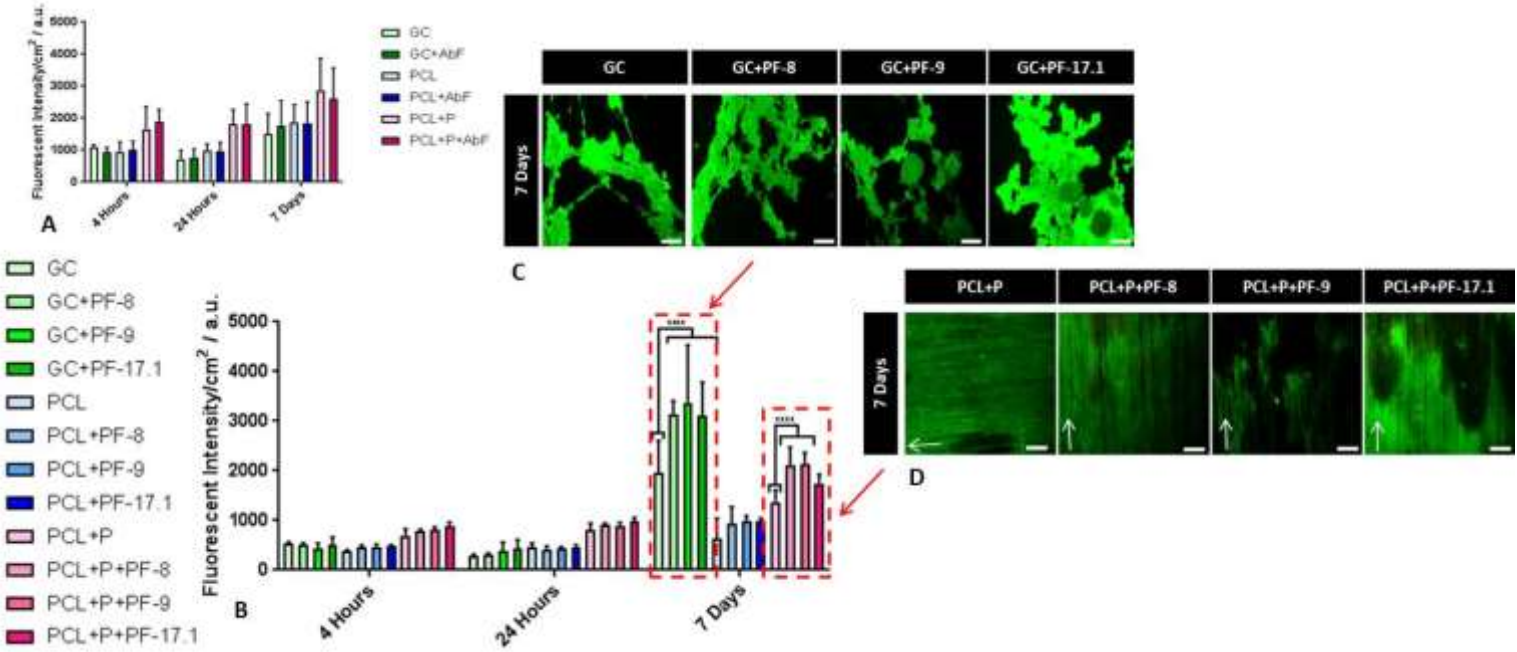
ACKNOWLEDGMENTS:

Funded by UK Regenerative Medicine Platform Hub and EPSRC UK.



Picture 1: Caption 1: Figure 1 Metabolic (A/B) and live/dead (C/D) assays show responses of cACLs cultured with fibrillin-1 fragment coatings.

Engineering orthopedic tissues 10:45 - 12:15 Auditorium I 13/09/2018

Oral presentation

390 Anisotropic direct current field stimulation significantly promotes the synthesis of cartilage extracellular matrix

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INTRODUCTION:

The extracellular matrix (ECM) of articular cartilage has zonal specific properties associated with collagen orientation, glycoprotein density, and gene expression of chondrocyte. These constructive and compositional inhomogeneity elements lead to generate anisotropic transduction of mechanical, chemical, and electrical stimuli, which strongly involve in the chondrocyte metabolism. We focused on this anisotropic stimulation for *in vitro* cartilage formation and developed a novel bioreactor which was able to apply anisotropic direct current field (DCF) to cultured

cartilage tissue without mechanical deformation[1]. In this study, we evaluated the effects of anisotropic DCF stimulation on ECM formation of cartilage tissues.

METHODS:

The shape and the design dimensions of the bioreactor were simulated by finite element analysis and determined to produce the 1.8-fold intensity of DCF between the surface (125 mV/cm) and the bottom (68mV/cm) of the specimen. These specimens were installed in the chamber filled with culture medium, and DCF stimulation was applied to them via agar salt bridges, Hanks' solution as an electrolyte and platinum electrodes. Chondrocytes isolated from the distal femur of 6-month-old pigs were seeded in agarose scaffold at 1.5×10^7 cells/ml and cultured for 7 days, while being exposed to DCF for 3 hours per day. ECM morphology was evaluated by using immunohistochemical staining for type-II collagen (green) and DAPI (blue) on frozen section. mRNA expression of *COL2A1*, major anabolic gene for cartilage ECM, and *MMP13*, catabolic gene for type-II collagen, was measured by using quantitative real-time PCR (N=3, Welch's *t*-test).

RESULTS AND DISCUSSION:

Figure 1 (a) shows the cross-section images of cultured cartilage with immunohistochemical staining. Much higher amount of type-II collagen was synthesized in the specimens with DCF than in those without DCF. Moreover, the specimens with DCF exhibited deviation of collagen synthesis between the edge and the inner part of tissue. Figure 1 (b) shows the results of relative expression of *COL2A1* and *MMP13* mRNA. The expression of *COL2A1* gene with DCF was 50 times higher than that without DCF. In addition, *MMP13* gene with DCF was approximately one hundredth as many as that without DCF. Although there might not be an obvious relation between the intensity of anisotropic DCF and the tissue distribution, this experimental condition led to a significant increase of collagen synthesis in cultured cartilage.

CONCLUSION:

Anisotropic DCF stimulation had a potential to significantly promote ECM synthesis of cultured cartilage tissue.

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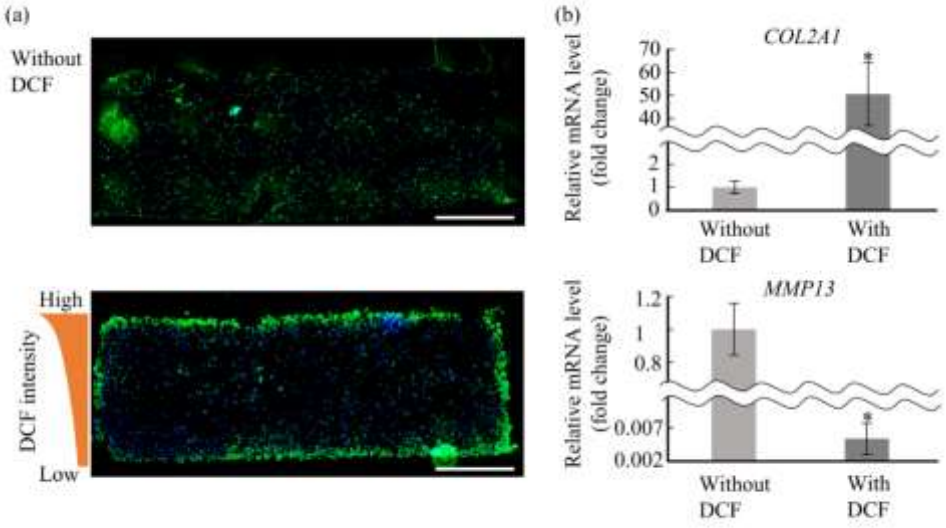

Picture 1: Caption 1: Fig.1 (a) Immunohistochemical staining for type-II collagen(×100, Bar = 1mm), (b) mRNA expression levels (mean ± s.d., ∗ :P < 0.01).

Engineering orthopedic tissues 10:45 - 12:15 Auditorium I 13/09/2018

Oral presentation

125 Nanocomposite chemically modified hyaluronic acid hydrogel based on sol-gel methods for osteochondral regenerations.

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[3]National Engineering Research Center for Biomaterials, Sichuan University, China

INTRODUCTION:

Hyaluronic acid is widely used for numerous medical applications, such as viscosupplementation, eye surgery and drug delivery [1][2]. In this study, hyaluronic acid sodium salt (HAs) was chemically modified to obtain a photocrosslinkable hydrogel with tailored mechanical properties for osteochondral application. To this aim, to optimize the mechanical and biological properties a composite hydrogel was developed based on chemically modified HAs and *in situ* synthesized hydroxyapatite (Hap) by sol-gel approach [3].

METHODS:

Hyaluronic acid sodium salt (HAs, M=~340 kDa) was Methacrylated (MeHA) and Maleated (MaHA) using different degrees of substitution (DS) to obtain a photocrosslinkable hydrogel with tunable properties. Composite hydrogels have been synthesized by sol-gel method varying the amount of Hap from 25 to 50wt%. Composite disc shaped samples were photocrosslinked by UV radiation. Nuclear Magnetic Resonance (NMR), Fourier transform infrared spectroscopy (FTIR) were performed in order to to identify the functional groups on MeHA and MaHA. Dynamic mechanical analysis (DMA) was carried to study mechanical properties of neat and composite hydrogels. Morphology and distribution of nanoparticles were investigated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). *In vitro* cytotoxicity was evaluated by seeding L929 cell line onto neat and composite hydrogels.

RESULTS AND DISCUSSION:

The ^{1}H NMR characteristic peaks at 5.6 and 6.0 ppm for MeHA and at 6.1 and 6.7 ppm for MaHA indicate that photocrosslinkable groups were successful attached onto HAs. The DMA highlighted a strong correlation between the DS and the storage modulus meanwhile the composite samples with 50wt% of Hap showed the best mechanical properties. SEM analysis showed a good distribution of Hap particles into the hydrogel. Furthermore, the cytotoxicity demonstrated that after 3 days of culture, neat and composite hydrogels with 25wt% of Hap showed the best biological behaviour.

CONCLUSION:

Nanocomposite hydrogels based on chemically modified HAs and Hap particles were developed and characterized by their morphological, mechanical and biological properties. Results showed that the mechanical properties of the modified HAs can be controlled by adjusting the chemical reaction and through the sol-gel method it is possible to obtain a finely dispersed HAp into the hydrogels. Finally, both neat and composite hydrogels highlighted no cytotoxic effect when seeded with L929 cells.

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ACKNOWLEDGMENTS:

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Biomaterials based on colloidal building blocks 10:45 - 12:15 Auditorium II 13/09/2018

Oral presentation

Bioactive glass nanoparticles with enhanced functionalities: progress and opportunities in biomedical applications

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Bioactive glass (BG) is a versatile material for various biomedical applications including bone/soft tissue regeneration 1,2. Their nanoscale forms, such as nanoparticles (BGN), are particularly suitable building blocks for developing nanocomposites as well as being effective platforms for drug delivery 3. Due to the amorphous structure and tunable chemical composition, ions (e.g. Cu and Ca ions) that exhibit biological activity can be conveniently incorporated into BG 4. Considering the small size and uniform shape, sol-gel derived BGN have shown great potential as carriers in targeted or local delivery and for the release of biologically active ions and drugs 5.

We have developed a series of sol-gel based approaches to synthesize spherical BGN for various biomedical applications. In order to incorporate metallic ions into BGN by keeping homogeneity in size and shape of the particles, different strategies, such as the Stöber method and surface modification, have been adopted in our studies. The synthesis strategy depends in each case on the metallic ion ion(s) involved. Particle size is controllable by tuning processing parameters (e.g. solvent concentration) while the porosity of particles is also tailorable through introducing pore-forming templates during sol-gel processing. Highly dispersed ion-containing BGN have been successfully produced through the developed sol-gel based approaches. The bioactivity of BGN can be retained after the incorporation of metallic ions. Furthermore, osteogenesis, angiogenesis or antibacterial activity of BGN are successfully promoted by the introduction of suitable ions (e.g. Cu and Ag ions). The developed BGN are also able to release ions in a sustained manner. These BGNs thus show great potential, usually combined with biopolymers, in applications related to tissue regeneration, drug delivery or nanomedicine.

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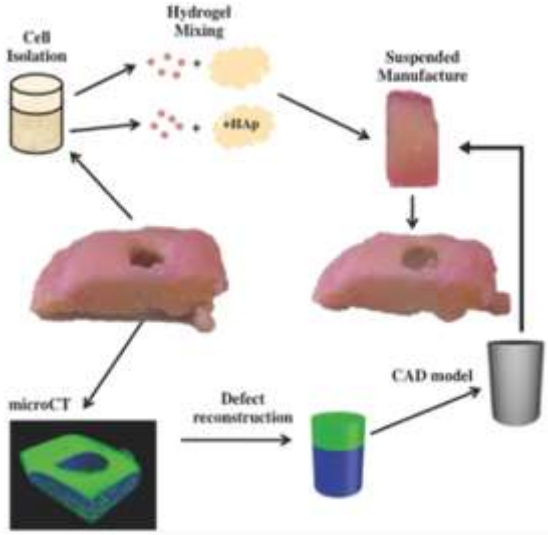
Biomaterials based on colloidal building blocks 10:45 - 12:15 Auditorium II 13/09/2018

Oral presentation

Structuring hydrogels to enable suspended manufacture of cell-loaded constructs

Liam M Grover
Director of Healthcare Technologies Institute, School of Chemical Engineering, University of Birmingham, UK

A major challenge in the 3D printing of soft tissues is that the materials that are used to support cell growth are typically too weak to support their own weight. Consequently, the soft materials must be supported by a second, more robust phase, during the manufacturing process. Researchers have used high viscosity liquids such as Pluronic F-1271 and slurries of gelatin microparticles2 to provide this support. There remain major drawbacks with the use of these materials, however, particularly since the supporting polymer is challenging to remove from the surface of the part. In order to address this drawback, we have generated supporting materials by applying shear to the hydrocolloids during gelation, resulting in what is known as a fluid gel. This material is formed from a mass of particles and ribbons which interact to form a structured soft solid in the absence of shear. When the material is locally deformed, it will flow and immediately heal when the shear is removed. These properties allow a secondary gelling phase to be deposited within the structure. This secondary phase may carry a population of viable cells or other sensitive entities, which may be localised within the structure. Importantly the support phase may be formed from the same compound as the gelling phase and can easily be removed from the final part using gentle agitation. We have demonstrated the utility of this process using by producing a full thickness chondral replacement using primary human osteoblast and chondrocyte cells. The cells were supported within a gellan gum matrix, which was modified with nanocrystalline hydroxyapatite within the boney region. The resulting material retained mechanical integrity over a period of 28 days of culture and importantly, the cells that were immobilised in the distinct regions of the cells retained either their predominantly osteoblastic or chondrocytic phenotype. This work demonstrates the power of the suspended manufacturing approach to produce geometrically complex cell-laden structures.



The suspended manufacture process allows for high-levels of spatial control over mechanical and chemical properties. The potential of this method for producing complicated tissues was demonstrated by manufacturing a complex hard/soft tissue interface and demonstrating that cell phenotype can be maintained over four weeks of culture.

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Biomaterials based on colloidal building blocks 10:45 - 12:15 Auditorium II 13/09/2018

Oral presentation

279 Composite colloidal gels with self-healing properties for regenerative medicine

Mani Diba[1], Huanan Wang[2], Thomas Kodger[3], Shima Parsa[4], Winston Camargo[5], Mariateresa Brindisi[6], Kambiz Farbod[5], Stephan Schmidt[7], Matthew Harrington[8], Lorenza Draghi[6], Aldo Boccaccini[9], John Jansen[5], Jeroen van den Beucken[5], Sander Leeuwenburgh[5]
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[5]Radboud University Medical Center, Netherlands
[6]Politecnico di Milano, Italy
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[9]University of Erlangen-Nuremberg, Germany

INTRODUCTION:

Colloidal gels are composed of particles that are assembled into three-dimensional networks. By exploiting the reversibility of non-covalent interparticle interactions, colloidal gels can be rendered self-healing [1]. Purely organic colloidal gels are, however, mechanically weak [1, 2]. Here, we present a novel class of composite colloidal gels with self-healing properties for application in regenerative medicine.

METHODS:

Preparation of building blocks:

Gelatin nanoparticles were prepared using a two-step desolvation method [1]. Silica nanoparticles were synthesized using a modified Stöber method. Bioactive glass particles (45S5 composition) were obtained from commercial sources. Bisphosphonate (BP) functionalization of gelatin nanoparticles was performed by aldehyde coupling of alendronate [3].

Preparation of hydrogels:

Hydrogels were prepared by mixing organic and inorganic building blocks at different ratios. The interparticle interactions in colloidal gel networks were adjusted by a gradual change of the pH using glucono delta-lactone or by the modification of gelatin particles with BP groups.

Characterization methods:

Structural and mechanical properties of the gels were evaluated using confocal microscopy, rheological, compressive and tensile tests. The in vitro and in vivo response to these materials were evaluated using MC3T3 osteoblastic cells and osteoporotic rats, respectively.

RESULTS AND DISCUSSION:

Evaluation of the self-assembly process and properties of the hydrogels revealed that the controlled assembly of the gel networks (Figure 1) resulted in hydrogels which combined high elasticity with a strong capacity for self-healing. The resulting composites completely self-healed after both shear- or cutting-induced mechanical damage. When BP-functionalized gelatin particles were combined with bioactive glass particles, the BP functionalization improved the elasticity of the resulting self-healing gels. *In vitro* cell culture experiments showed that these composite gels stimulated alkaline phosphatase activity and mineralization. The *in vivo* tests demonstrated that the composite gels

stimulated bone regeneration, while the release of BP from these composites increased the density of bone surrounding these implants.

CONCLUSION:

This study demonstrated the successful development of composite colloidal gels assembled from gelatin and silicate particles. These novel biomaterials were mechanically robust, exhibited a remarkable self-healing capability, and assisted the regeneration of osteoporotic bone defects.

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ACKNOWLEDGMENTS:

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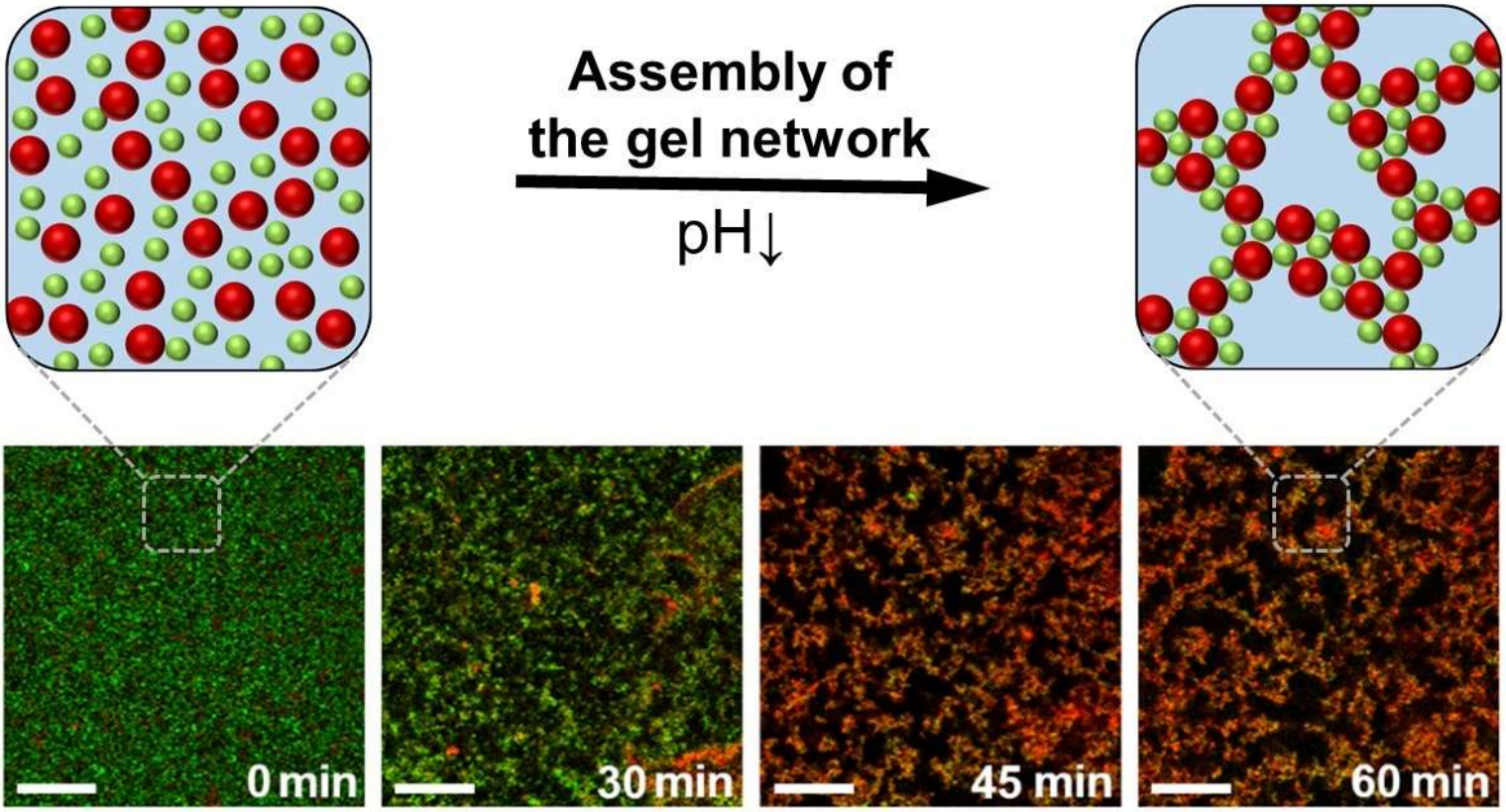

Picture 1: Caption 1: Figure 1. Schematic illustrations and confocal microscopy images showing the process of gel network formation. Red and green spheres indicate gelatin

Biomaterials based on colloidal building blocks 10:45 - 12:15 Auditorium II 13/09/2018

Oral presentation

197 Protein self-assembly at oil-water interfaces controls nanoscale mechanics, cell adhesion and stem cell fate decision

Julien Gautrot, Dexu Kong, Lihui Peng
Queen Mary University of London, London, United Kingdom

INTRODUCTION:

The mechanical behaviour of the extracellular matrix has an important impact on cell phenotype. It has been shown to regulate cell adhesion and spreading, cell motility, proliferation and differentiation in a wide range of cells, stem cells as well as cancer cells[1]. Recently, we reported that keratinocytes and mesenchymal stem cells do not seem to respond to the bulk mechanical properties of silicone materials, in contrast to their behaviour on acrylamide hydrogels[2]. We now show that HaCaT and primary keratinocytes can adhere, spread and proliferate at the surface of non-viscous liquids containing surfactant molecules.

METHODS:

We characterised cell proliferation and adhesion via fluorescence microscopy and scanning ion conductance microscopy. We used AFM and interfacial rheology to study the nanoscale mechanical properties of protein assemblies generated at liquid-liquid interfaces. We used AFM and SEM to characterise the morphology and thickness of deposited protein nanosheets.

RESULTS AND DISCUSSION:

We show that the cell behaviour observed (spreading and proliferation on non-viscous liquids, Fig. 1A/B) is directly correlated to the concentration of surfactant present in the oil (Fig. 1C; evolution of interfacial shear modulus before (red) and after (blue) protein adsorption as a function of [S] and associated surfactant concentration within the protein layer, in green). This is supported by our interfacial rheology data and XPS experiments. We identify that protein self-assembly at the oil-water interface is the main driver of this behaviour. We show that the type and concentration of surfactant as well as the proteins self-assembled control the nanoscale mechanical properties of the resulting interfaces and associated cell behaviour. We show that cell spreading at such liquid interfaces is mediated by integrin ligation, focal adhesion formation and acto-myosin contractility (Fig. 1E). In addition, we show that this behaviour depends on the interfacial mechanical properties of the protein layer assembled. We show that keratinocyte differentiation is not initiated by spreading at the surface of liquids, despite the absence of bulk mechanical properties. Finally we apply such interfaces for the culture of cell in emulsions (Fig. 1F).

CONCLUSION:

Our results suggest that nanoscale mechanical properties of biomaterials may dominate over bulk physical properties. This concept has important implications for the design of biomaterials in the field of regenerative medicine.

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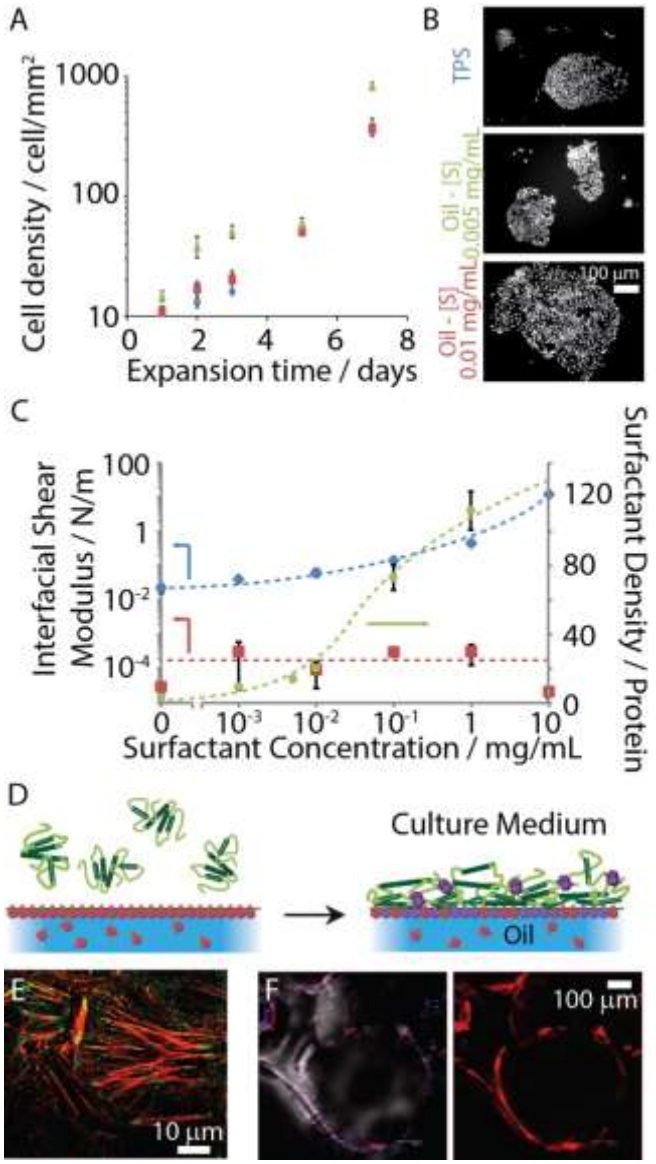
ACKNOWLEDGMENTS:

We would like to thank Khai Nguyen for help with interfacial rheology measurements and Dr John Connelly for discussion of our results.



Picture 1: Caption 1: Figure 1. Cell culture at liquid-liquid interfaces relies on a self-assembled protein layer

Interactions in and with 3D scaffolds 10:45 - 12:15 Room 0.4 13/09/2018

Oral presentation

798 Platelet lysate based hydrogels with tunable mechanical properties as platforms for 3D cell culture

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[2]Universidade de Aveiro- CICECO - Aveiro Institute of Materials, Portugal
[3]Universidade de Aveiro-CICECO - Aveiro Institute of Materials, Portugal

INTRODUCTION:

Platelet-rich plasma (PRP) and human platelet lysates (PL) are attractive sources of growth factors and other molecules involved in the tissue regeneration process. PRP and PL have been mainly explored as a promising non-

xenogenic supplement designed for the expansion of human cells that replaces animal derived serum. Recently, PRP/PL based scaffolds have been investigated as cell culture platforms. Despite all the promising results, in most cases this type of materials suffer from poor mechanical properties and also poor stability *in vitro*. In an attempt to overcome such limitations, we developed PL based hydrogels with tunable mechanical properties.

METHODS:

Methacryloyl platelet lysates (PLMA) were synthesized by reaction with methacrylic anhydride and purified by dialysis. NMR analysis was performed to verify the functionalization of original PL. Mass spectrometric identification of proteins and modified proteins from PL, was also performed. To prepare hydrogels, lyophilized PLMA was dissolved in PBS with a photoinitiator to final concentrations of 10% or 15% (w/v). Hydrogels were made by pipetting the polymer solution to PDMS molds followed by UV irradiation (1.54 W/cm2) during 60s. The mechanical behavior and water uptake of PLMA hydrogels was characterized. Quantification of protein and GFs release from the hydrogels was performed by Micro BCA Protein Assay Kit and ELISA assay. The biological performance of PLMA hydrogels was assessed using the L929 Cell Line and human adipose stem cells (hASCs).

RESULTS AND DISCUSSION:

Our results showed that the achieved materials own increased mechanical properties that can be easily adjustable by changing PLMA concentration or PLMA modification degree. Additionally, PLMA hydrogels enabled sustained release of VEGF and fibrinogen for up to 250 hours and were resistant to protease degradation. hASCs and L929 were successfully encapsulated in the hydrogels, exhibiting high cell viability, adhesion and proliferation.

CONCLUSION:

These results support the use of a PLMA hydrogel as a scaffold for stem cell culture and growth factor release. The material here developed could have an autologous origin, being adequate to produce customized robust matrices to be used as general platform for 3D cell culture with no risk of cross reactivity, immune reaction or disease transmission.

ACKNOWLEDGMENTS:

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Interactions in and with 3D scaffolds 10:45 - 12:15 Room 0.4 13/09/2018

Oral presentation

632 Polyurethane foam scaffolds interpenetrated with crosslinked gelatin hydrogel for adipose tissue regeneration

Nicola Contessi[1], Andrea Cochis[2], Rita Sorrentino[2], Serena Bertoldi[1], Barbara Azzimonti[2], Silvia Farè[1], Lia Rimondini[2]
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INTRODUCTION:

Adipose tissue (AT) augmentation is a clinical routine practice performed by using fillers, flaps or synthetic prosthesis, in case of third-grades burn, large resection or congenital defects[1]. Despite large needs, clinical approaches are still largely affected by limited functional regain, donor-site morbidity and/or systemic rejection. Here, we developed an innovative interpenetrated polyurethane-gelatin hybrid scaffold (PU/GEL) made of a poly-ether-urethane foam coated with a chemically crosslinked gelatin hydrogel, and we investigated its suitability as scaffold for AT regeneration .

METHODS:

PU foam was obtained by a one-step gas reaction by mixing optimized ratios of a polyol mixture, 4-4' difenyl-methan diisocyanate prepolymer, Fe-AcetylAcetonate, and water as expanding agent[2]. GEL was synthetized using Type A gelatin and N,N'-methylenebis(acrylamide) as crosslinker[3]. Interpenetrated PU/GEL specimens were obtained by using a home-made vacuum chamber that promoted the PU foam pores coating with GEL. Then, samples were disinfected in ethanol and dehydrated. The hybrid structure was observed at SEM, gelatin interpenetration investigated by Coomassie Blue staining, physical properties were analysed by weight variation test, density (UNI EN ISO 845), mechanical compression tests were performed using a Dynamic Mechanical Analyser. Adipogenesis was investigated *in vitro* using murine adipose-derived stem cells (ASC), and biocompatibility was evaluated *in vivo* by subcutaneous implantation into wild-type mice. Accordingly, scaffolds tissue ingrowth, neo-vascularization, adipose tissue infiltration and immunological reaction were evaluated 1 and 2 months after surgery.

RESULTS AND DISCUSSION:

Interpenetration of gelatin was proved by Coomassie Blue-stained gelatin observed in the PU foam pores. Breast and subcutaneous biomimetic mechanical properties were proved for PU/GEL by compression tests[4]. Stem cells seeded onto hybrid scaffolds were successfully induced towards adipogenesis, confirming scaffold suitability to host AT ingrowth. Post-implant *in vivo* analysis demonstrated that the scaffolds were deeply vascularized and largely filled by naïve tissue infiltrated into the PU/GEL scaffold. Spleens T-lymphocytes evaluation confirmed no severe immunological reaction was induced by PU/GEL implantation.

CONCLUSION:

An innovative PU/GEL hybrid scaffold for AT regeneration was successfully fabricated. PU/GEL showed mechanical properties comparable to human AT and supported hMSC adipogenesis *in vitro*. The excellent *in vivo* integration and neo-vascularization observed for PU/GEL supported the hypothesis that the hybrid scaffold represents an optimal candidate for AT engineering.

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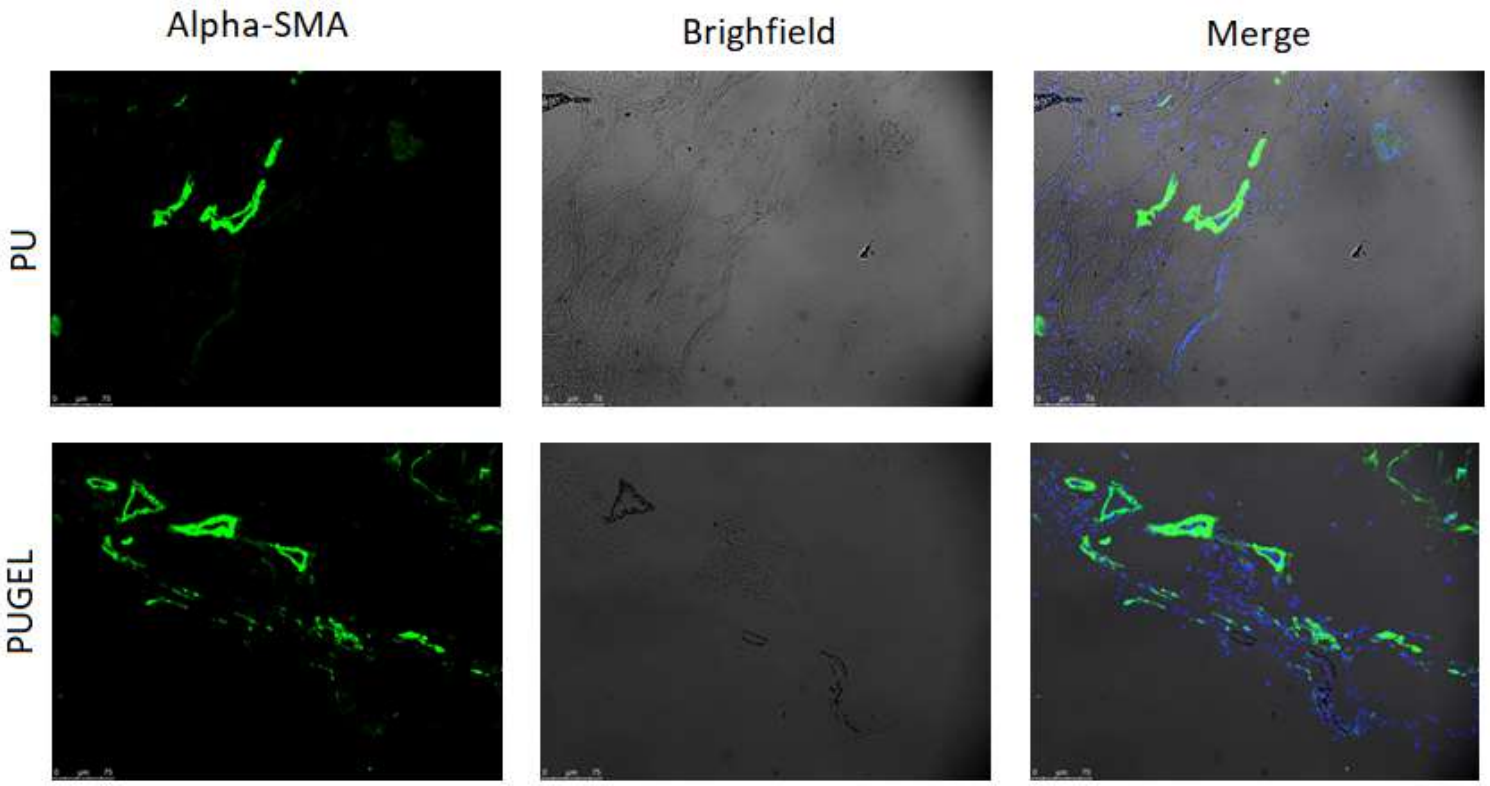

Picture 1: Caption 1: PU and PUGEL immunofluorescence analysis

Interactions in and with 3D scaffolds 10:45 - 12:15 Room 0.4 13/09/2018

Oral presentation

440 Mechanobiology-enhanced tissue engineering: a collagen-scaffold-based delivery system for accelerating bone repair by activating JNK3 in stem cells

Arlyng dr Gonzalez-Vazquez[1], Suzan msc Günbay[1], Dylan J mr Murray[2], Fergal J prof.dr O'Brien[1]
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[2]Temple Street Children's University Hospital, Ireland

INTRODUCTION:

In bone tissue engineering, scaffolds provide microenvironmental cues that direct stem cell behavior and provide a template for matrix deposition. In our lab, collagen-nanohydroxyapatite (-nHA) scaffolds have proven potential for directing stem cells to promote bone repair in small defects. However, repair of very large defects still requires the incorporation of growth factors such as bone morphogenetic proteins (BMPs). However, the side-effects associated to BMPs highlights the need for identifying new therapeutics. We have recently identified c-Jun N-terminal kinase 3 (JNK3) as a key modulator of the increased mechanically-activated osteogenic potential of stem cells derived from young donors (C-MSCs)[1], hence suggesting JNK3 as potential therapeutic target for enhancing bone repair in stem cells from adult donors (A-MSCs). Building on this work, the aim of this study was to enhance the repair capacity of the collagen-nHA scaffold, using it as a delivery system to accelerate osteogenesis by activating JNK3 in adult-derived stem cells.

METHODS:

Firstly, C-MSCs (11-12y) and A-MSCs (20-30y) were isolated from bone marrow[1]. To facilitate JNK3 activation, we fabricated in-house positively charged nanoparticles of 200 nm by complexing nHA with a JNK3 activator tagged with FITC (JNK3*). Then, we treated C-MSCs and A-MSCs with different concentrations of JNK3* (0-5 µM) and the role of JNK3* on the acceleration of osteogenesis was assessed.

RESULTS AND DISCUSSION:

Results revealed a dose-dependent uptake of JNK3* in both C-MSCs and A-MSCs with the highest uptake, 88.4%, observed in MSCs treated with the 5µM dose. Therefore, we took forward the 5µM dose. Findings demonstrated that after 14 days of treatment JNK3* enhances ALP activity in A-MSCs to the same extent as C-MSCs, and more importantly, when JNK3 inhibitor was added, the increase in ALP activity was inhibited. Finally, A-MSCs cultured on the collagen-JNK3* scaffold (collagen-scaffold containing JNK3*) showed positive uptake of the JNK3* (arrows) and A-MSC genetic expression revealed a significant upregulation of ALP, PGF and JNK3 as early as day three. Furthermore, ALP activity and mineralization were significantly enhanced in A-MSCs cultured on the collagen-JNK3* scaffold (Fig.1). *In vivo* assays currently ongoing.

CONCLUSION:

In conclusion, we have successfully developed an off-the-shelf 3D collagen-scaffold-based therapeutic delivery platform which enhances the osteogenic capacity of stem cells by facilitating JNK3 activation – ultimately showing how mechanobiology research can improve the development of next generation therapeutics in tissue engineering.

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ACKNOWLEDGMENTS:

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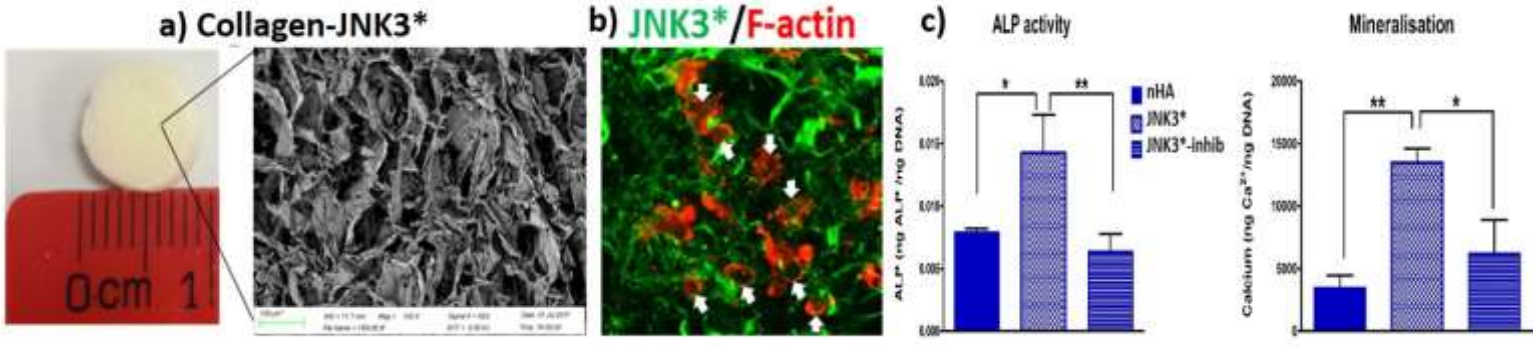

Picture 1: Caption 1: Collagen-JNK3* scaffolds (a) successfully delivers JNK3* to A-MSCs, arrows (b). ALP activity and mineralization reveals JNK3-mediated osteogenesis (c)

Interactions in and with 3D scaffolds 10:45 - 12:15 Room 0.4 13/09/2018

Oral presentation

160 Designing peptide / graphene derivatives hybrid hydrogels through fine tuning of molecular interactions

Jacek Wychowaniec[1], Maria Iliut[2], Mi Zhou[2], Jonathan Moffat[3], Wagner Pinheiro[4], Judith Hoyland[5], Aline Miller[2], Aravind Vijayaraghavan[2], Alberto Saiani[2]
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[2]University of Manchester, United Kingdom
[3]UK Asylum Research an Oxford Instruments company, United Kingdom
[4]Military Institute of Engineering, Brazil
[5]The University of Manchester, United Kingdom

INTRODUCTION:

Hydrogels which are highly hydrated materials have come to the fore front for the design of biocompatible and mechanically tuneable 3-dimensional (3D) scaffolds which offer opportunity for bio-functionalization[1, 2]. Recently strategy of incorporating graphene based nano-fillers in hydrogels has been used to tailor mechanical strength and conductivity and add binding sites for bio-functionalization to regulate cell behaviour, including proliferation, differentiation and protein synthesis to promote specific tissue regeneration[3]. As underlined by a number of authors the key challenge when designing hybrid materials is the understanding of the molecular interactions between the nano-filler and the matrix and how these affect the final properties of the bulk material[4]. In this work three peptides with varying physiochemical properties and five GDs with varying surface chemistries have been used to formulate selection of hybrid hydrogels (Figure 1).

METHODS:

XPS, UV-Vis, Raman spectroscopy, SEM, AFM and zeta-potential were used to characterise graphene derivatives. FTIR spectroscopy, AFM and SAXS were used to characterise peptide hydrogels. Molecular interactions in hybrid hydrogels were evaluated using AFM, oscillatory rheology and optical photographs of the hydrogels. Human mesenchymal stem cells (hMSC) were then 3D encapsulated in the FE, FE+ graphene oxide (GO) and FE+ reduced graphene oxide (rGO) hydrogels prepared at 26.8 mM and 0.5 mg mL^{-1} GO/rGO, and cultures were maintained for 14 days.

RESULTS AND DISCUSSION:

The interplay of various molecular interactions between nano-fibres and graphene-based materials was investigated. In particular hydrophobic and electrostatic interactions were found key to the design of bulk hydrogels with tuneable physicochemical and biological properties. Using Live-Dead staining, it was clear that cell viability was maintained at >95 % over 14-day culture period for both control FE, FE+GO and FE+rGO. This indicated non-cytotoxicity of the 3D culture systems for hMSC cells.

CONCLUSION:

Overall the incorportion of graphene-based nanofillers into the peptide hydrogels created biocompatible 3D scaffolds for 3D-culture of stem cells with the potentials to tune mechanical strengths and to further bio-functionalize through the adsorption of active biomolecules. We endevour to use this versatile platform for further biomedical/tissue engineering research.

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ACKNOWLEDGMENTS:

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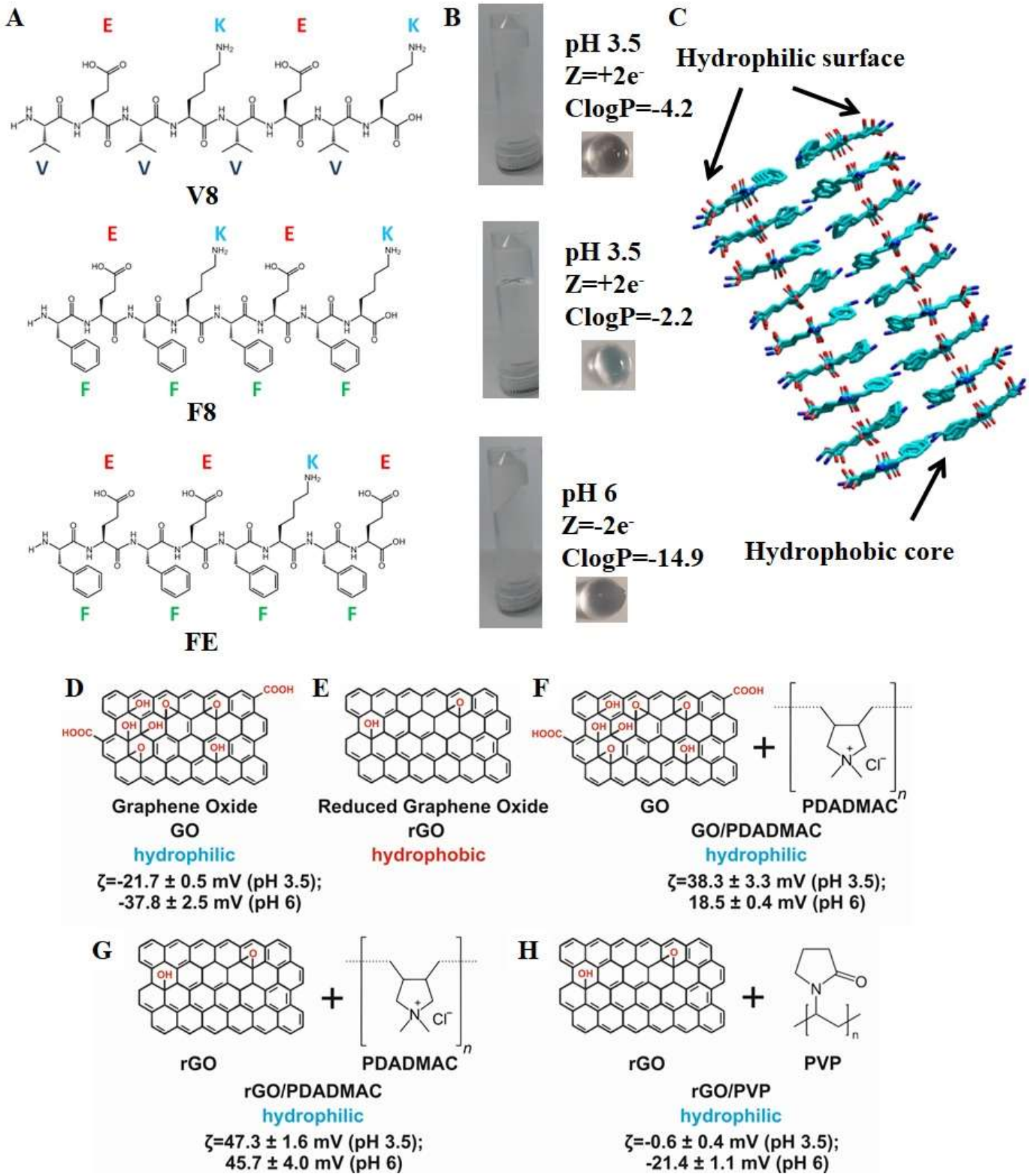

Picture 1: Caption 1: Schematic diagram of the three used peptides and five graphene derivatives used and their physicochemical properties.

Interactions in and with 3D scaffolds 10:45 - 12:15 Room 0.4 13/09/2018

Oral presentation

237 Novel hierarchical silk fibroin-based scaffolds incorporating Sr- and Zn-ions for osteochondral tissue engineering

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INTRODUCTION:

Osteochondral (OC) tissue engineering has been proposing bilayered scaffolds consisting of a cartilaginous and an underlying osseous layers. These scaffolds hold unique composition, structural strength and specific biological properties according to the target tissues.[1] Among natural biopolymers, silk fibroin (SF) exhibits high chemical versatility, biocompatibility and tunable mechanical properties.[2] On the other side, bioresorbable inorganic materials, such as β-*tricalcium phosphate* (β-*TCP)* have outstanding osteoconductivity.[2] The incorporation of ionic dopants into β-TCP enhance osteogenesis and neovascularization of scaffolds.[3] In this study, we aim to produce novel monolithic bilayered scaffolds composed by a combined enzymatically cross-linked SF hydrogels (HRP-SF) and β-TCP powders incorporating Sr and Zn, with enhanced viscoelastic properties for OC tissue repair/regeneration.

METHODS:

Hierarchical bilayered scaffolds were prepared with HRP-SF for the cartilage layer, and 80/20 (w/w) HRP-SF/undoped and ZnSr-doped β-TCP for the bone layer, using salt-leaching and b freeze-drying techniques (Fig.1a). Physicochemical characterization was assessed through FTIR, XRD, SEM, and micro-CT. Structural integrity was assessed by degradation profile studies and the mechanical properties were determined after immersion in PBS. Scaffolds bioactivity was assessed by immersion in SBF up to 30 days. The *in vitro* cell adhesion and proliferation were evaluated by co-culturing human chondrocytes and human osteoblasts in the scaffolds up to 14 days. Biochemical characterization of ALP activity and GAGs production were also performed.

RESULTS AND DISCUSSION:

The results showed porosity index of 50-60% and highly interconnected pores of 130-140 mm. A homogeneous distribution of the β-TCP into the HRP-SF on the osseous *layer was also observed. The mechanical properties of ZnSr-doped bilayered scaffolds were superior than the undoped scaffolds. Co-cultured cells adhered and proliferated on the bilayered scaffolds (Fig.1b) and higher ALP activity was detected on the monocultured* HRP-SF/undoped and ZnSr-doped β-TCP *constructs. A positive effect was induced by the co-culture system for GAGs production and deposition (Fig.1c).*

Figure 1.(a) Macroscopic image and (b) SEM micrograph of the cell-seeded ZnSr-doped bilayered scaffolds. (c) Safranin-O staining of GAGs matrix produced in the HRP-SF/ZnSr- β-TCP layer.

CONCLUSION:

The structural adaptability and suitable mechanical properties of the hierarchical tissue engineered OC scaffolds, combined with the biological performance achieved using co-culturing systems, make these constructs a viable strategy for OC defects regeneration.

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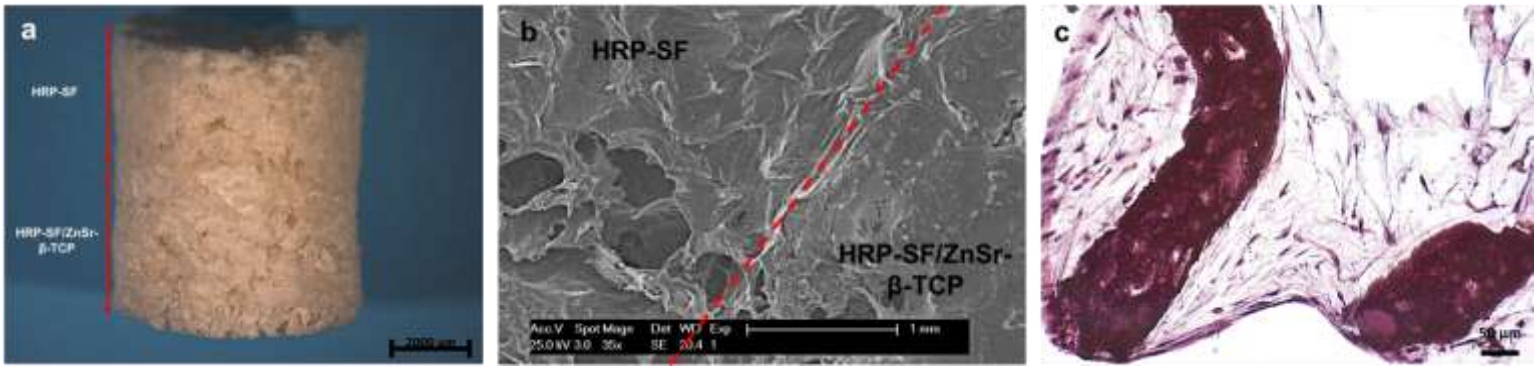
Investigador FCT program (IF/00423/2012, IF/01214/2014 and IF/01285/2015).

Picture 1:



Interactions in and with 3D scaffolds 10:45 - 12:15 Room 0.4 13/09/2018

Oral presentation

726 Design of a perfusion bioreactor for the high-throughput study of combinations of extracellular matrix proteins on human mesenchymal stem cells performance in dynamic 3D conditions

Diana Lopes[1], Mariana Oliveira[2], João Mano[2]
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INTRODUCTION:

By combining human cells, materials and engineering to induce biological responses, tissue engineering seeks to promote the rapid and accurate healing of damaged tissues. Using three-dimensional (3D) matrices that support cellular growth, instead of the traditionally used two dimensional (2D) materials, the structural organization of biological tissues can be emulated[1]. Not only the architectural layout of the extracellular matrix (ECM) affects cell response. Phenomena such as cell phenotype maintenance, cell differentiation and proliferation are stimulated by biochemical cues that are presented to cells by, for example, ECM proteins[2]. Currently, *in vitro* studies made on this field often lack physiological-like cues that include slow fluid dynamics that occur, for example, in native bone[3].

METHODS:

Here, we designed a system to unveil the effect of 32 different ECM protein combinations on the adhesion, proliferation and alkaline phosphatase (ALP) quantification of bone marrow-derived mesenchymal stem cells (MSCs). We created a novel bioreactor that enables a high-throughput study, allowing the collection of data from 32 biomaterial-cell combinations in one single test. This bioreactor was assembled from widely available affordable

labware. Cells were seeded onto chitosan porous scaffolds covalently modified with bone ECM proteins, as well as cell-cell contact proteins and enamel components.

RESULTS AND DISCUSSION:

By performing a factorial analysis study, we were able to detect correlations between the presence of single and combinations of proteins with improved cell adhesion to biomaterials, as well as improved ALP production after a 24 hours and 5 days of culture. This data was analyzed both for static culture conditions and for the presence of a slow perfusion rate.

CONCLUSION:

The developed system has proven to be useful in the interpretation of the wide complexity of cells-ECM interactions, and may find application in the development of biomaterials for tissue regeneration.

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ACKNOWLEDGMENTS:

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Toward tissue-specific application of biomaterials 10:45 - 12:15 Room 0.5 13/09/2018

Oral presentation

807 Importance of microenvironment pH in the design of biomaterials used for osteoporosis patients

Haobo Pan
Shenzhen Institute of Advanced Technology, Chinese Academy of Science, Shenzhen, China

INTRODUCTION:

Acid-base equilibrium is one of the most important factors that influence behaviors of bone cells. In scenario of osteoporotic fracture, significantly higher activity of osteoclasts than osteoblasts may lead to continuous loss of bone in fracture/defect site. In that case, we propose modulating the microenvironment pH (μe-pH) of that milieu, which is influenced by implants surface chemistry and biodegradation, to re-establish normal bone regeneration at the fracture site.

METHODS:

In our series studies, the measurement of material interfacial pH was realized by using the pH microelectrode. In vitro and in vivo examinations were conducted for evaluating the pH's effect on defect regeneration process in both normal/pathological conditions.

RESULTS AND DISCUSSION:

We demonstrated that the pH at the material surface is different from that of a homogeneous bulk extract at an early stage in vitro, and the altered local pH affects significantly the proliferation and ALP activity of MG-63 osteoblast-like cells. We have further revealed that the weakly-alkaline surface pH (pH 8–8.5) showed most stimulated effect on MC-3T3 cells viability and activity, thus further facilitating apatite nucleation. The increased culture environment pH has also been proved to stimulate osteogenic differentiation of osteoporosis bone marrow stromal cells (pH 7.73-7.94), and suppress the differentiation and pit-formation activity of RAW 264.7 osteoclast precursors (pH 7.59-8.02). In vivo, alkaline biodegradable materials generated a μe-pH which was higher than the normal physiological value, in particular, at the initial stage. Higher μe-pH is associated with better overall performances: greater new bone formation, suppression the activity of TRAP+ osteoclast-like cells, as well as the formation of an intermediate 'apatitic' layer. The osteoclastic associated enzymes have been proved to be greatly involved in the pH-related suppression effect towards osteoclasts.

CONCLUSION:

To conclude, implants with an alkaline microenvironment pH exhibited a promising healing effect for the repair of osteoporotic bone defects. As one of the indices for biomaterial evaluation, μe-pH may be used to better guide the design of biomaterials used for osteoporotic patients.

Toward tissue-specific application of biomaterials 10:45 - 12:15 Room 0.5 13/09/2018

Oral presentation

744 A comparison of the linking arm effect on the biological performance of a CD31 agonist directly grafted on L605 CoCr alloy by a plasma based multi-step strategy

Sergio Diaz-Rodriguez, Caroline Loy, Pascale Chevallier, Diego Mantovani
Université Laval, Quebec, Canada

INTRODUCTION:

Stents are cardiovascular devices implanted in atherosclerotic patient that help to reopen the narrowed artery, support and avoid its collapse. Nevertheless, post-implantation complications persist and the risk of the regeneration of the plaque is latent. Thus, enhanced properties are mandatory requirements for clinics[1]. A novel approach that allows the direct grafting of bioactive molecules has been developed. It involves the direct functionalization with primary amines (-NH_2) on L605 alloy surface by plasma-based techniques that serve as anchor points to covalent graft bioactive molecules. For this study, the impact on the biological activity of two different linking arms (LA) on a CD31 peptide (P23) with pro-endothelialization and anti-thrombotic properties was assessed[2]: Glutaric anhydride (GA), a short chain spacer and polyethylene glycol (PEG) with antifouling properties.

METHODS:

L605 specimens were cleaned in acetone/water/methanol, and electropolished (EP) to remove impurities. These samples were plasma treated using a Microwave-plasma reactor fed with N_2/H_2 in a two-step procedure to create -NH_2 on the surface. For the covalent grafting, aminated samples reacted first with one LA, followed by the terminal -COOH activation with EDC and the P23 grafting. XPS and ToF-SIMS assessed the surface chemical composition. Biological performance of the functionalized surfaces was evaluated using a culture-insert 2 well onto samples allowing to follow endothelial cell (EC) adhesion and proliferation. For hemocompatibility, the hemoglobin-free colorimetric assay was performed on endothelialized samples. Characterization and analyses were performed on 3 different samples with 3 points per samples.

RESULTS AND DISCUSSION:

XPS confirmed 1.5±0.4% -NH_2 after plasma treatment. Amines reacted with GA and PEG to graft the peptide by its lysyl moieties. An increase on %C, %N and %O in XPS confirmed the grafting, complimented by ToF-SIMS imaging (Figure 1a and 1b). Regarding the biological performance, the grafting of the peptide promoted cell adhesion compared to EP (Figure 1c). Moreover, EC formed a complete monolayer at the surface of the sample preventing clot formation (hemoglobin-free higher than 80%).

CONCLUSION:

The direct covalent grafting of a CD31 agonist on L605 by using two different LA was confirmed by XPS and ToF-SIMS analyses. The biological performance of these functionalized surfaces showed that, compared to the bare alloy, grafting the P23 with both LA increases EC adhesion and proliferation. Therefore, by using this novel plasma-based procedure, L605 was granted of properties desired in cardiovascular applications.

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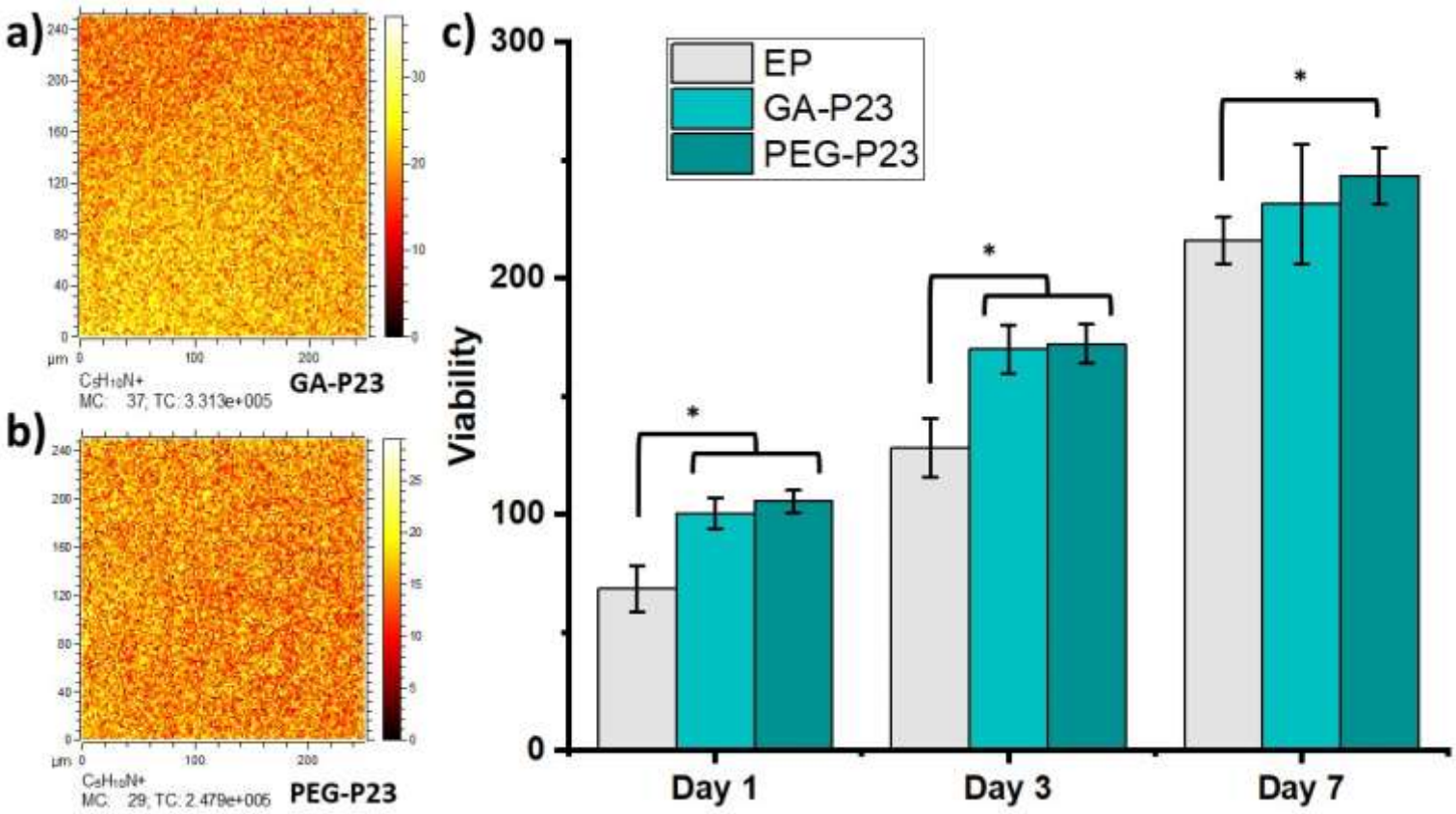

Picture 1: Caption 1: Figure 1 ToF-SIMS imaging of the specific lysine fragment: C5H10N+, m/z 84.085 of a) GA-P23 and b) PEG-P23 c) HUVEC viability assays of EP, GA-P23 and

Toward tissue-specific application of biomaterials 10:45 - 12:15 Room 0.5 13/09/2018

Oral presentation

158 Multimodal Porogen Platforms for Calcium Phosphate Cements

Irene Lodoso-Torrecilla[1], Nicole van Gestel[2], Eline-Claire Grosfeld[1], Luis Diaz-Gomez[3], Floris Stumpel[1], Kjell Laperre[4], Joop G.C. Wolke[1], Brandon T. Smith[5], Jacobus J. Arts[6], Antonios G. Mikos[7], Sandra Hofmann[2], John A. Jansen[1], Jeroen J.J.P. van den Beucken[1]
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INTRODUCTION:

Calcium phosphate cements (CPCs) show great potential as bone substitute materials due to their high resemblance to native bone and their favorable biological properties. However, their slow degradation and lack of macroporosity limits their applicability, as it hinders bone regeneration. The introduction of macroporosity is a straightforward approach to enhance the degradation of CPCs[1]. Poly(DL-lactic-co-glycolic acid) (PLGA) has shown to be a suitable porogen, as its degradation produces acidic by-products that further enhance CPC degradation. However, PLGA degradation only starts a few weeks after implantation, and hence bone regeneration is delayed until then[2]. We here explore the incorporation of multimodal porogen platforms within CPC to combine early water soluble porogen dissolution with late PLGA porogen degradation. We hypothesized that 1) the combination of water-soluble porogens and PLGA porogens in CPCs would enhance early stage macroporosity as well as late stage macroporosity and CPC degradation, and 2) the incorporation of water-soluble porogens would enhance bone formation at an early stage due to fast porosity formation.

METHODS:

Sucrose or polyvinylpyrrolidone (PVP) were added to CPC and CPC/PLGA as water-soluble porogens (10 or 20 wt.%). In vitro degradation in PBS at 37°C was evaluated for up to 6 weeks. Further, the biological performance of sucrose-containing CPC-formulations was analyzed in a rat femoral condyle bone defect model using implantation periods of 2 and 8 weeks.

RESULTS AND DISCUSSION:

In vitro degradation revealed that water-soluble porogen dissolution occurs within one week (Figure 1a and b). While sucrose was dissolved within one day, PVP progressively dissolved over 4 days, which avoids partial porogen dissolution during CPC setting. In contrast, PLGA degradation started after 2 weeks of incubation. In vivo implantation of CPC/porogen formulations (Figure 1c) showed similar biological performance in terms of material degradation and new bone formation for CPC/PLGA and CPC/PLGA/sucrose formulations. PLGA porogen incorporation significantly increased both material degradation and new bone formation.

CONCLUSION:

Incorporation of water-soluble porogens in CPC induced an early stage macroporosity and mass loss in vitro. However, this early stage porosity did not enhance biological performance compared to CPC/PLGA. Future work needs to focus on optimization of water-soluble porogen size and the creation of interconnected porosity to improve biological performance.

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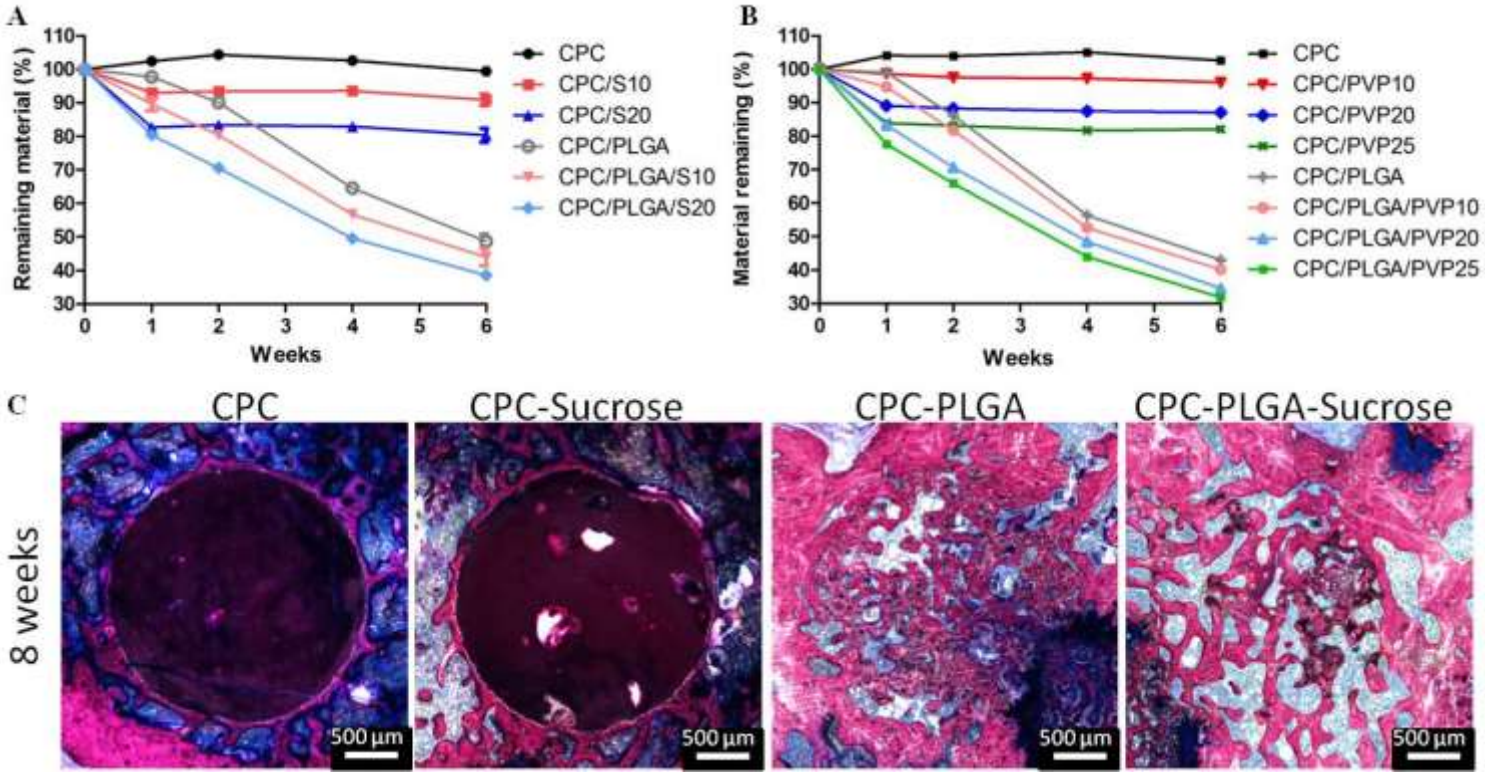

Picture 1: Caption 1: Mass loss of (A) sucrose- and (B) PVP-containing formulations. (C) Histological overview of CPC formulations implanted in a rat femoral condyle

Toward tissue-specific application of biomaterials 10:45 - 12:15 Room 0.5 13/09/2018

Oral presentation

471 Development of new methods for implant/tissue bonding and next generation of dental/bioadhesives

Edward mr Cozens
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INTRODUCTION:

Interfaces between tissues and devices or implants are playing an important role in the design of novel scaffolds for tissue engineering and other healthcare applications, such as dental or bio-adhesives. The mechanical properties of the implants and devices are important, but the bonding between an implant and the surrounding tissue should also be engineered to ensure optimal mechanical coupling. This is essential when the adhesive should sustain mechanical loads, but also to avoid detachment of the implant and formation of fibrotic or necrotic tissue. This project explores novel methods and materials for optimizing bonding between implants and soft tissues and protocols to characterise the mechanical performance of the bonding materials.

METHODS:

Bioadhesives based on functionalised Poly(Acrylic acid) were developed. A variety of functionalisation strategies, such as DCC/DMAP and DMTMM coupling[1], were used to conjugate various biomolecules to our polymers. The functionalisation levels were subsequently characterised through ^{1}H NMR. In order to enable multi-scale characterisation of the properties of the PAA-based adhesives two strategies were developed: functionalisation of free polymers allowing the bulk properties of the materials to be characterised, whereas synthesis of a polymer brush layer[2] enabled the interaction between individual polymer chains and biomolecules to be probed. Macroscale tests on the free polymer include indentation, lap shear testing, tensile bond strength tests, and rheometry. Tests on the microscale were via colloidal probe AFM, ellipsometry, and surface plasmon resonance.

RESULTS AND DISCUSSION:

PAA was functionalised with tyramine and boronic acid residues and the functionalisation levels confirmed through ^{1}H NMR, ellipsometry, XPS, and FTIR. Adhesion to model substrates, as well as cells (primary keratinocyte and HACAT cell monolayers), and soft tissues (porcine gingiva and epicardium) was studied on the microscale and macroscale. AFM adhesion tests between these surfaces and the functionalised polymer brush layers indicate that molecular interactions with the cell glycocalys dominate adhesion to cells and tissues.

CONCLUSION:

This study has identified how an understanding of the interactions between implant and soft tissue across different length scales enables a more effective and rational design of bioadhesives. Potential applications of this research include the adhesion of dentures to the oral mucosa or within the field of cardiac tissue engineering, such as the adhesion of hydrogel-based scaffolds to promote regeneration of cadiomyocytes following myocardial infarction.

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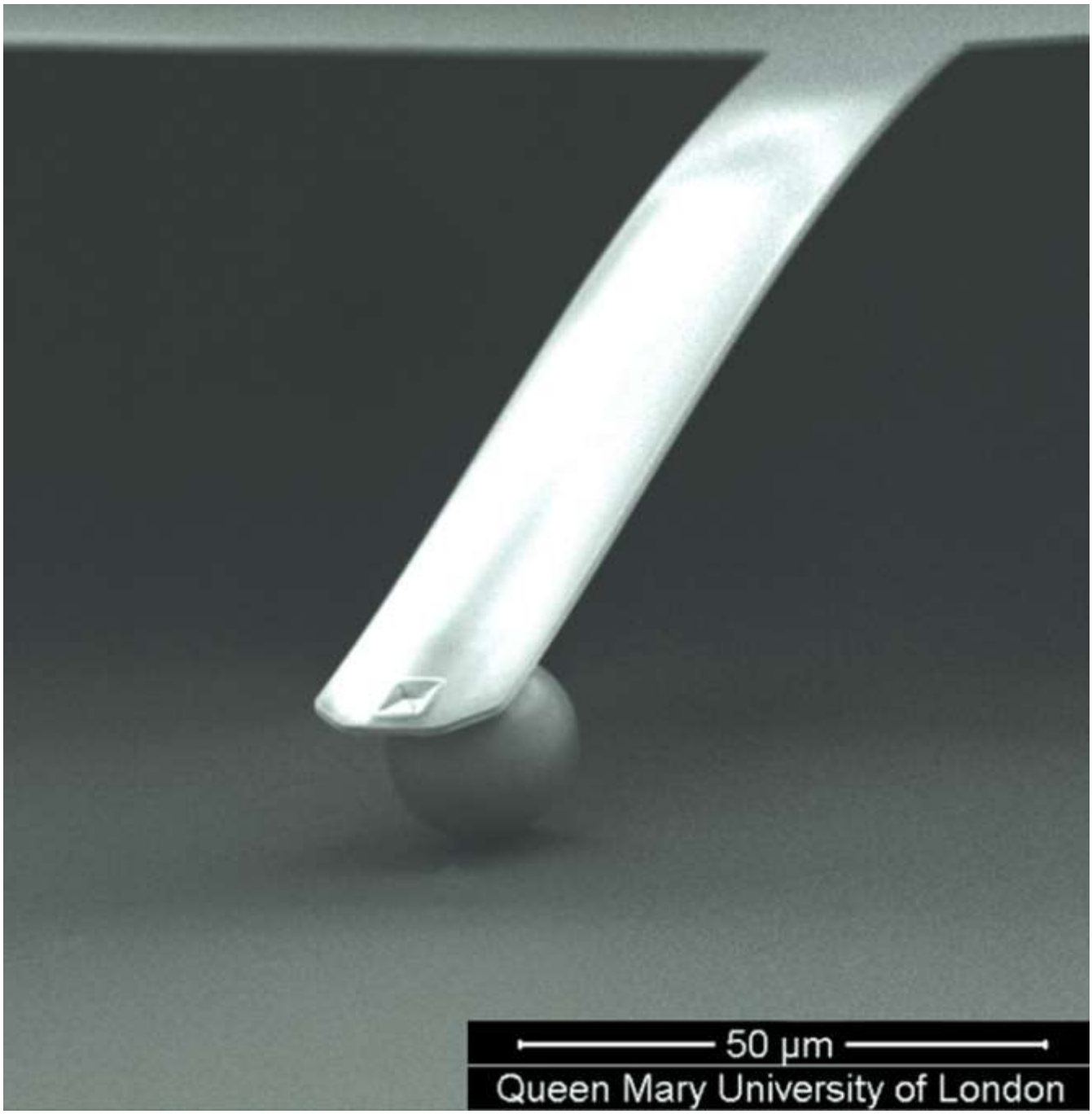
ACKNOWLEDGMENTS:

I would like to acknowledge the EPSRC (grant EP/M507532/1).



Picture 1: Caption 1: Colloidal probe AFM technique characterising the interaction between a polymer brush-functionalised probe and a functionalised silicone substrate

Toward tissue-specific application of biomaterials 10:45 - 12:15 Room 0.5 13/09/2018

Oral presentation

124 The development of multi-substituted apatite for bone tissue repair.

Naomi Lowry, Stephen Mckillop, Yisong Han, Brian Meenan, Adrian Boyd
Ulster University, Belfast, Northern Ireland

INTRODUCTION:

Global incidences of bone disorders are due to increase as populations continue to age, emphasising the need for enhanced bone substitute biomaterials[1]. The aim here was to produce multi-substituted nanoscale hydroxyapatite (nHA) biomaterials, to optimize osteoblast cell proliferation, differentiation and increase the rate of osseointegration between natural bone and nHA. The nHA was substituted with strontium (Sr^{2+}), magnesium (Mg^{2+}) and zinc (Zn^{2+}) ions in varying concentrations. Strontium increases the rate of bone formation by activating the Wnt/β-catenin signaling pathway, inducing osteoblastic differentiation and reducing osteoclast activity[2]. Zinc decreases osteoclast number and has potential antimicrobial properties[3]. Magnesium plays a role in the initial stages of osteogenesis[4]. Multi-substituted CaP systems could provide improved osteogenic potential[3, 4].

METHODS:

Single and Multi-Substituted Apatites

An acid-base reaction synthesised nHA, nHA substituted with 10/20/50wt%Sr, 10/20/50wt%Zn and 10/20/50wt%Mg, along with multi-substitutions of Sr5/Mg2/Zn2wt%nHA, Sr2.5/Mg1/Zn1wt%nHA and Sr10/Mg4/Zn4wt%nHA, with Ca/P or (Ca+M)/P ratios kept at 1.67. Suspensions were prepared using $Ca(NO_3)_2.4H_2O$, $Sr(NO_3)_2$, $Zn(NO_3)_2.6H_2O$ and/or $Mg(NO_3)_2.6H_2O$ in deionised H_2O. A solution of H_3PO_4 (85wt%) was added to each suspension and stirred for 2 hours at 60^0C with the pH kept above 9. The precipitate settled overnight, was washed 3 times with diH_2O and dried in an oven at 60^0C. All samples were characterised using x-ray photoelectron spectroscopy (XPS), Fourier transform infra-red spectroscopy (FTIR), x-ray diffraction (XRD) and transmission electron microscopy (TEM).

RESULTS AND DISCUSSION:

FTIR analysis indicated the crystallinity and purity of the nHA is as expected, XRD data confirmed that the nHA samples conform to the International Centre for Diffraction Data (ICDD) for pure HA (File# 09-0432), indicating peak shifts to the left for Sr^{2+} and to the right for Mg^{2+} and Zn^{2+}, confirming the presence of the ions, within the nHA lattice. TEM analysis confirmed the HA is nano-sized with a nano-porous appearance. XPS results confirmed a Ca/P ratio of 1.64 for nHA before substitution.

CONCLUSION:

Both single and multi-substitutions of Sr^{2+}, Mg^{2+} and Zn^{2+} have been successfully achieved within the nHA lattice, with each being fully characterised. The suitable candidate materials will undergo extensive in-vitro testing and will be brought forward to develop scaffolds using 3D printing methods.

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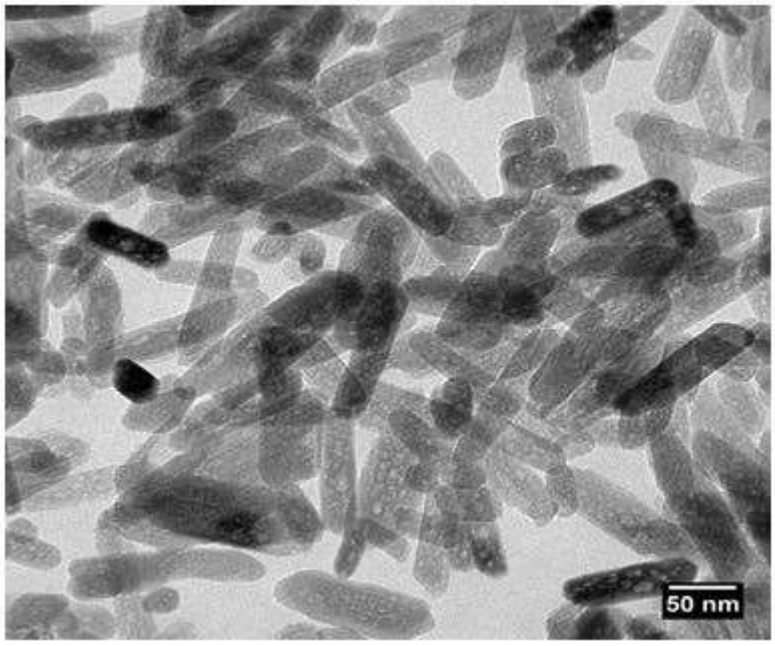
ACKNOWLEDGMENTS:

The authors would like to thank DFE for providing financial support to this project.



Picture 1: Caption 1: Figure 1 nHA TEM analysis

Toward tissue-specific application of biomaterials 10:45 - 12:15 Room 0.5 13/09/2018

Oral presentation

102 Novel in vitro method for the evaluation of bioactive materials

Weitian Zhao[1], David Michalik[2], Stephen Ferguson[3], Willy Hofstetter[4], Brigitte von Rechenberg[2], Jacques Lemaitre[1], Paul Bowen[1]
[1]EPFL, Lausanne, Switzerland
[2]University of Zurich, Switzerland
[3]ETHZ, Switzerland
[4]University of Bern, Switzerland

INTRODUCTION:

Understanding biomineralization processes carries an important medical significance. As one of its applications, the *in vivo* deposited calcium phosphate layer on bone implants can help to avoid the typical adverse host response and achieve a direct bonding with the bone. In fact, the prediction of *in vivo* implant behaviors by the use of easy-to-perform *in vitro* experimental methods is of great interest in the biomaterials research.

Previously, Kokubo proposed an *in vitro* method using simulated body fluid (SBF) by observing calcium phosphate crystal formation on implant surface after certain duration of immersion into this SBF. However, the nucleation is a subtle process that is sensitive to many external factors, often leading to inconsistent results using this method.

In this project, we proposed a new *in vitro* protocol based on a titration procedure, and the results were compared with in vitro cell culture experiments as well as in vivo animal experiments using a pelvic sheep model.

METHODS:

For the titration method, calcium solution is constantly added to the phosphate solution containing the implant material until nucleation takes place, as monitored by a calcium electrode. The degree of supersaturation needed for the nucleation provides a good indication of the biocompatibility of the material. For the animal experiments: four titanium-based surfaces with different chemical treatments were tested using a pelvic model. Bone-implant-contact area as well as torque values were measured.

RESULTS AND DISCUSSION:

The novel *in vitro* method provides a new perspective in evaluating the biocompatibility of implant materials. The collaborative *in vitro* cell culture experiments and *in vivo* experiments showed that the chemical treatments by NaOH are critical in obtaining a higher biocompatibility of the materials. There are also slight differences in cell culture experiment results and animal experiment results which can be understood by considering the different aspects each method was targeted.

CONCLUSION:

The results show that the proposed new titration method is a promising method for the evaluation of the biocompatibility of implant materials.

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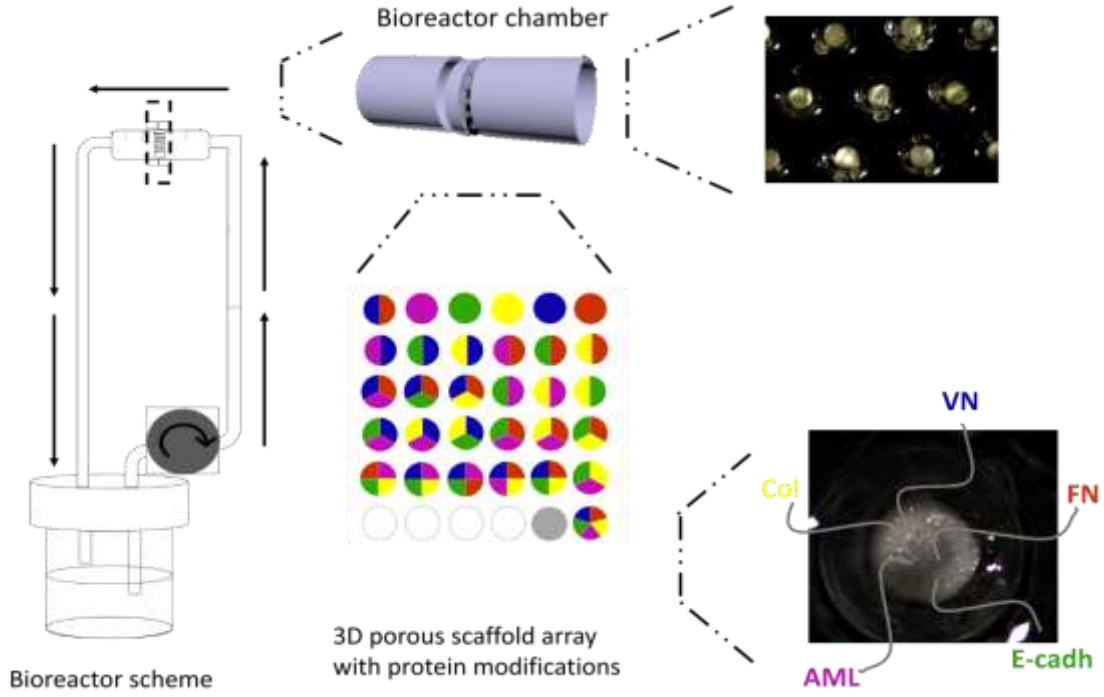
ACKNOWLEDGMENTS:

The authors would like to thank Swiss National Science Foundation (Project 150193) for providing financial support for this work.



Picture 1: Caption 1: DianaLopes_Figure

Instructive synthetic biomaterials 10:45 - 12:15 Room 0.2/0.3 13/09/2018

Oral presentation

83 Towards corneal endothelium repair using synthetic polymer membranes

Jasper van Hoorick[1], Bert van den Bogerd[2], Jürgen van Erps[3], Hugo Thienpont[3], Carina Koppen[2], Nadia Zakaria[2], Peter Dubruel[1], Sandra van Vlierberghe[1]
[1]Polymer Chemistry & Biomaterials research group, Ghent University, Ghent, Belgium
[2]Department of Opthalmology, University of Antwerp, Belgium
[3]Brussels Photonics, Vrije Universiteit Brussel, Belgium

INTRODUCTION:

Over 10 million people worldwide suffer from corneal blindness with a yearly prevalence of 1.5 million. One important cause of corneal blindness is related to dysfunction of the corneal endothelium. This monolayer of cells controls corneal hydration via a pump-and-leak mechanism. When the monolayer is damaged, these cells migrate and enlarge to cover the tissue defect since they cannot undergo mitosis. Following ageing, trauma or disease, cell density drops below a critical threshold leading to insufficient pumping, thereby causing edema. Concomitant loss of the organized collagen structure results in opacity, thereby impairing vision. Currently, the only treatment is corneal endothelial transplantation using cadaveric donor eye tissue. Unfortunately, supply does not meet demand, thereby underlining the need for synthetic ex vivo manufactured biomimetic grafts.

METHODS:

Transparent membranes were produced using multi-layer spincoating. A Gelatin base layer enables post-production membrane release. Poly (D,L-lactide)(PDLLA) was applied in combination with a UV-crosslinked functionalized gelatin. An argon plasma treatment was performed prior to gelatin deposition enabling covalent attachment. Different gelatin derivatives were evaluated using radical and thiol-ene crosslinking mechanisms. The membranes were characterized using glucose diffusion assays, optical profilometry, transparency measurements, X-ray photoelectron spectroscopy (XPS) and *in vitro* biological screening using primary corneal endothelial cells.

RESULTS AND DISCUSSION:

Amorphous PDLLA was selected to provide mechanical integrity while exhibiting proper transparency. *In vitro* biological assays indicated that PDLLA does not provide cell interactivity. Therefore, a crosslinked gelatin coating was applied to mimic the extracellular matrix (ECM), which was confirmed using XPS. The membranes were transparent throughout the visual spectrum (> 92%) with thicknesses ranging from 0.8 to 3.6 µm thereby complying to the dimensions of natural Descemet's membranes (<10 µm). Furthermore, membrane glucose diffusion (P = 0.0616 cm/s) was observed, an important prerequisite for corneal endothelial grafts. Finally, the membranes were seeded with primary corneal endothelial cells to assess their phenotype. A characteristic hexagonal shape was observed while immunocytochemistry showed the expression of Na^+/K^+ ATPase and ZO-1, hallmark proteins of healthy functional endothelial cells.

CONCLUSION:

In the present work, ultrathin membranes were constructed using both PDLLA for mechanical integrity and gelatin as ECM mimic. The membranes exhibited proper transparency, glucose permeability and cytocompatibility. The membranes should allow corneal microsurgery and hold promise to alleviate donor shortage.

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ACKNOWLEDGMENTS:

JVH and BVdB are both FWO SB-fellowship students.

Instructive synthetic biomaterials 10:45 - 12:15 Room 0.2/0.3 13/09/2018

Oral presentation

625 Degradable Hydrogels Based on Star Shaped Copolypeptides: From Synthesis to 3D printing

Andreas Heise, Robert Murphy, Sally-Ann Cryan
Royal College of Surgeons In Ireland, Dublin, Ireland

INTRODUCTION:

The development of defined three-dimensional (3D) scafolds for tissue engineering has been a recent emergence within the field of regenerative medicine[1]. However, the limited number of suitable bio-inks has been identified as the major barrier to progress[1]. We have previously reported on the excellent hydrogel properties of amphiphilic star polypeptides[2]. Here we present a star copolypeptide based hydrogel ink capable of structural microfabrication using 3D extrusion printing.

RESULTS AND DISCUSSION:

Star block copolypeptides were obtained by the polymerization of amino acid N-carboxyanhydrides (NCA) from 32-arm initiator composed of a 35mer of glutamic acid, chain extended with a 5mer of valine. After deprotection, these amphiphilic star block copolypeptides spontaneously form hydrogels. After subjecting the hydrogel to steps in strain (between 0.1% and 100%), the modulus recovered rapidly to 1522 ± 10 Pa. Once strain is reduced, rapid self-recovery of the hydrogel network occurs (30 seconds) as the bulk flow of the material is unhindered by the strain process. 3D printing of hydrogels was carried out using a 3D printer comprised of a precision positioning system and an ink extrusion system yielding well resolved constructs. All tested hydrogel formulations could form self-supporting micron sized structures at heights of up to 12 layers demonstrating their remarkable mechanical robustness. Hydrogel inks were loaded with doxorubicin hydrochloride (DOX.HCl) and 3D extrusion into rings was carried out. DOX.HCl loaded rings exhibited an initial burst release (28 ± 3 %) followed by a steady release up to 350 hours. The cytotoxicity of polymer constructs was investigated by incubating Balb/3T3 cells in the presence of extracted leachates. It was found that the cells do not suffer from a reduced metabolic state for the optimised materials.

CONCLUSION:

Novel degradable, non-toxic hydrogels based on amphiphilic star block copolypeptide capable of structural microfabrication using 3D extrusion printing were disclosed. The versatility of the material can allow for modulation of hydrogel ink properties, thus providing a plethora of possibilities for optimized tissue construction using 3D printing.

REFERENCES:

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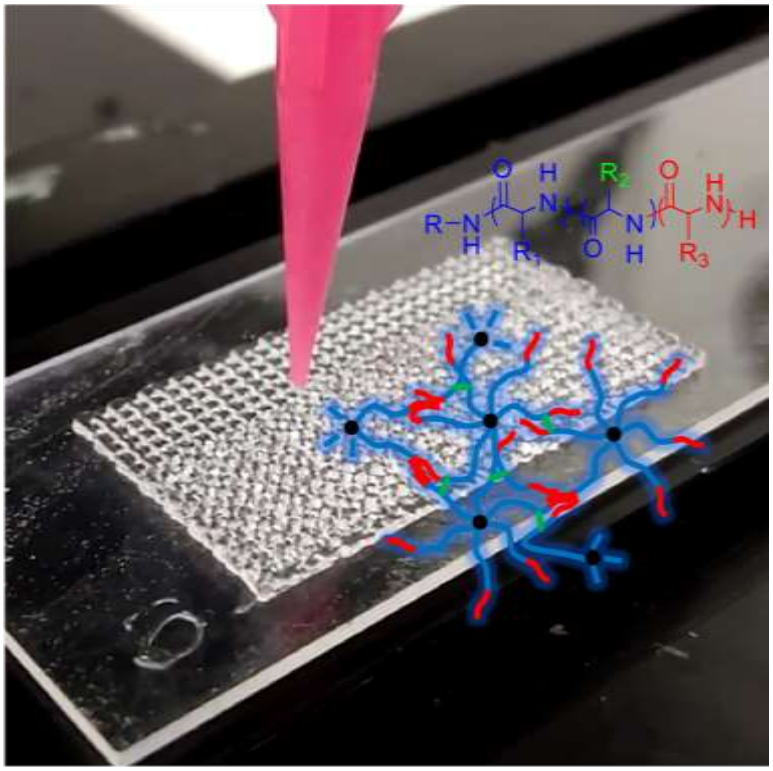

Picture 1: Caption 1: 3D printing of star polypeptide hydrogel

Instructive synthetic biomaterials 10:45 - 12:15 Room 0.2/0.3 13/09/2018

Oral presentation

544 Hyperbaric oxygen-generating hydrogels for facilitating wound healing process

Kyung Min Park[1], Heungsoo Shin[2], Sujin Park[1]
[1]Incheon National University, Incheon, South-Korea
[2]Hanyang University, South-Korea

INTRODUCTION:

Recently, it is demonstrated that hyperbaric oxygen plays pivotal roles in facilitating wound healing process and vascular development. Herein, we report hyperbaric oxygen-generating (HOG) hydrogels that serve as an injectable and dynamic matrices for enhanced wound healing as well as neovascularization. We designed tiolated-gelagin (GtnSH) that can form hydrogel networks *via* calcium peroxide (CaO_2)-mediated crosslinking reaction with oxygen generation. We assessed the effect of HOG hydrogels on human umbilical vein endothelial cells (HUVECs) activities *in vitro* as well as neovascularization *in vivo*.

METHODS:

We synthesized GtnSH by conjugating Traut's reagent (TR). The HOG hydrogels were fabricated by simply mixing GtnSH and CaO_2 solutions. To measure oxygen levels, we monitored dissolved oxygen (DO) levels using commercially available oxygen sensor. For cytocompatibility study, we cultured human dermal fibroblasts (HDFs) with the hydrogel eluate and analyzed cell viability by WST-1 and live/dead assay after 24 hours of culture. To investigate the effect of HOG hydrogels on cellular activities, we cultured HUVECs with different types of hydrogels. To evaluate *in vivo* effect of HOG hydrogels, we implanted hydrogels under mice subcutaneous tissues and incubated them up to 7 days.

RESULTS AND DISCUSSION:

We demonstrate that the HOG hydrogels formed *via* CaO_2-mediated oxidative crosslinking reaction and the hydrogels rapidly generate the hyperbaric oxygen levels (up to 91.2% pO_2). We next evaluated the cytotoxicity of HOG hydrogels using HDFs, showing excellent cell viability except G5C0.75. Based on the results, we selected optimized hydrogel samples for *in vitro* and *in vivo* studies (G5C0.25: hyperbaric gel, HG; G5C0: normoxic gel, NG). In addition, we investigated the effect of oxygen release on HUVEC proliferation, resulting in enhanced cell proliferative activities (107.4% for HG) compared to the control groups (100% for tissue culture polystyrene and NG). Furthermore, to analyze *in vivo* angiogenic effect, we transplanted the hydrogels into subcutaneous tissues of mice for 7 days. Interestingly, we observed that HG matrices could promote tissue infiltration and neovascularization from the host tissue compared to control groups (NG).

CONCLUSION:

We developed gelatin-based HOG hydrogels as dynamic matrices that can stimulate surrounding cells or tissue for facilitating wound healing as well as neovascularization. We suggest that our HOG hydrogels hold a great potential as advanced injectable matrices for the treatment of wound healing, vascular disorders as well as tissue regeneration.

REFERENCES:

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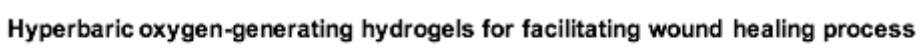
ACKNOWLEDGMENTS:

This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government (MSIP) (NRF-2015R1C1A1A01054498)

Picture 1:



Instructive synthetic biomaterials 10:45 - 12:15 Room 0.2/0.3 13/09/2018

Oral presentation

579 Oxygen-generating alginate hydrogels for enhanced wound healing process

Jeonil Kang, Kyung Min Park
Incheon national university, Incheon, South-Korea

INTRODUCTION:

Alginate hydrogels are widely used as an injectable material for a broad range of biomedical applications due to their biocompatibility and easy fabrication *via* ionotropic interaction[1]. Recently, growing evidence have demonstrated that hyperbaric oxygen (O_2) facilitates wound healing process and neovascularization through acute oxidative stress[2]. Herein, we present injectable and hyperbaric O_2-generating alginate (OGA) hydrogels *via* calcium peroxide (CaO_2) mediated crosslinking. We characterized their physico-chemical and biological properties, and investigated *in vivo* effect on wound healing process.

METHODS:

We fabricated OGA hydrogels by simply mixing alginate and CaO_2 solutions. Elastic modulus (*G'*) of OGA hydrogels were measured by using rheometric fluid spectrometer. We monitored dissolved O_2 (DO) levels using commercially available O_2 senor. Cytocompatibility of OGA hydrogels was investigated using human dermal fibroblasts (HDFs) incubated with the diluted hydrogel eluates, and WST-1 and live/dead assay. *In vivo* effects on wound healing were evaluated by subcutaneous injection and wound closing test for 2 weeks. Hydrogel explants with the surrounding tissues were obtained for histological analysis.

RESULTS AND DISCUSSION:

The OGA hydrogels formed *via* CaO_2-mediated ionotropic crosslinking (Fig. 1a). We measured the time-course *G'* of the OGA hydrogels depending on alginate (0.5-3.0 wt%) and CaO_2 (0-0.9 wt%) concentrations, showing controllable mechanical properties (2-3900 Pa). We next assessed cytocompatibility of OGA hydrogels, resulting in excellent cell viability (105.4-110.2% compared to control group). To investigate the *in vivo* applicability, we monitored *in vivo* O_2-releasing behavior depending on CaO_2 concentrations, demonstrating hyperbaric gels (HG, A2C0.75) could rapidly generate hyperbaric oxygen (up to 97.2% pO_2) and maintain for 5 hours (Fig. 1b). In addition, we performed wound closing test using critical wound defect models. Interestingly, we found that HG group facilitated wound closing with tissue infiltration and neovascularization compared to the normoxic gels (NG, A2C0) (Fig. 1c, d).

CONCLUSION:

We developed an *in situ* crosslinkable and oxygen-generating alginate hydrogels *via* ionotropic crosslinking reaction using CaO_2. Our hydrogels promoted wound healing process with tissue infiltration and neovascularization, suggesting that it has a great potential as an injectable and oxygen-generating material for treatment of vascular disorders and tissue regenerative medicine.

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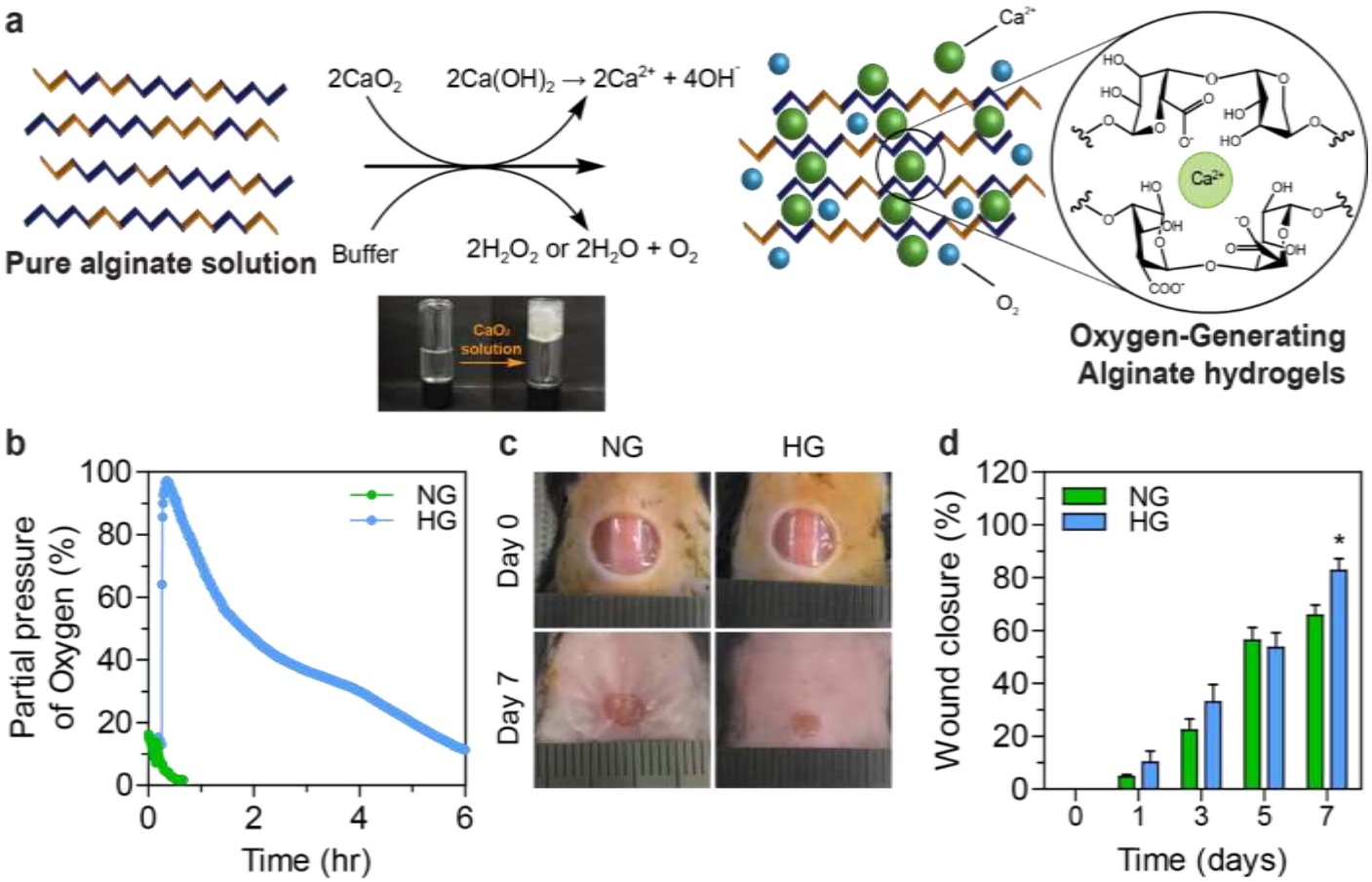
ACKNOWLEDGMENTS:

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Ministry of Science, ICT & Future Planning (2015R1A2A1A14027221) and the NRF grant funded by the Korea government (MSIP) (NRF-2015R1C1A1A01054498).



Picture 1: Caption 1: Schematic representation of OGA hydrogel system (a). In vivo O2-releasing behavior (b). In vivo effects of OGA hydrogel on wound healing (c,d).

Instructive synthetic biomaterials 10:45 - 12:15 Room 0.2/0.3 13/09/2018

Oral presentation

73 Synergistic effect of polymer molecular weight and Laponite concentration to tune the cell response of synthetic hydrogels

Arn Mignon[1], Daniele Pezzoli[2], Emilie Prouvé[1], Diego Mantovani[2], Sandra van Vlierberghe[1], Peter Dubruel[1]
[1]Universiteit Gent, Gent, Belgium
[2]Laval University, Canada

INTRODUCTION:

Despite being biocompatible, synthetic polymers are often not cell-interactive. By varying the physico-chemical properties of synthetic hydrogels and introducing additives, the cell response can be tuned. Acrylate-endcapped urethane-based poly(ethylene glycol) precursors (AUPs) [1] with a varying backbone molecular weight (2000-8000 g/mol), in combination with the nanoclay Laponite were selected herein. Both backbone MW and Laponite concentration were varied with the aim to tune the mechanical and *in vitro* cell response of the formed hydrogel networks.

METHODS:

Laponite was introduced into the AUP solution (0.5 or 1 wt%) prior to hydrogel formation. Gel fraction and swelling tests were performed on crosslinked films. Mechanical properties were determined by tensile tests , stress relaxation and rheology. Primary neonatal human dermal fibroblasts were used to perform indirect cytotoxicity tests along with cell adhesion and proliferation assays (using methacrylamide-modified gelatin as positive control) [2].

RESULTS AND DISCUSSION:

High gel fractions close to 100% were obtained. Swelling properties ranged between 4 and 11 $g_{water}/g_{polymer}$ with only a small significant reduction upon addition of Laponite. An increase in backbone MW induced a decrease in the storage modulus (from 58 down to 20 kPa). Young's moduli ranged between 0.1 and 0.6 MPa. The addition of Laponite influenced the modulus due to the distribution of nanoparticles as well as shear thinning behavior [3, 4]. Finally, no cytotoxicity was observed. Laponite led to a substantial increase of both cell adhesion and proliferation, but only at a low MW backbone (2000 g/mol), thereby demonstrating the tunability of the cell-adhesion properties, confirmed by fluorescence microscopy.

CONCLUSION:

Addition of Laponite and variation of the MW of the AUP backbone resulted in a synergistic effect on the cell-adhesive properties. This creates opportunities for non-adherent biomedical applications such as wound dressings or cell adherent applications including tissue engineering scaffolds.

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Instructive synthetic biomaterials 10:45 - 12:15 Room 0.2/0.3 13/09/2018

Oral presentation

425 Synthetic Light-Curable Polymeric Materials Provide a Supportive Niche for Dental Pulp Stem Cells

Adam Celiz[1], Kyle Vining[2], Jacob Scherba[2], Alaina Bever[2], Morgan Alexander[3], David Mooney[2]
[1]Imperial College London, London, United Kingdom
[2]Harvard University, United States of America
[3]University of Nottingham, United Kingdom

INTRODUCTION:

Dental disease annually affects billions of patients, and while regenerative dentistry aims to heal dental tissue after injury, existing polymeric restorative materials do not directly participate in the healing process in a bio-instructive manner. Thus, there is a need for restorative materials that can support native functions of dental pulp stem cells (DPSCs), which are capable of regenerating dentin.

METHODS:

High-throughput screening and scaling-up polymer chemistries: Briefly, 119 commercially available monomers of 250-400 μm diameter were printed and photo-polymerized on polyHEMA coated slides in microarrays, with six replicates per array. For scale-up, candidate monomers from the screen were prepared with thiol-ene crosslinking reagents at a molar ratio of 1.4:1 and 1% w/v of photo-initiator 2,2-dimethoxy-2-phenylacetophenone.

RESULTS AND DISCUSSION:

We use high-throughput screening using polymer microarrays formed from commercially-available monomers to identify materials that support DPSC adhesion[1]. Based on these findings, we employ thiol-ene chemistry to achieve rapid light-curing and minimize residual monomer of the lead materials. Several triacrylate bulk polymers support DPSC adhesion, proliferation, and differentiation in vitro, and exhibit stiffness and tensile strength similar to existing dental materials (Figure 1). Conversely, materials composed of a trimethacrylate monomer or BisGMA, which is a monomer standard in dental materials, do not support stem cell adhesion and negatively impact matrix and signaling pathways. Furthermore, thiol-ene polymerized triacrylates are used as permanent filling materials at the dentin-pulp interface in direct contact with irreversibly injured pulp tissue. These novel triacrylate-based biomaterials have potential to enable novel regenerative dental therapies in the clinic by both restoring teeth and providing a supportive niche for DPSCs.

CONCLUSION:

Triacrylate-based materials have significant promise as bio-instructive restorative materials for regenerative dentistry. They can be cured rapidly into bulk polymers without detectable residual monomer, provide robust mechanical properties, support DPSC regenerative-related functions, and can be placed directly at the dentin-pulp interface in a severe model of dental pulp injuries.

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ACKNOWLEDGMENTS:

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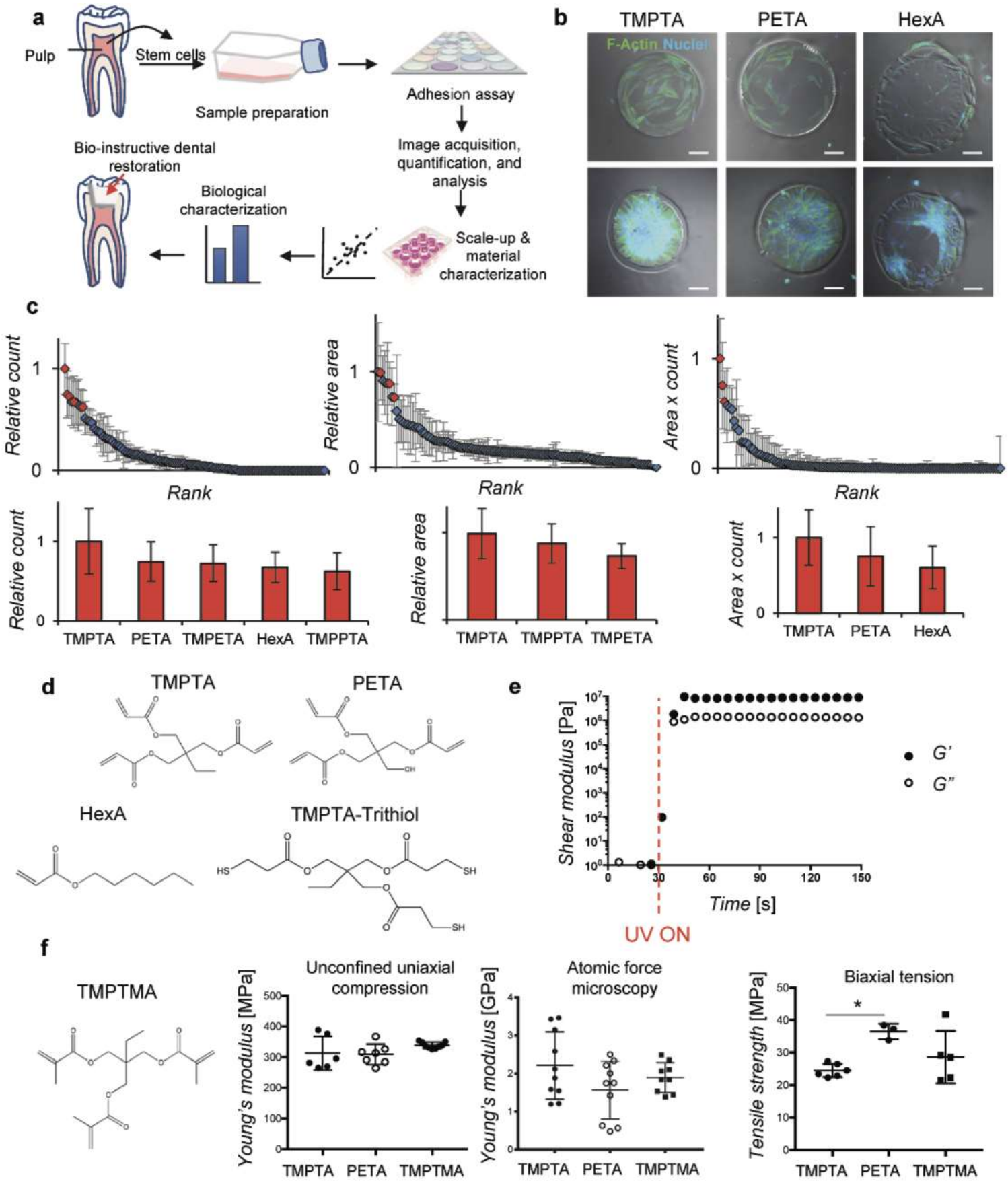

Picture 1: Caption 1: Figure 1. Rapid identification, scale up and characterization of triacrylate polymers as candidate bioinstructive dental restorative materials.

Poster session A

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

3 A rat study for various cross-linked collagen scaffolds in tissue regeneration of wounds

Pei-Ni Huang[1], Yi-Ting Wang[1], Hsiao-Chen Yen[1], Kwang-Yi Tung[2]
[1]HAN.BIOMEDICAL INC., New taipei city, Taiwan
[2]Mackay Memorial Hospital, Taiwan

INTRODUCTION:

Repair of wounds is a dynamic and complicate process and its pathways are including “scar” tissue formation and “regeneration”. Regeneration means the architecture of repaired tissue would be almost same with the preexisting tissue. The key factors for accelerating tissue regeneration are including cells, growth factors as well as an optimal structural scaffold[1]. The architecture of the porous scaffold should be similar to normal extracellular matrix, that it could provide an optimal template for cell growth and also a mechanical support for controlling wound closure; both characteristics result in tissue regeneration of wounds[2]. Collagen can induce angiogenesis and tissue growth during wound repair process that it is one of the optimal materials for producing porous scaffolds [3]. The study was investigating the efficacy of various collagen scaffolds with different cross-linked process in tissue regeneration in rats.

METHODS:

Rats were divided into three groups for three collagen scaffolds with different cross-linked method including DHT, UV and EDC[4]. Each rat was created two wounds of 2*2 cm size on its dorsal site, right side was as control covered with Tegaderm, and left side was implanted with 2.5*2.5*0.3 cm collagen scaffold and then covered with Tegaderm. The data of wound size from three rats of each group were recorded and tissue of the wound site was collected for pathology check after 28 days of operation. ANOVA and T-test were used for statistical analysis.(Significant variance: P value< 0.05)

RESULTS AND DISCUSSION:

For the wound contraction rate of day 28, two physical cross-linked scaffolds were same with controls and the EDC group is 20-30% lower than controls. The pathology results showed that inflammation and angiogenesis in the EDC group. Besides, the newly-formed collagen randomly distributed in the scaffold groups which were similar to normal tissue.(Fig 1.).

CONCLUSION:

Our data showed that collagen scaffolds with right structure and mechanical properties could promote the tissue regeneration of wounds.

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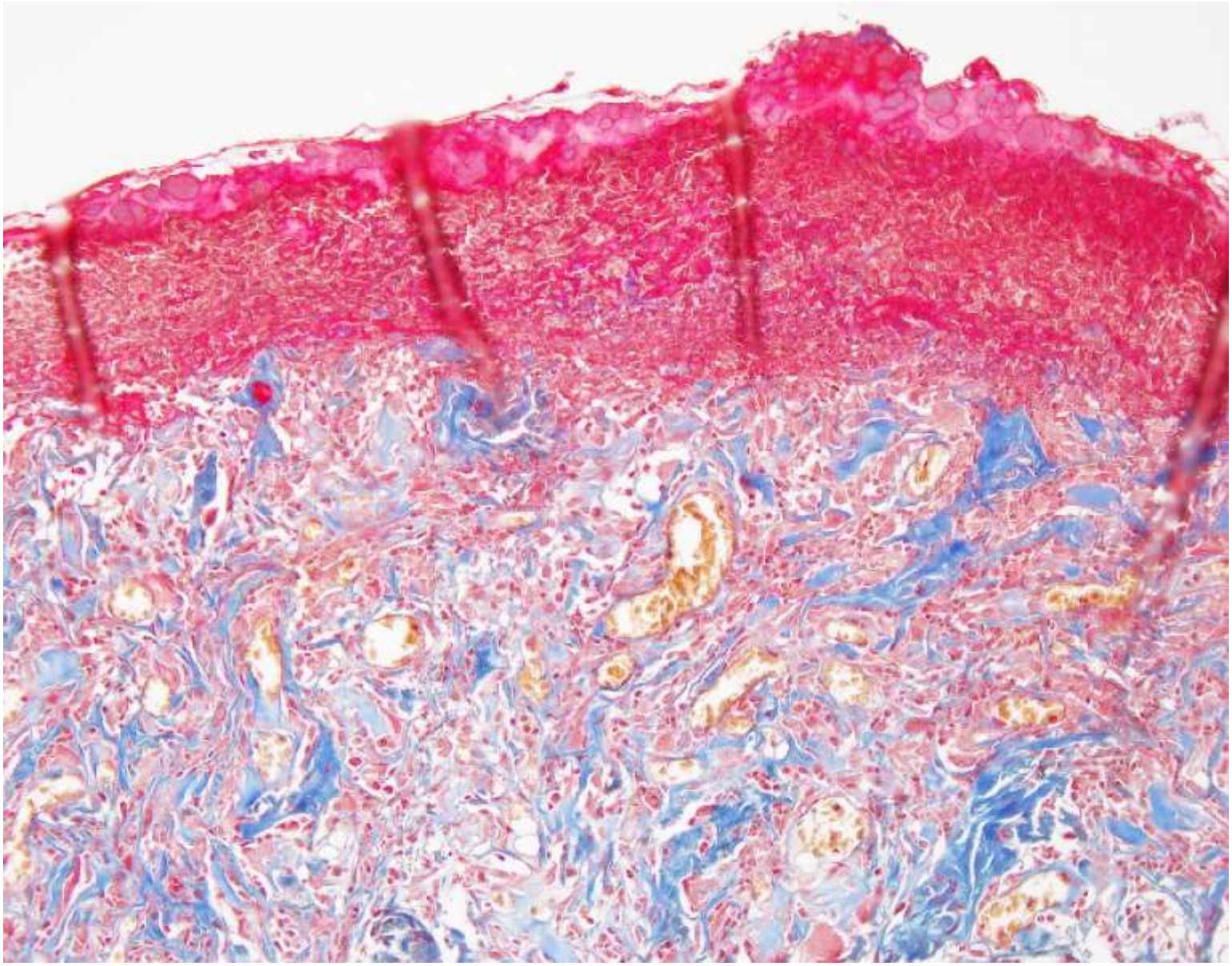
ACKNOWLEDGMENTS:

The authors would like to thank Mackey Memorial Hospital Laboratory Animal Center and

Mackey Memorial Hospital Dr.Chin-Lung Chang for providing support to this project.

Picture 1:



Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

10 Controlled release of drug using gold cluster-labeled liposomal doxorubicin into chitosan hydrogel by NIR irradiation for cancer therapy

Ji Eun Won[1], Min Gi Kim[2], Tae In Wi[3], Yeongseon Byeon[3], Ga Hee Kim[3], Jea Myeong Lee[3], Byung Cheol Shin[4], Yeong Min Park Park[3], Hee Dong Han[3]
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INTRODUCTION:

Liposomes has been widely studied as potential carrier for drug delivery systems[1]. Although the delivery system has been developed, an effective carrier system for controlled drug release is needed to achieve therapeutic efficacy[2]. Therefore, we developed gold cluster-labeled thermosensitive liposomal doxorubicin (GTSL-DOX) entrapped into chitosan hydrogel (CH-HG) system for controlled release of drug by NIR irradiation.

METHODS:

Size and zeta potential of GTSL-DOX were measured by light scattering with a particle size analyzer and Zeta Plus. Release of DOX from GTSL in CH-HG was measured by fluorescence spectrophotometry. The cytotoxicity of GTSL-DOX with or without NIR irradiation was determined by MTT assay. Diffusion distance for DOX release from GTSL with NIR irradiation was determined in 10% agarose gel. Therapeutic efficacy was examined in B16F10 tumor-bearing mice.

RESULTS AND DISCUSSION:

Size and surface charge of GTSL-DOX was 169 ± 6 nm and –50.5 ± 0.5 mV, respectively. Release of DOX from GTSL-DOX was increased with NIR irradiation (1.00 W/cm^2) compared to non-NIR irradiation. Cell cytotoxicity of GTSL-DOX was highly increased combined with NIR irradiation in B16F10 cells compared to non-NIR irradiation. Distance of released DOX from GTSL-DOX with NIR irradiation (3.00 W/cm^2) was extended 83% compared to non-NIR irradiation group. Therapeutic efficacy of CH-HG containing GTSL-DOX combined with NIR irradiation resulted in significant inhibition of tumor growth compared to CH-HG containing DOX ($p < 0.005$) and CH-HG containing GTSL-DOX without NIR irradiation ($p < 0.001$) against B16F10 tumor model.

CONCLUSION:

Taken together, CH-HG containing GTSL-DOX has the potential for controlled drug release by NIR irradiation in tumor microenvironment, and may be applied to a broad range for cancer therapies.

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ACKNOWLEDGMENTS:

This work was supported by Basic Research Laboratory Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (No.2013R1A4A1069575).

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

11 Enhanced antitumor immunity using a tumor cell lysate-encapsulated CO2 generatiEnhanced antitumor immunity using a tumor cell lysate-encapsulated CO2 generating liposomal carrier system and photothermal irradiationng liposomal carrier system and photothermal irradiation

Tae In Wi, Ji Eun Won, Yeongseon Byeon, Jae Myeong Lee, Yeong-Min Park, Hee Dong Han
Konkuk university, Seoul, South Africa

INTRODUCTION:

Dendritic cell (DC)-based cancer immunotherapies have been extensively studied to eradicate cancer[1,2]. In cancer immunotherapy, the key first step is delivery of the tumor antigen, leading to maturation and activation of DCs. To enhance antigen delivery, liposome-based delivery of tumor-specific antigens has been extensively explored. Although liposomes provide a promising strategy to increase delivery efficiency, triggered release of payload from liposomes is required to achieve an optimum immune response. Here, we developed whole tumor cell lysate (TCL) encapsulating bubble-generating themosensitive liposomes (BG-TSLs), which are capable of triggered release of TCL by near-infrared (NIR) irradiation in tumor microenvironment.

METHODS:

Physical properties of liposomes were measured by light scattering with a particle size analyzer and Zeta Plus. Release of DOX or TCL from TSLs or BG-TSLs with NIR irradiation was determined by UV-spectroscopy or BCA protein assay kit. The intracellular uptake of TSL and BG-TSLs in B16F10 cells was assessed by flow cytometry and confocal microscopy. Cell viability of TSL and BG-TSLs was determined by MTT assay. CO_2 generation into B16F10 cells containing BG-TSLs by NIR irradiation was monitored using ultrasound imaging system with a 7 MHz transducer. To determine the effectiveness of therapeutic efficacy of combination for DOX-BG-TSL and TCL-BG-TSL, we assessed the antitumor effects using the B16F10 melanoma tumor model.

RESULTS AND DISCUSSION:

TCL-BG-TSLs and DOX-BG-TSLs were 115.23 ± 6.78 nm and 134.45 ± 1.42 nm in mean particle size, and surface charge was -21 mV and -29 mV, respectively. The TCL-BG-TSLs and DOX-BG-TSLs enhanced burst release of TCL or DOX by bubble generation into liposomes stimulated with NIR irradiation compared to non-BG-TSLs. Moreover, the releases of TCL from TCL-BG-TSLs promoted dendritic cell maturation and activation, leading to emergence of antigen-specific cytotoxic CD8+ T cells. Combination therapy of TCL-BG-TSLs and DOX-BG-TSLs showed significantly greater antitumor efficacy in B16F10 tumor-bearing mice compared to control ($p< 0.01$).

CONCLUSION:

We demonstrate that a novel liposomal delivery system combined with NIR irradiation leads to potent immunotherapy efficacy in tumor-bearing mice. This approach has broad utility for enhancing the therapeutic effects on cancer immunotherapy.

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ACKNOWLEDGMENTS:

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

15 Synthesis of polysaccharides conjugated copolymers with intelligent CD44 receptor targeting

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INTRODUCTION:

Poly(D,L-lactide-co-glycolide)-poly(ethylene glycol) (PLGA-PEG) copolymer has been applied as drug and gene delivery nano-carriers with passive targeting character. The nanoparticles (NPs) with active targeting property are usually designed by chemical conjugation of targeting ligands which recognize the specific high-expressed receptors on the cancer cell surface.[1] Hyaluronic acid (HA) and chondroitin (CD) are endogenous polysaccharides existed in the extracellular matrix and have been found possessing specific binding to CD44 receptors which are overexpressed in several types of tumors.[2]

METHODS:

The PLGA-PEG copolymer possessing CD44 specific binding function was designed via chemical conjugation of HA and CD. The major functional groups of synthesized HA-PEG-PLGA and CD-PEG-PLGA copolymers were identified by FTIR and the compositions were confirmed by ^{1}H-NMR. The cytotoxicity was investigated in L929 normal cells and CD44-positive U87 cancer cells. The competition study between polysaccharide-conjugated NPs and polysaccharides was further performed in U87 cells.

RESULTS AND DISCUSSION:

The HA-PEG-PLGA and CD-PEG-PLGA copolymers were synthesized and confirmed by ^{1}H-NMR and FTIR. The negative-charged HA-PEG-PLGA-D/P and CD-PEG-PLGA-D/P NPs had particle size 270.2 ± 13.8 nm and 186.8 ± 21.7 nm, respectively, with narrow size distribution. The CD-PEG-PLGA-D/P NPs had cellular viability similar to HA-PEG-PLGA-D/P NPs in U87 cells (78.03 ± 8.39% vs 83.13 ± 6.82%) but higher than HA-PEG-PLGA-D/P NPs in L929 cells (113.08 ± 26.96% vs 82.44 ± 16.73%). The cellular transfection of HA-PEG-PLGA-D/P and CD-PEG-PLGA-D/P NPs in the presence of HA or CD were significantly reduced to 22.8 ± 0.8% and 8.1 ± 0.3%, respectively, in CD44 high-expressed U87 cells.

CONCLUSION:

The synthesized polysaccharide-conjugated PLGA-PEG copolymers exhibited comparable cellular viability higher than 70%. Through the competition study provided the evidence of cellular uptake of NPs via specific binding of HA and CD to CD44 receptors on U87 cancer cells.

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

22 Biocompatibility of porous PHB/CHIT scaffolds and using the CAM model

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INTRODUCTION:

Animal models play a key role in basic medical research. Advances in the area of development of biomaterials and regenerative medicine to a great extent depend upon in vitro and following in vivo experiments, which lead to the creation of new therapeutic approaches[4]. This study describes a new approach for testing the biological activity of porous PHB/CHIT biomaterial in ovo using the chorioallantoic membrane (CAM) of the quail embryo, as an alternative to the traditional mammalian models[1].

METHODS:

Fertilized Japanese quail eggs were incubated at 38.2°C and 55% relative humidity. On embryonic day 10 (ED10), Dent´s solution was applied directly on the CAM with implant and surrounding tissue. Subsequently, implant was removed and processed with histological and immunohistochemical methods. For the evidence of CAM microvilli as well as proliferating cells inside of implant, we used light, electron (SEM method), and confocal microscope.

RESULTS AND DISCUSSION:

The biomaterial was incorporated within CAM quite well 7 days after the implantation on the top of CAM. Hyperplasia of the CAM tissues under the implanting scaffolds was observed. Epithelial cells from the endoderm of the CAM proliferate and migrate into the scaffold forming fusional boundaries between the scaffolds and CAM tissues. At the same time, CAM model allows the continual monitoring of the biomaterial tested, which makes this method very attractive for rapid in ovo/ex ovo evaluation of the tested biomaterial[2]. But there are just a few studies in relation to the chitosan-based scaffolds and angiogenesis in the field of bone tissue engineering[3].

CONCLUSION:

Our observation suggested to biocompatibility of PHB/CHIT biomaterials using the quail CAM animal model. Thanks to these methods we can show an evidence of CAM tissue incorporation with porous PHB/CHIT biomaterial. Further, proliferating cells were present inside of biomaterial pores.

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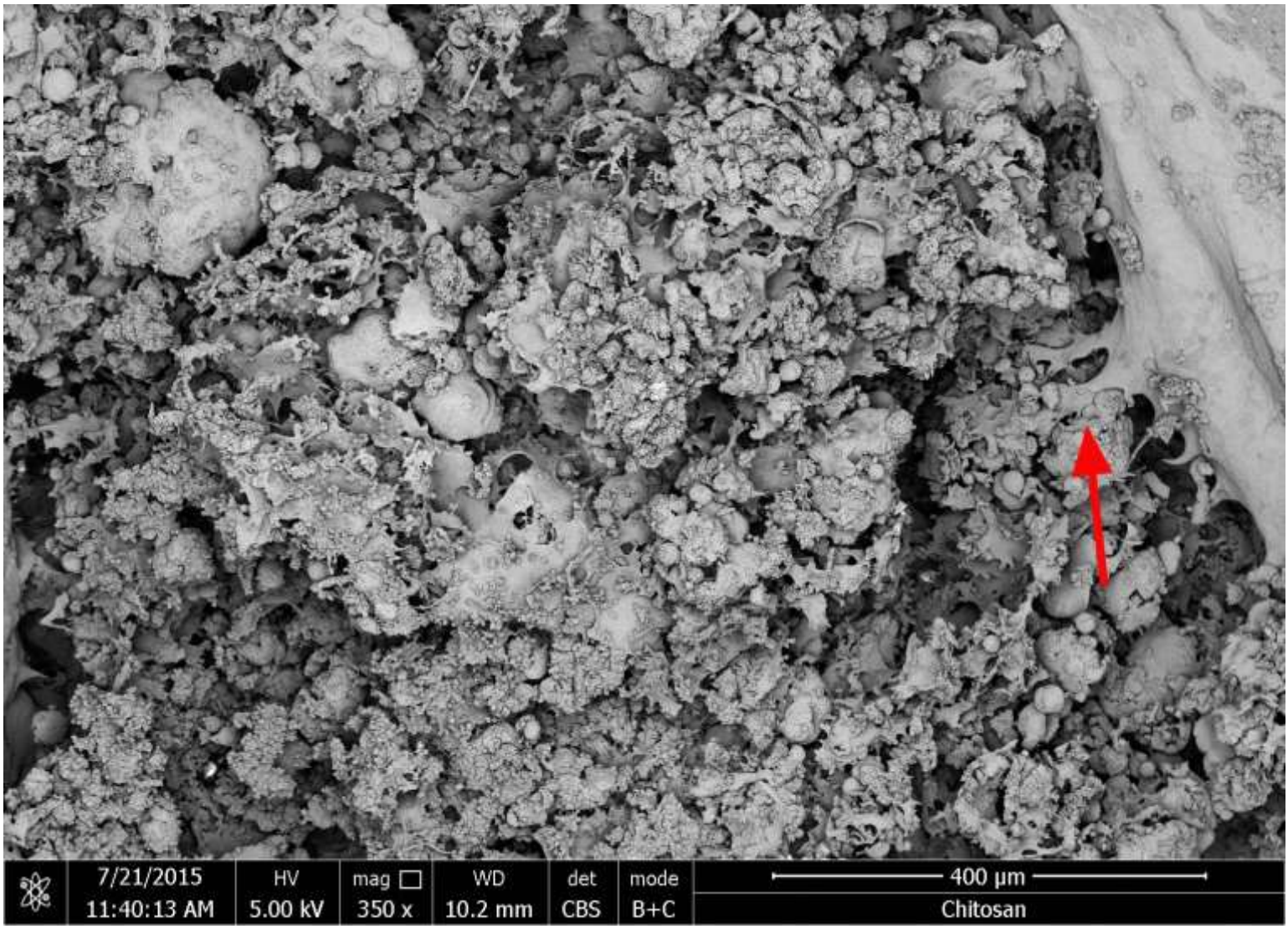
ACKNOWLEDGMENTS:

This work was realized within the framework of the project VEGA No. 1/0046/16.



Picture 1: Caption 1: Evidence of CAM microvilli using electron microscope (SEM), scale bar 400 µm

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

23 Angiogenesis evaluation inside of porous PHB/CHIT scaffolds using the CAM model

Lenka Luptakova, Eva Petrovova, Jana Teleky, Marek Tomco, Ludmila Balazova
University of Veterinary Medicine and Pharmacy, Kosice, Slovak Republic

INTRODUCTION:

Angiogenesis is a characteristic sign in the process of regeneration of normal tissue and hence is one of the important factors for the safe and successful use of biomaterials in regenerative medicine[1]. The chorioallantoic membrane (CAM) has a very dense capillary network, and it is used to study *in vivo* angiogenesis[4]. In our study, we used the quail CAM animal model for the first evidence of implantation modified porous PHB/CHIT biomaterial that is using in bone regenerative medicine.

METHODS:

Fertilized Japanese quail eggs were incubated at 38.2°C and 55% relative humidity. On embryonic day 10 (ED10), implant was removed and processed with immunohistochemical methods. For the evidence of vessels and endothelial cells outside/inside of implants, we used the stereomicroscope Leica MZ125, and confocal microscope Olympus FV-1000 BX61.

RESULTS AND DISCUSSION:

Using the marker of endothelial cells (QH1)[3], we could observed the presence of endothelial cells as well as vessels inside of porous biomaterial. Probably number and size of pores has an important effect on the sprouting of vessels inside the biomaterial. Our results shown that the porous biomaterial has satisfactory angiogenic properties. The CAM assay is a low-tech method, which makes it possible to continuously monitor angiogenesis, to easily and quickly obtain results, and to evaluate them in a relatively short time[5]. The response of CAM to implanted biomaterial is similar to the mammalian animal model[2].

CONCLUSION:

Our observation suggested to biocompatibility and bioactivity of PHB/CHIT biomaterials using the alternative *in vivo* animal model. Thanks to these methods we can show an evidence of blood vessels overgrowth of biomaterial before *in vivo* testing, and thus evaluate its biocompatibility from the view of angiogenesis at the site of implantation.

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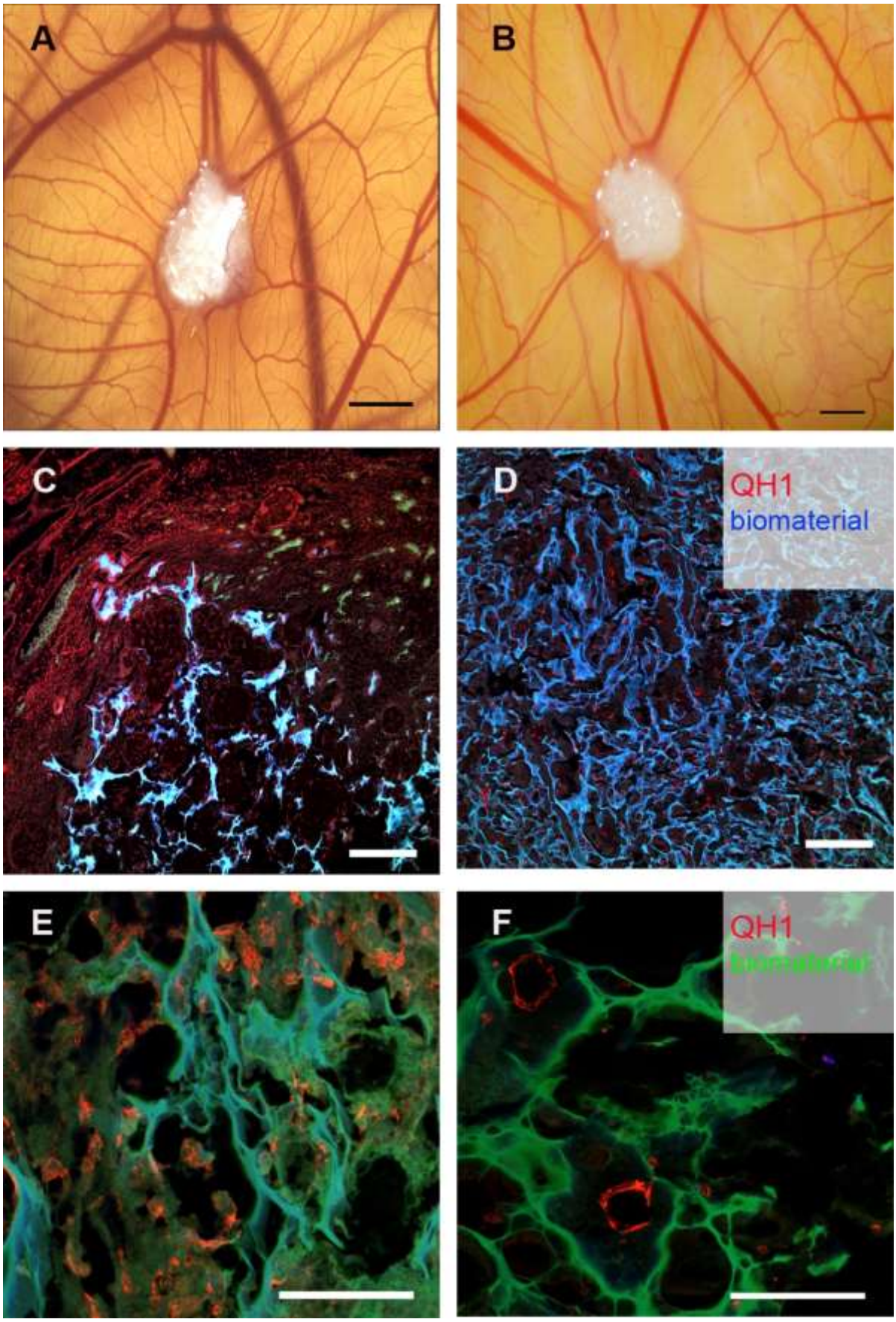

Picture 1: Caption 1: A, B: Macroscopic evidence of blood vessels converging toward the scaffold. C, D: Evidence of endothelial cells inside of scaffold

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

24 Fluid simulation method to study the effect of macroporous structure of 3D porous hydroxyapatite scaffolds on osteogenic differentiation of rBMSCs

Feng Shi[1], Yumei Liu[2], Wei Zhi[3], Jie Weng[3]
[1]Collaboration Innovation Center for TRMET, China West Normal University, Nanchong, China
[2]College of Environmental Science and Engineering, China West Normal University, China
[3]School of Materials Science and Engineering, Southwest Jiaotong University, China

INTRODUCTION:

Many studies have found that macroporous structures of tissue engineering scaffolds affect their physiological behaviors, vascular growth and bone formation[1-2]. However, conventional cellular culture systems are under static condition *in vitro*, in which it is difficult to characterize the effect of macroporous structures on cells[3-4]. Herein, we designed a novel 3D perfusion culture system which perfused culture medium through 3D porous constructs to investigated the roles of the macroporous structure of hydroxyapatite (HAp) scaffolds to rat bone marrow mesenchymal stem cells (rBMSCs) under the dynamic microfluidic environment.

METHODS:

HAp scaffold was prepared by the solvent casting/sugar spheres template leaching technique, and was seeded with rBMSCs. The 3D perfusion culture system was developed which facilitated controlled perfusion of a defined culture medium through cell-scaffold construct. The present study investigated the cell proliferation and gene expression of rBMSCs in this perfusion culture system compared with that under static condition (Fig. 1).

RESULTS AND DISCUSSION:

The fluid shear strength in the perfusion culture promoted osteogenic differentiation of BMSCs cultured on the scaffolds compared with those cultured under static conditions. In the same interconnectivity, the effect of pore size of scaffold on the cell response presented a critical dimension: when the macropore size of scaffold was reduced from 1300 to 800 μm, the increased internal fluid shear force promoted the expression of ALP, Col-I, OCN and OPN and the secretion of osteogenic protein on the scaffolds. However, when the macropore size of scaffold was reduced from 800 to 500 μm, internal fluid shear of scaffold was so great that reduced the levels of osteogenesis-related gene and protein.

CONCLUSION:

Fluid shear stress introduced some external stimuli into the cell nucleus, thereby causing a cascade of biological reactions. Microfluidic flow simulation revealed that the pore structure of the scaffold could affect the redistribution of the perfusion flow field, expanding the high velocity flow zone in the scaffold and intensifying the internal shear force distribution and shear force level. These mechanical signals influenced the biological behaviors of cells on different scaffolds even under the same level of stimulation.

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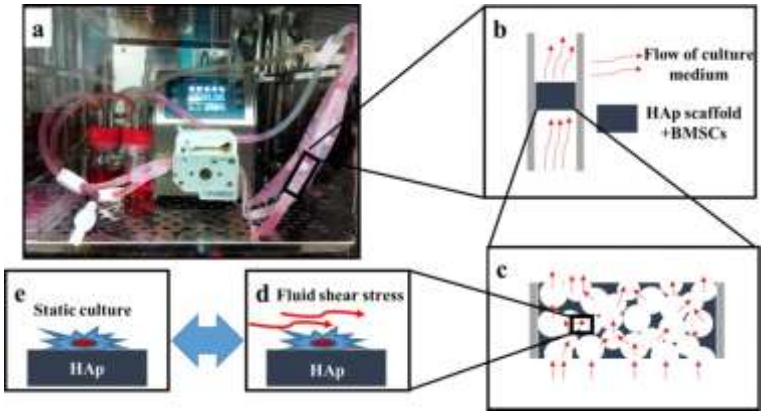
ACKNOWLEDGMENTS:

This work was supported by the National Key Research and Development Program of China (2016YFB0700803), the National Natural Science Foundation of China (51572228, 31400809).



Picture 1: Caption 1: Fig. 1. (a) Perfusion culture system in CO2 incubator. (b-c) Schematic diagram showed culture medium flowing in the chamber and through the scaffold.

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Poster presentation

25 Change in mechanical properties of biomaterial Ti-6Al-7Nb with various volume fractions of martensite phase.

Shota Ino, Toshikazu Akahori, Mitsuo Niinomi
Meijo University, Nagoya, Japan

INTRODUCTION:

Recently, biomaterial α + β type Ti alloys with low Young's modulus and high specific strength have been widely used all over the world. The martensite (M) phase in α + β type Ti alloy has been reported to improve the toughness, therefore, there is high possibility of improvement in the mechanical properties easily by controlling the volume fraction of M phase. In this study, the change in mechanical properties of α + β type Ti-6Al-7Nb (Ti67) with various volume fractions of M phase were systematically investigated.

METHODS:

Materials used in this study were hot-forged Ti67 bars subjected to solution treatment (ST) at 1123K, 1173K, 1223K, 1243K and 1273 K for 3.6 ks in vacuum followed by water-quenching (WQ). Optical microscopy, SEM and XRD spectroscopy were used to identify the constitutional phases. Vickers hardness (HV) test, Young's modulus measurement, tensile and fatigue tests were carried out to evaluate the mechanical properties.

RESULTS AND DISCUSSION:

Figure 1 shows relationship between tensile strength and Young's modulus of Ti67 subjected various ST. The Young's modulus decreases with an increase in the volume fraction of M phase. That of Ti67 subjected to ST at 1243 K is the lowest value (around 75 GPa). However, the Young 's modulus increase again after ST over 1243 K. On the other hand, the tensile strength of Ti67 subjected to various ST increases simply with an increase in the volume fractions of M phase.As the volume fraction of M phase increased, the fatigue strength showed an upward

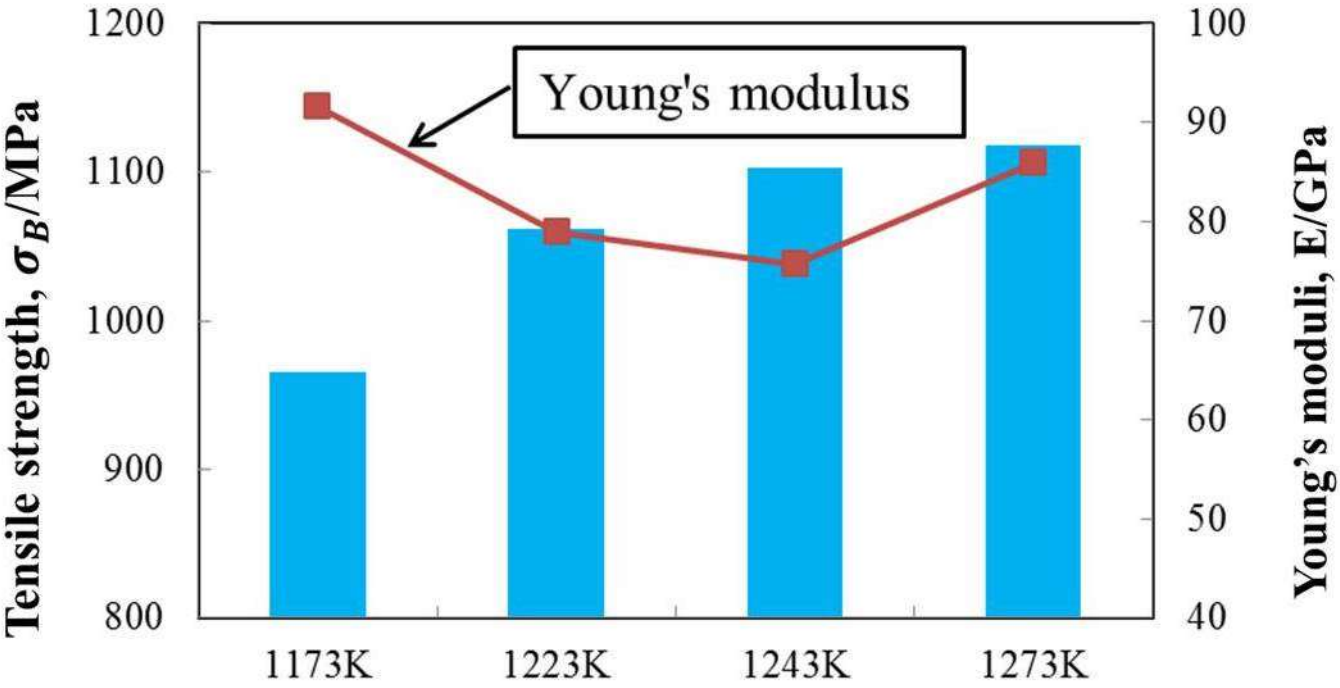
tendency, and the fatigue limit of Ti67 subjected to ST at 1243 K showed the highest value (985 MPa). This value was much greater than that of annealed Ti-6Al-4V, which is one of representative biomedical α + β type Ti alloys.

CONCLUSION:

Microstructures of Ti67 subjected to ST at 1173 K to 1273 K below the temperature of β transus were composed of martensite and primary α phases. The volume fraction of M phase increased with an increase in ST temperature.

Tensile strength increased simply with an increase in the volume fraction of M phase, while Young's modulus showed a reverse trend up to ST at 1243K. The Young's modulus of Ti67 subjected to ST at 1243 K was around 75 GPa.

Fatigue limit of Ti67 subjected to ST at 1243K showed the highest value of 985 MPa



Picture 1: Caption 1: Relationship between tensile strength and Young's moduli of Ti67 subjected to 1123 to 1273K/WQ and 1243K/AC.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

28 Multifunctional peptides containing integrin and heparin binding motives for improved biocompatibility of bioresorbable scaffolds

Franziska Clauder, Mareen Pagel, Annette G. Beck-Sickinger
Leipzig University, Leipzig, Germany

INTRODUCTION:

Biodegradable polymers based on polycaprolactone or polylactic acid bear a number of advantages for the treatment of bone defects, whereas the ability to fill-in critical size defects might be one of the most important. Even

though the porosity of the material enables infiltration of cells, direct interaction with the surface remains limited. Using specific peptide sequences to present cell adhesion motives can help to overcome these restrictions and improve implant integration.

METHODS:

A DOPA containing surface binding peptide deduced from the blue mussel was functionalized with a cyclic integrin binding sequence (c[RGDfK]) as well as a heparin binding sequence (FHRRIKA) by orthogonal ligation chemistry[1]. Surface binding to different biodegradable polymers was measured with a biotin-ELISA-like assay in comparison to a tyrosine containing control peptide. Cell adhesion, viability and proliferation of osteoblast-like cells were investigated in comparison to blank surfaces and fibronectin coating.

RESULTS AND DISCUSSION:

The presented mussel derived peptide enables not only coating of titanium dioxide surfaces as shown previously[2], but also of polymeric substrates like polystyrene or polycaprolactone. The RGD-motive of the coating greatly improved cell count and spreading in hBMSCs as well as the formation of focal adhesion contacts. Cell viability and proliferation of SaOS-2 cells were likewise increased on polymeric substrates, demonstrating the improved cell-surface-interaction.

CONCLUSION:

The application of DOPA-containing peptides in combination with adhesion sequences represents a versatile tool for the tuning of implant surface properties and can thereby positively influence implant integration.

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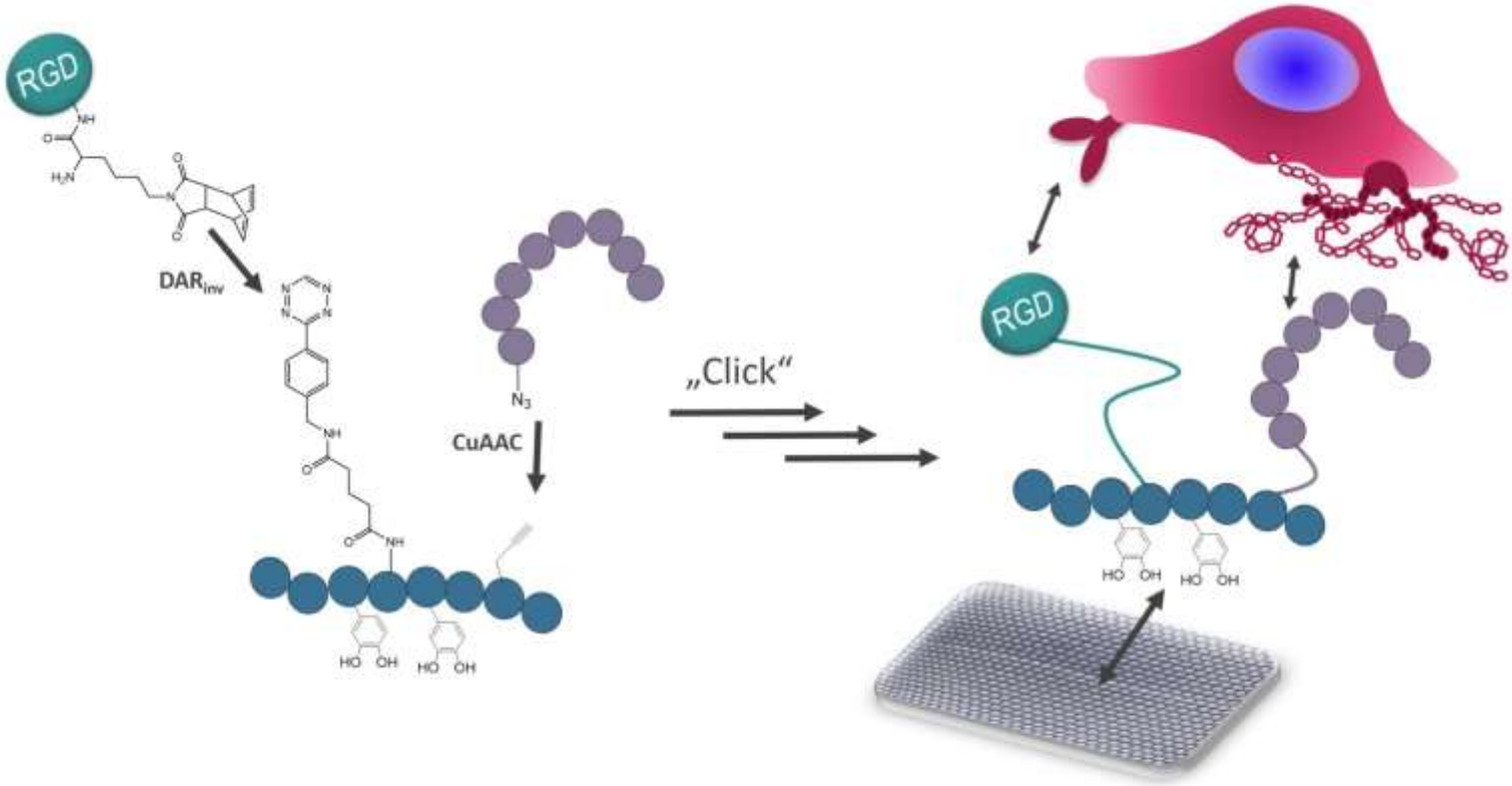
ACKNOWLEDGMENTS:

The authors like to thank Transregio 67 and the DFG for providing financial support to this project.



Picture 1: Caption 1: Modification of surface binding peptides with cell adhesion sequences for improved cell-implant-interactions.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

30 In situ preparation of brushite/alginate hydrogel composites with improved mechanical characteristics for tissue engineering applications

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[2]DIBRIS - University of Genoa, Italy
[3]DICCA - University of Genoa, Italy

INTRODUCTION:

Among natural hydrogels, alginate has been widely studied due to its biocompatibility, biodegradability, and low cost[1]. However the hydrogels present several limitations, such as low tensile strength, high water content and large pore size, which could result of low efficiency as scaffold biomaterial[2] as all as drug delivery support. The challenge is therefore to produce a composite with higher mechanical characteristic, but without affect the structure and the morphology of the Alginate and also without yield cytotoxic result.

METHODS:

Powder of Na Alginate was before added in a di-ammonium hydrogen phosphate solution and then put in a crosslinking solution of Calcium Nitrate (0.2M) maintaining a pH equal to 7.5 using HCl and NaOH (0.1 M). The spheres produced, of about 5 mm in diameter, were maintained in the solution for 24h to permit the grow of the brushite crystals inside the hydrogel matrix and then washed in distilled water to remove the Ca ions excess. The mechanical characteristic of the hydrogel spheres was evaluated through DMA (Dynamic Mechanical Analysis) in the range of 5–100 Hz and with an initial pre-deformation of 20% of the sample diameter by using a prototype apparatus developed at DICCA laboratory of University of Genova. The microstructure of the phosphate crystals was evaluated by SEM, after samples drying in oven at 50 °C for 12 h.

RESULTS AND DISCUSSION:

The increase of Alginate concentration from 1.5% to 2% produced an enhancement in the storage modulus of about 35 KPa, explainable with a high density in the crosslinks among the polymeric chains. The effect of Brushite crystals inside the alginate matrix can be evaluated by the comparison between the storage modulus of Alg 1.5 (30 KPa) and Alg 1.5-P 0.1 (58 KPa) samples, which were composed of equal Alg concentration and that revealed more than 20 kPa improvement. This relates to the fact that Brushite component has higher mechanical properties than Alginate and the polymeric chains movements are restricted by the crystalline component.

CONCLUSION:

The current study was carried out to evaluate the effect of Brushite crystals synthetized in-situ on the mechanical properties of alginate hydrogel. The hybrid material produced, with an evident increase in the stiffness with respect to the alginate hydrogel, can be useful in many biomedical applications as the tissue engineering and the drug delivery.

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Poster presentation

32 Polycaprolactone / bioactive glass hybrid scaffolds with controlled porosity

Cédric Bossard[1], Henri Granel[2], Édouard Jallot[1], Yohann Wittrant[2], Jonathan Lao[1]
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[2]INRA, France

INTRODUCTION:

Bioactive glass (BG) is a bone substitute first developed by Hench in 1969 [1]. It has been the focus of much research as it strongly bonds to bone, it resorbs as the bone regenerates and its dissolution products stimulate the synthesis of bone by osteoblasts [2]. However, BG are brittle which limits their use to low load-bearing applications. In the early 1990s, the first sol-gel BG were prepared [3], which opened new perspectives. Indeed, the low temperature of the sol-gel process allows the addition of polymers in the synthesis, thus introducing toughness to the material. In these hybrid materials, the interpenetration of the organic and inorganic phases at a molecular scale

provides more homogeneous properties compared to composites [4]. In the present work, 3-dimensional polycaprolactone (PCL) / BG hybrid scaffolds were successfully synthesized with well-controlled porosity.

METHODS:

Homogeneous organic-inorganic hybrid containing 70 wt.% PCL and 30 wt.% BG were obtained and scaffolds were prepared with the microsphere leaching technique. The BG was a binary system of 75 wt.% SiO_2 and 25 wt.% CaO. The pore size, the interconnection size and the ability to form bone-like apatite in Simulated Body Fluid (SBF) were evaluated.

RESULTS AND DISCUSSION:

The pore size was similar to the size of the microspheres. This means it can be readily controlled through the granulometry of the porogen microspheres. Interestingly, the interconnection size – a key parameter to angiogenesis and cell migration – can also be finely tuned by pre-heating the microspheres for different times; here interconnections ranging from 100 µm to 200 µm were obtained. The hybrid scaffolds rapidly formed apatite when soaked in SBF at 37°C. Their biocompatibility was successfully investigated *in-vitro.*

CONCLUSION:

Bioactive 3-dimensional PCL / BG hybrid scaffolds were successfully synthesized by sol-gel chemistry. Both the pore and interconnection sizes were well-controlled and tuneable thanks to the microsphere leaching technique. The biocompatibility of scaffolds proved to be similar to that of bovine bone, with the advantage that the materials are synthetic and that their porosity can be varied accordingly.

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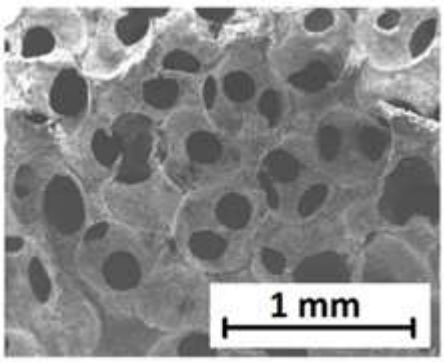

Picture 1: Caption 1: PCL / BG hybrid scaffold with pores of 350 µm and interconnections of 150 µm

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

34 The content of collagen in Collagen-polyester pad and hemostatic research

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INTRODUCTION:

Collagen plays an important role in the hemostatic process. When collagen exposes in wounds, it rapidly aggregates and activates platelets. It also indirectly causes fibrinogen and tissue fluid to react with thrombin and form blood fibrin[1]. However, the cost of a collagen-based pad is relatively higher than other pads for wound handling. In this study, we used a novel method to produce 1% collagen-polyester pad, and measure the hemostasis performance as well as tissue repair outcome in comparison with collagen-free pad. The results showed that the 1% collagen-polyester pad can significantly reduce the time of hemostasis. We conclude that the pad of 1% collagen-polyester is better than other collagen-free pad in terms of hemostasis and wound healing, and it also a cost effective way for wound handling.

METHODS:

The pad of 1% collagen-polyester was produced, and the collagen content was measured and calculated by optical-density of hydroxyproline. The hemostatic effect was tested by SD rats. Total six SD rats are equally divided into test-group and control-group. After the process of anesthesia, rats were underwent the muscle-incision surgery. The right femoral artery of rats were punctured with 24G needle[3]. To stanch bleeding, the wounds of test-group are covered with 1% collagen-polyester pad; and the wounds of control-group are covered with collagen-free pad (40×40×2mm). We record the duration of time until the hemostasis was completed[3]. Student's t-test is used as a statistical analysis (α=0.05) to analyze the data of experiment.

RESULTS AND DISCUSSION:

The result shows that the collagen-polyester pad contained 1±0.4% collagen which was matched the pre-set goal of the pad. The hemostatic time of test-group and control-group were 53.00±8.88 and 137.66±11.01 seconds, respectively. The p-value calculated was 0.037. The results showed that 1% collagen-polyester pad exhibited a much better performance of hemostasis than collagen-free pad. Even though the collagen content was only 1%, the effect of enhancing platelet aggregation as well as accelerating the formation of blood clot was achieved.

CONCLUSION:

This study showed that 1% collagen-polyester pad had better efficacy of hemostasis than collagen-free pad. Also, 1% of collagen-polyester pad is cost effective for wound handling. Further studies is undergoing, more data will be present soon.

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ACKNOWLEDGMENTS:

We would like to thank Mackay Memorial Hospital and Dr. Chin-Lung Chang for providing support to this project.

Picture 1: Caption 1: Table 1. Hemostatic effect experiment (P=0.037)

sample	collagen-polyester pad	collagen-free pad(CSD®)
1	46	125
2	50	145
3	63	143
mean±SD	53.00±8.88	137.66±11.01

The duration of time until the bleeding is stopped. Student's t-test was used for statistical analysis (α=0.05).

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Poster presentation

35 Relationship between mechanical properties and microstructure of Ti-12Cr for spinal fixture applications

Saki Tanaka[1], Toshikazu Akahori[1], Masaaki Nakai[2], Mitsuo Niinomi[1], Tomokazu Hattori[1]
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INTRODUCTION:

Metallic materials with a relatively high Young's modulus are required to suppress spring-back by elastic and plastic deformation during implantation. Therefore, Young's modulus controlling by stress-induced transformation in a newly developed β-type Ti-12Cr for spinal fixture applications has been proposed by the present authors[1]. However, the relationship between the microstructure and mechanical properties of Ti-12Cr has not been fully investigated up till now.

Therefore, changes in the mechanical properties of Ti-12Cr were investigated through some heat treatments and fine particle bombarding process (FPB), which is one of mechanical surface modification processing, in this study.

METHODS:

Forged round bars made of Ti-12Cr alloy was used in this study. Some bars were solutionized for 3.6 ks at 1003 K above the β-transus (953 K) followed by water quenching. The as-solutionized bar was designated as Ti-12Cr$_{ST}$. Some Ti-12Cr$_{ST}$ were then aged at 573 K, 673 K and 723 K for various times, and they were designated as STA$_{573K}$, STA$_{673K}$, and STA $_{723\,K}$, respectively. Surface modification by a fine particle bombarding (FPB) machine was applied to some sample. Optical microscopy (OM), scanning electron microscopy (SEM), and X-ray diffraction (XRD) spectroscopy were carried out on each specimen to evaluate the microstructure. The Vickers hardness, Young's modulus, the tensile and fatigue properties were investigated for each specimen to examine the mechanical properties.

RESULTS AND DISCUSSION:

The Vickers hardness of STA_{673K} in the peak aging condition (PA) was around 90% (HV 524) higher than that (HV 294) of Ti-12Cr_{ST}. Both the 0.2% proof stress and tensile strength of STA_{673K} in PA were also around 50% higher those of Ti-12Cr_{ST} as shown in Fig. 1. However, the ductility of Ti-12Cr in PA at each temperature reduces significantly. Therefore, a solo-solution treatment was judged to be the optimal heat treatment for Ti-12Cr with an excellent combination of strength and ductility.

CONCLUSION:

Ti-12Cr subjected to some peak aging treatments indicated very high mechanical strength and low ductility. In this case, the Ti-12Cr subjected to a solo solution treatment indicated excellent balance between strength and ductility.

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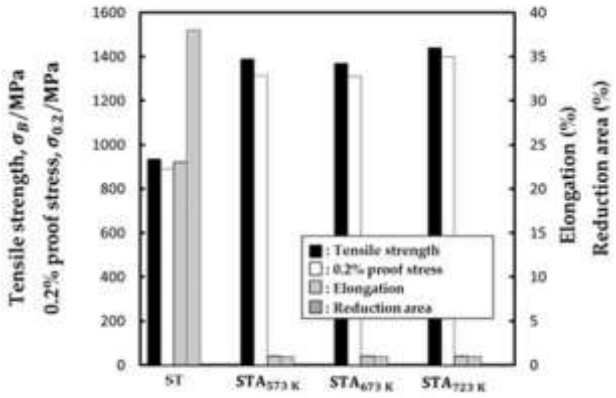

Picture 1: Caption 1: Tensile properties of Ti-12Cr subjected to each heat treatment.

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Poster presentation

36 Synthesis and characterization of novel antibacterial mesoporous silicate bioactive glass/polymer films as wound dressings

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[2]Universitätsklinikum Knappschaftskrankenhaus Bochum GmbH, Germany
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INTRODUCTION:

Preventing post-operative bacterial infection is essential due to subsequent complications in patients. Although strict hygienic protocols and preventive antibiotic treatments have drastically reduced the percentage of postoperative infections, many bacterial species have developed resistance against antibiotics. An alternative to antibiotics, is the

use of biomaterials with antimicrobial properties. In this context, antibacterial ion-doped mesoporous silicate bioactive glasses (MBGs) offer a potential alternative to antibiotics[1]. In this study, novel antibacterial silicate MBGs were combined with a biodegradable polyether urethane to form films for use as wound dressings.

METHODS:

The synthesis of MBGs doped with varying wt% of antibacterial ions (Ag, Cu, Zn and Ce) was carried out via sol-gel and surfactant assisted sol-gel methods. Disk diffusion tests were carried out to investigate the resistance of MBGs against *E. coli* and *S. carnosus.* Indirect cell tests were done with MBG extracts on L929 murine fibroblast cell line. MBG/polymer films were obtained by drop-casting and investigated via SEM/EDS, ATR-FTIR and elemental mapping. Further antibacterial and advanced cytocompatibility studies were carried out on these films.

RESULTS AND DISCUSSION:

Ag-MBGs showed the highest antibacterial performance followed by Cu-MBGs. In both cases, the antibacterial efficacy was dopant-ion concentration dependent; the higher the wt% of dopant present the better the antibacterial performance was up to 5 wt%. MBGs without surfactant were more antibacterial active than surfactant-added MBGs, due to their higher ion release and subsequent higher pH increase due to the dissolution products. All MBG extracts were viable against L929. According to the antibacterial and cell viability test results so far, Ag-MBGs without surfactant were used to prepare prototypic composite films. SEM/EDS and elemental mapping showed that Ag-BG did not cover the entire surface of the film homogeneously. The adsorption bands of BG and polymer were identified by ATR-FTIR.

CONCLUSION:

The largest inhibition zones for both bacteria were obtained with the non-surfactant Ag-MBGs. All MBGs showed high viability values tested with L929. Moreover, polymer/Ag-BG films were successfully produced (fig. 1). ATR-FTIR confirmed the presence of Ag-BG and polymer in films. Further antibacterial and cell studies will be performed on these films.

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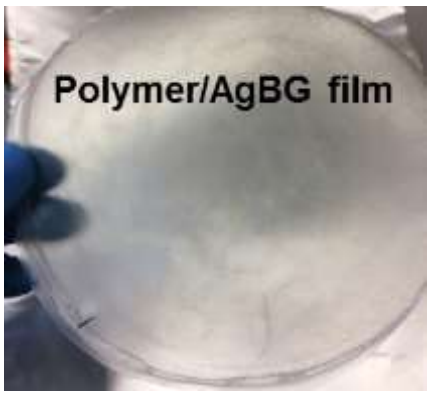
ACKNOWLEDGMENTS:

The authors thank the EU Horizon 2020 Marie Curie project [grant number 643050] “HyMedPoly” for providing financial support.



Picture 1: Caption 1: Figure 1. Image of a polymer/Ag BG composite film.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

37 novel nano-microspheres containing chitosan, hyaluronic acid, and chondroitin sulfate deliver gdf-5 plasmid for osteoarthritis gene therapy

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INTRODUCTION:

Osteoarthritis (OA) is a common disease in worldwide, despite recent progress, has no curative treatment[1]. Herein, we aimed to prepare nano-microspheres (NMPs) with chitosan(CS), hyaluronic acid(HA), and chondroitin sulfate (CHS) as a vector to deliver GDF-5 plasmid for gene therapy treatment of OA.

METHODS:

The nanoparticles were prepared by electrostatic adsorption theory under 55. Physical properties and biological function of the nanoparticle were tested in vitro, and the nanoparticles were injected into the rabbit articular cavity to observe the influence on the occurrence of osteoarthritis.

RESULTS AND DISCUSSION:

The early pathological changes that occur in OA are increased water content and cartilage matrix degradation[2]. In this study, GDF-5 was chosen as a therapeutic gene, because it can effectively promote secretion of the ECM in chondrocytes. More importantly, HA and CHS also regulate chondrocytes growth and differentiation. The introduction of HA and CHS into the vector may have directly improved degradation of the chondrocyte ECM and relived the symptoms of OA. In this study, NMPs exhibited good physicochemical properties and low cytotoxicity. The average diameter was 0.61 ± 0.20 μm, and its encapsulation efficiency was 38.19 ± 0.36% . According to CCK-8 assay, relative cell viability was 75-99% when the total NMPs weight was less than 560 μg. The immunohistochemical staining results suggested that NMPs can successfully transfect chondrocytes and stimulate extracellular matrix (ECM) protein expression in vitro. And NMPs had similar transfection efficiency with liposome 2000. Biochemical composition of chondrocytes ECM analysis reslut also proved this point. When NMPs were injected into OA model rabbit, the expression of ECM proteins in chondrocytes was significantly promoted and the progression of OA had been slown down.

CONCLUSION:

The nanoparticles loading GDF-5 with excellent physicochemical properties, low cytotoxicity and high transfection efficiency could effective slow down the occurrence of osteoarthritis in animal model.

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ACKNOWLEDGMENTS:

This work was supported by the Natural Science Foundation of China (81201407)

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

39 Surface modification of electrospun vascular grafts made of polycaprolactone

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INTRODUCTION:

Nanofibers are able to form a large surface-to-volume ratio which improves performance for many applications. They can be made from different materials and various fibrous assemblies with unique abilities by electrospinning[1]. One of the polymers widely used in the biomedical field in the form of nanofibers is polycaprolactone. Polycaprolactone (PCL), due to its unique properties such as biocompatibility and biodegradability, opens new ways to solve problems related to the coronary artery diseases which is one of the leading causes of death in the developed world[2]. PCL vascular grafts made by electrospinning, chemically modified by polydopamine and grafted by heparin[3] were successfully used as a new type of scaffold for bypass grafting with reduced thrombogenicity.

METHODS:

Nanofibrous vascular grafts were prepared by electrospinning of polycaprolactone (PCL) solution on rotating mandrel collector. Samples were pre-treated with 0.01 M solution of sodium hydroxide for 5 minutes, then washed and sonicated for 2 minutes. Subsequently, the pre-treated grafts were immersed into the polydopamine (PDA) solution for 4h/ 250 rpm/ r.t., finally washed 3x with distilled water. Afterwards, the samples were functionalized with heparin for 12h/ 250 rpm/ r.t. Such prepared samples were dried and stored in the desiccator for further experiments. Prepared samples were successfully characterized by different techniques including X-ray photoelectron spectroscopy (XPS) see Figure 1, FT-IR, UV-VIS spectroscopies, SEM microscopy and *in-vitro, in-vivo* cell experiments.

RESULTS AND DISCUSSION:

Nanofibrous vascular grafts were produced from biodegradable polyester. Its surface was chemically modified with polydopamine and heparin. Polydopamine is a mussel-inspired adhesive which enables binding of heparin responsible for the reduction of thrombus formation. X-ray photoelectron spectroscopy successfully confirmed the presence of nitrogen and sulfur coming from polydopamine and heparin coatings.

CONCLUSION:

Nanofibrous vascular grafts made of polycaprolactone seem to be a promissive material for bypass grafting. Although, these new types of nanomaterials have a high surface-volume ratio which is connected with many advantages. On the other hand, such properties could activate thrombocytes leading to dangerous thrombus formation. We have proposed solution related to this problem via successful immobilization of polydopamine followed by heparin bonding on the nanofibrous grafts.

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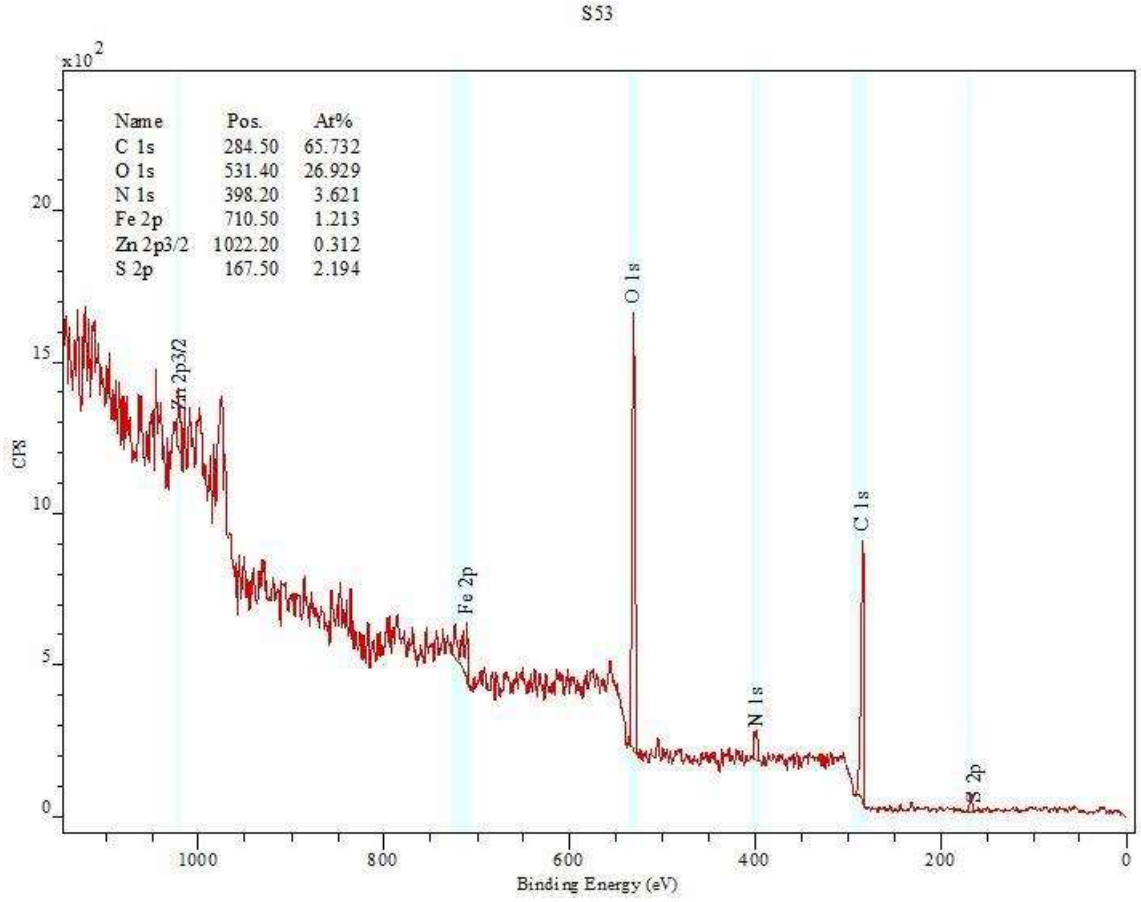

Picture 1: Caption 1: Figure 1: XPS graph confirming presence of sulfur coming from the heparin on the modified vascular graft.

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Poster presentation

41 Solid antimicrobial compositions containing hyaluronic acid, triiodide and a stabiliser

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INTRODUCTION:

The aim of this presentation is to describe solid antimicrobial compositions containing hyaluronic acid (HA) or its derivatives, triiodide and a stabiliser. Applications of the mixture of native HA and triiodide in the form of water solution is known more than a decade (Hyiodine®)[1]. Combination of non-selective, antimicrobial triiodide with biocompatible HA gave the material enormous potential in the field of wound healing which was successfully used for many types of open wounds and ulcers. New requirements for storage, maintenance and safety transport

focused our research activities on the development of various solid materials (without solvent) with similar content of HA and triiodide.

METHODS:

Absorption spectra of dissolved samples were recorded using UV-Vis spectrophotometer Varian V1002M169. NMR spectra were recorded on Bruker 500MHz.

RESULTS AND DISCUSSION:

All antimicrobial compositions containing triiodide anion show significant release of volatile iodine molecule. This loss of antimicrobial activity can be decelerated either with presence of solvent or with presence of some stabiliser with capability to bind triiodide anion in its anionic (non-volatile) form. As required solid form cannot contain any solvent, many biocompatible molecules were considered as a potential stabiliser of triiodide anion. It was disclosed that some heterocyclic compounds with permanent positive charge showed very interesting results. Biocompatible thiamine (vitamin B1) was studied in detail due to its great biocompatibility and availability. Results demonstrated that the solid compositions containing HA, vitamin B1 and trijodide showed suitable stability and antimicrobial activities[2] against *Candida albicans, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis and Staphylococcus aureus.*

CONCLUSION:

Results showed that the suggested concept of stabilisation of triiodide anion with heterocyclic cations was fully successful. Thiazolium cycle of thiamine (vitamin B1) was efficient to avoid of significant release of molecular iodine from the mixture of HA and triiodide. The final composition was successfully tested *in vitro* therefore it is very promising material for applications in the field of wound healing.

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

43 Study of interactions between molecular iodine and hyaluronic acid derivatives

Radovan Buffa, Daniel Švadlák, Veronika Štepánková, Tomáš Pitucha, Petra Faltusová, Vladimír Velebný
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INTRODUCTION:

Chemical modification of hyaluronic acid (HA) can change its basic physical characteristics dramatically. The most frequent are modifications based on acylation reactions of hydroxy groups or on alkylation reactions of carboxylic groups of the polymer. Final less polar materials are promising substrates for adsorption of less polar active compounds. Iodine is simple, non-polar and very efficient antimicrobial molecule used for many applications related to wound healing. Main disadvantage is its volatility and instability[1], but this problem can be solved using suitable polymer matrix (povidone-iodine). The aim of this work was to test basic antimicrobial properties of HA-I_2 composition and describe interaction of molecular iodine with several types of hyaluronic acid (HA) derivatives and forms in order to localise structural features responsible for the binding of iodine.

METHODS:

FT-IR spectra were recorded on Nicolet 6700.

NMR spectra were recorded on Bruker 500MHz.

RESULTS AND DISCUSSION:

NMR and FT-IR spectra of various modified and non-modified HA forms[2] with and without loaded iodine were recorded to find out the differences between shifts of signals of iodinated and non-iodinated materials. Localisation of the most significant changes involve anticipating of certain positions with the most intensive interaction among iodine and polymer. Data obtained from ^{1}H, ^{13}C NMR and FT-IR were in good correlation therefore the final interpretation should be relevant. According to results it is possible to summarize, that -CH- or -CH_2- groups attached on aprotic polar atom or group of atoms (oxygen, carbonyl) are the most attractive parts of the polymer for interaction with iodine. On the other hand -CH- or -CH_2- groups with neighbouring polar protic groups like -OH or -NH are shifted minimally. It was also observed, that in the case of oligomers, inner -CH- or -CH_2- groups of oligomers interacts more than -CH- or -CH_2- groups close to the end of the chain. Polymeric compositions containing HA and iodine were tested and antimicrobial activities against Klebsiella pneumoniae*, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus epidermidis and Staphylococcus aureus* were studied.

CONCLUSION:

This study showed differences in loading capacity between various types of materials based on hyaluronic acid. More precise localisation of chemical groups attractive for interactions with molecular iodine could help to design new materials with higher loading capacity and higher stability. Polymeric compositions with iodine showed antimicrobial properties against several types of microorganisms.

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

56 Osteoconductive behavior of a scaffold ceramic: Correlation between histomorphometric versus micro-CT image analysis techniques.

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[3]University of Murcia, Spain
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INTRODUCTION:

The use of xenografts in scaffolds for the replacement of bone is an important research field [1]. The addition of demineralize bone matrix (DBM) to ceramics can improve stability, provide better structural properties and stimulate

new bone formation. The aim of this study was to fabricate and characterize calcium silicophosphate (CaSiP) and DBM-coated CaSiP ceramic as candidates for scaffolds with improved osteogenic properties and compare their physical and in vivo behavior.

METHODS:

The scaffolds were inserted into 15 NZ rabbits, randomized into 3 groups (n=5). Characterization, histological, histomorphometric, and micro-CT analysis were performed at 1, 3 and 5 months.

RESULTS AND DISCUSSION:

The results show a correlation between micro-CT and the histomorphological results.The results showed disintegration of the biomaterial and the presence of free ceramic fragments dispersed inside the host bone's medulla over the 5-month study period. After 3 months the implants has lost its boundaries, increased its porosity, and begun a process of neo-trabeculation. After 5 months, the implant has gradually disintegrated and the presence of free ceramic fragments dispersed inside the host bone's medulla has become more evident. No difference was found between DBM-coated and not coated implants. The new bone tissue inside of the implants increase as the implantation time pass. A little less new bone formation has been found in the DBM-coated samples, but it was not statistically significant. In relation with the connective tissue (granulation tissue) produced inside the implants showed an increase until the third month and a high decrease in the fifth month in both implanted materials. This granulation tissue can be considered as a precursor to the formation of new bone. Thus, the decrease in connective tissue overlaps with the increase of new woven bone and mature bone in both materials.

CONCLUSION:

The comparison of micro-CT and histological images demonstrates that the new tissue between remaining ceramic fragments (hypodense material between the hyperdense biomaterial shown in mCT images according to the Hounsfield units), was identified histologically as osteogenic and fibroblastic tissue (granulation tissue). This finding made it possible to establish a correlation between the visual appearance in 3D, raw data, and the type of tissue in histological images.

On the other hand there is no statistically difference between the DBM-coated and non coated scaffold in vivo behavior. Both materials are biodegradables, bioactives and present osteoconductive properties.

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

57 Comparison of micro-CT and SEM analysis of tissue engineering scaffolds structure - dry versus hydrated state.

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INTRODUCTION:

The 3D structure and porosity of tissue engineering bone scaffolds influence importantly tissue-scaffold interaction[1]. Their implantation into bone defect leads to scaffold hydration, which may change its structural and mechanical properties[2]. However, scaffold structure is usually evaluated in its dry state, because hydrated structure analysis is complicated by common methods[2]. Recently, micro-CT came to this field and both dry and hydrated scaffolds can be analysed. This brings new questions: Is dry-state analysis sufficient? How does hydration change the 3D structure of scaffolds? How can micro-CT and SEM results be combined?

METHODS:

Eight collagen-based scaffold of different composition were prepared. Cylindrical scaffolds (*n*=*3*) were scanned in dry and hydrated (for different time interval) state using micro-CT (SkyScan1272, Bruker, Belgium) and obtained data were processed and analysed using Bruker software (NRecon, CTVox, CTAn). The same scaffolds were scanned in dry stated using SEM (Quanta 450 Microscope, FEI, USA) and the images were analysed using ImageJ (Bethesda, Maryland, USA).

RESULTS AND DISCUSSION:

Micro-CT enables non-destructive analysis and visualization (fig. 1) of specimen's 3D structure in dry and hydrated state[3] in contrast to SEM, which can work only with dry samples. However, micro-CT drawbacks (e.g. resolution, binarization) should be considered[3]. Tested scaffolds´ 3D structure significantly differed based on their composition. Surprisingly, statistically significant differences between SEM and micro-CT pore size values were found in dry state. Hydration was expected to strongly influence scaffolds inner structure[2]. However, only minor changes in pores structure and size were found.

CONCLUSION:

Micro-CT analysis is relatively new but very important imaging method for 3D scaffold structure analysis in dry and hydrated states, when hydration resulted in minor structural changes. However, using only 2D imaging (SEM) of 3D structures was shown to mislead the determination of pore size in comparison to micro-CT analysis.

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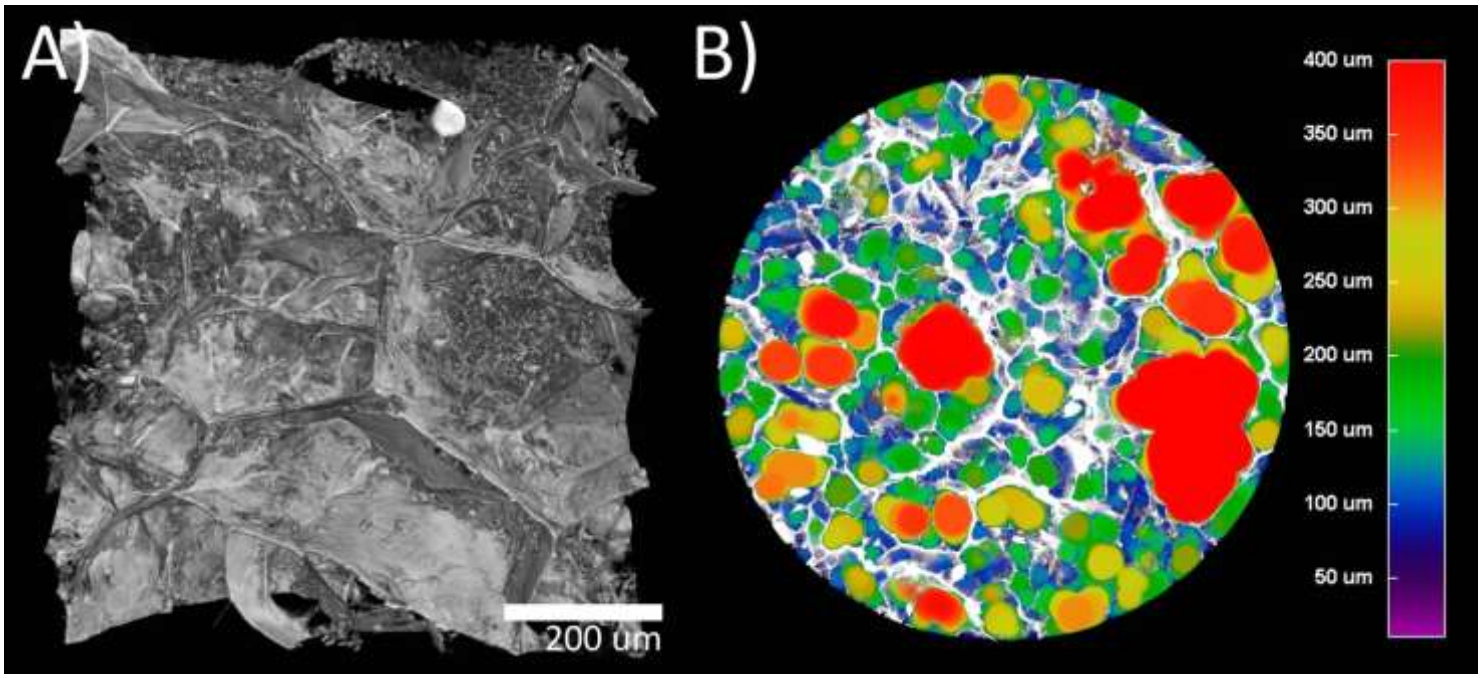

Picture 1: Caption 1: Fig. 1: Micro-CT visualization of collagen-based scaffold in dry state: A) Grayscale B) Color-coded pore size visualization.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

60 Culture and characterisation of beta-cells pseudo-islets in HA-TA hydrogel

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INTRODUCTION:

Type 1 diabetes is an autoimmune disease which manifests by loss of insulin-producing β-cells and thereby loss of insulin production. One treatment option is implantation of allogenic β-cells in form of spheroid clusters which are called pseudo-islets (PI). After implantation of allogenic PI, the biggest challenge is to overcome the adverse reaction of the patient´s immune system. The problem can be solved by encapsulation of PI [1]. Here we describe a preparation of functional PI, their encapsulation and culture in hydrogel based on hyaluronic acid-tyramine conjugate (HA-TA) [2].

METHODS:

PI were allowed to form spontaneously from INS-1E β-cells in 96U-well plates with super low attachment surface for 72 h. PI were then encapsulated in HA-TA hydrogel which was prepared by horseradish peroxidase mediated crosslink reaction. Cell viability, shape of PI, central necrosis development and glucose-responsiveness of encapsulated PI were monitored by Live/Dead staining, GSIS assay and ELISA and compared to non-encapsulated PI.

RESULTS AND DISCUSSION:

Encapsulation of PI into the hydrogel matrix did not affect their viability and shape stability. Central necrosis (expected result of insufficient nutrient and oxygen supply in the centre of PI) developed in encapsulated PI later than in non-encapsulated. GSIS assay confirmed that encapsulated PI retained their ability to produce insulin in the response to increasing glucose level.

CONCLUSION:

HA-TA-based hydrogels are biocompatible, promote viability of encapsulated β-cells and allow sufficient nutrient and oxygen diffusion *in vitro*. Since encapsulated PI retained their glucose-responsiveness, we can conclude that the process of hydrogelation did not cancelled their basic physiological functions. Promoting high cell viability and slowing down central necrosis development, this system can be used for preservation and prolonged *in vitro* culture of PI.

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ACKNOWLEDGMENTS:

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

67 Antibacterial property of the surface oxide layer containing antibacterial elements on titanium formed by electrochemical treatment

Yusuke Tsutsumi, Masaya Shimabukuro, Risa Yamada, Kosuke Nozaki, Peng Chen, Maki Ashida, Hisashi Doi, Akiko Nagai, Takao Hanawa
Tokyo Medical and Dental University, Tokyo, Japan

INTRODUCTION:

The problem of infection disease was recently recognized as one of the major reason of failure in implant surgery. In this study, we focused on micro arc oxidation (MAO) as a prospective solution. MAO treatment can easily alter the surface properties of metals and is also effective to improve hard tissue compatibility of Ti. We tried to introduce the antibacterial elements (Ag, Cu, and Zn) into the Ti surface by MAO treatment. The ion-release behavior from the oxide layer was evaluated by immersing in a simulated body fluid. The antibacterial property of the samples was evaluated using both Gram-positive/negative facultative anaerobic bacteria.

METHODS:

A commercially pure Ti disks were prepared in this study. The specimen was positively charged in the electrolyte containing calcium glycerophosphate, calcium acetate, and small amount of $AgNO_3$/$CuCl_2$/$ZnCl_2$. The applying positive voltage was limited up 400 V in maximum and the treatment time was 10 min. 0.9mass% NaCl solution was used as a simulated body fluid to evaluate the metal ion release from the MAO-treated specimens.

To evaluate the antibacterial property of the MAO-treated specimens, the bacterial adhesion test was performed using *Escherichia coli* and *Staphyrococcus aureus* according with the standard test method (ISO22196:2011). The bacteria was dropped on the specimen surface and incubated at 35 °C for 24 h.

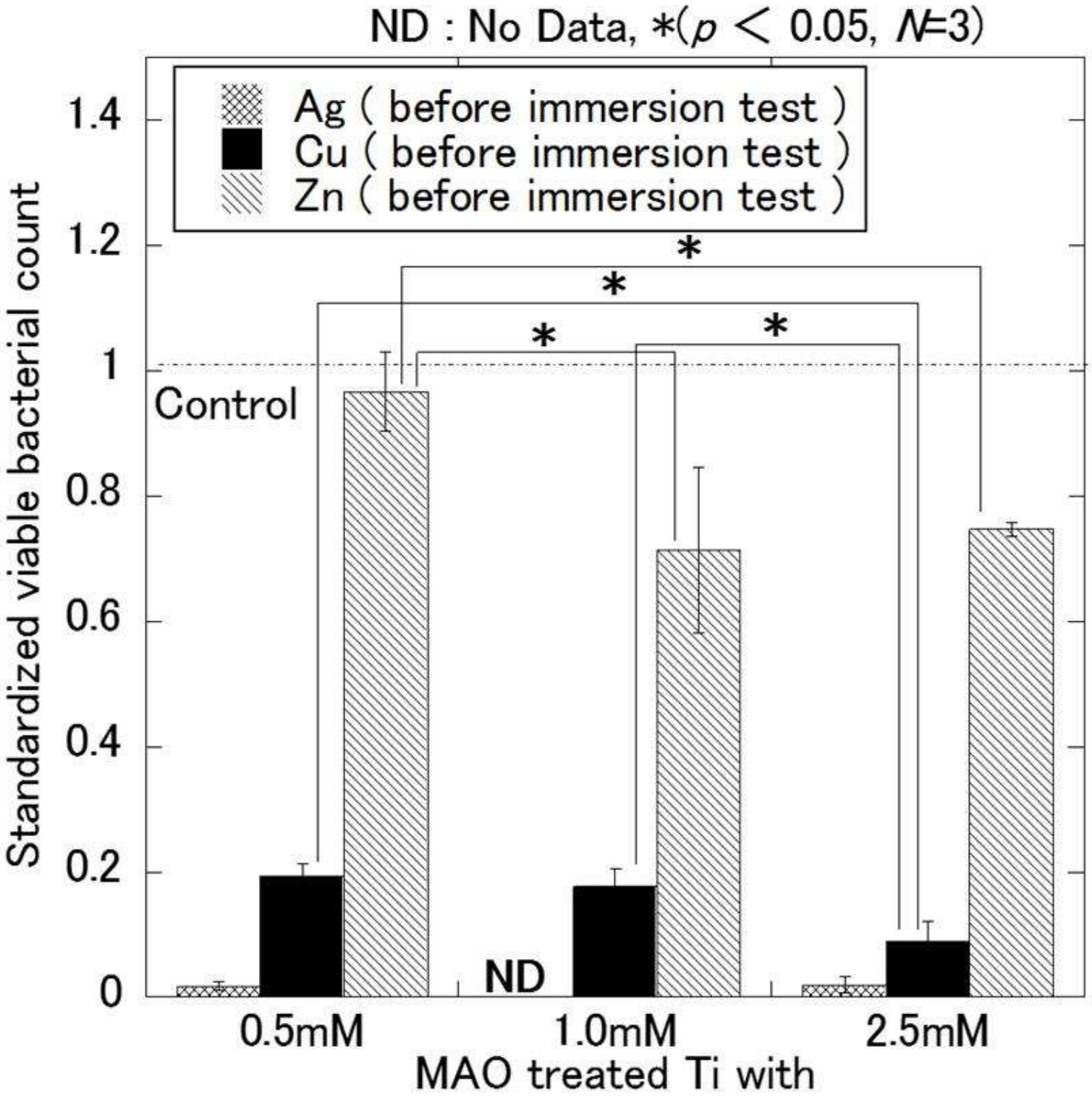
RESULTS AND DISCUSSION:

The amount of Ag-ion showed the largest in the tasted specimens while Cu and Zn showed almost no released ionsduring the immersion period.

The results of bacterial adhesion test are shown in Fig. 1. The MAO-treated specimens with Ag and Cu showed inhibition effect on the proliferation of both *E. coli* and *S. aureus*. MAO-treated Ti with Ag showed excellent antibacterial property due to the released Ag-ion from the oxide layer. The MAO-treated Ti with Cu showed good antibacterial property due to contact effect on the Cu species exposed to the surface of the oxide layer. The MAO-treated Ti with Zn showed unique tendency that the antibacterial property had been enhanced during the immersion period in the physiological saline. It might be derived from the accmmulation of the Zn products on the oxide layer.

CONCLUSION:

MAO treatment enables to introduce antibacterial elements into the resulting oxide layer on Ti surface. MAO-treated Ti with antibacterial elements showed excellent antibacterial properties against both *E. coli* and *S. aureus* bacteria.



Picture 1: Caption 1: Antibacterial properties of MAO-treated specimens against S. aureus

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

82 Bioactive coatings for cardiovascular tissue engineering

Johanka Kucerová, Zuzana Riedelová, Eduard Brynda, Tomáš Riedel
Institute of Macromolecular Chemistry, Czech Academy of Sciences, Praha 6, Czech Republic

INTRODUCTION:

Cardiovascular diseases are one of the main causes of death in the world. Deprivation of blood supply due to vessel blockage leads to ischemia in the affected place (myocardial infarction). In order to restore the blood flow into the ischemic tissue, it is necessary to replace damaged or blocked blood vessel with a synthetic/autologous vascular graft or with a stent. The main challenge faced by many research groups is to decrease acute as well as late thrombosis of the artificial surface and to promote endothelialization of the scaffold. A promising approach how to improve the biocompatibility of the synthetic graft is to coat their inner surface with fibrin network[1,2]. Fibrin is a natural biopolymer that is formed at the place of vessel injury to stop bleeding and later it serves as a provisional matrix for various cells and takes crucial part in wound healing. Moreover, fibrin network serves as a reservoir of various extracellular matrix proteins; coagulation cascade proteins; and growth factors (GF) *e.g.* fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). These GF are important during angiogenesis or blood vessels formation.

RESULTS AND DISCUSSION:

In this study, we present a novel method of cardiovascular scaffold surface modification with a thin layer of fibrin network with covalently attached heparin that can be subsequently used for attachment of GF via their heparin-binding domains. The effect of the coatings on viability, cell growth and morphology was tested using human umbilical vein endothelial cells (HUVEC). Both heparin and GF bound to the fibrin in dose dependent manner. FGF significantly promoted cell grow, especially in higher concentrations, while the effect of VEGF was less visible. The morphology of EC was dependent on the GF used. In case of FGF the shape of EC was prolonged, while with VEGF the cells had cobblestone-like structure. Best result were obtained when both GF were used at the same time confirming their synergic effect on the EC.

CONCLUSION:

The results presented here demonstrate that we are able to modify synthetic surfaces with fibrin network containing heparin and GF to promote EC viability, proliferation and differentiation.

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ACKNOWLEDGMENTS:

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

85 cBiT: The Compendium for Biomaterial Transcriptomics

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Maastricht University, Maastricht, Netherlands

INTRODUCTION:

The MERLN Institute at Maastricht University is a tissue engineering lab where we develop new techniques to help the human body repair damage. A key aspect of our research is the development of new biomaterials that integrate better with the surrounding tissue. For years already, we have been using transcriptomics, a technology that generates Big Data, to understand how the body reacts to certain biomaterials and how we can improve them. However, the generation of such large amounts of data required a proper data handling strategy, which was never before systematically implemented in our institute. Data were scattered, inadequately and inconsistently coded, and difficult to compare. This motivated us to develop a publically accessible data repository to systematically store data while simultaneously making them available to other researchers: The Compendium for Biomaterial Transcriptomics (cBiT).

METHODS:

The cBiT repository is accessible through a web interface and comprises a central warehouse containing data from transcriptomics studies. Studies are prepared in ISA-Tab format and include all relevant study details and data[1]. After archive preparation, data are first imported into the institutional DataHub repository (doubling as a back-up)[2], followed by import into cBiT where the data are processed and indexed to enable search queries and downloads.

RESULTS AND DISCUSSION:

cBiT was recently launched (https:\\cbit.maastrichtuniversity.nl) and gives users the opportunity to search through and download biomaterial-based transcriptomics data sets. Queries can be initiated using free text search or selection menus. Downloads can be customized to only include the samples of interest. New studies will be added on a regular basis and we are actively approaching owners of biomaterial-based transcriptomics studies to contribute to cBiT.

CONCLUSION:

We present the cBiT repository as a new tool to help researchers in finding unique and standardized knowledge on the interaction of commonly used biomaterials with different cell types and insight into the underlying biological responses. We invite other researchers to add their data to cBiT, thereby becoming the go-to resource for biomaterial-associated data. In doing so, we expect to make a major contribution to a more efficient development of new and better materials that show improved integration in the human body.

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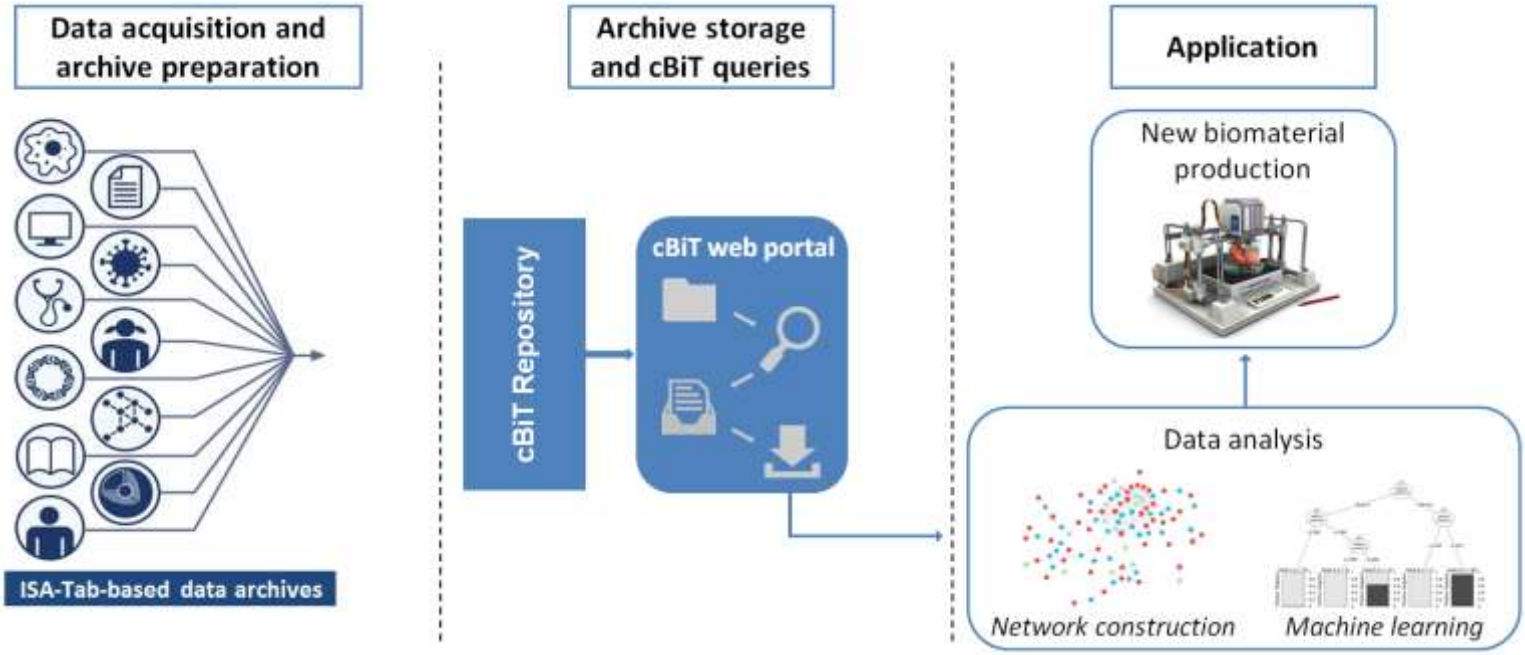
ACKNOWLEDGMENTS:

The authors would like to thank the Dutch province of Limburg (“Limburg INvesteert in haar Kenniseconomie” (LINK), Grant no: FCL67723) and the Dutch Science Foundation (NWO, grant no: 15075) for providing financial support to this project.



Picture 1: Caption 1: Figure: Data in cBiT are archived in a standardized way, allowing for efficient data analysis strategies.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

88 Friction and wear properties of additively manufactured open-cell porous titanium structures

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[2]Dpt. of Industrial Engineering, University of Trento, Italy

INTRODUCTION:

One of the main advantages of Additive Manufacturing (AM) technologies is the possibility to fabricate open-cell porous structures specifically designed for biomedical applications.

While many studies focus on assessing their mechanical stiffness and strength[1] few data can be found on friction and wear properties. A high friction surface is desirable in order to improve initial fixation while wear loss must be limited in order to reduce the formation of loosen metal particles[2].

The present work aims to compare the friction and wear characteristics of several Ti6Al4V open-cell porous structures fabricated by AM.

METHODS:

30 different structures were designed by changing shape and arrangement of the unit cell (10 different geometries), pore size (700 µm, 1500 µm) and strut thickness (340 µm, 640 µm), resulting in 15 regular, 9 irregular and 6 fully random geometries.

Specimens of various dimensions were fabricated via laser powder-bed fusion technology with a gas atomized Ti6Al4V powder.

Actual pore sizes and strut thicknesses were assessed by means of optical and electron microscopy, porosity was calculated by weighing. The static friction coefficient was measured in analogy with ASTM D4518, using a flat block of synthetic bone as counterpart[3]. The wear resistance of seven selected structures was evaluated with a taber abraser according to ASTM F1978.

RESULTS AND DISCUSSION:

The 30 different structures showed statistical differences in terms of porosity (ranging between 22.2±0.5% and 82.6±0.2%) and friction coefficients (ranging between 0.37±0.03 and 0.93±0.21). The higher the ratio between actual pore size and strut thickness, the higher the friction; however, for fully random samples the friction was less dependent by this ratio, while regular geometries showed the largest variation.

All measured weight losses were lower than 30 mg after 100 cycles, below the limit recommended by FDA for biomedical coatings (65 mg)[4].

CONCLUSION:

In terms of good primary fixation, a correct design of open-cell porous structures shall take into account the influence of pore size, strut thickness and unit cell geometry on friction coefficient.

The low values of wear loss, less influenced by design parameters, indicate an adequate abrasion resistance.

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ACKNOWLEDGMENTS:

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

92 Dual crosslinked hyaluronan-pectin cell-laden bioink for 3D bioprinting

Mariana I Neves[1], Rúben F Pereira[1], Miguel Js Ferreira[1], Aureliana Sousa[1], Pedro Granja[2]
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[2]Portuguese Catholic University, Porto, Portugal

INTRODUCTION:

Developing bioinks with good printability features and cell-friendly environments is a challenging task in extrusion-based bioprinting. The aim of this work is to create a heteropolymeric bioink with improved printability features while enabling cell viability during and after the printing process, for skin tissue engineering applications. Hyaluronan is a

component of the extracellular matrix that produces highly viscous solutions and is already used in skin regeneration products[1]. Pectin is a plant-derived polysaccharide used in the food industry and recently explored in the Tissue Engineering field[2,3,4]. In this work, hyaluronan and pectin were modified with methacrylate groups and combined in different concentrations so that proper rheological and mechanical properties could be achieved.

METHODS:

Hyaluronan and pectin were modified with methacrylate groups. Hydrogels were produced by exposing different formulations to UV-light in the presence of the photoinitiator Irgacure 2959. For biological studies, both polysaccharides were modified with thiolated-RGD peptides by a Michael addition type reaction to render the polymer cell adhesive. Human dermal fibroblasts were embedded and cell-laden discs were cultured for 15 days in standard cell culture conditions. Cell morphology and cytoskeletal integrity was assessed by F-actin staining and DNA counterstaining with DAPI. Cell viability was evaluated by Live/Dead assay.

RESULTS AND DISCUSSION:

Hyaluronan formulations were highly viscous, but shear storage modulus values of hyaluronan gels were far lower than gels co-incorporating pectin. On the other hand, despite producing low viscosity solutions, pectin strongly enhanced the stiffness of the produced hydrogels during the time of incubation, as the presence of divalent cations led to a secondary ionic crosslinking between carboxylic groups in pectin chains. Preliminary studies on cellular response show that cells remained viable throughout the culture time, as verified by Live/Dead and that cell spreading was improved by higher pectin contents.

CONCLUSION:

This dual crosslinked heteropolymeric system explores the intrinsic characteristics of both materials by using hyaluronan to tailor bioink viscosity and modulate cell response while pectin provides the possibility to adjust mechanical properties of hydrogels by secondary ionic crosslinking events.

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ACKNOWLEDGMENTS:

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

99 Inhibition of melanin synthesis and melanosome transfer by chitosan biomaterials

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[2]Institute of Biomedical Engineering, Taiwan

INTRODUCTION:

Decreasing skin pigmentation is desired in various medical or cosmetic conditions. Although some pharmaceutical agents are available for inhibiting melanogenesis, their effects are not ideal. Chitosan is a natural compound with good biocompatibility and anti-bacterial property. The present research aimed to investigate the skin whitening effects of chitosan on melanin synthesis and melanosome transfer.

METHODS:

Chitosan with different molecular weight and different degree of deacetylation were added into the medium for culturing B16F10 melanoma cells in the basal or α-melanocyte-stimulating hormone (αMSH)-stimulated conditions, and the synthesis of melanin was evaluated [1]. Moreover, we established a co-culture system using permeable inserts of transwell plates to study the interaction between melanocytes and keratinocytes. Melanosomes release and uptake between human melanocytes and human HaCaT keratinocytes was evaluated with the presence of chitosan in the medium.

RESULTS AND DISCUSSION:

Our results revealed that chitosan can significantly inhibit melanin synthesis and melanosomes release in melanocytes, and it also lessen melanosome uptake in keratinocytes. The inhibitory effect of chitosan on αMSH-stimulated melanogenesis was more significant than those without αMSH stimulation. Molecular weight of chitosan did not affect melanogenesis, while increasing the degree of deacetylation of chitosan enhanced the depigmentation effect (Figure 1). Moreover, chitosan was found to effectively reduce basal and αMSH-stimulated melanogenesis through suppression of the expression of melanogenic-related proteins (microphthalmia transcription factor (MITF), tyrosinase, tyrosinase-related protein (TRP)-1 and TRP-2) and inhibition of tyrosinase activity.

In a co-culture system to analyze the melanocyte-keratinocyte interaction, chitosan could decrease the melanosomes release from melanocytes. It also decreased the melanosomes uptake by keratinocytes phagocytosis by inhibiting the protein expression of protease-activated receptor 2 (PAR2). Numerous skin-whitening agents can modulate the process of melanogenesis, but few have been shown with the capability to decrease the melanosome transfer process. The depigmentation effect of chitosan may associate with its amino residues or positive charges [2].

CONCLUSION:

We demonstrated that chitosan exhibits a robust effect on depigmentation by inhibiting both melanogenesis and melanosome transfer. Therefore, chitosan represents a potential therapeutic approach for hyperpigmentation disorders.

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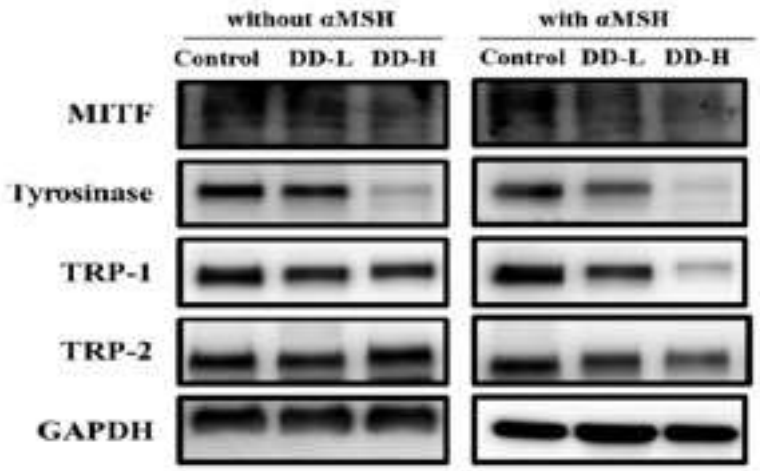
ACKNOWLEDGMENTS:

The authors would like to acknowledge National Taiwan University Hospital for funding this work.



Picture 1: Caption 1: Chitosan with a high degree of deacetylation (DD-H) inhibited the expression of melanogenesis-related proteins in B16F10 melanoma cells.

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Poster presentation

104 Relationship between ion release, durability and cytocompatibility of B2O3 containing phosphate based glasses

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[2]University of Nottingham, United Kingdom

INTRODUCTION:

Various formulations of Phosphate based glasses (PBGs) have been studied extensively for their biocompatibility which is affected by their solution degradation and associated ion release.Therefore, the biocompatibility of PBGs is expected to be affected by the addition of different metal oxides, which are known to alter the chemical durability of the glasses [1-2]. The aim of this current study was to establish the relationship between the ion release, degradation rate and cytocompatibility of PBGs.

METHODS:

Three PBGs formulations were prepared by melting the precursors for 2 hours at 1150^{o}C. The P_2O_5 content was fixed at 40 mol%. 5 and 10 mol% B_2O_3 was added at the expense of Na_2O. The ion release study was conducted using the inductively coupled plasma atomic emission spectroscopy . The cell culture study was conducted using MG63 cell lines. The metabolic activity and proliferation was measured using the alamar blue assay and DNA (Hoechst 33258) assay, respectively. The degradation study was conducted in Phosphate Buffer Solution at 37^{o}C.

RESULTS AND DISCUSSION:

The durability of the glasses increased with the addition of 5 (P40B5) and 10 (P40B10) mol% B_2O_3 which was mainly due to the replacement of P-O-P bond with more hydration resistant P-O-B bond. Although, the durability of P40B10 glasses were significantly higher than P40B5 glasses, the B^{3+} release from P40B10 glasses were significantly higher than that from P40B5 glasses which could be attributed to the presence of higher amount of B_2O_3

in P40B10 glasses. The lowest metabolic activity was observed for the cell cultured on P40B10 glasses, whereas, P40B5 glasses showed the highest metabolic activity. Similarly, the DNA concentration of the cells cultured on P40B5 glass samples was significantly higher than both P40B0 (glasses with no B_2O_3) and P40B10 glasses.

CONCLUSION:

A linear relationship was observed between the ion release and the degradation profile of the glasses. The cells cultured on P40B10 glasses showed lower metabolic activity and cell proliferation as compared to P40B5 glasses. This was attributed to the higher amount of B^{3+} ion release from the P40B10 glasses. Therefore, it could be concluded that the amount of B^{3+} ion release from these glasses should be carefully controlled in order to achieve a suitable end result.

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ACKNOWLEDGMENTS:

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

106 FUNGAL JUNGLE - THE CHEMISTRY OF SURFACE-GRAFTED POLYMERS AFFECTS BIOFILM FORMATION OF CANDIDA ALBICANS

Thomas Michl[1], Carla Giles[1], Piotr Mocny[2], Kathryn Futrega[3], Michael Doran[3], Harm-Anton Klok[2], Hans Griesser[1], Bryan Coad[1]
[1]University of South Australia, Mawson lakes, Australia
[2]EPFL Lausanne, Switzerland
[3]QUT, Australia

INTRODUCTION:

Fungal infections are an underappreciated killer, being the fourth-leading cause of hospital-acquired bloodstream infections. Even worse, systemic infections such as candidiasis have mortality rates of up to 40%. Fungi have proved to be formidable foes, partly due to being eukaryotic like human cells and partly due to their polymorphic adaptability, arguably making them harder to fight than bacteria. For biomedical devices the problem is exacerbated by the small pool of knowledge as to how fungi adhere to and interact with surfaces. Thus, exploring interactions of fungi with surface-grafted polymers of the same thicknesses but different chemical moieties, gives us viable insights into how to combat fungal biofilm formation. Surface-initiated ARGET-ATRP is a well-controlled polymerization process with a broad monomer choice and precise control over the brush thickness. However, laborious chemistry is necessary to install the required ATRP initiator onto the substrate.

In contrast, we here report the use of an industrially viable process, plasma polymerization, to generate a thin and well-adherent covalent coating which then is used to immobilize an ATRP initiator. This was then used to grow poly-(Hydroxyethyl)methacrylate (pHEMA) or poly-methacrylic acid (pMAA) polymer brushes off the surface via surface-initiated ARGET-ATRP under ambient conditions, up to thicknesses of 100nm. These coatings were then further modified by covalently attaching the antifungal drug caspofungin. Upon challenging the surfaces with the pathogen

Candida albicans, biofilm with different morphologies formed. Caspofungin modified surfaces reliably resisted fungal attachment and colonization, and no biofilm formation took place. Our novel combination of plasma polymerization and ARGET-ATRP holds the promise of providing a versatile platform to selectively vary the surface chemistry. This surface chemistry in turn can then be used for studying the differing adhesion and biofilm formation mechanisms of pathogenic fungi. Furthermore, the facile covalent attachment of antifungals to the polymer brushes also enables screening for drugs that can be covalently surface-tethered, for their ability to prevent biofilm formation, which also provides information on membrane targeting.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

110 Improvement in mechanical strength of dental Ag alloy subjected to simple solution treatment

Toshikazu Akahori[1], Tsubasa Mizuno[1], Tomokazu Hattori[1], Mitsuo Niinomi[1], Hisao Fukui[2]
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[2]Aichi-Gakuin University, Japan

INTRODUCTION:

Ag-Pd-Cu-Au type alloy, which was one of representative commercial dental silver type alloys, shows the unique hardening mechanism after a simple solution treatment (ST) at relatively high temperature[1]. In this study, the relationship between ST and mechanical properties of G12 after ST at various temperatures was investigated systematically in this study.

METHODS:

Materials used in this study were hot-rolled plates of Ag-20Pd-17.7Cu-12Au alloy (G12). All samples were conducted with the solution treatment (ST) at 1023 to 1173 K for 3.6 ks in an Ar gas atmosphere followed by water quenching (WQ). Hereafter, the samples of G12 subjected to ST were designated by using temperature. (e.g. G12/1123 K). In addition, some samples subjected to aging treatment (AT) at 673 K for 1.2 ks in a vacuum followed by air cooling after ST(G12/AT673 K). BSE images from SEM and EDS were used for the observation of microstructures and the identification of phase constitutions. The Vickers hardness (HV) and tensile tests were performed to evaluate the mechanical strength.

RESULTS AND DISCUSSION:

Figure 1 shows the microstructures of G12/1173 K and G12/AT673 K. G12/1173 K is composed of three kinds of phases with Ag-rich α_2 phase (white area), Cu-rich α_1 phase (gray area) and β phase (black area) of Pd-Cu intermetallic compound. Although nominal melting point of G12 is around 1233 K, the high Cu concentration area like α1 phase is partially dissolved and then the α_1 and β phases re-precipitate during cooling. On the other hand, the microstructure of the G12/AT673 K was composed of two kinds of phases with α_2 and β phases, which is a typical microstructure of AT after ST.

CONCLUSION:

Microstructure of G12 subjected to ST at relatively high temperature of 1173 K was composed of three kinds of phases with α_2, α_1 and β phases, and had partially dissolved at around high Cu concentration area.

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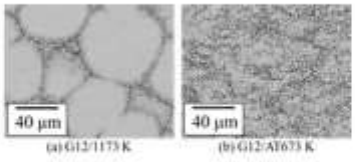

Picture 1: Caption 1: Fig. 1 BSE images of G12/1173 K and G12/AT673 K.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

112 The impact of composite surface roughness on multi-species biofilm composition

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[2]Department of Orthodontics, Seoul National University Dental Hospital, South-Korea

INTRODUCTION:

Surface properties, including surface roughness (SR) and surface free energy, affect oral biofilms formation because they can influence the adhesion and retention of oral microorganisms[1]. The aim of this study was to investigate the effects of SR of composite resin on the composition of multi-species biofilm.

METHODS:

Composite disks were prepared using a polytetrafluoroethylene mold with a glass slide and were randomly assigned to SR180, SR400, SR1500, and SRGlass. Disks in SR180, SR400, and SR1500 were manually roughened with 180-, 400-, and 1500-grit silicon carbide papers, respectively. Disks in SRGlass had an intact surface without surface roughening. SR was analyzed using confocal laser scanning and the surface texture was examined using scanning electron microscopy.

Thirteen strains of bacteria (*Streptococcus mutans* [Sm], *Streptococcus sobrinus* [Ss], *Streptococcus sanguinis*, *Streptococcus salivarius*, *Streptococcus oralis*, *Actinomyces naeslundii*, *Lactobacillus rhamnosus*, *Veillonella dispar*, *Neisseria subflava*, *Fusobacterium nucleatum*, *Prevotella nigrescens*, *Porphyromonas gingivalis* [Pg], and *Aggregatibacter actinomycetemcomitans* [Aa]) were prepared to simulate biofilm formation in the oral cavity. The multi-species biofilms were grown on composite surfaces in the CDC biofilm reactor (BioSurface Technologies, Bozeman, MT, USA) using a modified McBain medium[2]. Adhesion of Sm, Ss, Pg, Aa, and total bacteria were determined at day 1 (T1) and day 4 (T2) using real time polymerase chain reaction. Repeated measures analysis of variance was used to determine time-related changes in bacterial composition with respect to SR.

RESULTS AND DISCUSSION:

There were significant differences in SR among the four groups. The order of SR, from highest to lowest, was SR180, SR400, SR1500, and SRGlass (SR180 > SR400 > SR1500 > SRGlass, $p < 0.05$). Adhesion of total bacteria, Sm, and Ss to SR180, SR400, and SR1500 was higher than to SRGlass, but there was no significant difference in adhesion among SR180, SR400, and SR1500. However, adhesion of Aa and Pg to composite resin was not significantly influenced by SR. Incubation time significantly influenced the composition of biofilms. Adhesion of total bacteria, Sm, Ss increased with time from T1 to T2, whereas the adhesion of periodontopathogens decreased from T1 to T2 (Table).

CONCLUSION:

Because surface roughening significantly promoted bacterial adhesion, specifically cariogenic bacteria (Sm and Ss) to composite surfaces, regardless of incubation time, this study suggests that periodic repolishing may help to minimize cariogenic biofilm formation around composite surfaces.

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Table. Differences in biofilm composition with respect to surface roughness.

	Day 1 (T1)	Day 4 (T2)	Multiple comparisons[†]	
			Time	Surface treatment
Total bacteria (Log_{10}/unit area)				
SR180[a]	7.25 ± 0.19	7.80 ± 0.38		
SR400[b]	7.20 ± 0.24	7.77 ± 0.33	(T1 < T2)*	(SR180 , SR400, SR1500 > SRGlass)*
SR1500[c]	7.10 ± 0.21	7.70 ± 0.36		
SRGlass[d]	7.06 ± 0.24	7.62 ± 0.39		
Streptococcus mutans (Log_{10}/unit area)				
SR180	3.88 ± 0.42	4.24 ± 0.60		
SR400	3.81 ± 0.46	4.12 ± 0.48	(T1 < T2)*	(SR180 , SR400, SR1500 > SRGlass)*
SR1500	3.76 ± 0.42	4.06 ± 0.27		
SRGlass	3.60 ± 0.36	3.81 ± 0.33		
Streptococcus sobrinus (Log_{10}/unit area)				
SR180	4.10 ± 0.20	4.97 ± 0.61		
SR400	4.05 ± 0.37	4.75 ± 0.62	(T1 < T2)*	(SR180 , SR400, SR1500 > SRGlass)*
SR1500	3.94 ± 0.38	4.80 ± 0.58		
SRGlass	3.78 ± 0.22	4.44 ± 0.61		
Aggregatibacter actinomycetemcomitans (Log_{10}/unit area)				
SR180	4.31 ± 0.50	3.93 ± 0.38		
SR400	4.17 ± 0.27	3.88 ± 0.29	(T1 > T2)*	SR180 = SR400 = SR1500 = SRGlass
SR1500	4.12 ± 0.20	3.91 ± 0.27		
SRGlass	4.02 ± 0.23	3.87 ± 0.46		
Porphyromonas gingivalis (Log_{10}/unit area)				
SR180	2.15 ± 0.98	1.96 ± 0.67		
SR400	2.02 ± 1.16	1.69 ± 0.91	(T1 > T2)*	SR180 = SR400 = SR1500 = SRGlass
SR1500	2.08 ± 0.88	1.71 ± 0.86		
SRGlass	2.21 ± 0.61	1.74 ± 0.70		

[a]The composite surface roughened with 180-grit silicon carbide paper.

[b]The composite surface roughened with 400-grit silicon carbide paper.

[c]The composite surface roughened with 1500-grit silicon carbide paper.

[d]The composite surface prepared with a glass slide.

[†]Repeated measures analysis of variance was used to determine significant differences between the two time points using the Bonferroni corrections at a significant level of $\alpha < 0.05$.

* $P < 0.001$

Picture 1: Caption 1: Table. Differences in biofilm composition with respect to surface roughness.

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Poster presentation

113 Age-related properties of human dental pulp stem cells cryopreserved for regenerative medicine

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INTRODUCTION:

There is a limited number of studies on long-term cryopreserved dental pulp stem cells (DPSC) regarding their applicability in correlation with donors age[1-4]. The aim of this study was to investigate stemness properties, proliferative and differentiation capacity of DPSC in correlation with donors age and cryopreservation protocol.

METHODS:

DPSC were isolated from third molars extracted from healthy patients during orthodontic treatment, with their consent, according to ethics norms. Fifteen cultures were divided in 3 groups (n=5): I (16-21 years), II (23-29 years) and III (32-43 years). DPSC before freezing and DPSC cryopreserved in 10% glycerin for 1 year were cultured for 9 passages and comparatively studied for cell viability by Live/Dead assay and population doubling time (PDT) by cell counting using a Burker Turk hemocytometer. Differentiation of DPSC in osteogenic medium for 21 days was investigated by quantitative analyses of calcium ions secretion, alkaline phosphatase activity and collagen secretion. Statistical analysis was performed using one-tailed paired Student's t-test on each pair of interest.

RESULTS AND DISCUSSION:

DPSC cultures from all tested groups presented fibroblast-like morphology, adhesion capacity and mesenchymal immunophenotypic profile, regardless of donors age. After cryopreservation, DPSC cultures presented similar viability (~83%) at 24 h of cultivation. PDT increased from one passage to another, indicating slowdown of DPSC proliferative capacity in each tested group, but at higher speed in group III (Fig. 1). Moreover, thawed DPSC proliferation was prolonged even with 10 h, compared to DPSC before freezing. Metabolic activity of osteogenic differentiated DPSC from group III was influenced by donors age and cryopreservation, significantly decreasing ($p<0.05$) the quantity of secreted markers, compared to groups I and II.

CONCLUSION:

DPSC cultures could be expanded and preserved at any age, but long-term viability, proliferative and differentiation capacity were optimal for young (groups I and II, under 30 years old) samples.

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ACKNOWLEDGMENTS:

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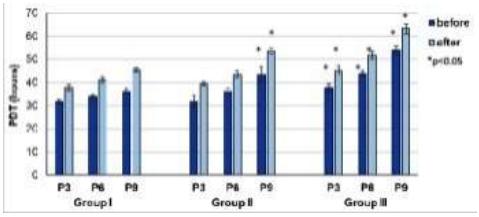

Picture 1: Caption 1: Population doubling time (PDT) of DPSC from 3 tested groups (I-III, n=5), cultivated for 9 passages, before and after cryopreservation in 10% glycerin

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Poster presentation

121 Impact of autoclave sterilization of gelatin on endotoxin level and physical properties compared to surfactant purified gelatins

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INTRODUCTION:

Endotoxins are found in the outer membrane of gram-negative bacteria and have profound in vitro and in vivo responses. They can trigger strong immune responses and negatively affect various cellar activities at levels as low as 5-20 pg/ml#_ftn1 (0.01-0.05 EU/ml). They are therefore unwanted contaminants of biomaterials sourced from natural raw materials and their activity must be as low as possible. Hence, FDA defined 0.06-0.5EU/ml as application limits depending on the type of application. Collagen and gelatin are natural extracellular matrix components and have, due to their low allergenic potential, suitable biological properties, and tunable physical characteristics, high potential in biomedical applications. The purpose of this study was to determine the influence of autoclave sterilization of gelatin on physical properties and endotoxin level compared to surfactant purified gelatin.

METHODS:

Type A gelatin from Sigma-Aldrich (G1890) with endotoxin level of 35000 endotoxin units (EU) per gram gelatin and type A gelatins from Rousselot Gent with endotoxin activity of 30000 EU per gram were used. A 10 w/w% G1890 gelatin solution was autoclave sterilized during 30 minutes at 121°C and 1 bar over pressure. The physical properties and the endotoxin level of the sterilized G1890 gelatin was compared to a type A gelatin from Rousselot purified with Triton X100 surfactant#_ftn1. The endotoxin levels of the gelatins were measured using the Endozyme recombinant factor C method from Hyglos GmbH (Germany).

RESULTS AND DISCUSSION:

Autoclave sterilization significantly affect the physical properties of gelatin. Molecular weight of G1890 decreased from 140 to 50kDa and gelstrength decreased from 300 to 40g. The endotoxin level of the gelatin reduced after sterilization from 35000 EU/g to levels of 400-500 EU/g. These endotoxin levels are however still far above the upper endotoxin level of 5EU/g to avoid cell proliferation alteration based on a 1% gelatin solution. Molecular weight and gelstrength of Rousselot gelatin was not altered after Triton X100 purification and remained 150kDa and 300g respectively. The endotoxin levels of Triton X100 purified Rousselot gelatin was <5EU/g.

CONCLUSION:

Autoclave sterilization of gelatin is, in comparison to Triton X100 purification, not efficient to inactivate endotoxin levels in gelatin to levels below the upper limit to avoid cell proliferation alteration. Autoclave sterilization gave in a significant decrease in molecular weight and gelstrength which makes autoclave sterilized gelatin, in comparison to Triton X100 purified gelatin, not suitable for 3D printing.

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Poster presentation

130 Fabrication and Antibacterial Activity of Pectin-Ag Hydrogel

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INTRODUCTION:

As antibacterial resistance has become a global issue, there is a pressing need to develop new bactericidal agents. The polymer-inorganic composite materials, such as, biopolymeric hydrogels containing AgNPs could be an answer to drug-resistant bacteria.

METHODS:

Nanocomposites were synthesized by chemical reduction of Ag^+ by pectins with different degree of methoxylation (including containing amide groups)[1]. The polysaccharide/metal ratio in nanocomposites was 10:1 and 25:1, the concentration of Ag^0 was ~ 1.7 mM. Hydrogels were prepared by ionotropic gelation[2] of pectin-Ag nanocomposites by Ca^{2+}. Kanamycin (KAN) entrapped into a hydrogel by sorption from its aqueous solutions (2-4 mg/ml). Antibacterial activity against test strains of *B. pumilus, B. subtilis, E. coli, Ps. aeruginosa* was studied by serial dilutions and well diffusion methods.

RESULTS AND DISCUSSION:

The synthesized pectin-Ag nanocomposites are AgNPs with pectin shell. These AgNPs have a spherical shape (with diameter from 10 to 40 nm), a negative charge (ξ = – (50÷68) mV) and an maximum absorption with in 400-443 nm, depending on the type of pectin. The pectin-Ag hydrogels were characterized by a ξ-potential of -30 mV, a water content of 98wt.% and a high kanamycin capacity (ca. 0.6 mg per 1.0 mg of polymer). The entrapped kanamycin in the pectin-Ag hydrogel does not reduce its bioavailability and allows prolonging release in physiological saline of 0.9% NaCl pH 5.5 and PBS at pH 7.4 at 37 ° C to 100 and 150 min, respectively.

The antibacterial activity of nanocomposites and their hydrogels depends on the type of pectin in composition and its mass fraction. Thus, nanocomposites and hydrogels based on high-methoxylated citrus pectin (pectin: Ag = 25: 1) have showed the greatest activity: MIC_{Ag} was 0.40-1.55 mM and 0.02-0.2 mM, respectively. For kanamycin-containing hydrogels a synergistic antibacterial effect was observed. Thus, for E.coli MIC for KAN and Ag^0 were $7{,}8 \cdot 10^{-3}$ mg/ml and $1{,}8 \cdot 10^{-2}$ mM, correspondingly. However, when used in combination, reduced quantities of kanamycin ($3 \cdot 10^{-4}$ mg/ml) and Ag ($<1 \cdot 10^{-3}$ mM) were required to achieve the same growth inhibition effect.

CONCLUSION:

Thus, combining silver nanoparticles (AgNPs) with antibiotics is consider to be a potential method to overcome bacterial drug resistance.

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ACKNOWLEDGMENTS:

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

132 Development of a multilayer-based chitosan and cyclodextrin-polymer wound dressing with dual therapy to treat infected wounds

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INTRODUCTION:

Due to their large-spectrum antibacterial activity, silver ions are today largely used in wound dressings. But their main negative side effect is cytotoxicity. To overcome this effect, we functionalized a textile on the basis of chitosan and cyclodextrin polymers as a multilayer structure with silver incorporated only on the first layer. As chronic wounds are also characterized by inflammation, a second anti-inflammatory compound, ibuprofen, was also incorporated yielding a device with a dual action.

METHODS:

Polyester (PET) textile was functionalized by crosslinked citric acid-β-cyclodextrin polymer (PCD) under curing conditions to form a thermofixed negatively charged layer (PET-PCD) and then it was loaded with a silver sulfate solution (10 g/L) (PET-PCD-Ag) to obtain an antibacterial layer. In a second step, a polyelectrolyte multilayer system (PEM) was built on the PET-PCD-Ag by alternating self deposition of chitosan (CHT, low Mw, 0.5% w/v) as cationic polyelectrolyte and water soluble PCD as anionic polyelectrolyte (0.3% w/v) yielding PET-PCD-Ag-PEM samples.

A curing process was further applied to stabilize the multilayer system. The swelling ratio was determined in a solution mimicking wound exudates. Silver loading and silver release in acidic media was determined by atomic spectroscopy.

PET-PCD-Ag-PEM textiles were finally impregnated in ibuprofen (IBU) solution (1g/L). The IBU loading and IBU release in PBS pH 7,4 profiles were determined by HPLC-UV. The antibacterial activity of the wound dressing was evaluated on *S. aureus* and *E. coli* by *Kill-Time* test and agar diffusion assays.

RESULTS AND DISCUSSION:

The weight increase of the textile after thermofixation was 33% and increased up to 58% after PEM construction. The swelling ratios of the wound dressing reached 50%w/w. The silver sorption on the first thermofixed PCD layer (600 mg/cm^2) did not affect the linear PEM build-up. The IBU sorption on PET-CD-Ag-PEM textile was 0.6 mg/cm², and the drug release was prolonged up to 6 hours in PBS medium. *Kill time* test revealed a 3 Log_{10} reduction on PET-CD-Ag-PEM on both strains for up to 24 hours and agar diffusion test demonstrated that IBU did not interfere with the antibacterial activity of silver.

CONCLUSION:

The wound dressing developed provided antibacterial activity. We used the advantages of PCD in the PEM system to incorporate IBU in order to treat wound pain and excessive inflammation. In addition, an "*in vivo*" evaluation of this wound dressing is in progress on infected mice to evaluate the antibacterial efficacy of the wound dressing and the wound healing process after 3 days.

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Poster presentation

135 Influence of different cyclodextrin polymers on the properties of chitosan-hydrogels for tissue engineering application

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INTRODUCTION:

Hydrogels are 3D polymeric networks swelled by water, that mimic extracellular matrix topography, support cell proliferation and can release biomolecules. Recently, injectable hydrogels have attracted increased attention in the field of tissue engineering (TE) because of their potential for mini-invasive procedures and consequently, less traumatic recovery for patients[1]. Chitosan (CS) physical hydrogels are of particular interest because of the absence of cross-linker. Polymers of cyclodextrines (PCD) crosslinked with citric acid could form a polyelectrolyte complex with CS that then could be used as injectable hydrogels in TE. Both types of PCD: water soluble (PCDs) and water insoluble (PCDi) have same chemical structure but different physicochemical properties[2], which could affect the characteristics of hydrogels. Based on this, the aim of the study was to evaluate the properties of hydrogels based on CS, PCDs and PCDi with various proportions.

METHODS:

Three CS:PCDi:PCDs ratios were studied for forming hydrogels: 3:0:3$_{w/w}$, 3:1.5:1.5$_{w/w}$ and 3:3:0$_{w/w}$. The powders of CS, PCDs and PCDi were first co-milled in a mixer mill, then suspended in ultrapure water and acidified with lactic acid by continuously mixing with two interconnected syringes. Hydrogels were characterized by assessing their viscoelastic properties using a rheometer and measuring their cohesion in phosphate-buffered saline (PBS, pH 7.4). Hydrogels cytocompatibility was evaluated by indirect contact with pre-osteoblast cells (MC3T3-E1) by using AlamarBlue® assay

RESULTS AND DISCUSSION:

Rheology revealed that hydrogels 3:0:3$_{w/w}$ and 3:1.5:1.5$_{w/w}$ had similar viscoelastic properties and a low damping factor (G"/G'), which indicates that these hydrogels are elastic. The ratio 3:3:0$_{w/w}$ (only PCDi) showed different viscoelastic properties and a higher damping factor than the others (Fig. 1). Among all, the hydrogels 3:1.5:1.5$_{w/w}$ showed the best cohesion and stability in PBS; the hydrogel 3:3:0$_{w/w}$ disintegrated quickly at 10 min after injection

and was discarded for further tests. Cytocompatibility of hydrogels 3:0:$3_{w/w}$ and 3:1.5:$1.5_{w/w}$ were acceptable with 77% and 74% of cell survival, respectively.

CONCLUSION:

The proportion of PCDs and PCDi showed a significant impact on the properties of CS/PCD hydrogels. Among all the ratios, hydrogel 3:1.5:$1.5_{w/w}$ appeared to be the most suitable as injectable hydrogel for TE. Incorporation of biomolecules into this hydrogel will be studied for improving their properties.

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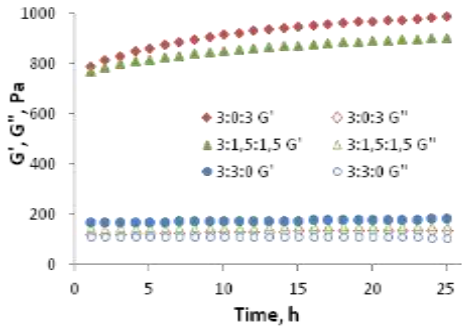

Picture 1: Caption 1: Fig.1. Storage and loss modulus (G' and G") of 3 hydrogels.

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Poster presentation

144 The immunomodulatory effects of surface chemistry on macrophage polarisation

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INTRODUCTION:

Macrophages are major effectors in eliciting foreign body reactions against biomedical implants. A potential strategy to limit these reactions is developing immunomodulatory biomaterials, to direct macrophage polarization away from the pro-inflammatory (M1) phenotype and towards the wound healing phenotype (M2) phenotype[1]. Biomaterials surface chemistry has been shown to influence the function of different cell types including macrophages[2,3]. In this study, using a high-throughput screening strategy, we investigated the immunomodulatory effects of 283 acrylate or acrylamide polymers on macrophage polarisation, with the purpose of identifying immune-instructive polymers as well as understand the basis of the cell-materials interactions.

METHODS:

Polymer microarray formation

283 polymer spots, with diameters of up to 300 μm, were printed in triplicates onto pHEMA coated glass slide.

Monocyte isolation and cell culture

Monocytes isolated from buffy coats of 7 healthy human donors were seeded uniformly on the polymer microarray slides (n≥3). Incubation was done at 37^{O}C for 8 days.

Immunofluorescence staining

Cells were stained for M1 and M2 markers, calprotectin and mannose receptor (MR) respectively, followed by imaging using a fluorescent microscope. Cells which stained positively for either marker were counted and M2/M1 ratios were calculated for each polymer. Polymers amongst the top or bottom 30 percentile of M2/M1 ratios were considered as M2 or M1 polarising polymers and were selected for further functional studies.

RESULTS AND DISCUSSION:

To determine the modulatory effects of biomaterial surface chemistry on macrophage polarisation, we quantified expression of M1 and M2 associated markers on macrophages cultured on different polymers. A high M2/M1 ratio is indicative of a less pro-inflammatory microenvironment and vice-versa. Results generated showed that a few polymers consistently biased cells from up to 4 biological donors either towards the M1 or M2 phenotypes.

The micro-array format presents a time and cost efficient approach for screening a wide range of materials for beneficial macrophage responses. Different material chemistries had varying effects on the expression of phenotypic markers by macrophage with some polymers inducing distinct pro or anti-inflammatory phenotypes.

CONCLUSION:

Our findings show that polymer choice influence macrophage behaviour. Future work will focus on consolidating these observations and also elucidating the molecular basis of material induced macrophage polarisation.

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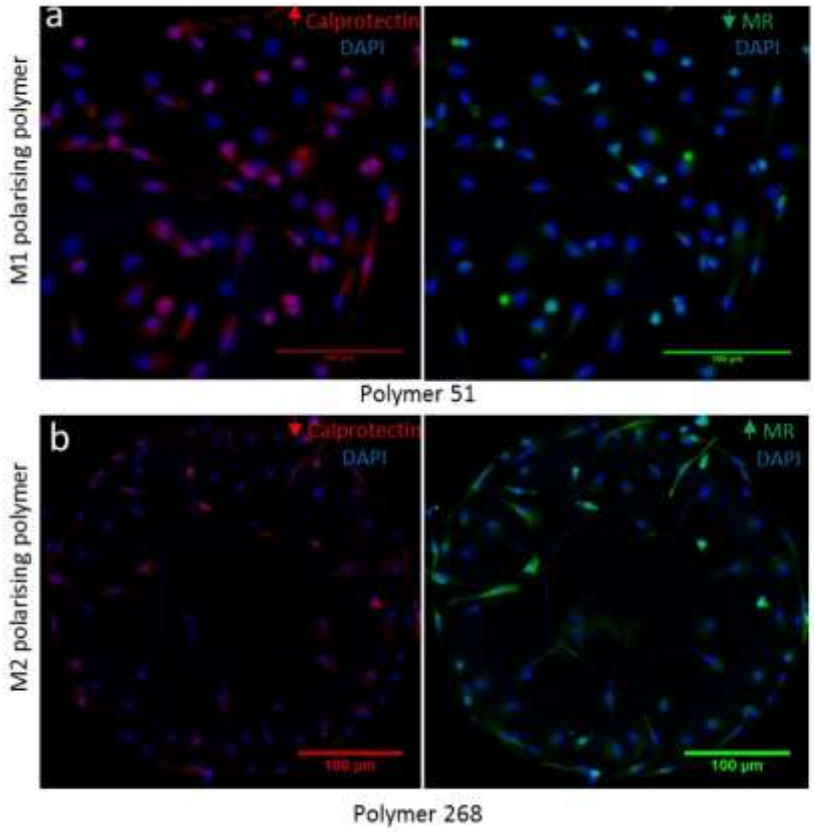
I would like to thank the University of Nottingham, UK for funding my PhD studies.



Immunofluorescence analysis of macrophage phenotypic markers on different polymers. Images show differential expression of calprotectin and mannose receptor by cells on M1 and M2 polarising polymers. A) polymer 51 B) polymer 268. Representative images are shown from n ≥ 3 technical replicates.

Picture 1: Caption 1: Immunofluorescence analysis of macrophage phenotypic markers on different polymers.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

150 Hypotrochoid-based scaffolds for osteochondral tissue engineering

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INTRODUCTION:

Osteoarthritis remains a serious clinical problem and current treatments are limited. Tissue engineered constructs can act as a solution to this problem. Collagen type II is the main structural protein found in articular cartilage and the fiber architecture follows pathways that can be described with a section of a hypotrochoidal geometry[1]. To the extent of our knowledge the application of mathematical hypotrochoid-based design has not been reported in designing scaffolds for osteochondral tissue regeneration. The hypotrochoid scaffold can be parametrically designed to mimic the architectural properties of the collagen fibers that compose the osteochondral tissue.

METHODS:

A custom algorithm was developed to allow the parametric calculation of the hypotrochoidal patterns which where sectioned to a region of interest and used as printing pathway for fused layer manufacturing (FLM) technique. Scaffolds of poly(ε-caprolactone) were manufactured with a screw extrusion based FLM equipment (Bioscaffolder,

SYSeng). The hypotrochoid scaffolds were mechanically characterized under compression and compared to a regular 0°/90° degrees pattern scaffold. Agarose collagen hydrogel containing primary chondrocytes were deposited inside the pores of the scaffolds through drop-on-demand bioprinting.

RESULTS AND DISCUSSION:

The custom written algorithm showed the feasibility to explore and manufacture hypotrochoidal patterns (Figure 1). Mechanical data shows that the arches formed with the hypotrochoid scaffold have a higher Young's Modulus compared to a regular 0°-90° degrees scaffold. The scaffolds proved to be cytocompatible and the cultured cells followed the fibers architecture of the scaffold.

CONCLUSION:

The preliminary data from this study shows the possibility to create a scaffold that mimics the collagen fiber orientation found in human articular cartilage. Despite the fact that the fibers produced with FLM are not in the same scale as collagen fibers, the algorithm could potentially be used in different techniques like melt electrospinning. This method of editing the printing pathway in FLM enables to create even more complex structures with intricate filling patterns.

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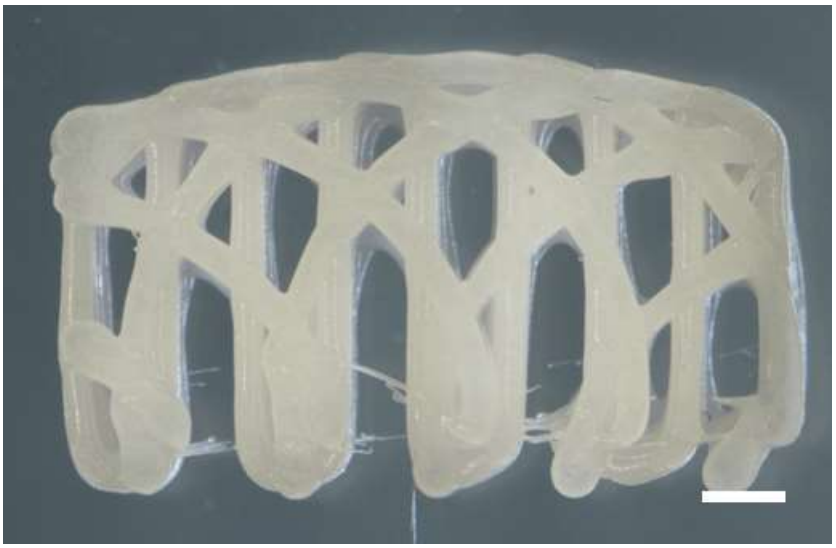

Picture 1: Caption 1: Figure 1. Macroscopic image of the hypotrochoidal based design scaffold mimicking the collagen fibrillar-like architecture found in articular cartila

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Poster presentation

151 3D printed hydrogel scaffolds based on bioconjugated alginate and graphene oxide nanoparticles

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INTRODUCTION:

3D printed scaffolds of hydrogel materials have been focus of interest due to their unique properties that mimic the extracellular matrix (ECM).[1] Photocrosslinkable hydrogels based on natural polymers like alginate (ALG), chondroitin sulfate (CS) and gelatin (GEL) are highlighted due to the increase biocompatibility and bioactivity of scaffolds.[2,3] Graphene oxide otherwise has been added to hydrogels for mechanical reinforcement and for rendering other functionalities with superior properties.[4] In this work it is shown novel biocompatible nanocomposite hydrogel inks for 3D printing of scaffolds, based on photocrosslinked ALG-CS-GEL biopolymer and graphene oxide (GO).

METHODS:

ALG and CS were functionalized with 2-aminoethyl methacrylate group and GEL was modified by reaction with methacrylic anhydride, producing ALG-MA, CS-MA and GEL-MA methacrylated biopolymers. GO was synthesized using modified Hummers method. Three inks were formulated varying the concentration of GO. Scaffolds were fabricated by 3D printing using calcium chloride solution as primary ionic crosslinker and UV radiation for covalent crosslinking of the methacrylated biopolymers. Cytotoxicity of scaffolds was studied by MTT assay of human adipose tissue derived mesenchymal stem cells (hAD-MSCs). For statistical comparison, one-way ANOVA followed by Tukey's test was employed.

RESULTS AND DISCUSSION:

A methacrylation degree of ~30% for ALG-MA and CS-MA and 100% for GEL-MA (relative to lysine amino groups) was determined by NMR. FTIR spectra of GO shows the typical bands of oxidized functional groups, besides the XRD diffractogram display the characteristic reflection near to 12° of GO and HR-TEM images confirm the layered morphology. The presence of GO into the hydrogel inks improved the printing quality and the scaffold resolution (see Figure 1). The three inks showed to be cytocompatible with hAD-MSC, the viability was higher than 80% on day 7 in MTT assay in each scaffold, there is not statistical difference regarding viability between scaffolds and control ($p < 0.05$).

CONCLUSION:

The addition of GO in nanocomposite hydrogels enhance the printability of the ink, allowing builds scaffolds with higher resolution than inks without GO. The scaffolds are cytocompatible with hAD-MSCs becoming potential candidates for soft tissue engineering.

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ACKNOWLEDGMENTS:

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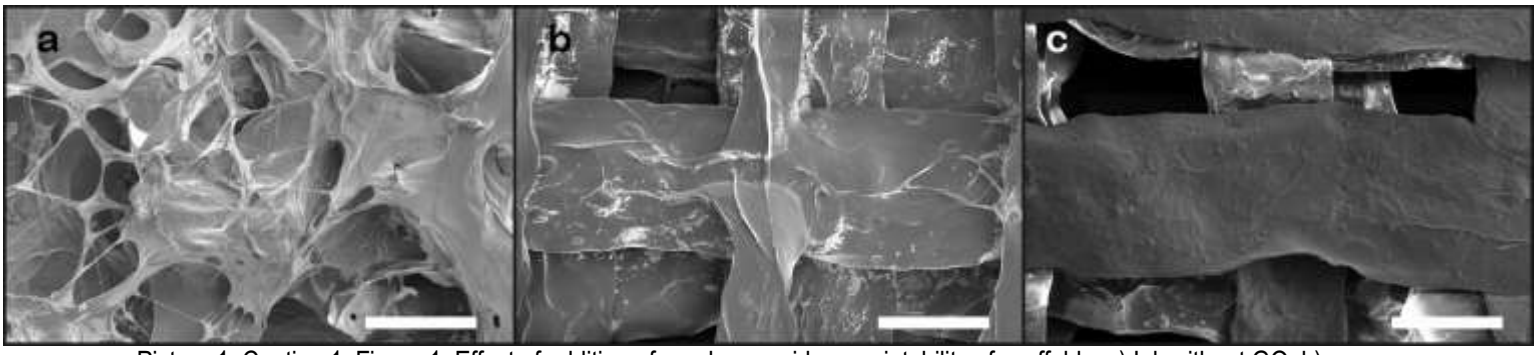

Picture 1: Caption 1: Figure 1. Effect of addition of graphene oxide on printability of scaffolds: a) Ink without GO, b) 0.1 mg/mL GO and c) 1 mg/mL GO.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

157 PIM-structured biomaterials for optimized bone regeneration

Frédéric Velard[1], Wissame Arabi-Taourirt[1], Christine Guillaume[1], Julien Braux[1], Justin Roche[2], Gaëlle Dangla[2], Bruno Cauwe[2], Delphine Auzène[2], Gangloff Sophie C.[1]
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INTRODUCTION:

One fundamental problem for bone engineering is the requirement of adequate biodegradable, porous and osteoinductive scaffolds. Calcium-phosphate biomaterials and bioactive glasses are commercially available and currently used in clinic[1] but reaching the goal to ideal structured biomaterial has not been yet achieved[2]. In this context, we proposed to elaborate a porous scaffold using powder injection molding (PIM). This approach appears to be particularly relevant for the challenging production of foams-like porous parts that could not be achieved using conventional sintering processes in an industry-compliant manner. The present work aims to develop and validate, in vitro, porous bone substitutes intended to accelerate bone regeneration.

METHODS:

Bioactive glass (Noraker) was embedded by Technical Centre for Ceramics and Glass, Portugal, before inclusion of NaCl spacer. Thermogravimetric analysis (TGA), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDXS) and microcomputed tomography were performed to characterize extruded materials. Raw material, the desorbed one (24h in cell culture media) and supernatants from desorption were tested for their impact on human primary osteoblasts culture.

RESULTS AND DISCUSSION:

TGA indicated that less than 1.5% of polymer remains after aqueous and thermal debinding and EDXS confirmed absence of remaining NaCl. This validated the debinding procedure. Tomography highlighted that closed porosity was reduced by 80% in parallel with a doubled open porosity volume when comparing 44% spacer and 73% spacer feedstocks. SEM analysis clearly demonstrated the presence of disseminated pores (mean diameter 500 µm) inside the 44 % material which were connected in the 73% material. These results suggested that 73% material could be the most suitable for bone regeneration purpose[3] (Figure 1A and B). First analyses on primary human osteoblasts behaviour demonstrated that nor the particles (desorbed or not) neither the desorption supernatants were cytotoxic, underlying the expected biocompatibility of the materials.

CONCLUSION:

Our data indicated that highly porous bioactive glass-made structure may be used with PIM process, which could be promising for bone tissue engineering. Further analyses are ongoing to determine the osteoconductive/osteoinductive properties of the biomaterials in vitro and in vivo in long bone critical defect model.

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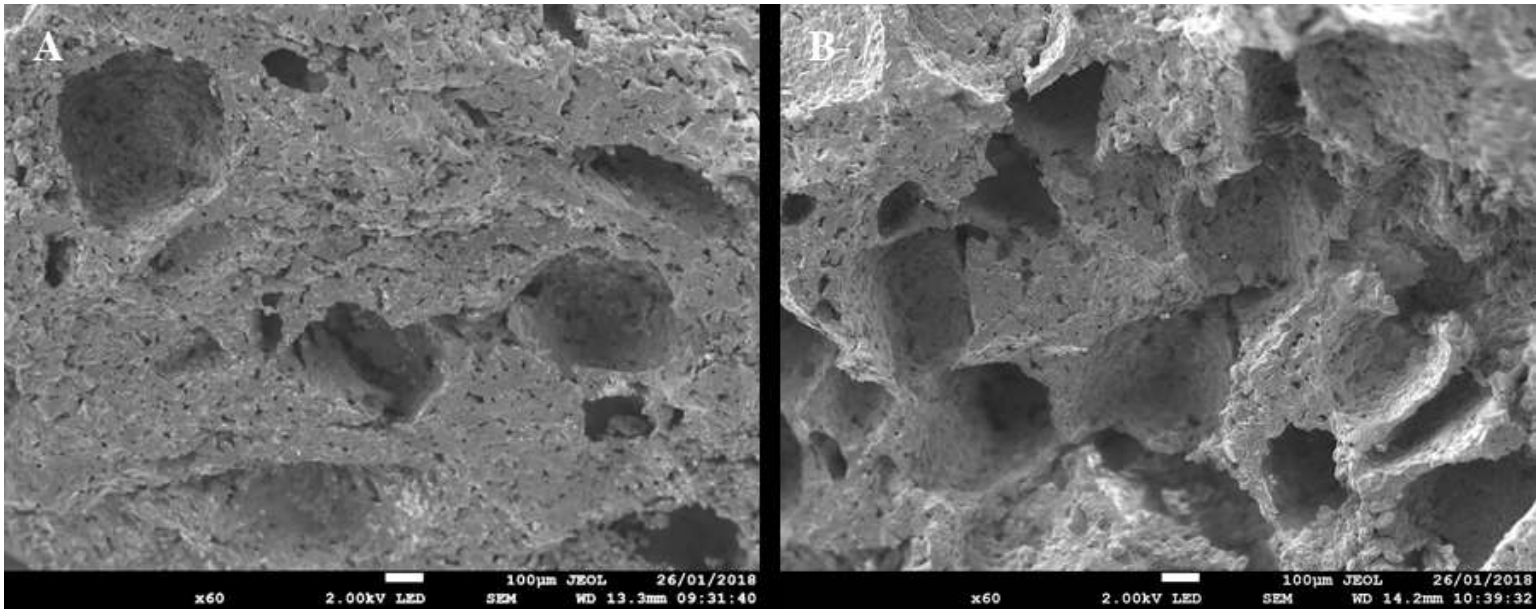
ACKNOWLEDGMENTS:

Authors would like to thank PICT platform for imagery and Institut Carnot MICA for funding the project (BiomateriOs exploratory program 2017).



Picture 1: Caption 1: SEM micrographs showing 44% (A) and 73% (B) spacer containing materials. JEOL JSM-7900F.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

159 Surface modification and biomechanical evaluation of of Ti-Nb-Sn alloy implants with a low Young's modulus

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INTRODUCTION:

Titanium metal (cp-Ti) and titanium alloys are widely used in various types of implants in orthopaedic and dental fields, because they have the greatest biocompatibility with bone. Ti-Nb-Sn alloy (Ti-35mass%Nb-7.9mass%Sn alloy.) is a novel β-type Ti alloy with a 40 GPa Young's modulus, which is similar to that of human cortical bone (30 GPa)[1]. However, the availability for dental implant is unknown.

On the other hand, it is known that the biocompatibility of Ti will change with the condition of the surface oxide (titania: TiO_2) film of implant Ti alloy. Electron cyclotron resonance (ECR) plasma oxidation is effective surface modification of metal Ti for biocompatibilities, which became clearly in previous study [2]. Our previous studies indicated that implantation of octacalcium phosphate [$Ca_8H_2(PO_4)_6 \cdot 5H_2O$, OCP] was efficiently enhanced bone regeneration compared to HAp [3].

The purpose of this study is to investigate the biomechanical behavior, biocompatibility and the effect of ECR plasma oxidation conditions on structure and precipitation behaviour of OCP in Ti-Nb-Sn alloy.

METHODS:

Commercially pure titanium Grade 2 and Ti-Nb-Sn alloy were used as substrate materials. Cell Culture and Proliferation (Mouse bone marrow stromal ST-2), Alkaline Phosphatase (ALP) Activity, and push-in tests by SD rats were measured.

ECR plasma oxidation apparatus was used for oxidation of Ti-Nb-Sn alloy surface. OCP was precipitated in phosphate-buffered solution on TiO_2 film deposited Ti substrate[3].

RESULTS AND DISCUSSION:

The cell proliferation assay showed that the propagated cells grew equally well on the Ti-Nb-Sn alloy and cpTi throughout the experiments. No significant difference in ALP activity was apparent in the cells grown on both surfaces. The push-in values of Ti-Nb-Sn alloy were relatively higher than that of cpTi in the early stages of healing.

Crystalline rutile-type TiO_2 films were obtained after oxidized by ECR plasma above 500 °C . Mixtures of OCP and dicalcium phosphate dihydrate (DCPD) peaks were observed after calcification. The amount of OCP and DCPD increased with increasing total pressure (PT) from $3.3x10^{-3}$ - $1.5x10^{-2}$ Pa, and showed the maximum at PT = $1.5x10^{-2}$ Pa.

CONCLUSION:

Our results suggest that Ti-Nb-Sn implants have a similar biological potential as cpTi. It was suggested that ECR plasma oxidation method is effective surface modification for the Ti-Nb-Sn alloy, because of their calcification ability.

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

166 Shear-thinning poly(β-cyclodextrin)/Tetronic-adamantane hydrogels as injectable biomaterial

Hyo Jin Lee, Ho Jun Kwon, Ki Dong Park
Ajou University, Suwon-si, South-Korea

INTRODUCTION:

Supramolecular hydrogels based on host-guest interaction has been extensively developed for biomedical applications. Cyclodextrin exhibits unique complex-forming ability with hydrophobic guest molecules in aqueous solutions. Particularly, β-cyclodextrin(host molecules, β-CD) and adamantane(guest molecules, Ada) has been widely employed as it has strong binding ability in water and reversible interactions.[1] The materials based on β-CD and Ada interactions has shear-thinning properties, showing viscous flow under shear stress that enable allowing injection through a syringe or catheter. It also has self-healing properties that recover elastic modulus after shear.[2] This study developed a shear-thinning hydrogel using poly(β-CD) and thermosensitive Tetronic-adamantane conjugate(Tet-Ada) and investigated the physicochemical properties such as shear-thinning and recovery of this hydrogels.

METHODS:

Poly(β-CD) was synthesized by grafting β-cyclodextrin using epichlorohydrin and toluene. Tet-Ada polymer was synthesized by conjugating adamantylamine with amine-reactive Tet-$(PNC)_4$. The structure of each polymers was characterized by ^{1}H NMR. Poly(β-CD)/Tet-Ada hydrogel was prepared by simply mixing two components. The mechanical properties of hydrogels were measured by performing a time sweep at 1.0 strain% and 10㎐. The shear-thinning and recovery properties of poly(β-CD)/Tet-Ada hydrogel were investigated under the various conditions (shear rate sweep :0.01-1.0 s^{-1}, cyclic deformation measurements :100% and 1% strain; 10㎐).

RESULTS AND DISCUSSION:

The chemical structure of Tet-Ada was confirmed by ^{1}H NMR ($CDCl_3$) : δ 1.5-1.85 (t, 2H, -CH_2- of adamantane). The formation of hydrogels was demonstrated by the increase of elastic modulus(G') after mixing two component solutions (from 0 to over 200 Pa). In addition, hydrogel showed higher G' value at 37℃ than that at 25℃, due to the thermosensitive nature of Tetronic. The continuous flow experiments showed that the viscosity of poly(β-CD)/Tet-Ada hydrogels decreased as increasing the shear rate, demonstrating the shear-thinning properties. The cyclic deformation test showed a clear decline in moduli and concurrent gel-sol transition at the onset of high strain conditions. At the transition from high to low strain conditions, rapid recovery to initial mechanics was observed.

CONCLUSION:

Shear-thinning poly(β-CD)/Tet-Ada hydrogels based on the host-guest interactions were developed. This hydrogels exhibited shear-thinning behaviors, rapid recovery properties and higher elastic modulus at 37℃ due to the thermosensitive nature of Tetronic. From the obtained results, the poly(β-CD)/Tet-Ada hydrogels can be a potential for injectable biomaterials.

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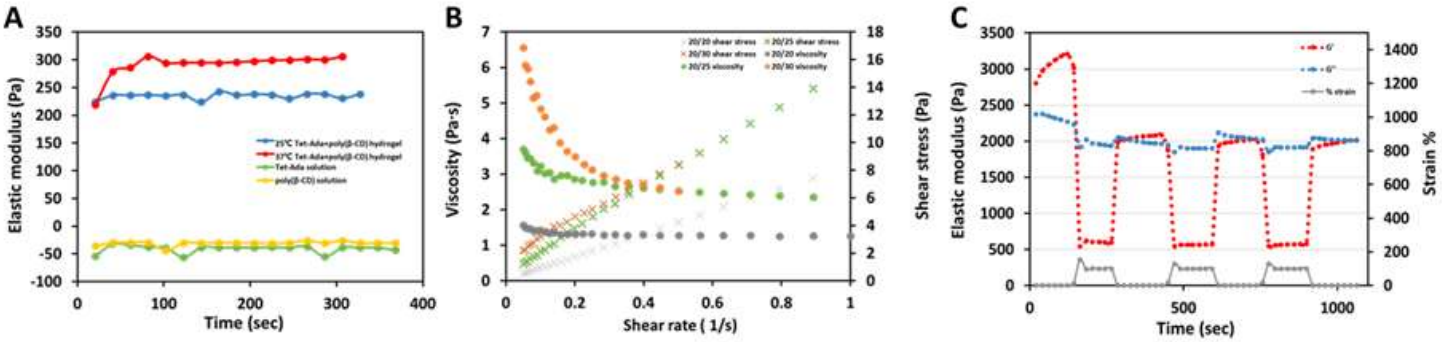
ACKNOWLEDGMENTS:

This study is supported by the National Research Foundation of Korea (NRF) grand funded by the Korea government (MSIP) (NRF-2015R1A2A1A14027221).



Picture 1: Caption 1: Figure 1. Rheological properties and relaxation behavior of hydrogels

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

167 Development of siRNA delivery system by EpCAM-targeting lipid nanoparticles using non-standard macrocyclic peptide.

Yu Sakurai, Wataru Mizumura, Hideyoshi Harashima
Hokkaido University, Sapporo, Japan

INTRODUCTION:

For developing an efficient siRNA delivery system, siRNA must be delivered to a cytosol of target cells by drug delivery systems, such as lipid nanoparticles (LNPs). Polyethyleneglycol (PEG) is used for functionalizing LNPs with regard to biocompatibility. However, PEG modification severely deteriorated a delivery of its cargo to target cells due to limited internalization to target cells via steric hindrance. To achieve an efficient siRNA delivery to cancer cells, we modified LNPs with non-standard macrocyclic peptide against epithelial cell adhesion molecule (EpCAM), which is highly expressed in cancer cells, previously identified. In this study, we tried to develop EpCAM-targeting LNPs.

METHODS:

To display EpCAM-targeting peptide on the surface of LNP, peptide was conjugated to the end of PEG-lipid via amide bond. The conjugation was confirmed by MALDI TOF-MS. Anti-polo-like kinase 1 (PLK1)-siRNA, which is well-validated gene for proliferation of cancer cells, was encapsulated in LNPs by alcohol dilution method as previously reported[1]. The gene silencing effect was measured by quantitative RT-PCR. For *in vivo* gene silencing, human ovary cancer SK-OV-3 cells were inoculated into nude mice on the right flank or intraperitoneally injected.

RESULTS AND DISCUSSION:

When non-targeting LNP (NT-LNP) or EpCAM-targeting LNP (ET-LNP) was added to the cells in the *in vitro* condition, ET-LNP exhibited over 10-fold cellular uptake compared to NT-LNP. To examine whether ET-LNP delivered its cargo, *PLK1* expression was determined 24 hour after the addition. As a result, IC50 of ET-LNP was 10-30-fold lower than that of NT-LNP. For *in vivo* evaluation, NT-LNP and ET-LNP was injected into SK-OV-3-bearting mice. Intravenous injection of ET-LNP exhibited approximately 60% gene silencing while NT-LNP did no silencing effect. Besides, we applied ET-LNP for peritoneal dissemination model. After ET-LNP was administered into peritoneal at a dose of 2.5 mg/kg, mRNA expression in floating cancer cells and solid tumor adhered on mesenteric was measured by qRT-PCR. ET-LNP showed a significant knockdown both in floating cells and in the solid tumor.

CONCLUSION:

ET-LNP demonstrated a prominent siRNA delivery both in subcutaneous model and in peritoneal dissemination model. In the future, we applied this system for various cancer models.

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ACKNOWLEDGMENTS:

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

168 Development of hyaluronic acid derivative-modified nanoparticle HAL-PoCL for malignant cells expressing CD44

Akari Kato, Yu Sakurai, Hideyoshi Harashima, Shoshiro Yamamoto
Hokkaido University, Sapporo, Japan

INTRODUCTION:

Since CD44 is expressed in many types of cancers, CD44 is one of promising target for active targeting system with nanoparticles. Although hyaluronic acid (HA) is well-known as a ligand for CD44, there is no effective nanoparticles functionalized through CD44-HA interaction. One possibility for this failure is difficulty to control topology of HA for sufficient interaction with CD44 on the membrane of nanoparticles. In this study, we aimed development of comprehensive system for cancer therapeutics using nanoparticles through a synthesis of a new HA derivative as a specific ligand.

METHODS:

In general HA modification methods to nanoparticles, carboxylic acid of HA is used for binding with nanoparticles via covalent bond or electrostatic interaction. However, the carboxylic acid is an active site for CD44-HA interaction[1]. In order to present HA to CD44 without using carboxylic acid for binding nanoparticle, we developed “Hyaluronic Acid-Lipid conjugate: HAL” by conjugating lipid with reductive terminal sugar in HA by reductive amination. Here, we evaluated the efficacy of HAL-PoCL that is Positive Charged Lipid nanoparticle (PoCL) modified with HAL to malignant pleural mesothelioma (MPM), which highly expresses CD44.

RESULTS AND DISCUSSION:

The cellular uptake of nanoparticles in MPM cells was significantly increased by HAL in the presence of cationic lipids while the amount was not by HA. The mechanism of this synergistic effect has not be unviled. However, since the interaction of CD44 and HA is known to be weak (Kd = 86.8 μM for 31 kDa HA)[2], there are some possible mechanisms as follows; First, cationic lipid enforces CD44-HA interaction itself. Second, cationic lipid mediates endocytosis of cells and nanoparticles while CD44-HA interaction is the starting point of cellular uptake of nanoparticle. Optimized HAL-PoCL encapsulating cisplatin (CDDP) exhibited stronger cytotoxicity than free CDDP to MPM cells in vitro. In addition, HAL-PoCL encapsulating CDDP exhibited substantial anti-cancer effect with low adverse effect with MPM orthotopic transplant model in vivo.

CONCLUSION:

Nanoparticle modified new HA-lipid conjugate was efficiently delivered to cancer cells highly expressing CD44. These results indicate that it is also necessary to take the structural arrangement of ligands into the account for modification on nanoparticles.

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ACKNOWLEDGMENTS:

The authors would like to express our appreciation to Dr. Hida Y., Hida K. and Maishi N. for providing MPM cells. This study was supported by The Mochida Memorial Foundation for Medical and Pharmaceutical Research.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

177 Release of silver from nanostructured surface of alfa-beta titanium alloy

Ludek Joska, Eva Pruchova, Petra Jarolimova, Vojtech Hybasek, Jaroslav Fojt
UCT Prague, Prague 6, Czech Republic

INTRODUCTION:

Titanium and its alloys are common materials in production of implants. Surface of any implanted material is preferred place for bacterial adhesion. Bacterial attack is a serious problem that can result in the failure of implant. One of the most important demands is therefore to create a bioactive surface with antibacterial properties. The goal of this work was to prepare specific silver containing nanosurface of Ti6Al4V alloy able release silver in a programmable way.

METHODS:

Experimental work consists of nanostructures preparation, development of method able to deposit silver into specific areas of nanosurface and electrochemical study of the release of silver. Nanostructures were prepared by potentiodynamic-potentiostatic method in electrolyte consisting of $(NH_4)_2SO_4$+NH_4F. Silver was deposited from $AgNO_3$+KNO_3 deaerated electrolyte by potentiostatic pulse method. Surface state was checked by SEM and XPS methods. Electrochemical exposures were done in aerated physiological saline solution (9,0 g/l NaCl) at 37°C. Standard electrochemical characteristics and electrochemical impedance spectra were measured. Amount of released silver was detected with the help of ICP-MS method.

RESULTS AND DISCUSSION:

Ti6Al4V alloy is alfa-beta alloy. The goal of nanostructures preparation was to remove grains of beta alloy from the surface and to create homogenous nanostructure on remaining surface. Nanotubes with diameter between 40-50 nm and length 523±41 nm were prepared. The use of pulse reduction method allowed for deposition of silver mainly into vacant areas after beta phase removal with open nanotubes just “coloured” by silver. The dosage is quantitative and programmable. Silver oxidation is possible to monitor by polarization resistance trend nevertheless because this process is not the only electrochemical reaction on the nanosurface its value is inapplicable for silver release rate calculation. According to results of corrosion measurements and environment analyses during exposures dissolution of silver was stepwise. Firstly higher amount was released from the whole nanostructure surface, continuing by maintaining dosage from smaller surface areas of silver grains in former vacancies.

CONCLUSION:

Preparation of nanosurface with beta-grains removed allowed for specific deposition of silver into vacant areas and onto the whole nanosurface. Electrochemical measurements and analytical results acknowledged two phase silver release mechanism - higher amounts at the beginning able to cover initial bacterial attack followed by decrease during the second long-term period.

ACKNOWLEDGMENTS:

The authors would like to thank Czech Health Research Council (project no. 15-27726A) for providing financial support to this project.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

178 Biogenic hydrogels for tissue engineering of cartilage

Andrea Liedmann[1], Juliane Strätz[2], Steffen Fischer[2], Thomas Groth[3]
[1]Martin Luther University Halle-Wittenberg, Halle (saale), Germany
[2]Dresden University of Technology / Institute of Wood and Plant Chemistry, Germany
[3]Martin Luther University Halle-Wittenberg / Institute of Pharmacy, Germany

INTRODUCTION:

In situ cross-linking hydrogels are useful for minimal invasive treatment of tissue defects. Our previous studies could demonstrate a tremendous bioactivity of sulphated cellulose towards growth factors comparable to that of Heparin[1,2]. Hence we propose here semisynthetic sulfated and oxidized cellulose derivatives for the generation of hydrogels with tuneable mechanical properties, degradation behaviour and release of growth factors for tissue engineering of cartilage.

METHODS:

Cellulose was sulphated first, followed by a Malaprade oxidation that leads to a cleavage of the C2-C3 bond of glucose monomers.

RESULTS AND DISCUSSION:

The obtained cellulose derivatives (CS) had different degrees of sulfation from 0.7 to 1.5 and oxidation degrees from 0.1 to 0.4. The biocompatibility of these derivatives was analysed *in vitro* studying the metabolic activity and lactate dehydrogenase release of mouse fibroblasts exposed to different concentrations of CS dissolved in tissue culture

medium (DMEM). Figure 1 shows the metabolic activity of fibroblasts treated 24 h with oxidized CS in dependence on concentration and aldehyde content. The results show that all CS were non-toxic at lower concentrations (0,5 mg/ml), but with rising oxidation degree and concentrations, toxic effects were observed particularly for highly oxidized CS. By contrast, carboxymethyl chitosan, which was later used as second component for gel formation was not toxic even at highest concentration of 5 mg/ml.

When oxidized CS (concentration: 20 mg/ml) were mixed 1:1 with carboxymethyl chitosan hydrogels were formed within a few minutes. The hydrogels were stable for more than 14 days under cell culture conditions. First studies with encapsulated mouse fibroblasts and adipose-derived stem cells (ADSC) showed maintenance viability over a period of 7 days. In ongoing studies, the ADSCs encapsulated in the hydrogels are differentiated up to 21 days with soluble and immobilized TGF-ß3 to analyse the bioactivity of hydrogels regarding the chondrogenic differentiation of cells.

CONCLUSION:

We conclude that hydrogels composed of semisynthetic oxidized CS might be suitable as building blocks for *in situ* cross-linking hydrogels for tissue engineering of cartilage.

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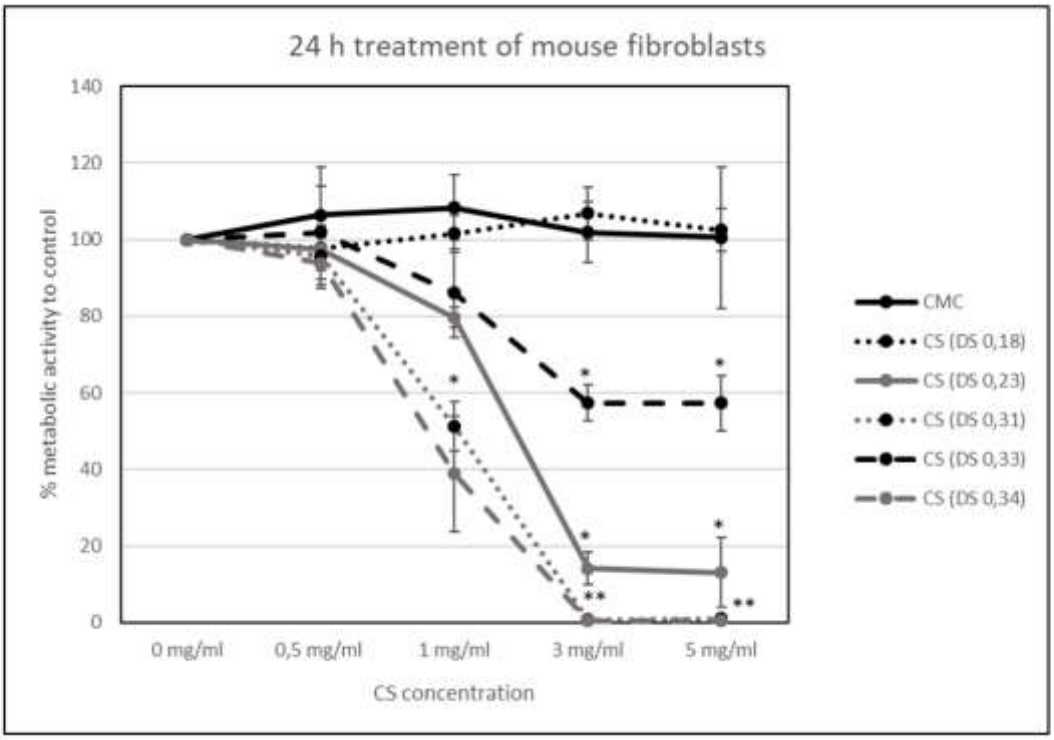

Picture 1: Caption 1: results

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Poster presentation

185 developing nanomaterial-based strategies for remediation of biological tissue contaminants

Tochukwu miss Ozulumba, Ganesh dr Ingavle, Patrick dr Dyer, Susan dr Sandeman
University of Brighton, Brighton, United Kingdom

INTRODUCTION:

Contaminant removal from biological tissues is hindered by several challenges including rising antimicrobial resistance and long treatment durations[1] prompting a search for innovative solutions. The properties of graphene nanoplatelets (GNP), graphene oxide (GO), graphene oxide-silver (GO-Ag) and multilayer Ti_3C_2 MXene are investigated to test whether these nanomaterials and their composites can be synthesised for biomedical remediation.

METHODS:

Nanomaterial biocompatibility was assessed in 3T3 fibroblasts with MTS, LDH and Live/Dead assays. Apoptotic potential was investigated in Jurkat cells with flow cytometric detection of FITC/Annexin V. Antimicrobial activity was studied in *Escherichia coli* and *Staphylococcus aureus* with colony count and ATP assays. Adsorptive potential was evaluated using ELISA detection of residual pro-inflammatory cytokines (IL-6, IL-8, TNF-α) after 2-hour incubation of nanomaterials with spiked plasma (1000 pg/mL). Experiments were repeated thrice. Statistical analysis was performed by one-way ANOVA (GraphPad Prism 7). Polycaprolactone (12%, w/v) was electrospun from chloroform/dimethylformamide (3:1) at 0.5 mL/hour, 15 cm and 16 kV. Nanomaterials were incorporated onto the electrospun nanofibres via chemical coupling.

RESULTS AND DISCUSSION:

GNP, GO and MXene did not impact 3T3 cell viability. GO-Ag caused membrane damage, attributed to the immobilised silver nanoparticles on the sheet surface. GO-Ag induced apoptosis in Jurkat cells within 1 hour. Only GO-Ag reduced *E. coli* and *S. aureus* viability, attributed to enhanced contact between the silver nanoparticles and bacteria[2]. The graphenes adsorbed more IL-6 and IL-8 than MXene. The nanomaterials removed less TNF-α due to its large molecular weight[3]. Nanomaterials were successfully incorporated onto the nanofibres to preserve their functional activity without uncontrolled release.

CONCLUSION:

GNP, GO and MXene supported cell growth and had adsorptive activity. GO-Ag had both antibacterial and adsorptive activity suggesting potential for dual function. Studies are ongoing to investigate retention of activity in the nanomaterial-nanofibre composites.

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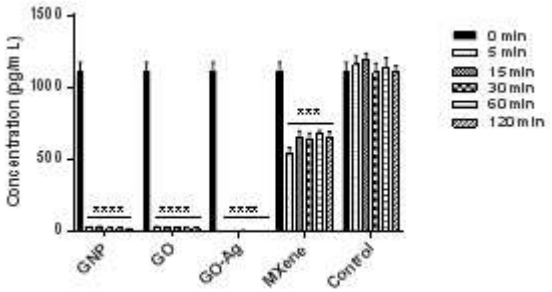

Picture 1: Caption 1: Figure 1: IL-8 adsorption by GNP, GO, GO-Ag and Ti3C2 MXene (mean ± SEM, n = 3) (***p < 0.001, ****p < 0.0001).

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

188 Controlling blood-material interaction by tuning the composition of amorphous metals

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[2]ETH Zurich, Switzerland

INTRODUCTION:

While metals have become a standard material class for blood-contacting devices, their high thrombogenic potential usually requires complex coatings of the material's surface and systemic anticoagulation therapy, which both bare the risk of bleeding complications. Therefore, new metallic materials with similar or advanced mechanical properties but reduced thrombogenic risk are desired. Metallic glasses are gaining increasing attention as promising materials for blood contacting devices due to the increased strength and elasticity as well as superior corrosion and wear resistance in comparison to their crystalline counterparts. This study aims to understand the impact of material surface physicochemical properties and material bulk atomic order on the blood response towards metallic glasses in comparison to state-of-the-art medical grade crystalline Ti64 and diamond like carbon (DLC) coated Ti64 surfaces.

METHODS:

Pd-, Ti-, and Zr-based metallic glasses with different elemental compositions were prepared and characterized via XRD, XPS and WCA measurements. Ti64 and DLC-coated Ti64-samples were used as reference materials. Samples were incubated for 8, 16 and 48 min in partially heparinized human whole blood obtained from healthy volunteers (with ethical approval), washed with PBS and fixed for subsequent analysis. Supernatants were collected for analysis via ELISA. Platelet adhesion and activation, fibrin formation as well as expression of biomarkers specific for the blood coagulation cascade (PF4, TAT, F1/F2) were analyzed by SEM, CLSM, and ELISA.

RESULTS AND DISCUSSION:

Upon incubation with human whole blood, a reduced thrombogenic potential of Pd-glass in comparison to Ti64 was observed. No significant differences were observed between Ti64 and Ti- or Zr-based metallic glasses. Interestingly, an elevated platelet adhesion but reduced fibrin formation and lowered expression of further blood coagulation

cascade biomarkers were found on the surface of Pd-based metallic glass in comparison to Ti64 and Ti- or Zr-based metallic glasses, which was however at a similar level as observed on the surfaces of the DLC-coated reference material. Evaluating the influence of the metal atomic order on the blood response by comparing the same Pd-based alloy in its amorphous with its crystalline state, no differences were observed.

CONCLUSION:

The blood response is dependent on the chemical composition of metallic glasses, being similar for Pd-based metallic glass and the state-of-the-art DLC coated surface. Notably, not the metal atomic order, but the surface chemistry is decisive for controlling the blood"'material interaction.

ACKNOWLEDGMENTS:

Funding from *Uniscientia Foundation,* project title: "Einfluss der Oberflächeneigenschaften von Herzimplantaten auf die Thrombogenese." is gratefully acknowledged.

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Poster presentation

194 Toughness of photo-crosslinked gelatin/PEG hybrid hydrogels

Jia Liang, Dirk Grijpma, André Poot
University of Twente, Enschede, Netherlands

INTRODUCTION:

Hybrid hydrogels made of natural and synthetic polymers may have interesting biological and mechanical properties for biomedical applications. Methacrylated gelatin (Gel-MA) is synthesized by reaction of gelatin with methacrylic anhydride. Modification of amine groups of gelatin to methacrylate groups makes gelatin photo-crosslinkable without losing its biological properties. Poly(ethylene glycol) (PEG) is a water-soluble synthetic polymer, which can also be methacrylated. Its hydrogels are popular for tissue engineering applications because they are non-toxic and exhibit favorable hydration and nutrient transport properties[1,2]. The aim of this work is to prepare tough gelatin-PEG hybrid hydrogels with potential for tissue engineering applications.

METHODS:

Gelatin (50-100 kg/mol) and PEG (4 kg/mol) were functionalized by reaction with methacrylic anhydride. The degrees of functionalization of Gelatin-methacrylate (Gel-MA) and PEG-dimethacrylate (PEG-dMA) were 37% and 99%, respectively[3]. Gel-MA and PEG-dMA were dissolved at concentrations of 20% (w/v) in de-ionized water at 50 °C. Irgacure 2959 (0.05% (w/v)) was used as photo-initiator. Solutions with different ratios of Gel-MA:PEG-dMA were stirred at 50 °C until they became homogeneous. The solutions were then transferred into quartz glass molds (13x3x0.1 cm) and photo-crosslinked by irradiation at 365 nm for 30 min at 50 °C. The mechanical properties of the Gelatin-PEG hydrogels were investigated by tensile testing according to ASTM D 638.

RESULTS AND DISCUSSION:

Tensile curves of the Gelatin-PEG hydrogels are shown in Figure 1. Different ratios of Gel-MA and PEG-dMA in the solutions during crosslinking have an effect on the mechanical characteristics of the formed networks. The 25%:75% Gelatin:PEG hydrogel showed the highest toughness (area under the curve, 10.40 N/mm^2).

CONCLUSION:

The toughness of the 25:75 and 50:50 Gelatin:PEG hybrid hydrogels was higher than of the 100% gelatin and 100% PEG hydrogels.

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ACKNOWLEDGMENTS:

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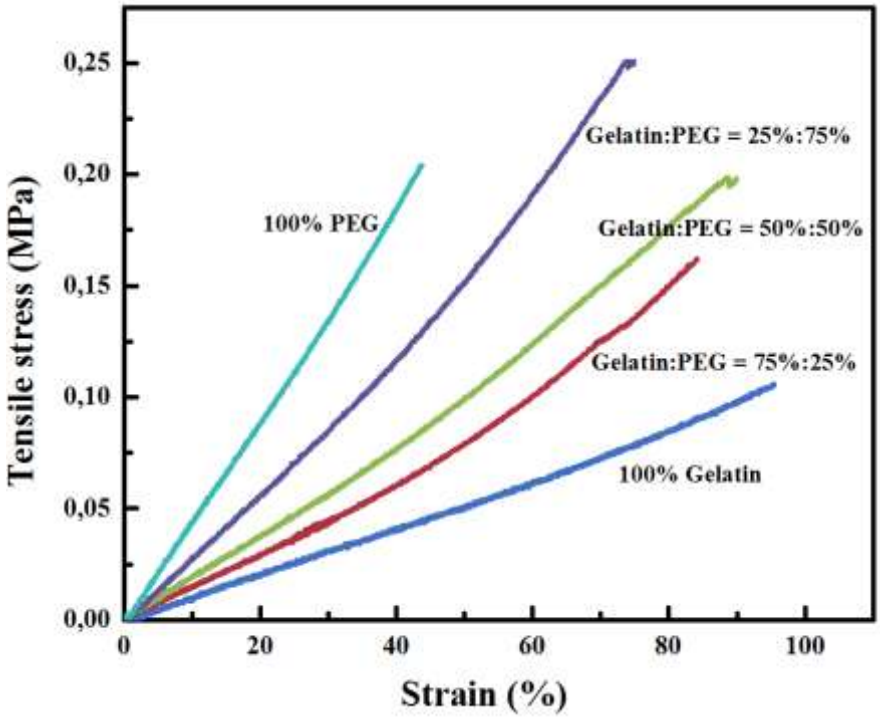

Picture 1: Caption 1: Figure 1. Stress-strain curves of Gelatin-PEG mixed hydrogels.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

201 The microscale architecture of the acto-myosing cytoskeleton regulates nanotopography sensing

Julien Gautrot, Stefania di Cio, John Connelly
Queen Mary University of London, London, United Kingdom

INTRODUCTION:

Cells can sense and respond to chemical and physical stimuli coming from their surroundings (the extracellular matrix -ECM) ultimately deciding whether to adhere or not to them[1]. Surface modification of materials at the

nanoscale via chemical[2] or topographical[3,4] patterning has demonstrated that cells sense surface chemistry and nanotopography. However, processes enabling nanotopography sensing remain unclear. We have recently developed a novel technique (Electrospun Nanofibre Lithography (ENL))[5] to produce fibre-shaped quasi 2D nanopatterns with varying sizes and controlled chemistry on large scales. In this work we show that the microscale architecture of the acto-myosin network regualtes nanotopograhy sensing.

METHODS:

We characterised cell and focal adhesion morphology via epifluorescent and confocal microscopy. Our nanopatterning approach was designed to be compatible with high resolution live fluorscence microscopy. We followed the dynamics of actin and proteins regulating actin assembly (α-actinin, myosin, cofilin) via live imaging.

RESULTS AND DISCUSSION:

The patterns tested presented fibre ranging from 200 to 1000 nm. Preliminary results showed that cell morphology and focal adhesion assembly are correlated to the size of the fibre, with spreading and cytoskeleton organization improving with the increase of the fibre sizes. Vinculin recruitment dynamics was not significantly affected by the nanofibres dimension. However, cytoskeleton dynamics in cells spreading on the nanofibres was significantly faster and resulted in frequent actin misassembly (foci), prior to collapse of the actin network. Live imaging further highlighted the role of myosin contractility in the dynamics of these foci, which were positive for α-actinin and cofilin.

CONCLUSION:

These results suggest that focal adhesions are not the primary sensing sites of nanoscale topography of the ECM and that actin dynamics plays an important role in regulating such sensing, hence providing a long range mechanism to nanotopography sensing. These observations shed light on the mechanisms underpinning the design of biomaterials with controlled surface chemistry and topography.

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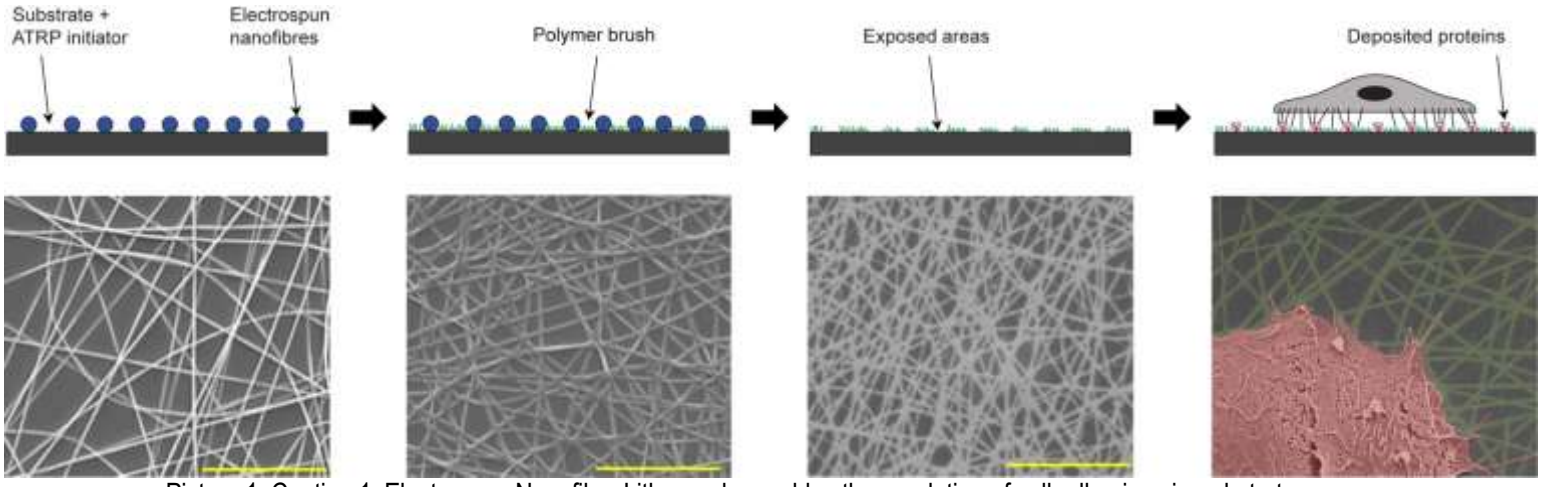
ACKNOWLEDGMENTS:

Funding from the Engineering and Physical Sciences Research Council (EP/J501360/1) is gratefully acknowledged.



Picture 1: Caption 1: Electrospun Nanofiber Lithography enables the regulation of cell adhesion via substrate nanotopography and the study of mechanisms via live imaging

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Poster presentation

204 Micro and nano silver deposition into TiAlV nanostructure leading to antimicrobial efficiency

Eva Pruchová, Ludek Joska, Jaroslav Fojt, Petra Jarolímová, Vojtech Hybášk
University of Chemistry and Technology, Prague, Prague 6, Czech Republic

INTRODUCTION:

Bacterial contamination and the biofilm formation is quite a big threat for metallic biomaterials. Almost 80 % of all complications associated with implants in hospitals are attributed to the bacterial infections[1].

Bio-activation of titanium surface can be achieved e.g. by anodic oxidation which results in formation of nanostructure in fluorine contained solution. To suppress the rise of infection, it is possible to use large surface area of nanostructure as a substrate for anchoring antibacterial agents[2]. Silver is well known for its strong anti-bacterial activity against wide range of micro-organisms[3]. Silver deposition could be done by various methods[4].

METHODS:

Titanium alloy Ti6Al4V was used to prepare nanostructure. Silver was deposited on the surface of nanostructured samples by photochemical reduction and electrochemical pulses deposition. Another set of the nanostructured samples was irradiated by UV. Antibacterial efficacy was evaluated by using the quantitative assessment and the indicator strain of bacteria was *Staphylococcus aureus*. The silver release rate in the model solutions was monitored electrochemically.

RESULTS AND DISCUSSION:

Cathodic pulses enabled microsilver particles reduction and its localization mainly inside the cavities created after beta phases, and into the mouth and partly by the length of the individual nanotubes. Silver particles were almost absent on the bottom of the tubes with the usage of this method. The photoreductive method of silver reduction enabled the filling of nanotubes along its whole length. Silver nanoparticles were present in many different sizes

compared to the quite uniform and larger particles produced by cathodic pulses. Photoreducted silver particles had obviously smaller and more varied size.

Antibacterial activity was verified on samples with different silver amount. Tested samples had sufficient lethal effect on *S. aureus*.

CONCLUSION:

Obtained results showed the promising modifications, which meet requirements for direct antimicrobial efficiency. Surfaces with the minimum deposited amount of silver inhibited bacterial activity, even after 48 hours. The deposited silver was still available in the nanostructure after this time. Biofilm formation has been also suppressed.

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ACKNOWLEDGMENTS:

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

206 Stem cell culture on nanosheets assembled at the surface of liquid microcarriers for applications in regenerative medicine

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Queen Mary University of London, London, United Kingdom

INTRODUCTION:

Adherent cell culture is typically thought to require cell spreading on rigid substrate. However, it was proposed that cells may be able to sense nanoscale physical and mechanical properties rather than directly bulk mechanics[1]. In fact, several reports indicate that cell adhesion and culture may be possible at the surface of liquids[2,3]. Here, we show that mesenchymal stem cells (MSCs) can be cultured at the surface of liquids and demonstrate that this behavior is regulated by protein self-assembly at the liquid-liquid interface and associated nanoscale mechanical properties.

METHODS:

The formation of poly(L-lysine) (PLL) nanosheets at liquid-liquid interface were studied by X-ray photoelectron spectroscopy (XPS), fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and atomic force microscopy (AFM) and their mechanical properties were characterised by interfacial rheology. Fluorescence microscopy was used to study the spreading and proliferation of MSCs on oil substrates.

RESULTS AND DISCUSSION:

Interfacial rheology demonstrated the assembly of PLL and fibronectin nanosheets, with mechanical properties controlled by protein concentration, pH and the presence of surfactants. Interfacial shear moduli ranged over several orders of magnitude (from 10^{-4} to 10^{0} N/m). We propose that such variation results from variations in the surfactant functionalization level of PLL, as indicated by XPS. Immunostaining shows stable focal adhesion formation and F-actin assembly, despite the absence of bulk mechanics of oil substrates used. MSCs expansion was also observed (Figure 1) and their proliferation profile was correlated to the nanoscale mechanical properties of the interfaces they were cultured on.

CONCLUSION:

Cell adhesion and proliferation is regulated by nanoscale mechanical properties of protein interfaces assembled on oils, rather than their bulk mechanical properties. The interfacial mechanics can be optimized for cell culture by controlling nanosheet chemistry and self-assembly. Such platform can be applied to the design of emulsion-based 3D bioreactors allowing stem cell expansion.

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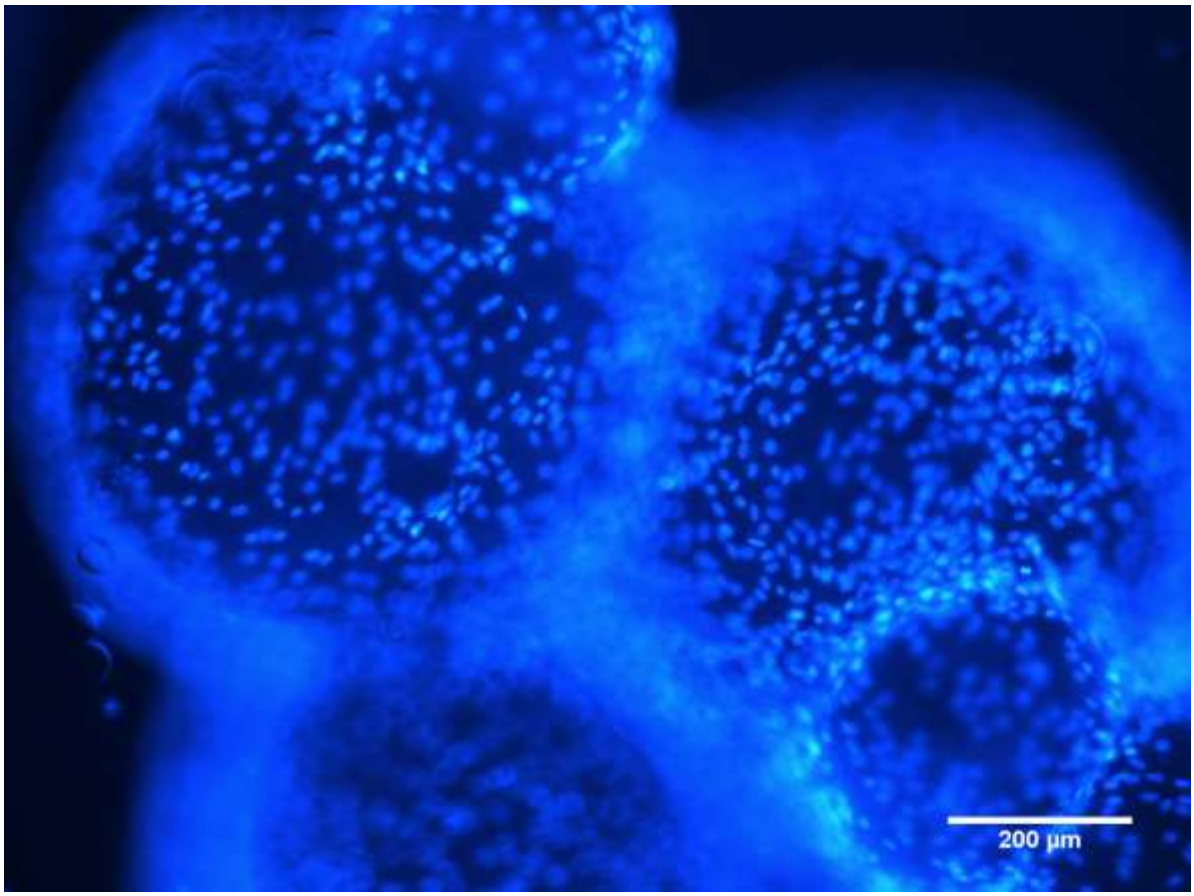

Picture 1: Caption 1: Hoechst staining of MSCs cultured on fluorinated oil-based emulsions for 7 days.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

211 Ultrafine grained titanium alloys for biomedical applications - what of their oxide layer properties?

Dieter Scharnweber[1], Melanie Franz[2], Bernhard Mingler[3], Krystian Maciej[3], Laszlo Sajti[3], Cornelia Wolf-Brandstetter[2]
[1]TU Dresden / Institute of Materials Science, Dresden, Germany
[2]TU Dresden / Max Bergmann Center, Germany
[3]AIT Austrian Institute of Technology, Austria

INTRODUCTION:

Titanium and its alloys are widely used for biomedical applications due to their excellent corrosion behavior and biocompatibility. Both are primarily determined by the properties of a few nanometers of semiconducting n-type, air-formed oxide layer[1]. Equal channel angular pressing (ECAP) offers the unique possibility to improved mechanical properties by drastically reducing the grain size of large metallic samples[2], but the consequences for the oxide layers are still unclear. The focus of our work was to determine such potential consequences for titanium based materials and to discuss the biological relevance.

METHODS:

ECAP resulted in grain size reduction from several micrometers to a few hundred nanometers for both CP titanium and Ti6Al4V. As received (AR) and ECAP samples (diameter 20 mm; thickness 3.5 mm) were used for electrochemical impedance spectroscopy (EIS) and anodic galvanostatic polarization in phosphate and perchlorate electrolytes and cell culture medium containing fetal calf serum in order to study oxide layer properties.

RESULTS AND DISCUSSION:

EIS measurements followed by Mott-Schottky analysis showed a strong increase of the oxide layer defect density N_d for ECAP treated materials for all electrolytes (s. table 1), most pronounced for Ti6Al4V. The effect may be due to the large increase in grain boundary density and its influence on the oxide layer properties above the grain boundaries; for the alloy, vanadium may be an additional effector. The strongest effects of the ECAP treatment were observed for the perchlorate electrolyte, possibly because of specific adsorption of either phosphate ions or proteins in the other electrolytes. Current densities for anodic polarization during the EIS measurements and in anodic galvanostatic polarization experiments confirmed the expected influence of N_d on oxide layer electronic conductivity.

CONCLUSION:

ECAP treatment of titanium-based materials substantially affects the semiconductor properties of their air-formed oxide layers, resulting in an increased electronic conductivity at potentials positive from the flat-band potential. This may impact the biocompatibility due to enhanced redox reactions with adsorbed proteins that lead to conformational changes and antigen formation. Potential countermeasures will be discussed and evaluated in their efficiency.

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ACKNOWLEDGMENTS:

The authors appreciate the financial support of the Austrian Research Promotion Agency (FFG), ‘HighPerformBioMat’, Research Studios Austria.

Electrolyte		CP Ti		Ti6Al4V	
Type	pH	AR	ECAP	AR	ECAP
Phosphate	4.4	$5.6 * 10^{21}$	$14.3 * 10^{21}$	$7.8 * 10^{21}$	$397 * 10^{21}$
Phosphate	7.4	$4.2 * 10^{21}$	$14.5 * 10^{21}$	$5.0 * 10^{21}$	$457 * 10^{21}$
Phosphate	9.2	$4.1 * 10^{21}$	$33.2 * 10^{21}$	$4.1 * 10^{21}$	$1069 * 10^{21}$
Perchlorate	7.4	$5.6 * 10^{21}$	$112.7 * 10^{21}$	$3.3 * 10^{21}$	$2285 * 10^{21}$
Cell culture medium	7.4	$3.6 * 10^{21}$	$29.2 * 10^{21}$	$3.8 * 10^{21}$	$939 * 10^{21}$

Picture 1: Caption 1: Table 1: Defect densities of air formed oxide layers in cm-3

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Poster presentation

219 Impact of copper on osteogenic induction strongly depends on osteogenic supplements and differentiation status of human mesenchymal stromal cells

Keerthana Srinivasan[1], Dieter Scharnweber[1], Claus Moseke[2], Cornelia Wolf-Brandstetter[1]
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[2]University of Applied Sciences Gießen, Germany

INTRODUCTION:

Copper ions are supposed to improve osseointegration in vivo when incorporated into implant coatings. However, own preliminary and published data[1,2] on *in vitro* characterization differ much with respect to cell growth and osteogenic potential which might be based on differences in cell types and osteogenic supplements. This work is dedicated to identify the role of the differentiation status of human mesenchymal stromal cells as well as different osteogenic supplements on in vitro cell behavior in presence of copper ions.

METHODS:

Copper ions (0 - 50 µM) were added either directly after cell seeding or after 11 days of pre-differentiation. Osteogenic induction was started at day 3 with 0 - 300 µM ascorbic acid (ASC), 5 and 10 mM b-glycerophosphate and 0 - 10 nM dexamethasone. Additional experiments were performed using a stable ASC phosphate (50 – 300 µM). Cell growth was analyzed via DNA and as differentiation markers activity of alkaline phosphatase (ALP) and deposited mineral (calcium) were determined.

RESULTS AND DISCUSSION:

While addition of copper to basal medium had no adverse impact on cell growth, in presence of standard osteogenic induction with 300 µM ASC growth was inhibited and only ~70% of initially seeded cells survived (Fig. 1A). If copper was added to pre-differentiated cells they further proliferated but slower than control. In presence of copper, ALP level remained comparable to the starting level with or without osteogenic supplements (Fig. 1B), while for pre-differentiation a slight decrease compared to the much higher level at the time point of copper addition occurred. Mineralization was decreased in presence of copper when added from day 0 but in case of pre-differentiation copper addition even topped the control (Fig. 1C).

Stable ASC phosphate in lower concentration compared to ASC provoked similar osteogenic potential and reduced the adverse effects, but a concentration dependent impact of copper was still visible. Reactive oxygen species were

identified as potential reason for impaired cell behavior and their concentration varied depending on actual ASC and copper concentration (4 times as high for 50 µM Cu and 300 µM ASC than in basal medium).

CONCLUSION:

The impact of copper ions on osteogenic differentiation is tremendously affected by the type and concentration of ASC and differentiation status of the multipotent stromal cells.

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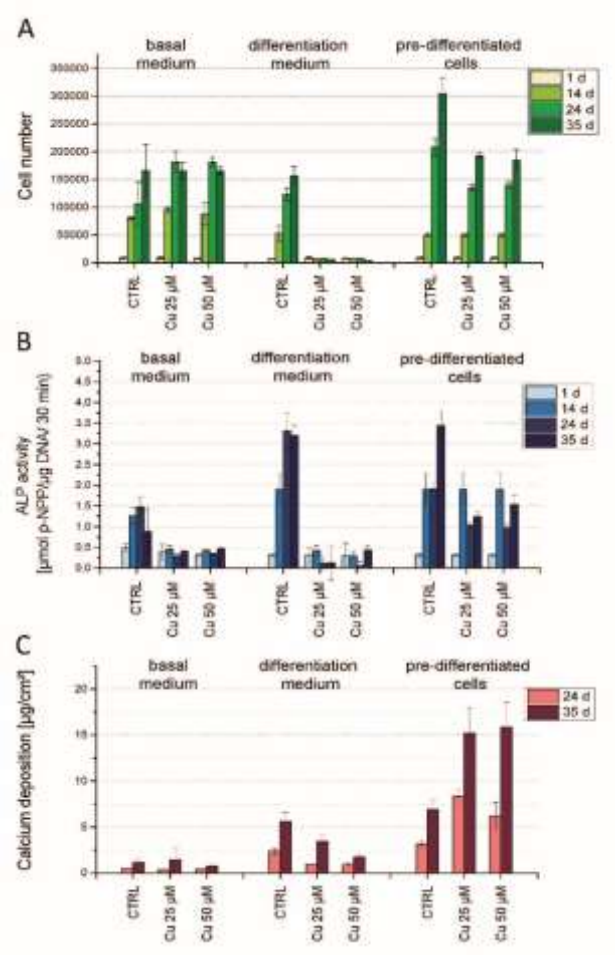
ACKNOWLEDGMENTS:

We thank the German Research Foundation (Grant no: WO 1903/2-1) for financial support.



Picture 1: Caption 1: Fig. 1 Proliferation and differentiation behavior of hMSC in presence of copper ions

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

223 Adverse effects of semi-permanent fillers. Determination of macrophage response and gene induction.

Wim de Jong[1], Marlon Jetten[2], Peter Keizers[1], Jolanda Vermeulen[1], Hennie Hodemaekers[1], Danyel Jennen[2], Jos Kleinjans[2], Rob Vandebriel[1]
[1]National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands
[2]University of Maastricht, Netherlands

INTRODUCTION:

Injectable tissue fillers applied for aesthetic purposes can lead to severe complications including product migration, unexpected late swelling and inflammatory reactions such as granulomas. An increasing number of semi- and non-permanent fillers show long-lasting effects, that may lead to similar problems as observed in cases where permanent fillers were applied.

METHODS:

We investigated whether the level of cross linking in hyaluronic acid based fillers could be linked to induction of adverse effects after administration. Four different fillers with different levels of hyaluronic acid cross linking were experimentally prepared in the laboratory. Also commercial hyaluronic acid based fillers with different composition were obtained. Macrophage responses in vitro were determined after exposure to these hyaluronic acid fillers. Gene profiling of filler exposed macrophages was performed.

RESULTS AND DISCUSSION:

Macrophage responses showed induction of various inflammatory cytokines for the different fillers. In addition, for both commercial hyaluronic acid filler and the experimentally prepared fillers were evaluated for their gene induction after injection in reconstructed human epidermis as a model for human skin responses.

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Poster presentation

225 Phosphate Glass Fibres for wound healing applications

Agata Lapa[1], Mark Cresswell[2], Phil Jackson[2], Andrew Parsons[3], Ifty Ahmed[3], Aldo Boccaccini[1]
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[2]Lucideon Ltd., United Kingdom
[3]University of Nottingham, United Kingdom

INTRODUCTION:

Phosphate glass fibres (PGF) are soluble, bioactive materials supporting hard and soft tissue regeneration [1]. Due to their solubility PGF, can be used as a delivery system for therapeutic or antibacterial ions like cerium which has been proven to exhibit not only antibacterial properties but also support healing of severe burns [2]. Moreover, bioactive glass and glass fibre materials have potential for wound healing application [3].

METHODS:

Ce-doped PGF (diameter 26 mm) were drawn from the melt using a specially designed fibre pulling rig. PGF have been characterized using FTIR, SEM, XRD and ICP and mechanical features have been measured. A random mesh type structure has been manufactured from short pieces of PGF and cellulose solution. Potential biological properties have been evaluated using simulated body fluid (SBF) and antibacterial evaluation was performed against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli*.

RESULTS AND DISCUSSION:

Novel continuous phosphate glass fibres doped with cerium oxide were successfully produced and used to fabricate 3D random meshes. Mechanical properties of the PGF were as follow: tensile strength 427 MPa and elastic modulus 45 GPa. The random meshes have shown high solubility (fully dissolved after 4 days), creation of a pH environment suitable for a wound and an ability to absorb water in order to enhance moisture environment. ICP-MS studies have shown high levels of cerium ion release (227 ppm after 48h). An in vitro bioactivity evaluation in simulated body fluid (SBF) has shown mineral deposition on the material's surface which is expected to accelerate the blood clotting [3]. Antibacterial studies suggest antibacterial resistance against gram-positive and gram-negative bacteria strains up to 10 days.

CONCLUSION:

GF have the potential to be clinically useful materials as soluble dressings for wound healing applications.

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

228 Non-invasive tracking and manipulation of 3D printed nanoparticles gelatin methacrylate hydrogel

Tiziano Serra[1], Adriana Augurio[1], Paolo Cortelletti[2], Mauro Alini[1], David Eglin[1], Adolfo Speghini[2]
[1]AO Research Institute Davos, Davos platz, Switzerland
[2]Nanomaterials Research Group, Department of Biotechnology, University of Verona, Italy

INTRODUCTION:

3D printed gelatin methacrylate (GelMA) hydrogels have been used for biomedical applications because of their biological and physical properties approaching those of the extracellular matrix (ECM)[1]. *In vitro,* the photo-polymerization process under UV light irradiation allows the manufacturing of stable and tailored GelMA construct. However, low penetration depth of UV light restricts *in vivo* application and manipulation. Here, we have optimized the printability of a 3D GelMA hydrogel doped with upconverting nanoparticles (UCNPs), which emits UV-vis light upon near-infrared (NIR) excitation source via multiphoton absorption process allowing control of radical species production in depth not achievable with UV light [2].

METHODS:

The Yb^{3+}/Tm^{3+} co-doped strontium fluoride UCNPs (SrF_2: Yb, Tm UCNPs) were prepared using hydrothermal method, with sodium citrate as capping agent[3]. The procedure proposed by Kan Yue *et al.* was used for the synthesis of GelMA[1]. Dynamic light scattering (DLS), transmission electron microscopy (TEM) and upconversion

spectroscopy were performed to characterize UCNPs physical and photo-luminescence properties. UCNPs were added (10%w/v) and vigorously mixed in a PBS solution of GelMA and photo-initiator IRGACURE 2959 to obtain a homogeneous dispersion. 3D printing of GelMA/UCNPs was carried out with a 3D Discovery™ (RegenHU Ltd), using FreeCad program and MMconverter to design and slice the printed architectures. The 3D printed GelMA/UCNPs hydrogel was photo-polymerized under UV light excitation for 10 minutes. Then, the 3D GelMA/UCNPs scaffolds were exposed to NIR light source (980 nm) and light emission visualized with stereo microscope.

RESULTS AND DISCUSSION:

The upconversion spectrum upon 980 nm excitation source showed typical UV-vis emission bands, due to energy transfer mechanism between Yb^{3+} and Tm^{3+}. DLS analysis showed UPNCs with an average dimension of 10 nm with PDI=0.25, as confirmed by TEM results. 3D GelMA/UCNPs scaffolds printed and cross-linked by UV light, as indicated in Fig.1. The scaffolds showed an intense blue visible emission upon NIR excitation light, which allowed imaging of the nanocomposite hydrogel in tissue and constructs with low UV light penetration.

CONCLUSION:

This study demonstrates the ability to produce a 3D printable GelMA hydrogel loaded with UCNPs and to perform imaging with the light emission of the UPNCs under NIR excitation. This nano-composite ink could be used for *in vivo* tracking via deep tissue imaging and for detecting of degradation upon NIR exposure with potential clinical translation.

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Picture 1: Caption 1: Schematic illustration of UCNPs doped GelMA, 3D-printed and photo-crosslinked through UV light.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

235 Nonwoven textiles made from hyaluronic acid staple microfibers

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INTRODUCTION:

Hyaluronic acid has been used in a broad range of biomedical applications in different forms for more than 20 years[1–3]. The aim of this contribution is to describe the possibility of processing native hyaluronan into nonwoven textiles based on staple microfibers and describe their basic properties.

METHODS:

Wet spinning method was used to prepare hyaluronic acid microfibers. Then, microfibers were shortened and dispersed in alcohol-based liquid. A layer of staple fibers was formed by sieving in a similar way as in paper-making process. Finally the fiber layer was dried, which resulted in a formation of a compact nonwoven textile[4].

A series of different hyaluronan based nonwovens was then analysed. SEC-MALLS was used to determine molecular weight of hyaluronic acid. Gas chromatography (GC) was used to determine residual solvents. Scanning electron microscope (SEM) was used to study surface morphology of the nonwoven textiles. Weight per square meter of the nonwoven textiles was determined according to the ISO 9073-1:1989 norm. MTT assay was used to assess cytotoxicity effect of the prepared material.

RESULTS AND DISCUSSION:

Staple fibers and nonwoven textiles was successfully prepared from hyaluronic acid of the molecular weight in the range 350 – 2 700 kDa. Depending on the process parameters, the weight per square meter of these textiles was within the range 18 – 80 grams per square meter. By using the image analysis of SEM pictures, we confirmed that changing of process parameters allow us to influence the thickness and homogeneity of fibers of the prepared nonwoven textiles. The presence of residual solvents was determined to be less than 0.02 weight percent and the cytotoxicity of the prepared material was excluded.

CONCLUSION:

Unique nonwoven textiles from native hyaluronic acid staple microfibers were prepared. They have soft touch and they are strong enough to allow common manipulation. Parameters of their characterisation and results of the safety verification indicates them to be a promising material for use e.g. in surgery, wound healing, or other medical area.

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

241 In vivo evaluation of toxicity and efficacy of nanospheres made of bioengineered spider silk for targeted drug delivery.

Tomasz Deptuch[1], Anna Florczak[1], Karolina Penderecka[1], Anna Lewandowska[2], Ewa Leporowska[3], Andrzej Marszalek[2], Andrzej Mackiewicz[1], Hanna Dams-Kozlowska[1]
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[2]Greater Poland Cancer Centre, Department of Oncologic Pathology, Poland
[3]Greater Poland Cancer Centre, Department of Laboratory Diagnostics, Poland

INTRODUCTION:

Drug delivery systems are aimed at efficient drug distribution and reduction of unwanted side effects of the therapy. Such system should not be toxic and should allow for selective drug delivery, what is especially important in the case of cancer treatment with chemotherapeutics. Previously we published *in vitro* study of drug delivery system that was based on bioengineered silk nanospheres targeting Her2+ breast cancer cells[1]. The bioengineered silks were functionalized with H2.1 peptide possessing binding properties towards the Her2 receptor. This study aimed to examine the toxicity and biodistribution of these carriers as well as their efficiency as doxorubicin delivery system in *in vivo* murine breast cancer model.

METHODS:

The bioengineered silk MS1 and its Her2-targeting variant H2.1MS1 were designed based on repetitive, alanine-rich motif of Major Ampulatte Spidroin 1 (MaSp1) of *Nevilia clavipes*. Proteins were overexpressed in the microbial system and purified by thermal method. The silk nanospheres were formed in the presence of the chaotropic agent. The maximum tolerated dose of silk spheres was examined based on the survival and behavior of mice 24 hours after administration of spheres. Long-lasting toxicity of particles was evaluated by i) clinical symptoms, ii) the morphological and biochemical parameters of peripheral blood, and iii) organ histopathology after multiple intravenous administrations of the silk particles. Distribution of fluorescently labeled nanospheres was determined by using IVIS imaging system. The therapeutic effect of doxorubicin delivered by silk carriers was studied in mouse model based on the measurement of the tumor size.

RESULTS AND DISCUSSION:

The highest tested dosage of silk nanospheres (20 mg/kg) was not lethal and did not affect the mice behavior. The repeated intravenous administration of the silk particles into mice did not elicit the morphological changes in internal organs and did not modify the biochemical markers and morphology of blood. Her2-targeting spheres (H2.1MS1) were observed at Her2-positive tumor site indicating site-specific accumulation of silk particles. Doxorubicin delivered in functionalized silk carriers provided its therapeutic effect; the growth of the Her2-positive tumor was inhibited in mice.

CONCLUSION:

The obtained results indicated the great potential of functionalized spider silk-based carriers for the delivery of a therapeutic agent to the specific tumor microenvironment.

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ACKNOWLEDGMENTS:

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

247 Mechanical and morphological evaluation of 2D and 3D electrospun structures for the manufacture of tissue engineered ligaments

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INTRODUCTION:

2D and 3D polyvinyl alcohol (PVA) electrospun scaffolds were studied to evaluate their suitability for use as anterior cruciate ligament (ACL) grafts. ACL *in-vivo* mechanical properties were previously determined [1,2]. The aim was to establish which electrospun structure would provide the morphology and mechanical properties most comparable to the natural ligament, in dry/wet, tensile and shear cyclic loading conditions (not previously investigated).

METHODS:

2D electrospun scaffolds were manufactured using 12% w/v of PVA dissolved in distilled water. 2D samples were cut with a dogbone cutter (25 x 4 mm). To fabricate the 3D structures, the 2D electrospun meshes were cut in rectangles of 2 x 15 cm and manually twisted clockwise to form scaffolds of: one twisted filament, three twisted filaments and three twisted/braided filaments. 2D and 3D structures were crosslinked with 25% glutaraldehyde for 24 hours.

Cyclic tensile and shear tests with crosslinked and non-crosslinked samples were performed in dry and wet conditions (n=8 samples per condition). The tests were performed using an Instron H10KS, 10 cycles to 13% strain and then tested to failure, with a 100 N load cell and 5 mm/min test speed.

Fibre diameter and thickness of the fibre bundles and fascicles were measured from SEM images using AxioVision SE64 Rel. 4.9.1. 20 fibres, 3 fibre bundles and fascicles (as relevant) were measured for each sample and test condition

RESULTS AND DISCUSSION:

Three twisted/braided filaments scaffolds produced a maximum tensile stress of 38.0 ± 3.0 MPa and a Young's modulus after 10 loading cycles of 148.7 ± 13.0 MPa in dry conditions, being the closer structure to the ACL mechanical properties (Fig.1) [1-3]. Wet PVA scaffolds exhibited elastic behaviour with a well-defined toe region. Three twisted/braided crosslinked filament structure showed the highest maximum shear stress, allowing the samples to bear higher shear loads than the other structures. The manufacturing process did not damage the nanofibres or alignment. The dimensions of the PVA fibres, fibre bundles and fascicles were in agreement with the diameters of the collagen fibrils, fibres and fascicles in the natural ligament

CONCLUSION:

Three twisted/braided crosslinked filament structures mimicked the ACL mechanical and morphological properties, showing significant potential for use in ACL reconstruction

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ACKNOWLEDGMENTS:

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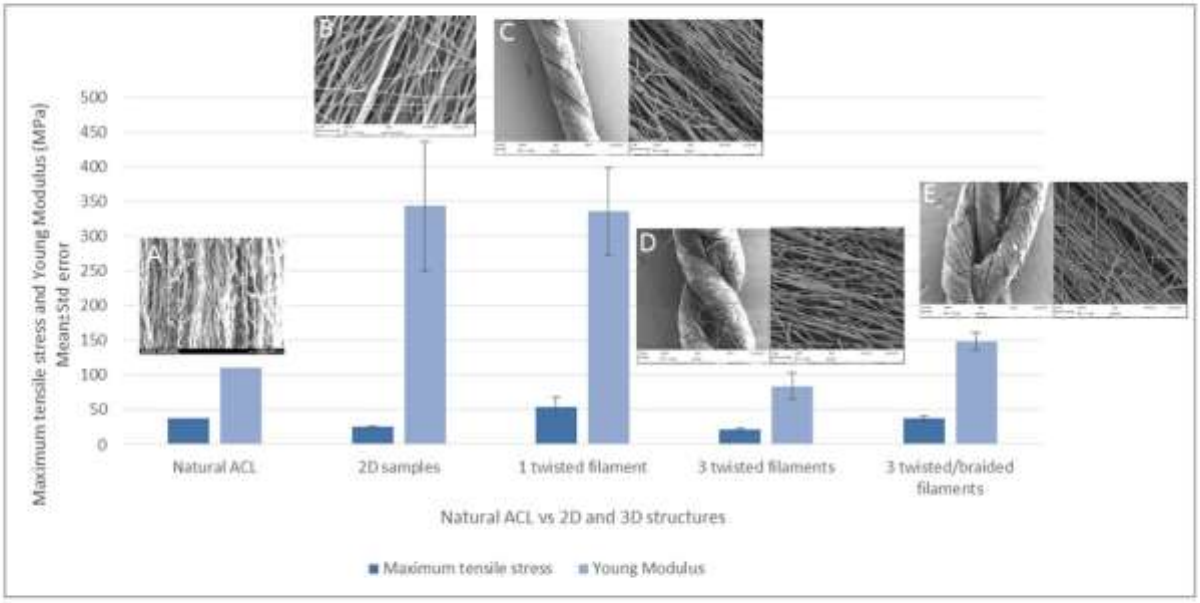

Picture 1: Caption 1: Fig. 1- Mechanical properties and SEM images of natural ACL vs 2D and 3D structures A) Natural ACL B) 2D samples C) 1 twisted filament D) 3 twisted fi

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Poster presentation

262 Time-dependent drug release from a highly porous ZnO network in an in-vitro tumor resection model of Glioblastoma multiforme

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INTRODUCTION:

Glioblastoma multiforme is known to be a poorly curable disease due to its heterogeneity that partly enables single cells to survive treatment regimen and initiate tumor regrowth[1]. Therefore, new therapy strategies are currently developed to enhance survival of affected patients[2,3]. Especially zinc-oxide (ZnO) nanoparticles have been already shown to decrease tumor-cell viability selectively[4]. Furthermore, tetrapodal ZnO (t-ZnO) has lately been used to create highly porous materials which represent suitable templates for scaffolds and drug-delivery systems[5].

METHODS:

The t-ZnO powders were produced by a simple flame-transport synthesis[6]. Subsequently, the obtained loose powder was pressed into pellets. Then, the t-ZnO network was infiltrated with drug solution (e.g. AT101). To control drug release with a barrier layer, the network was again infiltrated with different polymers (e.g. PLCL, PLGA). Finally, cellular responses to varying concentrations of both, released ZnO and the drug, were tested in glioblastoma tumor cells as well as astrocytes and microglia in a long-time co-culture model.

RESULTS AND DISCUSSION:

Tetrapodal ZnO networks could be fabricated with porosities of over 90% and large surface-to-volume ratio, enabling the deposition of high amounts of drug. Further infiltration with polymers created a form of barrier layer on the ZnO-drug network which caused a delayed release of drug and ZnO in surrounding media depending on barrier thickness and degradation time of the polymer. Accordingly, cells could be treated with small doses of drugs combined with ZnO over long time periods which ensured prolonged cell death of tumor cells but survival of astrocytes and microglia.

CONCLUSION:

Long-time drug release established by polymeric barrier layers on t-ZnO networks, together with the use of minimal drug concentrations to protect healthy tissue, form a promising basis for local therapy strategies in glioblastoma brain tumors.

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ACKNOWLEDGMENTS:

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Poster presentation

265 A novel ECM mimicking migration platform

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INTRODUCTION:

More and more evidence shows the differences in cell migration between a 2D and a 3D environment. However, good 3D extra cellular matrix (ECM) mimicking migration models are scarce. Electrospun (ESP) scaffolds can mimic the architecture of natural ECM, but generally allow for little cell penetration due to small pores.

METHODS:

Here, ESP scaffolds were produced using 20% or 35% (w/v)PEOT45/PBT55 in 70% chloroform and 30% hexafluorisopropanol, using a flowrate of 3ml/hr in an environment of 25 C and 30% humidity. We have optimized the pore size and fiber diameter by increasing the polymer concentration and using sacrificial polymers of 1,5% PEO(900.000)+50% PEG(3.350) (w/v) in 75% methanol and 25% water. Scaffolds fiber network was analyzed by scanning electron microscopy. Cell migration through the scaffolds was tested by seeding human mesenchymal stromal cells (hMSCs) on top of the 20% or 35% scaffolds at a density of 26.000 cells/cm^2 and cultured in proliferation media for 1, 4 and 7 days. Cell migration was analyzed by cryosectioning and by imaging the top and the bottom of the scaffolds using fluorescent microscopy.

RESULTS AND DISCUSSION:

By increasing the polymer concentration from 20% to 35%, fiber diameter increased from an average of 1±0,2µm, to an average of 3,5±0,4µm. With this also the pore size increased, from an average of 8,3±2,3µm^2 to an average of 20±6,1µm^2. The pore size was further increased by the addition of sacrificial polymer fibers in the scaffolds, increasing the pore size to 23,7±5,7µm^2 and 43,5±10,2µm^2 for 20% and 35%, respectively. Using the scaffolds with optimized porosity, we show that hMSCs are able to migrate through the entire scaffolds. Not only are hMSCs able to infiltrate into the scaffolds, but also migrate all the way through and populate both of their sides. Currently, we are further optimizing the scaffolds by incorporating an SDF-1a gradient, a potent migration chemokine, to further increase migration and to study the effect of chemokines in 3D.

CONCLUSION:

We developed an ESP scaffold that allows hMSCs to migrate all the way through the scaffold and populate both sides of it. These scaffolds could be used to help unravel the complex mechanisms of 3D migration in an ECM mimicking environment

ACKNOWLEDGMENTS:

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Poster presentation

268 Modulating human monocyte-derived dendritic cell phenotype through controlling surface topography

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INTRODUCTION:

Dendritic cells (DC) are known as the bridge between the innate and the adaptive arm of the immune system, as such, they are quintessential in the facilitation and regulation of an immune response due to their capability to prime

naïve T cells. During steady-state conditions, they maintain immune tolerance against self-antigens. While in the past biomaterials have been designed as practically inert materials, in recent years research in this area has seen a 'paradigm shift' in the engineering and design of 'bio-biomaterials' with a particular interest in materials that can direct an immune response in a favoured direction.

Biomaterial properties such as, topographic features, have previously been shown to influence immune cells however not much is known about how different topographies could influence function[1-3]. DC phenotype (particularly their maturation status) affects T cell activation and polarization. Assessment of changes in phenotype, are therefore important to show the influence of biomaterial properties on DCs. This project aims to investigate whether topographical features are able to influence DC phenotype and function, therefore directing the ensuing immune response.

METHODS:

Monocytes were isolated from human peripheral blood (after obtaining informed consent) and differentiated with IL-4 and GM-CSF supplementation into DCs. DCs were then cultured on 28 topographical features selected based on their ability to induce diverse cell shapes. DCs were harvested after 24 hours of culture and stained for surface receptors (CD83, CD86, CCR7 and PD-L1) followed by flow cytometry to give an overview on the maturation level induced by each topographic feature.

RESULTS AND DISCUSSION:

ur data clearly show that some topographies are able to induce DC maturation and potentially their migratory behaviour as evidenced by an increase in the expression of CD83, CD86 and the chemokine receptor CCR7. Future work will focus on confirming the reproducibility of these observation and better characterisation of DC responses including the impact of topographies on their cytokine profile and endocytic ability.

CONCLUSION:

Our data indicates the feasibility of modulating DC phenotype and potentially function via surface topographies. If confirmed, these observations provide a strong rationale for using materials with different surface topographies as novel tools for immune modulation through controlling DC phenotype and function.

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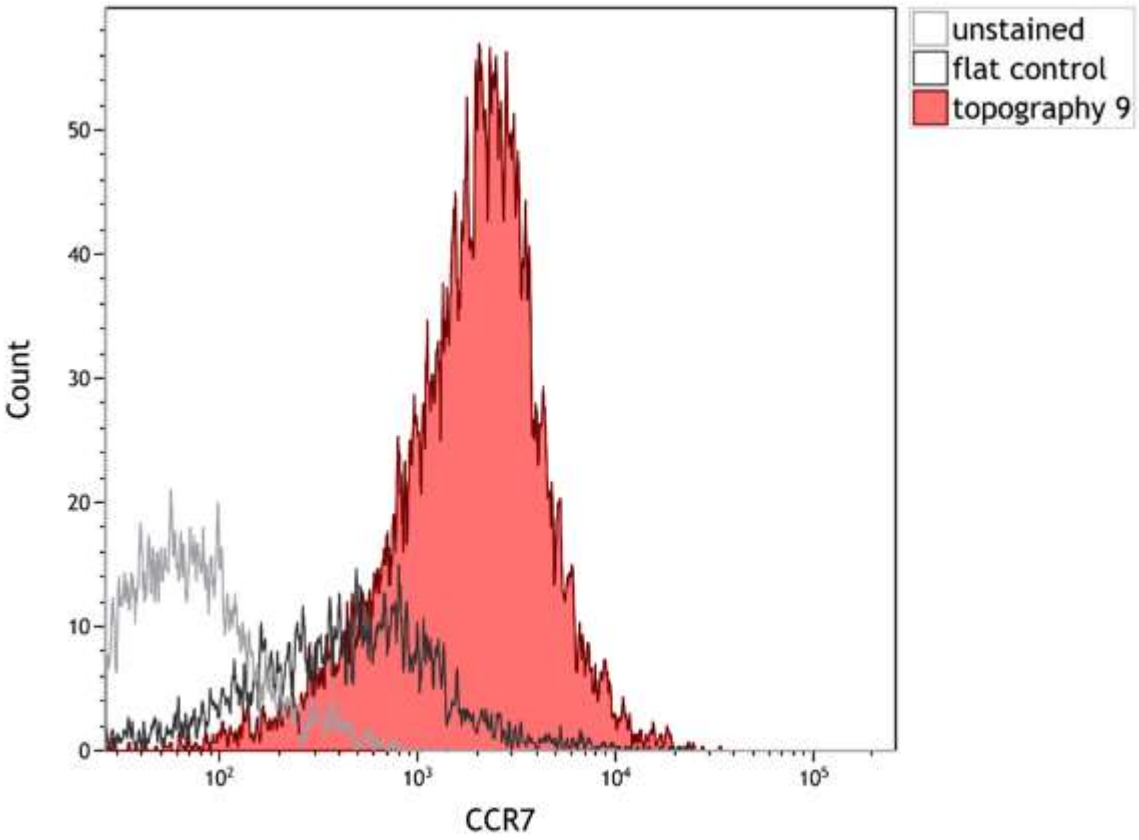

Figure 1: Histogram of DCs cultured on a flat surface (black line) and topographical feature 9 (red line). An unstained control is represented with a grey line. Cells were stained with a CCR7-PE-Cy7 monoclonal antibody.

Picture 1: Caption 1: Topography9_CCR7_Histogram

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Poster presentation

272 Detection of specific IgG antibodies against bovine collagen type I in patients with vascular prostheses

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INTRODUCTION:

Collagen, a connective tissue structural protein, is widely used as biomaterial. It is typically of bovine or porcine origin and thus constitutes a xenoprotein with immunogenic potential in humans. However, this issue received little

interest so far, with inconclusive results regarding an immune response against collagen used for vascular graft impregnation[1,2]. As we found anti-collagen IgG antibodies following implantation of vascular prostheses impregnated with bovine collagen in pigs[3] but not in rats[4], the present study aimed at examining sera from patients with polymeric vascular grafts regarding specific anti-collagen type I antibodies.

METHODS:

Two patient groups were examined: The PET group included n=26 patients with polyester-based vascular prostheses impregnated with native or denatured (gelatine) bovine collagen, while the PTFE group included n=15 patients with non-coated PTFE-based vascular prostheses. All patients in the PTFE group and 24/26 patients in the PET group additionally received antibiotic-releasing collagen sponges wrapped around the anastomosis for infection prevention. One pre-OP blood sample and 2-8 post-OP blood samples were collected from each patient during follow-up examinations. The differing post-OP sampling days were combined into the following periods: days 1-7, 8-14, 15-28, 29-56, 57-84, 85-170, and 171-365. Anti-collagen IgG antibodies were detected using an ELISA with solid-phase coated bovine collagen type I as previously described[3].

RESULTS AND DISCUSSION:

The prevalence of anti-collagen antibodies was 68.8% in the PET group and 62.5% in the PTFE group after one week, and 100% in the PET group after eight weeks and in the PTFE group after three weeks post-OP. A subsequent decrease occurred earlier in the PTFE group than in the PET group. However, most patients in both groups remained positive throughout the study. While the prevalence in both groups was overall comparable, the anti-collagen antibody response was stronger and long-lasting in the PET group as demonstrated by a significantly higher delta OD within the second and the fifth/sixth month post-OP.

CONCLUSION:

The results demonstrate that biomedical bovine collagen has a pronounced immunogenicity in human patients. Patients in the PET group had a stronger anti-collagen IgG response than patients in the PTFE group because they received a higher collagen amount from two different sources (collagen-impregnated prostheses and antibiotic-releasing collagen sponges). The immunogenicity of bovine collagen should be kept in mind especially for multiple applications within the same patient.

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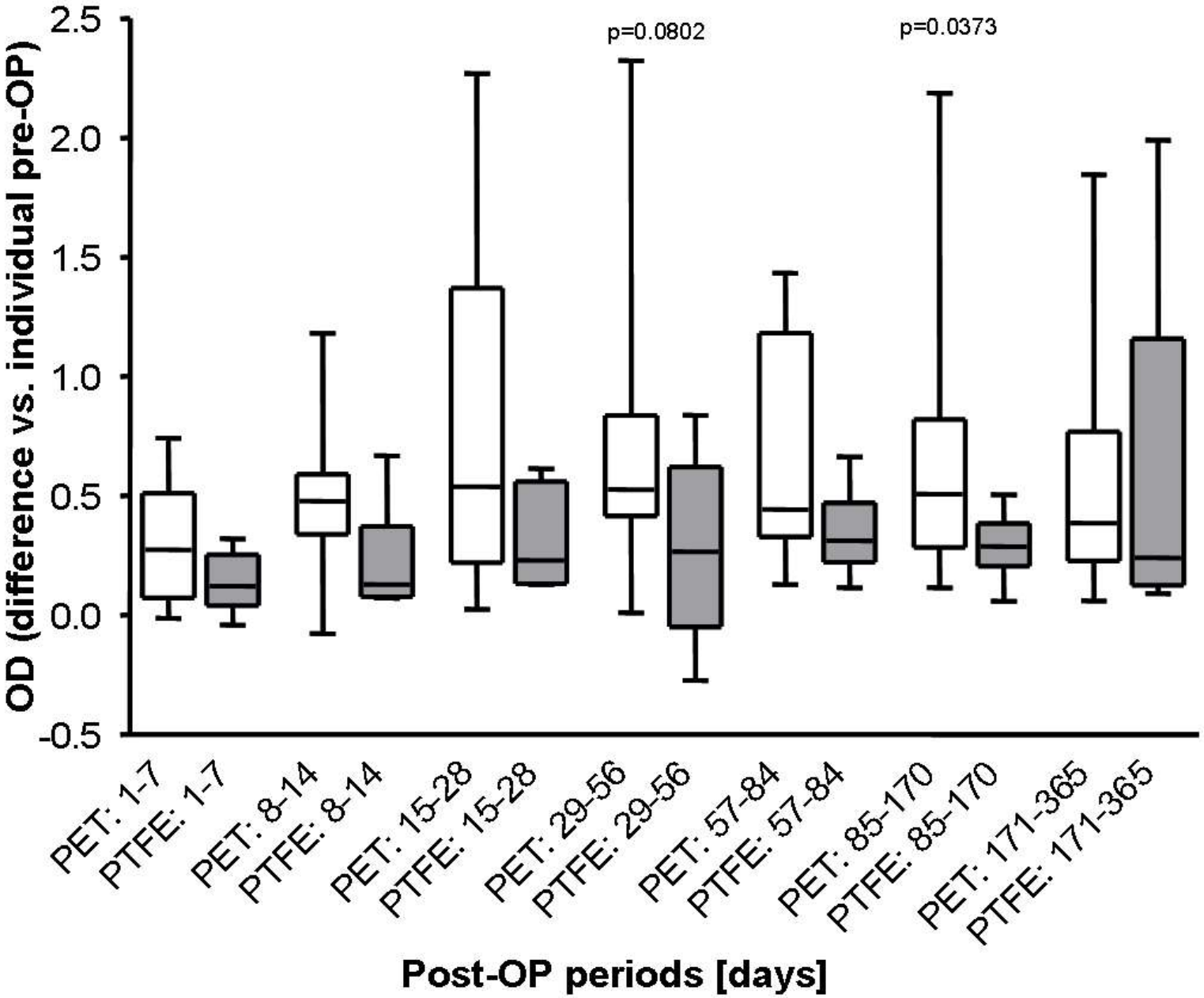

Picture 1: Caption 1: Time course of anti-collagen type I antibodies in the PET group (open boxes) and in the PTFE group (gray boxes).

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Poster presentation

273 Examination of the humoral immune response against synthetic polymers in patients with vascular prostheses and hernia meshes

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INTRODUCTION:

Synthetic polymers like polyethylene terephthalate (PET) and polypropylene (PP) are used for vascular prostheses and hernia meshes, respectively. Despite their clinical success, structural failure due to inflammation-induced biodegradation can occur. Concerning this, immune reactions including formation of material-specific antibodies should be considered. In different animal models, we found PET-specific IgG antibodies after implantation of PET vascular prostheses[1,2]. This prompted us to investigate in the present study sera from patients with PET-based vascular grafts or PP-based hernia meshes regarding polymer-specific antibodies.

METHODS:

Patients in the PET group (n=26) received PET-based vascular prostheses impregnated with bovine collagen or gelatine, while patients in the PP group (n=17) received PP-based hernia meshes. From each patient, one pre-OP and 2-8 post-OP blood samples were obtained over a period of up to one year. Varying post-OP sampling days were categorized into intervals as follows: days 1-7, 8-14, 15-28, 29-56, 57-84, 85-170, and 171-365. PET-specific antibodies were detected as previously described using a customized immunoassay with particles from a homogenized PET prosthesis as target[1]. Unmodified PP microtiter plates were used as target in the immunoassay for PP-specific antibodies.

RESULTS AND DISCUSSION:

In the PET group, 18/26 patients (69.2%) had at least one post-OP serum sample positive for anti-PET antibodies. A biphasic time course was observed in a number of patients (figure 1), with a first maximum one week post-OP (prevalence: 62.5%, median delta OD: 0.128). Following a decline, a second increase occurred 3-4 weeks post-OP (prevalence: 66.7%, median delta OD: 0.265). The anti-PET antibody response began to decline about eight weeks post-OP. In contrast, the overall prevalence of anti-PP antibodies was only 2/17 (11.8%). Additionally, sera from five PET group patients were tested in the PP assay and sera from seven PP group patients in the PET assay, with all of those controls being negative.

CONCLUSION:

Previous studies with human patients gave conflicting results regarding antibodies against synthetic polymers like silicone used for medical implants[3,4]. The present study demonstrates a specific anti-PET IgG antibody response in patients with PET-based vascular grafts, confirming our earlier results in experimental studies. In contrast, the immunogenicity of PP seems to be low, probably due to its hydrophobic nature. The possible clinical relevance of these antibodies and their potential utility for evaluating the immunogenicity of polymeric biomaterials remains to be examined in future studies.

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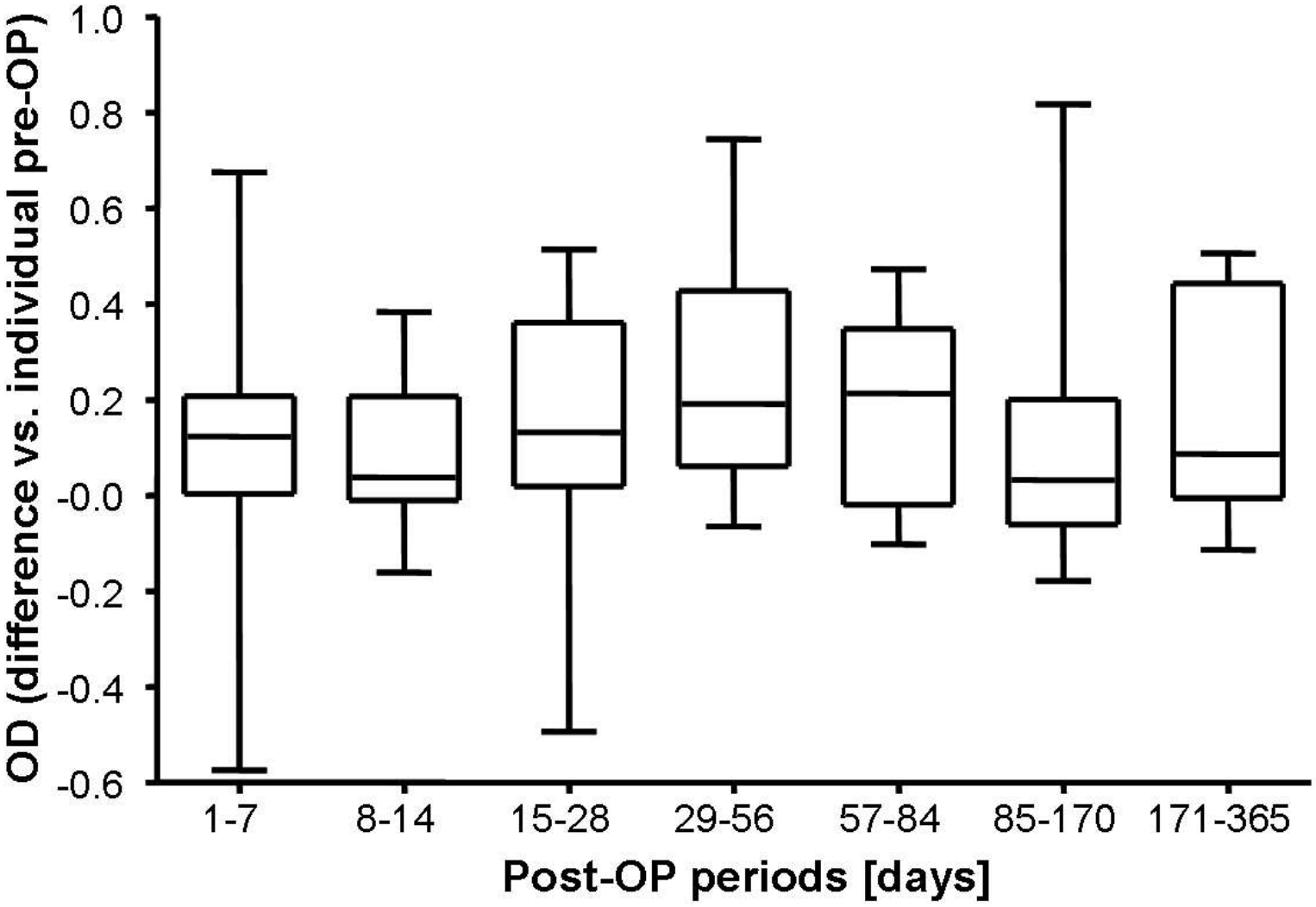

Picture 1: Caption 1: Time course of antibodies against PET in patients with a PET vascular prostheses

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Poster presentation

274 Soft non-fouling hydrogel systems for additive manufacture of prosthetic socket components

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INTRODUCTION:

Residual limbs are prone to dimensional changes, which leads to poorly fitted sockets resulting in discomfort. We are exploring reducing this discomfort using additive manufacture to custom fit the sockets, this requires development of new materials. This work is attempting to develop a soft hydrogel which would allow changes in dimension of the residual limb to occur without altering force distribution, as well as having the ability to guide neurons for applications within next generation prosthetics. Nano-clay will be used as a crosslinker in this work to improve mechanical properties, as previous research demonstrates[1]. To achieve neural guidance, a non-fouling

hydrogel will be developed which will later be coated using a cell adhesive material. Non-fouling and non-cytotoxic monomers such as 3-sulfopropyl-methacrylate-potassium salt (KSPMA)[2] crosslinked using a synthetic hectorite clay, Laponite (biocompatible[3]) are being explored.

METHODS:

Laponite XLG (4wt%) was dispersed in deoxygenated deionised water, then the monomer (10wt%) and Irgracure 2959 (0.5wt%) were added and the suspension was placed in moulds and UV-cured for 1h 50 min. Gels were also prepared using a chemical crosslinker, N,N'Methylenebis(acrylamide) (MBA), by replacing Laponite with 1 molar % of MBA in relation to the monomer.

2M HCl was used to keep the pH of the hydrogel mixture to below the pKa of KSPMA (pH 3).

RESULTS AND DISCUSSION:

KSPMA-Laponite gels were found to have high turbidity and did not form cohesive gels after UV-curing, while gels crosslinked with MBA showed high transparency and formed cohesive gels. The high turbidity observed may indicate an ionic interaction between K^+ and negatively charged surfaces on Laponite-platelets. To prevent these interactions, pH was reduced to lower than the pKa of KSPMA.

Turbidity was not reduced by reducing pH, and the viscosity of the mixtures were reduced. We believe that the latter effect is due to ionic interactions interfering with Laponite's "house of cards structure".

CONCLUSION:

KSPMA cannot be crosslinked using Laponite due to a problem with charge interactions. Changing pH to prevent this problem did not work as Laponite reacted with HCl. Other monomers with non-fouling properties must be further explored.

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ACKNOWLEDGMENTS:

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

277 Lipid modified mesoporous silica nanoparticles as dual tools for stem cell tracking and drug delivery

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INTRODUCTION:

Mesoporous silica nanoparticles (MSNs) have been proposed as suitable platforms for many diverse biomedical applications, including for controlled drug delivery in cancer therapy and as trackers in the bioimaging field. MSNs can be functionalized with multiple chemical entities and carry high cargo loads into cells. MSNs can be further

surface modified by for example supported lipid bilayers (SLBs) as a capping system to prevent unwanted cargo release. These hybrid nanoparticle systems combine the advantages of liposomes, namely low toxicity, immunogenicity and longer circulation times with those of MSNs, which include high loading capacity, stability, and their labelling properties. Furthermore, liposome instability and cargo leakage can be overcome by this system as the adhesion energy between the SLB and MSN suppresses fluctuations in the membrane bilayer. [1] In this project the use of MSN-SLB as potential stem cell drug delivery and tracking tools is explored.

METHODS:

Surface and core modified MSNs were synthesized through a multistep, delayed co-condensation method.[2] To synthesize MSNs with supported lipid bilayers (LIP) previously reported solvent exchange methods were used.[3] The MSNs were characterized by dynamic light scattering, thermo-gravimetric analysis, and scanning and transmission electron microscopy. Cargo release was measured by incorporating fluorescent dyes within the MSNs (plate reader, flow cytometry and microscopy). Mesenchymal stem cells (MSCs) from different human donors were used for toxicity screenings, uptake experiments, proteomics, biochemical assay and microscopy studies to determine the interaction of the MSN-SLB with MSCs.

RESULTS AND DISCUSSION:

Monodisperse MSNs and MSN-SLB were successfully synthesized and characterized. MSNs were non-toxic to hMSCs and internalized 2h after exposure by up to 95% of the hMSC population. Interestingly, MSN-LIP particles were taken up to a much higher extend compared to non-surface functionalized particles. Furthermore, MSN-LIP could controllably release cargo into MSCs. Combining this SLB-functionalization with an additional PEG-2000 layer did not affect this behavior but resulted in less differences in the proteome compared to control cells. In addition, the MSNs had no significant effect on the osteogenic differentiation potential of the MSCs.

CONCLUSION:

MSN-SLB nanoparticles and their PEGylated derivative represent interesting candidates for stem cell tracking and as cargo delivery tools in terms of their non-toxic behavior and internalization behavior.

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

278 Biphasic osteoconductive membranes for bone reconstruction

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INTRODUCTION:

Electrospinning is a simple and low cost method to produce membranes that mimic extracellular matrices. Polystyrene (PS), poly-ε-caprolactone (PCL) and bovine gelatin were electrospun with/without beta tricalcium phosphate (β-TCP) elementary grains inside the fibers to enhance osteoconductivity of the membranes. β-TCP is a synthetic ceramic chemically close to the mineral phase of bone and possess excellent osseointegration properties [1]. It is widely used for filling bone defect and is known to increase osteoconductivity when added to polymers [2, 3].

METHODS:

PS, PCL and gelatin with β-TCP were dissolved in dimethylformamide (DMF), acetone and methanol/water respectively. Solutions were loaded to a syringe placed in the electrospinning device facing the grounded collector and the needle was connected to a high power supply and a worm pump controlled the flow rate. β-TCP grains were added in amount ranging from 1 to 20 % (w/v). Fibers formed membranes that were collected from the collector and observed by SEM in the secondary and backscattered electron modes. Osteoblast-like cells were cultured on these membranes.

RESULTS AND DISCUSSION:

PS and PCL microfibers (from 1 to 10 µm) and gelatin nanofibers (from 40 µm to 500 µm) were successfully obtained by electrospinning. β-TCP grains repartition was homogenous along PS and PCL fibers. Gelatin fibers were more difficult to prepare and presented beads in which most β-TCP grains were agglomerated. PS and PCL fibers had an ideal fiber diameter (up to 2 µm) that facilitates cells infiltration through interconnected pores [4]. Unlike PCL and gelatin, electrospinning of PS requires a highly toxic solvent.

CONCLUSION:

An alternative to monophasic membrane for bone reconstruction scaffolds is proposed. PS, PCL and gelatin biphasic fibers doped with β-TCP elementary grains were successfully produced by electrospinning and favored cell proliferation. PCL/β-TCP membranes had a homogenous repartition of β-TCP grains. They do not require toxic solvent and have the ideal fiber diameter for osteoconduction.

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β-TCP granules were provided by Kasios SAS, France.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

283 Development of Light-Responsive Hydrogels Using Plasmonic Nanoparticles

Mani Diba, Patricia Dankers
Eindhoven University of Technology, Eindhoven, Netherlands

INTRODUCTION:

Hydrogels are three-dimensional interconnected networks dispersed in an aqueous phase. Their viscoelastic nature, high water content and possibility to include bioactive moieties in their composition are among the main factors which render these materials suitable for tissue engineering and regenerative medicine applications[1]. An important feature of natural tissues, is their dynamic and adaptive nature, which plays a key role in cellular functions and tissue regeneration processes[1]. This study aims to exploit supramolecular chemistry and plasmonic gold nanoshells to develop in-situ adaptable hydrogels.

METHODS:

Preparation of nanoparticles:

Gold nanoshells were synthesized using silica cores through a seeded growth strategy[2]. Samples were denoted as SX, where X indicates the volume (μL) of the dispersion of seed particles (gold-decorated silica cores) used for the growth of nanoshells.

Preparation of polymeric phase:

Hyaluronic acid and poly(ethylene glycol) were functionalized with four-fold hydrogen bonding ureido-pyrimidinone (UPy) units[3] and/or gold-binding thiol groups[2].

Preparation of hydrogels:

Hydrogels are prepared using different compositions of polymer solutions. The gelation is induced by changing pH, temperature and/or addition of gold nanoshells to the polymer solutions.

Characterization methods:

Scanning electron microscopy (SEM) and UV–vis–NIR spectrophotometry are used to investigate the formation of gold nanoshells. Viscoelastic properties of hydrogels are studied using rheological measurements. The responses of hydrogels to environmental changes are evaluated using confocal microscopy, rheological measurements and nano-indentation methods.

RESULTS AND DISCUSSION:

SEM images of gold nanoshells synthesized using different concentrations of seed particles in the growth reaction show differences in morphology (Fig. 1A). When a high concentration of seed particles (e.g. S20) was used for the synthesis, the resulting nanoparticles only contained gold clusters on their surface (Fig. 1A). However, the use of lower concentrations of seed particles (e.g. S5) resulted in the complete coating of silica cores with gold shells. Consequently, the optical properties of the nanoshells could be adjusted by changing the concentration of seed particles in the growth reaction (Fig. 1B and 1C).

CONCLUSION:

Gold nanoshells were synthesized and their plasmonic properties were successfully tuned. As the next step, these nanoshells are used for the synthesis of hydrogels, and their plasmonic properties are exploited to control the properties of these gels.

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ACKNOWLEDGMENTS:

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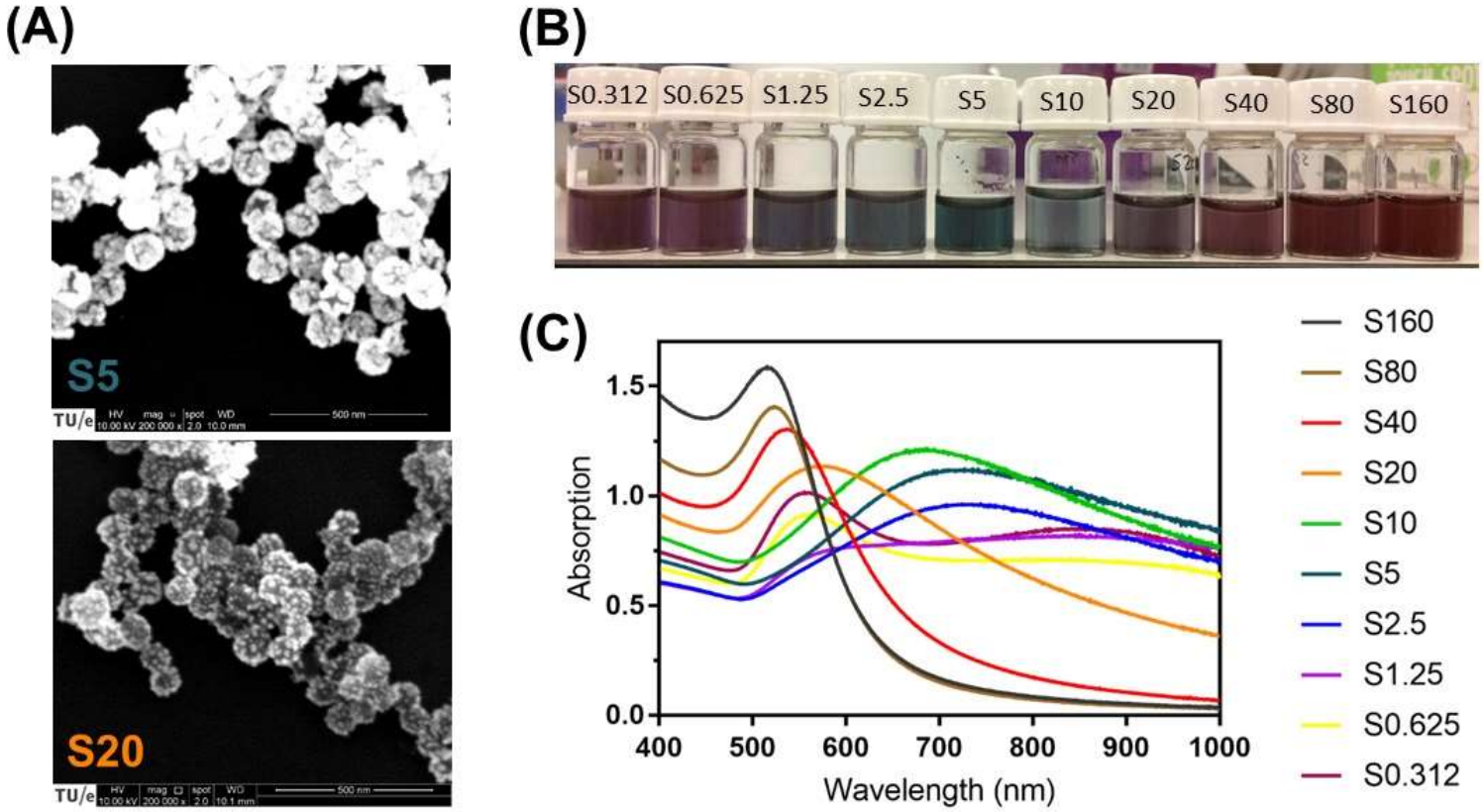

Picture 1: Caption 1: Figure 1. A) SEM images, B) digital photograph, and C) optical extinction spectra of gold-coated silica cores.

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Poster presentation

284 Engineered biopolymer scaffolds trigger a secretory phenotype of Wharton´s jelly Mesenchymal Stromal Cells for bone regeneration

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INTRODUCTION:

Bone tissue engineering has focused on the development of biopolymer-based scaffolds that promote biologically active responses and improved mechanical support of cells in order to form functional tissue [1,2]. However, little is known on the effect of synthetic or natural biopolymers in the release of growth factors or cytokines produced by cells cultured on these polymers [3]. In this work, we evaluated whether scaffolds fabricated with polylactic acid (PLA), polycaprolactone (PCL) or biological polymers (atellocollagen) impact on the secretion profile of Wharton´s jelly Mesenchymal Stromal Cells(WJ-MSC) for bone regeneration.

METHODS:

PCL scaffolds were prepared using the electrospinning technique, PLA scaffolds by 3D printing, and 3D Atelocollagen microsphere obtained from commercial vendors. Each scaffold was analyzed by scanning electron microscopy (SEM) and seeded with 1x10e5 WJ-MSC. These constructs were cultured in DMEM-low glucose with 10 % human platelet lysated (hPL) at 37 °C, 5 % CO2 till 70% confluence was reached. On 70 % confluence, medium was changed and after 72h collected for the determination of factors involved in bone tissue regeneration (TGF-B, VEGF, PDGF, FGF-2, Angiopoietin) using the Luminex technology

RESULTS AND DISCUSSION:

Scanning electron microscopy analysis of PLC scaffolds prepared by electrospinning demonstrated the presence of fibers with sixes (THIKNESS??) 858±96–1033±16 nm, also showed a high degree of nanofiber orientation with smooth and homogeneous surfaces. SEM analysis of PLA-based 3D scaffolds fabricate revealed porosities of 68% and 600±66–900±69 μm pore size. Atelocollagen beads were 200-400 microns in diameter and moderately rough surface. WJ-MSC grew in all scaffolds and showed significant enhanced levels of bFGF and TGFβ1 in PLA-constructs as compared to PLC and atelocollagen constructs. In contrast, moderate increase of angiopoietin-1 and PDGF levels occurred in all constructs. We did not observe differences in VEGF levels.

CONCLUSION:

In conclusion, biopolymers used in scaffolds modulate the expression of growth factors by WJ-MSC. According to our results, PLA is the best biopolymer to promote the expression of growth factors involved in repair of bone tissue by WJ-MSC.

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ACKNOWLEDGMENTS:

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Poster presentation

289 In vivo antibacterial properties of biodegradable ZK60 alloys by dual plasma immersion ion implantation

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INTRODUCTION:

Magnesium-based metals, which have good biocompatibility, osteogenic activity and antibacterial ability, have become the focus of biodegradable medical implants[1]. However, high rate of degradation of magnesium alloys and

potential surgical infection limited its clinical applications. In order to enhance antibacterial property *in vivo*, a layer of protective oxide film was fabricated on magnesium surfaces by dual zirconium and oxygen plasma immersion ion implantation (PIII).

METHODS:

The as-cast ZK60 alloys was cut into 10×10×5 mm^3 block and mechanically ground using up to 1200 grit SiC paper. Then the samples were treated by dual zirconium and oxygen PIII (Zr&O PIII). Adult SD rats (200-220g) were anesthetized with pentobarbital sodium (40 mg/kg) by intraperitoneal injection. Subcutaneous pockets were created on the back of each rat, then one sample co-cultured with 10^6 CFU/mL *staphylococcus aureus* (*S.aureus*) was implanted in subcutaneous pocket through one incision. No *S.aureus* group was used as a negative control. The rats were sacrificed at 3 and 7 days, number of viable *S.aureus* on the sample surface was analyzed by using the spread plate method. Histological evaluations of paraffin section of skin were evaluated by Gram staining.

RESULTS AND DISCUSSION:

With the increase of implantation time, the number of *S.aureus* on the ZK60 and Zr&O PIII ZK60 sample surfaces increased gradually (Fig.1a). However, the number of *S.aureus* on Zr&O PIII ZK60 surface was lower than that on ZK60 surface both at day 3 and 7. After 7 days implantation, the number of *S.aureus* on Zr&O PIII ZK60 surface was still lower than that in ZK60 after 3 days implantation, indicating that Zr&O PIII ZK60 had good antibacterial activity *in vivo*.

As shown in Fig.1b, no *S.aureus* was found on the paraffin section of skin of control and Zr&O PIII ZK60. However, there were many *S.aureus* on the paraffin section of skin of ZK60 (green circle). The number of *S.aureus* at day 7 wasmore than that of day 3. This was in good accordance with the results of spread plate method.

CONCLUSION:

This study indicates that Zr&O PIII can significantly improve antibacterial properties of ZK60 magnesium alloy *in vivo*.

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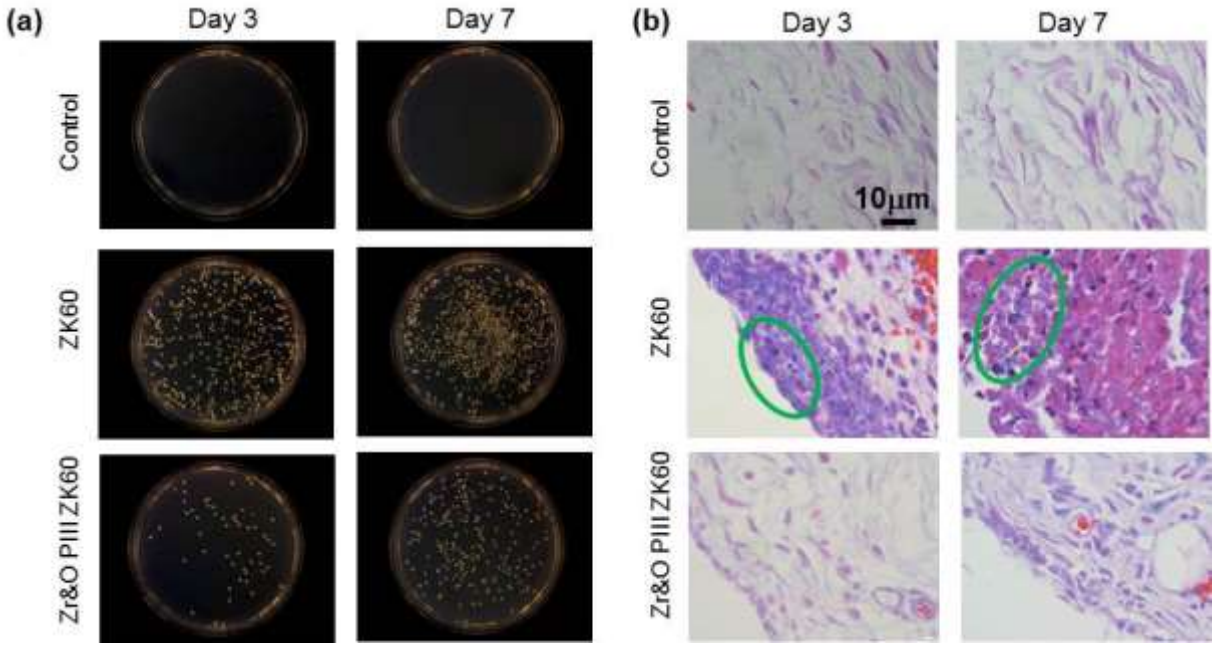
ACKNOWLEDGMENTS:

The study was financially supported by the National Natural Science Foundation of China (Nos.51501218, 81572113), Shenzhen Peacock Programme (110811003586331), Shenzhen Science and Technology Research Funding (JCYJ20160229195249481, JCYJ20160608153641020).



Picture 1: Caption 1: Fig.1 (a) Representative images of adherent S.aureus and (b) histological analysis for ZK60 and Zr&O PIII ZK60 groups

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Poster presentation

292 Nanostructure of acyl-modified hyaluronan in the solid state

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INTRODUCTION:

Hyaluronan (HA) is a naturally occurring polysaccharide that is an important constituent of our body. Due to its biodegradability and non-toxicity, HA is a promising material for applications in medicine. However, HA readily dissolves in water, which limits its use in applications requiring insolubility in aqueous environments. To overcome this, HA can be modified by long acyl side-chains[1]. This contribution explores the structure of acyl-HA in the solid state, studying powders (reaction product) and solution casted films[2] as a selected application form.

METHODS:

Native HA and acyl-HA samples were provided by Contipro a.s. The acyl-HA samples included various substituents (hexyl to oleyl), substitution degrees and molecular weights. For analysis, wide- and small-angle X-ray scattering (WAXS/SAXS) and extra-high resolution scanning electron microscopy (XHRSEM) were used.

RESULTS AND DISCUSSION:

The SAXS profiles of native HA did not show any scattering peaks, while all studied acyl-HA samples exhibited distinct peaks corresponding to structures with dimensions on the order of nanometres. Analogous results were obtained by XHRSEM. The SAXS data showed only minor influence of sample form (powder or film), so the observed nanostructure is inherent to the materials, i.e., not a result of sample preparation. The size of the nanostructures increased with the acyl side-chain length, while the degree of substitution and molecular weight had negligible effects. WAXS profiles showed that the samples were non-crystalline.

Considering the chemical structure of acyl-HA, we suggest that the solid-state nanostructure corresponds to a distribution of hydrophobic domains in a hydrophilic matrix of unmodified HA segments. The hydrophobic domains act as physical cross-links and provide insolubility or limited solubility to the material similarly to covalent cross-linking.

CONCLUSION:

We showed that acyl modified HA contains nano-structures corresponding to a distribution of hydrophobic domains that act as physical cross-links. These findings contribute to our understanding of the macroscopically observed behaviour of acyl-HA materials.

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Poster presentation

294 Study of the degradation of polyester nanofibrous materials for drug-delivery systems

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INTRODUCTION:

Electrospun fibres provide high surface-to-volume ratio and high porosity which is promising structure of scaffolds for the tissue engineering[1]. Additionally, the electrospinning technology used allows the preparation of drug-containing fibres[2]. Scaffold degradation[3,4] generally affects tissue regeneration and, in the case of materials with incorporated active substances, plays a crucial role in drug release kinetics. In presented work we tested degradation behaviour of polyester electrospun materials prepared from different polyesters and with various morphologies.

METHODS:

Fibrous structures of different morphologies) were prepared from polycaprolactone (PCL) and/or copolymer of polylactide and polycaprolactone (PLCL) by needleless electrospinning method (NANOSPIDER technology). Degradation of prepared materials was accelerated using enzymes for catalysis (Proteinase K and Lipase). Samples were further analyzed for the weight loss, morphology (SEM and subsequent image analysis), crystallinity (DSC) and molecular weight loss (GPC).

RESULTS AND DISCUSSION:

With the exception of materials prepared from polymer blends, no change in molecular weight was observed in residual materials during degradation. Crystallinity was evaluated for PCL presented in fibrous layers. A change was observed depending on the degree of degradation, however not observed only decrease as reported in the literature[3]. The increasing fibre diameter as well as increasing molecular weight of used polymer has the negative effect on the degradation rate. Obtained data define the mechanism of degradation of tested fibrous materials as the surface erosion mechanism[4]. The morphology of tested materials changes significantly during degradation (fig. 1). As well as speed of degradation, it is specific for each kind of material including polymer ratio in blends which demands the dependence of the degradation behaviour on the very specific composition of the polymeric fibrous material.

CONCLUSION:

The degradation of polyester nanofibrous scaffolds can be well influenced by the selection of the input polymer and the morphology of the resulting materials. In this way, the release of incorporated substances can be driven due to controlled material degradation.

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ACKNOWLEDGMENTS:

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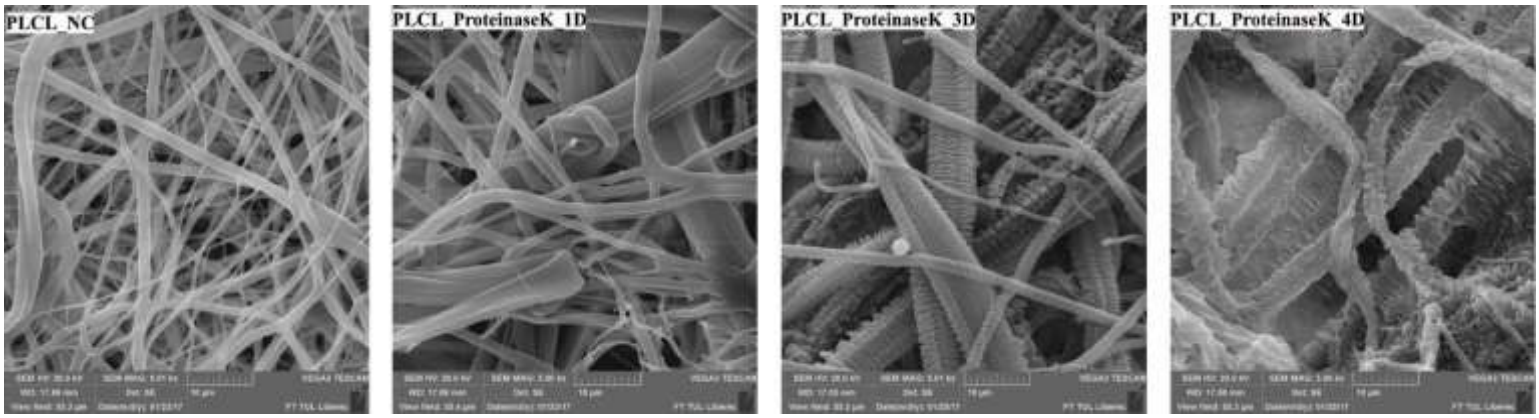

Picture 1: Caption 1: Figure 1: SEM pictures of PLCL fibres during degradation by Proteinase K, magnification 5000X

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Poster presentation

297 Low voltage electrospinning production of decellularised matrix laden nano-micro fibres and membranes

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INTRODUCTION:

Utilising decellularised matrix (ECM) to recreate organ-specific microenvironments *in vitro* has long been of interest. Although prior studies have produced ECM fibres using far-field electrospinning, the content of ECM is generally low and the patterning of soft fibres are technically demanding [1]. Here, we aimed to address the above challenges by fabricating ECM-laden fibres and membranes using low-voltage electrospinning (LEP). Cell study was performed to investigate the biofunction of this ECM-laden membrane.

METHODS:

ECM fibres and membranes were fabricated utilising a modified LEP process [2]. FTIR was used to reveal the ECM protein structures after fabrication. Mechanical properties of single fibres were measured using micropipette cantilever method. The biofunction of this ECM-laden membrane was evaluated by culturing podocytes and glomerular endothelial cells on it where their interactions with the ECM-laden membrane were examined by confocal and SEM imaging.

RESULTS AND DISCUSSION:

By utilising a modified LEP process [2], we were able to fabricate fibres and membranes with high content (up to 50wt%) of ECM directly patterned on targeted substrate. The Young's modulus of fibres increased with its ECM content, ranging from 600 KPa to 50 MPa. FTIR study confirmed that this fabrication method did not impose a significant denaturation to ECM protein. Interestingly, when co-culturing podocytes and endothelial cells on a membrane, aggregation of endothelial cells was seen, and the intended tri-layer structure did not maintain after 7 days of culture. Finally, with SEM imaging, a confluent layer of cells interconnected with neighbouring cells was observed on the membrane rather than cell-fibre interconnection which is known to be observed in thermoplastic fibres [3].

CONCLUSION:

We present a method to fabricate ECM fibres and membranes with tuneable biochemical, mechanical and topographical properties using a low-voltage electrospinning technique without imposing a significant denaturation to ECM proteins. Some of the key challenges reported from previous studies were addressed, including low content of ECM in fibres and difficulty of patterning soft membranes. Our results show the importance of using ECM and membrane mechanical properties in affecting the interconnection and reorganisation of neighbouring cells.

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ACKNOWLEDGMENTS:

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Poster presentation

300 PP meshes covered with PCL nanofibers functionalized by cold plasma for biomedical application

Malo Dufay[1], Maude Jimenez[1], Stéphanie Degoutin[1], Frédéric Cazaux[1], Séverine Bellayer[1], Mathilde Casetta[1], Grégory Stoclet[1], Ludovic Janus[1], Feng Chai[2], Nicolas Blanchemain[2]
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INTRODUCTION:

A large variety of processes are used nowadays to develop biomedical textile implants. Among them, electrospinning has gained interest these last years. This technique offers advantages for biomedical applications especially due to the nanoscale diameter range of electrospun fibers, and to their high porosity and surface area[1]. In addition, cold plasma process can be used to activate and/or functionalize electrospun fibers. In the literature, cold plasma is indeed mainly used to enhance hydrophilicity or to graft polymer at the nanofiber's surface[2]. In this study, polypropylene implants coated with polycaprolactone (PCL) electrospun nanofibers, functionalized by acrylic acid (AA) thanks to a cold plasma technique were prepared and the process was then optimized.

METHODS:

Polycaprolactone, in a mixture of formic/acetic acid, was electrospun onto polypropylene meshes (PPM) and the adhesion between PPM and PCL nanofibers was evaluated through peeling tests. Then, samples were immersed in acrylic acid solution and a cold plasma treatment was performed in order to graft-copolymerize acrylic acid on PCL nanofibers surface. Adequate mechanical and physico-chemical characterizations were performed (FTIR, SEM, TGA, peeling test …) at each step of the process. Finally, cytotoxicity was evaluated for a potential application in biomedical devices.

RESULTS AND DISCUSSION:

First, electrospinning of PCL solutions in a mixture of formic/acetic acid was optimized in terms of concentration, voltage, flow, tip-to-collector distance. The electrospun PCL nanofiber membranes were also analyzed by SEM and the mean nanofiber diameter was around 300 nm. Adhesion between PPM and PCL was also evaluated by peeling test, and different treatments (thermal or chemical) were tested to enhance this adhesion. The graft-copolymerization of AA was optimized to obtain cytocompatible nanofibers rich in COOH groups at the surface.

CONCLUSION:

We have successfully optimized the electrospinning of PCL and the adhesion of these nanofibers onto PP meshes. The graft-copolymerization of AA onto PCL nanofibers induced by cold plasma was achieved and the cytotoxicity of the functionalized implants was studied. Future work will focus on the grafting of bioactive monomers such as antibacterial ones in order to extend the field of potential biomedical applications.

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ACKNOWLEDGMENTS:

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

303 Tridimensional fibrous scaffolds from PCL/chitosan blends for tissue engineering

Andreia Leal Pereira, Samuel Guieu, Maria Helena Vaz Fernandes
University of Aveiro, Aveiro, Portugal

INTRODUCTION:

Tissue engineering (TE) is a treatment strategy that combines materials engineering, cell biology and growth factors to restore biological functions of damaged tissues and organs. Usually, cells are seeded in a temporary material architecture (scaffold), that mimics the extracellular matrix (ECM) and allows the attachment, proliferation and differentiation of cells. Ideally, these scaffolds should be biocompatible, with high interconnected porosity, suitable mechanical properties and controllable degradability.

Polycaprolactone (PCL) and chitosan are two biocompatible polymers widely used in TE, because their physical properties adequately complement each other. They have been successfully processed by electrospinning, producing nanofibrous bidimensional architecture. In order to mimic the ECM, the scaffolds should nevertheless be tridimensional, porous, and fibrous, and this was still missing for PCL/chitosan blends. Here we report the production of such scaffolds.

METHODS:

Blends of PCL/chitosan were dissolved in acetic acid/formic acid mixed solvents and the solution was charged in an electrospinning machine to obtain composite fibers. Those were then cut and subjected to the thermally induced self-agglomeration (TISA) process in order to obtain tridimensional architectures. Freeze drying of these structures produces tridimensional fibrous scaffolds.(1)

RESULTS AND DISCUSSION:

Concentrations of PCL/chitosan solutions in acetic acid/formic acid (1/1 to 1/3) between 5% and 15% w/w (5-50% w/w of chitosan / PCL) were used to prepare electrospun composite fibers. Beads-free fibers were obtained for the following settings: distance 10-20cm, voltage 20-30 kW, flux 500-1000 $\mu L.h^{-1}$, with average diameters in the range 50-600 nm (Figure 1). The fragmented fibers submitted to TISA and freeze drying retained their dispersion and morphologies in the final tridimensional scaffold. Density, porosity and pore structure of the scaffolds are within the suitable ranges for an appropriate cellular response in a tissue regeneration context.

CONCLUSION:

This work paves the way to the production of tridimensional scaffolds from PCL/chitosan blends. These architectures present a porous and fibrous structure, closely mimicking a tridimensional ECM, which is very promising for the *in vitro* growth of various cells, and will then be tested *in vivo* for regeneration of damaged tissues.

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ACKNOWLEDGMENTS:

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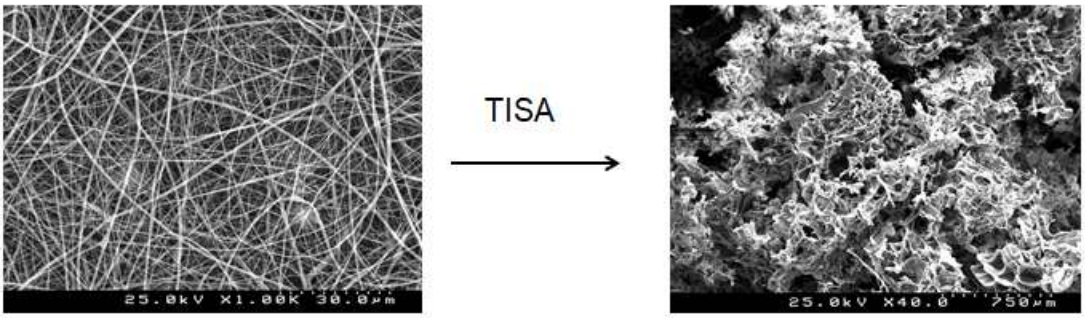

Picture 1: Caption 1: Figure 1. Synthesis of the scaffold

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Poster presentation

306 Self-assembly of electrospun nanofibers to fabricate gradients honeycomb structures for bone regeneration

Tianyu Yao[1], Matthew B Baker[2], Lorenzo Moroni[2]
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[2]MERLN Institute, Netherlands

INTRODUCTION:

A new phenomenon showing that electrospun nanofibers self-assemble into honeycomb-patterned nanofibrous structures have been previously reported. Controlled by the surface tension and electrostatic repulsion, wet nanofibers have been shown to self-assemble into honeycomb structures. Here, we used electrospinning to fabricate honeycomb structural meshes with different diameters. Furthermore, we have now shown for the first time how this technique can be controlled to create a gradient honeycomb pattern in the meshes. The aim of the present investigation is to study the effect of a honeycomb gradient built-in microfibrillar meshes for the adhesion, proliferation, and differentiation of bone marrow mesenchymal stromal cells, and also to evaluate the use of these scaffolds for bone regeneration.

METHODS:

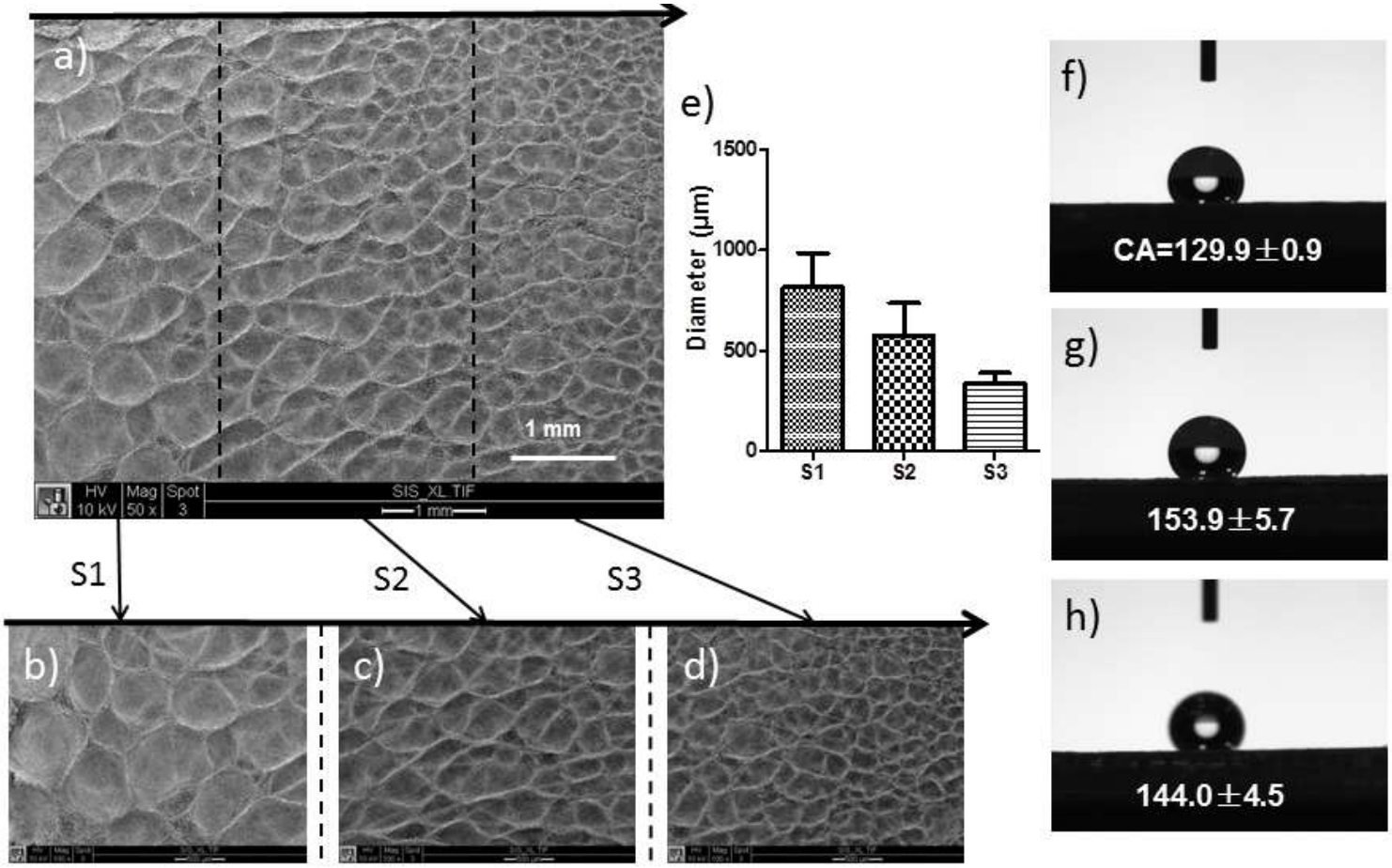
Poly(caprolactone) (Mn= 45,000) was dissolved in DMF:$CHCl_3$ (1:4) to prepare different concentrations for electrospinning. The morphology of the honeycomb-patterned scaffolds was observed by scanning electron microscopy (SEM). The wettability of the scaffolds was calculated by a contact angle (CA) goniometer.

RESULTS AND DISCUSSION:

Fig 1a shows the SEM images of a well-defined gradient honeycomb structure. The honeycomb structure of the left segment was comprised of large pores, whereas the honeycomb structure of the right segment was comprised of smaller pores (Fig. 1b–d). These observations are hypothesized to be due to the formation of a gradient electric field. A higher electric field in the central part of the collecting plate can result in a higher electric repulsion, so a larger honeycomb is formed in the center. As showed in Fig. 1f–h, the fibrous meshes, which contained honeycomb structures, had a higher CA value than normal PCL fibrous meshes. This indicates that the hydrophobicity of honeycomb meshes increased as honeycomb pores was added.

CONCLUSION:

In summary, a new process to produce gradient honeycomb structures was first demonstrated by using electrospinning. By adjusting the electrospinning conditions, it is possible to form honeycomb-patterned meshes with different diameters and induce the formation of gradient honeycomb structures. Furthermore, the geometry of honeycomb pattern provides a unique structure and environment for cell attachment and differentiation. These scaffolds may hold potential as an efficient platform for tissue regeneration.



Picture 1: Caption 1: Fig. 1 SEM images of the entire gradients honeycomb structures (a) and highly magnified images (c-d) of the three different regions

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Poster presentation

318 Surface free energy predominates in cell adhesion to ceramic biomaterials

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[2]Tokyo Medical and Dental University, Japan
[3]Nihon University, Japan
[4]University of Oulu, Finland

INTRODUCTION:

The initial adhesion of cells to biomaterials is key in the regulation of the subsequent proliferation, differentiation, and formation of extracellular matrix after cell spread and migration. The surface characteristics of ceramic biomaterials are reportedly determined by surface topography, roughness, crystallinity, grain size, constituent elements at the surface, and the incorporation of ions. The purpose of this study was to investigate a mechanism through which the surface wettability of biomaterials can be improved and determine the effects of biomaterial surface characteristics on cellular behaviors.

METHODS:

We prepared various types of hydroxyapatite (HA) after sintering in different atmospheres; sintered in a saturated water vapor atmosphere at 1250 °C for 2 h (wHA) and in air at 1250 °C for 2 h (aHA). The highly crystalline HA specimens were electrically polarized in d.c. electric fields of 5 $kV \times cm^{-1}$ in air at 400°C. The surface characteristics, including roughness, grain size, hydroxide ion content, zeta potential, surface free energy, and wettability, were investigated. The cell adhesion was evaluated using mouse mesenchymal stem cells.

RESULTS AND DISCUSSION:

The surface free energy of polarized wHA was approximately 1.6 times higher than that of conventional wHA. The contact angles of water significantly decreased on polarized wHA. On the other hand, the surface free energy of aHA did not change before and after polarization. These changes in the contact angle of water and surface free energy on polarized wHA surfaces indicated that the wettability of the polarized wHA surfaces was improved by the increase in surface free energy. Cellular morphological observation and quantitative analysis revealed that the spread of cells on polarized wHA was accelerated compared with that on conventional wHA. These results demonstrated that the improvement in wettability that accompanied the increase in surface free energy affected the number of initial spread cells and the degree of cell spread on wHA surfaces (Figure).

CONCLUSION:

Compared with HA sintered in air, HA sintered in saturated water vapor had a higher polarization capacity that increased surface free energy and improved wettability, which in turn accelerated cell adhesion. We determined the optimal conditions of HA polarization for the improvement of surface wettability and acceleration of cell adhesion.

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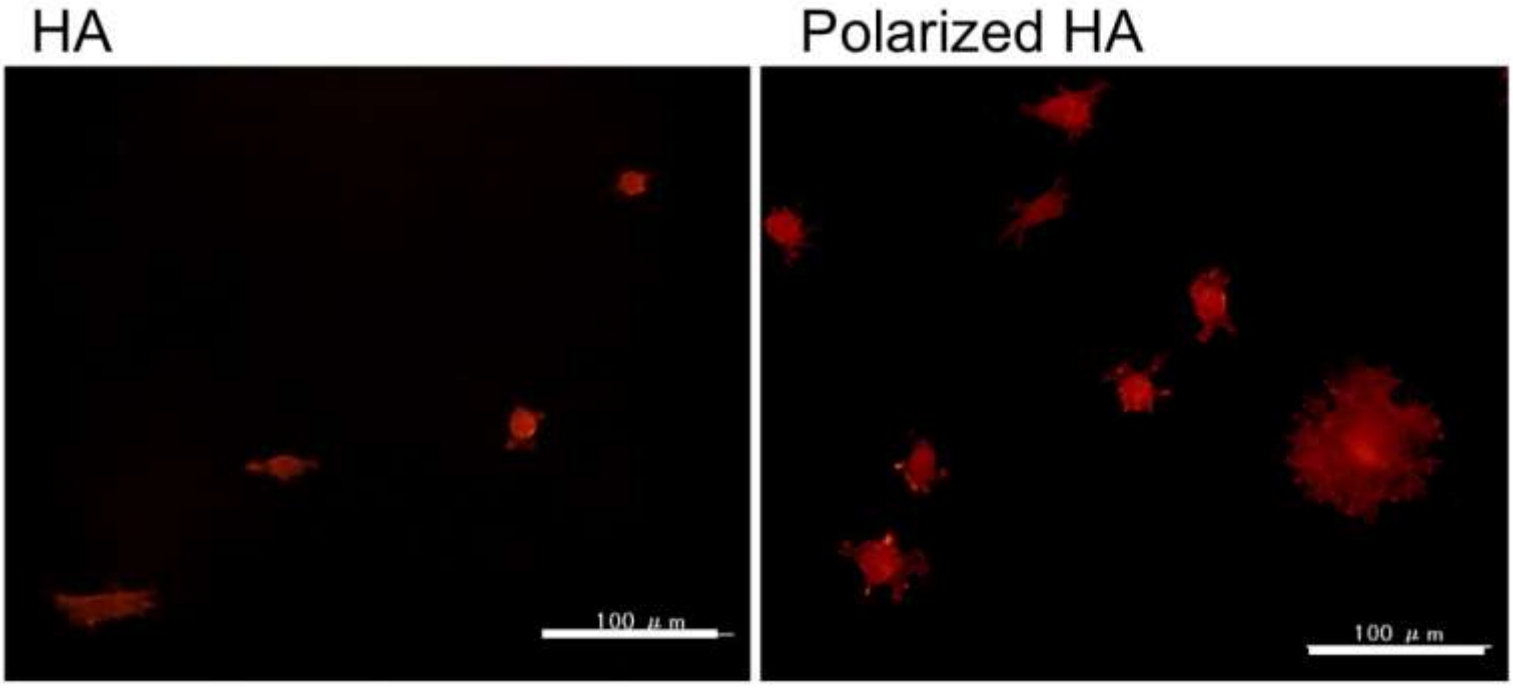
ACKNOWLEDGMENTS:

This study was supported in part by Grants-in-Aid for Young Scientists (B) (15K20472 and 15KK0299) and Grant for Basic Science Research Projects from The Sumitomo Foundation.



Picture 1: Caption 1: Cell adhesion on conventional and polarized HA samples

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Poster presentation

321 Enhancing fibroblast attachment onto bioresorbable glass through surface modification

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[2]University of Tampere, Finland

INTRODUCTION:

Phosphate glasses were developed to substitute silicate bioactive glasses. Their dissolution is, generally, congruent leading to a controlled therapeutic ion release and their thermal properties enable hot-forming into scaffolds and/or fibers. However, the fast degradation of some phosphate glasses inhibit proper cell attachment.

Therefore, we present an optimized functionalization procedure to graft proteins (albumin and fibronectin) at the surface of the glasses. The impact of surface chemistry on the protein grafting and change in fibroblast cell attachment, upon protein grafting, will be discussed.

METHODS:

Silicate- and phosphate-bioactive glasses were processed by melt-quenching. The glass discs were cut, polished and washed using buffer solution with pH from 5 to 9. Post-washing, part of the discs were functionalized using APTES. The washed and washed&silanized discs were exposed to either fibronectin or albumin. The protein attachment efficiency was assessed by fluorescence microscopy utilizing fluorescently labelled proteins. The most efficient functionalization protocol was then repeated and fibroblasts were cultured for 24 hours on samples (1)

washed, (2) washed&silanized and (3) washed&silanized&protein-grafted. Cells attachment and viability were assessed using live cell recording (EVOS system) and by staining of the cells at the end of the culture period, followed by confocal microscopy.

RESULTS AND DISCUSSION:

While proteins could bind to the surface of the silicate-based bioactive glasses, they did not bind efficiently to the phosphate glass surfaces. Washing of the surface improved slightly the protein adhesion while silanization was the most efficient in facilitating binding of both fibronectin and albumin. Fibronectin binds preferentially to the surface of glasses washed using an alkaline buffer solution, while albumin binding was more efficient when acid buffer solution was employed. Within 24h of cell culture, the fibroblasts clearly attached and proliferated better on silicate-based bioactive glass, as expected, whereas minimal cell attachment was recorded on the phosphate-based glasses. Samples washed&silanized enhanced significantly the ability of cells to firmly attach to the biomaterial surfaces. Confocal microscopy revealed that protein grafting improved cells attachment by increasing the number of focal adhesions as can be seen in Figure.

CONCLUSION:

Limited cell attachment, at the surface of phopshate glass, can be improved by modification of the materials surface chemistry and/or by binding proteins at the materials' surface. Similar cell attachment than at the silicate surfaces was reached. Surface modified phosphate glasses have potential in hard tissue engineering.

ACKNOWLEDGMENTS:

The authors would like to acknowledge the Academy of Finland as well as the Jane and Aatos Erkko foundation for financial support.

Picture 1:

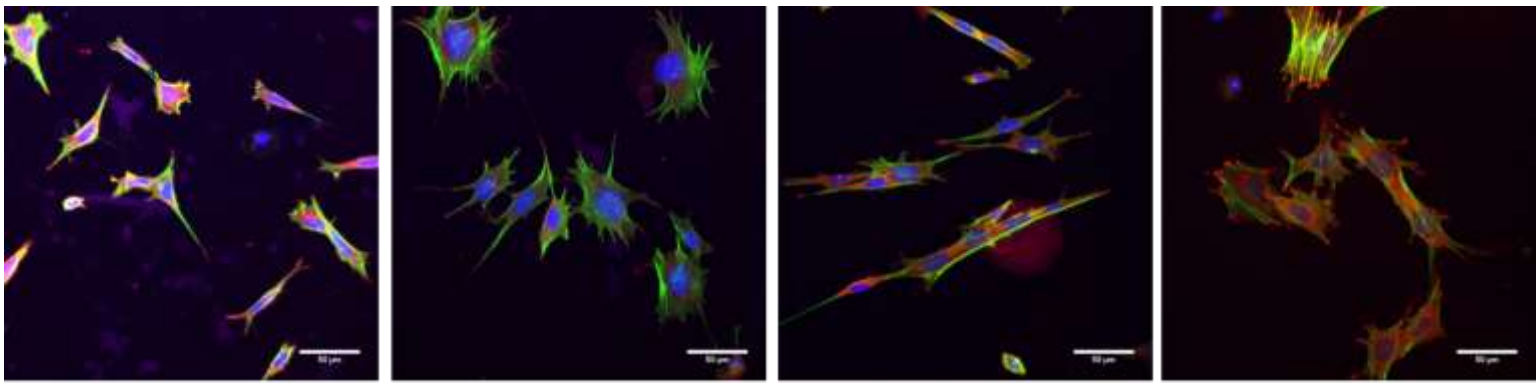

Figure: Confocal images showing stained fibroblasts at the surface of silicate glass S53P4 post silanization (a), S53P4 post silanization&fibronectin coated (b) as well as at the surface of phosphate glass Sr50 post silanization (c), Sr50 post silanization&fibronectin coated (d). (Blue: nuclei; Green: Actin filaments; red: Vinculin)

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Poster presentation

323 The effect of nanostructured Ti39Nb on osteogenic differentiation of human mesenchymal stem cells

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INTRODUCTION:

Titanium alloys are widely used in bone replacements and in dentistry. Except for widely used alpha/beta alloys, e.g. Ti6Al4V, novel beta alloys are being tested[1]. Nanotubes prepared by anodic oxidation of Ti6Al4V showed improved adhesion and bone-specific extracellular matrix (ECM) formation[2]. In this study, we tested novel beta alloy Ti39Nb and modified its surface with nanotubes and then tested their effect on human MSCs' adhesion, growth and differentiation.

METHODS:

Ti39Nb plates were modified by anodic oxidation using 10V, 20V, or 30V. Nanotubes were visualized by SEM and their diameter was measured. Modified, smooth plates and glass were seeded with hMSCs at a density 12 $\times 10^3$ cells/cm^2. Immunofluorescence staining of vinculin was performed in three samples/group and cells were visualized using confocal microscope ZEISS LSM 5 DUO. On day 1,7,14, and 21, RNA was isolated from 3 samples/group, and mRNA expression of bone markers RunX2, type I collagen, and osteocalcin was measured using qRT-PCR. Type I collagen immunostaining and visualization of three samples/group was done on day 7 and 14. Fluorescence intensity and cell densities were calculated from ten photomicrographs/group. Statistics was performed using One-way ANOVA and Student-Newman-Keuls Method, and for PCR evaluation, non-parametric Kruskal-Wallis test and Dunn's Multiple Comparison test were used.

RESULTS AND DISCUSSION:

Higher applied voltage increased the diameter of nanotubes. Contrary to random vinculin distribution on smooth surfaces, the distribution of vinculin was dot-like on all nanostructured Ti39Nb plates. We found no differences in relative expression of mRNA of RUNX2, type I collagen, and osteocalcin among Ti39Nb plates during the whole culture. We have shown increased type I collagen synthesis on nanostructured Ti39NB compared to smooth plates and glass on day 14 and 21. The highest type I collagen was found in 20V Ti39Nb plate on day 21, but also showed decreased cell number than other samples. The improved bone differentiation of Saos-2 cells on nanostructured Ti6Al4V at 10V and 20V was also proved by Filova et al.[2].

CONCLUSION:

Nanostructured Ti39Nb modified adhesion of hMSC and improved type I collagen synthesis, mainly on 20V and 30V Ti39Nb, and thus represent a useful tool to control hMSC differentiation.

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ACKNOWLEDGMENTS:

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Poster presentation

327 The effect of microtopography on endothelial cell specification using a novel high-content imaging system

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[2]University of Padova, United Kingdom

INTRODUCTION:

Research has shown the importance of biochemical factors in combination with the physical topography of the extracellular matrix in directing cellular behaviour1. Understanding the effect of physical cues is imperative for the fabrication of biomaterials to be used in tissue engineering applications. Achieving proper vascularisation is a primary aim of any tissue engineering strategy. This study aims to determine the effect of microtopographic cues on endothelial cell (EC) phenotype; focusing primarily on EC specification (artery, veins, capillaries) to further our understanding of the mechanisms of vascular development and to provide a solid basis to develop innovative biomaterials.

METHODS:

A range of PDMS substrates were manufactured with defined micropatterned surfaces that differed in their arrangement, spacing and aspect ratio in a combinatorial approach. Induced Pluripotent Stem Cell derived Endothelial Colony Forming Cells (iPSC EFC) were cultured onto the substrates. To evaluate EC phenotype, we performed immunostaining for selected EC markers (e.g. VE cadherin, CD 31) and transcription factors (e.g. COUP TFII, HEY2). Cells were imaged using a high content imaging system (Perkin Elmer Operetta CLS) and analysed by image analysis. To this aim, we are developing an automated pipeline for image analysis using Perkin Elmer Harmony and Cell profiler which will provide an unbiased method of evaluating and quantifying phenotypical data.

RESULTS AND DISCUSSION:

Preliminary data employing stimuli known to affect EC phenotype (i.e. VEGF, SDF-1) demonstrated the viability of the proposed strategy. As reported for Human Umbilical Vein Endothelial Cells, VEGF induced phenotypic changes in iPSC ECFCs, i.e. acquisition of a spindle shape indicative of migratory phenotype. In addition, where iPSC ECFCs were cultured onto differently functionalised surfaces, YAP/TAZ (involved in mechanosensing) was differentially activated thus providing a molecular basis to interpret phenotypical switch (e.g. migratory/proliferative phenotype).

CONCLUSION:

The phenotyping strategy we are designing and validating is a viable tool to assess EC phenotype under different culture conditions in a robust and unbiased manner. This will allow more in depth investigations of how microtopographic cues drive EC phenotypical specialisation and thus allow the design of novel materials driving desired EC phenotypes (e.g. activated/angiogenic ECs for tissue re-vascularisation and regeneration).

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

334 Coaxial electrospun scaffolds for dual delivery of bioagents for bone tissue engineering

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Middle East Technical Uni., Ankara, Turkey

INTRODUCTION:

Bone scaffolds can be modified to perform local controlled release of bioactive agents[1]. Scaffolds with fibrous structures can be developed from natural/synthetic polymers by electrospinning methods and wet-electrospinning can produce highly porous 3D structures. Here, a coaxial wet electrospinning system, creating two different environments for bioactive agent loading and release was developed for dual delivery of vitamin-K2 and calcitonin. They were also loaded to single phase wet-electrospun scaffolds and compared for their bone scaffold potency.

METHODS:

Coaxial wet-electrospun scaffolds were compared with single phase wet-electrospun groups collected in water/ethanol. Gelatin and Poly(caprolactone) (PCL) based fibers produced using surfactants (PLF and PVA) were loaded with VitK2 and/or Calcitonin. Degradation, pore size distribution and mechanical properties were characterized. Release properties of bioactive agents were investigated. Viability of SaOS-2 cells on scaffolds was compared with alamar blue assay. Morphology of proliferated cells was observed by scanning electron microscopy (SEM) and confocal microscopy.

RESULTS AND DISCUSSION:

Scaffold groups had ideal pore size distribution and coaxially electrospun scaffolds collected in ethanol group had the highest porosity (67.63%). After 10 days of degradation analysis, most stable groups were single phase electrospun scaffolds collected on water (≈28%) and coaxially electrospun scaffolds collected in water (≈32%). Mechanical strengths of all scaffold groups were low, therefore, they were considered suitable for non-load bearing bone tissues. Coaxial collected in water, showed steady and slowest release of agents. After 7 days, amount of released was about 10 fold for protein and 4 fold for VitK2 from single phase electrospun scaffolds compared to coaxially electrospun scaffolds collected in water. After 7 days of incubation highest cell viability was observed on coaxially electrospun scaffold collected in water. Cell morphology analysis showed that cells were well spread through scaffolds (Figure 1).

CONCLUSION:

This study focused on development and characterization of coaxial scaffolds that can support dual release of vitamin K2 and calcitonin for bone tissue engineering. Scaffolds produced by coaxial electrospinning showed more steady and controlled release of agents compared to single phase electrospun groups. Coaxial scaffolds collected in water has provided low degradation, controlled release and high cell proliferation, therefore, being a good candidate as a bone scaffold.

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Authors would like to thank METU BIOMATEN (Center of Excellence in Biomaterials and Tissue Engineering) for financial support and laboratory facilities.

Picture 1:

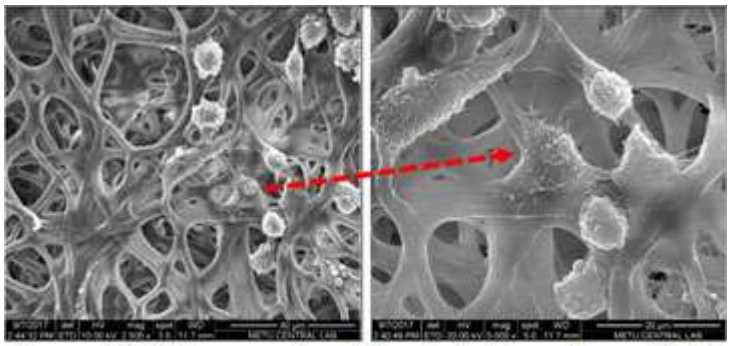

Figure 1. SEM images of proliferated cells on the scaffold after 7 days of incubation

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Poster presentation

336 Surfactant mediated hydrophilicity of porous degradable copolymer scaffolds enhance bone regeneration

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[2]Fibre and Polymer Technology, KTH Royal Institute of Technology, Stockholm, Sweden
[3]Department of Clinical Dentistry, University of Bergen, Norway
[4]KTH Royal Institute of Technology, Stockholm, Sweden

INTRODUCTION:

Aliphatic polyesters such as poly(L-lactide-co-ε-caprolactone) (poly(LLA-co-CL) has been extensively used in tissue engineering field. However, hydrophobicity and absence of natural recognition sites greatly limits their further applications[1-2]. Herein, we used Tween-80; a nonionic surfactant with different concentrations 0.5 - 3% (w/w) to enhance the hydrophilicity. The modified scaffolds were assessed *in vitro* and *in vivo* towards bone tissue formation.

METHODS:

Scaffolds were prepared using different Tween-80 concentrations by a salt leaching technique. Surface morphology and hydrophilicity were analyzed by SEM and contact angle, respectively. Microcomputed tomography (μ-CT) was used to evaluate 3D morphology of scaffolds. Different biological characterizations were employed to validate the bone formation *in vitro* and *in vivo*.

RESULTS AND DISCUSSION:

The pores in the scaffold were interconnected and not affected by the surface modification. The contact angle was significantly reduced after modifying with 3% Tween-80. Hence potentially higher cell attachment and proliferation were observed. Result revealed substantial *de novo* bone formation in 3% Tween-80 scaffolds compared to pristine poly(LLA-co-CL), which was corroborated through μ-CT, gene expression and histology analysis after 8 weeks, in a subcutaneous rat model.

CONCLUSION:

Surface modification with Tween-80 has a positive impact on surface properties without distorting its microstructural properties. The porous scaffolds with 3% Tween-80 enhanced osteogenic differentiation and bone formation *in vitro* and *in vivo*.

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Poster presentation

349 Human-based cellular models to predict biomaterial immunocompatibility - an alternative to animal testing

Burkhard Schlosshauer[1], Sören Segan[2], Sascha Klimosch[2], Meike Jacobi[2], Nicole Schneiderhan-Marra[2], Hanna Hartmann[2], Ulrich Rothbauer[2], Thomas Joos[3], Christopher Shipp[2]
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[3]Germany, Germany

INTRODUCTION:

The biocompatibility of implanted medical devices is heavily influenced by interactions with the immune system, resulting either in successful biointegration, implant failure or undesirable patient side effects[a]. It is known that different physical and chemical properties of implant materials results in distinct types of immune responses[b], but a fundamental understanding of how implants can be designed to produce appropriate immune responses for each type of implant remains elusive[1]. The aim of this study is to gain an understanding of the biomaterial properties that result in specific types of immune responses.

METHODS:

We employ three distinct human cell systems to model biomaterial-immune interactions *in vitro*: 1) a reductionist model based on pre-differentiated macrophages derived from the human THP-1 cell line which is complemented by two more complex models of the immune system: 2) primary human peripheral blood mononuclear cells (PBMCs) and 3) human whole blood. Upon contact with biomaterials with different physicochemical characteristics and topography, immune reactions are assessed in all three *in vitro* models by quantifying a spectrum of immune signalling proteins (>30 cytokines) using Luminex technology. This is correlated with cytoskeletal re-arrangements monitored by high content imaging (macrophage cell lines) and analysis of immune cell surface markers with flow cytometry (T/B/NK/myeloid cells) (primary PBMC model).

RESULTS AND DISCUSSION:

We have found that diverse immune responses are elicited in model systems by biomaterials with distinct properties (for example hydrophobic vs hydrophilic, anionic vs cationic). Current studies involve the systematic modification of biomaterial properties such as roughness / surface topography in order to develop an understanding of how subtle modification of a material's properties can affect immune response.

CONCLUSION:

Interactions between biomaterial and the human immune system can feasibly be modelled and monitored *in vitro*. Our results show that distinct biomaterial properties results in diverse responses by the immune system.

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

354 Molecular characterization of genes involved in cell-to-cell communication during the chondrogenic differentiation of a human mesenchymal stromal cell line

Cristina Rodríguez-Pereira[1], Mercedes Fernández-Moreno[2], Carolina Guiance-Varela[1], Cristina Ruiz-Romero[1], Francisco J. Blanco[1], Joana Magalhaes[1],
[1]INIBIC, A coruña, Spain
[2]CIBER, Spain

INTRODUCTION:

Cartilage has limited repair capacity and actual clinical treatments for osteoarthritis (OA) are insufficient. Cell therapy using mesenchymal stromal cells (MSC) is one of the most promising approaches being currently explored for cartilage regeneration[1]. The first steps of chondrogenesis involve cell aggregation and condensation and a large number of cell-to-cell communications occur[2]. This study aims to study the genes involved in cell-to-cell communication processes during the chondrogenic differentiation using a human MSC immortalized cell line.

METHODS:

3a6 hMSC cell line was cultured under conventional pellet culture with basal medium or commercial chondrogenic medium supplemented with 10 ng/ml TGF-β3 3 for 3, 9 and 21 days. Total RNA was isolated and real-time quantitative PCR was performed using custom-made primers: connexin -43 (Cx43), -45 (Cx45), -40 (Cx40), -46 (Cx46) and -32 (Cx32); integrin subunit alpha 5 (ITGA5); collagen type-II (col2a1), -X (col10a1) and -I (col1a1), SRY (Sex Determining Region Y)-Box9 (Sox9), aggrecan (acanb) and RPL13a as HKG. Immunofluorescence labelling of Col-II and Cx43 as primary antibodies was undertaken and the positive area was measured using ImageJ. The experiment was performed in triplicate and significant differences ($p<0.05$) were judged using Student t-test.

RESULTS AND DISCUSSION:

3a6 hMSC cell line expresses a panel of connexins (Fig.1A) previously described to be found for MSCs (Cx40 and Cx43) or chondrocytes (Cx32, Cx43, Cx45 and Cx46). Connexins' expression in chondrogenic-differentiated pellets was found progressively increased with time, except for Cx45, which had a peak at 9 days. We observed an up-regulation in ITGA5 in chondrogenic-differentiated pellets compared to basal condition. For chondrogenic related genes, Sox9 expression was significantly up-regulated over time for the chondrogenic-differentiated pellets, although acanb was not detected. Furthermore, col2a1 was not expressed whilst col1a1 and col10a1 were highly expressed.

Even though Col-II immunofluorescence positivity was detected as early as 3 days, the area of positivity decreased with culture time (Fig.1B). A similar result was observed for the area of positive cells for Cx43.

CONCLUSION:

The sequential expression of connexins found suggests the implication of these genes in cell-to-cell communication events during 3a6 chondrogenic differentiation. In addition, an up-regulation of ITGA5 upon chondrogenic stimulation suggests an important early migration and cell guidance during early chondrogenic differentiation.

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ACKNOWLEDGMENTS:

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Picture 1:

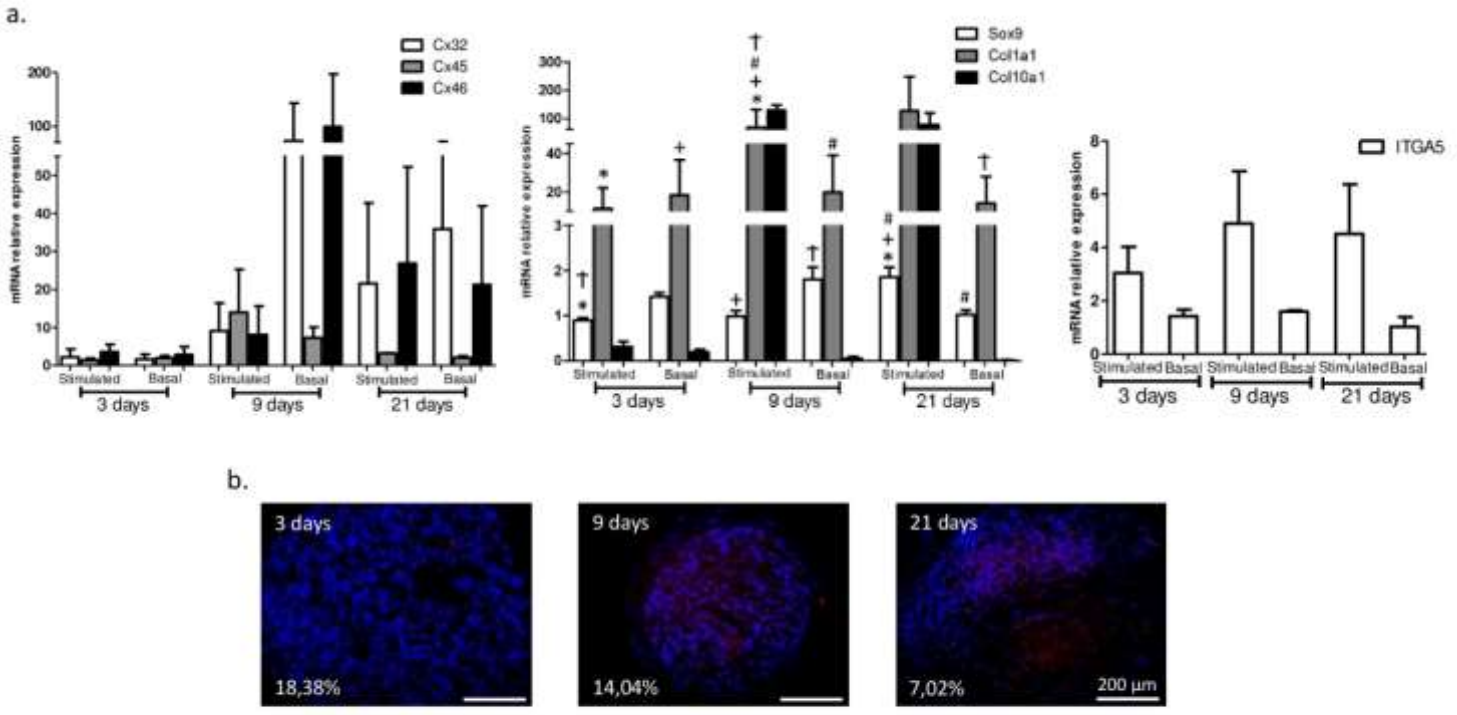

Figure 1. (a) 3a6 mRNA expression during chondrogenic differentiation or basal condition, after 3, 9 and 21 days culture. * † + # p<0.05. **(b)** Immunofluorescence of col-II (red) in chondrogenic-differentiated pellets; DAPI was used as counterstaining of the nucleus. Percentage of positivity (%).

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

355 Optical biomarkers for the early diagnosis of osteoarthritis

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[2]CIBER, Spain
[3]Universidad de Vigo, Spain

INTRODUCTION:

Osteoarthritis (OA) is a rheumatic disease characterized by articular cartilage degradation[1]. On its early stages, OA is asymptomatic and its current gold-standard diagnosis[2] (X-Rays), focused on changes on the adjacent tissue – bone – presents a limitation to the early diagnosis of this disease. Raman spectroscopy (RS) has been recently described as a non-invasive tool to detect molecular changes in biological tissues, producing a unique fingerprint. For this reason, its application for the diagnosis of different diseases offers high potential. Beyond its clinical application, RS offers value as a molecular quantification technique for tissue engineered cartilage characterization. The aim of this work was to evaluate the potential of RS for the early diagnosis of OA.

METHODS:

Human hip cartilage explants (n=14), from healthy (H) and OA donors, with Kellgren-Lawrence (K-L) radiological grades from 0 to IV, were obtained after informed consent. Raman analysis was performed on fresh tissue, using a Bruker RFS100 Spectrometer with a Nd:YaG laser (ʎ=1064). Main peaks were assigned according to literature[3], following their area's measurement after a normalization process. One-way ANOVA statistical analysis was performed and differences considered significant for $p<0.05$. We further analyzed correlations (Pearson's coefficient) between peaks and K-L grade.

RESULTS AND DISCUSSION:

RS cartilage spectra (Fig.1A) revealed the following assignments: 1245-1270cm^{-1} amide III doublet (random coil and α-helix collagen), 1063cm^{-1} (sulfated glycosaminoglycans, GAGs), 1377cm^{-1} (proteoglycans, PG), 1450cm^{-1} (nonspecific signal of lipids and proteins) and 1668cm^{-1} (carbonyl group in proteins). For higher K-L grades, a peak appeared at 960cm^{-1} (apatite phosphate), related to tissue mineralization. After quantitative analysis (Fig.1B) we observed the main molecular changes: GAGs and PG peaks showed a significant decrease with OA severity ($p<0.01$), supported by high correlation coefficients ($R^2=0.7361$ and $R^2=0.7999$, respectively), related to GAGs' degradation; an increase in 1245/1270 ratio (defective/functional collagen) could reveal collagen arrangement loss, although there was a low correlation vs K-L ($R^2=0.3764$); an indirect lipid index (IL), calculated as A1450/A1668, showed an increase of lipids in OA tissues.

CONCLUSION:

RS analysis revealed a hip cartilage molecular fingerprint. Variations found between H and OA tissue are representative of the molecular changes during OA progression. A set of parameters is suggested as an optical biomarker panel: defective/functional collagen (1245-1270cm^{-1}), GAGs (1063cm^{-1}), PG (1377cm^{-1}) and IL (1450/1668).

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Picture 1:

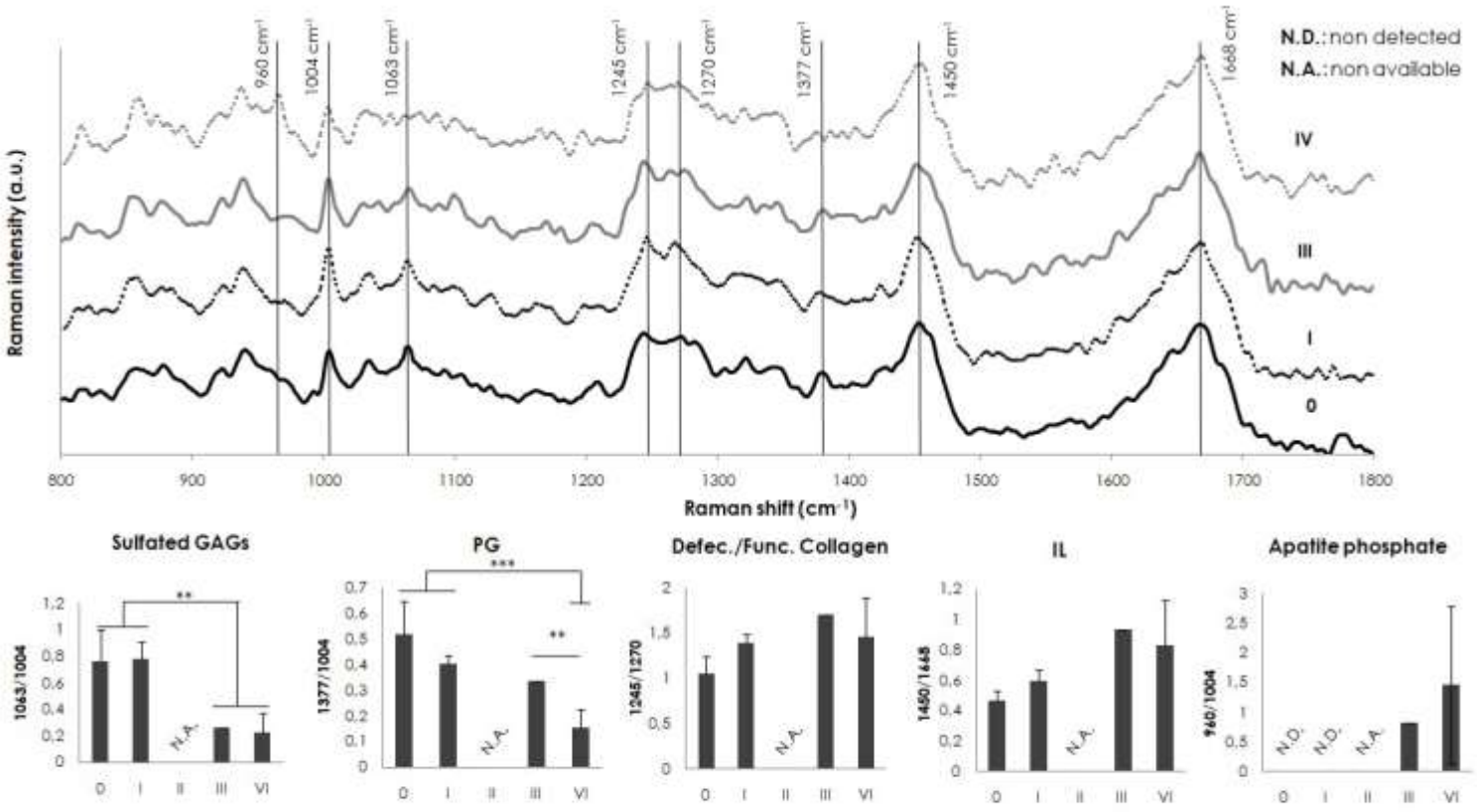

Fig.1. A. Raman spectra for H and OA cartilage (K-L: 0 to IV) and assigned peaks.**B.** Quantitative analysis of the main components found: glycosaminoglycans (GAGs), proteoglycans (PG), Defective/Functional Collagen, lipid index (IL) and phosphate apatite. Significant differences are represented as **p<0.01 or ***p<0.001.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

360 Environmental friendly solvents for the fabrication of poly(glycerol sebacate)/poly(e-caprolactone) electrospun fibers for cardiac tissue engineering

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[3]West Pomeranian University of Technology, Polymer Institute, Poland

INTRODUCTION:

The electrospinning is a method to obtain fibrous scaffolds resembling the morphology of the native extra cellular matrix[1,2]. In this study, blends of poly(glycerol sebacate) (PGS) and poly(ε-caprolactone) (PCL) were developed using benign (environmental-friendly) solvents. These blends were already studied extensively and showed promising results for many applications in soft tissue engineering, such as cardiac[3] and corneal[4] tissue engineering. However, the use of such environmental friendly solvents requires the optimization of blend ratio, solution properties and fabrication parameters.

METHODS:

PGS prepolymer and mildly crosslinked PGS (at 120°C and 2.5·10-2 Torr for 24 hours) were conventionally synthesized according to the protocol of Wang et al.[5]. For the electrospinning, blends of different ratios and concentrations of PGS to PCL (80kDa, Sigma Aldrich) in concentrated acetic acid (VWR) were obtained. Electrospinning parameters were optimized to an applied voltage of 15kV, a flow rate of 0.3-1.1mL/h and a tip-target distance of 11cm. Neat PCL fibers and films (bulk) were fabricated and used as a control for all the characterizations. The fiber mats and films were investigated regarding their morphology (SEM), chemical (ATR-FTIR) and mechanical properties. Furthermore, their degradation behaviour in Phosphate Buffered Saline was analysed.

RESULTS AND DISCUSSION:

Homogenous defect-free fibrous morphology was obtained for neat PCL. Fibers were not obtained for all the investigated ratios, beads and fused structures were observed during the optimization process, as reported in Figure 1. The fiber average diameters of the different blends were in a range between 1-2µm, whereby the addition of PGS could be correlated to an increasing fiber diameter. Chemical characterization confirmed the presence of both polymers in the blends (fibers and bulk). The degradation study revealed the beneficial effect of mildly crosslinked PGS regarding the release of acidic degradation products of PGS and degradation rate.

CONCLUSION:

After the optimization of various parameters, homogeneous PGS-PCL fiber mats using benign solvents have been obtained by electrospinning. The reported results showed suitable properties for further investigations of those materials in cardiac tissue engineering applications.

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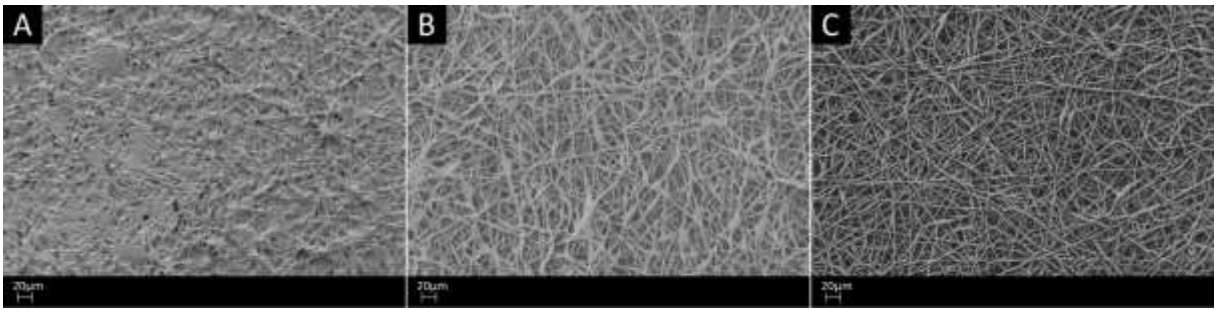
ACKNOWLEDGMENTS:

The authors would like to thank the DAAD Programme “Projektbezogenen Personenaustauschs with Poland (DAAD-PPP)” (Grant no: 57325706) and the Ministry for Science and Higher Education, Poland (Grant no: 002/2017/2018) for providing financial support to this project.



Picture 1: Caption 1: Figure 1 SEM micrographs of PGS-PCL fused structure (A), PGS-PCL beaded fibers (B), optimized PGS-PCL fibers (C).

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

364 Crosslinked extracellular matrix hydrogels developed for neural tissue repair

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INTRODUCTION:

Extracellular matrix (ECM) scaffolds prepared from decellularization of different tissue have been demonstrated to have important effects upon cell adhesion and proliferation. The ECM scaffolds reserve the composition, mechanical integrity, and biological activity of the native ECM suggesting that the molecular composition of these materials is an active factor in remodelling events. We previously develop ECM hydrogel derived from human umbilical cord tissue (UC-ECM) as natural injectable material suitable for neural tissue repair. However, fast in vivo hydrogel degradation and pure mechanical properties are limiting factors for neural tissue reconstruction.

The aim of this study is to stabilize UC-ECM by crosslinking using non-toxic concentration of genipin and N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC).

METHODS:

ECM hydrogel was prepared by the decellularization of human umbilical cord and crosslinked by genipin or EDC in concertation from 0.1mM to 10mM. Both gels were characterized in terms of their crosslinking degree, rhinometry, and enzymatic degradation caused by long-term collagenase presence. The biocompatibility of crosslinked ECM hydrogels was evaluated via viability and proliferation of human mesenchymal stem cells (MSCs). The gel contraction in 3D MSC culture was measured as well. The neurotrophic properties of crosslinked ECM hydrogels were assessed using dorsal root ganglion (DRG) dissociated culture.

RESULTS AND DISCUSSION:

Both crosslinkers improves gel endurance to enzymatic degradation and gel volume shrinkage in 3D MSC culture. Genipin but not EDC increased storage modulus at the concentration 1mM. Crosslinking in a concentration of 1mM did not show any changes in MSC proliferation as well as axonal sprouting of DRG neurons, when compared to uncrossliked ECM hydrogel.

CONCLUSION:

We optimized crosslinking of ECM hydrogel by genipin or EDC as biocompatible material for neural tissue repair and demonstrated significant improvement in gel degradation and volume contraction.

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ACKNOWLEDGMENTS:

This project was supported by MEYS of the Czech Republic LO1309.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

367 Presentation of extracellular matrix peptides at biomaterials via different supramolecular building blocks

Ronald van Gaal, Gitta Buskermolen, Bastiaan Ippel, Sabrina Zaccaria, Peter-Paul Fransen, Carlijn Bouten, Patricia Dankers
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INTRODUCTION:

Biomaterials draw inspiration from the extracellular matrix (ECM) to achieve function for their application. It is proposed that a synthetic ECM can be achieved by introducing ECM mimicking peptides at the biomaterial surface.[1] Supramolecular biomaterials based on hydrogen bonding ureido-pyrimidinone (UPy) or bis-urea (BU) functionalities are eminently suitable for this. The supramolecular nature of the biomaterials allows for a modular approach in which compounds, such as bioactive peptides, with the same supramolecular motif can be mixed and matched with various base materials resulting in biomaterials with various chemical properties.[2,3] Both the UPy and the BU system are based on hydrogen bonding interactions and form nano-fibers. However it remains unknown, if the two systems have different peptide presenting properties at the surface, and if this influences cell behavior.

METHODS:

Supramolecular polymers based on UPy or BU modified polycaprolactone (PCLdiUPy and PCL-BU) were cast into films with 0, 1 and 4mol% of UPy or BU modified cyclic- and linear-RGD peptides. Peptide presentation was assessed with WCA and AFM measurements. Moreover functional presentation was determined by focal adhesion size and number, and cell migration quantification.

RESULTS AND DISCUSSION:

Our investigation showed aggregates on peptide functionalized surfaces (Fig.1a). Interestingly, aggregates were globular on UPy-based materials, while fibrous aggregates were observed in BU-based materials. Additionally, more surface coverage by aggregates in the BU-system. In line herewith the PCL-BU hydrophobicity decreased more after peptide functionalization than for PCLdiUPy.

Focal adhesions adopted a smaller morphology and increased in number with increasing incorporated cRGD amounts in the corresponding UPy and BU biomaterials (Fig.1b), similarly found for other materials.[4] Focal adhesion behavior was more pronounced on functionalized BU-biomaterials than the corresponding UPy-materials. In addition, cell migration was reduced on (c)RGD functionalized materials compared to both pristine materials. However, the BU-based system showed a greater reduction in velocity than in the UPy system.

CONCLUSION:

The UPy- and BU-system distinctively presented peptides, in respectively globular or fibular aggregations. The surface morphology, with the hydrophobicity, suggested that the peptide is presented more at the BU-system surfaces. Moreover, focal adhesions increased in number and decreased in size, and cell migration reduced more promptly in the BU-system, further strengthening the suggestion of more functional peptides at the surface. Overall this research helped to understand the difference between supramolecular systems, and considerations required when choosing one of the systems.

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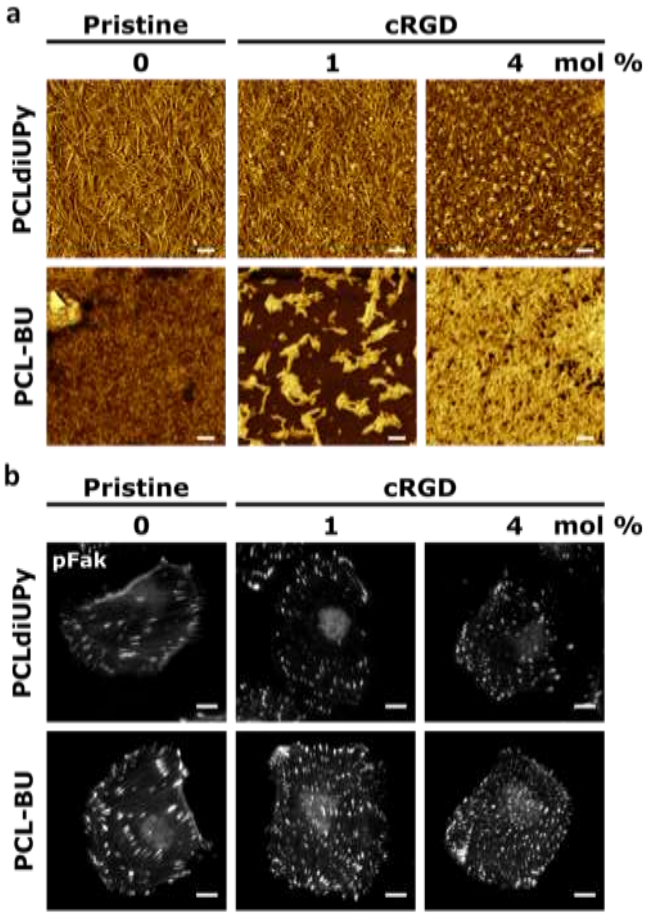

Figure 1: Surface morphology and cell response to increased amount of cRGD functionalization. a) AFM phase images of PCL-BU and PCLdiUPy with increasing amounts of mol% cRGD, scale bar = 100 nm b) Focal adhesion kinase staining of HK-2 cells cultured 24 h on the materials, scale bar= 15 µm.

Picture 1:

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Poster presentation

373 Functionalization of alginate-based hydrogels with ketoprofen reduces fibrosis in transplantation of insulin producing cells

François Noverraz[1], Luca Szabó[1], Elisa Montanari[2], Daniel Ortiz[1], Léo H. Bühler[2], Sandrine Gerber-Lemaire[1]
[1]Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland
[2]University Hospital of Geneva, Switzerland

INTRODUCTION:

Cell microencapsulation in hydrogels is used to develop cell therapy products. For this purpose hybrid alginate-based hydrogels [1-3] have been developed and exhibit excellent properties such as biocompatibility, permselectivity and good mechanical resistance. Still, transplantation of cells without immunosuppressive treatment have to face immune response as inflammation and fibrosis which leads to necrosis of the encapsulated cells and loss of

functionality of the graft. The present study intends to reduce pericapsular fibrotic overgrowth in living organisms by tuning the composition of the polymeric components of the hydrogels with anti-inflammatory agents.

METHODS:

The strategy relies on the conjugation of anti-inflammatory agents (ketoprofen) on the backbone of alginate to obtain microspheres with active components that are released in a controlled manner. Ketoprofen was first conjugated to polyethylene glycol (PEG) through ester or amide bond and grafted on the hydroxyl of alginate using CDI activation. Empty microspheres and microspheres containing MIN6 cells where formed with these alginate derivatives. The release of ketoprofen was measured by withdrawing samples from the supernatant at regular intervals (one week). The amount of ketoprofen in these samples was quantified by LC-MS. Microspheres were transplanted in mice and retrieved after 30 days. Analysis of fibrotic tissues around the microcapsules was performed to evaluate the anti-fibrotic effect ketoprofen conjugated to alginate-based hydrogels.

RESULTS AND DISCUSSION:

Quantification of cumulative release of ketoprofen from MIN6 containing capsules indicated continuous delivery of the active ingredient over one week, with faster kinetics in the case of ester linkage. Following transplantation in mice and retrieval of empty and MIN6 containing microcapsules after 30 days, pericapsular fibrotic overgrowth was significantly reduced in the case of ester linkage, as compared to pure Ca-alginate microcapsules.

CONCLUSION:

The release of ketoprofen from the surface of functionalized alginate-derived hydrogels was monitored under different conditions. The release kinetics was dependent on the nature of the covalent bonds used to graft ketoprofen on the PEG. Quantification of fibrotic tissues surrounding the microcapsules demonstrated that the hydrogel containing ketoprofen had a beneficial effect on the inflammation and could improve cell therapy.

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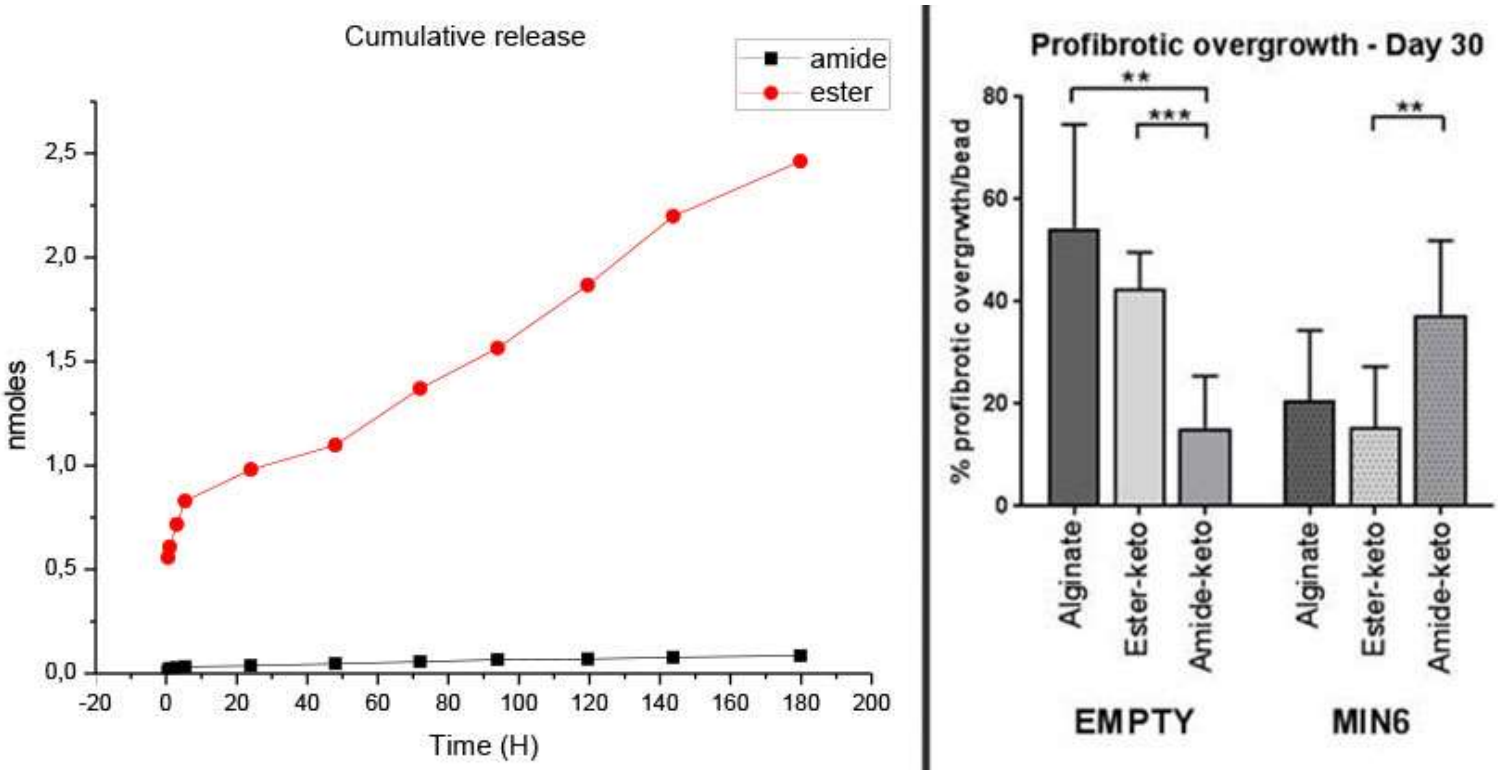
ACKNOWLEDGMENTS:

We thank the Swiss National Science Foundation (grant N° CR23I2_152974 and 310030E-164250) and the Foundation Insuleman for financial support. We are grateful to Dr Christine Wandrey for helpful scientific discussions.



Picture 1: Caption 1: Left: Cumulative release of ketoprofen. Right: profibrotic overgrowth around the microspheres after 30 days transplanted in mice.

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Poster presentation

375 Development of a 3D in vitro model of vascularised breast tumour using self-assembling peptide hydrogels

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[2]School of Materials, University of Manchester, United Kingdom

INTRODUCTION:

The tumour microenvironment (TME) is a diverse interplay between cancer cells, vasculature, extracellular matrix (ECM) and other cell types which provides external barriers to chemotherapeutic drugs [1]. *In vitro* models are vital in pre-clinical drug development, providing preliminary data indicating if a compound is efficacious. The majority of *in vitro* models are 2D, which are restricted by limited cell-cell and cell-ECM interactions, resulting in overestimation of the efficacy of drugs tested [2]. 3D *in vitro* models can bridge the gap between 2D and animal models, providing realistic responses to drugs by mimicking features of *in vivo* tumours. One group of promising biomaterials are self-assembling peptide hydrogels (SAPH) using ionic-complementary peptides. These are popular due to their chemical definition, ability to spontaneously self-assemble into 3D fibrillar scaffolds, biocompatibility and tuneable mechanical properties, thus mimicking many biological tissues [3]. This study aims to mimic the TME *in vitro* using SAPH as a 3D model for drug screening.

METHODS:

MCF-7 breast adenocarcinoma cells were encapsulated within SAPH up to 14 days. Cell viability was assessed using LIVE/DEAD and Ki67 and hypoxia staining were used to observe cell proliferation and oxygen depletion. Human umbilical vein endothelial cells (HUVECs) and mesenchymal stem cells (MSCs) were cultured in 2D to assess tubule formation capacity. Oscillatory rheometry was used to measure the stiffness of SAPH using frequency sweeps between 0.1 – 10 Hz at a strain of 0.2%.

RESULTS AND DISCUSSION:

Viability and phenotype of MCF-7 cells were maintained in SAPH and proliferation and centralised hypoxia of spheroids were investigated. By altering the stiffness of SAPH and incorporating laminin and fibronectin, proteins naturally found within the ECM, tubule formation by HUVECs and MSCs was assessed compared with collagen I. Tubule length, density, the number of tubules and branch points was thus quantified. The mechanical properties of SAPH were observed to mimic the stiffness of breast tumour (10-30 kPa). SAPH can therefore recapitulate important features of the TME.

CONCLUSION:

This work highlights the potential of SAPH for 3D modelling of cancer and pathogenic angiogenesis.

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ACKNOWLEDGMENTS:

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Poster presentation

380 Prospective biomaterial from Colombian Gracilariopsis tenuifrons as food additive

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INTRODUCTION:

Gracilariopsis tenuifrons, abundant specie in the Caribbean Sea, has not been evaluated from the physicochemical point of view. In addition, polysaccharides are one of the most important components present in the algae with potential applications as additive in food products[1,2]. In this research, were study the chemical characteristic of extracts in order to be use as natural bioactive ingredient for functional foods.

METHODS:

G. tenuifrons was harvested by Fundación Terrazul in June 2017 and was thoroughly washed with distilled water, dried at 45°C, for 6 h. 25 g of dried material was subjected to reflux at 65°C in ethanol-6 h. Then, the solution was filtered and the residue was collected and keep at 4°C. The crude extract was deproteinizated by Sevag-reagent. The enriched-polysaccharide extract was further purified by ultracentrifugation using filters of 3, 10, 50 and 100 kDa MWCO. The separation by molecular weight was evaluated by gel electrophoresis using dextran sulfate, chondroitin-6-sulfate, chondroitin-4-sulfate, heparin and enoxaparin as standards. The content of neutrals, acid and sulfated sugars, humidity, total protein, carbohydrate, ash were determined. Spectroscopy analysis was done by UV, FTIR-ATR and size distribution/zeta potential by DLS. Analysis of variance were performed with one-way ANOVA.

RESULTS AND DISCUSSION:

Sulfated and non-sulfated polysaccharides were identified. FTIR-ATR analysis showed characteristics absorption bands (1640, 1370, 1250, 1220, 1012, 930, 900, 845, 805 y 705 cm^{-1}) for carrageenans and agar, both polysaccharides identified in red seaweeds. The sugar content ranged from 6.4 to 9.3 for neutrals, 5.8 to 53.5 for acid and 16.9 to 31.5 for sulfated. The nutritional value showed 8.7% of relative humidity, 15.58% of total protein and 69.81% of total carbohydrate, comparable to that reported on similar genus*3*. According to this results, red seaweed can be easily utilized for producing functional ingredients or designing new functional foods to support reducing the diet related chronic malfunctions.

CONCLUSION:

Overall observations suggest that *G. tenuifrons* seaweed is a feasible candidate for further study as a potential commercial cultivar for human consumption.

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

384 Assessment of the parameters influencing the fibre characteristics of electrospun P(LA-co-GA) and P(CL-co-GA) membranes

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[1]Centre for Biomaterials and Tissue Engineering, Univ. Politècnica de València, Spain
[2]Centre for Biomaterials and Tissue Engineering, Spain

INTRODUCTION:

Poly(lactic) acid (PLA), poly(ε-caprolactone) (PCL) and poly(glycolic) acid (PGA) are biocompatible and biodegradable polymers approved by the FDA, and they are widely used in tissue engineering.[1] Nevertheless, the

handling of PGA is complex because of its high degree of crystallinity, high melting point and insolubility in common organic solvents.[2] To avoid these problems, PGA is thus usually copolymerized.

In this study, we obtained membranes of these copolymers by means of the electrospinning technique.[3] Electrospun membranes of P(LA-co-GA) have been obtained in the past [4], however, P(CL-co-GA) has begun to be studied recently, so the electrospinning procedure of this copolymer has not been reported yet.

METHODS:

PGA was heat-treated and next scanned by DSC to determine its crystallinity.

Then, P(LA-co-GA) with acid and ester terminations in equal proportions and P(CL-co-GA) were solved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and in chloroform to obtain electrospun membranes, which were observed on a Scanning Electron Microscope to determine the effect that the main electrospinning parameters had on the fibre diameter.

RESULTS AND DISCUSSION:

PGA crystallinity was still above 40% after the heat treatment, which prevents its solution in HFIP. However, P(LA-co-GA) and P(CL-co-GA) were successfully solved in HFIP and in chloroform.

In HFIP, a concentration of 10%w/v (*Figure 1*) resulted in fibres for P(LA-co-GA) not for P(CL-co-GA). By increasing voltage, the beads on P(LA-co-GA) samples were reduced and fibres could be obtained of P(CL-co-GA). These results were consistent with our previous work [3] in that the voltage is a main parameter.

Chloroform allows reducing the concentration of the polymers while keeping an adequate viscosity for electrospinning.

CONCLUSION:

The use of P(LA-co-GA) and P(CL-co-GA), rather than PGA, facilitate solution in HFIP. Moreover, these copolymers are soluble in chloroform, which allows reducing the concentration of the polymers. HFIP is a proper solvent for P(LA-co-GA), but the solution of P(CL-co-GA) in HFIP is less viscous than that in chloroform, which hinders the formation of fibres during the electrospinning process.

By obtaining polymer solutions, it was possible to manufacture electrospun membranes with a sub-micron diameter of fibres.

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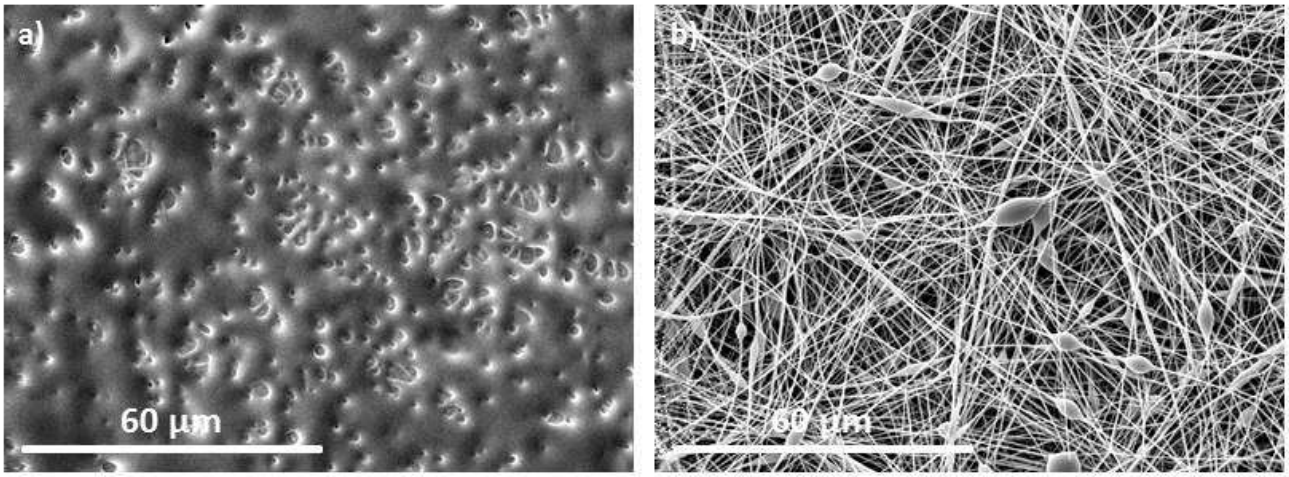

Picture 1: Caption 1: Figure 1. SEM images of a) P(CL-co-GA) and b) P(LA-co-GA) membranes prepared in HFIP.

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Poster presentation

386 Fabrication of polymer-free super-hydrophobic titanium surface

Hao Wu, Weidong Tian, Li Xie
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INTRODUCTION:

Super-hydrophobic surfaces are finding increased use in biomedical arena and titanium(Ti) is also widely used as biomaterial. The combination of them is a promising way for better application, but few literatures were focused on this field. Furthermore, super-hydrophobic Ti surface were often fabricated by introducing external polymers. Up to now, in the field of biomaterials, we haven't found literatures where polymer-free super-hydrophobic Ti surface could be achieved. Herein, we aimed to fabricate a polymer-free super-hydrophobic Ti surface, which can be promising for further application.

METHODS:

The preparations of pure titanium (PT) samples and anodized (A) samples were consistent to the literature we had published before [1]. Then A samples were immersed in 30ml 10%H_2O_2 for 2h in room temperature with a gentle shake every 0.5h. Finally, samples were rinsed with ultrapure water for 3 times, dried in an oven at 60 °C for 3 h and stored in dark for 2 weeks. The as-prepared samples were named as AH.

RESULTS AND DISCUSSION:

Structures of craters were formed after anodizing, which was ascribed to gradual field-assisted dissolution by electrolyte [2]. H_2O_2 treatment could brought slight corrosion to A surface. Anodization increased roughness values of Ti surface greatly, while H_2O_2 treatment couldn't change them. The XRD patterns indicated all treatments didn't change samples' crystalline structure. After anodizing, contents of C-O and TiO_2 increased, while C-H and OH decreased and the peak of H_2O could be found on A surface. After H_2O_2 treatment and aging, C-H occupied the most of C1s spectrum and no H_2O could be found, while OH content also decreased. pT, A and AH samples had their CAs of 46.1±8.0°, 25.1±7.0°, and 151.9±2.4°, respectively. After H_2O_2 treatment, the great change of wettability should be ascribed to chemical changes. Haitao Liu et al. found absorption of airborne hydrocarbon would make surface more hydrophobic [3]. Therefore, the super-hydrophobic surface was resulted from airborne hydrocarbon, water loss and decrease of OH group, as well as crater-like structures.

CONCLUSION:

A novel method was developed to fabricate polymer-free super-hydrophobic Ti surface, which may be promising for further application.

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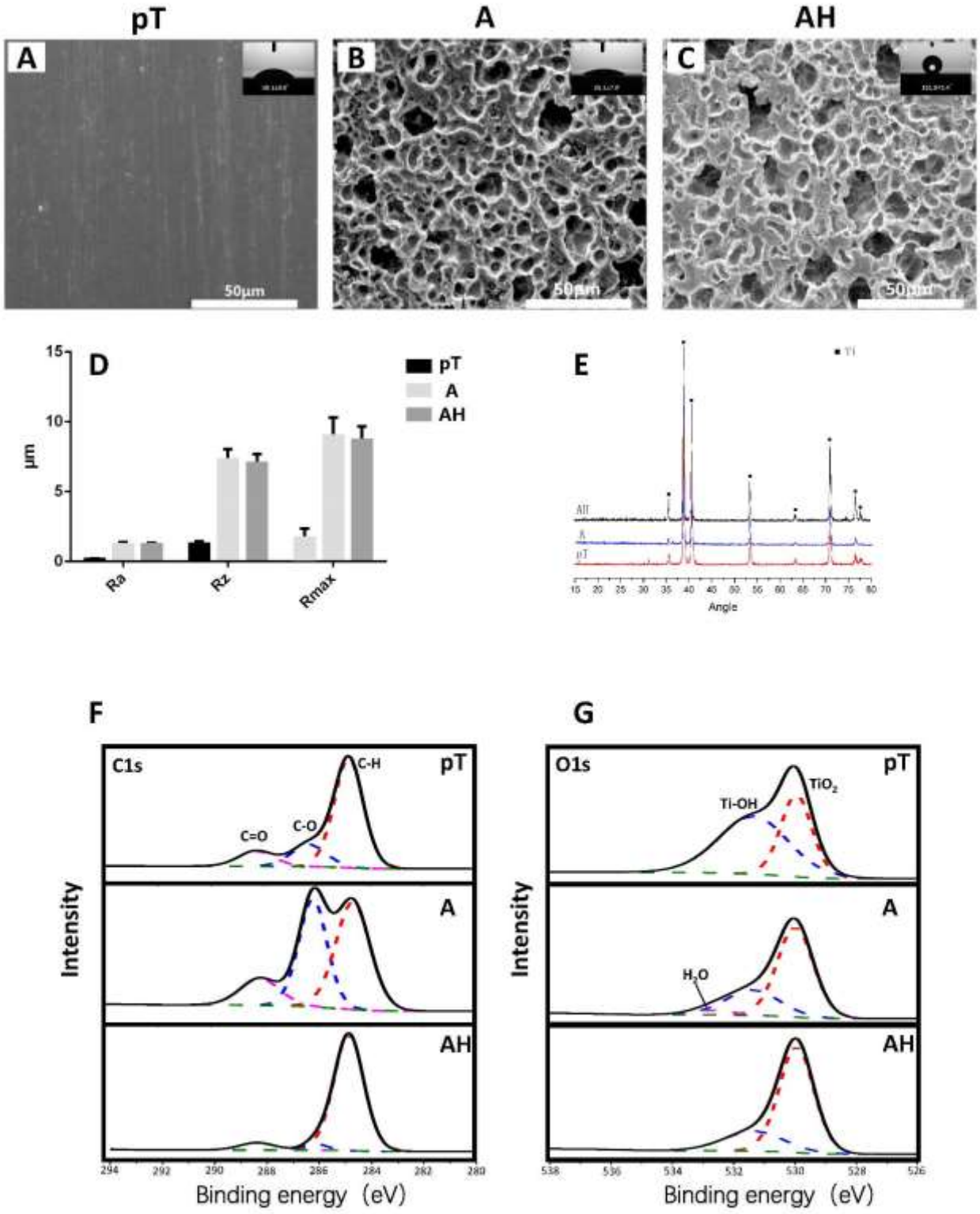

Picture 1: Caption 1: SEM images, wettability, roughness, XRD spectra, C1s and O1s narrow spectra of all samples

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

393 multi-functional microspheres with controllable microstructures fabricated by microfluidics for drug delivery applications

Jun Wu[1], Weichen Qi[2], Feihong Liu[1]
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INTRODUCTION:

Biodegradable polymeric drug delivery devices (DDDs) have been widely applied for improved therapeutic effects, reduced dose frequency and suppressed side effects. However, fragile drugs are difficult to be effectively delivered due to poor loading efficiency and unstable bio-activities. Microfluidic technology provides a method for fabricating DDDs with complex microstructures under mild conditions. In this study, poly(lactic-co-glycolic acid) (PLGA) microspheres with controllable microstructure were fabricated by microfluidic technology to achieve higher loading efficiency and prolonged therapeutic activities of drugs.

METHODS:

Fabrication of PLGA microspheres with controllable microstructure

The injection capillary and the collection capillary were inserted into a square glass tube to assemble the coaxial microfluidic chip. The inlet tubes, outlet tubes and capillaries were fixed on a glass slide using epoxy. PLGA solutions and hexane were used as oil phases, while aqueous alginate solution and aqueous poly (vinyl alcohol) (PVA) solution were used as water phases. Different types of emulsion droplets (oil in water, water in oil in water, oil in water in oil) were generated in microfluidic chip by injecting water phases and oil phases into different tubes. By solidifying droplets, PLGA microspheres, hollow PLGA microspheres and PLGA microspheres coated with alginate were obtained. Rifampicin was loaded in PLGA as a model drug.

Characterization of loading efficiency, drug release properties and long-term antimicrobial activity

Shape and microstructures of microspheres with different microstructures were investigated by microscope. Drug loading efficiencies was evaluated by *in vitro* release test. Long-term antimicrobial effects were evaluated by minimal inhibitory concentration (MIC) test using staphylococcus aureus.

RESULTS AND DISCUSSION:

Microscopic images demonstrated that PLGA microspheres, PLGA hollow microspheres and PLGA microspheres coated with alginate were successfully fabricated (figure 1 a-c). In vitro release test showed that, by microfluidic method, drug loading efficiency can be significantly enhanced. Drug release profiles can be partly modulated by changing microstructures. Interestingly, loaded drugs may gradually lose activity due to harsh environment. In this study, it was proven that the alginate coating could partly protect loaded drug, indicated by higher apparent MIC compared with bare PLGA microspheres (figure 1d).

CONCLUSION:

Microfluidic method is a promising method to fabricate drug delivery devices with controllable microstructures so as to incorporating multi-functions, including modulating drug release properties and prolonging therapeutic activities.

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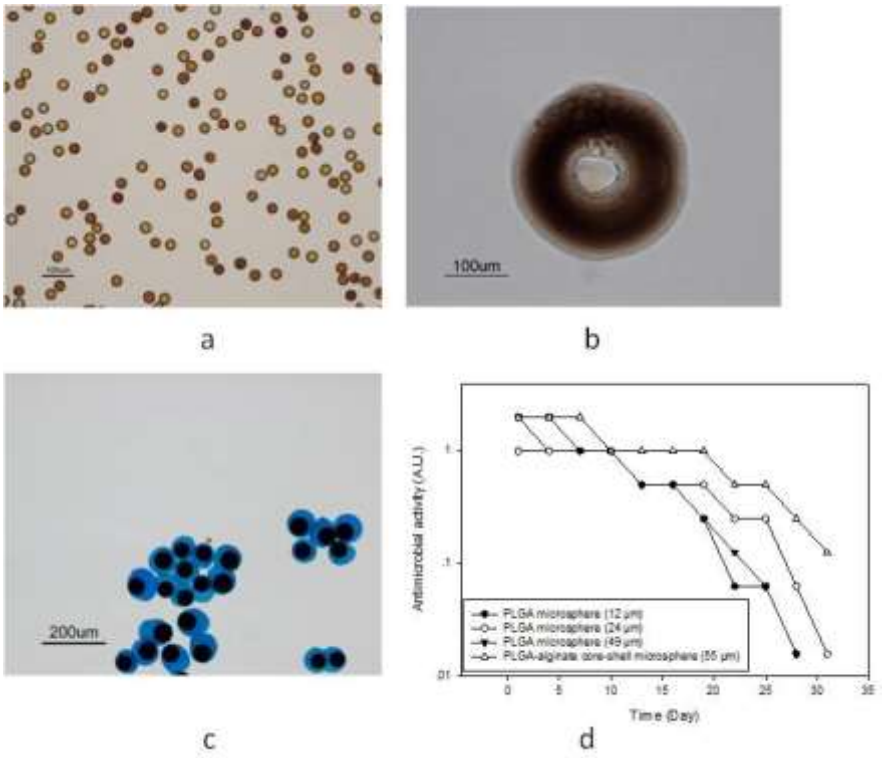

Picture 1: Caption 1: Figure 1. Microscopic images (a-c) and minimal inhibitory tests (d) of PLGA microspheres with different microstructures

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

395 Bioinert and Bioactive Polymer Coatings to Regulate Biological Responses

Chiaki Yoshikawa[1], Helmut Thissen[2]
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[2]CSIRO, Australia

INTRODUCTION:

A biomaterial is often used in direct contact with biological components such as tissue, blood and body fluids. Therefore, it is significantly important to regulate biological responses at interface. In particular, bioinert property is essential for *in vitro* and *in vivo* applications of biomaterials.

Recently, we have demonstrated that concentrated polymer brushes (CPBs)[1] obtainable by surface-initiated living radical polymerizations (SI-LRPs) showed excellent bioinert properties compared with semi-dilute polymer brushes and cast films.[2] The CPBs would be beneficial for medical device applications that require bioinert surfaces, however SI-LRP techniques typically require multi-step, cumbersome processes and have limitations in regard to the choice of device materials and shapes. In order to overcome these problems, we aimed to develop simple and versatile coatings with the bioinert property of CPBs. Meanwhile, we also have developed bioactive coatings using the bioinert coatings as a base.

METHODS:

Polymers (bottle brush and CPB-grafted particles) were prepared by RAFT polymerization and/or atom transfer radical polymerization (ATRP). The polymers were coated on substrates and cross-linked by UV irradiation (< 10sec) or heating. Protein adsorption on the coatings was quantified by quartz crystal microbalance. L929 fibroblast cells were seeded on the coatings.

RESULTS AND DISCUSSION:

We have developed two different types of coatings using silica particle with CPB and bottle brush having CPB structures. Herein we focus on the bottle brush coating. Bottle brushes were prepared by ATRP of poly(ethylene glycol) methyl ether methacrylate using poly(2-(2-bromoisobutyryloxy)ethyl methacrylate) (PBIEM) as a macro-initiator (Figure 1). Coatings of the bottle brush and a cross-linker were prepared on substrates (silicon wafer or polymer sheet) by spin-coating or casting. Subsequently, the coatings were cross-linked. We examined protein adsorption, cell adhesion, and bacterial adhesion on the coatings. The bottle brush having shorter chains which has a CPB structure showed excellent non-biofouling properties compared with that having longer chains (a semi-dilute brush structure). This result indicates that it is essential to control physical structure for non-biofouling properties. Further, we introduced antibodies in these bottle brush coatings. ELISA test confirmed that the coatings containing antibodies selectively captured target proteins.

CONCLUSION:

By optimizing the physical and chemical properties of a new class of polymers, we have been able to achieve coatings that provide bioinert as well as bioactive properties.

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Picture 1:

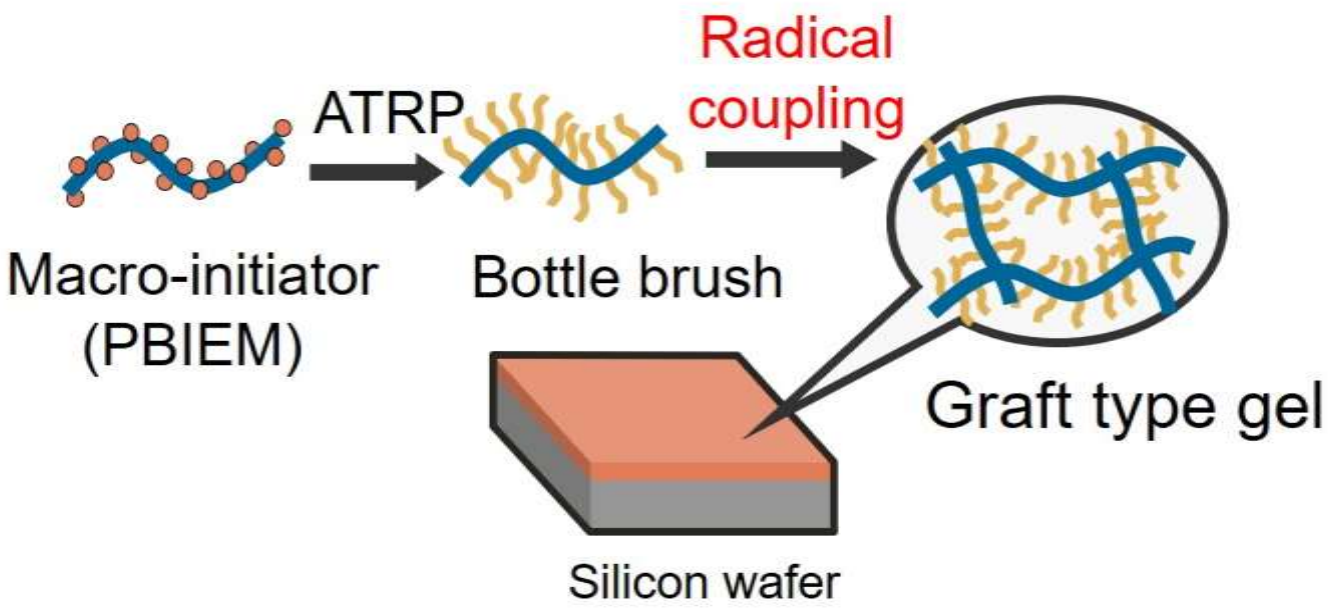

Figure 1. Schematic illustration of bottle brush polymer network.

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Poster presentation

397 Functionalization of bioengineered spider silk to target cancer cells and control doxorubicin delivery

Karolina Penderecka, Tomasz Deptuch, Kamil Kucharczyk, Edyta Jakubowska, Bogna Juskowiak, Anna Florczak, Katarzyna Jastrzebska, Andrzej Mackiewicz, Hanna Dams-Kozlowska

Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland

INTRODUCTION:

Spider silks are biocompatible, biodegradable and self-assemble proteins of potential biomedical application. Bioengineered silks can be used to form a drug delivery vehicles. Moreover, the bioengineered silks can be functionalized through the addition of sequence that encodes peptide responsible for desirable function[1]. Such functionalization can be used to control the binding of silk vehicles to cancer cells or to regulate the drug loading and release. The aim of this study was the development of the drug delivery system that targets Her2-positive cancer cells and controls the loading and release of doxorubicin.

METHODS:

The MaSp1- and MaSp2 based bioengineered silk proteins (MS1 and MS2, respectively) and their hybrid functionalized variants (H2.1MS1 containing the Her2-recognizing peptide and DOXMS2 comprising doxorubicin binding peptide) were designed and produced in *E. coli* expression system. The proteins were purified using thermal denaturation method. The silk nanospheres were formed using high-pressure syringe pumps in the process of salting out the proteins with potassium phosphate. The morphology of particles was observed by using scanning electron microscopy. Nanoparticles were loaded with doxorubicin by the diffusion method. Loading efficiency and release kinetics of the drug was analyzed using spectrophotometry. The toxicity towards Her2+ cancer and control cells of the doxorubicin-loaded spheres was examined by using MTT assay.

RESULTS AND DISCUSSION:

Bioengineered silks MS1, H2.1MS1, MS2, and DOXMS2 were produced, purified and processed into spheres. The loading of doxorubicin into DOXMS2 particles was slightly higher than into MS2 spheres however, the difference was not significant. Doxorubicin release from all silk particles was pH dependent, and in a pH of 7.4, the release from DOX functionalized spheres was significantly the lowest. To develop drug delivery system that targets the cancer cells and controls the drug release, three types of blended nanospheres were formed: H2.1MS1/DOXMS2, MS1/DOXMS2 and H2.1MS1/MS2. The doxorubicin loaded into H2.1MS2/DOXMS2 spheres were the most promising for targeted therapy due to their high cytotoxicity towards cancer cells and the lowest release of doxorubicin in a pH that corresponds to a pH of blood.

CONCLUSION:

The blending of functionalized silks can be a way to control the property of drug carriers. H2.1MS1/DOXMS2 spheres can efficiently deliver the drug to cancer cells and minimalize the drug release in the blood.

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Poster presentation

398 Surface Functionalization of Iron oxide and Gold Nanoparticles for Controlled Drug Release

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INTRODUCTION:

Superparamagnetic iron oxide nanoparticles (SPIONs) and gold nanoparticles (AuNPs) have recently emerged as novel imaging and thermotherapeutic agents[1] in the field of regenerative medicine. Furthermore surface modifications for drug or peptide conjugation enhances their multimodal functionalities. In this presentation, we demonstrate that SPIONs and AuNPs can be functionalized with maleimide-containing linkers to construct nanoparticle-drug or targeting peptide-conjugates for remotely controlled thermoresponsive release system

METHODS:

SPIONs were functionalized with APTES-maleimide through silanol groups on surface as described previously[2] (Fig1). Gold nanoparticles were modified with an analogous disulfide-maleimide linker. To quantify surface modification and thermoclevable release, fluorescent furanyl coumarin derivative was synthesized and attached to

the modified particles with conventional heating (Fig1, attached furanyl coumarin drawn in red).Thermal release was induced by heat dissipation under AMF (784kHz, 30 min) or by conventional heating, and the resulting increase in coumarin concentration was measured by fluorescent spectrophotometer

RESULTS AND DISCUSSION:

Our initial results show that under AMF, SPIONs functionalized with the maleimide-furan linker undergo 17,5% release of the furanyl coumarin probe. Heating with a conventional oil bath (110 °C, 30 min) also triggered the release mechanism, but at only 11%. This demonstrates our concept of controlled drug release through the reversible pericyclic reaction of maleimide and furan, and shows that the efficiency of thermo-cleavage is excellent when using an external magnetic field.

CONCLUSION:

We have shown that SPIONs modified with maleimide linkers can undergo reversible thermochemical conjugation with a furan-containing coumarin probe. Furthermore, the release of this probe is efficient (17,5%) when irradiated with AMF. This demonstrates that our remotely controlled thermoresponsive linkers may be useful in the design and application of nanoparticle-assisted drug delivery systems

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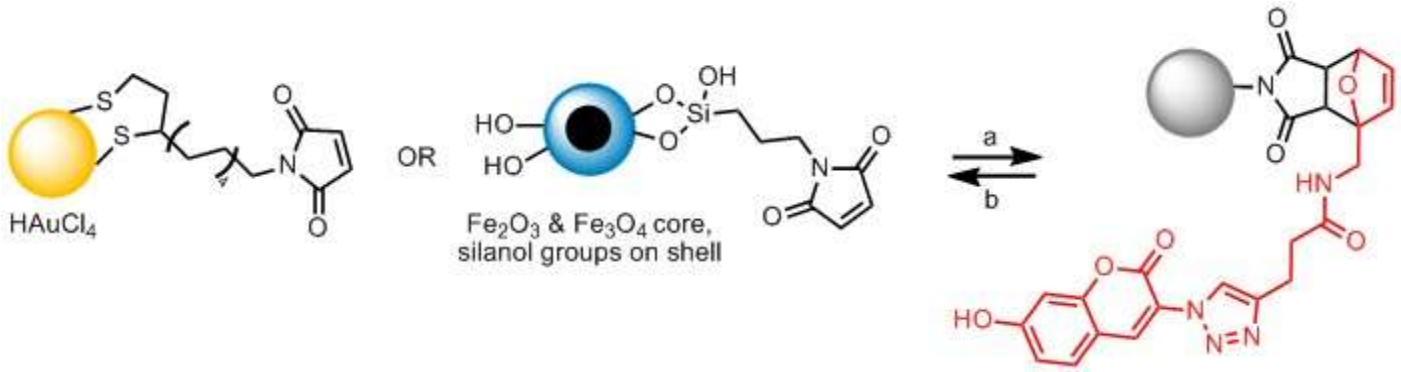
Picture 1:



Caption 1: Reversible thermochemical conjugation of modified SPION and AuNP with a fluorescent coumarin probe. a) Loading assay b) Release assay under AMF or hv

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Poster presentation

413 Mechanisms of internalization of HA-based carriers into skin cells

Jana Šógorková, Martin Cepa, Kristina Nešporová, Daniela Šmejkalová, Gloria Huerta-Angeles, Vladimír Velebný

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INTRODUCTION:

Dermal drug delivery is an interesting approach for the treatment of many diseases. In our previous study we observed the ability of nanocarriers made of hydrophobized hyaluronic acid to enclose active ingredients and

transport them across the skin tissue barrier[1]. It was the aim of this study to characterize processes at the moment when the carrier gets into the contact with skin cells.

METHODS:

The carrier HAC18:1-NB was synthesized as described in Ref. 1. Curcumin (CUR) was incorporated into the carrier by solvent evaporation method. The mechanism of transport into the fibroblasts was characterized by flow cytometry (MASCQuant, Miltenyi Biotec) and confocal microscopy (TCS-SP8-X, Leica). The inhibition of active transport in 4°C or after treatment with NaN_3 was evaluated, subsequently, specific chemical inhibitors (pitstop, rottlerin, methyl-β-cyclodextrin) of different endocytosis pathways were tested. Confocal microscopy and fluorescently labeled lysosomes (LysoTracker®, Thermo Fisher) were used to track the colocalization with the carrier.

RESULTS AND DISCUSSION:

The dual staining enabled us to describe the fate of the carrier and its payload after the contact with cells. The carrier with its content enters the cells by endocytosis, as confirmed by the decrease of fluorescent signal after the cultivation in 4°C (40% decrease for NB and CUR in HAC18:1-NB+CUR carrier compared to control) and also after the treatment with NaN_3 (20-30% decrease). On the other way, free CUR and NB molecules entered the cell passively which indicates that the carrier is the main driver of the active transport. The pitstop and rottlerin, but not the methyl-β-cyclodextrin inhibited the transport of carrier through the clathrin-mediated endocytosis (50% decrease) and micropinocytosis (40% decrease), but not the caveolae-mediated endocytosis, resp. That means that the receptor mediated transport is not the only one involved, but also the non-selective uptake took part. The colocalization of HAC18:1-NB but not CUR with lysosomes showed that the fate of this carrier and curcumin is separated after the uptake (Fig. 1).

CONCLUSION:

These results helped us to better understand the fate of the carrier system which could be used for transport of hydrophobic drugs or cosmetics ingredients into the skin cells.

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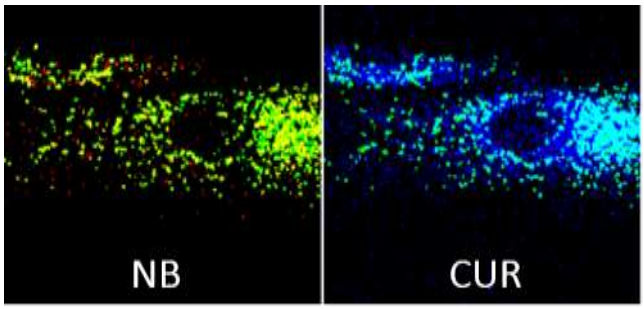

Picture 1: Caption 1: Fig. 1: The colocalization of HAC18:1-NB+CUR with lysosomes in NHDF cells. Lysosomes were stained by lysotracker (green).

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Poster presentation

420 Evaluation of magnetic nanoparticles as pulmonary drug delivery systems

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[2]The University of Sydney, Faculty of Pharmacy, Australia

INTRODUCTION:

Superparamagnetic iron oxide nanoparticles (SPIONs) are good candidates to induce hyperthermia or deliver drugs in cancer treatment[1]. However, it was shown that iron ions stimulate cancer cells proliferation and iron supplementation can increase the risk of further growth of cancer cells[2]. Thus, the aim of this study was to modify Fe_3O_4 nanoparticles to reduce potential release of iron ions. Modified nanoparticles were characterised and cytotoxicity was evaluated using human lung epithelial cells in order to assess their suitability in the treatment of lung cancer for which there are no effective therapies.

METHODS:

Fe_3O_4 nanoparticles (NP) were coated with SiO_2 layer (NP@SiO_2) using a sol-gel method with TEOS as a precursor. Presence of SiO_2 was studied by FTIR. Nanoparticles size and morphology were assessed using nanoparticle tracking analysis (Nanosight), STEM and AFM. Iron release was studied for 24 h in ultrahigh purity water and iron concentration was measured with AAS electrothermic method. Nanoparticles uptake and its influence on cell viability and migration were evaluated in contact with malignant (A549) and non-malignant (BEAS-2B) human lung epithelial cells (Prussian blue staining, AlamarBlue, LDH, real-time impedance measurement – xCelligence system, cell count – CyQuant, real-time phase contrast images – IncuCyte ZOOM).

RESULTS AND DISCUSSION:

Silica layer was successfully deposited on Fe_3O_4 nanoparticles as shown by microscopic studies. FTIR spectrum of NP@SiO_2 showed peaks in the range of 1000-1200 cm^{-1} corresponding to Si-O-Si bonds, which were not present for NP (Fig.1A). Increase in median size of nanoparticles was also observed (NP=20 nm, NP@SiO_2=30 nm) (Fig.1B). Deposition of silica layer on nanoparticles significantly decreased iron release (Fig.1C). Both NP and NP@SiO_2 were efficiently internalised by lung epithelial cells (Fig.1D) and they did not show any influence on viability of malignant lung epithelial cells at 10 µg/ml concentration. In the case of non-malignant lung epithelial cells reduced proliferation was observed, which was correlated with more intensive cell migration as shown by real-time phase contrast analysis by IncuCyte.

CONCLUSION:

NP@SiO_2 are promising materials for pulmonary drug delivery systems as they are stable in aqueous media, do not show significant cytotoxic effects towards lung epithelial cells and further can be easily modified with different drugs or biologically active molecules.

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ACKNOWLEDGMENTS:

This study was supported by National Science Centre, Poland (No2014/14/M/ST5/00649). Authors would like to acknowledge the Bosch Institute at the University of Sydney for support in characterisation.

Picture 1:

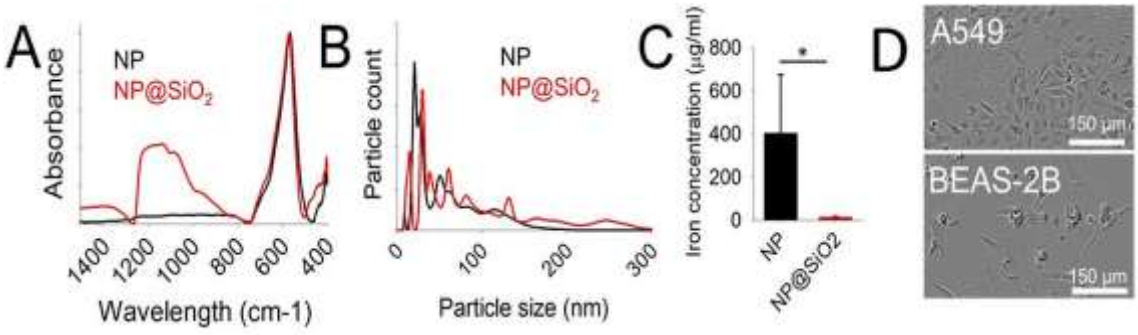

Figure 1. FTIR spectra (A), particle size (B) and iron release (C) from NP and NP@SiO_2. Phase contrast images of cells incubated for 3 days with NP@SiO_2 (D).

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Poster presentation

421 Plasma-modified 3D additive manufactured scaffolds for cartilage/bone interfacial tissue engineering

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INTRODUCTION:

Interface tissue engineering is a rapidly developing field aimed at repairing and regenerating damaged tissue at the interface of different tissue types with the help of artificially fabricated 3D constructs [#_ENREF_1]. Examples of common interfacial tissues found in the human body are cartilage-bone, tendon-bone and ligament-bone [#_ENREF_2]. The regular occurrence of irreversible damage to these interfacial tissues as well as the lack of true integrative solutions makes interface tissue engineering a significant clinical challenge. In the last decade, intensive research has therefore been performed on the development of anisotropic scaffolds to tackle this issue.

RESULTS AND DISCUSSION:

In this study, the possibility of depositing a combination of coatings with a high anisotropic character onto 3D scaffolds is explored. 3D interporous scaffolds were fabricated from 300PEO-PEOT/PBT 55/45, a biodegradable copolymer that is known for its excellent mechanical properties, good biocompatibility and controllable degradation rate [#_ENREF_3]. With the aid of additive manufacturing technology, reproducible scaffolds were obtained and their porosity was determined via scanning electron microscopy (SEM). After fabrication, the scaffolds were coated in a newly-designed dielectric barrier discharge (DBD) plasma system operating at medium pressure (50 kPa) that allowed for the simultaneous deposition of 2 coatings consisting either out of primary amines or carboxylic acid functionalities. The influence of discharge power, gas feed rates and monomer concentrations on the anisotropic character of the deposited coatings as well as their respective stability were analyzed using localized mapping techniques based on X-ray photo-electron spectroscopy (XPS) and infrared spectroscopy (FT-IR). Finally, a series of in-vitro studies was performed using adipose derived mesenchymal stem cells (ADSC) and different fluorescent stains were used to identify differences in cell behavior according to their chemical surroundings.

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Poster presentation

429 Dynamic supramolecular thermoresponsive scaffold with controlled modularity and motion

Dan Jing Wu, Patricia Dankers
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INTRODUCTION:

3D processing techniques have been widely used for the development of biomaterials. However, the objects produced are rather static and inanimate. Therefore, stimuli-responsive materials for 3D processing are explored to induce motion due to external stimuli.[1,2,3] An interesting example is the thermoresponsive polymer, poly(N-isoproylacrylamide) (PNIPAM), that swells or shrinks due to temperature dependent phase separation in water.[4] However, these stimuli-responsive polymers lack modularity in the combination with bioactive components to create a bioactive biomaterial. Therefore, we propose to use supramolecular chemistry to produce a supramolecular thermoresponsive material to control scaffolds in a dynamic way.

#_ednref1 METHODS: Ureido-Pyrimidinone-PNIPAM (UPy-PNIPAM) was synthesized via reaction of 3.3 mmol UPy-hexyl-isocyanate and 1.2 eq. PNIPAM (M

$_n$ 2500 g/mol) in anhydrous tetrahydrofuran in the presence of 3 eq. triethylamine overnight. Ninhydrine staining was used to confirm that the primary amines had reacted. The access of UPy-hexyl-isocyanate was removed using NH

$_2$-resin by stirring for 3 hours at RT. The solution was filtered and precipitated in DCM/hexane and dried overnight.

^{1}H-NMR spectroscopy was performed to confirm that the reaction was completed. The compound was dried overnight, resulting in a white solid (~yield 97%). RESULTS AND DISCUSSION: The synthesis of the UPy-modified PNIPAM was successfully performed. Two approaches were taken to produce versatile scaffolds based on a hydrogel and an elastomer. The UPy-PNIPAM was mixed into other supramolecular UPy-based polymer in a controlled approach to create a modular system. The materials were printed into 3D objects and motion was induced using temperature switches in aquous surrounding. Shape transformation over time was observed and analyzed. CONCLUSION: This supramolecular thermoresponsive material can be used for a wide range of potential biomedical applications. The temperature change can induce efficacy, programmability and speed in transformation of material geometry. Supramolecular UPy-UPy systems provide a modular approach to mix and match different polymers and bioactive compounds in a controlled manner and therefore versatile scaffolds for various applications can be produced. REFERENCES: 1. Wu DJ, Bouten CVC, Dankers PYW:

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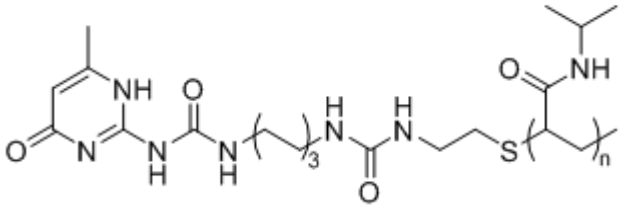

Picture 1: Caption 1: Chemical structure of the synthesized UPy-PNIPAM

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Poster presentation

435 Influence of biomaterial and Mesenchymal Stem Cells delivery on macrophage phenotype in the early inflammatory process after bone defect in rats

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INTRODUCTION:

Large bone loss may be a consequence of tumours or traumas and is still a great challenge to reconstructive surgery. An inflammatory response occurs quickly and plays a crucial role in bone healing, mainly characterized by the recruitment of circulating monocytes into the injured tissue where they differentiate into macrophages, that can be activated into M1 (conventional) and M2 (alternatively activated) populations according to their functions. Mesenchymal Stem Cells (MSC) are multipotent cells widely studied in tissue regeneration, known to modulate inflammatory response and promote tissue recovery. However, the precise mechanisms of this modulation are still unclear. Hydrogels are attractive scaffolds for cell transplantation, and currently exhibit positive interactions with host tissue to promote healing.

The success of the bone tissue engineering strategy depends on the repair micro-environment area; therefore, implantation of biomaterials with or without MSC might modulate the early immune response, and have an impact on bone healing.

METHODS:

In the present study, we assessed the effects of a pullulan/dextran osteoinductive hydrogel with or without MSC on the early inflammatory response (two days) when implanted in a rat femoral defect. We evaluated T cell and macrophage responses, using representative CD68, CD163 and CD11b-c markers in bone marrow to discriminate M1 and M2 populations.

RESULTS AND DISCUSSION:

Inflammatory response in this model is characterized by innate immune response activation since only macrophages had increased. The systemic and local molecular profile is mainly pro-inflammatory and pro-angiogenic. M1-like cells represent the largest population which is consistent with inflammatory circulating cytokines, chemokines and growth factors.

Hydrogel increased the percentage of the whole macrophage population ($CD45^+/CD68^+$ cells) without changing the percentage of the resident macrophage population ($CD45^+/CD68^+/CD163^+$cells). On the other hand, hydrogel did not modify the M1-like population percentage, whereas it increased M2-like population. This result is consistent with cytokine analysis showing that M-CSF and MCP-1 (polarizing macrophages to the pro-healing M2-like phenotype) increased in bone marrow from hydrogel-treated rats.

CONCLUSION:

In our model, on day 2 after the lesion, exogenous MSC did not bring any advantages in comparison to hydrogel; they did not participate to M2 polarization since their addition did not change M-CSF and MCP-1 levels, but they clearly exhibited a paracrine modulating role by lowering some cytokine levels in the contralateral non-injured femur. Moreover, we showed in our previous work that they improved later bone mineralization in comparison to hydrogel alone, suggesting that MSC therapeutic effect is to enhance the bone repair process establishment.

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Poster presentation

449 A preclinical 3D model for testing nerve guidance conduits using high throughput light-sheet microscopy

Mehri Behbehani, Frederik Claeyssens, John Haycock
The University of Sheffield, Sheffield, United Kingdom

INTRODUCTION:

Clinically used nerve guidance conduits (NGCs) fail to promote peripheral nerve regeneration in >3cm nerve gaps in humans. NGC scaffolds are currently smooth tubular constructs, but incorporating intraluminal structures could provide additional guidance to axons and Schwann cells (SCs) to prevent misguidance and enhance nerve regeneration success. In this study, we designed a standardized *in vitro* testing model of NGCs to assess the success of different NGC designs before *in vivo* testing. This model is inexpensive and straightforward to replicate and provides an *ex-vivo* model for nerve regeneration[1]. Herein we explored the effectiveness of using aligned electrospun fibres as intraluminal scaffolds and studied the influence of fibre diameters, packing densities and surface modifications in our model.

METHODS:

Aligned PCL fibres were electrospun to 1, 5, 8, 10 and 13 µm diameters. Poly(ethylene glycol) NGCs were fabricated by microstereolithography, fibres were inserted inside NGCs and evaluated. Samples were surface-modified with air-plasma. Embryonic chick dorsal root ganglia (DRGs) were placed on top of the fibre-filled conduits. Immunocytochemistry was performed and light-sheet microscopy used to visualise and measure neurite and SC outgrowth from the DRG body towards the end of the 6 mm tube.

RESULTS AND DISCUSSION:

All DRGs on air-plasma deposited microfibres showed axon and Schwann cell outgrowth (see attached figure). DRGs on non-deposited samples failed to grow out. The minimum outgrowth over 7 days was 2 mm on surface modified 1-13 µm fibres. Fibres of 10 µm performed best with an average axon and SC outgrowth of 2.8 and 3.2 mm, respectively. A detailed comparison of the model with *in vivo* results is ongoing and results will be summarised in the presentation. This model enabled the direct comparison of different guide designs in a single *ex vivo* setup and would enable a reduction of downstream nerve injury models. The model; based on DRG outgrowth, is more sophisticated than standard cell (co)culture. Additionally, our experimental setup has a highly increased success rate to standard DRG-based models.

CONCLUSION:

10 µm PCL fibres were found to be the best physical guide in NGCs among those tested here and showed the importance of air-plasma treatment when using PCL as a material in nerve regeneration. Furthermore, DRGs demonstrated their effectiveness for evaluating internal NGC scaffolds as they simulated the proximal nerve stump with primary neurons and SCs after nerve injury.

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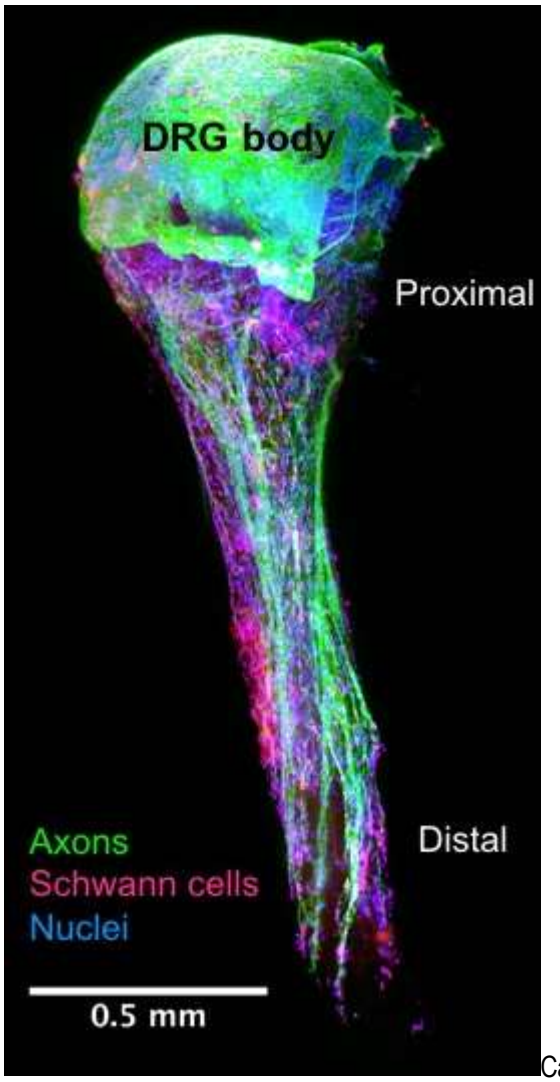
[1] Behbehani et al., Int J Bioprint, 4(1):123, 2018

ACKNOWLEDGMENTS:

We are grateful to the EPSRC (UK) for the funding

Picture 1: 

Caption 1: Axon and SC outgrowth along air-plasma deposited PCL microfibres inside a nerve conduit from the DRG explant (proximal) towards the tube end (distal).

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Poster presentation

450 Impact of standardized platelet-derived growth factor lyophilisates on the mesenchymal differentiation potential of human MSC in vitro

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[1]Rostock University Medical Center, Rostock, Germany
[2]Department of Cell Biology, Rostock University Medical Center, Germany
[3]Biomechanics and Implant technology Research Laboratory, Germany
[4]Department of Sports Medicine, Berlin Charité University Medical Center, Germany
[5]DOT GmbH medical implant solutions, Rostock, Germany

INTRODUCTION:

Platelets (thrombocytes) affect many physiological and pathological processes by the provision of coagulation factors, adhesion molecules, fibrinolytic factors, growth factors and cytokines. The application of platelet-derived

growth factors is currently one of the promising approaches in regenerative therapies in orthopedics, dentistry and many other subjects. The panel of the platelet released biologically active factors is long, among them platelet-derived growth factor (PDGF), transforming growth factor-ß (TGF-ß), vascular endothelial growth factor (VEGF) and insulin-like growth factor-I (IGF-I). Several preparation methods exist to obtain platelet-derived growth factors (e.g. platelet-rich plasma/PRP). However, these methods are limited by their inhomogeneous composition and their elaborative production protocols. In this respect, the need for a standardized preparation of platelet-derived growth factors is obvious.

METHODS:

For the stimulation of human mesenchymal stem/stromal cells (MSC) of two donors, a standardized, lyophilized platelet growth factor preparation was used. The application was by cultivation of MSC up to 5 weeks with a single or a multiple addition of different concentrations of growth factor lyophilisates in non-differentiating, in a specific osteogenic or adipogenic differentiation medium, respectively. These cultures were analyzed for the proliferation and differentiation status at different times. Cell proliferation was analyzed by cell number determination and metabolic activity. The adipogenic differentiation was monitored by quantification of lipid accumulation, the osteogenic differentiation was proven by the analysis of the enzyme activity of alkaline phosphatase (ALP) and the calcium content of the extracellular matrix.

RESULTS AND DISCUSSION:

MSC stimulated with growth factor lyophilisates showed a dose- and time-dependent increase in cell number in the non-differentiating cultures and osteogenic differentiation medium. Especially repeated stimulation with growth factor lyophilisates resulted in enhanced proliferation rates. Additionally, in adipogenic differentiation the growth factor application led to a stagnation of cell number and a reduction of adipogenic differentiation. In osteogenic differentiation MSCs showed an increase of the osteogenic marker ALP.

CONCLUSION:

Thus, an impact on the differentiation status of MSC by the treatment with growth factor lyophilisates was obvious. Hence, our results underline the substantial potential of standardized growth factor lyophilisates to affect proliferation and differentiation of human MSC. Since these issues enable a wide range of possible applications in the field of regenerative medicine further investigation will be necessary.

ACKNOWLEDGMENTS:

This work was financially supported by the European Union and the Federal State Mecklenburg-Vorpommern (EFRE, ESF, ELER, project-No. TBI-V-1-141-VBW-050).

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Poster presentation

453 Biomaterial surface charges and their influence on cell signaling in living osteoblasts

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[1]University Medical Center, Rostock, Germany
[2]Dept. of Cell Biology, University Medical Center Rostock, Germany
[3]Leibniz Institute for Plasma Science and Technology e.V. (INP) Greifswald, Germany
[4]Institute of Physics, Ernst-Moritz-Arndt-University, Greifswald, Germany

INTRODUCTION:

Bone implant surfaces are being continuously improved to achieve faster cellular attachment. Therefore physico-chemical material surface characteristics play an essential role as they are known to affect the cell physiology[1]. It was determined that a positively charged, amino-group containing titanium (Ti) surface strongly increased osteoblast adhesion, cytoskeleton formation (Fig. 1) and spreading[2-4]. However, it is unclear whether a single surface property or a combination results in this influence. To clarify this, we currently investigate the cell physiology and signaling on differently modified Ti surfaces.

METHODS:

We chemically modify silicon-Ti arrays (ZfM, TU Chemnitz) by (i) polyelectrolyte multilayers (terminated with poly(styrenesulfonate), poly(allylamine hydrochloride) and polyethyleneimine) and (ii) amino functionalization via plasma polymerized allylamine (PPAAm). We determine the surface properties by zeta potential and water contact angle measurements. Human MG-63 cells (ATCC) are cultured on the modified Ti substrates to evaluate the surface property-dependent cell responses, e.g. initial cell adhesion processes and the mobilization of intracellular calcium ions (Ca^{2+}).

RESULTS AND DISCUSSION:

Our previous data have shown, especially for the positively charged PPAAm surface, an improved initial cell adhesion and spreading[2-4]. In addition, MG-63 osteoblasts showed after 24 h of cultivation on Ti PPAAm a high increase of intracellular Ca^{2+} ions following an adenosine 50-triphosphate stimulation (mean fluorescence intensity (MFI) peak at 188–246 s, 96±5 mean±s.e.m.) compared to cells on uncoated Ti (MFI peak at 188–246 s, 44±2 mean±s.e.m.) (n=30)[5]. Hence, we assume that the influence of positive surface charges on the cellular behavior dominates other surface properties like wettability.

CONCLUSION:

The understanding of cell behavior at interfaces is crucial for the development of new biomaterials and surface modifications. It is important to extend these studies to investigate which properties are favored by osteoblasts.

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ACKNOWLEDGMENTS:

We are grateful for the financial support of the German Research Foundation (DFG) CRC 1270 ELAINE (for MG, SN) and DFG graduate school welisa 1505/1-2 (for SSt).

Picture 1:

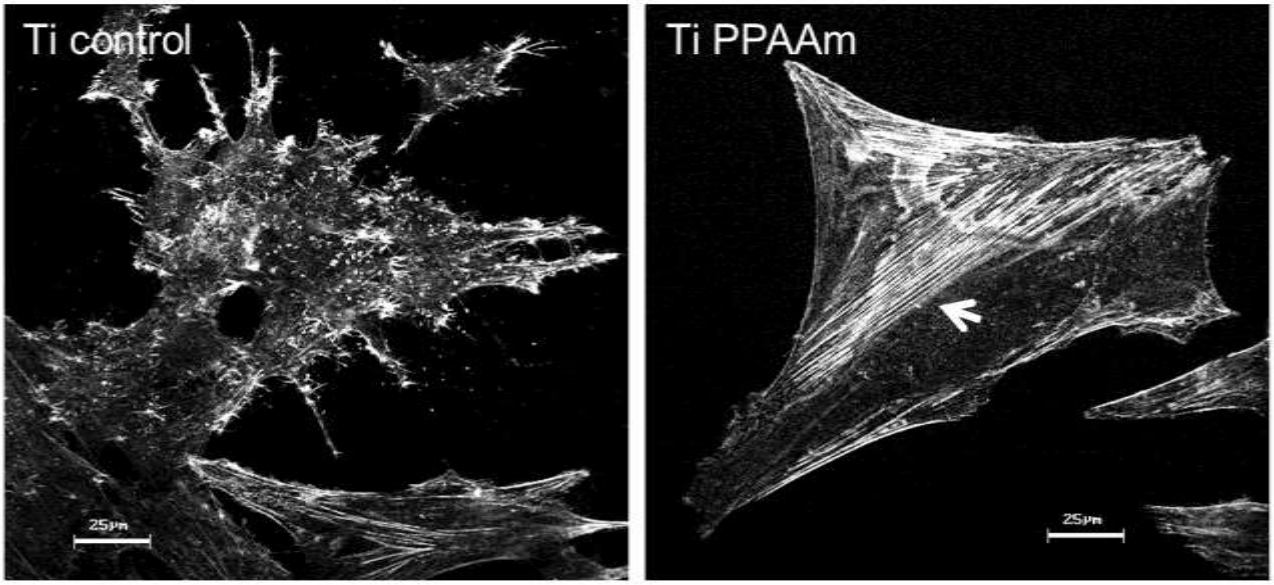

Figure 1: Actin cytoskeleton of MG-63 osteoblasts on polished titanium (Ti). Note that after 21 days of cultivation under extreme conditions – serum free DMEM – cells on Ti PPAAm demonstrate a well spread morphology and express actin stress fibers (arrow) throughout the entire cell. Method: phallacidine BODYPI, confocal microscopy, LSM 410 (Carl Zeiss), scale bars 25 µm.

Caption 1: Actin cytoskeleton of MG-63 osteoblasts on polished titanium

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

454 Multiple ionic substitutions enhancing antibacterial properties of nano-hydroxyapatite

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[2]National Research Council-Institute of Science and Technology for Ceramics, Italy
[3]National Research Council - Institute of Science and Technology for Ceramics, Italy
[4]University of Ferrara - Institute of Microbiology, Italy
[5]Institute of Science and Technology for Ceramics, Italy

INTRODUCTION:

The steady increase of serious bone diseases requires the development of bioactive materials with bone-forming ability. However, the occurrence of infections during surgery can seriously impair the biomaterial effectiveness and the bone healing process[1]. Given this scenario, multifunctional biomaterials associating therapeutic and anti-infective ability are a clear research target today. Ion-doped hydroxyapatites (HA) are considered as elective drivers of bone regeneration, due to their close similarity with the bone mineral. Previous studies reported the antibacterial properties of HA doped with ions such as gallium[2] and zinc. In the present work, multiple ions doping was carried out for the first time by introducing Mg^{2+} and CO_3^{2-} ions into the HA lattice in co-substitution with gallium or zinc.

METHODS:

Single- and multi-doped HA phases were synthesized by a neutralization method. Stoichiometric HA was synthesized as a reference material. To introduce foreign ions in the final product, zinc, gallium and magnesium ions were added to the alkaline suspension, whereas carbonate ions were added to the acidic solution. The new materials were evaluated by physico-chemical and crystal analysis, by cell culture of Human Adipose-Derived Mesenchymal Stem Cells (ASCs) to detect cell viability, cell morphology, gene expression, and by antibacterial tests to evaluate inhibiting effects on a yeast (*Candida Albicans*), gram negative (*Escherichia Coli, Pseudomonas Aeruginosa*) and gram positive bacteria (*Staphylococcus Aureus)*.

RESULTS AND DISCUSSION:

Physico-chemical analyses confirm that multiple ions substitution occurs into hydroxyapatite lattice, consistently altering cell parameters and crystal size. The new materials show composition-dependent ability of stimulating new bone formation and largely inhibiting the proliferation of the infective pathogens. Single substitution with gallium or zinc, and more intensively multiple substitutions, resulted in enhanced both osteogenic and antibacterial ability than the undoped HA. Both effects could be supported by continuous ion release along 14 days, whereas it was shown that antibacterial effect could be also related to new active functional groups related to doping ions and exposed on the apatite surface, thus providing long-term protective effects (Figure 1).

CONCLUSION:

Multi-doped hydroxyapatite nanophases are elective material for bone regeneration, and also present inherent antibacterial ability. The present work demonstrates that the synergistic contribution of ions known for their osteogenic character also enhances antibacterial ability against various bacterial strains and pathogens.

ACKNOWLEDGMENTS:

N/A

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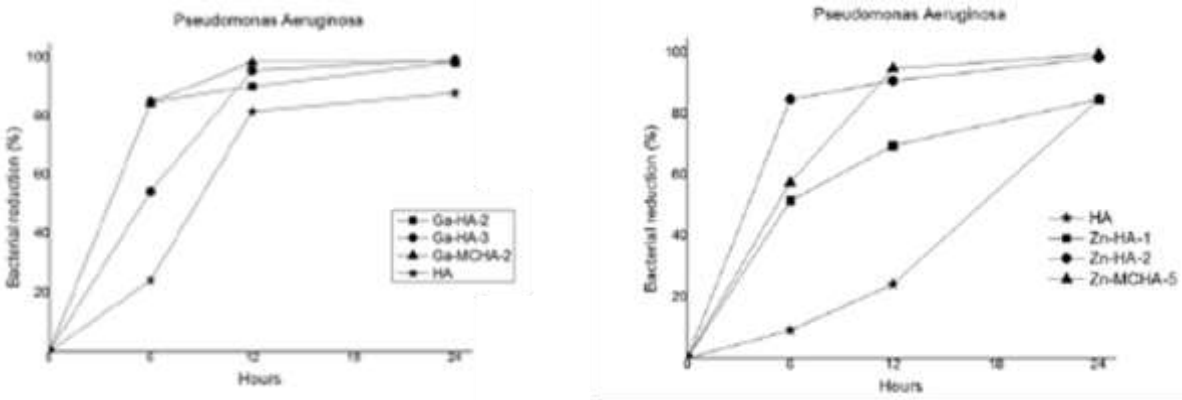

Caption 1: Antibacterial effect of multi-doped apatite containing Ga (left) and Zn (right), expressed by materials pre-conditioned for 14 days.

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Poster presentation

464 Development of cryopreserved cell-laden scaffold using a cell printing system supplemented with low-temperature processing method

Jaeyoon Lee, Wonjin Kim, Jiun Lee, Minseong Kim, Geunhyung Kim
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INTRODUCTION:

One of the specific issues of ideal wound healing scaffold is that it should be prepared within a short period. Because rapid healing could prevent the possibility of infection, scar and irreversible loss of damaged tissue[1]. Premade and preserved scaffold is one of the solution for this issue. In this study, we fabricated a cryopreserved cell-laden scaffold with hollow structure.

METHODS:

We used coaxial nozzle for dispensing cell and cell preservation solution in the core region, and gelatin methacrylate (GelMA) in the shell region for structural support and rapid gelation. To obtain optimum processing conditions, various low temperatures from -5°C to -30°C were applied to the working stage. In addition, the rapid temperature change during processing was a critical parameter affecting initial cell viability. To reduce this effect, we used various cooling temperature before the cell-laden solution was printed onto the low-temperature plate.

RESULTS AND DISCUSSION:

Following two weeks of cryopreservation, the cells (osteoblast-like-cells or human adipose stem cells) in the scaffold showed good viability (over 90%), steady growth, and similar mineralization to that of a control scaffold fabricated using a conventional cell-printing process without cryopreservation.

CONCLUSION:

In this study, an innovative cell printing process supplemented with a microfluidic channel, a core/shell nozzle, and low temperature working stage was proposed to obtain a cell-laden collagen scaffold for cryopreservation. By controlling various processing factors, such as the temperature of microfluidic channel, working plate temperature, flow rates in core and shell region, and nozzle moving speed, a porous cell-laden scaffold (core: cell-laden collagen/DMSO, shell: GelMA) with high cell viability could be successfully fabricated.

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ACKNOWLEDGMENTS:

This study was supported by a grant from the Ministry of Trade, Industry & Energy (MOTIE, Korea) under Industrial Technology Innovation Program (No. 10063541: Development of bioceramic 3D printing materials and low temperature (<40 °C) process customized by implant sites) and was supported by a fund by Research of Korea Centers for Disease Control and Prevention.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

468 Silicone implants enabled with the controlled delivery of triamcinolone for the prevention of fibrosis with minimized drug side effects

Beom Kang Huh[1], Beom Su Jeon[1], Byung Ho Shin[1], Byung Hwi Kim[1], Se-Na Kim[1], Han Bi Ji[1], Seung Ho Lee[1], Kyu Sang Lee[2], Chan Yeong Heo[2], Young Bin Choy[1]

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[2]Seoul National University Bundang Hospital, South-Korea

INTRODUCTION:

Silicone implants have been widely used in clinical settings for augmentation and reconstruction surgery; however, capsular contracture around inserted implants is still one of the most serious complications [1]. Steroidal drug is known to suppress the inflammatory cells, such as mast cell and macrophage, but also various cytokines from those cells, which can regulate the inflammatory responses, hence eventually reducing capsular contracture.

METHODS:

To prepare triancinolone (TA)-coated implants (TA_IM), we cut the shell of the silicone implant with 2 cm in diameter, and sprayed the TA/acetone solution on its surface with 0.005 and 0.01 % w/v TA (TA_IM_1 and TA_IM_2, respectively). For *in vitro* drug release tests, each implant was immersed in PBS (pH 7.4) at 37 °C. At predetermined periods, the aliquot was collected and evaluated by high-performance liquid chromatography. For *in vivo* experiments, the prepared samples were implanted in rats and the tissue samples were biopsied at scheduled times, which were stained to assess the capsule thickness, collagen density, various inflammatory cells and cytokines for evaluation of fibrosis, and also the muscle and skin thickness for evaluation of drug side effects.

RESULTS AND DISCUSSION:

For TA_IM_1 and TA_IM_2, the loading amounts of TA were 2.77 ± 0.13 μg/cm^2 and 5.21 ± 0.25 μg/cm^2, respectively, and both TA_IM_1 and TA_IM_2 released the drug in a sustained manner for 12 weeks (Fig. 1). For the *in vivo* experiment, the drug-coated implants decreased the capsule thickness and collagen density compared with those of the non-coated implant. Because of the effect of TA, inflammation and the expression of pro-inflammatory cytokines were downregulated, thereby decreasing the number of monocytes. This effect in turn decreased the number of macrophages at the later stage of inflammation, leading to the expression of less TGF-β and consequently fewer fibroblasts. Our findings also revealed that with an appropriate dose control, skin and muscle atrophy could be avoided while still effectively reducing fibrosis.

CONCLUSION:

We conclude that the local, sustained release of an appropriate dose of a glucocorticoid drug can be a promising strategy for safely preventing fibrosis around silicone implants.

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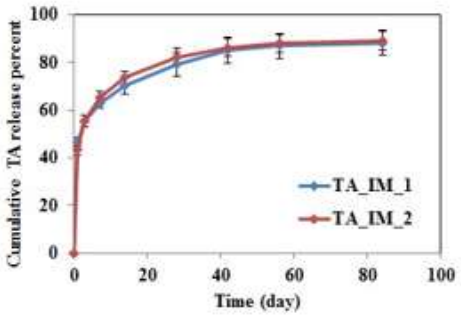
ACKNOWLEDGMENTS:

This research was supported by grants from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare, Republic of Korea (Grant Number: HI15C1744)



Picture 1: Caption 1: Figure 1. In vitro TA release profile of TA_IM_1 and TA_IM_2

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Poster presentation

475 Amine-Functionalized Metal-Organic Framework as Carriers for Ophthalmic Drug Delivery

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[2]Sungkyunkwan University, South-Korea

INTRODUCTION:

Ophthalmic drugs are generally formulated as eye drops and administered topically to the eye because of the ease of administration and high patient acceptance. However, eye drops are eliminated very rapidly from the surface of the eye (<3 min), thereby limiting their ocular bioavailability (<5%). To resolve this limitation, we proposed amine-functionalized metal-organic framework (NH_2-MIL-88(Fe)) as mucoadhesive carriers for sustained delivery of brimonidine, as a model ophthalmic drug.

METHODS:

2-aminoterephthalic acid (0.126g) and $FeCl_3 \cdot 6H_2O$ (0.187 g) were dissolved in 15 mL N,N-dimethylformamide (DMF)[1]. The substrate mixture was heated in a convection oven at 110 °C for 24 h. The solid brown product was collected and washed with DMF and ethanol to remove excess reactants. To encapsulate brimonidine (BRM) into the porous NH_2-MIL-88(Fe) (i.e., NH_2-MIL-88(Fe)/BRM), 50 mg BRM was dissolved in 5 mL deionized water. To this solution, 200 mg NH_2-MIL-88(Fe) was added with continuous stirring at 100 rpm for 24 h. Next, the suspension was filtered through a 200-nm polytetrafluoroethylene membrane filter to obtain NH_2-MIL-88(Fe)/BRM, which was washed with ethanol three times. In vivo experiments were conducted on New Zealand White rabbits.

RESULTS AND DISCUSSION:

The loaded amount of brimonidine was measured as 121.3 μg/mg NH_2-MIL-88(Fe). The NH_2-MIL-88(Fe)/BRM exhibited a sustained drug release pattern for 12 h (Figure 1A). To examine the ocular drug efficacy, we measured the change in the intraocular pressure (IOP) after the administration of brimonidine formulations. As shown in Figure 1B, for Alphagan P (brimonidine eye drops already approved for clinical use), a decrease in IOP was observed for 6 h. In contrast, when the same dose of brimonidine was administered as NH_2-MIL-88(Fe)/BRM, the period of IOP reduction persisted for 12 h, which was an increase of approximately two-fold.

CONCLUSION:

A biocompatible Fe-based MOF, NH_2-MIL-88(Fe) was successfully synthesized by using a solvothermal method and used for the first time as a carrier for the topical drug delivery of an ocular drug, brimonidine, to the eye. *In vivo* evaluations showed that NH_2-MIL-88(Fe)/BRM remained on the preocular surface for a prolonged period, leading to a prolonged duration of IOP reduction and the promotion of the ocular bioavailability of brimonidine.

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ACKNOWLEDGMENTS:

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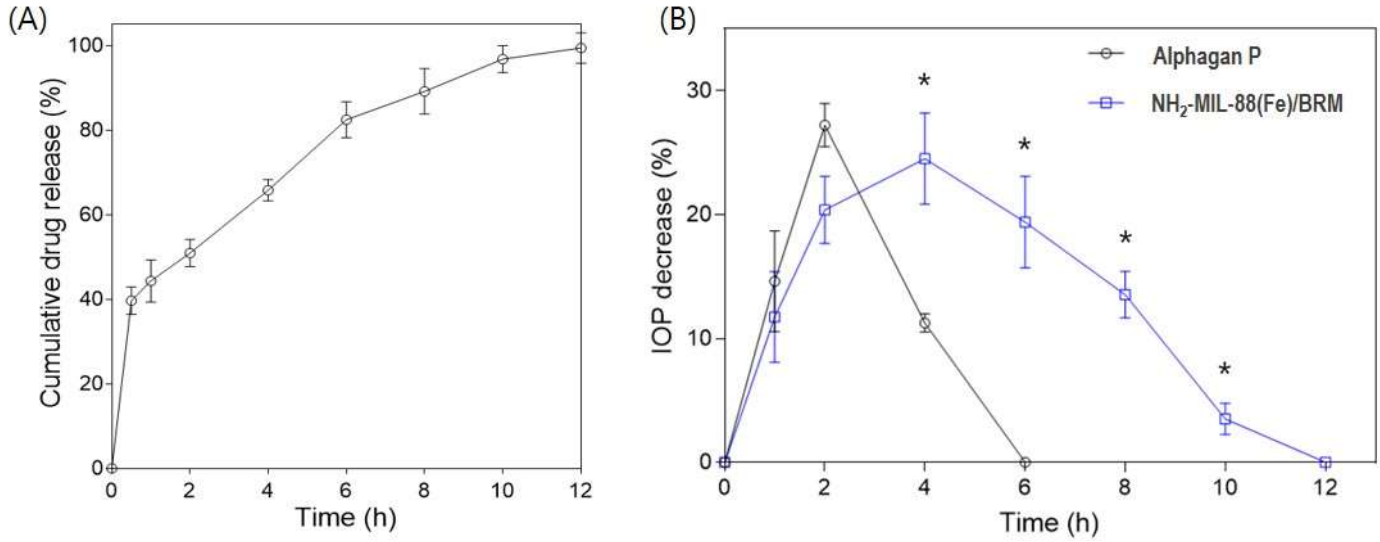
Picture 1:



Caption 1: Figure 1. (A) In vitro release profile of brimonidine from NH2-MIL-88(Fe)/BRM in PBS (pH 7.4). (B) Brimonidine concentration in aqueous humor.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

479 Gene expression in NTA-hyaluronan hydrogel particles: A strategy for in situ separation and purification of His-tagged proteins

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INTRODUCTION:

Within the last decades, droplet microfluidics has been established as a highly modular method allowing for the fabrication of hydrogel particles of micrometer size for diverse biological purposes. One key biological function is *in vitro* transcription/translation (IVTT), where genetic information is transferred into a gene product, usually proteins, through transcription of a gene into messenger RNA (mRNA) followed by mRNA translation on ribosomes into the encoded polypeptide. In contrast to protein production in cells, cell-free protein expression (CFPE) mostly based on *Escherichia coli* enables precise control over single reaction components and post-translational processing.[1] CFPE has become a powerful tool for tailored protein production and protein folding studies. On this account, we perform IVTT in droplet encapsulated micron-sized hydrogel particles to systematically study diffusion processes and reaction kinetics on cellular basis to gain insight in the effects of cellular structure on biological function.[2]

METHODS:

PDMS-based microfluidic devices were prepared by combined photo- and soft lithography. These devices were used to fabricate hydrogel precursor droplets, whereby a dispersed phase was emulsified in a continuous medium.

Once microdroplets were formed, their gelation was triggered *via* Diels-Alder click chemistry. For further CFPE and selective protein binding studies, gelled particles were separated from continuous medium by breaking the emulsion and transferring the as-fabricated polymer particles into an aqueous medium.

RESULTS AND DISCUSSION:

We perform the synthesis and characterization of nitrilotriacetic acid (NTA)-modified hydrogel particles as well as their application in IVTT, to selectively catch and separate expressed proteins *in situ*. Droplet microfluidics was employed to fabricate hydrogel particles based on furan-functionalized hyaluronic acid and maleimide-modified poly(ethylene glycol) (PEG) crosslinkers. As a proof of principle, we showed selective conjugation and release of His-tagged green fluorescent protein (GFP) within the particles by reversible binding *via* Ni(II)-complexation. Thereafter, we utilized these particles as experimental platform in IVTT by additional functionalization with DNA encoding for His-GFP. *In situ* formed proteins were selectively separated from the complex IVTT reaction mixture and controllably released from the gel matrix after purification.

CONCLUSION:

Our NTA-hyaluronan microgels serve as experimental platform for cell-free synthesis and purification of His-tagged proteins.

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ACKNOWLEDGMENTS:

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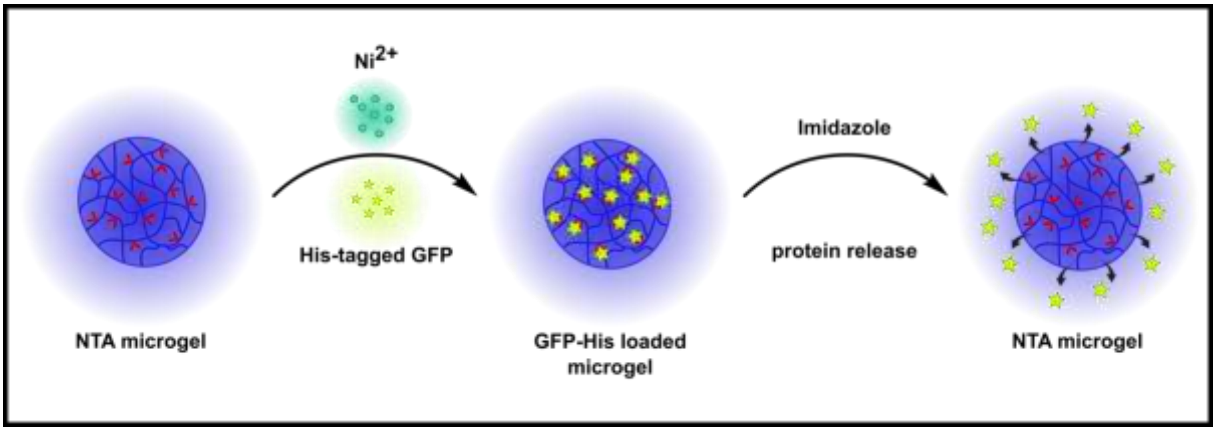
Picture 1:



Caption 1: The concept of binding His-tagged proteins on NTA microgels followed by the release via imidazole treatment.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

489 Surface functionalization of bacterial cellulose for wound healing applications

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University of Westminster, London, United Kingdom

INTRODUCTION:

Bacterial cellulose (BC) based wound dressings have been attracting increasing interest thanks to their unique features, and are now available in the market. BC presents an inherent hydrogel-like structure that enables it to incorporate more than 90% of its weight of water, providing an optimal level of moisture where applied. Its higher degree of crystallinity compared to plant cellulose results in better mechanical properties, i.e. higher tensile strength and Young's modulus values. Moreover, BC is highly pure as it does not contain lignin and hemicellulose, ensuring excellent biocompatibility and biodegradability.[1] However, there are limitations to its use due to the lack of antibacterial properties. Bacterial resistance to traditional antibiotics is a major threat for modern medicine, with the rise of "superbugs" capable to genetically modify themselves in order to deactivate or pump out the drug.[2] In this context, the need of novel strategies has become crucial. The aim of this study is to investigate different chemical methods for the surface modification of BC to develop an intrinsically antibacterial material for wound healing purposes.

METHODS:

BC was produced by *Gluconacetobacter xylinus* in static conditions using glucose as the main carbon source and purified at high temperature in order to remove the residual biomass. Antibacterial groups were introduced by wet and dry chemistry. The functionalization degree was assessed by EDX and XPS analyses. The antibacterial activity on contact was tested against *Staphylococcus aureus* following a modified ISO22916 procedure. The cell compatibility was evaluated using HaCat cells and quantified by Alamar blue assay.

RESULTS AND DISCUSSION:

A water-based reaction was performed to substitute the surface hydroxyl groups of cellulose. Antibacterial studies showed a decrease of 53% in the bacterial growth upon 24 hours of contact with the samples. The direct/indirect cytotoxicity evaluation produced no adverse effect for both modified and unmodified materials. The membranes were also functionalized in dry conditions to improve the yield of functionalization, and their biological behaviour is currently under investigation.

CONCLUSION:

A promising wound dressing material was developed by surface modification of BC. The intrinsic antibacterial groups introduced will ensure long-term effect without interfering with metabolic pathways, hence reducing the risk of development of bacterial resistance.

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ACKNOWLEDGMENTS:

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

493 Enzyme-mimetic Luminescent Hybrid Nanoaggregates as Ratiometric Hydrogen Peroxide Biosensors

Georgios Sotiriou
Karolinska Institutet, Stockholm, Sweden

INTRODUCTION:

Hydrogen peroxide (H_2O_2) is an abundant molecule associated with biological processes and reacts with natural enzymes, such as catalase. Thus, H_2O_2 quantification constitutes a powerful tool for detection of disease biomarkers linked to enzyme-based assays such as the plasmonic ELISA. However, the optical H_2O_2 biosensing without organic-dyes in biological media and at low, submicromolar, concentrations has yet to be achieved. The target of this work is to design biomimetic artificial enzymes based on antioxidant CeO_2 nanoparticles that become luminescent upon their Eu^{3+} doping [1,2]. CeO_2 nanoparticles have received a lot of attention recently due to their antioxidant enzyme-like (nanozyme) properties.

METHODS:

Here, europium-doped cerium oxide (CeO_2:Eu^{3+}) nanoparticles with well-controlled size (d = 4 – 16 nm) are prepared by flame aerosol technology and characterized in regards to H_2O_2 sensor response in physiologically-relevant solutions. Temporal stability was compared to a commercially available fluorescent dye in a peroxidase coupled reaction. Furthermore, we combine luminescent enzyme-mimetic CeO_2:Eu^{3+} nanoparticles with optically stable Y_2O_3:Tb^{3+} nanophosphors [1,2] that serve as concentration-independent reference in the same nanoaggregate.

RESULTS AND DISCUSSION:

The developed biosensors are coupled to enzyme-based assays that consume or generate H_2O_2 aiming the detection of other important bioanalytes, such as alcohol and glucose, using alcohol and glucose oxidase enzymes, respectively [3]. Upon the addition of a luminescent material with no H_2O_2 sensitivity, ratiometric sensors can be made for the facile and fast H_2O_2 detection *in vitro*. The sensing performance of the nanoparticles was further evaluated with hydrogen peroxide producing *S. pneumoniae* bacteria in biological media.

CONCLUSION:

The biomimetic artificial enzyme developed here could serve as a starting point of sophisticated *in vitro* assays towards highly sensitive detection of disease biomarkers.

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ACKNOWLEDGMENTS:

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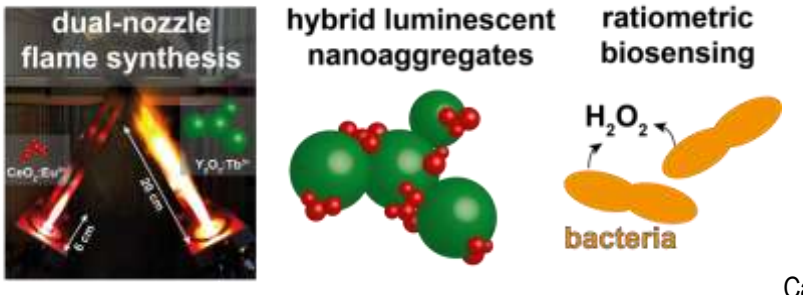

Picture 1: Caption 1: Hybrid luminescent nanoaggreates are made in a single step by double-nozzle flame nanoparticle synthesis for H2O2 biosensing generated by bacteria

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

502 Towards systemic delivery of an orally-administered biopharmaceutical: Exploitation of intestinal neonatal Fc receptor (FcRn) with mutant albumin-coated, polymeric nanoparticles

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[2]I3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal
[3]Oslo University Hospital Rikshospitalet and University of Oslo, Norway
[4]University of Applied Sciences Kaiserslautern, Site Zweibrücken, Germany
[5]Oslo University Hospital Rikshospitalet and Universtiy of Oslo, Norway

INTRODUCTION:

Systemic delivery of biopharmaceuticals by oral administration remains a challenge due, in part, to their limited transport through the intestinal epithelium. Nanoparticles (NPs) are promising drug delivery systems for the treatment of many diseases, and functionalization of NPs with ligands that can target specific surface receptors may improve the penetration of biological barriers (e.g. intestine). Exploiting the neonatal Fc receptor (FcRn) transcytosis pathway[1], whose interaction is strongly pH-dependent is of particular interest in the potential delivery of a biopharmaceutical across the intestinal epithelium (binding at acidic pH at the basolateral side, transcytosis in acidic vesicles and release at physiological pH at the apical side)[2]. Because albumin is as well as IgG a ligand of FcRn, fusing a drug to albumin is known to increase the drug's half-life[3]. Therefore FcRn might be a promising target molecule and promotor for the intestinal drug delivery.

METHODS:

We produced FcRn-targeted, poly(lactic-co-glycolic acid) (PLGA) NPs encapsulating insulin by double emulsion/evaporation[4] and conjugated with either wildtype (wt) or one of two variant (var) human serum albumins (HSA) with either higher (K573P) or lower (K500A) binding affinity towards FcRn compared to wt. The physical-

chemical properties of the NPs were characterized by dynamic light scattering, laser Doppler anemometry and electron microscopy, and insulin encapsulation efficiency was monitored by UV/Vis-spectroscopy. The transport and FcRn binding properties of the functionalized NPs were investigated using *in vitro* model systems of intestinal FcRn-presenting Caco-2 cells and recombinant FcRn binding ELISAs, respectively, as well as uptake and recycling through human endothelial recycling assay (HERA).

RESULTS AND DISCUSSION:

Our NPs had a slightly negative zeta potential and insulin encapsulation efficiency over 80%. FcRn-binding of HSA at pH 5.5 is retained post conjugation and the HSA-NPs bind with expected (compared to naked albumin) binding hierarchy to hFcRn in ELISA: HSA K500A (weak binder) < HSA WT < HSA K573P (12-fold stronger binder than WT). In addition, results from HERA suggest that NPs with conjugated albumin is recycled in an FcRn-dependent manner where HSA K573P-NPs recycled more efficiently than HSA K500A-NPs. Current efforts are focused on development and improvement of permeability assays of FcRn receptor presenting cells.

CONCLUSION:

The results indicate that, targeting the FcRn seems to be a promising drug delivery route that needs to be analyzed more deeply in the future.

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ACKNOWLEDGMENTS:

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

505 RGD-immobilized microcavity hydrogels for the chondrogenesis of ATDC5 cells

Lei Zeng, Xiaodong Cao
South china university of technology, Guangzhou, China

INTRODUCTION:

Hydrogels have been widely used for cartilage tissue engineering because they can be a good mimic of the morphology of the in vivo microenvironment. However, an appropriate mimic micro-environment and signaling molecules are both required in the process of chondrogenesis[1]. Arginine-Glycine-Aspartate(RGD) tripeptide as a cell-binding sequence which can promote cell survival, adhesion, motility and proliferation[2]. Therefore, in this work, we developed a novel RGD-immobilized microcavity alginate hydrogel(RGD/MSA) model and apply it to incubated with ATDC5 cells for possible in vitro induction of chondrogenic differentiation that could hold promise for cartilage repair. In our previous work, we have concluded that the microcavity alginate hydrogels(MSA) can enhance the proliferation of chondrocytes and promote the expression of cartilaginous genes[3].

METHODS:

ATDC5 cells and gelatin microspheres were suspended in a sterile RGD-immobilized alginate solution and drops of this solution were dispensed in a culture dish with $CaCl_2$ solution. After the alginate bead was well gelled and washed in DMEM and transferred to standard 24 well culture dishes. Then, the effects of RGD immobilized microcavity alginate scaffolds induced ATDC5 chondrogenesis were evaluated compared with MSA. The scaffolds of the two groups were collected and investigated by RT-PCR, Live-Dead staining and HE staining. Data were presented as mean ± SD. Where appropriate, ANOVA was performed to analyze results and $P< 0.05$ was considered to indicate a statistically significant difference.

RESULTS AND DISCUSSION:

We have therefore covalently modified microcavity alginate hydrogel with RGD at a percent grafting of 1.4%-3.5%. The RGD immobilized microcavity hydrogel showed good cell survival and proliferation (Figure. A and Figure. C). Chondrogenic expression levels of the genes were higher in the RGD-immobilized cell constructs compared to those in pure micro-cavitary alginates (Figure. B). After 32 days of cultivation in vitro, rich-ECM and lots of lacuna was found in the RGD-immobilized cell hydrogels (Figure. D).

CONCLUSION:

The obtained results supported the idea that the RGD peptides conjugated to microcavity hydrogels provided a better 3D environment for ATDC5 adhesion, survival, migration and chondrogenic differentiation. Our results highlighted the importance of RGD participating in the chondrogenesis and provide a candidate solution for cartilage tissue engineering.

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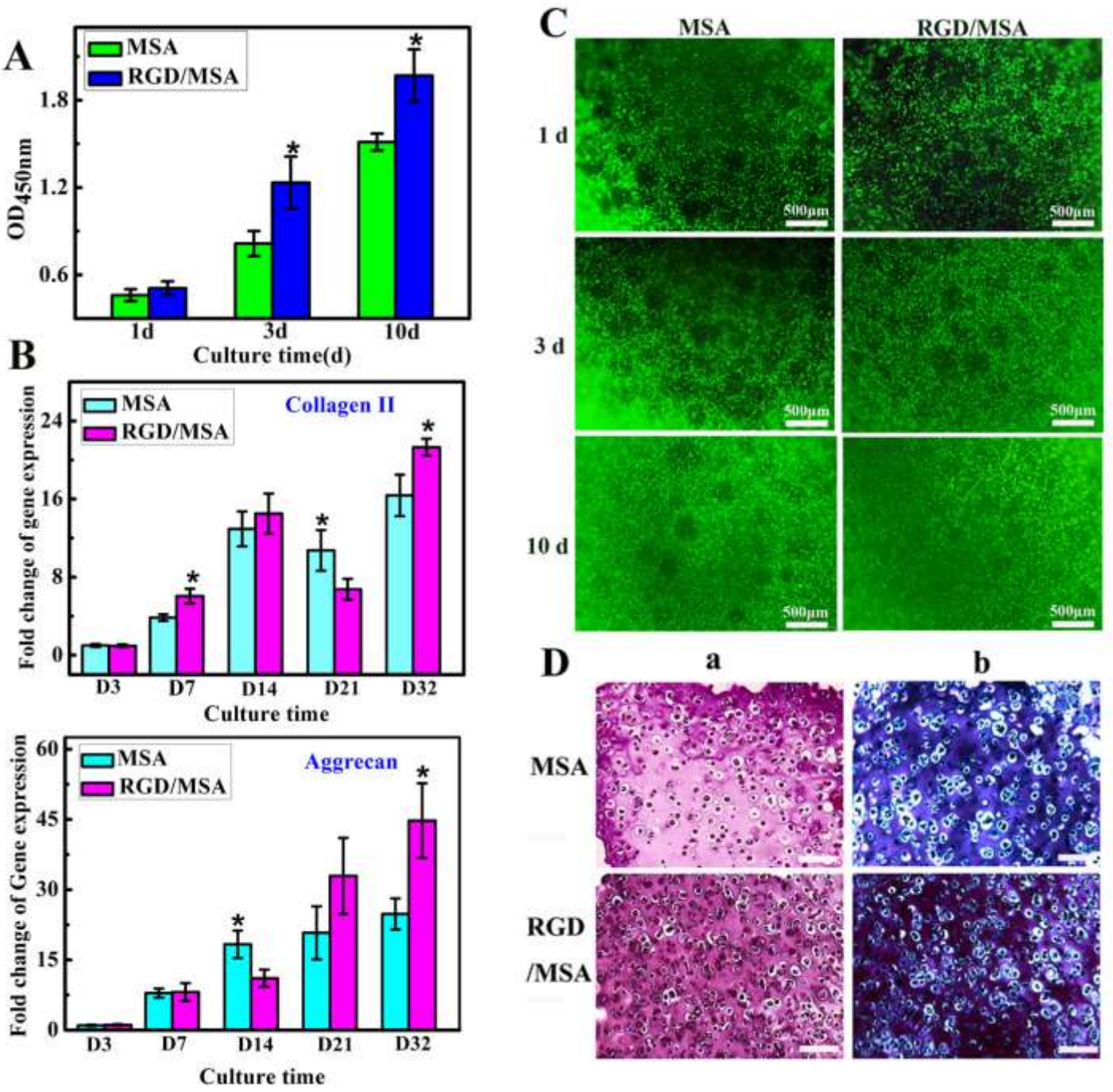

Picture 1: Caption 1: Figure. (A) The ATDC5 cells proliferation in the hydrogels was quantified by CCK-8 assay; (B) Real time-PCR detected the chondrogenic differentiation

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Poster presentation

508 ECAD-Fabrication of Strontium-doped Struvite Coatings on Titanium

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INTRODUCTION:

Electrochemically assisted deposition (ECAD) is a well-proven method for the fabrication of ceramic coatings on metallic surfaces and can be applied to a variety of sample geometries and surface topographies. The deposition principle is based on the pH-dependent solubility of several calcium phosphates (e.g. brushite, hydroxyapatite) and could also be successfully applied to the deposition of calcium hydroxide and – as shown in the study at hand – the magnesium phosphate struvite ($(NH_4)Mg[PO_4]\cdot 6H_2O$), a mineral which is found e.g. in kidney stones.

METHODS:

The standard ECAD electrolyte was composed of ammonium dihydrogen phosphate and magnesium oxide, supplemented with nitric acid in order to dissolve all components and to achieve an acidic pH-value in the bulk electrolyte. Sr doping of the coatings occurred by addition of strontium nitrate to the electrolyte. Coating characterization was carried out by scanning electron microscopy, X-ray diffraction, ICP mass spectrometry, energy-dispersive X-ray analysis, and surface milling with a focused ion beam (FIB). In addition cell-biological studies were performed using osteoblasts (MG 63) and osteoclasts (OC, RAW 264.7), in order to evaluate the biocompatibility of the struvite coatings on one hand and the effects of Sr doping on osteoclast activity on the other. Analytical methods comprised WST tests, TRAP staining, phalloidin staining, and PCR.

RESULTS AND DISCUSSION:

The variation of the coating parameters current density, deposition time, and strontium concentration enabled the fabrication of coatings with a broad range of Sr contents, as was confirmed by EDS and ICP-MS analysis. FIB milling revealed hollow structures within the struvite crystals, which most probably served as a reservoir for Sr-containing phases. Depending on the Sr concentration in the coatings, significant effects of the coatings on osteoclast activity could be observed. Apparently the process of osteoclast fusion and growth was decelerated with rising Sr contents. Furthermore, the formation of the OC-specific actin sealing ring was noticeably disturbed on Sr-doped coatings. The clearest correlation of rising Sr content with decreased OC activity was revealed by the significant reduction of TRAP activity.

CONCLUSION:

ECAD is a suitable method for the fabrication of dense struvite coatings. Doping with strontium significantly inhibits osteoclast formation and activity and hence provides a promising strategy for the improvement of implant healing in osteoporotic bone.

ACKNOWLEDGMENTS:

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Poster presentation

510 Topography memory effects of human mesenchymal stem cells

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INTRODUCTION:

Mesenchymal stem cells (hMSCs) are used in regenerative medicine and tissue engineering. Recent reports conclude that stem cells possess mechanical memory and store information from past physical environments and influences the cells fate[1]. Topography can control cell behavior, such as morphology, proliferation, migration and differentiation[2]. Therefore, the question arises whether stem cells remember past topography stimuli and could this memory effect be used to influence stem cell fate.

For a better understanding of possible topography memory effects, substrates with aligned nano- and microfeatures were designed [3]which elicit an elongation and orientation effect. After culture on different topography, the hBMSCs were transferred to flat substrates and cellular behavior and gene expression were analyzed to investigate a possible topographical memory effect.

METHODS:

PDMS wrinkle gradients preparation.

PDMS substrates with aligned topography were prepared as described in previous work using unidirectional stretching with subsequent plasma oxidation. Release of strain will result in surface wrinkles[4].

Cell culture

First, hMSCs (passage 3) were seeded at a density of 1000 cells/cm^2 on three topography surfaces. The cells were cultured for 7 days and 14 days (medium was changed every two days). After receiving the topography stimulus, the cells were transferred to flat PDMS and cultured for another 7 days. ALP and collagen I gene expression was analyzed.

RESULTS AND DISCUSSION:

The unidirectional gradients were obtained with wavelengths of 400 nm and 10 μ m. From the present gene expression analyses, we found that ALP gene expression was significantly higher on the nanotopography and is increased from 7 to 14 days of culturing.

CONCLUSION:

Although, topography is shown to have an influence on the hMSCs, from the present gene expression analyses, a memory effect could not be established. Further analyses is needed to assess if there is a topographical memory effect.

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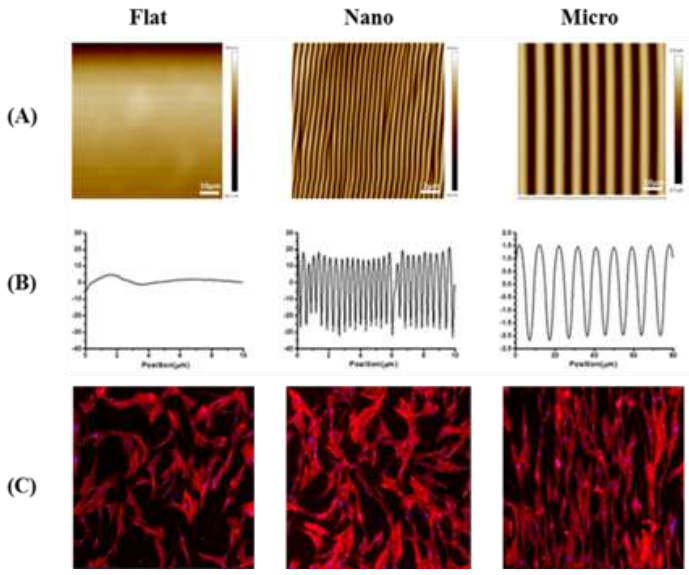
Picture 1: 

Caption 1: (A) AFM images and (B) Amplitude curves of the PDMS surfaces (C) Confocal laser fluorescence microscopy images of hMSCs cultures 7d on surfaces.

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Poster presentation

565 Spheroidal hydrogel particles as vehicles for cell and drug encapsulation

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INTRODUCTION:

Natural systems, such as bacteria and phytoplankton, present varying geometry and preferentially adopt geometries other than spherical, highlighting the relevance of this parameter in evolution. Considering this and the need to mimic native tissues, development of new platforms for cell and drug encapsulation with diverse material geometries has been gaining momentum. Even though microspheres, commonly-used encapsulation systems, allow for efficient nutrient diffusion and metabolite removal, increasing particle dimension compromises diffusion and results in lower cellular viability within the core. For drug delivery purposes, particle geometry is an impacting factor in opsonisation and targeted therapy. Moreover, spheres present the lowest surface area to volume ratio, which is an impacting factor in diffusion, whether of nutrients for cell survival, or kinetic profile of drug delivery. Thus, aiming to investigate how increasing surface area and varying particle shape could impact drug release and cell viability, a novel method was developed to produce spheroidal hydrogel particles with tuneable volume and geometrical parameters.

METHODS:

Spheroidal particles were produced by sandwiching droplets of hydrogel precursor between two superamphiphobic surfaces separated by spacers with different height, and photo-crosslinked to maintain the acquired shape after “de-sandwiching”. Methacrylated chitosan was used as proof of concept and, for each applied deformation, particle circularity and surface area were characterised, and numerical modelling studies were performed. Model protein bovine serum albumin was used for the controlled release from the hydrogel network (n=3), and results were

modelled used the Korsemeyer-Peppas equation. Additionally, viability of encapsulated L929 cells upon 24-hour culture was assessed for each deformation.

RESULTS AND DISCUSSION:

Spheroidal particles were successfully produced and characterised. Likely due to their higher surface area to volume-ratio, spheroids presented an improved viability of encapsulated cells due to an enhanced nutrient diffusion to the core, and lead to a significantly faster drug release rate from the polymer network (figure 1).

CONCLUSION:

Spheroidal particles with varying thickness were successfully created using photocrosslinkable chitosan and superamphiphobic surfaces. As an encapsulation vehicle for either drugs/proteins or cells, spheroids demonstrated a significantly faster BSA release, and an enhanced cell viability. Thus, the described method can be used to produce spheroidal particles with tailored thickness that can be broadly applied in the biomedical field, including for drug delivery or as cell encapsulation platforms.

ACKNOWLEDGMENTS:

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Picture 1: https:\\www.eventure-online.com/parthen-uploads/40/18903/add_1_428967_47764aca-c9d8-44f8-a0c8-84250eda89fb.jpeg
Caption 1: Figure 1.Cumulative BSA release from 2% M-CHT spheres and spheroids (62% deformation), up to 216 hours, with a zoom-in for the initial 2 hours (n=3).

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Poster presentation

572 Manufacturing and characterization of resorbable microspheres for cell culture

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INTRODUCTION:

Recently there is an increasing interest in development of tissue constructs by ‘bottom-up’ approach[1].This includes application of additive manufacturing techniques such as 3D printing with the use of bioplotters[2]. In our studies we explore different methodology i.e. assembly of small quasi-tissue modules made of cells cultured on resorbable microspheres.

The aim of this study was to develop a method of manufacturing poly(L-lactide-co-glycolide) microsphreres (MS) with different topographies and to test their usefulness as cell carriers.

METHODS:

MS were obtained by oil-in-water emulsification followed by solvent evaporation. PLGA (85:15, M_n=100 kDa, M_w=210 kDa) solution in dichloromethane (20%wt) was added to 1.5% polyvinyl alcohol (PVA, Mowiol, Sigma-Aldrich) aqueous solution. Two types of MS were prepared: without and with addition of 20% poly(ethylene glycol) (PEG, M_w=400 Da, Sigma-Aldrich) to oil phase. After formation MS were vacuum filtered, washed and dried at 37°C

for 24 h. Diameter and microstructure of MS were assessed with optical (Keyence VHX-900F) and scanning electron microscopy (SEM, Nova Nano SEM 200), respectively. Cytocompatibility tests were performed for 1, 3 and 7 days with osteoblast-like MG-63 cells. Live/dead, hematoxylin-eosin (H-E) staining and Alamar Blue assay were done.

RESULTS AND DISCUSSION:

Obtained MS had diameters in the range of 100-200 μm. MS produced with PEG were smooth as shown by SEM, while those without PEG modification were rougher and had small pores (2-5 μm in diameter) on their surface. Both carriers were not cytotoxic and supported adhesion and growth of cells as shown by viability tests and live/dead staining. H-E staining revealed that the cells were homogenously growing on the MS. Differences in cell topography were found not to affect cell culture.

CONCLUSION:

Our research proved that it is possible to obtain MS with defined diameter and morphology with oil-in-water emulsification method. All samples are cytocompatible, support cell adhesion and growth. The next step will be creation of bigger cell-material modules by self-assembly or by suspending MS with cultured cells in the hydrogels.

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Poster presentation

576 Mouse embryo culture on in vitro endometrium model

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INTRODUCTION:

Recent progress of in vitro fertilization technology has much improved the infertility. However, the reason for infertility caused by uterus or after embryo transplantation is still unclear. In this study, in vitro endometrium model has been constructed to investigate embryogenesis mechanism and to improve the infertility treatment in mouse model.

METHODS:

Mouse fertilized eggs were prepared by IVF technique. Ova were collected from 10 to 20-week-old ICR mouse with injection of 0.2mL PMSG and hCG at 48-hour interval. Then the fertilized eggs were cultured in IVC1 and IVC2 medium until blastocysts, embryo implantation, and egg cylinder [1][2].

Endometrium tissue was harvested and both of endometrial epithelial and stromal cells were isolated by M199 containing collagenase and dispase, respectively. The isolated cells were cultured with ACM medium containing EGF or FGF [3]. As a control, endometrium tissue of non-pregnant or pregnant mouse were harvested and observed histologically by HE and Picrosirius Red stain.

RESULTS AND DISCUSSION:

Embryo adhesion and extension of trophoblasts were observed after 72 hour of culture on polystyrene culture dish and collagen gel. Some blastocysts generated egg cylinders, however they were not same shape as native and do not develop after egg cylinder.

Endometrial epithelial and stromal cells were isolated by enzyme treatment, however it was not easy to be purified from each other. HE and Picrosirius Red staining indicated a change of endometrium collagen type drastically after pregnancy. Adhesion and implantation of embryo onto isolated endometrium cells are also investigated.

CONCLUSION:

Adhesion of embryo was observed on culture dish and collagen gel. Farther improvement of culture environment using native-like endometrium will be necessary for development after egg cylinder in vitro.

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Poster presentation

578 Cardiac progenitor cell mechanoresponse: cell-ECM and cell-cell adhesions

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INTRODUCTION:

Mechanical stimuli from the cellular microenvironment, transmitted through cell-extracellular matrix (ECM) or cell-cell adhesions, can determine cell functions and behavior. Cardiac progenitor cells (CPCs) hold potential for cardiac repair and regeneration; however, their interaction with external mechanical stimuli is largely unknown. In previous work, we showed that human CPCs develop cell-ECM adhesions upon early cardiomyogenic differentiation, together with the ability to reorient perpendicular to uniaxial cyclic strain (strain avoidance) [1]. At the same time, cell-cell adhesions are recognized mechanosensors [2], and allow mechanical signaling between adjacent cells. Here, we aimed at uncovering the role of cell-ECM and cell-cell adhesions in the mechanoresponse of CPCs.

METHODS:

To investigate the role of cell-ECM adhesions, uniaxial cyclic strain (10%; 0.5Hz) was applied to early differentiated CPCs in the presence of inhibitors of the activity of cell-ECM adhesion components: integrinβ1, integrin-linked kinase (ILK), Rho kinase (ROCK). Secondly, the contribution of cell-cell contact and adhesions in CPC strain avoidance response was evaluated by culturing undifferentiated CPCs at increasing seeding densities ($1x10^4$, $2.5x10^4$, $5x10^4$ cells/cm^2). Additionally, cell-cell adhesion protein N-cadherin was inhibited to analyze the role of cadherin-mediated adhesions in CPC mechanoresponse.

RESULTS AND DISCUSSION:

Our results indicate that all three cell-adhesion component inhibitions led to reduced strain avoidance response in CPCs, without affecting the early differentiated state of these cells. This suggests that the activity of all three investigated components is required for a proper mechanoresponse of these cells. Higher cell density led to enhanced strain avoidance of the undifferentiated CPCs, without affecting their stemness or their cell-ECM adhesions. Inhibition of the cell-cell adhesion protein N-cadherin resulted in reduced strain avoidance of the highly dense CPCs, indicating that cadherin-mediated cell-cell adhesions indeed play a role in CPC mechanoresponse.

CONCLUSION:

The presented study aimed to investigate how human CPCs respond to biomechanical stimuli in 2D. Our results shed light on the contribution of cell-ECM and cell-cell adhesions to mechanical signaling in CPCs, and provide fundamental knowledge that can ultimately aid in the development or optimization of CPC-based regeneration strategies in 3D biomaterial systems.

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ACKNOWLEDGMENTS:

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Poster presentation

589 Development and Characterization of a Novel Bioconjugated Hydrogel Scaffold Composed of Plasma Proteins and Aminated Hyaluronic Acid

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INTRODUCTION:

Platelet rich plasma (PRP) is a concentrate of platelets suspended in a small amount of blood plasma, derived from the centrifugation of the whole blood. PRP, an autologous product with no risk of allergic potential, contains a large amount of fibrinogen, high concentration of growth factors and cytokines, plays an important role in the tissue repair process[1]. Hyaluronic acid (HA) is an anionic, nonsulfated glycosaminoglycan involved in cell division, migration, differentiation and tissue regeneration. We hypothesized that by bioconjugating HA-NH_2 with PRP, it is possible to develop a hydrogel scaffold with improved regenerative properties. The aim of this study was to develop a scaffold based on a protein-polysaccharide conjugate rich in growth factors, using PRP, HA-NH_2 and Genipin, a natural crosslinker.

METHODS:

Human PRP was obtained from AU Ibni Sina Hospital Blood Center with ethics committee approval. HA-NH_2 was synthesized according to standard methods. PRP/HA-NH_2 was fabricated using different concentrations of HA-NH_2,

genipin and cross-linking temperatures. The surface and pore structure of PRP/HA-NH_2 was characterized by SEM and porosimetry analysis. The crosslinking degree was determined by the ninhydrin assay. The compressive strength, biodegradability and water retention capacity of the scaffolds were assessed. The PDGF, VEGF, TGF-β1 contents of the scaffolds were quantified by ELISA. In-vitro cytotoxicity and hemocompatibility studies were also carried out. The viability and proliferation of adipose mesenchymal stem cells (ASCs) seeded on the scaffolds was determined by Alamar Blue assay.

RESULTS AND DISCUSSION:

The results of the SEM analysis, mercury porosimetry, ninhidrin assay and mechanical testing indicated that the pore structure of the bioconjugated hydrogel could be tuned by modifying either the HA-NH_2 concentration, crosslinker concentration, or the crosslinking temperature. ELISA results indicated the presence of PDGF, VEGF and TGF-β1 in the scaffolds. Viewed overall, in-vitro cytotoxicity, hemocompatibility and cell culture findings indicated that the porous hydrogel scaffold showed good biocompatibility, supported the adhesion and proliferation of ASCs in-vitro.

CONCLUSION:

We have shown that PRP/HA-NH_2 hydrogel scaffold can be fabricated in desired porosity and mechanical properties with preserved growth factor content. This cytocompatible and hemocompatible scaffold permits the proliferation of stem cells in culture. Thus, prospective in-vivo studies will show the regenerative potential of PRP/HA-NH_2 alone, and/or in combination with cells.

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Poster presentation

591 IMPROVED PHYSICAL AND OSTEOINDUCTIVE PROPERTIES OF DEMINERALIZED BONE MATRIX (DBM) BY GELATIN METHACRYLOYL (GelMA) FORMULATION

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INTRODUCTION:

The demineralized bone matrix (DBM) is the most widely used bone allograft since it is able to induce bone matrix regeneration, thanks to a set of proteins such as bone morphogenetic proteins (BMPs). DBM is obtained by removing the mineral component from the bone by an acid treatment that leads to exposure of the protein

component of the bone. For the clinical application of DBM, it is necessary to formulate it with a carrier that provides consistency and if possible that stimulates bone regeneration.

METHODS:

In this study three DBM formulations with glycerol (Gly), hyaluronic acid (AH) and gelatine methacryloyl (GelMA) were evaluated, measuring their physicochemical properties (microstructure, compressive strength and serum cohesivity), together with the study of their *in vitro* osteoinductive capacity in human mesenchymal stem cells obtained from umbilical cord (HUC-MSCs). In order to measure the effectiveness of DBM in inducing the differentiation of hUC-MSCs into osteoblasts, different markers have been used, including alkaline phosphatase (ALP) activity and expression levels of specific marker genes by real-time RT-PCR. The osteoinductive potential of the different formulations was also assessed in an ectopic bone formation model with an intramuscular implantation in athymic mice. Results were assessed by histological and micro-computed tomography analysis.

RESULTS AND DISCUSSION:

In the compression assay, as well as in the serum cohesive assay, the DBM formulation with GelMA has shown its superiority over the other formulations. In addition, by analyzing its microstructure by scanning electron microscopy the GelMA showed to have a more compact structure. In the *in vitro* studies, the cytotoxicity assay showed that Gly increased cell toxicity against the control, while GelMa and AH showed very low toxicity. Although in the ALP assay all formulations significantly improved the differentiation into osteoblasts compared to the control group, no significant differences were found in the gene expression studies. In the in vivo studies, GelMA showed the greatest osteoinductive potential.

CONCLUSION:

In conclusion, GelMA proved to be the best carrier from the ones studied, being able to improve the osteoinductive activity of the DBM, being also the carrier with the best physicochemical properties.

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Poster presentation

599 Bisphosphonic acid functionalized hydrogels for biomimetic mineralization

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INTRODUCTION:

Mineralization of hydrogels is a fundamental step for their utilization in bone regeneration. The hydrogels with designed chemical functionalities have the potential to control type, size, organization of the inorganic crystals and also cell binding during mineralization. Anionic functional groups such as phosphate, phosphonate, carboxylate on their surfaces act as nucleating centers for the deposition of hydroxyapatite-like calcium phosphate and enhance mineralization; and bisphosphonic acid (BP) groups have excellent binding ability to Ca^{2+} ions. Therefore, BP-

functionalized dimethacrylates with different hydrophobicity were used as crosslinkers to prepare HEMA hydrogels as potential scaffolds for tissue engineering.

METHODS:

Two BP-functionalized dimethacrylates (1, 2) were synthesized[1] by reaction of two bisphosphonated amines (prepared from the reactions of 1,4-butanediamine or 4,9-dioxa-1,12-dodecanediamine with tetraethyl vinylidene bisphosphonate) with 2-isocyanatoethyl methacrylate followed by the dealkylation of the bisphosphonate groups. They were incorporated into hydrogels by copolymerization with HEMA in water at different ratios (5, 10 and 20 w% crosslinker). Mineralization of hydrogels was performed in SBF and studied using SEM, XRD, EDX and calcium assay. The mechanical properties of hydrogels were evaluated through compression measurements.

RESULTS AND DISCUSSION:

SEM micrographs indicated that the hydrogels were covered with a mineral layer of spherical mineral clusters. EDX analysis of the mineral layers showed (Ca+Mg)/P ratios of 1.08 and 1.40 for hydrogels containing 10 w% 1 and 2. The amount of calcium reached after 4 weeks of incubation was 677 ± 40 and 554 ± 36 mg/mg of dry hydrogels containing 10 w% of 1 and 2. The XRD spectra of mineralized hydrogels were similar to those of biological apatites, especially with respect to the peak at 32^0. Non-covalent interactions between calcium ions and BP-functionalized hydrogels were shown by an improvement in mechanical properties.

CONCLUSION:

The use of BP-functionalized dimethacrylates as crosslinkers in the synthesis of biomaterials for mineralization was shown in HEMA-based hydrogels. The presence of BP groups regulates calcium binding to the hydrogels and results in mineral growth in SBF. The composition of the mineral was a carbonate apatite similar to bone mineral. The crosslinker structures strongly influence the extent of mineralization. The different mineralization results are probably due to BP content and degree of crosslinking as well as hydrophophilicity of the hydrogels. These results imply that the BP-containing crosslinkers are good candidates for use in tissue engineering applications.

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Poster presentation

613 Ugi four-component reaction as versatile tool for preparing multifunctional polysaccharide microgels

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INTRODUCTION:

Polysaccharide-based microgels are of special interest in cell biology and tissue engineering, drug synthesis and delivery as well as cell-free biotechnology, since they are renewable, non-toxic, biodegradable, and easily available

from natural sources.[1] However, hydrogel synthesis from polysaccharides often requires pre-functionalization of respective building blocks for subsequent crosslinking as well as for introducing other functionalities (e.g. cell-binding sites, DNA, fluorophores). In this context, it is particularly disadvantageous that conventional polymer-analogous coupling reactions often cannot simply be applied for hydrogel formation due to an insufficient number of crosslinking sites that can be achieved, for instance. Thus, additional, time-consuming synthesis steps are necessary, before polysaccharide-based materials can be processed into defined microgels, e.g. by droplet-assisted microfluidic fabrication.[2]

METHODS:

We employ photo- and soft lithography for manufacturing of PDMS-based microfluidic flow cells for droplet-assisted templating of the polysaccharide microgels. The characteristics of resulting hydrogel particles are studied by rheology, atomic force microscopy (AFM), fluorescence recovery after photobleaching (FRAP), and fluorescence correlation spectroscopy (FCS).

RESULTS AND DISCUSSION:

To address aforementioned challenges of polysaccharide-based microgel production, we exploit multicomponent reactions, which allow for functionalization with bioactive compounds or fluorescent dyes, and crosslinking of hydrogel precursors in parallel without time-consuming modifications of precursors. We focus on the Ugi four-component reaction, since this one-pot synthesis utilizes a carboxylic acid, a carbonyl compound, an amine, and an isocyanide to yield an α-(acylamino)amide. Varying the length and composition of the diamine crosslinker allows for a tuning of porosity and mechanical properties of microgels, and tailor these characteristics for numerous applications. Based on carboxylic acid containing hyaluronic acid and different diamines as bifunctional crosslinkers, we form microgels with variously functionalized aldehydes *in situ* in one step.

CONCLUSION:

Depending on the type of functionalization produced microgels can serve, for instance, as experimental platforms in cell biology and cell-free biotechnology. We evolve individual microgel systems for the use as microcompartments for cell-free expression of proteins and in an aptamer-based approach for the detection of pollutants in waste water.

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ACKNOWLEDGMENTS:

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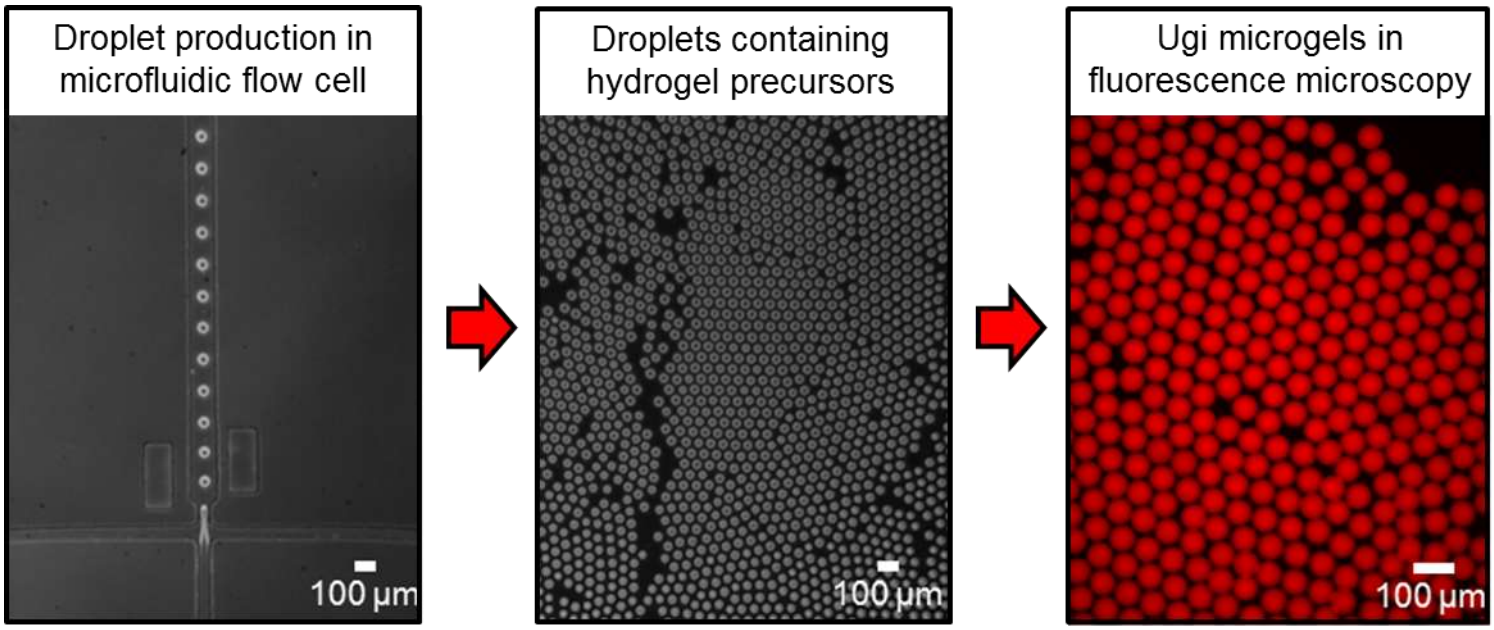

Picture 1: Caption 1: Individual steps of microfluidic generation of Ugi four-component reaction microgels

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Poster presentation

619 Vitreous humor as an alternative bioactive matrix for cartilage tissue engineering

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INTRODUCTION:

A challenge in tissue engineering is to develop bioactive scaffolds that mimic tissues extracellular matrix (ECM)[1]. A logical choice is to exploit decellularization strategies to remove cellular and antigenic components from native ECMs while preserving their bioactive cues. Due to the dense ECM and avascular nature of cartilage, its decellularization requires harsh treatments that often compromise the ECM bioactivity[2].

Vitreous humor (VH) has been described as a loose and acellular tissue with a biochemical composition that closely resembles the cartilage one[3]. The aim of this study was to evaluate whether VH can be applied as a chondro-supportive ECM for cartilage tissue engineering.

METHODS:

Glycosaminoglycan (GAG) (Dimethylmethylene blue), total protein (Pierce™) and DNA (CyQUANT) retention were compared when VH was extracted from freeze-thawed or fresh samples (n=9). Changes in VH architecture were determined histologically (picrosirius red/alcian blue, collagen IX/II). Cytotoxicity was evaluated by monitoring the metabolic activity of human mesenchymal stromal cells (hMSCs) (AlamarBlue) after incubation with VH. Changes in

U937 (pro-monocytic cell line) phenotype after exposure to VH were analysed to determine the inflammatory response. To establish VH capability to support chondrogenesis, VH hydrogels were seeded with human articular chondrocytes (hACs) (0.25-0.75x10^6 cells/50µl VH) and cultured in chondro-inductive medium (TGF-β1) for 3 weeks. Pellets without VH (0.25x10^6 hACs) were used as control. Metabolic activity, GAG and DNA were quantified and matrix deposition was visualised using (immuno)histochemistry (Safranin-O, collagen I/II).

RESULTS AND DISCUSSION:

VH was successfully extracted from freeze-thawed or fresh samples. The latter emerged as the optimal candidate for further analysis, since it exhibited lower amount of DNA residues (0.4±0.4µg/mg dry weight) and higher preservation of ECM components (156±90µg GAG, 68±21µg protein) compared to the freeze-thawed samples (1.06±0.4µg/mg dry weight DNA, 146.93±69.36µg GAG and 68±21µg protein). MSCs proliferation was enhanced after exposure to VH, indicating that the extracted VH was not cytotoxic. Furthermore, VH did not promote an inflammatory response as observed from the U937 studies. Encapsulated hACs self-assembled the VH hydrogel into spheroid structures with a uniform distribution of GAGs, collagen type II and with significantly higher GAG/DNA content (0.75x10^6 cells: 21.5±5.2 g/g, 0.25x10^6 cells: 14.1±2.5 g/g) compared to the hAC pellet controls (7.0±1.3 g/g).

CONCLUSION:

This study demonstrated for the first time that VH could be safely used as a bioactive matrix for cartilage tissue engineering.

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ACKNOWLEDGMENTS:

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Picture 1:

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Poster presentation

623 A Silk Fibroin Conduit to improve Peripheral Nerve Regeneration: in vitro and in vivo evidences.

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[2]Silk biomaterials srl, Italy

INTRODUCTION:

Peripheral nerve lesion are common injuries often caused by trauma or accident at work and can lead to loss of motor and sensory function. Despite the ability of the peripheral nerve to regenerate and re-innervate denervated target organs has been recognized, clinical and experimental evidences show that the regeneration is usually far from satisfactory, especially after severe injuries. In this study a Silk fibroin scaffold was used to performed different *in vitro* and *in vivo* experiments in order to study the regenerative potential of this biomaterial.

METHODS:

In vitro experiments were performed using glial RT4-D6P2T and neuronal NSC34 cell lines seeded on Silk fibroin. At this purpose, different parameters were evaluated: for RT4-D6P2T, proliferation and adhesion were evaluated 2-4-6 days after culture, for NSC34, differentiation and neurites elongation were investigated 5 days after seeding.

For *in vivo* experiments, rat median nerve was repaired by two different Silk fibroin conduits: 10 mm hollow tube and 10 mm Silk fibroin tube filled with fibroin fibers. Samples were harvested at early (14, 21 days after nerve repair) time points, and morphological analyses by Trasmission Electron microscopy (TEM), Scanning Electron microscopy (SEM) and light microscopy were carried out.

RESULTS AND DISCUSSION:

Morphological analysis carried out on median nerves at early time points show the presence of cell types and first fibers appearance, typical of the first regenerative phase. Results obtained on RT4-D6P2T and NSC34 cell lines show that Silk fibroin represents a permissive substrate in term of proliferation and adhesion of glial cells, and differentiation and axonal elongation of NSC34 neuronal cell.

CONCLUSION:

Tissue-engineering technology is receiving more and more attention in the treatment of peripheral nerve injury. Among the various biomaterials used, silk fibroin is increasingly interest thank to its biocompatibility, mechanical properties and easy processing as a promising alternative to autologous nerve grafts.

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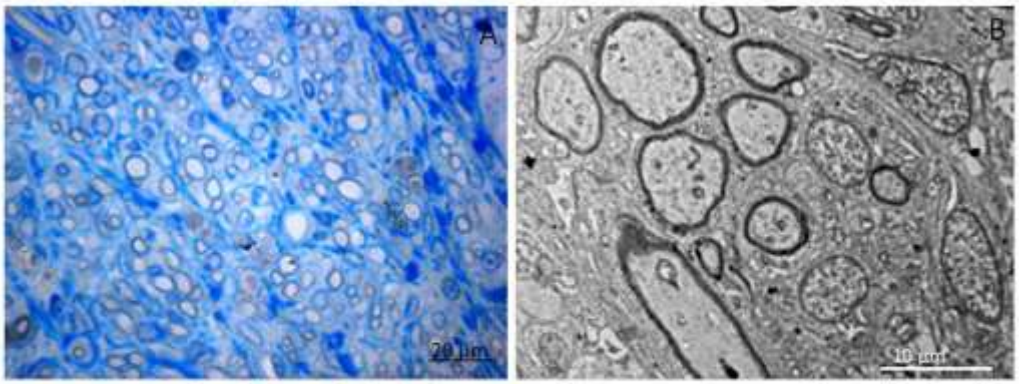

Figure 1: Presentation of the morphological appearance of the regenerating nerve fibers within the silk conduits 3 weeks after surgery. Photomicro- graphs illustrate the morphology of the proximal stump using light microscopy (A) and trasmission electron microscopy (TEM) (B).

Picture 1: Caption 1: Figure 1: Presentation of the morphological appearance of the regenerating nerve fibers within the silk conduits 3 weeks after surgery. Photomicro gr

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Poster presentation

630 Hyaluronan based hydrogel as a matrix for 3D culture of pancreatic β-cells

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INTRODUCTION:

Transplantation of allogeneic pancreatic islets is one of method for type I diabetes treatment. Design of ECM-like biomaterials which could provide sufficient delivery, retention and maintaining survival, engraftment and functioning of newly implanted islets is one of challenges in the field of bioartificial pancreas development.[#_ENREF_1]

Hyaluronan-tyramine conjugate (HA-Tyr), which is capable to undergo horseradish peroxidase (HRP) mediated crosslinking, have been designed as material for development of hydrogels suitable for 3D cell culture. Aim of this study was to evaluate the influence of gelation process on the viability of the INS-1E cells entrapped within the hydrogel structure.

METHODS:

HA-Tyr conjugate was synthesized according previously reported protocol.[#_ENREF_2] The structure and DS of the HA-Tyr conjugate was confirmed by ^{1}H NMR spectra. Molecular weight of conjugate was determined by SEC-MALLS. Gelation time was measured by TA Instruments AR-G2 rheometer. During viability study, INS-1E cells were cultivated both in the form of monolayer culture and as cell clusters (pseudoislets - PIs). ATP assay was used to evaluate viability of β-cells in the presence of H_2O_2.Viability of PIs during 3D culture was confirmed by LIVE/DEAD staining.

RESULTS AND DISCUSSION:

HA-Tyr conjugate with molecular weight of 300 kDa and DS 2 % was synthesized for the purpose of this study. Influence of H_2O_2 (initiator of hydrogelation) on viability of ISN-1E cells was described. Concentrations of peroxide ranging 0,17 to 1 µmol/ml were tested. Cells cultivated in the form of pseudoislets exhibited significantly higher resistance in comparison with monolayer culture. Viability of PIs was not influenced even at concentration of peroxide 0,5 µmol/ml.

Precursor hydrogel solutions containing HA-Tyr (10 mg/ml) and horseradish peroxidase (0,12 U/ml) was used for preparation of PIs suspension. Peroxide concentration of 0,5 µmol/ml enables sufficiently fast rate of hydrogelation (less the 1 minute). Results confirmed that cells in the form of PIs kept their viability at least for six days after the encapsulation.

CONCLUSION:

Results of this study confirmed that HA-Tyr based hydrogel could be a suitable material for encapsulation and 3D culture of pseudoislets prepared from INS-1E cell line. Additional studies, describing long term viability and retention of cell functionality (e.g. glucose dependent insulin production) are necessary to proof the value of this material for further research.

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ACKNOWLEDGMENTS:

ACKNOWLEDGMENTS: This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement no. 645991.

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Poster presentation

636 New experimental studies on the potential of chitosan-based medical device for improving functional recovery after radical prostatectomy.

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INTRODUCTION:

Chitosan (CS) based nerve grafts are employed to promote neural repair after injury raising more and more interest among basic and clinical research. *In vitro* and *in vivo* studies have shown that this biomaterial has biocompatible and biomimetic properties to improve the regeneration process of the peripheral nervous system.

Prostate cancer (PCa) is the most common cancer among men. The surgical treatment for PCa is represented by the radical prostatectomy, which is the gold standard in the treatment of localized disease. Patients who underwent a radical prostatectomy frequently are subjected to iatrogenic damage to the peri-prostatic nerve bundles leading to erectile dysfunction (ED).

The aim of this in vitro and ex-vivo study is to assess the simultaneous anti-proliferative and pro-regenerative properties of a CS film, which has already achieved a clinical use for the peri-prostatic nerve plexus protection.

METHODS:

CS-anti-proliferative properties were tested on different human prostate cancer cell lines (PC-3, DU145, LNCap) seeded on two different experimental condition: dissolution products of CS and CS coating. Since the prostatic plexus is innervated by sympathetic, parasympathetic and somatic fibers, the regenerative potential of CS films was assessed through primary neuronal cultures and ex vivo explants derived from autonomic ganglia.

RESULTS AND DISCUSSION:

The dissolution products of CS on proliferation assay performed after 1, 3, 6 days determined a significant lower proliferation of cancer cells, accordingly the same cells in direct contact with CS coating showed a substantial change in morphology, but also a significant decrease in proliferation. Regarding the regenerative potential, primary organotypic cultures derived from sympathetic ganglia were cultured on CS films and results showed that CS films significantly stimulate axon elongation in comparison to controls.

CONCLUSION:

An increasing number of young men have an early prostate cancer diagnosis, and ED caused by radical prostatectomy is associated with distress and impaired quality of life. The clinical application of new techniques and new materials in the field of peripheral nerve regeneration would result in minor inconvenience for patients and allow to extend the treatment also for applications in oncology.

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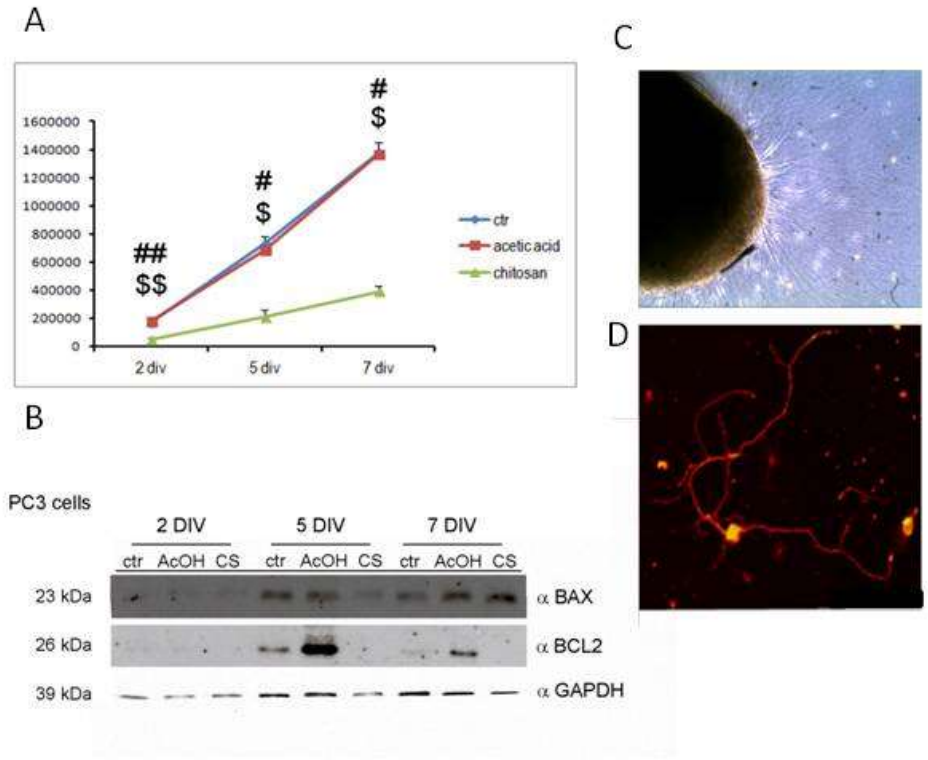
Picture 1:



Caption 1: Figure 1: A) PC3 cancer cell line on CS coating, effect on cell proliferation and on Bcl2/Bax expression (B). (C) Pro-regenerative effect of CS on Aut

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Poster presentation

642 Sonochemical modification of electrospun fibres with hydroxyapatite nanoparticles

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INTRODUCTION:

Electrospun nonwovens are considerated by various authors as a scaffold for bone tissue regeneration. In order to increase biocompatibility and promote osteoblast phenotype of the cells, fibres may be coated with ceramic component [1]. Various methods were suggested, eg. mineralisation from simulated body fluid (SBF) [2]. However, this method is time consuming and lead to precipitation of calcium phosphates, which crystal form is not under control during procedure. In our work we propose application of ultrasounds for hydroxyapatite depositions on fibres surface. The aim of this research was to tailor the surfaces properties of nonwoven by sonochemical deposition of hydroxyapatite and properties analysis.

METHODS:

Poly(lacide-*co*-glicolide)(PLGA) fibres were formed via electrospinning technique. Ultrasonic covering with nHAp took place in water bath containing nHAp component per: 3, 15 and 30 minutes. The samples were imaged by scanning electron microscopy (SEM) with EDS mode. The particles embedding in the fibres' surface was visualised by transmission electron microscopy (TEM). For structure characteristic wide angle X-ray scattering (WAXS), gel permeation chromatography (GPC) and differential scanning calorimetry (DSC) was used. Mechanical properties of the nonwoven were analysed in tensile test.

RESULTS AND DISCUSSION:

SEM imaging confirmed presence and homogenous distribution of nHAp particles on fibres surface. TEM analysis confirmed that nHAp particles are embedded only in outer layer of the fibres. EDS analysis, as well as WAXS measurement confirmed presence of calcium/phosphorous and nHAp crystal structures, respectively. DSC revealed no significant changes in glass transition temperature nor melting temperature of PLGA. TGA analysis proved increasing amount of embedded nHAp with increasing time of sonochemical procedure, as well as significant shift of half decomposition temperature of PLGA into higher values. GPC analysis revealed slight decrease of PLGA average molecular weight and increase of chain polydispersity.

CONCLUSION:

Ultrasonic deposition of nHAp particles is quick and efficient method of particles incorporation into electrospun fibres' surface. This method do not change structure and mechanical properties of electrospun nonwovens. Due to those advantages it is suitable for use in industry scale.

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ACKNOWLEDGMENTS:

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Poster presentation

647 Polyphenols surface functionalization strongly reduce ROS/RONS production and selectively affect the viability of cancerous cells

Rita Sorrentino[1], Nicola Contessi[2], Sara Ferraris[3], Martina Cazzola[3], Andrea Cochis[4], Lia Rimondini[4], Enrico Prenesti[5], Silvia Spriano[3], Enrica Vernè[3]
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INTRODUCTION:

A recent evolution in biomaterials science is represented by multifunctional surfaces able to promote healthy tissue integration and decreasing adverse effects such as inflammation. Accordingly, here we introduce innovative surfaces grafting composed by well-known multispectral biomolecules such as polyphenols. In fact, polyphenols are known to be successful in reducing the production of reactive species commonly related to inflammation; moreover, some polyphenols have been shown to hold a certain target killing activity towards tumorigenic cells.

METHODS:

Bioactive glass (CEL2) has been prepared by melt and quenching technique[1] while Ti6Al4V has been chemically treated (CT) to obtain an oxide surface with nanotextured morphology enriched by hydroxyls groups[2]. Polyphenols were extracted from green tea leaves (TPH) and red grape skins (GPH) by solvent extraction, freeze-dried, resuspended in water and used to soak activated substrates. Finally, surfaces coating was chemically activated by UV exposure. Surface analysis was performed by means of Folin&Ciocalteu test, XPS and fluorescence analyses. Polyphenols selective antitumor activity was investigated on non-tumorigenic progenitor hFOB and tumorigenic U2OS osteoblast cells by means of direct and indirect cellular viability tests. The anti-inflammatory activity was checked by means of ROS/RONS quantification, while the DNA damage and repair were evaluated by nuclear immunolocalizing 53BP1 and cyclin B1.

RESULTS AND DISCUSSION:

The Folin&Ciocalteu tests, XPS analysis and fluorescence microscope observations demonstrated the presence and the activity of polyphenols on the surface of the samples after the functionalization procedure. The targeted activity of the functional groups exposed by polyphenols was highlighted during the direct viability assay where only the viability of bone cancer cell line (U2OS) was significantly affected in comparison with hFOB cells. Moreover, ROS/RONS levels were significantly higher for the U2OS cells in comparison with hFOB, thus suggesting a certain protective effect to the healthy counterpart. Finally, tumorigenic U2OS cells cytology revealed the presence of DNA-damage foci established by 53BP1 analysis; the further nuclear localization of cyclin B1 confirmed that the DNA-damage cannot be repair (Figure 1).

CONCLUSION:

Natural polyphenols were successfully grafted onto bioactive surfaces without losing polyphenol activity. The biological characterization evidenced polyphenols ability to selectively induce ROS mediated DNA-damage within cancer cells preserving the proliferation rate and of normal osteoblastic progenitor cells.

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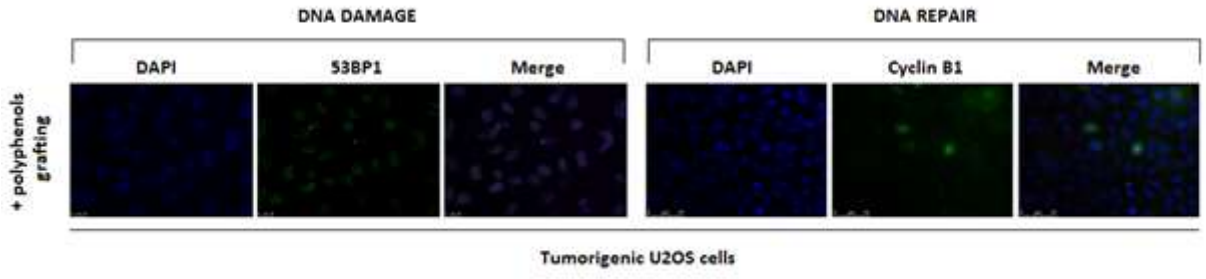
Picture 1:



Caption 1: Polyphenols induced U2OS DNA damage (53BP1) and repair lacks (Cyclin B1)

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Poster presentation

653 Development of a novel multifunctional bioglass-based coating for the next generation of prostheses

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INTRODUCTION:

With an ever ageing population, there are an increasing number of patients requiring medical devices, such as artificial joints and dental implants to enable everyday activity. An improvement of current implants will offer tremendous benefits. In particular, there is an urgent need for technologies to improve the fixation of implants/devices in bone without infection occurring. These will contain doped nano-sized bioactive glass to enable strong integration with bone and anti-microbial properties.

The project is funded through Innovate UK and is there are three project partners Johnson Matthey, Promethean Particles and Queen Mary University, London and the project has 4 key deliverables: 1- Synthesis of novel nano-materials by state of the art manufacturing processes; 2- Development of optimum formulations with these novel materials; 3 Selection of optimum coating technique for application onto implant substrates and 4- Biological testing of coated implant prototypes.

METHODS:

A range of synthesis methods have been used including Promethean's patented method and traditional melt/quench and sol gel routes to produce nano sized bioactive glass materials.

Furthermore, a range of coating methods have been employed including traditional spray coating and Electrohydrodynamic Atomisation (EHDA) spraying[1] to produce coated titanium plates which can then be tested for osteoblast proliferation and also antimicrobial effects.

RESULTS AND DISCUSSION:

Simple spray coating methods lead to highly cracked surfaces which adhere badly to the surface and are prone to delaminating during bioactivity testing and skewing the results. This led to significant work to improve the adhesion in terms of the support and coating method.

Various compositions have been investigated and characterised to assess the bioactivity and antimicrobial effects these might have.

CONCLUSION:

Improvements have been made to the coating technique to increase adhesion and maintain bioactivity. Compositional modifications have been made to assess antimicrobial effects.

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ACKNOWLEDGMENTS:

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Poster presentation

662 Pore dynamics in silk-elastin-like recombinamer-based hydrogels

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INTRODUCTION:

Porosity and pore size are key parameters in the design of hydrogels for tissue engineering and regenerative medicine. In this regard, it is widely accepted that hydrogels require a high porosity and interconnectivity to achieve an optimal cell proliferation[1]. In this work, we have developed a silk-elastin-like recombinamer (SELR) that self-assembles into hydrogels above the transition temperature (Tt), and whose pore size undergoes an evolution over time.

METHODS:

The SELR was obtained by recombinant DNA technology and bioproduction in *E. coli*. Characterization techniques included MALDI-TOF and SDS-PAGE for molecular weight and purity evaluation, and differential scanning calorimetry (DSC) to determine the Tt. Real-time fluorescence microscopy was used to evaluate the evolution of pore size over time. ImageJ was used for image analysis. DSC and infrared spectra allowed the determination of the formation of silk β-sheets[2].

RESULTS AND DISCUSSION:

The automatic processing through real-time fluorescence microscopy and imaging software allowed the determination of pore sizes at different conditions. For instance, we observed that at a concentration of 175 mg/mL, pore size increases from a few μm to approximately 70 μm over 16 h (Fig. 1). At a first stage, this pore dynamics is driven by a phase separation between water and the SELR, due to the inverse temperature transition (ITT) described for the elastin-like domains[3], which occurs fast when the temperature is raised above the Tt. However, the presence of stable β-sheets between the silk-like domains slows down this phase separation, although with slower kinetics. Subsequently, the water phase adopts a droplet morphology in order to reduce the interfacial tension/free energy of the system. Then, the microstructure evolves by coalescence of the droplets inside the hydrogel until, eventually, they become trapped at a non-equilibrium stage due to the arrest of the hydrogel structure as a consequence of silk crystallization[2], which highlights the relation between the nano- (i.e. formation of hydrogen bonds in β-sheets) and the microscale (i.e. pore size evolution).

CONCLUSION:

In summary, pore dynamics within SELR-based hydrogels is driven by several consecutive phenomena. The control of pore size may lead to hydrogels suitable for different applications in tissue engineering and regenerative medicine, due to the inclusion of RGD cell-adhesion sequences within the SELR molecule.

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ACKNOWLEDGMENTS:

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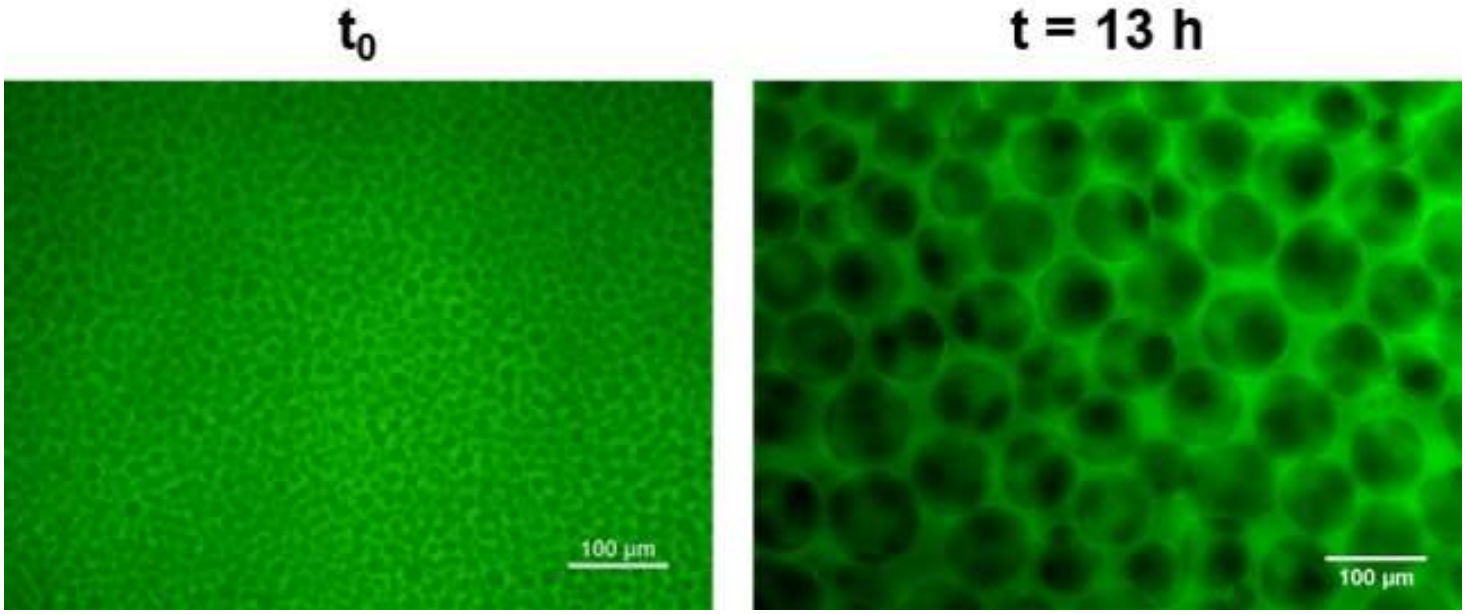

Picture 1: Caption 1: Fig. 1. Start (left) and endpoint (right) of the gelation process undergone by a SELR-based hydrogel.

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Poster presentation

666 Differences in biological lung scaffolds derived from healthy and IPF donor tissues affect cellular response of human primary fibroblasts

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INTRODUCTION:

The use of biological scaffolds, i.e. decellularized tissue, in the field of lung tissue research allows us to specifically study cell-ECM interaction and tissue regeneration. In diseases such as idiopathic pulmonary fibrosis (IPF), a devastating lung disease associated with heterogeneous and abnormal build-up of extracellular matrix (ECM) proteins, we believe that the altered material properties of lung tissue is one of the driving forces in the disease. Our aim is to find a correlation between structural alterations of the ECM and cellular mechanisms associated with disease pathology such as increased proliferation, ECM synthesis and mechanotransduction.

METHODS:

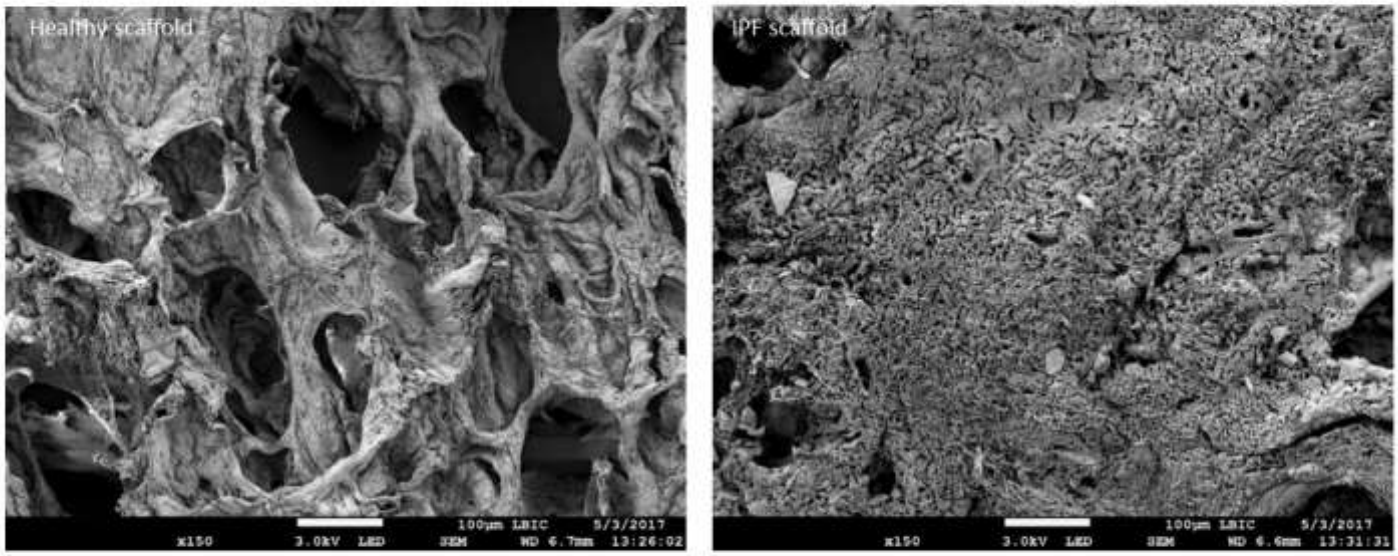
Thinly sliced distal tissue (350 µm x 1 cm^2) from healthy (n=4) respective IPF (n=4) subjects was decellularized using a detergent-based protocol to produce acellular scaffolds. The scaffolds were characterized with histology, SEM, mass spectrometry, measurements of DNA content, tissue density and tensile strength. Next, the scaffolds were mounted in a holder, seeded with primary human fibroblasts from healthy donors and cultured for 9 days. Metabolic activity and DNA content was measured after 1, 3, 6, and 9 days, with collection of histology samples at day 1 and 9. Tensile strength was measured after 3, and 9 days.

RESULTS AND DISCUSSION:

The material properties differed between scaffolds derived from healthy and IPF tissue. The IPF-derived scaffolds displayed denser morphology with higher matrix stiffness in comparison to healthy tissue scaffolds. Mass spectrometric characterization of scaffolds, separated IPF and healthy scaffolds into two cluster groups. Despite differences in tissue function and composition, both healthy and IPF derived scaffolds supported the attachment and growth of fibroblasts. Repopulated scaffolds displayed increased number of fibroblast over time for both groups. Interestingly, cells cultured on healthy scaffolds seemed to alter the material properties to become stiffer, whereas cells cultured on stiff IPF scaffolds did not seem to alter the material properties much.

CONCLUSION:

This leads us to think that the material properties play an important role in cellular response and ultimately in disease progression. Further analysis is being done to elucidate the underlying mechanisms using mass spectrometry, analysis of medium released factors, mRNA expression, and immunohistochemistry.



Picture 1: Caption 1: SEM images of lung scaffolds derived from healthy donor tissue (left) and IPF patient material (right).

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

671 Development of personalized anti-infective medical devices by a EU training consortium

Clara Mar Guarch Pérez, S.A.J Zaat, Martijn Riool

Academic Medical Center, Amsterdam, Netherlands

INTRODUCTION:

PRINTing Anti-Infective Devices (PRINT-AID) is a multidisciplinary European training partnership for the development of personalized anti-infective medical devices combining printing technologies and antimicrobial functionality. Six academic groups and four industrial partners in six European countries have come together in collaboration to address the challenge of alternative antimicrobial strategies.

Antimicrobial resistance (AMR) and Healthcare-Associated Infections (HAI) are the most serious public health concerns globally. The European Center for Disease Prevention and Control determined that almost 4 million patients acquire HAIs each year in the EU resulting in close to 37,000 deaths/year. Biofilm infections in medical

devices are responsible for a considerable number of HAIs. It is essential to study novel antimicrobial methods to comfront the problem at hand. One strategy to prevent or decrease medical device-associated infections is altering the surface properties of these devices by incorporating antimicrobial compounds.

PRINT-AID aim consists in an educational platform for the development of novel generation of 3D-printed medical devices with antimicrobial functionalities. Experimental drugs which inhibit bacterial colonization or kill bacteria will be added in the printed implant. These compounds will be added in the medical device structure itself during the 3D printing process. The use of 3D-printing technology makes possible the customization of personalized devices that fit the needs of the patients.

PRINT-AID is structured in four consecutive work packages with the contribution of each institution. The first package consists of the identification and characterization of anti-biofilm compounds. Subsequently, the development and printing devices takes place. Consequently, the implants are evaluated with in vivo studies. Finally, the data is integrated and standardized in the forth step.

As part of PRINT-AID, my study will focus on the development, characterization and in vivo evaluation of novel 3D-printed femur implants by incorporating newly developed highly potent synthetic antimicrobial and anti-biofilm peptides to prevent Staphylococcus (S.) aureus and S. epidermidis infection in a murine model. Furthermore, anti-biofilm activity and possible influence on the host immune response and tissue repair using invasive and non-invasive techniques will also be evaluated.

CONCLUSION:

Overall, the consortium aims to raise the young scientists for the study and development of novel anti-infective implants in order to prevent persistent infections related to medical devices.

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Poster presentation

682 The effect of hydroxyl ion concentration on the degree of polarization in thermally sprayed hydroxyapatite

Darta Ubele, Liene Pluduma, Karlis Gross
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INTRODUCTION:

Bone healing can be stimulated by biological factors, chemical cues and even by an electrical field. Since electrical stimulation can improve bone healing after bone fracture[1], implants are being produced with charged surfaces for faster bone growth and better osteointegration.[2] Previous work has focused on altering the electric field during polarization, but another approach is to identify the dipoles that present a charge and vary the concentration of charge generators.

Thermally sprayed hydroxyapatite (HAp) contains dipoles, has a good biocompatibility[3], and so is suitable for this study. Hydrothermal treatment (HT) increases the depleted hydroxyl ion (OH^-) concentration by incorporating OH^- in the HAp structure.[4] In this study, we show how the OH^- concentration influences the degree of polarization.

METHODS:

Hydroxyapatite powder was flame-sprayed onto titanium plates. Coatings were hydrothermally treated in a Teflon-lined custom-made pressure vessel at 250°C for 12h. The OH^- content was determined from the intensity of the OH^- librational peak in the FTIR specra after peak fitting.

Coatings were polarized between two electrodes, in a 1kV/cm electric field, heated to 300°C for 1h, then cooled to room temperature. Thermally stimulated depolarization current (TSDC), that is related to the charge, was measured with Keithley 6487 picoammeter.

RESULTS AND DISCUSSION:

The increase in OH^- content from 25±3% (in the as-sprayed coating) to 92±1% (after hydrothermal treatment) represents a large range in the OH^- ion content, shown in Fig 1, supporting the inquiry on the effect of OH^- concentration. The maximum current density, determined from TSDC spectra, was 0,65nA/cm^2 for the low OH^- content polarized coating and 1,09nA/cm^2 for the high OH^- content coating. Previous work from others reported 0.41nA/cm^2 for a coating polarized at the same conditions[5], but we achieved a higher current density to show the contribution from the higher OH^- content. Polarization conditions including temperature, time and field strength influence the generated charge[2,5], and will be further studied.

CONCLUSION:

HAp coatings displayed a higher current density from high OH^- content (92±1%) coatings showing that the OH^- content is an important contributor to the generation of surface charge.

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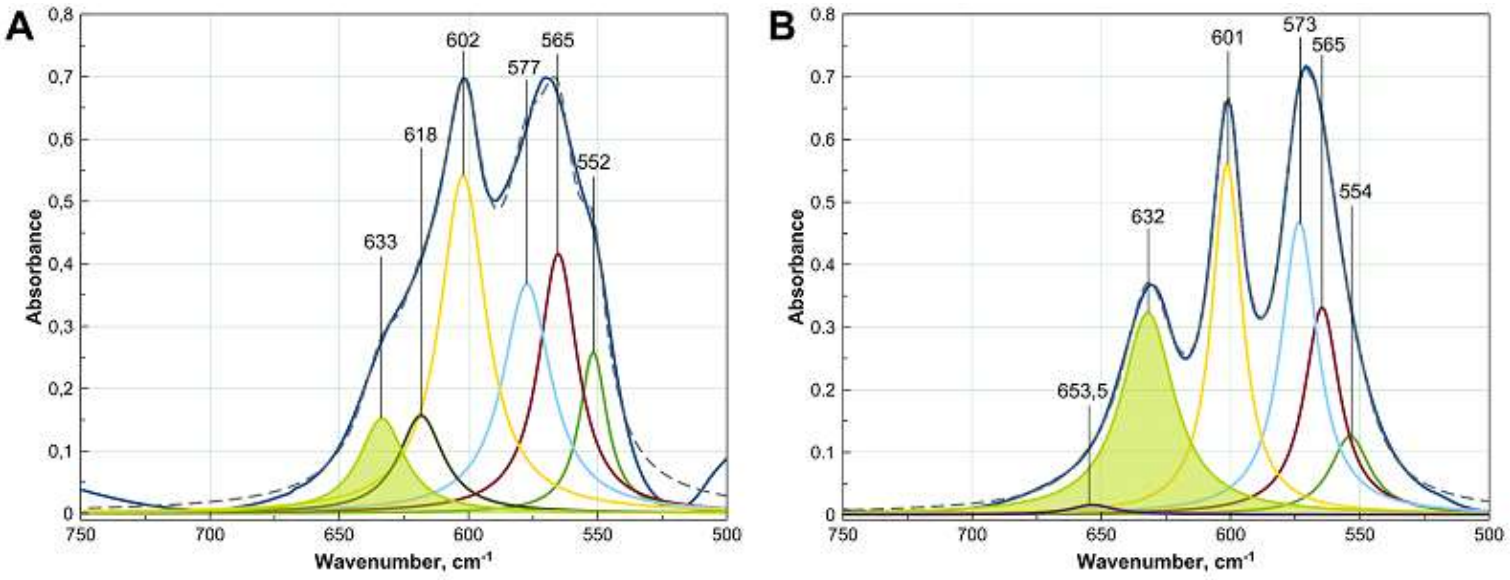
ACKNOWLEDGMENTS:

The authors thank the M-ERA.NET “Signaling Implant” project (Grant no: ESRTD/2017/4) for providing financial support to this project.



Picture 1: Caption 1: Figure 1. Spectral decomposition of FTIR spectra for coating with low OH- content (A) and high OH- content (B).

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Poster presentation

690 Development of a tough polycaprolactone-based photo-curable resin

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INTRODUCTION:

Polycaprolactone (PCL) is a versatile resorbable semi-crystalline polymer used in a variety of biomedical and tissue engineering applications[1]. The mechanical properties of previous photo-curable PCL networks have been poor due use of low molecular weight PCL triol (1500 g/mol) being processed in the melt[2]. We hypothesised that using appropriate solvents would enable to formulate photo-curable resins containing higher molecular weight PCL macromers, hence producing networks with greatly improved mechanical properties through stereolithography 3D printing (SL).

METHODS:

Photo-crosslinkable macromonomers were prepared by methacrylating 2,000 and 10,000 g/mol PCL diol oligomers. Macromer was dissolved in benzyl alcohol and mixed with diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO) UV photo-initiator. Networks of intermediate molecular weights were produced by blending the original macromers. Resin was dispensed into moulds and cured in a UV crosslinker. Samples were removed and extracted in isopropyl alcohol using Soxhlet apparatus. Following drying, an Instron mechanical tester was used to assess the tensile properties of the samples.

Resins for stereolithography also contained 0.1 wt% 2,5-Bis(5-tert-butyl-benzoxazol-2-yl)thiophene (BBOT) UV absorber. To increase the photo-reactivity of the resins, the addition of pentaerythritol tetraacrylate was explored as well.

RESULTS AND DISCUSSION:

The samples indicated that tensile strength, elongation at break and energy absorption all increase with average molecular weight of the crosslinked resins. The tensile strengths ranged from 1.2 MPa to 15.6 MPa, elongation at break from 51 % to 303 % and the total energy absorbed by the samples over the tensile method ranged from 0.3 to 44.6 J/mm^3. Previously reported PCL networks had a tensile strength of 2.87 MPa, and a 78.5 % elongation at break[2].

A stereolithography resin was formulated with benzyl alcohol, pentaerythritol tertraacrylate, TPO and BBOT. Printing of pyramids, and rectangles with 1 mm^2 channels has been performed.

CONCLUSION:

Using solvents has enabled production of tough photo-cured networks of PCL.

Additionally, by using a combination of benzyl alcohol and pentaerythritol tetraacrylate it has been possible to use SL to print objects using over six times higher molecular weight PCL than previously demonstrated, while using an unmodified budget SLA printer.

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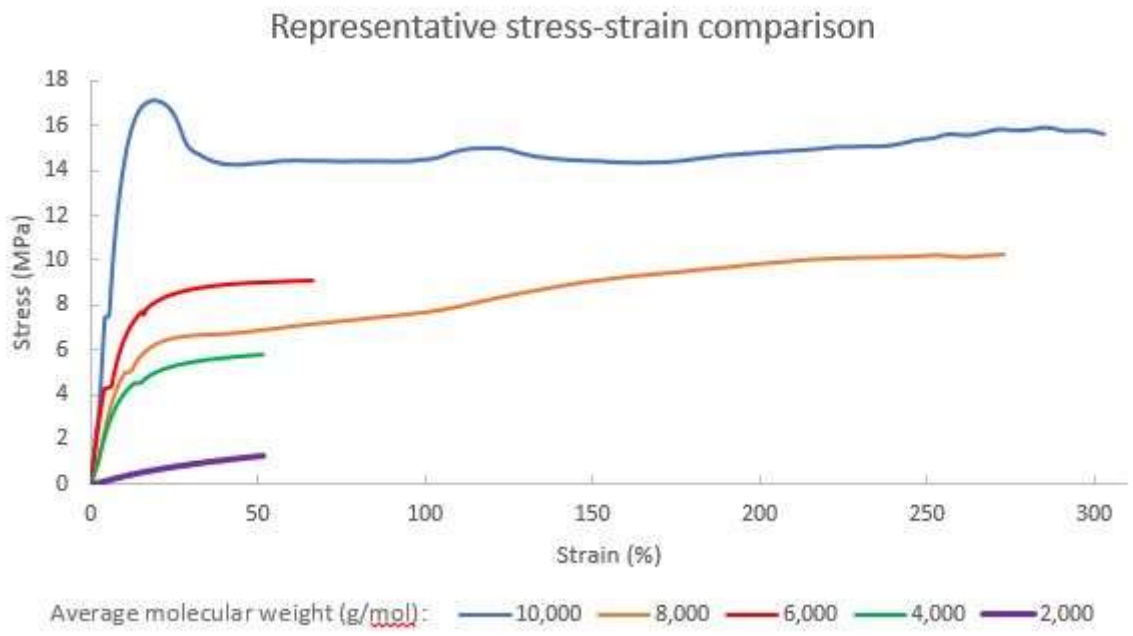
ACKNOWLEDGMENTS:

Thanks to The Engineering and Physical Sciences Research Council (EPSRC) for funding the Doctoral Training Partnership.

Picture 1:



Caption 1: Figure 1: Tensile testing results

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Poster presentation

696 Optimization of supercritical fluid-based decellularization

Rabea Loczenski, Cameron Alexander, Steven Howdle, Lisa White
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INTRODUCTION:

Organ transplantation comes with many risks, such as organ rejection, lifelong immune suppression, and drastically reduced life expectancy. Using an extracellular matrix (ECM) derived tissue scaffold to engineer a new personalised organ is a promising alternative that can minimize these risks due to the biochemical and biophysical properties of the ECM [1]. Current decellularization methods used to generate ECM scaffolds utilize a combination of chemical and biological agents that disrupt the ECM architecture and surface structure and may leave residual detergents and toxicity behind [2]. Herein, supercritical carbon dioxide was investigated for its decellularization efficacy as a non-toxic and safe alternative.

METHODS:

Pig liver (1g), stored at -20°C before use, was exposed to supercritical carbon dioxide ($scCO_2$) in a 20ml autoclave at ~2900psi at 37°C for varying durations. Batch and flow systems were compared. A post-$scCO_2$ exposure wash step was also tested. Residual DNA content was used as a metric of decellularization. DNA concentration (ng/ml) was determined using the Quant-iT Pico Green (Invitrogen) assay kit and normalized to dry tissue weight. DNA fragmentation was assessed by gel electrophoresis. Histology was performed to evaluate morphological changes. Statistical analysis was performed using PRISM (GraphPad). Significance was accepted if $p<0.05$.

RESULTS AND DISCUSSION:

Exposure to $scCO_2$ for >5 hours reduced liver DNA content compared to controls. Short exposure to $scCO_2$ reduced DNA content only when followed by a 24-hour wash. Previous publications on supercritical fluids for decellularization have utilized ethanol [3] [4] or a hybrid detergent/$scCO_2$ [5] method to successfully reduce DNA content.

CONCLUSION:

$scCO_2$ alone reduced cellular content (as measured by DNA content), which is required for effective decellularization. Future studies will investigate the full potential of $scCO_2$ in combination with low concentrations of $scCO_2$ soluble detergents to further improve the level of DNA reduction whilst maintaining low residual toxicity. To the best of our knowledge, this is the first report of successful removal of DNA content using $scCO_2$ alone.

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ACKNOWLEDGMENTS:

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Picture 1:

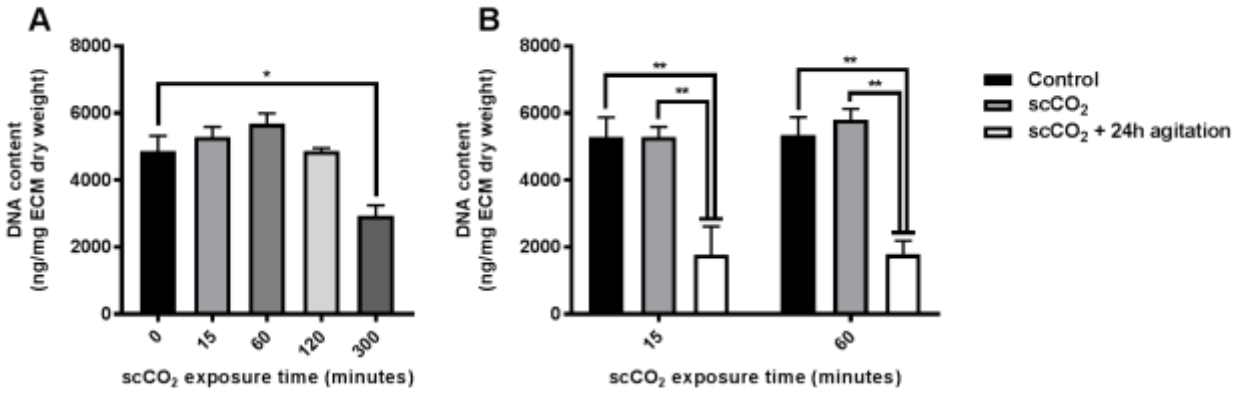

Figure 1. Effect of $scCO_2$ exposure on liver DNA content
(A) Effect of $scCO_2$ exposure time on liver DNA content. **(B)** Effect of combined $scCO_2$ exposure followed by agitation in water. All data are mean +/- SEM (n=3). Significance is indicated by * p<0.05, ** p<0.01

Caption 1: Effect of scCO2 exposure on liver DNA content

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

699 Magnesium powder reinforced PLA filaments for 3D printing process

Iulian Antoniac[1], Diana Popescu[1], Aurora Antoniac[1], Claudia Milea[1], Aura Andreea Gheorghe[1], Aurel Mohan[2]
[1]University Politehnica of Bucharest, Bucharest, Romania
[2]University of Oradea, Romania

INTRODUCTION:

Since 1988 a large number of applications, especially in the medical field, were developed using Additive Manufacturing (AM) technology: personalized surgical guides, tissue-engineering scaffolds, drug dosing, customized implants, etc[1,2]. Some of these applications are based on Fused Deposition Modeling (FDM) process, which usually uses thermoplastic polymers. Literature shows several studies on developing new composites for FDM process, two of them being considered: (1) Producing feedstock filament by mixing matrix and reinforcements[3,4]; (2) Inserting continuous carbon fibers into the matrix or inserting continuous natural fibers in different polymers inside the extrusion nozzle by "in-nozzle impregnation"[5,6]. The purpose of this study was to prove the viability of obtaining Mg reinforced PLA as filament feedstock for AM based on FDM process.

METHODS:

Two compositions of PLA-Mg particles were prepared using different amount of Mg particles. Using this compositions and vitamin E as precursor, we obtain, by extrusion, filaments for 3D printing process. In order to analyze both PLA filament and the two new PLA-Mg filaments we used a scanning electron microscope type Philips ESM XL40SEM. In order to analyze the integration of the Mg particle in PLA matrix, a SEM analysis was performed on the filaments after their fracture.

RESULTS AND DISCUSSION:

The diameter of the filament containing Mg was less constant; it varied between 1.11 mm and 1.17 mm. According the SEM analysis in the fracture area it can be observed a good integration of Mg particles due to the use of vitamin E as precursor. The implants screws were manufactured at a extrusion temperature of 200^{o}C and 50^{o}C bed temperature, these values being chosen after analyzing the samples obtained by varying process parameters.

CONCLUSION:

Our results proved that Mg reinforced PLA filaments can be produced and used for 3D printing process. Another aspects revealed by the study is related to the use of vitamin E for ensuring Mg powder adherence to the PLA pellets.

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ACKNOWLEDGMENTS:

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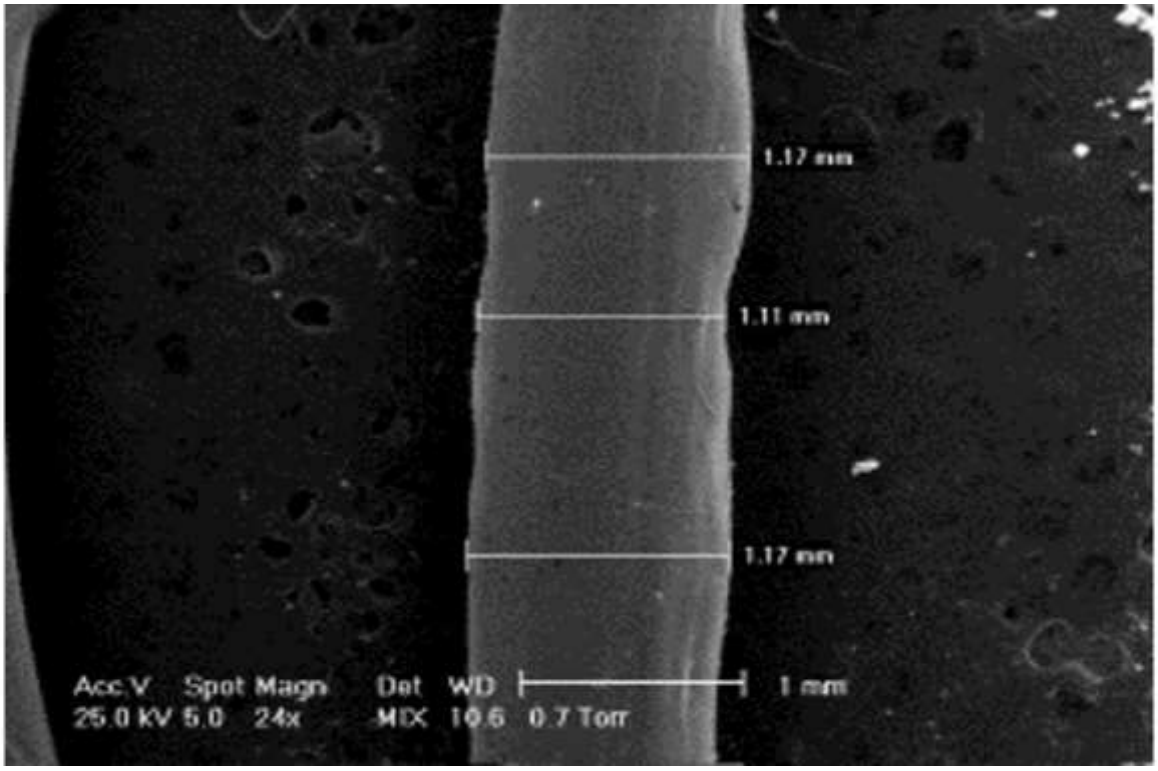

Picture 1: Caption 1: SEM image of the experimental PLA-Mg filament

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Poster presentation

707 Effect of the sandblasting process on the surface properties of Co-Cr alloy

Aurora Antoniac[1], Iulian Antoniac[1], Petrica Vizureanu[2], Mirabela Minciuna[2], Claudia Milea[1], Anton Budici[1], Ileana Mates[1], Aurel Mohan[3]
[1]University Politehnica of Bucharest, Bucharest, Romania
[2]Gheorghe Asachi Technical University of Iasi, Romania
[3]University of Oradea, Romania

INTRODUCTION:

Metallic dental alloys are knowns due to their very good durability, mechanical resistance, high elastic modulus and biocompatibility characteristics compared to other conventional alloys. Dental alloys became more diverse in time, depending on the technology applied and the characteristics required for a specific type of dental prosthesis[1,2]. This paper presents the effect of the sandblasting process on the surface properties of some cobalt-chromium alloys for dentistry before and after the immersion in simulated body fluid (SBF).

METHODS:

Sandblasting process was used to create micrometer-sized pores on the surface of cobalt-chromium (Co-Cr) alloys[3]. Surface modifications of the experimental samples was performed for 15 s with Al_2O_3 particles (average particle size 75μm, respectively 125 μm) at a pressure of 2 bars, using a SAB- Caloris equipment. Scanning Electron Microscopy (SEM) coupled with Energy Dispersive Spectroscopy (EDS) was used for the surface morphology characterization of the samples. The wettability assessment by determining the contact angle and free surface energy was performed using a Drop Shape Analyzer DSA30, manufactured by KRÜSS GmbH. To highlight the bioactive character of the Co-Cr alloys, the samples were soaked in SBF and heated. In order to evaluate the surface of the investigated samples before and after immersion in SBF, Scanning Electron Microscopy was used.

RESULTS AND DISCUSSION:

In this study, SEM analysis revealed the formation of micrometer-size pores on the surface of the sandblasted samples, pores in which the apatite nuclei were precipitated.

CONCLUSION:

Based on our results, we consider that the bonelike apatite layer was formed on samples of Co-Cr alloys after these were soaked in SBF. As analyzed by SEM and EDS, all the samples formed apatite crystals on their surfaces after 7 days of immersion in SBF and an homogeneous apatite layer after longer immersion times. The results indicate that the rate of apatite formation increases after the sandblasting process.

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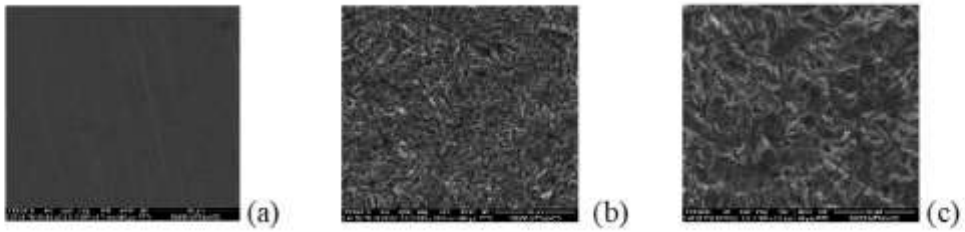

Picture 1: Caption 1: SEM images of the Co-Cr samples before soaking in SBF: (a) Co-Cr before sandblasting; (b) sandblasted (size 75μm); (b) sandblasted (size 125μm)

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

717 Hydroxyapatite coatings by electrophoretic deposition on biodegradable magnesium alloys

Claudia Milea[1], Iulian Antoniac[1], Aurora Antoniac[1], Corneliu Munteanu[2], Bogdan Istrate[2], Daniel Pop[1], Elisa Plopeanu[1], Ionut-Bogdan Balan[1], Aurel Mohan[3]
[1]University Politehnica of Bucharest, Bucharest, Romania
[2]Gheorghe Asachi Technical University of Iasi, Romania
[3]University of Oradea, Romania

INTRODUCTION:

Biodegradable implants must meet a number of specific properties including an adequate stability, full bone regeneration within 12–15 months, a moderate degradation and biocompatibility[1,2]. However, Mg-based alloys can corrode too rapidly in the high-chloride environment of the physiological system, and losing their mechanical properties before the tissue has sufficiently healed. Hydroxyapatite coating was proposed by different research group to be used in order to decrease the corrosion rate and improve the bioactivity of Mg-based alloys[3,4,5,6,7]. Electrophoretic deposition is a method to obtain uniform coatings, even with complex geometries. The aim of this study was to increase the corrosion resistance and to reduce the biodegradation of some Mg-based alloys by coating them with bovine hydroxyapatite using electrophoretic deposition.

METHODS:

The biodegradable magnesium alloys used in this study was ZMX 410 and ZM 21. Electrophoretic deposition experiments were performed at 37 V using a regulated DC power supply, for 3 minutes. The experiment took place at ambient temperature and constant applied voltage. After coating process, the samples were sintered at 400 ^{o}C for one hour to densify the material and improve the coating adhesion. The electrochemical behaviour of samples was investigated by potentiodynamic polarization tests conducted in simulated body fluid (SBF). For characterization of bovine hydroxyapatite before and after coating was used X-ray diffractometry. In addition, the surface morphologies of the samples before and after corrosion were analysed using scanning electron microscopy.

RESULTS AND DISCUSSION:

Between the two studied magnesium alloys, ZMX410 and ZM21, we can see that ZMX410 alloy has a better corrosion response, SEM images SEM images highlight a pitting-type corrosion for the uncoated ZMX410 alloy and a crevice corrosion for the hydroxyapatite-coated ZMX410 alloy.

CONCLUSION:

Based on the experimental results, we observe the stability and uniformity of the coating. Also, the corrosion properties was improved after the coatings. Future studies must be made in order to establish clearly the advantage of this deposition technique in the case of hydroxyapatite coatings on Mg-based alloys.

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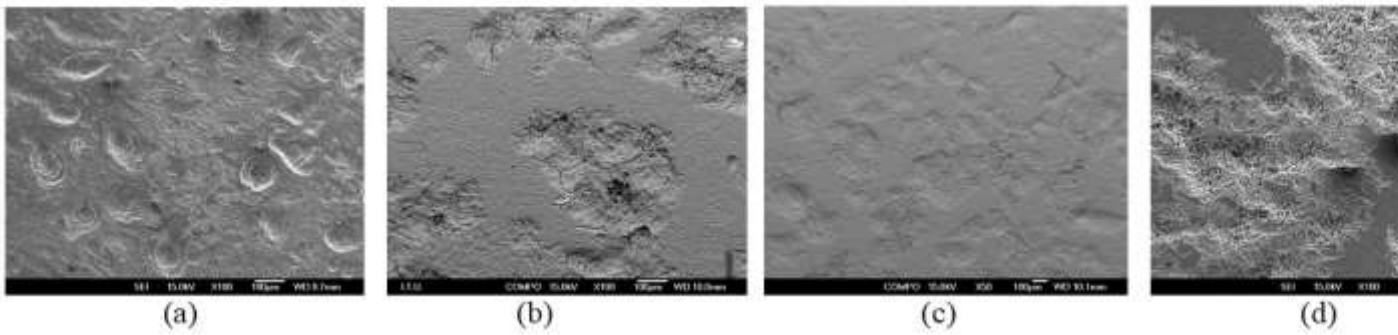

Caption 1: SEM images after corrosion test in SBF of: (a) uncoated ZMX410 alloys; (b) coated ZMX410 alloys; (c) uncoated ZM21 alloys; (d) coated ZM21 alloys

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

727 In vivo evaluation of an injectable hybrid system for strontium local delivery in a sheep vertebrae model

Cláudia Ribeiro-Machado[1], Ana Henriques Lourenço[2], Nuno Neves[2], Nuno Alexandre[3], Meriem Lamghari[2], Mário A. Barbosa[2], Cristina C. Ribeiro[2]
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[2]I3S - Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Portugal
[3]Departamento de Zootecnia da Universidade de Évora, Portugal

INTRODUCTION:

When bone regeneration is required beyond the normal potential for self-healing, such as in the case of lesions caused by trauma or tumor resection, or when the regenerative process is compromised, as for instance in osteoporosis, the use of bone substitute materials is necessary. In our group, we developed an injectable hybrid system for bone regeneration composed of Sr-doped hydroxyapatite microspheres embedded in a Sr-crosslinked alginate matrix. Strontium (Sr), is described as a promoter of bone formation while inhibiting bone resorption[1]

Previous results from our group showed that this system is able to promote bone regeneration in a rat critical-sized defect model[2]. The present work investigates the effect of the Sr-hybrid system in an *in vivo* vertebrae sheep model.

METHODS:

A critical sized-defect (4.5x3.6 mm), adapted from Lamghari et al[2], was made in the body of lumbar vertebrae of 5 years old Merino Branco sheeps and filled with the Sr-hybrid system. Vertebrae were evaluated at one and eight weeks post-implantation by micro computed tomography and histological analysis after methylmethacrylate embedding of bone specimens. Soft tissues histology and ICP-AES analysis of microwave digested organs (liver and kidneys) were performed to evaluate strontium systemic effects.

RESULTS AND DISCUSSION:

After one week, inflammatory cell migrated towards the center of the defect and newly formed bone at the periphery was observed. Eight weeks post-implantation, a thick mature bone structure in the surroundings of the defect was found, as well as extended granulation tissue infiltration into the center, including neovascularization. Functional newly formed bone was confirmed by mineralization regions with osteoblasts and bone resorption pits with osteoclasts. No structural changes or measurable Sr levels were detected in the retrieved organs indicating that the hybrid material acts only as a local Sr delivery system.

CONCLUSION:

The hybrid system provided a scaffold for cell migration and promoted local bone formation, without detectable systemic effects. Our results suggest that this material may be a promising alternative for bone regeneration, particularly in osteoporotic patients.

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Poster presentation

728 Bioengineering of silk to enhance its capacity to bind the iron oxide nanoparticles

Kamil Kucharczyk, Aleksandra Smigielska, Andrzej Mackiewicz, Hanna Dams-Kozlowska
Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland

INTRODUCTION:

Spider silks are biomaterials which combine unique mechanical properties, biocompatibility and biodegradability. Silk can be processed into different morphological forms such as fiber, film, hydrogel, scaffold, capsules, micro- and nanospheres. Moreover, the genetic engineering enables to obtain silk of modified properties, which may be useful for composite materials production[1]. Iron oxide nanoparticles can be applied in the biosensing, target drug delivery, as contrast agents in MRI, and as therapeutic agents for hyperthermia-based cancer treatments. The study aimed to develop iron oxide/silk composite material for the theragnostic application. We genetically modified the bioengineered spider silk with sequences that encode peptides responsible for metal ions binding.

METHODS:

Three bioengineered spider silks were constructed – $(MS1Fe1)_2$, $(MS1Fe2)_2$ and MS1Fe1MS1Fe2. The proteins consisted of silk MS1(12mer) (based on the repetitive motif of MaSp1 spidroin from Nephila clavipes) and two peptides capable of binding the metal ions named Fe1 and Fe2. The functionalized and control (sMS1) bioengineered silks were produced in E. coli expression system, purified using thermal denaturation method and analyzed using SDS-PAGE electrophoresis. Silk films were cast on coverslips and then incubated with iron oxide nanoparticles. Spheres were formed by mixing high concentration of potassium phosphate buffer with silks solution and iron oxide nanoparticles (NPs) suspension (of positive or negative charge). The morphology and elemental composition of obtained films and spheres were determined using SEM/EDS microscopy. Prussian blue assay analyzed iron oxide nanoparticles presence.

RESULTS AND DISCUSSION:

The functionalized and control sMS1 bioengineered spider silks were successfully constructed, produced and purified. The functionalization of silk did not impede its self-assembly property, and films and spheres were obtained. The analysis of silk films indicated that Fe1 peptide enhanced binding and decreased the release of iron oxide NPs. The SEM/EDS analysis demonstrated that the negatively charged NPs were faster bounded to the $(MS1Fe1)_2$ film than positively charged NPs. The SEM/EDS analysis of composite silk/NPs spheres indicated that both functionalized silks increased the binding capacity of NPs comparing with control silk, and the Fe1 peptide bounded more NPs than Fe2 peptide. The binding of negatively charged NPs resulted in the formation of spheres with more separated and more spherical morphology than spheres with positive NPs.

CONCLUSION:

Functionalization of silk with peptides responsible for metal ions binding increased the affinity of magnetic nanoparticles to the silk. The silk/NPs composite material can be a promising tool for theragnostics.

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

732 Development of Well-Defined Biodegradable Microparticles Based on Poly(trimethylene carbonate)

Gozde Sahin[1], Seha Tirkes[2], Erhan Bat[1]
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[2]Atilim University, Turkey

INTRODUCTION:

Biodegradable microparticles are promising carriers for controlled release applications. Recently, it has been shown that aspect ratio and edge geometry have high influence on *in vivo* circulation time of particles[1]. One of the most promising particle fabrication methods is Particle Replication in Non-wetting Templates (PRINT). With this technique, it has been tried to produce particles from polymers frequently used in biomedical applications[2]. Poly(trimethylene carbonate) (PTMC) is a biodegradable polymer with surface erosion behavior that is desirable for drug delivery applications. Herein, PTMC based microparticles were produced using PRINT technique.

METHODS:

Three-armed methacrylate functionalized PTMC oligomers were synthesized to use as resin. Perfluoropolyether (PFPE) was spread onto silicon master which have indentations and photocrosslinked. PTMC pre-particle resin was poured into PFPE mold cavities and exposed to UV radiation. After photocrosslinking, free particles were harvested from the mold by sonication.

RESULTS AND DISCUSSION:

The synthesized three-armed PTMC oligomers were characterized via ^{1}H NMR analysis. PFPE mold was fabricated and morphology was observed by scanning electron microscopy (SEM). An example of a silicon master with rectangle protrusions, replicated PFPE mold with indentations and isolated rectangular prism harvested on a filter paper was shown in Figure. Approximately, silicon master with 1.6 x 7 µm rectangle patterns was obtained and PFPE mold with the same dimensions with 2 µm depth was replicated. It has been observed that PTMC particles mainly retain the shape and dimensions of the mold since there is no considerable cure shrinkage. In this way, cylindrical and rectangular prism isolated particles with 100 µm, 50 µm, 25 µm, 8 µm, 5 µm and 2 µm characteristic length have been produced.

CONCLUSION:

PTMC based microparticle fabrication by using PRINT technique have been demonstrated. Non-wetting molds based of perfluoropolyether were produced. The mold cavities could be filled with functionalized oligomers without formation of any scum layer. Cylinder and rectangular prism shaped PTMC particles have been fabricated. These isolated particles have the potential to use in controlled drug delivery applications.

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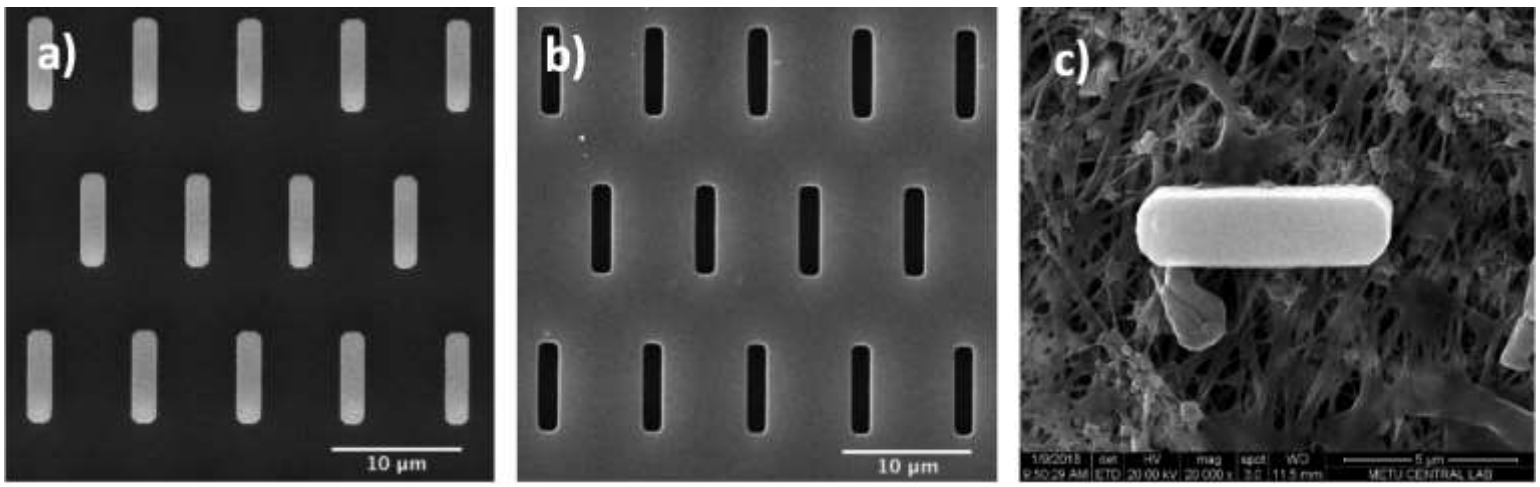

Picture 1: Caption 1: 1.6 x 7 µm a) rectangle protrusions produced on silicon wafer, b) replicated PFPE mold with rectangle indentations, c) isolated rectangular prism

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Poster presentation

745 Surface modification of tissue carriers by partial enzymatic degradation

Kristýna Havlícková, Vera Jencová, Katerina Strnadová, Jana Hlavatá
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INTRODUCTION:

This study was focused on the modification of micro/nanofibrous surface of planar layers prepared from biodegradable aliphatic polyesters by partial degradation. The aim was to positively influence the interaction of these fibrous tissue carriers with cells.

METHODS:

Fibrous layers were prepared by electrospinning of PCL, PLCL and blends of both polymers in different ratios. The surface morphology was modified by enzymatically catalyzed hydrolysis. The used enzymes were responsible for the hydrolytic cleavage of the polymeric chains of the aforesaid polymers. The degradation was catalysed by two enzymes. Lipase from *Pseudomonas cepacia* was used to cleave PCL, proteinase K from the *Tritirachium album* was used to cleave PLCL. The degradation experiment lasted for five days. The surface analysis and morphology characterization (SEM, BET analysis, determination of contact angle and wettability), molecular weight change analysis (GPC) and thermal analysis (DSC) were performed with all materials. The materials were also subjected to *in vitro* testing with 3T3 mouse fibroblasts.

RESULTS AND DISCUSSION:

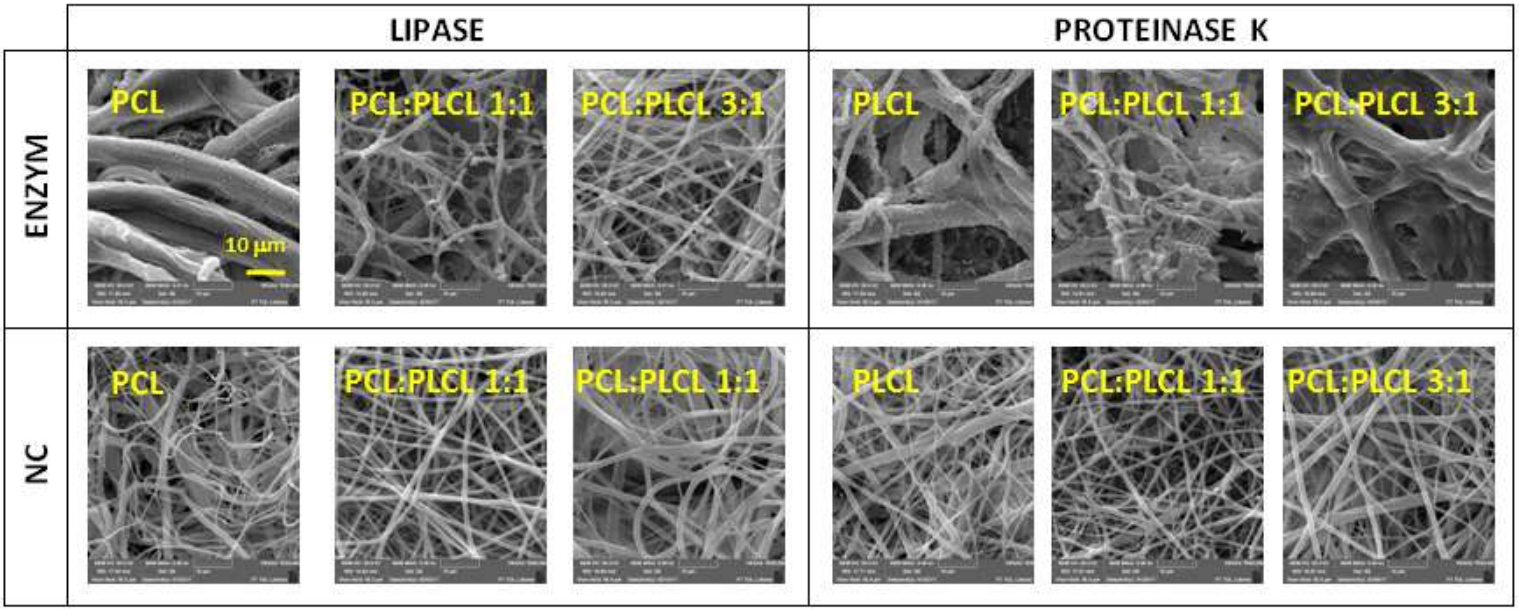
Significant changes in the morphology of the fibrous layers can be clearly seen on the SEM images (Fig. 1). These results are also supported by the surface analysis, GPC and DSC analysis and it is important to point out the very different behavior of the fibrous layers of polymer blends made in different ratio of PCL and PLCL (Fig. 1). The interaction of fibrous carriers with cell was subsequently affected by the type of material.

CONCLUSION:

The process of partial enzymatic degradation, i.e. degradation, which is controlled by accurate loading of enzyme units, so that polymer chains can be cleaved only from the surface of the fibrous layers, can affect the fibre morphology. This targeted modification may, along with the appropriate type of polymer or polymer blend, provide favorable conditions for the interaction of the fibrous supports scaffolds with cells.

ACKNOWLEDGMENTS:

The authors would like to thank to Student Grant Competition for 2018 at Technical University of Liberec for providing financial support to this project.



Picture 1: Caption 1: Fig. 1: Scanning electron micrographs (SEM) of PCL, PLCL, PCL:PLCL(1:1; 3:1) blends after four days of partial degradation process by lipase from Pseu

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Poster presentation

747 3D bioprinting of nanocomposite hydrogel bioinks based on agarose with enhanced bioactivity

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Sabanci University, Istanbul, Turkey

INTRODUCTION:

Agarose is a bioinert, non-interacting with cells hydrogel due to the lack of necessary cell binding sites in the backbone, and previous studies showed its incompatibility with extrusion-based 3D bioprinting processes. Recently, nanocomposite hydrogels based on nanosilicates showed a great potential in formulation of bioinks and incorporation of Laponite nanosilicates resulted in enhancement of printability of polysaccharides[1]. In this study, a novel approach in synergistic tuning of biocompatibility and printability of agarose hydrogels was investigated. Here, Laponite nanosilicates were used to modify flow properties to meet extrusion-based 3D bioprinting process requirements and at the same time to induce bioactivity.

METHODS:

Bioink formulations were prepared by dispersion of Laponite RD nanosilicates (2 wt%, BYK) in distilled water followed by addition of agarose powder (3 wt%, Lonza) and autoclaving at 115 ˚C for 15 min. Rheological characterizations were performed using MCR 302 rheometer (Anton Paar). 3D bioprinting was performed by a three-axes robotic platform equipped with a pneumatic dispensing unit through a 22-gauge needle at 37 ˚C. The metabolic activity of the encapsulated NIH-3T3 cells were assessed at different days of culture using PrestoBlue Cell Viability Reagent normalized to those of agarose cell laden hydrogel samples. A cell spreading assay was performed by confocal imaging of NIH/3T3 cells seeded on nanocomposite hydrogel discs. Data were evaluated by two-way analysis of variance followed by Tukey multiple comparison tests ($p<0.05$).

RESULTS AND DISCUSSION:

Rheological characterization showed a significant increase in yield stress and an enhanced shear thinning behavior prior gelation of the nanosilicates based bioinks, while the gelation temperature increased from 31 to 33 ˚C. This resulted in a highly printable bioinks which could be printed to form 3D structures with high integrity and fidelity. The role of Laponite nanosilicates in adjusting flow behavior and gel formation was mainly attributed to its unique heterogeneous charged structure which can interact with a wide range of anionic, cationic and neutral organic molecules. The encapsulated cells in nanocomposite hydrogels showed significant increase in metabolic activity compared with agarose hydrogel while Laponite nanosilicates induced cell spreading and morphogenesis at short incubation times due to their charged nature[2].

CONCLUSION:

Incorporation of Laponite into agarose hydrogel matrix resulted in significant enhancement of bioactivity and flow behavior of nanocomposites. Our findings showed that combination of enhanced shear thinning behavior with improved bioactivity resulted in formation of nanocomposite hydrogels which can be adapted to extrusion-based 3D bioprinting with excellent printability.

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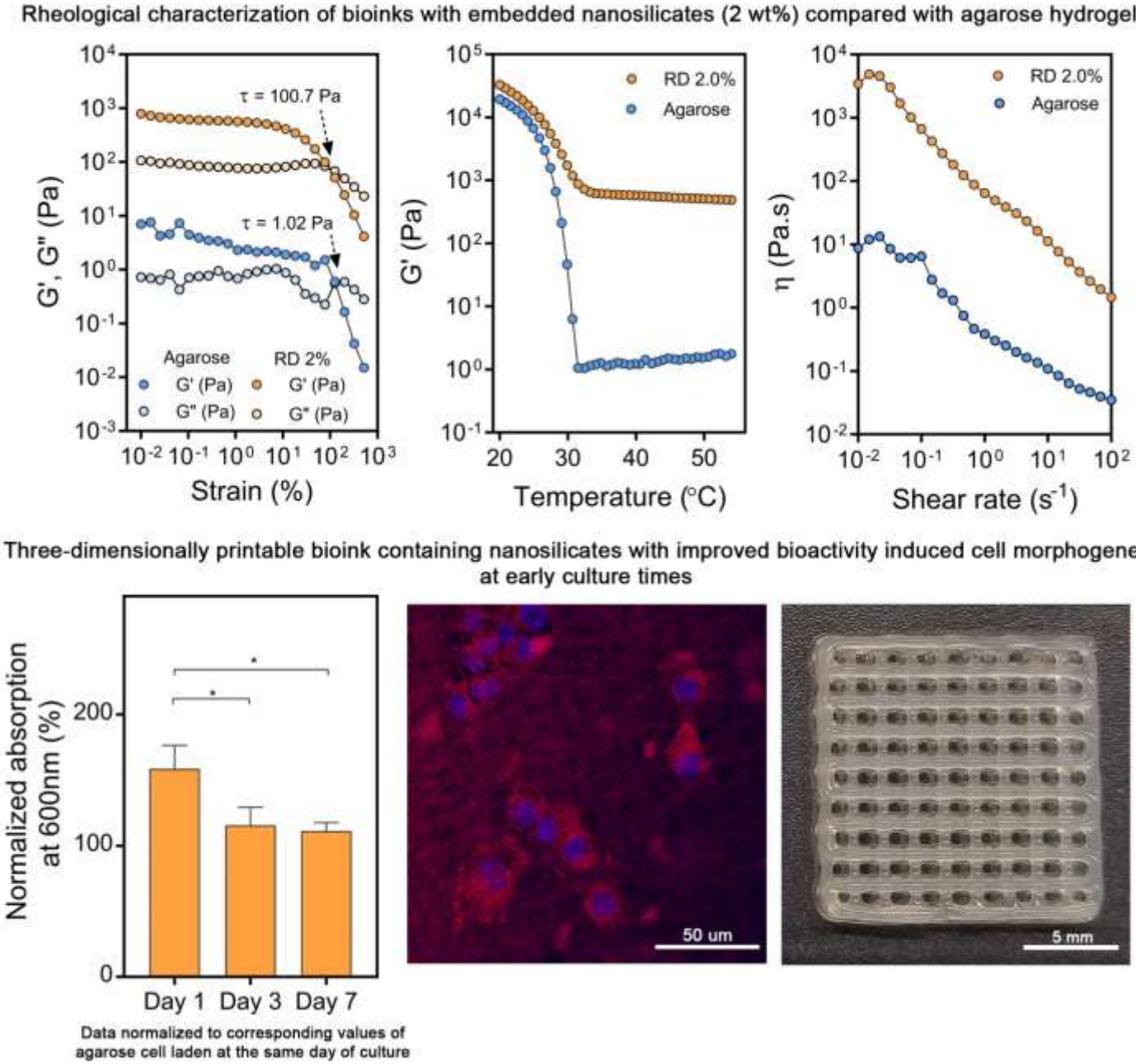

Picture 1: Caption 1: Rheological characterization and bioactivity of bioinks containing nanosilicates in comparison with pure agarose hydrogel

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Poster presentation

750 Simulation of the Vroman effect at a biomaterial interface using Brownian dynamics

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INTRODUCTION:

When a medical implant comes into contact with the human bio-system, proteins from the body fluid adsorb to the material surface in one of the first steps. The formed protein layer is frequently considered to be a key factor for regulation of the subsequent cell adhesion and consequently, many studies focus on protein adsorption. However, the interpretation of protein data from a living body is complex, because the body fluid contains a cocktail of different proteins which all adsorb in competition with each other. During this competing adsorption, the implant surface is initially occupied by small and mobile proteins, which are later replaced by proteins with a more attractive interaction to the substrate (Vroman effect)[1]. Interestingly, the reordering process is slow and proceeds on about the same time scale as the initial cell adhesion. Therefore, it may be of great importance for the biocompatibility of an implant material, e.g. if a cell adhesion inhibiting protein is exchanged by a cell adhesion promoting protein.

METHODS:

To further improve the understanding of the influence of the Vroman effect on biocompatibility, a software algorithm based on Brownian dynamics[2,3] was developed and the competitive adsorption of lysozyme and serum albumin was simulated in a preliminary study. The simulation box consists of the implant located at the bottom and a large protein reservoir at a great distance above it. Periodic boundary conditions were used for all other directions. In addition to steric repulsion, electrostatic and Van-der-Waals interactions between the proteins and the material surface as well as for the mutual interactions between the proteins were considered. The proteins were initially located in the protein reservoir and the movement trajectory of each protein was calculated during the relaxation of the system. The ions of the body fluid were not explicitly calculated, but their influence on the interaction potentials was considered.

RESULTS AND DISCUSSION:

The calculations demonstrate that protein rearrangement at a biomaterial surface can be basically understood with the help of Brownian dynamics simulations. First results show a significant dependence of the Vroman kinetics on the surface charge of the biomaterial and on the concentration of ions.

CONCLUSION:

The computer simulations demonstrate that the rate at which protein rearrangement occurs at a biomaterial surface depends on material properties like the surface charge. In the future the developed algorithm can help to better interpret protein adsorption data.

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ACKNOWLEDGMENTS:

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

761 Advancement of clickECM technique by metabolic glycoengineering using synthetic dienophile-modified monosaccharide derivates

Svenja Nellinger[1], Mareike Rapp[2], Valentin Wittmann[2], Monika Bach[3], Petra Kluger[1]
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INTRODUCTION:

Due to its biological complexity natural extracellular matrix (ECM) represents an ideal biomaterial for tissue engineering and regenerative medicine. However, there is a need for specific addressable functional groups to achieve individual chemical and physical properties. This was achieved by the clickECM technique using metabolic glycoengineering with azide-modified monosaccharide derivates [1]. In this study, synthetic dienophile-modified monosaccharides were used instead of azide-modified monosaccharides. The modification of the ECM with dienophiles makes it possible to create a new ECM with alternative addressable functional groups using diels alder reaction. This opens new perspectives to create ECMs as a smart biomaterial with individual chemical and physical properties.

METHODS:

Adipose-derived stem cells (ASCs) were isolated from adipose tissue obtained from patients undergoing plastic surgery. To investigate the cytotoxicity and the influence on ASC viability and proliferation LDH assay, WST I assay and BrdU assay were performed. For generation of modified ECM, dienophile-modified monosaccharide derivates were added into the cell culture medium. Their incorporation efficiency into the ECM was proven by biorthogonal ligation (diels alder) with fluorescent labeled tetrazine. Modified ECM was characterized by immunohistochemistry of collagen I, collagen IV and fibronectin. Additionally histochemical staining were performed. Further the influence of the modified ECM on adhesion, viability and proliferation of ASCs was investigated.

RESULTS AND DISCUSSION:

LDH assay revealed no cytotoxicity of the monosaccharides. Further no significant differences of their influence on viability (WST I assay) and proliferation (BrdU assay) of ASCs can be observed. Therefore all the used monosaccharide derivates are suitable for clickECM technique by glycoengineering. Incorporation efficiency of the dienophile-modified monosaccharides into the ECM was successfully proven by diels alder reaction with fluorescent labeled tetrazine. Immunohistochemically staining of matrix proteins collagen I, collagen IV and fibronectin and histochemical staining alcian blue revealed no significant changes in composition of these protein between modified and unmodified ECM. Influence of dienophile-modified ECM on ASCs was successfully proven by live-dead staining, cell viability assays (WST I assay, LDH assay) and proliferation assay (BrdU assay).

CONCLUSION:

We successfully modified ECM of ASCs with dienophile-modified monosaccharides using clickECM technique of metabolic glycoengineering. This dienophile-modified ECM can be used to address further issues in tissue engineering and regenerative medicine.

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ACKNOWLEDGMENTS:

The authors would like to thank the “Ministerium für Wissenschaft, Forschung und Kunst” in Baden-Wuerttemberg, Germany for providing financial support to this project.

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Poster presentation

767 Fabrication of a bioengineered 3D printed calciumalkaliorthophosphate- bone graft with homogenously distributed osteoblasts and mineralizing bone matrix in vitro

Francesca Giacomini[1], Doaa Khattab[2], Barbara Peleska[3], Renate Gildenhaar[4], Cynthia Gomes[4], Ulf Linow[4], Jens Guenster[5], Micheal Stiller[5], Alexander Rack[6], Christine Knabe-Ducheyne[5]
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[6]European Synchrotron Radiation Facility, France

INTRODUCTION:

Over the last decade there have been increasing efforts to develop 3D scaffolds for bone tissue engineering from bioactive ceramics. Previously, we showed that a silica containing CAOP material displayed a greater stimulatory effect on osteoblast differentiation and bone formation *in vitro* and *in vivo* than β-TCP, bioactive glass. [1,2] The aim of the present study was to generate a tissue engineered synthetic bone graft with homogenously distributed osteoblasts and mineralizing bone matrix *in vitro,* which mimics the properties of autogenous bone grafts.

METHODS:

3D scaffolds were developed from a silica containing CAOP utilizing a replica technique (SSM) and a 3D printing (RP) technique. Mechanical and physical properties (porosity, compressive strength, solubility) and the potential to facilitate homogenous colonization by osteogenic cells and extracellular bone matrix formation were examined. Osteoblastic cells were dynamically cultured for 7d and 14d on both scaffolds with a concentration of $3x10^6$ cells/ml utilizing a TEB 1000 Ebers perfusion flow bioreactor. Morphology of the cells cultured for 7 days were examined by Scanning Electron Microscopy. The amount of cells, bone matrix formed and osteogenic markers expression were evaluated using histomorphometric, immunohistochemical analysis, Western Blot and qPCR. Confocal Laser Scanning microscopy of osteoblasts cultured on both scaffolds was performed after 7 days utilizing phalloidin labelling for detection of actin fibers and vinculin staining for analyzing cell-cell, cell-matrix interaction.

RESULTS AND DISCUSSION:

SSM scaffolds (SSMS) displayed greater total porosity (86.9%) than RP scaffolds (RPS) (50%). RPS had more open micropores (38%) than SSMS, in addition to greater compressive strength and silica release. RPS displayed greater cell and extracellular matrix formation and mineralization, higher osteocalcin and osteopontin expression than SSM scaffolds. SEM revealed that RP scaffolds displayed more evenly distributed cells when compared to SSM scaffolds. CLSM showed that RPS exhibited a higher cell concentration with cells displaying a more organized actin cytoskeleton and more cell-cell contacts than identical cells grown on SSMS. RPS displayed superior mechanical and biological properties. Analysis of osteogenic markers expression showed that cells cultured in RPS are in an advanced maturation state, while cells cultured in SSMS showed a delay in matrix maturation.

CONCLUSION:

RPS facilitated generating a tissue engineered synthetic bone graft *in vitro*, which mimics the properties of autogenous bone grafts. Therefore is suitable for subsequent *in vivo* implantation for regenerating segmental discontinuity bone defects.

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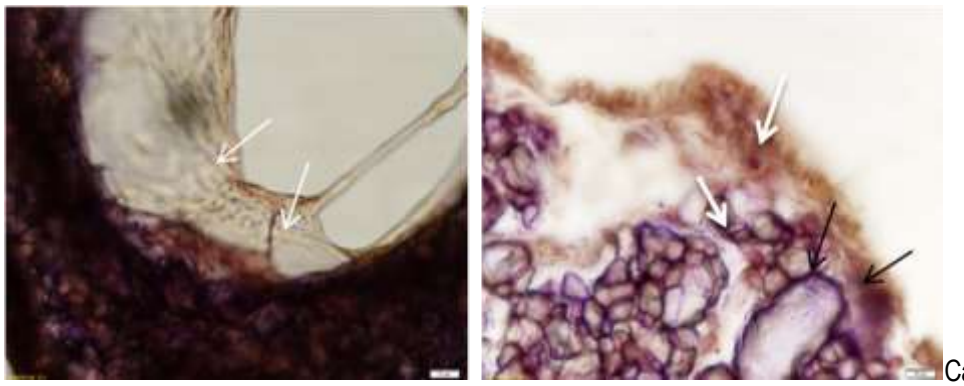
ACKNOWLEDGMENTS:

Funding by DFG Grant KN 377/8-1.

Picture 1:  Caption 1: Figure2. Histomicrographs of deacrylated sawed sections of osteoblasts cultured dynamically on (a) SSM scaffolds, immunohistochemically stained for ty

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Poster presentation

773 Evaluation of ceramic-based 3D-printed scaffolds for in-vivo bone regeneration: a systematic review

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[1]University of Bergen, Bergen, Norway
[2]KTH Royal Institute of Technology, Sweden

INTRODUCTION:

3D-printing of ceramic based scaffolds has been heavily introduced in different aspects of craniofacial surgeries in the past few years. In the current systematic review, we are analyzing the added value of 3D-printed scaffolds used to regenerate calvarial bone *in-vivo* during the last fifteen years. Only the potentially biodegradable ceramic passed printed scaffolds were reported; with and without cells or growth factors.

METHODS:

Database collection was completed by January 2018 to all relevant peer-reviewed journal publications, based in PubMed/MEDLINE and the web of Science (ISI), and all other cited references, and relevant review articles. The database collection strategy was kept broad. The inclusion criteria were all titles and keywords combining 3D-printing, Ceramic-based scaffolds and bone regeneration in calvarial defects. The data about the scaffold's type, porosity and their *in vivo* histomorphometric analysis (mean ± standard deviation), per each time point, were

extracted from each included study. All Clinical trials were excluded as well as poorly-documented *in-vivo* studies. Data collected were statistically analyzed.

RESULTS AND DISCUSSION:

It was found that the field of 3D-printed scaffolds in calvarial bone regeneration, is rapidly growing with most of the included studies published within the past five years. Within each animal species used, the size of bone defects varied, however, the printed ceramic-based scaffolds always revealed the highest amount of bone regeneration when compared to negative controls or polymeric scaffolds. The growth of new bone depended considerably on the available space given by the printed scaffolds and further regulated by each scaffold's degradation.

CONCLUSION:

Various types of printed ceramic-based scaffolds were used *in-vivo*, and achieving noticeable results in promoting bone regeneration. The application of such customized scaffolds is endorsing the future bone tissue regeneration strategies.

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Poster presentation

777 Implant manufacturing by Electron Beam Melting: From preclinical to clinical experience

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[2]Arcam, Sweden

INTRODUCTION:

Additive manufacturing has shown great potential for the production of complex shaped materials, where the freedom in design is not as restricted as in subtractive manufacturing. Electron beam melting (EBM) is one powder bed fusion technique which uses an electron beam to locally melt the powder in a layer-wise manner to build a 3D object and has great potential in the biomedical field.

METHODS:

The current paper describes the development and knowledge buildup on the bone response to EBM produced titanium alloy implants. Previous experimental studies in Sheep (1, 2). Furthermore, an on-going human study with implantation of experimental implants in the jaw of patients undergoing dental implant reconstruction will be presented as well as a current clinical update of the pilot patient treated in 2014 with a customized implant after jaw-resection (3).

RESULTS AND DISCUSSION:

The preclinical studies have shown that large amount of mature bone grow into the porous implants, where larger amount of bone is typically found at the periphery as compared to the center of the porous network. However, the bone-implant contact remains high through-out the porous network. Preliminary microCT data of explanted experimental implants from the human study show bone ingrowth throughout the porous network, which is in line with previous clinical retrieved EBM manufactured fixation plate used for in a fibula reconstruction after jaw-resection(4).

CONCLUSION:

The experimental studies show that electron beam melting is a safe method for implant production, where the capacity for computer controlled manufacturing further enables patient customized designs.

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ACKNOWLEDGMENTS:

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Poster presentation

781 Development of bio-based membranes for guided (periodontal) tissue regeneration: functional grading, multi-layering and micro-structuring aspects

Selestina Gorgieva
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INTRODUCTION:

Periodontitis is a chronic inflammatory disorder that affects ~750M people worldwide, being the 4th highest cost disease with up to 10% consumption of healthcare resources, being close related to diabetes, osteoporosis and other systematic cardio-, cerebrovascular and respiratory diseases. Guided tissue regeneration (GTR) is promising therapy, which utilizes the (non) bioresorbable barrier membranes for suppressing the epithelial cells' invasions in alveolar bone defects by providing space for osteoprogenitor cells migration for regeneration process accomplishment.

This project focus on new GTR membranes development by the systematic engineering of bio-based and functionally graded, multilayer composites. The leading idea is site specific introduction of the structural, morph-chemical and mechanical triggers within a single material, which would closely meet the periodontal complex tissue´ self-regeneration requirements.

METHODS:

New GTR membranes are envisaged to provide superior performance of their *state of the art* counterparts, starting from their basic barrier function, up to a) simultaneous regeneration function due to the application biopolymer (gelatin, chitosan, bacterial cellulose, mixtures) layers with specific composition and micro-structure, and b) bacteria infections' management function by incorporation of antimicrobial peptides being active against periodontal pathogens. Project will focus on:

Identification of biopolymers and multilayer membrane processing protocols and modification routes;

Physicochemical evaluation;

Assessment of physiological degradation profile(s) and antimicrobial peptides release kinetics of/from multilayer membranes;

Morphological and mechanical evaluation and

Biological assessment: the antimicrobial activity and biocompatibility impact towards selected cell lines.

RESULTS AND DISCUSSION:

Systematically engineered, biobased, functionally graded, multilayer composites were developed within presented project (Fig.1). Introduction of natural antimicrobial peptides combinations, i.e. the εPolyLlysine and Nisin Z, both with GRAS status (Generally Recognised As Safe), as an nontoxic to eukaryotic cells, broad pH range stability and activity against a diverse group of periodontal-relevant pathogens were examined by this project for first time. Their simultaneous introduction *in situ* processing of multilayer membranes is expected to allow broader and prolonged effectiveness, compensating use of high cost *on site* prophylactic antibiotics administration performed by surgeons

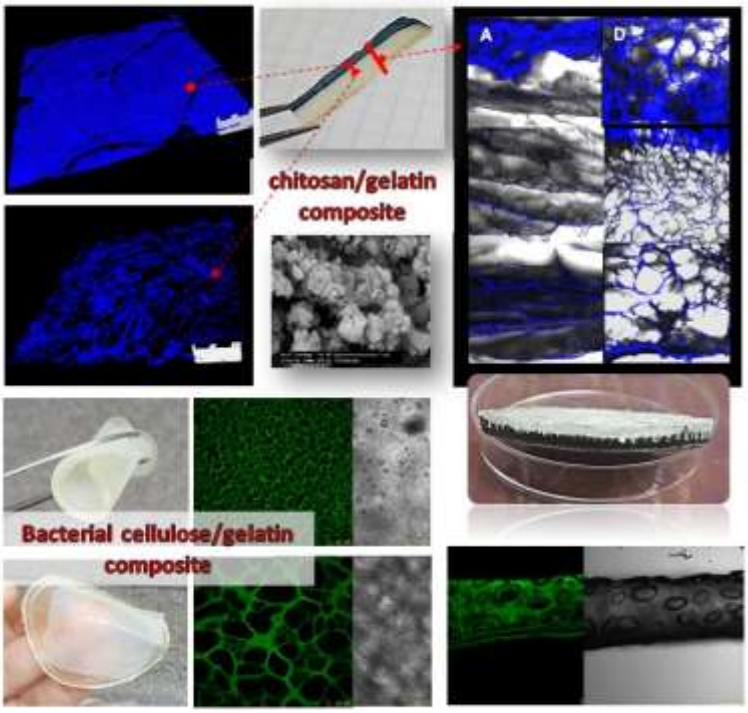
CONCLUSION:

The presented innovative concept in GTR membranes' processing might serve in future as a platform for developing cellular scaffolds for different tissue interfaces' regeneration, while gained knowledge for different modification/ stabilization routes of biopolymers as well as their graded 3D structuring may be further exploited within other applications such as filtration, separation, sensing, and drug delivery systems.

ACKNOWLEDGMENTS:

This work was supported by the Slovenian Research Agency (postdoctoral grant No. Z7-7169).

Picture 1:



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Poster presentation

785 Electrospun thermoresponsive fibers for cell sheet engineering

Maria Gabriella Fois[1], Lorenza Draghi[1], Dencho Gugutkov[2], Salima Nedjari[2], George Altankov[3]
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INTRODUCTION:

Cell sheet engineering (CSE) is an innovative scaffold-free technique for tissue repair. It became possible with the development of non-enzymatic methods for cell harvesting. To date, cell-releasing substrates are generally based on grafting of thermo-responsive moieties to the planar cell culture substrata [1]. However, due to the crucial role that play the ECM-like fibrillary structures in controlling cells fate [2], the development of fibrous thermo-responsive substrata not available yet are highly desirable. Here, we used poly(N-isopropylacrilamide) (PNIPAm) to electrospun highly biocompatible thermo-responsive microfibrous membranes considered for CSE application.

METHODS:

PNIPAm was electrospun using Benzophenone as photo-initiator and the N,N'-methylenebis(acrylamide) as crosslinking agent added to the electrospinning solution in different ratios. To initiate cross-linking, dry fibers were

exposed to UV light. To characterize the sample's stability, SEM and fluorescence microscopy were used. Water contact angle (WCAº) as function of temperature and volume phase changes were investigated.

To evaluate biocompatibility of PNIPAm fibers, Alamar Blue® assay was performed using HeLa cells. Cell morphology was further evaluated using human fibroblasts (HDFs) and adipose derived mesenchymal stem cells (ADMSCs) addressing focal adhesion formation, cytoskeleton development and early ECM deposition.

RESULTS AND DISCUSSION:

By adjusting polymer composition and UV exposure we succeeded in producing thermo-responsive microfibers stable in water up to 7 days.WCA° varied significantly from 21.9°±5.8° at 15ºC (hydrophilic) to 97.23°±16.9° (hydrophobic) at 37ºC. Average fibers diameter increased from 1.76 mm at 15°C to 2.45 mm at 37°C.

Cell viability was measured between 95% and 83% of controls (glass slides) after 24 and 48 h incubation, respectively. Morphological studies showed that HDFs seeded for 4h on plain fibres show delayed cell spreading and developed stellate-like morphology. When fibres were coated with fibrinogen, the cells spread significantly better possessing well developed actin cytoskeleton and focal adhesion formation. No appreciable differences in cell density vs. the plane substrata were observed. ADMSCs showed a similar trend. Cell adhesion to the microfibers was strongly dependent on the temperature. Detachment of cells at temperatures below LCST was observed in correlation with cell geometry and size.

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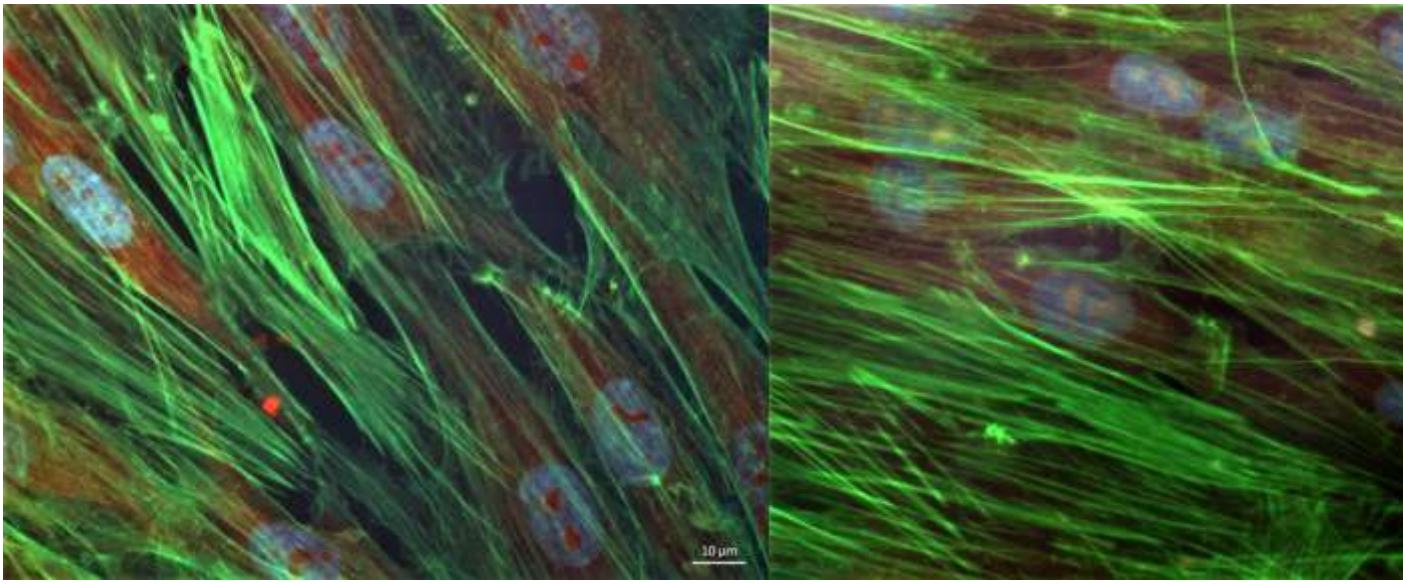
Picture 1:



Caption 1: FIG 1 HDFs on PNIPAm on glass (control) (right), on fibers (right) at 72h. OM: 63X

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

786 Allergic reaction evaluation of nitride surface layer on TiAl6Nb alloy and zirconium oxide-yttria stabilized ZrO2 · Y2O3

Barbara Zawidlak-Wegrzynska[1], Karolina Janiczak[1], Maciej Gawlikowski[1], Malgorzata Gonsior[1], Roman Kustosz[1], Michal Tarnowski[2], Tadeusz Wierzchon[2]
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INTRODUCTION:

In the clinical prototype of Polish implantable rotary blood pump ReligaHeart® ROT (RH ROT) [1] the motor divider is made from ceramic composite, ZrO2-Y2O3, with high hardness, to improve device wear resistance. The other pump parts are made of titanium alloy Ti6Al7Nb titanium nitride TiN diffusive layer modified in order to increase corrosion and wear resistance in contact with ceramic parts as well as device's biocompatibility [2]. This paper presents biomaterials allergic reaction evaluation according to PN-EN-ISO-10993 standard.

METHODS:

The skin sensitization test was performed by the closed-patch test (Buehler method). Titanium alloy (TiAl6Nb), TiAl6Nb modified by TiN and zirconium(IV) oxide-yttria stabilized (ZrO2 · Y2O3) were investigated. Titanium alloy surface was modified by forming of TiN+Ti2N+αTi(N) diffusive layer using active screen plasma nitriding process. Biomaterials were sterilized with ETO. Healthy adult albino genuine pigs (n=51) of either sex where utilized (17 animals for each material including 12 tests and 5 control animals). Animals underwent 10 days quarantine and acclimatization. One day before induction phase and then weekly, fur on the back of animals was shaved. In the induction phase biomaterials samples where administrated on the left side of animal spine and covered with occlusive dressing. Animals from control groups received only the occlusive dressing. Biomaterials application lasted 6h and the procedure was performed 3 days a week for following 3 weeks. After 14 days from the last application, all animals received biomaterials samples and occlusive dressing (challenge phase). Biomaterials were administrated to skin on the right side of animal spine for 6h. After 24h the animal skin was inspected to evaluate the erythema and/or swelling occurrence, in accordance with Magnusson and Klingman scale (MKSc).

RESULTS AND DISCUSSION:

No signs of swelling or erythema was observed on animals skin after contact with investigated biomaterials. The biomaterials did not induce any skin sensitization in allergic reaction of animals (value 0 regarding MKSc). Both biomaterials,Ti6Al7Nb titanium alloy with TiN+Ti2N+αTi(N) surface layer produced by active screen plasma nitriding process and zirconium(IV) oxide-yttria stabilized, were classified as a non-sensitizer within Buehler method performed according to EN ISO 10993-10 standard.

CONCLUSION:

No allergic reaction was observed for TiN manufactured on TiAl6Nb alloy as well as ZrO2 · Y2O3. The complete biomaterial biological in-vitro and in-vivo evaluation will be performed to confirm its biocompatibility and hence usability in ReligaHeart® ROT device.

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Gonsior, et a.., Engineering of Biomaterials, 143, Vol. XX, 2017, p.65

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

790 Ti6Al7Nb alloy long-term degradation resistance improvement in biological environment

Malgorzata Gonsior[1], Barbara Zawidlak-Wegrzynska[1], Roman Kustosz[1], Michal Tarnowski[2], Tadeusz Wierzchon[2]
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INTRODUCTION:

Modification of the well-known glow discharge assisted nitriding process called active screen plasma nitriding has been used for enhancing the biocompatible properties of Ti6Al7Nb titanium alloy through production of TiN+Ti_2N+αTi(N) diffusive surface layers [1]. This paper presents the improvement of Ti6Al7Nb surface biodegradation resistance.

METHODS:

Two biomaterials, Ti6Al7Nb titanium alloy and Ti6Al7Nb after plasma nitriding process (with outer surface zone - TiN), with roughness of Ra=80nm, were sterilized with ETO. Surface microstructure and topography were examined using TEM, SEM and AFM. Biodegradation assessment was performed in accordance with PN EN ISO 10993-9 standard, in SBF (simulated body fluid) at the temperature of 37°C, with constant stirring (100 rpm) for 30, 90 and 180 days. Immersion test was carried out according to 10993-15 standard (T = 37°C, 100 rpm) for 7 days in 0,9% NaCl. After degradation test, surface morphological change was investigated with SEM utilization and titanium anion concentration in degradation medium was analyzed using ICP-OES (Inductively Coupled Plasma - Optical Emission Spectrometers).

RESULTS AND DISCUSSION:

The microscopic evaluation of biomaterials surface quality performed after 30, 90 and 180 days in biological environment showed that the TiN+Ti2N+αTi(N)- type layer was stable during degradation process. No significant surface degradation and no mass change was observed after test. Titanium anion concentration in degradation medium after 30, 90 as well as 180 days was below 0,05 mg/l for TiN+Ti_2N+αTi(N)- type layer. For Ti6Al7Nb the titanium anion concentration in degradation medium after 30 and 90 days was below 0,05 mg/l, however after 180 days the increase of 0,067-0,187 mg/l was observed.

CONCLUSION:

Active screen plasma nitriding process allowed to produced TiN+Ti_2N+αTi(N) diffusive surface layers on Ti6Al7Nb titanium alloy and improved its long-term biodegradation resistance.

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ACKNOWLEDGMENTS:

The authors would like to thank NCBIR (Grant no: STRATEGMED-2/ RH-ROT/266798/15/NCBR/2015) for providing financial support to this project.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

792 Cell allocation in 3D scaffolds

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INTRODUCTION:

Tissue engineering is an emerging approach to replace the human body by an artificial living tissue. However, it has been successful only in the repair and preparation of some limited organs and tissues. One of the reasons is that cells cannot be placed at desired positions in a scaffold for the target tissue. Since organs and tissues have a complicated structure, it is important to arrange the cells at an appropriate position. Cells demonstrate directional migration or orientation change in response to physiological DC electric fields in both in vitro and in vivo settings [1]. The purpose of this study is to allocate cells to the correct positions in a scaffold using electric fields.

METHODS:

Two types of devices were made using 3D printer to induce electric field in culture medium. One was for making electric field directly in the culture medium through two platinum electrodes placed in the medium at 2.5cm apart. The other was via salt bridge filled with 1.5% agar dissolved in D-PBS (-) and a foundation immersed with PBS. Electric potential was applied by a DC power supply. Cell suspension of cultured L929 cells were seeded in the culture dish and then electric fields was applied soon using both devices.

RESULTS AND DISCUSSION:

It was suggested that suspended cells may be placed by applying electric field by the both devices. However, the cells were damaged by electrolysis through the electrodes in the direct application device and the application time was limited. On the other hand, electric field was able to be applied longer in the salt bridge device because the medium part and the reaction part containing PBS were separated. However, the cell moving direction was not stable probably because of some leak from the salt bridge.

CONCLUSION:

It was suggested that there was a possibility of cell placement and introduction into the scaffold by the electric field, however a further improvement is necessary.

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

803 Fine tuning of collagen cross-linking to obtain micro-patterned scaffolds

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INTRODUCTION:

Collagen is the basic building block of extracellular matrices, and as such it contains cell-binding domains essential for tissue homeostasis and regeneration[1]. Collagen is extensively extracted from animal tissue and polymerized to obtain scaffolds that are generally considered to have low mechanical properties and random fibril orientation[2]. Recently, we developed a simple technique to create collagen type I scaffolds with well-defined surface micro-patterns. These collagen constructs have proven to induce cell alignment *in vitro* and to support cyclic mechanical loadings, two features that make them suitable as substitutes for highly anisotropic and mechanically loaded tissues such as cornea or tendon.

METHODS:

Porcine collagen solution was mixed with PBS10X, NaOH and one of the following cross-linkers: GTA, Genipin and 4-arm PEG (4SP). The resulting collagen mixture was casted on micro-grooved (2x2x2µm) PDMS moulds and allowed to dry in a laminar flow hood to obtain 5mg/ml collagen films. Different pH and temperatures (T°) were tested in the process. Collagen gelation kinetics was analysed with rheometry and surface topography was assessed with scanning electron microscopy (SEM). Human bone marrow stem cells (HBMSCs) were seeded on the films and cell alignment was analysed by F-actin staining and imaged with fluorescence microscopy.

RESULTS AND DISCUSSION:

From the three collagen cross-linkers tested, only 4SP cross-linked scaffolds showed a well-defined micro-grooved pattern. By altering pH and temperature, 4SP cross-linking kinetics was modified. Higher pH and temperatures decreased collagen gelation time, which resulted in the complete inhibition of the imprinted structures, suggesting that low viscosities are required for the correct infiltration of the collagen in the micro-patterns (Fig 1A). A range of gelation times for which collagen scaffolds presented well-defined micro-grooves was defined. hBMSCs seeded on top of the optimised scaffolds aligned along the pattern for fourteen days in culture (Fig 1B). Gene and protein expression analysis are on going to further characterize the effect of these micro-patterned collagen scaffolds on hBMSC lineage commitment.

CONCLUSION:

We developed a fast and simple technique to engineer collagen scaffolds with micrometric surface topographies able to modify stem cell morphology *in vitro.* Micro-patterned collagen scaffolds could induce specific stem cell lineage commitment while being biocompatible and biodegradable, two features that would make them extremely attractive as implantable devices.

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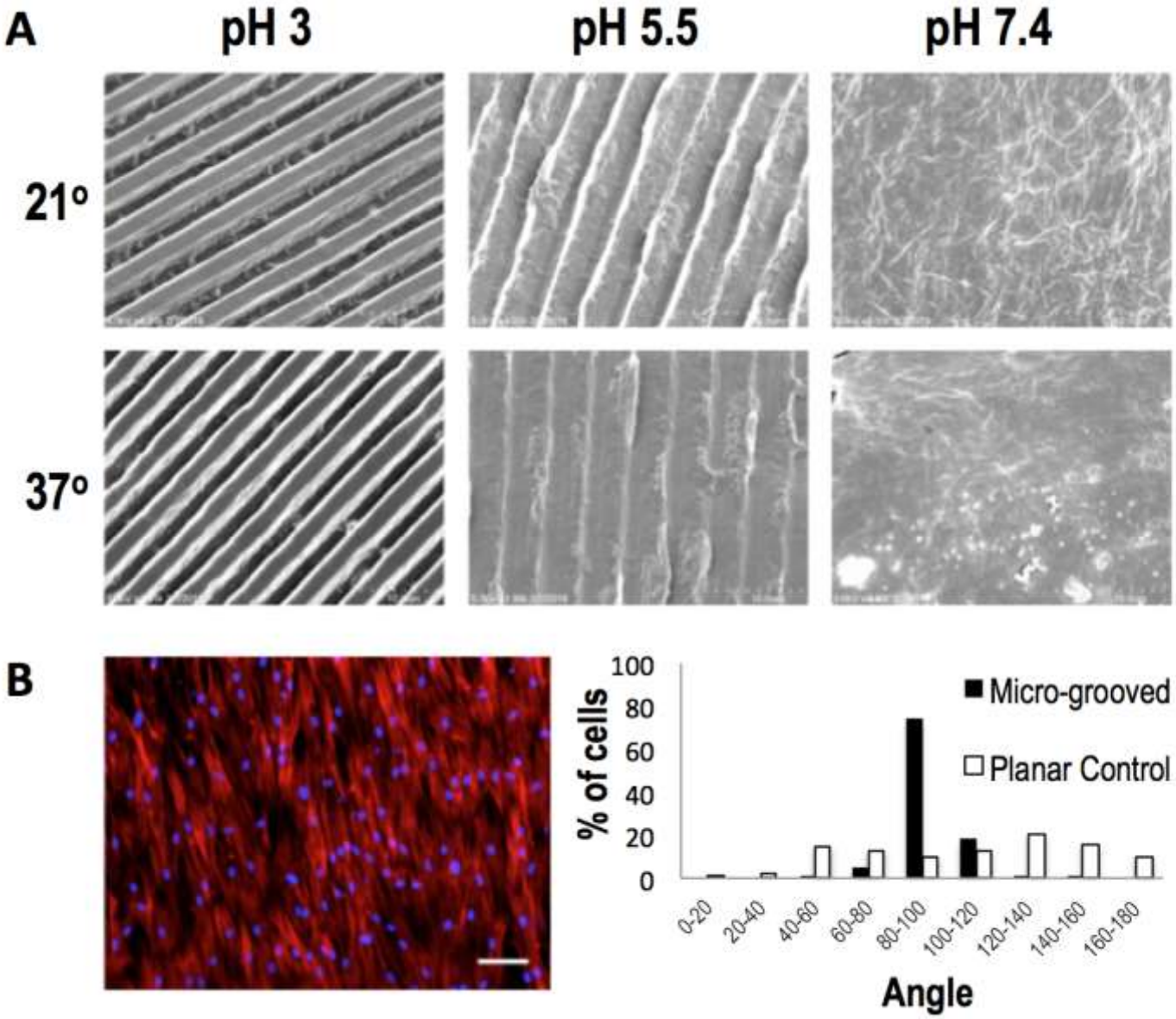
ACKNOWLEDGMENTS:

The authors would like to thank the Tendon Therapy Train Programme (Grant no: 676338) for providing financial support for this project.



Picture 1: Caption 1: Fig1. SEM images of the collagen films (A) and cell alignment on pH 3 and 21° cross-linked micro-grooved scaffold.

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Poster presentation

809 Hybrid biodegradable scaffolds of piezoelectric polyhydroxybutyrate and conductive polyaniline: piezocharge constants and electric potential study

Roman Chernozem, Maria Surmeneva, Roman Surmenev
Tomsk polytechnic university, Tomsk, Russian Federation

INTRODUCTION:

There is growing interest in piezoelectric materials due to their potential of providing electrical stimulation to cells to promote tissue formation without external source. A recent study has shown that the most significant effect on fibroblasts has been revealed in case of scaffolds with the largest piezoelectric constants such as polyvinylidene fluoride [1]. Polyhydroxybutyrate (PHB) is biodegradable and piezoelectric polymer. However, PHB possesses reduced piezoelectric properties compared with PVDF. Polyaniline (PANi) is conductive biocompatible polymer [2] and increase piezocharge constants of piezoelectric materials [3]. However, the influence of PANi on the piezoelectric properties of PHB scaffolds has not been studied so far. Thus, the present study is aimed at fabrication and investigation of the piezoelectric properties, chemical and phase compositions of hybrid scaffolds based on PHB and its blends with PANi.

METHODS:

Scaffolds were fabricated using electrospinning. PHB and PANi have been mixed as follows: pure PHB (100-0), PHB–1%PANi (99-1), PHB-2%PANi (98-2), PHB-3%PANi (97-3). The piezoelectric charge coefficient (d_{33}) of the prepared scaffolds was tasted using a Wide-Range d_{33} Tester Meter. Surface electric potential of the scaffolds was measured under mechanical loading using a custom-made set contained: two electrodes, oscilloscope and power amplifier. FTIR spectra and XRD patterns were recorded.

RESULTS AND DISCUSSION:

FTIR and XRD analysis showed the presence of PANi in the structure of the scaffolds. Figure 1 represents the dependence of d_{33} on the PANi mass fraction as well as electric potential of the scaffold surface. It can be seen that the trend was the same for d_{33} and surface electric potential, i.e. the maximum of the d_{33} and surface electric potential were observed at the 2% PANi mass fraction in the scaffolds.

CONCLUSION:

The addition of conductive PANi leads to significantly increase the piezoelectric charge coefficient and surface electric potential of PHB fibrous scaffolds.

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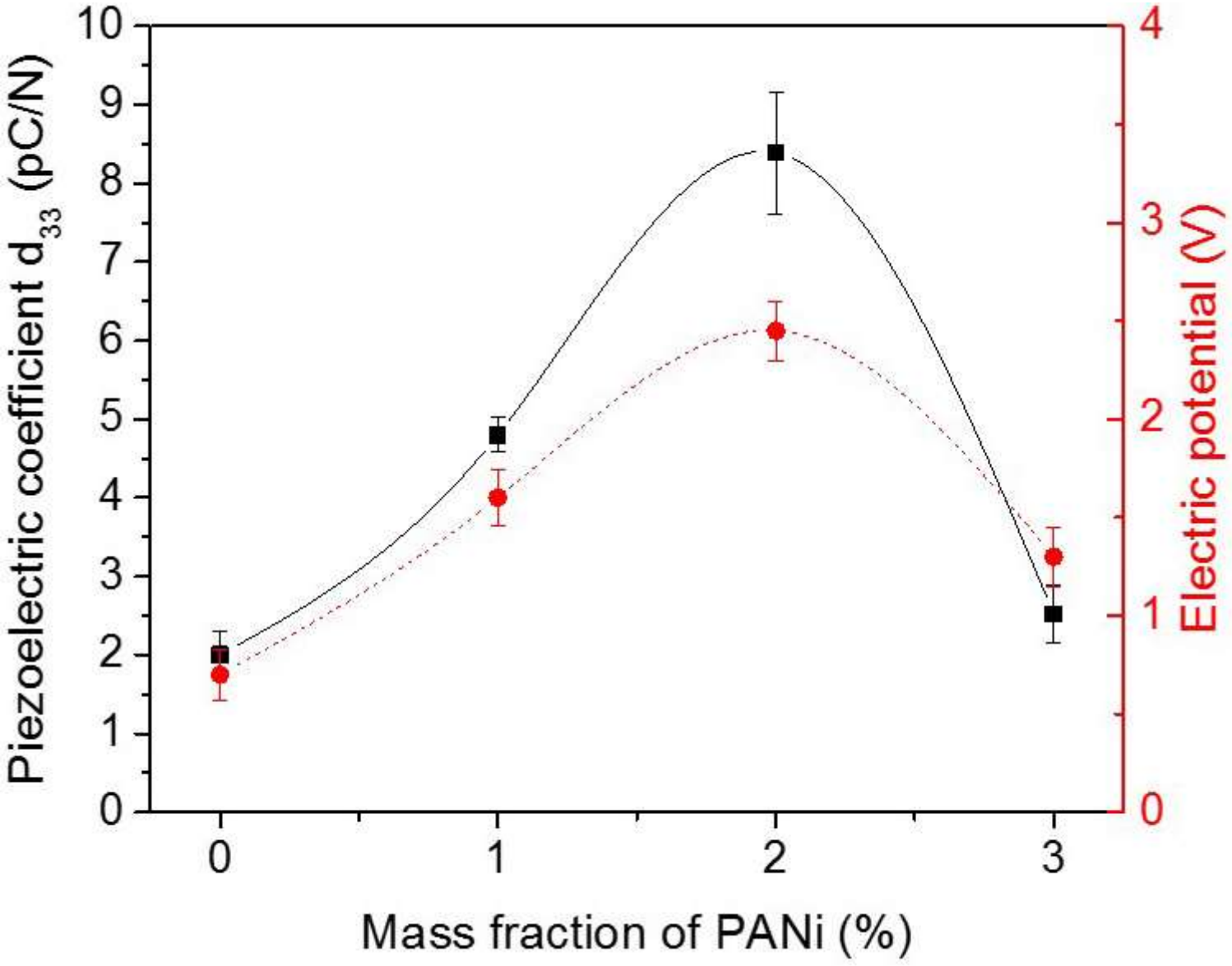

Picture 1: Caption 1: Figure 1. Dependence of d33 and surface electrical potential of PHB scaffolds on the different PANi content.

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Poster presentation

819 Effect of strontium content in bioactive borosilicate glass bone cement on physiochemical and biological properties

Wenhai Huang[1], Xu Cui[2], Yadong Zhang[3], Deping Wang[1], Wenlong Liu[2], Haobo Pan[2], Jian Lin[1]
[1]Tongji University, SHANGHAI, China
[2]Center for Human Tissue and Organs Degeneration, Shenzhen Institute of Advanced, SHENZHEN, China
[3]Department of Orthopaedics, Shanghai Fenfxian Central Hospital, South Campus of, SHANGHAI, China

INTRODUCTION:

A kind of novel bone cements composed of bioactive borosilicate glass particles containing Sr and an aqueous chitosan solution has many desirable physicochemical properties, such as workability, degradation, mechanical strength and bioactivity. In this cement, the Sr-contained glass particles enhanced the osteogenic capacity of the cement *in vitro* and *in vivo*.[1-3] However, the optimal Sr content in glass particles to achieve a cement with desirable properties is not determined. The objective of this work was to evaluate the effect of Sr content on the physicochemical properties and osteogenic capacity of this kind of bone cements.

METHODS:

Cements composed of the glass particles substituted with varying amounts of Sr (0 to 12 mol% SrO) and chitosan solution were created and evaluated *in vitro* and *in vivo*. The injectability, setting time, degradation rate and bioactivity of the cements were evaluated *in vitro*. The ability of Sr ions released from the cements to modulate the proliferation, differentiation and mineralization of human bone marrow stem cells (hBMSCs) was studied *in vitro*. Cements were implanted for up to 8 weeks in a rabbit femoral condyle defect model *in vivo* and evaluated for their capacity to stimulate the healing of bone defects. The evaluation was arrived by the Van Gieson's picrofuchsin stain to identify new bone formation at the bone-cement interface. All quantitative data are presented as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA and the Student's t-test, with the level of significance set at $p < 0.05$.

RESULTS AND DISCUSSION:

An increase in Sr substitution (0 to 12 mol % SrO) resulted in an increase in the setting time of the cement but little change in its compressive strength. Sr ions released from the cements modulated the proliferation, differentiation, and mineralization of human bone marrow stem cells (hBMSCs) *in vitro*. When implanted for up to 8 weeks into rabbit femoral condyle defects *in vivo*, the 2B6Sr cement supported better peri-implant bone formation and significantly higher bone-implant contact area than cements substituted with 0 or 9 mol % SrO.

CONCLUSION:

Optimal enhancement of these osteogenic characteristics was achieved for the cement (designated 2B6Sr) composed of glass particles substituted with 6 mol % SrO substitution.

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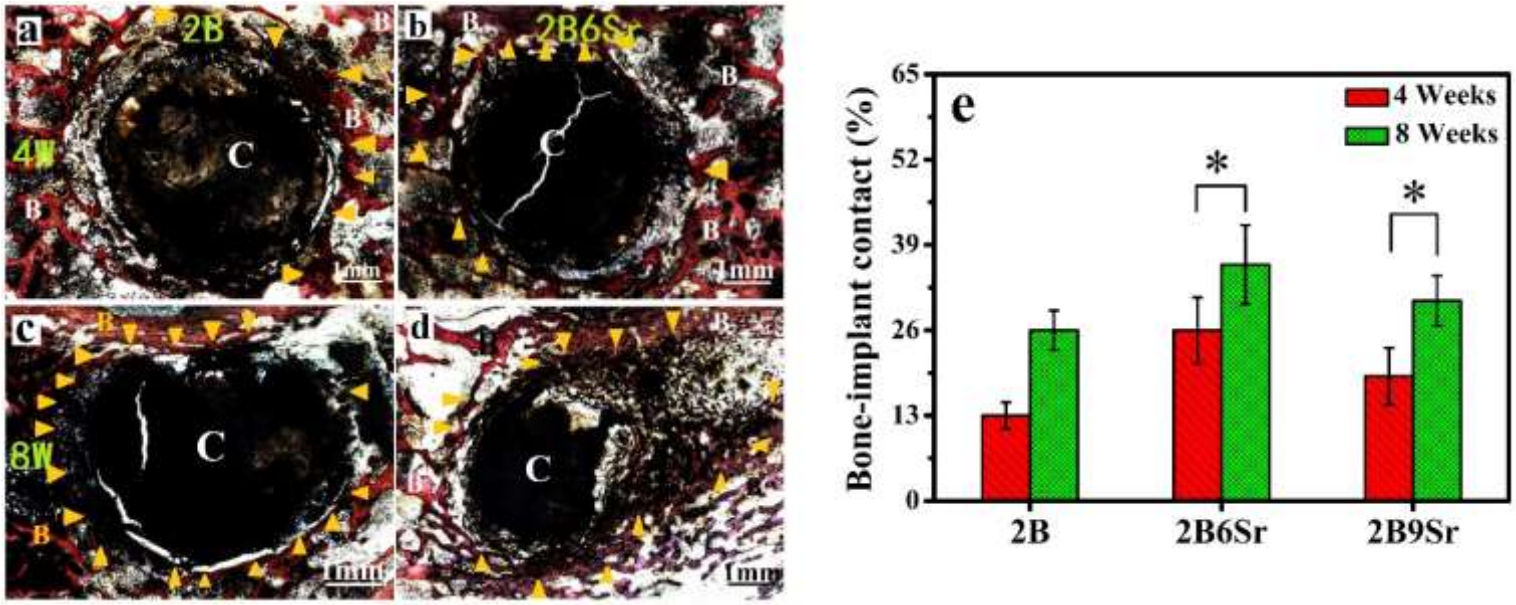
ACKNOWLEDGMENTS:

The authors would like to thank the support from the National Key R&D Program of China (Grant Number 2017YFC1105000), the National Natural Science Foundation of China (Grant Numbers 81672227, 51672191, 51372170, 31771041 and 51772210 to this work.



Picture 1: Caption 1: Figure (a-d) Optical images of undecalcified sections stained with Van Gieson's picrofuchsin, and (e) bone implant contact (BIC) for rabbit femoral co

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

820 Engineering an in vitro organotypic model for neonatal rat cardiomyocyte culture toward understanding cardiac hypertrophy

Aditi miss Aditi Jain, Jafar dr. Jafar Hasan, Desingu dr. Perumal Arumugam Desingu, Nagalingam dr. Nagalingam R. Sundaresan, Kaushik prof. Kaushik Chatterjee

Indian Institute of Science, BANGALORE, India

INTRODUCTION:

Diseases of the heart are the leading cause of deaths in the world. It is mainly due to the inability of the myocardium to repair the damaged heart. This often leads to hypertrophying of the heart [#_ENREF_1]. Prolonged exposure to hypertrophic agents compromises cardiac function, ultimately leading to death [#_ENREF_2]. To study cardiac failure of diverse origin, neonatal cardiomyocytes cultured on flat surfaces are commonly used as a model. A major drawback of such a system is that the cardiomyocytes do not exhibit alignment, organization and calcium transients, similar to the heart. Therefore, there is a need to develop *in vitro* platforms that recapitulate heart microenvironment as models to study cardiovascular diseases. The objective of this study was to engineer a culture platform for the organization of cardiac cells to closely mimic the heart and to demonstrate its utility to understand cardiac hypertrophy.

METHODS:

Here we report an engineered platform that mimics cardiac cell organization and function of the heart. For this, microscale ridges were fabricated on silicon using UV-lithography and reactive ion etching techniques. Physical characterization of the microstructures was done using SEM and AFM. Live cell imaging was done to track the

calcium currents in cardiomyocytes grown on ridges using Fluo-4. Development of cardiac hypertrophy upon Phenylephrine treatment (PE) was confirmed by atrial natriuretic peptide (ANP) expression using immunofluorescence.

RESULTS AND DISCUSSION:

Cardiomyocytes grown on micro-ridges showed global parallel alignment and elliptical nuclear morphology as observed in the heart. Calcium currents traversed in coordinated and directional manner in cardiomyocytes grown on ridges. These cardiomyocytes were found to be responsive to hypertrophic stimuli, as observed by the expression of ANP and increase in calcium transients upon PE treatment. These data clearly establish that cardiomyocytes cultured on ridges are a close representation of the cardiac milieu under both normal and hypertrophic conditions. Such a model system can be useful to understand the molecular basis of the cardiovascular diseases.

CONCLUSION:

This work demonstrates that micro-ridges can be reliably used to grow cardiomyocytes *in vitro*, which closely resembles mammalian heart [3].

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Jain, A., et al., *Engineering an in vitro organotypic model for studying cardiac hypertrophy.* Colloids and Surfaces B:Biointerfaces, 2018. **165**: p. 355-362.

ACKNOWLEDGMENTS:

We acknowledge funding from Wellcome Trust/DBT, DST and DBT, India.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

821 MG-63 cell proliferation and differentiation induced by dynamic stretch stimuli on PDMS with different surface modified layers

Jui-Che Lin[1], Chi-Hui Cheng[2], Shen-Jui Tseng[1]
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INTRODUCTION:

Performing in vitro investigation under an environment mimicking native biological system has become the major focus in biomedical studies. Besides the chemical ques on the cell culturing substrates, the dynamic mechanical stretching stimuli could provide a bio-mimic environment experienced by the adherent cells.

A continuous stretching system, by which, the biomimetic stretching force exerted onto various elastic polydimethylsiloxane (PDMS) substrates modified with different covalently bound biomolecules was utilized to assess the likely chemical and physical effects on MG-63 cell proliferation and differentiation.

METHODS:

The PDMS substrates were modified with five different biomolecules, namely, alginate, type I collagen, fibronectin (FN), poly-l-lysine (PLL) and laminin. The PDMS substrates were first treated with O_2 plasma and then soaked in the glutaraldehyde solution. Subsequently the substrates were immersed in the modification solution. Finally all substrates were cleaned with de-ionized water.

All substrates were UV-sterilized. The dynamic culture parameters were: 10% (strain), 0.5Hz (frequency). The MG-63 cells were first statically cultured on PDMS substrate for 24 hours. Then the substrates were placed onto dynamic cell culture system (Figure 1) within an incubator under 5% CO_2, 37°C for 12 hr.

RESULTS AND DISCUSSION:

The XPS analyses indicated that the oxygen concentration of PDMS-Alginate was increased than O_2 plasma treated one. In addition, the nitrogen atoms were noted on the PDMS modified with the other four proteins. All modified PDMS substrates exhibited more hydrophilic characteristic than the non-treated one.

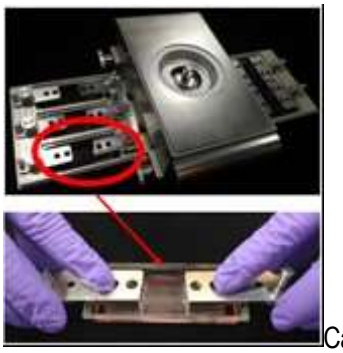
After 24-hr static culture, only MG-63 cells adhered on PDMS-Alginate and PDMS-Collagen exhibited spindle phenotype similar to the one cultured on petri dish. After additional 12-hr dynamic culture on these two substrates, the adhered cells aligned themselves to be perpendicular to the stretching direction to maintain intracellular tension equilibrium. Higher cell proliferation and higher differentiation markers such as alkaline phosphatase and osteocalcin were noted on PDMS-Collagen after dynamic culturing. This could be due to the surface bound type I collagen, an extracellular matrix-like protein, enhancing MG-63 proliferation and differentiation.

CONCLUSION:

The elastic PDMS substrate was successfully modified with various biomolecules via covalent bonding. In addition, PDMS with surface layer exhibiting extracellular affinity (i.e. PDMS-Collagen) can induce more cell proliferation and differentiation.

ACKNOWLEDGMENTS:

The financial support from Ministry of Science and Technology Taiwan, R.O.C (MOST 105-2221-E-006-254) and Chang Gung Memorial Hospital (CMRPG3D1283) are acknowledged.

Picture 1:  Caption 1: Figure 1. ATMS Dynamic cell culture system

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Poster presentation

823 One-Step Synthesis of pH- and Redox-Responsive Degradable Poly(Beta-Amino Ester) Hydrogels

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[2]Koc University, ISTANBUL, Turkey

INTRODUCTION:

Biodegradable hydrogels are extensively studied materials for a wide range of potential applications in tissue engineering, drug and gene delivery[1]. Poly(beta-amino ester)s (PBAEs) are an important class of polymers for the formation of biodegradable hydrogels due to their pH sensitivities, biodegradabilities and high biocompatibilities[2]. Herein, a one-step synthesis strategy based on aza-Michael reaction was employed to fabricate injectable, biocompatible and degradable novel PBAE hydrogels. The obtained hydrogels are responsive to both pH and redox state which enables the control of degradation and release properties by external triggers.

METHODS:

The hydrogels were synthesized by one-step aza-Michael reaction of diacrylates 1,6-hexanediol diacrylate (HDDA) and poly(ethylene glycol diacrylate) (PEGDA) with cystamine at physiological conditions. The phosphonated/bisphosphonated analogues of these hydrogels were synthesized by addition of diethyl vinylphosphonate or tetraethyl vinylidinebisphosphonate to the reaction mixture with the aim of improving biocompatibility and interactions with hydroxyapatite (HAP)-based tissues such as dentin, enamel, and bone. Properties such as water uptake, morphology, degradability, toxicity of the degradation products, pH and redox dependent drug release profiles of the hydrogels were investigated.

RESULTS AND DISCUSSION:

The selection of the diacrylate enabled the tailoring of the hydrophobicity which in turn determined the swelling, degradation and release properties. The degradation was monitored in neutral and acidic conditions and degradation products were found to be compatible with human osteosarcoma (Saos-2) cells. The disulfide bonds within the hydrogel structure provided an effective tool to enhance the rate of degradation in the presence of dithiothreitol (DTT). Methylene blue, a photosensitizer, was used as a model compound to demonstrate the pH and redox dependent release profile of hydrogels.

CONCLUSION:

The synthesized hydrogels with tailored degradation and release properties have the potential to be used as tissue engineering scaffolds as well as platforms for controlled delivery of therapeutic agents.

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

824 Serum protein adsorption profiles on BCP ceramic and the regulation of the preferentially adsorbed adhesive proteins on MSCs behavior

Xiangdong Zhu, Jing Wang, Xingdong Zhang
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INTRODUCTION:

Protein adsorption, as an initial event after implantation of a biomaterial into the body, plays a key role in mediating cell function and the implant's fate [1]. In the present study, a proteomics technology, iTRAQ was used to evaluate the adsorption profiles of serum proteins on BCP ceramic, and the regulation of the preferentially adsorbed adhesive proteins on MSCs behavior was investigated.

METHODS:

Adsorption of rat serum proteins was performed on porous BCP ceramic. The eluted protein samples were subjected to iTRAQ analysis. The preferentially adsorbed vitronectin (VN) and Laminin (LN) were selected to investigate their roles in MSCs behavior. Four types of BCP samples, i.e. BCP without any treatments (BCP), BCP with pre-adsorption of serum proteins (SP+BCP), BCP with pre-adsorption of serum proteins and VN blockage (SP-VN+BCP) and BCP with pre-adsorption of serum proteins and LN blockage (SP-LN+BCP) were prepared. The attachment, growth and osteogenic gene expressions of MSCs were evaluated.

RESULTS AND DISCUSSION:

The results of iTRAQ proteomic analysis showed that a total of 137 proteins showed the elevated adsorption, and 144 proteins exhibited the decreased adsorption on the BCP ceramic. In the signaling pathway of ECM-receptor interaction, VN and LN showed elevated adsorption on the ceramic.

The CLSM observation showed that more dead cells but fewer live cells appeared on BCP group than other groups. The results of CCK-8 analysis indicated that SP+BCP group presented the significantly increased attachment of MSCs than BCP group. After VN or LN blockage, no significantly decreased MSCs attachment could be found on SP-VN+BCP or SP-LN+BCP group, as compared to SP+BCP group. The gene expressions of ALP, BMP-2 and RUNX2 in MSCs were evaluated by PCR analysis. The results showed that as compared to BCP group, all the other groups up-regulated the expressions of the three osteogenic genes. However, after VN or LN blockage, the expressions of the three genes were influenced to varying degrees.

CONCLUSION:

With pre-adsorption of serum proteins, the BCP ceramic promoted the attachment and osteogenic gene expressions of MSCs. Although VN or LN blockage had certain impact on the behavior of MSCs, other adhesive proteins, such as FN could still play the positive role in the attachment and osteogenic differentiation of MSCs.

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ACKNOWLEDGMENTS:

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Poster presentation

825 Combating biofilm formation by diffusive release of the antifungal drug fluconazole from heptylamine plasma polymer coating

Javad Naderi, Solmaz Saboohi, Hans Griesser, Bryan Coad

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INTRODUCTION:

Combating microbial pathogens by coating medical device materials with antimicrobial agents requires different immobilisation strategies. While some agents can be effective when covalently bound onto surfaces, another promising strategy is to fabricate coatings that release compounds. Drug release allows for diffusion towards and into pathogenic cells to meet intercellular targets enabling a wide range of antimicrobial agents to be used. With fungal pathogens it is particularly important that compounds have low toxicity which is why our focus has been on FDA-approved compounds which selectively interfere with fungi-specific targets.

METHODS:

Loading fluconazole from approved class of Azole which are able to target cell membrane and intracellular parts of fungal cell offers an avenue for producing coatings that can selectively deter fungal colonization while supporting mammalian cell attachment.

As a carrier coating for releasing drug, heptylamine plasma polymer is a good option which has simple deposition technique, good absorption and compatibility with innate immune cell function[1].

RESULTS AND DISCUSSION:

Fluconazole is a highly effective antifungal drug widely used to combat infections due to yeasts from the Candida genus. It is known to inhibit an intracellular pathway for producing ergosterol – an essential component of fungal (but not mammalian) cell membranes.

CONCLUSION:

In this research we show prevention of biofilm formation from surface coatings that release fluconazole. The compound was loaded into heptylamine plasma polymer coatings and characterized using surface analytical techniques. Results of characterization analysis and microbiological assays such as depth profiling, modified ISO22196 and diffusion zone of inhibition assays will be discussed.

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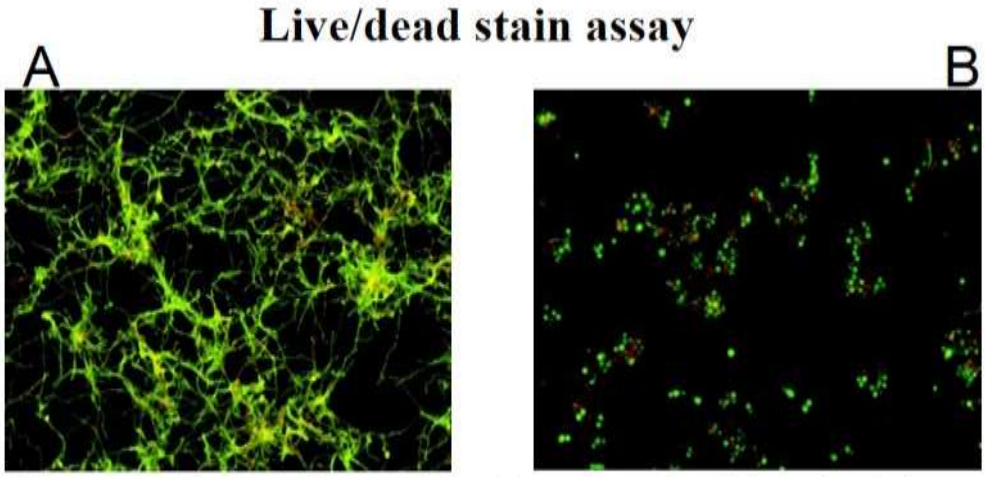
ACKNOWLEDGMENTS:

“The authors would like to thank Australian Research Council Discovery Program (grant DP150101674) for providing financial support to this project”.



Fluorescence microscopy images of *Candida albicans* after 24 hours incubation on A) Control Heptylamine plasma polymer surface with hyphae (intermediate stage of biofilm formation) and B) Heptylamine-fluconazole treated surface with no hyphae formation; Magnification 20X.

Picture 1:
Caption 1: Live/dead stain assay

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Poster presentation

827 Antimicrobial nitric oxide peptide contact lens bandages

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INTRODUCTION:

Bacterial keratitis is often contracted through the improper use of contact lenses and treatment regimes often include broad spectrum antibiotic drops, and sometimes the application of a bandage lens to protect the wound. [1] This method of delivery of the drug however is not efficacious as less than 7% of the active agent reaches the site of injury due to the method of administration. Moreover, in recent years, there has been a drive to reduce the use of antibiotics owing to the growing epidemic of antimicrobial resistance.

Nitric oxide (NO) acts as an antimicrobial agent by interacting with simultaneously produced reactive oxygen species such as hydrogen peroxide (H_2O_2) and superoxide (O_2^-) to generate reactive nitrogen species such as peroxynitrite ($OONO^-$), S-nitrosothiols (RSNO), nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3), and dinitrogen tetroxide (N_2O_4).[2,3] It has been shown that these reactive intermediates target DNA, causing deamination, oxidative damage, strand breaks, and other DNA alterations.

Compounds containing the diazeniumdiolate $[N(O)=NO]^-$ functional group have shown great potential in a variety of medical applications requiring the controlled and sustained release of NO [4] Described herein is a method to develop diazeniumdiolate functionalised contact lenses capable of releasing a controlled and sustained dose of NO to target biofilms on infected wounds

METHODS:

Poly-ε-lysine (pεK) is cross-linked with bis-carboxy fatty acids and functionalised with diazeniumdiolate to produce nitric oxide releasing hydrogel contact lens bandages. The mechanical properties of the gels are tailored by altering the density of the polymer, the molecular length of the cross-linker and the cross-linking density. The chemical properties of the gels were determined using X-ray photoelectron spectroscopy and fourier transform infrared spectroscopy. The NO payload released was determined using a chemiluminescent NO detector.

RESULTS AND DISCUSSION:

NO release from the functionalised contact lens bandages was evaluated at varying pHs and the gels demonstrated a burst release at pH 4, and a lower and more sustained release profile at pH 7. The antimicrobial efficacy of the contact lenses was observed as reduction of the adhesion of Staphylococcus aureus using the Resazurin assay. An indirect cytotoxicity assay was carried out to determine if released NO negatively affected a human corneal epithelial cell line (HCE-T cells).

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ACKNOWLEDGMENTS:

Supported by EPSRC grant EP/P023223/1

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Poster presentation

828 Electrospinning effect on the relaxation molecular dynamics of poly(D,L-lactide) via dielectric relaxation spectroscopy

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INTRODUCTION:

Despite the analytical potential of DRS, limited research has been conducted so far on polymer fibers produced by electrospinning[1]. Here, changes in the molecular features of amorphous PDLLA chains after electrospinning are investigated by performing DRS on PDLLA fibers and films. Differences in terms of dielectric relaxations, interfacial phenomena and DC conductivity are recorded. The applicability of different formalisms and the resulting relaxation molecular dynamics of the observed processes are discussed[2].

METHODS:

PDLLA (4060D, Nature Works LLC) fibres were produced by electrospinning PDLLA solutions in acetone (concentration of 14% w/v) using a voltage of 12 kV, a flow rate of 0.7 mL/hour and a needle-collector distance of 15

cm. The dielectric properties of the samples were examined at time varying applied voltage with constant amplitude of 1 V and frequency and temperature ranges of 10^{-1} to 10^{6} Hz and 30 to 100^{o}C (in steps of 5^{o}C), respectively. The temperature was controlled by the Novotherm system and the dielectric cell employed was the BDS-1200 parallel gold-plated electrodes all supplied by Novocontrol Technologies (Germany). PDLLA film and fibrous mat had a disk shape with a diameter of 2 cm and 1.5 cm, respectively.

RESULTS AND DISCUSSION:

In the low frequency spectrum edge of the PDLLA fibers, it is visible a process assigned to MWS IP between the partially ordered amorphous and the amorphous parts of PDLLA, with no signs of DC conductivity. Between 65 and 85^{o}C close to the high frequency edge, the imaginary part shows a peak which is attributed to fluctuations of the main polymer chains. The frequency loss peak position with temperature follows the Arrhenius equation.

CONCLUSION:

In conclusion, BDS and relaxation molecular dynamics have proven to be paramount to highlight the effect of the electrospinning process on the molecular characteristics and chain dynamics of PLA. Compared to the PDLLA film, the fibres presented a stronger α*-mode, which was attributed to polarization phenomena in the partially ordered amorphous parts of the chains. As expected, the order increased after the electrospinning process, in agreement with previous works reported in the literature using different techniques.

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Picture 1: https:\\www.eventure-online.com/parthen-uploads/40/18903/add_1_458859_a2e609cf-5da5-4341-97a3-7dc5f0d180b0.1.jpeg
Caption 1: Figure 1. The imaginary part of dielectric permittivity as a function of frequency and temperature of the PDLLA fibers specimen. Inset the Arrhenius d

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Poster presentation

832 Targeting the Neurogenic Niches of Adult Brain by Peptide Conjugated Nanoparticles

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INTRODUCTION:

Stimulation of adult neurogenesis by targeting the endogenous neural stem cells, located in hippocampus and subventricular zone (SVZ), has been proposed for brain repair in cases of neurodegenerative diseases [1]. One major drawback for the treatment of these diseases is the incapacity of drugs/carriers to cross efficiently the BBB [2]. Studies have demonstrated that nanoparticles (NPs), upon a intracerebroventricular administration, can deliver active molecules at the SVZ region, triggering the neurogenic process [3]. Nevertheless, this type of administration is

very invasive and requires specific medical facilities. So far, it is relatively unknown the required properties to facilitate NPs accumulation in the neurogenic niches.

METHODS:

Different densities of transferrin (Tf) peptides were covalently conjugated on spherical Au NPs and Au nanorods (Au NRs) using maleimide chemistry**.** Human BBB model was used to analyze the permeability of Tf conjugated NPs and NRs. ICP-MS measurement was done to quantify the amount of NPs transcytosed across the in-vitro BBB and different niches of brain was quantified using ICP-MS.

RESULTS AND DISCUSSION:

We have screened AuNPs formulations having variable morphology (spherical and rod shape), surface chemistry (different density of Tf peptide) and responsiveness to near infrared (NIR) light for their capacity to cross the BBB and to accumulate preferentially in the neurogenic niches. Results obtained in a human *in vitro* BBB model showed that AuNPs and AuNRs conjugated with Tf peptides between 169 (AuNRs-Tf_{169}) and 230 (AuNPs-Tf_{230}) crossed more efficiently than formulations with higher or lower number of Tf peptides per formulation, without affecting the BBB properties. The transcytosis of AuNPs-Tf_{230} and AuNRs-Tf_{169} depend on their avidity to Tf receptors as compared to the other formulations. We further show that AuNRs-Tf_{169} administered intravenously in mice and activated by NIR light had the highest accumulation in the neurogenic niche (SVZ), due to a transient opening of the BBB, induced by local heat generated by Au NRs.

CONCLUSION:

We show that the neurogenic niches of the brain can be targeted more effectively by modulating the properties of NP formulations. Our results open the possibility of targeting effectively the neurogenic niches by controlling the properties of the nanoformulations.

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ACKNOWLEDGMENTS:

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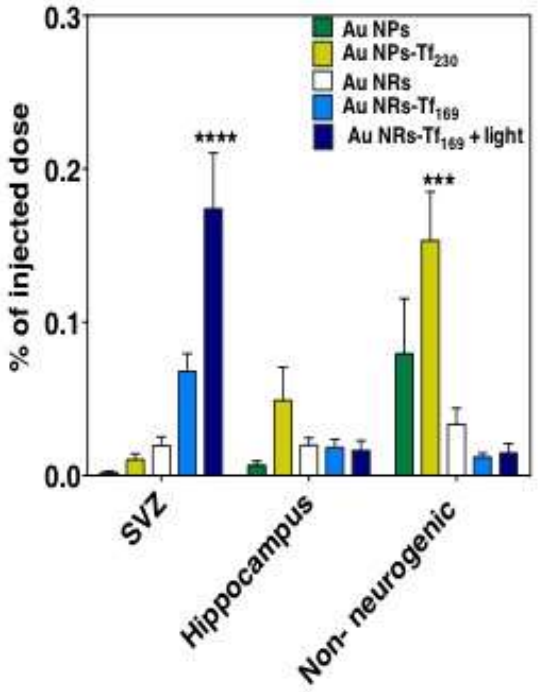

Picture 1: Caption 1: Quantification of Au content in the different regions normalized to the initial Au administrated. Results are average ± SEM (n=6-9 animals).

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Poster presentation

833 In situ silver/Hyaluronan Bio-nanocomposite Fabrics for Wound and Chronic Ulcer Dressing: In Vitro and In Vivo Evaluations

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INTRODUCTION:

Wound healing is a complex, multi-step process including the activation of a variety of cell types. Re-epithelization during the early phase of wound healing, occurs only after migration and proliferation of keratinocyte cells in the epidermal layer from the wound edge as well as by differentiation of stem cells residing in the bulge of hair follicles (Tjong, 2012; Zanette et al., 2011). Bio-nanocomposites represent a fascinating interdisciplinary area that brings together biology, materials science and nanotechnology. Generally, polymer nanocomposite results from the combination of a suitable biopolymer and filler particles at the nanometer scale

METHODS:

Hyaluronan/silver nanocomposite (HA/Ag-NPs) with different Ag nanoparticles contents were synthesized with no external reducing or stabilizing agents. Sodium hyaluronate was used as the reducing and capping agents at the same time. 40 mg of sodium hyaluronate (HA) molecular weight 700-900 KDa was dissolved under stirring in 1 ml of demineralized water into homogenous, viscous solution of suitable for wet-spinning

RESULTS AND DISCUSSION:

The functional fibers/fabrics were synthesized by wet-dry-spinning technique (WDST) in the biologically benign hyaluronan/water/silver nitrate/sodium hydroxide system. The coagulation bath byproduct was mainly sodium acetate and sodium chloride. Therefore, this bath procedure was a green bio-friendly technology

CONCLUSION:

In-situ silver nanoparticles (Ag-NPs) were synthesized by using hyaluronan as stabilizing and capping agents in absence any external reducing agent. Uniform dispersion of almost monodisperse *in-situ* formed nanoparticles was obtained by the novel process. The HA/Ag-NPs nanocomposite fabrics wound cover exhibits the desired antibacterial activity against gram negative bacterial (*E.coli*), has no effect on the keratinocyte's cell line growth (*HaCaT*) and was highly biocompatible. The non-woven HA/Ag-NPs fabrics wound cover has higher wound healing efficacy compared to the plain HA fabrics covers and control sample, and histological measurements, demonstrated its applicability in wound and chronic ulcer treatment.

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Poster presentation

834 Optimization of Platelet Lysate-Derived Exosomes Isolation for Tissue Engineering and Regenerative Medicine Applications

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INTRODUCTION:

Platelets are blood components involved in wound healing that have attracted a great interest in tissue engineering and regenerative medicine (TERM). Particularly, platelets-derived extracellular vesicles (EVs) (microvesicles and exosomes), are an important source of bioactive molecules that actively contribute for several homeostatic multicellular processes and also for tissue repair[1]. Recently, platelet-derived exosomes (PEx) (30-100 nm), have emerged as important mediators in cell-cell communication once they are capable of binding and exchanging their cargo (proteins, miRNAs, growth factors) in neighboring or distant cells[2]. The paracrine effect of the PEx leads to metabolic and epigenetic modulation of target cells and, consequently, influencing physiological and/or pathological pathways[3]. Such evidences suggest PEx as ideal candidates as biomarkers and as well as therapeutics. This study aimed at optimizing the isolation of exosomes from platelet lysate (PL) through different methods for future TERM applications.

METHODS:

PL was obtained by freeze/thaw cycles disruption of the platelets contained in platelet concentrate batches. PL-derived exosomes were isolated by differential centrifugations and using the total exosome isolation commercial kit. Size distributions were determined by zetasizer and electron microscopy, and exosomes total protein was quantified by micro-BCA kit.

RESULTS AND DISCUSSION:

Our preliminary results show that differential centrifugations yield a more homogeneous exosomes' population (size-average: 135 nm; PDI: 0,2) when compared with the commercial kit (size-average: 235 nm; PDI: 0,5). Nevertheless, the protein amount was significantly lower when exosomes were isolated with differential centrifugations (11 mg/mL) than when were isolated with the kit (131 mg/mL).

CONCLUSION:

Isolation of the EVs subpopulations requires developing reliable isolation methods of pure fractions of exosomes. Moreover, given the limited basic knowledge of PL-derived exosomes, biology and mode of action, additional comprehension of the mechanisms that underline the secretion and uptake of exosomes, as well as further proteomics and transcriptomics analysis will be addressed within the next steps of this study. The understanding of all these aspects will open new perspectives in translational medicine and exosomes might become the next-generation tool in TERM.

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Optimization of Platelet Lysate-Derived Exosomes Isolation for Tissue Engineering and Regenerative Medicine Applications

Picture 1:

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Poster presentation

835 Three types of zirconia particle addition to calcium phosphates cement for strengthening

Yeeun Kim, Jiyoung Bae, Yumika Ida, Kazumitsu Sekine, Fumiaki Kawano, Kenichi Hamada
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INTRODUCTION:

Calcium phosphate cements (CPCs) are widely used for bone substitutes. However, their application is limited because of the insufficient mechanical properties. Zirconia powder is known as a reinforcement of CPC without biocompatibility degradation. The objective of this research is to investigate the effects of three types of zirconia reinforcement on strength of CPC.

METHODS:

3 types of zirconia reinforcement were prepared. eZR: β tricalcium phosphate (βTCP) powder after ball milling using zirconia jar/balls (mβTCP) was immersed in hydrochloric acid and residual zirconia particles were extracted. nZR: particles of 100 nm diameter were extracted from a commercial zirconia dispersion. fZR: commercial stabilized zirconia fiber of 6–10 μm diameter with very little agglomeration. βTCP powder was mixed with the above reinforcements at ratios of 1 to 6 mass% using ball milling (mβTCPZ). mβTCPZ powder was mixed with $CaCl_2$ solutions and NaH_2PO_4 solutions, and the mixed paste was filled into a mold to produce specimens for diameter tensile strength (DTS) test. Fracture surface of specimen after DTS test was observed using SEM.

RESULTS AND DISCUSSION:

The DTS value of specimen with eZR and nZR increased with increasing zirconia ratio from 1%, and then decreased. While that with fZR did not change clearly with zirconia ratio. The highest value of specimen with eZR and nZR were 7.8 MPa at 4% and 7.5 MPa at 3%, respectively, and they were significantly higher than that with fZR of 5.4 MPa at 4%. Rough agglomerated granular structure was observed on the fracture surface of specimen with nZR, while the fracture surface of specimen with eZR and fZR was flatter. Many large size pores were observed on that with eZR and fZR, while only small size pores were observed on that with nZR. Since higher porosity usually reduces the strength of porous material, and flatter fracture surface suggests brittle fracture which leads to lower strength, simultaneous higher porosity and flatter fracture surface potentially reduced DTS of specimen with fZR.

CONCLUSION:

Fiber zirconia reinforcement addition to CPC in this research showed less effect on DTS than particle zirconia reinforcement. Further research was required to clarify the effects of fracture surface morphology on mechanical properties of mβTCPZ.

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ACKNOWLEDGMENTS:

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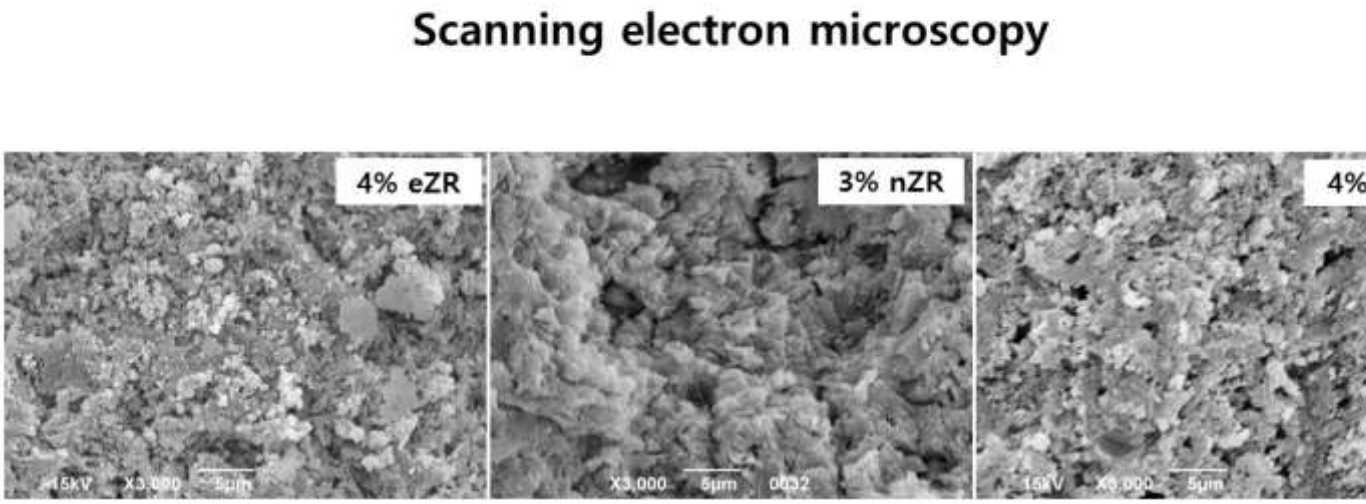
Picture 1:



Caption 1: SEM Image

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Poster presentation

839 Chondrocyte differentiation on an eggshell membrane 3D scaffold

M. Soledad Fernandez, Hernan Vargas, J. Ignacio Arias, Liliana Ortiz, Jose Luis Arias
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INTRODUCTION:

The non-mineralized and acellular eggshell membranes (ESM) surrounding the egg white, are a natural fibrillar biopolymeric network composed mainly of type X collagen[1-3]. We have previously used ESM as a substrate for culturing chicken primary calvaria-derived osteogenic cells[4]. Additionally, pieces of ESM as an *in vivo* scaffold to regulate bone regeneration have been used[5]. Here we explored the ability of ESM to support the culture of rat bone marrow mesenchymal stem cells (MSC) induced to differentiate to chondrocyte in a 3D scaffold.

METHODS:

Rat bone marrow MSC cultured on sterilized ESM in DMEM High Glucose Medium with or without chondrogenic inductors for 7, 14 and 21 days at 37ºC in a cell incubator were used. Cell viability, total protein secretion, collagen II secretion, cartilage-specific matrix proteins (aggrecan and type II collagen) expression, and cell morphology were analyzed by MTT, Bradford, ELISA, immunoperoxidase and immune-beads, and scanning electron microscopy (SEM) respectively.

RESULTS AND DISCUSSION:

Viability was lesser in MSC cultured in the presence of chondrogenic inductors. However, at 21 days of culture, these cells secrete to the medium almost double amount of proteins. By using monoclonal antibodies for immunolabelling cartilage-specific proteins in ESM cultured with MSC, the occurrence of type II collagen and aggrecan was observed, but only in those membranes cultured in the presence of chondrogenic inductors. SEM observation showed that in ESM cultured with MSC in the absence of chondrogenic inductors, cells spread as fibroblast-like shape on the ESM. On the other hand, spherical or cylindrical aggregates of cells located inside the eggshell membranes was observed in ESM cultured with MSC in the presence of chondrogenic inductors. There was a positive immunobeads-labelling for type II collagen and aggrecan around these aggregates.

CONCLUSION:

It is safe to conclude that eggshell membranes could be used as promising natural fibrillary scaffolds to produce a 3D cartilage implants.

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Poster presentation

842 Injectable and magnetic responsive hydrogels with bioinspired ordered structures for the regeneration of anisotropic tissues

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INTRODUCTION:

We have been developing a range of nanocomposite hydrogels exploring the unique properties of naturally derived rod-shaped cellulose nanocrystals (CNC) to produce new nanostructured biomaterials for high performance applications in tissue engineering and regenerative medicine (TERM). In the present study, we hypothesize that by combining CNC with magnetic nanoparticles (MNP@CNC) it would be possible to control their spatial distribution with external magnetic fields to generate bioinspired ordered structures within injectable hydrogels. The combined magnetic responsiveness and 3D ordered textures of these biomaterials, typically unavailable in other homogeneous nanocomposite systems, are envisioned to engineer anisotropic and mechanosensitive tissues (e.g. tendon), synergistically guiding cell into anisotropic organizations crucial for the functionality of such highly ordered tissues. Moreover, by using MNP@CNC as remote actuators within injectable hydrogel matrices, these design strategies and stimulation approaches might additionally be extended to *in vivo* settings.

METHODS:

Polydopamine coated MNP@CNCs grafted with polyethylene glycol polymer brushes were first synthetized to improve the colloidal stability of the nanoparticles in biological solutions and to prevent the fast oxidation of iron oxide MNPs in biological fluids. Gelatin hydrogels based on enzymatic crosslinking (microbial transglutaminase) were incorporated with coated MNP@CNC (C-MNP@CNC, 0.1 - 0.5 wt.%.). Magnetically responsive hydrogels were formed without (isotropic) and under (anisotropic) the influence of uniform magnetic fields (100 to 400 mT). The physicochemical and magnetic properties of nanoparticles and hydrogels were thoroughly characterized. The biological performance of selected nanocomposite hydrogels formulations was studied using human adipose-derived stem cells (hASCs).

RESULTS AND DISCUSSION:

Remarkably, high degrees of MNP@CNCs alignment within the hydrogel matrix were obtained under uniform magnetic field as low as 108mT, facilitating the implementation of the concept in clinical scenarios. As hypothesized, nanoparticles alignment within nanocomposite hydrogels resulted in biomaterials with anisotropic microstructure and mechanical properties. hASCs encapsulated in nanocomposite hydrogels have shown high rates of viability demonstrating that the nanocomposite biomaterials are not cytotoxic. Moreover, the microstructural patterns stemming from nanoparticles alignment induced the directional growth of seeded and, to some extent, encapsulated cells within the hydrogels.

CONCLUSION:

Overall, these results demonstrated that the proposed concept widen the design space of injectable hydrogel and suggest that it might find broad applications as minimal invasive TERM strategies of anisotropic tissues.

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Poster presentation

844 3D printing of flexible bioactive glass containing nanocomposite hydrogels

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INTRODUCTION:

Bioactive glasses (BGs) are well-known biomaterials that can bond with bone tissues and promote osteogenic/angiogenic activities towards tissue regeneration[1]. Hydrogels can mimic some of the physiochemical properties of the extracellular matrix (ECM) because of their structural similarity to tissues which make them suitable materials for tissue engineering[2]. The mechanical and biological properties of hydrogels can be improved by the inclusion of BGs[4]. Nanoscale BGs (BGNs) are suitable fillers for enhancing the performances of hydrogels towards regenerating tissues, considering their morphological advantages (e.g. large surface-area-to-volume ratio)[5]. A combination of BGNs and hydrogels is thus expected to induce an advanced nanocomposite hydrogel for tissue regeneration.

METHODS:

We used a 3D printing method to fabricate BGNs/gelatin-cellulose nanocomposite hydrogels for tissue regeneration applications[6]. BGNs (SiO_2-CaO composition system) were synthesized using a sol-gel based method and they were added to gelatin-cellulose gels before printing. The influence of mass ratios of BGNs/gelatin-cellulose were investigated in terms of printability and physicochemical properties.

RESULTS AND DISCUSSION:

Fig. 1 shows representative optical images of printed nanocomposite hydrogels that exhibits a well-defined pore structure. Their pore size and thickness could be conveniently controlled by tuning the processing parameters of printing. These hydrogels also exhibited good bendability and flexibility (Fig. 1) which are of great interest to certain applications. The mass ratio of BGNs in the hydrogels could be varied in a large range without significantly affecting the printability. The addition of BGNs induced hydroxyapatite formation on hydrogels in body fluid. The nanocomposite hydrogels are degradable and also able to release bioactive ions.

CONCLUSION:

This study provides a 3D printing strategy to fabricate flexible BGNs-containing nanocomposite hydrogels that show great potential in the applications of tissue regeneration.

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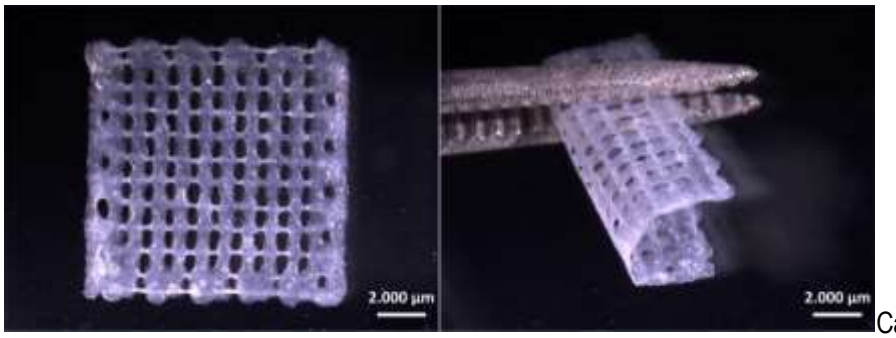
S.R. would like to thank the German Academic Exchange Service (DAAD) for financial support.

Picture 1: 

Caption 1: Fig. 1. Representative optical images of 3D printed BGNs (0.1 wt.%)/gelatin-cellulose nanocomposite hydrogel.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

845 Drug loaded coaxial hydrogel fibers as drug delivery depots for cancer treatment

Sepehr Talebian[1], Javad Foroughi[1], Samantha J. Wade[2], Kara Perrow[2], Gordon Wallace[1]
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INTRODUCTION:

Hydrogels are soft materials that possess 3D network structure with tuneable physical and chemical properties[1]. They have been investigated for their use in drug delivery, as gel solutions can be loaded with biologically active compounds, such as chemotherapeutic drugs. Howbeit, pristine hydrogels came up short in cancer drug delivery due to their intrinsic burst release profile and low loading capacity [2]. In this study, in an attempt to address such short comings, we have designed a core-shell wet-spun fiber, where the core was comprised of bioinspired catechol conjugated alginate loaded with the chemotherapeutic drug (Gemcitabine or Doxorubicin) and the shell contained a UV-cross linkable alginate-methacrylate. Such specific composition is expected to allow controlled release of the drugs while providing a high encapsulation efficiency.

METHODS:

Alginate-dopamine synthesis

Carbodiimide chemistry was used to synthesize alginate-dopamine.[3] The yielded alginate dopamine was further characterized by H-NMR and FTIR spectroscopies.

Alginate-methacrylate synthesis

Alginate-methacrylate was synthesized accordingly to a previously established method.[4] The samples were characterized using FTIR and HNMR spectroscopies.

Wet-spinning of drug loaded coaxial fibers

A coaxial fibers were fabricated based on a established method by our group.[5]

Drug release and in-vitro cell studies

Drug release was measured based on a method by our group.[6] the in-vitro studies were done of MDA-MB-231 breast cancer cells and Mia-paca-2 pancreatic cell lines.

RESULTS AND DISCUSSION:

The core-shell fibers showed acceptable mechanical properties in both dry and wet states, besides these fibers were capable of controlled delivery of anti-cancer drugs. the in-vitro results proved the anti-cancer performance of the fibers.

CONCLUSION:

The specific composition of these core-shell fibers allowed controlled release of drugs.Besides these wet-spun fibers are capable of being processed into 3D implnatble textiles which further adds to their value when it comes to clinical studies.

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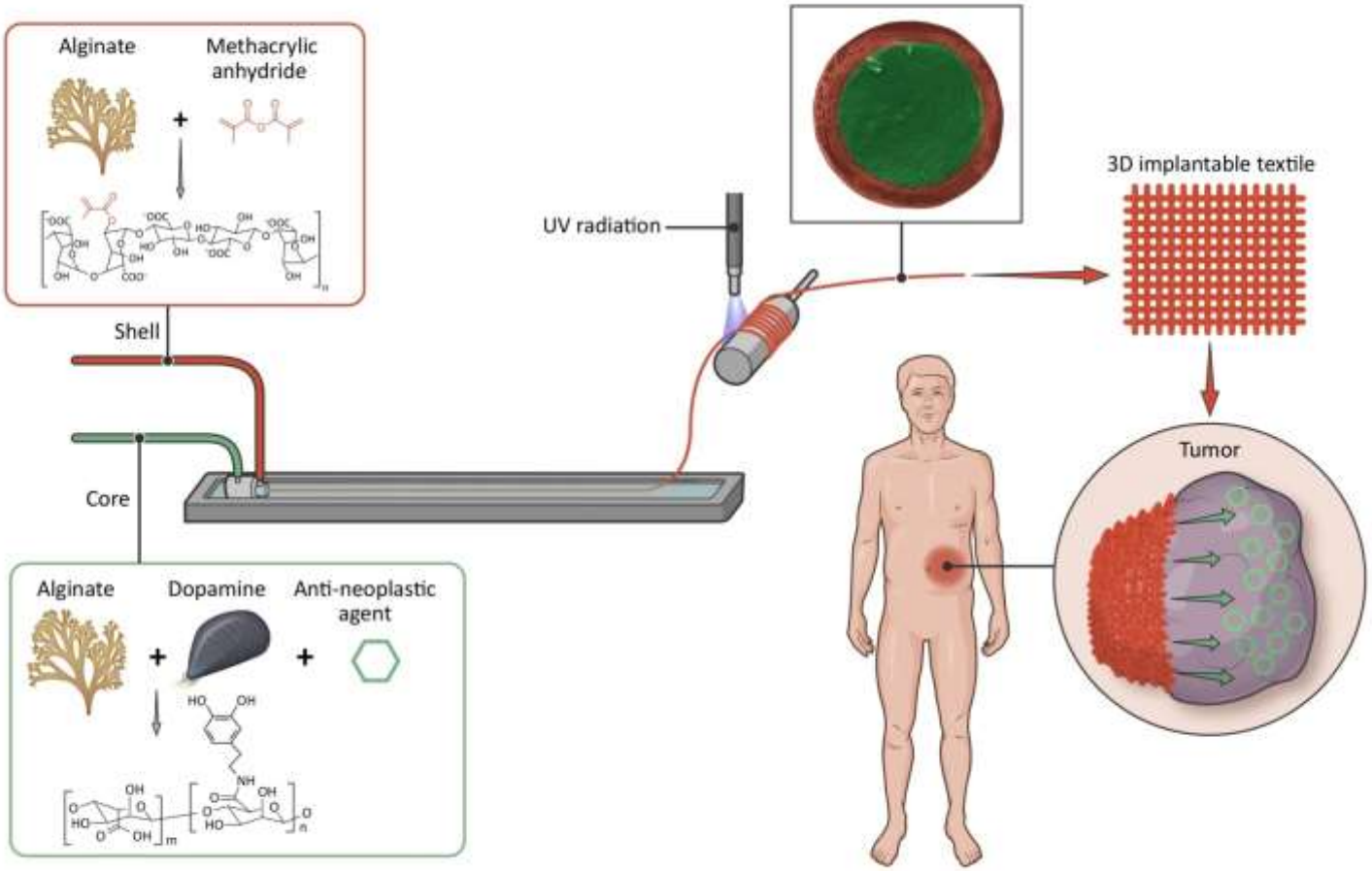
Picture 1: Caption 1: Graphical abstract



Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

848 Crystallographic texture control of biomedical beta-type Ti-15Mo-5Zr-3Al alloy by laser powder bed fusion process

Takuya Ishimoto, Koji Hagihara, Shi-Hai Sun, Takayoshi Nakano
Osaka University, SUITA, Japan

INTRODUCTION:

Biomedical implant materials with low Young's moduli have been demanded to suppress stress shielding [1]. b-type Ti–15Mo–5Zr–3Al (mass%) alloy is a promising candidates because of its reduced Young's modulus (85 GPa). Interestingly, the modulus can be further reduced into 44 GPa in a single crystalline <001> [2]. In this study, we aimed at fabricating single crystalline-like highly texturized Ti–15Mo–5Zr–3Al products by a selective laser melting (SLM).

METHODS:

Ti–15Mo–5Zr–3Al powder produced by the gas-atomization was used for SLM. The specimens with dimensions of 5 mm × 5 mm × 10 mm were built under the two-types of scanning strategies (SSs): one was a bidirectional scanning without any rotation between the layers (SS_X); the other was a bidirectional scanning with rotation of 90˚ between layers (SS_XY). The melt pool morphology was observed using an optical microscope, and inverse pole figures (IPFs) was taken using an electron backscatter diffraction (EBSD) system.

RESULTS AND DISCUSSION:

In the product fabricated by SS_X, <001> and <011> orientation arose in the building direction (z) and the scanning direction (x), respectively. On the other hand, the SS_XY formed the <001> texture along the x, y and z directions. In this process, solidification occurred from the melt-pool wall or bottom inheriting the crystal orientation of underneath and/or neighboring pre-solidified parts. In the sample fabricated by SS_X, two directional cell growths elongated along −45° and 45° from the building direction in the melt pools. The elongated direction of cellular microstructure almost corresponds to <001>. For SS_XY, cellular microstructure growth in two directions is observed for a melt pool generated by an X-scan; the <001> elongated cells generated around the melt pool bottom grew along the building direction, and other <001> elongated cells generated around the upper wall of the pool grew vertically with respect to the building direction. The texturized products obtained here are promising as biomedical implants that possess biocompatible low Young's modulus.

CONCLUSION:

We clarified that the scan strategy in the SLM process is an important controlling parameter for crystallographic texture. The SLM is a good tool to fabricate products not only with complicated 3D structures but with crystallographic textures.

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Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

850 Biodegradable Polyurethane-Fibrinogen Elastomers in Skeletal Muscle Tissue Engineering

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[2]Hacettepe University, ANKARA, Turkey

INTRODUCTION:

Functional skeletal muscle substitutes are highly demanded since there is no effective drug therapy to induce muscle healing and the availability of suitable donor tissues are limited. A key challenge in skeletal muscle tissue engineering is scaffold design, as the material should withstand and support the dynamic loading of the tissue

microenvironment[1,2]. In this study, we tested the applicability of a novel biodegradable elastomer to fulfill these requirements.

METHODS:

Novel biodegradable polyurethane-fibrinogen (PU-Fib) elastomers were synthesized by two-step condensation polymerization and the chemical, thermal, viscoelastic and biodegradation properties of PU-Fib were characterized. Aligned PU-Fib microfibers were produced, seeded with mouse myoblasts and uniaxial cyclic stretch was applied using a custom made bioreactor system for 21 days to mimic the native tissue architecture and dynamic microenvironment. Cell proliferation, viability and the expression of muscle-specific markers (immunofluorescent staining for MyoD1, myogenin, MHC) were assessed.

RESULTS AND DISCUSSION:

FTIR-ATR spectrum showed amide peaks specific to PU and Fib and DSC thermograms showed the suitable integration between the components. Dynamic mechanical analysis revealed Tg ve Tα* transitions at 64.5°C and 38.4°C, respectively. PU and Fib had shown chemically compatible interactions and PU-Fib possessed suitable viscoelastic properties with the native tissue.

Myoblasts proliferated well on PU-Fib fibers; aligned parallel to the axis of the fibers, and express myogenic markers (including MHC) under biomimetic dynamic culture (Figure 1).

CONCLUSION:

It was possible to culture myoblasts with high viability on PU-fib elastomeric fibers mimicking native muscle architecture and structure.

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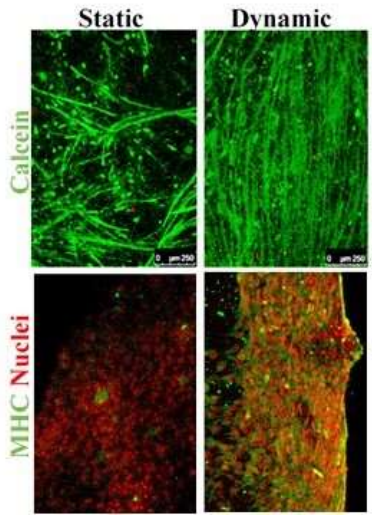
ACKNOWLEDGMENTS:

The authors would like to thank Ankara University Research Fund (Grant no: 16L0443010) for providing financial support to this project.

Picture 1:



Caption 1: Figure 1. Mouse myoblasts on PU-Fib fibers on day 21.

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

873 Quantitative collagen arrangement of bovine pericardium with micro CT

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INTRODUCTION:

Bioprosthetic heart valves constructed from bovine pericardial tissue are used for the replacement of diseased heart valves. These valves are limited in their durability, showing complications in 50% of patients after 10 years[1]. Failure rates may be improved by better characterisation of tissue mechanical properties[2]. Collagen is the main load bearing component in pericardium and is thought to give rise to its complex mechanical properties[3]. Bovine pericardium has been shown to have a high intra- and inter-specimen variability of its collagen fibre architecture[4].

A non-destructive optical imaging technique has been recently developed for quantifying the bulk anisotropic and heterogeneous structural arrangement of collagen in soft tissue membranes, such as bovine pericardium[5]. The measurement of bulk tissue optical properties, which are accumulated over the interrogated depth, must be compared with full volume imaging of pericardium. The objective of this work is to develop a protocol for full volume imaging of pericardium that gives the 3D arrangement of collagen.

METHODS:

Bovine pericardia, harvested post mortem from Angus-Hereford steers aged 18 to 24 months, were fixed with 0.625% glutaraldehyde in phosphate buffer solution (PBS) for 24 hours at room temperature and pressure, then stored in PBS prior to staining. Randomly selected regions were laser cut into squares and stained in 1% (w/v) PTA in 70% ethanol for 12 hours then stored in 70% ethanol prior to imaging. Samples were imaged in a micro-CT scanner (Bruker, SkyScan 1172) at a pixel resolution of 1.08 μm with X-ray source set at 47 kV and 201 μA.

RESULTS AND DISCUSSION:

The figure below shows a reconstructed volume for a sample of pericardium with dimensions of 4.3 mm × 2.7 mm × 0.50 mm. The fibrous structure on the epipericardial surface of the pericardium is highly resolved with good contrast between the stained tissue and the surrounding ethanol.

CONCLUSION:

This work demonstrates the first full volume imaging of collagen in bovine pericardium and will be used to assess the efficacy of an optical imaging technique for tissue selection of pericardial bioprosthetic heart valves.

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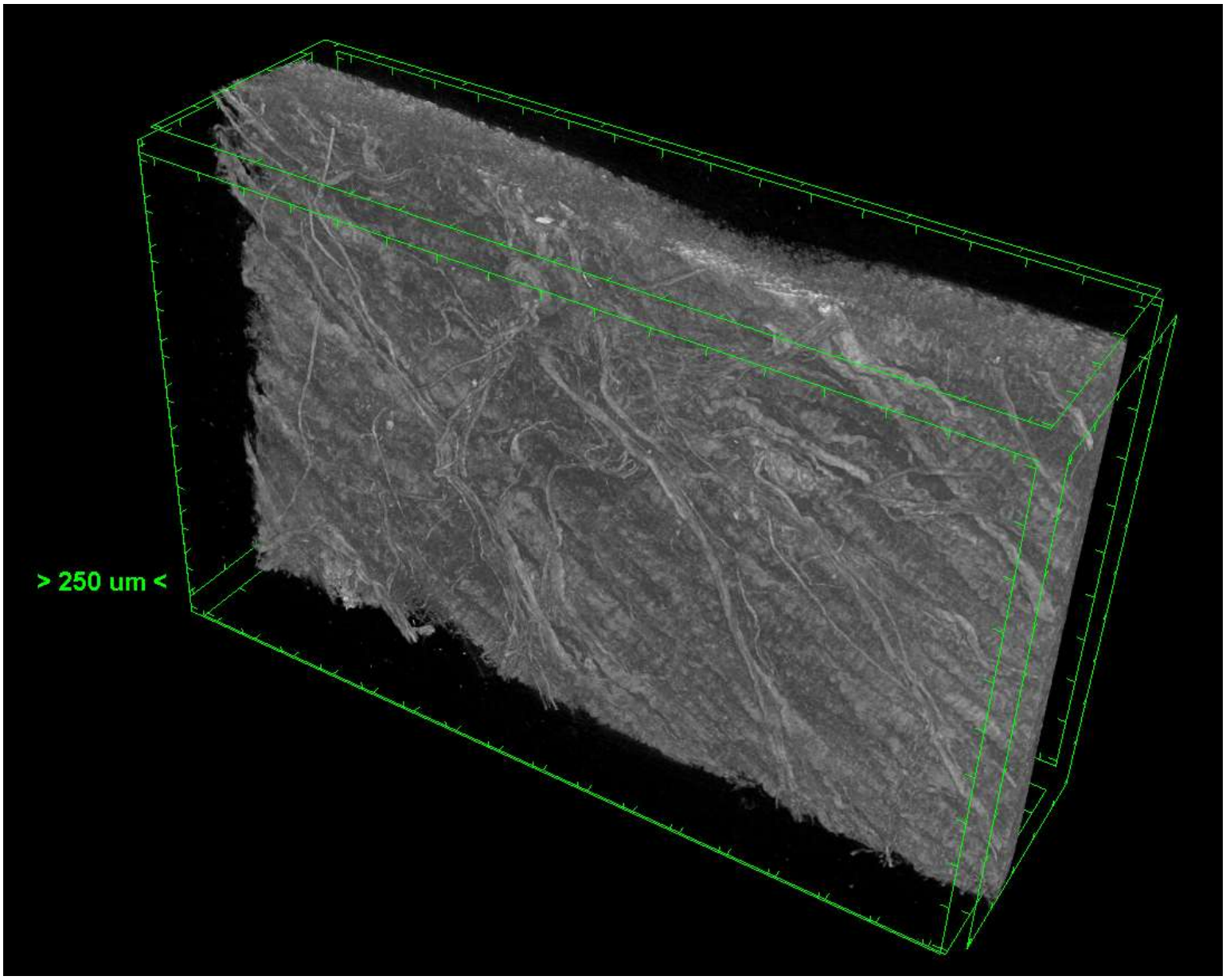
ACKNOWLEDGMENTS:

The authors would like to thank Dane Gerneke for assistance with micro-CT operation.



Picture 1: Caption 1: Reconstructed volume from micro-CT imaging of bovine pericardium. Sample dimensions: 4.3 mm × 2.7 mm × 0.50 mm

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

846 Deposition of polysulfobetaine via pyorgallo deposition for anti-fouling applications

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National Taiwan University, TAIPEI, Taiwan

INTRODUCTION:

In recent years, poly-zwitterionic materials, such as poly(sulfobetaine methacrylate)(SBMA), have been applied to modify the surface of a wide range of biomaterials for anti-fouling applications due to the polymers' high hydration capacity. Many approaches aim to anchor zwitterionic polymers on the surface to obtain a well-packed antifouling surface. In this work, we used a simple immerse process based on pyrogallol deposition to a wide variety of materials. A copolymer of SBMA and aminoethyl methacrylate (AEMA) was synthesized and co-deposited with pyrogallol on various substrates. The antifouling efficacy of the coatings was evaluated.

METHODS:

pSBMA-co-AEMA was synthesized from SBMA and aminoethyl methacrylate (AEMA) via free radical polymerization. Pyrogallol (PG) in PBS (pH7.4) was mixed with pSBMA-co-AEMA solution and then added onto substrates for incubation at 45°C under constant agitation. After coating, substrates were rinsed with deionized water and air-dried. The coatings were characterized via field-emission scanning electron microscope, Fourier-transform infrared spectrometry, atomic force microscopy, water contact angle measurement and X-ray photoelectron spectroscopy. The anti-fouling efficacy of the coatings was evaluated via the attachment of L929 cells and the adsorption of fibrinogen.

RESULTS AND DISCUSSION:

Poly(SBMA-co-AEMA) was deposited on various substrates such as polystyrene and PDMS, along with PG deposition. The surfaces conjugated with poly(SBMA-co-AEMA) resisted cell adhesion and protein adsorption. The resistance against cell adhesion was increased with increasing coating time and is positively correlated with the surface hydrophilicity and film thickness. Protein adsorption was also greatly reduced on the SBMA copolymer-coated surfaces. The surface coating is robust to resist harsh sterilization conditions.

CONCLUSION:

This work developed a simple one-step coating method that could be applied on various substrates. Zwitterionic pSBMA-co-AEMA was successfully immobilized on a variety of substrates and resisted cell adhesion and protein adsorption. The positive correlation between anti-fouling ability and surface hydrophilicity indicates that surface hydrophilicity plays a major role in antifouling efficacy. This study fabricate a transparent and antifouling film, providing a simple route to endow biomaterials with high antifouling ability. We expect that the strategy for anti-fouling coatings is applicable in manufacturing medical devices.

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Picture 1:

Caption 1: The scheme of cell and protein adhesion resistance by polySBMA-modified surface

Poster presentation session A 13:15 - 14:15 10/09/2018

Poster presentation

162 Evaluation of Boron Modified Bioactive Glass Nanoparticles Incorporated Nanobiocomposite Scaffolds for Dentin Tissue Engineering

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[1]Middle East Technical University, Ankara, Turkey

[2]Oral and Maxillofacial Surgery, Topraklik Mouth and Teeth Health Center, Turkey

INTRODUCTION:

Dental caries is a disease affecting public health with socio-economic consequences. Dentin layer is the most damaged part in tooth caries (1). Dentin tissue engineering is a new strategy for regenerating damaged dentin (2). In this study, we aimed to develop boron modified bioactive glass nanoparticles (BG-NPs) containing cellulose acetate/oxidized pullulan/gelatin (CA/ox-PULL/GEL) three dimensional scaffolds for dentin tissue regeneration.

METHODS:

Pure and borate modified BG-NPs with different ratios (7, 14, 21%) were synthesized by quick alkali-mediated sol–gel method (3). Thermally induced phase separation/porogen leaching method was used to prepare three dimensional scaffolds containing (CA/ ox-PULL/GEL) and 10 or 20% BG-NPs. Human Dental Pulp Stem Cells (hDPSCs) were isolated from human third molars by enzymatic digestion. For scaffolds structural analysis, in vitro degradation, water, SEM analysis, in vitro bioactivity, porosity and mechanical tests and for biological evaluation, Alamar blue assay, and tests for evaluation of odontogenic differentiation (ALP activity, intracellular calcium measurements, and etc.), were done. Statistical analysis was done with (ANOVA) with Tukey's post hoc test for multiple comparisons.

RESULTS AND DISCUSSION:

Three-dimensional porous scaffolds with tubular pore structures were successfully produced. Bioactivity tests showed higher apatite-like depositions on boron modified BG containing scaffolds. Scaffolds without BG possessed the highest porosity percentage (94.3± 1.5%) (n=3). 10%BG addition improved the mechanical properties of scaffolds. Presence of boron modified BGs increased viability of hDPSCs on scaffolds. On day 14, cells in B14-10 and B14-20 groups had the highest ALP enzyme activity (Fig.1a) and intracellular calcium amounts of cells on B14-10 and B14-20 scaffold groups were significantly higher than all groups (Fig.1b).

Picture 1:

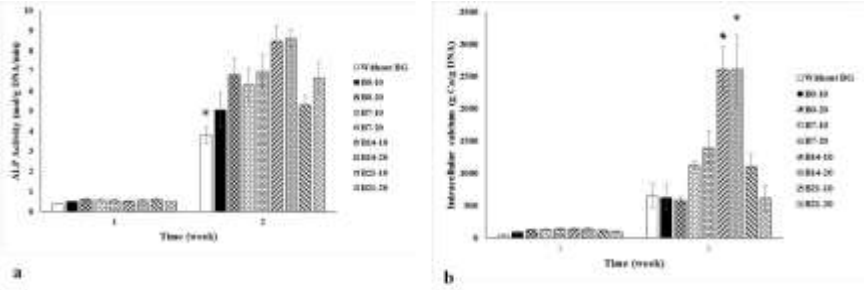

Figure 1. a) ALP enzyme activity, b) Intracellular calcium amounts of hDPSCs seeded on scaffolds (n=4). * designates groups that are significantly higher ($p<0.05$) than other groups at week 2.

Poster session B

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

14 New zirconium alloy to decrease MRI artifact

Takao Hanawa[1], Kou Honma[2], Maki Ashida[1], Yusuke Tsutsumi[3], Naoyuki Nomura[1], Hisashi Doi[1], Masayuki Shimojo[1]
[1]Tokyo Medical and Dental University, Tokyo, Japan
[2]Shibaura Institute of Technology, Japan
[3]Tokyo Medical and Detal University, Japan

INTRODUCTION:

Metals implanted into the humanbody have sometimes higher magnetic susceptibility than the tissues. Therefore magnetic resonance imaging (MRI) diagnosing is disturbed by artifacts appearing on the MR images. In our previous study, Zr-1Mo alloy with low magnetic susceptibility is developed [1] and generates much smaller artifact tahn Ti-6Al-4V ELI alloy. In this study, with d-electron alloy design method, Zr alloy is designed and in the resultant composition alloy mechanical properties, magnetic susceptibility, corrosion resistance, and cytocompatibility are evaluated.

METHODS:

Employing d-electron alloy design method, Composition of alloys were decided. The designed ally was fabricated by induction skull melting. The ingot was forged at 1050°C. A rod with 5 mmφ was obtained through electrical discharge machining. Then, cold swaging was performed to 56% and 97%. In addition, heat tretment was performed to all sepecimens: heated at 400°C for 45 minand quenched in iced water. The crystal phase structure was characterized using XRD and TEM. Mechanical property was evaluated by tensile test and Vickers handness test. Moreover, corrosion resistance and cytocompatibility were evaluated.

RESULTS AND DISCUSSION:

According to d-electron alloy design method, composition of alloys were determined as Zr-14Nb-5Ta-1Mo (mass%). There is no macro-segregation in the uingot of the alloy. Hot forging and cold swaging are performed without any farcture of the specimen. The alloy was consituted of β pahse with a smaal amout of α pahse and/or ϖ pahse. In the case of 97% cold swaging, tensile strength was 1054 MPa, 0.2% proof strength was 1011 MPa, elongation to farcture was 16%, Vickers hardness was 258, and Youngs' modulus was 67 GPa. The mass magnetic susceptibility was 18.1 x 10^{-9} m^3kg^{-1}. After heat treatment, Tensile strength 0.2% proof strength, and Vickers haedness increased reminning Youngs modulus and magnetic susceptibility. in addition, this alloy did not show any cytotoxicity.

CONCLUSION:

Zr-14Nb-5Ta-1Mo alloy has exllent balance of mechanical property with low magnetic susceptibility, high corrosion resistance, and no cytotoxicity. This alloy achieves karge strength and elongation with small Youngs modulus that is not aquired in conventional titanium alloys. In addition, magnetic susceptibility was half of titanium alloys. Therefore, Zr-14Nb-5Ta-1Mo alloy is a useful metallic materials for medical use.

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ACKNOWLEDGMENTS:

This research was supported by by Grant-in-Aid for Scientific Research (B) 15H03018 from Japan Society for the Promotion of Science (JSPS).

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

20 Human adipose stem cell viability and proliferation on magnesium and strontium containing bioactive borosilicate glasses

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[2]BioMediTech, University of Tampere, Finland

INTRODUCTION:

Bioactive glasses are promising materials utilized in regenerative medicine, mainly in bone tissue engineering applications.[1] Borosilicate bioactive glasses are especially interesting as they have fast conversion to hydroxyapatite, which enables the material to degrade more completely in vivo.[2] However, in some experiments boron containing glasses have been shown to reduce cell proliferation.[3] Alternatively, magnesium (Mg) and strontium (Sr) elements in bioactive glass are known to be beneficial for bone formation.[2,4,5] In this study, the impact of Mg and Sr substitution of Ca in borosilicate glasses was studied on human adipose stem cells (hASCs) viability and proliferation.

METHODS:

Bioactivity of a borosilicate glass series was tested with hASCs. In investigated glasses, varying amounts of Ca were replaced with Mg and/or Sr, following the molar composition $47.1SiO_2$-$6.7B_2O_3$-$22.7Na_2O$–(21.8-x-y)CaO-$1.7P_2O_5$–xMgO–ySrO, where x,y varied from 0 to 10 mol%. The objective was to assess hASCs viability and proliferation when cultured on the surface of glass discs, in basic medium (BM).

RESULTS AND DISCUSSION:

In the literature, it is noticeable that borosilicate glasses can lead to poor proliferation of cells. Our results with B12.5 glass (x,y=0) support this finding (Fig.1). B12.5 glass showed limited hASC proliferation, while substitution of Ca with Sr and/or Mg greatly improved both cell viability and proliferation (Fig.1). Results will be correlated to the release of ions in the medium, measured by ICP-OES. In the coming experiments, in addition to cell viability and proliferation, the differentiation towards osteogenic lineages will be studied.

CONCLUSION:

Based on preliminary results, substitution of Ca for Mg/Sr increased hASC viability and proliferation on borosilicate glass. This could be due to either a decrease in the glass reactivity, a decrease in [B] in the medium and/or a decrease in the [Ca] burst from the glass surface.

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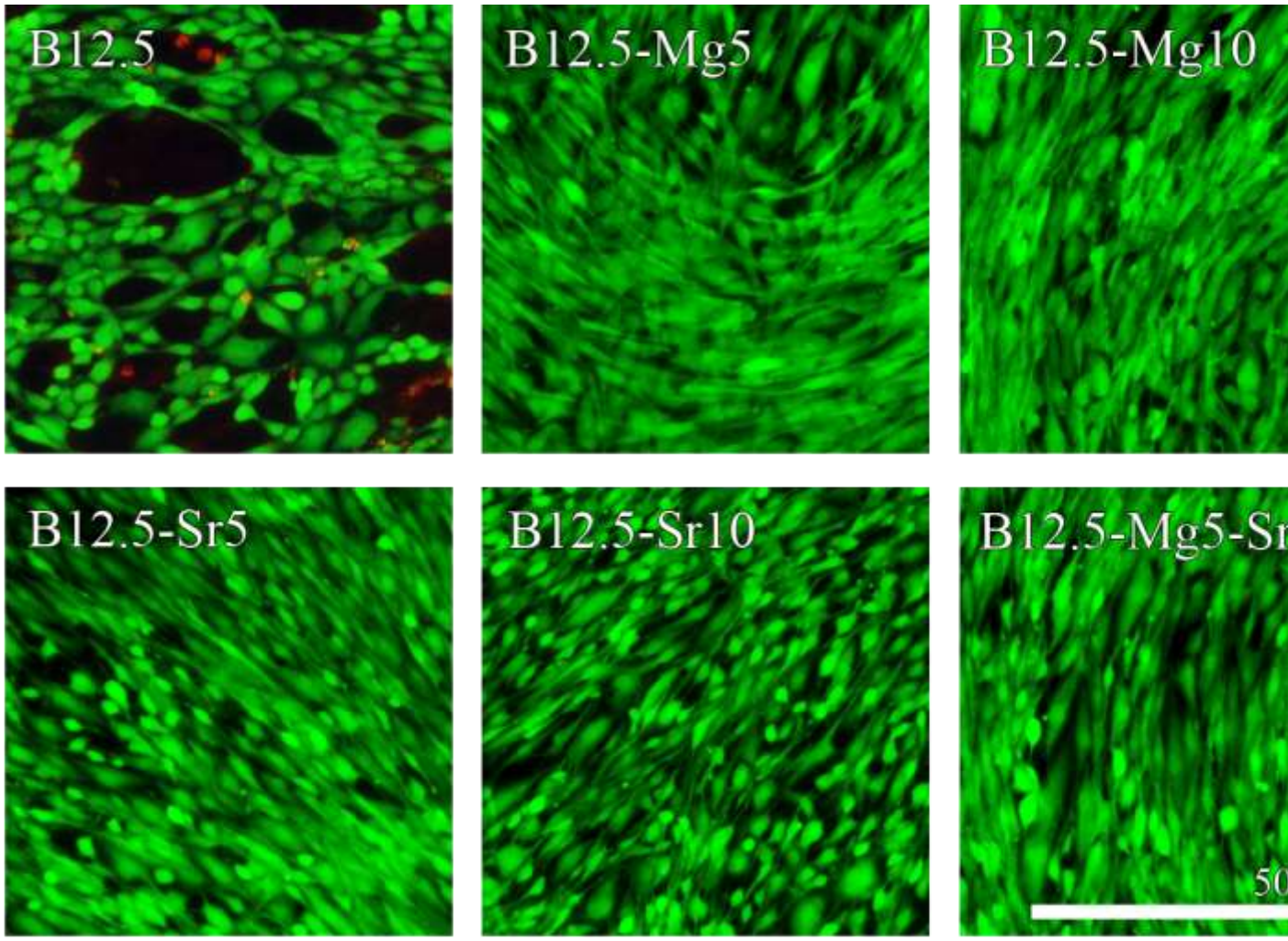
ACKNOWLEDGMENTS:

The authors would like to thank Jane and Aatos Erkko foundation (AGATE project) for providing financial support to this project.

Picture 1:



Caption 1: Figure 1. The viability of hASC (via live/dead staining) on bioactive borosilicate glass discs, in BM, after 7 days of culturing.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

33 Facilitating wound healing in the splinted nude mouse model with an engineered living modular construct

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[1]Regenerative, Modular and Developmental Engineering Laboratory (REMODEL), Galway, Ireland

[2]Sofradim Production, France

INTRODUCTION:

Advanced therapeutic medicinal products for wound healing applications are an

evolving option, aiming to improve wound healing outcomes by reducing wound healing closure

time by delivering cells, medical compounds and biologics. Matrix – rich tissue equivalents can be fabricated in vitro by employing biophysical, biological and biochemical cues. Our work is focused on the accelerated production of matrix-rich tissue equivalents with the utilisation of macromolecular crowding (MMC) which has been shown to enhance matrix deposition in vitro.

METHODS:

In this work, we investigated the effect of MMC on the fabrication of
a matrix rich tissue equivalent for a wound healing application.
A collagen-based film has been utilised for the fabrication of a modular, matrix-rich cell carrier for wound healing. The structural, mechanical and thermal properties of the material were assessed with electronic microscopy, uniaxial mechanical testing and differential scanning calorimetry respectively. Human dermal fibroblasts and stem cells (bone marrow and adipose tissue derived) were expanded up to passage 3 in DMEM and MEM media, supplemented with 10% fetal bovine serum and 1% penicillin / streptomycin. For the enhancement of extracellular matrix deposition, a macromolecular crowding agent (Carrageenan) was utilised at all time points. Matrix deposition was assessed with immucytochemisty. A splinted wound healing model in athymic nude mice was utilised to assess wound healing in vivo. Wound healing closure ratio was assessed on day 3,7 and 14. Tissues were harvested 14 days post implantation for histology.

RESULTS AND DISCUSSION:

Extracellular matrix deposition has been enhanced at all time points
when carrageenan was used as a MMC agent in the in vitro regime.The 30k/cm2 cell seeding density was found the most suitable for the fabrication of a cell and matrix rich construct in the 7day culture period prior in vivo implantation.Modular constructs grown in vitro in the presence of carrageenan,facilitate improved and accelerated wound healing in vivo when implanted in the athymic nude mouse model.

CONCLUSION:

Collagen – based matrix-rich tissue equivalents for cutaneous tissue engineering fabricated with collagen and MMC facilitate the enhanced matrix deposition in vitro, and improved and accelerated in vivo wound closure. Further assessment is under way.

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ACKNOWLEDGMENTS:

Irish Research Council and Health Research Board for financial support. KS, DG, SK and DZ have no conflict of interest. YB is an employee of SP

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

52 A smart fluorescent nanoprobe to visualize the pathway of nanoparticles in living cells

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[2]Rhine-Waal University of Applied Sciences, Germany

[3]University of Duisburg-Essen / Imaging Centre Campus Essen (ICCE), Germany

[4]University of Duisburg-Essen / Molecular Biology and Centre, Germany

[5]University of Duisburg-Essen / Molecular Biology, Germany

INTRODUCTION:

Calcium phosphate naturally occurs in bone tissue and teeth and is generally considered to have a good biocompatibility and a high biodegradability in biological applications.[1] Due to these properties of calcium phosphate, nanoparticles based on this material have become a potent delivery system of a vast range of cargo molecules, including proteins, for *in vitro* and *in vivo* experiments.[2,3] The aim was to create a smart tool to visualize the intracellular pathway and the fate of nanoparticles.

METHODS:

The nanoparticles were synthesized according to Sokolova et al.[4] and loaded with a fusion protein consisting of the two fluorescent units mRFP1-eGFP.[5] The particles were characterized by dynamic light scattering (DLS) and scanning electron microscopy (SEM). HeLa cells were incubated with protein-loaded nanoparticles (1 µg of protein per well) for 6 h, washed and then stained with 75 nM LysoTracker™ Deep Red for 1 h. Live-cell confocal laser scanning microscopy (CLSM) was performed on a TCS SP8 system (Leica Microsystems) using a 63x/1.2 water immersion objective.

RESULTS AND DISCUSSION:

The protein-loaded nanoparticles were taken up by the cells by endocytosis and then directed into lysosomes.[2] At a physiological pH of 7.4, the fluorescence of both proteins (red: mRFP1 and green: eGFP) is detectable. Under the conditions of an acidic pH of about 4.5-5 inside of lysosomes (Fig. 1), the green fluorescence will disappear[6] due to the protonation of the chromophore eGFP. Thus, only the red fluorescence of mRFP1 will remain detectable.

CONCLUSION:

The CLSM images showed the successful transport of protein-loaded nanoparticles, with some mRFP1 fluorescence co-localizing with lysosomes. However, eGFP fluorescence is only detectable outside of lysosomes, inside of recently build endosomes or at the cell membrane coming in from the periphery.

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ACKNOWLEDGMENTS:

The authors thank the Deutsche Forschungsgemeinschaft (DFG) for funding this project within the framework of the CRC 1093: Supramolecular Chemistry on Proteins and the ICCE for access to confocal laser scanning microscopy.

Picture 1:

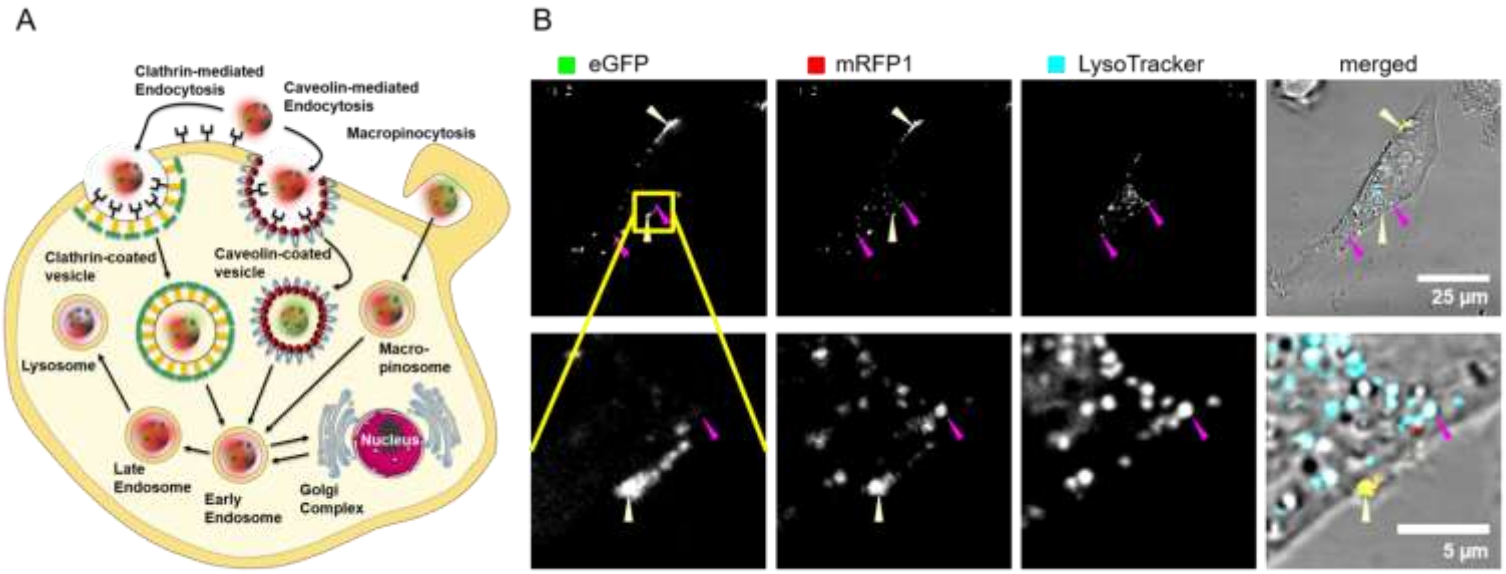

Fig. 1: A: Scheme of endocytic pathways of a nanoparticle uptake into eukaryotic cells.
B: CLSM images of CaP-NP mediated protein transport in living HeLa cells. Fluorescence signals are shown in grayscale and coloured in the merged images (upper and lower row). Green is representing eGFP, red for mRFP1 and cyan for Cy5 fluorescence. Lysosomes were stained with LysoTracker™ Deep Red (Cy5). The Magenta arrows are depicting the co-localization of mRFP1 fluorescence with Cy5 (lysosomes). There is no eGFP signal detectable inside of lysosomes (see magenta arrows in the eGFP channel) but green and red fluorescence at the periphery of the cell membrane (pale yellow arrows).

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

69 Fabrication of Carbonate Apatite Honeycomb and its Tissue Response

Kunio Ishikawa, Melvin Munar, Koichiro Hayashi

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INTRODUCTION:

Artificially fabricated carbonate apatite (CO_3Ap) granules fabricated through dissolution-precipitation reaction using calcium carbonate ($CaCO_3$) granules as precursor are replaced to bone and demonstrated higher osteoconductivity when compared to hydroxyapatite. To accelerate replacement of CO_3Ap to bone, introduction of the interconnected porous structure is one of the ideal methods. In the present study, feasibility to fabricate CO_3Ap honeycomb (HC) was evaluated using $CaCO_3$ HC as a precursor. Also, tissue response to CO_3Ap HC at bone defect was evaluated using experimental animals.

METHODS:

$Ca(OH)_2$ powder was mixed with a wax based binder, and extracted through a HC mold. The $Ca(OH)_2$ and binder HC was heated to burn out the wax based binder and carbonate $Ca(OH)_2$ in the tubular furnace under O_2-CO_2 atmosphere. $CaCO_3$ HC, thus prepared was immersed in sodium phosphate solution at 80°C for 7days for compositional transformation from $CaCO_3$ to CO_3Ap. Tissue response to the CO_3Ap HC was evaluated by reconstructing the bone defect made at the femur of rabbit and examined histologically.

RESULTS AND DISCUSSION:

When $Ca(OH)_2$ mixed with wax-based binder was extracted through HC mold, $Ca(OH)_2$ with wax-based binder HC was made. When the HC was heated at 700°C under O_2-CO_2 atmosphere in a tubular furnace, wax-based binder was debindered. The HC structure was maintained even after debinder process as shown in Fig (a). XRD analysis revealed that composition of the $Ca(OH)_2$ with binder became $CaCO_3$ after exposure to O_2-CO_2 atmosphere at 700°C. In other words, $CaCO_3$ HC was successfully fabricated.

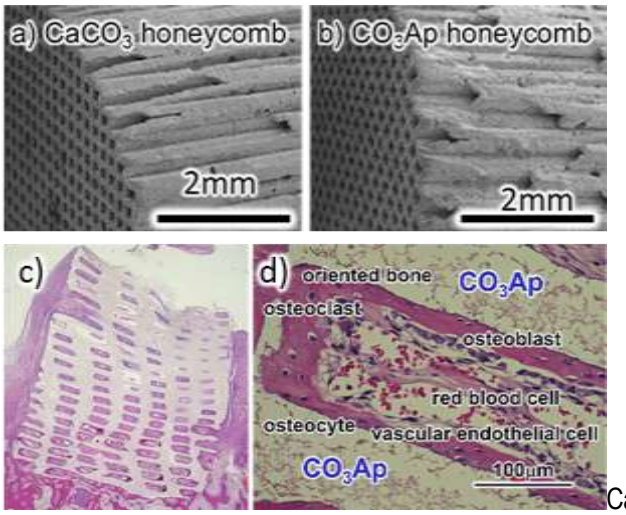
Then, the $CaCO_3$ HC was immersed in 80°C Na_2HPO_4 solution for compositional transformation. No macroscopic change was observed even after immersed in 80°C Na_2HPO_4 solution as shown in Fig. (b). In contrast, the composition of the $CaCO_3$ HC became CO_3Ap after immersion in 80°C Na_2HPO_4 solution based on dissolution-precipitation reaction.

When bone defect at the femur of the rabbit was reconstructed with the CO_3Ap HC, no inflammatory response was observed during the implantation period. At 4 weeks, all pore of the CO_3Ap HC was filled with the new bone as shown in Fig (c). Larger magnification, Fig (d), revealed that the new bone was formed on the inside surface of CO_3Ap HC tube. In addition to osteoblasts and osteoblasts, vascular endothelial cell and red blood cells were observed.

CONCLUSION:

CO_3Ap HC has a good potential value to be an ideal artificial bone replacement.

ACKNOWLEDGMENTS:

Picture 1: 

Caption 1: Figure Typical SEM images of (a) CaCO3 HC, (b) CO3Ap HC, and (c) histological images of CO3Ap HC, after implanted for 4 weeks (d) lager magnification.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

71 Cross-linking hyaluronan with unsaturated fatty acids for biomedical applications

Gloria Huerta-Angeles, Tomas Medek, Martina Brandejsova, Katerina Kopecka, Ondrej Zidek, Jaromir Kulhanek, Hana Vagnerova, Vladimir Velebny

CONTIPRO a.s., Dolni dobrouc, Czech Republic

INTRODUCTION:

Hydrogels are hydrated polymeric networks with unique properties such as high-water content, softness, flexibility, and biocompatibility with many applications in tissue engineering. Up to now, one of the most studied macromers for cross-linking is methacrylated hyaluronic acid (MeHA)[#_ENREF_1]. Despite the many applications of the material [#_ENREF_2]. its degradation products are irritant [#_ENREF_3]. Therefore, new chemical modifications of hyaluronic acid (HA) are required to address increasingly demanding applications.

METHODS:

In this work, hyaluronan (HA) was chemically modified in order to cross-link the polysaccharide. Undecylenic acid, a fatty acid containing- double bonds was grafted by esterification reaction on the primary hydroxyl groups in HA by mixed anhydrides [#_ENREF_4]. The methodology produces a precise control of the degree of substitution by controlling the molar ratio of mixed anhydride in the reaction feed, temperature, and solvent polarity. The structure of the grafted copolymer was confirmed by IR, NMR, and SEC-MALLS. Chemically modified HA was photo cross-linked by Irgacure 2959 or DMPA, showing the feasibility of the macromer for the preparation of 3D polymeric networks.

RESULTS AND DISCUSSION:

The unsaturated fatty acid was activated by benzoyl chloride while forming a mixed anhydride. The second reaction is the esterification of HA. One of the most typical problems for chemical modification of HA, is to carry out reactions in water. As mixed anhydrides are stable in aqueous solution, the reaction process is suitable for up-scale. Advantageously, this methodology produces relatively high DS values, hence a considerably higher density of cross-

linkable substituents is accessible. UV-induced cross-linking was carried out at 365 nm in the order of seconds. The influence of experimental parameters such as reagent molar amount, concentration, reaction time, were systematically studied. In addition, several parameters such as concentration (1.5-7.5 % w/v), the molecular weight of the substrate (15-450 kDa) and degree of substitution (DS=7-42 %) were investigated. Finally, the derivative and the cross-linked hydrogel proved to be biocompatible.

CONCLUSION:

The esterification reaction is reproducible, yields high purity, and the conjugate retains the non-cytotoxicity of HA. The new HA derivative can be used as a water-soluble hydrogel precursor for fabricating single component pure HA hydrogel without the need of another polymer precursor and cross-linkers for scaffolds.

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

77 Supramolecular antimicrobial 'nanobullets' for intracellular bacteria targeting

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Institute for complex molecular systems, Eindhoven University of Technoloby, Eindhoven, Netherlands

INTRODUCTION:

Bacterial infections are common disease in human beings. Although endless effort has been spent to combat infections, complex and persistent infections, e.g., intracellular infections[1], are still emerging. With these infections, the bacteria can survive and persist inside the professional phagocytic cells, e.g., macrophages and neutrophils[2], as well as non-professional phagocytes e.g., epithelial and endothelial cells[3]. These bacteria shield themselves from the host immune system and antibiotic treatment, which may cause antibiotic resistance and induce chronic and recurrent infections[4]. To address this problem, intracellular delivery of antimicrobial peptides via a supramolecular method is proposed as a powerful strategy. To this end, supramolecular ureidopyrimidinone (UPy)-based materials are used as carriers since they have been successfully used for intracellular delivery purpose[5].

METHODS:

The UPy molecules were functionalized to obtain various assemblies (Figure 1a). The “UPy-nanobullets” were prepared through assembling of the UPy-assemblies in aqueous phase (Figure 1a). The formation, ζ-potential and morphology of these aggregates were fully characterized. The UPy-aggregates containing UPy-Cy5 as a reporter were then incubated with human kidney cells (HK-2) and THP-1 derived macrophages, respectively. Internalization of these aggregates was examined under a Leica SP5 confocal laser scanning microscope.

RESULTS AND DISCUSSION:

The formation of “UPy-nanobullets” containing antimicrobial peptide was confirmed by intensive Nile Red signals with strong hydrophobic pocket formation and the positively charged properties of these “nanobullets” are indicated by ζ-potential values (Figure 1, b-c). These aggregates are rod-like fibrils as confirmed by stochastic optical reconstruction micrograph (Figure 1d) and can be internalized by HK-2 cells and THP-1 derived macrophages (Figure 1, e-f).

CONCLUSION:

Supramolecular antibacterial “nanobullets” containing antimicrobial peptide has been successful prepared. These “nanobullets” can be internalized by professional and non-professional phagocytes, which indicates their potential to treat intracellular infections.

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ACKNOWLEDGMENTS:

We acknowledge the support from the Netherlands Cardiovascular Research Initiative (CVON 2012-01). The Dutch Heart Foundation, Dutch Federation of University Medical Centers, the Netherlands Organization for Health Research and Development and the Royal Netherlands Academy of Sciences. Additionally, this work was financially supported by the European Research Council (FP7/2007–2013) ERC Grant Agreement 308045, the Ministry of Education, Culture and Science (Gravity program 024.001.03).

Picture 1:

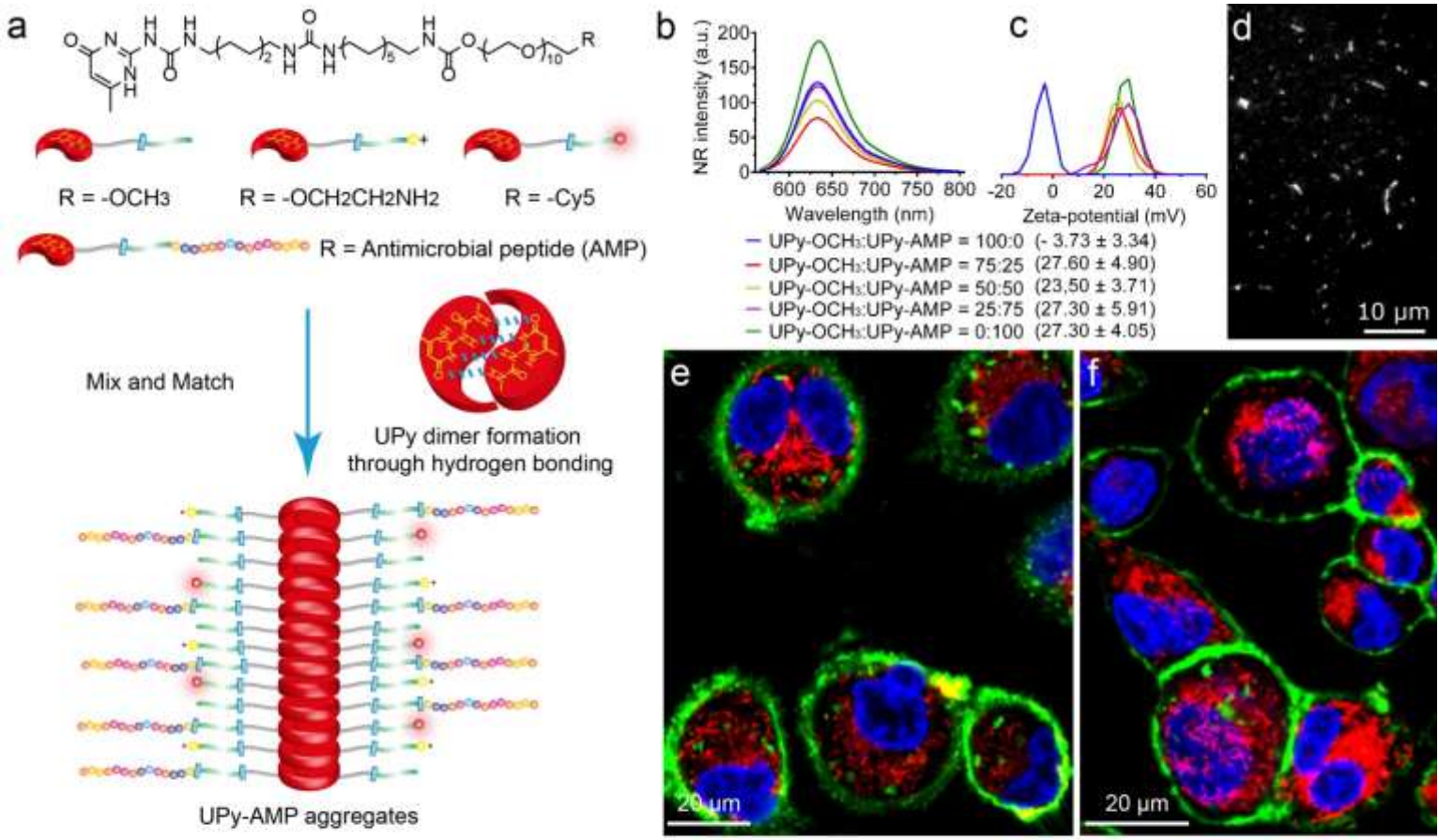

Figure 1. *Schematic illustration of fabrication of ureidopyrimidinone - antibacterial peptide (UPy-AMP) aggregates (a), characterization of these aggregates (b-d) and their internalization by mammalian cells (e-f). The formation of aggregates is confirmed by intensive Nile Red (NR) signals which indicate strong hydrophobic pocket formation (b), positive charge properties presented by zeta-potential (c), and morphology of aggregates containing 75% of UPy-AMP examined by stochastic optical reconstruction microscopy (d). The 75% UPy-AMP containing aggregates (red staining, 10 µM) can be internalized by human kidney cells (e) and THP-1 derived macrophages (f), with nuclei and membranes stained blue and green, respectively.*

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

80 Tailoring the mechanical properties of poly(1,3-trimethylene carbonate) networks

Iris Allijn[1], Marcelo Ribeiro[2], Mike Geven[3], André Poot[3], Dirk Grijpma[3], Robert Passier[2], Dimitrios Stamatialis[3]

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[2]Applied Stem Cell Technologies, University of Twente, Netherlands

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INTRODUCTION:

Liver, lung and heart are often used in organ-on-chips (OOCs).[1] The elastic modulus (E) of these tissues is < 50 kPa and increases under pathological conditions, like fibrosis[2–4] Here, we aim at developing a range of biomaterials resembling the elastic properties of human tissues, which can be used for cell supports in OOCs.

To achieve this, we prepared networks based on photo-crosslinked macromers of poly(1,3-trimethylene carbonate) (PTMC), a flexible and biocompatible polymer,[5] which however, has a relatively high E, with poly(ethylene glycol) (PEG), which has a lower E in the crosslinked and hydrated state.

METHODS:

PTMC 10 kg/mol was synthesized and functionalized with methacrylate end-groups (dMA) as described before.[5] PEG (6 and 10 kg/mol) was functionalized to PEG-dMA. Molecular weight and degree of functionalization (df) were determined by NMR.

For network formation, PTMC-dMA and PEG-dMA were dissolved in propylene carbonate (PC) (25% or 50% (w/w)) with a photo-initiator. Mixtures hereof were made at various ratios, cast and photo-crosslinked (365 nm, 20 min). PC was extracted using ethanol. The networks were dried at RT. E of the PBS-swollen networks was measured using a tensile tester. All data is from >5 tensile measurements ± stdev.

RESULTS AND DISCUSSION:

All prepared macromers have a df >85%. Photo-crosslinked PTMC-dMA networks made from a 50% (w/w) macromer solution have an E of 2.6 ± 0.1 MPa. Mixed networks with a 50% (w/w) PEG-dMA 6 kg/mol macromer solution gradually lowered the E in the wet state to 0.4 ± 0.02 MPa for a 90:10 PEG-dMA:PTMC-dMA ratio (Fig.1A). When preparing 25% (w/w) macromer solutions with the same 90:10 blend ratio, the value of E in the hydrated state was reduced to 0.08 ± 0.01 MPa. By keeping the same conditions, but using PEG-dMA 10 kg/mol, E could be further lowered to 0.04 ± 0.01 MPa (Fig.1B), which is in the physiological range of tissues. All networks are well crosslinked with a gel content >80%, are transparent (Fig.1C) and easy to handle.

CONCLUSION:

Here, we tailored the mechanical properties of PEG-dMA:PTMC-dMA photo-crosslinked networks in a range of elastic moduli resembling the mechanical properties of human tissues.[2–4] Selected networks will be applied in OOCs.

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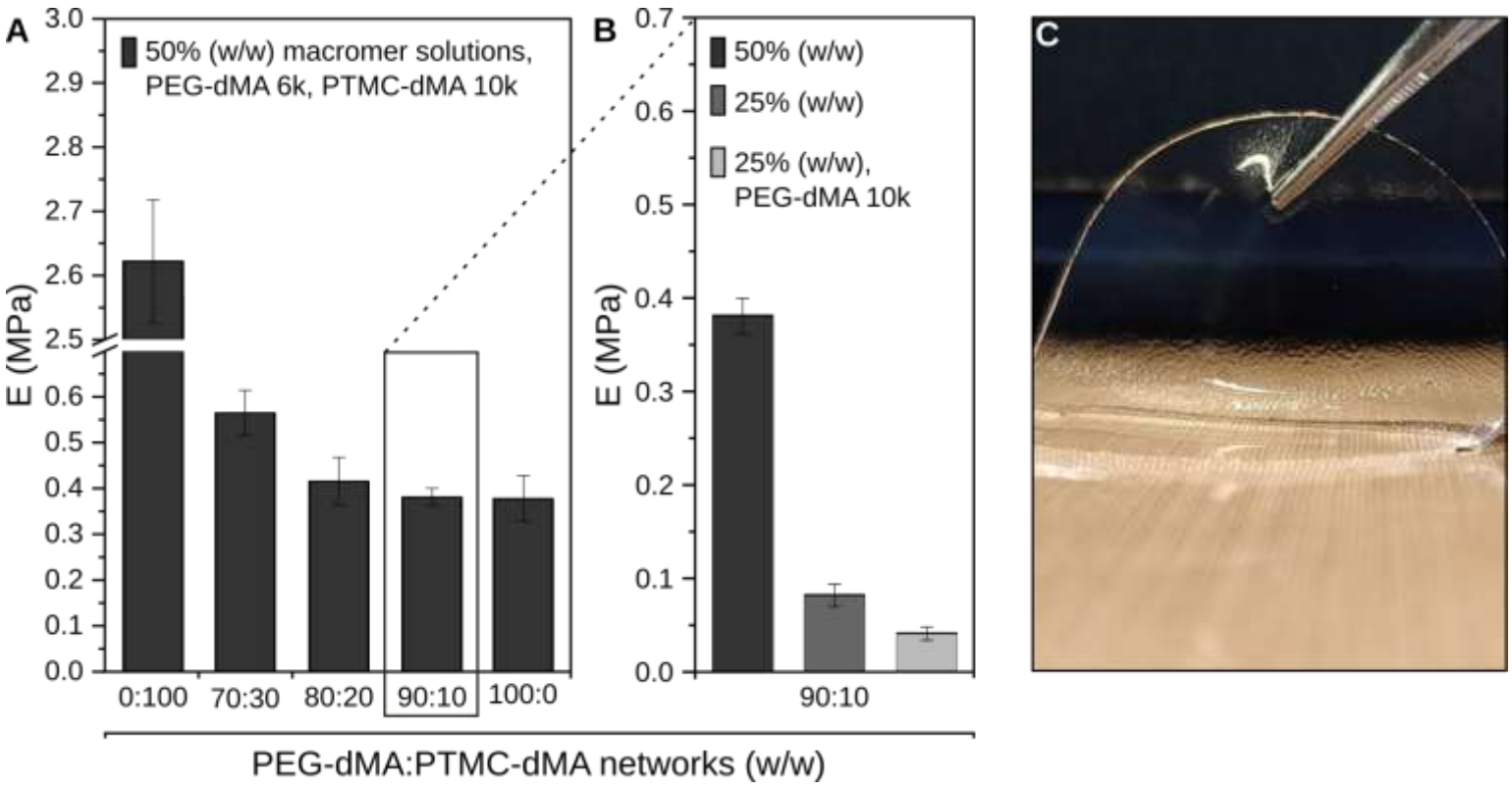
ACKNOWLEDGMENTS:

The authors thank ZonMW MKMD-114022507 for providing financial support.



Picture 1: Caption 1: Fig. 1. A) E of 50% (w/w) mixed networks in various ratios. B) E of different 90:10 mixed networks. C) Typical swollen appearance of the networks.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

81 Molecular control of tenocyt phenotype through matrix-mediated mechanotransduction

Aysegul Dede-Eren

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INTRODUCTION:

During *in vitro* sub-culturing, tenocytes lose their phenotype which ultimately affects their functioning[1]. As spindle-shaped fibroblasts, tenocytes have a unique thin elongated phenotype and they possess more spread-out shape through phenomena named dedifferentiation[1]. Given the link between cell shape and cell function, in this studye first aimed to dedifferentiate tenocytes through *in vitro* sub-culturing in order to have a model system for dedifferentiation and hypothesize that they can be redifferentiated through matrix-mediated mechanotransduction and regain their morphology and function.

METHODS:

Healthy human flexor tendon cells were isolated from healthy female flexor digitorum longus. Cells were seeded at 5000 cells/cm^2 cell density, passaged every two days for six passages. In order to assess cell phenotype, cells were fixed with 4% paraformaldehyde and stained with phalloidin and DAPI to visualize the actin cytoskeleton and DNA respectively.

RESULTS AND DISCUSSION:

We noted that in each passage, cells lost their spindle-shaped phenotype and became more pancake-shaped. At passage 1 and 2, the main cell phenotype is spindle-shaped. However, as the cells are further passaged, the phenotype of the cell population becomes more heterogeneous and at passage 5 and 6, they already display a more spread-out shape (Figure 1). In the future experiments, in order to get more insight in the change of phenotypes through each passage, quantification of the changes will be performed. Additionally, in order to evaluate the changes in the tenocyt function, tenocyt specific genes will be analysed.

CONCLUSION:

In this study, we aimed to dedifferentiate human tenocytes through *in vitro* sub-culturing. In future experiments, we will use decellularized tendons as a matrix to culture cells and apply physiological levels of mechanical loading to obtain differentiated tenocyte function and shape through redifferentiation. This study will provide information regarding the phenotypic and functional maintenance of tencytes and provide insights on the link between tenocyt morphology and function

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ACKNOWLEDGMENTS:

The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement no.289720.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

86 Lipid-based magnetic nanovectors for the targeted treatment of glioblasgtoma multiforme through combinatory chemotherapy and magnetic hyperthermia

Christos Tapeinos[1], Matteo Battaglini[2], Attilio Marino[1], Gianni Ciofani[3]

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[2]Italian Institute of Technology / Scuola Superiore Sa, Pontedera, Italy

[3]Italian Institute of Technology / Politecnico di Torino, Italy

INTRODUCTION:

Glioblastoma multiforme (GBM) is considered one of the most aggressive malignancies in the brain due to its invasiveness and rapid growth, resulting in more than 90% 5-year mortality[1]. Current treatment fail to successfully treat GBM due to various reasons, including the location and the pathophysiology of the tumor and the blood brain barrier (BBB) that hinders an efficient delivery of various chemotherapeutics[2]. In view of this, we hypothesized that

the fabrication of a biomimetic drug delivery system able to cross the BBB and controllably deliver temozolomide (TMZ), as well as the simultaneous magnetic hyperthermia treatment, will result into a tumor regression increasing the life expectancy of patients suffering from GBM.

#_ftnref1#_ftnref1 METHODS: The lipid-based magnetic nanovectors (LMNVs) were fabricated using a combination of hot ultra-sonication and high pressure homogenization (HPH), and they were characterized using various material characterization techniques. Loading and release studies were performed using an HPLC and the magnetic hyperthermia effect was demonstrated using alternating magnetic field (AMF) stimulation. The targeting ability of the LMNVs and their ability to cross the blood brain barrier (BBB) were assessed

in vitro after their functionalization with specific peptides, using flow cytometry and confocal microscopy. Viability studies were performed using metabolic activity and proliferation assays. RESULTS AND DISCUSSION: LMNVs were successfully synthesized and characterized (

Fig. 1A-B). The LMNVs demonstrated high stability (

Fig. 1C), as well as good loading and release profiles. Their ability to increase temperature above 42°C was assessed using an AMF, and the results demonstrated (

Fig. 1D) that LMNVs need 8.5 min to increase the temperature from 37°C to 42°C. Preliminary viability studies on U87&U251 cells demonstrated that plain LMNVs are not toxic (500 µg/ml, 72h), while their toxicity increases when TMZ-loaded LMNVs are used. Preliminary targeting studies also demonstrated that peptide-functionalized LMNVs are able to pass an

in vitro BBB model (

Fig. 1 E-I). CONCLUSION: LMNVs loaded with TMZ and functionalized with specific targeting peptides were shown to pass an

in vitro BBB model and be successfully internalized by U87 cells. These LMNVs present high stability and a good drug release profile, resulting in an enhanced anti-proliferative effect against U87 cells. REFERENCES: [1] S.K.Carlsson,

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ACKNOWLEDGMENTS: This project is funded by the European Research Council (ERC) under the EU's Horizon 2020 research and innovation program (grant agreement N°709613, SLaMM).

Picture 1:

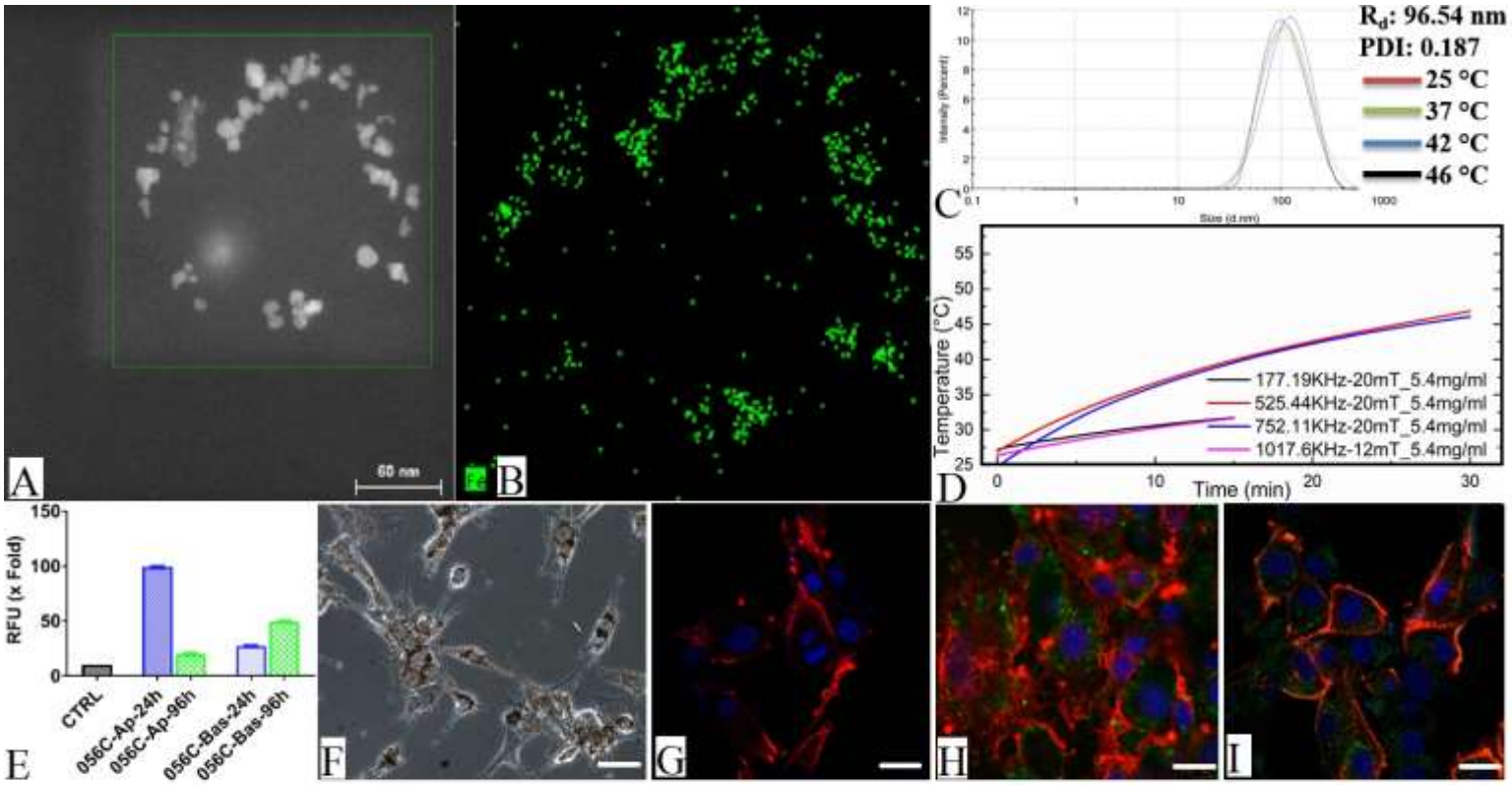

Figure 1. Morphological and physicochemical characterization of LMNVs: A) TEM & B) EELS of LMNVs, C) DLS measurements presenting the stability of the LMNVs at various temperatures (25, 37, 42 & 46 °C), D) LMNVs' temperature increase in response to an AMF at different frequencies (177, 525, 752 & 1017 KHz), E) fluorescent intensity of FITV-labeled LMNVs in the medium of the apical and basolateral space of an *in vitro* BBB model after 24 & 96h, F) optical microscopy image of U87 cells 24h after treatment with LMNVs, and confocal microscopy images of internalized LMNVs, 24h after treatment: G) control, H) LMNVs internalized by BENd3 cells (apical part) and I) LMNVs internalized by U87 cells (basolateral part).

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

90 Comparison of In-Vivo Digital Image Correlation and Tissue Doppler Analyses of Right Ventricle Strains

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[2]Tampere University Hospital Heart Center, Finland

INTRODUCTION:

During open-heart surgery, it is difficult to acquire accurate data from the right ventricle (RV) because of anatomical reasons as well as technical limitations of the Speckle tracking Echocardiography (STE) [1, 2]. As an alternative method, we have used Digital Image Correlation (DIC) to monitor and characterize the functions of the RV and compared the obtained data to STE measurements.

METHODS:

Two cameras were installed in the operating room, overlooking the patient's heart with a slanted angle. After the sternotomy, the visible surface of the heart was marked with a sterile medical marker to create a surface contrast pattern for DIC. Approximately 400 images were taken at four stages of the surgery, covering approximately 20 heartbeats. These images were analyzed using the Davis software package from LaVision LTD to extract the relevant data such as strain. As representative examples, the results from two heartbeats are presented in this paper.

RESULTS AND DISCUSSION:

Fig. 1 presents the strain of the outer surface of the heart obtained with DIC together with Free Wall strain obtained with STE. Starting from the diastole, or maximum volume, the heart compresses during systole, reaches the maximum compression, and returns towards zero strain. The measurements were not synchronized, and they may correspond to different heartbeats within a time window of approximately one minute. The measurements for DIC and STE show very similar results, including the post systolic strains (the peak compression value) of 15% for DIC and 16% for STE.

CONCLUSION:

Longitudinal strains of the outer surface of the RV were obtained with DIC and STE during open heart surgery. The study shows that optical photography and DIC can resolve the RV function including the values typically used for characterization of the functioning of the heart. In this case the post systolic strains obtained with the two methods are essentially identical, and the overall shape of the strain vs. time plots is very similar.

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ACKNOWLEDGMENTS:

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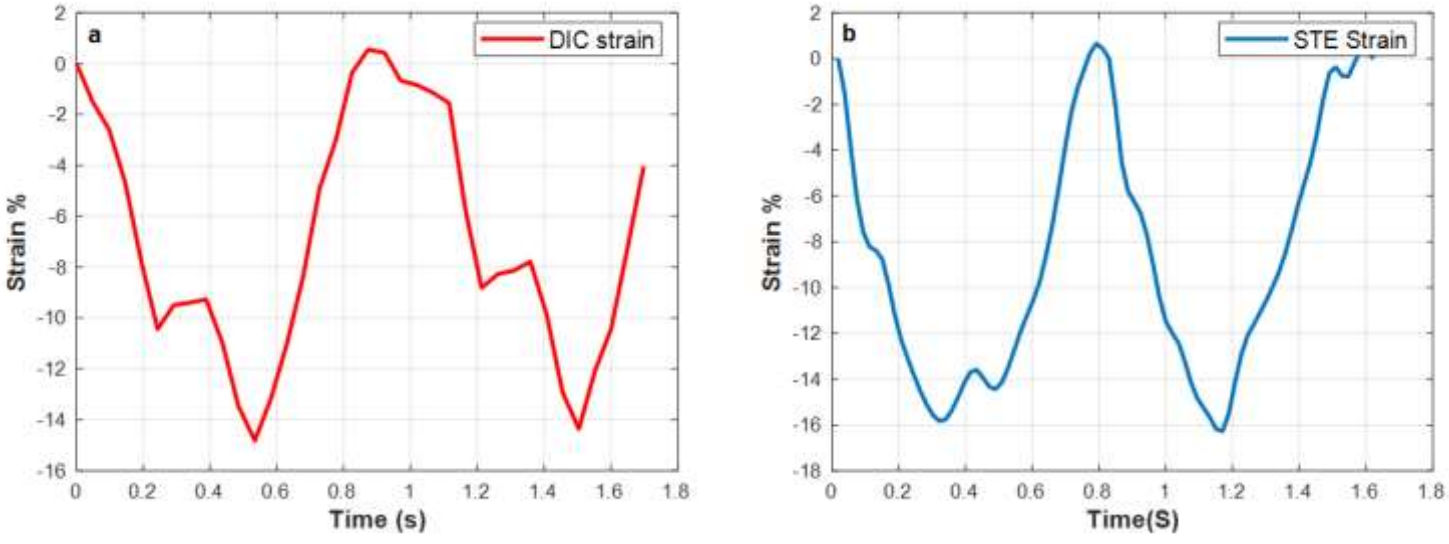
Picture 1: Caption 1: Figure 1. a) DIC and b) STE strains as a function of time



Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

91 Click chemistry as new approach for the selective surface modification of calcium phosphate nanoparticles

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[2]Universität Duisburg-Essen/Inorganic Chemistry and CeNIDE, Germany

INTRODUCTION:

Calcium phosphate nanoparticles are a well-known biomaterial and frequently used as carrier for biomolecules, drugs, and applied, e.g., for transfection, siRNA gene silencing *in vitro* and *in vivo*.[1] Surface modification methods of silica-terminated calcium phosphate nanoparticles based on thiol and amine chemistry have been reported,[2] but this approach is limited by its selectivity for specific applications. To extend the applicability and selectivity of calcium phosphate nanoparticles, the “click reactions”, copper-catalyzed azide-alkyne cycloaddition[3] (CuAAC) and the strain-promoted azide-alkyne cycloaddition[4] (SPAAC), were established for covalent attachment of molecules to azide-terminated calcium phosphate nanoparticles.

METHODS:

Calcium phosphate nanoparticles stabilized with polyethyleneimine (PEI) and an outer silica shell were synthesized as reported earlier.[2] Azide groups were attached to the silica shell coating using 3-(Azidopropyl)triethoxysilane as precursor. The surface reactivity for click reactions was studied using model alkyne-modified and dibenzylcyclooctyne-modified dyes.

RESULTS AND DISCUSSION:

The azide-terminated calcium phosphate nanoparticles had an average hydrodynamic diameter of 196 nm (dynamic light scattering; DLS) and an average diameter of the solid core of 50 nm (scanning electron microscopy; SEM). The azide group on the nanoparticles was detected by FT-IR spectroscopy at 2105 cm^{-1}.

Both click reaction approaches (CuAAC and SPAAC) were successful under aqueous conditions and up to 30% DMSO. Additionally, this method allowed to attach two different dye molecules to the nanoparticle surface, thus generating a dual-labeled nanoparticle. Fluorescence spectroscopy and UV-Vis spectroscopy were used to quantify the amount of attached dye molecule. The uptake of the nanoparticles by HeLa cells was studied by super-resolution light microscopy.

CONCLUSION:

As a new approach for calcium phosphate nanoparticles, its surface has been successfully modified using CuAAC and SPAAC click reactions. As demonstrated with different dyes, both methods are suitable for the covalent functionalization of calcium phosphate nanoparticles. Particles are enabled for a selective surface functionalization (e.g. by proteins or antibodies) which enhances their potential for imaging and cellular targeting.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

94 Impact of conditioning process related properties on material properties of methacryloylated type A and type B gelatin

Lisa Sewald[1], Christiane Claaßen[1], Tobias Götz[1], Marc H. Claaßen[2], Vincent Truffault[2], Günter E. M. Tovar[1], Alexander Southan[1], Kirsten Borchers[3]

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INTRODUCTION:

Gelatin type A (G_A) and gelatin type B (G_B) are utilized for synthesis of methacryloylated gelatin (GM) or methacryloylated and acetylated gelatin (GMA), which are investigated for various applications in the field of regenerative medicine.[1] However, the impact of gelatin type on resulting polymer and material properties was rarely investigated, so far. In this study, we compared five chemically modified G_A and G_B derivatives with regard to their physico-chemical properties.

METHODS:

Chemical modification of G_A and G_B as well as determination of degree of methacryloylation (DM) and acetylation (DA) was done following the procedure reported by Claaßen *et al.*[2]. The isoelectric points (IEP) of GM(A) were determined by a deionization method. Molecular weight distributions were investigated using size exclusion chromatography (SEC). Viscosity, gelation and melting temperature of GM(A)-solutions and storage moduli (G') of GM(A)-hydrogels were obtained by rheological measurements.

A two-side student *t*-test was used for statistical analysis. Presented data show the mean of at least three independently repeated experiments using three different gelatin batches of each derivative.

RESULTS AND DISCUSSION:

DM and DA of GM(A)s increased with increasing molar excess of anhydride used for modification, consistent with previous reports[2,3]. Additionally, they were indistinguishable for G_A and G_B derivatives. Differences in SEC elugrams were assigned to different hydrophilicity of derivatives. The IEP of G_AM was generally higher compared to G_BM.

Dynamic viscosities, gelation and melting temperature decreased with increasing degree of modification. Viscosities were indistinguishable for all highly-modified gelatins, see Figure 1. Low-modified G_AM showed lower viscosity compared to G_BM derivatives, which was traced back to different standard viscosities of the raw materials. G' of cross-linked GM(A) hydrogels were dependent on the raw material as well as of the degree of chemical modification.[4]

CONCLUSION:

Material properties of modified gelatins are not only determined by the overall degree of modification (DMA) but also by properties of the raw material. The higher the DMA the lower the impact of raw material properties and the type of gelatin used.

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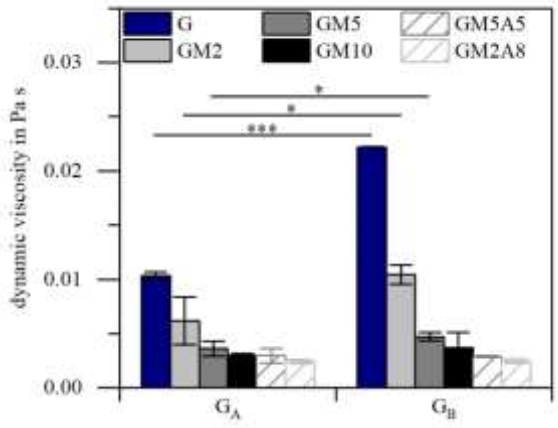
ACKNOWLEDGMENTS:

L. S. thanks the *Evonik Stiftung* for financial support. C. C. and K. B. thank the Deutsche Forschungsgemeinschaft [grant ID BO 4252/1-1] for financial support. A. S. thanks the Carl Zeiss Foundation and the University of Stuttgart for financial support within the *Projekthaus NanoBioMater*.



Picture 1: Caption 1: Dynamic viscosity of 10 % (w/w) GA and GB derivative solutions determined at 50 s-1 and 37 °C.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

95 Process of physical gelling influences the mechanical characteristics of chemically cross-linked gelatin methacryloyl hydrogels

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INTRODUCTION:

Gelatin methacryloyl (GM) hydrogels are investigated as promising scaffolds for tissue engineering. With view to load bearing tissues, controlled strengthening of GM hydrogels is of great interest.[1] Utilizing a second polymer network, e. g. collagen[2], is one possibility. We investigated the impact of preceding gelling of different GM precursor solutions on mechanical properties of the resulting hydrogels. The solutions were able to form physical gels to a more or less pronounced state, depending on the degree of modification.

METHODS:

Acetylated and methacryloylated gelatin derivatives (GM2, GM10 and GM2A8) were synthesized and the degrees of methacryloylation (DM) and acetylation (DA) quantified as reported by Claaßen *et al.* [3].

Photo-induced radical cross-linking of GM(A) was performed using the precursor solutions either in liquid or gel state. Compression testing of chemically cross-linked hydrogels was executed in confined or unconfined mode in buffer at 37 °C, at 0.2 N pre-load and a compressive strain rate of 0.5 %/s. Cytocompatibility of the new hydrogel preparation procedure including physical gelling at various conditions was tested using primary porcine chondrocytes.

We present results from three independently repeated experiments using three gelatin derivative batches.

RESULTS AND DISCUSSION:

GM2 and GM2A8 possessed indistinguishable DM, while the DM of GM10 was significantly higher. On the other hand, the total degree of modification (DMA = DM+DA) of GM10 and GM2A8 were also indistinguishable and significantly higher than the DMA of GM2.[4] Thereby, derivatives with equal DM and derivatives with equal DMA were available for comparison. Preceding gelling of hydrogel precursor solutions before chemical cross-linking increased mechanical properties of resulting hydrogels.

The maximum compressive stress of gelled and chemical cross-linked gels was higher compared to simple chemical cross-linked hydrogels (Figure 1). The influence of gelation was more pronounced in low modified derivatives, since they are able to form physical gels while gelation is vastly impaired in gels with high DMA.

Additionally, we will present data on the cytocompatibility of the presented hydrogel preparation procedure.

CONCLUSION:

GM hydrogels can be strengthened by physical gelation of hydrogel precursor solution before chemical cross-linking.

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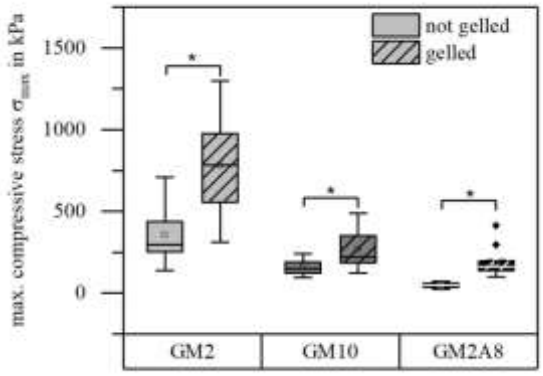
ACKNOWLEDGMENTS:

The authors kindly thank Birgit Claasen (IOC, University of Stuttgart) for the ^{1}H-NMR. L.S. thanks the *Evonik Stiftung* for financial support.



Picture 1: Caption 1: Maximum compressive stress of GM2, GM10 and GM2A8 hydrogels.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

100 Development of a new generation of bioresorbable cardiac stents - Investigation of phosphate glass and toughened PLLA/PLCL-PEG blends

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[2]Lucideon Ltd., United Kingdom

INTRODUCTION:

Modern cardiac stents based on polylactide are resorbable, however their mechanical and degradation properties still need improvement[1]. Composites of polymers and phosphate glass are promising candidates to meet these requirements, and our aim is to develop composite materials with appropriate strength, ductility, and degradation profile for cardiovascular applications. Here, the behaviour of the constituent phosphate glasses and polymer blends are investigated.

METHODS:

Ternary $(P_2O_5)_{50-x}(CaO)_{40+x}(Na_2O)_{10}$ phosphate glasses were produced using melt quenching. After characterisation, their dissolution behaviour in deionised water at 37°C was monitored by mass loss, pH, and Ca^{2+} ion activity. Blended polymer films were solvent cast and injection moulded, using poly-L-lactide (PLLA) and polyethylene glycol-functionalised poly(lactide-co-caprolactone) copolymers (PLCL-PEG). Mechanical testing of polymer blends was carried out under immersion in deionised water at 37°C, and degradation tests were conducted in phosphate-buffered saline at 37°C.

RESULTS AND DISCUSSION:

Dissolution tests of phosphate glass indicate a two stage process, where diffusion controlled dissolution occurs first, and once the solution pH and ion concentration are appropriate, a hydration layer forms on the glass surface (Fig. 1.b-c) and dissolution proceeds according to the phosphate chain hydrolysis surface reaction. Increased Ca reduces

the dissolution rate (Fig.1.a), caused by Ca blocking interstitial diffusion pathways, and crosslinking phosphate chains. The release of ions during dissolution produces a buffering effect, which could help control degradation of polymer glass composites. Mechanical testing of polymer blends emphasises the importance of testing under physiologically relevant conditions, and shows the addition of PLCL-PEG copolymers to PLLA in blends has a plasticising effect, increasing ductility while decreasing strength (Fig. 1.d).

CONCLUSION:

The glass dissolution mechanisms revealed not only enhance our understanding of how these phosphate glasses dissolve, but also inform the design of polymer glass composites, especially in terms of overall composite mass loss, and control of polymer degradation using the buffering effect. Mechanical and degradation testing of polymers indicates that certain blend compositions show significant promise for production of polymer glass composites for cardiovascular applications.

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ACKNOWLEDGMENTS:

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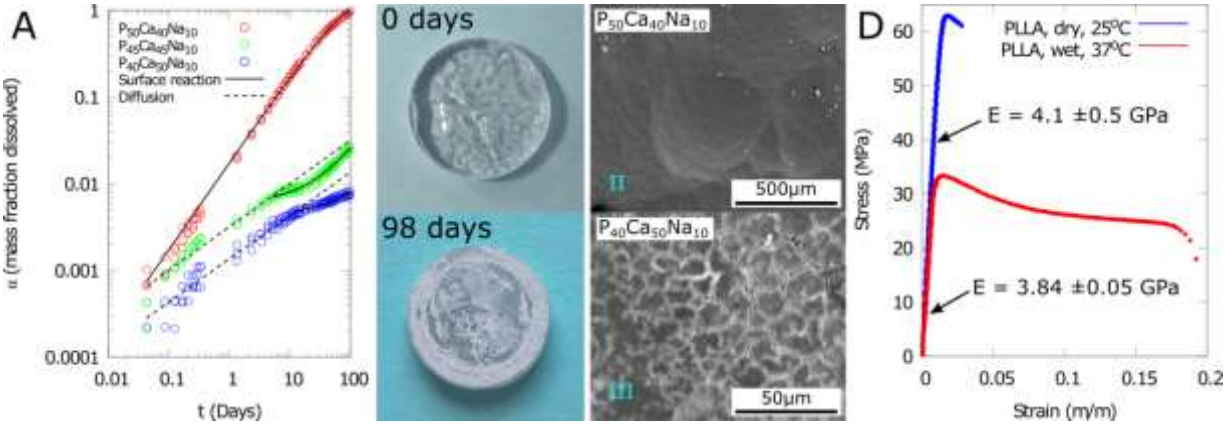
Picture 1:



Caption 1: Figure 1: Glass mass loss (a), hydration layer and pitting dissolution (b,c). PLLA stress-strain at normal and simulated body conditions (d).

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

109 Late stage differentiation of osteobastic cells with synthetic octacalcium phosphate material

Osamu Suzuki, Yukari Shiwaku, Takahisa Aanada, Yuko Sai, Kaori Tsuchiya, Tetsu Takahashi

Tohoku University Graduate School of Dentistry, Sendai, Japan

INTRODUCTION:

Octacalcium phosphate (OCP) has stimulatory capacities on bone formation[1)] and osteoblastic activity under 2D[1,2)] and 3D in vitro environments[3)]. Stimulatory capacity of OCP could be induced during OCP hydrolysis into Ca-

deficient hydroxyapatite (HA) with chemical dissolution under physiological conditions[4,5]. However, it is still unclear whether the structural changes of OCP affect the differentiation of osteoblastic cells to osteocytes related to the structural changes from the view point of phosphate ions involvement. The aim of this study was to investigate the structural changes of OCP associated with osteoblastic cell differentiation to osteocytes in vitro.

METHODS:

OCP was synthesized in a wet method[6]. OCP was incubated in a culture media (αMEM) at 37°C for 35 days and subjected for structural analysis of X-ray diffraction (XRD). Curve fitting analysis of Fourier transform infrared (FTIR) spectra was carried out to analyze the HPO_4 and OH. FTIR and Raman spectra were correlated with HPO_4 content in OCP[7]. Mouse osteoblastic cell strain IDG-SW3[8] was cultured in osteogenic differentiation media in the presence of OCP using the transwell inserts and its differentiation into osteocytes were measured by real-time PCR.

RESULTS AND DISCUSSION:

XRD and FTIR analyses showed that OCP tended to change to low crystalline HA during the incubation. HPO_4 content of OCP was about 36%, a non-stoichiometric composition[7], and decreased to about 26% until 35 days. Detailed FTIR analysis indicated that apatitic and non-apatitic HPO_4^{2-} and apatitic OH^- peaks were observed. Raman spectra of OCP also suggested the structural changes upon the incubation. OCP brought forward the appearance of alkaline phosphatase (ALP) in the IDG-SW3 cells compared to the control without OCP and increased osteocyte differentiation markers SOST/sclerostin and FGF23 gene expression until 35 days of incubation.

CONCLUSION:

The results showed that OCP facilitates osteoblastic differentiation to osteocytes and suggests that the capacity of OCP is associated with the detectable phosphate-related physicochemical changes.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

111 Osteoclasts can play a role in the degradation of S53P4 bioactive glass

Nicole van Gestel[1], Rienk Schuiringa[1], Keita Ito[1], Jacobus Arts[2], Hofmann Sandra[1]

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[2]Maastricht University Medical Centre, Netherlands

INTRODUCTION:

S53P4 Bioactive glass (BAG) granules are promising in the treatment of osteomyelitis[1,2]. However, in vivo degradation is slow and hinders long-term regeneration of the bone[3]. Directly after contact with (body) fluids, BAG releases ions, its surface remodels, and calcium phosphate (Ca-P) precipitates[2]. We studied the ion release, Ca-P precipitation, and degradation of the Ca-P layer by osteoclasts.

METHODS:

Release of sodium (Na^+), calcium (Ca^{2+}) and phosphate (PO_4^{3-}) from BAG granules in buffer solutions (pH=4.5 and pH=7.3) was determined after 24h, 72h and 1 week of submersion, using ion chromatography (n=3 per group. Energy-dispersive X-ray spectroscopy (EDX) was performed to confirm Ca-P precipitation. Scanning electron microscopy (SEM) was used to identify resorption pits, as a measure for osteoclastic activity of differentiated human monocytes. The monocytes were seeded on BAG surfaces, pre-soaked in PBS for 3 days (n=5), and control hydroxyapatite (HA) (n=5). EDX was performed to analyze the elements at the surface of the resorption pits.

RESULTS AND DISCUSSION:

Significantly more Na^+, Ca^{2+} and PO_4^{3-} was released in the buffer with pH=4.5. Osteoclasts can produce a low pH under their ruffled border and therefore it was believed that they can resorb BAG. This was confirmed with the observation of active osteoclasts on the BAG (Figure 1). The surface of the resorption pits on BAG discs was more uniform and smooth than those on HA, which suggests that further resorption was hindered. Possibly because of the silica underneath the Ca-P[4]. EDX confirmed Ca-P precipitation after fluid contact (PBS and buffer solutions) and showed an incomplete osteoclastic resorption of this layer, but de Ca-P might have precipitated back during sample preparation.

CONCLUSION:

Active osteoclasts were observed on BAG surfaces with precipitated Ca-P, but could only (partially) degrade the precipitated Ca-P. This suggests that osteoclasts may only play a small role in the degradation of BAG, which might explain the slow in vivo degradation.

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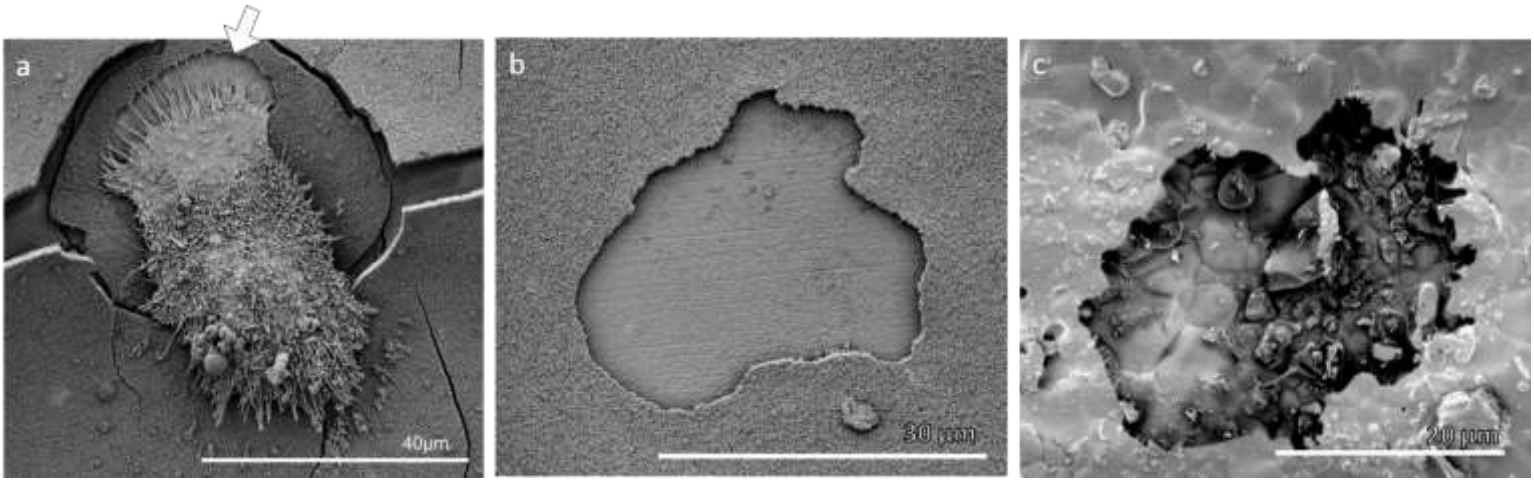
Picture 1: Caption 1: Figure 1. a) An osteoclast moving from a resorbed area (arrow) on BAG, b) a resorption pit on BAG, c) a resorption pit on HA.



Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

117 Investigation on osteogenic HA-based upconversion material in anti-autofluorescence and long-term biomedical tracking

Xiyu Li, Wei Li

Sichuan University, Chengdu, China

INTRODUCTION:

Hydroxyapatite (HA) has been extensively used to repair bone defects or as bioactive component of tissue engineering scaffolds. [1] HA material will be long-standing once implanted in human body, [2] yet no one knows how long HA material will remain in bone tissue after implantation. Therefore, how to long-term trace implanted HA material is still a technical and clinical challenge. In this study, we prepared a lanthanides-doped upconversion HA material to endow HA with traceable fluorescence and investigated its characteristics in anti-autofluorescence and distinctive biomedical tracking.

METHODS:

The HA:Yb/Ho nanoparticles were prepared according to our previous reports. [3] Fresh tissues of pig skin, muscle and bone in 3 mm thickness were used to investigate the interference of tissue autofluorescence. The HA:Yb/Ho powder was also implanted into the defects (Figure 1b) of rabbits and harvested with surrounding tissue at 2, 4 and 6 months after implantation.

RESULTS AND DISCUSSION:

The results showed that lanthanides Yb/Ho co-doping could endow the HA with long-term fluorescence tracking ability without affecting its osteogenesis. The HA:Yb/Ho material with hexagonal structure and good cytocompatibility possessed stable green upconversion luminescence at 546 nm. The HA:Yb/Ho material could effectively avoid the

autofluorescence of various tissues as thick as 3 mm (Figure 1a), and reveal the interrelation between implanted HA material and new bone tissue (Figure 1c).

CONCLUSION:

We used for the first time the HA:Yb/Ho upconversion material to show its effect on anti-autofluorescence, and clearly reveal the material-tissue interrelation and osteointegration. The lanthanides-doped upconversion HA material will exhibit their versatility in future biomedical applications for both bone repair and lifelong tracking.

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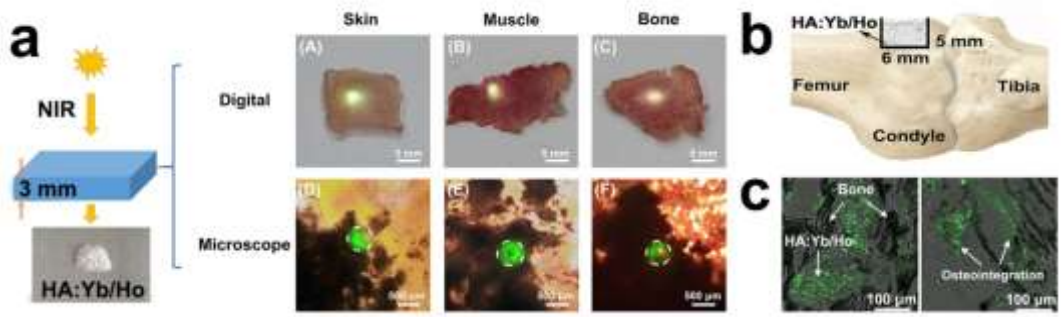

Picture 1:

Caption 1: Figure 1. HA:Yb/Ho covered by tissues emitted green light (a), HA:Yb/Ho was implanted in bone defect (b), and the confocal fluorescence images (c).

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

118 3D printing scaffolds of poly-(e-caprolactone), polyvinyl acetate and hydroxyapatite for bone regeneration

Yubao Li, Jingqi Ma, Yi Zuo, Qin Zou

Sichuan University, Chengdu, China

INTRODUCTION:

The development of 3D printing as a fast additive manufacturing technique offers a new choice for orthopedic surgeons to meet the requirement of individual-based treatment to specific bone defects[1-2]. In the study, poly-(ε-

caprolactone) (PCL), polyvinyl acetate (PVAc) and hydroxyapatite (HA) were used to construct bone scaffolds by 3D printing technique; the degradable PVAc component was selected to promote the blend of HA particles and the hydrophilicity of the composite scaffolds.

METHODS:

Various 3D printing scaffolds were fabricated including PCL, PCL/PVAc, PCL/HA and PCL/PVAc/HA, their physicochemical properties and biological characteristics were tested via XRD, FTIR, SEM, *in vitro* cell culture and *in vivo* animal experiment. Statistical analyses were performed using Originpro 2017 (USA). $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION:

The 3D printing scaffolds have a porous structure with a pore size of ~375 - 475 μm and a porosity of 74 - 76%. The surface of PCL/PVAc/HA scaffold was rougher than others, demonstrating better cell adhesion and proliferation. The micro-CT image and the histological sections showed that bone reconstruction on and within the scaffold was better than PCL/HA scaffold and significantly superior to the PCL and PCL/PVAc scaffolds after implantation for 12 weeks. New bone was found to closely bond with the scaffold, and a structure similar to Haversian system in the pores of the PCL/PVAc/HA scaffold could also be observed clearly. The good results should be attributed to the blending of degradable PVAc in the matrix to improve the exposure of HA particles on the surface and the hydrophilicity of the scaffold.

Figure 1. SEM photo, cell adhesion and 12-week bone formation of PCL/PVAc/HA scaffold.

CONCLUSION:

The blending of PVAc promotes the exposure of HA particles and the hydrophilicity of PCL scaffolds, demonstrating better cell adhesion and new bone reconstruction. The composition and regular tunnel structure of the tri-component PCL/PVAc/HA scaffold are beneficial to the bone formation. The tri-component scaffold has promising prospect for bone repair application.

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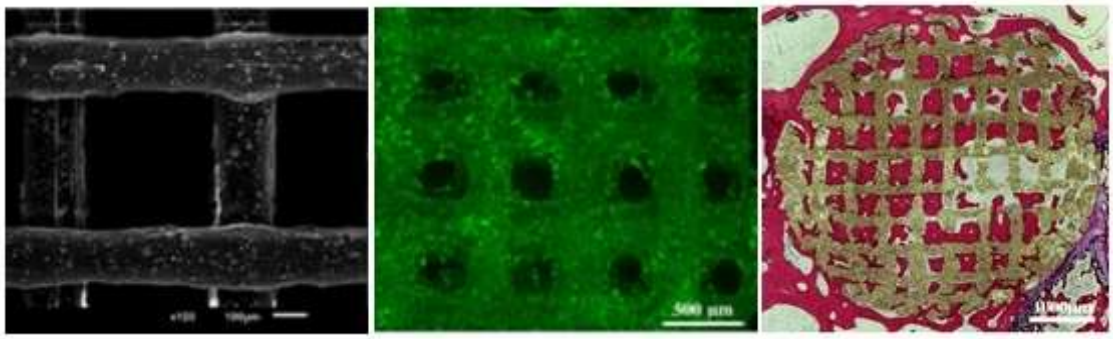
Picture 1:



Caption 1: Figure 1

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

123 Porous poly(dimethylsiloxane) as substrate for mesenchymal stem cells adhesion

Vladimir Agabekov[1], Irina Paribok[1], Aliaksandr Kraskouski[1], Sergei Pinchuk[2], Irina Vasilevich[2], Kirill Matievski[2], Igor Volotovski[2]

[1]Institute of Chemistry of New Materials of NAS of Belarus, Minsk, Belarus

[2]Institute of Biophysics and Cell Engineering of NAS of Belarus, Belarus

INTRODUCTION:

Poly(dimethylsiloxane) (PDMS) is widely known as a biocompatible substrate for cell adhesion[1] but its inherent hydrophobicity is unfavourable for cell culture, causing cells to eventually dislodge from the surface[2]. High degree of PDMS porosity has clearly been shown to improve cell survival and functions[3]. It has been proven that mesenchymal stem cells (MSCs) adhere effectively to multilayer chitosan/pectin films formed on the glass surface using Layer-by-layer technique[4]. The aim of the present work was to deposit similar polysaccharide films on porous PDMS substrates and to check whether they affect MSCs adhesion and survival.

METHODS:

For porous PDMS fabrication a porogen material (sieved sugar) was added to the mixture of commercial prepolymer and catalyst (SYLGARD® 184). The mixture was diluted by hexane and cured at 100°C. Porous PDMS substrates were obtained after sugar leaching in water heated to 50-60°C. Maximal water absorption by porous PDMS was about 40% by mass of the substrate (according to gravimetric analysis). Multilayer ultrathin (<100 nm) polyelectrolyte films were deposited onto PDMS surface by alternating adsorption of chitosan and pectin.

RESULTS AND DISCUSSION:

In the case of smooth (non-porous) PDMS substrates the possibility to improve polysaccharide multilayer adhesion on their surface by modification with (3-aminopropyl)triethoxysilane (APTES) and glutaraldehyde (GA) was shown. The coatings morphology, thickness and wear stability were analyzed by the atomic force microscopy. It was found that on the surface of native PDMS non-uniform wrinkled polyelectrolyte multilayers were obtained while on PDMS substrate modified with APTES and GA the uniform coatings with relatively high wear stability were formed. Fluorescence microscopy was used to estimate cell adhesion on porous PDMS. It has been shown that MSCs adhere on non-modified porous PDMS surface worse than on the substrates modified with viscoelastic chitosan/pectin multilayers. In the latter case cells form a monolayer culture of fibroblast-like cells with high viability (Figure).

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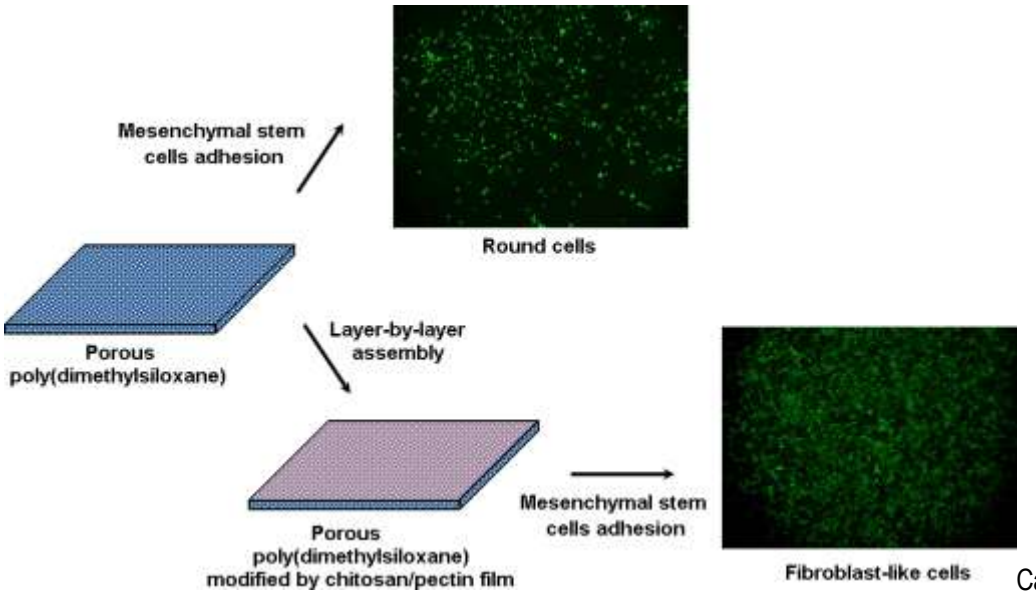

Picture 1: Caption 1: Mesenchymal stem cells adhesion on porous poly(dimethylsiloxane)

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

127 Application of bimineralization mechanisms to develop novel biomaterials for bone regeneration

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MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, Netherlands

INTRODUCTION:

The need for synthetic replacements for bone autograft continues to spur research into materials for bone regeneration. Advances in the field of bone biomineralization, in particular regarding the intrafibrillar mineralization of collagen[1], have led to the development of materials that show mechanical properties similar to those of bone[2]. However, the bioactivity of these materials has been evaluated only to a limited extent. A critical bioactivity aspect of an artificial bone graft substitute is its ability to be resorbed, allowing its replacement by newly formed tissue. This study aimed to evaluate the osteoclasts-driven degradation of PILP-mineralized collagen materials.

METHODS:

Type-I collagen gels, composed of fibrils exhibiting native D-spacing banding, were mineralized by immersion in a solution consisting of 4.5 mM $CaCl_2$ and 2.1 mM K_2HPO_4, with poly(L-aspartic acid). Mineralized gels were characterized by TGA, ICP-MS, SEM-EDS and TEM-SAED. Optimal differentiation conditions to derive osteoclasts from RAW 264.7 murine macrophage cell line were assessed by quantifying their number and area from fluorescence images of cells cultured on tissue culture plastic or hydroxyapatite (HA) coatings. Osteoclast activity on

HA coatings was assessed by quantifying tartrate-resistant alkaline phosphatase (TRAP) activity and resorbed area from SEM images.

RESULTS AND DISCUSSION:

Characterization of the mineralized gels showed signs of calcium phosphate infiltration (SEM-EDS and ICP-MS), with a total mineral content around 10% (TGA), which is significantly lower than that of bone (60-70%). TEM confirmed the presence of mineralized collagen fibrils (Figure 1), but diffraction data (SAED) were still inconclusive, suggesting that further optimization of the method is needed. To optimize cell culture, osteoclasts were cultured on HA coatings. Large osteoclast-like cells were found on the coatings (SEM imaging) but after 7 days of culture, no signs of active resorption were observed.

CONCLUSION:

In conclusion, mineralization of the collagen gels was not yet optimal, possibly due to the nature of collagen used. In addition, more data is needed to verify whether mineralization is intrafibrillar. While osteoclast differentiation was observed, there was no resorption after 7 days of culture on HA coatings. The differentiation protocol may need to be extended to obtain resorbing osteoclasts. Further developments will be aimed at increasing the mineral content of collagen gels, and optimizing the conditions for osteoclast resorption.

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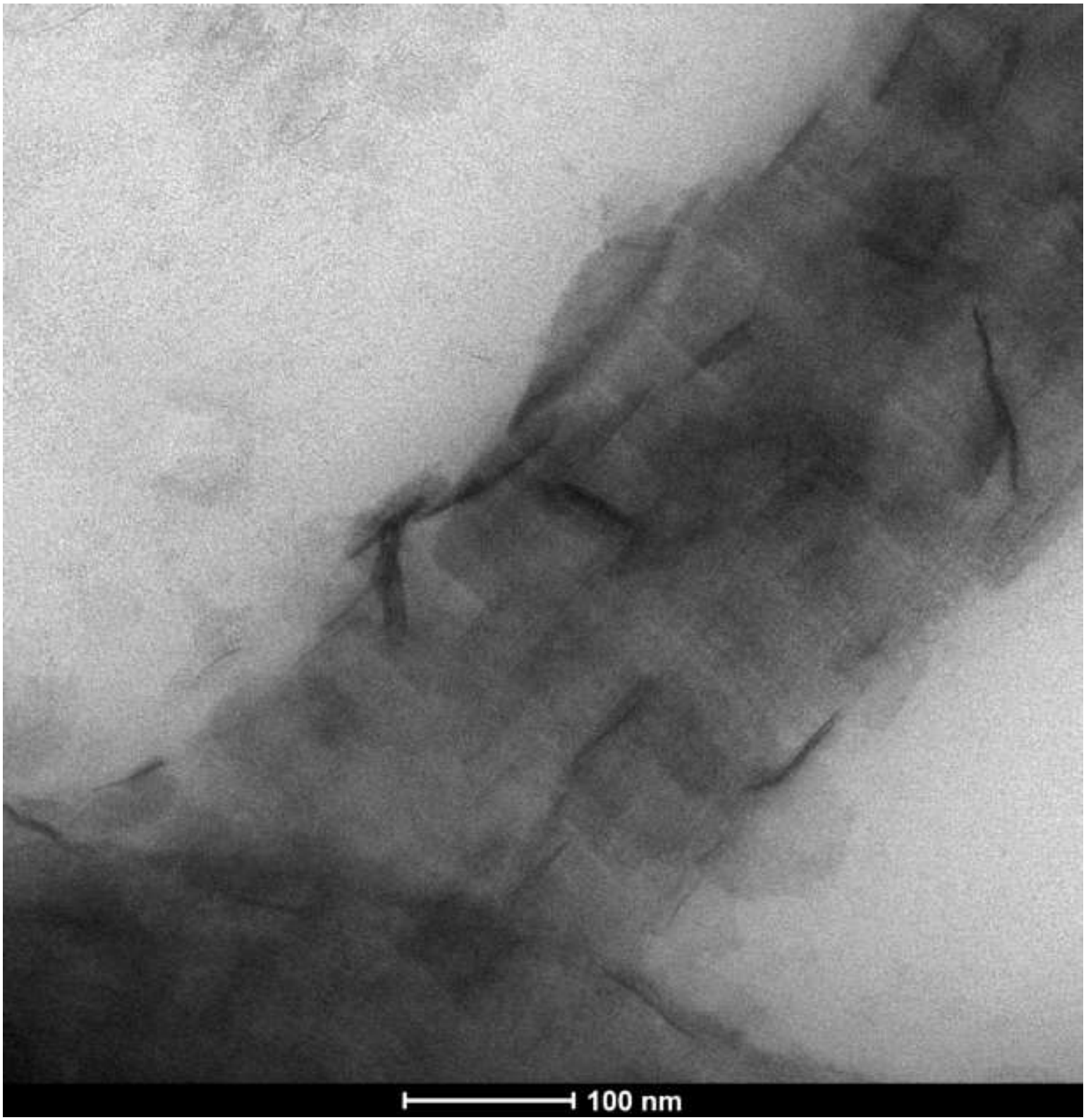
ACKNOWLEDGMENTS:

This project was made possible with the support of the Dutch Province of Limburg.



Picture 1: Caption 1: Figure 1. TEM image of mineralized collagen fibril.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

128 Strength degradation, phase and morphological changes of calcium phosphate scaffolds in acidic environment simulating osteoclastic activity

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[2]Institute of Experimental Medicine of the Czech Academy of Sciences, Czech Republic

[3]Czech technical university in Prague, Czech Republic

INTRODUCTION:

Resorbable porous ceramic materials with customized shape are promising category of materials for healing of bone defects with critical size[1]. Resorption rate of the ceramic structure must correspond to the bone tissue regeneration. The only mechanism of degradation of sintered ceramic materials in a human body is via dissolution under acidic conditions produced by osteoclastic cells[2]. Dissolution of the ceramics correspond to changes in its mechanical strength. In our research we described structural and mechanical changes of different types of calcium phosphate ceramic materials under acidic conditions simulating osteoclastic activity. Results will help to develop bioceramic scaffolds with controlled resorption rate with good mechanical strength during healing period.

METHODS:

Hydroxyapatite (HA), tricalcium phosphate (TCP) and multiphasic calcium phosphate (MCP) porous ceramic samples were prepared by direct foaming method. Samples were exposed to buffered, slightly acidic environment (pH 5.5) at a temperature of 37°C for 0, 2, 7 and 14 days. Compressive strength of samples were measured before and after the exposition. Changes in the phase composition and morphological characterisation of samples before and after exposition were evaluated by X-ray diffraction (XRD), scanning microscope analysis (SEM) and electron back-scattered diffraction analysis (EBSD). Bioactivity of the materials used were investigated by measuring metabolic activity of cells (MTS assay), DNA quantification (PicoGreen assay) and SEM observations.

RESULTS AND DISCUSSION:

Low ability of HA scaffolds to be dissolved compared to MCP or TCP has been shown. The dissolution of TCP was very rapid and the degradation of TCP ceramics led to a fast disintegration of structure and a steep drop-off of mechanical strength after an exposition to acidic conditions. Dissolution of MCP composed of TCP and HA was found somewhere between pure HA and pure TCP samples corresponding to continual drop-off of mechanical characteristics. Biological evaluation of samples has confirmed MCP as a material with the highest potential for bone defect healing.

CONCLUSION:

Mechanical, phase and structure changes during dissolution of calcium phosphate ceramic has been described. MCP ceramic has shown promising mechanical, structure and biological behaviour for a development of the customized resorbable ceramic scaffold for bone regeneration.

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ACKNOWLEDGMENTS:

This research was supported by Ministry of Health of the Czech Republic under the grant 17-31276A and by CEITEC Nano RI, MEYS CR, 2016–2019.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

129 Facile Hydrogel Coatings for Silicone-Based Biomaterials

Damla Keskin, Henny van der Mei, Henk Busscher, Patrick van Rijn

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INTRODUCTION:

Polydimethylsiloxane (PDMS) is used in clinical applications such as biomedical devices, implants, catheters and insulating coatings. PDMS is a transparent, chemically inert, non-toxic and biocompatible silicone elastomer-based material combined with a good mechanical resistance [1]. However, PDMS surfaces lack many desirable properties such as being anti-fouling or anti-microbial. For many applications, hydrogels offer possible solutions as these may resemble tissue-like environments, are easy to functionalize, are adaptive and deformable, and can respond to external stimuli. Although, polymer brushes have offered many possibilities so far this approach still requires too complex synthetic approaches and undesired Fe or Cu catalysts and do not resemble true hydrogel layers[2]. Easy modification approaches are needed to facilitate clinical translation of these hydrogel coatings. Therefore, in this work we present a hydrogel coating approach on PDMS surfaces via UV polymerization using benzophenone. We developed this approach in such a way that it gives us control over coating properties. Particularly, we related coating properties to changes in bulk properties as these need to be maintained.

METHODS:

UV polymerization is a clinically approved method often used in dentistry to cure the polymeric resins for which benzophenone is often used. Moreover, UV polymerization technique is very easy to apply. In this work, acrylate-based monomers were selected to perform the polymerization on PDMS surfaces under various reaction conditions. Surface confined benzophenone is used to initiate the polymerization.

RESULTS AND DISCUSSION:

The surface properties were affected by applying the hydrogel coatings that depended on the chosen monomers. The reaction kinetics differed between monomers and hence optimization is needed for every monomer. Varying the UV polymerization time, the type of monomer, and the monomer concentration resulted in different morphologies with different roughness and thickness as well as alteration in bulk material stiffness. Long UV polymerization time and high monomer concentration affected the bulk material properties making PDMS brittle after the coating procedure.

CONCLUSION:

Hydrogel coatings with different thicknesses and morphologies were achieved by changing the UV polymerization conditions and the monomer concentration. Additionally, the reactions conditions affected the bulk material properties illustrating the importance of connecting coating properties and bulk material characteristic for optimal functional biomedical materials.

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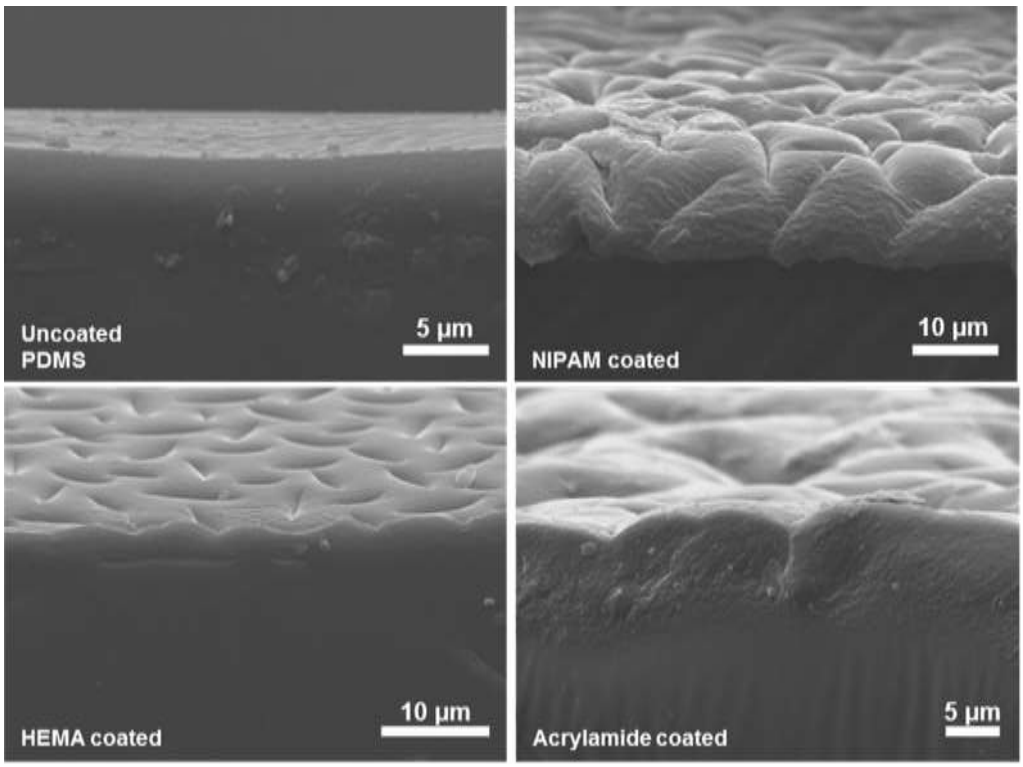
ACKNOWLEDGMENTS:

Authors kindly thank H2020 Marie Curie COFUND program (ALERT) for financial support.

Picture 1: 

Caption 1: Figure 1 SEM cross section images of PDMS surfaces coated with different monomers

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

133 Development of chitosan/poly-β-cyclodextrin sponges for bone tissue engineering application

Carla Palomino Durand[1], Alice Gauzit-Amiel[1], Marco Lopez[1], Frédéric Cazaux[2], Bernard Martel[2], Nicolas Blanchemain[1], Feng Chai[1]

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[2]Université de Lille, ENSCL UMR8207 UMET, France

INTRODUCTION:

Bone defect repair using bone tissue engineering (BTE) approach is considered as an efficient alternative to the use of traditional bone grafts. Hydrogels and sponges can be used as BTE scaffolds to mimic extracellular matrix topography and to deliver bioactive agents. Chitosan (CS), a natural cationic polymer, is an excellent excipient to prepare hydrogels due to its non-toxicity and biodegradability. In this context, physical CS-hydrogels are interesting since they are cross-linker free and therefore, less cytotoxic. Cyclodextrin polymer (PCD) is crosslinked with citric acid and demonstrates an anionic character, which is ideal for the formation of polyelectrolyte complexes with CS[1]. The aim of this study was to develop a CS:PCD sponge from a physical hydrogel and to evaluate its feasibility as a carrier of cells or bioactive molecules for BTE application.

METHODS:

CS and PCD powders were first co-milled in a mixer mill. Hydrogels with CS:PCD ratios of $3{:}3_{w/w}$ and $3{:}5_{w/w}$ were obtained using two interconnected syringes. One syringe contained the solid phase (CS:PCD) and another a 1% lactic acid solution. Sponges were obtained by freeze-drying of hydrogels, and heat treated at 160°C. Microstructure of sponges was evaluated by scanning electronic microscope (SEM). Swelling and enzymatic degradation of sponges were evaluated by weight change in a precision balance. *In vitro* cytocompatibility of the sponges was evaluated by indirect and direct contact with pre-osteoblasts (MC3T3-E1) and human umbilical vein endothelial cells (HUVEC) by AlamarBlue® method and LIVE/DEAD® assay with confocal laser microscopy (CLSM), respectively.

RESULTS AND DISCUSSION:

Both CS:PCD ratios obtained sponges with a high swelling ~550%. Enzymatic degradation revealed a slow degradation profile up to 7 days and reached a plateau (~12% weight loss) up to 21 days. Sponges had an interconnected macroporous structure and a good cytocompatibility with more than 95% of cell survival (indirect method). A good cell adhesion for pre-osteoblastic and endothelial cells was also observed by CLSM (Fig.1) and SEM.

CONCLUSION:

The two CS:PCD ratios showed similar characteristics. Due to its good properties and cytocompatibility, the developed CS:PCD sponges could be promising 3D scaffolds in BTE. Incorporation of growth factors *e.g.* VEGF into the sponge will be studied for enhancing angiogenesis and bone regeneration.

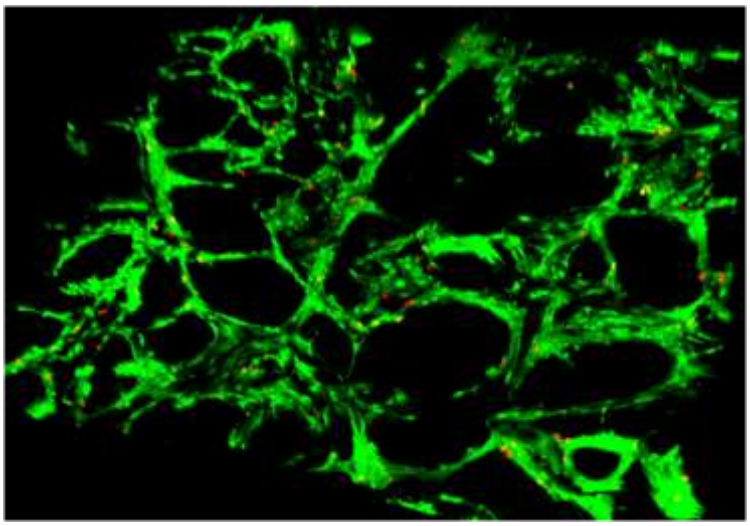
REFERENCES:

Flores C. et al., Carbohydrate Polymers. 173: p. 535–546, 2017

ACKNOWLEDGMENTS:

This work was developed thanks to the financing of Cienciactiva (CONCYTEC-PERU).

Picture 1:
Caption 1: Fig. 1. CLSM image of adhered MC3T3 cells at the sponge by using the LIVE/DEAD® assay. Live cells (green). Dead cells (red).



Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

134 Effect of metal ions on type I collagen production of L929 fibroblasts.

Seiji Yamaguchi, Yuuki Niwa, Morihiro Ito, Seine a. Sintani, Hiroaki Takadama

Chubu University, Kasugai, Japan

INTRODUCTION:

Micofibrous and nanofibrous bioactive 13-93B3 borate glasses have been attracting growing interest in healing soft tissue wounds because of ease of handling and shape flexibility [1]. The glass fibers have shown the capacity to heal soft tissue wounds in humans [2]. Although the released metallic ions are considered to play an important role for healing, the healing process with the bioactive borate glasses are still unclear.

In the present study, the effect of borate, potassium and calcium ions that are main component of the 13-93B3 borate glass on type I collagen production was investigated using L929 fibroblasts.

METHODS:

L929 fibroblast cells are cultured in DMEM medium supplemented with 5 % FBS, 2mM L-glutamine, 100 U/ml penicillin and 100 µg/ml under a CO_2(5%) atmosphere and 37 ˚C. The effect of metallic ions on proliferation was examined using the medium added with H_3BO_3, KCl or $CaCl_2$. The type I collagen production of the cells was assessed by fluorescent labeling, and gene expression of type I collagen was investigated by real-time PCR.

RESULTS AND DISCUSSION:

The metallic ion concentrations of 25 mM K^+ and 4 mM Ca^{2+} significantly increased proliferation of the cells after 3 days culture whereas those more than 25 mM $B(OH)_4^-$ suppress or decreased the proliferation significantly. Real-time PCR revealed that 25 mM K^+, 4 mM Ca^{2+} and 5 mM $B(OH)_4^-$ upregulated gene expression of type I collagen after 3 days culture. Figure 1 shows the result of the fluorescent labeling that showed limited increase of fluorescence of type I collagen near the nuclei of the cells by the same concentrations of metallic ions after the same culture period. It has been reported that the production of type I collagen plays an important role in a new tissue formation and remodelling process [3]. The results in the present study imply that the healing process is

acceleralated by increasing production of type I collagen by the metallic ions such as K^+, Ca^{2+} and $B(OH)_4^-$ released from bioactive borate glass fibers *in vivo*.

CONCLUSION:

The metallic ions of K^+ and Ca^{2+} significantly increased proliferation of L929 fibroblasts, while the $B(OH)_4^-$, K^+ and Ca^{2+} ions exhibited upregulation of gene expression of type I collagen and increased the type I collagen production.

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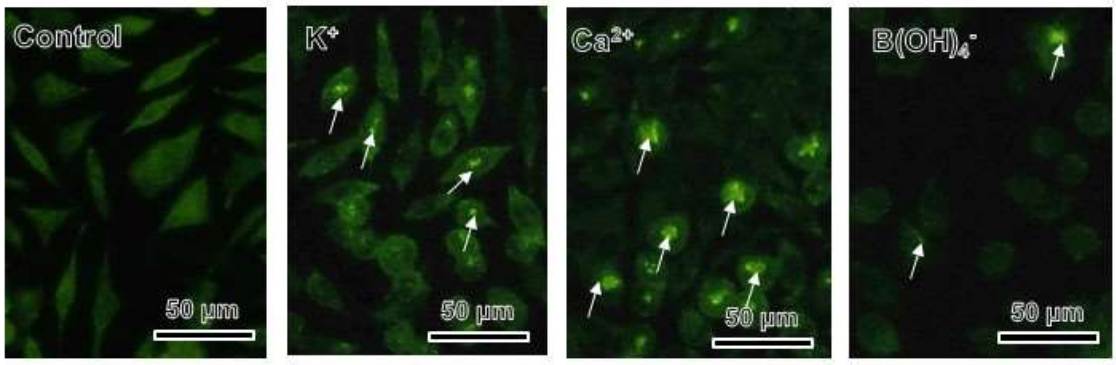

Picture 1: Caption 1: Figure 1 Fluorescent labeling of type I collagen in L929 fibroblast cultured without or with the additives of KCl, CaCl2 and H3BO3.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

139 Titanium nanostructures for the control of osteoblast cell response

Shannon Wilson, George Burke, Brian Meenan, Adrian Boyd

Ulster University, Jordanstown, United Kingdom

INTRODUCTION:

Titanium is used universally in dental and bone implants due to several positive attributes, including a bioactive oxide layer. The surface roughness of titanium has shown significant influence over osteointegration in several studies[1,2], including those by Dalby et al. 2007[3] and Minagar et al.2013[4]. Hydroxyapatite (HA) is chemically very similar to that of naturally occurring bone bioapatite. However, bone bioapatite contains several calcium phosphate phases and various trace ions including zinc (Zn), magnesium (Mg) or Strontium (Sr). This work aims to investigate the chemical and physical response of osteoblasts to hydroxyapatite and substituted apatite thin film coated nanostructured titanium, without the addition of osteogenic media or growth factors.

METHODS:

The two types of titanium nanostructures used in this project were polycrystalline titanium, synthesised through radio frequency magnetron sputtering, and titanium nanotubes, achieved through electrochemical anodisation. These different nanostructures were fully characterised using XPS, FTIR, XRD, AFM and SEM before and after being coated with hydroxyapatite thin films. These substrates were then subjected to 21-day dissolution studies in water, PBS and SBF, each respectively, before carrying out any *in vitro* work.

RESULTS AND DISCUSSION:

XPS, FTIR and XRD data all show clearly that a thin film coating is present on all the substrates. As shown in Fig.1, the presence of the Ti 2p and O 1s peaks show that titanium and titanium oxide are present in the coating. XRD results confirm that the surfaces are polycrystalline titanium. The polycrystalline titanium surfaces showed granular nanostructures in the range of 80-100nm, whilst the titanium nanotube substrates showed homogenous tubular topography with very small interspacing in the range of 30-50nm. After apatite coating, XPS confirmed the presence of a calcium phosphate (CaP) coating. Coatings with Sr substituted HA showed clear Sr peaks, indicating that the strontium was indeed present on the substrate. As the apatite coatings were amorphous, they largely dissolved off the surfaces during the dissolution investigation, as expected. However, there was residual CaP material still on the surfaces after dissolution, as reported by XPS.

CONCLUSION:

Titanium nanostructures were successfully synthesised and fully characterised to investigate the physical and chemical attributes. The results gathered suggest that these structures will have a positive response on osteoblast cells, and this will be investigated through *in vitro* studies.

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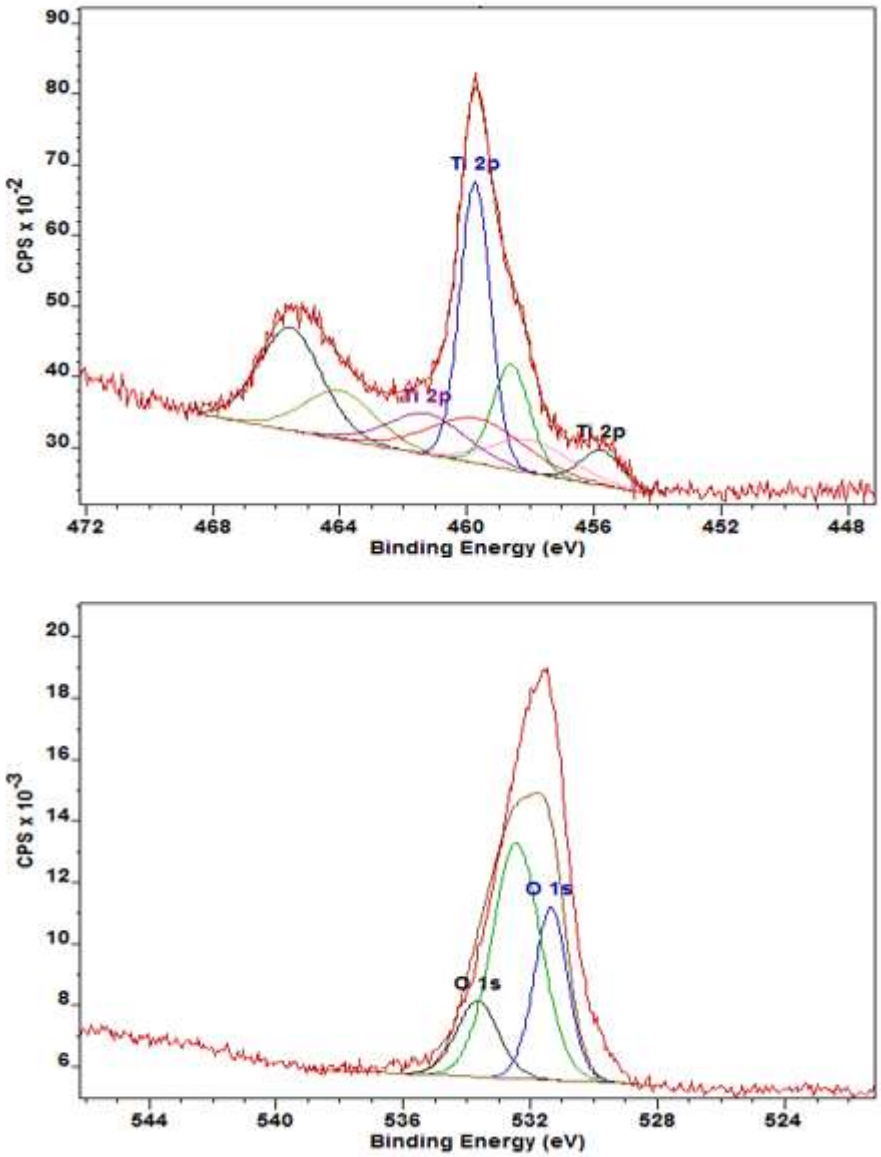

Picture 1: Caption 1:
Figure 1. XPS peaks showing Ti 2p and O 1s

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

143 The interaction of bone-like cells with electrodeposited calcium phosphate coatings

Taraneh Mokabber[1], Patrick van Rijn[2], Antonis Vakis[1], Yutao Pei[1]

[1]University of Groningen, Groningen, Netherlands

[2]University of Groningen, University Medical Center Groningen, Netherlands

INTRODUCTION:

Calcium phosphate (Ca-P) ceramics coated on titanium are extensively used as orthopaedic implants. This is attributed to the advantages of titanium offering desirable mechanical properties and to the biocompatibility of Ca-P ceramics. Electrochemical deposition (ECD) is the most frequently used technique to produce Ca-P coatings. Recently, we described parameters of ECD that significantly affect the thickness and morphology of the deposited coatings which in turn influences the interaction with tissue cells [1-4]. The present work discusses methods for controlling the morphology of the coatings in order to enhance the biocompatibility. Tissue compatibility is investigated by assessing the interactions of bone-like cells with the Ca-P coatings.

METHODS:

ECD was performed in a conventional electrochemical cell. An electrolyte solution of 0.042 M $Ca(NO_3)_2.4H_2O$, 0.025 M $NH_4H_2PO_4$, and 1.5 wt.% H_2O_2 was used. The deposition time was varied (1, 3 and 30 min) in order to investigate its influence on the coating morphology. SaOs cells with a cell density of 4×10^4 were seeded and cultured on the various coatings with distinct morphology. The cellular behaviour was observed via SEM and fluorescence microscopy after 2 days and 7 days culture and complemented with viability studies and protein expression such as ALP and Collagen I.

RESULTS AND DISCUSSION:

Altering the deposition time results in different morphologies with different roughness and affects the cellular behavior. A 1 min deposition time resulted in a flat and smooth coating with minimum roughness on which cells adhere very well and exhibit high spreading area. By increasing the deposition time to 3 min, Ca-P plates start to grow on the coating surface. On this coating, cells were less viable. Finally, the coating deposited for 30 min had the highest roughness due to the formation of elongated ribbons and many dead cells were observed. The cellular behaviour of cells is accordingly discussed.

CONCLUSION:

Ca-P coatings with different morphologies were achieved by altering the deposition time. The most favourable surface for adhesion and growth of the cells is smooth surface with low roughness. As the roughness of the surface increased, cell adhesion, spreading and viability were compromised.

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R. Schmidt, Surface and Coating Technology, 2016, 294.

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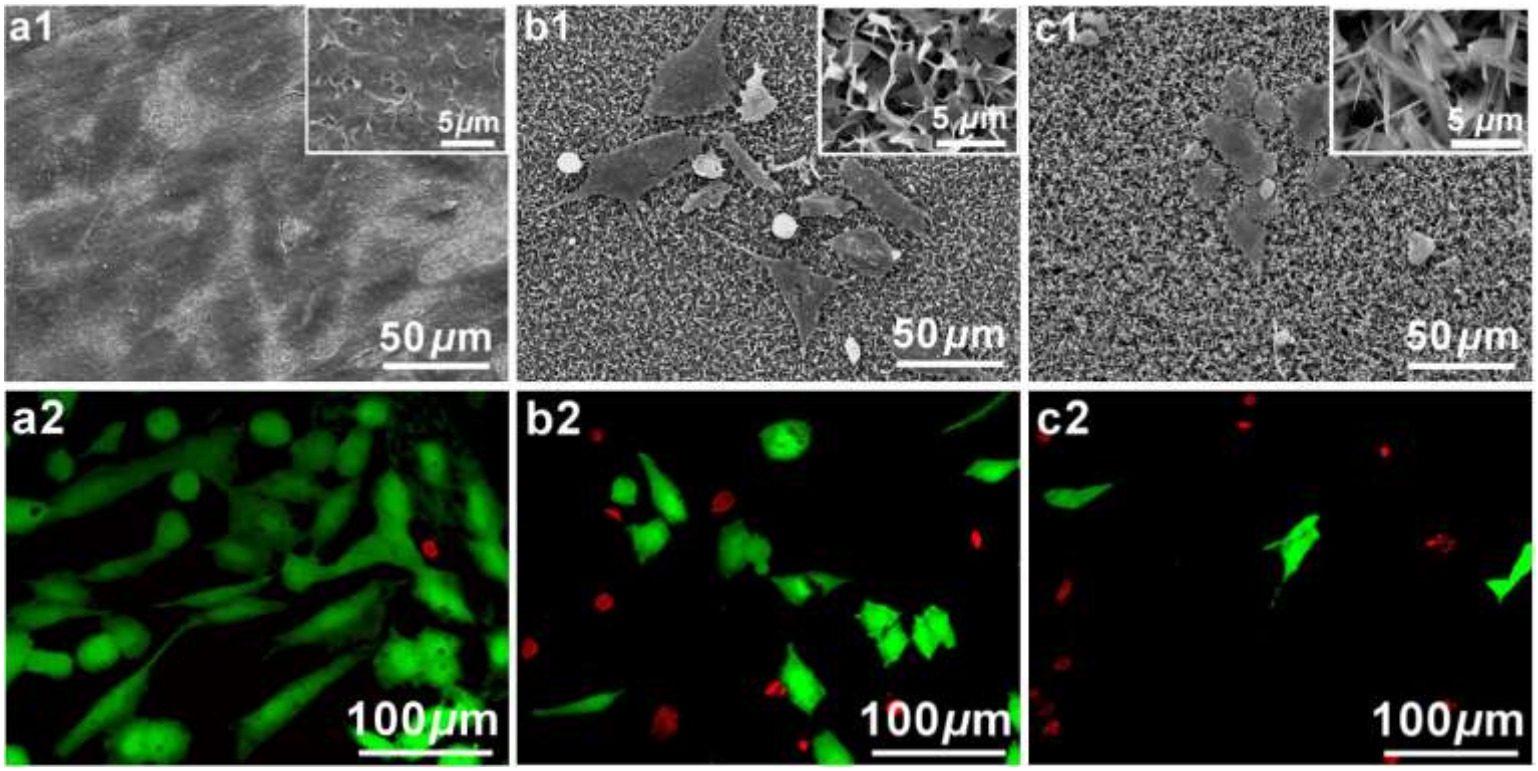
Picture 1: Caption 1: Fig. 1. (a1-c1) SEM images and (a2-c2) confocal microscopy images of SaOs cells on Ca-P



coatings deposited at (a) 1 min, (b) 3 min, and (c) 30 min.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

145 In vitro cellularized porous hydrogel scaffolds with flow perfusion for bone defect reconstruction.

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[2]Laboratoire MSSMat, UMR 8579 - CentraleSupelec, France

[3]Laboratoire LGPM, CentraleSupelec, France

[4]Laboratoire LVTS, INSERM 1148 - Université Paris Diderot, France

INTRODUCTION:

Natural biomaterials are a promising alternative to autograft or allograft for restoring bone defects. Our goal is to rationalize results in vitro to improve the formulation of natural biomaterials for bone tissue regeneration in vivo. We focus on the multiscale characterization of a porous hydrogel polysaccharide-based 3D scaffold that showed osteogenic and osteoinductive properties in rat and goat [1]. As an in vitro model of bone defect, we chose an underflow cell culture (bioreactor [2]) to mimic a physiological-like 3D environment.

METHODS:

The polysaccharide-based (75% pullulan – 25 % dextran (w/w)) porous scaffold was prepared with porogen agent (NaCl or Na_2CO_3) and different concentrations of cross-linker (STMP) from 1.5% to 5% (w/v) before freeze-drying. Porosity and pore size distribution were quantified with scanning electron microscopy (ZEISS Quanta 200) and confocal microscopy (ZEISS LSM700). Then 3%-STMP scaffolds (8 mm in diameter and 1.5 mm thick) were seeded with 100 000 osteoblast MC3T3-E1 cells and perfused with 10 $mL.min^{-1}$ and 20 $mL.min^{-1}$ flow rate within the 5mL-bioreactor under standard cell culture conditions.

RESULTS AND DISCUSSION:

In the bioreactor, osteoblasts proliferation until at least 17 days for 10 $mL.min^{-1}$ flow rate was observed. An increased in flow rate from 10 $mL.min^{-1}$ to 20 $mL.min^{-1}$ results in spontaneous cell organization in spheroid after 17 days. This 3D organization offers favorable conditions to achieve osteoblasts differentiation [3]. Using either NaCl or Na_2CO_3 as porogen agent showed no significantly different results for osteoblasts proliferation or organization in this perfusion conditions. Porosity and average pore size were not significantly different depending on the porogen agent. Results showed that the increase of cross-linking concentration from 1.5% to 5% decrease the porosity of the hydrated scaffold from 23.5±1.5 % to 5.5±0.5 % and pore sizes from 140±60 µm to 25±13 µm. This indicates that pore formation after freeze-drying can be modulated with scaffold stiffness.

CONCLUSION:

Since our bioreactor allowed in vitro qualification and quantification of proliferation and organization of osteoblasts in porous scaffolds, future works will be devoted to the optimization of scaffold formulation and perfusion conditions to achieved bone-like tissue generation. Mathematical modelling of the flow perfusion conditions shall be developed to link biological results with hydrodynamics conditions.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

147 Functionalized Polymeric Implants For Controlling The Migration Of Glioblastoma Cells And Their Subsequent Trapping

Muhammad Haji Mansor[1], Mathie Najberg[2], Jean-Michel Thomassin[3], Carmen Alvarez-Lorenzo[4], Emmanuel Garcion[2], Christine Jérôme[3], Frank Boury[2]

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[2]Cancer and Immunology Research Centre Nantes-Angers(CRCINA), Université d'Angers, France

[3]Center for Education and Research on Macromolecules (CERM), Université de Liège, Belgium

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INTRODUCTION:

Glioblastoma (GBM) is the most common form of brain cancer. The diffusive nature of GBM tumours makes them impossible to be removed completely by surgery. Consequently, the residual GBM cells contribute to ≥90% rate of tumour recurrence[1]. To counter this treatment deficiency, our team is pursuing an approach to develop implants that gradually release chemoattractant molecules and support cell infiltration that are suitable for inducing chemotaxis and trapping of the residual GBM cells, which will subsequently enable their selective killing (Figure 1)[2].

METHODS:

Stromal cell-derived factor-1α (SDF-1α) chemoattractant was encapsulated into biodegradable poly-lactic-co-glycolic acid (PLGA)-based particles by a phase separation method to produce a chemoattractant reservoir. The SDF-1α-loaded nanoparticles were then embedded within a chitosan scaffold by electrospinning to obtain nanofibrous implants that mimic the brain extracellular matrix (ECM) structure to encourage GBM cell trapping.

RESULTS AND DISCUSSION:

Spherical SDF-1α-loaded nanoparticles of 282 ± 37 nm in size with a narrow size distribution and high encapsulation efficiency (76%) were successfully synthesized. There was no significant difference between the bioactivity of encapsulated SDF-1α and its native counterpart, as determined by their ability to induce migration in GBM cells[3]. The nanoparticles were also conveniently co-electrospun with chitosan to encapsulate the nanoparticles within chitosan nanofibres.

CONCLUSION:

A non-denaturing SDF-1α encapsulation process was developed. The SDF-1α-loaded nanoparticles were incorporated into chitosan scaffolds to establish SDF-1α concentration gradients that may induce chemotaxis of GBM cells for their trapping. In future work, focus will shift towards coating the nanoparticle-containing nanofibres with ECM molecules such as hyaluronic acid to maximize cell-scaffold interactions. The physicochemical properties of the scaffolds, such as their porosity, will also be optimized to maximize GBM cell trapping.

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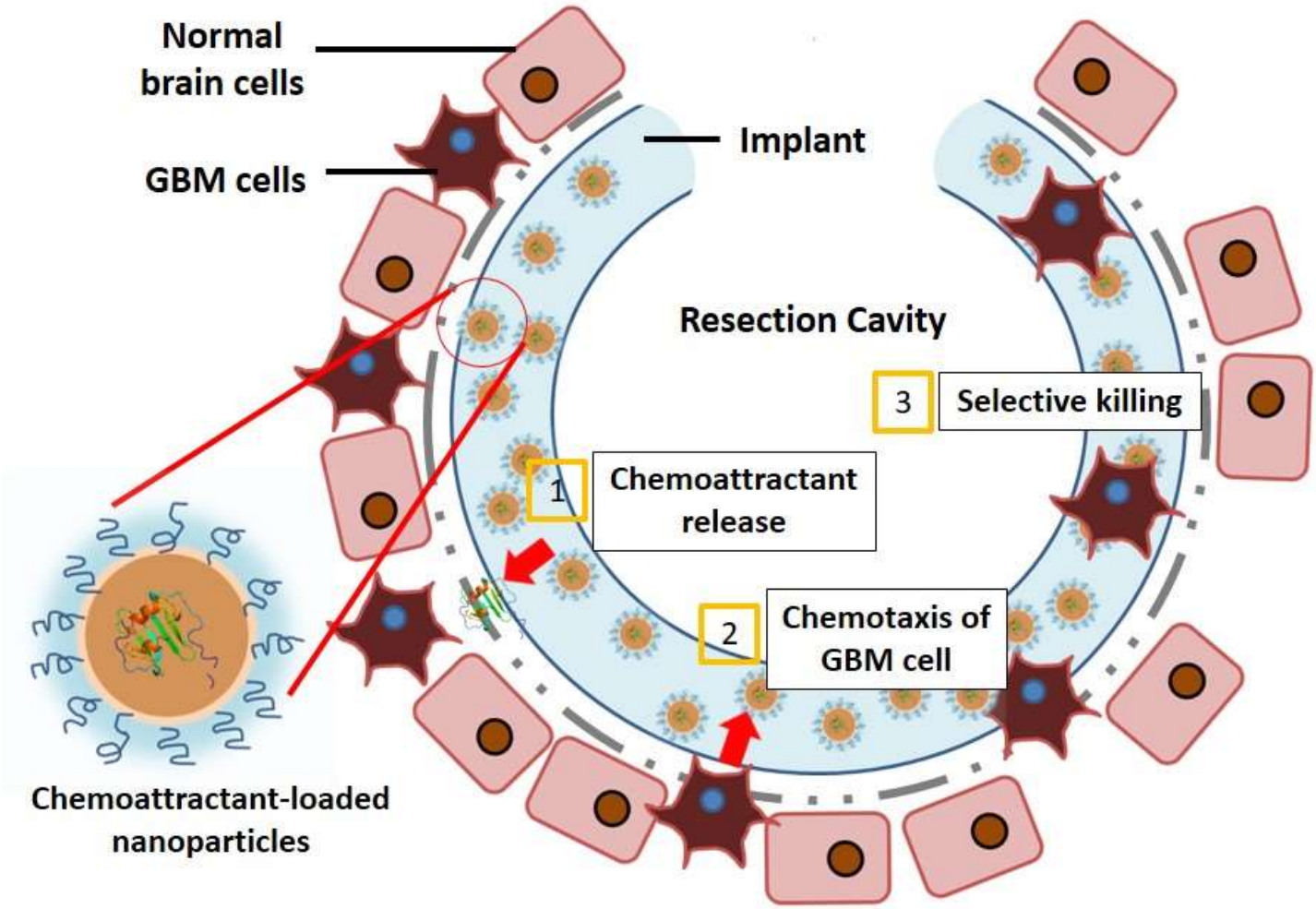
ACKNOWLEDGMENTS:

Authors would like to thank the French Ministry of Higher Education and Research (MESR), the University of Angers & Liège and the Erasmus Mundus Joint Doctorate (NanoFar) for their financial support.



Picture 1: Caption 1: Fig. 1. Design of a polymeric implant for the entrapment of GBM cells

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

154 In situ linkable hydrogel for cardiac tissue regeneration

Samhita Banerjee[1], Andreas Kirschning[2], Gerald Dräger[2]

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[2]Leibniz Universität, Hannover, Germany

INTRODUCTION:

One of the strategies to improve the conventional treatments of myocardial infarction is to develop a less invasive injectable hydrogel which would serve as a favourable matrix for cell delivery and tissue regeneration.[1] Thereby, we

focus on applying the Aldehyde/Hydrazine condensation method to generate chemically characterised biocompatible and biodegradable hydrogels using various polysaccharide as the building block.

METHODS:

The Aldehyde/Hydrazide modified polysaccharides were prepared by procedures done previously in our laboratory[2]
Procedure for RGDfK-polysaccharide: Carboxymethylated polymer (100mg) in dest. water (50ml) was treated with EDC (8.2mg), HOBt (6.0mg), DIPEA (0.02ml) RGDfK (1.0mg) and stirred overnight at rt. It was further converted to the hydrazide using the above method.

Hydrogel formation: Equal volumes of hydrazide component (20mg/ml) and aldehyde component (20mg/ml) in isotonic saline each were mixed at room temperature.

RESULTS AND DISCUSSION:

In situ gelation was achieved within few minutes by the hydrazone formation between the functionalized polysaccharide. The polymers are also modified with the cyclic peptide RGDfK in order to enhance biocompatibility of the hydrogel. Furthermore, the RGDfk modified hydrogels were also tested as potential three dimensional scaffold for generating cardiac tissue in presence of cardiomyocytes.

CONCLUSION:

The Aldehyde/hydrazine condensation reaction can be used to generate hydrogels *in situ*. Preliminary data suggest that RGDfk modified hydrogels based scaffolds can be used a template for cardiac tissue regeneration

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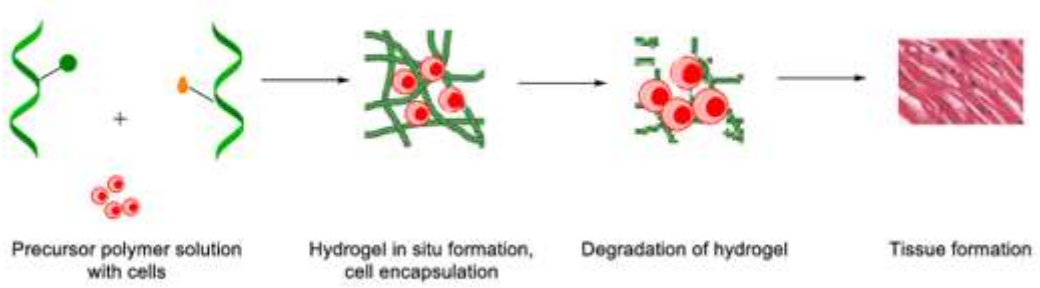

Picture 1: Caption 1: Overview of the Aim of the project

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

155 Layer-by-layer implant coating of cationic polymer drug nanoparticles and heparin with dual anti-inflammatory properties

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INTRODUCTION:

Poor integration and short-term failure of implants is mainly caused by the uncontrolled activation of macrophages in their immediate surroundings[1]. Therefore, the reduction of early inflammatory process and the generation of a long-lasting anti-inflammatory microenvironment around implants may be of clinical and economic significance[1,2]. This work is aimed to design a novel anti-inflammatory coating combining the immediate anti-inflammatory activity of heparin[3] and the long-term release of naproxen, a well-known NSAID.

METHODS:

A naproxen-containing prodrug was obtained and copolymerized by free radical copolymerization with 2-vinylimidazole. Copolymers were extensively characterized and nanoparticles were prepared via nanoprecipitation method. The preparation conditions were optimized to favor polyelectrolyte multilayer formation and inflammation-site accumulation (i.e. NPs diameter between 100-150 nm and positive surface charge)[4].

Multilayered films of heparin (polyanion) and nanoparticles (polycation) were prepared by dip coating. Film formation was assessed using D-QCM and profilometry and surface properties were studied by SEM, WCA and Z-potential measurements. Finally, surfaces were evaluated for macrophage adhesion and Foreign Body Giant Cells (FBGCs) formation by Giemsa staining on days 1 and 15, respectively. Moreover, levels of IL-1β and nitric oxide (NO) were correspondingly quantified by ELISA and NO assays.

RESULTS AND DISCUSSION:

The naproxen-containing prodrug and the amphiphilic copolymers were prepared successfully. After optimizing the preparation conditions, nanoparticles presented 130 nm in diameter and a surface charge of +30 mV favoring film formation and cell internalization.

D-QCM data revealed effective film growth presenting 80 nm thickness, high roughness and heterogeneous coverage according to profilometry and SEM (***figure 1***). The surface was highly hydrophilic and positively charged contributing to a reduction in cell adhesion after 24 hours in first tests. Moreover, preliminary results showed a long-term decrease in NO levels.

CONCLUSION:

NPs present hydrodynamic properties that favor film formation and NPs retention at inflammation site[4]. Moreover, preliminary results demonstrate *in vitro* dual anti-inflammatory capacity (short-term and long-term) of the coating being a good candidate to improve implants half-life[1].

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ACKNOWLEDGMENTS:

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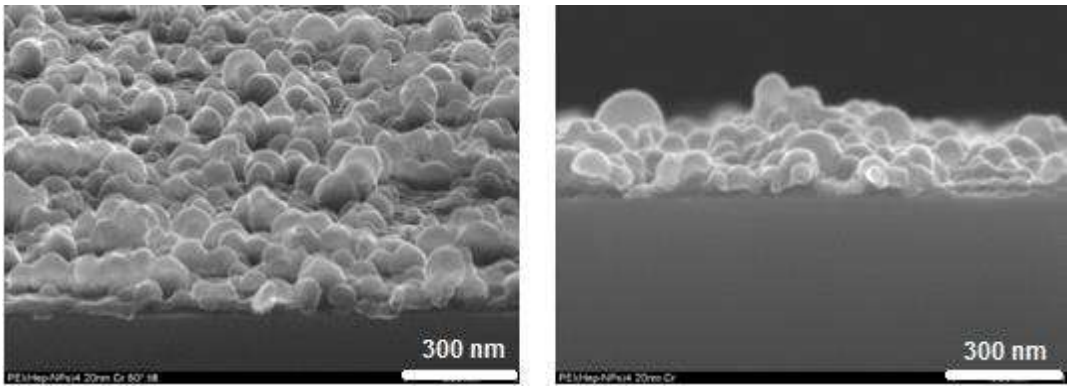
Picture 1: 

Caption 1: Figure 1. SEM micrographs of multilayered films' top layer.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

161 Decontamination of PEGDMA with Cold Atmospheric Plasma for Cell Encapsulation Purpose

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INTRODUCTION:

Hydrogels are biocompatible polymeric structures that can maintain high amounts of water or biological fluids and crosslink with covalent, ionic, hydrogen bonding, or van der Waals forces and can deliver both controlled and sustained releases of nutrients, drugs or proteins[1]. Poly (ethylene glycol) dimethacrylate (PEGDMA) is a PEG-based polymer and has minimal toxicity and biocompatibility. Plasma is ionized gas composed of ions, electrons and neutral particles[2]. Free radicals from plasma application can alter the mechanical and/or chemical properties of the biomaterial. Antimicrobial effect of cold atmospheric plasmas (CAP) is very well known and widely studied[2]. Sterilization techniques such as ethylene oxide and gamma radiation are the most common methods for sterilization of scaffolds. However, biodegradable scaffolds might show sensitivity to those sterilization techniques. The aim of this study was to decontaminate PEGDMA with CAP treatment, and also to examine the effect of CAP treatment on L929 fibroblast cells that are encapsulated in PEGDMA.

METHODS:

In decontamination experiments, Different concentrations of PEGDMA was mixed with 10^5 CFU/ml *E. coli* and *S. aureus* cultures in equal volumes and then treated with CAP for 1, 2, and 3 minutes separately. After treatment, serial dilutions were made and samples were plated and incubated at 37ºC, for 24 hours. In antimicrobial activity acquisition experiments, first, only different concentrations of PEGDMA was treated with CAP and then plasma

treated PEGDMA was mixed with 10^6 CFU/ml *E. coli* and *S. aureus* cultures in equal volumes. Then plasma treated PEGDMA mixed with bacteria, plated and incubated. In cytotoxicity experiments, L929 fibroblast cell at concentration of $5x10^6$ cells/ml were mixed with untreated PEGDMA solution and CAP treated PEGDMA solutions separately. PEGDMA-cell mixture was polymerized. Polymerized PEGDMA-cell mixture was transferred to 24-well plate and MTT and LIVE/DEAD assays were carried out at day 1st, 4th and 7th days of incubation.

RESULTS AND DISCUSSION:

In antimicrobial tests, no antimicrobial activity was determined on control groups for two different sets of experiments. In plasma groups for both decontamination and acquisition of antimicrobial activity tests, 5-log reduction of *E. coli* and *S. aureus* was determined for all different concentrations of PEGDMA. Cytotoxicity experiments reveled that, only plasma treated PEGDMA with 10% w/v concentration was capable of not exerting cytotoxic effect on L929 cells.

CONCLUSION:

In conclusion, CAP could be an alternative method for decontamination of PEGDMA with no detectable toxicity on L929 cells.

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

164 Interactions of metallic nanoparticles with 2D and 3D human skin models

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INTRODUCTION:

Metallic nanoparticles provide the basis for the design and application of drug delivery vectors with tuneable surface chemistry.[1] The interactions of metallic nanoparticles with respect to their stability, cytotoxicity and uptake mechanisms following their administration is yet to be fully understood.[2] Skin is an organ and target at the forefront of therapies to combat antimicrobial resistance and accelerate wound healing, an area where the application of polymer coated metallic nanoparticles is growing.[3] Here we investigate the nanotoxicology of metallic nanoparticles in 2D and 3D human skin models for use as drug delivery vectors.

METHODS:

A variety of standardised biophysical techniques and commercially available *in vitro* biochemical assays have been utilised in order to characterise the physical and chemical properties of polymer coated metallic nanoparticles and subsequent impact on cells *in vitro* in 2D. The development of a 3D model focussed on the optimisation of culture conditions to ensure controllable and reproducible cell behaviour prior to the administration of tailored metallic nanoparticles inferred from 2D screening studies.

RESULTS AND DISCUSSION:

We demonstrate the effect of size (10 nm - 100 nm), coating (+/- polymer) and concentration (up to 100 $\mu g.mL^{-1}$) of silver and gold nanoparticles with respect to cytotoxicity (MTT, LDH & Annexin-V), uptake (ICP-OES), immunogenicity (ELISA; IL-6) and cell motility (scratch assays) in human skin fibroblasts, keratinocytes and epithelial cells in 2D. Furthermore we are developing an optimised 3D co-culture human skin model that acts as a replacement for *in vivo* explants in order understand the interaction, distribution and nanotoxicology of metallic nanoparticles in a controlled manner. This allows for the identification of factors that permit the rational design of metallic nanoparticle based drug delivery vectors as novel therapeutics.

CONCLUSION:

The data presented here supports the utilisation of polymer coated metallic nanoparticles as drug delivery vectors in a 3D human skin model to address ongoing challenges surrounding antimicrobial resistance and wound healing.

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

165 Effect of surface topography on epithelial cell migration

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INTRODUCTION:

Collective cell migration plays a crucial role in many fundamental biological processes, such as in wound healing and cancer metastasis. Different extracellular cues orchestrate cell migration and, recently, mechanical signaling has

received considerable attention. Here, we focus on the effect of topography on collective migration of epithelial cells. This will provide new insights into epithelium mechanotransduction and into the plasticity of cellular phenotypes for future therapeutic interventions.[1]

METHODS:

Substrate preparation.

Glass coverslips were spin-coated with photosensitive Disperse Red 1 molecular glass (DR1-glass, Solaris Chem.), patterned by interference lithography and characterized by AFM.

Cell culture.

MDCK cells were seeded on patterned DR1-glass. In wound healing experiments, PDMS rectangular stencils were placed on the substrates before seeding and peeled off at confluence. Pharmacological inhibitors were used to perturb ROCK and PI(3)K pathways during migration. Cells were fixed and immunolabeled.

RESULTS AND DISCUSSION:

It is well known that topography plays an important role in guiding single-cell migration through cell-material interaction.[2] Geometrical confinement of epithelial cells influences their collective migration.[3] We therefore hypothesized that topographical cues could also guide the collective displacement of epithelia.

We chose different micron-sized linear topographies photoinscribed on DR1-glass. The image analysis of the migrating cells revealed that cells moved most coherently on 1 μm-pitch pattern and pharmacological inhibitors showed that both the PI(3)K and ROCK pathways are involved in the process.

Finally, we proposed that this topographical guidance could be exploited in a wound healing experiment in vitro, where the pattern was positioned orthogonally or parallel to the wound. We show that the orthogonal configuration promotes the wound closure, whereas the parallel one hinders it. This result was attributed to topography-induced focal adhesion confinement, as the pFAK orientation analysis shows.

CONCLUSION:

Topography has a prominent role in defining the collective migration of MDCK cells through ROCK pathway enhancement induced by focal adhesion confinement. For this reason, in in vitro wound healing assay a topography “linking” wound boundaries was able to promote wound closure.

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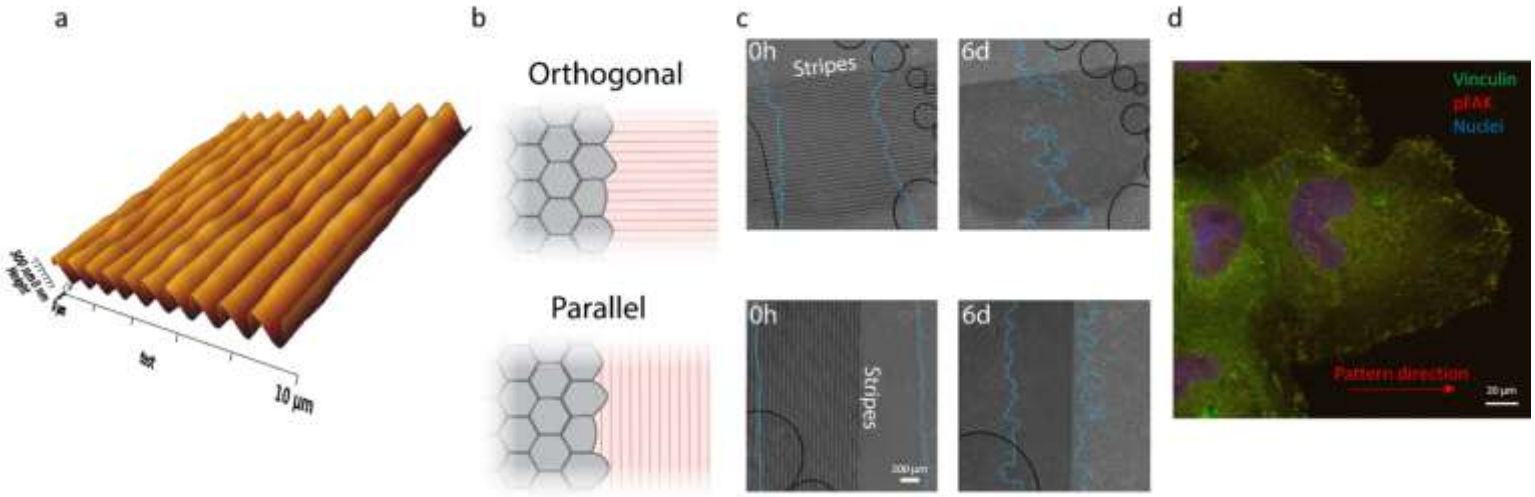

Picture 1: Caption 1: a) AFM of the pattern. b) Sketch of wound configurations and c) their healing. d) Immunostained wound edge in orthogonal configuration.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

181 Ferroelectric coatings on metallic implants for guided mineralization and protein adsorption

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INTRODUCTION:

A wide range of synthetic materials has been proposed as bone substitutes[1], but due to their superior mechanical strength metals are still the most employed[2]. Austenitic stainless steel 316L-SS is routinely used in total hip replacements [2]. However, its poor bioactivity leads to problematic osseointegration that may result in failures, as aseptic loosening and implant infection[3-5]. Within this context, the concept of a biologically active platform composed of ferroelectric $LiTaO_3$ (LTO) and 316L-SS is here proposed. Taking advantage of ferroelectric and semiconductor character of LTO, surface functionalization of coatings is suggested as an effective method to induce the appropriate stimuli capable of rendering the surface biologically responsive.

METHODS:

LTO-functionalized coatings were characterized by GI-XRD, FTIR, XPS and SEM. Surface roughness and wettability were also evaluated. In vitro acellular assays were conducted in SBF. UV-Vis spectrometry was used to study protein adsorption.

RESULTS AND DISCUSSION:

Functionalization of LTO on 316L-SS by external stimuli, electric field or UV-light, favor the formation of polar groups on its surface leading to increase of (i)surface wettability, (ii)rate of CaP formation and (iii)BSA protein adsorption. CaP precipitates as a consequence of Li lixiviation and functional groups attach to the surface due to UV-light and corona discharge. The type of functionalization affects protein conformation. UV-light produce an increase of α-helical structures percentage whereas β-ones are reduced. The opposite is observed for electrical functionalized surfaces. The decrease of the α-helical structures in corona charged surfaces is attributed to a higher content of carboxyl groups and a high ability to promote H bonds reactions. Cells response will correspondingly profit from these protein conformational features.

CONCLUSION:

We proved that platforms electrically functionalized can modulate protein conformation for further adequate cells response and tissue growth stimulation. This will ultimately contribute for a direct implant integration and a reduction of implant failure.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

182 Tissue cells can win the race for the surface on gold-nanoparticle coatings between tissue cells and pathogenic bacteria with the aid of macrophages

Henny van der Mei[1], Yafei Luan[1], Melissa Dijk[1], Yijin Ren[1], Hong Chen[2], Henk Busscher[1]

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INTRODUCTION:

Biomaterials implants and devices are indispensable once disease, injury and wear have caused irreparable damage to the human body. However, biomaterials implants and devices fail at an unacceptable rate of around 5% across different types of implants and devices.[1] Biomaterials associated infections caused by bacterial colonization is one of the main reasons for this failure of the implants. Bacteria are introduced during implantation and can cause

early or late infections.[2] Nanotechnology often designs a surface for a specific biologic response,[3] but.in biomaterial associated infections bacteria, tissue cells and immune all play a role. Therefore, we have investigated if tissue cells can win the race for the surface from pathogenic bacteria on randomly nanostructured gold-nanoparticle coatings[4] with the aid of macrophages.

METHODS:

Three bacterial strains, two different tissue cells and macrophages were used in tri-cultures experiments, which mimic the *in vivo* situation. Gold-coated surfaces with gold-nanoparticle coatings with different surface roughnesses and wettabilities have been prepared. Tri-culture experiments were performed in static conditions.

RESULTS AND DISCUSSION:

The gold-nanoparticle coating with nanoscale roughness decreased the biofilm thickness. These surfaces also showed a much higher tissue cells surface coverage on contaminated gold-nanoparticle coated surfaces than on smooth gold surfaces when contaminated with bacteria. Tissue cells did not survive when gold-nanoparticle coated surfaces were contaminated with *S. aureus*, but with the aid of macrophages tissue cells can survive on bacterially contaminated gold-nanoparticle-coated surfaces.

CONCLUSION:

Gold-nanoparticles with a nanoscale roughness showed an advantage above smooth gold surfaces in the race for the surface between bacteria and tissue cells, but only with the aid of macrophages. Tri-culture systems are needed when the race for the surfaces is studied on newly developed nanomaterials.

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Poster presentation

184 Antibiotic-free antibacterial coating of resorbable surgical sutures: a design of experiment approach

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[2]Lucideon Ltd, United Kingdom

INTRODUCTION:

Antimicrobial drugs are progressively losing efficacy worldwide due to excessive use or misuse. For instance, triclosan is an antibacterial and antifungal agent used in a variety of products, such as toothpaste, soaps and detergents. It is also used in clinics, for instance in surgical sutures. As for many other drugs, the widespread exposure of bacteria to triclosan can promote the development of resistance[1]. In this study we developed a new

antibacterial biomaterial meant to be used as coating of surgical sutures. The antibacterial activity is based on the properties of copper ions, an ion that due to its fast and multifaceted antibacterial effect is known to trigger little to no resistance[2].

METHODS:

Copper(II)-chitosan complexes were prepared and their antibacterial activity and cytocompatibility were confirmed[3]. The composition of the complexes was optimized with plasticizing additives to reduce their brittleness and reach desired mechanical properties for the targeted application. Plasticized copper(II)-chitosan was then used to coat polylactide-co-glycolide surgical sutures by dip-coating. A design of experiment (DOE) approach was used to identify the best combination of solution concentration, type of solvent, dipping and retrieving speed, number of dips and post-processing to optimize the morphological and mechanical properties of the samples against a selected benchmark (i.e. Vicryl™ sutures by Ethicon®).

RESULTS AND DISCUSSION:

The addition of plasticizer to copper(II)-chitosan results in a twofold increase of the elongation at break and a 50% ction of the Young's modulus. This behaviour is ideal for a complying coating. The DOE performed on sutures was successful in identifying a promising competitor to the current market benchmark. A device with similar morphological, degradation and mechanical properties, but offering an antibacterial effect with low risk of resistance development was fabricated.

CONCLUSION:

With this work we showed the fabrication, proof of concept and optimization through DOE of a newly developed material towards its clinical application as antibiotic-free antibacterial suture coating. The results achieved so far confirmed that this technology could reduce the risk of bacterial resistance development that characterizes current commercial products.

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

186 Implant design based on bone tissue anisotropy

Takayoshi Nakano, Takuya Ishimoto, Takahashi Hiroyuki

Osaka University, Suita, Japan

INTRODUCTION:

It is known that crystallographic orientation strongly dominates the mechanical and/or functional properties of biomaterials . The biological apatite (BAp) crystal, for example, is a major inorganic component of bone that belongs to a hexagonal system, which normally has greater anisotropy than the cubic crystal[1].

In this study, the implants design such as artificial hip joint, dental implant and vertebral fusion cage which are suitable for the preferential orientation of BAp and the related collagen fiber in bone tissues was proposed.

METHODS:

The BAp *c*-axis orientation and the related properties were analyzed using the biological techniques, μXRD, pQCT, nano-indentation, etc. Subsequently, implants design was developed based on bone microstructural anisotropy.

RESULTS AND DISCUSSION:

BAp crystal and collagen fiber in bones possess unique crystallographic anisotropy depending on the anatomical bone position. The contribution of the BAp *c*-axis orientation to the mechanical property of bone was understood using the bone regeneration model, which was expected to recover the BAp orientation as well as the mechanical property in defective rabbit ulna[2]. The orientation of the BAp *c*-axis should be a determinant of the mechanical property of the regenerated bone.

The degree of BAp *c*-axis orientation significantly changes and positively correlates with the mechanical parameter of bone in the regenerated bone and in other bones such as intact and pathological bones.

To arbitrarily control or enhance the mechanical properties during bone regeneration, artificial induction of the oriented BAp structure is effective. Artificial control of the BAp orientation has been tried on the basis of mainly two strategies: 1) control of the principle stress and subsequent continuous stress transfer between host bone and metal implants (Fig. 1) by facilitating an adaptive response to change the degree of BAp orientation and 2) usage of anisotropically-patterned substrates to align bone forming osteoblasts for secreting an oriented extracellular matrix.

The osteoblast alignment could be achieved using metallic substrates with unidirectional periodic aligned steps[3,4] and a collagen substrate with an anisotropic molecular arrangement.

CONCLUSION:

Future artificial implants should be developed based on the control of BAp/collagen arrangement for clinical use in the orthopedic[5] and dental fields[6].

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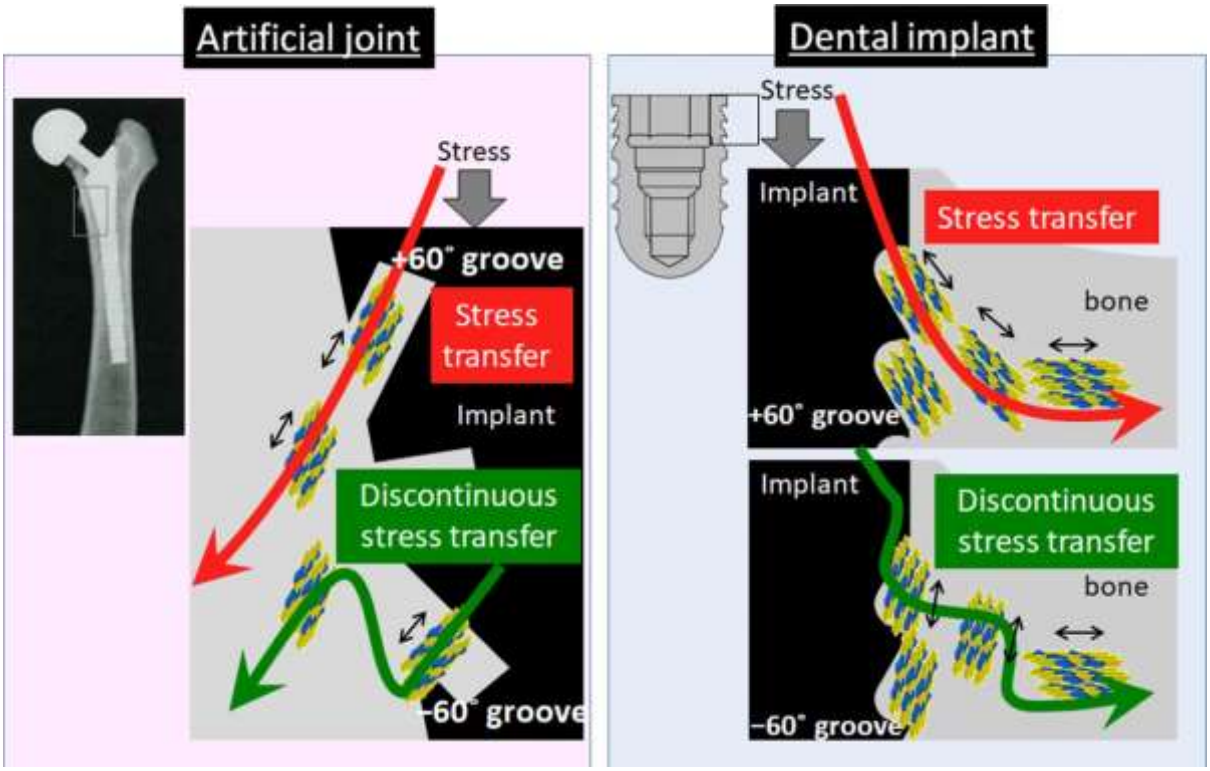

Picture 1: Caption 1: Fig. 1 Development of artificial Implants based on anisotropic bone microstructure. Reproduced from refs. [5,6].

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

187 Artificial analogues of antimicrobial peptides: synergetic activity of nano-gold simultaneously functionalized with different amino acids

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INTRODUCTION:

Antimicrobial peptides are characterized by a range of properties which rank them very high on the list of potential alternatives to currently available antibiotics.[1] There is, however, a list of their limitations which significantly affect their usage.[1,2] A considerable effort has been paid to improve their bioavailability and stability identified as the primary obstacles for their practical applications.[2] Aside from designing new synthetic antimicrobial peptides, there is a high effort in developing innovative nanotechnological approaches that combine well-known antimicrobial peptides with nanoparticles.[3] Gold nanoparticles (Au NPs) functionalized with cationic arginine molecules [5] enable more stable structures with the potential to provide better bioavailability.[4] In this work we investigated possibility to use a mixture of charged amino acids to functionalize Au NPs to combine better stability with stronger antimicrobial activity.

METHODS:

AuNPs were synthesized using sonochemical method [4] and tested for their antimicrobial activity against *Escherichia coli* (MG1655), *Pseudomonas aeruginosa* PAO1 (ATCC 15692) and *Staphylococcus aureus* (ATCC 12600).

RESULTS AND DISCUSSION:

Chemistry at the surface of Au NPs was regularly tailored using functionalization with different, cationic amino acids providing morphologically similar, 20-nm sized particles. The antimicrobial activity of the different AuNPs, illustrated for PAO1 (Fig. 1), confirmed surface chemistry dependence: for functionalization with single amino acid (Au(his(100 wt.%))) MIC_{100} was higher than 0.1 mg/ml; mixture of two cationic amino acids (Au(his-arg(50:50 wt.%))) decreased MIC_{100} value to the range of 0.04-0.05 mg/ml while three amino acids (Au(his-arg-lys(33:33:33 wt.%)) provided further MIC_{100} decrease to 0.03-0.04 mg/ml. The same trend of decreasing the MIC values was observed for *E. coli* and *S. aureus*.

CONCLUSION:

Bonding various amino acids to the bioinert AuNPs give them the possibility for specific interactions with bacterial membranes that enable increased antimicrobial activity, provides synergy and boosts efficiency.

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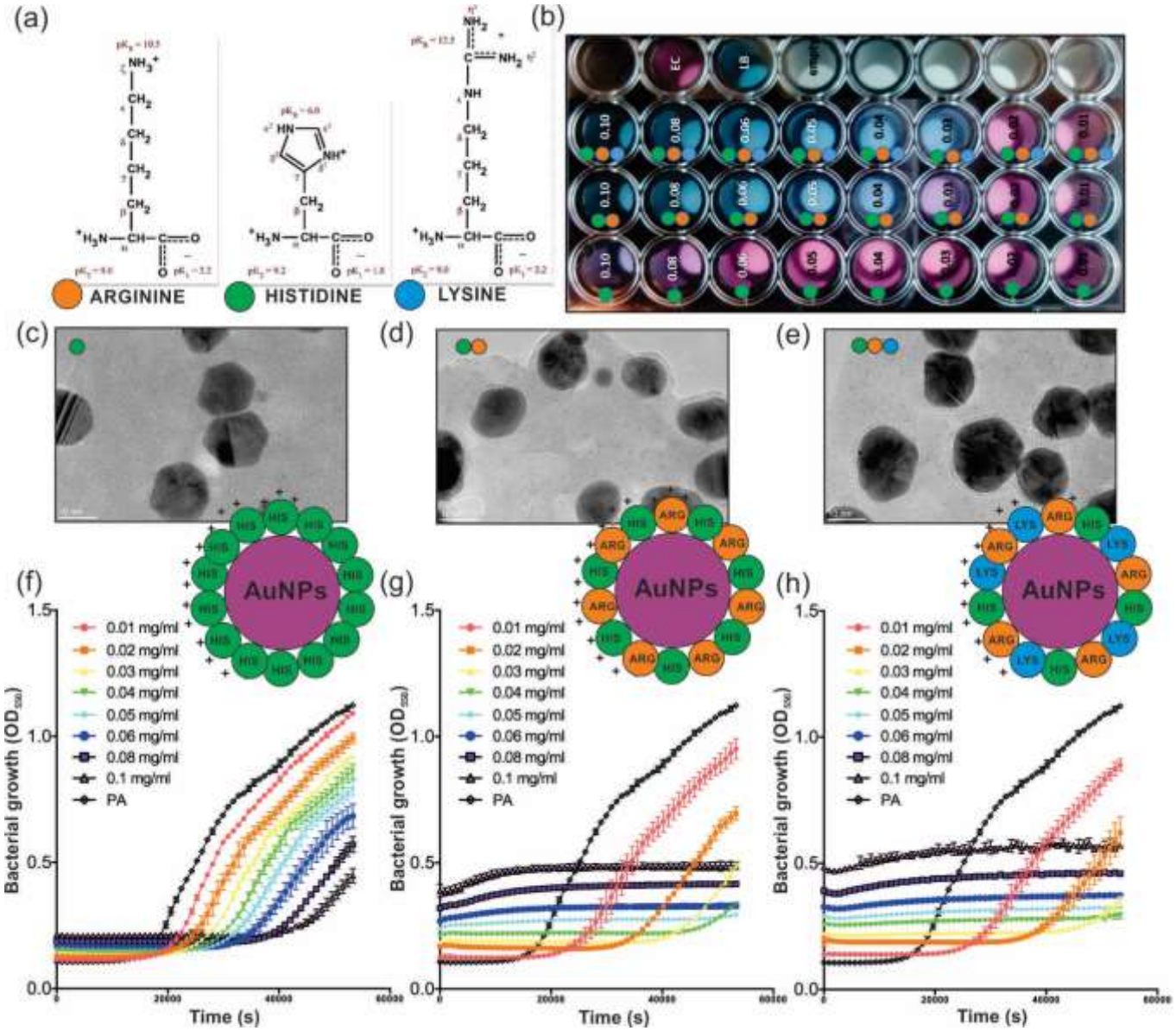

Picture 1: Caption 1: Amino acids used for functionalization (a); Presto blue test for PAO 1 viability (b), morphology of various AuNPs (c-e); and PAO 1 growth kinetics

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

190 In vivo 3D bioassembly of cellularized 3D printed scaffolds supports vascularization within entire tissue engineering construct

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INTRODUCTION:

Conventional tissue engineering (TE) approach is based on cell seeding on the surface of macroporous 3D scaffold. The main issue associated with this approach is an insufficient vascularization in the inner parts of the scaffold (1). The Layer-by-layer (LBL) bioassembly approach aims to obtain a homogeneous cell repartition inside such composite biomaterials by assembling small seeded blocks (2), leading to more efficient cell proliferation and differentiation comparing to conventional TE approach. The aim of this work was to evaluate the advantage of LBL *in vitro* and *in vivo* using poly(lacic) acid (PLA) 3D printed membranes seeded with human primary cells.

METHODS:

PLA microporous membranes were 3D printed by fused deposition modelling. Membranes were seeded using mono-cultures of human bone marrow stroma cells (HBMSCs) or co-cultures of these cells and human endothelial progenitor cells (EPCs) isolated from cord blood. LBL constructs were prepared by assembling 4 seeded membranes stabilized with PLA clips. Early osteoblastic and endothelial differentiation markers were evaluated for the each layer by the expression of alkaline phosphatase (ALP) and von Willebrand's factor (vWF) respectively. Then, these constructs were implanted subcutaneously in immunodeficient mice, as well as cellularized 3D printed blocks (conventional approach). The implants were harvested after 8 weeks, embedded in resin and labelled with Goldner Trichrome. Immunohistochemistry staining was performed with anti-Mitofilin antibody to label human cells. Quantitative analyses were performed to evaluate the number of newly formed blood vessels per mm^2 of PLA.

RESULTS AND DISCUSSION:

In vitro results showed higher level of ALP expression in co-culture systems comparing to mono-cultures of HBMSCs. Histological analyses showed human cells presence at the periphery of blocks and in the inner parts of LBL. The highest host tissue penetration was observed in LBL co-culture samples. This was confirmed by statistical analyses. As expected, the addition of endothelial cells improved the vascularization inside the implanted scaffolds (3).

CONCLUSION:

LBL bioassembly provided more efficient cell repartition in 3D comparing to TD, especially when co-culture system was used. It led to higher host tissue penetration and increased blood vessel formation inside the implants.

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ACKNOWLEDGMENTS:

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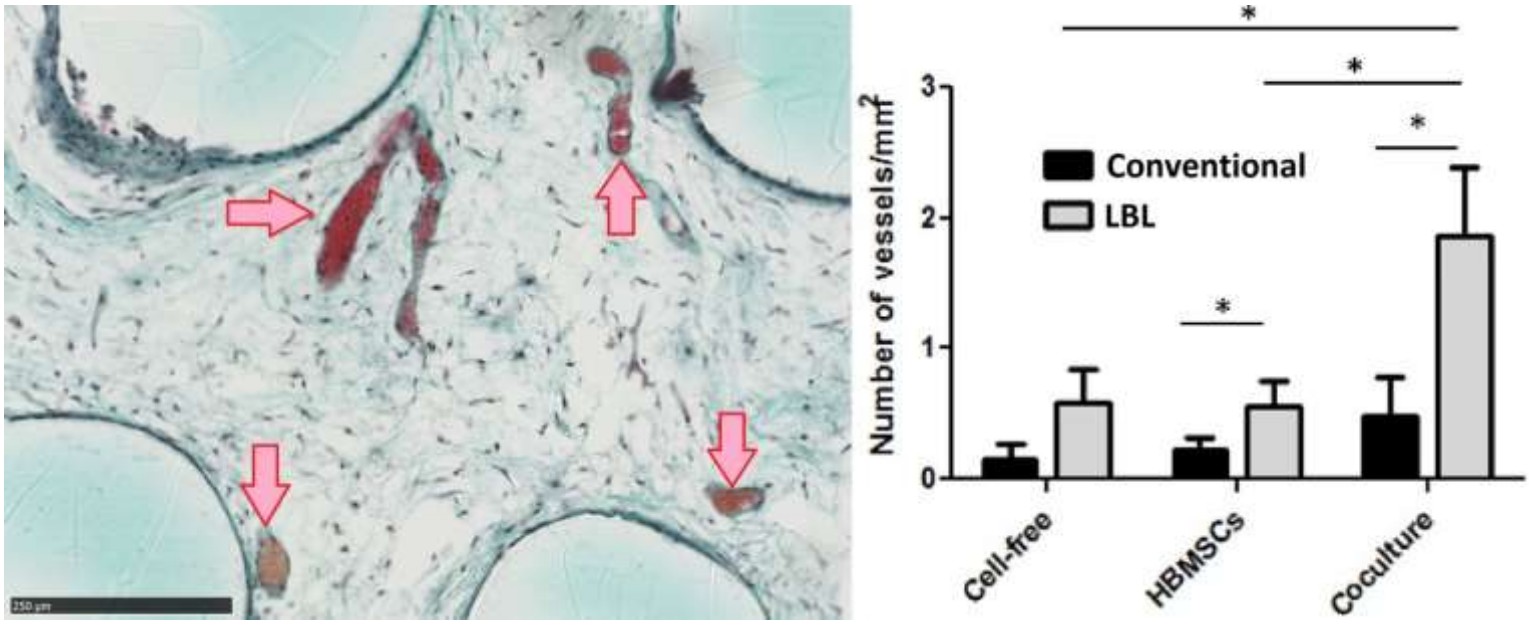

Picture 1: Caption 1: Blood vessels formed in the inner layers of LBL constructs after 8 weeks in vivo and statistical analysis of number of vessels observed in all samples

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

195 Cell delivery by chemically crosslinked gelatin hydrogel microspheres

Nicola Contessi Negrini[1], Maria Veronica Lipreri[1], Mariacristina Tanzi[2], Silvia Fare'[1]

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INTRODUCTION:

Delivery of cells by microcapsules or microspheres is useful in cell therapy-based approaches to achieve a controlled release of cells in space and time, compared to direct cells injection [1]. Advantages in using microspheres as cell delivery systems include a higher surface for cells growth and direct nutrients supply to cells. Here, we describe the production of gelatin microspheres useful as vehicles for the controlled release of cells.

METHODS:

Microspheres (MS) were produced by a gelatin hydrogel crosslinked using methylene(bis-acrylamide) as crosslinker [2]. A 15% w/v gelatin solution was dropped in soybean oil under stirring at 50 °C and allowed to crosslink for 24 h. MS were collected in a filtered syringe (Ø = 35 mm), washed with acetone, disinfected with 70% v/v ethanol and allowed to dry in sterile condition. MS stability at 37 °C was investigated by weight variation tests in culture medium and the crosslinking degree was measured by ninhydrin assay. *In vitro* cytotoxicity tests were performed by culturing L929 cells with eluates obtained by contact with MS up to 7 days. Cells were seeded on MS by swelling anhydrous MS in a cells suspension under stirring. Viable cells adhesion was investigated by LIVE/DEAD staining. Seeded MS

were collected in Cell Strainers and the proliferation of cells released from MS on tissue culture plastics (TCPS) was investigated by Alamar Blue™ assay.

RESULTS AND DISCUSSION:

The average diameter of MS after production was 89.8 ± 27.1 μm. MS were stable in culture medium up to 21 days; the MS average weight quickly increased in the first 6 h of swelling, reaching stable percentage weight variation (150% weight increase) after 24 h. The crosslinking degree was 86.3 ± 0.1%, confirming the efficiency of synthesis procedure. The viability of cells cultured in medium eluates extracted until 7 days of contact with the MS was >90%. Viable cells (>90%) adhered and proliferated on MS; moreover, cells were successfully released from MS on TCPS, colonized it and proliferated onto it, thus proving the efficiency of the produced MS as cell delivery vehicles.

CONCLUSION:

Chemically crosslinked gelatin hydrogel MS were successfully produced by water/oil emulsion. MS with controlled dimension able to promote cells adhesion and proliferation were obtained; moreover, viable cells were released from MS and were able to colonize and proliferate on the TCPS.

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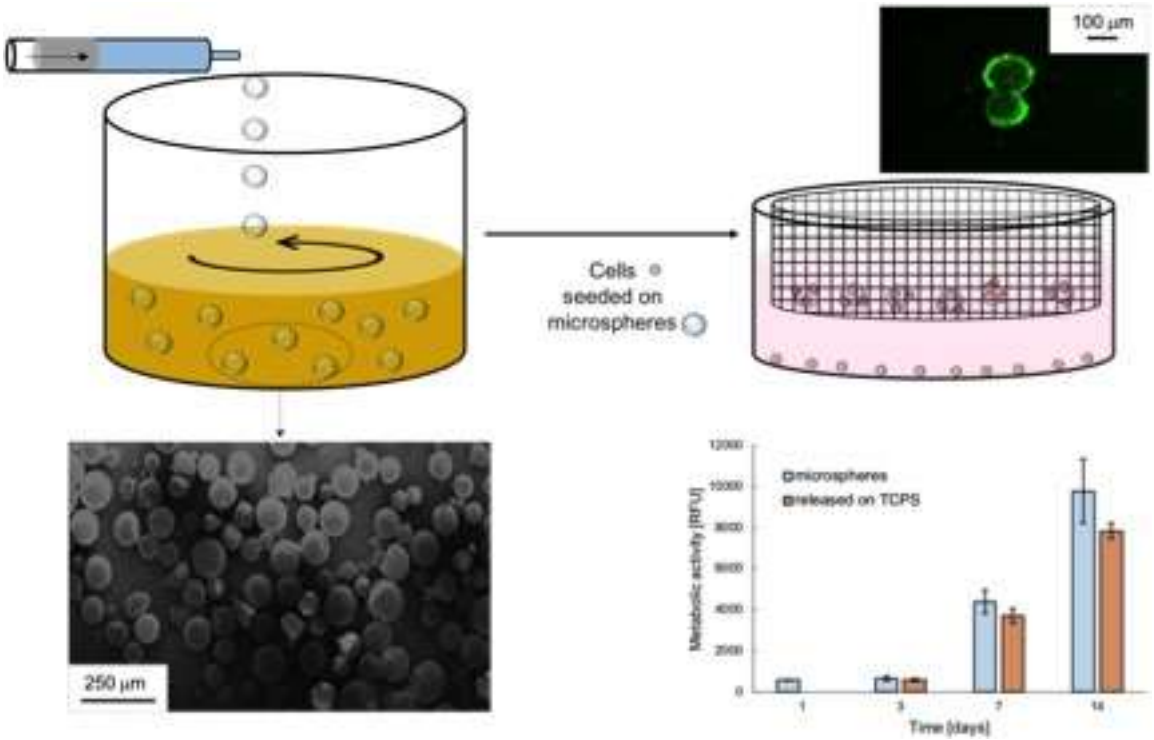
Picture 1:



Caption 1: Scheme of the protocol to obtain chemically crosslinked microspheres as cell delivery vehicles and results of in vitro cell delivery tests

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

199 Highly Stable RNA Uptake by Dense Cationic polymer brushes for the design of cytocompatible, serum-stable siRNA delivery vectors

Julien Gautrot, Danyang Li

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INTRODUCTION:

The high density of polymer brushes confers to these coatings unique physico-chemical properties, in particular for the regulation of biomolecular interaction and the design of highly selective coatings for biosensors and protein patterning.[1] Here we show that high density poly(dimethylaminoethyl methacrylate, PDMAEMA) cationic polymer brushes enable the stable uptake of high levels of oligonucleotides.[2] This is proposed to result from the high degree of crowding and associated increase in entropic driving force for the binding of polyelectrolytes such as nucleic acid molecules. We further demonstrate the ease with which such coatings allow the design of highly structured nanomaterials for siRNA delivery using block copolymer-brush based nanoparticles that allow the protection of oligonucleotides by a protein resistant outer block. In particular these nanomaterials display a high serum stability and low cytotoxicity whilst retaining excellent knock down efficiencies. Polymer brush-based nanomaterials therefore appear particularly attractive for the rational design of a new generation of high performance theranostics and RNA delivery probes.

METHODS:

We used ellipsometry, SPR, DLS and confocal microscopy to study RNA infiltration within polymer brushes. Cationic brush-functionalised nanoparticles were generated via ATRP and characterised via DLS, electrophoretic light scattering, TEM and FTIR. Transfection was monitored via immunostaining and western blotting. Particle and RNA fate via confocal microscopy.

RESULTS AND DISCUSSION:

We observed that the binding of smaller oligonucleotides (e.g. 10 - 25 bp) is significantly enhanced within dense cationic brushes . Interestingly, we observed that on dense brushes, dsRNA is absorbed to higher levels than for dsDNA . Our results indicate that dense polymer brushes should be more suitable for delivery of smaller RNA oligonucleotides such as siRNA or microRNA than larger DNA (e.g. plasmid DNA).

Based on the brush-gene interaction behaviour, we designed dense block copolymer brush coated silica nanoparticles (SiO_2-block copolymer) vectors for siRNA delivery, displaying a PDMAEMA brush core to capture siRNA and a PEG methacrylate brush shell, conferring protein resistance and improving cytocompatibility of the vectors whilst preserving high transfection efficiencies.

=CONCLUSION:

In summary, cationic polymer brush coated biomaterials, displaying controlled brush architecture (thickness, density, block *et al*), chemistry and bioactivity, are attractive systems for the study of oligonucleotide-polymer interactions and efficient transfection.

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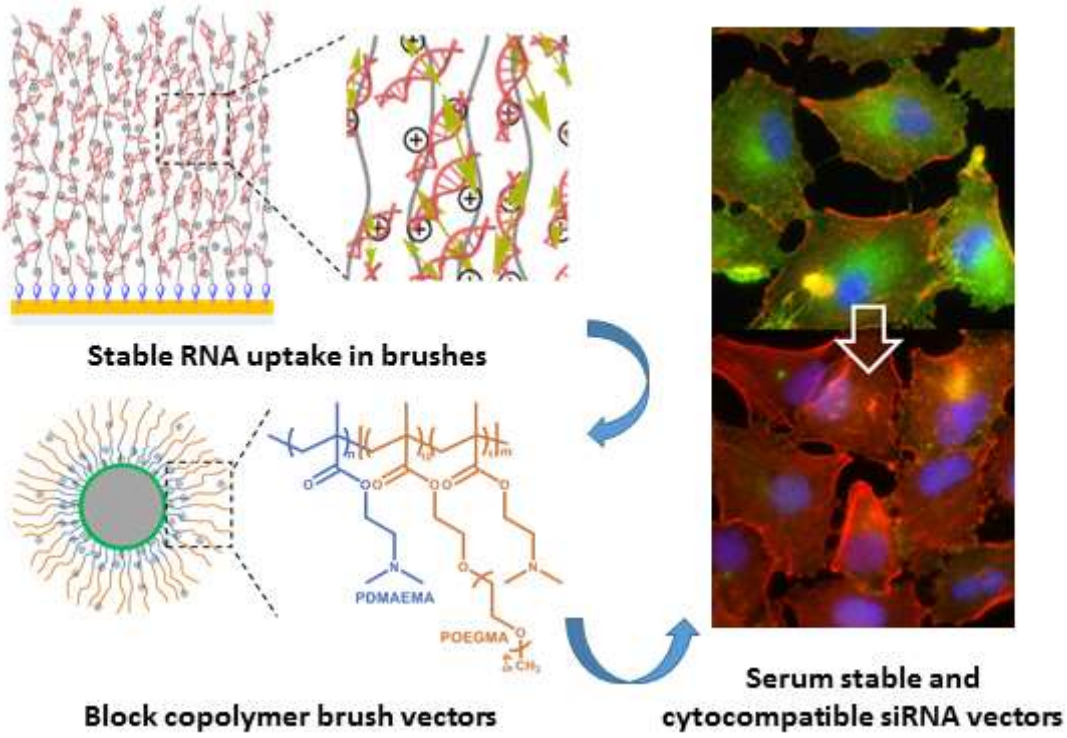
ACKNOWLEDGMENTS:

Danyang Li thanks the China Scholarship Council (No. 201406240022) for funding.

Picture 1:



Caption 1: Design of dense cationic polymer brushes vector for Cytocompatible, Serum-Stable siRNA Delivery.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

203 Electrospun polyvinyl alcohol - cyclodextrin nanofibers for biomedical applications

Marco Lopez, Dyhia Kersani, Justine Mougin, Nicolas Tabary, Frédéric Cazaux, Stéphanie Degoutin, Benoit Hue, Ludovic Janus, Mickaël Maton, Feng Chai, Jonathan Sobocinski, Nicolas Blanchemain, Bernard Martel

University of Lille, Lille, France

INTRODUCTION:

Electrospinning (ES) has gained importance for biomedical applications[1]. Thanks to their high surface-to-volume ratio and their open and interconnected porosity, electrospun nanofibers (ENFs) can be applied as scaffolds and/or delivery systems for drug, growth factors and cells. ES can be used with a large range of organic and inorganic polymers such as polyvinyl alcohol (PVA), a water-soluble polymer. Cyclodextrins can be used for host-guest interactions and hence, help to fine tune the release of loaded drugs. This work aims to study the feasibility and effect of different PVA-cyclodextrin ENFs as drug delivery systems.

METHODS:

ES solutions consisted of PVA (8%wt), citric acid (CA, 0.8%wt) and *hydroxypropyl* betacyclodextrin (HPB, 0 to 3.0 wt%). The dynamic viscosity and conductivity of the PVA-HPB solutions was preliminary measured. After ES, NFs were thermal treated (TT; 160°C, 1 h). NFs degradation was followed in PBS (pH 7.4) at 80 rpm up to 21 days. The morphology of NFs was observed with SEM, the weight was followed and chemical changes were monitored by Fourier Transform Infrared (FTIR) Spectroscopy. Methylene Blue (MB) was used as drug model for loading and release capacity of the NFs. BM release was studied under dynamic conditions (USP IV, SOTAX) coupled with a UV spectrometer.

RESULTS AND DISCUSSION:

The viscosity and conductivity values were around 0.6 Pa·s and 0.7 mS, respectively. No significant differences on the fiber diameter after ES and TT were found for the different HPB amounts. No degradation, *i.e.* change in weight, was observed in PBS up to 21 days of immersion. FTIR analysis indicated some differences on the chemical reaction bands for ENFs at the different time points as well as a change on the C-H wagging[2]. After 1 day of immersion a reduction on intensity of the HPB bands was observed. HPB and CA had a speed and amount tuning release effect of MB[3].

CONCLUSION:

We obtained ENFs with an enhanced effect of HPB on MB loading and release. Hence, PVA-HPB ENFs could be applied on supports, such as stents or implants, for cardiovascular and bone regeneration applications with tuned drug loading and release capacities.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

217 High-content screening identifies pro-survival microRNAs

Hugo Fernandes[1], Alessandra Zonnari[2], Sezin Aday[2], Cátia Seabra[2], Tânia Barata[2], Ermelindo Leal[2], Ricardo Abreu[3], Eugénia Carvalho[2], Lino Ferreira[4]

[1]University Coimbra, Cantanhede, Portugal

[2]University Coimbra; Center Neuroscience and Cell Biology, Portugal

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INTRODUCTION:

Cell-based therapies have shown promising results in several clinical trials but the poor survival and/or engraftment of the transplanted cells hinder their clinical efficacy. To improve their survival and engraftment, several strategies have been used but, given the multifactorial nature of cell death, single target approaches are unnecessarily restrictive and thus multi-target approaches are needed.

METHODS:

uman CD34+-derived endothelial cells (ECs) were transfected with a library of 2080 miRNAs mimics and high-content screening assays were used to identify miRNAs capable of enhancing their survival in ischemic conditions (0.1% O2 and growth factor depleted medium during 48h). Upon hit identification, secondary assays were used to validate the hits and two miRNAs were selected for extensive in vitro and in vivo testing. To that end, in vitro angiogenic assays (tube formation, migration, and proliferation) and an in vivo wound healing model in STZ-induced diabetic mice were used to validate the effect of the selected miRNA on the survival of human ECs upon transplantation. Furthermore, western blot, 3´UTR luciferase-based assays and RNA-Sequencing analysis were employed to identify and validate the putative targets of the selected miRNA.

RESULTS AND DISCUSSION:

Twenty-five miRNAs were identified on the primary screen and, using secondary assays, fifteen were confirmed as pro-survival miRNAs (7 previously identified and 8 new pro-survival miRNAs). Besides improving survival in ischemic conditions, the selected miRNAs did not affect the tube forming potential of human ECs and, notably, enhanced their migration. Mechanistically, in-silico target prediction and RNA-Seq analysis showed that, upon transfection of human ECs with the selected miRNAs, classical pro-survival signalling pathways were modulated ultimately contributing to the enhanced survival of the transplanted cells in vivo.

CONCLUSION:

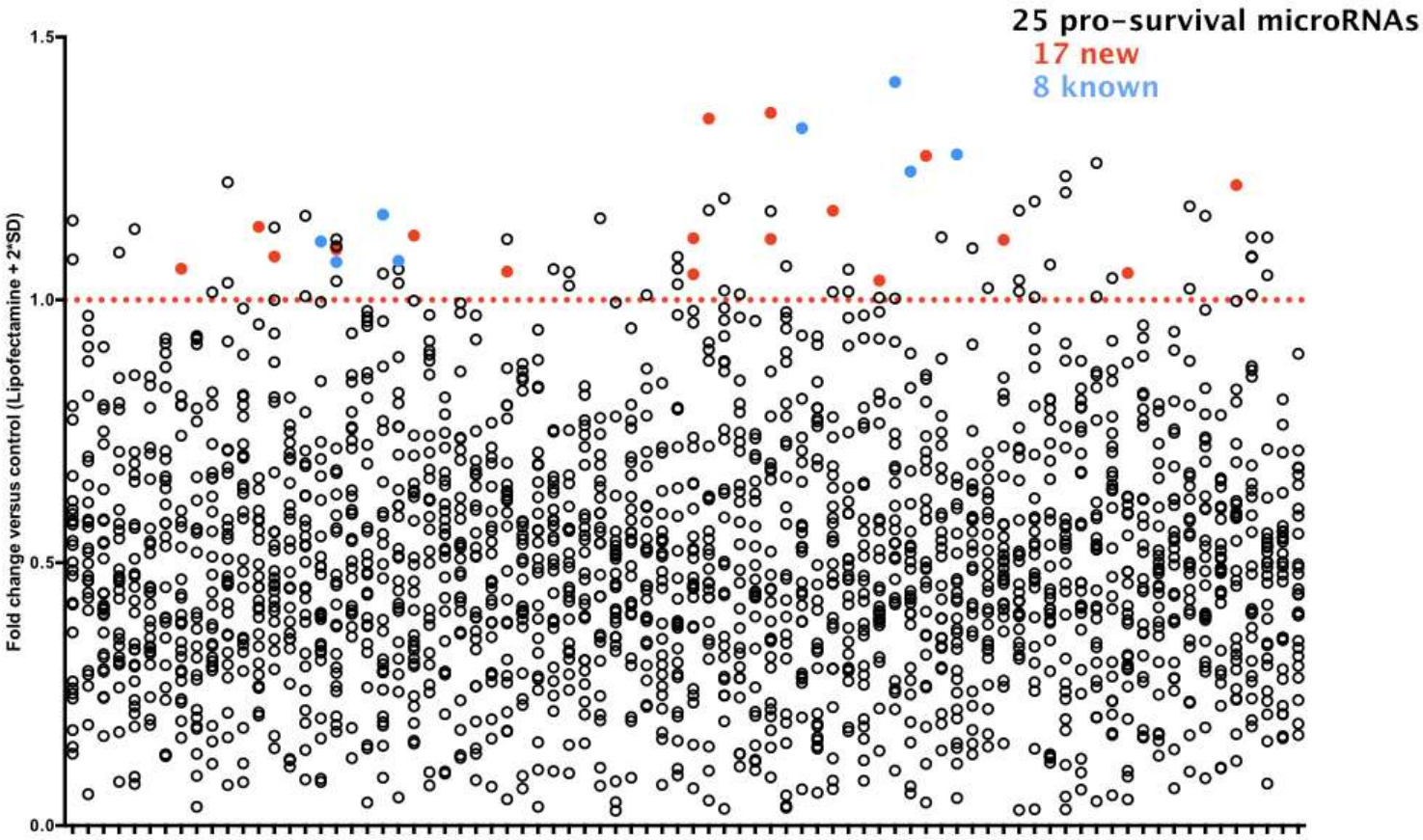
Using this strategy, we identified novel pro-survival miRNAs and showed that they are capable of enhancing cell survival. In vivo, we showed that transplantation of ECs transfected with one of the novel pro-survival miRNA into a diabetic wound healing mouse model improved wound closure compared with scramble-transfected cells.

These results pave the way for the use of miRNA as novel tools in the field of cell-based therapy.

ACKNOWLEDGMENTS:

This work was supported by funds from FEDER through COMPETE program and PTDC/BIM-MED/1118/2012 and MITP-TB/ECE/0013/2013 as well as the European project ERAatUC (ref. 669088).

Picture 1:
Caption 1: High-content screening identifies microRNAs enhancing survival miRNAs



Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

218 Bioactive glass nanoparticles: Optimization of the synthesis protocol in order to increase the Ca content

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[2]ICCF, France

INTRODUCTION:

Bioactive glass is a promising material for bone tissue repair thanks to its ability to dissolve and precipitate into apatite when immerged in simulated body fluid **[1]**. The mineralization process is accelerated when the quantity of calcium ions inside the silica network is high and with a large specific surface area. SiO_2-CaO bioactive glass nanoparticles obtained by sol-gel chemistry are potential candidates for this purpose. In this study, we synthesized particles with different size and calcium content to unravel their impact on the particle's bioactivity.

METHODS:

In a first step, TEOS was hydrolyzed in an alkaline hydro-alcoholic solution and in a second step, calcium nitrate was added under mixing. The timing of Ca precursor addition (H) and the total reaction time were varied. Also, initial ratios of Ca:Si have been used. After washing, silica nanoparticles present calcium ions adsorbed on their surface. The bioactive nanoparticles are finally obtained after a calcination step which allow the Ca ions to diffuse into the silica network.

RESULTS AND DISCUSSION:

he Ca content measured by EDS in the obtained nanoparticles decreases when the addition timing of $Ca(NO3)_3$ is delayed. At H=0.5h and 1h, almost all the calcium in the media has been incorporated. Nevertheless, the particles are aggregated. At H=3h, the particles are spherical, monodispersed in size and well separated. However, the Ca quantity inserted is reduced. Different initial Ca:Si molar ratios have been tested. The best result in terms of particles shape and dispersity and calcium content was obtained for a ratio of 2:1.

For reaction times smaller than 3h, the particles are not fully formed and present a highly reactive surface which led to their agglomeration after calcination. This high specific surface area is also the reason why more calcium was inserted.

CONCLUSION:

Monodispersed bioactive glass nanoparticles were obtained when the Ca salt was added 3h after the beginning of the reaction. It is possible to insert more calcium by tailoring the Ca:Si molar ratio. The quantity of calcium inserted was maximal for an initial ratio of 2:1. Beyond this value, Ca insertion does not increase more. Also, particles of 55-250nm were obtained by varying the base concentration during the synthesis.

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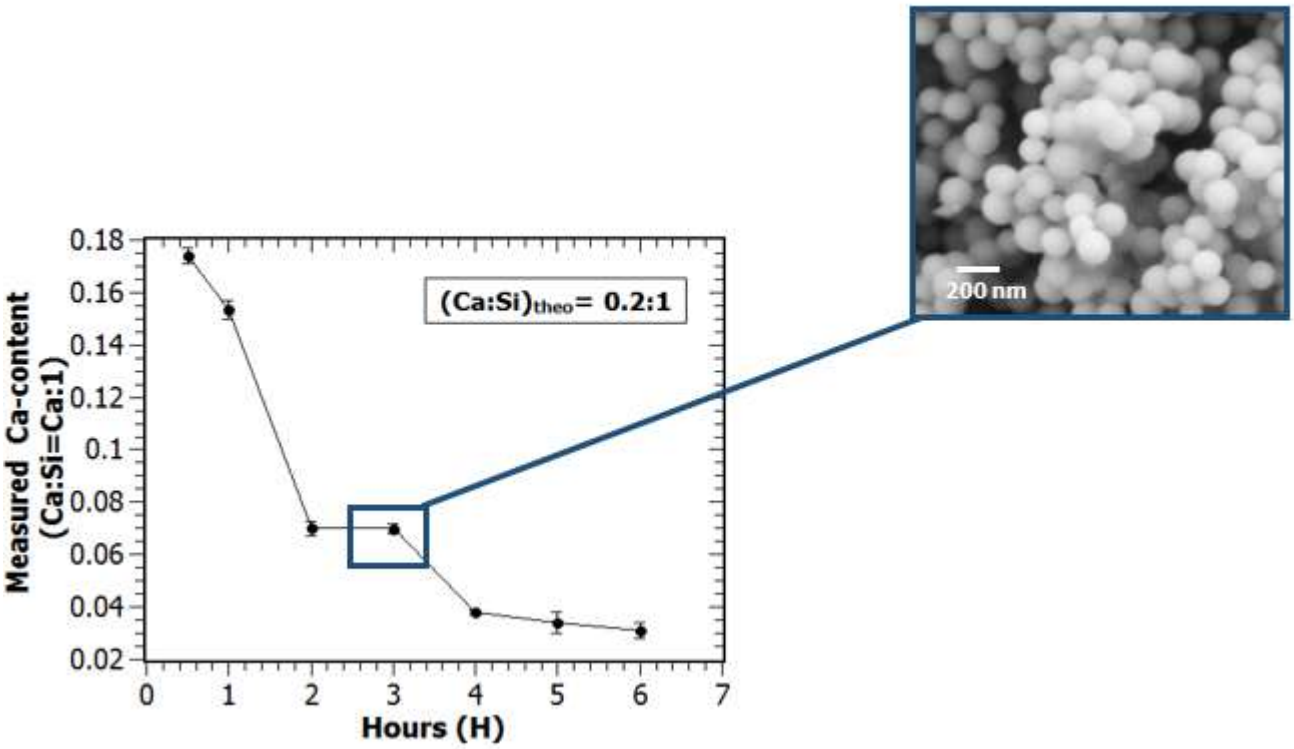
CKNOWLEDGMENTS:

Conseil Régional Auvergne Rhone Alpes is gratefully acknowledged for funding under project "Nanoparticules bioactives et magnétiques pour le traitement de cancer et la régénération de tissus osseux "



Picture 1: Caption 1: Evolution of the quantity of calcium inserted vs the timing of precursor addition (H) and SEM photograph of particles (H=3h)

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

243 Nanofabricated bone replica as a 3D model to study bone regeneration

Víctor P Galván[1], David Barata[1], Jiaping Li[1], Bernhard Hesse[2], Marc Bohner[3], Pamela Habibovic[1]

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INTRODUCTION:

Currently a few million bone graft procedures are performed worldwide each year[1], as a treatment of bone defects caused by trauma, tumor removal or spinal fusion. Synthetic biomaterials, such as calcium phosphates (CaP), are widely applied for repair and regeneration of diseased bone, however, their performance is generally still considered inferior to that of autologous bone. In an attempt to design new, improved bone graft substitutes, in this work, we propose a model to understand how individual properties of bone (i.e. chemical and structural properties) affect its performance in bone repair and regeneration[2], looking towards miniaturization and increased throughput of the bioactivity assesment.

METHODS:

Starting from phase-contrast nano-computed tomography (nanoCT) scans of natural trabecular bone, a 3D model was developed and optimized to allow additive manufacturing. Using a direct-writing nanofabrication method, replicas in polymer of the bone structure were produced. In order to provide the replicas with surface chemistry similar to that of bone, they were coated with CaP using various methods (ultrasound , biomimetic, enzymatic). Scanning electron microscopy (SEM), optical profilometry and microCT images of the replicas were taken to assess the reliability of the reproduction in polymer (Fig. 1).

RESULTS AND DISCUSSION:

A 3D model of the structure of natural bone was optimized for additive manufacturing (Figure, left). The SEM, profilometry (Figure, center) and microCT (Figure, right) images of the polymeric replicas showed the reliability of the reproduction of the bone structural features in polymer and how the different coating techniques modified the surface properties. Further optimization of the coating technique will enhance the potential of this method for screening on fundamental aspects of osteogenesis and bone repair.

CONCLUSION:

In this work we successfully replicated the structural features of bone, characterized using nanoCT, in a polymer. The coating methods allowed to introduce CaP on the surface of replicas, without significantly changing the surface structural properties. The resulting material could potentially be used as a model to mimic the microenvironment of bone and to study how individual material properties influence bone regeneration.

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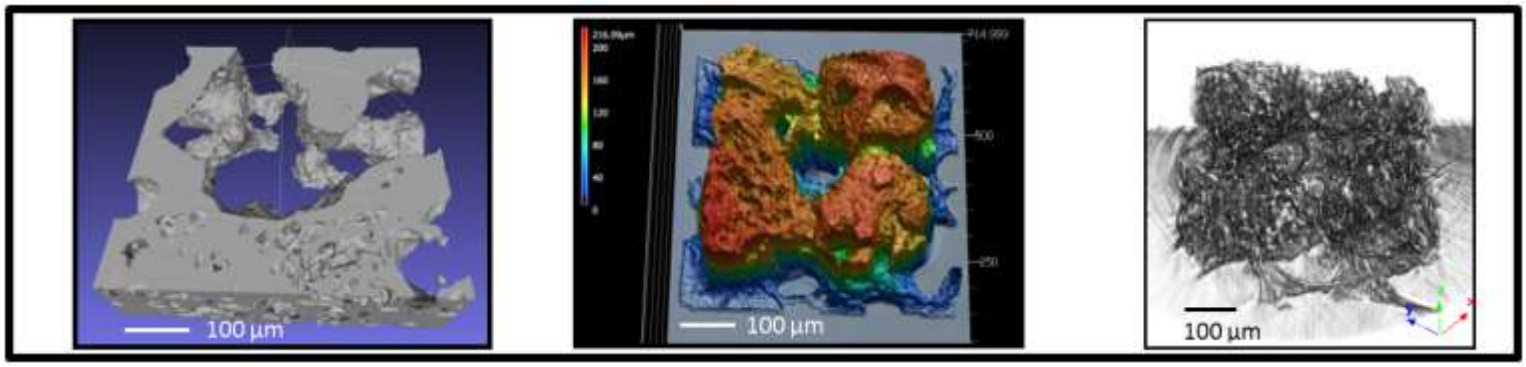
ACKNOWLEDGMENTS:

This research has been in part made possible with the support of the Dutch Province of Limburg.



Picture 1: Caption 1: Replica of the bone structure in polymer. Left) 3D model used to reproduce the structure. Center) Profilometry image. Right) MicroCT reconstruction.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

258 Multifunctional hyaluronan/collagen hydrogels for improving the regeneration of vascularized tissues

Sandra Rother[1], Vanessa D. Galiazzo[1], Karen M. Fiebig[1], Ute Hempel[1], Stephanie Moeller[2], Matthias Schnabelrauch[2], Dieter Scharnweber[1], Vera Hintze[1]

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INTRODUCTION:

The demographic development accounts for an increasing number of multi-morbid patients with reduced healing capacities. Hyaluronan (HA)-based hydrogels are favorable biomaterial systems because they are biodegradable and non-immunogenic. Furthermore, their biofunctionality can be modulated by adding further extracellular matrix components like collagen and sulfated glycosaminoglycans (sGAGs). In particular, sulfated HA (sHA) is reported to control the bioactivity of growth factors as well as to stimulate the proliferation of cells relevant to wound healing such as fibroblasts[1]. This study aimed to develop and characterize bioinspired HA/collagen-hydrogels as cellular 3D environments as well as scavenger or carrier systems for pro-inflammatory cytokines or mediator proteins such as angiogenic growth factors.

METHODS:

HA and sGAG acrylates were photocrosslinked[2] in the presence of collagen fibrils followed by freeze-drying to obtain porous scaffolds. Their composition and stability were biochemically analyzed and their structural and mechanical properties were investigated via scanning electron microscopy and compression tests. Lysozyme and vascular endothelial growth factor-A (VEGF-A) were used to characterize their protein interaction profiles. The biological effects of hydrogel composition and GAG-bound VEGF-A were analyzed *in vitro* using endothelial cells. The metabolic activity, cell proliferation as well as functional endothelial cell morphology were examined by biochemical methods and immunofluorescence staining.

RESULTS AND DISCUSSION:

Morphological analysis reveals a homogeneous porous structure of the scaffolds with embedded collagen fibrils after freeze-drying. Biochemical studies confirmed the incorporation of all components during the crosslinking process. In addition, the gels are completely degradable via hyaluronidase. The presence of collagen fibrils leads to an increased enzymatic stability compared to pure HA gels, while it reduces the elastic modulus of the gels. Gels containing sGAG derivatives show a sustained release of lysozyme and VEGF-A. This indicates that sGAGs are able to control the interaction with mediator proteins within the gels. In addition, all sHA-containing gels strongly stimulate the endothelial cell proliferation compared to gels with chondroitin sulfate (CS) or HA alone even in the absence of hydrogel-bound VEGF-A[3].

CONCLUSION:

HA/collagen hydrogels with sGAGs as biomimetic materials are able to bind and release angiogenic factors and to directly enhance the endothelial cell proliferation *in vitro*, which might translate into an improved healing of injured vascularized tissues.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

266 Cryogel scaffolds as a delivery device for creating a focally demyelinated ex-vivo Multiple Sclerosis brain slice model

Dimitri Eigel[1], Sowmya Sekizar[2], Lida Zoupi[2], Petra B. Welzel[3], Carsten Werner[3], Ben Newland[4]

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[2]MRC Centre for Regenerative Medicine, The University of Edinburgh, United Kingdom

[3]Leibniz Institute of Polymer Research Dresden, Germany

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INTRODUCTION:

Multiple sclerosis (MS) is a chronic neuroimmunological induced disease of the central nervous system (CNS), which is characterized by focal areas of demyelination, axonal damage and atrophy of the white and grey matter [1,2]. After demyelination, a response mechanism is triggered to repair/remyelinate the damaged axons, which isn't very efficient, poorly understood and eventually always fails [1,2]. In order to understand and improve this repair process, a combination model of demyelination and remyelination has been investigated [1,2]. Current models typically use lysophosphatidylcholine (LPC) to induce global demyelination in ex-vivo cultures. This does not represent the patchy nature of the pathology in vivo.

The aim of this study was to develop small macroporous cryogel scaffolds [3], which are capable of acting as a controlled delivery device for LPC, in order to induce focal areas of demyelination within an otherwise healthy tissue slice of mouse brain.

METHODS:

Cylindrical cryogels were synthesized in polystyrene templates of 0.5 mm to 2 mm diameter (Figure 1) via photo-crosslinking of a poly(ethylene glycol) diacrylate (PEGDA) precursor solution with 2-hydroxy-2-methylpropiophenone as photoinitiator at -20 °C. The capability of these cryogel materials to induce focal demyelination was tested by soaking them LPC solution (5 mg/mL to 20 mg/mL) for 5 min. Afterwards, the cryogels were placed next to the mouse brain slides and the demyelination progress was analysed by fluorescence microscopy using specific cell and tissue stainings.

RESULTS AND DISCUSSION:

Macroporous PEGDA based cryogel scaffolds with three different diameters and with high surface to volume ratios were successfully developed (Figure 1).

The cryogels could be loaded with LPC, and, after placing in contact with mouse brain slices, caused focal demyelination only in close proximity to the scaffold (Figure 1). The contralateral side of the brain slices (no cryogel) was myelinated as normal.

CONCLUSION:

PEGDA based cryogel sponges allow loading and subsequent release of LPC to mouse brain slices. Thereby the process of demyelination and remyelination can be studied in a context that more closely represents the in vivo situation than the previous global demyelination models. Ongoing studies are analysing the use of these scaffolds with sections of spinal cord, to represent patchy demyelination in other areas of the CNS

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Picture 1:

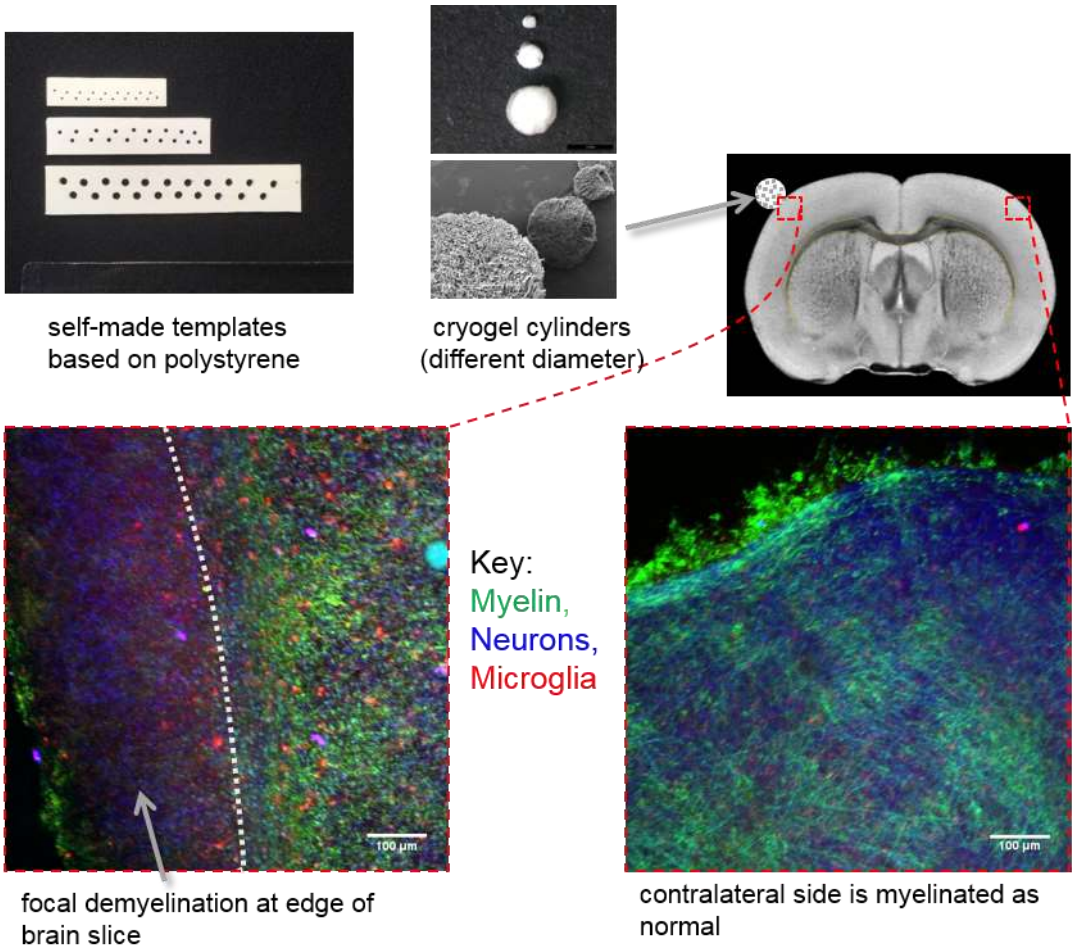

Figure.1 - SEM and digital images of the cylindrical cryogels and fluorescent microscopy images of neurons (neurofliament staining - blue,), myelin (myelin basic protein – green) and microglia (Iba1 staining - red) after applying LPC loaded cryogel material to brain slices

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Poster presentation

276 Multifunctional mesoporous silica nanoparticles as new building blocks for biomaterial design

Sabine van Rijt, Pichaporn Sutthavas, Pamela Habibovic

Maastricht University, Maastricht, Netherlands

INTRODUCTION:

Mesoporous silica nanoparticles (MSNs) are particles in the size range of 50-200 nm with a uniform porous structure. MSNs can be easily functionalized throughout their structure. This feature combined with their high surface area allows efficient crosslinking of various types of organic molecules such as hydrophilic polymers. Although relatively new in the field of regenerative medicine, studies have shown that the incorporation of MSNs within

biomaterials can result in improved biological and material properties. For example, even small loading ratios of MSNs within polymers significantly increased the mechanical properties compared to the polymers alone.[1, 2] The addition of MSNs to polymers also enhanced initial cell adhesion, cell viability, cell spreading, and metabolic activity of stem cells. [3-5] In this project, we aim to use MSN surface chemistry to create new types of hybrid inorganic-organic biomaterials for tissue regeneration.

METHODS:

Surface and core modified MSNs (amines on the surface and thiols in the core) were synthesized through a multistep, delayed co-condensation method.[6] Polymer-surface coupling was possible through the formation of thiol bridges and NHS coupling strategies. The (functionalized) particles were characterized by dynamic light scattering, Fourier transform infra-red spectroscopy, thermo-gravimetric analysis, and scanning and transmission electron microscopy.

RESULTS AND DISCUSSION:

Core and surface functionalized MSNs could be successfully synthesized with colloidal stability and in sizes ranging from 100 to 200 nm. Further surface modifications with hydrophilic polymers using thiol and amine-NHS coupling techniques could be achieved in high yield. All surface modified MSNs were stable homogeneous colloidal suspensions (pdi <0,3). In neutral to basic conditions the functionalized particles could self-assemble into hydrogel-like materials which highly depended on weight % of MSNs present in the mixture.

CONCLUSION:

Polymer functionalized MSNs form stable colloidal suspensions and can be used as precursors for the formation of new types of hybrid inorganic-organic materials for tissue regeneration

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ACKNOWLEDGMENTS:

We would like to thank the province of Limburg (LINK) for providing financial support to this project.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

280 Antibacterial modified PHA based biomedical materials

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INTRODUCTION:

Currently, one of the biggest challenges of biomaterials used in the medical field is the prevention of bacterial adhesion and consequent biofilm formation on medical devices. Hence, the importance of research on combating biofilms has increased[1]. The modification of biomaterials using natural bactericidal substances has been considered as a promising strategy to mitigate the above problem[2]. In this study, the combination of polyhydroxyalkanoates and innovative bioactive agents was explored for the development of antibacterial and antibiofilm PHA based coatings and medical devices[3].

METHODS:

Polyhydroxyalkanoates were produced by bacterial fermentation. Polymer sheets and meshes were made by solvent casting technique and electrospinning, respectively. The scaffolds were loaded with a set of bioactive agents including a small molecule, macromolecules and enzymes, independently. The physical and chemical properties of the materials were evaluated. The antibacterial activity was checked against some of the most frequent medical device colonizing bacteria. The cytocompatibility of the materials were tested against L929 murine fibroblasts.

RESULTS AND DISCUSSION:

Different antibacterial performances were shown for the selected bioactive agents. The small molecule exerted a bactericidal effect and showed antibiofilm properties against the selected bacteria. The macromolecules also showed a bactericidal effect against bacteria and the enzyme showed not only a preventive effect on biofilms but also a curative effect on pre-established biofilms without interfering with the bacterial growth. A slight modification to the physical properties of the materials was observed in the presence of the agents. Both the macromolecules and the enzyme showed excellent cytocompatibility against L929 cell line, whereas the small molecule exhibited a smaller cytocompatibility window.

CONCLUSION:

Antibacterial PHA based materials have been proven to be promising candidates for the development of antibacterial medical devices which would also target bacterial biofilms, a step change in the current antibacterial material technology. The synergistic antimicrobial and antibiofilm activity of the bioactive agents will be evaluated in the future to incorporate a dual effect in the materials developed: Prevention of bacterial growth allied with the prevention and destruction of pre-established biofilms.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

281 GELATIN-GRAPHENE OXIDE AEROGELS synthesized BY MICROWAVE-ASSISTED REACTION

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University of Concepcion, Concepcion, Chile

INTRODUCTION:

Graphene and its oxidized forms (GO) are materials that are currently used in the biomedical field because they have biological, chemical, physical, mechanical, thermal properties [1], that support to use it as a loading platform to load small molecules such as antibodies, DNA, genes and proteins. An application of this material is its capacity to be functionalized with biocompatible polymers to develop aerogels. Within these polymers, is possible to form composites GO-gelatin (GO-G). The traditional processes of covalent synthesis of aerogels with gelatin take a long time. Besides it has been reported that the use of microwave in the synthesis of aerogels improves the functionalization [2]**.** In this work, aerogels were developed based on GO-G through a microwave-assisted reaction process, with the aim of compare the synthesis and the characteristics of these porous sponges in relation to the traditional process.

The synthesis of the GO-G aerogels was set up at different pH conditions and GO-G ratio. The formation of these aerogels was evaluated at different reaction times, analyzing the influence of these factors on the properties of the materials synthetized. The reaction temperature was controlled in all the experiments, since the gelatin can be denatured by temperature changes [3]. The obtained aerogels were characterized chemically and physically by Raman, FTIR, XRD, thermogravimetry, AFM and SEM and was also evaluated their mechanical properties by compression assays.

RESULTS AND DISCUSSION:

The properties of the aerogels obtained were compared with those obtained by the traditional synthesis. With the microwave use, the reaction time was reduced from 24 h (for the traditional synthesis) to 30 min, in cycles of 5 min each. The resulting aerogels were more porous and absorbed more water than the aerogels synthesized by the traditional method. Also, There was an influence of GO-G ratio and pH in the hardness and the absorption capacity of the sponges.

CONCLUSION:

The effectiveness of microwave use in the synthesis of GO-G aerogels was validated.

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ACKNOWLEDGMENTS:

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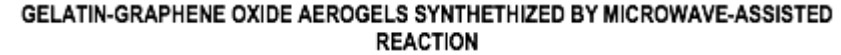
Picture 1:



Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

285 Development of a PHA-based stem cell patch for the treatment of heart failure

Qasim A. Majid[1], Gabor Foldes[1], Thusharika Kodagoda[1], Pooja Basnett[2], Christopher Grigsby[3], Molly M. Stevens[4], Ipsita Roy[2], Sian E. Harding[1]

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INTRODUCTION:

Myocardial infarction (MI) is a debilitating condition arising from occlusion of the coronary artery. The failure of current therapies to replenish the lost population of cells post-MI often leads to heart failure. Although cellular therapies are being investigated, they are plagued by poor cellular retention and cellular immaturity[1]. Therefore, biomaterial based approaches are being studied to address these concerns. Medium chain length Polyhydroxyalkanoates (MCL-PHAs) are a family of bioresorbable polyesters produced by a multitude of bacteria under nutrient limiting conditions, whilst in the presence of excessive carbon. Owing to their controllable surface degradation, resulting in the production of non-inflammatory, natural constituents of the human body, coupled to their high elastomeric nature, MCL-PHAs have great potential for cardiac tissue engineering[2]. Using melt electrospinning writing (MEW) we aim to generate innovative porous scaffolds with appropriately sized pores to accommodate stem cell-derived cardiomyocytes (CMs). Our key objectives are to obtain a scaffold with appropriate degradation kinetics to compliment the *in-vivo* timeline of regeneration whilst also retaining and structurally maturing the seeded CMs thus improving post-MI cardiac function.

METHODS:

Here 3 MCL-PHAs, P(3HO), PHA1 and PHA2 were produced via nitrogen limited batch fermentation of *Pseudomonas mendocina* CH50 whilst in the presence of varying carbon sources. Synthesised MCL-PHA granules were extracted using soxhlet extraction and subsequent characterisation to determine molecular weight (M_w), thermal and mechanical properties were completed via GPC, DSC and DMA respectively. Additionally, structural characterisation was completed via FT-IR, GC-MS and NMR. PHAs were electrospun under optimised thermal, pressure and voltage conditions to generate PHA-MEW scaffolds. Cellular investigation was completed using CMs produced via a small molecule differentiation protocol.

RESULTS AND DISCUSSION:

DSC analysis revealed the T_m values of the MCL-PHAs to be in the range of 45-50^{o}C, which rendered them highly suitable for MEW. PHA1 was most conducive to fabrication by MEW, although a significantly higher heating temperature was required to generate an electrified jet. This could be explained by PHA1's relatively high M_w resulting in a highly viscous melt at their T_m, however at temperatures greater than the T_m the apparent viscosity reduced, thereby allowing for the fabrication of reproducible scaffolds (Figure.1). Subsequent cellular investigations revealed good cell attachment with CMs displaying a propensity to span across pores.

CONCLUSION:

PHA-MEW scaffolds have great potential to generate cardiac patches with mature CMs, potentially allowing for cardiac regeneration.

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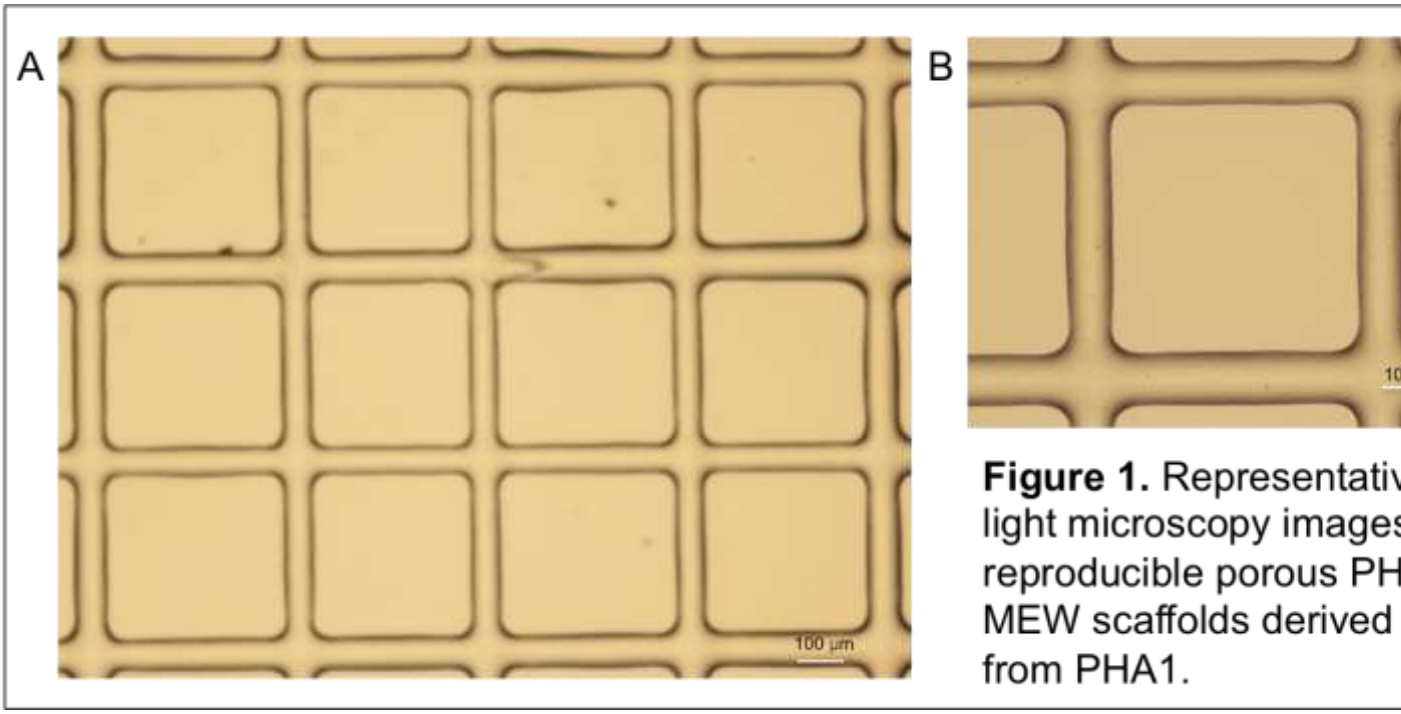

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

291 Design of poly e-caprolactone/zein blends for delivery of essential oils as antimicrobial biomaterials

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INTRODUCTION:

Essential oils are recognized as one of the most effective natural antimicrobial agents. Here, we report the development of antimicrobial biomaterials, using biodegradable poly ε-caprolactone (PCL) and natural corn protein – zein to deliver and control the release of selected essential oils. The concept of a combination of PCL and zein improves the overall biodegradability and mechanical properties for coating applications.

METHODS:

Essential oil (cinnamon bark oil, peppermint oil)-encapsulated-zein nanoparticles (NPs) were prepared by the liquid-liquid dispersion method. The blend films of PCL/zein and PCL/NPs (zein ≤ 30wt%) were prepared by the solvent - casting method.

The morphologies were characterized by Scanning Electron Microscope (SEM). The encapsulation efficiency and release were quantified by Gas Chromatography-Mass Spectrometry (GC-MS). The biological activity was tested by Halo tests and cytotoxicity assays. The films were further characterized by water-uptake, mass loss and tensile strength.

RESULTS AND DISCUSSION:

The porosity of the blend films was dependent on the choice of solvent for preparation. The encapsulation efficiencies were up to 60% and 100% for NPs and PCL/zein blend, respectively. The Halo test showed significant antimicrobial inhibitions of blends containing cinnamon bark oil (Figure 1). EO-encapsulated-PCL/zein exhibited fast release over the first 6h, resulting in a higher cytotoxicity and in potential applications. However, the PCL/NPs blends showed the prolonged release of essential oils.

Moreover, the addition of zein significantly increased the water-uptake and promoted the mass loss of the blend films over 14 days. The increase of zein in the blends resulted in a significant lower Young's modulus in PCL/zein blends, but the presence of EOs had only a minor influence.

CONCLUSION:

Zein nanoparticles improved the preservation of volatile essential oils in these blend systems. The antimicrobial effect of the films containing cinnamon bark oil was much higher than the ones containing peppermint oil. The physical properties can be controlled by varying the solvent and the content of zein. In summary, the concept blends, especially PCL/NPs, are promising candidates for the development of drug–free antimicrobial biomaterials.

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ACKNOWLEDGMENTS:

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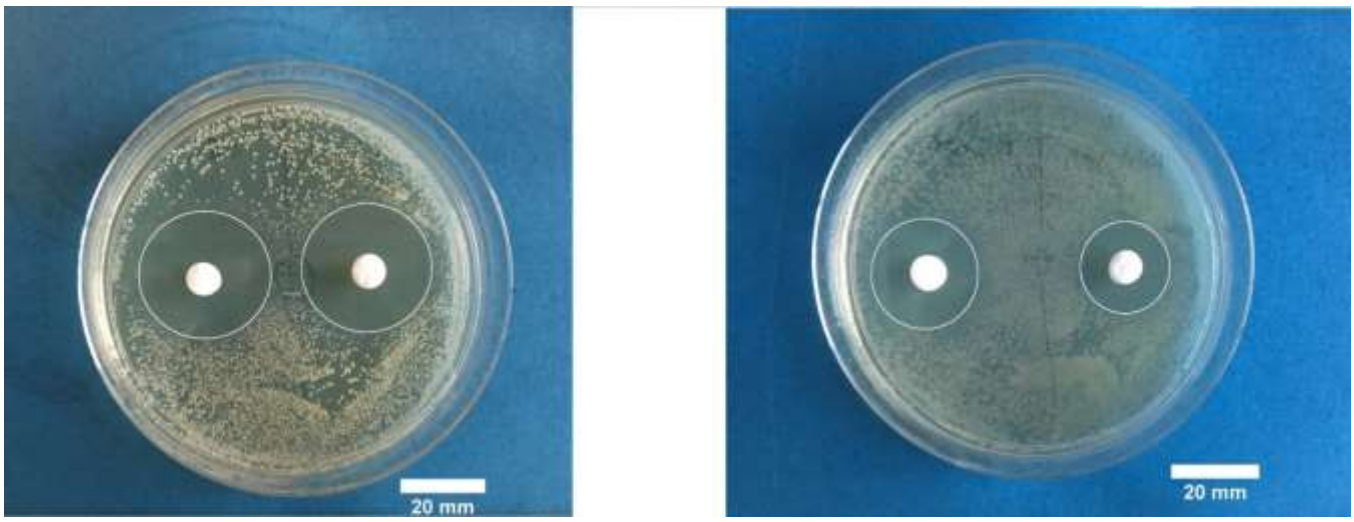

Picture 1: Caption 1: Figure 1: Halo images of PCL/zein blends containing 15 w/w% CO against (left) S. carnosus, (right) E.coli. Scale bar = 20mm.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

302 Assessment of the Biodegradable Synthetic Films Potential for Tissue Engineering

Sofia Ribeiro[1], Vit Novacek[1], Emanuel m. Fernandes[2], Manuela e Gomes[2], Rui l. Reis[2], Yves Bayon[1], Dimitrios i. Zeugolis[3], Yves Bayon[1]

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[2]3B's Research Group,ICVS, University of Minho, Portugal

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INTRODUCTION:

Degradable polymers have been studied and applied in biomedical application, such as tissue engineering applications.[1]

To repair damaged tissue it is vital to ensure that the biomaterial is able to mimic the complex elasticity of the native tissue. Huge efforts have been invested into the development and design of appropriate elastomeric biomaterials to match the tissue of choice.[2]

It has been demonstrated substrate stiffness has a huge influence on cellular growth, motility and phenotype maintenance.[3] The main goal of the present study is to characterize extensively a set of polymeric films with a wide range of mechanical properties.

METHODS:

A range of synthetic biodegradable polymers was selected according to the physico-chemical intrinsic properties of aliphatic polymers. They have similar chemistry (ie. absorbable polyesters made from lactic acid, glycolic acid, trimethylene carbonate, dioxanone & e-caprolactone), however show different mechanical and degradation properties. The films were manufactured by thermal presser and then characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), nuclear magnetic resonance spectroscopy (NMR) and Fourier transform infrared spectroscopy (FTIR). The mechanical properties of the films were assessed by uniaxial tensile tests in wet conditions and also by atomic force microscopy (AFM). *In vitro* assays were performed using human dermal fibroblasts (hDFs) to assess the cell cytocompatibility and proliferation of the films. Human adipose stem cells (hASCs) were cultured on polymeric films to access their differentiation potential using differentiation media. The differentiation ability was determined by histological and biochemical analysis.

RESULTS AND DISCUSSION:

It was possible to successfully produce polymeric films using a large range of polyesters using a thermal presser. The chemical properties of the films were characterized using appropriate methods. The mechanical properties of the materials are within the range intended for musculoskeletal tissue repair. Biological assays showed good cell adhesion, cell proliferation and cell viability. Stem cells were able to differentiate into adipogenic, osteogenic, chondrogenic and tenogenic lineages.

CONCLUSION:

Overall the selection of polymers gives us good options for a potential tissue repair scaffolds. In the future, the combined effect of stiffness and topography will be assessed on cell phenotype maintenance.

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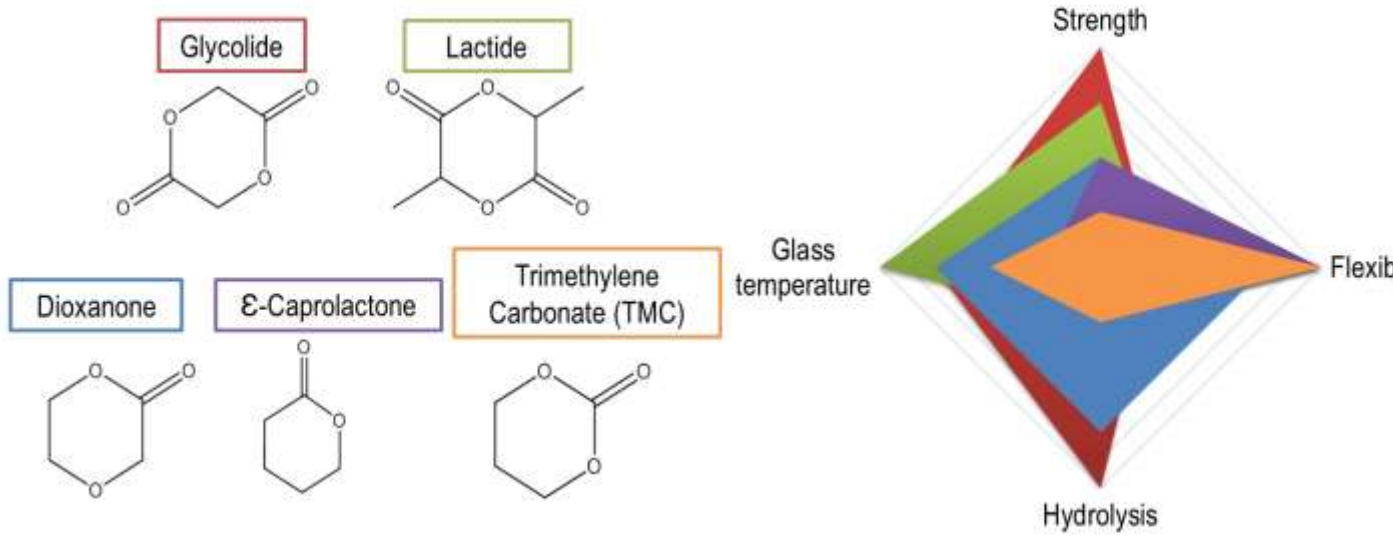
Picture 1:



Caption 1: Fig.1-Monomers selected (Glycolide, Lactide; Dioxanone, Ɛ-Caprolactone and Trimethylene Carbonate) and the representation of their intrinsic propertie

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

309 Reduction of specific surface area of amorphous calcium phosphate during gradual heat treatments

Jana Vecstaudza, Janis Locs

Riga Technical University, Riga, Latvia

INTRODUCTION:

High specific surface area (SSA) of biomaterials is linked to improved reactivity, solubility and adsorption of biologically relevant substances. Poorly crystalline calcium phosphate (CaP) present in bone has high SSA of 40-240 m^2/g[1,2]. Meanwhile commercial CaP grafts have rather low SSA (0.5-2 m^2/g)[3-5] and their performance could be improved by increasing SSA. Aim of the study was to describe reduction of SSA of amorphous calcium phosphate (ACP) during heat treatment alongside with a set of physicochemical properties and an *in vitro* assessment.

METHODS:

ACP nanopowder with initial SSA of 115 m^2/g was used[6]. ACP was heated in temperature range of 100 - 1000 °C with interval of 100 °C, and with two hold times (5 or 60 min) at each temperature. SSA, particle size d_{BET} and mesoporosity was analysed with BET technique. Additionally, FT-IR, XRD, SEM and TEM analysis were performed. For *in vitro* cytotoxicity tests with MG63 cells two types of scaffolds were used – one with higher (78±5 m^2/g) and other with lower SSA (28±2 m^2/g).

RESULTS AND DISCUSSION:

Obtained results showed gradual reduction of SSA of ACP starting around 200 °C and ending at 700 °C (see Fig.). Different heat treatment hold times had negligible effect on SSA. Several pathways for SSA reduction of ACP nanopowder were proposed: 1) coalescence of ACP particles during sintering; 2) decrease of the size of mesopores at 600-1000 °C (mesoporosity data not shown); 3) final reduction of SSA after crystallization is governed by further growth in size of the previously coalesced particles. ACP scaffolds with higher SSA were non-cytotoxic and demonstrated 2.7 times better cell viability *in vitro* than the crystalline ones with lower SSA.

CONCLUSION:

The decrease of SSA of ACP during heat treatment is gradual and allows to adjust SSA to desired value. Reduction of SSA of ACP powders proceeds through mixture of pathways: particle coalescence and decrease in mesoporosity. Further studies should be devoted to separate possible overlapping effects in *in vitro* experiments of SSA and differences in phase compositions.

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ACKNOWLEDGMENTS:

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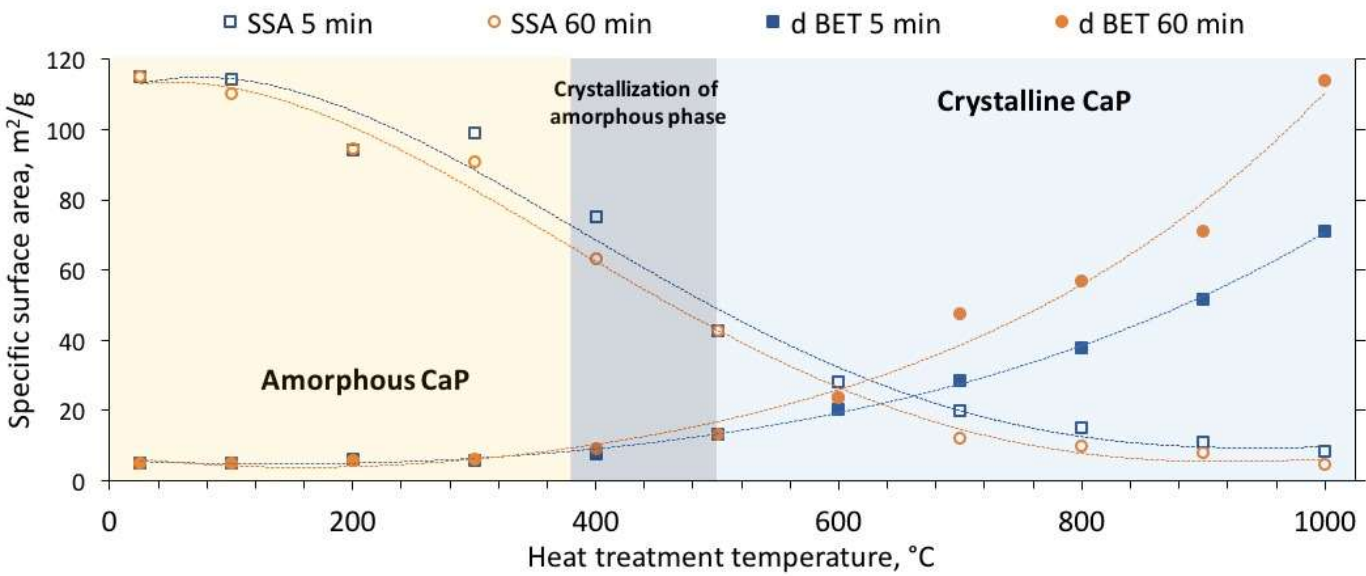
Picture 1:



Caption 1: Dependence of specific surface area and particle size dBET on heat treatment temperature and hold time

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Poster presentation

317 Submicron surface structure enhancing osteogenic differentiation of human bone marrow stromal cells

Huipin Yuan[1], Davide Barbieri[2], Florence de Groot[2], Joost d de Bruijn[2]

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[2]KUROS Bioscience BV, Netherlands

INTRODUCTION:

With their presences in bone and their possible roles in bone metabolism, trace ions are doped into synthetic materials to improve bone formation [1]. It was shown that silicon-containing bioceramics enhanced osteogenic differentiation of mesenchymal stem cells [2]. Next to the chemical modification of bioceramics, physical modification of surface structure improved bone forming ability of calcium phosphate ceramics[3,4]. The influence of a submicron surface structured CaP ceramic on the human bone marrow stromal cells was thus evaluated in the current study, as compared to silicon-containing hydroxyapatite.

METHODS:

CaP ceramic (namely NCS-CaP) with submicron scaled needle-crystal surface was fabricated in granule form (1-2mm) with a thermal treatment. Actifuse (1-2mm) was employed as a reference of Si-HA. Human bone marrow stromal cells were isolated from 3 biopsies (Lonza; D01: 18yr, male; D02: 23yr, female; D03: 46yr, male) and expanded. Cells ($3X10^5$ cells, 2nd or 3rd passage) was seeded onto 200ul ceramic granules and cultured in basic medium (aMEM+15%FBS+0.2M ASAP+1000ng bFGF/ml+pen/strep) at 37C, 5% CO_2. Meanwhile cells were

seeded onto glass coverslips and cultured with Dexamethasone (10^{-8}M, positive control) or without (negative control). At Day 14, the samples were harvested with 3 times PBS rinsing and subjected to biochemical assays for DNA, ALP and OCN.

RESULTS AND DISCUSSION:

Given the same number of cells seeded onto the materials, the DNA value showed the influence of Dex (PC vs NC) and materials on cell proliferation, with less cells at Day14 in positive control and Magnetos for all donors (Figure 1). The donor (most likely the age) affected slightly proliferation, but greatly the function of DEX in ALP production (as seen in ALP/DNA in positive control) and the function of materials in OCN production. Neither Actifuse nor NCS-CaP increased ALP production as compared to the negative control, while Osteocalcin production was significantly enhanced by both Actifuse and NCS-CaP, with NCS-CaP the greatest.

CONCLUSION:

Slower proliferation on NCS-CaP surface indicated that hBMSC underwent differentiation. As its specificity to osteoblasts, osteocalcin production confirmed the osteogenic differentiation of hBMSC on both Actifuse and NCS-CaP surface. Moreover, the higher osteocalcin production in the case of NCS-CaP than Actifuse demonstrated a superiority of submicron surface structured CaP ceramic to the silicon-doping one (Actifuse) in instructing osteogenic differentiation of hBMSCs.

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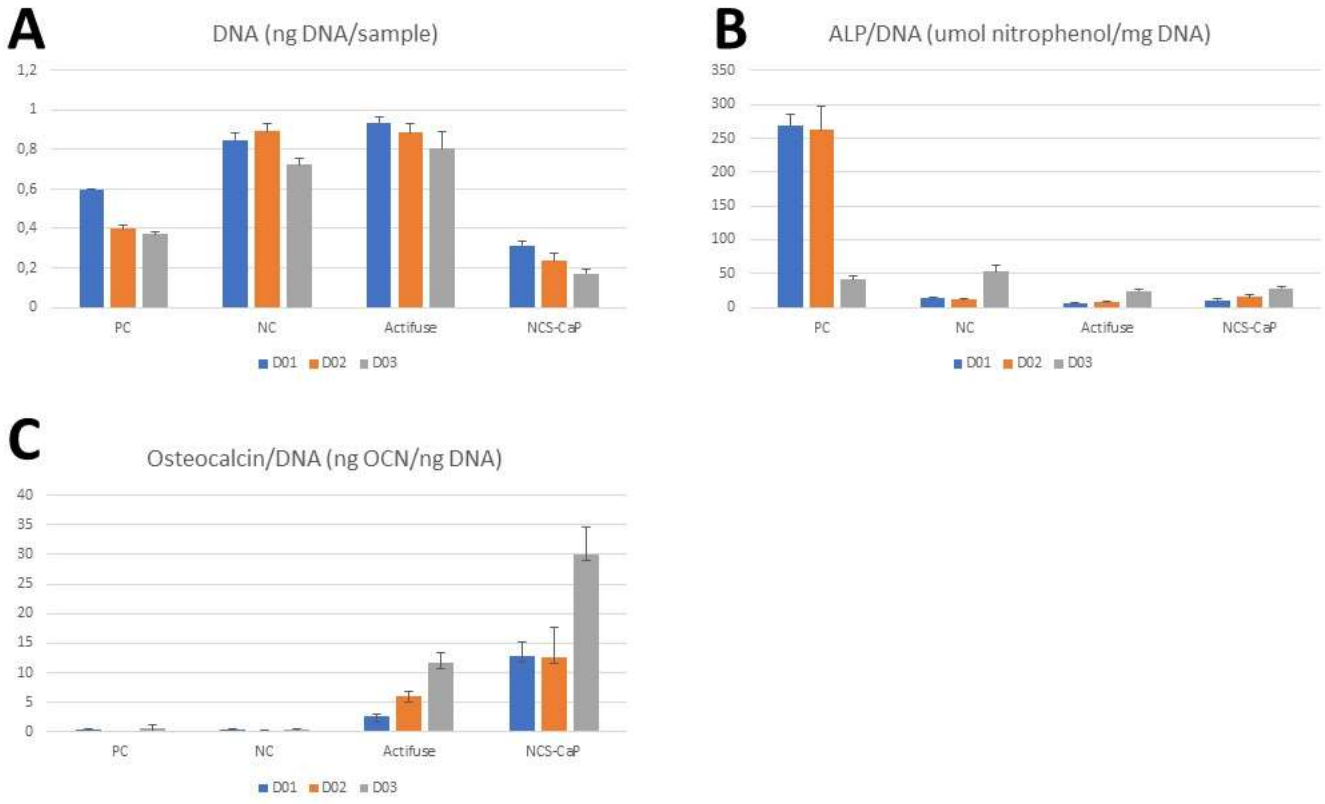
Picture 1:



Caption 1: DNA (A), ALP/DNA (B) and OCN (C) of hBMSCs at day 14

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Poster presentation

326 Characterization of collagen/lipid nanoparticle aerogels for wound healing applications

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[2]Department for Innovation in Biological, Agrifood and Forestry Systems (DIBAF),, Italy

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[5]University of Pisa, Italy

[6]DIBAF, Tuscia University, Italy

INTRODUCTION:

Collagen is a material of choice for wound contact in medical devices[1]. Curcumin has strong antioxidant, antiseptic, and anti-inflammatory properties, which recently aroused much interest for wound healing[2]. However, when directly loading curcumin into collagen hydrogels, drug aggregated and clogged matrix pores, an essential feature for efficient cell growth[3]. Therefore we designed an innovative aerogel comprising lipid nanoparticles (LNP) coated with a PEG shell. LNP can easily load curcumin and be transferred into the hydrophilic collagen matrix (Figure A). Thorough characterization of collagen/LNP aerogels is herein described.

METHODS:

Collagen I (equine Achilles tendon) was a gift of Euroresearch (Italy). LNP (50 or 120 nm diameter) were synthesized according to previous protocol[4]. Collagen/LNP aerogels were obtained by freeze-drying. FRET LNPs were used to assess particle stability and release. DSC studied material thermal stability. Aerogel structure was characterized by fluorescence microscopy, SEM, AFM, and TEM. NIH 3T3 cells were seeded on the materials to study their cytotoxicity and their potential to act as scaffolds for tissue reparation.

RESULTS AND DISCUSSION:

Collagen/LNP aerogels presented a fibrous structure with 100 µm average pore size, an ideal scaffold to promote cell growth and tissue reconstruction (Figure B)[3]. Nanoparticles were homogeneously distributed along the collagen fibers (Figure C). The ultrastructure of fibers, and particularly their typical band spacing, was maintained (Figure D). This was confirmed by DSC that showed no alteration in the thermal stability of collagen. About 30% to 50% of the nanoparticles were released in 24h when the materials were immersed in saline buffer, followed by a prolonged release to achieve 70 % to 100 % after 25 days. No material cytotoxicity was observed when in contact with NIH 3T3 fibroblasts. A good level of interaction and adhesion of cells with the scaffolds was evidenced as early as 4 h after cell seeding (Figure E).

CONCLUSION:

The positive features presented by the collagen/LNP aerogels make them promising materials for use in wound healing.

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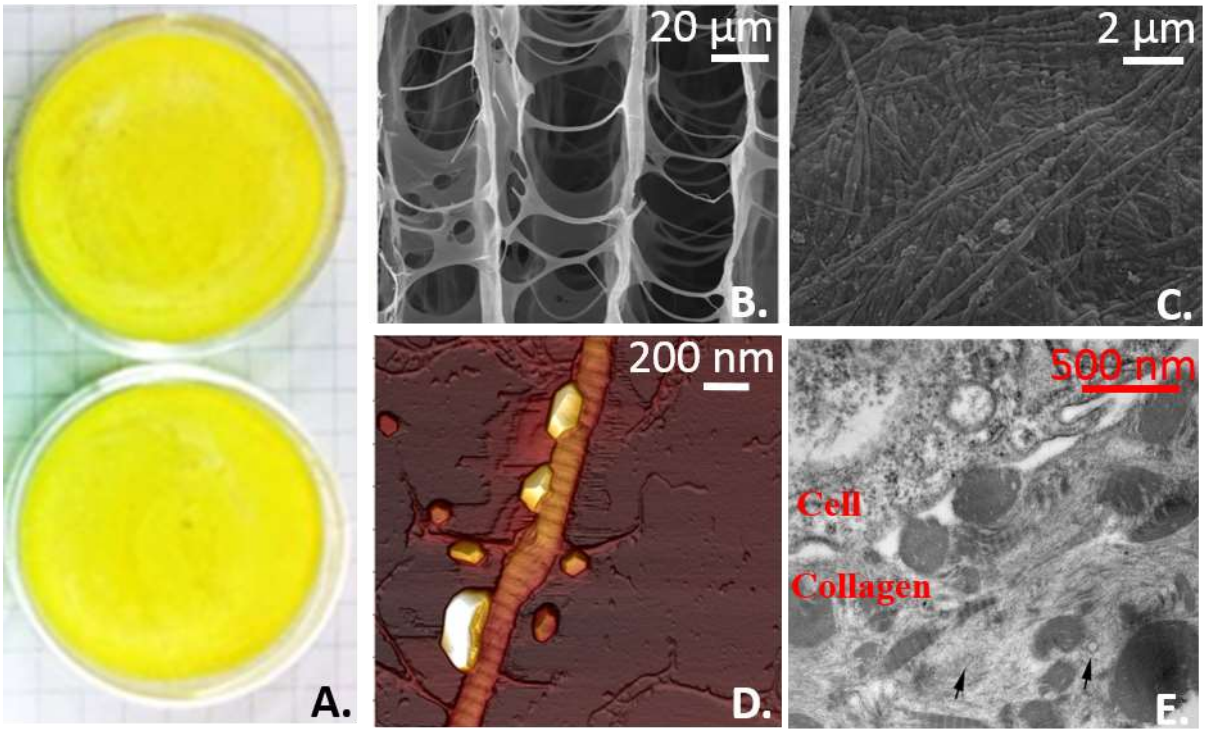
Picture 1:



Caption 1: Characterization of collagen/LNP (50 nm) aerogels (A) by SEM (B,C), AFM (D), and TEM when in interaction with NIH3T3 fibroblasts for 4 h (E).

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Poster presentation

330 Graded implants releasing two growth factors with spatio-temporal control

Henning Menzel[1], Dominik de Cassan[1], Steffen Sydow[1], Julius Sundermann[1], Heike Bunjes[1], Nadeschda Schmidt[2], Alexandra Satalov[2], Peter Behrens[2], Yvonne Roger[3], Laura Burmeister[3], Andrea Hoffmann[3], Anna Lena Hoheisel[2], Birgit Glasmacher[2], Robert Hänsch[1]

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[2]Leibniz University Hannover, Germany

[3]Medical School Hannover, Germany

INTRODUCTION:

Functionalization of implants with drug delivery systems that allow a spatio-temporal control of growth factor release is an approach towards in-situ tissue engineering. Such a tactic seems to be particularly interesting for implants placed in regions where different tissues merge, for example bone-tendon-transitions.

METHODS:

Polycaprolacton (PCL) fiber mats were prepared by electrospinning. Chitosan was grafted with PCL and the graft polymer was used to modify the PCL fiber mats.[1] Nanogels were prepared by mixing chitosan and tripolyphosphate (TPP) solutions.[2] Furthermore, nanoporous silica nanoparticles were used as drug delivery system. The nanoparticles were applied to the fiber mats by Layer-by-Layer dipping (LbL).

RESULTS AND DISCUSSION:

We have developed methods to modify the surface of electrospun PCL fiber mats via a simple dipping process using a chitosan-graft-caprolactone polymer. The modified fiber mats show improved cell adhesion *in vitro* and *in vivo* and have cationic charges, which can be used for functionalization with drug delivery systems.[1]

Nanoparticular hydrogels can be formed spontaneously from chitosan and tripolyphosphate[2] or alginate and poly-L-lysine, respectively. Proteins like BMP2 or TGF beta can be incorporated with very high efficiency. These nano-particular release systems have been characterized thoroughly and their release profiles were studied in detail. The dispersions of the nanoparticular hydrogels were used to functionalize the modified fiber mats by a LbL-process. The functionalized fiber mats release the growth factors with controlled rates and the factors are still biologically active. The functionalization can be carried out in a way that the different factors are installed in gradients on the fiber mats; thus having the BMP2-releasing hydrogels on the side directed towards the bone, while the TGF beta-releasing region is directed to the cartilage or tendon side.

CONCLUSION:

Modification and functionalization of PCL fiber mats can be carried out in an easy and scalable method using chitosan-g-PCL for surface modification and different nanoparticle suspensions for functionalization with drug delivery systems. In this way PCL fiber mats can be transferred into bioactive implants for in-situ tissue engineering applications.

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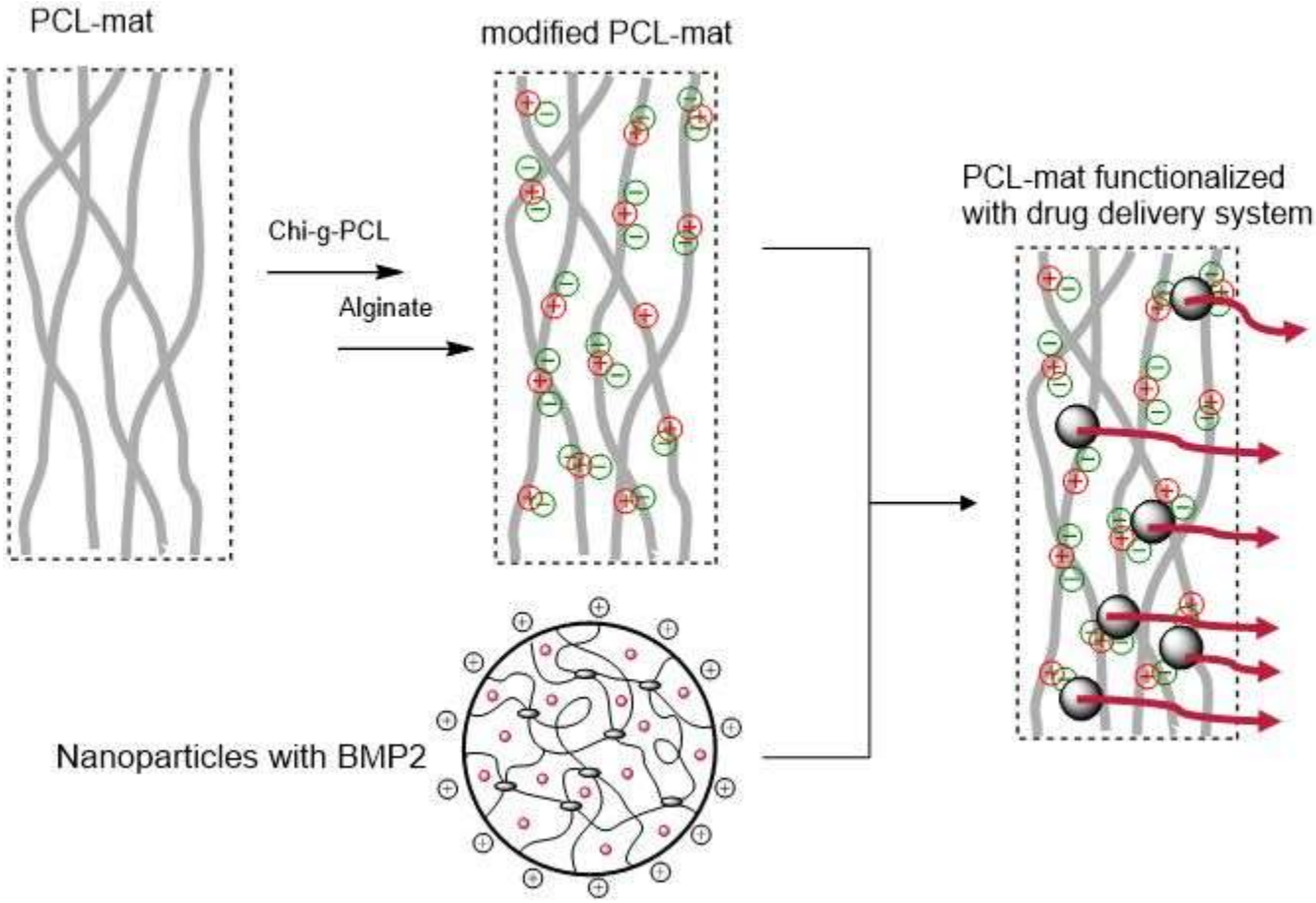
ACKNOWLEDGMENTS:

The research project has been supported by the DFG in the framework of the Research Unit 2180 “Graded Implants for Tendon-Bone Junctions”.

Picture 1:



Caption 1: Flow chart for the preparation of graded implants capable of spatio-temporally controlled release of growth factors

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

338 Collagen scaffolds functionalized with copper-eluting bioactive glass for the treatment of infection and regeneration of vascularized bone

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[2]University of Erlangen-Nuremberg, Germany

INTRODUCTION:

The bone infection osteomyelitis (typically *Staphylococcus aureus*) requires surgical debridement, long-term systemic high-dose antibiotics, and often bone grafting. With antibiotic resistance becoming increasingly concerning, alternative approaches are urgently needed. Herein, we develop a one-step treatment for osteomyelitis that combines local, controlled release of non-antibiotic antibacterials (copper) within a proven regenerative scaffold. To maximize efficacy we utilized bioactive glass, an established osteogenic material with immense capacity for bone

repair, as a copper ion delivery reservoir. Copper ions have been shown to be involved in collagen maturation, angiogenesis, and can induce MSC differentiation down an osteogenic lineage[1]. By delivering the copper locally, it can be carefully dosed to maximize antibacterial activity, while limiting mammalian cell death. To eliminate grafting requirements, the copper-doped BG was incorporated into our previously developed collagen scaffolds[2]. The resulting device is a multifunctional antibacterial, osteogenic, and angiogenic scaffold.

METHODS:

Scaffolds were fabricated by freeze-drying a co-suspension of collagen and bioactive glass particles (+/- copper doping, referred to as CuBG and BG, respectively) at a range of different concentrations (0-300% w/w bioactive glass to collagen). Scaffold mechanical and microarchitectural properties were characterized. The antibacterial activity of the scaffolds was assessed against *Staphylococcus aureus*. The scaffolds were then assessed for their ability to support osteogenesis and angiogenesis using PicoGreen®, calcium assay, alizarin red, Matrigel®, and VEGF ELISA (all ≥n=3).

RESULTS AND DISCUSSION:

CuBG scaffolds were successfully fabricated, demonstrating a 2.7-fold increase in compressive modulus (300% CuBG vs. 0%; $p<0.01$), whilst maintaining >98% porosity. The 300% CuBG scaffold reduced *Staphylococcus aureus* growth ($p<0.001$; Fig.1A). In terms of osteogenesis, 100% CuBG and 300% CuBG scaffolds increased cell-mediated calcium deposition on the scaffolds ($p<0.05$ and $p<0.001$; Fig.1B). Alizarin red staining confirmed the superior levels of well-distributed cell-mediated mineral deposition in comparison to the control (Fig.1C). 100% CuBG scaffolds enhanced angiogenesis by significantly increased tubule formation ($p<0.01$) and VEGF protein production ($p<0.001$; Fig.1D).

CONCLUSION:

Herein, we successfully produced CuBG scaffolds capable of antibacterial activity, without the use of antibiotic, which also stimulated osteogenesis and angiogenesis. CuBG scaffolds showed increased antibacterial activity, superior cell-mediated calcium production, and enhanced endothelial cell tubule formation and VEGF production. In summary, this single-stage, off-the-shelf treatment for osteomyelitis shows potential to eliminate the need for antibiotics and bone grafting, while reducing hospital stays and costs.

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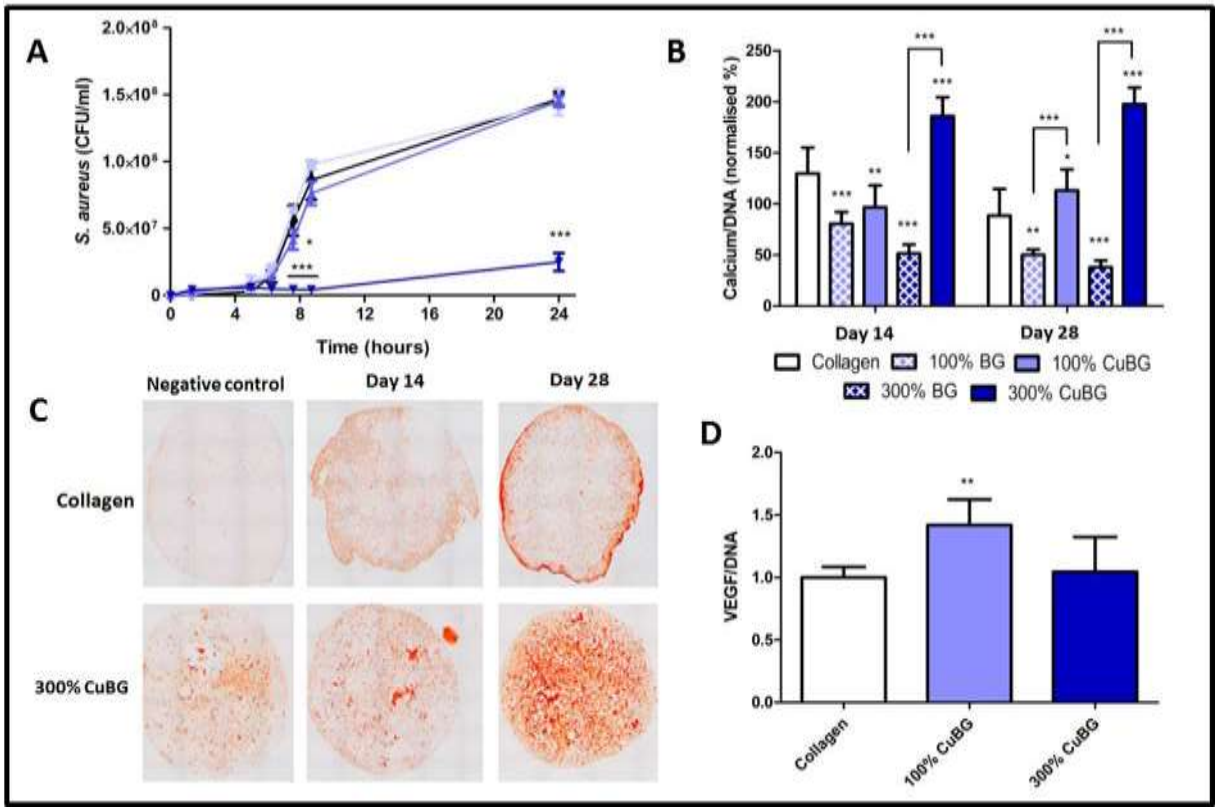
ACKNOWLEDGMENTS:

Research funded by Irish Research Council (GOIPG/2015/3044).

Picture 1:



Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

343 Enhanced cellular proliferation and metabolic activity of MC3T3-E1 cells on piezoelectric polymeric substrates/scaffolds

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INTRODUCTION:

Piezoelectric materials are attracting attention from the tissue engineering community [1]. This study demonstrates the efficacy of polyvinylidene fluoride (PVDF) piezoelectric films and fibres for bone repair applications.

METHODS:

Solution blow spinning is utilised to fabricate aligned fibre mats of PVDF and PVDF/graphene oxide (GO). Scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and X-ray diffraction (XRD) measurements are performed on fibres. Commercial piezoelectric and non-piezoelectric PVDF films along with PVDF and PVDF/GO fibres have been used for cell culture studies. Resazurin reduction assay is utilised for quantification of metabolic activity of cells. A four point bending bioreactor has been fabricated to mechanically deform piezoelectric films to stimulate cells electrically. Modelling and simulation of 4 point bioreactor system is carried out in Abaqus to discuss the limitations of the device.

RESULTS AND DISCUSSION:

SEM images reveal average fibre diameter of ~1.5 μm. FTIR data is utilised to quantify the amount of β phase (~75% for fibres), DSC shows that the fibres are more than 50% crystalline and XRD qualitatively confirms this.

Metabolic activity of MC3T3-E1 cells in static culture (no mechanical stimulation) of PVDF fibres on day 7 is significantly higher than non-piezoelectric films but the piezoelectric PVDF films display significantly higher metabolic activity than PVDF fibres. GO incorporation on PVDF fibres results in higher metabolic activity than fibres which is similar to those of piezo films. Four point bending bioreactor fabricated for mechanical and consequent electrical stimulation of cells on piezo films is validated through modelling, simulation and experiments to deliver up to 5000 με of uniaxial tensile strain.

CONCLUSION:

Static cell culture experiments reveal that poling PVDF significantly contributes to increased metabolic activity which can be further enhanced by adding fillers and utilising dynamic mechanical stimulation of PVDF scaffolds. A uniaxial tensile strain of 1000 με will be applied to assess the effect of piezoelectricity on cells.

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ACKNOWLEDGMENTS:

The authors would like to thank the Commonwealth scholarship commission for their support.

Picture 1: Caption 1: a) SEM images of fibres taken at 500x magnification. b) Metabolic activity of MC3T3E1 cells

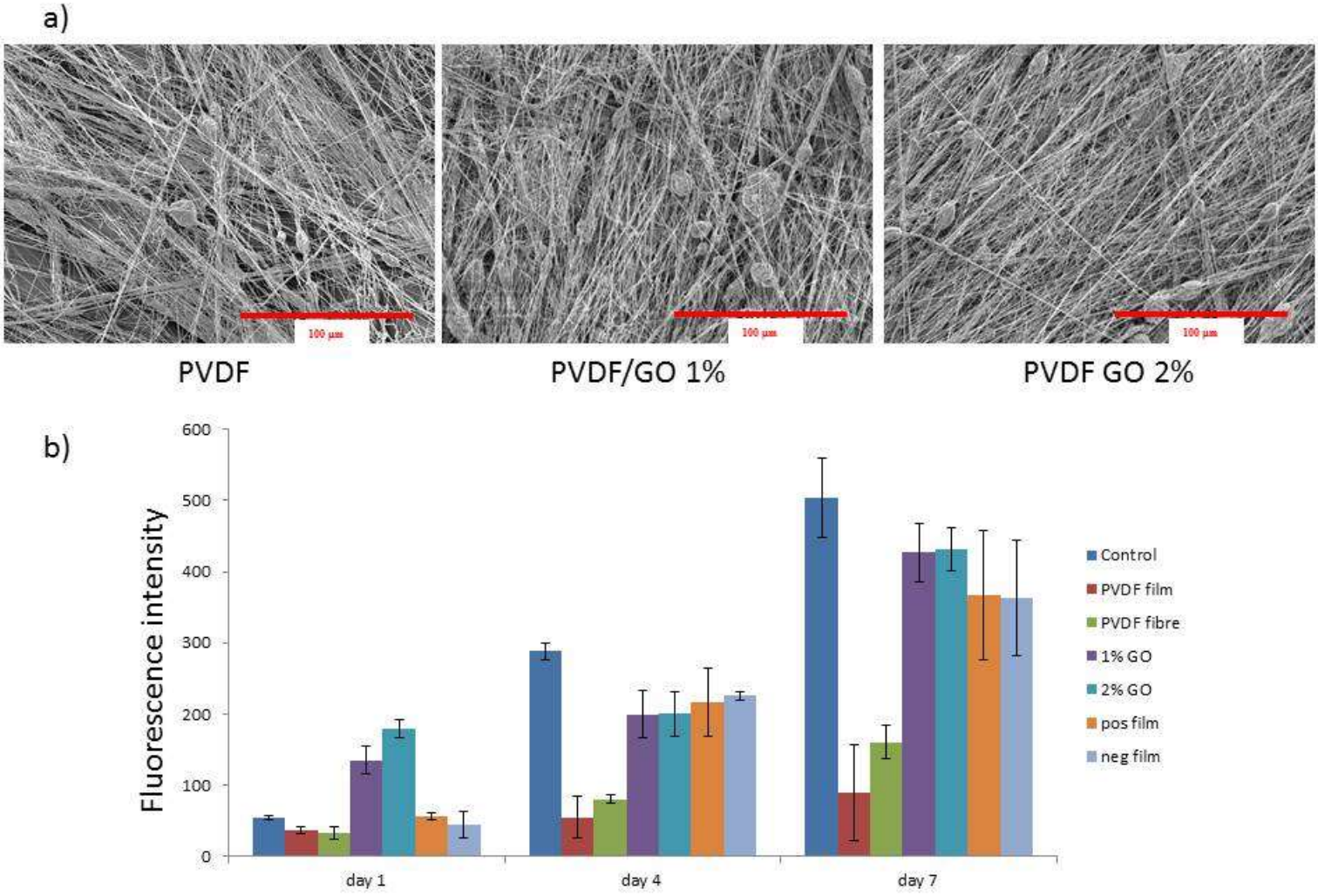

Figure 1 a) SEM images of fibres taken at 500x magnification. b) Metabolic activity of MC3T3E1 cells seeded on different material substrates

seeded on different material substrates

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

346 Designing and high throughput screening biomaterials for tissue engineering based on Materials Genome Initiative (MGI) project

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INTRODUCTION:

Biomaterials are generally defined as artificial or natural materials used to replace, repair body tissues and organs, which can perform, enhance or replace certain lost function. After implantation into human bodies, an abundance of

proteins adsorption on the surfaces of biomaterials occur instantaneously, which lead to a series of subsequent effects, such as complement activation, platelet activation, coagulation activities, and adherence of cells and bacteria. Therefore, understanding the interaction mechanism between biomaterial surfaces and biomolecules, such as proteins/peptides/amino acids, is crucial to the successful design of biomaterials in clinics.

The Materials Genome Initiative (MGI) project that was announced by American president Obama in 2011. This project aims to greatly reduce the cost and the time cycle of new material development by integrating the high-throughput experimental screening and computational data with established material databases containing comprehensive material properties.

METHODS:

COMPUTER SIMULATIONComputer simulation is an effective way to study the interactions between biomolecules and biomaterial surfaces, because it can provide information about the interaction at the atomic level that cannot be obtained directly from experiments. Quantum mechanics (QM) and Molecular mechanics/dynamics (MM/MD) are two widely used molecular modeling methods. QM mainly solves the Schrodinger equation. We have conduct a large amount high quality computational work on biomaterials during the preceding years, which provide considerable theoretical guidance for the design of new biomaterials. Here we present the computer simulation of biomolecules interaction with three most popular biomaterials, including hydroxylapatite, TiO2 and graphene [1].

RESULTS AND DISCUSSION:

HIGH-THROUGHPUT SCREENING

As for high-throughput study, we reported a conductive, stretchable, self-adhesive, and self-healable hydrogel, which is able to regulate BMSC activity though high throughput electrical stimulation [2]. In addition, we also reported an electroresponsive and conductive polydopamine-polypyrrole microcapsules (PDA-PPy-MCs) that is able to stimulate BMSC behaviors through high throughput strategies [3].

CONCLUSION:

CONCLUSIONUnder the strategy of MGI, integration of HT screening and computational research could be applied to accelerate the development of biomaterials,

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

351 Staged tumorigenesis-mimicking matrices for mechanisms analysis of chemoresistance acquisition

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INTRODUCTION:

Chemoresistance is one of the biggest barriers for chemotherapy. It is clinically known that chemoresistance increases according to tumor progression. Also, extracellular matrix (ECM) is remodeled during tumor progression. However, the role of ECM in chemoresistance has been unclear. Recently, I have prepared staged tumorigenesis-mimicking matrices which mimic native ECM at each malignant level (high malignant, low malignant and normal level). And tumor cells exhibited the highest chemoresistance on high malignant ECM by increase of *ABCB1* expression. Here, ECM-induced chemoresistance acquisition mechanisms were assessed. It is reported that chemoresistance acquisition is related to epithelial-mesenchymal transition (EMT). Thus, I focused on EMT on the matrices to unveil the mechanisms of ABCB1 expression induction on the high malignant ECM.

METHODS:

Three colorectal tumor cell lines, high malignant HT-29, low malignant SW480, and normal CCD-841-CoN cells, were cultured for 2 weeks to form ECM beneath the cells. After the culture, cellular components were specifically removed by the treatments of detergent and nucleases to obtain decellularized matrices (dECM) as “staged tumorigenesis-mimicking matrices”. Fresh HT-29 cells were cultured on the dECM for further analysis of 5-fluorouracil resistance acquisition mechanisms.

RESULTS AND DISCUSSION:

5-fluorouracil (5-FU), can induce the EMT of tumor cells via TGF-β-Smad signaling. Together, *ABCB1* expression increased via TGF-β-Smad signaling, suggesting that *ABCB1* expression was associated by EMT. Compared EMT gene expression on staged tumorigenesis-mimicking matrices, high malignant ECM exhibited the highest EMT gene expression, indicating that high malignant ECM strongly induced EMT. Also. high malignant ECM exhibited the highest *ABCB1* expression. However, this expression was suppressed to similar levels with low malignant and normal ECM by the addition with a TGF-β-Smad signal inhibitor. It was reported that chondroitin sulfate (CS) chain can bind TGF-β to regulate their activity. Thus, I compared the amounts of CS chains in the matrices. The highest CS chains were detected in high malignant ECM. These results suggested that CS chains in high malignant ECM increased *ABCB1* expression to acquire chemoresistance by the promotion of TGF-β-induced EMT.

CONCLUSION:

Conclusively, staged tumorigenesis-mimicking matrices might be suitable *in vitro* ECM models for tumor biology and pharmacological researches.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

353 Modification of poly-e-lysine hydrogels with recombinant perlecan domain V for bandage contact lenses

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INTRODUCTION:

Keratoconus is characterised by thinning and coning of the cornea, resulting in distorted vision. Treatment by corneal crosslinking controls progression but requires removal of the corneal epithelium, so bandage contact lenses can be applied to aid comfort and healing.

Peptide hydrogels, based on poly-ε-lysine (PεK), have been designed with properties similar to commercially available soft contact lenses. Free amine groups allow for the addition of moieties to the hydrogel[1]. Perlecan is a large, extracellular matrix proteoglycan found in the basement membrane of many tissues, including the cornea, where it has been implicated in wound healing[2]. Perlecan domain V (PDV) is the C terminal domain of perlecan. It is hypothesised that the addition of recombinant PDV (rPDV) to PεK hydrogels has the potential to enhance wound healing of the cornea after corneal crosslinking.

METHODS:

rPDV was covalently or electrostatically bound to the PεK hydrogels for 2.5 hours at 37°C. Binding of rPDV was investigated using quartz crystal microbalance (QCM-D) and ELISA. Human corneal epithelial cells (HCE-T) were seeded onto hydrogels with and without bound rPDV. CyQuant assays were performed, at days; 1, 4 and 7, to quantify the total amount of DNA on the substrates and infer cell adhesion and proliferation. Scratch assays were performed (reduced 1% FCS) to measure wound closure.

RESULTS AND DISCUSSION:

ELISA confirmed that rPDV bound to the PεK hydrogels. An estimate of 6.1 μg/cm^2 of rPDV remained on PεK-hydrogels after washing with 2% SDS as determined by QCM-D. Previous research has shown a lower amount of binding of rPDV to silk films still had a beneficial effect on endothelial cell growth and prevented platelet adhesion[3] demonstrating the potential bioactivity of bound rPDV. rPDV PεK hydrogels were not cytotoxic, demonstrated by CyQuant analysis. At early time points cell attachment and growth was increased on gels treated with rPDV compared to bare gels but this was not significantly different. Wound closure times were similar.

CONCLUSION:

We have successfully bound rPDV to PεK hydrogels with no cytotoxic response. Further work is being conducted into an ex vivo corneal model to mimic a keratoconus wound to study re-epithelialisation.

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

357 Modulating the race for the surface: Femtosecond laser micro/nanopatterning of alumina toughened zirconia to improve osteogenic response and diminish bacterial adhesion

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[1]INEB - Instituto de Engenharia Biomédica, U. Porto, Portugal

[2]Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

[3]Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Lisboa, Portugal

[4]: Instituto Superior Técnico, Universidade de Lisboa, Portugal, Lisboa, Portugal

[5]Laboratory for Bone Metabolism and Regeneration, Faculdade de Medicina Dentaria, U. Porto, Portugal

INTRODUCTION:

Micro and nanoscale surface topographies have been shown to induce a positive effect on bone regeneration, with improved cellular adhesion and implant osteointegration. Although, increased surface roughness is usually associated with increased bacterial adhesion. [1,2]

In this work, a femtosecond laser ablation technique was used to develop Alumina toughened Zirconia (ATZ) surfaces structured at the micro and nanoscale. The main goal was to enhance the surface functionality of the ceramic, for improved osteogenic differentiation of MSCs and diminished bacterial adhesion.

Femtosecond laser is a versatile tool for surface modification with high reproducibility, commonly used on metals. However, there are rare reports on the use of this technique on hard ceramics for biomedical applications. [3]

METHODS:

Materials characterization was performed before and after laser treatment using SEM/EDS, FTIR, XRD and contact angle measurements and the influence of the topographical stimuli on the osteogenic differentiation of hMSCs and adhesion of *Staphylococcus aureus* was assessed.

RESULTS AND DISCUSSION:

Microfeatures were successfully developed with overlapping of high-frequency laser induced periodic surfaces structures (LIPSS), that nanotextured the ceramic surface in a direction perpendicular to those of the micropattern features. Materials characterization revealed that no changes occurred in the chemical composition of the materials due to laser treatment.

hMSCs metabolic activity and proliferation increased with the time of culture and cells alignment and proliferation on the laser treated surface showed to be modulated mostly by the microtopography (Figure 1). After 21 days of culture, the laser treated ATZ displayed a higher expression of osteogenic-related markers and mineralized extracellular matrix.

Regarding the bacterial adhesion, CFU counts revealed that there was a significantly lower *S. aureus* attachment on the laser treated surface, when compared to the untreated ATZ. These results were confirmed by CSLM and SEM microscopy.

CONCLUSION:

Surface modification by femtosecond laser constitutes a simple, non-chemical, single-step process to create precise and reproducible micro- and nano- textures on ATZ. These ceramic biomaterials with improved surface functionality may be used in load-bearing applications, as an alternative to metals, to enhance osteogenic response and decrease infections risks.

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ACKNOWLEDGMENTS:

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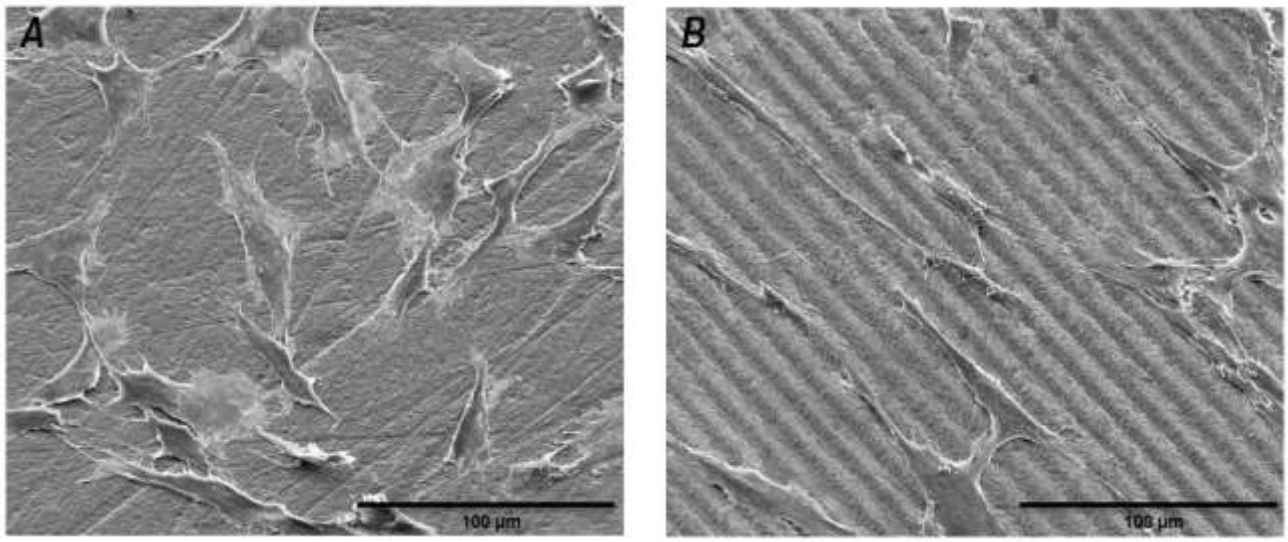
Picture 1:



Caption 1: Figure 1 - SEM images of hMSCs morphology adhered to the untreated (A) and micro/nanostructured ATZ (B) at 7 days of culture.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

370 Ag-doped mesoporous bioactive glass particles as filler in pasty bone cements for the application in peri-implantitis treatment

Matthias Schumacher[1], Rudi Walther[2], Anja Lode[2], Michael Gelinsky[2]

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[2]Technische Universität Dresden, Germany

INTRODUCTION:

Peri-implantitis, an inflammation of soft tissue around a functioning dental implant accompanied by a loss in supporting bone, is estimated to occur in one of five patients within five years after implantation.[1] This loss of supporting bone frequently results in loosening and finally loss of the implant. Here, we develop a calcium phosphate cement (CPC) with anti-bacterial properties to be applied around dental implants with beginning bone loss. The aim is to (a) fill the peri-implant defect area, provide sufficient mechanical stability to preserve the implant, allow bone regeneration and (b) release antibacterial silver ions[2] into the defect area that help preventing recurrence of the bacterial infection.

METHODS:

Pasty CPC (INNOTERE GmbH) was used as base material. Silver-doped mesoporous bioactive glass (Ag-MBG) particles were synthesised using a modified template-induced, self-assembling method described by Zhu *et al.*[3] Ag-doping was achieved by partial substitution of $Ca(NO_3)_2$ with $AgNO_3$ in a TEOS-based sol (Ag/Ca = 0.143) containing Pluronic P123 as structure-directing agent. Ag-release from Ag-MBG particles was studied in water (ICP-OES). CPC/Ag-MBG composites with 2.5, 5 and 10 wt-% Ag-MBG were characterised regarding their extrudability and used to prepare samples for compression strength and porosity measurement after setting in humid atmosphere.

RESULTS AND DISCUSSION:

Ag-MBG particles were successfully synthesised (Fig. 1a). During immersion in water, a sustained release of silicon and calcium indicates degradation of the glass, accompanied by an initially accelerated release of silver ions (Fig. 1b) that resulted in Ag-concentrations of approx. 7 mg/l in the supernatant. Upon mixture with CPC, up to 10 wt-% of Ag-MBG did not significantly alter the extrudability of the pastes (Fig. 1c), retaining the excellent micro-invasive applicability. Compressive strength of composites was in the range of 40-50 MPa after 7 days ageing, and set cements had an open porosity around 30 vol-%. Maximum strength was found for composites with 10 wt-% Ag-MBG, suggesting a strengthening effect of Ag-MBG on the cement matrix as described earlier.

CONCLUSION:

Up to 10 wt-% Ag-MBG could be mixed into pasty CPC without impairing its applicability or mechanical strength. Ag-release from the composites indicates their potential applicability in peri-implantitic bone defects.

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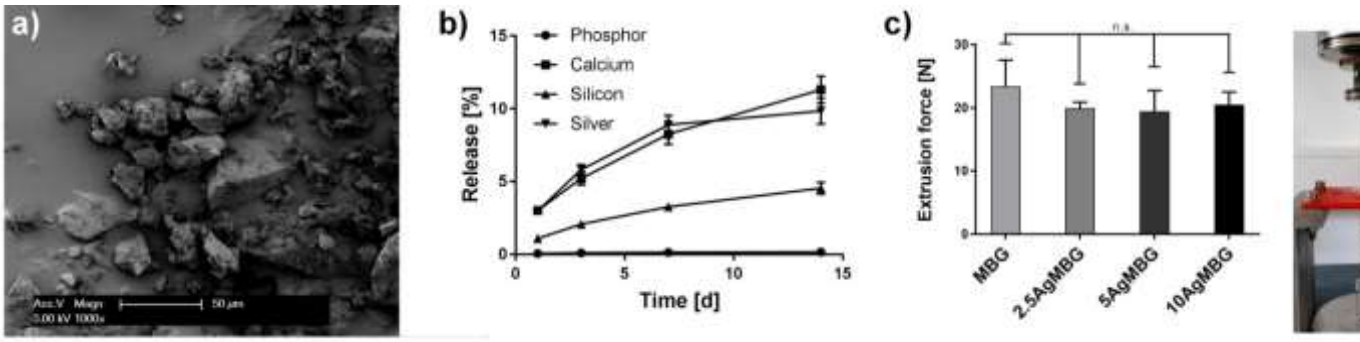
ACKNOWLEDGMENTS:

This study was funded by the Roland Ernst Foundation for Medical Research. The authors thank Andrea Voß (IFW Dresden).

Picture 1:



Caption 1: SEM micrograph of Ag-MBG (a), ion release from Ag-MBG in water (b) and extrusion force of CPC/Ag-MBG composites (c).

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

371 Mechanical characterization of a PEOT-PBT polymer containing bioactive fillers for producing functionally graded bone tissue engineering scaffolds

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[2]Fundación Tecnalia Research and Innovation, Health Division, Spain

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INTRODUCTION:

Thermoplastic polymers with resorbable degradation products are well suited for producing additively manufactured bone tissue engineering scaffolds, due to their sufficient mechanical strength and ease of manipulation. The addition of particulate fillers can often give them additional beneficial properties, such as further improvement in mechanical strength or desired stem cell differentiation. In this work, the beneficial effects of three types of fillers, namely hydroxyapatite (HA), lamellar compounds (layered double hydroxides, LDH and zirconium phosphate, ZrP) with intercalated antibiotics, and reduced graphene oxide (rGO), are being considered for bone tissue engineering. The thermoplastic polymer of choice is a block co-polymer of poly(ethylene oxide terephthalate) and poly(butylene terephthalate) (Polyvation B.V.). The filler loaded polymer was characterized with a goal of printing continuous filler concentration gradients with a newly developed print head with this capability.

METHODS:

LDH/ZrP and rGO composites with the polymer were prepared by twin screw compounding, while the HA composites were produced by solvent blending. Each composite was tested for compressive and tensile mechanical properties, melt viscosity and extrusion on the additive manufacturing platform. COMSOL modeling is being used to optimize scaffold designs.

RESULTS AND DISCUSSION:

With increasing filler concentration, we observed increasing compressive and tensile moduli, decreasing tensile strength, increasing compressive strength, and increasing viscosity at low shear rates. Based on the ability to retain its integrity and the possibility to extrude, the maximum useful filler loadings were determined for each filler (20% for LDH/ZrP, 45% for HA, and 15% for rGO). The compressive moduli obtained were in the range 100-260 MPa, similar to the values of cancellous bone properties (Figure 1a). All composites showed extrusion ability from 400 µm and 250 µm diameter extrusion needles. The models suggest that by printing in ways to increase fiber overlap[1], a higher scaffold modulus can also be obtained even at high porosities (Figure 1b).

CONCLUSION:

Several useful polymeric composites have been prepared and characterized for printing continuous material composition gradients. Production of gradient scaffolds and characterization of the gradients are currently being carried out.

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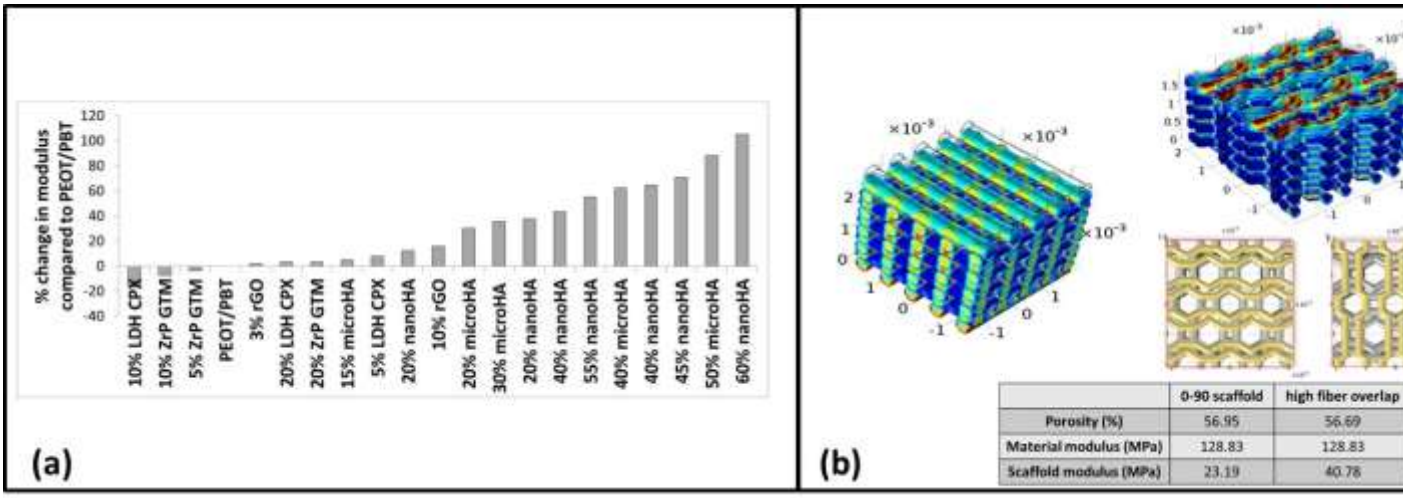
ACKNOWLEDGMENTS:

The authors would like to thank the European Union (H2020 grant #625825) for providing financial support to this project.

Picture 1:



(a) Change in compressive modulus on filler (HA micro particles, HA nanoparticles, gentamic intercalated ZrP, ciprofloxacin intercalated LDH, or rGO) loading compared to polymer witho filler (modulus of polymer without filler = 128.83 MPa), (b) A scaffold with a higher fiber ove (right) has almost double the modulus for the same porosity when compared to a scaffold pr with a commonly used 0-90 pattern (left).

Caption 1: Figure 1

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

376 Citrate-induced fibrillogenesis of fibrinogen

Karsten Stapelfeldt, Xin Guo, Nikos Molochidis, Dorothea Brüggemann

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INTRODUCTION:

Fibrinogen nanofibers are often produced *in vitro* by electrospinning to be used as cell culture scaffolds[1]. Other methods which induce fibrillogenesis of fibrinogen *in vitro* include the use of ethanol[2], acidic pH conditions[3] or oxidative stress[4]. However, these methods employ rather harsh buffer conditions, which might interfere with the biofunctionality of the resulting fibrinogen nanofibers. Here, we present a novel approach to prepare fibrinogen nanofibers *in vitro* under physiological conditions by using sodium citrate with fibrinogen in varying buffers.

METHODS:

Fibrinogen was dissolved in different buffers containing sodium citrate at varying concentrations and incubated on glass slides. The dried protein films were analyzed using scanning electron microscopy (SEM) and atomic force microscopy (AFM). Furthermore, different pH ranges were used to study the influence of pH on the fibrillogenesis mechanism.

RESULTS AND DISCUSSION:

SEM analysis revealed that fibrinogen dissolved in sodium citrate buffer assembled into nanofibrous scaffolds upon drying (Fig. 1). The morphology of the nanofibrous fibrinogen scaffolds was confirmed with AFM.

Adjusting the fibrinogen concentration from 2 to 5 mg/ml led to a significant increase of the fiber amount in the nanofibrous scaffolds. The citrate concentration was also found to influence the fiber density and the hierarchical assembly of the fibrinogen nanofibers between 5 and 40 mM sodium citrate. When sodium citrate was added to fibrinogen in phosphate or carbonate buffers we could also induce fibrillogenesis. The highest fiber yield was achieved at a pH of 8.8. Below a pH of 5.8 no fibrinogen fibers formed.

When fibrinogen nanofibers were rehydrated and dried again we observed a degradation of the fibrous scaffolds over 24 hours. Cross-linking of dried fibrinogen nanofibers with glutaraldehyde vapor, however, resulted in fibrous scaffolds, which were stable for several days.

CONCLUSION:

Our new approach of citrate-induced fibrillogenesis is a simple method to prepare fibrinogen nanofibers *in vitro* using mild buffers and physiological pH conditions. These novel citrate-induced fibrinogen scaffolds will be highly attractive scaffolds for future cell culture studies.

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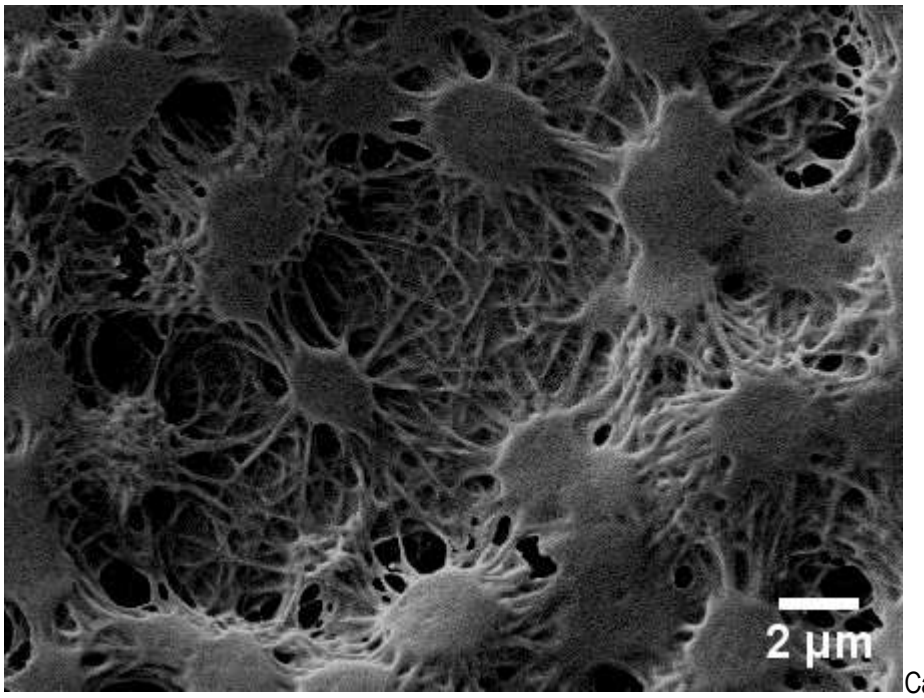
ACKNOWLEDGMENTS:

We gratefully acknowledge funding from the Emmy Noether Programme of the Deutsche Forschungsgemeinschaft under grant number BR 5043/1-1.

Picture 1: 

Caption 1: Figure 1: SEM image of citrate-induced fibrinogen nanofibers, which were dried on a glass slide.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

379 Sargasumm sp. from Colombia: Characterization of potential medical biomaterial

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INTRODUCTION:

The brown seaweed is a common invasive species found on the coasts of Colombia, and are valuable natural resources of polysaccharides for developing new medical products[1]. Sulfated polysaccharides known as fucoidans, can support bone cell growth and may be a promising scaffold for progenitor cells to create a bone graft substitute for orthopedic surgery[2], however the structure of fucoidans isolated from Colombia algae, is still poorly investigated; this study is devoted to structural characterization of fuocidans originated from Colombian biodiversity.

METHODS:

Sargassum sp. was collected in October 2017 in Santa Marta-Colombia, washed and dried at 50°C for 6 h. 10 g of dried material was allowed to stand in HCl 0.1 M for 24 h at 4°C. The solution was filtered and the residue was collected and keep at 4°C. Then, the solution was filtered and the supernatant was neutralize using NaOH 1 M. The crude extract was obtained by precipitation with ethanol. The precipitate was redissolved in water, dialyzed at 4°C in

water for 48 h and then freeze-dried. The crude fucoidan extract was further purified by ultracentrifugation using filters of 3, 10, 50 and 100 kDa MWCO. The separation by molecular weight was evaluated by gel electrophoresis using dextran-sulfate/chondroitin-6-sulfate/chondroitin-4-sulfate/heparin/enoxaparin as standards. The content of humidity, total protein, carbohydrate were also determined. Spectroscopy analysis was done by UV, FTIR-ATR and size distribution and zeta potential by DLS. Analysis of variance were performed with one-way ANOVA.

RESULTS AND DISCUSSION:

The purified fucoidan composition was shown to be mainly of fucose, galactose and xylose. Furthermore, neutral and sulfated sugars was detected. From spectroscopy analysis fucoidan was found to be a linear (1→3)-linked a-L-fucan, sulfated and partially acetylated. In addition, FTIR spectra of the extract showed typical absorption bands for sulfated polysaccharides and sulfate groups. The findings in the assessment of the nutritional contribution showed 12.27% of relative humidity, 6.0% of total protein and 59.06% of total carbohydrate similar to that reported previously[3].

CONCLUSION:

Sargasumm sp. evaluated in this study can be easily utilized as source fucoidan-based hydrogels, and suggested as potential biomaterial as tissue regenerating agents, which could improve material biocompatibility or as nutritional supplement.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

381 The thermal Treatment of tricalcium phosphate by means of calcination influences the cellular reaction and enhances its resistance to degradation in vivo

Shahram Ghanaati[1], Sarah al-Maawi[1], Mike Barbek[2], Stevo Najman[3], Robert Sader[1], C James Kirkpatrick[1], Marc Bohner[4]

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[2]Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Charité-Universität, Germany

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INTRODUCTION:

The chemical composition of biomaterials is significant for the evoked tissue reaction after implantation[1]. The degradation pattern of bone substitute materials (BSM) should timely correspond with the biological process of new bone formation to prevent premature connective tissue ingrowth[2]. The present study evaluated the modification of synthetic bone substitute materials (alpha and beta tricalcium phosphate (TCP) by means of thermal treatment i.e. calcination in Wistar rats. A subcutaneous implantation model was used to investigate its influence on the tissue reaction and vascularization. A Femur-model was used to analyze the regenerative capacity on bone regeneration and degradation.

METHODS:

Four TCP-based BMS were evaluated (control groups: A= α-TCP, B= β-TCP, C= sham operation; Test groups: D= α-TCP calcined at 500°C and E= β-TCP calcined at 500°C). The biomaterials were implanted subcutaneously and in femoral defect model (n=4 per group and timepoint). Subcutaneous implantation areas were evaluated histologically after 3, 10, 15, 30 and 60 days. The femoral implantation areas were evaluated after 15 days. The results were quantified histomorphometrically and analyzed statistically.

RESULTS AND DISCUSSION:

Subcutaneous tissue reaction over the observation period showed the induction of multinucleated giant cells (MNGCs) in all evaluated test groups. Whereas no MNGCs were found in the sham operated group. After 60 days, MNGCs/mm² was significantly higher in A and B compared to C, D and E ($p<0.001$). The vessel density correlated with the number of the MNGCs. It was significantly higher in the groups A and B compared to C, D and E ($p<0.001$).

In the femur model, high degradation rate of the uncalcined groups A and B correlated with higher amount of newly formed bone as compared to D and E. Accordingly, in the latter groups the BSM underwent less degradation and occupied significantly higher amount of the total implantation area compared to A and B ($p<0.01$). The amount of connective tissue was the highest in the control group.

The number of induced MNGCs, as a foreign body reaction in the subcutaneous implantation model was in concert with the degradation observed in the femoral implantation model. These findings underline the importance of the induced cellular reaction for the regenerative capacity of the biomaterials.

CONCLUSION:

These results suggest that thermal treatment of TCP-based BSM is a potential approach to enhance their resistance to degradation and modulate their inflammatory pattern to shift the foreign body reaction towards a less expressed reaction by reducing the number of the induced MNGCs.

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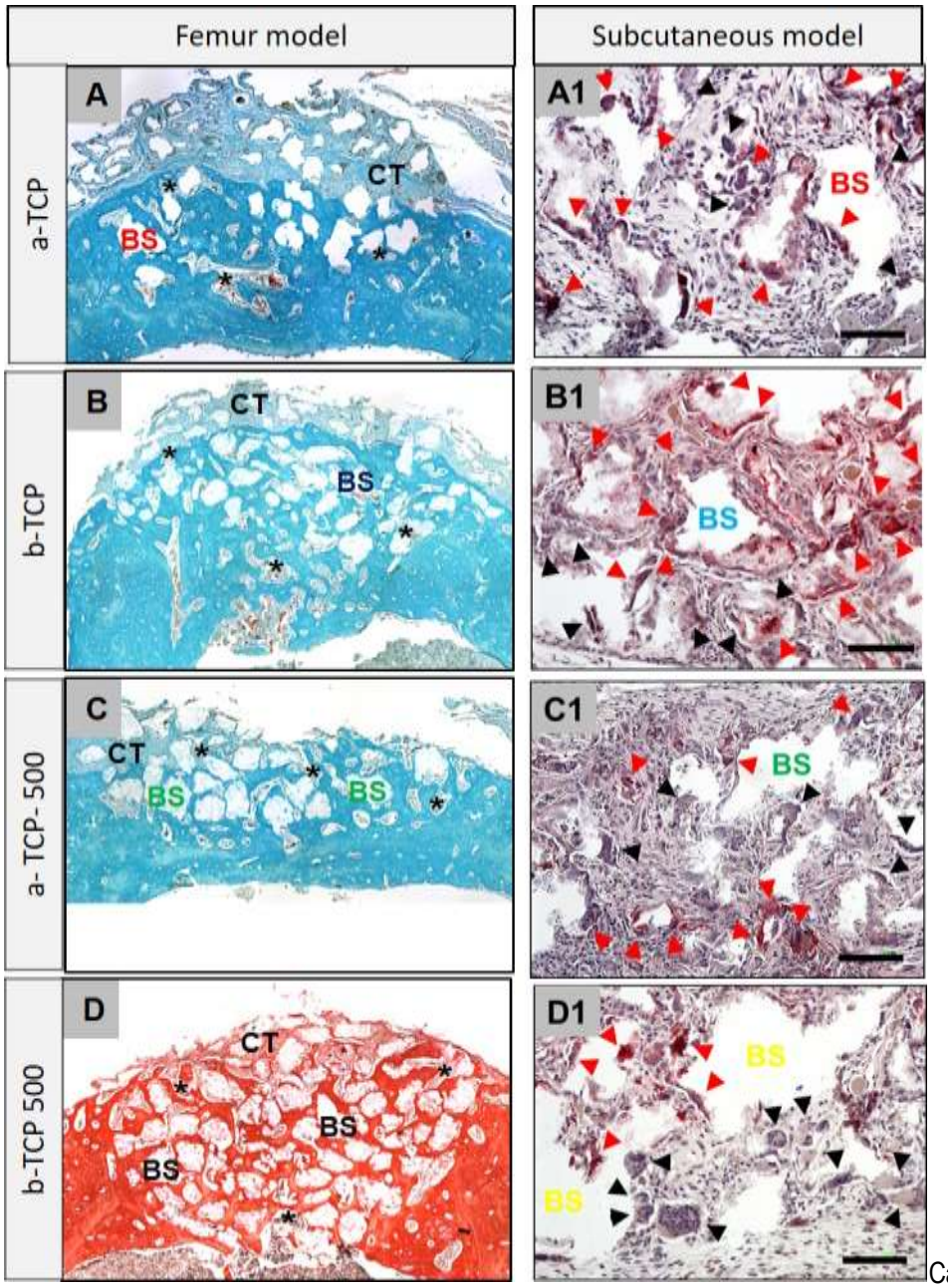
Picture 1: 

Caption 1: The cellular reaction after 15 days within the femural model and the subcutaneous implantation model

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

382 The use of high-sulfated hyaluronan to modulate the inflammatory pattern and vascularization of collagen-based biomaterials.

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INTRODUCTION:

The interaction between the biomaterial's surface and the host tissue results in specific inflammatory pattern dependent on its physico-chemical properties[1] that massively influences the vascularization[2,3]. This study investigated the functionalization of a collagen-based biomaterial using high-sulfated hyaluronan (sHA3) and its influence on the cellular reaction in vitro, the inflammatory and vascularization patterns in vivo.

METHODS:

A clinically used collagen-based biomaterial was functionalized with sHA3. The test groups were A= association of sHA3 via electrostatics to collagen and B= covalent binding of sHA3 via amine coupling and. The control groups included C= collagen crosslinking via amine coupling without sHA3 and D= non-functionalized biomaterial.

In vitro: platelet-rich-fibrin (PRF), a platelets- and leukocytes-rich concentrate gained from human peripheral blood was cultured with the biomaterials for 3 days. The concentrations of epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) were measured via ELISA.

In vivo: biomaterials were subcutaneously implanted in Wistar rats (n= 4/timepoint). After 3, 15 and 30 days, the implantation areas were evaluated histologically and histomorphometrically. Immunohistological detection analyzed the vessel density (CD-31), pro-inflammatory (CCR-7 M1) and anti-inflammatory (CD-206 M2) cells.

RESULTS AND DISCUSSION:

In vitro, EGF and VEGF release was significantly higher in A-C compared to D. For EGF, the difference between B and C was not statistically significant, both groups were significantly higher than A ($P < 0.01$). VEGF concentrations were significantly higher in A and C compared to B ($P < 0.01$). No statistical significant difference was found between A and C.

In vivo, the cellular reaction over 30 days was mediated by only mononuclear cells for B and D leading to their physiological integration. A and C induced a foreign body reaction by a high number of multinucleated giant cells (MNGCs). The number of MNGCs/mm² was the highest in C leading to premature biomaterial degradation and disintegration. The induction of MNGCs correlated with the rate of vascularization. No vessels were found within the biomaterials in B and D. The vessel density was significantly higher in C compared to A, B and D ($P < 0.001$). Most of MNGCs were of proinflammatory type (M1).

CONCLUSION:

PRF, a clinically highly relevant concept, was appropriate to analyze the initial cellular reaction towards different biomaterial surfaces in vitro. The functionalization of biomaterials with sHA3 modulated the inflammatory pattern in vivo by preventing a foreign body giant cell formation, which would have led to collagen disintegration. Both parameters are essential for sufficient tissue regeneration.

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ACKNOWLEDGMENTS:

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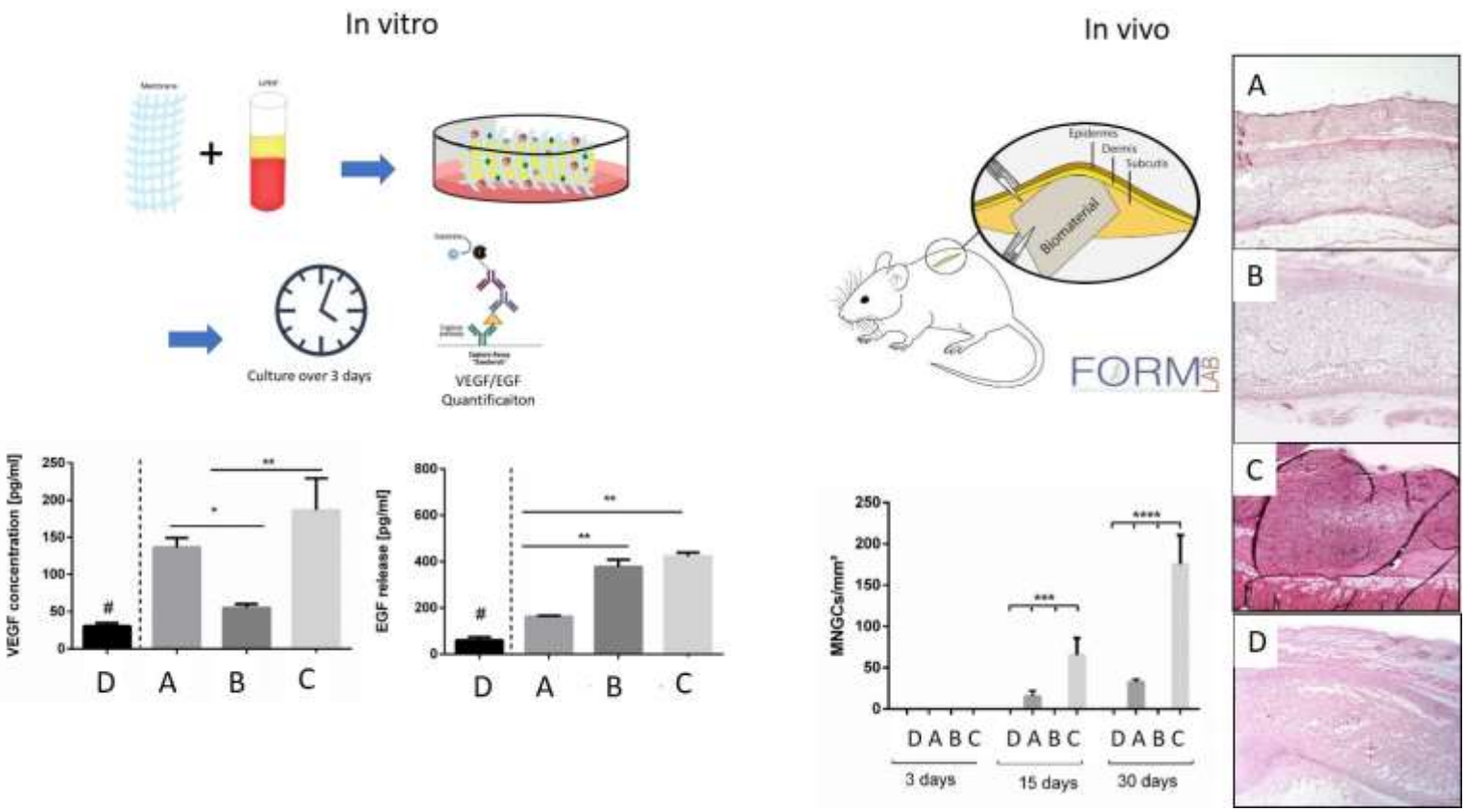

Picture 1: Caption 1: Graphical abstract of the used materials and the most significant results. A-D show the cellular reaction on day 30 in H and E

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

388 Research on Extramembranous Osteogenesis Based on Osteoinductivity of 3D Printing Bioactive Glass Scaffolds

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[2]Foshan RegenLand Bio-Tech Co. Ltd., China

INTRODUCTION:

In recent years, micro-nano bioactive glasses (MNBG) have caused much attention due to its superior bone repair performance. However, it is not clear whether MNBG have the ability to form new bone out of cortical bone without any bone defect. In some clinical requirements, bone repair materials are demanded to form the new bone out of cortical bone for achieving bone augmentation. Unlike the intramembranous osteogenesis in bone defects repair[1], we propose the concept of extramembranous osteogenesis, in which the bone formation occurs outside the cortical bone and the osteoprogenitor cells show reversed migration.

METHODS:

MNBG powder were prepared by the combination of sol-gel method and template selfassembly technique using dodecylamine as template agent. Then 3D printing bioactive glass scaffolds were prepared by using MNBG. To investigate whether MNBG scaffolds have extramembranous osteogenesis property, the scaffolds were equally planted in right back subcutaneous and skull subcutaneous of rats which marked as back group and head group, respectively (Figure. A). The scaffolds and surrounding tissues of the two groups were collected and investigated by micro-CT, SEM and histology after 6 weeks of implantation. All results were expressed as mean ± standard deviation (SD). The statistical analysis was carried out using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION:

The results showed that the scaffolds were closely attached to the skull surface without any space from the 3D reconstruction image of head group (Figure. B). Active osteogenesis process could be observed outside the cortical bone without adding any biomolecules or destroying the alveolar ridge. While in the head group the residual scaffolds were just colonization by loose connective tissue (Figure. C). Osteogenesis difference indicated that the osteoblasts derived from the reverse migration of cranial cells. The osteoinductive property of MNBG played a vital role in extramembranous osteogenesis.

CONCLUSION:

We confirmed the bone augmentation ability of MNBG in an intramembranous microenvironment by implanting in skull subcutaneous of rats without making any bone defect. This study may provide a new strategy to induce bone augmentation and extramembranous osteogenesis mechanism stimulated by MNBG.

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ACKNOWLEDGMENTS:

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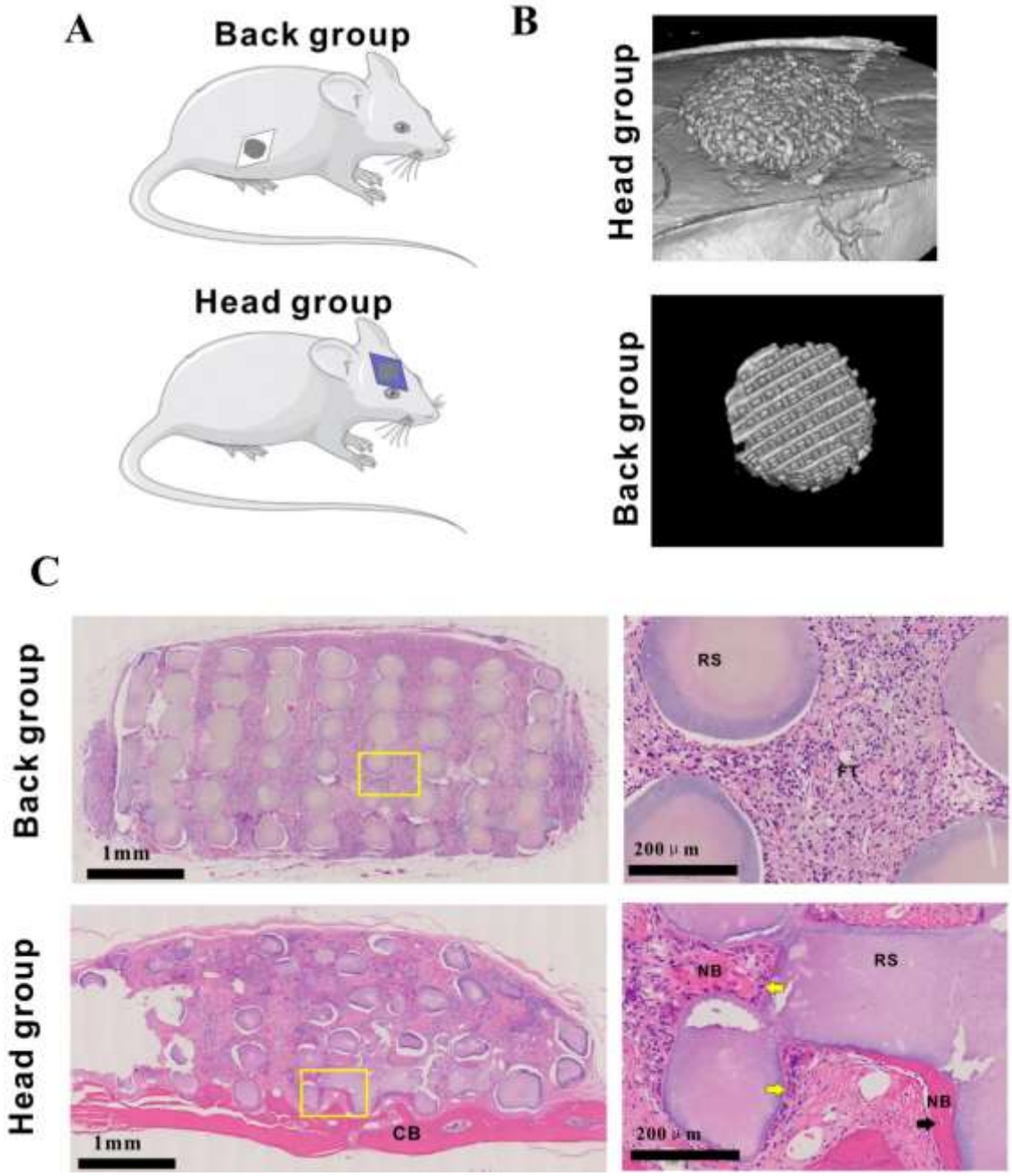
Picture 1:



Caption 1: Figure. (A) Schematic illustration of MNBG implanted in different position; (B) 3D reconstruction images; (C) histological images at 6 weeks.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

389 Multifactorial approaches towards tenogenic phenotype maintenance, trans-differentiation and differentiation

Dimitrios Zeugolis[1], Christina Ryan[1], Naledi Sholugu[1], Diana Gaspar[1], Paedar Rooney[1], Manus Biggs[1], Matthew Griffin[1], Alan O'Riordan[2], Andrea de Pirie[1]

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[2]Tyndall Institute, Ireland

INTRODUCTION:

Cell-based therapies require removal of cells from their optimal *in vivo* tissue context and propagation i*n vitro* to attain suitable numbers. However, bereft of their optimal tissue niche, cells lose their phenotype and with it their function and therapeutic potential. Biophysical, biochemical and biological signals have been shown to maintain permanently differentiated cell phenotype and to precisely regulate stem cell lineage commitment [1-3]. Herein, we developed and characterised substrates of various stiffness with controlled nanotopographical features and assessed these substrates in culture in combination with macromolecular crowding (MMC) on various cell sources to determine their suitability for the *in vitro* fabrication of tendon-like tissue.

METHODS:

Substrates of varying stiffness and controlled surface topographical features were created using a silinated silicon wafer. Substrates were then plasma treated and assessed with/without collagen type I coating (0, 0.5 mg/ml). The simultaneous effect of MMC / substrate stiffness / surface topography on cell phenotype and function was assessed using human dermal fibroblasts, tenocytes and bone marrow stem cells (hBMSCs) cultured with 100 μg/ml of carrageenan (MMC) for 3, 7 and 14 days. Detailed protein synthesis / deposition and gene expression analyses were conducted.

RESULTS AND DISCUSSION:

The nano-topographical features on the substrate surface induced cellular alignment in all cell types, which was not affected by MMC or substrate stiffness. Immunocytochemistry analysis revealed that when MMC was used, cells showed increased deposition of collagen types I, III, V and VI. FACS analysis in hBMSCs indicated a significant reduction in surface marker expression as a function of MMC, but not as a function of surface topography or stiffness. Gene analysis made apparent that MMC had a greater influence on phenotypic markers in all cell types compared to topography or stiffness.

CONCLUSION:

This study provides insight into modulation of cell behaviour and phenotype using microenvironmental cues and can have a significant contribution to the development of cell-based therapies for tendon repair and regeneration.

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

392 Development of an additive manufacturing print head for production of continuous polymeric gradients for bone tissue engineering

Ravi Sinha[1], Lorenzo Moroni[1], Carlos Mota[1]

[1]Complex Tissue Regeneration group, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, The Netherlands.

INTRODUCTION:

Additive manufacturing of biodegradable and bioresorbable thermoplastics is a preferred way to prepare scaffolds for bone tissue engineering. It provides a simple way to obtain highly controlled shapes and porosities, and the materials have sufficient mechanical strength. However, in tissue engineering, there is often a need to produce scaffolds with continuous gradients in composition and mechanical properties matching those found in the body. For example, the bone to cartilage interface in the body has a smooth transition from the hard bone with a high calcium mineral content to the softer cartilage with a lower mineral content. Such continuous gradients are currently not achievable using existing thermoplastic additive manufacturing platforms, and are thus often replaced with step gradients by switching material after depositing a few layers. We have developed a new print head that can process two materials, mix them and control each material in the extrusion output, all during the manufacturing process. Thus, continuous gradients can now be printed.

METHODS:

A dual material print head capable of producing continuous gradients of material compositions has been designed. Computational modeling using COMSOL was utilized to optimize the mixing. Particle tracing was added to visualize the mixing zone. Using a dyed and non-dyed polymer (block co-polymer of poly(ethylene oxide terephthalate) and poly(butylene terephthalate), Polyvation B.V.), gradients of the dye were produced during the printing (Figure 1).

RESULTS AND DISCUSSION:

The print head prototype was manufactured and consists of two reservoirs and a mixing zone. The mixing zone was designed according to the obtained COMSOL results. The thermoplastic materials in each reservoir are supplied to the mixing zone. The mixed materials are pushed out through an extrusion needle. The mixing concept was optimized to allow a fine control and enhance mixing.

After showing gradient printing ability, tests are underway to print gradients of filler concentrations, for fillers useful for bone tissue engineering, such as hydroxyapatite, reduced graphene oxide and layered double hydroxides with intercalated antibiotics.

CONCLUSION:

The developed printing head adds an important capability, which is the possibility to manufacture continuous gradient scaffolds by changing the material compositions. Several applications have been identified and will be explored, starting from scaffolds for bone tissue engineering.

References

ACKNOWLEDGEMENTS:

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Figure 1: Demonstration of a continuous material composition gradient production using a dyed and a non-dyed polymer.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

394 Contribution of Chitosan (Ch) and poly(gamma)-glutamic (PGA) to the immunomodulatory role of Ch/PGA-based nanoparticles

Flávia Castro, Rui Almeida, Catarina Leite Pereira, Marta Pinto, Mário Barbosa, Maria José Oliveira, Raquel Goncalves

INEB, Porto, Portugal

INTRODUCTION:

Immunomodulatory biomaterials have new perspectives in the field. Modulation of immune response by Ch and fibrinogen-based scaffolds enhanced new bone formation [1,2]. Ch, as a cationic polymer, has also been combined with the anionic PGA to form nanoparticles (NPs), that were shown to modulate macrophages from M2 into M1 phenotype and through this, hinder cancer cell invasion [3]. Despite this, Ch/PGA NPs with anti-inflammatory-drug counteracted pro-inflammatory markers of an intervertebral disc organ culture [4]. In this study, we addressed the individual immunomodulatory role of Ch and PGA. Furthermore, we investigated Ch/PGA NPs stabilization at physiological pH and how this affects their immunomodulatory properties.

METHODS:

Ch, PGA and Ch/PGA NPs produced as previously described [5], were added to primary human M2 macrophages (Mac) (IL-10 stimulated [3]) at similar concentrations (0.7mg/ml). Control experiments with unstimulated Mac and M1 Mac (LPS) were performed in parallel. After 72h, Mac metabolic activity, phenotype and inflammatory cytokine production were evaluated. In addition, Ch/γ-PGA NPs were cross-linked with Genipin (GN) at different concentrations during different time points. GN-crosslinked NPs were then incubated at pH 7.4 and their size and polydispersion was characterized by DLS.

RESULTS AND DISCUSSION:

Mac metabolic activity was maintained although a higher number of cells detached in the presence of Ch. Ch-stimulated Mac presented reduced CD163 and CD40 and increased CD86 expression and did not produce IL-12p40. PGA by itself seemed to induce an elongated Mac morphology and expression of CD14, similarly to M1. Nevertheless, contrarily to LPS stimulus, PGA promoted CD40 and CD163 and the secretion of IL-12p40. Ch/PGA NPs were successfully crosslinked using GN, maintaining NPs lower size, particularly at pH 7.4. Different concentrations of GN (1,10,20,40 mM) and different time-points (1,2,24h) were then tested. A concentration of 20 mM GN and 2h of incubation was selected as the most adequate conditions to preserve NPs.

CONCLUSION:

Ch and PGA present a distinct immunomodulatory effect than Ch/PGA NPs and PGA induced a higher pro-inflammatory response than Ch. The stability of Ch/PGA NPs at pH 7.4 was increased by a GN crosslinking during 2h a 20mM. Evaluation of the immunomodulatory role of GN-crosslinked NPs is being conducted.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

402 Embedding of functional pancreatic islets in structured alginate-based hydrogels by 3D bioprinting

Sarah Duin[1], Kathleen Schuetz[2], Anja Lode[2], Barbara Ludwig[3], Michael Gelinsky[2]

[1]TU Dresden, Centre for Translational Bone, Joint and Soft Tissue Research, Dresden, Germany

[2]Centre for Translational Bone, Joint and Soft Tissue Research, University Hospit, Germany

[3]Department of Medicine III, University Hospital Carl Gustav Carus at the Technis, Germany

INTRODUCTION:

Diabetes type 1 is characterized by insulin deficiency due to autoimmune derived destruction of the insulin producing beta cells in the pancreas. Exogenous insulin treatment is the standard therapy, however, for a subgroup of patients with highly unstable blood glucose control transplantation of islets and reconstitution of endogenous insulin secretion would be preferable. For successful islet transplantation it is necessary to ensure protection from the immune system (by immunosuppressive agents or through encapsulation techniques), while still providing an environment in which the beta cells can adequately react to changes in blood glucose levels.

It has been shown previously that islets remain fully functional in alginate hydrogels.[1] 3D plotting, an additive manufacturing technique capable of producing scaffolds of clinically relevant dimensions and incorporating biological agents, was used to produce macro-porous alginate based scaffolds containing islets with the aim to improve supply of oxygen and nutrients, and hormonal exchange.

METHODS:

The hydrogel used was a paste of alginate and methylcellulose (MC)[2] into which freshly isolated rat islets had been incorporated prior to plotting. 3D plotting was carried out on a BioScaffolder 3.1 from GeSiM (Radeberg, Germany).

For assaying cell viability samples were stained with calcein AM/ethidium homodimer or MTT. Presence of insulin and glucagon in the islets was shown through immunofluorescence staining of cryosections. Glucose stimulated insulin response of plotted islets was analysed by ELISA. Glucose diffusion was measured indirectly via release from cellfree scaffolds.

RESULTS AND DISCUSSION:

Release assays showed that inclusion of MC does not impair diffusion of small molecules compared to pure alginate. Islets can be incorporated into the material and tolerate the plotting process well. Insulin and glucagon are continuously produced and adequately located in encapsulated islets. Our data also suggest that after a short initial period of stress, the encapsulated islets release appropriate amounts of insulin in response to low high low glucose stimulation.

CONCLUSION:

This study constitutes a proof of concept for the 3D plotting of fully functional pancreatic islets from rat.

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ACKNOWLEDGMENTS:

The authors thank Susann Lehmann for helping with the islet preparation and the Paul Langerhans Institute Dresden of Helmholtz Centre Munich for providing financial support.

Picture 1:

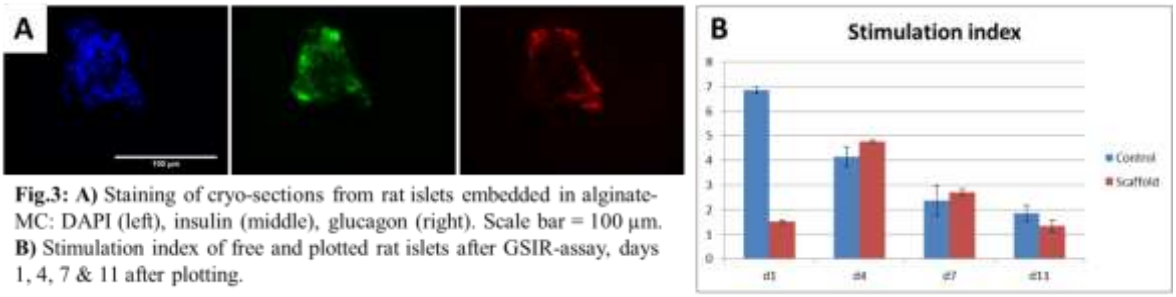

Fig.3: A) Staining of cryo-sections from rat islets embedded in alginate-MC: DAPI (left), insulin (middle), glucagon (right). Scale bar = 100 µm. **B)** Stimulation index of free and plotted rat islets after GSIR-assay, days 1, 4, 7 & 11 after plotting.

Caption 1: Functional pancreatic islets plotted in alginate/methyl-cellulose

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

404 Bioinspired engineering of bi-layered electrospun blood vessels

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[2]University of Würzburg, Germany

[3]University Medical Center Utrecht, Netherlands

INTRODUCTION:

When aiming for tissue engineering of clinically relevant sized tissues (*e.g.* for bone), a functional vasculature is desired[1]. Most blood vessels consist of a tunica intima with an endothelial monolayer, and tunica media containing multiple layers of vascular smooth muscle cells (vSMCs) in a circumferential orientation[2,3]. Hence, a small diameter

(3 mm) vascular graft was engineered with a bi-layered architecture by combining solution electrospinning (solES) and melt electrowriting (MEW).

METHODS:

solES was used to fabricate nonwoven tubular scaffold as luminal layer. A second outer layer was deposited on top of it with MEW to generate oriented fibers. Poly-e-caprolactone (PCL) was used to create both layers.

Human Endothelial Colony Forming Cells (cb-ECFCs) and/or Multipotent Mesenchymal Stromal Cells (bm-MSCs) were seeded on the inside and onto the outer layer of the scaffolds, respectively. Samples were analyzed for the vSMC markers α-smooth muscle actin (α-SMA), calponin, smooth muscle 22α (SM22a), smooth muscle myosin heavy chain (SMMH) and extracellular matrix proteins (tropo)elastin, collagen type IV and laminin subunit α4&α5. Also, the endothelial marker CD31, VE-cadherin and von Willebrand Factor (vWF) were analyzed.

RESULTS AND DISCUSSION:

Combining MEW and solES techniques was proven to be a feasible fabrication technique for the engineering of coherent bi-layered scaffolds. A randomly oriented luminal layer (fiber thickness ~1-1.5 µm) was created with solES, and a second oriented layer of MEW fibers was deposited (fiber thickness 20-30 µm)[4]. The orientation of the MSCs was influenced by the winding angle (α) of the MEW fibers, resulting in a near circumferential multi-layered second layer (α=70°). The MSCs differentiated into vSM-like cells: they were elongated, α-SMA$^+$/ calponin$^+$ and showed gene expression of SM22α and SMMH. Moreover, the cells produced their basement membrane containing (tropo)elastin, laminin subunits α4&α 5, and collagen IV.

In ECFC mono and co-cultures, a confluent monolayer of VE-cadherin, CD31 and vWF-positive ECFCs was found on the luminal layer.

CONCLUSION:

Overall, the feasibility of combining melt and solution electrospinning techniques to engineer one construct was demonstrated. The bi-layered electrospun tubular scaffold resembled the spatial organization of the layers of a native vessel, and provided a scaffold that fostered the differentiation, orientation and infiltration of the vSM-like cells, while also supporting endothelialization.

Future directions are aimed at applying fluid shear stress with a flow-perfusion bioreactor.

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

424 Multi-domain collagen-based scaffold as multi-cargo delivery vehicle for the enthesis tissue engineering

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INTRODUCTION:

The enthesis is a specialised zonal tissue interface between tendon and bone, essential for adequate force transmission. After injury, the native structure is often not re-established and a mechanically weaker fibrovascular scar tissue is formed[1], which is the result of an excessive inflammatory response, followed by increased cell proliferation and matrix deposition. Traditionally used monotherapies have often failed to be effective, posing the need for multi-cargo sustained and localized delivery vehicles. We hypothesize that controlling the healing response by targeting initial inflammation and later remodeling phases can enhance function regeneration of the enthesis.

METHODS:

In this approach, a multi-compartment collagen-based scaffold will be cross-linked to different extents in order to mimic the mechanical properties of the different layers of native enthesis and enable the early release of infliximab, a Tumor Necrosis Factor inhibitor, to target the inflammation response and platelet-derived growth factor-β (PDGF-BB) at later stages. Three-layer collagen scaffolds were fabricated with porcine collagen type I, cross-linked with polyethylene glycol succinimidyl succinate (PEG-SS) and loaded with infliximab in the outer layers and PDGF-BB internally. The scaffolds were characterised in terms of cross-linking efficiency, degradation, mechanical properties, biomolecule release. Anti-inflammatory properties and tendon cell proliferation were investigated *in vitro*. Ultimately, loaded collagen scaffolds will be transplanted *in vivo* patellar enthesis defect model in rats.

RESULTS AND DISCUSSION:

Collagen type I scaffolds were successfully cross-linked with PEG-SS, were shown to be stable in collagenase over 48h and had a compression modulus of approximately 50 kPa. Infliximab release over the first 48h was approximately 60%, whilst PDGF-BB had a slower release over 1 week (up to 80%). *In vitro* studies confirmed proliferation of tendon cells seeded on the scaffolds and reduced expression of pro-inflammatory cytokines.

CONCLUSION:

The multi-compartment collagen system may provide an appropriate environment to regenerate the enthesis defect by the synergistic effect of an anti-inflammatory and a pro-healing biomolecule.

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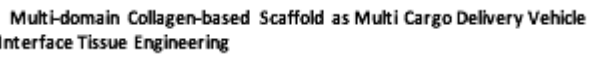
ACKNOWLEDGMENTS:

We thank the European Union, Horizon 2020 Programme, Grant Agreement 676338

Picture 1: 

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

426 Chitosan based scaffolds as potential biomaterials to control angiogenesis in vitro

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INTRODUCTION:

Angiogenesis involves the proliferation, migration, and maturation of endothelial cells in the process of tube formation. Investigations into angiogenic mechanisms require *in vitro* experimental models, which stimulate the important steps of angiogenesis to assess therapeutic agents' efficacy. In this context, HUVECs offer an important *in vitro* model to study angiogenesis. The present work aims to investigate the angiogenic properties of bioactivated chitosan (CS)-based scaffolds, in terms of cell proliferation, migration and tubes formation.

METHODS:

The CS-based scaffolds were bioactivated by using organic (BMP-2 peptide) and inorganic (hydroxyapatite nanoparticles) cues and their effects on angiogenesis were investigated. *In vitro* live-dead assay was performed to study the cytotoxic effects of scaffolds at contact with HUVECs. HUVECs were cultured onto neat and bioactivated CS scaffolds and the proliferation was checked by using PicoGreen assay for 1, 3 and 7 days. The migration capability of HUVECs was assessed using a scratch-wound assay and the reduction of wound areas was analysed with *Image J*1.48i software. Moreover, tube formation was determined with Matrigel and conditioned media at 4 and 8 hours. Statistical analysis of the data was conducted using one-way ANOVA.

RESULTS AND DISCUSSION:

Angiogenesis was monitored *in vitro* by measuring the growth and migration of HUVECs with media conditioned by CS-based scaffolds. The *in vitro* cytotoxicity results suggested that both neat and bioactivated scaffolds were not cytotoxic for HUVECs. Furthermore, HUVECs proliferation showed that all CS scaffolds (with and without bioactivation) supported cell proliferation over culture time (7 days). Moreover, CS scaffolds bioactivated by hydroxyapatite nanoparticles showed a higher proliferation than CS neat. This behaviour was due to a potential differentiation of HUVECs [1]. The scratch-wound assay demonstrated that both neat and bioactivated scaffolds stimulate and support HUVECs migration. Moreover, all scaffolds with Matrigel induced tubes formation at 4 and 8 hours of cell culture. However, chitosan-BMP2 conditioned medium induced tubes formation without Matrigel at 18 hours of culture time.

CONCLUSION:

The present study demonstrated that materials have a desirable effect on angiogenic response in terms of cell proliferation, migration and tube formation. Our work supports the concept that CS scaffolds with bioactive signals may be a source of novel implants to promote angiogenesis in different tissue engineering applications.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

432 Electrophoretic deposition of Chitosan/Selenium-strontium co-substituted hydroxyapatite composite coatings for antibacterial and osteoinductive applications

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INTRODUCTION:

Metallic implants used in reconstructive surgery face the potential issue of bacterial infections [1]. Antibacterial properties, osteoinductivity, osteoconductivity and bioactivity of hydroxyapatite (HA) coatings on metallic implants can be improved by substitution of various metal ions [1]. Selenium (Se), a nutritive trace element, has antimicrobial as well as anti-tumour properties [2]. Strontium (Sr) promotes bone formation and reduces bone resorption, in vivo [3]. In this study, bioactive and antibacterial composite coatings of HA co-substituted with Se and Sr (Se-Sr-HA)/polyether ether ketone (PEEK) on stainless steel substrates were deposited by electrophoretic deposition (EPD). PEEK was used to improve mechanical properties and corrosion resistance of the coatings.

METHODS:

Nanoparticles of HA, and Se, Sr co-substituted HA with an atomic ratio of Se/[Se+P] (xSe) from 0 to 0.2 and Sr/[Ca+Sr] (xSr) from 0 to 0.2 were both prepared by a chemical precipitation method. PEEK/Se-Sr-HA composite coatings were deposited by EPD and heat treated at 375 °C to densify the coating and enhance the adhesion to the substrate.

RESULTS AND DISCUSSION:

Co-substitution of SeO_3^{2-} and Sr^{2+} ions in the HA lattice was confirmed by XRD analysis (Figure 1) and Reitveld refinement which indicated an increase in the HA lattice parameters and unit cell volume. XRF and EDX analysis validated the required composition while FTIR spectra confirmed the presence of essential functional groups. Substitution caused changes in particle morphology, as revealed by SEM. Inductively coupled plasma optical emission spectroscopy (ICP-OES) investigations showed good trends in ion release for 5 different time points. The obtained coatings induced fast apatite formation in simulated body fluid, indicating potential higher bioactivity than pure HA. Antibacterial properties examined by a zone inhibition method revealed that Se-Sr-HA coatings had superior antibacterial activity against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) compared to pure HA. The cell culturing results showed that the Se-Sr-HA coatings could enhance adhesion and proliferation of osteoblastic cells.

CONCLUSION:

Antibacterial efficacy was enhanced with an increase in Se content. It is concluded that exceptional antibacterial properties and good biological activity can be accomplished by balancing the amount of Se and Sr.

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ACKNOWLEDGMENTS:

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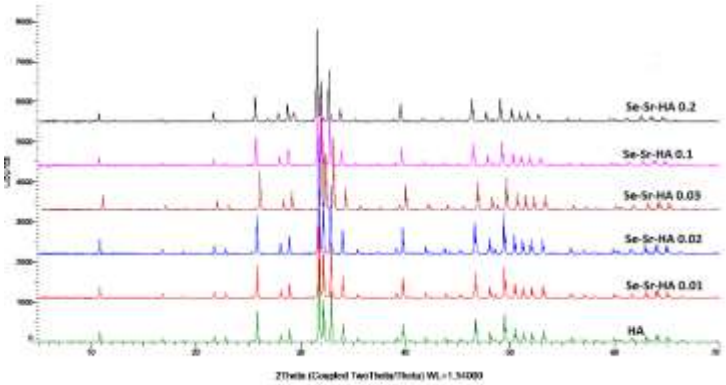

Picture 1: Caption 1: XRD Spectra of Se-Sr-HA (Se/Se+P and Sr/Sr+Ca ratios ranging from 0.01 to 0.2)

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

438 Functionalisation of a protein/peptide system towards a more complex and functional biomaterial

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INTRODUCTION:

The use of protein and peptides to build functional materials is of great interest in the field of tissue engineering (TE) and regenerative medicine (RM). While great work has been done[1,2], there is still a need for more complex systems mimicking the micro-environments of the ECM.

We had explored the self-assembling capabilities of elastin- like-polypeptides (ELPs) and peptide amphiphiles (PAs) in order to develop a complex and bioactive membrane showing a hierarchical architecture and dynamic properties[3]. This system offers an attractive platform for TE and RM applications because of the capacity to engineer with molecular control while creating controlled macroscopic scaffolds. In this work we aim to include bioactive PA molecules to enhance cell adhesion and the ECM biomolecule heparan sulphate (HS) to increase complexity and delivery of growth factors[4].

METHODS:

Cell attachment study was conducted. ELISA immunosorbent assay was used to quantify growth factor's retention and release rates. Student t-test was used to analyse data (n = 6). Significant differences between the groups were looked for at $p < 0.05$ and at $p < 0.005$.

RESULTS AND DISCUSSION:

We have shown the successful incorporation of HS within the ELP/PA membrane without disturbing its stability or hierarchical structure (Fig.1A). By conducting an ELISA assay we were able to demonstrate that the membrane can retain and release a growth factor (VEGF) (Fig.1B).

In order to tune membranes' cell adhesion properties, a bioactive peptide was incorporated. Figure 1C shows morphology of membranes formed with non-bioactive ELP and bioactive peptide PA-RGDS. There is a significant increase in cell adhesion between the conditions (0%, 5% and 10% RGDS) as shown in figure 1D.

CONCLUSION:

In this study, we were able to improve the functionality of ELP/PA membranes by using a PA with a cell adhesive epitope and by incorporating a third biomolecule (HS) which enabled retention and release of VEGF overtime. This work demonstrates the possibility to engineer complex scaffolds for TE and RM that can be fabricated with multiple functional biomolecules.

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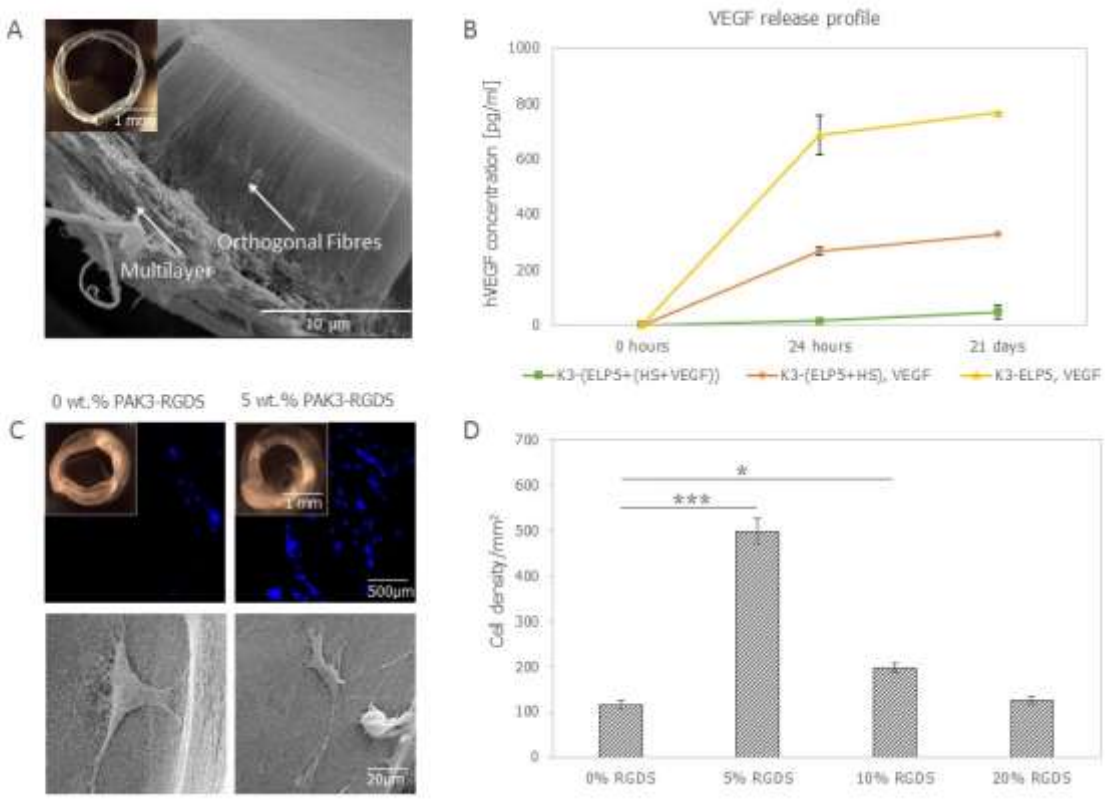
ACKNOWLEDGMENTS:

We wish to thank ERC Starting Grant STROFUNSCAFF, Biomorph, LSI and QMUL for funding.

Picture 1:



Caption 1: Figure 1.A. ELP/PA/HS membrane. B. VEGF release profile from the membrane. C. Cell attachment to the membrane D. Cell density

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

444 Converging of melt electrowriting and extrusion-based bioprinting for cartilage tissue regeneration

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University Medical Center Utrecht, Utrecht, Netherlands

INTRODUCTION:

Extrusion-based bioprinting enables hierarchical printing of cell-encapsulated hydrogels, whereas melt electrowriting (MEW) enables fabrication of (sub)-micrometer-scale fibres[1]. These techniques were recently combined in a two-step approach for the fabrication of fibre-reinforced soft hydrogels, *i.e.* fabrication of the fibre scaffold and then crosslinking a cell laden hydrogel inside. Our group pioneered the reinforcement of gelatin methacryloyl (gelMA) with a box-structured micro fibre scaffold obtained by MEW, which resulted in composite constructs with compressive properties similar to those of articular cartilage and a beneficial environment for cartilage matrix formation[2]. However, by using a two-step fabrication approach it was not possible to achieve functional cartilage constructs with adequate mechanical integrity and zonal organization of cartilage. Here, we combine these two techniques in a single-step

manufacturing process, to fabricate constructs that further mimic the composition and zonal organization of articular cartilage.

METHODS:

Polycaprolactone (PCL) MEW boxes of 400 x 400 µm, and 10% gelMA containing Equine derived Mesenchymal Stromal Cells (MSCs) (density = 20* 10^6/ml), were converged printed using a Bioarchitect bioprinter device (3DDiscovery, regenHU). Printed constructs were cultured for 4 weeks. Viability, metabolic activity, chondrogenic differentiation, and compressive properties (peak and equilibrium) were investigated using a LIVE/DEAD assay, Alamar Blue assay, GAG/DNA assay, and dynamical mechanical analysis, respectively.

RESULTS AND DISCUSSION:

High resolution (MEW fiber diameter = 10µm), multilayered zonal constructs were successfully fabricated in a single-step manufacturing process (Figure 1A-C). Viability, metabolic activity, and chondrogenic differentiation (Figure 1D) of MSCs were not compromised by the fabrication process that required high voltages to generate the MEW micro fibre scaffolds. The compressive peak- and equilibrium modulus varied from 19.85 ± 7.51 kPa (gel alone), to 246.84 ± 66.42 kPa (converged, reinforced gel), and from 11.90 ± 4.09 kPa (gel alone) to 53.02 ± 8.73 kPa (converged, reinforced gel), respectively, show that the reinforcing effect of MEW is still present after converged printing.

CONCLUSION:

This single-step manufacturing approach results in micro control over construct architecture, fibrous and gel component, allowing for the creation of scaffolds that closely resemble the native tissue composition and architecture. These are the first steps in moving towards biofabrication of larger constructs that could possibly replace or repair large defects in joints.

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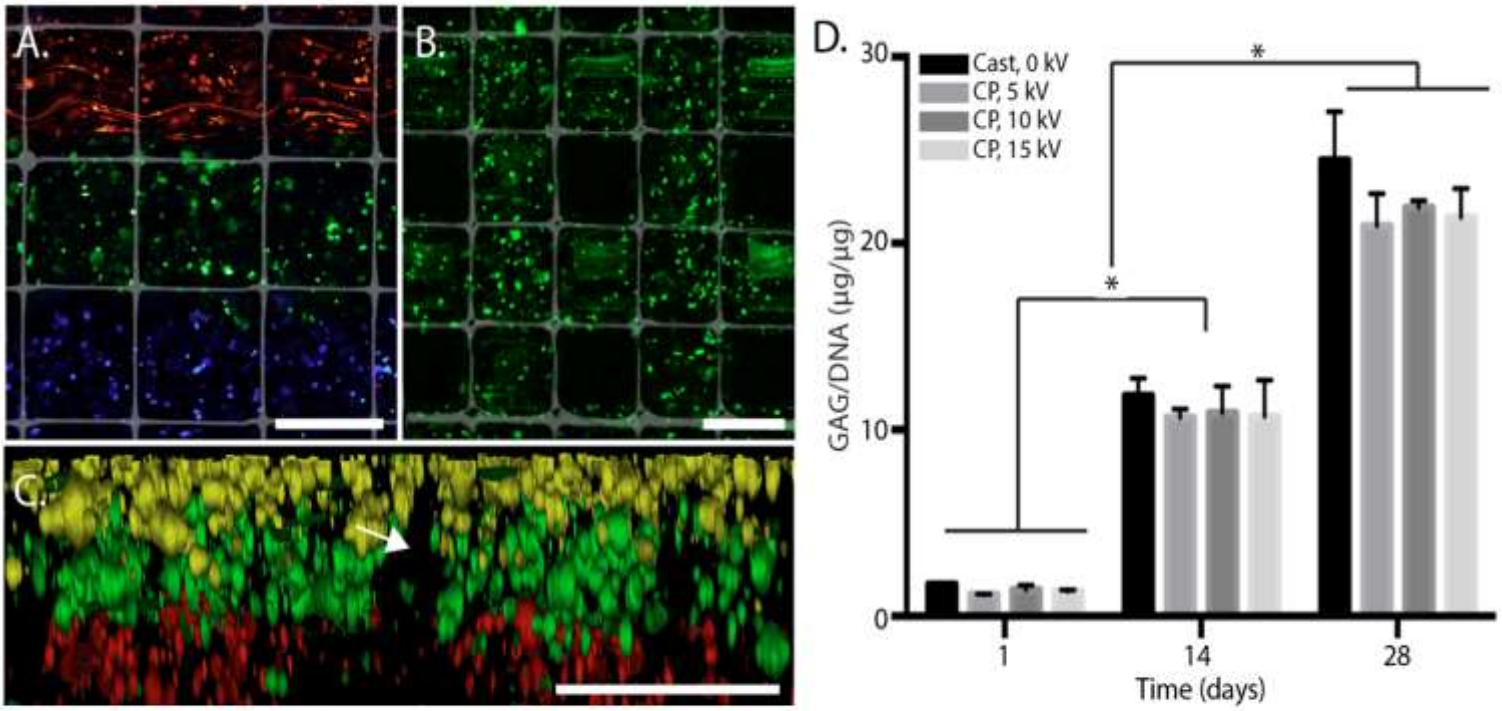

Figure 1. Converging MEW and extrusion-based bioprinting allows for multilayered constructs (A), porous constructs (B) and zonal distribution of cells while including MEW (C). Membrane-labelled eMSCs, scale bar = 400 µm. Chondrogenic differentiation (D) was not compromised by the fabrication process, that included high voltages (CP = converged printed).

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

448 Perlecan domain V functionalized blood bags for improved platelet storage

Keerthana Chandrasekar[1], Kieran Lau[1], Brooke Farrugia[1], John Whitelock[1], Jelena Rnjak-Kovacina[1], Lacey Johnson[2], Denese Marks[2], David Irving[2], Marcela Bilek[3], Megan Lord[1]

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INTRODUCTION:

Platelets for transfusion are routinely stored in polyvinyl chloride (PVC) bags at room temperature under constant agitation. This limits their shelf life to 7 days due to deterioration in function. Perlecan, a major multidomain basement membrane proteoglycan present in blood vessels, has shown to resist platelet adhesion via its heparan sulfate (HS) chains[1], which when removed, promoted platelet adhesion to the protein core. The C-terminal Domain V of perlecan contains an α2β1 integrin binding site[2], which is thought to be the major cell adhesion site within the core protein. This region was recombinantly expressed in Human Embryonic Kidney cells (HEK)-293 as a proteoglycan decorated with both chondroitin sulphate (CS) and HS. The orientation and presentation of domain V

and its CS and HS chains on surfaces have shown to be dependent on the immobilization technique, which dictates the cell responses[3]. The aim of this study was to explore domain V coated on PVC to inhibit platelet adhesion and activation and thus support prolonged platelet storage.

METHODS:

The proteoglycan and protein core forms of domain V were coated on PVC, via either passive adsorption or covalent crosslinking using plasma ion immersion implantation (PIII). Enzyme-linked immunosorbent assay (ELISA) was used to confirm the presence and orientation of domain V on PVC. Platelet adhesion and activation on domain V coated PVC was verified by the number and morphology of adhered platelets by confocal microscopy following actin staining.

RESULTS AND DISCUSSION:

More C-terminal epitopes were exposed when domain V was covalently immobilized on PVC. PVC coated with domain V via either passive adsorption or PIII significantly ($p<0.05$) reduced platelet adhesion and activation compared to uncoated PVC/PIII-PVC. The presence of HS or CS had no effect on the level of platelet adhesion when domain V was passively adsorbed. However, the domain V protein core coupled using PIII promoted platelet adhesion and activation. This indicated that the protein core of domain V was immobilised in a different orientation on PIII-PVC compared to PVC coated with domain V passively, possibly enabling access to the α2β1 integrin binding site at the C-terminal for platelet adhesion.

CONCLUSION:

Together these data demonstrate that the presentation of proteoglycans on PVC is dependent on the functionalization technique and the presence of the HS and CS enables control of platelet-surface interactions. This is a promising approach for improving platelet storage beyond 7 days.

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ACKNOWLEDGMENTS:

The authors would like to thank the Australian Research Council under the Linkage Project (LP140101056) for providing financial support to this project and the Australian Red Cross Blood Service for the supply of platelets.

Perlecan functionalized blood bags for improved platelet storage

Picture 1:

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

455 Preventing dental and orthopaedic infections with a bone substitute of nanohydroxyapatite and magnesium oxide

Catarina Coelho[1], Rita Araújo[1], Paulo Quadros[2], Susana Sousa[3], Fernando Monteiro[4]

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INTRODUCTION:

Bone fractures are a major concern presently in society and this problem will increase in the coming years. Consequently, there is a great demand for products capable of filling bone defects and ensure bone regeneration, namely bone graft substitutes[1]. However, most of these products are not able to inhibit bacterial colonization, leading to tissue infection and consequent implant failure[2]. The antibacterial effect that few bone graft products have is conferred by antibiotics, but the rise of antibiotic resistance makes these products ineffective in many cases[3]. Metal oxides are an interesting alternative for antibacterial bone grafts, as these possess strong antibacterial activity and high stability[2,4]. For that purpose, spherical granules of nanohydroxyapatite aggregates integrating different amounts of magnesium oxide (MgO) were produced and their antibacterial activity was evaluated towards *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)*.

METHODS:

Nanohydroxyapatite spherical granules with different percentages of MgO were produced and characterized using SEM, FTIR and zeta potential. To determine their antibacterial potential, granules were incubated with *S. aureus* and *E. coli.* The resultant suspension was placed onto Tryptic Soy Agar and the number of planktonic and adherent bacteria was determined in terms of colony-forming units. MTT assay was performed to evaluate the biocompatibility towards L929 fibroblasts.

RESULTS AND DISCUSSION:

The produced granules presented a spherical morphology adequate for both dental and orthopaedic applications since they present a particle size between 0.5 and 1.0 mm (Figure 1). The spectra obtained by FTIR revealed the characteristic peaks for the composite material. Moreover, zeta potential analysis showed that the inclusion of MgO in the granules did not induce a relevant modification in terms of surface charge. Microbiological assays showed that granules containing MgO were able to reduce *S. aureus and E. coli* growth both for planktonic and adherent bacteria. Finally, cell culture tests with L929 fibroblasts revealed that all granules were biocompatible.

CONCLUSION:

Nanohydroxyapatite spherical granules containing different percentages of MgO were successfully produced and the presence of this metal oxide inhibited bacterial growth, without compromising cell viability.

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ACKNOWLEDGMENTS:

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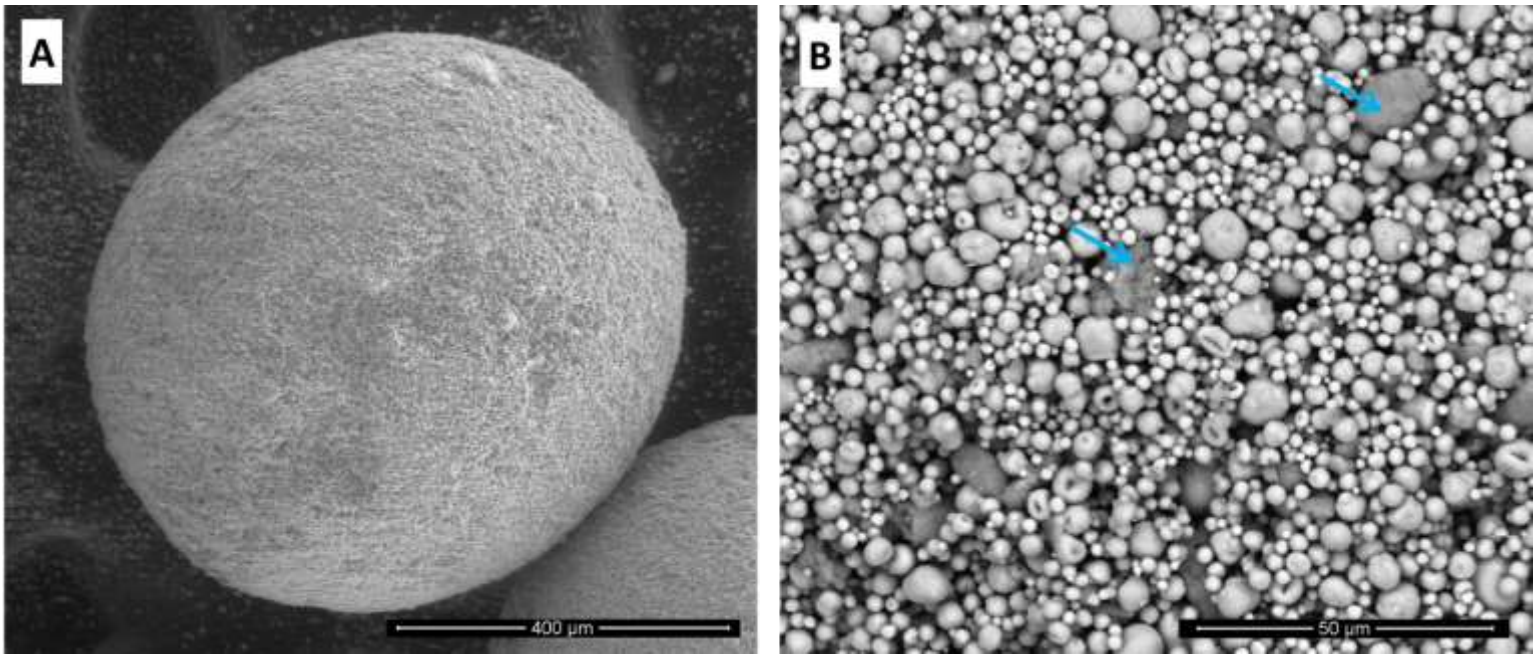

Picture 1: Caption 1: Figure 1: SEM images of nanohydroxyapatite spherical granules. A) General view. B) Back scattering image showing MgO particles (blue arrows).

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

466 Fibroblast interaction with nanofibrous collagen scaffolds

Naiana Suter, Sophie Stebel, Dorothea Brüggemann

University of Bremen, Bremen, Germany

INTRODUCTION:

Cells are embedded into the extracellular matrix (ECM), a complex network of protein nanofibers and polysaccharides. Collagen is the most abundant protein in the ECM and therefore particularly interesting as nanofibrous biomaterial. It has previously been processed into nanofibers using electrospinning [1], self-assembly [2] or template-assisted extrusion [3]. We here present the first-time combination of collagen self-assembly with ultrasonication to prepare nanofibrous collagen scaffolds with a high surface coverage as synthetic ECM.

METHODS:

Self-assembly of collagen type I into nanofibers was induced by adding collagen in acetic acid into phosphate buffered saline (PBS). The morphology of the collagen scaffolds was analysed with scanning electron microscopy (SEM). Subsequently, 3T3 mouse fibroblasts were cultivated on collagen nanofibers for up to 72 hours. Live-dead staining and fluorescence microscopy were used to analyse the cell viability. Nuclei and actin filaments were fluorescently labelled to analyse the cell area and cell shape index (CSI).

RESULTS AND DISCUSSION:

SEM analysis showed that collagen was reproducibly self-assembled into nanofibrous scaffolds with concentrations between 0.75 and 5 mg/mL (Fig. 1). The fibre diameter varied between 100 and 150 nm. By introducing an ultrasonication step during the self-assembly we could significantly increase the coverage with collagen nanofibers from 50% to 90%.

3T3 fibroblasts on collagen nanofibers proliferated well in comparison to planar collagen and glass. Using live-dead staining we observed a good overall viability on collagen nanofibers up to 72 hours. Analysis of the CSI revealed that fibroblasts on nanofibrous collagen exhibited a more elongated shape than on glass. Interestingly, the area of immunostained fibroblasts on collagen nanofibers was smaller than on planar collagen. Using SEM analysis 3T3 fibroblasts were found to grow partly into the nanofibrous scaffolds, thus indicating a close interaction with the nanofibers.

CONCLUSION:

With the first-time combination of self-assembly and ultrasonication we reproducibly prepared nanofibrous collagen scaffolds with high surface coverage, which induced a positive cellular response. In future, these synthetic ECM scaffolds will be a very attractive platform for tissue engineering and wound healing applications.

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ACKNOWLEDGMENTS:

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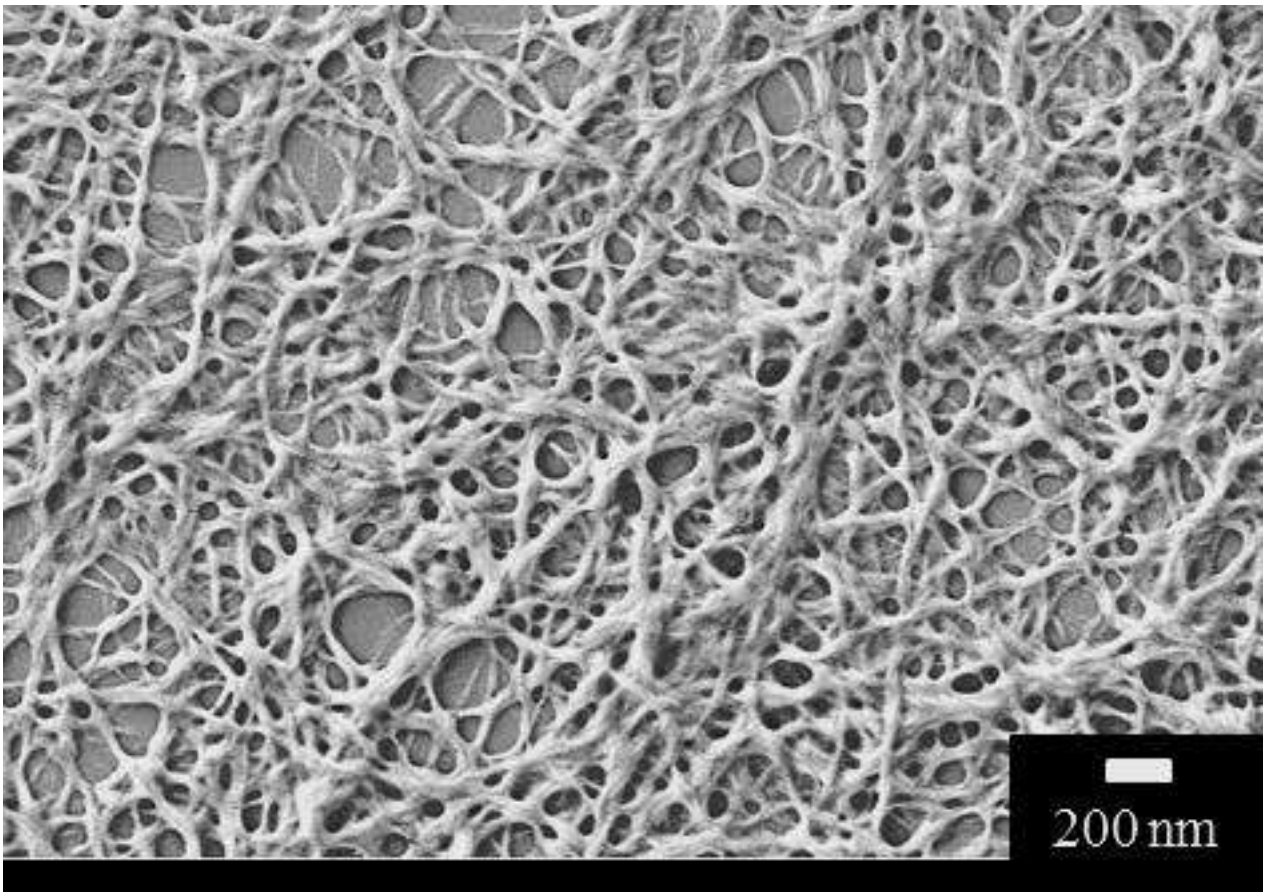

Caption 1: Nano-fibrous collagen scaffold on glass prepared by self-assembly and ultrasonication.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

470 New improved cp Ti obtained by laser additive manufacturing for dental implants

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INTRODUCTION:

Titanium and titanium alloys are widely employed in biomedical applications by virtue of their remarkable corrosion resistance, biocompatibility, exceptional specific strength and relatively low elastic modulus. These properties lead to less stress shielding in comparison to other metallic biomaterials.

Laser cladding is a method widely employed in the industry, able to apply coatings of different materials over different surfaces[1]. By controlling the cooling rates, the material microstructure can be tuned in order to improve their mechanical/chemical properties. Due to its advantages, additive manufacturing based on laser cladding (AMLC) allows the generation of functional parts with enhanced mechanical/chemical properties and therefore optimize their performance with regard to standard manufacturing methods[2].

METHODS:

Laser cladding by blowing powder method was employed to generate the parts. A high power near-infrared continuous wave laser was used as energy source. Precursor commercially pure (cp) titanium powder was coupled with the laser beam via a coaxial processing head. Parts were produced on a CNC controlled 3-axis positioning system enclosed in a controlled-ambient inert chamber.

Precursor material employed was grade 4 cp-titanium in powder form injected with argon as carrier gas. The substrates required to perform the deposition of the first layer were plates of cp-titanium as well.

RESULTS AND DISCUSSION:

The cross-sections of the generated parts were inspected in order to characterize the grain morphology employing Scanning electron Microscopy (SEM), etching techniques, and optical microscopy (OM). Samples obtained are free of cracks, interstitial oxygen or pores. The generated material (FIGURE 1a) presents a fine acicular microstructure while the standard material (FIGURE 1b) shows a typical coarser equiaxed grains. This modification of the microstructure is due to the much higher cooling rate achieved during laser cladding in comparison to standard methods.

Evaluation of mechanical properties by tensile test and microhardness corroborate those findings. These improved properties will allow reducing the size of implants without jeopardizing their reliability in service.

CONCLUSION:

The generation of commercially pure grade 4 titanium tridimensional parts for dental implants by means of additive manufacturing based on laser cladding is demonstrated. The improved mechanical properties of this material allows producing smaller implants, helping patients with low bone mass to recover oral functions.

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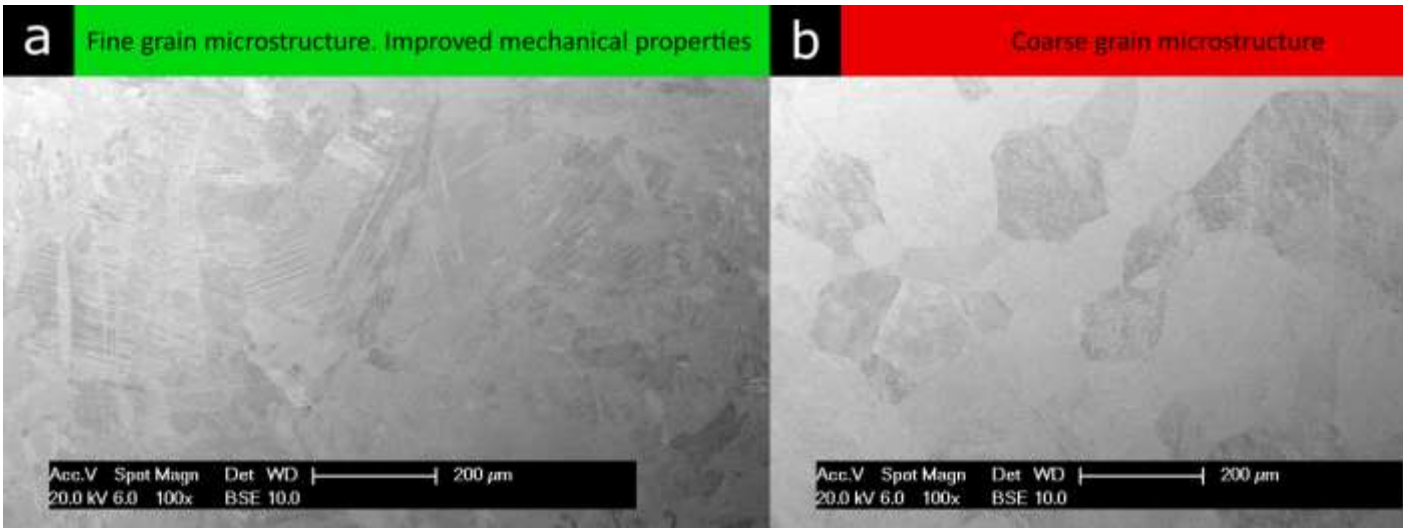
ACKNOWLEDGMENTS:

This work was partially supported by Government of Spain (MAT2015-71459-C2-1-P (MINECO/FEDER) and FPU13/02944), and by Xunta de Galicia (ED431B 2016/042, ED481D 2017/010, ED481B 2016/047-0).

Picture 1:



Caption 1: Figure 1. a) Cross-section of sample generated by AMLC. b) Cross-section of standard cp-Ti material.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

476 Natural Polymers with Antibacterial Properties

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INTRODUCTION:

The introduction of a medical device inside the human body is inherently associated with the risk of infection, defined as foreign-body related infection. Hence, researchers have been focusing on the development of new therapeutics to prevent bacterial attachment. One of the main strategies is to develop inherently active antibacterial materials using polymers possessing bactericidal effect or introducing antibacterial groups through chemical modification.

Polyhydroxyalkanoates (PHAs) are a family of biodegradable polyesters that have received great attention for a range of medical applications due to their biocompatibility[1].

The main aim of this project is the production of PHAs with inherent antibacterial properties and their functionalization to allow their use for bone tissue engineering.

METHODS:

For PHA production, *Pseudomonas putida* KT2440 was cultured in 0.1 N M63 nitrogen-limited minimal medium supplemented with decanoic acid and 6-acetylthiolhexanoic or decanoic acid (control condition) as carbon sources[2]. The antibacterial activity was investigated using ISO 22196 against different strains.

RESULTS AND DISCUSSION:

To develop antibacterial PHAs, the production of polyesters containing thioester groups (S -PHAs) in the side chains was investigated. Two strategies were explored to fulfill this objective. For the first strategy, S-PHAs were successfully produced through bacterial fermentation using *P. putida* KT2440. S-PHAs exhibited antibacterial activity against *Staphylococcus aureus* 6538P, showing a decrease in the number of cells growing on the surface of the material. This activity is most likely due to the presence of thioester groups in the polymer as the polymer obtained from the control condition was inactive against *S. aureus.* However, S-PHAs were inactive against *Escherichia coli* 8739.

The second strategy involves the chemical functionalization of PHAs to introduce thioester groups. Optimization of the production of the starting material using *P. putida* KT2440 and decanoic acid was studied. The functionalization of the material using UV-grafting is currently under investigation[3].

CONCLUSION:

In this study, S-PHAs produced by bacterial fermentation showed bactericidal properties against Gram-positive bacteria. Chemical functionalization of PHAs is currently ongoing to develop a new family of inherently active antibacterial materials. Future work will involve the investigation of the potential of these polymers as scaffolds for bone regeneration.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

480 Development of three-dimensional Cell-Derived Matrices using PLA microcarriers as in vitro tumor models

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INTRODUCTION:

The use of cell-derived matrices (CDMs) is a promising alternative to decellularized tissues/organs because these are bioactive and biocompatible materials consisting of a complex assembly of proteins, matrix macromolecules and growth factors. The ability to create different CDM based on the cell source and culture methods, offers the possibility to tailor-made bioactive materials for desired applications as the creation of a 3D printable bioink, for

tissue engineering applications. Cell cultured microparticles that favor its adhesion, proliferation and CDM production enhanced by the Macromolecular Crowding (MMCs) effect will be used for the creation of colon tumor CDM[1].

METHODS:

Poly-lactic acid (PLA) microcarriers are made by jetting this polymer through a coaxial needle[2] and coating them with Fibronectin to enhance cell adhesion.

hAMSCs (human Adipose Mesenchymal Stem Cells), colon tumor cells and CAFs (Cancer Associated Fibroblasts)[3] cells are seeded in co-culture at microparticles' surface in spinner flasks with intermittent agitation to enhance nutrient transfer and avoid dead areas. Then, cell-seeded particles are cultured for 21 days with MMCs and with permanent agitation. Results are analyzed in terms of Total DNA, Total protein production, surface properties by SEM, mechanical properties by AFM and protein expression by immunostaining and confocal microscope imaging and qRT-PCR.

RESULTS AND DISCUSSION:

MMCs cultured tissues present significantly higher amounts of total protein in comparison to the same tissues cultured in absence of this substance. Fibrillary proteins (such as Collagen types I and III) present in colon tumor extracellular matrix (ECM) are highly expressed after 21 days of culture. Therefore, tissues density and size is greater, more protein is observed when topography is studied at the SEM and tissue stiffness is increased (Figure 1).

CONCLUSION:

PLA microcarriers and MMCs help cell adhesion, proliferation and ECM deposition in a 3D environment to create CDMs.

Furthermore, CDMs can be used to understand various mechanisms promoting cancer progression by decellularizing bioengineered tissues and by the recellularization of them, as a tumoral model to develop drug screening assays or identifying therapeutic targets.

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ACKNOWLEDGMENTS:

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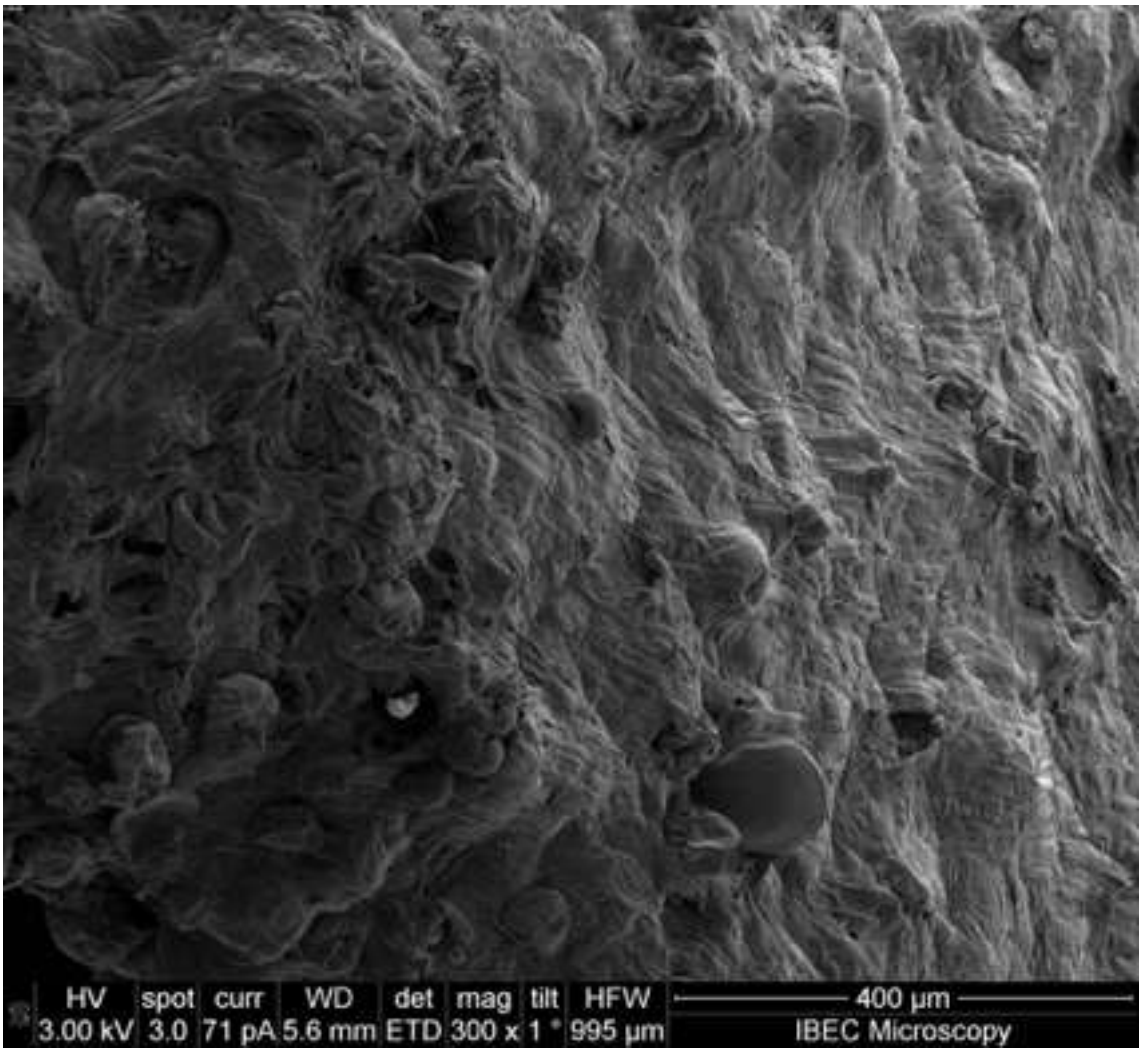
Picture 1:



Caption 1: Figure 1 CDM deposition in Cell-seeded PLA microcarriers

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Poster presentation

482 Borax induces osteogenesis and inhibits adipogenesis in MSCs

Hayk Mnatsakanyan[1], Victoria Gutierrez Salazar[1], Manuel Salmerón-Sánchez[2]

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[2]Division of Biomedical Engineering, School of Engineering, United Kingdom

INTRODUCTION:

Boron (B) plays a key role in plants and animals metabolisms[1,2]. It has been reported that B is involved in bone mineralisation[3]. However, its use in the area of Tissue Engineering has been limited to works involving bioactive glasses but its mechanism of action is unknown to date. We report for the first time the novel effects of B on mouse Mesenchymal Stem Cells (mMSC). We have analysed MSC behaviour by examining both differentiation potential

along three different lineages (osteogenic, adipogenic and myogenic) or analysing its capability to induce sustained self-renewal, culturing cells in absence and presence of differentiation factors. Multiple phenotypic features including cell morphology, gene/protein expression and functional differentiation were examined.

METHODS:

Spin casted films were obtained from a PLLA 2% (w/v) solution in chloroform. PLLA and glass substrates were functionalised with fibronectin (20 µg/ml) and used for cell culture. We have tested two different borax concentrations corresponding to 0.59 and 1.47 mM as additive in the culture medium. After seeding cell morphology and myogenic/osteogenic/adipocyte commitment were assessed by immunofluorescence for lineage-specific markers and qPCR quantification. In cell western was used for determination of key proteins involved in MSC fate and intracellular signalling.

RESULTS AND DISCUSSION:

Borax effect on mMSC using basal (without supplements) and differentiation media was evaluated. Adipogenic (Oil red O) and immunofluorescent detection of myogenic commitment (α-sarcomeric actinin) showed minimum levels after 15 days of culture under basal conditions. However, osteogenic (Runx2 and OPN) markers presented greater expression only using borax in basal media. mMSCs stimulation using differentiation media showed similar results in both immunofluorescent and qPCR analysis, resulting in an increase in osteogenic and a proportional decrease in adipogenic markers only in borax presence (Figure 1-A). Interestingly, besides enhancing osteogenic commitment, borax presence inhibits adipocyte formation even in the presence of adipogenic defined media at phenotypic, protein and mRNA levels (Fig. 1-B).

CONCLUSION:

We report that borax is able to direct osteogenic differentiation in mMSCs in absence of defined factors in the culture media. Furthermore, in the presence of defined media, borax maintain its effect enhancing osteogenesis and inhibiting adipogenesis. We propose borax as a novel bioactive molecule to direct osteogenic mMSC differentiation

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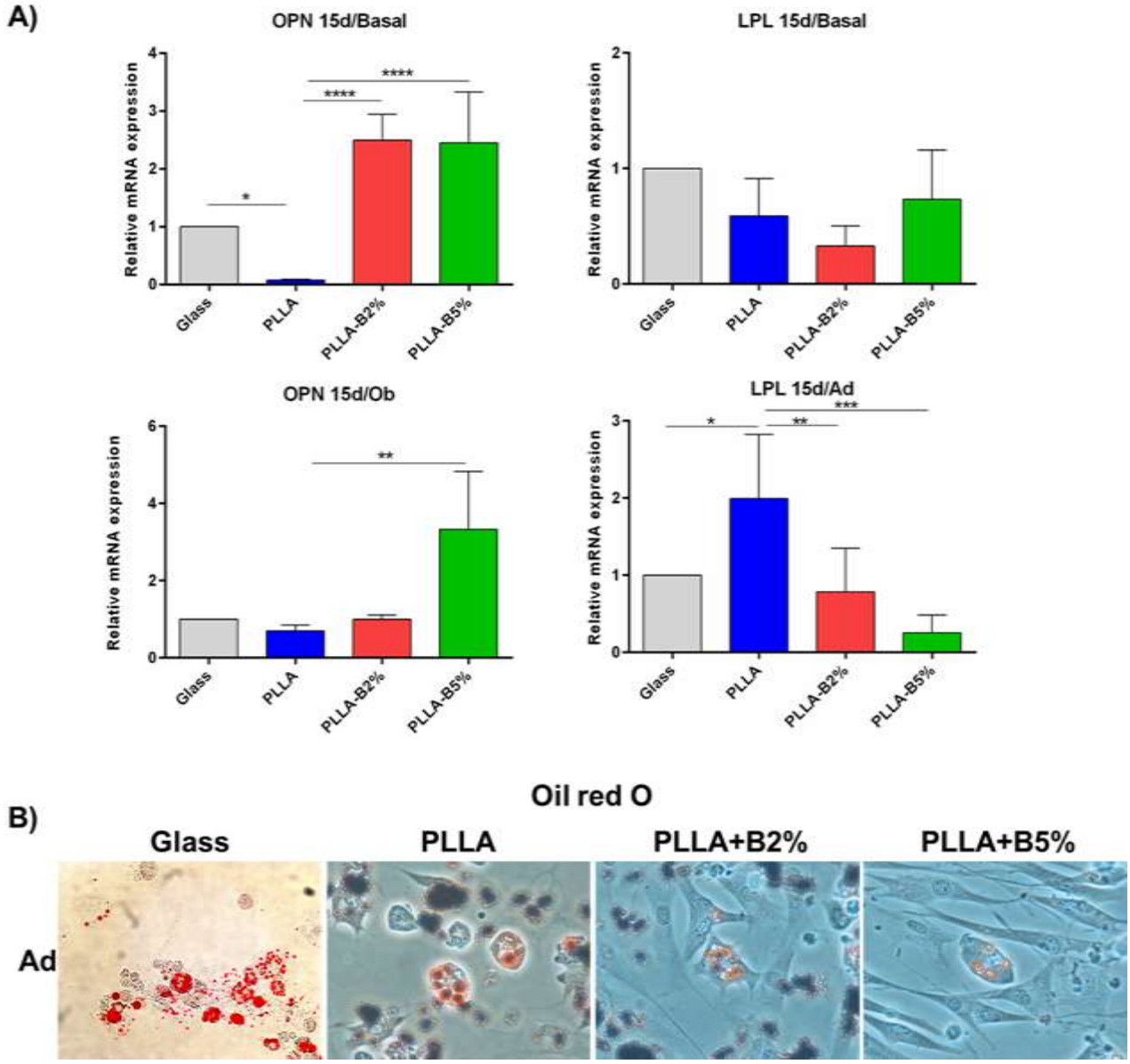
Picture 1:



Caption 1: Fig 1A) qPCR expression of OPN and LPL under basal and differentiation conditions.B) mMSCs under adipogenic conditions, B inhibits adipocyte formation

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Poster presentation

483 Printable Materials with Enhanced Functionality for use in 3D Tissue Models

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INTRODUCTION:

New materials for the provision of functional printable inks are essential if the potential of 3D printing of human tissue models is to be fully released. This study reports the properties of a range of novel conductive polymers formulated from hydroxypropyl-cellulose (HPC), PEDOT:PSS and graphene nano-platelets. Data on their suitability for 3D printing and response to external conditions such as variation in temperature and electrical stimulus are presented.

METHODS:

Formulations containing 10% and 12% w/w HPC and 6% and 7% w/w PEDOT:PSS were prepared. Graphene nano-platelets in aliquots of 1%, 2% and 3% w/w were added to the formulations. Temperature sweep rheograms were conducted in the range 20°C to 60°C using a AR200 Rheomoeter (TA instruments, UK). Cyclic voltammetry (CV) was carried out in a custom-built cell using a screen-printed carbon electrode immersed in in Britton-Robinson (BR) buffer (pH 7.0) using a sweep rate of 100 $mV.s^{-1}$. Conductivity was calculated from the electrical resistance of each gel, as measured with a Sinometer multimeter (RS Components UK). Electro Optical Analysis was performed using insulated tin-plated electrodes (RS Components UK) to which voltages of 0.5V, 1V and 2V were applied and optical images captured at 5 s intervals over a 4 min sweep period.

RESULTS AND DISCUSSION:

All HPC and graphene loaded gel formulations underwent shrinkage and a change in turbidity when heated above the Low Critical Solution Temperature (LCST) which occurred between 35°C and 41°C. The CV data confirmed that applying voltages in the range -0.3V to 1V modified the properties of the PEDOT:PSS and HPC formulations. An increase in current was noted at 200mV and deemed to result from a structural change within the HPC material. Electro-optical analysis confirmed that was a phase transition from a soluble to insoluble material state, figure 1.

CONCLUSION:

Formulations containing HPC, PEDOT:PSS and graphene nano-platelets have been identified as functional materials to be used for 3D-printing with the potential to induce cellular responses through external stimuli such as temperature and electricity [1]. Their response to external stimuli have shown them to offer the potential to induce specific cellular responses in 3D printed matrices.

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ACKNOWLEDGMENTS:

Funding from Invest Northern Ireland under the BioDevices Lab Award is gratefully acknowledged.

Picture 1:  Caption 1: Figure 1: Electrically induced phase transition of 10% w/w HPC HXF

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

494 Cold atmospheric plasma assisted deposition of nanostructured coatings to reduce biofilm adhesion and proliferation

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INTRODUCTION:

In recent years, innovative solutions able to prevent bacterial adhesion to blood-contacting biomaterials have raised significant interest. Beside the reduction of biofilm proliferation, these novel approaches have to preserve the bio- and hemo-compatibility, avoiding blood clots formation on the surface of the biomaterials. Among the innovative strategies developed to reduce bacterial proliferation and biofilm adhesion on biomaterials, the deposition of nanostructured coatings by means of cold atmospheric plasma (CAP) has been demonstrated to be a promising technology[1]. In this work, a CAP assisted process to produce nanostructured coatings composed by silver nanoparticles (AgNPs) embedded in a plasma polymerized HMDSO (ppHMDSO) matrix with antibiofilm and anti-clot properties is presented.

METHODS:

Nanostructured coatings were deposited by means of a CAP jet operated in argon and driven by a high voltage generator working at 10 kV and 14 kHz. The produced coatings were composed of three distinct layers: a first layer of ppHMDSO, working as buffer layer; a second layer composed of AgNPs, directly synthetized in the plasma region through the reduction of $AgNO_3$ aerosol droplets; a third thin protective layer of ppHMDSO, to prevent the dispersion of the nanoparticles and to increase the coating's biocompatibility. The chemo-morphological analysis of the coatings was carried out by means of Fourier-Transform-Infrared (FTIR) spectroscopy and Scanning Electron Microscopy-Energy-Dispersive X-Ray (SEM-EDX) analysis. In order to evaluate the biocompatibility of the deposited coatings, hemocompatibility tests were performed by dynamic blood contact and blood cell lysis was evaluated by haemoglobin free assay according to ISO10993-4. After blood contact the biomaterials were stained by haematoxylin/eosin to detect clots. The antibiofilm performance of the coating was evaluated with a contact-test of a 4 strains bacterial culture, and crystal violet staining was used to evaluate the presence of biofilm on the coated surface[2].

RESULTS AND DISCUSSION:

FTIR and SEM-EDX analysis confirmed the presence of organo-silicon coatings and of AgNPs incorporated between the buffer and protective ppHMDSO layers. The biological assays outlined that deposited coatings were able to reduce bacterial adhesion and biofilm formation, preserving the hemocompatibility and avoiding clots formation compared to pristine substrates.

CONCLUSION:

Nanostructured coatings deposited by means of CAP assisted processes are able to prevent biofilm adhesion and clots formation, maintaining a physiological level of blood haemolysis.

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

503 Cell-free expression of MatB and immobilization in polysaccharide microgels for production of polyketide building blocks

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INTRODUCTION:

Secondary metabolites, as structurally complex polyketides are assembled by consecutive steps of decarboxylative Claisen condensations. The enzymes catalyzing these condensation reactions, known as polyketide synthases (PKS), are commonly consuming coenzyme A (CoA)-activated acyl groups like acetyl-CoA and malonyl-CoA, which can be formed by acetyl-CoA synthetase and malonyl-CoA synthetase (MatB), respectively (1).

METHODS:

To support the cell-free synthesis of non-natural polyketides, which may serve as novel approach in drug development, we provide a microfluidics-based approach to synthesize key building blocks, particularly malonyl-CoA in a tailored, cell-free environment. For this purpose, we construct a fusion protein of a deletion mutant of the deGFP and MatB, and clone it into a pET28a vector, which enables expression of fluorescent MatB with a polyhistidin (His)-tag. We then prepare polymer microgels with tailored size and porosity by droplet microfluidics cross-linking furan-functionalized hyaluronic acid and poly(ethylene glycol) dimaleimide via Diels-Alder cycloaddition. The integration of a nitrilotriacetic acid moiety loaded with nickel ions into the gel particles enables immobilization of His-tagged deGFP-MatB by forming a metal-chelate complex. To investigate the enzymatic activity, we establish an enzymatic assay based on pyrophosphate (PP_i) detection (2) as well as a HPLC-protocol monitoring the two-step reaction of MatB.

RESULTS AND DISCUSSION:

The MatB-functionalized microgels are filled into a microfluidic reaction chamber to perform the enzyme-catalyzed reaction to malonyl-CoA in a defined environment under continuous flow, where reaction conditions (e.g. temperature) can be tuned. Different binding studies indicate the high binding affinity of the His-tagged protein to Nickel-NTA-modified gels. The immobilized enzyme shows still activity and determination of reaction product malonyl-CoA via HPLC-MS is feasible within a detection limit of 25 nmol. Also, the established PPi assay based on the formation of a blue molybdate complex allows for detecting PP_i in the range of 1-5 nmol.

CONCLUSION:

By employing a fluorescent fusion protein of MatB, we can determine encapsulation efficiency and enzyme distribution within microfluidically prepared microgels via fluorescence intensity. The use of microreactors (Figure 1) to study optimal conditions of immobilization efficiency and enzyme activity in the conversion of malonate to malonyl-CoA is an important step towards cell-free production of novel non-natural polyketides on the scale of a few microliters.

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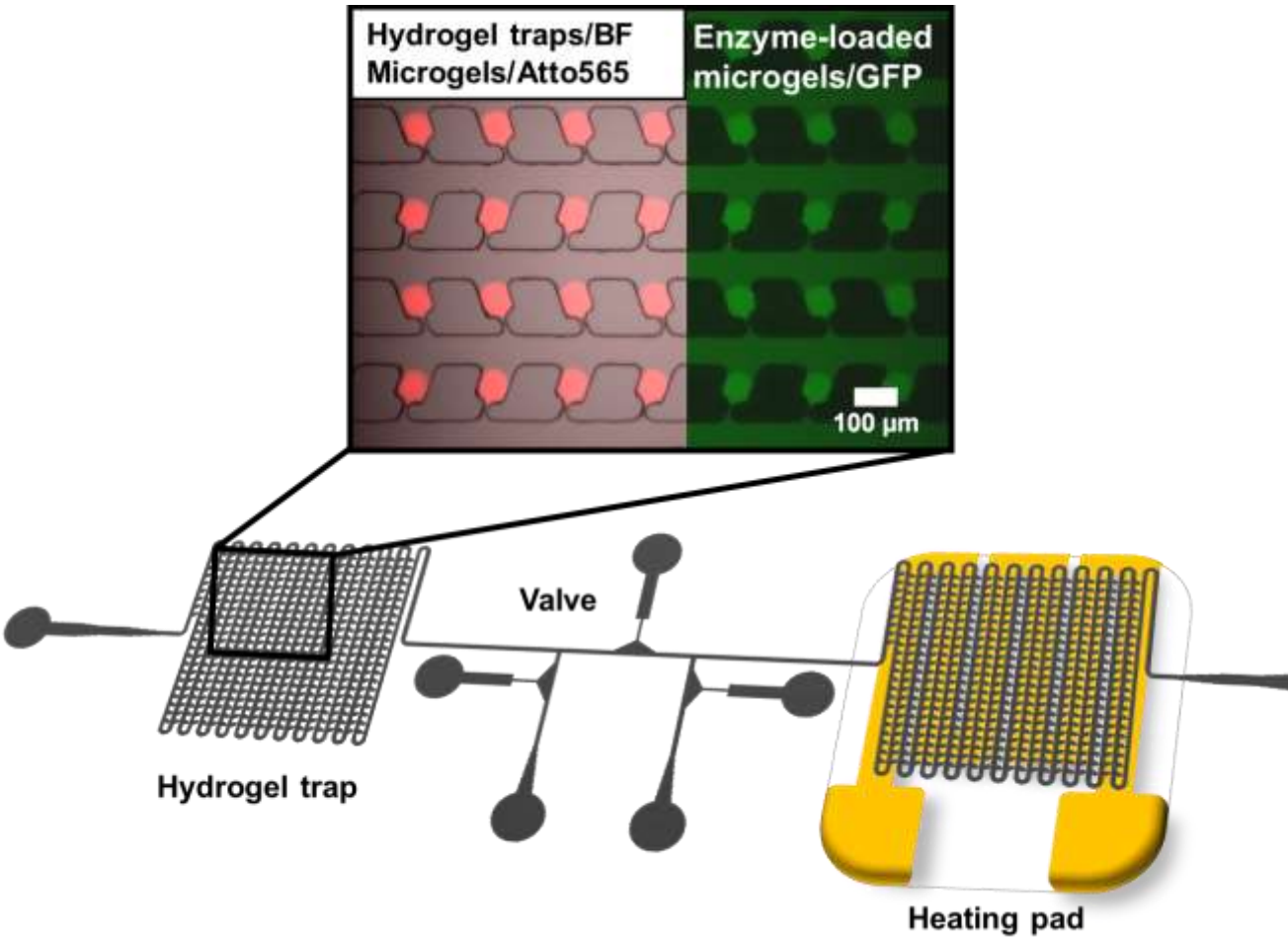
Picture 1:



Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

506 Injectable reactive oligomer/gelatin hydrogels

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INTRODUCTION:

Gelatin-based hydrogels mimic extracellular matrix (ECM) due to their inherent cell-adhesive properties, degradability, immuno- and biocompatibility. Chemical cross-linking of gelatin is one strategy to make these hydrogels mechanically resilient in physiological environments. Our previously developed anhydride-containing oligomers can be used to formulate versatile prefabricated hydrogels.[1,2] Here, we strive to synthesize a new class of

these oligomers with increased hydrophilicity to formulate injectable gelatin-based hydrogels cross-linked via amine-anhydride conjugation.

METHODS:

Oligomers were synthesized by free radical polymerization of maleic anhydride (MA) and hydrophilic comonomers: acryloylmorpholine (Mo), *N*-vinylpyrrolidone (Vp) and hydroxypropyl acrylate (Hp) in defined ratios with and without the addition of pentaerythritol diacrylate monostearate (P).[1] The oligomers were characterized by acid-base titrations, proton NMR, gel permeation chromatography (GPC) and dissolution experiments. Injectable formulations of these oligomers with aqueous solution of gelatin (type A, 300B) were characterized rheologically. Human adipose tissue derived stem cells (hASC) were encapsulated and cytocompatibility was assessed by Live/Dead® staining and confocal microscopy. Differentiation and mineralization capability of gel encapsulated cells and incubated in osteogenic medium was also investigated until day 21.

RESULTS AND DISCUSSION:

Three sets of hydrophilic oligomers (oPHpMoMA, oPVpMoMA and oPHpVpMA) and their PEDAS free analogues (oHpMoMA, oVpMoMA and oHpVpMA) were synthesized. Titrations confirmed controlled anhydride incorporation with high chemical intactness (> 80%). ^{1}H-NMR proved integration of all comonomers in predefined ratios. GPC analysis revealed molecular weights (M_n) in the range of 2-3 kDa. PEDAS free oligomers dissolved in water faster than their PEDAS analogues. All hydrophilic macromers dissolved faster than previously established derivatives[1,3]. Hydrogel formulations from the new oligomers and gelatin were established for in situ cross-linking under pH control using a multi-step programmable pipette. Hydrogel stiffness depended on presence of PEDAS in oligomers (*G'P* > *G'*) and comonomer composition (oPHpMoMA < oPVpMoMA < oPHpVpMA). Viable hASC were incorporated in hydrogels upon cross-linking and proliferated effectively over 7 days. Cell-laden gels showed good mineralization at day 21 in osteogenic culture.

CONCLUSION:

Three sets of hydrophilic oligomers (oPHpMoMA, oPVpMoMA and oPHpVpMA) and their PEDAS-free analogues (oHpMoMA, oVpMoMA and oHpVpMA) were synthesized and characterized physico-chemically. These hydrophilic oligomers presented high reactivity and controlled comonomer incorporation. Oligomer based cross-linked gelatin hydrogels encapsulated live cells and presented good gel mineralization after 21 days. These oligomer cross-linked gelatin networks represent a material platform that can further be covalently modified with pharmacologically active substances and are promising for cell delivery and potentially control of cell fate in biomedical applications.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

515 Improvement of functional properties of extruded films made with milk proteins

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[2]Lactips, France

INTRODUCTION:

Among bio-based materials, proteins have been widely studied. Soybean or zein, for example, are sources of proteins which have been studied for years [1][2]. Other studies suggest that some natural additives can be added into films to modify their functional properties. However, these reviews concern the formation of films by solution

casting method, inadequate for industry. The present work deals with the **formulation** and the **plasticization** of milk proteins films produced by **blowing-extrusion** of thermoplastic pellets. This new biosourced material has the ability to be **soluble in water** and to be biodegradable.

METHODS:

In order to modify functional properties, two ways have been followed. In one part, an alternative plasticizer, **triethanolamine** (TEA), has been studied in presence or not of glycerol. In another part, a specific additive '*A*' used to increase the water content into the film has been introduced in the matrix to study its effects in terms of mechanical properties. The interactions between the two plasticizers and the additive have been studied through physicochemical properties, mechanical properties, measures of water sorption isotherms and dissolution times of films.

RESULTS AND DISCUSSION:

Mechanical studies reveal that blends of TEA and glycerol show a **better plasticizing effect than each polyol alone**. The same trend is observed when 'A' is added in the matrix. Anterior works revealed that TEA was hardly as good plasticizers as glycerol [3], but no studies mentioned any synergic effect of the two components. Likewise no study has been revealed about this phenomena between glycerol and 'A'. Further studies has demonstrated that TEA and 'A' are **not compatible** each other in terms of mechanical properties. A final experimental design has been successfully used to optimize the formulation.

CONCLUSION:

In this protein matrix, glycerol shows a **synergic effect** with TEA in one part, and with 'A' in another part. These results are due to **specific interactions** between plasticizers and additives, revealed by **physicochemical measurements**. Further works will help to understand the relations between structuration, mechanical properties and wettability. The experimental design allows an improvement of the properties of a promising material in terms of mechanical resistance and solubility.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

516 Elastin-like based grafts as off-the-shelf small vessel substitutes

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[2]Univeristy of Valladolid/ Cibber-BBN, Spain

INTRODUCTION:

Current research in vascular tissue engineering[1] strives to develop off-the-shelf grafts able to support cell infiltration in situ, i.e. once implanted in the body. A key issue to bring this idea to success, is the appropriate selection of the biomaterial. In this work, we aim to develop macroporous vascular grafts for in situ tissue engineering by using Elastin-Like Recombinamers (ELR)[2,3], a novel class of biomaterials with immune tolerant properties, elastic performance and low thrombogenicity.

METHODS:

Macroporous ELR-based vascular grafts were fabricated by combining salt-leaching gas foaming, injection moulding and electrospinning techniques. SEM and Two Photon Microscopy were used to investigate the porosity. Cellular studies were carried out with smooth muscle cells. Cellular infiltration and extracellular matrix (ECM) production were investigated by immunohistochemistry. Mechanical properties were evaluated by burst strength and suture retention measurements.

RESULTS AND DISCUSSION:

ELR-based vascular grafts with controlled porosity were successfully produced. Importantly, the resulting porous vascular grafts were proved to be suturable and able to withstand the physiological pressure conditions. Cellular studies revealed cell infiltration and concomitant ECM production, which makes this system of high interest for in situ tissue engineering.

CONCLUSION:

We have successfully engineer macroporous ELR-based grafts, here proposed for vascular endogenous tissue generation. Importantly, the ELRs used here and the bioprocessing techniques presented here can be employed for a variety of applications in the field of regenerative medicine. Specifically, the capability of creating a desired porosity that supports cell infiltration makes this system of high interest.

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ACKNOWLEDGMENTS:

START-Program of the Medical Faculty of RWTH Aachen University– Fond #691713. MAT2013-42473-R, MAT2015-68901-R, MAT2016-78903-R, VA313U14, VA015U16 and PCIN-2015-010.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

518 Soft collagen scaffold provides a mimicking MSCs niche environment

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INTRODUCTION:

Maintenance of mesenchymal stem cells (MSCs) features requires a tissue-specific microenvironment (*e.g.* niche), which is poorly represented by the typical plastic substrate used for 2D growth of MSCs in tissue culture flask. The objective of this study was to address the potential use of collagen-based medical device (Hemocollagene®) as MSCs mimetic niche with the ability to preserve human MSCs stemness *in vitr*o.

METHODS:

The characterization of Hemocollagen® foams (1 cm^3, Septodont, France) was performed by scanning electron microscopy (SEM), Fourier transform infrared (FTIR), ion exchange chromatography, mercury intrusion porosimetry and micro-indentation. Human MSCs from bone marrow (BM-MSCs), dental pulp (DP-MSCs) and Wharton's jelly (WJ-MSC) were cultured within the foam for 4, 7 and 10 days and the secretory profile of these cells was analysed by ELISA quantification.

RESULTS AND DISCUSSION:

With a chemical composition similar to type I collagen, a major component of connective tissue such as dental pulp, bone marrow and umbilical cord derived Wharton's jelly, Hemocollagene® foam presented porous and interconnected structure (> 90%) and a relative low elastic modulus (about 60 kPa). All these criteria meet basic requirements of a biomaterial for tissue engineering field. ELISA experiments showed that MSCs were able to release in supernatant cytokines/chemokines such as IL-6, IL-8 whatever their origin. No statistical differences were observed over the studied time. However, we observed a higher amount of IL-6 and IL-8 released by WJ-MSCs compared to BM- and DP-MSCs ($p<0.001$, $n=8$, *Mann Whitney)*. Moreover, we noticed the absence of IL-1ß and TNF-a pro-inflammatory cytokines as well as IL-10 anti-inflammatory cytokine for all MSCs source. Despite VEGF release in supernatants, no b-FGF, TGF-ß and BMP-2 production was detected in our experimental conditions. BM-MSCs released a higher amount of VEGF compared to WJ- and DP-MSCs ($p<0.05$, $n=8$, *Mann Whitney).* Gene regulation of the corresponding proteins is under investigation.

CONCLUSION:

Hemocollagene® 3D foams seem to present an inert environment for MSCs since no pro-inflammatory cytokines have been detected in supernatants. Furthermore, a basal secretion of both IL-6 and IL-8 over the time suggests that Hemocollagene® foams could present *in vitro* MSCs niche model.

ACKNOWLEDGMENTS:

This work was partially supported by SEPTODONT. L. Entz is supported by "Interreg V – France-Wallonie-Vlaaderer program: TEXTOS" and Marie Dubus is supported by a doctoral fellowship from the High Education Minister.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

528 Effect of different RGD peptide concentrations on Vasculogenesis of HUVECs in 3D Micro-tissue

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[2]Izmir Katip Çelebi University Biomedical Engineering Department, Turkey

INTRODUCTION:

Vascularization is vital period for viability. It provides the delivery of nutrient and oxygen and helps to remove waste products of cells. In vascularization process, HUVEC reproduces in microenvironment with ECM proteins and differentiate to vascular structures[2]. 3D systems are used to mimic natural cell behaviors especially *in vitro* vascularization. RGD is effective units of fibronectin which is located in ECM. It has capability of enhancing cellular attachment, proliferation and growth in monolayer culture. In this study, effect of different concentration of RGD peptide units on vascularization of HUVECs in 3D micro-tissues are investigated by evaluating expressions of key vasculogenic gene markes.

METHODS:

GRGDS was synthesized in solid phase peptide synthesis as previousy decribed [1]. 4mM, 2mM 1mM and 0mM concentrateion of RGD peptides were dissolved in EGM-2 (Lonza). Micro-tissues were constructed with 100,000 cells by 3D Petri Dish as described previously [2]. Micrographs were taken and rt-PCR analysis for VEGF, Tie-1 and Tie-2 were conducted at 1st 4th and 7th days by StepOnePlus (AppliedBioSystems).

RESULTS AND DISCUSSION:

Figure 1 presents effect of different RGD concentrations over vascular markers. 2mM RGD peptides increase VEGF expression, it may promote cellular attachment and proliferation in microvascular tissue in early and late time points. The expression of Tie-1 may affect Tie-2. RGD peptide may stabilize vascular network formation via *Tie-1:Tie-2* complex in late time points.

For each vascular markers, 2 mM RGD groups have been shown that it contributes to vascular period because of overexpression of those markers. Optimum ratio of RGD was 2 mM RGD group for vascularization. 4 mM and 1 mM groups were either non-adequate or letal dosage. Optimum RGD ratio may permit to cellular connection and matrix-cell interaction via 3D microtissue systems.

CONCLUSION:

The study showed that 2 mM RGD peptide concentration enhances proliferation of HUVECs and supports expression of vascular markers and vascular structures.

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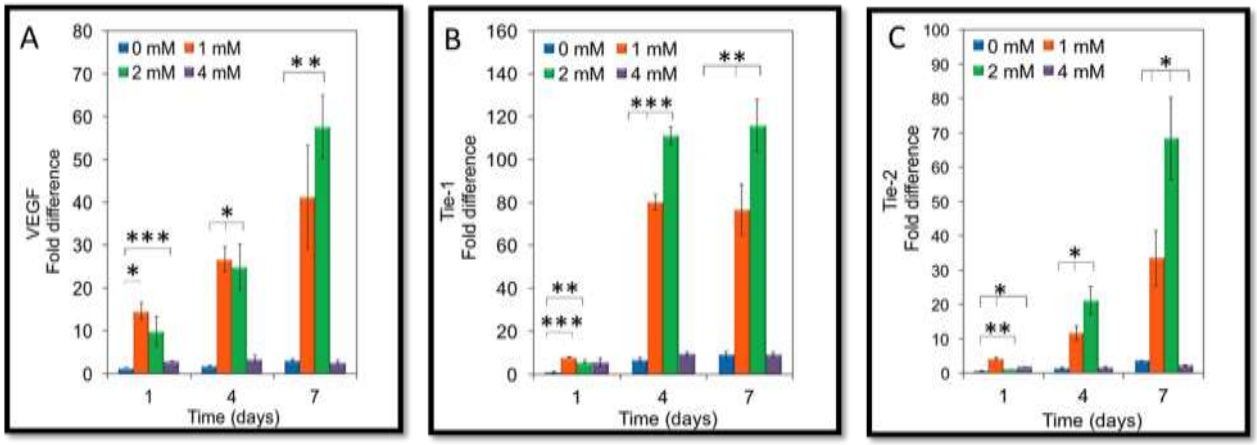
Picture 1:



Caption 1: 0mM, 1mM, 2mM and 4 mM at 1st 4th and 7th days Fold Differences of A) VEGF B) Tie-1 C) Tie-2

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

535 Fine tuning neuronal targeting of nanoparticles for nucleic acid delivery

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INTRODUCTION:

Poly(ethylene glycol) (PEG) has been extensively used to coat the surface of nanocarriers to improve their physicochemical properties and allow the grafting of targeting moieties. Still, to date there is no common agreement on the ideal PEG coverage-density or length to be used for optimum vector performance. In this study, we aimed to investigate the impact of both PEG density and length on the vectoring capacity of neuron-targeted gene-carrying trimethyl chitosan (TMC) nanoparticles that we have been exploring to deliver therapeutic genes to peripheral neurons [1].

METHODS:

Polymer-pDNA nanocomplexes were prepared by mixing equal volumes of pDNA and thiolated TMC (prepared as previously described [2]) solutions. The non-toxic fragment from the tetanus toxin (HC) was coupled to a 5 kDa heterobifunctional PEG (HC-PEG_{5k}) reactive for the thiol groups of TMC_{SH} and grafted onto the resulting nanoparticles (NPs). For the ligand density optimization, NPs functionalized with PEG (Fig 1.A) in 1, 2 or 4 mol% of the TMC primary amino groups were produced. For the ligand exposure optimization, mixtures of HC-PEG_{5k} and a 2 kDa MeO-PEG-MAL (MeO-PEG2k) were used at 1:1, 2:1 and 1:2 molar proportion (HC-PEG_{5k}:MeO-PEG_{2k}) (Fig 1.B). To determine the best NP formulation in terms of cell-specific interaction, we took advantage of imaging flow cytometry [2], that allowed us to evaluate the impact of the fine-tuning functionalization of the developed NPs on their internalization and pDNA delivery in different cell lines.

RESULTS AND DISCUSSION:

Internalization and transfection studies on neuronal versus non-neuronal cell lines allowed to determine the PEG density of 2 mol% of PEG chains the one with superior biological performance. To enhance HC exposure and maximize cell-nanoparticle specific interaction, NPs containing different ratios of HC-PEG_{5k} and 2 kDa MeO-PEG at the same grafting density were produced. By intercalating HC-PEG_{5k} with MeO-PEG_{2k} we attained the best performance in terms of internalization (higher payload delivery into cells) and transfection efficiency, using twice lower amount of HC.

CONCLUSION:

In summary, our study showed that precise adjustment of the PEG grafting density presented a significant impact on the biological performance of the developed NPs. This outcome emphasizes the need for the fine-tuning of PEG-modified NPs to attain optimal pDNA specifically delivery and bioactivity, as well as endorses the critical importance for the spatial control of the targeting moieties for the successful development of the next-generation nanomedicines.

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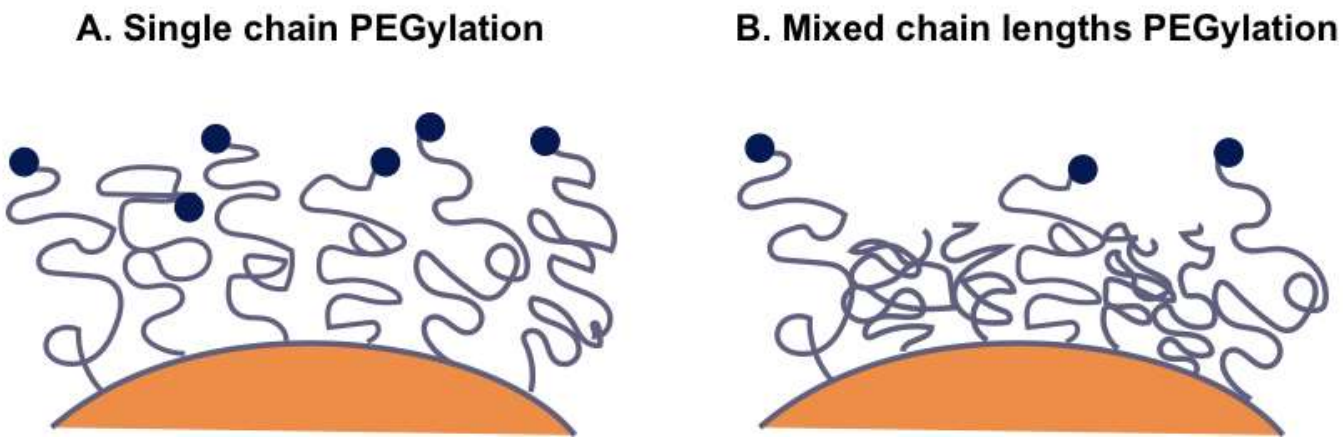
ACKNOWLEDGMENTS:

FCT (PTDC/CTM-NAN/3547/2014)



Picture 1: Caption 1: Figure 1. Targeting moieties grafting strategies.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

538 Biocompatibility study on tunable polyester urethane acrylates

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INTRODUCTION:

Synthetic polymeric biomaterials have been used for medical applications due to the wide range of physical and chemical properties that can be engineered based on monomer units, polymerization reaction, and formation of different co-polymers at adjustable concentration[1]. Despite their common use in the biomedical field, complications may occur due to an interference of residual monomers, degradation-products or additives affecting biochemical pathways[2]. Hence, extensive biocompatibility studies are still necessary. Surface modification techniques provide the possibility to optimize biocompatibility properties of the surface[3] independently of the bulk mechanical properties. Here, we examined as library of biodegradable polyester urethane acrylates (PEUAc) with different molecular weights (Mw) and modified surface properties, by undergoing an *in vitro* biocompatibility study to analyse the suitability of these newly synthesized polymers for tissue engineering purposes.

METHODS:

PEUAc with different Mw (2000, 5000, 8000, 12000 and 20000g/mol) was synthesized by a ring-opening polymerization of ε-caprolactone (CL) and D,L-lactide (LA) with 75:25 feed ratio at 140°C and stannous octoate catalyst at a 2000:1 co-monomer : catalyst ratio. Mw and polydispersity was confirmed with nuclear magnetic resonance and gel permeation chromatography. Mechanical testing measured young's modulus, tensile strength and fracture strain. *In vitro* studies using L929 and human dermal fibroblasts (HDFs) seeded on PEUAc films were analysed with PrestoBlue and DNA assay for metabolic activity, adhesion and proliferation studies, live/dead assay for cell viability, and phalloidin and dapi staining and scanning electron microscopy imaging for actin filaments and cell morphology. Solvent etching and plasma treatment was done to 5000 and 8000g/mol PEUAc films.

RESULTS AND DISCUSSION:

The overall conversion range of the synthesized polymers was 95%-99%. Despite no specific trend seen for young modulus and tensile strength, an increase in Mw of PEUAc increased strain at break capability. PEUAc films of 8000-20000g/mol (Mw) showed higher cell attachment, metabolic activity, and live cells compared to 2000 and 5000g/mol films. Cell spreading was properly seen in 8000-20000g/mol films, with express actin filaments. Variation in cell compatibility on different Mw PEUAc films could be caused by changes in degradation rate and crystallinity, as well as wettability effecting protein absorption. Nevertheless, surface modification techniques were able to improve cell adhesion, proliferation, and spreading on treated PEUAc films, verifying optimized biocompatibility

CONCLUSION:

Synthesis of different Mw PEUAc caused alteration to strain at break and cell behaviour. Surface modification on films provided an optimization to surface biocompatibility, thus improving cell viability.

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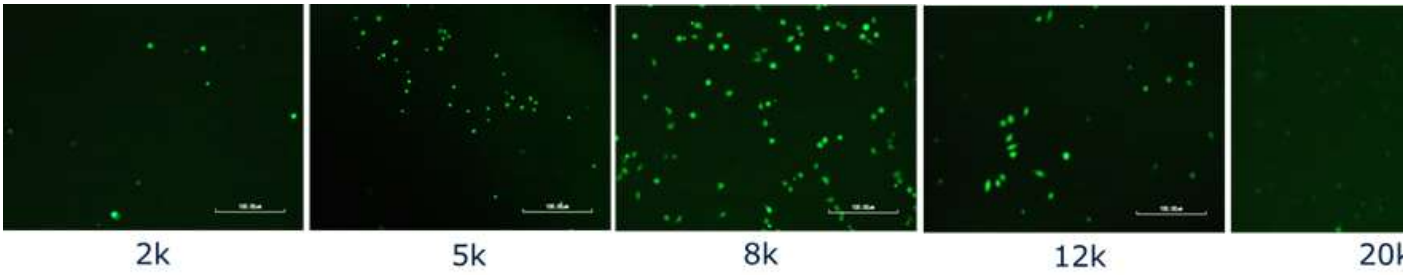
ACKNOWLEDGMENTS:

We thank Brightlands Materials Center for financial support

Picture 1:



Caption 1: Live/dead assay of different Mw PEUAc films, live cells displayed in green

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

556 Natural polymers based antimicrobial thermosensitive nanocomposites hydrogels for wound healing applications

Assunta Borzacchiello[1], Pooyan Makvandi[1], Ghareib W Ali[2], Wafa I. Abdel-Fattah[3], Luigi Ambrosio[1]

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INTRODUCTION:

Thermosensitive based hydrogels has attracted significant research attention in wound healing as they can be easily fill the wound cavities and become gel *in situ* at body temperature (Tb). Thermosensitive amphiphilic block copolymers (Pluronic or Poloxamers), due to their thermo-sensitivity have been applied in biomedical field despite lack of biological clues. Hyaluronic acid (HA) is natural occurring polysaccharide that demonstrated to promote dermal regeneration. used in wound dressings. Being infection a crucial and generally unsolved issue in wound healing, frequently Ag nanoparticles (AgNPs) have been applied to inhibit or decline infections. However AgNps synthesis often doesn't follow green technologies to avoid toxic and non-biocompatible compounds. Corn silk extract (CSE), a waste material of the crop, has been used for AgNPs biosynthesis as both a reducing and stabilizing/capping agent [1]. In this context, we propose novel and green thermosensitive nanocomposites hydrogels based on HA, Pluronics and AgNPs for wound healing applications.

METHODS:

The corn silk were heat extracted. AgNPs were synthesized within CSE by microwave. The hydrogels were prepared by dissolving different amounts of Pluronics F127 and F68 in silk extract with and without AgNPs. Subsequently HA was added. The hydrogels composition was optimised by rheological analysis. The formation of AgNPs was confirmed by UV-Vis surface plasmon band while their morphology was evaluated by DLS and HRTEM. Antibacterial activity and cytotoxicity were investigated *in vitro*.

RESULTS AND DISCUSSION:

Separate Pluronic F127 and F68 solutions do not show an appropriate Tgel but by formulating Pluronic F127/F68 blends at specific concentrations, it was possible to obtain a medium with a gelification temperatute (Tgel) close to Tb (fig.1A). The addition of HA slightly affects Tgel, but very interestingly, improved significantly the final gel viscoelastic properties. AgNPs were spherical with average diameter of 8 nm (fig.1B). The presence of AgNPs didn't alter the hydrogels rheological properties. The AgNPs hydrogels showed an excellent bactericidal activity against the tested Gram-positive and Gram-negative bacterial strains (fig.1C). The results confirmed the bactericidal activity of the samples in comparison with control. The cytotoxicity results showed the AgNps ability to promote vitality and proliferation of L929 fibroblasts.

CONCLUSION:

Novel and green thermosensitive nanocomposites hydrogels with promising bactericidal activity were synthesized for potential wound healing applications.

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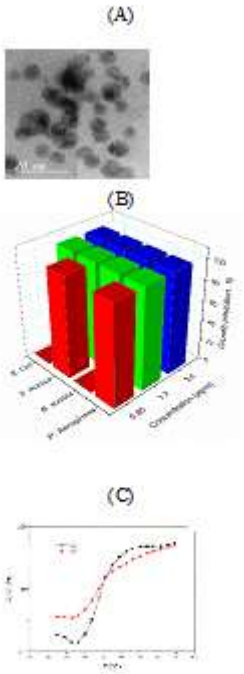
ACKNOWLEDGMENTS:

Italian and Egyptian Ministry of Foreign Affairs and International Cooperation in the frame of the Executive Program of Scientific and Technological Cooperation between Italy and Egypt for the years 2016-2018.



Picture 1: Caption 1: Figure 1. (A) Dependence of viscoelastic moduli of hydrogel upon temperature (B) TEM images of AgNPs (C) antibacterial activity of Ag NPs.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

559 High-throughput screening of optimal osteogenic differentiation conditions in Gellan Gum hydrogel microfibers

Andreia F Carvalho[1], Raquel S Ribeiro[1], Luca Gasperini[1], Lucília P Silva[1], Alexandra P Marques[2], Rui L Reis[2]

[1]3B's Research Group_UM; ICVS/3B's, Guimarães, Portugal

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INTRODUCTION:

The development of improved bone tissue engineering strategies is highly dependent on the evolution of the biomaterials field. The demanding task of continuously assessing the response of cells to new biomaterial-cell reinforces the need to develop screening methods for fast evaluation of experimental readouts. Thus, we have previously developed a multiparametric high-throughput screening platform based on hydrogel microfibers, where parameters such as cell viability and differentiation into defined lineages can be easily assessed, ultimately to define the ideal biomaterial, cellular and culture conditions for a specific tissue engineering application[1]. This work explores our system to screen the influence of marine-origin materials, and its blends with gellan gum (GG), on the osteogenic differentiation of human adipose-derived stem cells (hASCs) aiming at generating an improved bone-like tissue.

METHODS:

Cell-laden hydrogel microfibers of GG, or GG combined with gradients of hyaluronic acid and chondroitin sulphate, were produced and subjected to standard osteogenic differentiation conditions. To evaluate cell responses, cell viability was assessed 24h post-encapsulation as well as at 28d of differentiation to guarantee long-term cell survival. To evaluate mineral deposition, Alizarin Red staining was performed at 14d, 21d and 28d. Osteogenic differentiation was also confirmed and quantified by the OsteoImage® Kit as well as by immunohistochemistry, with osteogenic-related markers Osteocalcin, osteopontin and Alkaline Phosphatase at several time points. Rheological assays were used to determine mechanical properties of GG hydrogel blends at different time points.

RESULTS AND DISCUSSION:

The gradient of materials was successfully established in the GG hydrogel microfibers. High levels of viability were maintained in the generated cell-laden constructs up to time-point 28d. The highest degree of osteogenic differentiation, assessed by deposition of mineralized matrix, was obtained with GG. These results were further confirmed by scaling-up of specific microfiber hydrogels combinations into hydrogels of defined macro-dimensions.

CONCLUSION:

We have successfully generated cell-laden hydrogel microfibers encompassing gradients of materials for bone TE applications. Overall, our platform allows the screening of multiple 3D cell-laden hydrogel compositions and selection of the most favourable combination for osteogenic differentiation. Furthermore, the versatility of the microfluidic system can be extrapolated to other TE scenarios, allowing for a high-throughput/fast assessment of both material and cell combinations.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

571 pH sensitive multi-functional coating for porous tissue scaffold

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[2]School of Pharmacy, Queens University Belfast, United Kingdom

[3]Faculty of Engineering, University of Southampton, United Kingdom

[4]School of Mechanical & Aerospace Engineering, Queens University Belfast, United Kingdom

INTRODUCTION:

In this work, a pH sensitive coating consists of alternative layers of chitosan (CS) and graphene oxide (GO) has been deposited onto a porous polyurethane (PU) scaffold through an electrostatic layer-by-layer (LbL) assembly technique. Such hybrid coating is expected to offer various multi-functionalities to the tissue scaffold, such as degradability, electrical conductivity, drug delivery, etc.

METHODS:

The fabrication process is illustrated in Fig1. PU foam templates (12.7mm in diameter, 10 mm in height) were immersed in 1M NaOH solution for 10 mins, rinsed with DI water. The foams were then dipped in 0.01 M PEI solution for 60 min to generate a primer layer followed by rinsing with DI water. The LbL coating was formed by immersing the foams into GO (2mg/ml) and 0.5 wt% CS solution (in 2% (v/v) acetic acid aqueous solution) alternatively. The immersion time in both solutions is 20 min, and the samples were rinsed and dried between immersions. This procedure was repeated until a desired number of layers were formed, namely (GO/CS)n. For drug release test, GO loaded with model drug FL (Fluorescein Sodium, 1.2 wt%)[1], CS loaded with MB (Methylene blue, 0.2 mg/ml).

RESULTS AND DISCUSSION:

The average coating thickness of single layer is about 65 nm based on SEM and AFM measurements. FTIR results show that the characteristic peaks of PU substrate gradually decrease as the number of bi-layers increases, indicating thicker coating has formed as the LBL assembly process continues. Our multilayer coatings also demonstrated pH dependent drug release, FL can only release at pH 7 and without release at pH 4 even after 9 days. MB can release both in pH4 and pH 7 environment. The diffusion of the FL drug in an acidic solution is unfavorable because GO sheets tend to form tightly packed aggregates[2]. In addition, the hybrid coatings has an electrical conductivity (0.056 S/m) similar to that of ventricular muscle blood, and skeletal muscle[3].

CONCLUSION:

Electrostatic layer-by-layer assembly technique has been successfully deployed to deposit a novel hybrid coating with alternative layers of negatively charged GO nanosheets and positively charged CS onto a porous polyurethane scaffold. The pH sensitive drug release, electrical conductivity and the potential antibacterial properties of the multifunctional coating would enable its broad applications in fields including biomedical/healthcare, environmental and sensing.

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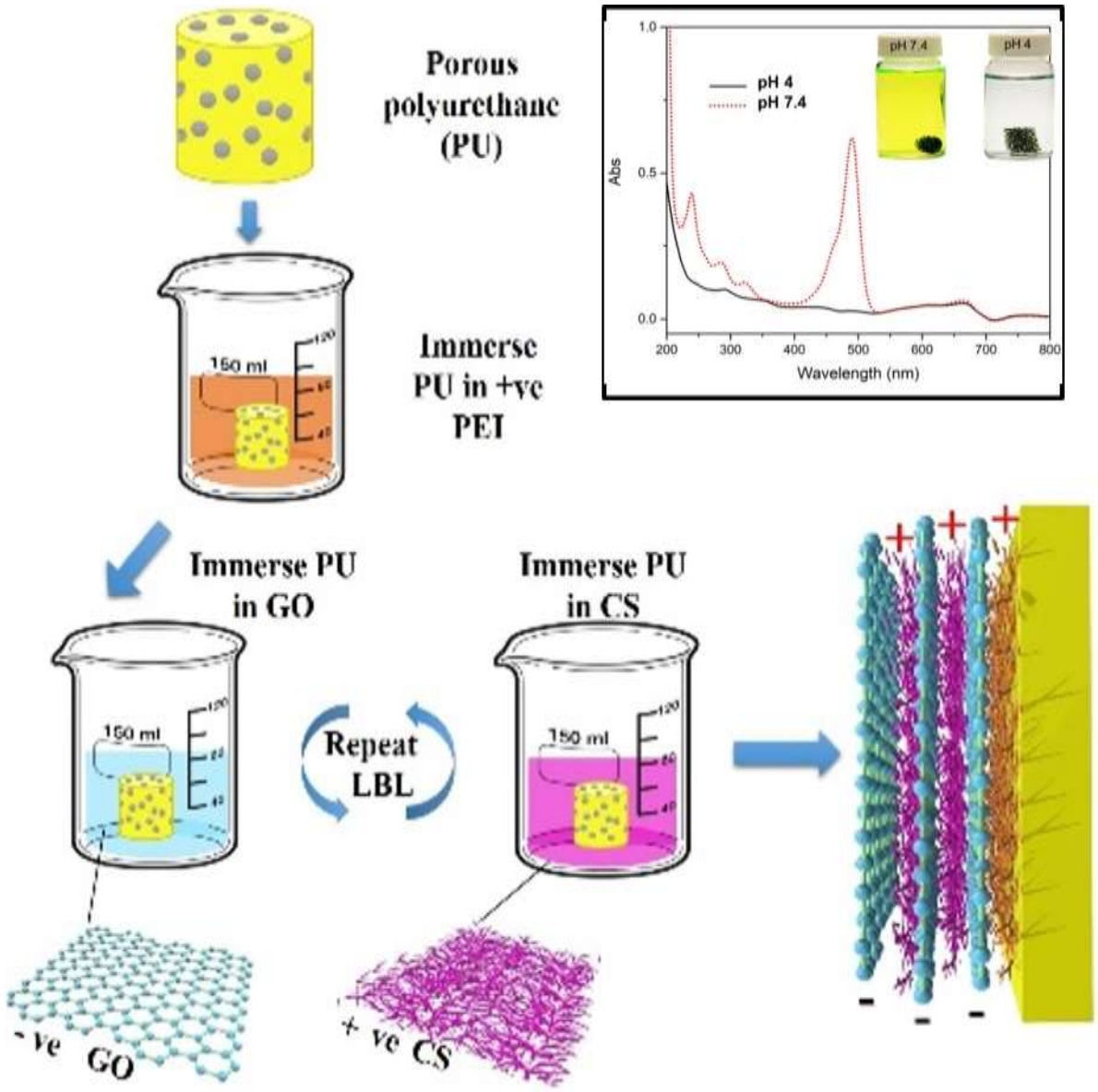
Picture 1:



Caption 1: Schematic of layer-by-layer assembly of CS/GO composites coating on porous PU scaffold

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

596 New design for mechanically resistant Silk Fibroin nerve guides

Marco Biagiotti[1], Giulia Bassani[1], Valentina Vincoli[1], Federica Fregnan[2], Stefano Geuna[2], Antonio Alessndrino[1], Giuliano Freddi[1]

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[2]University of Turin, Italy

INTRODUCTION:

Peripheral nerve damages often lead to permanent disability. Gaps larger than 5mm are treated surgically using artificial conduits[1]. The market is estimated at $1.9bn in the US and $1.8bn in Europe (projected growth at 10% CAGR in the next 5 years). Electrospun silk fibroin (ES-SF) demonstrated the potentiality to be used as guide for nerve regeneration[2], however ES-SF tubes showed very low mechanical properties[3] and their use is still limited to experimental phases. Using a patented technology, different designs of composite electrospun and textile Silk Fibroin (SF) devices were produced and tested as potential peripheral nerves conduits.

METHODS:

Pure SF from *Bombyx mori* cocoons was dissolved in Formic Acid (8% w/v) and electrospun to get tubular conduits (SF-ES). SF yarn (40 den) was used to braid tubular textile matrices (SF-TEXs). According to patented technology (WO2015IB58262 20151027) two SF-ESs were coupled to one SF-TEX to obtain SF NerveGrafts (SF-NGs), as is ($SF\text{-}NG^{-}$) or reinforced with SF thin film ($SF\text{-}NG^{+}$). Mechanical compression tests were performed both in dry state and submersed in water at 37°C (specimens length = 10mm). Morphology and structure of the devices were analyzed by Scanning Electron Microscopy (SEM), Fourier-Transform Infrared Spectroscopy (FT-IR) and Differential Scanning Calorimetry (DSC).

RESULTS AND DISCUSSION:

Compression tests showed that $SF\text{-}NG^{+}$s reinforced with SF film displayed higher compression resistance (Fig. 1.a-b), up to 40% deformation in dry tests and 60% in submersed tests. $SF\text{-}NG^{-/+}$ exhibited lower stiffness in submersed tests than in dry state (Fig. 1.c). SEM observations of $SF\text{-}NG^{-}$ and $SF\text{-}NG^{+}$ showed a multilayer tubular structure with SF-ES in the inner and outer layers and SF-TEX in the middle, with internal diameter of 1.4mm of and wall thickness of 350μm. In $SF\text{-}NG^{+}$ samples, SF microfibers were packed together by SF thin film. FT-IR showed that devices are 100% pure SF with high crystallinity, as confirmed by DSC results.

CONCLUSION:

Literature data demonstrated the high potentiality of Silk Fibroin grafts in nerve regeneration application, however mechanical properties of tested grafts were so far inadequate. Our $SF\text{-}NG^{+}$ showed compression behaviour optimized for the application. Actually, in dry state $SF\text{-}NG^{+}$ exhibited higher compression resistance than current commercially available nerve guidances[4], while in submersed tests stiffness decreased to guarantee a correct physiological flexibility.

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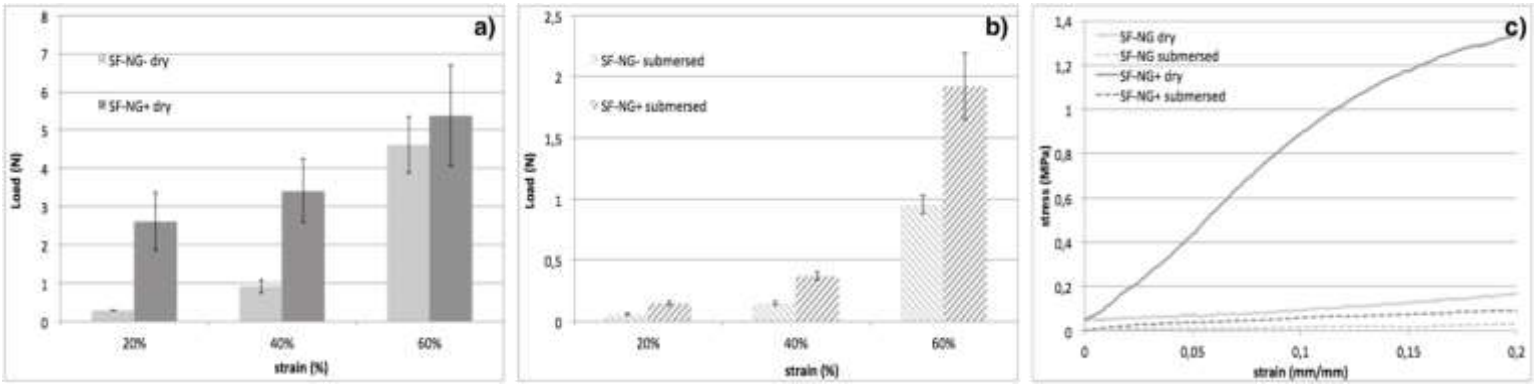

Picture 1: Caption 1: Fig 1: Load at different deformation for SF-NG-/+ in dry condition (a) and submersed (b) and a particular of the stress-strain curves (c)

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Poster presentation

602 Versatile porous hydrogels as matrix for the development of organotypic dermal and full skin models

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INTRODUCTION:

Full-thickness skin models created using 3D matrices are of particular interest for cosmetic screening, transdermal delivery systems or cell-to-cell and cell-to-matrix interactions studies[1]. However, the different matrices proposed still face setbacks such as contraction, inadequate viscoelastic properties and low complexity as compared to skin[2]. To circumvent these drawbacks, an innovative porous hydrogel composed of dendrimers of poly-L-lysine (DGL) and poly ethylene glycol (PEG-NHS) mimicking skin mechanical properties was developed and evaluated as a matrix for the development of dermal and skin equivalents.

METHODS:

Various 3D porous hydrogels (2x8mm discs) were obtained by reticulating DGL and PEG-NHS solutions within paraffin-microspheres templates of different pore size and ratios. Microspheres pores sizes and distribution was measured by image analysis and hydrogel porosity determined by pycnometry. Reconstructed dermis was obtained by seeding human fibroblast on the matrices while full skin equivalents were obtained by subsequent seeding of human keratinocytes on the fibroblast-colonized hydrogels, followed by an air-lift interface. Optimal cell seeding density and methods to obtain homogenous skin equivalents, as well as cell infiltration and proliferation in the 3D matrices was determined by cross section image analysis, DAPI staining and H&E staining. To fully characterize the reconstructed skins, IHC of ECM proteins and keratinocytes differentiation (collagen I, involucrin, K10 and K14) were performed.

RESULTS AND DISCUSSION:

Hydrogels pore-sizes distribution could be controlled by varying the paraffin microspheres diameters and fractions, reaching 75% of porosity. A mixed population of 50-180µm pores resulted in the most effective fibroblasts infiltration, reaching 1 mm after 14 days. Increasing cell seeding density did not increase cell colonization efficiency probably due to cell-cell contact inhibition and optimal density was found to be $1x10^5$ fibroblasts/hydrogel. A homogeneous

cell distribution over time was observed when seeding fibroblasts directly in hydrogel-mounted culture inserts without contraction (fig.1A)

The resulting skin equivalents confirmed the ability of fibroblast to synthetize ECM (collagen I) while a homogenous layer of differentiated keratinocytes and stratified epidermis was validated by the expression of involucrin, K10 and K14. (fig.1B-C).

CONCLUSION:

This versatile porous hydrogel, which is readily colonized by fibroblast, allows the formation of extracellular matrix and supports keratinocytes differentiation into a stratified epidermis, shows potential to develop more complex skin 3D-models. Evaluations with hydrogels varying elasticities are currently on-going to better mimic skin aging.

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ACKNOWLEDGMENTS:

The Rhone-Alpes and CONACyT are acknowledged for financial support.

Picture 1:

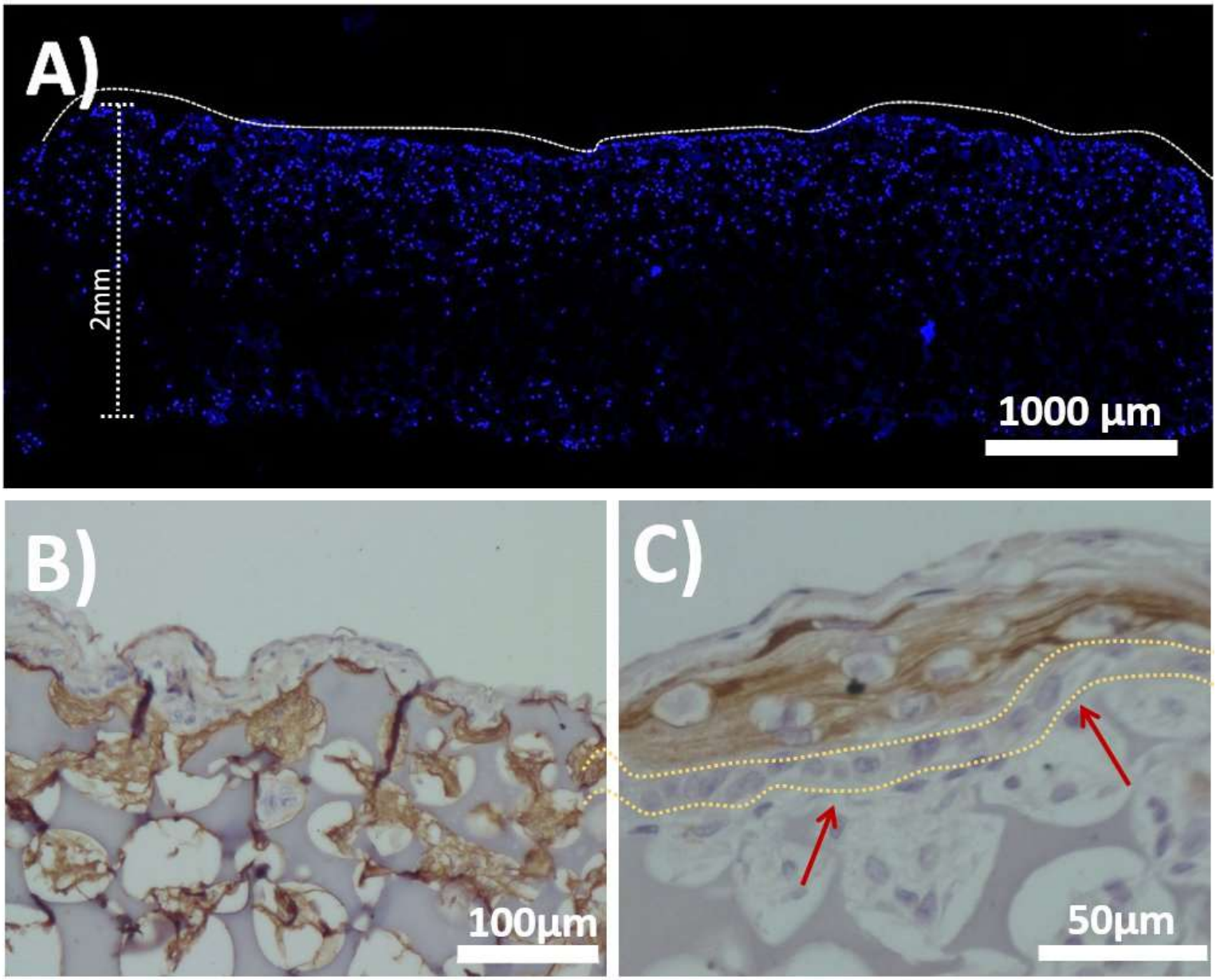

Figure 1. A) Cross sections of dermis equivalents after 21 days of cell culture with human fibroblasts (DAPI, nuclei in blue). B) IHC of collagen I of skin equivalents (brown staining) C) K10 expression by keratinocytes (brown staining) in stratum sipinosum and granulosum and lack of expression in the stratum basale (arrows) confirms a correct epidermis stratification.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

603 In Vivo Screening of Artificial Extracellular Matrices using High-throughput Multi-well Devices

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INTRODUCTION:

The design of artificial 3D-microenvironments that recapitulate key features of native extracellular matrix (ECM) is becoming increasingly relevant. Different types of ECM analogs have been explored so far, and sophisticated 3D matrices with tunable biochemical/physical properties are currently available[1]. Yet, assessing the combined effect of multiple cues in 3D systems is a challenging task. This motivated the development of high-throughput screening platforms to optimize these formulations and test cell response *in vitro*. Likewise, when moving to *in vivo* testing, more efficient screening settings are required to increase the throughput of animal experiments, while complying with the 3Rs policy. Here, hydrogel matrices of natural polymers were tested, using previously developed multi-well devices (MWDs)[2]. Specifically, the *in vivo* behavior of pectin and alginate were compared, which exhibit similar gelling mechanisms but different molecular structures. This allowed the analysis of 12 trial conditions in a single animal, using minimal quantities of materials, proving to be a helpful tool to test/compare different hydrogel formulations.

METHODS:

MWDs (3x3 wells) of poly(ethylene-oxide-terephthalate)/poly(butylene-terephthalate) were prepared by hot embossing (Fig.1A). Wells were filled with different formulations of ionically-crosslinked hydrogels of pectin and alginate (Fig.1B), with comparable molecular weights (M_w), different polymer concentrations (wt%), and different densities of RGD-containing peptides. Four hydrogel-loaded devices were implanted subcutaneously in mice (C57BL/6, n=4/time-point) (Fig.1C), retrieved after one and two weeks post-implantation, and processed by standard histological techniques.

RESULTS AND DISCUSSION:

The spatial confinement of hydrogels within the MWDs allows much easier identification when compared to conventional implantation, where hydrogels can be difficult to locate and retrieve for analysis due to their transparency and eventual degradation. From the provided example of histological analysis (Fig1.D), when comparing pectin and alginate hydrogels with similar M_w and wt%, pectin hydrogels clearly presented more pronounced degradation/fragmentation. This may be related to the highly branched nature of pectin as compared to linear alginate macromolecules, which results in less structured hydrogel networks with larger mesh size. Also, this likely facilitated host tissue ingrowth into pectin hydrogels. Similar degradation/fragmentation profiles were observed regardless of the RGD density used.

CONCLUSION:

The use of high-throughput screening devices for *in vivo* testing of hydrogels represents a promising strategy to refine animal experiments. The present study would have required 24 mice (4 conditions/animal) instead of 8 mice if performed with conventional implantation methods.

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ACKNOWLEDGMENTS:

NORTE2020, PORTUGAL2020, ERDF Programs (Norte-01-0145-FEDER-000012), FCT (PTDC/BBB-ECT/2145/2014, SFRH/BD/76995/2011), Dutch Province of Limburg.

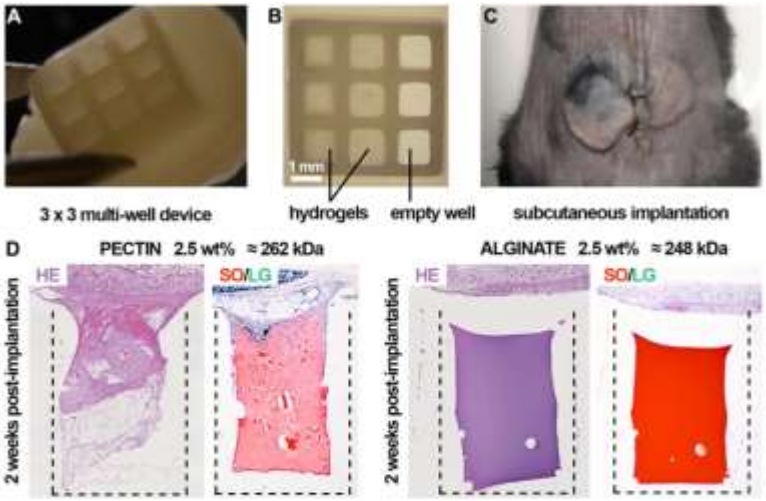

Figure 1. Photographs of (A) an empty multi-well device (MWD) (3x3 wells), (B) a device with hydrogel-filled and empty wells, and (C) devices implanted subcutaneously in the back of a C57BL/6 mouse. (D) Representative histological sections of MWDs loaded with pectin and alginate hydrogels, stained with haematoxylin and eosin (HE) and safranin-O/light green (SO/LG). The dashed lines indicate the walls of the wells.

Picture 1:

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

605 Sol-gel synthesis of porous phosphate-based glasses for tissue engineering applications

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INTRODUCTION:

Phosphate-based glasses are a new generation of biomaterials with great potential for orthopaedic applications[1]. Differently from the silicate-based glasses, the phosphate-based glasses are totally soluble in the body fluids with more controllable degradation rate and easily metabolised dissolution products [2, 3]. Being totally replaced by natural tissues after healing as occurred, they can be used as safe degradable temporary implants. The release of ions such as Ca^{2+} from these glasses can also stimulates cell proliferation and bone formation in bone tissue engineering

applications[3]. The sol-gel method offer homogeneous mixing of the reactants due to the use of precursors in solution and using the non-ionic block copolymer can be leading to highly porous and high surface area glasses for tissue engineering applications.

METHODS:

Sol-gel synthesis was initiated by mixing non-ionic block copolymer with calcium, sodium and phosphorous precursors. The mixture turned to gel after overnight keeping at room temperature. Sample was aged for 3 days, before the temperature was increased to 320 °C to remove the surfactant. The structure of the sample was characterized using wide angle X-ray diffraction (WA-XRD) and low angle X-ray diffraction (LA-XRD), ^{31}P magic angle spinning-nuclear magnetic resonance (^{31}P MAS-NMR), scanning electron microscopy (SEM) and N_2 adsorption surface analysis.

RESULTS AND DISCUSSION:

The sol-gel method allows to prepare glasses in any shape or form (porous foams, fibres, spheres, thin films). The WA-XRD pattern of the gel, shown in Fig. 1a, confirms the amorphous structure of the material. The structure of the phosphate network was investigated using ^{31}P MAS-NMR (Fig. 1b). The SEM image reported in Fig. 1c, shows the presence of extended porosity. Confirmation of porosity is also given by the peak observed in the LA-XRD (Fig. 1d). This is the first example of a porous bioresorbale phosphate-based glass in the P_2O_5-CaO-Na_2O system ever reported.

CONCLUSION:

Porous phosphate-based glass was successfully synthesized for the first time using sol-gel supramolecular chemistry and surfactants. These glasses have a great potential in hard and soft tissue engineering and their porous structure provides high surface area to promote cell adhesion and enable delivery of nutrients into the regenerating tissues. Future work will be undertaken on feasibility of attachment, growing and proliferation of MSCs on these glasses and their potential for drug delivery applications.

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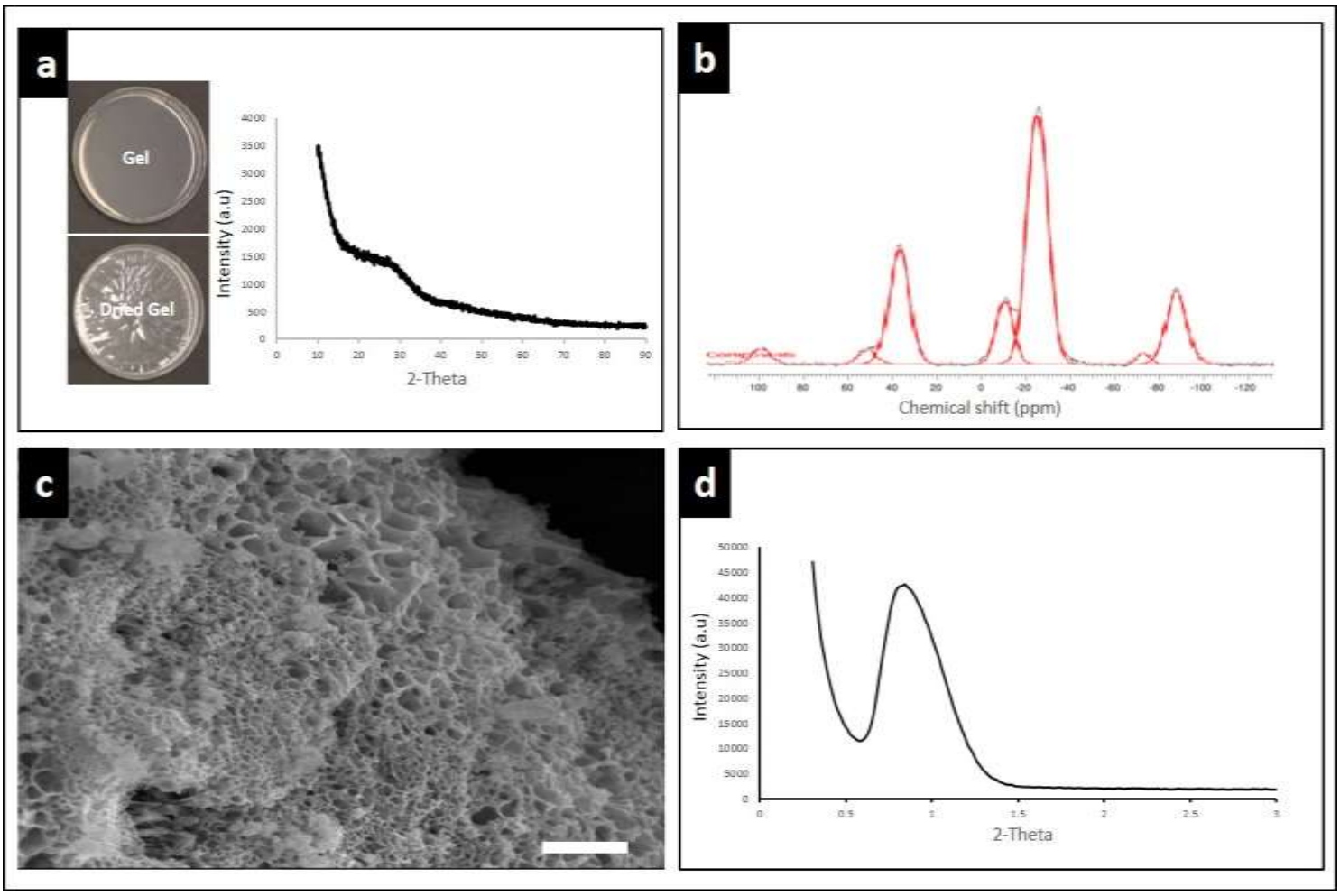
ACKNOWLEDGMENTS:

The authors would like to thank to EPSRC (EP/P033636/1) for providing financial support.



Picture 1: Caption 1: Figure 1: (a) Images of the as-prepared and gel dried at room temperature and WA-XRD after heath treatment, (b) 31P-MAS NMR, (c) SEM image (scale bar

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

608 Galloyl-terminated dendrimers block the formation of amyloid fibrils and rescue cell viability

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INTRODUCTION:

Amyloid plaques, deposits of beta-amyloid (Ab42) peptide, is a hallmark of Alzheimer's disease (AD). These plaques are generated by the self-assembling of Ab42 monomers into supramolecular nanofibrillar structures stabilized by the peptide's β-sheets. For several years these plaques have been considered the basis of cell toxicity that lead to

neuronal cell death in AD. However, recent studies suggest that the neurotoxic effect are derived from the smaller Ab42 protein aggregates, i.e. oligomers [#_ENREF_1, #_ENREF_2].

We have previously demonstrated that specific natural polyphenols (e.g. vescalagin/castalagin) can rescue cell viability affected by the cytotoxicity of Ab42 fibrils. Polyphenols (e.g. EGCG) as modulators of Ab42 fibre formation has been studied, and their ability to block the Ab42 self-assembly process has been reported [#_ENREF_3]. The activity of EGCG is reported to occur through the interference of the Pi-Pi stacking within the Ab42 supramolecular arrangement [#_ENREF_4]. In general, most of the natural polyphenols reported to modulate Ab42 self-assembly present galloyl-type moieties. Based on this observation, we designed dendrimers displaying this type of moiety on their surface and tested them for their ability to modulate Ab42 fibrillization.

METHODS:

Gallic Acid (GA) base dendrimers were synthetized from 3,4,5-trimethoxybenzoic acid and 2,2'-(Etilenodioxi)bis-ethylamine. The capacity of the dendrimers to modulate the Ab42 fibrillization was evaluated by CD, DLS and FS.

Cell studies were conducted with SHSY-5Y neuroblastoma cell line. The evaluation of the capacity to rescue cell viability in the presence of cytotoxic concentrations of Ab42 due to the reduced formation of nanofibers and/or oligomeric structures was executed by Live/dead assay, alamarBlue® assay and immunostaining.

RESULTS AND DISCUSSION:

Dendrimer GA-G1 with six galloyl units (fig1A) is able to reduce the aggregation of Ab42 (fig1B), while decreasing the β-sheet content of the Ab42 supramolecular assemblies (measured by CD), compared with GA-G0 (presenting only two gallate units). These results suggested that the activity is directlty correlated with the number of galloyl units present in the dendrimer. Cells studies, confirmed these assumption, since GA-G1 (and not GA-G0) has the capacity of rescue SH-SY5Y cell viability in the presence of Ab42, reducing the oligomeric Aβ42 assemblies in the cytoplasm of the cells (fig1C).

CONCLUSION:

Our results suggest that galloyl-terminated dendrimers are a promising versatile platform to design nanodevices able to reduce the toxicity of Ab42 assemblies in the AD context.

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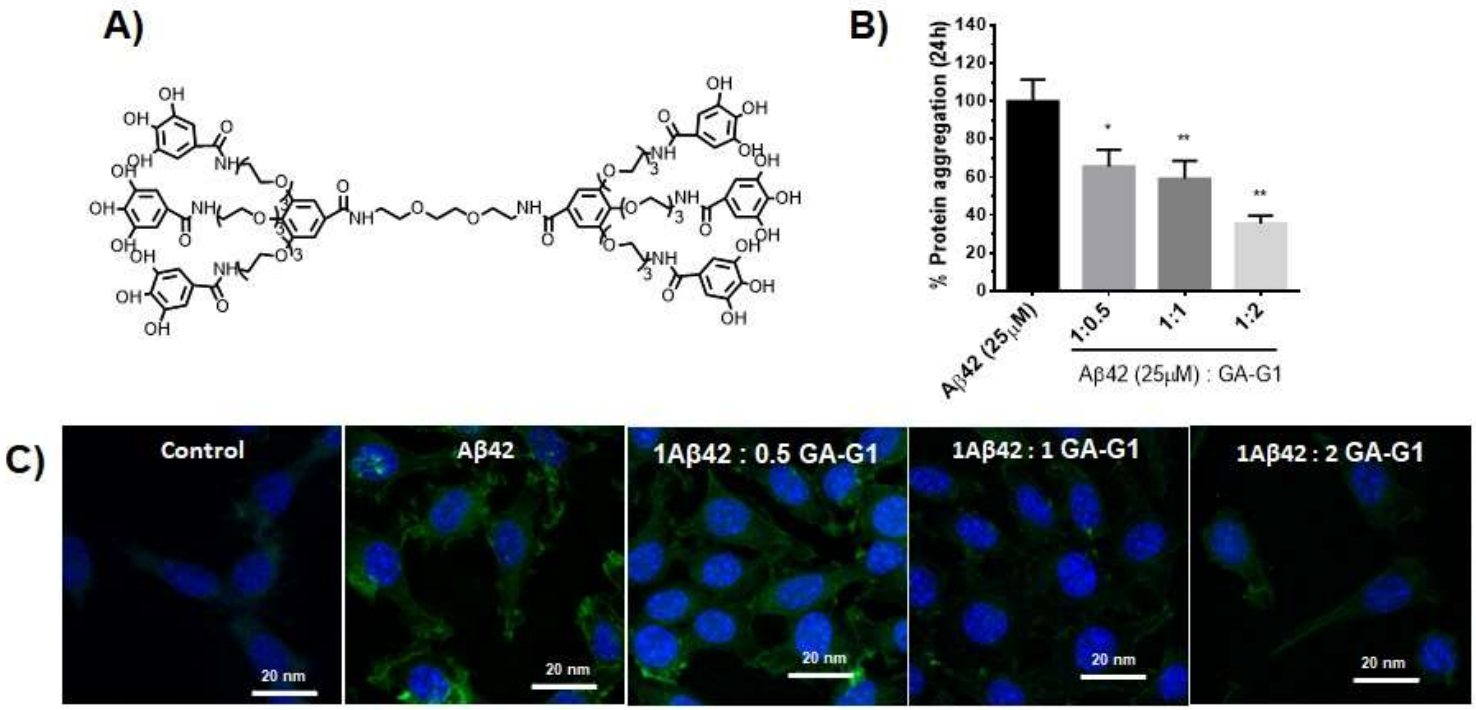
ACKNOWLEDGMENTS:

The authors acknowledge the financial support from:NORTE-08-5369-FSE-000037 - “Programa Operacional Norte 2020”; European Union: H2020-TWINN-2015-692333-CHEM2NATURE, H2020-WIDESPREAD-2014-2-668983-FORECAST. Spanish MINECO:CTQ2015-69021-R; GRC2014/040 cccreditation 2016-2019, ED431G/09 (ERDF).



Picture 1: Caption 1: Fig1 - A)chemical structure of GA-G1;B) Ab42:GA-G1 aggregation analysed by FS; C) Immunostaining with anti-Ab42_1-16 (green) for GA-G1.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

612 Efficacy of Biphasic Calcium Phosphate with Submicron-Scale Surface Topography as Autograft Extender in a Rabbit Posterolateral Lumbar Spine Fusion Model

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INTRODUCTION:

Posterolateral lumbar fusion (PLF) by autologous bone graft is a common procedure for the treatment of degenerative spine conditions. However, because autograft is associated with considerable disadvantages (e.g. donor-site morbidity[1]), alternative technologies are explored. Calcium phosphates (CaP) with submicron-scale

surface topography have attracted attention due to their excellent performance in regeneration of bone defects[2]. The current study investigates a novel CaP with submicron-scale surface topography as autograft extender in the 'Boden' rabbit PLF model.

METHODS:

Sixty skeletally mature New Zealand white rabbits were divided into three groups and underwent bilateral intertransverse process PLF at L4-5. Graft materials were BCP granules (HA/TCP 30/70) with submicron-scale surface topography, either alone or combined with a fast-resorbing polymer binder, mixed with iliac crest autologous bone (ABG) at a volume ratio of 50%:50%. The third group was treated with 100% ABG as reference. At 0, 6, 9 and 12 weeks post-surgery, spinal fusion was determined by manual palpation (MP), X-ray and micro-CT (Lenke scale), mechanical testing (range of motion testing at 12 weeks only) and histology.

RESULTS AND DISCUSSION:

All groups showed an equivalent and gradual progression in bone formation and implant resorption during time, leading to mature fusion masses at 12 weeks. Bilateral fusion rates by MP were consistent with literature (0-20% at 6 weeks; 60% at 9 weeks; 75% at 12 weeks) and similar trends were seen by radiographic assessment (both X-ray and micro-CT). Mechanical testing at 12 weeks demonstrated fusion rates consistent with MP results. By histology, from 6 weeks onwards, progressive bone formation was observed in all groups, with developing marrow spaces after 9 and 12 weeks. Resorption of BCP granules was evident with resorbing multi-nucleated cells on the material surface and a decrease in material proportion over time. Histological fusion, i.e. continuous bone bridge between the transverse processes was equivalent for all groups with 0-20% at 6 weeks to 20-40% at 9 weeks and 75-87.5% at 12 weeks.

CONCLUSION:

These results show that BCP with submicron-scale surface topography could achieve equivalent fusion rates to ABG when used as graft extender in a rabbit PLF model. During time, a progression in bone healing, graft resorption and remodeling was seen at all endpoints, with consistency between the groups.

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ACKNOWLEDGMENTS:

The authors thank the European Union's Horizon 2020 research and innovation program for the financial support to this project (grant agreement no. 674282).

Picture 1:

Table 1. *Fusion grades assessed according to Lenke scale using X-ray and micro CT scan. "A", "Gr" and "P" stand for autograft, granules and putty, respectively.*

		6 weeks				9 weeks				12 weeks			
		A	B	C	D	A	B	C	D	A	B	C	D
A	X-ray	-	1 / 5	4 / 5	-	2 / 5	3 / 5	-	-	6 / 8	2 / 8	-	-
	CT	-	1 / 5	4 / 5	-	2 / 5	3 / 5	-	-	7 / 8	1 / 8	-	-
Gr + A	X-ray	-	1 / 5	4 / 5	-	2 / 5	3 / 5	-	-	7 / 8	1 / 8	-	-
	CT	-	-	5 / 5	-	3 / 5	2 / 5	-	-	8 / 8	-	-	-
P + A	X-ray	-	2 / 5	3 / 5	-	3 / 5	2 / 5	-	-	6 / 8	2 / 8	-	-
	CT	-	-	5 / 5	-	2 / 5	3 / 5	-	-	7 / 8	1 / 8	-	-

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

616 Tuning nanopore diameter of titanium surfaces to improve human gingival fibroblast response

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INTRODUCTION:

Nowadays one of the main aspects in which dental implant science has focused is in the obtention of an effective integration of implant abutment in order to prevent periimplantitis, the most important cause of long-term failure of dental implants. Self-organized titanium dioxide (TiO_2) nanostructures arrays provide the possibility of improving the properties of these implants promoting soft tissue integration. To produce TiO_2 nanostructures, electrochemical anodization is the most widely used method. Many factors during anodization affect the growth and the diameter of nanostructure formation, which may affect the cellular response. The aim of the present study was to obtain an optimal pore diameter for improved human gingival fibroblast response.

METHODS:

Titanium dioxide nanoporous structures with different pore diameters (26 to 64 nm) were grown on Titanium (Ti) foils using an organic electrolyte containing fluoride by electrochemical oxidation, varying the applied voltage and the inter-electrode spacing. The obtained surfaces were characterized by SEM, AFM and contact angle.

Human gingival fibroblasts (HGF) culture assays were carried out in order to analyze the effects of the different nanoporous diameters by metabolic activity, cytotoxicity, cell adhesion, gene expression of genes related to the synthesis and organization of the extracellular matrix and collagen deposition.

RESULTS AND DISCUSSION:

Larger porous diameters were obtained by increasing the used voltage during anodization. In order to obtain the smallest diameter, apart from lowering the voltage a lower inter-electrode spacing was needed.

The in vitro results using HGFs showed that the larger diameter nanoporous (47 and 64 nm) had a better cellular behavior involving cell adhesion, proliferation, gene expression and collagen deposition compared to smaller diameter nanoporous (26 nm). However, these effects had a significant dependence on the cell donor.

CONCLUSION:

Nanoporous in the range of 47 - 64nm of diameter induce a better gingival fibroblast response than unstructured control Ti surfaces or nanostructured surfaces with a smaller (26nm) porous diameter.

ACKNOWLEDGMENTS:

This work was supported by the Osteology Foundation (13-069), the Ministerio de Educación Cultura y Deporte (contract to M.A. L.G; FPU15/03412), the Instituto de Salud Carlos III (contract to J.M.R;CP16/00124), and the Ministerio de Empleo y Seguridad Social with the Sistema de Garantía Juvenil (contract to M.M.F.C). The authors thank Dr. F. Hierro and Dr. J. Cifre (UIB) for their technical contribution with SEM and AFM respectively.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

622 Characterisation of mechanical properties of hydrogels for 3D haematopoietic stem cell culture

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INTRODUCTION:

Haematopoietic stem cells (HSC) are responsible of generating the full complement of blood cells. These stem cells, like many others, are capable of sensing their 3D environment and interact with other cells and extracellular matrix (ECM) [1]. Although widely accepted that physical stimuli are affecting Mesenchymal stem cell behaviour, surprisingly little is known about biophysical cueing of hematopoietic stem cells [2]. It has become clear that cell proliferation and differentiation are not just affected by soluble factors but also by physical cues such as matrix stiffness, characterised by the Young Modulus (E). Therefore it is of great importance to develop biomaterials capable to mimic the stiffness of natural soft tissue, in the range of kPa and below, to support stem cells. While the mechanical properties of hard scaffolds (E > 1MPa) are relatively straightforward to measure, little attention has been paid to soft scaffolds for cell culture (E < 30 kPa) due to the challenges imposed by the fragility of the material.

In an attempt to mimic the physiological properties of the niche microenvironment, we have prepared 3D hydrogels based on Polyethylene Glycol (PEG) with Young modulus values matching those of HSCs and studied their mechanical properties and interactions with stem cells. Since it is known the bone barrow niche stiffness ranges around 30kPa, this has been the target of our biomaterial.

METHODS:

In this work we probed the local properties of hydrogels by Atomic Force Microscopy (AFM). AFM is a valuable, non-destructive technique allowing obtaining stiffness maps while simultaneously providing morphological information with nanometer resolution [3]. Force-distance curves (FC) were recorded by vertically indenting the AFM tip onto the surface of the material within a selected area, while hydrogels were immersed in PBS at room temperature. To evaluate the mechanical properties influence on cells, bone marrow HSC were encapsulated and cultured in hydrogels. Cells were cultured within the gels for a period of 6 days and their proliferation was quantified with confocal microscopy, while the change in mechanical properties of the gels with cells was assessed by AFM.

RESULTS AND DISCUSSION:

The average Young Modulus obtained for hard PEG gels was 30 kPa while for soft samples, an average E of 0.5 kPa was obtained. PEG hydrogels exhibit a relatively flat surface with peak roughness of 500 nm. By studying the HSC culture in hard and soft PEG gels, we are evaluating the effect of the matrix mechanical properties on the fate of these cells.

CONCLUSION:

In this work, we present the mechanical properties of hydrogels prepared to mimic the HSC niche microenvironment and the preliminary data on cell viability and proliferation of HSCs embedded in these materials. These results contribute to the development of biomaterials capable of mimicking the physical parameters of the HSCs microenvironment, a step that to our knowledge, has not been considered for the in-vitro culture of HSC.

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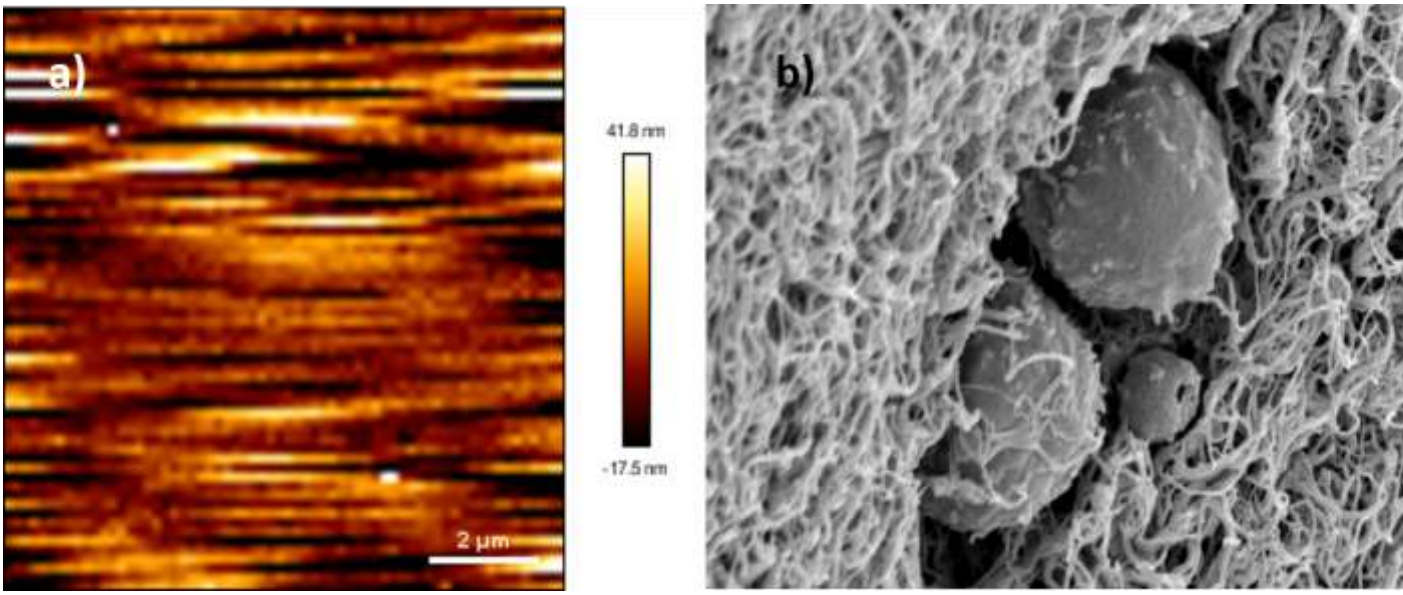
Picture 1:



Caption 1: Figure 1. AFM surface topography of PEG gels (a), electron micrograph of HSC cultured within fibrin gels (b).

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

631 Lung-on-a-chip platform recapitulating lung cancer pathology

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Ege University, Izmir, Turkey

INTRODUCTION:

Lung is one of the most challenging organ system to study *in vivo* due to its mechanically dynamic nature and presence of a variety of physical forces.**1** Lung-on-a-chip platform is especially suited for the *in vitro* simulation of physiological environments involving flow and shear stress. Flow-derived mechanical stimulation controls fundamental biological processes such as cell differentiation, proliferation, migration and adhesion. Cultivation of adherent cells under perfusion reflects their physiologic environment more closely than traditional static conditions.**2** Hence, flow stimulation is crucial for proper mimicry of vascular tissue as cells are strongly influenced by the blood flow.**3-4** This study aims to investigate the effect of a potential drug molecule on lung carcinoma cell lines using a dynamic microfluidic chip platform to mimic lung cancer.

METHODS:

In this study, the cytotoxic activity and IC_{50} values of panaxatriol were determined on A549 (human lung carcinoma cell lines) and MRC-5 (human normal lung fibroblast cell lines) cells by MTT assay. Peristaltic pump was used in order to generate microfluidic dynamic systems. For these, two Ibidi µ–Slide I Luer microchip were fed with drug-free medium (for control) and drug medium continuously. Cells were seeded into the channel of microchip at $1x10^5$ cell/100 ml and the flow was applied after three days of effective cell growth at 2 ml/min for 48h. Morphologies of cells were observed by light microscopy and cell counts were evaluated by ImageJ analysis. Cell viability of the groups was also compared with the MTT test at the end of the study period.

RESULTS AND DISCUSSION:

The cell viability, morphology and proliferation results in the dynamic system revealed higher cytotoxic effect of panaxatriol (97.26 µM) on cancer cells than the healthy cells in comparison to the stationary system (**Fig. 1**).

CONCLUSION:

Application of such mimetic dynamic systems will contribute to advancing basic research and increasing the predictive accuracy of potential drug molecules which may accelerate the translation of novel therapeutics to the clinic, possibly decreasing the use of animal models.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

634 Polymer microparticles and mechanical-biological approach to optimize the use of stem cells in neurodegenerative medicine

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INTRODUCTION:

Pharmacologically Active Microcarriers**1** are polymeric microcarriers providing a biomimetic 3-dimensional surface to enhance stem cell response. The marrow isolated adult multilineage inducible stem (MIAMI) cells, that can be differentiated towards a neuronal phenotype on a laminin (LM) surface**2** and secrete many repair factors are candidates of choice for cell therapy studies. Recently, it has been shown that MSCs located on the surface of PAMs composed of PLGA-P188-PLGA with a fibronectin survived 7 days longer than on the surface of PLGA**3**. However, it still remains unclear how the physico-chemical microcarries surface properties affect on the behavior of MIAMI cells.

METHODS:

The PAMs were functionalized with LM, and poly-D- Lysine (PDL), which provide them with a biomimetic surface to promote cell adhesion. In the current work, the PAMs were characterized for size, zeta potential, surface topography and roughness by atomic force microscopy, and surface morphology by scanning electron microscopy. Furthermore, the LM coating of PAMs was studied by fluorescent confocal microscopy imaging, and Time-of-Flight Secondary Ion Mass Spectrometry.

RESULTS AND DISCUSSION:

AFM and SEM results indicate that PLGA-P188-PLGA microcarriers, have a rough surface compared to the PLGA that have a smooth surface with the presence of holes. The microcarriers exhibit a negative zeta potential, and after coating with LM combined with PDL, a positive zeta potential was acquired for both types of polymer. According to our confocal microscopy results, the LM adsorption is decreased by the presence of the Poloxamer188, but is enhanced on the PAMs composed of PLGA alone. The E/F MIAMI cells adhered and presented a flattened morphology on the surface of PAMs-PLGA-LM; in comparison, the E/F MIAMI cells adhered less and maintain their round shape on the surface of PAMs-PLGA-P188-PLGA-LM, which could be explained by the low adsorption of LM on these surfaces. The cell survival results indicate that the E/F MIAMI cells have a higher survival on the surface made of PLGA-P188-PLGA than on the surface made of PLGA. Differentiation of E/F MIAMI cells analysis is underway

CONCLUSION:

In conclusion, the chemical composition and the surface physico-chemical properties of the microcarriers significantly influence the adsorption of the extra-cellular matrix molecules, and has an effect on the E/F MIAMI cells adhesion on their surfaces.

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ACKNOWLEDGMENTS:

The authors are grateful for the “La région des Pays de la Loire” for financial support in this project through the funded project MECASTEM

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

638 A new strategy for osteomyelitis treatment using nanohydroxyapatite/collagen biocomposite

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INTRODUCTION:

Osteomyelitis is a bone infection caused by a diversity of micro-organisms, being the *Staphylococcus aureus* the pathogen most commonly associated to this disease. The standard procedure to treat this clinical situation involves a surgery for debridement of the infected tissue followed by the parental administration of an antibiotic. Vancomycin is a common choice due to its efficacy for *S.aureus.* However, there are several flaws in this clinical approach, such as the need for at least two surgeries, intravenous administration of the drug in hospital during several weeks and up to 40% recurrence[1,2]. A local drug delivery system (vancomycin loaded nanohydroxyapatite/collagen biocomposite), that also works as a scaffold for bone regeneration, was produced to improve osteomyelitis treatment.

METHODS:

Materials were produced as granules as described by Coelho et al.[3] at two sintering temperatures, 830 °C and 1050 °C, and characterized using SEM, FTIR, Mercury Porosimetry, Micro-CT and mechanical tests.

Vancomycin kinetics release was assessed. The antibacterial activity was evaluated using methicillin resistant *Staphylococcus aureus* (MRSA).

RESULTS AND DISCUSSION:

NanoHA/collagen granules presented macro- micro- and nanoporosity (Figure 1). However, granules sintered at 830 °C possessed higher percentage of nanoporosity, thus generating a higher actual surface area. On the other hand, for 1050 °C, granules showed higher macroporosity and interconnectivity, better mechanical strength and better integrity when handled. For both materials, the vancomycin release profile obtained had a high initial burst followed by a sustained release for 19 days, with concentrations always above MIC for MRSA. As the granules sintered at 1050 °C had lower porosity and surface area, they released less vancomycin. However, in both cases, the antibiotic totally inhibited MRSA growth.

CONCLUSION:

The sintered granules at both temperatures proved to be adequate for osteomyelitis treatment. Both granules were able to release vancomycin in concentrations capable of eradicating MRSA. However, a balance between the

characteristics of the materials as handling properties and mechanical resistance that are crucial for surgical manipulation and the respective antimicrobial properties must be achieved. Therefore, granules sintered at 1050 °C were considered to be the most promising.

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ACKNOWLEDGMENTS:

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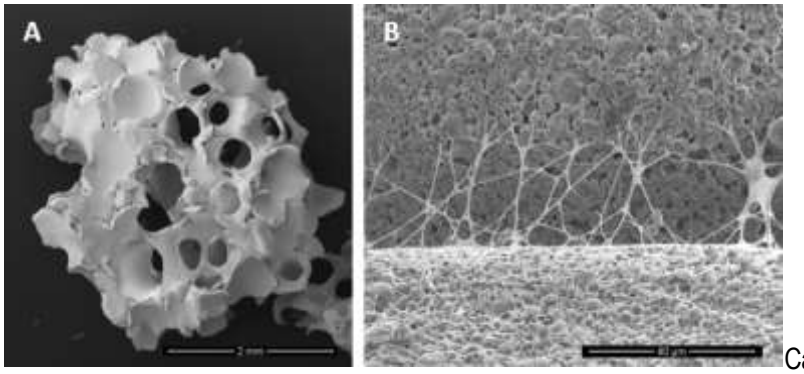
Picture 1: 

Caption 1: SEM images from nanoHA/collagen granules. (A) granule morphology evidencing the presence of interconnective macroporosity; (B) collagen distribution.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

643 Bone formation instructed by a novel epitaxial polygons surface structure

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INTRODUCTION:

Next to using growth factors and cytokines, the possibility to instruct specific histological responses for tissue regeneration through physical design of scaffolding materials has been shown [1]. By controlling the dimension of surface architecture to submicron scale, synthetic calcium phosphate ceramics could be made osteoinductive to

repair of critical-sized bone defects [2]. By keeping the surface structure dimension similar but varying the shape of the surface crystals, we address the possible role of surface morphology in material-driven bone formation.

METHODS:

Together with positive TCP-S and negative TCP-B [2], a material (namely NCS-CaP) with needle-like crystal surface structure, were characterized with their chemistry, surface structure, calcium release and protein adsorption, followed with intramuscular implantation in canine (n=8) for 12 weeks. Fluorochromes were used to monitor the onsets of bone formation in vivo. Explants were subjected to histology and histomorphometry regarding the amount of the bone formed at week 12 and the onset of bone.

RESULTS AND DISCUSSION:

The three materials varied with chemistry, surface morphology and surface structure dimension (Figure 1A), NCS-CaP had needle-like crystals with the similar size of the grains on TCP-S surface, and released less calcium ions and adsorbed similar amount of protein by volume, as compared with TCP-S. Histology and histomorphometry showed that similar amount of bone was found in all TCP-S and NCS-CaP (24.5±4.3% and 23.9 ± 6.3% res.), but in none of TCP-B explants at week 12. Fluorescent observation revealed earlier bone formation in NCS-CaP (Figure 1B), indicating NCS-CaP had higher osteoinductive potential as compared to TCP-S. With the similar protein adsorption between NCS-CaP and TCP-S, the less calcium release in NCS-CaP indicated that the earlier bone formation was not assigned to their different chemistry. Given the similar crystal size, the earlier bone formation in NCS-CaP was likely the outcomes of the unique surface morphology created by the needle-like crystals.

CONCLUSION:

The results indicate under the similar surface structure dimension, the surface morphology play a role in material-driven inductive bone formation.

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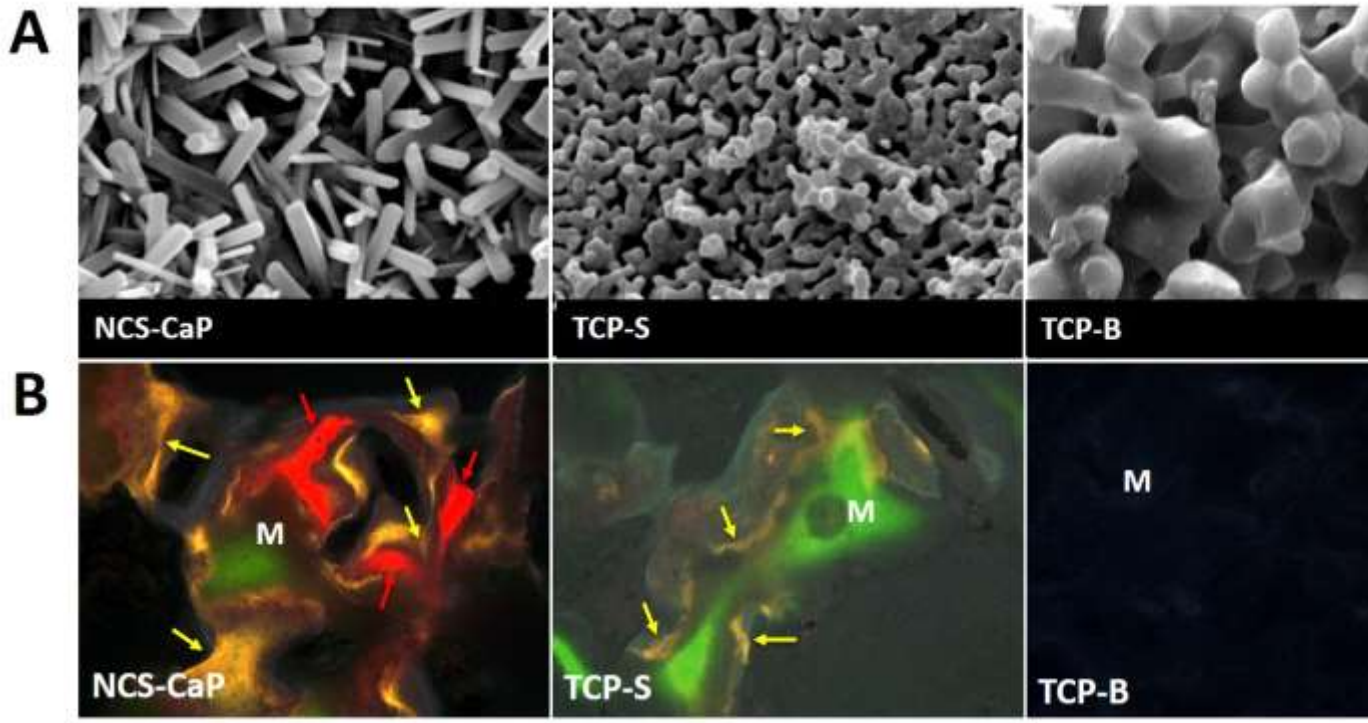
ACKNOWLEDGMENTS:

The authors would like to thank the European Union’s Horizon 2020 (no. 674282) for their financial support in this study.

Picture 1:



Caption 1: Figure 1. A: SEM images showing the surface structure of the ceramics; B: Onsets of bone formation shown with fluochrome labelings.

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Poster presentation

664 Macrophage-mediated degradation of poly-trimethylenecarbonate is influenced by the 3D environment

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UMCG, Groningen, Netherlands

INTRODUCTION:

The polymer poly-trimethylenecarbonate (pTMC) is readily degradable by macrophages[1]. This makes pTMC an ideal model polymer to investigate which conditions influence the degradation activity of macrophages, even with fibroblasts[2] and molecules that mediate inflammation. Although *in vitro* studies often involve the two-dimensional approach of culturing the cells on top of the material, it is recognized that a 3D environment may show more similarity with the *in vivo* situation. In this study macrophages and fibroblasts were cultured in a 3D collagen gel environment while potentially interacting with pTMC in order to determine if these conditions influenced macrophage-mediated degradation.

METHODS:

pTMC polymer discs were prepared from solution. J774 mouse macrophages and L929 mouse fibroblasts were used. Collagen type I gels (Purecol) were prepared. Gelation occurred within 1-2 hours. Based on this time the different steps necessary to add cells (on top, mixed in or directly on the sample with the gels added later) were performed in sequential order. Incubations were done for 6 days with medium changes at day 2 and day 4. Supernatants of days 2, 4 and 6 were stored for cytokine determination. At day 6 samples were fixed. Cell/gel combinations were stained with phalloidin (actin cytoskeleton) and DAPI (nuclei) and imaged with confocal microscopy. Afterwards, the end weights of the pTMC were determined and the percent degradation calculated.

RESULTS AND DISCUSSION:

The use of collagen gels on top of macrophages interacting with pTMC increased the degradation of pTMC. When macrophages were given on top of the gel degradation was strongly reduced. Macrophage migration into the gels did not occur at a significant level, whereas fibroblasts were more prone to move in the gels. With the presence of fibroblasts the macrophages on the pTMC degraded pTMC at clearly reduced rates. In the case of macrophage / fibroblast interaction the release of both IL-6 and TNF-α was enhanced with the exception of macrophages seeded on top of the gels (Fig. 1). Detailed confocal microscopy image stacks are available that shed more light on the behavior of both cell populations.

CONCLUSION:

The three-dimensional environment in which the macrophages are situated determines the level of pTMC degradation, even if the macrophages are in direct contact with the polymer.

REFERENCES:

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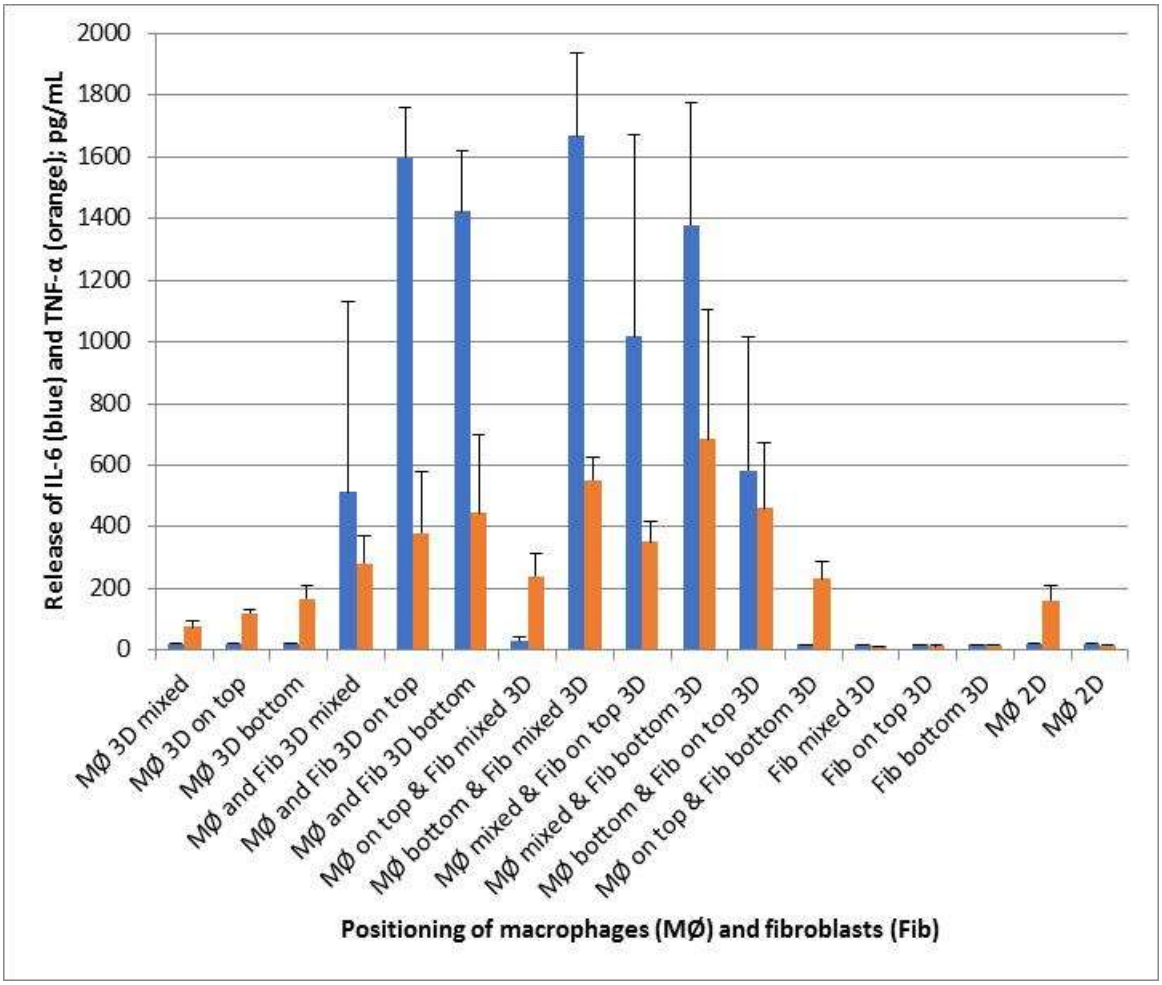
ACKNOWLEDGMENTS:

The author would like to thank the support of bachelor students medicine that performed part of the study in their science elective.

Picture 1:



Caption 1: Figure 1: Release of cytokines IL-6 and TNF-α as a function of positions of macrophages and fibroblasts, probed at day 6 of incubation, with a medium.

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Poster presentation

683 Mechanical stimulation promotes in vitro 3D bone tissue formation of human adipose derived stromal cells on silk fibroin scaffolds

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[2]Technische Universiteit EindhovenEindhoven University of Technology, Netherlands

INTRODUCTION:

Bone marrow derived mesenchymal stromal cells (MSCs) are commonly used in bone tissue engineering (BTE) due to their potential to promote bone regeneration. However, adipose derived MSCs (ASCs) demonstrate several advantages such as low age-dependent senescence and better angiogenic properties in vivo. Mechanical stimuli being increasingly important for BTE make the mechanosensitivity of ASCs a promising attribute. A challenge is to

induce 3D tissue deposition which comprises both cellular (osteoblasts/osteocytes) and extracellular matrix (ECM) components of bone. Especially osteocytes, major orchestrators of bone remodeling, are challenging to study due to their location. We used dynamic culture conditions, employing fluid-induced wall shear stress, to differentiate human ASCs on silk fibroin scaffolds into osteoblasts and further into bone matrix embedded osteocytes.

METHODS:

Porous silk fibroin scaffolds [1] were seeded with human ASCs (4 donors) and cultured statically or dynamically for 4 weeks in osteogenic medium in spinner flasks. Collagen, GAG and mineral deposition were determined with histological stainings and FTIR. Micro-computed tomography (μCT) was used to quantify mineral deposition. Histological sections were stained for osteocyte markers (podoplanin, DMP-1, sclerostin). Collagen and mineral deposition was imaged in 3D constructs using fluorescent microscopy.

RESULTS AND DISCUSSION:

μCT imaging showed static culture leading to low mineral deposition (0.02±0.02 mm^3) which was significantly increased by dynamic culture (0.48±0.32 mm^3) (Fig. 1). Histological analysis showed low ECM deposition in static constructs while dynamic culture increased collagen, calcium and GAG secretion (Fig.1). Only dynamic culture conditions showed osteocyte markers, as demonstrated by immunohistochemistry. 3D fluorescent microscopy showed mineral formation nucleating in a dense collagen network. The mineral was characterized as carbonated hydroxyapatite by FTIR.

CONCLUSION:

Human ASCs react to mechanical forces in a 3D setting, which is a crucial characteristic of the bone environment. Only under dynamic culture conditions, human ASCs differentiated to osteoblasts which deposited and embedded themselves into bone matrix and differentiated further into osteocytes. Our novel system allows investigating bone matrix deposition as well as osteogenic differentiation and demonstrates the remarkable potential of human ASCs to advance humanized 3D BTE systems.

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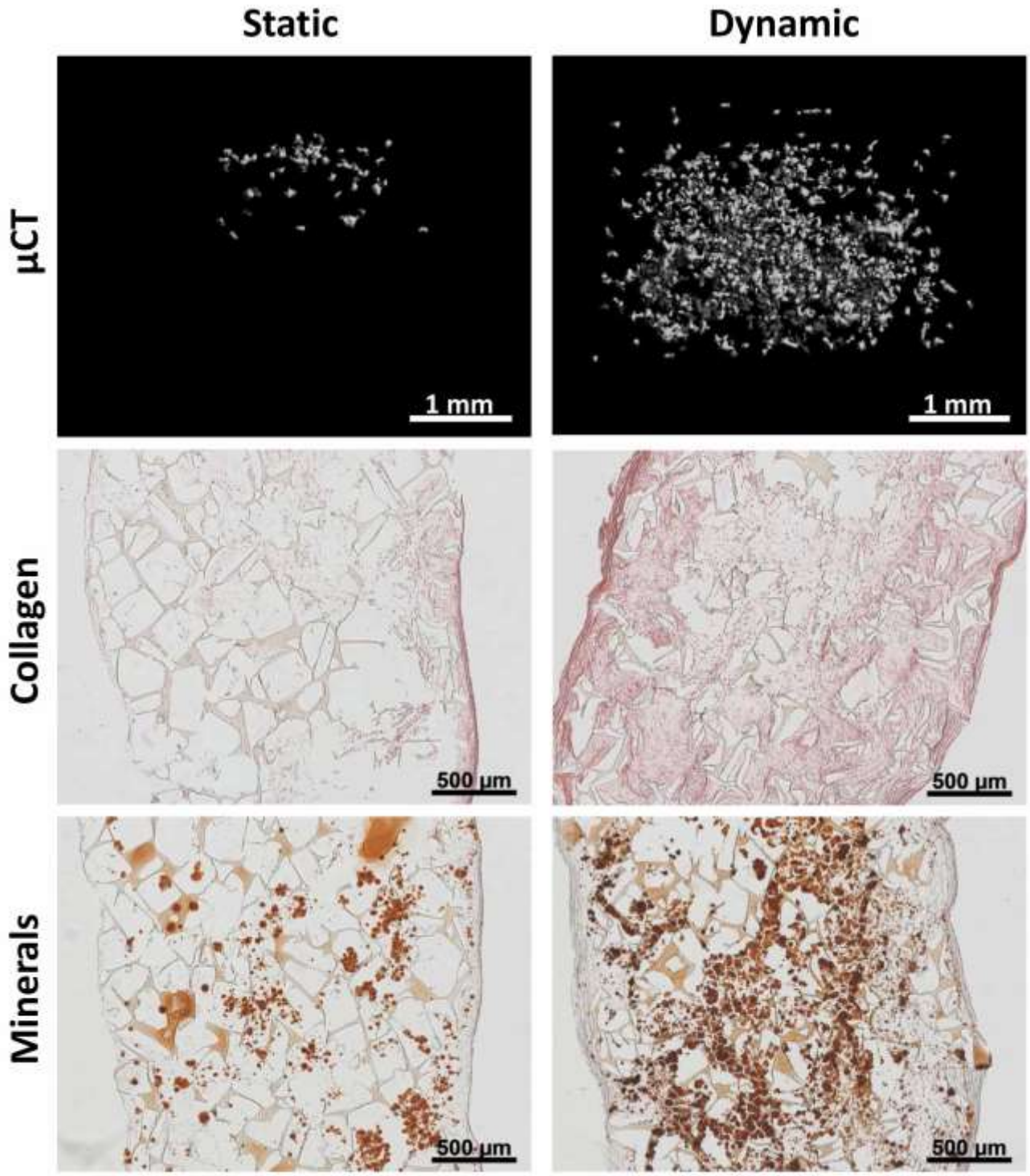
ACKNOWLEDGMENTS:

FP7/2007-2013 no. 336043

Picture 1:



Caption 1: Fig. 1: Bone-like tissue formation of human ASCs cultured statically or dynamically on 3D silk fibroin scaffolds in spinner flask bioreactors.

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Poster presentation

685 Assessing the potential of fucoidan-based hydrogels for pancreatic cells encapsulation

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INTRODUCTION:

Diabetes mellitus is a metabolic disorder that affects 450 millions of people in the world. It is the sixth most common cause of death. Therefore, it is crucial to develop new therapeutic strategies and models to treat this disease[1]. In this perspective, marine origin polymers represent a relatively untapped source that can be used in the creation of hydrogels to attend these needs. Fucoidan (Fu) is an important example, an underexploited sulfated polysaccharide extracted from the cell wall of brown seaweeds[2]. It has relevant properties namely reducing blood glucose, antioxidant and anti-inflammatory actions[2]. This study intends to assess the biomedical potential of Fu-based hydrogels for diabetes treatment, namely for encapsulation of insulin-producing pancreatic cells.

METHODS:

Hydrogels were produced by blending fucoidan (Fu) with agarose (Aga) through a thermal crosslinking reaction, from 3 or 5 wt% Fu aqueous solutions and adding 3 or 5 wt% Aga powder, using different proportions (50:50; 70:30 and 40:60). Chemical characterization of blends was addressed by FTIR, ^{1}HNMR and XPS. Moreover, the rheological behaviour was also characterized by submitting the hydrogels to rotational and oscilatory experiments using parallel plate as geometry. The ability of the developed materials to encapsulate human pancreatic cells (1.1B4) in a viable step was assessed using 1.1B4 cell line (1.5 x10^{6} cells/ml) during up to 21 days.

RESULTS AND DISCUSSION:

The chemical characterization confirmed the presence of the two polymers in the structure of hydrogels. In assessment of mechanical behavior we can observe that increasing the agarose concentration, we can increase the Young modulus of hydrogels (Figure 1B), while the rheological tests revealed a non-newtonian, viscoelastic behaviour. Furthermore, the biological assessment of pancreatic beta cells culture into the hydrogels demonstrated that hydrogels supported good cell adhesion, proliferation and tend to form pseudo-islets during the culture period studied (Figure 1 C, D1-D2).

CONCLUSION:

This work demonstrates the successful production of AgaFu hydrogels with a versatile mechanical, physical and biological performance. The proposed technology based on the thermal gelation of agarose and fucoidan establishes a method to obtained hydrogels, which may be applied in diabetes treatment.

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ACKNOWLEDGMENTS:

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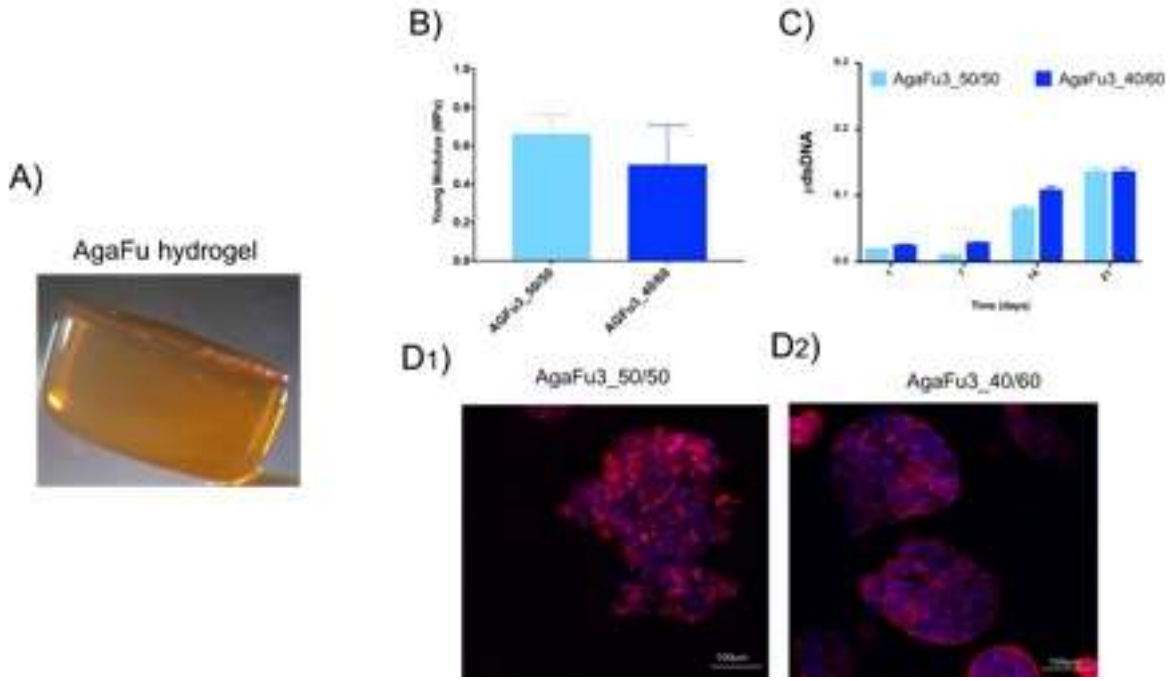
Picture 1:



Caption 1: FIGURE 1: A) AgaFu hydrogels, B) mechanical behavior, C) cell proliferation, D1 and D2) confocal microscopy images.

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Poster presentation

710 Chemical collagen cross-linking induces multinucleated giant cells and leads to membrane disintegration: a histological analysis in three different in vivo models

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INTRODUCTION:

Cross-linking techniques aim to enhance the biomaterial stability[1]. However, cross-linking changes the physico-chemical biomaterial properties that are crucial for the induced cellular reaction and regenerative capacity[2]. The present study evaluated the influence of chemical cross-linking on the inflammatory pattern, vascularization and degradation in vivo.

METHODS:

A clinically used non-cross-linked collagen membrane was modified using chemical cross-linking (A = high cross-linking, B = medium cross-linking, C = low cross-linking and D = no cross-linking). The membranes were subcutaneously implanted in Wistar rats. The implantation areas were analyzed after 3, 10 and 30 days. Femoral and calvarial implantation models were used to evaluate the barrier function of the biomaterials after 10 and 30 days. The cellular reaction, vascularization and degradation pattern were evaluated histologically.

RESULTS AND DISCUSSION:

In the subcutaneous model, A and B induced a high number of multinucleated giant cells (MNGCs) as a foreign body reaction leading to biomaterial disintegration and premature degradation over 30 days. In group C, few MNGCs were sporadically found on the biomaterial surface, while the membrane maintained its integrity. Only mononuclear cells (MNCs) were detected in D and the membrane preserved its native structure and was well integrated. The vascularization rate correlated with the presence of MNGCs. It was higher in A and B compared to C and D. This was reproducible in the femur and calvarial models. In the femur model, the defect region in A and B contained a higher amount of connective tissue compared to C and D. In group C, signs of membrane ossification were revealed over the evaluation time. The calvarial model showed similar results, thus small bone islands were found in the defect region in A and B, while C and D showed a higher rate of new bone formation over time.

Membranes that underwent disintegration by the formation of MNGCs in the subcutaneous implantation model resulted in less new bone formation in the femur and calvarial models and vice versa.

CONCLUSION:

The chemical cross-linking of collagen-membranes reduces their stability by inducing MNGCs that lead to premature degradation and loss of the native membrane-structure. These results underlined the importance of the cellular reaction and physical integrity for the regenerative capacity of barrier membranes in terms of guided bone regeneration, and question crosslinking techniques as a mean to prolong barrier function of collagen membrane.

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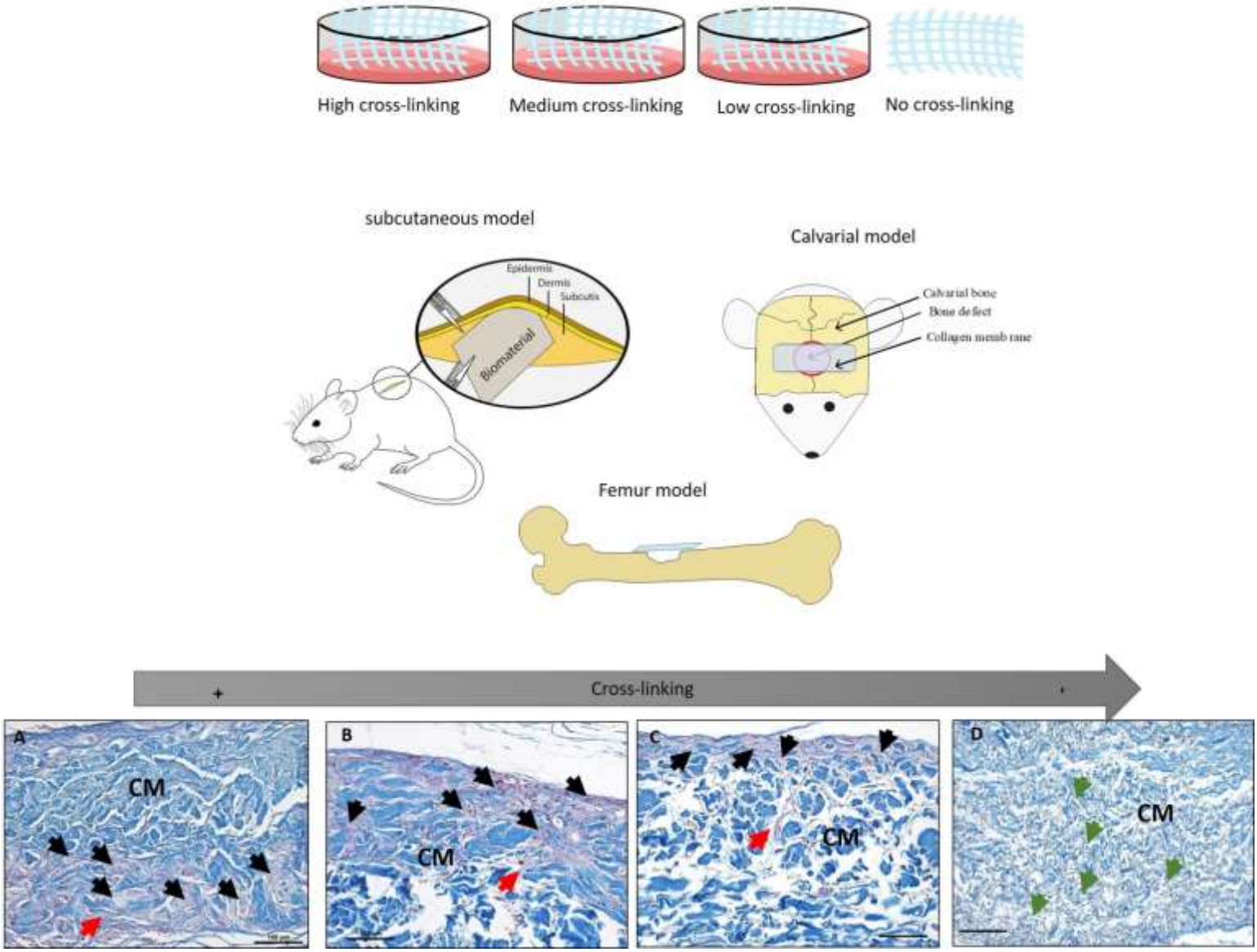

Picture 1: Caption 1: The implantation models and histological results in subcutaneous model after 30 days. black arrow= MNGC; red arrow=disintegration; green arrow=MNCs

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Poster presentation

715 DEVELOPMENT AND CHARACTERIZATION OF A POLY(LACTIC-CO-GLYCOLIC) ACID - HYDROXYAPATITE COMPOSITE MATERIAL TO FABRICATE 3D-PRINTED SCAFFOLDS FOR BONE TISSUE ENGINEERING

Joanna Babilotte[1], Vera Guduric[1], Reine Bareille[1], Damien Le Nihouannen[1], Thierry Cardinal[2], Manuel Gaudon[2], Jean-Christophe Fricain[1], Joëlle Amédée[1], Sylvain Catros[1]

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INTRODUCTION:

Current bone tissue engineering strategies are based on porous biocompatible scaffolds seeded with tissue-specific cells. Improvement in rapid prototyping technology, such as 3D printing, allows fabrication of custom-made 3D scaffolds with high resolution. We have developed a new material, made of medical grade Poly(lactic-co-glycolic) acid (PLGA) mixed with hydroxyapatite nanoparticles (nHA), in the shape of a filament for 3D printing by Fused Deposition Modelling (FDM). PLA has shown its osteoconductive capacities in many studies, and the addition of glycolide can reduce the degradation rate. nHA were included to improve the bioactivity of the material for bone tissue engineering applications.

METHODS:

PLGA was mixed with 5% or 10% (w/w) nHA to fabricate a filament. Characterization of the materials were done by XRD analysis, Micro-Raman spectroscopy and thermogravimetric analysis (TGA). Then, the materials were used to fabricate microporous membranes by FDM. The membrane topography was evaluate by Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). The membranes were seeded with human adipose-derived stem cells (ADSCs) or human bone marrow stem cells (HBMSCs). The cytotoxicity of different materials was assessed by MTT and Neutral Red tests on both cell types. Cell survival was evaluated by Live-Dead assay during 21 days of culture. Early osteoblastic differentiation was evaluated by qualitative expression of alkaline phosphatase (ALP).

RESULTS AND DISCUSSION:

The inclusion of nHA decreased the printing temperature relative to pure PLGA. XRD has confirmed the presence of HA in materials and Micro-Raman the homogeneous distribution of particles with different size. The TGA has shown that we succeeded to obtain a composite material containing 9% or 4% of HA. SEM revealed that the nHA particles were included regularly in the struts resulting in a rough appearance. AFM showed an augmentation of the material roughness correlated to increased nHA concentration. Cytotoxic assays revealed no adverse effects on cells after 24h culture: the composite material was non-toxic and the cells could be seeded directly after sterilization without rinse process. Live-Dead assays have shown adhesion for both cell type and cell survival after 21 days of culture. PAL activity seems higher with composite materials.

CONCLUSION:

These experiments have shown that it was possible to fabricate a PLGA-HA composite biomaterial for 3D printing by FDM. Futures studies will focus on evaluation of materials' biodegradation rate, mechanical tests and in vitro experiments will be completed to perform phenotypic assays.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

718 Polylactic vs. Composite interference screws from lab to clinic

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INTRODUCTION:

Resorbable osteosynthesis have been developed using bioresorbable polyesters in the early 1980s[1]. The addition of mineral charge usually calcium phosphate granules[2,3] was the next step to improve the osteointegration.

Focused on the knee ligament reconstructions, the purpose of this study is to compare the performances of composite interference screws containing Biphasic Calcium Phosphate (BCP) granules vs. Polylactic ones from mechanical, *in vitro* degradation and clinical point of view.

METHODS:

The basic mechanical properties of sterilized specimens were evaluated according to ISO standards. Failure torque measurements were directly performed on Osteotwin™ screws (Biomatlante).

In vitro degradation was followed during 73 months by inherent viscosity and compression measurements supplemented by pull-out tests.

A prospective clinical study was performed to evaluate the effect of BCP as osteoconductive additives on the Anterior Cruciate Ligament reconstruction by intrapatient comparison[4].

RESULTS AND DISCUSSION:

Whatever the standardized mechanical test, an increase of the Young modulus is observed, whereas a slight decrease of the maximal stress is noticed when the mineral charge content increases. This result is confirmed by failure torque measurements since a very slight decrease of the resistance of Composite was shown.

In vitro tests indicate that the Composite screws degrade slower than Polylactic ones thanks to a buffer effect of BCP granules; compression resistance drops at 6 months but the pull-out resistance is still effective.

Clinical radiographic assessment based on computed tomography scans confirms that the resorption of Composite is slower than of Polylactic. As expected, the ossification is better and more interesting, less tunnel widening is observed with Composite screws than with Polylactic ones.

CONCLUSION:

Even if a slight decrease of mechanical properties of composite material was measured, the use of composite screws remains as simple and safe as with polylactic ones and leads to better bone reconstruction thanks to BCP granules. This can be explained by the homogeneous distribution of the mineral charge inside the polymer matrix and the design of the internal imprint of the screw.

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

719 3D-Bioplotting of functional human chondrocytes in biopolymer hydrogels as a basis for volumetric individualized osteochondral constructs

David Kilian, Tilman Ahlfeld, Ashwini Rahul Akkineni, Rania Abdelgaber, Anja Lode, Michael Gelinsky

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INTRODUCTION:

Additive Manufacturing has gained large interest for biomedical application. Not only are researchers able to create individual metal implants but also to process viable cells in native matrix-mimicking hydrogels. For tissue engineering, this provides a promising opportunity to arrange various cell types in a spatially defined distribution which overcomes the limitations of conventional cell seeding of scaffolds. Therefore, it also offers potential for multiphasic tissue structures such as osteochondral interfaces. Here, we present a study on the plotting of chondrocyte-laden biopolymer-based hydrogels as 3D scaffolds, examining cellular fate and metabolism inside the 3D environment in response to external and internal stimuli.

METHODS:

Human primary chondrocytes were 3D-plotted after encapsulation inside a 3% alginate/9% methyl cellulose (alg-mc) hydrogel[1] using the BioScaffolder 3.1 (GeSiM, Radeberg, Germany). Viability and fate of chondrocytes reacting to biochemical stimulation were investigated. Biochemistry and histology were applied to detect matrix formation, glycosaminoglycan production and expression of chondrogenic markers on gene and protein level.

To provide local delivery of chondrogenic factors such as TGFβ-3 required for spatially defined supply inside a multiphasic osteochondral construct, core-shell structures[2] with a nanoclay-based core[3] for triggered release kinetics and a cell-laden shell receiving those stimuli were printed.

RESULTS AND DISCUSSION:

Volumetric structures (> 1cm³) were realized by applying alg-mc hydrogel for plotting of cells. Human chondrocytes encapsulated in this biopolymer-based bioink showed high cell viability during long-term incubation after both single-component and core-shell extrusion. Cells were observed to migrate out from hydrogel strands to the surface in de-differentiated state. After adhesion to the alginate surface, cells started proliferating. Non-adherent morphology was found for differentiated chondrocytes remaining inside the hydrogel. Active chondrogenic metabolism could be proven by the production of collagen II after chondrogenic stimulation. To spatially define chondrogenic and osteogenic differentiation, a local factor delivery system was established.

CONCLUSION:

The approach provides a possibility of including human chondrocytes in a process for volumetric 3D-bioprinting, maintaining their functional integrity. It offers potential as a basis for individualized osteochondral implants or for (co-culture) model systems that require triggering of cell fate via spatially provided biochemical factors.

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Poster presentation

720 Molecular biology-based breast implant surface classification

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[2]IS2M, France

INTRODUCTION:

As a result of the Foreign Body Reaction, breast prostheses are surrounded by a fibrotic capsule. To increase the biocompatibility of the implant surface, different texturation processes were designed. Thus, the highly structured implant surfaces present a better anchorage in the tissue.[1] However, the roughest surfaces were incriminated as a potential risk factor for some long-term complications, such as Anaplastic Large Cell Lymphoma (ALCL).[2] All the proposed etiologies for ALCL lead to a chronic inflammation in the capsular tissue.

The aim of this study is to determine whether different surface topographies mediate different fibrosis and inflammatory-involved gene activations by the periprosthetic cells.

Different genetic markers were reported in the transcriptomic studies of breast capsular tissues: some involved in the extracellular matrix remodeling and other responsible for the activation of some immune cells.[3,4] However, it is the first time that the impact of different silicone surface topographies on these cellular mechanisms is analyzed on only healthy breast tissues.

METHODS:

For this study, the breast implant surfaces were classified, according to biologically-relevant surface parameters into three categories: the peak and valley-based surfaces (PV), the open pore-based surfaces (OP) and the open cavity-based surfaces (OC).

At least 30 samples of capsules were collected during revision surgeries and divided into the three surface categories. This analysis aims at quantifying the expression rate of some genes of interest via qRT-PCR.

RESULTS AND DISCUSSION:

The OC surfaces elicit a specific response profile. They are the least fibrosis-inductive surfaces. For example, MMP12 was found highly up-regulated compared to PV and OP surfaces. This finding is consistent with the data published by Abramo et al.[5], which show a significantly reduced risk of fibrotic periprosthetic capsule thickening with OC surfaces.

CONCLUSION:

Periprosthetic cells sense the differences between the three surface categories. Moreover, the differences of expressions are consistent with the clinical data.

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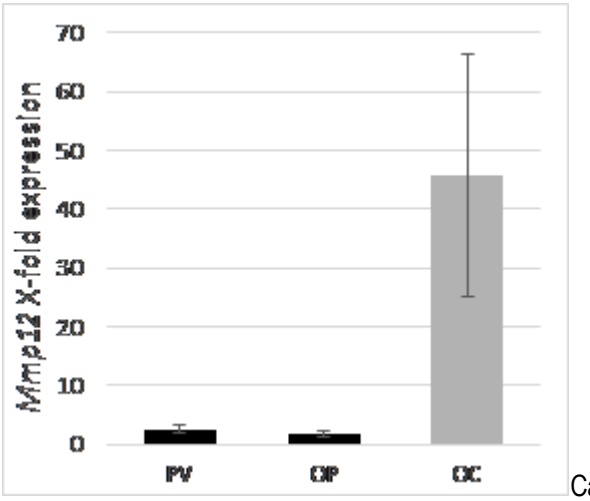
Picture 1: 

Caption 1: Comparison of the MMP12 expressions between the three surface groups.

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Poster presentation

729 Interplay between sensory neurons and endothelial cells in view of angiogenesis

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INTRODUCTION:

Innovative bone tissue engineering strategies have been focusing on the dialogue between different cell types for building and remodeling a functional tissue after severe lesion. We have recently assessed the influence of the sensory neurons (SNs) as important regulators of the osteoblastic differentiation of mesenchymal stem cells[1]. Here, we aim to evaluate the interaction between SNs and endothelial cells (ECs) to understand the interplay between angiogenesis and innervation in a bone repair and vascular remodeling scenario.

METHODS:

Rat primary SNs and ECs were cultured in microfluidic devices (2D) for 4 and 7 days, as depicted in Figure 1A. These devices separate SNs and ECs cell bodies and allow their interaction through microchannels with very precise dimensions. In addition, cell populations can be analyzed separately. ECs gene expression was evaluated by RT-qPCR for important markers involved in vascular development and remodeling. Differences between groups ($n \geq 5$) were analyzed by one-way ANOVA, followed by Bonferroni as post-hoc. We also encapsulated ECs in type I collagen gel and then seeded SNs on top of it, cultivating this construct for 15 days. Cells were labelled for specific antigens and observed in confocal microscopy.

RESULTS AND DISCUSSION:

After 4 days of indirect culture, SNs were able to form neurites towards the EC compartment (Figure 1B). For the ECs gene expression, a significant upregulation of *Tek1* and *Pecam1* was observed in ECs cocultured with SNs on

day 7 relative to day 4, indicating that SNs may induce vascular development. In addition, *Mmp2* was strongly upregulated in ECs cocultured with SNs after 4 and 7 days relative to monocultured ECs, suggesting a role of SNs on vascular remodeling and sprouting. For 3D evaluation, direct coculture demonstrated that ECs formed aggregates and SNs emitted long neurites, which surrounded them, reinforcing the regulated cellular organization (Figure 1C). In addition, macroscopically, the collagen matrix was intensively degraded when ECs are cocultured with SNs, which seems to support the upregulation of *Mmp2* detected in 2D.

CONCLUSION:

Our preliminary results suggest that SNs can closely interact with ECs, and may modulate ECs' gene expression in order to stimulate vascular development and remodeling.

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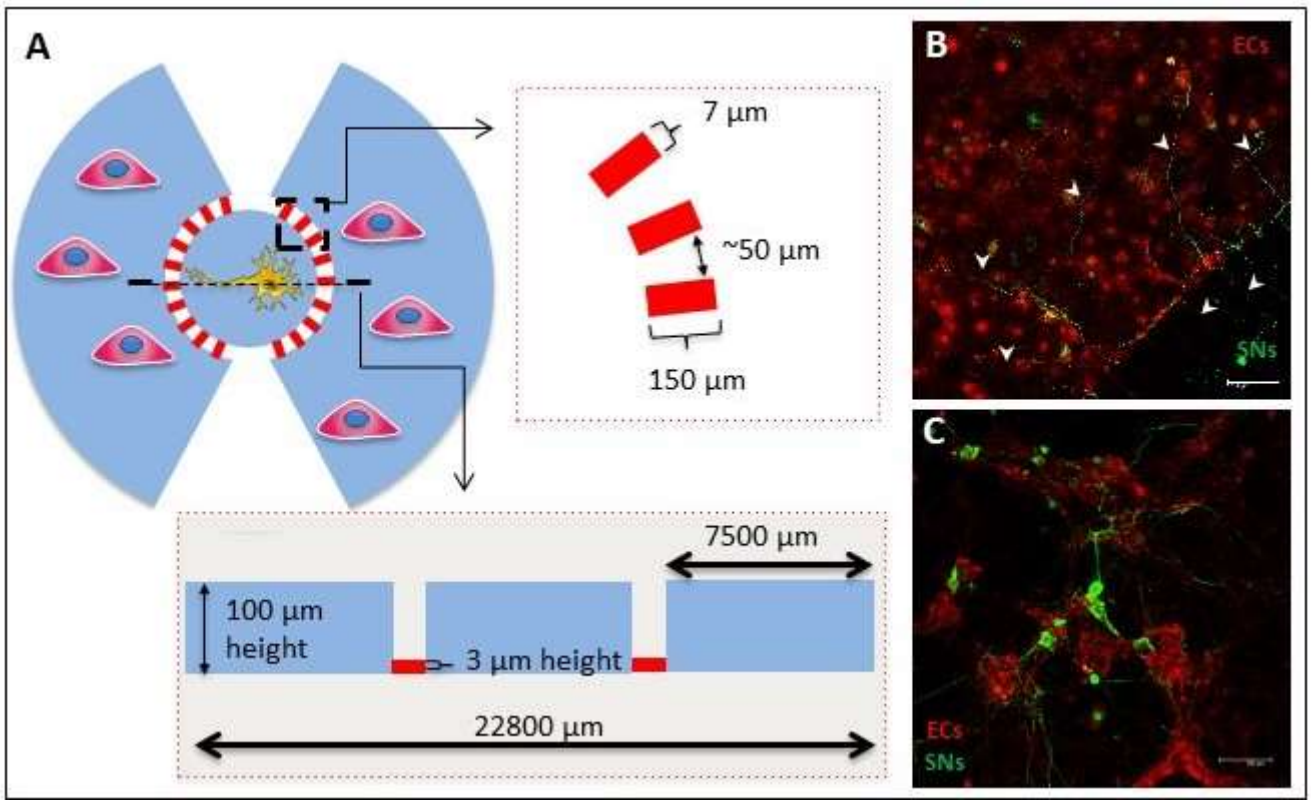
ACKNOWLEDGMENTS:

FrTecSan, BxCRM, Université de Bordeaux, and Conseil Régional de Nouvelle Aquitaine provided financial support.

Picture 1:



Caption 1: Figure 1. (A-B) Microfluidic devices design and dimension for ECs and SNs coculture. Arrowheads indicate neurites. (C) ECs and SNs direct culture.

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Poster presentation

731 Cerebral aneurysm tissue investigation: experiment and simulations

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[3]Institute of Theoretical and Applied Mechanics SB RAS, Russian Federation

[4]Federal Center of Neurosurgery, Russian Federation

INTRODUCTION:

Cerebral aneurysms (CA) are a very widespread disease, occurring in 2-5% of population. Although a CA ruptures in a mere 0.5% of cases, the consequences of a rupture are catastrophic and lead to death in 30% of observed cases[1]. A study of the causes of CA rupture is of current interest in hemodynamics, materials science and computer simulations [2,3]. The present study concerns the mechanical properties of cerebral aneurysm tissue and the impact of patient specific data on numerical simulations as well classification of aneurysm tissue by using laser induced fluorescence(LIF) phenomena.

METHODS:

Tissue samples were harvested during neurosurgical treatments at Federal Centre of Neurosurgery (Novosibirsk, Russian Federation) and following this the relationship between stress and strain for the tissues was investigated, and the collagen content of the tissue samples was determined."Firstly, the collagen content of 5 tissue samples was determined by means of fluorescent macroscopy. A laser unit of 210nm-350nm was used, with a laser spot of 5mmx14mm. Impulse energy was up to 200 microJ/impulse. The fluorescence spectra of these samples were investigated. Following this, the mechanical properties of the samples were determined using a Zwick/Roell Z100 rupture machine (Germany). Several stretch modes were applied to each of the 6 tissue samples. We apply Mooney-Rivlin model (both 3 and 5 parameters) for each of the tissue samples.

RESULTS AND DISCUSSION:

Our strain-stress experiments and calculations show that there exists a critical value of strain (1,8-2,6 with respect to specimen), where the values of Mooney-Rivlin model constants reach its extremum. Both 3 and 5-parameter models do not correspond to the tissues behavior within this critical strain value and while strain grows. LIF experements allow to classify CA tissues with respect to triptophan-collagen-tirosin ratio.

CONCLUSION:

Under LIF tissue investigation we have been able to classify which spectra corresponded to ruptured and unruptured aneurysm tissue samples respectively. We have also established the importance of using patient specific blood flow waveforms for the calculation of wall shear stress values. Furthermore, we have determined that there is no clear benefit of using patient specific tissue characteristics to simulate von Mises stress and blood vessel deformation in linear case.

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ACKNOWLEDGMENTS:

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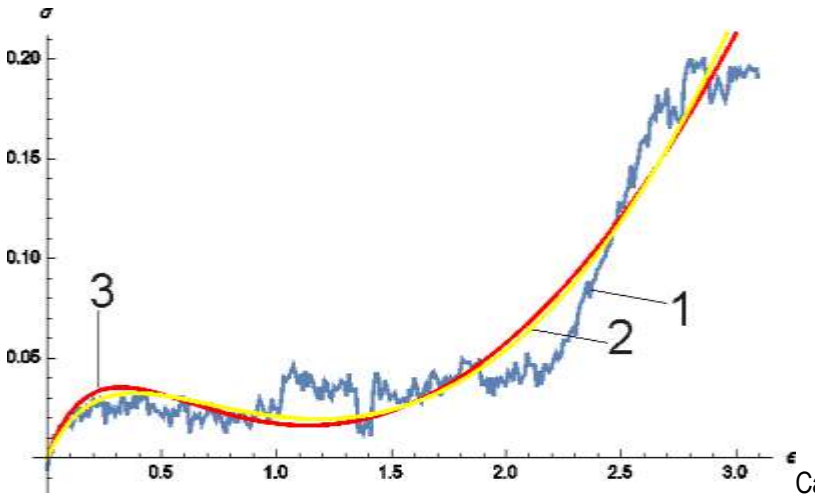
Picture 1: 

Caption 1: Strain-Stretch curve from experimental data (1-blue), 3-parameter Mooney-Rivlin model (2-yellow), 5-parameter Mooney-Rivlin model (3-Red)

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Poster presentation

733 Design of an immunoprotective macroencapsulation device for islets

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MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, Netherlands

INTRODUCTION:

Type 1 diabetes is an autoimmune disorder in which the insulin producing cells are destroyed. Commonly, daily insulin injections are used to control blood glucose in these patients. Clinical Islet Transplantation (CIT), involving intrahepatic beta cell transplantation, is a promising alternative therapy leading to good blood glucose control and insulin independency.[1] The main limitation of CIT is limited cell survival, due to the liver being a suboptimal beta cell environment and the immunosuppressive therapy to prevent rejection.[2] We report on the development of an immunoprotective islet encapsulation device for extrahepatic islet transplantation while preventing an immune response against the allogeneic tissue.

METHODS:

Porous (0.2 µm pores) PVDF (Bio-rad, #1620177) microwell membranes were produced with microthermoforming using a hot press (Specac manual hydraulic press) and stainless steel mould.[3] Membranes where moulded into microwell films using heat and pressure. The diffusion kintecs of different molecules such as glucose and insulin was determined using a standardized diffusion setup. Moreover, beta cell endocrine functionality and viability on the membranes was measured.

RESULTS AND DISCUSSION:

The mould used for microthermoforming has ~4400 openings with 400 µm diameter and 350 µm depth (fig. 1A). After microthermoforming the resulting microwells have a diameter of 388 ± 8 µm and depth ~ 200 µm (fig. 1C & D). Membranes have good permeability and have no significant effect on beta cell survival and glucose responsiveness.

CONCLUSION:

We were able to reproducibly manufacture microwell membranes containing 400 µm diameter and ~ 200 µm deep wells. By manipulating temperature and pressure we can tune the microwell depth, while retaining the membrane porosity.

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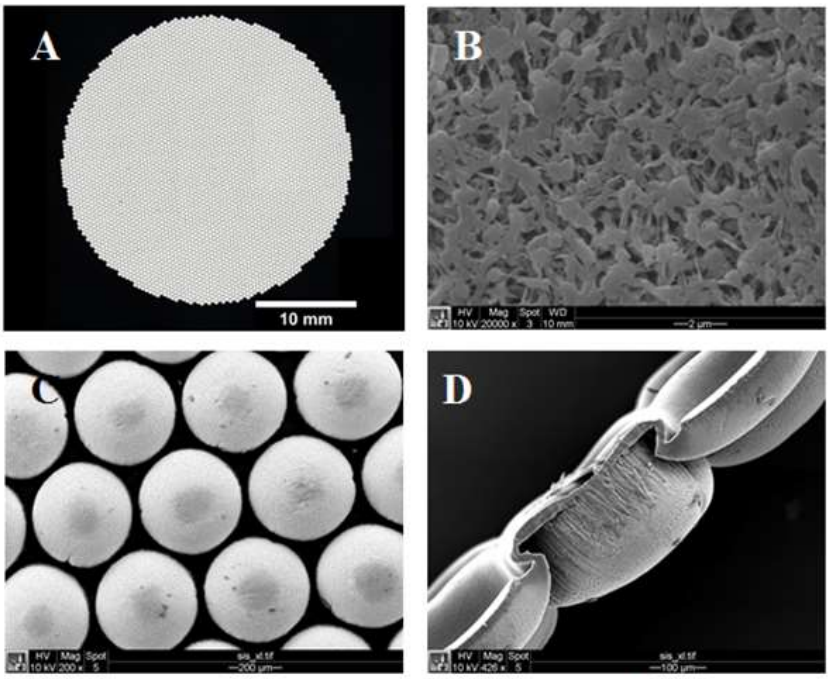

Figure 1: (A) Stainless steel mould with 400 μm diameter openings. Scale bar represents 10 mm. (B) SEM image of PVDF membrane with 0.2 μm pores. Scale bar represents 2 μm. (C) SEM image of microwells. Scale bar represents 200 μm. (D) SEM image of microwell cross-section. Scale bar represents 100 μm.

Picture 1: Caption 1: Figure 1

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Poster presentation

738 Gold nanosensing of toxic heavy metal ions to detect implant failure

Elena Zattera[1], Eepsita Priyadarshini[2], Nilotpala Pradhan[2], Brian G. Cousins[1]

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INTRODUCTION:

Early detection of toxic heavy metal ions (HMIs) in the blood (>7 µg/L) can indicate metallosis or corrosion surrounding metallic implants (e.g. Ni-Co-Cr alloy). Degradation and wear are associated with systemic toxicity (e.g. inflammation, osteolysis, pseudotumours) leading to implant failure. Currently, there are no simple diagnostic tests to detect toxic HMIs in physiological fluids. Gold nanoparticles (GNPs) physicochemical properties and localised surface plasmon resonance are effective colorimetric sensors, which can be tailored to study their interactions in blood.[1,2] The aim of this study was to develop efficient gold nanosensors for accurate and selective detection of toxic HMIs in physiological solution, where these metals present as contaminants.

METHODS:

GNPs were synthesised using the method of Frens to produce 16, 24 and 41 nm particles, and were treated with nickel (II) chloride, cobalt (II) chloride and chromium (II) chloride in a 1:2 ratio. We assessed GNP sensitivity and selectivity through aggregation in deionised water (dH_2O) and physiological fluids using UV-vis spectroscopy, TEM, DLS, and Zeta-potential measurements. Cytotoxicity studies were performed with L929 fibroblasts in DMEM supplemented with 10 % FBS, and 1% penicillin/streptomycin over a 72 h period after incubation with GNPs treated with Ni, Co, and Cr.

RESULTS AND DISCUSSION:

GNPs show selective detection of Cr in mixed HMI solutions in the parts per billion (ppb) range (Fig 1A). Increasing the concentration of Cr from 100 to 1000 ppb resulted in a colour change from red to violet due to GNP aggregation (Fig 1B). Aggregation was confirmed by UV-vis spectroscopy, DLS and TEM analysis. Preliminary cytotoxicity data indicates that toxicity was found to increase with GNP size and concentration. We are optimizing GNP size and ligand density to detect Cr within the physiological range.

CONCLUSION:

We have developed a simple, low cost, efficient GNP nanosensor for selective detection of Cr in the ppb range. This has great potential as a diagnostic test for toxic HMIs to measure implant failure and provide model substrates to study the effects of micro- and nanoparticle wear debris with a range of tissue types.

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ACKNOWLEDGMENTS:

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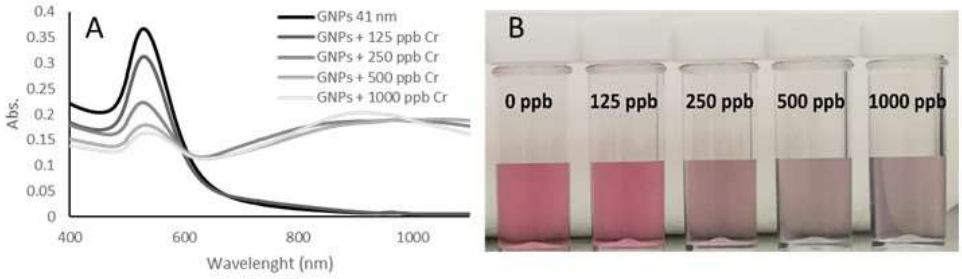

Picture 1: Caption 1:
Figure 1. UV-vis spectra of Cr detection with 41 nm GNPs (A) and colorimetric response (B) in dH20.

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Poster presentation

743 Production and biomedical potential of collagen from codfish swim-bladder

Rita O. Sousa, Ana L. Alves, Duarte Nuno Carvalho, Eva Martins, Ana Catarina Oliveira, Tiago H. Silva, Rui L. Reis

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INTRODUCTION:

Atlantic cod is processed industrially for food purposes, with several by-products being directed to animal feed and other ends. Looking particularly into swim bladders, the extraction of collagen can be a valuable strategy for by-product valorization, which was explored in the present work. Given the ubiquitous presence of this protein in the extracellular matrix of connective tissues, it has a relevant position as building block for the development of bioinspired biomaterials.

METHODS:

In this work, swim bladders removed from salt-cured Atlantic cod (*Gadus morhua*) were used to extract collagen using acetic acid (sbACol) and pepsin (sbPCol) based methodologies. Collagen was salted out with 2.6M NaCl in 0.05M Tris-HCl at pH 7.5. The solution was dialyzed first against 0.1 M acetic acid for 2 days, then against 0.02 M acetic acid for 2 days, and finally against distilled water until pH 7 and freeze-dried.

RESULTS AND DISCUSSION:

The extraction of collagen was possible by both methods with yields of 5.72% for sbACol and 11.14% for sbPCol, with both extracts being characterized by SDS-PAGE profiles (Figure 1) compatible with type I collagen.

According to the results from FTIR, circular dichroism and X-ray diffraction spectroscopy, sbPCol underwent a slight denaturation, while the sbACol structure remained intact, with preserved triple helix. Amino acid analysis revealed a

total proline-like amino acid content of 148/1000 residues for sbACol and 141/1000 residues for sbPCol, with hydroxylation degree of 36.5 % and 37.6%, respectively. All extracts exhibited a typical shear thinning behaviour, like hyaluronic acid, which might be interesting for the formulation of injectable biomaterials. The cytotoxicity studies have demonstrated the non-toxic nature of both sbACol and sbPCol.

CONCLUSION:

Overall, the obtained results support the efficiency of the proposed approach for the extraction of collagen from cod swim bladders and further enable the design of methodologies to address the use of sbACol and sbPCol in biomedical or cosmetic context.

ACKNOWLEDGMENTS:

Financial support from European Regional Development Fund (ERDF) through the Structured Project NORTE-01-0145-FEDER-000021 (Norte2020) and from European Union Seventh Framework Programme (FP7/2007-2013) through grant agreement ERC-2012-ADG 20120216-321266 (ERC Advanced Grant ComplexiTE). Soguima (Guimarães, Portugal) for the kind offer of Atlantic cod swim bladders.

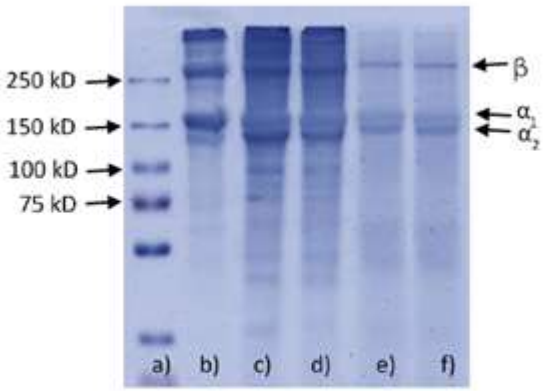

Figure 1- Electrophoresis analysis of collagen extracts: a) protein marker; b) type I collagen from bovine skin; c) acid soluble collagen from codskin; d) sbACol; e) pepsine soluble collagen from cod skin and f) sbPCol.

Picture 1:

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Poster presentation

746 Impedance spectroscopy as water pollutant detector

Gonçalo Magalhães-Mota[1], Flavio Makoto[2], Osvaldo Oliveira Jr.[2], Paulo Ribeiro[1], Maria Raposo[1]

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INTRODUCTION:

The detection of pollutants such as triclosan and ibuprofen in the environment is a major issue, particularly in water supplies, water courses and aquifers, which are known to be mainly contaminated by pesticides, industrial products and emergent pharmaceuticals and personal care products (PPCPs)[1,2,3]. No commercial sensors for monitoring and detecting these pollutants have been developed so far. In this regard, the electronic tongue concept, based on AC impedance measurements, can be a valuable tool towards contaminants probing[4]. Even so, this possibility presents difficulties, namely on the AC impedance spectra interpretation and on the application of mathematical methods which allow finding classification patterns with consequent contaminant detection.

METHODS:

Layer-by-layer (LbL) films were deposited onto gold interdigitated electrodes (IDEs) and dipped in aqueous solutions of different concentrations of triclosan and ibuprofen, both alone and mixed. The impedance spectra were acquired through the electronic tongue method.

RESULTS AND DISCUSSION:

Mathematical statistical methods were applied to the impedance spectra obtained from the electronic tongue data, evidencing the different concentrations.

CONCLUSION:

The impedance spectra from the IDEs showed that the developed polyelectrolyte-functionalized gold electrodes are selective and efficient to detect aqueous contaminants, such as triclosan and/or ibuprofen, in a wide range of concentrations.

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

748 Novel polysaccharide/graphene family materials/hydroxyapatite composite hydrogels as scaffolds for bone and cartilage engineering

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[2]Malopolska Centre of Biotechnology, Jagiellonian University, Poland

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INTRODUCTION:

New solutions for bone and cartilage tissue engineering are constantly investigated. One of the possible approaches is to use polysaccharide hydrogels as scaffold materials. This diverse class of materials offers biocompatibility, versatility, and resemblance to the native extracellular matrix (ECM)[1]. Moreover, their properties can be further tailored by developing composite systems in which nanomaterials, e.g. graphene family materials (GFM), and microparticles can be incorporated. Human umbilical cord Wharton's jelly derived mesenchymal stem cells (hUC-MSCs) are multipotent cells characterized by e.g. high proliferative and multi-lineage differentiation capacity *in vitro* and *in vivo*[2,3]. In this study, various composite hydrogels based on chitosan (CS) and hyaluronic acid (HA) modified with graphene oxide (GO), reduced GO, and hydroxyapatite (HAp) were developed and their physicochemical and biological properties were evaluated.

METHODS:

Composite matrices were based on either CS solution (2% (w/v) in 5% (v/v) lactic acid) or HA solution (1% (w/v) in distilled water). Various composite systems were developed by introducing different amounts of GO, rGO (0-3% (w/w); ITME, Poland), and HAp (0-30 % (w/w); MKN-HXAP, 12 μm) separately and then GO and HAp simultaneously with decrease/increase tendency. Structural (XRD, ATR-FTIR), thermal (DSC), surface (XPS, SEM, wettability), rheological, and mechanical (tensile test) properties of the obtained materials were evaluated. Next, the impact of selected composites on morphology and various functions of hUC-MSCs was studied.

RESULTS AND DISCUSSION:

Various chitosan and hyaluronic acid composites modified with GO, rGO, and HAp were screened for potential application in bone and cartilage engineering. The study showed that modification of polymer matrix with GO/rGO flakes or HAp particles largely influences its properties. Particularly, increase in mechanical properties, crystallinity, thermal and chemical stability was observed. Tribological characteristics were affected by the GO/rGO addition, while bioactivity was enhanced by the HAp addition. Biological studies revealed the impact of the polymer matrix and modifying phases on the hUC-MSCs morphology, viability, proliferative capacity, metabolic activity as well as chondrogenic and osteogenic differentiation capacity *in vitro*.

CONCLUSION:

The obtained results indicated that it is possible to tune properties of the polysaccharide-based composites by varying the type and amount of GO/rGO/HAp. The proposed composite hydrogels constitute non-toxic substrates for hUC-MSCs propagation.

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

752 A top-down approach to produce protein-functionalized cellulose nanofibers: Comparing chemical and enzymatic isolation

Franck Quero, Jeimy Fernández, Genesis Opazo

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INTRODUCTION:

Cellulose nanofibers (CNFs) are of great interest in fundamental and applied research. It is now considered as a biomaterial with excellent physical and biological properties. Very recently, a top-down approach has been proposed to obtain CNF naturally functionalized with proteins from microalgae, which were subsequently used to obtain cellulose nanopapers. Tunicates also offer the possibility to obtain CNF functionalized with proteins[2]. Up to date, the extraction of CNF from tunicates has been focusing only on chemical methods[2]. The use of enzymes can offer a milder alternative for obtaining CNF while preserving as best as possible the cellulosic component. This study compares some characteristics of CNFs extracted using chemical and enzymatic approaches.

METHODS:

Two extraction processes were applied: chemical and enzymatic chemical in separate experiments. The first involved three alkaline treatments and a bleaching step. The enzymatic process involved two alkaline treatments and one enzymatic treatment followed by a bleaching process.

RESULTS AND DISCUSSION:

The presence of CNFs associated to proteins was confirmed by Fourier-transform infrared spectroscopy. The isolation of CNFs was confirmed by scanning electron microscopy. Powder X-ray diffraction also confirmed the successful isolation of highly crystalline CNFs (Figure 1A). The results suggested that CNFs obtained using the enzymatic process had significantly larger crystallite size (Figure 1B). Thermogravimetric analysis showed that the presence of proteins obtained after the third alkaline treatment resulted in a peak degradation temperature as high as 374 °C. The peak degradation temperature was found to be significantly lower upon bleaching. When an enzymatic step was used followed by a bleaching step, the peak degradation temperature of the CNFs was found to be higher compared to the purely chemical extraction approach.

CONCLUSION:

CNFs naturally functionalized with proteins were successfully isolated from a tunicate source by two methods namely a chemical and an enzymatic method. Powder XRD results suggested that the crystalline structure of CNFs is better preserved when replacing the chemical method by an enzymatic method. Also, the enzymatic method

resulted in the extraction of bleached CNFs with significantly improved thermal stability compared to the purely chemical approach as suggested by thermogravimetric analysis.

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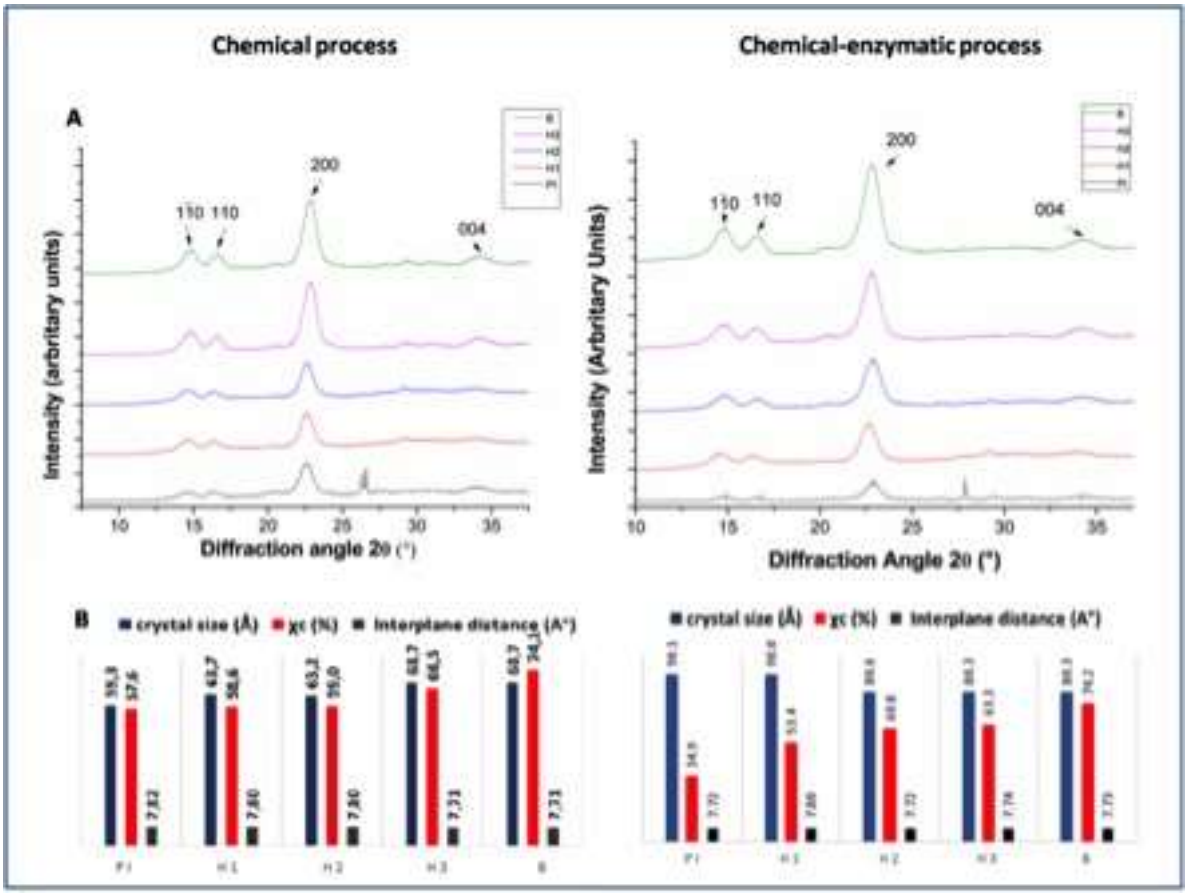

Caption 1: Figure 1 (A) Comparison of powder XRD diffractograms and (B) crystalline characteristics of cellulose nanofibers.

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Poster presentation

753 Nanostructured biomaterials for skeletal muscle engineering

Ana Maria Almonacid Suarez, Martin Harmsen, Patrick van Rijn

University Medical Center Groningen, Groningen, Netherlands

INTRODUCTION:

Despite recent breakthroughs in the culturing of contracting human muscle fibres [1], it is still unknown how to engineer a vascularized and innervated muscle. [2] Skeletal muscle has a highly aligned morphology. Recently, aligned gradient surfaces have allowed cell screening behaviour. [3] In our research, isolated human satellite cells (hSCs)[4] and developed gradient technology [5] were combined to evaluate hSCs response to gradient topography.

METHODS:

Gradient topography substrates were created by applying unidirectional strain during shielded surface oxidation of polydimethylsiloxane (PDMS) with air plasma. As a result, wrinkles ranging from 1020 nm to 6930 nm in wavelength and 74 nm and 2843 nm in amplitude were created within the same substrate. hSCs were cultured on PDMS wrinkle gradients for 12 days. Cell alignment, area and elongation during three days of proliferation and after four, six, and eight days of differentiation were evaluated.

RESULTS AND DISCUSSION:

Preliminary results showed that cells started to align after 16 hours of cell culturing in proliferation medium on the wrinkled gradients. Wavelengths between 4300 nm and 6930 nm and amplitudes between 1142 nm and 2843 nm were optimal for alignment and differentiation after 12 days in culture. It was observed that myofiber diameter changes across the gradient; larger diameters were obtained in larger wrinkle sizes. The diameter values ranged from 16 to 125 μm. Additionally, the cell area of differentiated myotubes varied significantly across the gradient. In differentiated cells, the cell area on the smallest wrinkles was significantly different from the rest of the gradient.

CONCLUSION:

In conclusion, satellite cell proliferation and differentiation into myotubes in PDMS wrinkled gradients was achieved. This preliminary study shows that cell behaviour is dependent on wrinkle size. Once cells start differentiating, they show a preference for larger wrinkle sizes. However, further analysis is necessary to determine the exact nature of this correlation.

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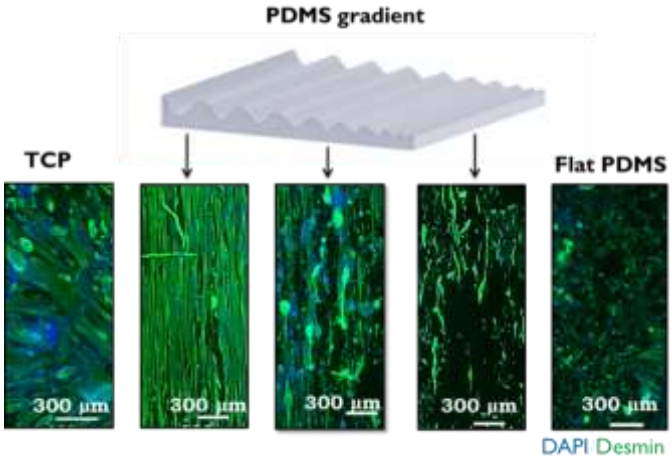

Picture 1: Caption 1: Cell culture in gradient after 8 days in differentiation medium. Controls are tissue culture polystyrene (TCP) and flat polydimethylsiloxane (PDMS)

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Poster presentation

757 Suitability of a liquid TEM cell to study calcium phosphate nucleation

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[2]Glaxo Smith Kline, United Kingdom

INTRODUCTION:

The use and assessment of an in-situ liquid TEM cell to study solution-phase self-assembly of calcium phosphate nanoaggregates from phosphate salt solutions and nucleation driven by the dissolution of a bioactive glass (45S5) is reported where glass dissolution and growth kinetics will be discussed.

METHODS:

In the nucleation driven experiments 5 µl of a calcium aqueous solution (20 mM) was dropped cast onto the nitride membrane of the liquid cell (LC) and a phosphate solution (10 mM) was flowed through the LC (5 µl min-1). For the bioglass experiments, the glass particles were either placed in the holder well in the fluid pathway or 50 nm thick slices of the bioactive glasses (FIB-prepared) were positioned across the nitride membranes. To help understand the in-situ TEM data parallel, and as equivalent as possible, time series invitro experiments were performed.

RESULTS AND DISCUSSION:

Figure 1 shows stills of an in-situ calcium phosphate nucleation from the series of salt experiments performed. Round amorphous calcium phosphate particles formed onto which nucleated a network of branches. Extensive nucleation and dissolution around the branches were visible as a function of time which lead to the formation of larger aggregates. The diffraction and chemical analysis of this and of the invitro experiments indicate that transitions from the amorphous to octocalcium phosphate to hydroxyapatite phases were occurring. Nucleation occurred around and onto the bioactive glass over time scales from a few seconds to minutes. Ordered arrays of hexagonal nanoparticles and micro-sized crystals were also observed to nucleate from the dissolution products. Diffraction and chemical analysis of these experiments indicated that the micro-sized formed crystals were

hydroxyapatite like and that growth bands of different chemical composition occurred around the glass. The material growth and dissolution were dependent on the imaging conditions being used (dose, water thickness) and this too will be discussed.

CONCLUSION:

This work has shown that the in-situ cell can be used to follow and study the nucleation of phosphates from salt precursors and from bioactive glasses and that the process is complicated by the radiolysis products from the electron beam breaking down the water.

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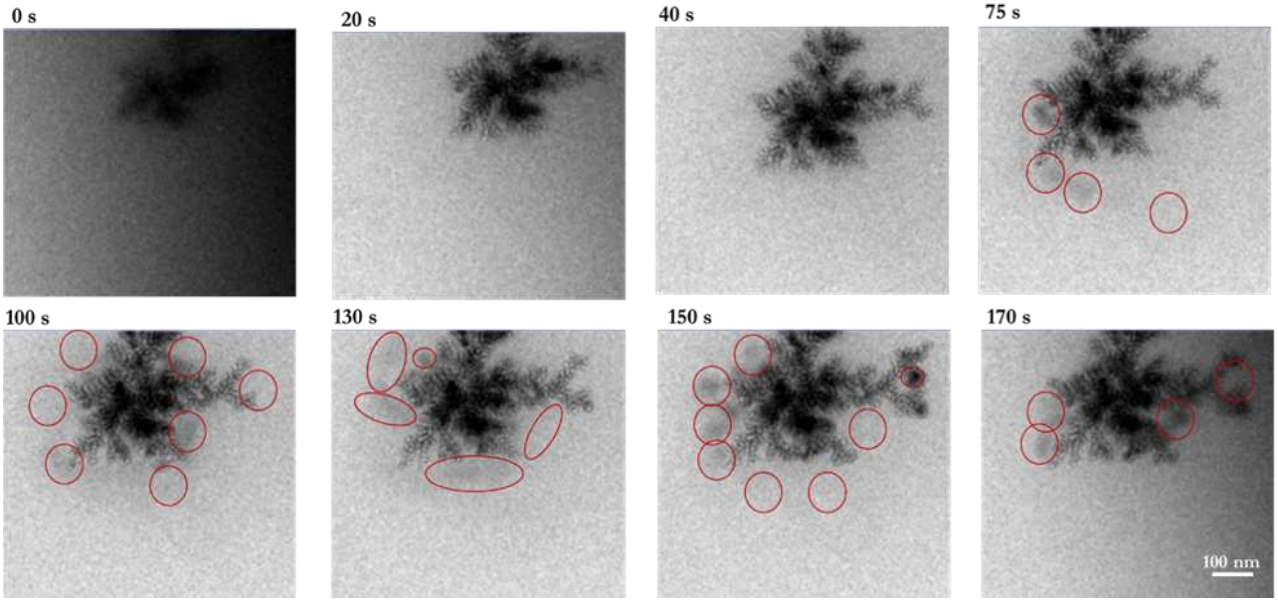
ACKNOWLEDGMENTS:

The authors would like to thank the Engineering and Physical Sciences Research Council (EPSRC) and Glaxo Smith Kline (GSK) for the funding provided to execute this project.

Picture 1:



Caption 1: Figure 1: time series in-situ BF TEM images showing in-situ calcium phosphate nucleation. Red circles show nuclei arriving or sometimes leaving the bi

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Poster presentation

758 3D Electrophoresis-Assisted Lithography (3DEAL): An avenue for creating high complexity functional materials

Gaston Primo, Michal Lipka, Juan Aguilar, Alvaro Mata

Queen Mary University of London, London, United Kingdom

INTRODUCTION:

The human body and its parts are complex, anisotropic, hierarchical, and three dimensional. There is a great need to obtain complex structures that resemble the human body in a cost-effective and easy manner. Specifically, the challenge is to fabricate materials[1], scaffolds, and environments[2] with specific molecular and chemical composition[3] and to do it with spatial control. We have developed a novel 3D-Electrophoresis-Assisted-Lithography (3DEAL) platform to create patterns of multiple functional molecules, and subsequently complex anisotropic environments, within readily available hydrogels.

METHODS:

The five main components of the 3DEAL and its connectivity are shown in **Figure 1A**. The key feature of this technology is the use of an electric field to precisely localize functional molecules into hydrogels by means of a templating mask (**Figure 1B**). Readily available hydrogels and a broad spectra of proteins can be used with the 3DEAL technology. For this work, polyacrylamide (PA) 6 and 3% hydrogels, and fluorescently labelled IgG [blue (b-), green (g-) and red(r-)], as patterned molecules, were used.

RESULTS AND DISCUSSION:

The 3DEAL enables to create complex patterns of functional molecules (**Figure 1D**) within 3-dimensional hydrogels. The technique has the capability to create chemically complex (**Figure 1E**) and anisotropic (**Figures 1C-E**) environments within hydrogels in an easy manner and with high spatial resolution. A major advantage of the technique is that it can be used with any type of hydrogel and with many type of molecules and that it is very affordable and simple.

CONCLUSION:

An affordable, easily tuneable, and versatile device and fabrication process designed to print multiple types of functional molecules within different kinds of hydrogels with high precision has been developed. The printing platform offers a simple, accessible, and practical molecular printing method with potential widespread applications in cell studies, in vitro models, drug screening, and tissue engineering due to its specific spatio-temporal location of proteins to recreate anisotropic environments.

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ACKNOWLEDGMENTS:

The authors would like to thank the STROFUNSCAFF Project (ERC Grant no: 306873) for providing financial support to this project.

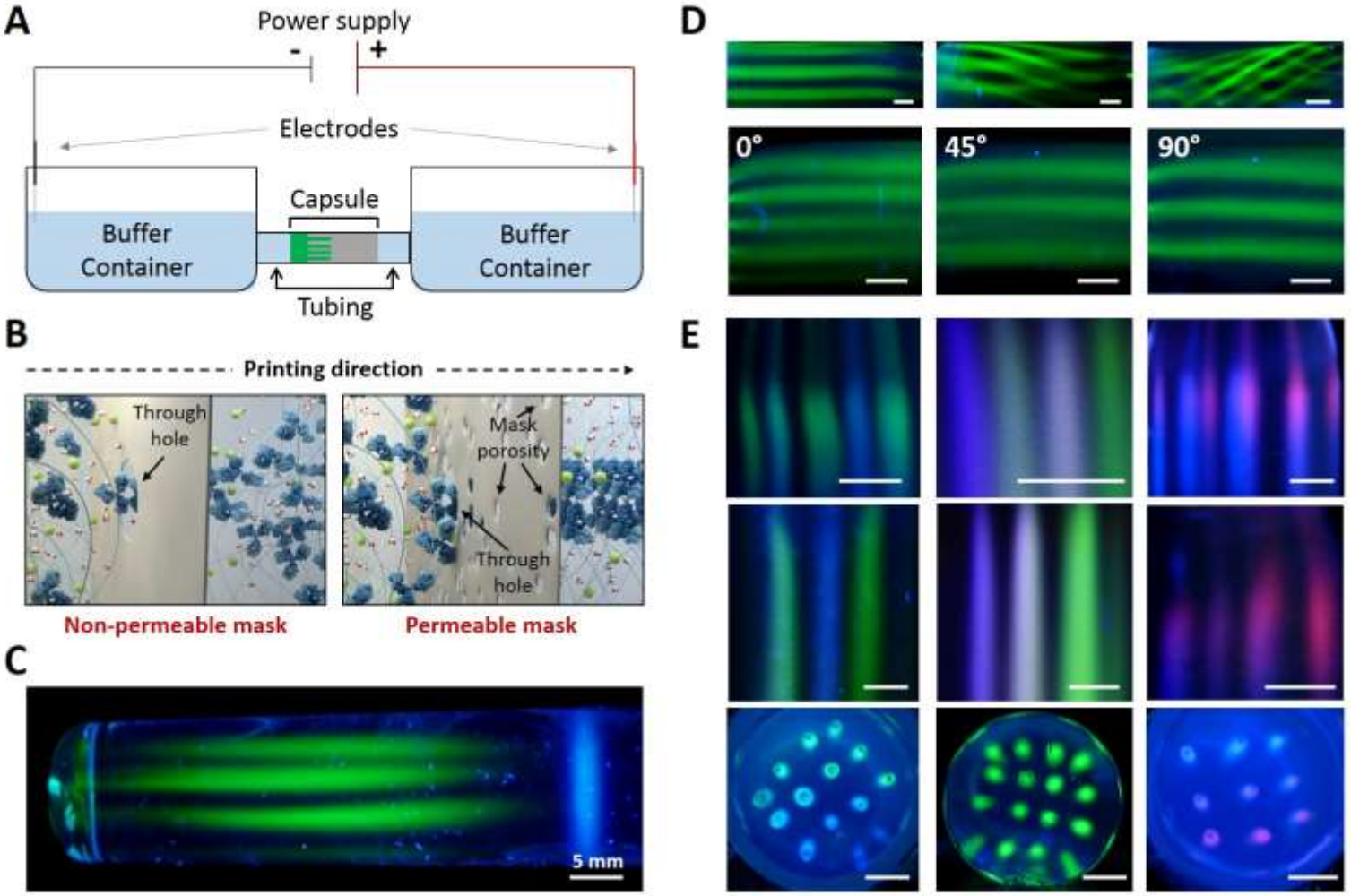

Figure 1. 3DEAL versatility. A, Scheme of the 3DEAL platform with its five components. **B**, Printing process through a non-permeable (*left*) and a permeable (*right*) mask. The inherent porosity of the mask is required for the patterns definition. **C**, PA 6% with 5 cm of gIgG defined and resolved patterns. **D**, PA 3% printed with gIgG. The mechanical properties of the hydrogel allow the moulding of the scaffold to form helicoidal structures without losing the patterns definition (*from left to right*). *Bottom panel*, spatial anisotropy. **E**, Different chemical gradients produced with the 3DEAL of either one, two or three proteins (g-, b- and r-IgG), in z and x-y directions. **Scale bar**: 2 mm.

Picture 1: Caption 1: Figure 1. 3DEAL versatility

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Poster presentation

760 Injectable Hyaluronic acid hydrogel in brain tissue engineering

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[2]Isfahan University of Technology, Iran

[3]Isfahan University of Medical Sciences, Iran

INTRODUCTION:

Brain tissue engineering is a promising way for repair of injured brain. [1]. One of the approaches of tissue engineering is fabricating the scaffolds that can mimic the structure and components of the extracellular matrix (ECM) of the injured tissue. Hyaluronic acid (HA) has long been recognized as an important component of the brain ECM and it plays a critical role in the formation of the brain [2]. The best way for application of HA is to make it injectable. There are many ways for making this gel injectable as: photo polymerization [3], Michael addition crosslinking [4] and Schiff base reaction crosslinking [5]. Also the other way, is to making a copolymer of the HA with another thermosensitive polymer such as Poly N-Isopropyl acrylamide (PNIPAAM). Tan et al. [6] reported the potential application of thermosensitive PNIPAAM grafted HA in Adipose tissue engineering. Here we tested the applicability of this injectable gel in brain tissue engineering and also we evaluate the role of HA in making new blood vessels.

METHODS:

First Hyaluronic acid sodium has been copolymerized with Poly (N- isopropyl acrylamide) (PNIPAAM) which is a typical paradigm of thermosensitive polymers that undergo a coil-to-globule phase transition at ~ 32 C. The copolymer was synthesized by coupling Aminated hyaluronic acid to carboxylic end capped PNIPAAM through amide bond linkages. The Schematic of the procedure is shown in Figure 1. The effect of this hydrogel on neural tissue regeneration was evaluated by culture of neural stem cells on the hydrogel.

RESULTS AND DISCUSSION:

The chemical structure of hydrogel was evaluated by H NMR. The physical structure of hydrogel and also the morphology of cells cultured on the hydrogel and tubulisation of the cells was evaluated by SEM. Viscosity of the hydrogel was determined by Rheometer.

CONCLUSION:

This hydrogel can be a promising material in regenerating the damaged brain tissue.

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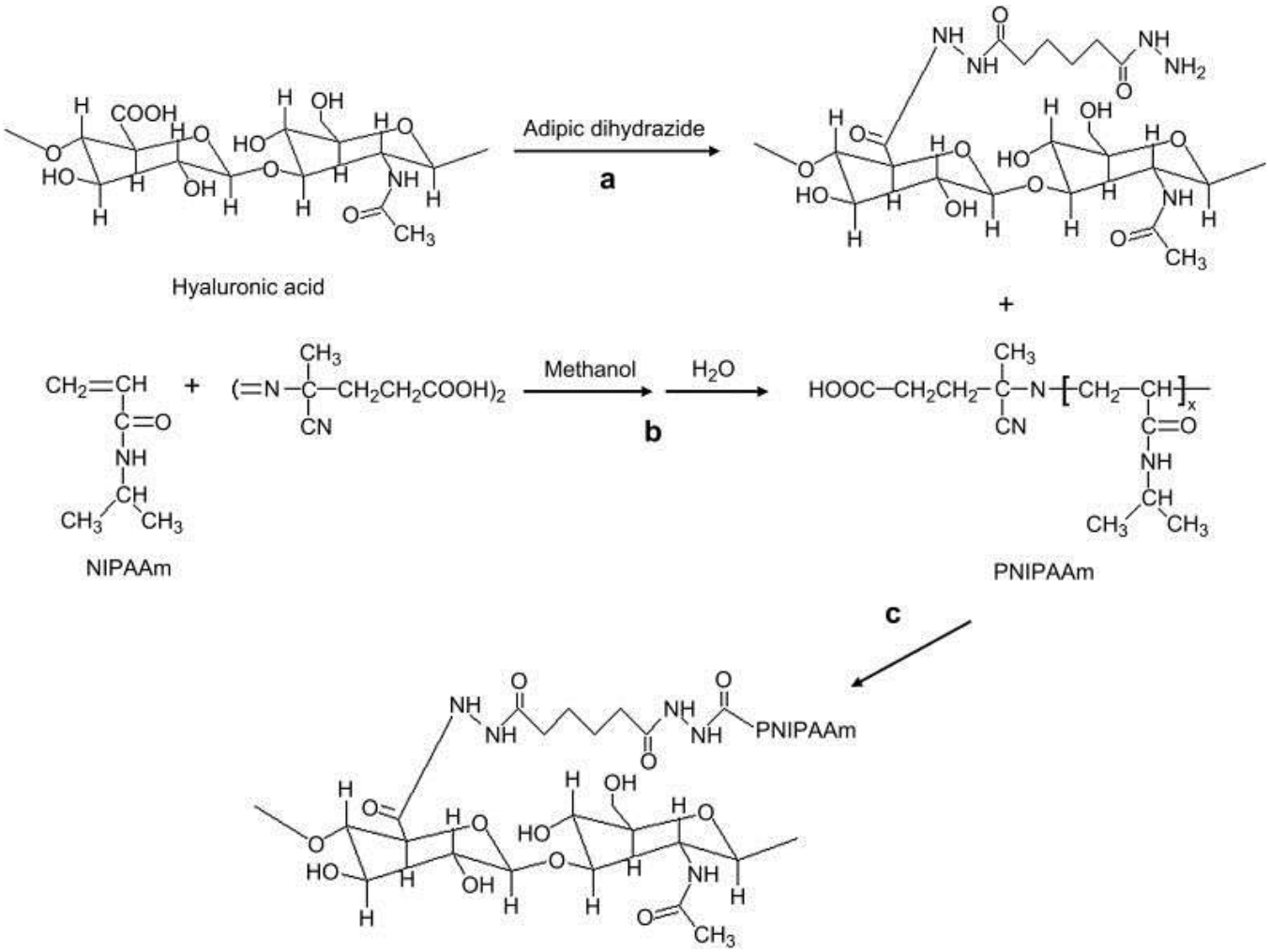

Caption 1: Figure 1. Schematic of HYA-PNIPAAM synthesis [6].

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Poster presentation

769 Engineering collagen based scaffolds for ex vivo culture of breast cancer tissue

John Redmond[1], Ellie Shoji[2], Simona Herbaj[1], Paul Buchanan[3], Tanya Levingstone[4], Nicholas Dunne[5]

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[2]Dublin City University;MEDeng, Ireland

[3]Dublin City University; NICB, Ireland

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[5]Dublin City University; MEDeng; Queens University Belfast;Trinity College Dublin, Ireland

INTRODUCTION:

Breast cancer is the most common cancer amongst women and is responsible for the 2nd highest number of cancer deaths among females[1]. Many current lab-based breast cancer models use immortalised breast cancer cell lines in simple 2D or 3D culture and do not accurately mimic the tumour microenvironment. As a result, the ability to advance understanding of the disease and to test the efficacy of drug therapies is limited[2]. This project proposes a significant advancement in breast cancer research and thus clinical outcomes by developing a novel 3D collagen scaffold model that can be adapted to grow fresh human tumour tissue outside of the body (*ex vivo*).

METHODS:

Scaffolds (Fig.1) were fabricated using a controlled rate freeze drying technique[3]. Scaffold components (type I collagen/gelatin) were blended at 10,000 rpm for 90 min with 0.05M acetic acid and freeze-dried at a constant cooling rate of 1°C min^{-1} to a final freezing temperature of −40°C[3]. Scaffolds underwent dehydrothermal crosslinking (5kPa at 105ºC for 24h). Scaffold architecture was analysed using scanning electron microscopy (SEM). Porosity was calculated using a gravimetric method, comparing scaffold relative density to the theoretical solid density[3]. Pore size analysis was conducted using ImageJ2 software. Mechanical and cytotoxicity assessment is ongoing. Statistical significance between groups will be accessed using one-way ANOVA.

RESULTS AND DISCUSSION:

Successful fabrication of biomimetic and highly porous collagen and collagen/gelatin scaffolds was achieved on a repeatable basis. SEM analysis and porosity measurements showed the scaffolds were highly porous (≥99.27%) with an interconnecting pore structure. Additionally, pores ranged between 100-350 μm in size, were homogenous and relatively equiaxed in shape. Interestingly, increasing the gelatin concentration from 0 to 0.25 wt.% significantly reduced the porosity.

CONCLUSION:

We have successfully fabricated a range of highly porous collagen and collagen/gelatin scaffolds with predominantly homogenous pore size that show promise for use in an *ex vivo* breast cancer culture model. Future work will complete characterisation studies to identity optimal scaffolds for use in *ex vivo* culture assessments.

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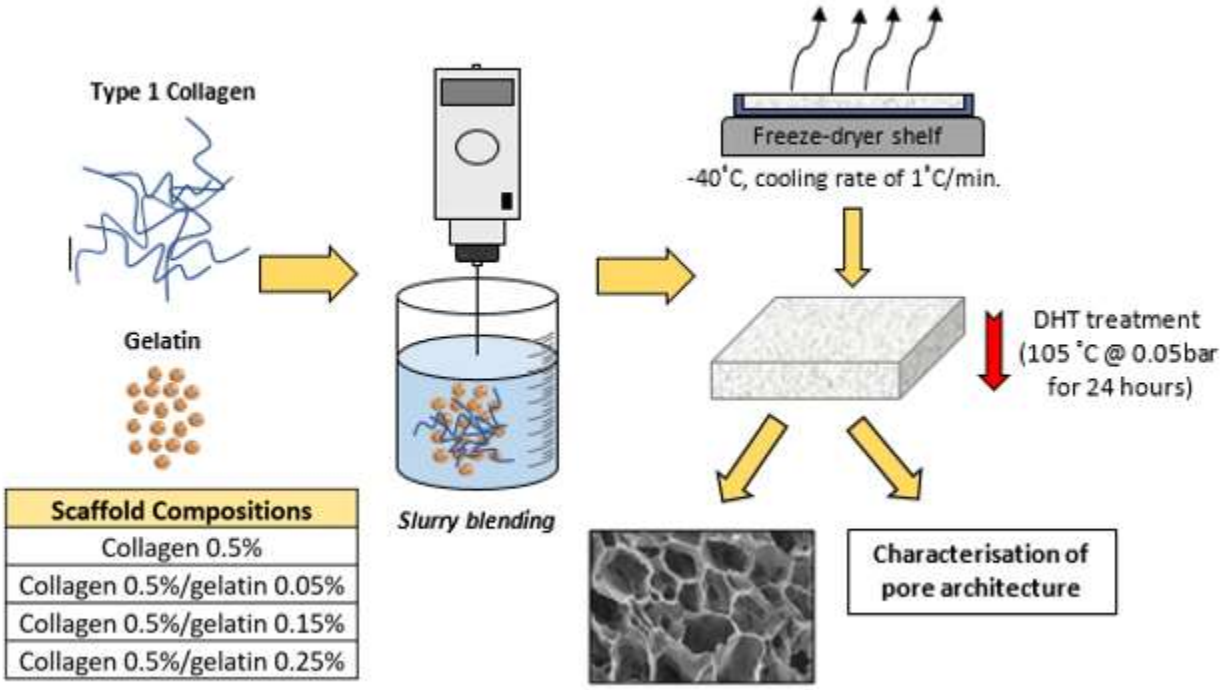
ACKNOWLEDGMENTS:

The authors would like to thank the DCU O'Hare Scholarship for their financial support.

Picture 1:



Caption 1: Figure 1: Schematic demonstration of the fabrication process for collagen/gelatin scaffolds.

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Poster presentation

778 Natural polymers and thiol-yne click-chemistry: synergy towards cytocompatible hydrogel scaffolds with enhanced features

Maria M. Pérez-Madrigal[1], Laura J. Macdougall[1], Maria C. Arno[1], Andrew P. Dove[2]

[1]University of Warwick, Coventry, United Kingdom

[2]University of Birmingham, United Kingdom

INTRODUCTION:

Within the context of biomaterials, the unique features displayed by hydrogels (high water content, suitable porosity, synthetic versatility, and biocompatibility) define them as powerful soft tissue engineering scaffolds. Specifically, through metal-free biorthogonal click reactions, we can now prepare under physiological conditions injectable hydrogels that display outstanding mechanical performance.[1] However, the design of robust hydrogels with accurately predefined properties is still a challenging task, and research efforts need to develop straightforward strategies for the precise control of their properties.

In that regard, we have satisfactorily reported the synthesis of robust poly(ethylene glycol) (PEG) thiol-yne click-hydrogels with tuneable stiffness and excellent mechanical properties achieved through the judicious choice of the polymeric precursors.[2] More recently, we have rendered them nonswellable - hydrogels retain their mechanical performance over time in a biological setting, thus enlarging their bioapplication.[3] Therefore, the current work focuses on further improving their performance by exploiting natural polymers using accessible strategies.

METHODS:

Thiol-yne hydrogels were formed in PBS (pH 7.4) with a 8 wt%. PEG content and a 1 wt%. of natural polymer. Besides, hyaluronic acid (HA) was chemically modified with thiol moieties to obtain a HA-based click-hydrogel. Rheological, mechanical, and biocompatibility testing was conducted to assess the enhanced properties of the natural polymer-containing click-hydrogels and verify their suitability as injectable tissue engineering scaffolds.

RESULTS AND DISCUSSION:

By simply blending commercially available unfunctionalized polysaccharides, our click-hydrogels are still mechanically robust in addition to self-healing and stretchable while providing a matrix which cells can interact with. Moreover, the thiol-yne click-reaction was envisaged as a suitable approach, with few synthetic steps, to prepare click-hydrogels based on HA. The mechanical response of the resulting systems, which displayed adequate swelling, was significantly improved by adding a Ca^{2+}-crosslinked alginate network. Specifically, the ionic noncovalent interactions played a remarkable role in increasing both the Young's modulus as well as the compression strength values of the hydrogels.

CONCLUSION:

The synergetic combination of natural biopolymers with thiol-yne click-chemistry, and hence exploiting the benefits of both elements, results in suitable robust scaffolds with enhanced features for load-bearing soft tissue regeneration.

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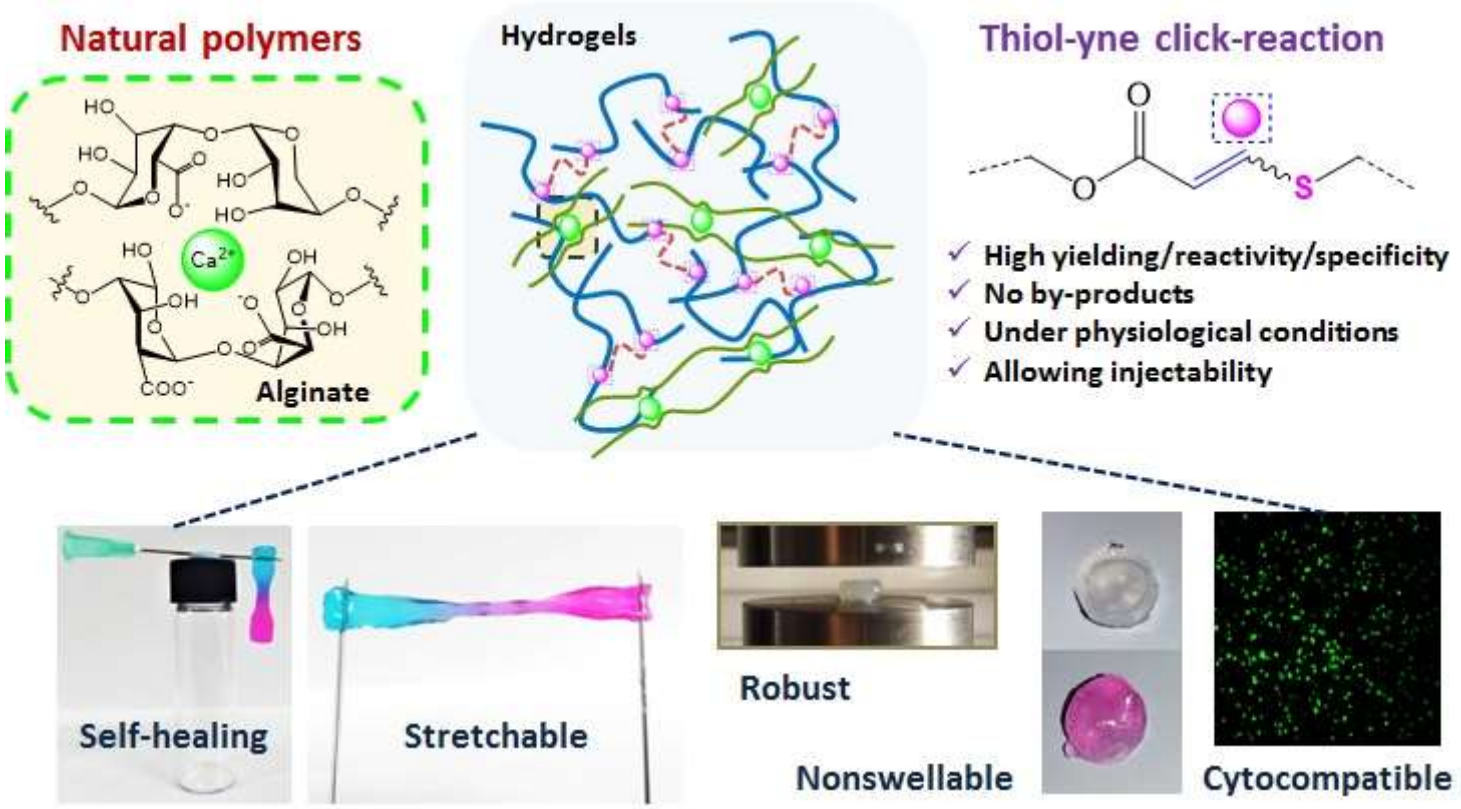
ACKNOWLEDGMENTS:

M.M.P.-M. thanks the EU Horizon2020 - Marie Sklodowska-Curie program (grant agreement 703415). EPSRC are thanked for the DTP studentship to L. J. M., and ERC for the support to M. C. A and A. P. D. (grant 681559).



Picture 1: Caption 1: Graphical abstract

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

783 In vitro toxicity evaluation of Ti6Al7Nb with diffusive nitrided surface layers

Malgorzata Gonsior[1], Karolina Janiczak[1], Barbara Zawidlak-Wegrzynska[1], Maciej Gawlikowski[1], Roman Kustosz[1], Tadeusz Wierzchon[2], Michal Tarnowski[2], Irena Bubko[3], Beata Gruber[3]

[1]Foundation for Cardiac Surgery Development, Zabrze, Poland

[2]Warsaw University of Technology, Poland

[3]National Institute of Medicines, Poland

INTRODUCTION:

Biocompatibility assessment of advanced implants like heart assist devices requires a multi-stage biocompatibility analysis to confirm the safety of use. A novel rotary ventricular assist device ReligaHeart® ROT [1] was developed. Construction of implantable blood pump is a huge challenge in the aspect of long-term contact with blood. Modification of the well-known glow discharge assisted nitriding process called active screen plasma nitriding has been used for enhancing the properties of titanium pump parts, made of Ti6Al7Nb alloy, through production of TiN+Ti_2N+αTi(N) diffusive surface layers [2]. This paper presents biomaterial cytotoxicity and genotoxicity evaluation according to PN-EN-ISO-10993 standard.

METHODS:

Ti6Al7Nb with TiN layer was prepared in a representative process for medical device and ETO sterilized. Genotoxicity assessment was performed with Ames test, with and without metabolic activation. Biomaterial extracts were prepared in PBS (T=37°C, t=72 hours), with final concentration of 0,2g/ml. Salmonella typhimurium strains were utilized: TA97, TA98, TA 100, TA 102. Positive and negative control was used. The test was performed in T= 37°C for t=72 hours. Revertants number was counted. Cytotoxicity analysis was performed by a qualitative test method on mouse fibroblast NCTC clone 929. Biomaterial extracts in culture medium with serum were prepared (T=37°C, t=24 hours). Live and necrotic cells number was counted.

RESULTS AND DISCUSSION:

Genotoxicity results shown as mean revertant number on plate (±SD) obtained for each bacteria strain was similar to the number of spontaneous revertants, in both tests: with and without metabolic activation. The positive control has confirmed proper test conditions. In any of the performed tests the number of obtained revertants was doubled in comparison to number of spontaneous revertants. In the cytotoxicity analysis no cell lysis, neither inhibition of coulter growth nor visible cytoplasmic graininess was observed.

CONCLUSION:

Titanium alloy Ti6Al7Nb with TiN+Ti_2N+αTi(N) surface layer produced in active screen plasma nitriding process reveals no geno- and cytotoxicity. The complete biomaterial biological in-vitro and in-vivo evaluation will be performed to confirm its biocompatibility and hence usability in ReligaHeart® ROT device.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

788 Synthesis of semi-interpenetrated networks of poly(2-hydroxyethyl methacrylate) and poly(e-caprolactone)

Rubén Martín-Cabezuelo, Alfredo Salgado-Gallegos, Guillermo Vilariño-Feltrer, Jose A. Gómez-Tejedor, Ana Vallés-Lluch

Center for Biomaterials and Tissue Engineering, Valencia, Spain

INTRODUCTION:

The goal of this study was to find the optimal synthesis conditions to achieve semi-interpenetrating polymer networks (IPN) of poly(2-hydroxyethyl methacrylate) (PHEMA) and poly(ε-caprolactone) (PCL). This procedure was developed with the intention of being applicable to electrospinning, for the interest of combining such different polymers in porous structures to be used as cell supports, and tailor hydrophilicity, degradability, molecular diffusion, cell adhesion, proliferation and differentiation[1], among other characteristics.

METHODS:

PHEMA synthesis was performed using dimethylformamide (DMF) or methanol (MetOH) as solvents at different concentrations to avoid cross-linking, with 5%wt of benzoyl peroxide (BPO) as thermal initiator. The reaction took place in an oven at 60ºC for a range of time between 12h and 24h. Subsequent cross-linking of PHEMA was checked by allowing the evaporation of the solvent and trying to dissolve PHEMA again. For PCL production, dilutions in a range of 10%wt and 25%wt in DMF and MetOH were performed. For synthesis of PHEMA and PCL semi-IPNs, different polymer proportions (between 10%wt and 25%wt) were tested to be dissolved with DMF, MetOH, and chloroform (CLF) as common solvent. Rheometric assays were conducted to obtain shear moduli and viscosity. Protein adsorption was performed from fetal bovine serum (FBS) 10%v. in PBS for 30 min on flat surfaces obtained by spin coating. Protein adsorption was quantified by means of Bradford protein assay and atomic force microscopy (AFM).

RESULTS AND DISCUSSION:

DMF turned out to be difficult to work with as a solvent whilst MetOH proved to be the optimal common solvent at a concentration of 25%wt for PHEMA and PCL mixtures, since DMF produced unstable. The best PHEMA/PCL solution for spin coating was 25%wt of the polymer mixture in CLF/MetOH (75:25) as solvent. The AFM preliminary results obtained for assessing the optimal timing for protein deposition are shown in Figure 1.

CONCLUSION:

A procedure for obtaining PHEMA/PCL semi-IPNs transposable to electrospinning was successfully set. MetOH:CLF (25:75wt) can be used for a mixture of PHEMA and PCL at 25%wt, as common solvent. Adsorbed proteins show a different pattern depending on the PCL/PHEMA ratio because of their hydrophobic/hydrophilic nature.

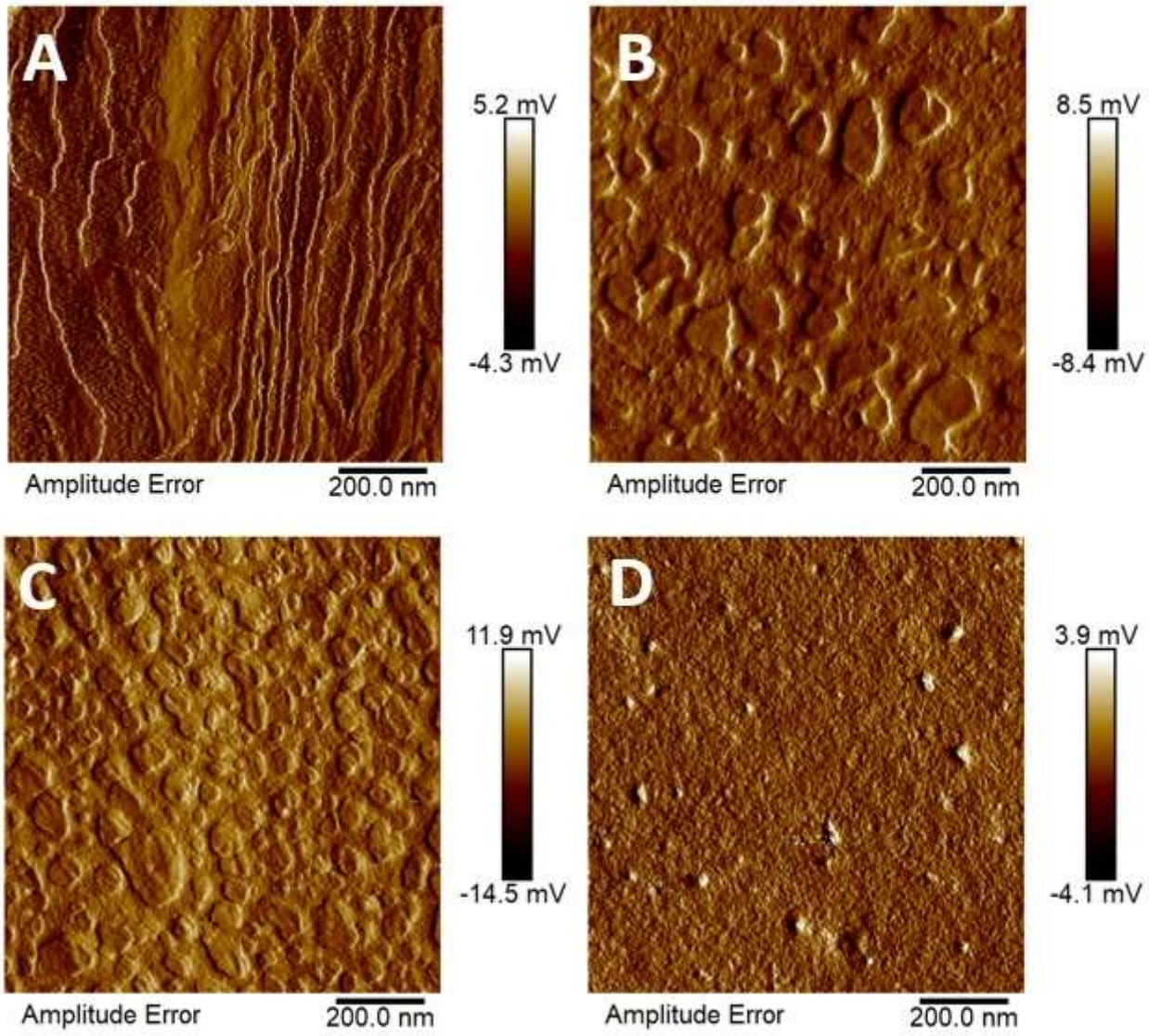
REFERENCES:

A procedure for obtaining PHEMA/PCL semi-IPNs transposable to electrospinning was successfully set. MetOH:CLF (25:75wt) can be used for a mixture of PHEMA and PCL at 25%wt, as common solvent. Adsorbed proteins show a different pattern depending on the PCL/PHEMA ratio because of their hydrophobic/hydrophilic nature.

ACKNOWLEDGMENTS:

This work was funded by the Spanish Ministerio de Economía y Competitividad through DPI2015-65401-C3-2-R project.

Picture 1:



Caption 1: Figure 1 AFM results showing the Amplitude error for protein adsorption test: A) t=0s, B) t=10s; C) t=30s; D) t=60s.

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Poster presentation

789 Zirconium(IV) oxide-yttria stabilized ($ZrO_2 \cdot Y_2O_3$) long-term degradation resistance improvement in biological environment

Barbara Zawidlak-Wegrzynska, Malgorzata Gonsior, Roman Kustosz

Foundation for Cardiac Surgery Development, Zabrze, Poland

INTRODUCTION:

In the clinical version of Polish implantable rotary blood pump ReligaHeart® ROT (RH ROT) [1] the motor divider is made from ceramic composite, ZrO2-Y2O3, with high hardness, to improve device wear resistance [1]. This paper presents the biomaterial long-term degradation resistance in biological environment.

METHODS:

Zirconium(IV) oxide-yttria stabilized (ZrO2 · Y2O3) was prepared in form of disks with the sintering of ceramic powder technology and ETO sterilized. Biodegradation assessment was performed in accordance with PN EN ISO 10993-9 standard in SBF (simulated body fluid) at the temperature of 37°C, with constant stirring (100 rpm), for three different time periods: 30, 90 and 180 days. After degradation test, surface morphological change was investigated with SEM utilization and titanium anion concentration in degradation medium was analyzed using ICP-OES (Inductively Coupled Plasma - Optical Emission Spectrometers).

Additionally the simulation test was performed in accordance with 10993-14 standard at the temperature of 37°C, with constant stirring (100 rpm) for 120 hours, in buffer solution with pH = 7,4 ± 0,1 (simulating the body's normal pH level). The solution was freshly prepared with TRIS-HCl buffer (dissolving 13,25 g of tris(hydroxymethyl)aminomethane in 500 ml of water). The pH was adjust with an appropriate amount of 1 mol/l hydrochloric acid to pH 7,4 ± 0,1 at a temperature of 37°C. After the degradation test, biomaterial microstructure was determined by X-ray diffraction method. The concentration of zirconia anion in degradation medium after the degradation process was investigated with ICP-OES analysis.

RESULTS AND DISCUSSION:

The results showed that the zirconium(IV) oxide-yttria stabilized is stable during the long term degradation process. The zirconium anion concentration level was below 0,05 mg/l. The long term degradation process did not affect the biomaterial structure. The simulation test showed that analyzed biomaterial is stable in pH = 7,4.

CONCLUSION:

ZrO2-Y2O3 is stable in the long term degradation process. The complete biomaterial biological in-vitro and in-vivo evaluation will be performed to confirm its biocompatibility and hence usability in ReligaHeart® ROT device.

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

793 Developing a predictive empirical model to optimize biomaterials characteristics for intra-oral bone regeneration

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[2]Prometheus, the R&D division for skeletal tissue engineering, KU Leuven, Belgium

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INTRODUCTION:

Facial trauma, bone resection due to cancer, periodontal diseases and bone atrophy following tooth extraction often lead to alveolar bone defects that requires bone regeneration in order to restore dental function. Guided bone regeneration using allogenic, xenogenic or synthetic biomaterials has been promoted as an alternative approach to autologous bone grafts. Efficiency of bone grafts is influenced by their physico-chemical characteristics, however, the debate is still ongoing on what constitutes these optimal bone substitute material characteristics. The purpose of this study was to develop a predictive empirical model allowing to assess the bone regeneration potential of new biomaterials on the basis of their physico-chemical characteristics, potentially giving directions of the design of a new generation of dental biomaterials.

METHODS:

A quantitative data set was built composed of morphological characteristics of 7 commercially available intra-oral bone biomaterials (BioOss®, BioOss®-Collagen, BoneCeramic®, Cerasorb®, MP3®, Natix® and Ostim®) and their *in vivo* response when implanted in a sinus augmentation model in rabbits. The morphological properties include chemical composition, micro-porosity and surface roughness parameters. To acquire the surface profile of bone grafts for surface roughness evaluation, we used an in-house developed protocol that allows non-destructive assessment of the micro-scale roughness of porous materials at the outer surface when it is applied on high resolution SEM images[1]. A partial least square regression (PLSR) model was applied to the data set in order to gain find out which (combination of) morphological characteristics would allow to predict the bone regenerative response after in vivo implantation, quantified by the bone to material contact that was evaluated from histology at 3 time points.

RESULTS AND DISCUSSION:

The empirical model based on the aforementioned data set, allowed identification of the construct parameters driving optimized bone formation i.e. (a) the percentage of chemical components, (b) micro-porosity and (c) surface roughness. A leave-one-out strategy was employed to avoid overfitting and assess the potential of the empirical model to be applied to other new materials not present in the training data set.

CONCLUSION:

The presented model provides a better understanding on the influence of driving biomaterial properties in the bone healing process as well as a robust tool for the design of (3D printable) bone biomaterials with more controlled and custom-made structure. This method appears to facilitate and improve clinical translation.

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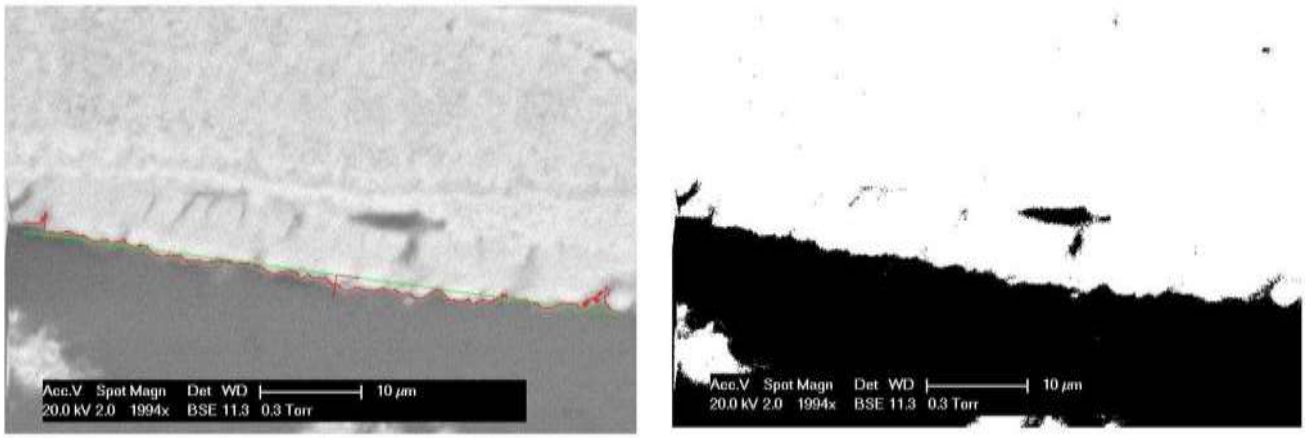
Picture 1:



Caption 1: SEM image analyzed by in-house developed MatLab tool

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

799 Nano-Microcarriers Mediated Naringin Delivery for Bioinstructed Mesenchymal Stem Cells Osteogenic Differentiation

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[1]Universidade de Aveiro, Aveiro, Portugal

[2]University of Aveiro, CICECO - Aveiro Institute of Materials, Portugal

INTRODUCTION:

Naringin is a naturally occurring hydrophobic flavanone present in Citrus fruits and is the main component in Drynaria fortunei, a traditional Chinese medicine for osteoporosis [1]. This natural compound has shown bone regeneration capacity. In this work, the delivery of Naringin to mesenchymal/stromal stem cells through biodegradable di-block methoxy-Poly(ethylene glyco-maleimide-thiol-Poly(L-Lactide) (mPEG-MS-PLA) polymeric micelles and layer-by-layer functionalized microparticles is explored in order to discover ist pro-osteogenic stem cell differentiation potential.

METHODS:

Microparticles/Nanoparticles Formulation and characterization

PLGA microcarriers were formulated by w/o/w emulsion. The oil phase was emulsified with the aqueous phase containing Naringin for the primary emulsification and then poured into the second aqueous phase of PVA to promote particle formation. Layer-by-layer (LbL) of poly(L-lysine) and Hyaluronic acid or poly(L-lysine) and Alginate-GRGDSP peptide functionalized polymer was promoted via sequential deposition-washing steps. Self-assembly of Naringin-loaded or blank mPEG-MS-PLA nanomicelles was performed by nanoprecipitation by extruding polymer solution + Naringin to water. Particle size, zeta potential and morphology were characterized by DLS, STEM and SEM. Nano and microparticles cellular uptake in human Adipose Derived Stem Cells (hASCs) was evaluated by Flow Cytometry and confocal microscopy, for this the particles were loaded with Coumarin-6 dye.

Osteogenic markers evaluation

The ability of particle-loaded Naringin to induce osteogenic differentiation was evaluated by quantification of alkaline phosphatase activity by using the p-nitrophenol method.

RESULTS AND DISCUSSION:

PLGA LbL Alginate-RGD surface nanostructured microcarriers formulated by w/o/w emulsification-solvent evaporation technique demonstrated a spherical shape, high Naringin loading efficacy and suitable size for cellular internalization (3668 ± 203 nm, 78%). Also mPEG-MS-PLA demonstrated colloidal stability and very small size (84.48 ± 2.44) as well as high Naringin Drug Loading (86%). The pro-osteogenic activity of micelle delivered Naringin was investigated by alkaline phosphatase assay. The obtained results suggest that the controlled delivery of Naringin promotes hASCs differentiation into bone cells. The pro-osteogenic activity of PLGA(PLL/ALG-GRGDSP) LbL microparticles was higher than that of PLGA(PLL/HA) microparticles.

CONCLUSION:

Overall, both the micellar carriers and PLGA(PLL/ALG-GRGDSP) LbL microparticles demonstrated suitable properties for the delivery of flavanones into hard to transfect hASCs and are envisioned to be used in the future for stem-cell based osteogenic therapies.

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ACKNOWLEDGMENTS:

The authors to acknowledge the support of the European Research Council grant agreement ERC-2014-ADG-669858 for project ATLAS and the financial support by the Portuguese Foundation for Science and Technology (FCT) vi a Post-doctoral grant (SFRH/BPD/119983/2016, Vítor Gaspar).

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

804 Laponite® crosslinked pNIPAM injectable hydrogels for regenerative medical applications.

Chris professor Sammon, Abbey dr Thorpe, Rasha ms Dosh, Christine professor Le Maitre

Sheffield Hallam University, Sheffield, United Kingdom

INTRODUCTION:

Hydrogels offer potential as a choice of biomaterial for musculoskeletal tissue engineering, due to their hydrated nature, excellent mechanical properties, biocompatibility and extensive structural framework which can be chemically tailored to mimic the *in vivo* extracellular matrix (ECM). Here we showcase an ideal hydrogel that can be injected as a low viscosity liquid, incorporating cells and regenerative factors administered as a minimally invasive injection where the hydrogel solidifies *in situ.* We show how environmental factors such as oxygen concentration, the nature of native tissue and mechanical stimuli influence the nature of the new tissue formed[1-4].

METHODS:

Laponite® crosslinked pNIPAM based hydrogels were synthesised using methods described in the cited literature[1-4] seeded with commercial bone marrow derived human adult mesenchymal stem cells or Caco-2 cells and subjected to standard oxygen (20%) and low oxygen (5%) incubation under static and dynamic culture conditions (orbital

shaker at 30rpm). Samples were characterised using SEM and DMA and subjected to a range of histological analyses including H&E, Masson's trichrome, alcian blue and alizarin red.

RESULTS AND DISCUSSION:

The morphology and nature of matrix and tissue types laid down after incubation was shown to be highly dependent upon the environment that the samples were subjected to. For example hMSCs incubated under 5% oxygen in L-pNIPAM-co-DMAc were shown to result in the laying down of collagen and proteoglycan ECM and cells differentiated towards nucleus pulposus like morphologies. Under the same conditions in the presence of low quantities of hydroxyapatite, osteogenic differentiation coupled with calcium deposition was observed. In the case of Caco-2 cells seeded onto L-pNIPAM incubated under dynamic conditions cells formed microvilli structures analogous to those observed in the small intestine.

CONCLUSION:

We have shown that it is possible to fully tailor the mechanical and morphological properties of cell constructs by careful selection of the hydrogel composition facilitating the differentiation of mesenchymal stem cells towards predetermined phenotypes for a range of clinical applications.

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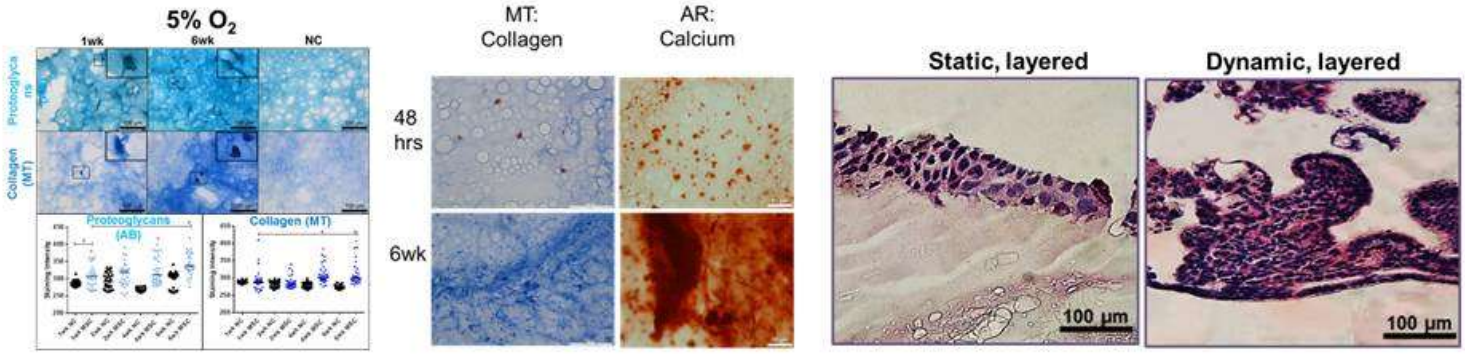
ACKNOWLEDGMENTS:

We would like to acknowledge the support of EPSRC (EP/H000275/1, EP/1016473/1) and Arthritis Research UK (grant number 21497) for supporting this research.



Picture 1: Caption 1: Figure 1: The influence of L-pNIPAM composition and environment on cell morphology and phenotype.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

811 A macroporous delivery device for transplantation of pancreatic islets

Rick de Vries[1], Elahe Hadavi[2], Sandra Smink[3], Clemens van Blitterswijk[1], Marcel Karperien[2], Paul de Vos[3], Aart van Apeldoorn[1]

[1]Maastricht University, Maastricht, Netherlands

[2]University of Twente, Enschede, Netherlands

[3]University Medical Center Groningen, Groningen, Netherlands

INTRODUCTION:

Current treatments for type 1 diabetes include exogenous insulin administration, which only treats symptoms, or islet transplantation which is associated with loss of implanted islets over time.[1] We have previously shown that the implantation of islet-loaded Polyetheylene terephtalate-polybutylene-terephtalate (PEOT-PBT) microwell array delivery device into mice lead to normoglycaemia and survival of islets over 30 days.[2] The current goal is to upscale the original design towards a rat-sized implant to open ways for upscaling towards human-sized implants.

METHODS:

Current treatments for type 1 diabetes include exogenous insulin administration, which only treats symptoms, or islet transplantation which is associated with loss of implanted islets over time.[1] We have previously shown that the implantation of islet-loaded Polyetheylene terephtalate-polybutylene-terephtalate (PEOT-PBT) microwell array delivery device into mice lead to normoglycaemia and survival of islets over 30 days.[2] The current goal is to upscale the original design towards a rat-sized implant to open ways for upscaling towards human-sized implants.

RESULTS AND DISCUSSION:

Animals within the control group reached normoglycaemia within a week, while both PUG and PEOT-PBT implanted rats still show hyperglycemia. Islets could only be detected in 0-10% of all wells in both PEOT-PBT and PUG implants, which are most likely already lost before implantation (figure 1). HE and trichrome stained sections showed blood vessels growing through the pores of the microwells. PUG scaffolds showed a decreased well diameter and increased interspacing, indicating that PUG structures had collapsed and lost their integrity. This is caused by a combination of fibrous tissue formation, wound contraction, relative low mechanical stability and low glass transition temperature of the PUG biomaterial.

CONCLUSION:

We found that PUG has unfavorable mechanical and degradation properties to serve as building block for a long term thin film islet delivery device. PEOT-PBT implants retained their initial shape during implantation. We observed that the rats did not show sufficient stable glycemic levels in contrast to the previously published mouse study. We hypothesize that the implant location, size and scaffold configuration might play an important role in transplant outcomes. We therefore recommend a number of important changes in the scaffold design, manufacturing process and surgical handling which are needed to improve future transplantations.

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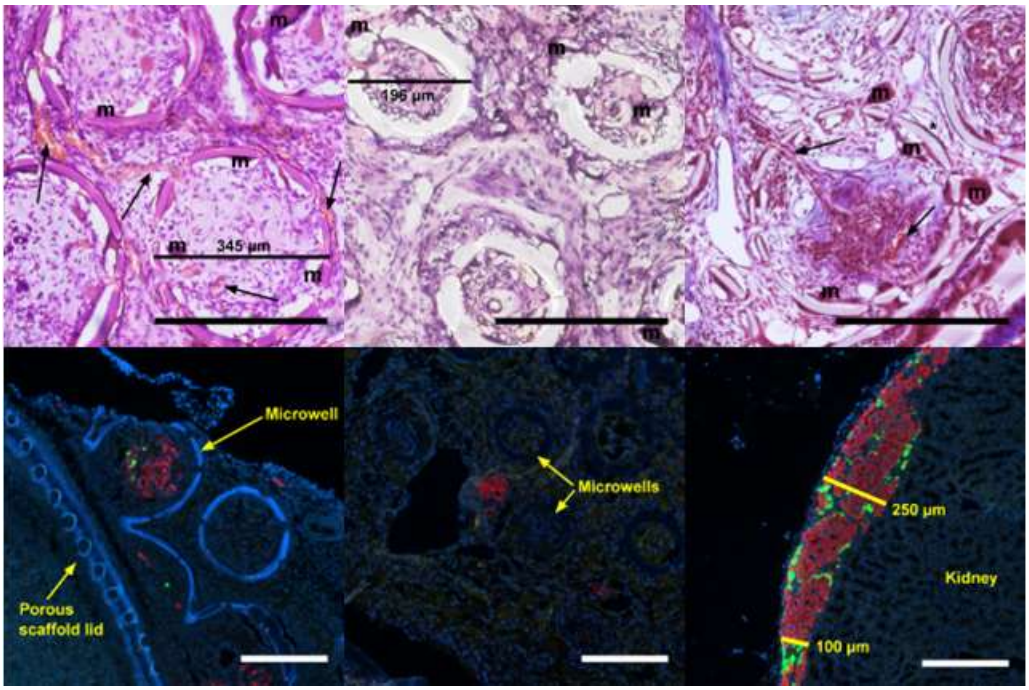
ACKNOWLEDGMENTS:

This project was funded by the Juvenile Diabetes Research Foundation, grant key 3-SRA-2016-256-S-B

Picture 1: 

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

813 Electrospinning as a method for biomaterial manufacturing: an industry perspective

Marco Thio, Rob Mckean, Matei Cirstea, Brendan Robb

The Electrospinning Company, Didcot, United Kingdom

INTRODUCTION:

Restoring damaged tissue through regenerative medicine strategies has gained significant momentum over the last decade, with broad portfolios of biomaterials available to the end user. However, the majority of commercially available regenerative medicine products are based on processed collagen, decellularized donor tissue and ceramic-associated processes, which may be detrimental to some tissue regeneration.

Reconstruction of bone defects is well established using these products. In most cases, these bone substitutes contain natural bone building blocks such as calcium phosphates. In contrast to hard tissue, however, soft tissue regeneration needs tissue extracellular matrix (ECM). For this reason, decellularised tissues such as skin, intestine, amniotic membrane and pericardium are used, and they do not always provide a suitable ECM, offering the surgeon a compromise rather than an indication-specific solution.

Electrospinning technology is widely acknowledged amongst the academic community as a method of producing unique scaffolds for cell culture & in vivo experiments. Features such as superior strength, controllable fibre diameter and fibre alignment, and variable degradability allow the generation of indication-specific biomaterials that, in theory, could outperform currently available medical devices. In addition, numerous publications describe the possibility of incorporating active pharmaceutical ingredients (API) within or surrounding the fibres providing a platform for drug delivery with potential use in major sectors such as wound care, orthopaedics and cell therapies. The technology

also inherently provides a micro layer of fibres, allowing for the modulation of tissue adherence to the implant, which could be used to develop smart implants.

In this brief review, we provide an overview of the status and position of electrospinning as a method for clinical-grade biomaterial manufacturing and share our insights on the challenges and opportunities as a centre of excellence in biomedical electrospinning.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

822 Thioester Linked Cationic Lipopolymers for co-delivery of TRAIL plasmid and its complementary siRNA targets.

Bindu Bindu, Remant K.C., Hasan Uludag

University of Alberta, EDMONTON, Canada

INTRODUCTION:

TRAIL induces apoptosis in variety of cancer cells without affecting normal cells but TRAIL protein therapy tested so far failed in patients due to resistance induction in malignant cells and its rapid renal clearance. An alternative approach to TRAIL therapy is gene based delivery. To improve therapeutic effects, one can also selectively target intracellular mediators that sensitizes the cells to TRAIL action. Hence, in this study, we aim (i) to use TRAIL plasmid, (ii) to identify novel small interfering RNA (siRNA) targets, which sensitize breast cancer cells against TRAIL, and (iii) to co-deliver TRAIL plasmid and identified siRNAs for synergistic activity. We, herein, proposed thioester linked cationic lipopolymers, which display sufficient binding and delivery capacity of individual as well as cocktails of these therapeutics agents.

METHODS:

A siRNA library against 446 human apoptosis-related proteins were screened in breast cancer MDA-MB-231 cells with TRAIL protein. A library of cationic lipopolymers was prepared by rational grafting of aliphatic lipids onto small molecular weight polyethyleneimine (PEI) with amide or thioester bond (composition determined by 1-H NMR). The ability of the polymers to bind to nucleic acids were tested in gel electrophoresis. Delivery efficiency was explored and potential polymer was identified.

RESULTS AND DISCUSSION:

Based on growth inhibition of MDA-MB-231 cells, 16 siRNAs were found to sensitize TRAIL-induced cell death. Novel and the most promising targets were BCL2L12 and SOD1. Silencing of both these targets significantly sensitized TRAIL-induced cell death in MDA-MB-231 cells and TRAIL-resistant MCF-7 cells. Thioester linked polymer resulted higher DNA transfection efficiency while employed in co-delivery of DNA/siRNA cocktail. A range of other polymers was found effective in delivery of individual molecules, but not in co-delivery simultaneously. We optimized the proper composition of plasmid DNA:siRNA as well as polymer/nucleic ratio to generate maximum effect using these particular polymers.

CONCLUSION:

Co-delivery of plasmid DNA and siRNA was feasible to include complementary therapy with a single carrier. It maximized the therapeutic benefit via enhancing each other's transfection efficiency and generating synergistic effects. The therapeutic benefit by dual delivery of TRAIL plasmid and its complementary siRNA targets with single carrier provided a more effective way to treat breast cancer *in vitro*.

ACKNOWLEDGMENTS:

Bindu Thapa is a recipient of Alberta Innovates-Health Solutions (AIHS) Graduate Studentships. This study was supported by CBCF and NSERC. The authors RBKC and HU are the founder and shareholders in RJH Biosciences Inc. that is developing the lipopolymers for medical applications.

Thioester Linked Cationic Lipopolymers for co-delivery of TRAIL plasmid and its complementary siRNA targets: One Stone Two Bird Approach for Cancer Therapy

Picture 1:

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

830 Nanovibrational stimulation (Nanokicking) for 3D osteogenesis in biphasic scaffolds; compositing of freeze dried collagen sponges with MSC seeded hydrogels for bone tissue engineering

Wich Orapiriyakul[1], Penelope M Tsimbouri[1], Peter Childs[2], R.M. Dominic Meek[3], Richard O.C. Oreffo[4], K. Elizabeth Tanner[2], Manuel Salmerón-Sánchez[2], Stuart Reid[5], Matthew J Dalby[1]

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[2]Division of Biomedical Engineering, School of Engineering, University of Glasgow, GLASGOW, United Kingdom

[3]Department of Orthopedics, Southern General Hospital, GLASGOW, United Kingdom

[4]Institute of Developmental Sciences, University of Southampton, GLASGOW, United Kingdom

[5]Department of Biomedical Engineering, Wolfson Centre, Stratclyde University, GLASGOW, United Kingdom

INTRODUCTION:

Nanovibrational stimulation(nanokicking) promotes osteoblastogenesis of human mesenchymal stem cells (MSCs) in 3D culture[1]. We are developing biphasic scaffolds compositing collagen hydrogels and freeze dried collagen sponges to allow 3D culture within nanokick bioreactor aimed at clinical application.

METHODS:

Stro1 selected human MSCs seeded in 1.8 mg/ml rat tail collagen type I hydrogels containing 0%,35%,58%,70% dry weight hydroxyapatite(HA) were prepared as gel phase. To prepare the biphasic scaffold,5% freeze dried collagen sponges were produced and integrated into MSC seeded gels as follows. MSC seeded gels were set with a sponge already in the well; the cells were then stimulated with nanovibration(90 nm,1000 Hz) for 7days. After this time, gels were detached from the well plate and allowed to contract onto the sponges to stiffen the construct. Gel,sponge and gel-sponge mechanical properties were tested by rheology, compression tests and nanodisplacement measurement(interferometry) under nanovibration stimulation. Biological responses was measured using ICP-OES, cell viability testing, qPCR, western blot analysis and metabolomic studies.

RESULTS AND DISCUSSION:

In the gel phase alone(no sponge), the mean elastic moduli of the HA-gels were 180, 194.6, 204.8, 182.8 Pa for 0%,35%,58% and 70% dry weight HA-gels as measured by rheology. At 1000 Hz frequency of nanovibrational stimulation, gel displacement amplitudes were consistency measured by interferometry at~90 nm. Alamar blue and live-dead staining showed that the HA-gels and nanovibration had no negative effect on cell viability. PCR showed a trend of osteogenic gene up-regulation(RUNX2,osteonectin,osterix). Western blotting showed phosphoRUNX2 vs totalRUNX2 up-regulation in the nanovibrated MSC scaffolds. Metabolomics demonstrated involvement of lipid metabolism(energy), and predicted activation of ERK1/2 pathway and inflammatory metabolites suggestive the nanovibrational technique enhanced osteogenesis through natural bone healing pathways.

In the biphasic gel-sponge scaffold, the average elastic modulus of dry sponges was 137.3 MPa(SD=71.61) measured by compression testing;SEM showed the average pore size was 227.74 mm(SD 72.93). Interferometry showed good fidelity of nanovibrational stimulation for the biphasic scaffolds. At day9, microscopy showed MSCs migrating from the gel into the sponge and Alamar blue showed nanovibration increased metabolic activity at day7. PCR showed enhanced osteogenic gene expression(osterix,osteonectin and osteopontin).

CONCLUSION:

Nanovibrational stimulation in the HA-hydrogels is safe for the MSCs and, with nanovibrational stimulation, promotes 3D osteoblastogenesis. Biphasic collagen scaffolds allowed nanovibrational force transmission, improved composite handleability for clinical use and enhanced osteoblastic phenotype.

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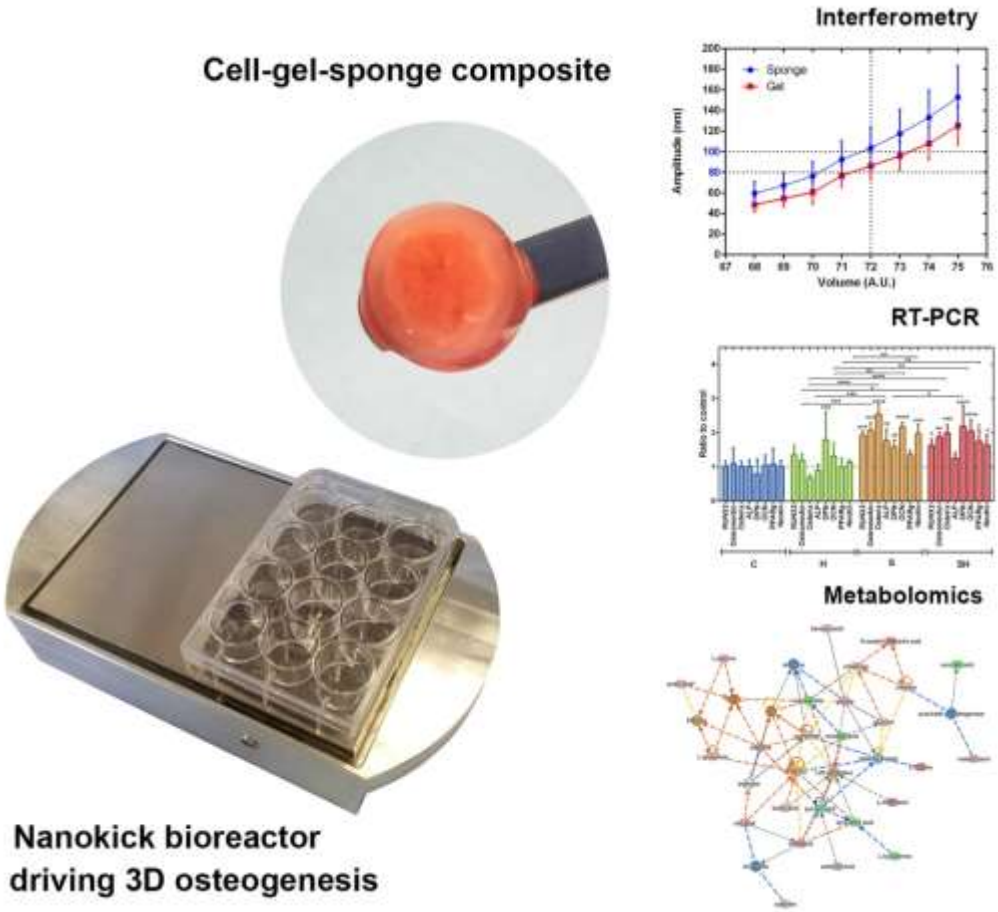
ACKNOWLEDGMENTS:

The authors thanks Royal Thai Government,Find a better way,BBSRC(grant no:BB/N012690/1),EPSRC(grant no: EP/N013905/1) and CA Smith for technical support.



Picture 1:
Caption 1: Nanovibrational stimulation in a cell-gel-sponge composite; reliable, enhance 3D osteogenesis

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

836 Electrospun hydrophobic polycaprolactone fibres for oil-water separation

Ioannis Kouparitsas, Elisa Mele, Sara Ronca

Loughborough University, LOUGHBOROUGH, United Kingdom

INTRODUCTION:

Hydrophobicity is a much sought-after attribute employed in materials science and engineering, with extensive literature describing and testing the multiple aspects through which it can be introduced. One such combination of aspects is the fluorine end-functionalization of polycaprolactone[1] coupled with electrospinning[2]. Polymers synthesized and manufactured in this manner show promise in producing low surface energy fibres with unique topographies[3,4]; optimal characteristics for oil-water separation.

METHODS:

Polycaprolactone was synthesized in-house by utilizing a coordination-insertion ROP with an Al-based catalytic system. Chemical functionalization was achieved via the introduction of fluorine atoms (perfluoro-octanol) to the

polymer chains. The produced polymers were dissolved in solutions of DCM/DMSO (6:1, 36%w PCL) and fibres were produced via horizontal electrospinning at a spraying rate of 4.5 ml/h under an 8 kV voltage. Oil-water separation capabilities were assessed via immersion of fibre mats in mixtures of water and i) dodecane, ii) olive oil, and iii) silicone oil. Absorption was calculated by using $(M-M_0)/M_0$ where M_0 is the initial mass before each cycle and M the mass after absorption.

RESULTS AND DISCUSSION:

Samples A through C, which underwent chemical functionalization via the incorporation of fluorine atoms, show increased oil-water separation as well as oil retention capabilities compared to sample D which was polymerized without this addition. Moreover, no significant differences in surface topography were observed via SEM between the two classes of sample fibres produced.

CONCLUSION:

In conclusion, the resulting highly porous (both externally and internally) micron-fibre mats showed a high degree of bulk oil-water separation capability which, in turn, was improved further with the introduction of fluorine-containing end groups in the polymerization stage. Such constructs are ideal candidates for antimicrobial wound dressings, for example coupled with antimicrobial essential oils, or for other oil-water separation applications.

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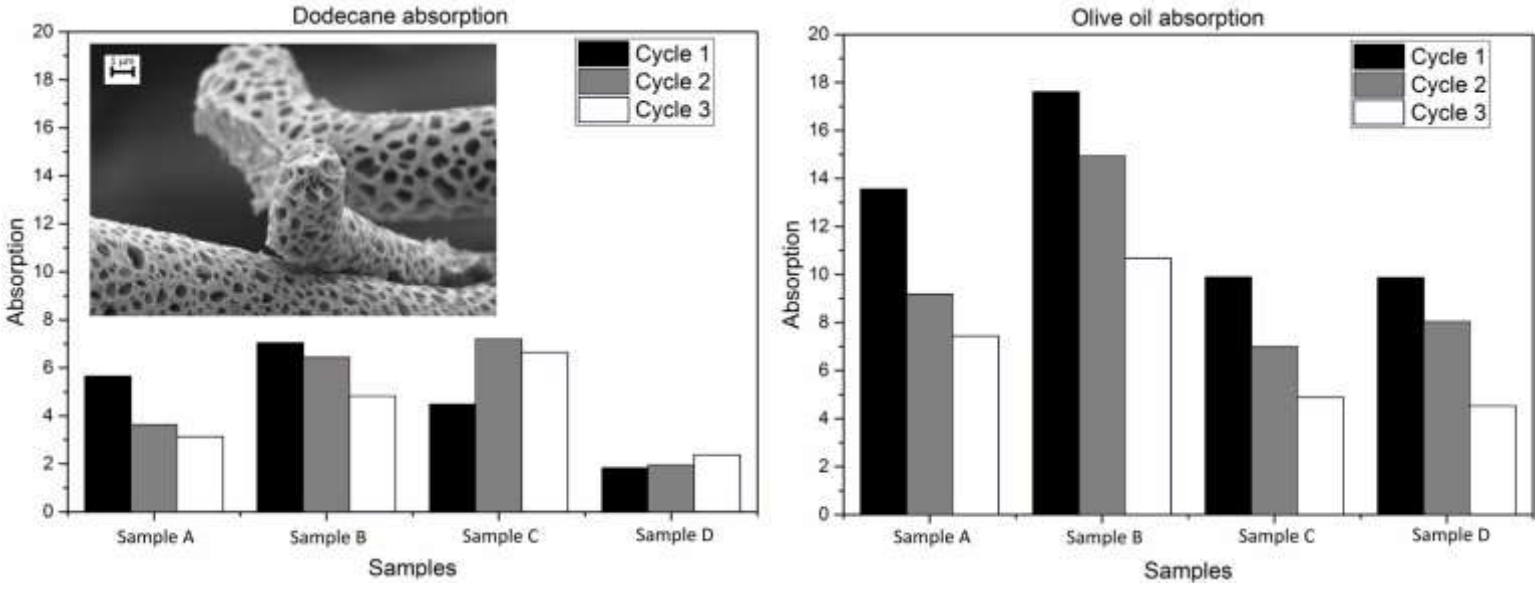

Picture 1: Caption 1: Bulk oil-water separation capabilities across three cycles in various oils. Insert shows an SEM image of sample C.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

837 Synthesis of antibacterial hydroxyapatite by a simple sol-gel method

Diogo Ramos[1], Carmo Lança[2], Margarida Lima[2], João Veiga[2], Manuel Graça[3], Célia Henriques[2], Eduardo Pires[4], Jorge Silva[2], João Borges[2]

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INTRODUCTION:

Hydroxyapatite (HA) is known for its *in vivo* osteoconductive and osteoinductive properties [1,2]. However, the physico-chemical characteristics that support osteoblast attachment and differentiation onto HA surfaces are also responsible for its proneness to bacterial colonization [3,4]. This study aims to take advantage of the antibacterial properties of silver and zinc ions to synthesize HA powders immune to colonization by Gram- and Gram+ microorganisms [5].

METHODS:

Samples of HA co-doped with Ag^{+} and Zn^{2+} were synthesized by a simple sol-gel method: M1 (2 mol% Ag and 0.5 mol% Zn), M2 (1.5 mol% Ag and 1 mol% Zn) and M3 (1.3 mol% Ag and 1.3 mol% Zn). Calcium nitrate, phosphorus pentoxide, silver nitrate and zinc nitrate hexahydrate were used as precursors. The synthesized powders, after sinterization, were analyzed for their structure (XRD), chemical composition (FTIR), cytotoxicity and antibacterial activity.

RESULTS AND DISCUSSION:

XRD analysis showed that the addition of the dopants does not change the crystalline structure of hydroxyapatite. Small changes of the degree of crystallinity and lattice parameters *(a, c)* were observed in doped samples, resultant from the ion insertion in the apatitic structure. FTIR spectra evidenced the presence of characteristic bands of phosphate and hydroxil groups, representative of an apatitic phase.

Cell culture studies performed on all samples, using Vero cells, showed that cell viability was higher than 95% revealing that the presence of dopants doesn't induce citotoxicity.

As demonstrated in figure 1, antibacterial assays revealed a synergistic effect between Ag^{+} and Zn^{2+} ions, resulting in antibacterial activity both for Gram- and Gram+ microorganisms.

CONCLUSION:

HA co-doped with Ag^{+} and Zn^{2+} ions was synthesized by a simple sol-gel method. The co-doped powders revealed antibacterial properties for both Gram+ and Gram- microorganisms.

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ACKNOWLEDGMENTS:

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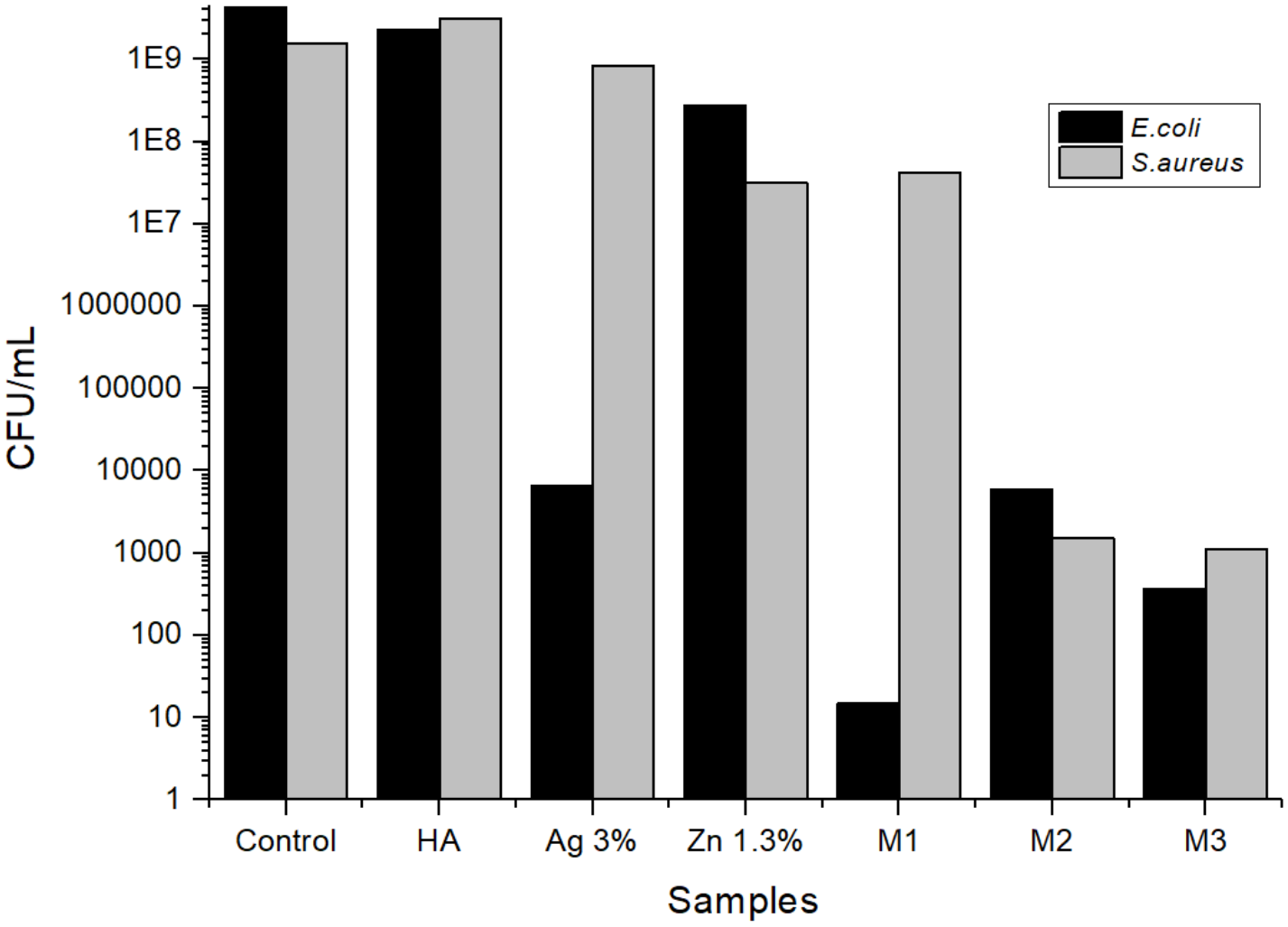

Picture 1: Caption 1: Antibacterial activity assays of synthesized powders.

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Poster presentation

838 Pancreatic beta cell differentiation of human tonsil-derived mesenchymal stem cells by regulating cell-matrix interactions

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INTRODUCTION:

Human tonsil-derived mesenchymal stem cells (T-MSCs) possess a great potential to differentiate into multiple lineages and self-renewal capacity, allowing them to be utilized as patient-specific cell-based therapeutics. Here, we aim to develop a novel cell culture platform that could regulate important cell-cell/cell-matrix interactions by introducing a growth factor immobilized matrix that can support the adhesion, proliferation, and differentiation of T-MSCs into insulin-producing cells.

METHODS:

A. Derivation of human tonsil-derived mesenchymal stem cells (T-MSCs). Tonsil tissues were minced and digested in DMEM containing 210 Unit/mL type I collagenase, 10 μg/mL DNase, and 3mM $CaCl_2$ for 30minutes 37°C with 5% CO_2. Cells isolated from digested tissues were washed with fresh medium for 3 times and plated into T175 flask with a seeding density of 10^8 cells per flask. After 48 hours of initial cell seeding, non-adherent cells were washed out and adherent cells were cultured until they reach 70-80% confluence.

B. *In vitro* differentiation of T-MSCs into Insulin-producing functional beta cells. T-MSCs were seeded into a growth factor-immobilized matrix with a seeding density of 10^5 cells/cm^2 and allowed to form self-assembled organoids. After 48 hours of organoid formation, these organoids were cultured with beta cell induction medium containing B27, N2 supplement, Activin A (100 μg/mL), VPA (Valproic acid, 2M), bFGF (50 μg/mL) and Exendin-4 (23.7 μM) for 2 weeks.

RESULTS AND DISCUSSION:

Our findings demonstrated that T-MSCs cultured on a growth factor-immobilized matrix were able to undergo spontaneous organoid formation through cell-matrix interactions. After 2 weeks of *in vitro* culture in beta cell induction medium, these T-MSCs demonstrated upregulation of pancreatic beta cell-specific genes, such as PAX4, PDX-1, Insulin, and Glut-2. In addition to upregulation of beta cell-associated genes, immunofluorescence staining of insulin and PDX-1 further confirmed successful production of insulin upon differentiation.

CONCLUSION:

This study provides a proof-of-concept that insulin-producing functional beta cells can be generated from T-MSCs. Such a cell culture platform can offer novel strategies to achieve functional pancreatic beta cells from a patient-specific cell source to treat diabetes mellitus.

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ACKNOWLEDGMENTS:

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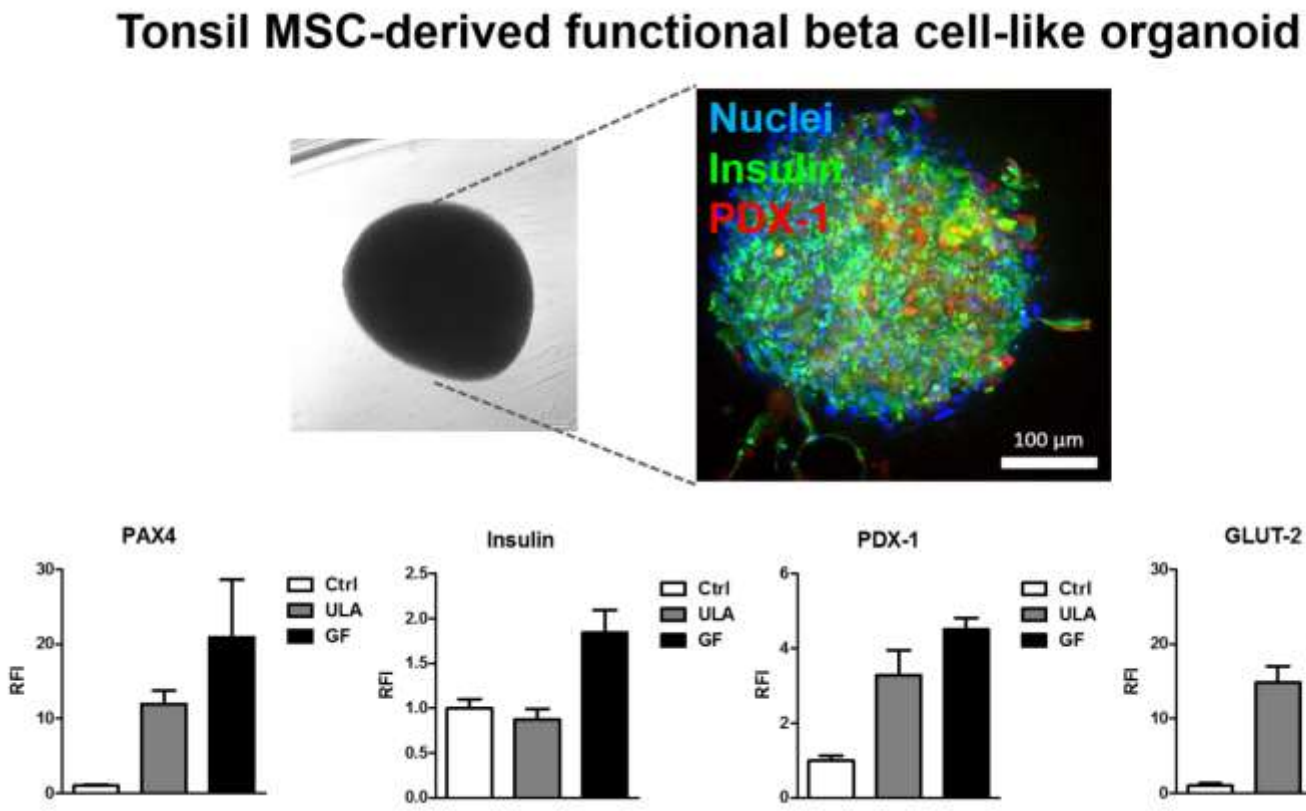
Picture 1:



Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

841 Synthesis of highly biocompatible phases Silicocarnotite and Nagelschmidtite from Hydroxyapatite/Bioactive Glass composites

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INTRODUCTION:

The current trend in bioceramics is focused on substituting replacement tissues by regenerating tissues, which are known as third generation biomaterials[1,2,3]. Ceramics obtained from mixtures of the ternary system formed by Ca_2SiO_4, $Ca_3(PO_4)_2$, and $NaCaPO_4$ have shown promising characteristics such as an improved apatite formation, cell infiltration, and osteogenic differentiation, also observed in silicate-based biomaterials and Si-doped hydroxyapatite (HAp)[3,4,5]. Within the phases of this complex system are the Silicocarnotite ($Ca_2SiO_4 \cdot Ca_3(PO_4)_2$) and Nagelschmidtite structure ($Ca_{7-x}Na_x(PO_4)_{2+x}(SiO_4)_{2-x}$; $x \leq 2$)[4,5]. In this work, a novel mechanism for the synthesis of

Silicocarnotite and Nagelschmidtite from bovine-derived HAp (BHAp) and 45S5-bioactive glass (BG) composites using design of experiments (DoE) methodologies is presented.

METHODS:

BHAp/BG mixtures were prepared using 2^3 factorial design to determine the milling parameters (time, ball-to-powder weight ratio and powder-to-vial volume ratio) to obtain powders with a homogeneous particle size between 5-15 μm as response. Additionally, a 2^2 factorial design was performed to evaluate the effect of temperature and BG content on ceramic densification, considering grain size, porosity and compressive strength as sintering responses. The effect of BHAp/BG ratio on the ceramic characteristics was studied by a compositional mapping from 0 to 30% BG content and subsequently characterized by scanning electron microscopy (SEM), Raman spectroscopy and Rietveld refinement of the X-ray diffraction patterns.

RESULTS AND DISCUSSION:

A combination of milling and sintering processing parameters was established by statistical analysis to improve the microstructural and mechanical properties of BHAp/BG composites. For instance, the sintering temperature with optimal simultaneous responses was 1220 °C.

Variations in BHAp/BG ratios lead to an isothermal phase transformation. Silicocarnotite and Nagelschmidtite, single crystalline phases, were respectively obtained with of 85/15 and 70/30, BHAp/BG ratios. Multiphased materials were obtained in ratios between 92.5/7.5 - 88.75/11.25 and 81.25/18.75- 77.5/22.5.

CONCLUSION:

A low-cost and efficient processing route to obtain Silicocarnotite and Nagelschmidtite phases was stablished by DoE using bovine-derived HAp and bioactive glass 45S5 as starting powders.

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ACKNOWLEDGMENTS:

The authors thank to Conacyt for the financial support. This project was funded by Conacyt Projects 272095 and 2293429 (National Laboratory program) and carried out partially at CENAPROT, LIDTRA and LISMA laboratories.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

843 fabrication of poly(L-lactic acid)/chitosan macroporous scaffolds for bone tissue engineering

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INTRODUCTION:

For bone tissue engineering, the hydrophobicity of PLLA makes it hard for cells to attach on the scaffold . Chitosan has been used to modify the PLLA to improve its hydrophilic, mechanical properties and degradation performance[1,2]. However the poor solubility of chitosan in organic solvents limiting its application with PLLA[4]. And, it is still a problem to maximize the contact area and interaction of chitosan and PLLA.

METHODS:

The porous scaffolds were fabricated by TIPS. Briefly, 4% (W/V) PLLA was dissolving in dioxane/water mixed solvents (86/14) at 70 °C for 2 h. Then the solution was transferred into -80°C for 2 h. Deionized water was used to solvent exchange at 4 °C and changed three times a day for 2 days. Chitosan solutions (1 to 3 wt.%) were prepared by dissolving chitosan in 1% acetic acid. Previously prepared PLLA scaffolds were immersed into a mixture of acetone/water (7:3) for 1 h. The treated scaffolds were then transferred to chitosan acetate aqueous solution and squeezed several times. The resultant sample was freeze-dried for 48 h and stored.

RESULTS AND DISCUSSION:

Twelve weeks after postoperation, as shown in Figure 1a, the large cavity defect in the blank group and control group still remains. In Figure 1b, c and d, with the increase of chitosan concentration, more bone tissue with higher density had been detected in the defect site than in the control group. The largest amount of regenerated bone tissues were found when the concentration of chitosan reaches 3%, with the regenerated bone tissues filling almost 90% of the defect. The regenerated bone volume in the defect and bone mineral density in the 3% group was much higher than in the other scaffolds (Fig. 1e, f).

CONCLUSION:

In vivo, the PLLA/CS scaffolds scaffold group healed the calvarial bone defect better than the control group, and the 3%CS group healed the defect best. Therefore, such PLLA/CS scaffolds show promise for bone tissue engineering applications.

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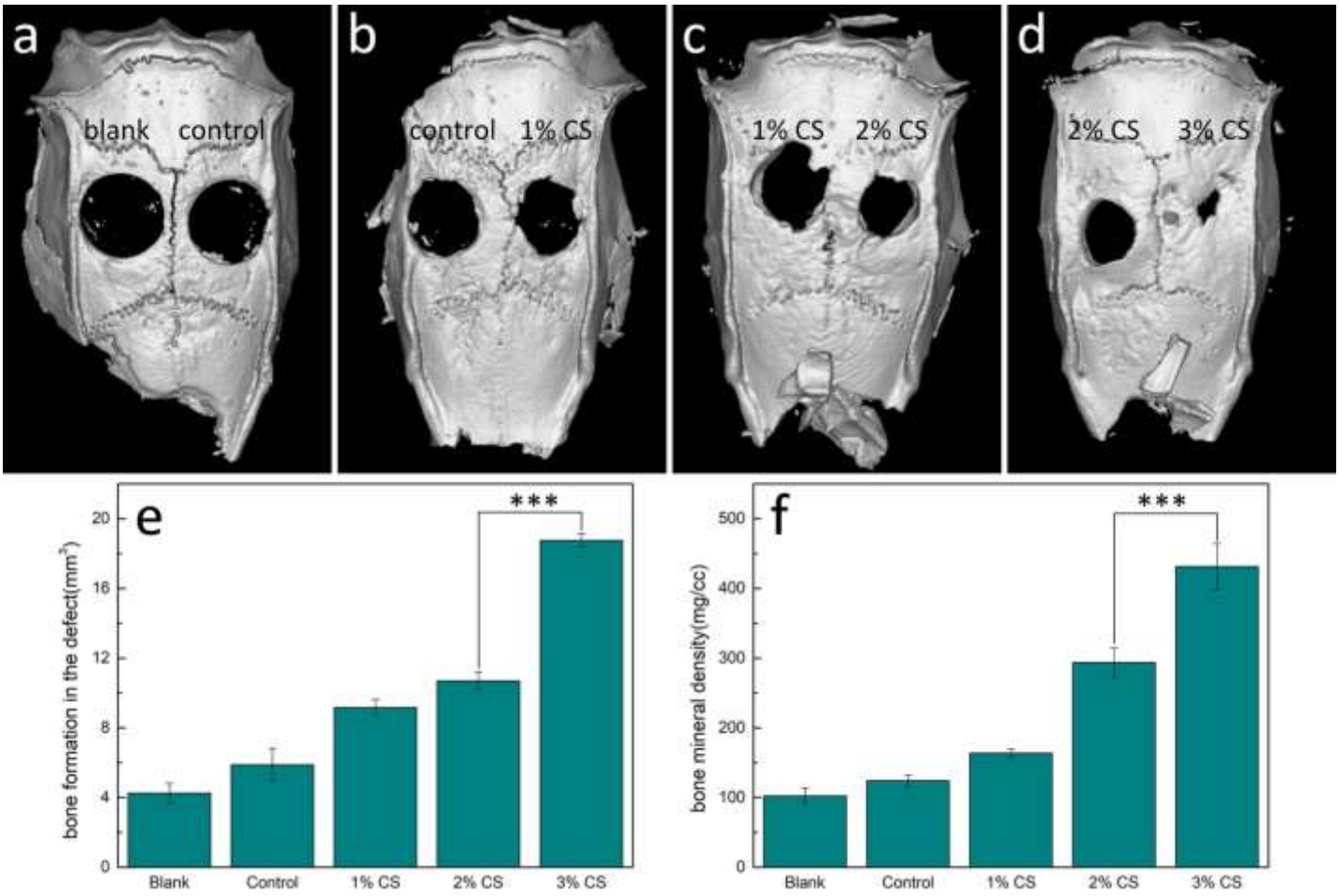

Picture 1: Caption 1: Fig. 1. Micro-CT results of the PLLA/CS scaffolds repair of rat skull defect for 12 weeks.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

849 Electrochemical behavior of Ti-Cu-Sn alloys in the 0.2 wt.%NaF mouthwashes

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INTRODUCTION:

Titanium and Titanium alloys are widely used for dental implants and restorations because of their good biocompatibility, enhanced mechanical behavior and excellent corrosion resistance in physiological media. The corrosion resistance of Ti-based alloys is due to the spontaneous formation of the nano-TiO_2 oxide film on their surface, which exhibits elevated stability in biological fluids[1]. In the literature, there are very few studies on the effect of Sn addition on the corrosion behavior of Ti-Cu alloys[2,3]. Therefore, the aimof thisworkwas to investigate themicrostructure and the mechanical properties of Ti-Cu-Sn alloys as a function of the Sn content and to discuss the alloys corrosion behavior in the 0.2 wt.% sodium fluoride (NaF) mouthwashes at room temperature using electrochemical techniques.

METHODS:

Ti7Cu*x*Sn(x = 0 and 2.5 wt. %) samples were prepared usingan arc melting furnace with water-cooled copper crucible under a high purity argon atmosphere from Ti (99.8%), Cu (99.99%), and Sn sheet (99.99%) as raw materials. The corrosion behavior of Ti7Cu*x*Sn alloys was evaluated through potentiodynamic polarization measurement in 0.2 wt.% NaF mouthwashes.

RESULTS AND DISCUSSION:

Fig. 1 presents the representative Tafel plots for the Ti7CuxSn samples in the 0.2wt.%NaF mouthwashes. Tafel results indicated that the corrosion current densities were in the magnitude of below 0.5 mA/cm^2. Anodic polarization curves demonstrated a wide passive region. Another, increasing the Sn content to 2.5wt.% improves the corrosion resistance of Ti7Cu*x*Sn alloy by increasing the corrosion potential (E_{corr}), passive range (ΔE) and slightly decreasing both the corrosion density (I_{corr}). In recent work[2,4], adding Sn can significantly refine Ti_2Cu particles and increase the volume fraction of the Ti_2Cu phase, thereby improving corrosion resistance.

CONCLUSION:

The low corrosion current and wide passive region suggest that Ti7Cu*x*Sn alloys have good corrosion resistance in the 0.2wt.% NaF mouthwashes. Another, the electrochemical corrosion behavior of Ti7Cu*x*Sn alloys can be improved by increasing Sn content.

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ACKNOWLEDGMENTS:

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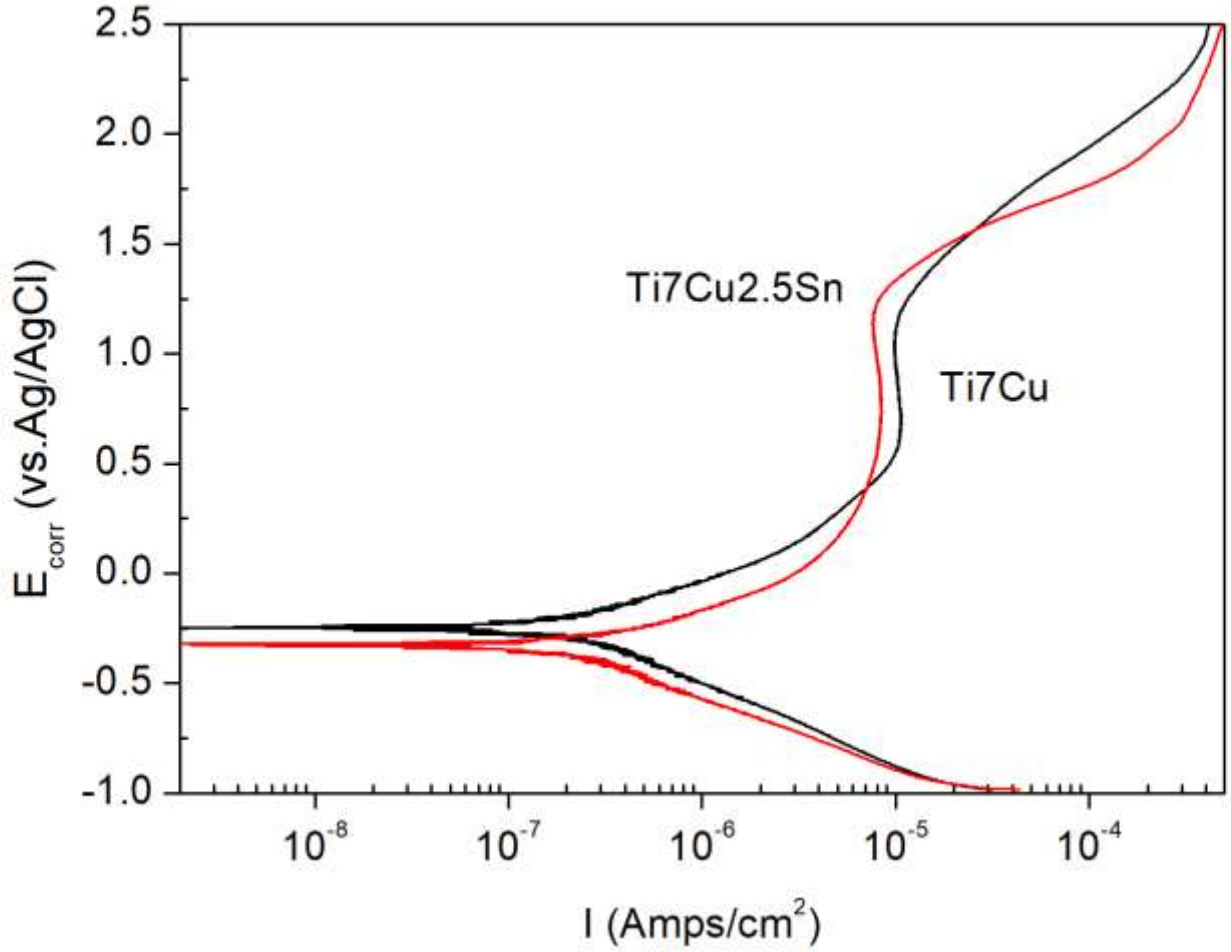
Picture 1:



Caption 1: Fig.1 Potentiodynamic polarization of Ti7CuxSn alloys. polarization of Ti7CuxS

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

851 Near-infrared light triggered release of bioactive molecules from supramolecular modified gold nanorods

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INTRODUCTION:

Previously, we demonstrated that small molecules such as retinoic acid (RA) released by nanoparticles may control the biological activity of neural stem cells[1,2] or leukemia cells[3] and the possibility to control *in vivo* the release profile of nanoparticles by a blue laser. Unfortunately, tissue penetration of the blue laser is relatively low[3]. Here, we

developed a near infrared-triggerable release system of RA that allows high tissue penetration. The nanoformulation is based in gold nanorods (AuNRs) conjugated with cucubit[6]uril (CB6) host to complex modified RA (Figure 1). After exposure to NIR laser, the AuNRs generate plasmonic heat which is enough to disrupt the host-guest interaction between CB6 and RA.

METHODS:

The functionalized AuNRs were synthesized using seed-mediated method with a stabilizer, followed by ligand exchange with CB6 modified hyaluronic acid (CB6HA). The ligand exchange was evaluated by UV-Vis, Z-Potential, FTIR and DLS.

RESULTS AND DISCUSSION:

We demonstrate the release of RA from the nanoformulation upon NIR irradiation by fluorescence spectroscopy. Since the complexation of RA with CB6HA@AuNRs is an exothermic process, the binding strength of the host-guest complex between RA and CB6 remarkably decreases with increasing the temperature[4]. Therefore, the increased temperature near the AuNRs surface due to photothermal conversion upon irradiation (2min, 780nm, 2W/cm^2) lead to the displacement of the RA from the CB6 cavity. Additionally, we confirmed the high drug loading capacity of the nanoformulation, 100µg of RA per mg of AuNRs.

In a NB4-RARE reporter cell line, confocal images evidenced the fast internalization of the AuNRs and their endolysosomal escape. We also evaluated the photothermal efficacy to deliver RA in the reporter cells exploiting activation at different times. The results show high levels of luminescence in cells that were light activated, in both cases, indicating the spatio-temporal control in the release of RA.

CONCLUSION:

We have developed a functional AuNR complex for precise delivery of RA upon activation with NIR light. The surface functionalization of the AuNRs with CB6 provides a mechanism to complex RA with high efficiency and with the advantage of the delivery in cells with spatio-temporal control.

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ACKNOWLEDGMENTS:

Authors would like to acknowledge ERA Chair project ERA@UC ref:669088 through EU Horizon 2020 program, Compete 2020 POCI-01-0145-FEDER-016390:CANCEL STEM project, POCI-01-0145-FEDER-029414:LIghtBRARY project and FCT (SFRH/BPD/102103/2014) for supporting this work.

Picture 1: https:\\www.eventure-online.com/parthen-uploads/40/18903/add_1_459920_663d7bee-42d7-4595-9ccf-92b11b94a2fa.jpeg
Caption 1: Schematic representation of the macrocycle conjugated gold nanorods for NIR light photothermal drug delivery.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

852 Bioactivity and degradation assessment of the Silicocarnotite and Nagelschmidtite ceramics synthesized from Hydroxyapatite/Bioactive Glass composites

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INTRODUCTION:

In-vitro and *in-vivo* biocompatibility of bioceramics composed by Ca_2SiO_4-$Ca_3(PO_4)$-$NaCaPO_4$ have attracted the interest in hard tissue regeneration[1,2]. Previous studies with Silicocarnotite-($Ca_5P_2SiO_{12}$) and Nagelschmidtite-($Ca_7Si_2P_2O_{16}$) have shown an excellent apatite mineralization as well as an improved proliferation, differentiation and osteogenic/cementogenic gene expression related to their ionic products[2,3,4]. The aim of this study is to assess the *in-vitro* bioactive behavior of non-stoichiometric Silicocarnotite and Nagelschmidtite phases, synthesized from bovine-derived-Hydroxyapatite/Bioactive-glass-(BHAp/BG) composites, to identify the influence of structure/composition on apatite formation kinetics, phase degradation rates and their products.

METHODS:

Silicocarnotite and Nagelschmidtite were synthesized from BHAp/BG composites with 85/15 and 70/30 ratios, respectively. Starting powders were mixed, pressed and sintered to obtain dense ceramics. The ability to form apatite layer in Hank´s solution was evaluated following the ISO/FDIS-23317:2007 standard. Ceramics exposed between 6 to 28 days were characterized by XRD, Raman spectroscopy and SEM, determining structural and microstructural variations compared to as-produced ceramics. Rietveld analysis of the XRD patterns were performed to characterize and quantify the apatite formation and the crystallographic changes in each sample. Ceramics degradation was evaluated according to ISO-10993-14 standard.

RESULTS AND DISCUSSION:

XRD patterns of samples immersed in Hank´s solution for different periods showed the formation of an apatite layer. For the Nagelschmidtite, a total phase transformation was observed after 21 days of immersion. Microstructural changes were observed on both ceramics surface starting with globular precipitates, followed by dune-like structures and ending with plate-like crystals. An increase in apatite layer thickness from 3 to 9 µm was observed for Silicocarnotite and Nagelschmidtite, respectively. The dissolution products measured for both phases are non-cytotoxic. These results were compared and discussed with the BHAp responses.

CONCLUSION:

The apatite layers formed on ceramics´ surface are a clear evidence of bioactivity with different kinetics as correlated with the original phase structures. Dissolution rates and products were different for each phase studied indicating a wide range of biomedical applications.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

854 Bioactivity and in vitro tests of hydroxyapatite based coatings for hard tissue engineering

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INTRODUCTION:

Hydroxyapatite (HA) is widely used in the coating of implants because of its biocompatibility and similarity to the hard tissue. To obtain HA coatings in titanium alloys, the most common commercial technique is plasma spray (PS) deposition, requiring high temperatures. A recent coating technique at room temperature, CoBlast™ (CB) consists of a mechanical micro-shot blasting process[1]. Because of the piezoelectricity of bone and its influence on the bone remodelling process, enhancing the piezoelectric characteristics of the coatings will improve the osteogenic capacity of the coatings. Barium titanate (BaTiO3) is a piezoelectric bioceramic, which has been successfully combined to HA in hard tissue studies[2]. In this work a comparison between coatings of HA obtained by PS and CB depositions was made and HA/BaTiO3 coatings by CB were investigated.

METHODS:

The substrate Ti-6Al-4V (medical grade) was coated by CoBlast™ with 2 different materials HA and the mixture of 80%/20% (m/m) of HAp/BaTiO3 (piezoelectric micropowders with tetragonal structure) and for comparative purposes, HA coatings were produced by plasma spray. The CB coatings used alumina has abrasive to create the necessary roughness for good HA adhesion. The coatings were analysed using the DRX, FTIR and SEM techniques to study the structural, chemical and morphologic composition. Bioactivity was investigated by coatings immersion in SBF. And the *in vitro* tests cytotoxicity, adherence, proliferation, metabolism and cellular morphology.

RESULTS AND DISCUSSION:

Average coating thickness by PS is 13 mm and by CoBlast™ 5 mm. DRX and FTIR showed for all coated materials the absence of subproducts and the presence of the typical crystalline structures of HA and BaTiO3. Higher crystallinity of HA was found in PS coatings. Cytotoxicity tests evidence that all materials are suitable for implants coating. By SEM and EDS it was concluded that plasma spray coating is more bioactive. The *in vitro* tests showed higher cell adhesion and proliferation for the piezoelectric mixture.

CONCLUSION:

By comparison with samples without coating, the coatings produced with PS were the most bioactive, and the least biocompatible, whilst the CB coatings with the piezoelectric mixture were the most biocompatible.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

856 In-vitro paraxial mesoderm morphogenesis in 3D artificial extracellular microenvironments

Gregorius Rustandi, Helena Ferreira, Abdel Rahman Abdel Fattah, Sergii Grebeniuk, Adrian Ranga

KU Leuven, LEUVEN, Belgium

INTRODUCTION:

Dysregulated skeletal muscle development can lead to severe pathological conditions. Current 2D in-vitro skeletal muscle models fail to recapitulate key events in developmental morphogenesis, thereby lacking the possibility to investigate the important role of the extracellular microenvironment[1]. Human pluripotent stem cells (hPSCs), particularly 3D organoids grown in suspension, provide a unique model to recapitulate the developmental stages of skeletal muscle and correlate the early morphogenetical characteristics with the cell fate specification. By embedding such organoids in highly defined poly(ethylene)-glycol (PEG)-based artificial extracellular matrices (aECM)[2] the biomechanical and biophysical role of the extracellular microenvironment at various points in development can be investigated.

METHODS:

Single-cell derived-hPSCs from genetically engineered transcription factor reporter cell lines were differentiated towards skeletal muscle in customized agarose-based microwells by introducing defined signaling factors already established in 2D culture for 12 days. Cell fate specification and early morphogenetical characteristics were assessed by daily live imaging of reporter expression, followed by unbiased ranking correlation of such metrics using hierarchical clustering analysis in MATLAB. To assess the role of the biomechanical and biophysical extracellular microenvironment in hPSC-derived skeletal muscle differentiation, organoids were embedded in synthetic matrices of variable stiffness (0,5kPA and 2kPa).

RESULTS AND DISCUSSION:

The early stages of human skeletal muscle development could be recapitulated up to the skeletal muscle progenitor fate using organoids derived from established reporter cell lines (**Figure 1a**). On average 92,9% (±6,8%) of organoids demonstrated paraxial mesodermal cell fate, which reached its peak expression levels at day 4 (94,4% (±5,4%) Msgn1+ organoids. Embedding organoids at early time points in defined aECMs resulted in similar cell fate specification (**Figure 1b**), but differing growth kinetics, including proliferation constraint compared to suspension model. Significant correlation between high Msgn1-expressing fraction area, high reporter mean intensity and low starting size and perimeter of the organoids were also observed (**Figure 1c**).

CONCLUSION:

Our preliminary results demonstrate for the first time the generation of hPSCs-derived skeletal muscle organoids which recapitulate the early stages of development. Further differentiation of these organoids to myoprogenitor cells and mature skeletal muscle by characterizing the morphogenetical characteristics along with the cell fate specification involved in this process will help in establishing the relationship between these key developmental processes. This will contribute to generation of a more robust and systematic platform to investigate skeletal muscle differentiation in normal and myopathic development and contribute to understanding morphogenesis at elusive early stages of human development.

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Picture 1:

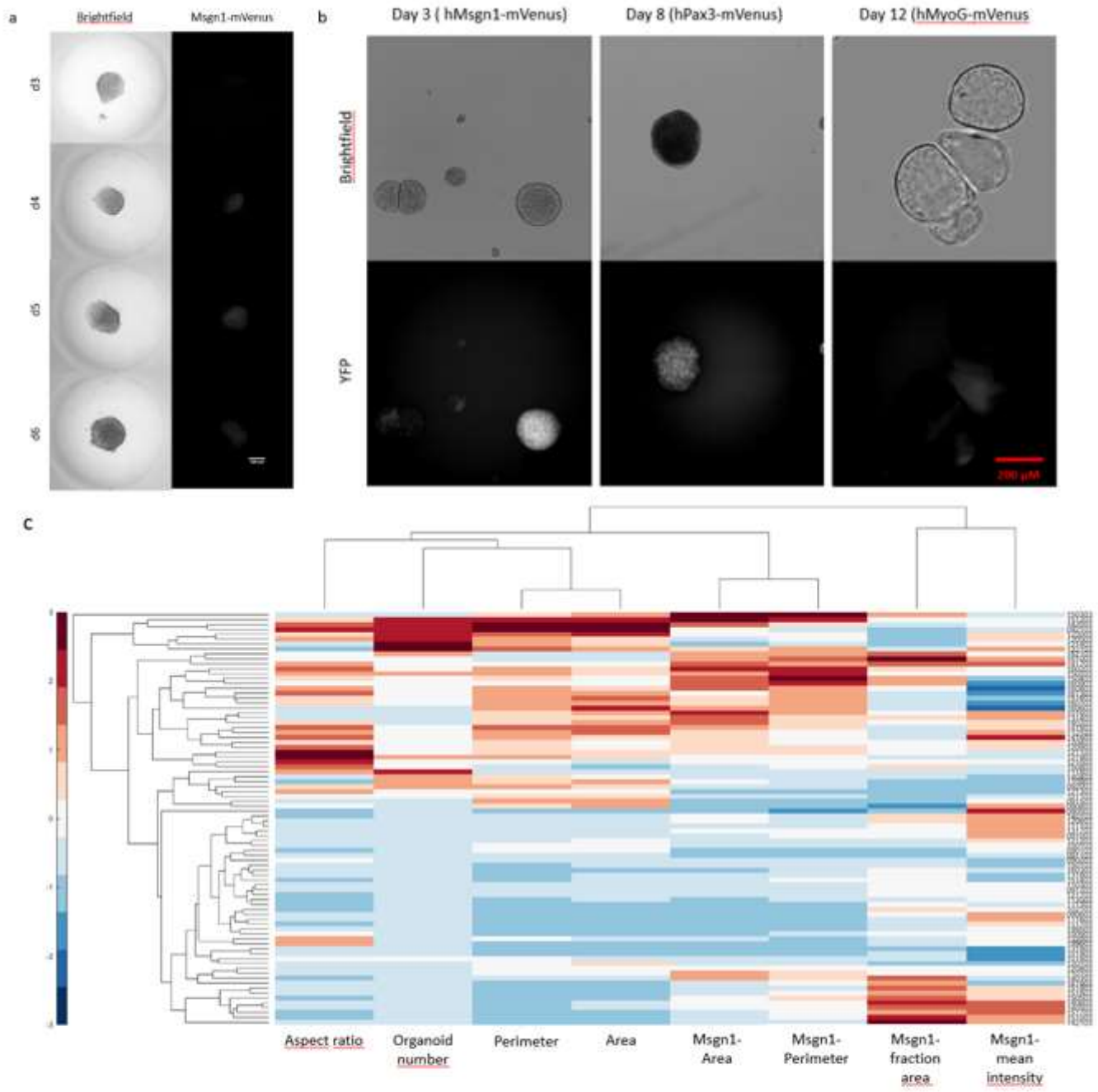

Figure 1. a. Expression of early pre-somitic mesoderm marker (*Mesogenin1*) in organoids grown in a microwell between day 3 and day 6. **b.** Expression of intermediate marker during skeletal muscle differentiation (*Mesogenin1, Pax3 and MyoG*) in PEG-based hydrogel of 0,5KPa stiffness. **c.** Hierarchical clustering analysis of 84 single organoids based on their morphogenesis characteristics and reporter expression on day 3.

Caption 1: Expression of early and intermediate skeletal muscle marker and hierarchical clustering analysis of skeletal muscle organoids

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

857 Shielded polymer hydrogel arrays for point-of-care diagnostics

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[3]Technische Universität Dresden, Dresden, Germany

INTRODUCTION:

Unexpected exacerbations in patients suffering from respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma represent sizable risks [1,2]. Early prediction and reliable diagnostics could improve prognosis and resource allocation for treatment. Here, we present a novel concept for hydrogel-based assays to be used in the point-of -care detection of endogenous and exogenous biomarkers associated with the onset of the exacerbation of the mentioned respiratory diseases.

METHODS:

Hydrogels were printed onto POMA-coated glass slides in a microarray format using three differently functionalized poly(ethylene glycol) (PEG) precursors, namely 4-arm PEG-thiol (MW 10,000 g/mol), linear PEG-maleimide (MW 2500 g/mol), and biotin-PEG-maleimide (2,500 g/mol). Streptavidin conjugated antibodies against IL6, IL8 and TNFα were pre-conjugated to biotin-PEG-maleimide and embedded in the sensorial active regions of the hydrogel arrays. An additional filter layer of 4-arm PEG-thiol and linear PEG-maleimide was subsequently casted on top of the microarrays to restrict the molecular diffusion into the microarray.

RESULTS AND DISCUSSION:

3D printing of hydrogels was successfully established, the obtained arrays were demonstrated to enable separation and detection of exacerbation markers from saliva and sputum. Adjusting the degree of crosslinking allowed for adjusting the polymer network properties and, by that, the molecular uptake characteristics of the hydrogels. Incubation of the antibody-conjugated gel arrays with different concentrations of antigens (IL-6, IL-8, and TNF-a) yielded different intensities of fluorescence signals after conditioning the gels with a fluorescence-labeled secondary antibody. Furthermore, it was shown that the double layer format of the gel array effectively restrains the penetration of larger molecules (diameters>10 nm) from biofluids while allowing for the unrestricted uptake of the smaller biomarkers.

CONCLUSION:

The introduced principle of shielded hydrogel arrays enables the detection of multiple respiratory disease markers from biofluids. The marker uptake characteristics and the sensitivity of the assay can be optimized by tailoring the mesh size of the polymer hydrogel network and the density of antibodies against IL6, IL8 and TNFα, offering unprecedented options for applications in point-of-care diagnostics.

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ACKNOWLEDGMENTS:

Leibniz Gesundheitstechnologien and BMBF: Projekt EXASENS

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

858 Chitosan modified with hydroxyapatite for bone tissue engineering - evaluation of the physicochemical properties

Patrycja Domalik-Pyzik, Aneta Kukulka, Jan Chlopek

AGH University of Science and Technology, KRAKOW, Poland

INTRODUCTION:

Chitosan (CS), a derivative of chitin, is one of the most promising candidates for bone tissue engineering. Structure and properties of CS are similar to those of glycosaminoglycans (GAGs) – major extracellular matrix (ECM) components. Moreover, chitosan is biodegradable and biocompatible, expresses antibacterial and biological activity[1]. However, a successful scaffold for bone tissue engineering should also direct osteogenic differentiation to facilitate healthy bone formation. Incorporation of bioactive ceramic particles into the polymer matrix can bring this functionality. As a result, composite material that mimics the composition of a natural bone (chitosan as a simplified analogue of ECM and inorganic part in the form of bioactive ceramics) can be obtained[2]. The aim of this study was to evaluate physicochemical properties of the hybrid composites based on chitosan matrix modified with hydroxyapatite (HAp).

METHODS:

Commercially available chitosan (Acros Organics, MW=100,000-300,000 and MW=600,000-800,000) was dissolved in an aqueous lactic acid solution (5% v/v) and combined with homogenous aqueous dispersions of HAp particles (MKN-HXAP, 12 μm) to finally obtain 2% (w/v) CS with various amounts of HAp (0, 1, 2, 5, 10, 15, 30% w/w of CS). Additionally, tannic acid was added. The degree of deacetylation of CS was determined by pH-metric titration; the structure of hybrid hydrogels was investigated by attenuated total reflection spectroscopy (ATR-FTIR) and X-ray diffractometry (XRD). Also, differential scanning calorimetry (DSC), scanning electron microscopy (SEM), uniaxial tensile test, and sessile drop method were used to investigate properties of the composites. Bioactivity was predicted on the basis of the Kokubo assay.

RESULTS AND DISCUSSION:

SEM analysis confirmed homogenous dispersion of the HAp particles in the CS matrix. The surface area and surface roughness increased with the increase of HAp content. In the case of CS/30HAp samples, ceramic crystals oriented perpendicularly to the polymer matrix surface were present. Hydroxyapatite introduction affected physicochemical properties of the hybrid composites - crystallinity, mechanical properties, and wettability were improved. Moreover, potential bioactivity of the CS/HAp composites was confirmed in the SBF immersion test.

CONCLUSION:

Chitosan/hydroxyapatite hybrid composites possess many interesting physicochemical properties and thus can be considered as promising materials for bone tissue engineering. In the next step, a detailed biological examination is necessary.

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

859 Strontium (Sr) is an important trace element in the human body. The released Sr ions can promote the osteogenesis stemmed from osteoblasts while inhibiting bone resorption resulted from osteoclasts. Besides these, Sr in the bioactive glass can be potentially substituted by the in situ mineralized C

Jingjing Wu

University of Erlangen-Nuremberg, ERLANGEN, Germany

INTRODUCTION:

Bioactive glasses (BGs) are promising bone tissue repair biomaterials, because of their good bioactivity, excellent ability of rapidly bonding to bone and stimulating new bone growth. Subsequently, sol-gel derived BGs have improved bioactivity and can enhance cell proliferation and differentiation due to their large surface area and unique surface textures. However, the problems for sol-gel BGs are still unsolved, such as the severe agglomeration and irregular morphology of BGs particles. In our previous studies, monodispersed mesoporous bioactive glasses spheres (MBG) with controllable size were successfully fabricated, and showed the some apatite-forming ability and cellular compatibility when cultured with MC3T3-E1 cells.

METHODS:

A series of BGN (SiO_2-CaO-SrO) in which Ca is partially substituted by Sr was prepared using sol-gel technique. In brief, proportional precursors including TEOS, calcium nitrate and strontium nitrate were reacted under aqueous conditions, followed by aging, drying and calcination to form glass nanoparticles.

RESULTS AND DISCUSSION:

1.SEM observations indicated that all samples exhibit regularly spherical morphology and good dispersion characteristics, which solves the agglomeration problem of conventional sol-gel BGs. Based on the results of SEM and particle size distributions, it is evident that doping different strontium amounts did not affect the morphology and particle size of MBG.

2.The spectrum of all MBG exhibited characteristic absorption bands corresponding to Si-O-Si bonding at 1060 (stretch vibration), 798 (bending vibration) and 480 cm-1 (bending vibration).

3. All the Sr-BGN possessed good apatite-forming ability, but substituting a certain amount of strontium for calcium would weaken the apatite-forming ability of BGN. Additionally, all the Sr-SBG extractions promoted proliferation.

CONCLUSION:

Our results demonstrated that Sr-MBG with different strontium substitution amounts were successfully fabricated by improved sol-gel method. This study effectively solved the problems of the severe agglomeration, irregular shape and uncontrollable size of the conventional BGs particles. Doping different strontium amounts did not affect the morphology and particle size of MBG. All samples possessed good apatite-forming ability, but substituting a certain amount of strontium for calcium would weaken the apatite-forming ability of MBG. Additionally, all the Sr-MBG extractions promoted proliferation.

REFERENCES:

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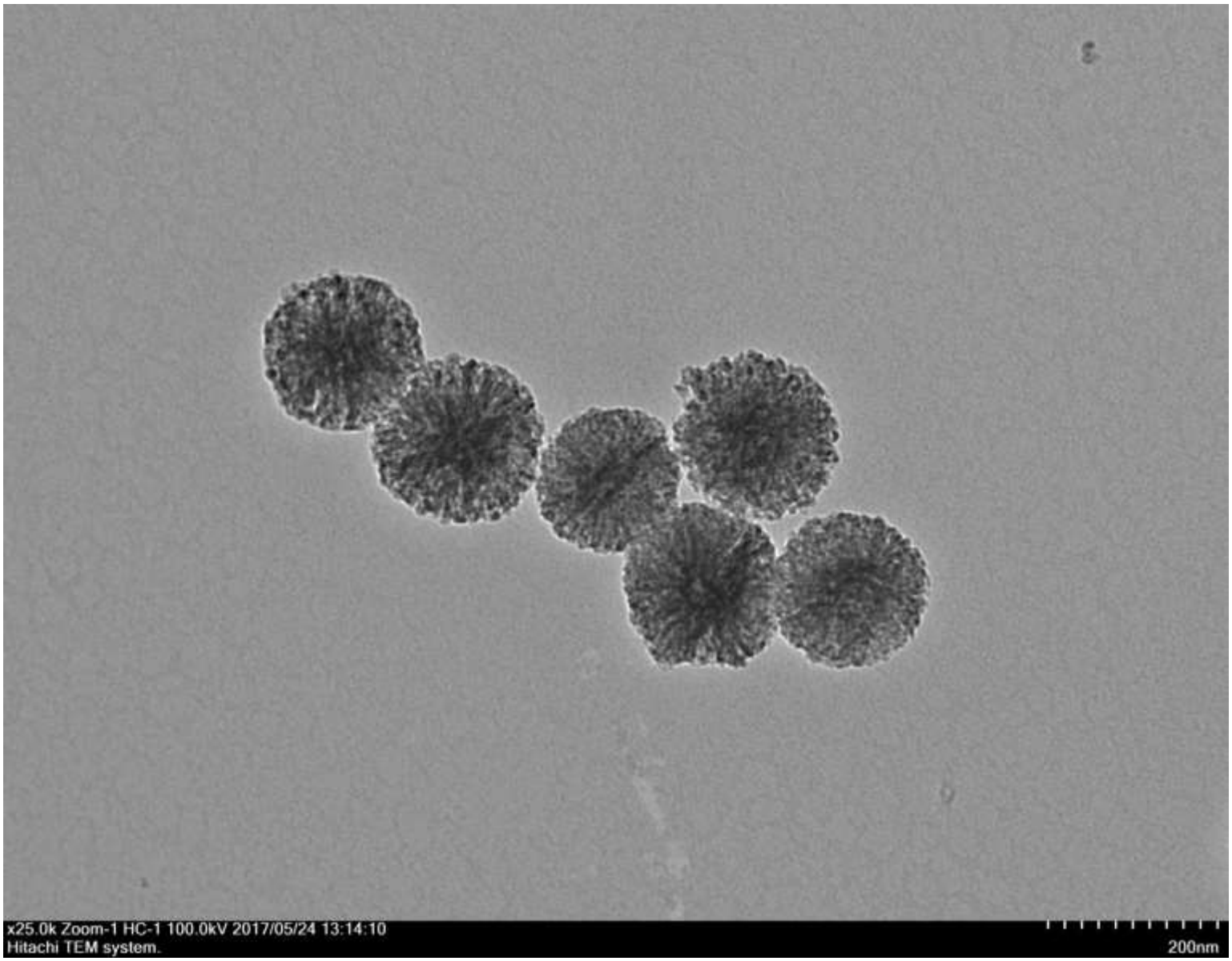
ACKNOWLEDGMENTS:

J.W. acknowledges the fellowship from the China Scholarship Council and Professor Aldo R. Boccaccini.



Picture 1: Caption 1: TEM of 0.2%MBG-Sr

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

861 3-D printable self-healing hydrogels for tissue engineering

Hyunho Roh, Kuen Yong Lee

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INTRODUCTION:

Three-dimensional (3-D) bioprinting has developed as an important method for tissue regeneration, with possibilities to construct functional biological structures for various medical applications.[1] Oxidized hyaluronate (OHA) can form hydrogels in the presence of glycol chitosan (GC) via Schiff base formation.[2] Interestingly, autonomous healing of OHA/GC hydrogels after damage can be achieved by incorporating adipic acid dihydrazide (ADH) into the gels. In

this study, alginate (ALG) was added to OHA/GC/ADH hydrogels to maintain their stability under physiological conditions, which may be potentially applicable to 3-D bioprinting for complex tissue regeneration.

METHODS:

Oxidized hyaluronic acid (OHA), glycol chitosan (GC), adipic acid dihydrazide (ADH) and alginate (ALG) were mixed to prepare hydrogels ([OHA]:[GC]:[ADH]:[ALG]= 2:1:0.3:0.3, weight ratio). A rotational rheometer equipped with a cone-and-plate fixture (Malvern) was used to measure the viscoelastic properties of gels. A 3-D printer (Bioinvivo-STD) was used to fabricate hydrogel scaffolds containing cells. ATDC5 cells were cultured in DMEM/F-12 media containing 10% FBS and 1% PS. MC3T3 cells were cultured in alpha-MEM media containing 10% FBS and 1% PS. A live/dead viability/cytotoxicity kit (Invitrogen) was used to evaluate cell viability.

RESULTS AND DISCUSSION:

Hydrogels composed of OHA, GC and ADH showed autonomous healing within a short time period after the gels were broken. OHA/GC/ADH/ALG gels also showed self-healing behavior both with or without cells in the gels. The addition of ALG to OHA/GC/ADH/ALG hydrogels was useful to maintain their stability under physiological conditions. ATDC5 and MC3T3 cells were used as model chondrocyte and osteoblast, respectively. These cells were encapsulated in each hydrogel disk and attached together to form an integrated structure owing to the self-healing properties. Also, hydrogel scaffolds encapsulating cells were fabricated using a 3-D printer (Fig. 1). The majority of the cells were alive, and it was confirmed that neither a use of ADH nor the 3-D printing process did significantly affect the viability of encapsulated cells in the gels.

CONCLUSION:

We demonstrated that OHA/GC/ADH/ALG hydrogels showed self-healing ability after gel breaking as well as the prolonged stability under physiological conditions. These gels may be useful to fabricate complex 3-D tissue structure using a 3-D printer.

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ACKNOWLEDGMENTS:

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Ministry of Science and ICT (NRF-2016R1A2A2A10005086).

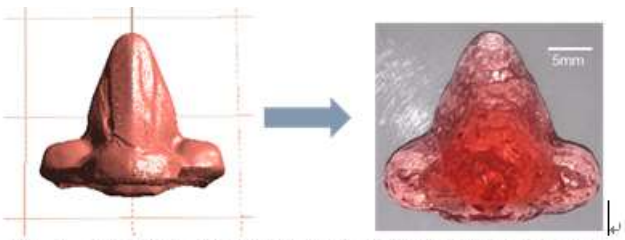

Fig. 1. Example of a hydrogel scaffold encapsulating ATDC5 cells fabricated by a 3-D printer.

Picture 1:

Caption 1: Fig. 1. Example of a hydrogel scaffold encapsulating ATDC5 cells fabricated by a 3-D printer.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

863 Investigation into bacterial biofilm formation of metal orthopedic scaffolds

Michal Zurawski, David Grant, Colin Scotchford, Atkinson Stephen

University of Nottingham, NOTTINGHAM, United Kingdom

INTRODUCTION:

Out of 228,861 Total Joint Replacement (TJR) surgeries carried out in the UK in 2017, approximately 9,154 resulted in biofilm infections at an approximate cost of £90m[1]. Once bacteria adhere to a surface they begin to secrete extracellular proteins that form a protective biofilm around them, which creates a physical barrier protecting the bacteria from the immune system and antibiotics.

METHODS:

Initial trial samples were obtained from Zimmer Biomet. These were 3D scaffolds (Tantalum [Trabecular

Metal] and Titanium [Osseo-Ti]) and flat Ti and Ta disks with varying treatments (anodized, iodized, grit blasted and polished). All titanium in this study is the alloy Ti6Al4V. All samples had a diameter of 10mm, with the height of 2mm and 6mm for flat and scaffold samples respectively.

Bacterial cell fixation for SEM was done using a modified human cell fixation protocol using 3% Glutaraldehyde to result in longer dehydration times using ethanol.

Bacteria used in this study were exclusively *Staphylococcus aureus* strain Newman, and the optical density of the starting culture (OD_{600}) was 0.1 and the incubation time for samples was 168h in Tryptic Soy Broth (TSB) media.

RESULTS AND DISCUSSION:

Bacterial cells have the correct morphology[2], meaning the dehydration and fixation was successful. All sample types, with the exception of sterile samples, have adhered bacteria present on the surface after an incubation period of 168h. Bacterial biofilm structures can be seen on the Ti scaffold and flat Ti disk, and individual bacterial cells can be observed on the Ta scaffold and iodized Ti disk samples.

Bacterial adherence to Tantalum has previously been shown to be weaker than to Titanium (Schildhauer *et al.*,2006).

ONCLUSION:

The SEM fixation protocol worked for both 2D and 3D samples. Bacterial growth was observed on all samples using SEM. Biofilm formation was only observed on a flat polished Ti disk and a Ti scaffold. Less bacterial growth was observed on Ta scaffolds than Ti scaffolds in initial testing.

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ACKNOWLEDGMENTS:

The author would like to thank Zimmer Biomet for providing funding this project.

Picture 1:

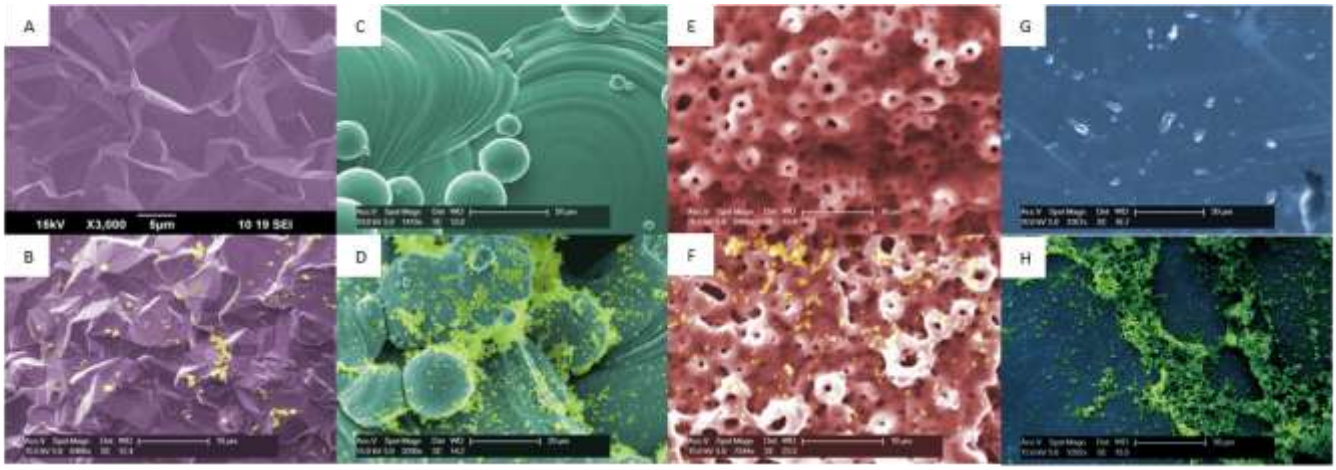

Figure 1. SEM micrographs colourised using a personally modified open source software (GIMP) before and after incubation for 168h with *S. aurous* strain Newman. A is a sterile Tantalum scaffold, at x3000 magnification, with the purple area representing the metal surface. B is a Ta scaffold that has been incubated for 168h with bacteria, at x6400 magnification; the purple area is the metal surface and the bacteria is yellow in colour. C is a sterile Titanium scaffold at a magnification of x1419, with the metal surface teal in colour. D is a Ti scaffold after incubation for 168h with bacteria, at 3200x magnification. The metal is teal and bacteria lime green. E is a sterile flat iodized disk sample, at 6400x magnification. The surface of the sample is red, with pores resulting from the anodising process visible. F is a flat iodized disk sample after incubation for 168h with bacteria, at a magnification of 7344x. The surface of the sample is red and the bacteria are yellow in colour. G is a sterile polished Ti disk sample, at a magnification of 1267x with the surface blue in colour and pits in the surface seen as white areas . H is a polished Ti disk sample at a magnification of 1292x with the surface blue in colour, and the bacteria is green/yellow.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

864 Design of a new instrument set up for the development of a special shape bone graft.

Helen Vavouraki[1], Eleftherios Kosmidis[2], Yiannis Ninios[1], George Arealis[3]

[1]National Center for Scientific Research „DEMOKRITOS„, ATHENS, Greece

[2]University of Crete Hospital, HERAKLEION, CRETE, Greece

[3]Red Cross Hospital, ATHENS, Greece

INTRODUCTION:

Human Tissue Bank of NCSR “DEMOKRITOS” collects and processes human tissues, producing grafts to be used in regenerative medicine. Among Tissue Banking research interests are the process of new tissues and the development of new grafts.

Based in this concept, we have designed a special set of instruments as in Fig 1(B), in order to produce a new innovative bone graft from living donors (allograft) especially shaped to be used with the TALOS milling head in Neurosurgery.

The TALOS system consists of a special configuration milling head , Fig 1(C), accompanied with one or more synthetic bone graft disks. It is used in all trepanning methods , adapted in any type of craniotomy. The new bone graft in the shape of disk Fig 1(D) , replaces the synthetic ones .

METHODS:

A) Collection of the raw materials- processing of the tissues.

Based on scientific collaboration protocols, the femour heads Fig1 (A),from orthopeadic departments of hospitals, have been collected according the national and European Directives.

Initial processing has been carried out under sterile conditions , where tissues underwent a series of physicochemical processing established in the Bank, to remove blood, fat, loose pieces , and enhance their appearance.

Final processing of the tissue parts, includes freeze- drying, optical control and packaging in HDPE vials or sealing STERI PEEL paper, labeling , γ- rays sterilization, sterility and pyrogen tests in samples from each donnor before the grafts are released.

B) Design of a set of instruments which operates in the Human Tissue Bank giving the special shape of the disk to the bone grafts.

RESULTS AND DISCUSSION:

We collected and processed 30 femour heads. We have produced over 60 disk shaped grafts, which have been fitted and tested with TALOS .

The designed set of instruments is given in fig 1(B) .

Optimization study confirmed the step of processing in which the use of the set of instruments has given the most beneficial results.

Talos system with human grafts was tested successfully in 15 patients, concerning the safety, efficacy, application difficulties, cosmetic and bone healing results.

CONCLUSION:

The new instrument configuration set up enabled us to develop the innovative human bone graft from living donors (allograft) , which is very well fitted with TALOS milling head. TALOS system provides in Neurosurgery, both coverage of the treppaning holes as well as a second fixed ossification matrix .

Picture 1:

Caption 1: Fig 1. (A) Femour Head, (B) special set of instruments , (C) TALOS milling head, (D) Disk shaped allograft.

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

866 The effects of Sr concentration on physicochemical properties, bioactivity and biocompatibility of bioactive glasses nanoparticles Jingjing Wu,1, 2 Kai zheng1,

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Jingjing Wu

University of Erlangen-Nuremberg, ERLANGEN, Germany

INTRODUCTION:

Bioactive glasses (BGs) are promising bone tissue repair biomaterials, because of their good bioactivity, excellent ability of rapidly bonding to bone and stimulating new bone growth. Subsequently, sol-gel derived BGs have improved bioactivity and can enhance cell proliferation and differentiation due to their large surface area and unique surface textures. However, the problems for sol-gel BGs are still unsolved, such as the severe agglomeration and irregular morphology of BGs particles. In our studies, mesoporous bioactive glasses nanoparticles (MBG) with controllable size were successfully fabricated, and showed the some apatite-forming ability and cellular compatibility when cultured with MC3T3-E1 cells.

METHODS:

A series of BGN (SiO_2-CaO-SrO) in which Ca is partially substituted by Sr was prepared using sol-gel technique. In brief, proportional precursors including TEOS, calcium nitrate and strontium nitrate were reacted under aqueous conditions, followed by aging, drying and calcination to form glass nanoparticles.

RESULTS AND DISCUSSION:

1.SEM observations indicated that all samples exhibit regularly spherical morphology and good dispersion characteristics, which solves the agglomeration problem of conventional sol-gel BGs. Based on the results of SEM and particle size distributions, it is evident that doping different strontium amounts did not affect the morphology and particle size of MBG.

2.The spectrum of all MBG exhibited characteristic absorption bands corresponding to Si-O-Si bonding at 1060 (stretch vibration), 798 (bending vibration) and 480 cm-1 (bending vibration).

3. All the Sr-BGN possessed good apatite-forming ability, but substituting a certain amount of strontium for calcium would weaken the apatite-forming ability of BGN. Additionally, all the Sr-SBG extractions promoted proliferation.

CONCLUSION:

Our results demonstrated that Sr-MBG with different strontium substitution amounts were successfully fabricated by improved sol-gel method. This study effectively solved the problems of the severe agglomeration, irregular shape and uncontrollable size of the conventional BGs particles. Doping different strontium amounts did not affect the morphology and particle size of MBG. All samples possessed good apatite-forming ability, but substituting a certain amount of strontium for calcium would weaken the apatite-forming ability of MBG. Additionally, all the Sr-MBG extractions promoted cells proliferation.

REFERENCES:

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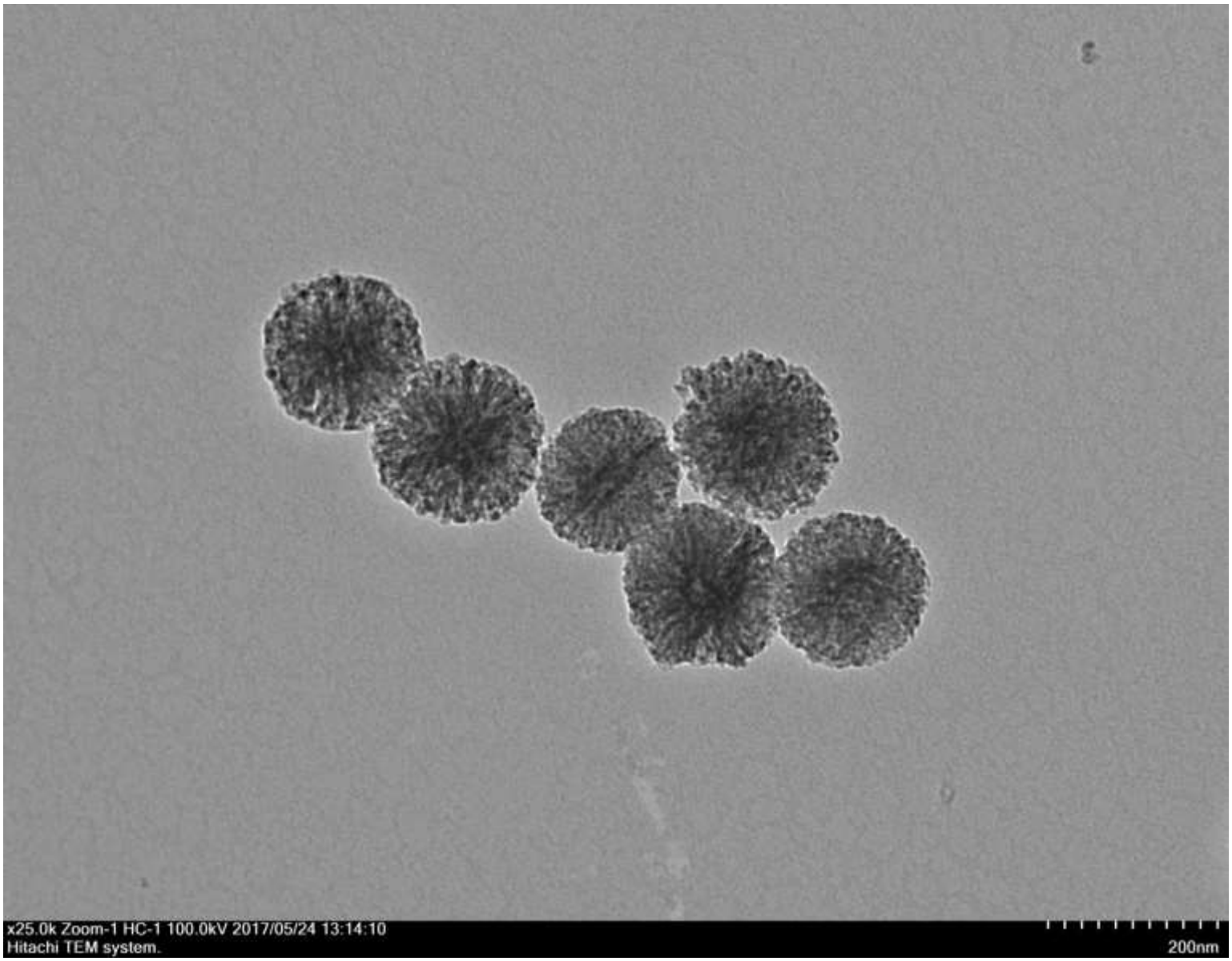

Picture 1: Caption 1: TEM of 0.2%MBG-Sr

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

871 Evaluation of electrospun polycaprolactone/egg white protein scaffolds interaction with adipose derived stem cell

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[2]Ankara University, ANKARA, Turkey

INTRODUCTION:

A recognized breakthrough in the fields of tissue engineering consists of mimicking natural tissue architectures to achieve a desired cell response for creating 3D tissue equivalents. Due to the architectural similarity of the

nonwoven electrospun nanofibers to collagen structure of ECM, the electrospinning processes have received substantial attention as a way to mimic the structure of natural ECM. Polycaprolactone (PCL) is a FDA approved and widely used raw material for scaffold production. However, the hydrophobic nature of PCL has been a major obstacle for initial cell attachment and it even causes a reduction in proliferation of many cells. Hen egg white (HEW) is a widely available protein resource and contains more than 100 types of soluble proteins at high concentrations. We herein propose a new polymer/protein composite nanofiber mats as matrix for culturing adipose derived stem cells (ADSCs).

METHODS:

Egg white protein was purified from hen eggs according to method described in literature[1]. For production of nanofiber membrane, the solution contains equal amount of PCL and HEW was electrospun through a plate collector by applying high voltage between tip and collector. The morphology of obtained mats was determined by SEM, where FTIR-ATR was used to confirm the presence of HEW on the surface of the membrane. ADSCs were cultured on the nanofiber mats for 14 days. Cell viability was assessed using Alamar Blue assay and Calcein-AM staining. The morphology and cytoskeleton organization of the cells on the mats was analyzed after phalloidin staining of the F-actin at day 14. Neat PCL nanofiber mats were used as a control for all experiments.

RESULTS AND DISCUSSION:

A nonwoven mesh structure was produced from PCL/HEW via electrospinning process without any bead formation on the nanofibers. FTIR-ATR analysis confirmed the presence of HEW on the nanofiber surfaces. Regarding cell culture experiments with ADSCs, cell cultured on PCL/HEW membranes showed significantly higher viability and increasing proliferation rate than those on neat PCL mats. Moreover, the phalloidin staining of actin filaments demonstrated that the cells were well spread and bridging to each other on the PCL/HEW membranes, whereas no viable cell observed on PCL samples.

CONCLUSION:

The results obtained from the present work indicate that PCL/HEW electrospun mats possess favorable morphological and biochemical properties to serve as an artificial matrix for stem cell growth and can be used as a scaffold for the regeneration of different tissues.

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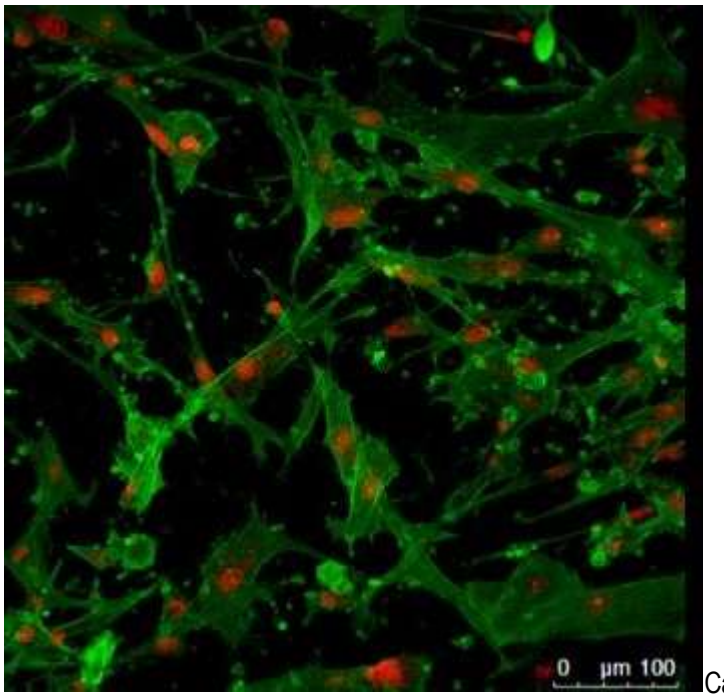
Picture 1: 

Caption 1: Staining of nuclei (red) and F-actin (green) of ADSCs cultured on PCL/HEW mats

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

881 Nanofunctionalized electrospun fibers for tympanic membrane regeneration

Diana-Maria Dragu?in, Andrada Serafim, Eugeniu Vasile, Horia Iovu, Izabela-Cristina Stancu

University Politehnica of Bucharest, BUCHAREST, Romania

INTRODUCTION:

The tympanic membrane (TM) is a thin tissue able collect and transmit sound vibrations across the middle ear due to the specific orientation of collagen fibers. Damage to the TM, such as from chronic otitis media (COM), or traumatic perforation, results in hearing loss due to ineffective sound transmission[1,2]. An appropriate method to replicate the morphology of the collagen fibers is electrospinning. Also, due to the capacity of the electrospun fibers to incorporate drugs, they could also be used as complex long-term drug delivery systems. The aim of this study was the production of nanofunctionalized electrospun meshes for the regeneration of TM with sufficient mechanical properties and able to sustain the release of antibiotics loaded in (2-Hydroxypropyl)-β-cyclodextrin (CD) to prevent the long-term infections.

METHODS:

Multicomponent precursors for the nanofibers were obtained using different concentrations of functionalized (OH, COOH) diamond nanoparticles (NDPs) suspensions (0.5 and 1% w/v). Various amounts of CD were solubilized in the NDPs suspensions at 50°C for 8 h using magnetic stirring, followed by sonication for additional 2 hours. After the complete solubilization of CD, gelatin from cold water fish skin (FG) was dissoluted in the NDPs-CD suspensions until a final concentration of 50% w/v FG. The fabrication of the fibrous mats was performed at 25°C and 45% humidity. Crosslinking was performed in ethanolic glutaraldehyde solution for 48 hours, at RT.

RESULTS AND DISCUSSION:

Rheological and injectability measurement of the NDPs-FG and NDPs-CD-FG suspensions have shown the influence of the components on the viscosity and injectability of the precursors. Thus, higher content of the functionalized NDPs led to an important increase of the viscosity when compared with simple FG solution. But, when introducing CD in the system, the viscosity of the 3 component systems decreases when compared with the 2 component systems. These indicated the formation of hydrogen bonds between the NDPs and FG, hindered by the presence of the CD. No significant differences were noticed between the synthesized nanocomposite materials, entangled into mats with interconnected porosity (before and after crosslinking).

CONCLUSION:

This work shows the potential of NDPs-CD-FG to produce advanced, personalized, and functional TM implants.

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ACKNOWLEDGMENTS:

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

883 Superporous microparticle alginate/halloysite beads for drug delivery application

Adi Ghebaur

University Politehnica of Bucharest, BUCHAREST, Romania

INTRODUCTION:

In order to enhance the drug entrapment efficiency and improve the swelling behaviors of drug delivery system, Ca^{2+} crosslinking and freeze-thawing cycle techniques were used to prepare sodium alginate/polyvinyl alcohol hydrogel beads. Freezing–thawing process is the most facile method to produce physically crosslinked polyvinyl alcohol gel because it does not require the presence of crosslinking agent that may cause toxicity. The high porosity that is induced by freeze-thawing process will induce a severe burst release of an active ingredient. The presence of clays in will act like a barrier and drastically reduce the burst release. Clay minerals like kaolin, halloysite, talc, sepiolite, montmorillonite are used in pharmaceutical field due to their high specific area and adsorption capacity. In drug delivery field halloysite is intensively used because it presents a lumen where different types of active substances could be loaded and so the clay acts as a carrier. Halloysite is an aluminosilicate with Si:Al ratio of 1:1 and a chemical composition similar to kaolinite but with a hallow tubular structure. On the internal and external surface halloysite is negatively charge but the ends are amphoteres[1]. The aim of this study is to obtain superporous hybrid beads on sodium alginate and halloysite using polyvinyl alcohol as a template.

METHODS:

The alginate/ halloysite beads were prepared by electrospraying technique. Briefly in a alginate/polyvinyl alcohol were dispersed the active ingredient and different amounts of halloysite. The obtained suspensions were electrosprayed in a $CaCl_2$ solution at 24 kW, and different material flows. The obtained beads were maintained for 2 h at freezeing followed by 1 h of thawing. The freeze-thawing cycle was repeated 3 times and then the beads were intensively washed with water in order to remove the polyvinyl alcohol. In order to be characterized the obtained alginate/halloysite beads were freeze dried. The microparticle were characterized by FTIR, DCS, DLS, optic microscopy, *in vitro* drug release.

RESULTS AND DISCUSSION:

In figure 1 are presented the optical microscopy indicate that after freeze thawinh the beads have a superporous morphology and the pores have diameters between 2-15 μm.

CONCLUSION:

Eletrospraying is a proper method to obtain microparticle that can be used in controlled drug delivery field.

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ACKNOWLEDGMENTS:

This work has been funded by University Politehnica of Bucharest, through the “Excellence Research Grants” Program, UPB – GEX 2017. Identifier: UPB- GEX2017, Ctr. No. 80/2017

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

378 Biopolymer composite fibers as versatile cell scaffolds

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INTRODUCTION:

Nanofibrous composites are particularly well-suited to replace nanostructured protein-based tissues like bone or tendon[1]. On a different length scale, composite microfibers can promote the hierarchical organization of cells in nerve conduits or artificial blood vessels [2]. Here, we present a versatile extrusion method for the fabrication of biopolymer composite fibers, which spans the nano- and the microscale.

METHODS:

We recently established a template-assisted extrusion approach through nanoporous alumina membranes.[3] This process has now been used to extrude blends of different biopolymers and biopolymers with nanoparticles. The fiber morphology was characterized using scanning electron microscopy (SEM). Extruded protein blends were also analyzed with immunostaining and fluorescence microscopy. The extrusion process was then scaled up to fabricate micron-sized composite fibers, which were precipitated in a coagulation bath. Subsequently, composite microfibers were used in cell culture studies with 3T3 mouse fibroblasts.

RESULTS AND DISCUSSION:

Using blends of different proteins facilitated the successful extrusion of composite nanofibers. SEM analysis showed that fibers either assembled into randomly oriented meshes or into highly aligned fiber bundles. Immunostaining of composite fibers containing collagen and fibronectin revealed that both proteins were present in the extruded scaffolds. Furthermore, we could extrude chitosan fibers with embedded iron oxide nanoparticles (Fig. 1). When chitosan solutions with magnetic nanoparticles were extruded on a larger scale using syringe needles and a coagulation bath with ethanol the composite solution directly precipitated into microfibers.

Cell culture studies with 3T3 mouse fibroblasts on such dried chitosan-nanoparticle microfibers showed a good overall cell growth up to 7 days in culture. No significant differences in cell viability and cell proliferation were found for chitosan-nanoparticle fibers and bare chitosan fibers, which indicates a good biocompatibility of the composite microfibers.

CONCLUSION:

Extrusion of blended biopolymer solutions provides a powerful platform to prepare fibrous composite biomaterials for versatile tissue engineering applications. The functionality of such fibrous scaffolds can be customized by tailoring the blend composition and by adjusting the dimensions of the extruded fibers from the nano- to the microscale.

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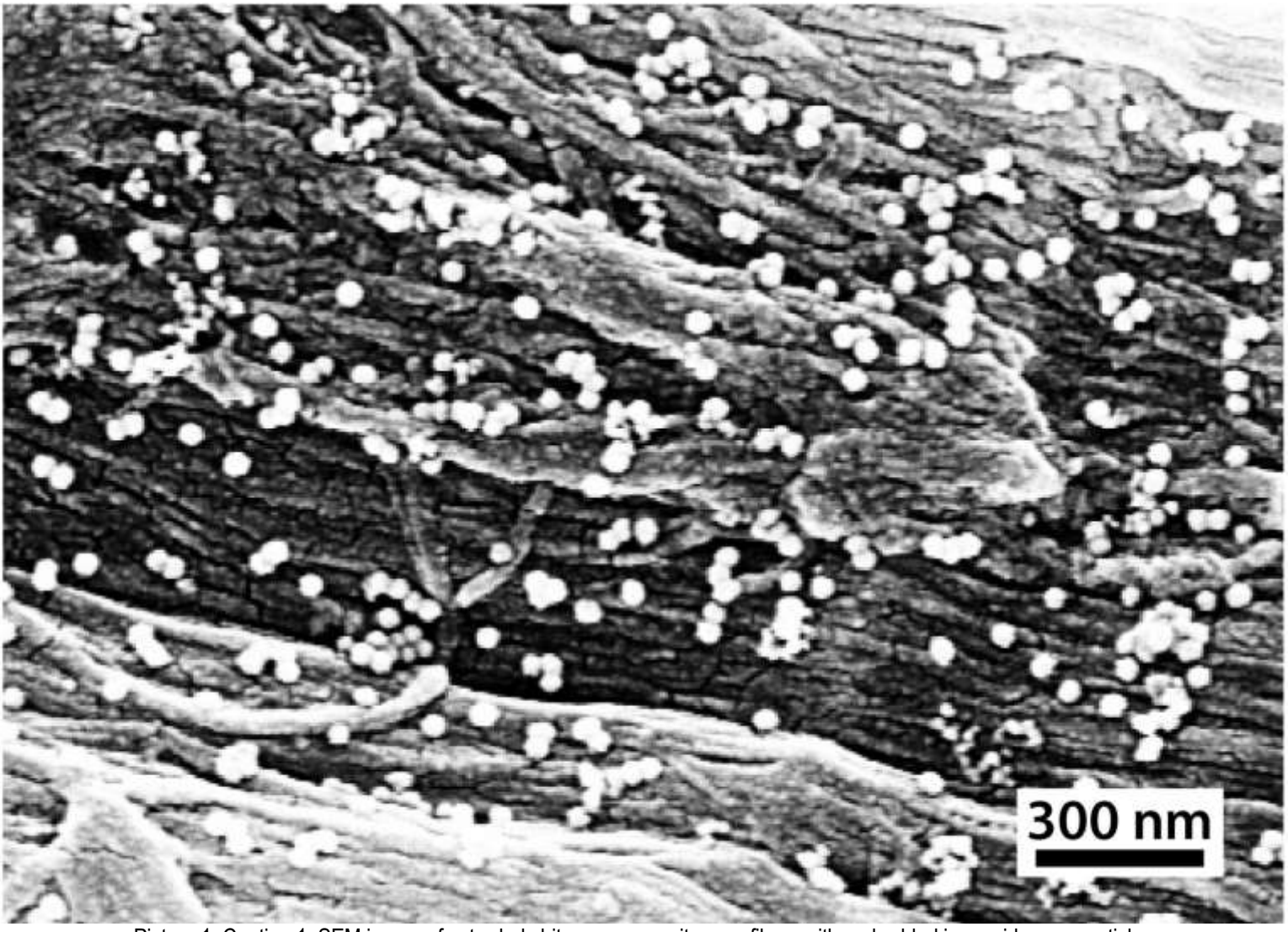
ACKNOWLEDGMENTS:

We gratefully acknowledge funding via the Emmy Noether Programme of the Deutsche Forschungsgemeinschaft (grant no BR 5043/1-1)



Picture 1: Caption 1: SEM image of extruded chitosan composite nanofibers with embedded iron oxide nanoparticles

Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

427 Eumelanin decorated polylactic acid electrospun micro fibers as bio-inspired cradle for neuroblastoma cells growth and maturation,

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INTRODUCTION

The regeneration of neurite network constitutes a strategy for the treatment of neurodegenerative disorders characterized by a loss and dysfunction of trophic factors such as BDNF and NGF. Clinical use of neutrophic factors is limited by their inability to cross the blood brain barrier. The fabrication of eumelanin-coated microfibrous structures represents a novel strategy in order to realize tissue-engineering scaffolds for neuronal cells growth and control by providing both mechanical support for growing cells and biological signals to direct the axonal growth cone to the distal stump.

METHODS

To realize the scaffolds, an appropriate protocol combining electrospinning, spin coating and solid-state polymerization process was established [1]. For biological analysis, a human derived cell line from neuroblastoma was used. Cell growth and differentiation on eumelanin microfibers both random and aligned were evaluated through Class-III β tubulin and GAP-43 expression, marker of differentiating neurons, by using confocal analysis. Furthermore, cell morphology by using SEM analysis and βIII tubulin expression was tested.

RESULTS AND DISCUSSION

3D matrices such as hydrogels, electrospun fibers and spheroid-based systems or porous materials are widely studied in vitro because they mimic more realistically 3D in vivo microenvironments thus preserving cell-cell interaction and promoting cell response in terms of viability and differentiation [2-3]. Biological results showed that both random and aligned eumelanin microfibers support biological response in terms of cell survival and adhesion. Meanwhile, eumelanin random microfibers induced the formation network of neuritic processes in cells over culture time compared to aligned microfibers. These random microfibers stimulated also GAP-43 expression over cell culture time thus confirming differentiation processes. Furthermore, morphological studies (SEM and confocal microscopy) revealed that eumelanin microfibers were able to induce a good cellular spreading.

CONCLUSION

Our results show that eumelanin coatings, eliminating the need of differentiating factors in the media, expand the scope of substrate driven cell culture growth and maturation. These findings suggest that eumelanin microfibers might be worthy of consideration for future evaluations of new therapeutic strategies for neurodegenerative diseases.

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ACKNOWLEDGMENTS

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Poster presentation session B 13:00 - 14:00 11/09/2018

Poster presentation

536 Antibacterial nanostructured polyurethane composite membranes

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INTRODUCTION:

The next decades will bring a significant increase in both the number of old citizens and their share of the world population. This increase in life expectancy demands the prevention of the decline in the functional abilities of the elderly induced by their prevailing diseases, namely those related with musculoskeletal and cardiovascular systems. In the context of regenerative medicine, and despite the advances in new materials for tissue engineering focused on those fronts, the danger of infections still persists.Thus there is a need to engineer novel architectures with both regeneration abilities and antimicrobial activity [1].

Polyurethane/nanostructured zinc oxide composites (PU/nZnO) hold promise if combining the attractive properties of both nZnO and PU: the biocompatibility and tunable mechanical behavior of PU and the antibacterial activity and selective toxicity of nZnO. The knowledge on PU/nZnO composites, particularly regarding biocidal response, is still scarce and thus requires further development.

This work focus the preparation and characterization of porous PU/nZnO membranes for tissue engineering applications able to offer antibacterial activity.

METHODS:

nZnO was synthesized by chemical precipitation. Crystal phase composition, morphology and specific surface area of nZnO were evaluated by XRD, FTIR, SEM, and gas adsorption.

Electrospinning technique was used to produce nZnO-filled PU membranes. Random fibers were collected and characterized by XRD, FTIR and SEM. Tensile properties of the composite meshes were evaluated and the membranes antibacterial activity against E. coli and S.aureus was also assessed.

RESULTS AND DISCUSSION:

nZnO consisting of assembled nanoplatelets was obtained. nZnO could be used in the production of composite membranes with PU matrix by electrospinning. The morphology of the produced composite fibers and their size distribution indicate the electrospun fibers as promising alternative to current synthetic matrices. The tensile strength of the composites depends on the ZnO content, being maximized for a ZnO amount of 10%. The biological tests confirmed the antibacterial properties of the composites while revealing their dependence on ZnO content of the composite.

CONCLUSION:

nZnO was combined with PU by electrospinning for producing porous membranes with antibacterial properties. Antibacterial activity and tensile strength of the composite may be tuned via ZnO concentration.

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ACKNOWLEDGMENTS:

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement

Antibacterial nanostructured polyurethane composite membranes

Picture 1:

Poster session C

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

561 Silk fibroin - hyaluronic acid sponges as interventional implants for trapping glioblastoma infiltrative cells

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INTRODUCTION:

Glioblastoma (GBM) is a devastating tumor of the central nervous system. Despite a heavy treatment, recurrence is inevitable, and the application of a significant and effective therapeutic strategy remains a challenge[1]. Among innovative approaches, the breakthrough concept of cancer cell trap offers new hopes and opportunities[2]. The goal is to attract these residual cancer cells surrounding the surgical cavity and to confine them in a biomimetic micro-nano-polymeric scaffold delivering chemo-attractive molecules. For this purpose, a biocompatible scaffold with appropriate physico-chemical properties has been designed. The natural polymers silk fibroin (SF) and hyaluronic acid (HA) have been selected as candidate materials because of their biocompatibility and bio-physicochemical properties.

METHODS:

Sponges have been prepared with mixtures of SF, HA, poly-l-lysine (PLL) and heparin by freeze-drying. Optimization of a crosslinking method with N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide sodium salt (NHS) have been performed on a mixture of HA and PLL and applied to the SF-HA sponges. Their swelling ability and degradation in PBS, morphology (porosity, pore size and connectivity) and texture have been evaluated.

RESULTS AND DISCUSSION:

Sponges with a low amount of cross-linking agent showed good porosity near 90% with a pore diameter between 34 µm and 120 µm depending on the formulation and can uptake up to ca. 17 times their weight in water. Cells should therefore be able to penetrate the sponges. Moreover, swollen sponges had a Young modulus between 14 kPa and 85 kPa which is close to the one of a brain tumor (26 kPa) and higher than normal brain (between 0.1 and 1 kPa)[3].

CONCLUSION:

We have been able to produce sponges presenting a porous morphology with high connectivity and pores large enough for cells to invade the interior of the sponges. Moreover, they presented a high water uptake with textures close to the one of the brain.

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Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

712 Hybrid polymer composite made from gelatin and cellulose nanofibrils: a nanobiomaterial with tunable degradability and mechanical performances

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INTRODUCTION:

In recent years, new regenerative approaches for the healing of diseased tissues and organs have aimed to recover the original functionality, reducing healthcare costs and patient’s pain. Furthermore, different studies show how biomaterials properties (stiffness, mechanical stimulation, surface topography, etc.) can influence cellular functions and direct stem cells differentiation.[1]

In the present work, natural polymers have been used to design scaffolds for tissue engineering, in particular Gelatin (Gel) has been selected for its biomimicry while cellulose nano-fibers (CNF) as structural reinforcement due to their biocompatibility, exceptional mechanical properties and availability.[2]

The best features of the selected biopolymers are combined through blending and cross-linking processes to obtain hybrid materials with improved mechanical performances without losing biocompatibility, chemical stability and flexibility

METHODS:

Several blend compositions were studied to obtain scaffolds with different mechanical properties and biodegradability in simulated body conditions, able to induce specific cell differentiation. The polymeric blends were obtained by dissolving porcine gelatin in CNF water suspension at 40°C under mechanical stirring, keeping the final blend concentration constant and varying the polymers weight ratio. Blends were than freeze-dried to achieve 3D porous structures and cross-linked by dehydrothermal cross-linking treatment (DHT). Evaluations of morphological, chemical-physical and viscoelastic properties were carried out. *In vitro* tests were performed to evaluate scaffolds cytotoxicity.

RESULTS AND DISCUSSION:

Different CNF/Gel weight ratio were evaluated, i.e. 1:0, 1:1, 2:1, 1:2, and 0:1 and were cross-linked by DHT. Scaffolds show similar interconnected porous structures and an adequate chemical stability in physiological conditions, proving that the polymeric ratio doesn’t affect significantly scaffold morphologies and stability. Data from DMTA demonstrate scaffolds with tunable mechanical performances (stiffness and Young’s modulus) can be obtained by changing the polymeric ratio. In vitro evaluations prove scaffolds biocompatibility and a good interaction with cells.

CONCLUSION:

In the present work, polymer composites with different CNF/Gel weight ratio were synthesized in order to obtain three-dimensional scaffolds for tissue engineering applications. Experimental data reveal improved and tunable mechanical performances and a biodegradability rate in simulated body conditions able to induce specific cell differentiation.

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Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

325 Preparation of bilayered scaffolds containing hyaluronic acid and Sr/Zn folates for ostechondral tissue engeneering

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INTRODUCTION:

Osteochondral tissue engineering is considered a worldwide challenge due to the increasing number of patients affected by degenerative cartilage conditions. However, it is still needed to develop biomimetic systems that consider the hieratical organization and composition of the osteochondral interphase. Hence, we propose the preparation of bilayered porous scaffolds containing novel bioactive hyaluronic acid and strontium and zinc folic acid derivatives (SrFO/ZnFO)1,2 forming 3D-supports with gradient composition and microstructure capable to drive cellular population towards a spatially-varying phenotype for osteochondral tissue engineering.

METHODS:

The new ZnFO derivative was prepared and characterized using an analogue procedure as reported for SrFO1. Moreover, a novel automatized large-scaled method was used for the preparation of methacrylated hyaluronic acid (HAMA) by reacting hyaluronic acid (5g) and methcrylate anhydride (39.3g) at pH 8.5 using a Titrando® equipment. Extent of methacrylation and Mn of HAMA was determined by NMR and SEC respectively.

Hierarchical scaffolds were produced by a two-step manufacturing procedure. Porous supports were obtained by cryopolymerization of PEGDMA in presence of PLGA, TCP and SrFO, followed by liophylization. Afterwards,

bilayered scaffolds were obtained by deposition of interpenetrated layers of HAMA (3-10%w-v), PEGDMA (17-10%w-v), ZnFO (1%w-v), IGARCURE-2959 (0.05%w-v) solutions on the top of the cryogels followed by photopolymerization. Composition and microstructure of scaffolds were analyzed by SEM-EDS. Degradation, swelling and ion released (PBS, pH=7.4, 37°C) were assessed gravimetrically, by ESEM and ICP analysis, respectively. hCs and hOBs cells were co-cultured within the scaffolds evaluating the biocompatibility by the AlamarBlue assay and confocal microscopy.

RESULTS AND DISCUSSION:

Spectroscopical analysis confirmed the structure proposed and coordination mode of the new ZnFO. derivative comparable to SrFO2. Automatized large-scaled synthesis of HAMA was achieved with 44% of methacrylate modification, supposing and increase of 30% compared to manual method. Biomimetic scaffolds presented homogeneous microstructure and interconnected pores with presence of EDS characteristic peaks: Zn(1.01KeV), Sr(1.80KeV), P(2.01Kev) and Ca(3.69KeV). In-vitro studies showed a sustained release of Sr and Zn over 21 days and good biocompatibility and cell colonization capacity through the structure.

CONCLUSION:

A novel bioactive ZnFO derivative and automatized large-scale method for HAMA synthesis has been reported for the first time. Hieratically-structured scaffolds based on HAMA/PLGA were obtained by two-step manufacturing method containing bioactive Sr/Zn folates and TCP. Microstructure of the scaffolds and cell colonization capacity showed evidences of biomimetic spatially-varying properties with potential to be used in osteochondral tissue engineering.

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ACKNOWLEDGMENTS:

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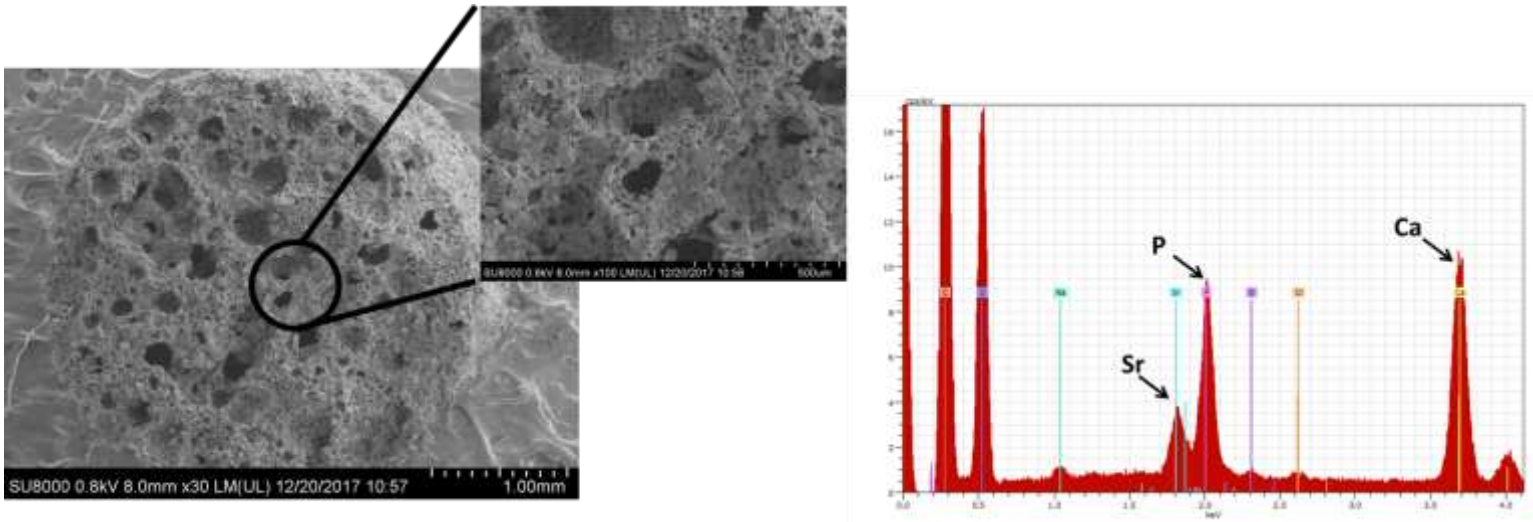

Picture 1: Caption 1: Figure. Microstructure and surface analysis by SEM-EDS of a scaffold containing HAMA/PLGA SrFO and TCP.

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

365 Prediction of effects of scaffold geometry fabricated by direct ink writing on tissue growth

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INTRODUCTION:

Direct ink writing(DIW)is a potential technique to produce scaffolds with controlled structural geometry.Numerous experiments support that the scaffold geometry contributes to control tissue formation. C.Bidan et al.[1]developed a mathematical model to predict the curvature controlled tissue growth based on Frette algorithm[2].In this study, the influence of pore geometry-various filament angle on the response of initial tissue formation was studied based on[1].The purpose is to predict initial tissue growth and further to optimization of pore geometries to improve the speed of ingrowth of bone tissue into porous scaffolds.

METHODS:

Scaffolds with various filament angles(30, 45, 60 and 90deg)with pore sizes of 400x120 m using 600 m filaments were fabricated with HA/PCL ink using the DIW technique(Fig2).The algorithm [1] was applied with Matlab code to the scaffolds digital images, and the local curvature associated with an interface pixel was then estimated from the ratio of the number of black to white pixels lying within a given radius from the interface as $k=3\pi/r(A/Atotal -1/2)$

Where A is the number of pixels in the mask on the outside of interface(the black area), A_{total} is the number of pixels in the mask and r is the mask radius. Assuming that growth occurs only on a concave surface, each white pixel where the effective curvature is positive was changed to black, representing tissue deposition. According[1], the time scale parameter $\alpha=17$ step.day^{-1} was applied.

Tissue grows at a constant rate λ-relates proliferation rate to curvature, directly proportional to the local surface curvature towards the center of curvature. Thus, the projected tissue area, PTA, was modeled over time t as $dPTA/dt =\lambda k$

RESULTS AND DISCUSSION:

Fig1Ashown the simulated project tissue growth behavior in 45deg scaffold at 8-time points (day 0 to day 7). Tissue deposition started in the corners whereas no growth occurs on flat surfaces until the surrounding tissue deposition modifies the local geometry, which has also been pointed out in[1].As shown figure 1B, the curvature controlled initial tissue growth is expected to be significantly affected by the geometry of scaffold filament angles. Fig1C shown the initial growth rates were calculated, which suggests that the PTA growth rate for scaffolds follow as 90deg>60deg>45deg>30deg.

CONCLUSION:

The tissue growth rate is affected by the scaffolds geometry changing. The results demonstrate pore geometry can be optimized to achieve the desired tissue deposition by predicting tissue growth advanced.

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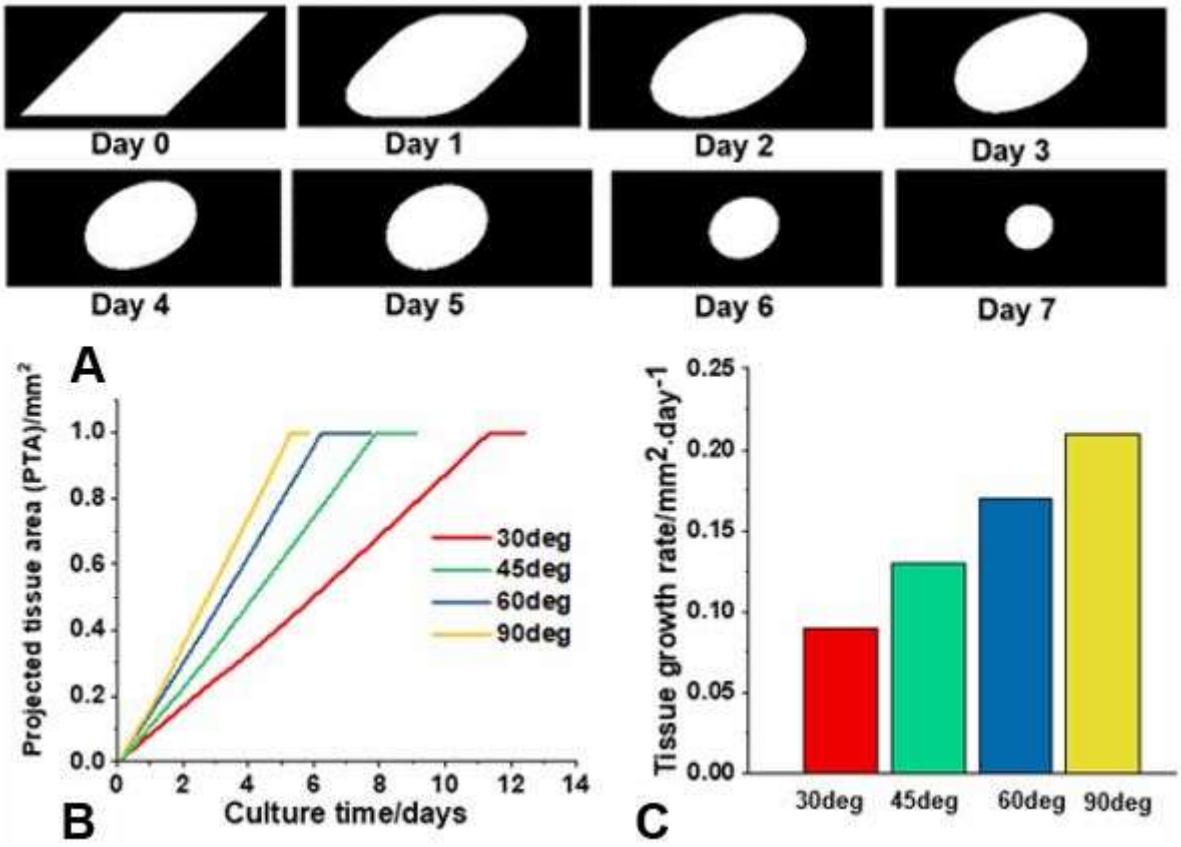
Picture 1:



Caption 1: Fig1 Numerical simulation of tissue formation within 45deg scaffold(A). Curvature controlled tissue growth area and rate for scaffolds (B and C)

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Poster presentation

670 Post-processing dependent microstructure, μCT based in silico modelling and in situ failure properties of 3D powder printed scaffolds

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INTRODUCTION:

For bone-tissue engineering scaffold, fabricated through 3D powder printing (3DPP), post processing has profound influence on attributes like microstructure, phase composition and mechanical properties[1-3]. A comprehensive knowledge of the resulting microstructure and the identification of the failure mechanism is necessary to improve the mechanical properties and adopt necessary modification of the 3DPP cycle[2]. Micro-computed tomography emerged as an attractive method to characterise complex 3D architecture produced by the additive manufacturing techniques. It's applicability can further be extended with *in situ* or modelling. The motivation was to model the scaffold micro architecture and correlate to the macro-scale mechanical behaviour, by *in silico* and *in situ* approaches.

METHODS:

A well-known POP-based model system for 3DPP technique was adopted, as the starting scaffold. Then two post-processing schedules, namely, chemical conversion and polymer infiltration, followed[3]. The evolution of phase composition, 2D and 3D microstructure along with printing accuracy, were monitored though X-Ray diffraction, scanning electron microscopy and μCT, respectively. The finite elemental (FE) modelling of compression behavior for the microstructure was done with μCT-based image. The compressive strength and failure behavior was obtained *ex situ* and *in situ*.

RESULTS AND DISCUSSION:

The efficacy of post-processing techniques was ensured by phase analyses. μCT image-based analysis protocol provided that infiltration leads to reproducible and accurate reproduction of geometric entities to micron level. Majority of the microscale model experience low level of stress and strain, except at few places, from uniaxial compression (Fig. 1a,b). This observation, was one of the keys to rationalise the discrepancy between elastic modulus at micro-scale and macro-scale. μCT imaging and *in situ* compression illustrates the distinct mechanical response obtained through two post-processing approaches (compare Fig. 1c-e with 1f-h).

CONCLUSION:

Although, the microstructural compression response cannot be scaled directly onto macrostructural behaviour, for the first time, *in silico* modelling of 3DPP scaffold was attempted and the probable causes of the discrepancy between *in silico* and *ex situ* reaction were identified. Furthermore, *in situ* compression coupled with μCT imaging could provide us with the necessary insight, to identify failure mechanisms, advantage and disadvantages, in relation to the unique microstructure obtained through 3DPP and post-processing.

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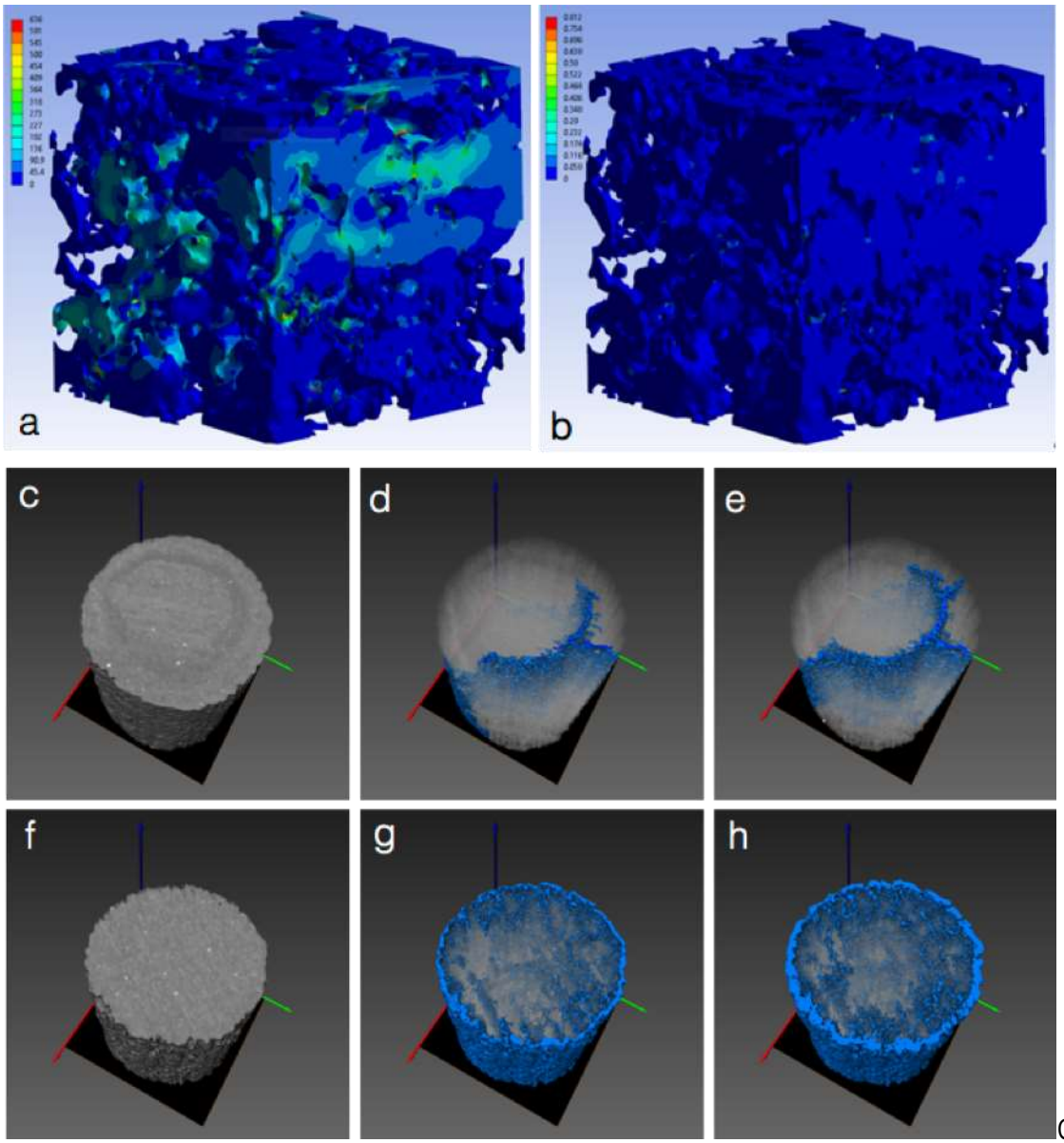
Picture 1:



Caption 1: In silico (a,b) and in situ (c,h) mechanical 3DPP scaffold models, under compression.

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Poster presentation

504 Temporal change in the mechanical properties of supramolecular electrospun vascular scaffolds during accelerated in-vitro degradation

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INTRODUCTION:

In-situ engineering of vascular tissues, starting from a degradable synthetic scaffold, requires an optimal balance between scaffold degradation and neo-tissue formation to maintain mechanical function. Due to their elastomeric properties, bis-urea (BU)-modified supramolecular polymers are attractive biomaterials for vascular tissue engineering[1,2]. The susceptibility of these polymers to enzymatic degradation can be easily tuned by varying the amount of ester groups in the polymer backbone. However, it is unknown if and how the degradation of the polymers affects the degradation of the scaffold and its consequent functional performance. Therefore, this study aims to characterize the mechanical performance during accelerated degradation of vascular scaffolds electrospun from candidate BU materials with varying susceptibility to degradation.

METHODS:

Polycaprolactone-BU (PCL-BU), polycarbonate-ester-BU (PC(e)-BU), and polycarbonate-BU (PC-BU) were selected as base materials and processed into vascular scaffolds using electrospinning (fiber diameter: 5µm; scaffold thickness: ~500µm, graft diameter: 3mm). Enzymatic degradation was induced by incubating the scaffolds in a lipase solution at concentrations ranging from 10U/ml to 500U/ml at 37°C. Scaffold degradation was evaluated in terms of changes in the chemical properties (differential scanning calorimetry, gel permeation chromatography), scaffold fiber morphology (scanning electron microscopy), and macroscopic properties (mass loss and scaffold dimensions) at 5 different time points up to 12 days. Functional performance was quantified by means of biaxial tensile tests and longitudinal ultrasound elastography measurements.

RESULTS AND DISCUSSION:

Large amounts of mass loss (up to 80%) and reductions in fibre diameter (up to 30%, accompanied by 30% mass loss) were observed following lipase incubation. Remarkably, the chemical properties (i.e., thermal properties and molecular weight) of the remaining material were barely affected, indicating that enzymatic degradation mainly occurs via surface erosion. Interestingly, whereas the mid-to-fast degrading materials (PC(e)-BU and PCL-BU) maintained their mechanical performance during degradation, the slow-degrading material (PC-BU) gradually became less stiff with degradation. This can be explained by water uptake by the scaffold fibres, resulting in swelling of the material, as the thickness of the PC-BU scaffolds remained largely constant with increasing mass losses.

CONCLUSION:

These data show that enzymatic degradation of BU scaffolds occurs mainly via surface erosion. However, the consequent functional performance is also governed by the susceptibility of the base material to degradation, which is mediated by differences in the swelling behavior.

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ACKNOWLEDGMENTS:

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Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

594 A 3D printed in vitro small intestine model

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Freie Universität Berlin, Berlin, Germany

INTRODUCTION:

Pathogen-small intestine interactions are preferably studied using *in vitro* human intestinal models instead of complex *in vivo* animal models. However, current *in vitro* models often lack physical intestinal 3D features, especially a villi structure that is needed for absorption and a barrier function. As 3D printing enables complex geometries, it is a great technique to fabricate tissue scaffolds with fine 3D features. We aimed to develop an *in vitro* tissue model by 3D printing intestinal-mimicking scaffolds, where epithelial cells and fibroblasts were cultured. These cell-containing tissue models will be further matured into multilayered tissue constructs in a dynamic bioreactor.

METHODS:

Biodegradable scaffolds consisting of a spike-containing lumen and a porous surrounding area were 3D printed with stereolithography using poly(ε-caprolactone)-based photocrosslinkable resin. Epithelial cells were seeded on the lumen side of the scaffolds, and their proliferation and differentiation were followed by measuring cells' metabolic activity, enzyme activity, microvilli formation, and tight junction formation. Fibroblasts were seeded on the porous area and their proliferation and migration were followed by fluorescence staining.

RESULTS AND DISCUSSION:

Tubular tissue scaffolds were successfully 3D printed by stereolithography to have villi-mimicking spikes on their lumen side. The spikes increased the surface area of the lumen and provided seeded epithelial cells with physical guidance and mechanical support. In *in vitro* culture, epithelial cells formed a confluent cell layer along the 3D spikes, showing increased alkaline phosphatase activity typical for enterocytes. Also formation of tight junctions and a microvilli structure on the lumen surface indicated differentiation of the cells into enterocyte-like cells. Fibroblasts attached and proliferated well in the porous area, forming a continuous cell layer on the outer scaffold surface.

CONCLUSION:

Use of SLA enabled high-resolution 3D printing of tubular scaffolds mimicking a native small intestine structure. The detected differentiation of Caco-2 epithelial cells into enterocyte-like cells indicated the suitability of our 3D printed scaffolds for epithelium formation. To achieve a matured *in vitro* tissue model, the cell-containing scaffolds will be further cultured in a dynamic bioreactor in physiologically relevant conditions.

ACKNOWLEDGMENTS:

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Poster presentation

611 Tailoring Substrate stiffness to investigate corneal epithelial cell behaviour

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INTRODUCTION:

Corneal blindness is the third leading cause of blindness worldwide [1]. Current treatments involve the use of donor corneas and also the use of well-established cell carriers (e.g. amniotic membrane) [2]. Although these treatments are successful to a degree, they present drawbacks including availability of tissue and the risk of disease transmission [3]. The use of synthetic scaffolds is a potential solution; recent research has demonstrated the importance of controlling mechanical properties when designing new approaches to corneal regeneration. The aim

of this project is to create synthetic electrospun scaffolds with different stiffness to explore corneal cell behaviour *in vitro*.

METHODS:

Metallic collectors were designed using Autodesk Inventor Professional 2017, then 3D printed in wax, and casted. Poly lactide-co-glycolide (PLGA) and Polycaprolactone (PCL) solutions were prepared in 100:0, 99:1, 95:5, 90:10, and 80:20 respectively using Dichloromethane and Dimethylformamide as solvents, then the solutions were electrospun using an in-house developed set up with a voltage of 17 kV and 14 cm distance.

Mechanical properties of the electrospun materials and biological tissues (cornea and amniotic membrane) were tested using a uniaxial tensiometer (BOSE). Scanning electron microscope was used to determine morphological characteristics.

RESULTS AND DISCUSSION:

We have shown that by using a wax 3d printer to later cast the wax to make metallic substrates is a reliable methodology to create intricate collectors for electrospinning. Well-defined topography was successfully incorporated into electrospun scaffolds using in-house designed collectors. Electrospun PLGA scaffolds and PLGA-PCL electrospun blends have similar morphological characteristics, but in terms of mechanical properties, the addition of PCL seems to reduce the stiffness of the scaffolds.

CONCLUSION:

We have shown a cost-effective strategy for the fabrication of mechanically-tailored scaffolds using FDA approved polymers (PLGA and PCL) for developing the next generation of electrospun substrates for corneal regeneration. Next steps will aim to explore the correlation of the tuneable mechanical properties of the substrates with the behaviour of corneal cells, both from human and rabbit sources.

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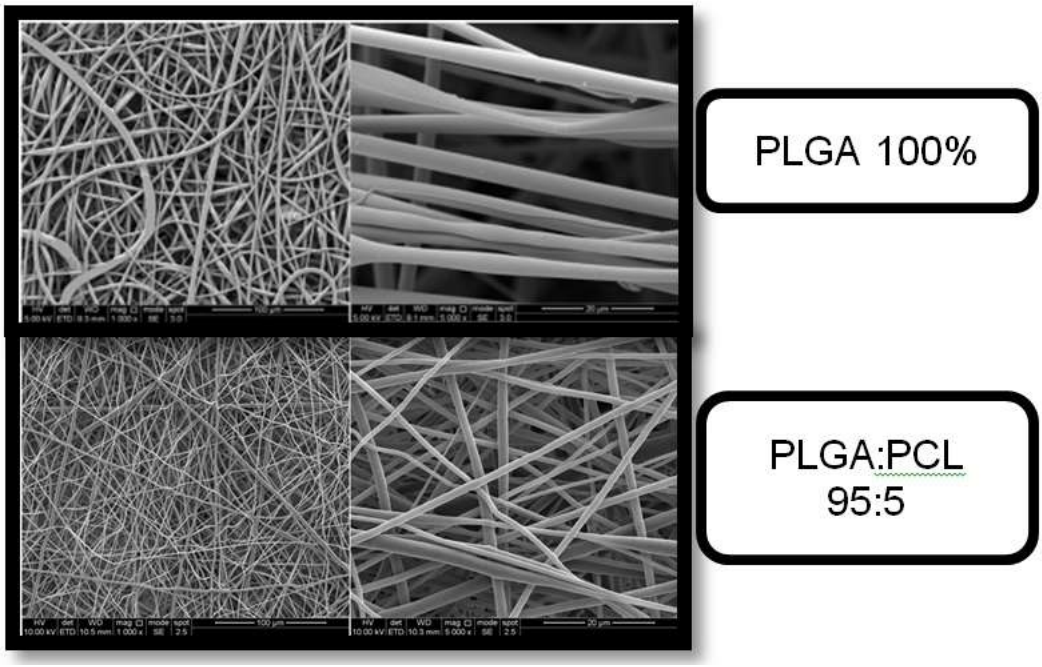
The authors would like to thank CONICYT for founding this research.



Picture 1:

Caption 1: Figure 1: comparison of SEM images of a PLGA scaffold versus a scaffold with 95% PLGA - 5% PCL. Both scaffolds were electrospun using the same solvent

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Poster presentation

1 Coral skeleton topography induces neural elongation and activation

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INTRODUCTION:

In recent years, several biodegradable materials were explored as scaffolds for tissue engineering and regenerative medicine. Topography modification of these scaffolds was shown to influence their bioactivity and regenerative capacity. In this regard, coral skeleton was shown to promote neuronal regeneration processes. However, neural response to different coral scaffold's topography was not yet examined.

METHODS:

In this paper, we assessed the response of dissociated neural cells to distinct coral scaffold topographies. We distinguished between three types of topography: smooth, ragged and ridged surfaces.

RESULTS AND DISCUSSION:

We observed that, with the increase of the surface curvatures, astrocytes' expression of glial fibrillary acidic protein was 10-fold higher compared to control. Moreover, astrocytes developed thin edged processes, which were found to be 20% longer when compared to control. Interestingly, we also showed that dendrites' expression of microtubule-associated protein 2 was also increased in response to ridged coral surface.

CONCLUSION:

Thus, we suggest that the topography induces an increase in neuronal cells activation and expansion. These effects of the coral scaffold's topography on neural cell behavior might play a key role in applications for neuronal tissue restoration.

ACKNOWLEDGMENTS:

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Poster presentation

9 Regulation of calcium phosphate crystal formation using small organic molecules and inorganic ions

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INTRODUCTION:

Synthesis of inorganic materials with specific morphologies and architectures has been attracting great attention due to their unique physical-chemical properties and biological effects. Calcium phosphate, as the main inorganic component of human hard tissue, has been widely used in the field of bone tissue reconstruction owing to its good biocompatibility and bioactivity. Calcium phosphate physical-chemical properties (e.g. solubility, thermostability and mechanical performance) and biological effects (e.g. protein adsorption and cell behavior) may significantly influenced by crystal morphology, size and chemical composition. Especially, calcium phosphate with nano- to micrometer sizes, with high specific surface areas, can enhance protein adsorption and promote cell growth. Meanwhile, inorganic ions substitutions have been a useful tool to improve the biological performance of calcium phosphate. Therefore, it is significantly important to regulate calcium phosphate architectures at the nano/micro-scale for improving its biological functions.

METHODS:

Herein, structurally symmetric small organic molecules with different functional group were used to modulate calcium phospahte growth under hydrothermal condition. In addition, inorganic ions were added in the reaction solution for synthesis inorganic ions-substituted calcium phosphate. Then, we studied protein storage/release behaviors of calcium phosphate particles based on their morphlogy using BSA and LSZ as a model protein. Finally, cell behaviors on micro/nano-structured calcium phosphate particles were investigated.

RESULTS AND DISCUSSION:

The results showed that calcium phosphate particles with various morphologies, including whisker, flower-like aggregates, nanostructured hollow microspheres were obtained with the assistance of small organic molecules. The particle microstructures changed with the small organic molecules concentration. Additionally, inorganic substituting also changed the morphology of calcium phosphate , generating flower-like microspheres. Then, protein adsorption results demonstrated that calcium phosphate with nano/micro-structures may have a selective adsorption and controlled release on proteins. Cell experiments showed that spherical calcium phosphate was more benefit for cell proliferation and differentiation comparing with plate-like calcium phosphate.

CONCLUSION:

In conclusion, small organic molecules and inorganic ions addition can effectively regulate calcium phosphate growth and guide synthesis of functional calcium phosphate particles with different nano/micro-structures. And, micro/nano-structured calcium phosphate has a selective protein adsorption and plays a key role in controlling protein release. Cell differentiation behavior can be effectively regulated by modulating calcium phosphate micro/nano-structures.

ACKNOWLEDGMENTS:

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Poster presentation

12 Genotoxicity and Photodynamic Properties of Heat-Treated Silicalite-1 Film

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INTRODUCTION:

Silicalite-1 films supported on the surface of stainless steel and TiAl6V4 alloy are prospective anticorrosive coating of materials for fabrication of prostheses[1]. Effect of the film calcination on genotoxicity and photodynamic properties of the film is investigated.

METHODS:

Surfaces of freshly prepared silicalite-1 films before and after calcination to 500°C were characterized in detail by SEM, angle resolved XPS, FTIR and GC MS. Luminescence of singlet oxygen ($O_2(^1\Delta_g)$) at 1270 nm was recorded after excitation of the sample using a COMPEX 102 excimer laser (wavelength 308 nm, pulse width ~28 ns). The osteosarcoma cell line U-2 OS (ATCC-LGC, USA) with wild-type p53 and Rb genes was used in vitro to investigate the potential DNA damage to the cells. The level of DNA damage was investigated by immunofluorescence staining of phosphorylated histone H2AX analyzed by flow cytometry.

RESULTS AND DISCUSSION:

Carbonaceous residues created in silicalite-1 films after calcination to 500°C were localized on their outer surface and in a shallow subsurface region as a mixture of aliphatic and polyaromatic hydrocarbons (*PAH*). Naphthalene, anthracene, pyrene, fluoranthene and phenanthrene were created in micromole concentrations, and they were found to be sufficiently volatile to release into the environment, where they can sensitize the production of $O_2(^1\Delta_g)$ upon irradiation by light. The evaluation of potential DNA damage of silicalite-1 films revealed increased induction of double-strand breaks in osteoblast-like cells cultured on calcined silicalite films but not on the films which were not calcined.

CONCLUSION:

Irradiation of *PAH*s present on the surface of silicalite-1 film in micromole concentrations led to the formation of singlet oxygen, which may act to sterilize of the material in its application for the coating of the metallic prosthetic materials. However, observed genotoxicity of calcined silicalite-1 film complicates its applicability in fabrication of prosthetic material.

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Poster presentation

31 Cytotoxicity and antibacterial assessment of chitosan-based membranes loaded with silver

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INTRODUCTION:

Antibacterial wound dressings can improve the wound healing by decreasing the bioburden.[1] Chitosan-based membranes containing silver was developed for this application.[2] The aim of this study is to further evaluate the cytotoxicity and antibacterial property of the chitosan/silver membranes.

METHODS:

Chitosan/silver membranes were prepared from chitosan/PEO solutions mixed with 0, 1, or 5 % $AgNO_3$ by electrospinning, and named as Ag0, Ag1, and Ag5, respectively. Samples were characterized by scanning and transmission electron microscopy. The silver release study was conducted by immersing the membranes in phosphate buffer saline (PBS). Cytotoxicity was evaluated by trans-well-culturing human foreskin fibroblasts (hFFs) with membranes. An agar diffusion test against Staphylococcus aureus ATCC 25923 was performed after the membranes immersed in PBS for predetermined time periods up to 28 days (n=3). All data were analyzed using one-way analysis of variance with statistical significance at $p < 0.05$ or less.

RESULTS AND DISCUSSION:

All the samples displayed a nanofibrous structure. Those made from the solutions containing AgNO3 showed that silver nanoparticles formed homogeneously in the nanofibers. The silver release showed first a burst release at day 1 and then followed by a sustained release for at least 4 weeks. The hFFs cultured with membranes demonstrated similar proliferation as cultured on the tissue culture plate. Ag0 group had no antibacterial effect as expected whereas Ag1 and Ag5 showed dose-dependent antibacterial activity.

CONCLUSION:

The chitosan-based membranes loaded with silver showed antibacterial efficacy without noticeable cytotoxicity and thus are promising for further exploration as wound dressings.

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Poster presentation

44 Antimicrobial and hemocompatible properties of PVA/CA films treated with LL37 and pexiganan

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INTRODUCTION:

The unique diverse function and architecture of antimicrobial peptides (AMPs) has attracted considerable attention as a tool in the design of molecular templates for new anti-infective drugs. AMPs are gene-encoded short amphipathic molecules, with a broad spectrum of activity over pathogens, that act rapidly at multiple sites within microbial cells, reducing their likelihood of developing resistance[1]. LL37 (37a.a.), the only cathelicidin-derived AMP found in humans, plays a central role in the innate immune response and inflammation[2]. On its turn, pexiganan (22a.a.) synthesized from magaining is known to reduce microbial burden without enhancing bacterial resistance[3]. In the present work these two AMPs were combined with biocompatible polymeric matrixes, poly(vinyl alcohol) (PVA) and cellulose-acetate (CA), endowed with versatile physical properties and wound-healing abilities,[1,4] with the purpose of testing their merged antimicrobial and hemocompatible properties for prospective wound-healing applications. The goal was to establish the most successful combination of PVA/CA+AMP for a possible wound dressing formulation, that would fight bacterial invasion and promote platelet adhesion while reducing clotting time.

METHODS:

PVA/CA films were produced by phase inversion at 100/0, 80/20, 50/50, 20/80 and 0/100%wt. Polymeric solutions were prepared at 10w/v% in DMSO, heated at 90ºC to promote dissolution, and combined with glutaraldehyde. All traces of DMSO were eliminated by periodic exchange of dH_2O bath. LL37 and pexiganan were prepared at 10-40µg/mL in pure water and functionalized using dopamine as binding agent or combined with the polymer in an “all-

in-one" approach. Vancomycin-antibiotic was used as control. AMPs functionalization was confirmed using sulfo-SDTB. SEM, ATR-FTIR, DMA and contact-angle techniques were used for characterization purposes. PVA/CA+AMP films physiological stability was evaluated in presence of proteolytic enzymes. AMPs antimicrobial performance was tested against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli*. Their hemocompatibility and clotting-time was determined using human-platelets and by following the loss of movement of re-calcified plasma, respectively.

RESULTS AND DISCUSSION:

AMPs presence on PVA/CA films was confirmed. Films were very hydrophilic (high hydration capacity), possessed an interconnected-porous structure and resisted to enzymatic degradation. Preliminary testing revealed LL37 and pexiganan functionalized films to reduce bacterial presence compared to control and to accelerate clotting time. Biological testing are still ongoing to establish the best combination PVA/CA+AMP and AMPs' functionalization method.

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Poster presentation

51 Cell biological response to clinically relevant micro-sized wear particles of ultra high molecular weight polyethylene loaded with alendronate sodium

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INTRODUCTION:

Ultra high molecular weight polyethylene (UHMWPE) has been used as a bearing material in joint prosthesis for more than half century[1]. Nevertheless, wear particles of UHMWPE, especially with the size ≤ 10 μm, have been currently recognized as one of the major causes of osteolysis and aseptic loosening of joint prosthesis due to its cell response[2,3]. Therefore, it is necessary to find a way to reduce the cell response caused by UHMWPE wear particles.

METHODS:

UHMWPE loaded with alendronate (ALN), a drug used to treat osteolysis, was developed by solvent evaporation and compression molding. Clinically relevant micro-sized (≤ 10 μm) UHMWPE-ALN wear particles were obtained by vacuum gradient filtration after preparing initial wear particles using a rough rubbing pair. The release behavior of ALN from UHMWPE-ALN wear particles was investigated *in vitro*. Alginate sodium (SA) beads were fabricated by

simply extrusion method to encapsulate cells and wear particles to evaluate the cell response to UHMWPE-ALN wear particles, and explore the mechanism of ALN-cell intactions.

RESULTS AND DISCUSSION:

In vitro release results showed that ALN could be released from UHMWPE-ALN wear particles. SA bead was proved to be used as a cell reactor to evaluate the long-term cell response to wear particles. The effect mechanism of ALN released from UHMWPE-ALN wear particles on macrophages and osteoblasts were revealed at the molecular level. Results showed that released ALN may act synergistically on macrophages and osteoblasts, inhibit osteolysis by interfering with the cytokine and RANKL / RANK signaling pathway.

CONCLUSION:

In this study, SA bead was first used as a cell reactor to encapsulate cells and wear particles to evaluate the cell biological response and mechanisms to UHMWPE-ALN wear particles. This novel co-culture method effectively overcame the difficulties of cells-wear particles contact in other co-culture methods, and avoided the disadvantages associated with the long-term evaluation of cells to wear particles by inverted culture technique. The results provide a scientific basis for the clinical application of UHMWPE-ALN, and open up a new way to evaluate the biological response to micro-nano particles.

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Poster presentation

59 Porcine-derived extracellular matrix for musculoskeletal tissue engineering

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INTRODUCTION:

Collagen materials are extensively used in regenerative medicine and clinics. However, they still present limitations including monodomain composition, poor mechanical properties, and need of crosslinking. On the other hand, tissue grafts overcome most of these limitations. Moreover, although numerous tissue grafts are employed in hernia and woundcare, their potential in musculoskeletal tissue engineering has not been fully investigated. Herein, we ventured to assess the potential of a bi-phasic porcine peritoneum for musculoskeletal application by comparing its characteristics with a commercial collagen scaffold employed in tendon

METHODS:

The mechanical, thermal, biochemical and biological properties and topography of the porcine peritoneum were assesed *in vitro*. To this end, tensile uniaxial test, DSC, free amine analysis, MMP degradation, SEM and AFM were carried out respectively. The microstructure and composition were also studied with histology and immunohistochemistry. The effects of porcine peritoneum on the behaviour of adult dermal fibroblasts, tenocytes and stem cells were analysed. Immune response in vitro was also assessed with THP-1 cell line. All studies employed a commercially available mono-domain collagen scaffold as control. Statistical significance was accepted when $p<0.05$.

RESULTS AND DISCUSSION:

Results indicated that the porcine peritoneum had higher mechanical properties and a lower crosslinking ratio. The porcine peritoneum was completely degraded by MMP-1 and MMP-8 after 24h, contrary to the collagen matrix, which suggests a faster remodelling *in vivo* of the tissue graft. The histology and immunohistochemistry analysis showed a multicomponent and organized structure in the porcine peritoneum, including basal membrane markers such as elastin, fibrin, and collagen; compared to only collagens type I/III and fibrin in the collagen matrix.

Cell studies on human tenocytes and fibroblasts showed the capability of the peritoneum to support cell growth. In addition, tenocytes had a slight higher proliferation on the basal membrane, meanwhile they did not proliferate on the collagen matrix. ADSCs were able to grow on both materials, however, proliferation was enhanced by the porcine peritoneum ($p<0.01$). Immune response by THP-1 showed an acute inflammatory response to the collagen matrix, contrary to that observed in the porcine peritoneum, which triggered a mild reaction. Currently, an *in vivo* study in a rabbit flexor tendon model is being carried out to elucidate the tissue graft potential for tendon repair.

CONCLUSION:

The present study shows the suitability of porcine peritoneum as an implantable device and its multifunctionality due to its heterogeneous composition and structure. Besides, its multifunctionality provides higher cytocompatibility than a mono-domain collagen matrix with human tenocytes and ADSC. Moreover, its lower immune response *in vitro* suggests better remodelling after implantation.

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Picture 1:

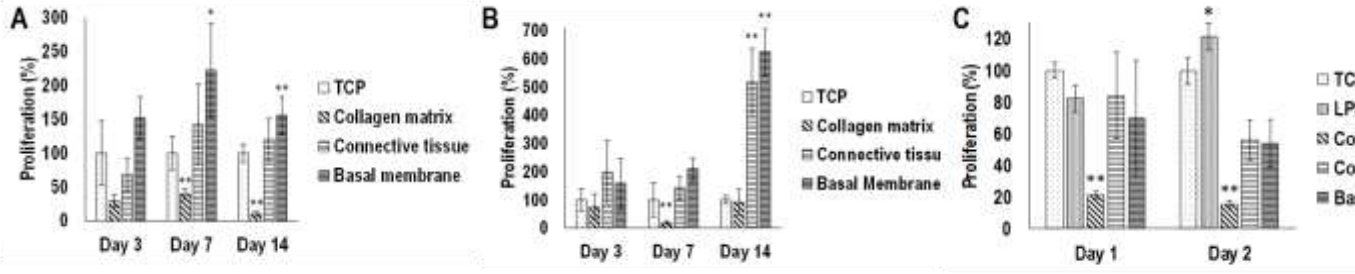

Figure 1. Proliferation of human tenocytes on the different materials and tissue culture plastic (TCP) as control (A). Prolifera ADSC on the different materials and TCP as control (B). Proliferation of differentiated THP-1 monocytes on the different materia TCP and TCP + LPS as negative and positive controls (C). * $p<0.05$, ** $p<0.01$.

Caption 1: Cell response in vitro

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Poster presentation

66 Fatigue properties of a low-modulus bone cement designed for the treatment of vertebral compression fractures

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INTRODUCTION:

The difference in stiffness between acrylic bone cement and trabecular bone tissue may facilitate the formation of new fractures after vertebroplasty[1]. This lead to the development of cements with more bone-compliant mechanical properties [2]. While the quasi-static mechanical properties of these cements have been well reported, data related to their fatigue performance are still missing in the literature. Therefore, the aim of the present study was to evaluate the *in vitro* compressive fatigue properties of acrylic bone cement modified with linoleic acid (LA).

METHODS:

V-Steady (VS) was used as the vertebroplastic base cement, and modified with 12 vol% linoleic acid (LA). Fatigue testing was performed in phosphate buffered saline solution at 37°C to better mimic the *in vivo* scenario. A small preload of -20N was applied to the sample, followed by a constant-amplitude cyclic compression-compression load at 2Hz. This test was performed until run-out at 2 million cycles was reached or when the specimen failed (defined as 15% deformation). The “up and down method” for small sample sizes was employed as it allows for a substantial reduction of the number of specimens to test. Additional quasi-static mechanical testing was performed to investigate the evolution of the mechanical properties over time.

RESULTS AND DISCUSSION:

The quasi-static mechanical properties of the unmodified cement were similar to other commercially available bone cements (σ= 100.7 ± 3.1 MPa; E= 2140.4 ± 128.8 MPa). The LA-modified cement showed bone-compliant properties after 24h of setting (σ= 28.3 ± 5.1 MPa; E= 494.7 ± 51.8 MPa). The unmodified cement exhibited a high fatigue strength (42.5-45 MPa), as expected. The fatigue strength of LA-modified cement, LA-VS, was reduced to approx. 3.8 MPa, which is however higher than the pressures experienced by the vertebral body during daily activities[3].

CONCLUSION:

Bone cement with bone-compliant mechanical properties had a lower fatigue limit than the corresponding standard cement, as expected, which is however still higher than the normal pressures experienced by the vertebral body. LA-modified bone cement is a promising material designed to prevent complications following vertebroplasty associated with a high cement stiffness.

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Poster presentation

76 Covalent Immobilization of Echinocandins and Polyenes as Antifungal Coatings

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INTRODUCTION:

Polymicrobial biofilms can be formed on surfaces by microbial colonization of materials such as biomedical implant devices and are caused by bacterial or fungal species, or both kinds of microbes. Although fungal infections related to biomaterials are increasing and threatening human lives, this field has received little interest compare to bacterial infection and anti-bacterial surfaces.

METHODS:

One strategy to combat fungal biofilms is to prepare coatings with covalently bound, FDA-approved antifungal agents such as echinocandins and polyenes on the biomaterial surfaces, for long-lasting effects that are non-toxic to human cells. Using plasma polymer interlayers is a convenient strategy to functionalize surfaces allowing straightforward immobilization of bioactive molecules. Amongst the echinocandin drug class, caspofungin possesses primary amine groups that facilitate surface immobilization to propionaldehyde plasma polymers. Micafungin and anidulafungin do not have convenient functional handles, so ethanol plasma polymer coating was activated with carbonyldiimidazole (CDI) and then covalently coupled to hydroxyl groups.

RESULTS AND DISCUSSION:

Results show echinocandins retain activity when covalently bound onto the surface which indicate they can disrupt the cell wall integrity of fungal cells. Also, it has been found that these coatings can be reused several times while still maintaining efficiency against fungal cells which is a highly desirable feature for medical device coatings. In contrast, polyenes do not have antifungal activity when are covalently attached onto the surface because surface attachment prevents them from reaching their cell membrane target.

CONCLUSION:

Results of surface characterization techniques like XPS and TOF-SIMS and also microbiological assays such as static biofilm assay and live/dead stain assay will be discussed.

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Poster presentation

84 Hyaluronic acid-based hydrogel with mechanical strength for tissue engineering

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INTRODUCTION:

Nowadays, loss of tissue and organ happens because of accidents, diseases and aging, and their demands are rapidly growing as one of the major parts of human health concerns. To meet these demands, tissue engineering has appeared as a therapeutic route that regenerates the defected tissue and its neighbouring environments. For this purpose, cells and bioactive molecules should be delivered to the injured irregular defected area through suitable scaffolding substrate such as hydrogel carriers and substracts having analogous characteristics similar to that of natural bone. Among many biocompatible hydrogels, hyaluronic acid has gained high interesting in injectable forms and biocompatibilyt, but its mecanical stability was yet to be developed.

METHODS:

. To achieve sufficient gel strength, elasticity, and well-mannered porous architecture, first, 2-hydroxyethyl acrylate has been grafted onto hydroxyl groups of hyaluronate, and then crosslinking using different amount of poly(ethylene glycol) diacrylate. The fabricated terpolymer [HA-g-p(2-HEA)-x-PEGDA] has been characterized by FTIR, ^{1}H HR-MAS-NMR, and TGA analyses.

RESULTS AND DISCUSSION:

SEM images indicate that the terpolymer contains interconnected porous network structure. The achievement of equilibrium swelling ratio, and higher value of elastic modulus in rheology study confirmed the gel nature of polymer in aqueous medium at 37 °C. The terpolymeric gel showed pH-dependent release of dimethyloxalylglycine, and tetracycline at 37 °C. The cell study results ascertained that the prepared gel supported excellent osteoblastic MC3T3 cell adhesion, proliferation, and viability *in vitro*, which are promoted by interconnected porous structure and 3D network of the gel. The histology results confirmed that the native gel itself provides excellent environment for the regeneration of extracellular matrix and collagen.

CONCLUSION:

The novel biocompatible HA-g-p(2-HEA)-x-PEGDA gel could be employed in dimethyloxalylglycine and tetracycline delivery, as well as in bone tissue engineering applications

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ACKNOWLEDGMENTS:

Authors also sincerely acknowledge the financial support of National Research Foundation of Korea (NRF) Grant (2015R1A2A1A10054592).

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

98 Application of octacalcium phosphate collagen composite for bone augmentation with sinus floor elevation in humans

Tadashi Kawai[1], Tetsu Takahashi[1], Keiko Matsui[1], Yushi Ezoe[1], Fumihiko Kajii[2], Yoshinaka Shimizu[1], Shigeto Koyama[3], Hiroyuki Kumamoto[1], Osamu Suzuki[1], Shinji Kamakura[4]

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INTRODUCTION:

Octacalcium phosphate (OCP) has become recognized as an osteoconductive bone substitute material based on *in vitro* and *in vivo* studies[1, 2]. It has also been demonstrated that OCP combined with collagen (OCP/Col) facilitates bone regeneration more effectively than OCP alone[3]. We recently reported the efficacy of OCP/Col in the treatment of human bone defects[4,5]. The current study reported the application of OCP/Col for bone augmentation with sinus floor elevation in patients with atrophic maxilla.

METHODS:

OCP was prepared according to a method of synthesis by mixing calcium and phosphate solution. Particle size of OCP was 300 – 500 mm in diameter. OCP/Col was prepared from pepsin-digested atelocollagen isolated from the porcine dermis and OCP. OCP/Col was molded in the shape of a disc, 9 mm diameter, and 1.5 mm thick. The current study was a part of the 'Prospective, Multi-center, Single-arm Study of OCP/Col for Guided Bone Regeneration' clinical trial, which were registered with the Medical Information Network in Japan (JPRN-UMIN000018192). The protocol of the clinical trial was approved by the Institutional Review Board of the Pharmaceuticals and Medical Devices Agency in Japan. The patients were healthy men or women from 20 to 65 years old. OCP/Col was applied to three patients for bone augmentation with sinus floor elevation. After operation, we evaluated subsequent bone formation radiographically, examined the newly formed bone histologically, and evaluated their progress until the final dental implant prostheses were fitted.

RESULTS AND DISCUSSION:

OCP/Col converted to hard tissue with radiopacity within 6 months of implantation. At the 6-month time-point, bone biopsy was performed in conjunction with dental implant placement. Histological examination confirmed normal bone tissue in a specimen and FTIR revealed characteristics of bone-like apatite. It has been approximately 12–18 months since final dental implant placement in the 3 cases, and no substantial adverse events have occurred. The dental implant treatment of these 3 cases was performed to the point of final prosthesis fitting without any problems.

CONCLUSION:

This study demonstrated that OCP/Col may be a candidate bone substitute material in bone augmentation procedures involving sinus floor elevation.

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Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

115 Influence of the heat treatment on the bioactivity of nanostructured beta titanium alloy

Jaroslav Fojt[1], Zdenek Kacenka[2], Petra Jarolimova[2], Eva Pruchova[2], Ludek Joska[2], Vojtech Hybasek[2]

[1]University of chemistry and technology, Prague, Prague, Czech Republic

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INTRODUCTION:

Titanium and Ti6Al4V alloy are widely used materials in orthopaedics and dentistry. However, because of biomechanical incompatibility, the new generation of less-problematic low modulus beta-titanium alloys has been developed. Surface nanostructuring can be used to enhance the bioactivity of the materials. Nanotubes have positive influence on cells osteogenic differentiations, adhesion and proliferation. The nanotubes heat treatment lead to the partial conversion of the amorphous titania to anatase or rutile form which will further increase bioactivity. The aim of this study is focused on influence of the surface treatment on the bioactivity of titanium beta alloy. The main parameters are nanotubes diameter and its crystal structure.

METHODS:

Experiments were conducted with specimens of Ti-39Nb alloy. Nanostructuring by anodic oxidation was realised in water based electrolyte. The three different potentials were used for obtain difference in tubes parameters. One set of the specimens were heat treated at 500°C. The bioactivity was studied in simulated body fluid (SBF) according to ISO 23317 standard. The electrochemical impedance response at 2 kHz was recorded during 168 h of the exposure. For the morphological characterisation of the samples and surface evaluation after exposure, a scanning electron microscope was used.

RESULTS AND DISCUSSION:

Nanotubes with diameter from 20 nm to 80 nm and length from 400 nm to 3 mm were successfully prepared. The tubes diameter increased with increasing anodic voltage, also the tubes length increased with time of the anodization process. The exposure tests in SBF show evident influence of the tubes diameter on the apatite precipitation. In the as prepared form, the diameter approximately 40nm was able to induce new layer precipitation. This was also detected by single frequency impedance measurement. The impedance time dependencies for heat treated specimens shown changes in trend in the case of all surfaces. The apatite-based layer precipitation on the all surfaces was proved by EDS analysis. The shortest precipitation period was detected in the case of 40 nm nanotubes. It was almost twice faster than in the case of specimen without heat treatment.

CONCLUSION:

The exposure test showed influence of tubes parameters on the layer precipitation from simulated body fluid. The most perspective diameter was approximately 40 nm. The appropriate heat treatment leads to increasing bioactivity of nanotubes. The apatite-based layer precipitation occurred on all heat treated surfaces.

ACKNOWLEDGMENTS:

The authors would like to thank Czech Science Foundation (Grant no: 6-14758S) for providing financial support to this project.

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Poster presentation

122 Bacterial nanocellulose as a cell culture platform

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INTRODUCTION:

Nanocellulose of bacterial origin (BNC) is a biopolymer naturally synthesized and secreted by several bacterial genera. BNC forms a 3D-network comprised solely of cellulose nanofibres that resembles the structure of collagen in the extracellular matrix. BNC has unique properties that make it a promising candidate for biomedical applications[1]:high liquid holding capacity, tensile strength, flexibility and porosity. On the other hand, there are still a limited number of biomaterials that meet all the requirements to fit in the regenerative medicine settings2. Thus, our goal is to investigate BNC as a novel, versatile and controllable support for cell-manipulation.

METHODS:

BNC pellicles were obtained from 3-day static cultures of *Komagataeibacter xylinus*. The BNC films were thoroughly washed and autoclaved before being used as a platform for culturing human dermal fibroblasts. The topography of some BNC films was patterned during biosynthesis using PDMS molds while others were functionalized with TiO_2 nanoparticles by a microwave-assisted method. Attachment, distribution and growth kinetics of the seeded cells were studied by cell viability assays (n=5 and ANOVA and Tukey's statistical tests) and confocal microscope.

RESULTS AND DISCUSSION:

All BNC films proved to be biocompatible and to support attachment and proliferation of fibroblasts (B). Growth kinetics on BNC was be very similar to that on culture plates with the advantage of representing a transportable platform for adherent cells (A). Moreover, BNC pellicles with TiO_2 nanoparticles facilitated cell attachment, probably by providing more anchorage sites. As for BNC with controlled topographies, we found that cells tended to follow the patterns on the BNC surface (C). As suggested by Yang et al.[3], patterned-BNC could be used to guide the growth of cells in tissue engineering.

CONCLUSION:

Our data reinforces that BNC substrates could both facilitate *in vitro* analysis (migration studies, drug testing) and open a door for future applications of BNC in tissue regeneration.

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ACKNOWLEDGMENTS:

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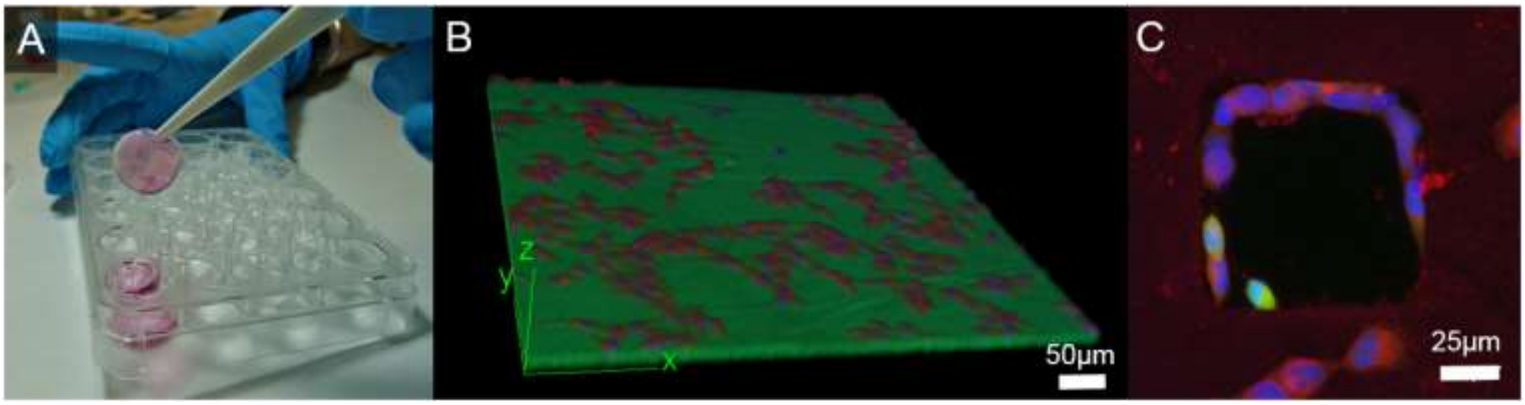

Picture 1: Caption 1: Figure: A) fibroblasts platted on BNC pellicles can be easily transfered without trypsinization. B) Human cells attached and proliferated on BNC. C) P

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Poster presentation

136 Characterization of an ocular drug delivery device

Christian Bertens[1], Chiara Martino[2], Marlies Gijs[1], Marty Osch[3], Aylvin Dias[4], Arno Lataster[3], Biggelaar van den Frank[1], Remco Tuinier[2], Rudy Nuijts[1]

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[2]Technische Universiteit Eindhoven, Netherlands

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[4]Eyegle bv., Netherlands

INTRODUCTION:

Most ocular drugs are administered via eye drops or ointments, but these delivery methods suffer from low drug bioavailability, rapid drug elimination, side effects (e.g. allergies) and low patient compliance. Therefore, a more efficient and convenient way to deliver ophthalmic drugs is needed.

We are developing an ocular device for sustained drug delivery. The ocular device consists of a coated stainless steel coiled wire with two terminal caps and a drug-loaded filling, comprising microspheres. The ocular coil is placed behind the lower eye lid, in the *cul de sac* (Fig.1). Two important characteristics of the ocular coil are flexibility and drug delivery capacity. In order to provide a comfortable product, the coil should be highly flexible in order to bend and adapt to the anatomical configuration of the *adnexae* (e.g. orbit, soft tissues) of the eye. Furthermore, individual anatomical boundaries may vary the bending of the coil and therefore could increase or decrease the space between the coil windings and modify the level of drug diffusion to ocular tissue. The aim of this poster is to describe the shape performance of the ocular coil with respect to the anatomical boundaries of the eye for future clinical applications.

METHODS:

The position and shape of the ocular coil were investigated by inserting a prototype of the ocular coil *post mortem* in the conjunctival bags of rabbits and a human cadaver.

RESULTS AND DISCUSSION:

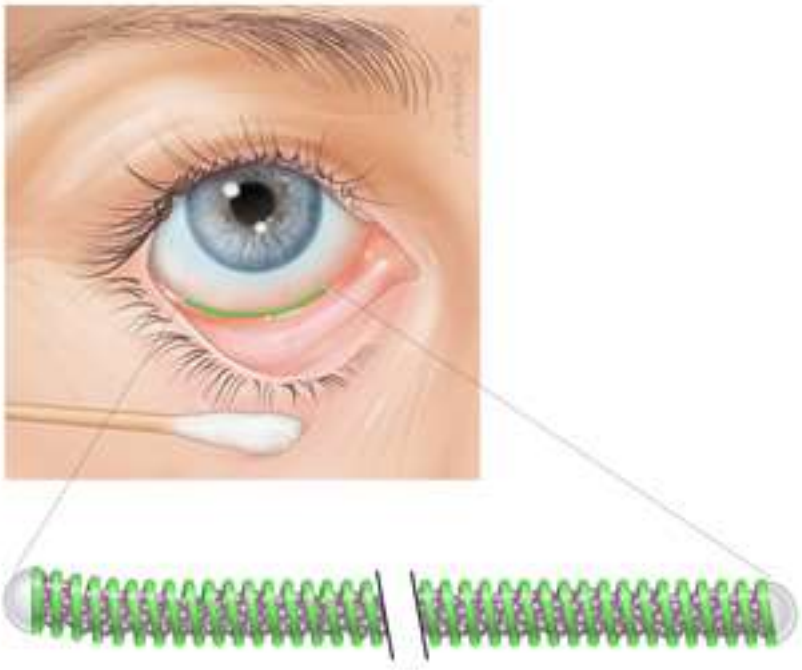
CT-scan imaging showed that the ocular coil is located deep in the fornix where the palpebral conjunctiva transits into the bulbar conjunctiva. The data suggest that the location of the ocular coil will not affect muscular movements of the eye. Thanks to the properties of the drug delivery matrix (microspheres), the ocular coil maintains its flexibility and is able to follow the anatomical boundaries of the eye.

CONCLUSION:

To conclude, preliminary data confirmed the preferred position, shape and flexibility of the ocular coil. First-in-human tests are planned to evaluate the comfort and tolerance of the ocular coil in healthy volunteers. In parallel, an animal study will explore the drug delivery capacity.

ACKNOWLEDGMENTS:

For this study the head of an intact human cadaver specimen was used. A handwritten and signed codicil from the donor, posed when still alive and well, is kept at the Department of Anatomy and Embryology, Faculty of Health, Medicine and Life Sciences, Maastricht University, The Netherlands. This is required by Dutch law for the use of cadavers for scientific research and education.



Picture 1: Caption 1:
Figure 1. Sketch from the ocular coil in the cul de sac, and filled with a matrix comprising microspheres, and capped on both sides with an UV-curable

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Poster presentation

138 Cell interaction with modified Ti6Al4V pre-exposed in simulated body fluid

Petra Jarolímová[1], Barbora Voltrová[2], Eva Filová[2], Jaroslav Fojt[1], Eva Pruchová[1], Vojtech Hybášek[1], Ludek Joska[1]

[1]University of Chemistry and Technology, Prague, Czech Republic

[2]Institute of Experimental Medicine of the Czech Academy of Sciences, Czech Republic

INTRODUCTION:

Titanium alloys are currently the most widely used metallic biomaterials. In principle, they are classified as bioinert. Bioactivity can be achieved by appropriate modifications, e.g. creating an ordered tubular nanostructure[1]. When contacting the body environment, a layer similar to bone mineral hydroxyapatite is formed on the surface of the bioactive material. This layer is responsible for the differentiation and proliferation of osteoblasts[2]. The aim of this work was to study the interaction of ground and nanostructured surface of Ti6Al4V alloy with simulated body fluid (SBF) and to compare a cell metabolic activity tested on the exposed and unexposed samples in SBF.

METHODS:

Disc specimens of Ti6Al4V ELI with ground and nanostructured surface were exposed in SBF at a temperature of 37 °C for 168 hours under monitoring of electrochemical impedance spectroscopy (EIS). For cell tests, exposed samples were sterilized using a cyclotron. Subsequently, human mesenchymal stem cells were seeded on the specimens and proliferation, osteogenic differentiation and viability were studied.

RESULTS AND DISCUSSION:

Figure 1 shows surfaces of the specimens after the exposure in SBF. 1.5 % at. of Ca and 0.9 % at. of P were detected on the ground surface. In case of nanostructured surface, the content of these elements was higher, 9.7 % at. of Ca and 6.1 % at. of P. Moreover, EIS monitoring demonstrated that a faster hydroxyapatite precipitation occurs on the nanostructured surface (induction period 15 hours) compared to the ground sample (30 hours). Cellular assays have shown that the resulting Ca a P containing layer has a positive effect on cell differentiation and proliferation. However, the difference between ground and nanostructured surface was not detected. In subsequent experiment, polymerase chain reaction (PCR) will be performed, providing information about bone marrow differentiation.

CONCLUSION:

The nanostructured surface accelerates the formation of hydroxyapatite-like layer as compared to the ground surface. It was found that this layer on the sample surface positively affects cellular activity.

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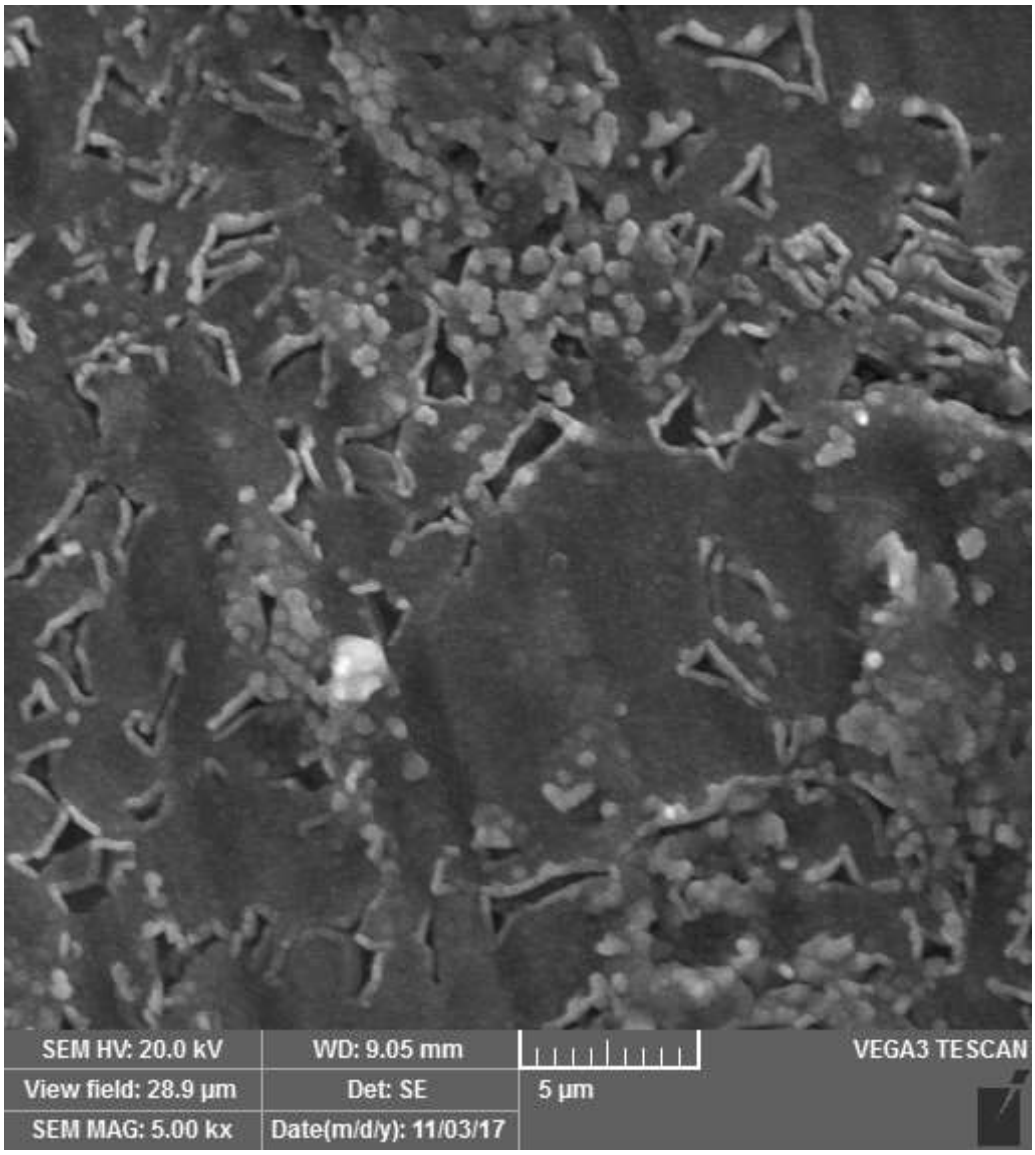
ACKNOWLEDGMENTS:

This work was realised under the financial support from the project no 16-14758S (Czech Science Foundation).

Picture 1: 

Caption 1: Figure 1: SEM image of nanostructured specimen after exposures in SBF.

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Poster presentation

146 New development of fibrin/collagen-based materials for musculo-tendinous junction reconstruction

Clément Rieu[1], Nicolas Rose[1], Bernard Haye[1], Gervaise Mosser[1], Fabien Le Grand[2], Léa Trichet[1], Thibaud Coradin[1]

[1]LCMCP, Sorbonne Université, CNRS, Paris, France

[2]Institut de Recherche en Myologie, INSERM, AIM, UMRS974, Sorbonne Université, France

INTRODUCTION:

Tendon injury is a clinical challenge due to the limited regeneration of the tissue, caused by poor vascularization and low cellularity, and to the mechanical constraints inside and at the junctions between muscle and bone [1]. Whereas

tendon repair has drawn much interest, the musculo-tendinous junction has been seldom addressed as a target. This may be explained by its complexity and the switch from a stiff (tendon >500MPa) to a soft tissue (muscle<3MPa) [2].

The idea here is to combine collagen and fibrin to prepare threads with specific characteristics for each tissue, that can be used as scaffolds for the co-culture of tendon- and muscle- related cells.

METHODS:

We developed a protocol (under patenting) to process fibrin(ogen) in the same conditions as collagen, and in presence or absence of thrombin, enabling production of single component or hybrid threads by co-extrusion or 3D printing of solutions, in the absence of thrombin. The mechanisms involved in fibrinogen self-assembly were studied by MALDI-TOF, TEM and rheology. By tuning buffer composition and extrusion rate, we obtained threads, 300 mm in length, with variable collagen/fibrin(ogen) composition that were used for the cultures of mice primary myoblasts and tenocytes, extracted from young mice lower limb skeletal muscles and Achilles tendon respectively.

RESULTS AND DISCUSSION:

Extensive studies carried out to characterize the mechanism of fibrinogen self-assembly without thrombin prove that this mechanism differs from the one activated by thrombin and can be used to extrude pure fibrinogen or to improve extrusion of fibrin.

Cultures of myoblasts and tenocytes showed distinct behaviors depending on materials composition. Myoblasts have higher proliferation and differentiation rates on fibrin and mixed fibrin/collagen threads over pure collagen threads whereas tenocytes demonstrated similar good adherence and proliferation on the different threads.

CONCLUSION:

The here-described mechanism and protocol open a wide field of possibility to design biomaterials from very popular proteins in tissue engineering, collagen and fibrin(ogen). Study of thread colonization on each side, mechanical stimulation and 3D printing are under way, allowing for a better understanding of the myo-tendinous junction and design of new scaffolds to help tendon reconstruction.

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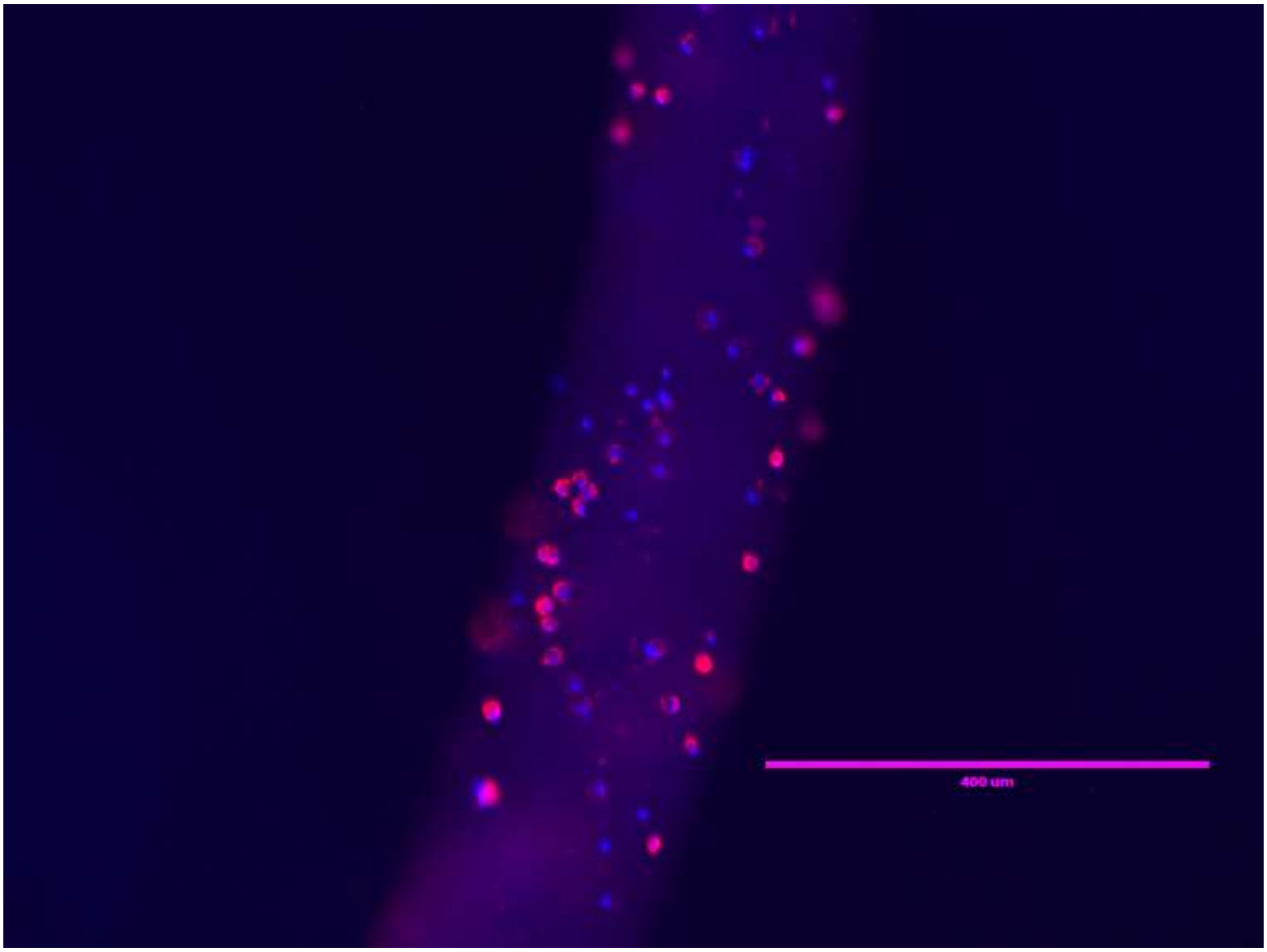

Picture 1: Caption 1: Optical fluorescence image of a fibrin thread after 7d proliferation and 2d differentiation of mice primary myoblasts (blue : Hoechst, red : MF-20)

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Poster presentation

170 Protein absorbtion into and release from glycosaminoglycan microgels for tissue engineering

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[1]Utrecht University, Utrecht, Netherlands

[2]Utrecht Universtity, Netherlands

INTRODUCTION:

Glycosaminoglycans (GAGs) represent a group of extracellular matrix components which are currently under investigation for use in hydrogel scaffolds for tissue engineering[1]. GAGs play a major role in retention of proteins including growth factors. In this study, the potential of methacrylated GAG microgels for protein absorption, retention and release was investigated. This work is of relevance for tissue engineering strategies utilizing controlled growth factor release in empty or cell-seeded scaffolds.

METHODS:

Methacrylated hyaluronic acid (HAMA) and chondroitin sulfate (CSMA) were synthesized according to established methods[2,3]. Covalently crosslinked HAMA and CSMA microgels (diameter: 500-700 μm, coefficient of variation<10%, fabricated using a microfluidic device) were used to study lysozyme (as a model cationic protein) absorption, gel (de)swelling, intra-gel protein distribution and diffusion in release buffers with varying ionic strengths of 20, 170 and 500 mM (pH 7.4).

RESULTS AND DISCUSSION:

High amounts of lysozyme were absorbed homogeneously in the microgels up to 3 mg/mg dry microspheres for HAMA and 4 mg/mg dry microspheres for CSMA with 100% loading efficiency. Lysozyme to GAG binding stoichiometries and microgel shrinking upon protein absorption indicated complex coacervation[4,5]. Lysozyme diffusion from GAG microgels was found to be dependent on buffer ionic strength and the negative charge density of the hydrogel networks. Diffusion coefficients of 0.027 in HAMA and < 0.006 $\mu m^2.sec^{-1}$ in CSMA were found in buffer of physiological ionic strength. Complete lysozyme release occurred in 10 (HAMA) or 24 days (CSMA) and the protein was found to be fully bioactive, showing the protein-friendly nature of the system. Finally, lysozyme-loaded CSMA microgels were embedded into a thermosensitve hydrogel scaffold. These composite systems showed complete lysozyme release in 58 days, as opposed to only 5 hours for GAG-free scaffolds.

CONCLUSION:

Our results show that HAMA and CSMA hydrogels can absorb high amounts of lysozyme through complex coacervation. Subsequent release of protein from GAG microgels in isotonic conditions occurs in timescales relevant for growth factor delivery in tissue engineering applications.

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ACKNOWLEDGMENTS:

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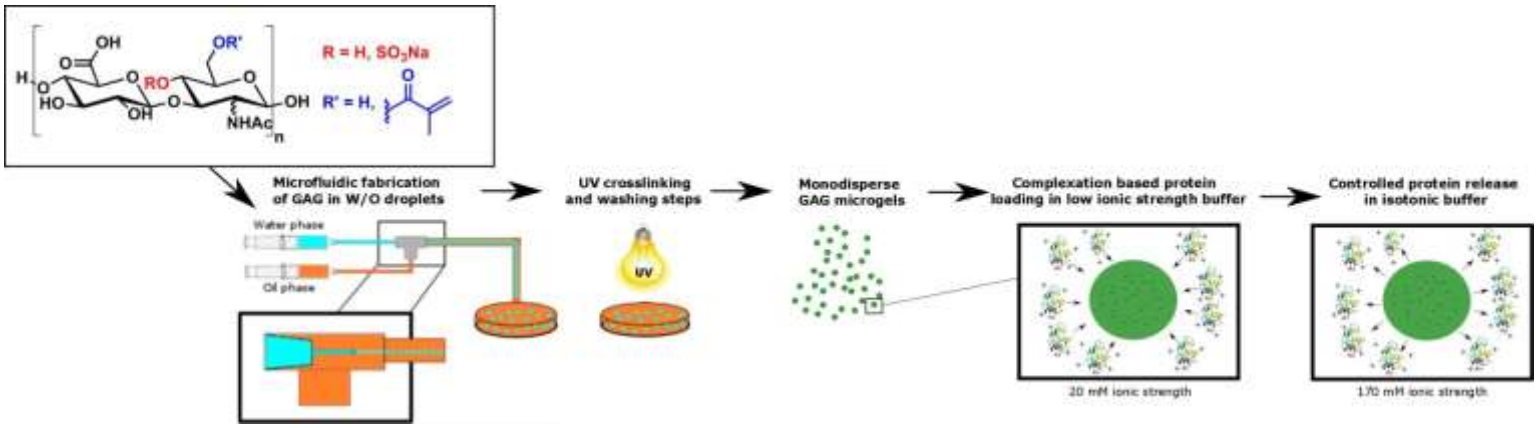
Picture 1:



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Poster presentation

172 Fabrication of Chitosan/PVA/Bioglass Trilayer Nanofibrous Membrane with Corresponding Function during Skin Wound Healing

Xiaoli Zhao[1], Qingchan Chen[1], Haobo Pan[1], William Lu[2]

[1]Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China

[2]The University of Hong Kong, Hongkong

INTRODUCTION:

The therapy of acute and non-healing wounds remains a challenging clinical problem. This is mainly due to the complexity of wound healing process and inadequate functions of wound dressings[1]. Therefore, it is highly desirable to develop innovative dressings that can function at proper stages in the healing process to accelerate wound healing[2]. In this study, a trilayer nanofibrous membrane based on chitosan, polyvinyl alcohol (PVA) and bioglass was fabricated and expected to function corresponding to the different healing stage.

METHODS:

The triple-layer nanofibrous membrane was fabricated via layer-by-layer co-electrospinning with the sub-layer of chitosan for hemostasis and antibacterial, mid-layer of PVA and chitosan for moisturization, and top-layer of nBG in PVA for promoting tissue generation. Nanobioglass (nBG) was synthesized by a sol-gel method. The morphology, physicochemical properties, antibacterial capabilities, biological activities of trilayer nanofibrous membranes were systematically evaluated. *In vivo* study was performed using a rat full-thickness skin defects model to study the promotion effect in wound healing.

RESULTS AND DISCUSSION:

Chitosan-PVA-bioglass trilayer nanofibrous membrane showed a “sandwich structure” in SEM images and fluorescence staining. Synthesized nBG exhibited spherical structure and narrow size distribution around 800 nm. The triple-layers membrane achieved excellent physicochemical properties and biological activity. The ion release mainly happened in the first two days. *In vitro* experiments on fibroblasts and endothelial cells showed the good cytocompatibility, cell adhesion and proliferation. Above 99% antibacterial effect was achieved in both *E. coli* and *S. aureus*. *In vivo* studies was performed on rat full-thickness skin defects, and the results suggested that multilayer fibrous membrane with 40% nBG exhibited a significantly better capacity in improving wound healing. Histological

assessments confirmed its effect in significant epithelialization, improved collagen formation and alignment. The component and architecture had the influences on the antibacterial effect and pH value of microenvironment, which was directly associated with the effect in wound healing.

CONCLUSION:

Chitosan-PVA-bioglass multilayer fibrous membrane could function corresponding to the different stages and accelerate wound healing. This provides a promising candidate for wound dressing application.

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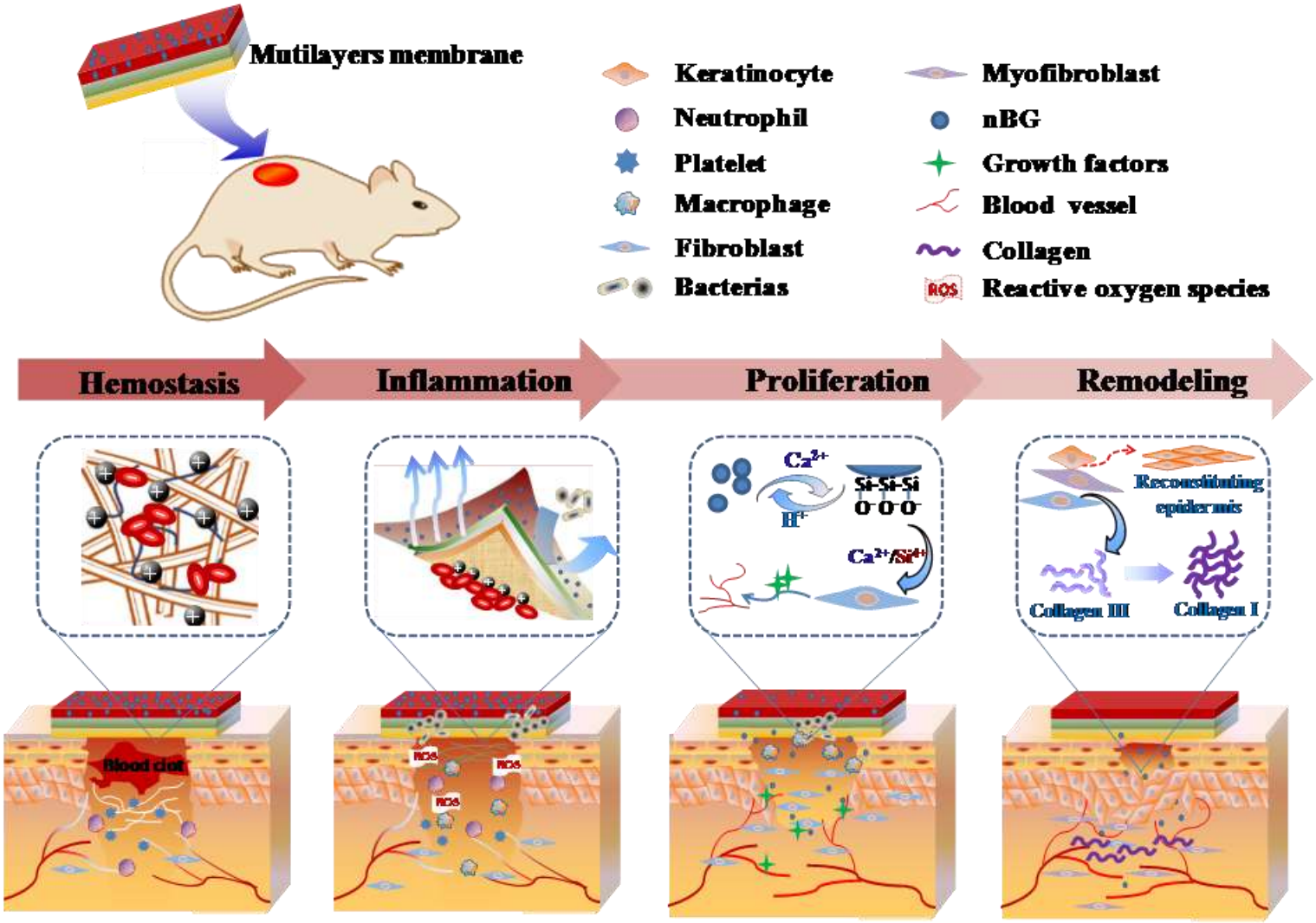
ACKNOWLEDGMENTS:

The authors would like to thank NSFC (81672226) and STRF of Shenzhen (JCYJ 20160531174634936) for providing financial support to this project.

Picture 1: Caption 1: Mechanism of trilayer nanofibrous membrane in accelerating wound healing.



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Poster presentation

175 Bioproduction of virus-like particles/RNAi nanocomplex for dual-gene silencing therapy in melanoma

Guan-Yu Chen, Hao-Han Pang, Hung-Wei Yang

National Sun Yat-sen University, Kaohsiung, Taiwan

INTRODUCTION:

Melanoma is a highly aggressive skin cancer with a potential of metastasis and responsible for the majority of skin-related deaths. [1,2] However, nucleic acid is prone to hydrolysis in physiological condition and its low permeability of the cell membrane due to the polyanionic nature, gene delivery systems are inevitably needed for gene therapy. Thus, we designed a new bioproduction procedure to rapidly obtain red fluorescent virus-like particles/RNAi (rVLPs/RNAi) nanocomplexes, which can efficiently enter cells for dual-gene silencing in melanoma.

METHODS:

DNA sequence of fluorescent protein (mCherry) and Qβ coat protein were cloned into pCDF-Duet-1 vector to form pCDF-Duet-mCherry-QβCP. The DNA sequences encoding $T7_p$-RNAi scaffold-$T7_t$ ($RNAi_{Akt1}$) were cloned into pET28-b(+) to generate QβCP binding RNAi scaffold expression vectors. Vectors were transformed to *E. coli* cell line BL21 competent cells for protein co-expression to generate $rVLPs/RNAi_{Akt1}$. [3] Then the $rVLPs/RNAi_{Akt1}$ surface were modified with branched polyethylenimine (bPEI) for Cys-TAT peptide immobilization and pSUPER-gfp-siCD147 adsorption to form $TAT@pSUPER@rVLPs/RNAi_{Akt1}$ (Figure 1A) to investigate cell uptake efficiency, gene silencing efficiency, cytotoxicity, et al.

RESULTS AND DISCUSSION:

In this study, we have successfully produced $TAT@pSUPER@rVLPs/RNAi_{Akt1}$ for gene therapy. By TEM measurement, the $rVLPs/RNAi_{Akt1}$ shown spherical morphology with a diameter of 33.5 ± 0.4 nm (Figure 1b). Then the pSUPER-gfp-siCD147 can be completely adsorbed on the bPEI-modified $rVLPs/RNAi_{Akt1}$ (lanes 7 and 8, Figure 1c) compared with non-modified $rVLPs/RNAi_{Akt1}$ (lanes 3-6, Figure 1c). Finally, we incubated the A375 cells with $TAT@pSUPER@rVLPs/RNAi_{Akt1}$, the results showed that the carried pSUPER-gfp-siCD147 could be transfected to cells expressing GFP and $siRNA_{CD147}$ (Figure 1d), and the Akt1 and CD147 were successfully knocked down to inhibit the cell growth.

CONCLUSION:

The current results showed that we are able to bio-produce $VLPs/RNAi_{Akt1}$ nanocomplexes and also carry pSUPER-gfp-siCD147 to express $siRNA_{CD147}$ for dual-gene silencing therapy in melanoma A375 cells.

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Picture 1: National Sun Yat-sen University

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Poster presentation

179 surface functionalization of titanium dioxide using bisphosphonates for biomedical applications

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INTRODUCTION:

Titanium it has been considered biocompatible, presenting good interaction with proteins present in human body, such as albumin and fibronectin[1]. However, there are still open problems associated to this allegedly biocompatibility. One strategy to enhance its physicochemical-surface properties is the deposition of a protective titanium dioxide[2] layer and then the use of biofunctionalization approaches with bisphosphonates.

The TiO_2 surface is terminated by OH groups, which may form covalent bonds with molecules, such as carboxylic acids, carboxylate salts and others, forming self-assembled monolayers[3]. Bisphosphonates are an interesting group for functionalization due its good clinical performance, increasing the osteoblast activity and cause osteoclast apoptosis[4].

In this study, TiO_2 were grown on commercial titanium surfaces (TiCp-4, Acnis) and subsequently biofunctionalized using two different Bisphosphonates-molecules: alendronate and risedronate. Surface physicochemical properties were analyzed and Cell-viability testes were also performed.

METHODS:

Titanium dioxide solutions was prepared by a sol-gel method. A thin film of 600 nm of thickness was deposited onto titanium by the spin-coating technique. The film was hydroxylated using UV-C light during fourth-five minutes. Functionalization occurred with a solution of 1 mg/mL of alendronate and risedronate. Each group was analyzed using x-ray photoelectron spectroscopy and contact angle. Further characterization using a quartz crystal microbalance is under progress.

Cell viability was performance using MC3T3-E1 cells, the time of incubation was 24 and 48 hours. The results were analyzed via MTT reduction.

RESULTS AND DISCUSSION:

The results indicated that, both alendronate and risedronate, forms covalent bindings via phosphate groups, in possible mono or bi-dentate configurations. Surface functionalized with alendronate showed to be more hydrophilic than with risedronate, however, values of surface energy for the surfaces at the same level. The cellular viability of TiO_2 similar than functionalized surface, this result indicates a super concentration of bisphosphonates.

CONCLUSION:

The molecules forms Ti-O-P binds on surface. The wettability and surface energy are determined by free groups of molecules, amine and pyridine ring. Cellular viability of TiO_2 and functionalized surface are close, however there are

studies relating high concentration of bisphosphonates and negative effects on hard tissue[4], so studies testing lower concentration must be realized.

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ACKNOWLEDGMENTS:

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Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

191 Human amniotic membrane mechanical properties cartographies

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INTRODUCTION:

The human amniotic membrane (HAM) is widely used for tissue regeneration/engineering research due to its low immunogenicity and proven clinical safety[1]. As mechanical properties are very important for some applications (vascular or bone regeneration for example), it is surprising to see that a detailed study of its mechanical properties distributions is not available. Thus, our objective was to perform a detailed cartography of the mechanical properties of the HAM.

METHODS:

Fetal membranes were collected from patients who had a cesarean delivery (n=2, on-going). Membranes were cut radially in 8 “petals”. Amnions were dissected from chorions. HAMs were sampled using a standardized pattern (fig.1). Thickness was measured using a laser micrometer. Tensile tests were performed at 1% length/s. Force, stress and Young’s modulus were recorded. Data are presented as mean ± SD. T-test or one way ANOVA were performed and were considered statistically significant when $p<0.05$.

RESULTS AND DISCUSSION:

Histology demonstrated that HAM is not a uniform tissue with a thin compact connective tissue layer and a thick loose tissue layer, which could be partially removed by scraping. Thickness of scraped HAM samples varied from 22 to 143 µm (n=4) but after dehydration, samples thickness varied only from 4 to 5 µm (n=4). This suggests that the loose connective layer greatly influences HAM thickness without contributing significant material. Consequently, while maximal stress and Young’s modulus are common values used to describe mechanical properties, these thickness-dependant values do not convey the actual strength of the material. So, maximal force before rupture (Fmax) was chosen to investigate HAM strength distribution. Placental (pl) was significantly stronger than peripheral (pe) HAM for both patients ($_{1pl}$=1.0 ± 0.3 N *vs.* $_{1pe}$=0.7 ± 0.2 N, p_1=0.00005, n_1=26-42; $_{2pl}$=1.1 ± 0.4 N *vs.* $_{2pe}$=0.6 ± 0.3 N, p_2=0.000002, n_2=28-71). There was important intra-tissue variability but there was no clear “weak zone” as

described in some studies[2]. Overall strength between patients was not significantly different ($_1$=0.9 ± 0.3 N *vs.* $_2$=0.8 ± 0.4 N, n=69-99). Additional HAMs are being tested to confirm these results.

CONCLUSION:

Since the amnion is not a uniform tissue, Fmax is a better criterion to investigate HAM mechanical properties. Cartographies showed that placental is stronger than peripheral HAM. When completed, this data set will help tissue engineers to develop a smart HAM sourcing strategy for products where mechanical properties are critical.

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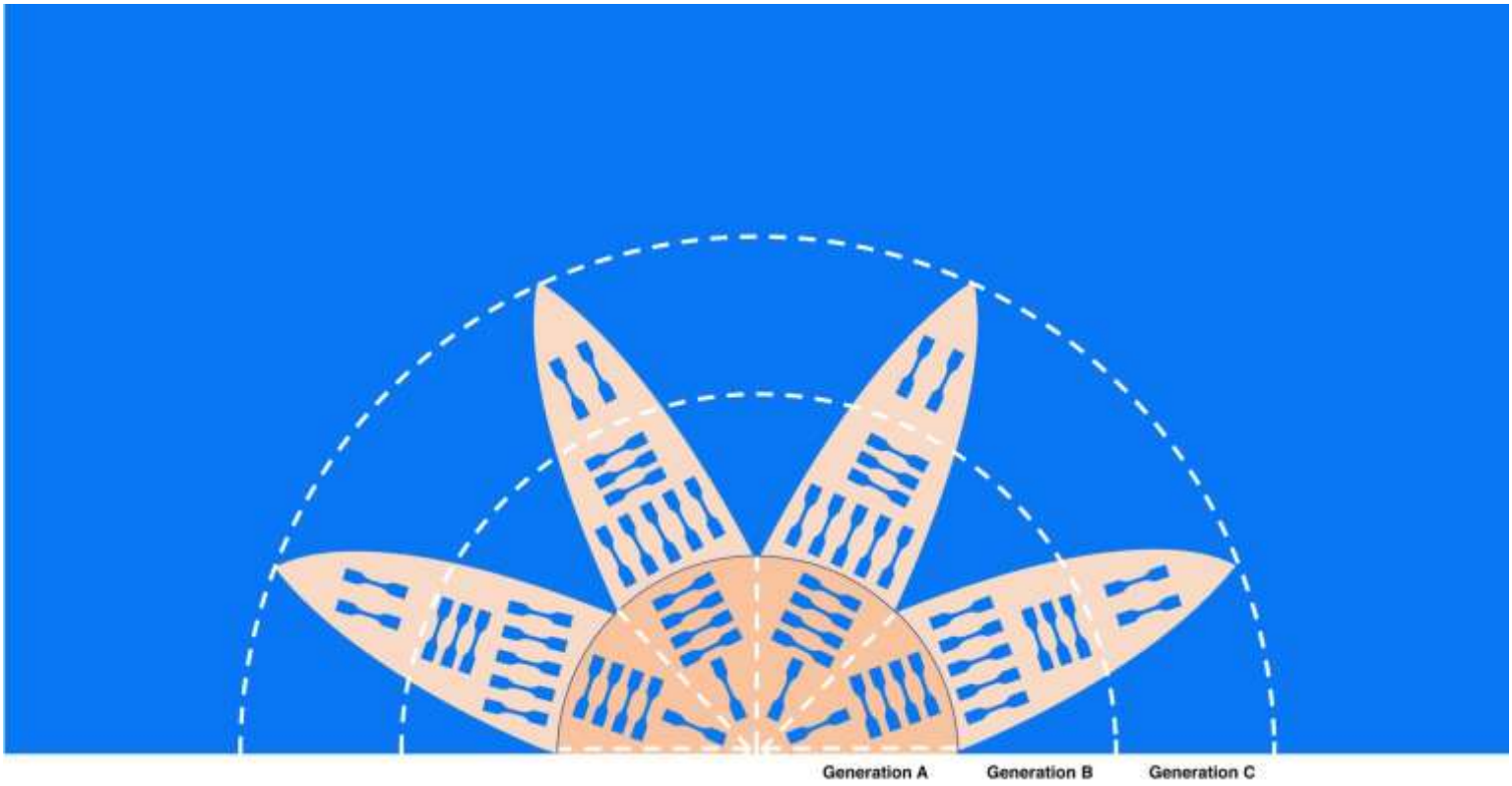
Picture 1:



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Poster presentation

192 Mussel-inspired interpenetrated scaffolds for wound healing regeneration

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INTRODUCTION:

Mussel-inspired tissue adhesive materials, characterized by the presence of catechol groups, are considered as a potential candidate for ideal wound dressings because of their excellent biocompatibility, biodegradability and their muco-adhesiveness, which allows adhering to body tissues in moist environments leading to a higher wound closure and tissue regeneration than the traditional invasive methods used for surgical closures, such as suturing or stapling[1,2,3]. The aim of this project is the preparation of a resorbable and biocompatible hydrogel system based on chitosan(Ch)/oxidized hyaluronic acid(HAox)/synthetic catechol conjugate(CMA), for the fabrication of a bioactive adhesive material applied for wound dressings.

METHODS:

The structural characterization and morphology of the interpenetrated network are studied by FTIR, UV and SEM, and the water absorption and biodegradability are evaluated in PBS. Fibroblasts and mesenchymal stem cells (MSC) proliferation of the processed membranes are analyzed by Alamar Blue test, Picogreen assay and confocal microscopy. Antioxidant and anti-inflammatory activities are evaluated in vitro with standardized protocols. Bioadhesion assays of the hydrogels are measured using porcine skin. Finally, experiments with rats are performed to assess in vivo biocompatibility.

RESULTS AND DISCUSSION:

The interpenetrated network is obtained from chitosan covalently crosslinked with the oxidized hyaluronic acid, and the ionically crosslinked catechol conjugate using Fe^{3+} ions, leading to a stable hydrogel system. The processed membranes swell and are stable until at least 90 days. Direct seeding of fibroblasts and MSC within the films reveals high biocompatibility and good colonization and proliferation. Additionally, hydrogels present good antioxidant and anti-inflammatory activities coming from the catechol moieties. Bioadhesion assays of the systems show maximum tensile strength values around 400 KPa, showing a great adhesion to the skin tissue. Lastly, satisfactory in vivo biocompatibility of hydrogel films is observed using a subcutaneous model in rats.

CONCLUSION:

Thus, these novel mussel-inspired interpenetrating hydrogels have an enormous potential of application as bioadhesive scaffolds for activate wound healing regeneration.

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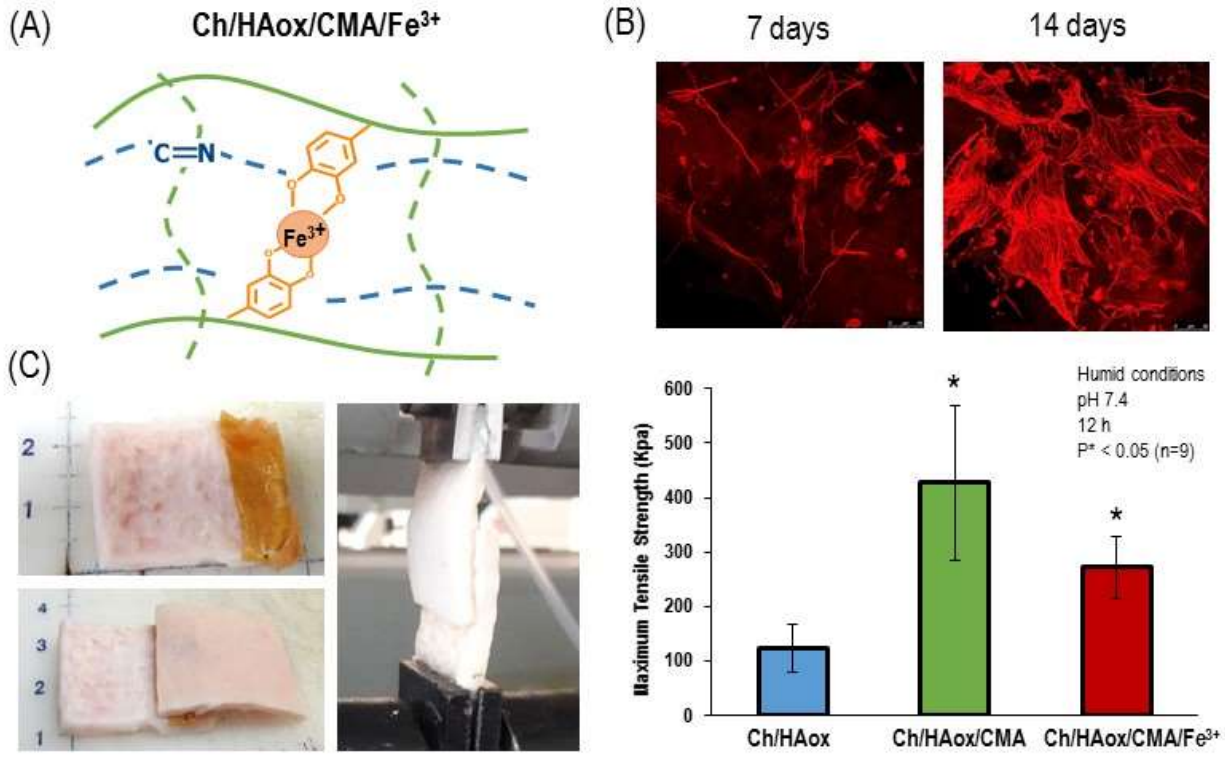

Picture 1: Caption 1: (A) Scheme of the Interpenetrated network. (B) MSC within the hydrogel stained with Rhodamine. (C) Bioadhesion test samples and results using UTM.

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Poster presentation

202 Development of a new method for decellularization and preservation of human amniotic membrane for bone regeneration: cytocompatibility and biocompatibility assessment

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INTRODUCTION:

Due to its biological properties and its low immunogenicity, human amniotic membrane (hAM) is widely studied in the field of tissue engineering. To reduce the risk of disease transmission related to the use of fresh hAM, several conditioning methods have been developed. To assess its potential for bone repair, a wide heterogeneity of preservation methods of hAM has been studied. However, there is no study comparing the biocompatibility of these membranes according to the preservation protocol.

The objectives of this study were: i) to develop a simple and reproducible method for decellularization of hAM ; ii) to compare its cytocompatibility and biocompatibility with conventional preservation methods.

METHODS:

Placenta were recovered after elective cesarean surgery and the amniotic membrane was peeled from the chorion (n=6). Four treatments were realized: hAM was used either fresh (F-hAM), cryopreserved (C-hAM), lyophilized (L-hAM) or decellularized and lyophilized (D-hAM). Histological analyzis was performed for morphological caracterization (HES staining). Decellularization was also assessed using DAPI staining and DNA extraction and quantification. *In vitro*, the effects of the four treatments on human bone marrow mesenchymal stem cells (hBMSCs) viability and activity were evaluated. Their biocompatibility was evaluated *in vivo* in a rat subcutaneous model. Histological analysis was performed on subcutaneous hAM implants one week and one month after the surgery (n=20).

RESULTS AND DISCUSSION:

Histological analysis showed that F-hAM and C-hAM had a similary morphology whereas L-hAM and D-hAM were thinner. Cells removal from D-hAM was observed with HES, DAPI staining, and this result was confirmed by DNA extraction (< 50ng/mg). *In vitro*, the decellularization process did not confer any cytotoxicity of the tissue compared to other preservation methods. *In vivo*, L-hAM and D-hAM were easier to handle compared to F-hAM and C-hAM. Histological analysis of explanted samples from the rat indicated that the acute inflammatory reaction was higher around F-hAM and C-hAM implants compared to L-hAM and D-hAM. One month after surgery, a complete resorption of F-hAM and C-hAM implants occured, whereas no resorption was observed for both L-hAM and D-hAM samples. A moderate inflammatory reaction was still observed around these two implants.

CONCLUSION:

Several decellularization methods of hAM have been reported. They usually are time-consuming or expensive. Here we developed a simple and reproducible method for effective decellularization of biocompatible hAM, without cytotoxic effects. This decellularization of hAM should be suitable for the field of bone regeneration.

Decellularized hAM

Non decellularized hAM

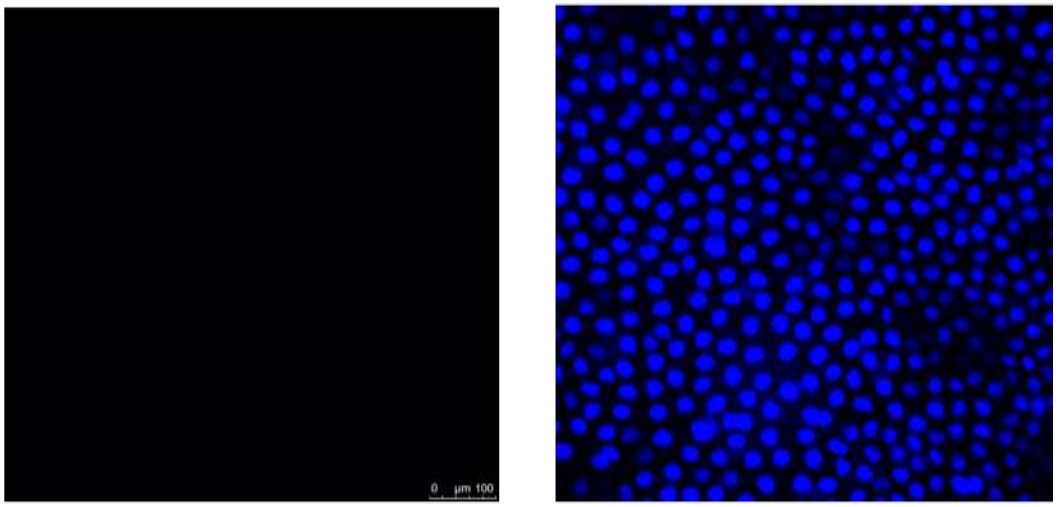

Fig 1. DAPI staining

Picture 1:

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Poster presentation

205 Microfluidic platform for combinatorial studies of flow, surface patterning and drug concentrations as a toolbox for understanding cell responses to biomaterials surfaces

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INTRODUCTION:

Recreating cellular microenvironments in vitro is a promising approach to studying the effects of chemical, physical and structural properties of biomaterials on cell behaviour. For example, micro- and nano-topographical physical cues have been shown determinant in controlling the differentiation of stem cells (1, 2).

In this work, we propose a microfluidic device that integrates biomaterials that are micro- or nanostructured to allow adherent cells culture. Furthermore, the cells inside the devices can be exposed to flow and/or to soluble factors in varying concentrations. Investigating the contribution of different cues in such systems is justified as improved knowledge of cell-material interactions at the microscale is expected to enhance and accelerate the developments in the field of regenerative medicine, in particular in the context of personalised medicine

METHODS:

Microfluidic devices housing poly lactic acid (PLA) surfaces patterned with parallel-aligned microgrooves with 100 µm width/spacing and 600 nm height were successfully produced to allow cell culture. MG-63 cells were cultured on

these surfaces and shape descriptors such as orientation and eccentricity were determined, in static condition or under flow.

RESULTS AND DISCUSSION:

The cells adhered and grew on the patterned surfaces, indicated by actin and nuclei staining (Figure 1).

In static conditions (no flow) cells elongated in the direction of the grooves. When the flow 8 µl/h was introduced in the direction perpendicular to the direction of the grooves, the elongation of cells in the microgroove direction remained. Nevertheless, when flow rate was increased to 16 µl/h, the extent of orientation in the microgroove direction decreased.

Figure 1 – Stained MG-63 cells (nuclei, actin) on 100 µm wide grooves on PLA substrate in microfluidic culture (after 24h) under static culture (A) and under perfusion regimes of 8 µl/h and 16 µl/h (B). Yellow arrow shows the perfusion direction, perpendicular to the grooves. Orientation histograms for static, 8 µl/h and 16 µl/h culture.

CONCLUSION:

The results showed that the platform microfluidic platform is useful for combinatorial screening of surface-bound topographical cues and flow/shear forces on adherent cells behaviour. Further studies will focus on screening of other topographies, in combination with flow and/or concentration gradients of cytoskeletal drugs (preventing/enhancing polymerization).

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ACKNOWLEDGMENTS:

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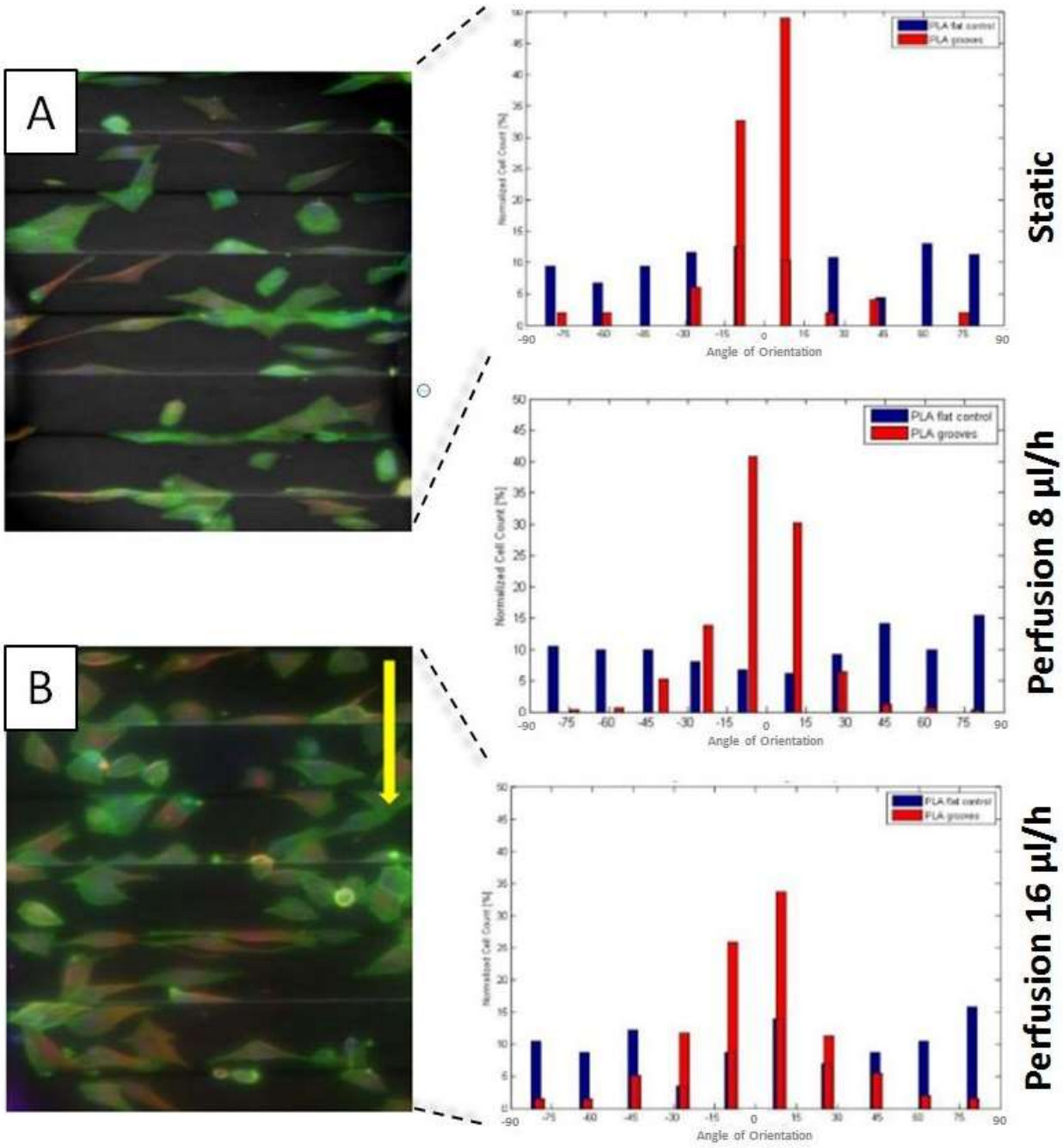
Picture 1:



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Poster presentation

207 Mechanism of the antibacterial effect of metal oxide nanosheets

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INTRODUCTION:

Metal oxide nanosheet has a highly anisotropic structure, which is very thin and wide. Consequently, nanosheets are able to intercalate other reagents/biomolecules between the sheets.[1,2] This has application in several fields including clinical engineering.[1,2] Some researchers use nanosheets as host materials for antibiotics.[2] However, it was noticed that nanosheets themselves inherently provided an antibacterial effect. Here we present the results and explain the mechanism of this efficacy.

METHODS:

Titanate nanosheets (TNS) were synthesized using a hydrolysis reaction with titanium tetraisopropoxide and tetramethylammonium hydroxide. The particle size of the resulting TNS was measured by dynamic light scattering (DLS). Microbial culture was performed for 24 h at 37^{o}C in darkness, and the antibacterial effect of the TNS solutions was evaluated by a colony forming unit (CFU) counting method. 0.9 % sodium chloride solution was used as a control.

RESULTS AND DISCUSSION:

TNS particle size was under 10 nm and its solution was transparent. The results of the antibacterial effect are shown in Fig. 1. The CFUs of the TNS samples suppressed proliferation of *Staphylococcus aureus* and *Staphylococcus epidermidis* compared to the control sample and the effect was related to the concentration of titanium. However, TNS was ineffective against *Enterobacter cloacae* and *Escherichia coli*. When the bacterial test was performed with different particle sizes of TNS, the effect clearly decreased with increasing TNS size. These results imply that the antibacterial effect of TNS was affected by the difference of cell wall structure clearly differentiating between Gram-positive and Gram-negative bacteria, and induced by the penetration of peptidoglycan cell wall through its small voids.

CONCLUSION:

The antibacterial/bactericidal effects of TNS without photocatalysis were examined. The effects increased with the concentration of titanium in TNS. However, the nanosheets were only effective against Gram-positive bacteria and this effect decreased with increasing TNS particle size. It is envisaged that only very small nanosheets could penetrate the net-like peptidoglycan structure, which induces the bacteria destruction. Furthermore, as the TNS colloidal solution is transparent, this material has potential as disinfectant and antibacterial coating for medical devices.

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ACKNOWLEDGMENTS:

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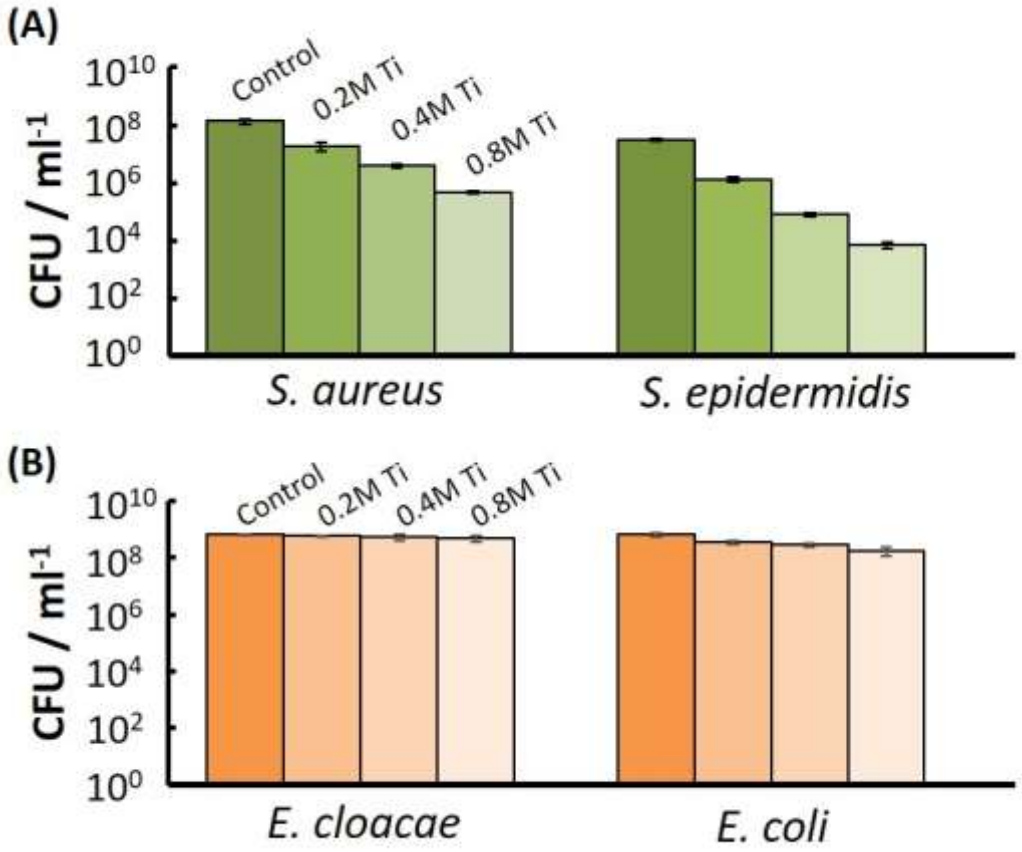

Picture 1:
Caption 1: Fig. 1 CFU results of TNS with different concentrations of titanium against (A) Gram-positive bacteria and (B) Gram-negative bacteria

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216 Engineering a microvascular 3D microenvironment of a colorectal tumor-on-chip

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INTRODUCTION:

Cancer has become a universal health problem, with colorectal cancer (CRC) in the top three leading causes of cancer-related deaths, mainly due to metastases [1]. 3D models integrating tissue engineering (TE) strategies with microfluidic technology have sparked the expectation on physiologically relevant *in vitro* models.

In this study, we aimed at establishing a microfluidic 3D *in vitro* model that emulates the human colorectal tumor microenvironment, enabling the reconstitution of the related microvascular tissue. By mimicking the native cancer microenvironment, this model also allows the assessment of the efficiency of targeted anti-cancer drug-loaded nanoparticles.

METHODS:

The microfluidic chip contains three compartments: a central chamber (for ECM-like hydrogel) and a pair of lateral perfused channels. Human colon cancer cells (HCT-116, ATCC) and human colonic microvascular endothelial cells (hCoMECs, Innoprot) were cultured according to suppliers' specifications. Carboxymethyl-chitosan/poly(amidoamine) (CMCht/PAMAM) dendrimer nanoparticles were synthetized [2] and labeled with Fluorescein-5(6)-isothiocyanate (FITC). Gemcitabine release profile from nanoparticles was performed over 5 days and determined by UV spectrophotometer (270nm). Permeability of the hydrogels for the drug-loaded dendrimer nanoparticles within the microfluidic platform was assessed statically (channels perfused with a pipette tip) and dynamically (channels perfused with continuous flow) by fluorescence imaging.

RESULTS AND DISCUSSION:

We achieved a stable gradient profile over time, more prominent in the dynamic setting (Figure 1A). As one of the main goals of this work was to assess the delivery efficiency of gemcitabine loaded nanoparticles, the drug calibration curve and release profile studies were performed (Figure 1B) with an average of 2 μg of drug being released per each mg of dendrimer nanoparticles after 5 days. A pre-vascularization process within the microfluidic chip was successfully achieved by co-culturing hCoMECs (yellow), and HCT-116 cancer cells (red) for 5 days (Figure 1C).

CONCLUSION:

The colorectal tumor-on-a-chip model described herein represents a promising tool to better understand cell migration and overall tumorigenesis. We were able to fabricate a new microfluidic device and achieve a stable co-culture comprising early sprouting of hCoMECS towards the hydrogel. Drug delivery gradients can be tightly controlled in this novel on-chip model, highly relevant for assessing the influence of chemoattractants and drugs.

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ACKNOWLEDGMENTS:

This project was possible with the support of the Dutch Province of Limburg. Mariana Carvalho acknowledges her PhD scholarship NORTE-08-5369-FSE-000044, by Programa Operacional Regional do Norte, Norte2020-TERM&SC, FROnTHERA NORTE-01-0145-FEDER-0000232 and FCT R&D Project IF/01285/2015.

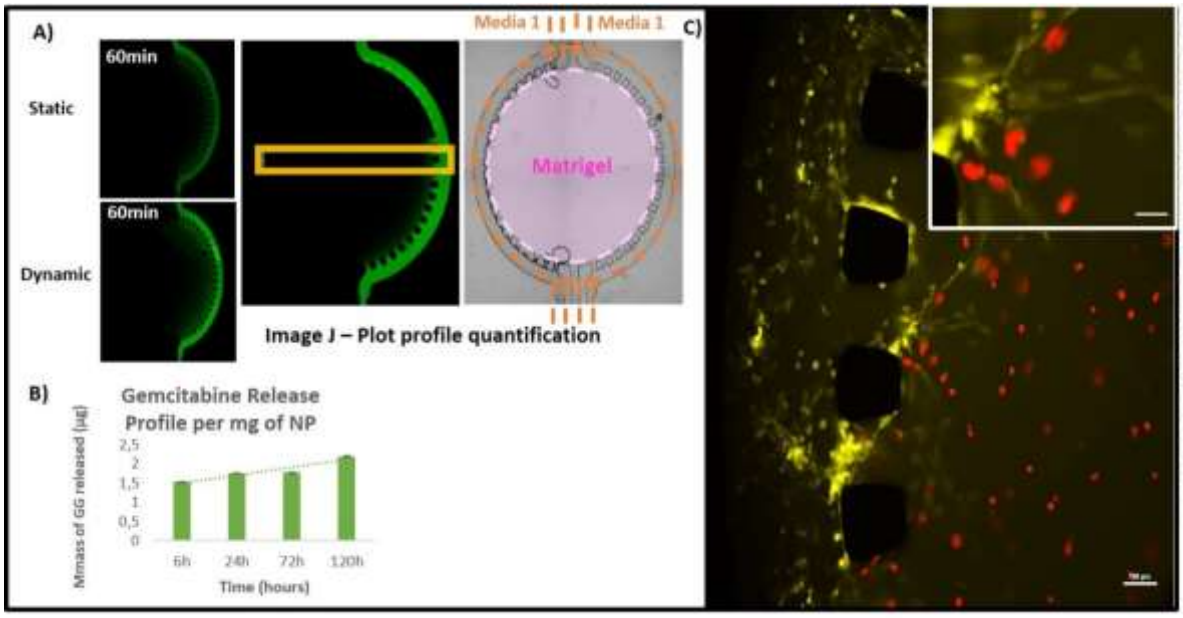

Figure 1 – A) Gradient of FITC-CMCht/PAMAM nanoparticles in static and dynamic conditions, and quantification method by ImageJ; B) Gemcitabine release profile from nanoparticles over 5 days in PBS buffer. The results are expressed as an average ± standard deviation, n=3; C) Fluorescence microscopy image of hCoMECs (yellow) sprouting towards the central chamber with HCT-116 cancer cells (red) embedded in Matrigel.

Picture 1: Caption 1: Figure 1 -A) Gradient of FITC labeled NP in static and dynamic. B) Gemcitabine release profile. C) Early sprouting of hCoMECs

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Poster presentation

220 Physical sponge based on chitosan and poly-β-cyclodextrin as an antibacterial drug release device

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INTRODUCTION:

Deep bone infections are difficult to treat. Among these, we focus on diabetic foot infections and joint infections due to public health impact. Drug delivery in the bone and the surroundings sclerotic tissues is difficult due to a low blood flow in the infected site. Therefore, traditional treatments are not always successful leading to amputations. We worked on a local drug release device to use in addition to the systemic route. We developed a lyophilized hydrogel, *i.e.* chitosan (CS) sponge and a cyclodextrin polymer (PCD) loaded with antibacterial molecules to raise local drug concentration without potential side effects outside the infectious site.

METHODS:

CS is cationic polymer while PCD obtained by a cross linking reaction between CD and citric acid is anionic one [1,2]. The two powders in CS:PCD ratios of 3:0 (control), 3:1, 3:3, 3:5 and 3:7 (w/v%) were mixed by co-milling,

suspended in distilled water and acidified with acetic acid to obtain a physical hydrogel characterized by rheology. Sponges were obtained by freeze-drying followed by a curing step to stabilize the system. Their microstructure and swelling rate were characterized by SEM and weight gain, respectively. Cytocompatibility was evaluated by the AlamarBlue® assay. Finally, sponges were impregnated in a ciprofloxacin solution to evaluate the drug release profile and the antibacterial activity.

RESULTS AND DISCUSSION:

Rheology indicated that ratios 3:1 and 3:3 were the only reproducible elastics hydrogels. SEM showed high porous structure without effect of the curing treatment on sponge's morphology. Nevertheless, curing step improved sponge's stability, swelling rate and drug sorption in aqueous medium. Highest drug sorption is related to the highest swelling rate (Figure 1). Two kinetic profiles were observed, one sustained without an initial burst for the 3:0 cured sponge and one fast with an initial burst for the other ratios. A prolonged antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was obtained up to 48 hours. High cytocompatibility over 70% of cell survival was observed for all types of sponges.

CONCLUSION:

Cured sponge 3:3 ratio gave suitable results for all 3 established conditions, *i.e.* cytocompatibility, antibacterial activity and stability for at least 5 days. Hence this ratio seems to be the most promising as a drug delivery system for the treatment of deep bone infections.

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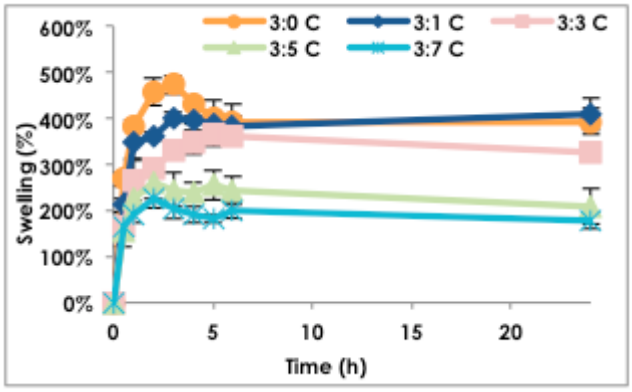
Picture 1:



Caption 1: Figure 1 : Swelling rate as function of time for the cured sponges

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248 Impact of composite substrates' modulus of elasticity on cell behavior

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INTRODUCTION:

Stiffness is a measure of the ability of a material to resist deformation. The influence of the substrate stiffness on human bone cells morphology, motility and differentiation has been known for decades and recently re-emphasized, as an essential *in vivo* control parameter in cells biochemical signaling[1,2]. In the body, tissue stiffness ranges several orders of magnitude, from adipose tissue (Young's Modulus E ~ several kPa) to bone (E ~ GPa). To evaluate the importance of substrate elasticity in biomaterial design, it is critical to test a wide variety of substrata that span physiologically relevant ranges of elasticity[3]. However, for valid results, the substrate's chemical profile and topography should be the same. In this context, traditional polymeric materials and composites have been manufactured and the stiffness, of the order of GPa, influence on cells behavior has been investigated.

METHODS:

Pellets of Poly-caprolactone (PCL) was reinforced with Carbon Nanotubes (CNTs). Thus, solvent casted composites with enhanced mechanical properties were manufactured. Titanium substrates were also used. The mean Young's modulus of elasticity of the substrates were determined.

Human mesenchymal stem cells were obtained from umbilical cord (hMSCs) and were cultured on the substrates. Cayman detection kits were used to determine Total Protein levels, as a measure of cell proliferation. Cell spreading was detected by staining with DAPI and Phalloidin.

RESULTS AND DISCUSSION:

The substrates were divided into two groups according to the magnitude of elasticity. The chemical profile of the substrates was kept constant by coating their surface with appropriate proteins. The modulus of elasticity of the substrates was increased up to three times due to the presence of CNTs in the PCL matrix. MSCs cultured on the substrates appeared to have similar Total Protein levels, which indicates same cell proliferation rates among the examined substrates. On the other hand, different cell spreading areas were noticed among the substrates with various Modulus of Elasticity.

CONCLUSION:

The CNTs are a remarkably good reinforcement for the PCL matrix. The cells' behavior was noticeably affected by substrate' s elasticity.

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ACKNOWLEDGMENTS:

Special thanks are due to Dr. S. Michalopoulos of the Biomedical Research foundation (Academy of Athens) for providing MSCs.

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

249 Influence of substrate topography on osteoblasts' behavior Comparing Solvent Cast and Electrospun Composite Scaffolds

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INTRODUCTION:

The topography of a scaffold appears to have a great impact on cell organization, proliferation, migration and differentiation process[1]. Electrospinng technique is widely used fabrication method for scaffolds with different topographic textures. Electrospun scaffolds can mimic the hierarchical organized fibrous structure found in the Extracellular Matrix and the fibrous porosity of the scaffolds facilitates nutrient and waste exchange[2]. Poly-caprolactone (PCL) can enhance osteoblasts' growth, differentiation and adherence with small manufacturing cost and easy manipulation. In addition, carbon nanostructures (CNTs) can be used for the improvement of the mechanical properties and the cells functions[3].

In this study, four different composite materials were manufactured, PCL film, PCL film reinforced with CNTs, PCL Electrospun and PCL Electrospun reinforced with CNTs, in order to investigate the impact of surface topography on cell behavior.

METHODS:

PCL pellets and CNTs were dissolved in glacial Acetic Acid and the solution was casted on glass petri dish. A certain operation window, referring to the distance between the syringe and the collector, the applied electric field strength, and the solution flow rate, was employed for the manufacture of electrospun scaffolds. The mean Young's modulus of elasticity was determined. SEM analysis was employed for the investigation of the substrate topography.

Human mesenchymal stem cells were obtained from umbilical cord (hMSCs) and were cultured on the substrates. Cayman detection kits were used to determine Total Protein levels, as a measure of cell proliferation. Cell spreading was detected by staining with DAPI and Phalloidin.

RESULTS AND DISCUSSION:

Randomly distributed fibers of the electrospun scaffolds were clearly shown at SEM micrographs (fig. 1). The Modulus of Elasticity of the solvent cast composites was significantly increased after the addition of CNTs, whereas in the case of electrospun composites, the CNTs had not a remarkable effect on the mechanical properties. As for the cell behavior, the total protein levels increased due to the electrospun fibers and the presence of CNTS.

CONCLUSION:

The mechanical properties of PCL were improved due to the CNTs reinforcement. Differences in topography of the substrates has a significant effect on cell behavior.

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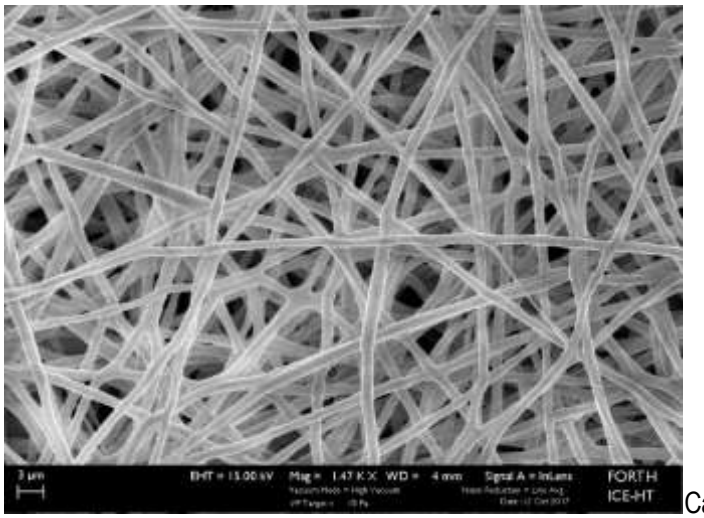
ACKNOWLEDGMENTS:

Special thanks are due to Dr. S. Michalopoulos of the Biomedical Research foundation (Academy of Athens) for providing MSCs.

Picture 1: 

Caption 1: Figure 1- SEM micrograph of PCL Electrospun substrate

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Poster presentation

250 A market surveillance study on dermal fillers

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INTRODUCTION:

Dermal fillers, or just fillers, are products that are injected into or under the skin for medical or cosmetic purposes. This could be to restore the natural contours of the body after an operation for example, but also to mask the visible effects of ageing. The National Institute for Public Health and the Environment (RIVM) has compiled an overview of 26 so-called non-permanent fillers that were marketed in the Netherlands in 2014, and has analysed these products in a laboratory. The technical files of the 14 manufacturers of these products were also investigated. Following a request through professional associations, 67 treating professionals completed a questionnaire about the fillers that they use and about their potential side effects.

METHODS:

75 samples were chemically analyzed by LC-MS, NMR spectroscopy and SEM-EDX. The same samples were evaluated for cytotoxicity and membrane integrity on RAW264.7 macrophages and L929 fibroblasts. The 14 technical files were assessed based on scoring using a preset assessment form.

RESULTS AND DISCUSSION:

All 26 products from 14 manufacturers proved to be harmless. In order to establish this, an internationally recognised laboratory test that measures harmful effects on cells was carried out. The composition of the products conforms

with the description in the technical files. According to the treating professionals, the products from the 14 manufacturers cause very few side effects.

CONCLUSION:

The quality of key sections in the technical files of the 14 manufacturers varied. It is important that manufacturers ensure their technical files are kept in good order. By keeping complete and correct files, manufacturers underpin the safety of the product for the patient, although a limitation in the files does not lead directly to a substandard product. Two sets of files were incomplete, meaning that the safety of the product for the patient is not well substantiated. Most of the inadequacies in the files were of an administrative nature, and are not expected to have any influence on the safety of the product for the patient.

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Poster presentation

252 Injectable hydrogel-mesoporous bioactive glass systems to deliver in situ therapeutic species for bone application

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INTRODUCTION:

Mesoporous bioactive glasses (MBGs) have been extensively studied for bone applications[1].

Their high surface area and tunable pore size allow the incorporation of therapeutic ions/drugs to impart specific biological functions. The incorporation of these carriers in a thermosensitive hydrogel, acting as vehicle phase, represents a strategy to release these therapeutic species in the pathological sites.

The aim of this work is to design a hybrid platform based on MBGs containing strontium (inhibition of the osteoclast differentiation potential[2]) and a Poloxamer 407-based poly(ether urethane) (PEU)[3]. In order to enhance the pro-osteogenic response, MBGs were also loaded with N-acetylcysteine (NAC).

METHODS:

A water-based spray-drying procedure[4] and a base-catalyzed sol-gel method[5] were adopted to produce Sr-MBG particles. The Poloxamer 407-based PEU (named CHP407) was synthesized via a two-step procedure in nitrogen atmosphere[3]. Sr-MBGs were incorporated with a final concentration of 20 mg/mL into the thermosensitive CHP407. Morphological and structural characterizations have been performed using FE-SEM and N_2 adsorption-desorption analysis. The hybrid systems were characterized in terms of sol-to-gel transition temperature and time, injectability and stability in aqueous environment at 37 °C.

Sr-MBGs and CHP407-based hydrogels loaded with Sr-MBGs were compared in terms of ion release kinetics in Tris-HCl, biocompatibility (MTT assay) and pro-osteogenic response (qRT-PCR test).

RESULTS AND DISCUSSION:

Spherical-shaped particles were characterized by different size (0.5-5 μm for spray-dryer samples and around 100 nm for sol-gel particles). The incorporated Sr^{2+} was released in a sustained way throughout 14 days from both MBG particles and from the hybrid systems. The MTT assay and qRT-PCR revealed an increase in cell viability and a pro-osteogenic effect of Sr-MBGs, respectively.

Some results are shown in Figure 1.

CONCLUSION:

A novel biocompatible and injectable system for the *in situ* delivery of pro-osteogenic species was developed by incorporating Sr-containing MBGs, loaded with NAC, into a thermosensitive polyurethane-based hydrogel.

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ACKNOWLEDGMENTS:

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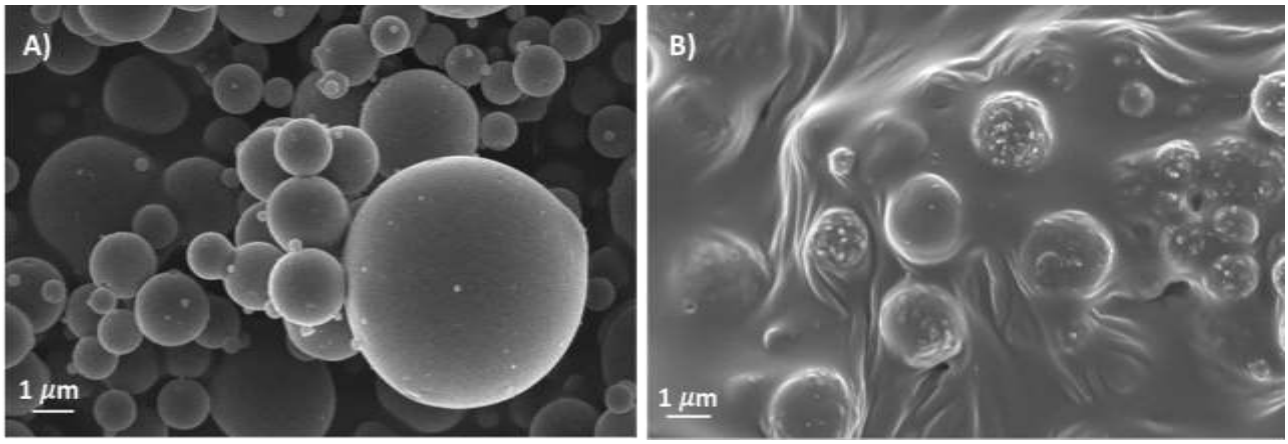
Picture 1:



Caption 1: Figure 1: FE-SEM images of Sr-MBGs (A) and of the hybrid system after 24h of Tris-HCl incubation (B).

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Poster presentation

254 Controlled delivery of non antibiotic antimicrobial agent from uncemented prosthesis

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INTRODUCTION:

The annual incidence of prosthetic joint infection (PJI) in the United Kingdom is around 0.5 to 2%[1]. This relatively low figure, however, should be taken as a concern since the demand for joint arthroplasty is increasing steadily. Moreover, most of PJI cases are caused by multidrug-resistant bacteria, which is difficult to eradicate[2,3]. Despite growing attention on development of prosthesis with antimicrobial activity [3,4,5], there is still a major problem of controlling release rate of an antimicrobial agent from the implant. Antimicrobial agent need to be released in a controlled manner so that sufficient concentration of antimicrobial agent can be reached at all times to prevent biofilm formation during osseointegration process. In this study, we have developed non antibiotic based antibacterial coating on titanium alloy implant. The coating was built employing Layer by Layer (LbL) technique incorporating antimicrobial agent (chlorhexidine diacetate) using patented polymer, B1.

METHODS:

TiO2 nanoparticles were amine functionalized and alternative layers of Sodium alginate (ALG) and patented B1 polyelectrolyte were deposited sandwiching Chlorhexidine diacetate (CL) as a drug in LbL system. During the coating process, some amount of coated nanoparticles were collected for evaluation (Zeta potential, TGA analysis, drug release and antimicrobial testing): from one quadruple layer (Q1), three quadruple layers (Q3), five quadruple layers (Q5), seven quadruple layers (Q7), and ten quadruple layers (Q10).

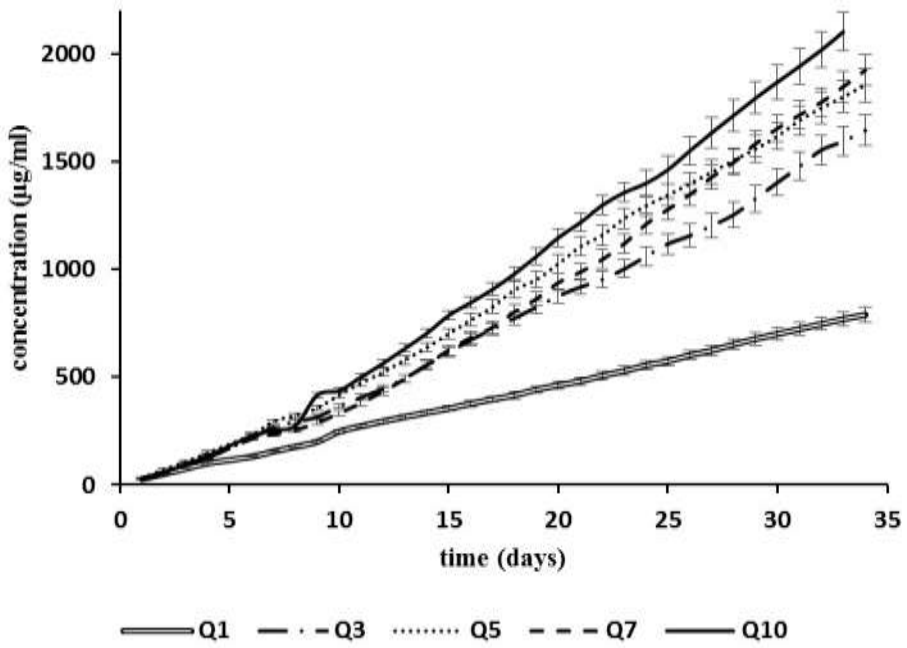
RESULTS AND DISCUSSION:

Zeta potential measurement showed zig-zag pattern indicating correct construction of the LbL system. TGA assay showed the total organic content from the highest to the lowest in the following order: Q 10 , Q7, Q5, Q3, Q1. This result indirectly indicated that the film thickness of Q10 was the highest, followed by Q7, Q5, Q3, and Q1. The total released amount of CL was found to be monotonically increasing with the number of quadruple layers, which means that the highest drug content was found in Q10, while the lowest drug content was in Q1 (Fig. 1). Antibacterial study showed that the daily release of chlorhexidine prevents the growth of MRSA and Staphylococcus epidermidis strains for more than three weeks. Also it suppresses the growth of Enterococci and Escherichia coli strains for more than two weeks.

Overall the prophylactic effect of our formulation (2 weeks min) is longer than the prophylactic effect achieved by using pre-operative antibiotics protocols, usually given as a single dose 60 min prior surgery.

CONCLUSION:

We constructed a drug elution system of antibacterial agent (chlorhexidine diacetate) from uncemented prosthesis using layer-by-layer technique.



Picture 1:

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Poster presentation

260 Naltrexone-loaded soft contact lenses for diabetes mellitus corneal complications

Fernando Alvarez-Rivera[1], Ana Paula Serro[2], Diana Silva[3], Angel Concheiro[1], Carmen Alvarez-Lorenzo[1]

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INTRODUCTION:

Antagonists of OGFr, such as naltrexone (NTX), can revert ocular surface complications[1] if an adequate delivery system is developed. In this sense, contact lenses (CL) attract interest as ocular drug delivery platforms allowing controlled release when adequately designed[2,3]. Thus, the aim of this work was to prepare NTX-loaded CL by molecular imprinting that can provide controlled release on the eye surface to address diabetic complications.

METHODS:

Synthesis of hydrogels

Hydroxyethyl methacrylate (HEMA)-based hydrogels were synthesized using acrylic acid (AAc) and benzyl methacrylate (BzMA) as functional monomers (table 1). Imprinted hydrogels were prepared adding NTX to monomer mixtures before polymerization.

Naltrexone loading and release

Discs were immersed in NTX aqueous solution at room temperature to evaluate loading capability. Then, drug-loaded discs were rinsed with water to remove non-loaded NTX. Release profiles were recorded in 0.9% NaCl at 36 ºC using both conventional test setup and also microfluidics mimicking lachrymal fluid turn over.[4]

Bovine corneal permeability test

Bovine corneas were placed in vertical diffusion cells and the flux of NTX (from CL or aqueous solution) through the cornea was measured for 6 hours. Carbonate buffer pH 7.4 was used as receptor medium to simulate aqueous humour. HET-CAM test was used to screening ocular compatibility.

RESULTS AND DISCUSSION:

Discs with AAc showed higher affinity for NTX, and those prepared applying the imprinting technology loaded higher amounts of drug than non-imprinted ones (7.28+0.41 mg/g and 7.29+0.28 mg/g for C1 and D1; 9.78+0.70 mg/g and 9.18+0.31 mg/g for C2 and D2 respectively). In addition, discs with AAc were able to provide sustained release. Finally, NTX into the cornea can be considered therapeutically efficient. All formulations successfully passed HET-CAM test.

CONCLUSION:

Incorporation of AAc endows the HEMA CLs with affinity for NTX due to weak interactions with the aliphatic nitrogen, hydroxyl and carbonyl groups of NTX. Imprinted hydrogels showed increased affinity, while maintaining the ocular compatibility. Functionalized CLs were able to control the release of NTX for several days and delivered therapeutic amounts to the cornea.

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Picture 1:

Formulation	HEMA mL	NTX mg	BzMA µL	AAc µL	EGDMA µL	AIBN mg
A1	3	0	0	0	45.20	4.93
A2	3	10	0	0	45.20	4.93
B1	3	0	25.41	0	45.20	4.93
B2	3	10	25.41	0	45.20	4.93
C1	3	0	0	10.30	45.20	4.93
C2	3	10	0	10.30	45.20	4.93
D1	3	0	25.41	10.30	45.20	4.93
D2	3	10	25.41	10.30	45.20	4.93

Caption 1: Table 1. Composition of the monomers mixtures.

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Poster presentation

263 Towards understanding the molecular basis of the foreign body response and biomaterial-associated infection

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Academic Medical Center (AMC) at the University of Amsterdam, Amsterdam, Netherlands

INTRODUCTION:

Infection of inserted or implanted medical devices ("biomaterials") can have disastrous consequences, including removal of the device. Implantation of a biomaterial provokes an inflammatory response known as the "foreign body response". *Staphylococcus epidermidis* is one of the major causes of biomaterial associated infection, which in absence of a foreign body hardly ever cause infection. Formation of biofilms on the biomaterial surface is generally considered the main reason for these persistent infections, but *S. epidermidis* has been shown to survive inside macrophages around biomaterials implanted in mice[1], and was retrieved from peri-catheter tissue in humans[2], showing that the tissue around implants is a second niche for infection. In order to understand the high infection-susceptibility of tissue around biomaterials, we aimed to unravel the molecular basis of the foreign body reaction and of biomaterial-associated infection. We assessed the gene expression underlying the foreign body response to titanium over time and the influence of *S. epidermidis* on this response.

METHODS:

Four experimental groups were compared in the biomaterial-associated infection mouse model: a) sham surgery (no implantation of a biomaterial), b) implantation of a titanium biomaterial, c) sham surgery with an *S. epidermidis* infection, and d) implantation of a titanium biomaterial combined with an *S. epidermidis* infection (4 mice per experimental group). At 1 and 6 hours and 2, 4, 9, 14 and 21 days, bacterial colonization, histology and gene expression were analyzed. To characterize multiple cellular immune responses in single microscopic slides of mouse tissue with both implant and bacterial infection, an immunohistological staining protocol using multiple spectral imaging was developed. Gene expression was recorded using Affimetrix Mouse Gene-ST microarrays.

RESULTS AND DISCUSSION:

The histology and gene expression patterns showed distinct differences between sham (i.e. only surgery) and biomaterial groups possibly related to the foreign body response, and between biomaterial without and with infection. The analysis of the expressed gene sets is presently ongoing.

CONCLUSION:

These results are a powerful start towards understanding the molecular basis of the foreign body response and biomaterial-associated infection.

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Poster presentation

269 Strontium induced peri-implant and generalized tissue response in constant osteoporotic experimental rabbit bone model

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[2]Institute of Anatomy and Anthropology, Riga Stradins University, Latvia, Latvia

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INTRODUCTION:

Despite that previous studies have shown the effectiveness of strontium (Sr) enriched biomaterials for enhancing bone regeneration in animals, there are controversial data among experimental models due to the relatively small number of control groups [1]. Therefore, the aim of our research was to analyze seven different rabbits bone conditions in one study.

METHODS:

Constant osteoporosis was induced in 20 rabbits by ovarectomy and methylprednisolone for 6 weeks. Hydroxyapatite (HAP) 30% and tricalcium phosphate (TCP) 70% granules with or without 5% of Sr were implanted in 7 rabbits each, while sham surgery was done in 6. Euthanasia was done after 12 weeks. Bone samples from non-operated leg were also obtained for generalized tissue response. Ten healthy rabbits were set as an intact tissue. Fifty bone samples were analyzed by routine hematoxylin and eosin and immunohistochemistry. Trabecular bone volume and appearance of NFkB 105, OPG, OC, BMP 2/4, Col-1α, MMP 2, TIMP 2, Il-1 and Il-10 immunopositive bone cells were evaluated.

RESULTS AND DISCUSSION:

Trabecular bone volume in healthy group was twice higher than in experimental groups, without difference among operated and non-operated tissue. Group of Sr demonstrated most noticeable increase of OC, OPG, NFkB 105, BMP 2/4, Il-1 and Col-1α ($p < 0.05$) in comparison with non-operated leg, while in pure HAP/TCP NFkB 105 and MMP 2 or in sham only Col-1α showed statistical difference. HAP/TCP/Sr bioceramics demonstrated numerous NFkB 105 ($p = 0.01$) and OPG ($p = 0.040$) positive bone cells, whereas only moderate was found in group of HAP/TCP and moderate number of OPG-containing osteocytes ($p = 0.023$) after sham surgery. Appearance of NFkB 105 and OPG in Sr group was similar to healthy bone. Considerable increase of BMP 2/4, TIMP 2, Il-1 and Col-1α was found after implantation of both biomaterials rather than in healthy tissue samples. Similar expression of factors was found in non-operated legs among experimental groups. Our findings partially coincide with the results reported by other authors, wherein a higher expression of different physiologically active factors was detected after

implantation of variety Sr enriched biomaterials [1,2]. However, contrary results are demonstrated regarding new bone formation around the implant due to the different experimental and control group models, type of biomaterial and duration of implantation [1,2,3].

CONCLUSION:

Twelve weeks are insufficient to increase new bone formation in constant osteoporotic rabbit bone, while significant increase of OC, OPG, NFkB 105, BMP 2/4, Il-1 and Col-1α positive cells was enhanced by Sr bioceramics. Simultaneously to the suppression of osteoclastogenesis, Sr seems to stimulate the mineralization, bone regeneration, cellular activity, and expression of extracellular matrix proteins.

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Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

287 Fabrication of porous poly(trimethylene carbonate) membranes for cell culturing using evaporation-induced phase separation

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INTRODUCTION:

Organs-on-chips (OOCs) can more accurately mimic *in vivo* situations than traditional *in vitro* models as they introduce mechanical forces and microfluidics to cells. The membranes on which the cells grow, however, often lack the necessary biocompatibility and mechanical properties for a given tissue due to the choice of material. Moreover, membranes do not mimic the shape of the tissue.

In this study, porous, microstructured membranes were fabricated from poly(trimethylene carbonate) (PTMC) by evaporation-induced phase separation (EIPS)[1] for cell culturing in Transwell® systems and ultimately a lung-on-a-chip.

METHODS:

PTMC was dissolved in chloroform together with hexanol and poly(ethylene oxide) (PEO)[2]. Pentaerythritol triacrylate (PETA) and Irgacure 2959 were added as crosslinking agents[3]. The polymer solution was cast on a silicon wafer or a microstructured PDMS mould, after which EIPS was performed. The membranes were then crosslinked by UV-light, washed in demineralised water and dried. Calu-3 (human airway epithelial) cells were cultured on flat membranes.

RESULTS AND DISCUSSION:

Porous PTMC membranes were made by EIPS. Increasing the hexanol amount increased the porosity, permeability and roughness of membranes. Cooling during UV treatment or UV treatment and phase separation decreased surface pore size and increased surface roughness and overall porosity of the membranes (Fig.1A-B). Microwells mimicking alveoli were obtained in the membranes (Fig.1B). Calu-3 cells were grown on flat, porous PTMC membranes at an air-liquid-interface (ALI) (Fig.1C). Cells grew best on membranes prepared at lower temperatures with high hexanol amounts, likely due to the higher porosity and permeability. Cells produced occludin at ALI (Fig.1C), indicating cell-cell interactions and the formation of a barrier.

CONCLUSION:

Fabricating PTMC membranes by EIPS is a versatile method to prepare biocompatible, porous and microstructured membranes that have great promise as cell culturing substrates in OOCs.

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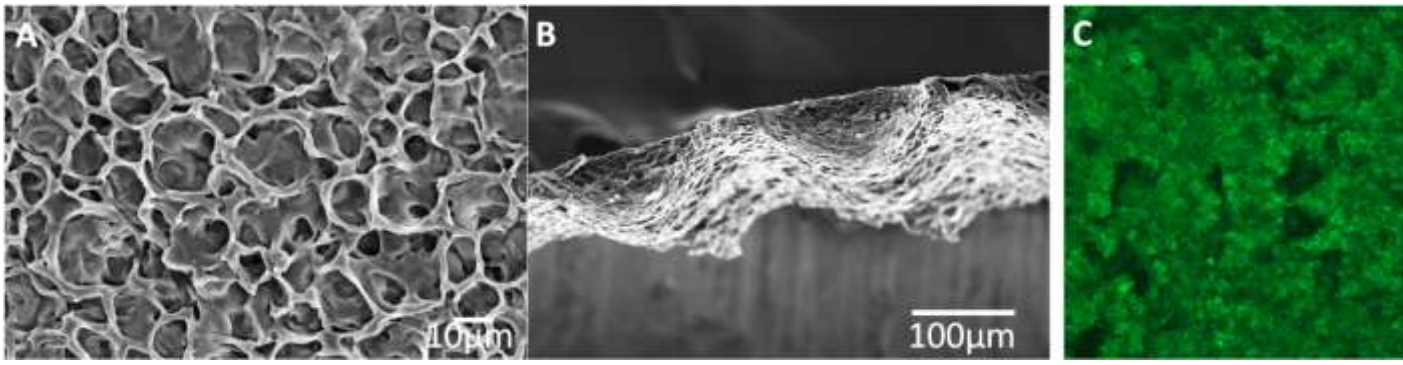
Picture 1:



Caption 1: Figure 1: (A) Flat and (B) microstructured membrane cooled during crosslinking and EIPS. (C) Occludin staining of Calu-3 cells on a flat PTMC membrane

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Poster presentation

288 Development of Antibacterial Nerve Conduits

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INTRODUCTION:

Peripheral nerve injuries (PNI) are a problem in level one trauma centres and one of the most challenging surgical problems. They have a critical impact in patient productivity caused by decrease in function[1]. Due to the limitations and drawbacks of techniques such as end-to-end repair and nerve grafts, researchers are investigating the use of nerve conduits as an alternative approach. Since most of the nerve injuries occurs due to an accident, the immune system becomes compromised and exposed to bacteria, leading to severe infections and limb loss. Polyhydroxyalkanoates (PHAs) are promising candidates for nerve conduit development due to their biocompatibility, biodegradability and versatile properties[2]. In this context, PHAs were loaded with antibacterial agents to develop antibacterial nerve conduits.

METHODS:

PHA polymers obtained via bacterial fermentation were characterized thermally using DSC and mechanically by tensile testing. 2D and 3D structures of neat polymers and blends was accomplished using the solvent-casting and dip moulding technique. The final composites containing the organic and inorganic antibacterial agents, were tested for their antibacterial activity, performing assays such as broth microdilution method and disk diffusion. The cytocompatibility of these materials was evaluated performing direct and indirect *in vitro* assays using multiplex CellTiter-Blue® (Promega) with respect to the neuronal cell line NG108.

RESULTS AND DISCUSSION:

Thermal and mechanical characterization of 2D and 3D structures using PHA blends loaded with antibacterial agents, demonstrated that these structures exhibited the desired properties required for their use as nerve conduits. Preliminary antibacterial evaluation of organic additive, resulted a MIC value of 35mg/ml and 52mg/ml against *S. aureus* and *E. coli* respectively whereas the antibacterial activity of inorganic additive was established according to ISO 22196. The cytocompatibility of the specimens were assessed using indirect contact studies, showing no cytotoxicity.

CONCLUSION:

The mechanical properties of the selected final composites confirm their suitability as nerve conduits, considering that peripheral nerves *in situ* have a tensile strength of 10 MPa[3]. In addition, antibacterial tests of the final structures confirmed antibacterial activity of the composites against both Gram-negative and Gram-positive bacteria. Based on the promising *in vitro* data, the biocompatibility of these nerve conduits will be investigated using *in vivo* tests in a sciatic nerve trauma model of the rat.

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Poster presentation

293 How pure a calcium-deficient hydroxyapatite can be synthetized by inorganic precipitation ?

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INTRODUCTION:

Many researchers have studied in the last decades the effect of doping agents (Sr, Na, Si, Mg, Cu, Li…) on the physical, chemical and biological properties of calcium phosphates (CaP). However, there are generally no comprehensive details provided on the purity of the CaP used as the control when assessing the effect of doping. In fact, CaP raw materials contain quite large amounts of impurities, which can affect their solubility and to some extent modify their physiological integrity/behavior [1]. The aim of this study was to determine how pure a CaP can be synthetized using a series of commercial starting products.

METHODS:

Calcium-deficient hydroxyapatite (CDHA) was obtained by precipitation in an aqueous calcium nitrate – diammonium phosphate solution [2]. Five calcium nitrates and two diammonium phosphate products were used (Table 1). Impurities were quantified by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) and the Ca/P molar ratio was determined by X-Ray Diffraction (XRD) after subsequent thermal treatment (>850°C).

RESULTS AND DISCUSSION:

The content of impurities varied between < 1 (detection limit) and 330 ppm depending on the raw materials. The impurity content varied also significantly when using the same products but different lot numbers (*e.g.* syntheses 1 and 2). The use of ultrapure products (≥ 99.95 %) decreased the impurity content (synthesis 3-6), but considerably increased the synthesis costs and up to 50 ppm impurities were still detected. Among the 45 tested elements (Zr, Si, Ti, Ge, Mo, W not included), only B, Fe, Al, and K were sometimes detected below 10 ppm. Ca/P molar ratios were determined by XRD and were equal to 1.50 ± 0.01 (number of samples, n=15).

CONCLUSION:

This study revealed that CDHA contain impurities, in amounts and types that are highly dependent on the raw materials. Both the article and the batch/lot numbers of the raw material can modify the impurity content. Herein, the purest CDHA still contained at least 30 ppm of impurities, which is quite a lot considering that for ceramics, an impurity with a concentration of 100 ppm is already a dopant.

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ACKNOWLEDGMENTS:

The authors would like to thank the Swiss National Science Foundation Funding (grant n°200021_169027).

Picture 1:

		ICP-MS				XRD
Synthesis n°	Raw Materials (brand, art. n°, lot. n°)	Cu content [ppm]	Mg content [ppm]	Na content [ppm]	Sr content [ppm]	Ca/P ratio [-]
1 (n=3)	**$Ca(NO_3)\cdot 4H_2O$:** Sigma, C4955, MKBV3330V, (≥ 99.0%) **$(NH_4)_2HPO_4$:** Fluka,09840,BCBP9558V, (≥ 99.0%) **NH_4OH:** Roth, P093.1, 216238252	< 1	14 ± 3	11 ± 19	327± 14	1.50 ± 0.01
2 (n=3)	**$Ca(NO_3)\cdot 4H_2O$:** Sigma, C4955, MKCC5930, (≥ 99.0%) **$(NH_4)_2HPO_4$:** Fluka,09840,BCBR7916V, (≥ 99.0%) **NH_4OH:** Roth, P093.1, 067253691	< 1	21 ± 4	9 ± 6	190 ± 8	1.50 ± 0.01
3 (n=2)	**$Ca(NO_3)\cdot 4H_2O$:** GFS, Art n°A1506, C795037 (99.999%) **$(NH_4)_2HPO_4$**: Fluka, Art n°43391, BCBR9912V (99.998%) **NH_4OH**: Sigma, Art n°33881-8, SZBF3200V (≥99.99%)	5 ± 6	31 ± 30	12 ± 2	9 ± 0	1.50 ± 0.01
4 (n=3)	**$Ca(NO_3)\cdot H_2O$:** Merck, Art n°13477-34-4, B1371623640 (99.95%) **$(NH_4)_2HPO_4$:** Fluka, Art n°43391, BCBL5076V (99.998%) **NH_4OH**: Sigma, Art n°33881-8, SZBF3200V (≥99.99%)	< 1	2 ± 2	12 ± 4	21 ± 0.1	1.50 ± 0.01
5 (n=2)	**$Ca(NO_3)\cdot 4H_2O$:** Alfa, Art n° 44515, 23388 (99.9995%) **$(NH_4)_2HPO_4$:** Fluka, Art n°43391, BCBR9912V (99.998%) NH_4OH: Sigma, Art n°33881-8, SZBF3200V (≥99.99%)	< 1	13 ± 3	7 ± 9	50 ± 5	1.51 ± 0.01
6 (n=2)	**$Ca(NO_3)\cdot H_2O$:** Sigma, Art n°202967, MKBM4437V (99.997 %) **$(NH_4)_2HPO_4$**: Fluka, Art n°43391, BCBL5076V (99.998%) **NH_4OH:** SigmaArt n°33881-8, SZBF3200V (≥99.99%)	28 ± 18	17 ±17	14 ± 1	13 ± 1	1.51 ± 0.01

Caption 1: Table 1: Influence of raw materials on CDHA Ca/P molar ratio and content in impurities.

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Poster presentation

313 Strategies for stabilising alginate gel beads with intermediate G-content

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INTRODUCTION:

Using alginate beads as an immunoisolation system for the entrapment of pancreatic islets for treating Type 1 diabetes presents several challenges, which include destabilisation of the alginate gel and fibrotic overgrowth *in vivo*. Alginate is a kelp derived heteropolymer, containing 1,4-linked β-**D**-mannuronic acid (M) and α-**L**-guluronic acid (G) residues, known for its gel forming properties in the presence of divalent cations. Alginates with a high G-content (>60%) produce mechanically stable beads with calcium and barium ions, as opposed to alginates with lower G-content (40%)[1]. Alginate beads of intermediate G-content induce minimal cellular overgrowth *in vivo*, but are unstable[2]. The present study investigates different strategies for stabilising intermediate-G alginate beads (46% G).

METHODS:

Alginate microbeads (340-650 µm), made from sodium alginate (Mw=118-222 kDa) of *L. hyp.* leaf (46% G) and stipe (68% G), were prepared using an electrostatic droplet generator. Different alginate formulations were studied by adding G-blocks (DP=30) or low-Mw alginates (33-34 kDa). Alginate beads were produced with concentrations of alginates from 1.8 to 2.8% (w/v) and gelling-ions (Ca^{2+} and/or Ba^{2+}) in concentrations of 1 mM to 50 mM. The size stability of the gel beads was investigated through treatments in 0.9% (w/v) NaCl. The polymer distributions of fluorescently labelled alginate beads were examined through CLSM.

RESULTS AND DISCUSSION:

The size stability of leaf alginate beads was significantly improved by using barium during gelation, and by the inclusion of free G-blocks or low-Mw leaf alginate (*Fig. 1A*). CLSM studies on leaf and stipe alginate beads revealed initial inhomogeneous polymer distributions for all gelling conditions, which were disrupted by the presence of non-gelling sodium ions (*Fig. 1B*). Solutions with physiological concentrations of calcium had a less disrupting effect on the alginate distribution.

CONCLUSION:

The size stability of leaf alginate beads with intermediate G-content improves with barium in the gelling solution, and through the inclusion of G-blocks or low-Mw leaf alginate. The polymer distribution of intermediate-G alginate beads is unstable in the presence of sodium ions, though it is partly stabilised by the presence of physiological amounts of calcium. The general stability of leaf alginate (46% G) gels is lower than stipe alginate (68% G) gels. However, different strategies can be used to increase the stability of leaf alginate gel beads, which could in the future allow for the use of a more biocompatible alginate for transplantation purposes.

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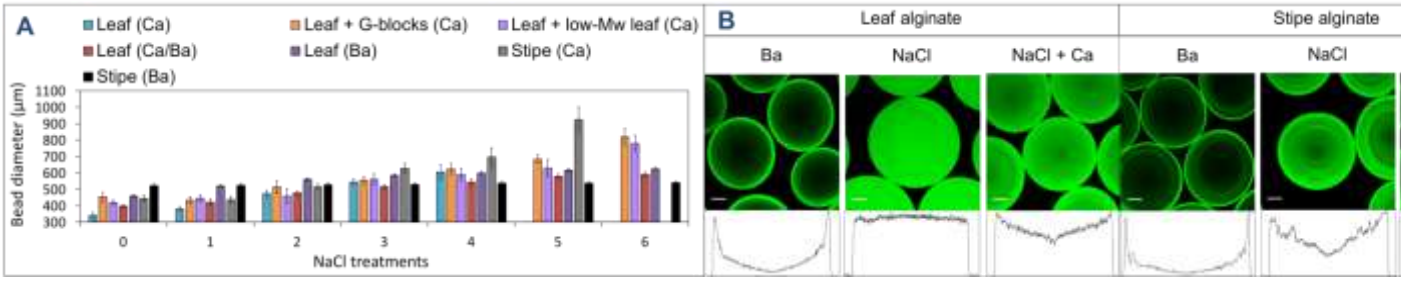
Picture 1:



Caption 1: Fig. 1: A) Size stability and B) polymer distributions of different preparations of leaf and stipe alginate gel beads.

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Poster presentation

314 Fabrication of tubular nanofiber structures for nerve tissue regeneration by a novel two pole air gap electrospinning system

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Research Unit Plasma Technology (RUPT), Ghent University, Gent, Belgium

INTRODUCTION:

Recently, electrospun nanofibers have drawn widespread attention in tissue engineering applications[1]. Despite the growing interest in these nanofibrous materials due to their similarity to ECM (extra cellular matrix), the fabrication of 3D structures made of nanofibers is still challenging. For example, conduit nanofibers suitable for nerve tissue regeneration demand a challenging structure - random nanofibers in the outer layer and aligned ones inside, which perfectly suits to mimic the nerve structure. This research is focused on designing tubular nanofibers possessing the required structure for nerve tissue regeneration by a novel two-pole electrospinning collector. Poly-caprolactone (PCL), mixtures of PCL/Gelatin and PCL/Gelatin/Chitosan were used as well-known biocompatible, biodegradable polymers[2,3].

METHODS:

Tubular nanofiber structures were fabricated by deposition of fibers across the air gap between two horizontal pole electrodes. PCL, PCL/Gelatin (80:20) and PCL/Gelatin/Chitosan (80:10:10) were used as material for nanofibers production. Morphological and mechanical properties of the electrospun nanofibers were evaluated by scanning electron microscopy (SEM) and tensile tests respectively.

RESULTS AND DISCUSSION:

The aim of this study is to create a conduit shape made of nanofibers with appropriate orientation by using a newly designed electrospinning device. SEM images shown in Figure 1 demonstrate that the used method is successful in obtaining the requested nanofibers arrangement of the fabricated tubular structure: random fiber orientation on outside layers and aligned fiber morphology on inside ones. This complex structure has been achieved through the novel design of the electrospinning collector in combination with optimized electrospinning operating parameters. Moreover, ultimate tensile strength, maximum elongation and breaking strength will be tested with tensile tests.

CONCLUSION:

A novel two-pole electrospinning system has been successfully employed to fabricate cylindrical structures from nano-scale fibers. Obtained randomly oriented and uni-axially aligned nanofibers on the outside and inside parts of the tubes respectively result in an excellent configuration for regeneration of damaged nerves. The combination of using biocompatible materials with this biomimetic nanofibrous structure ensures a bright future for these tubular structures in tissue engineering.

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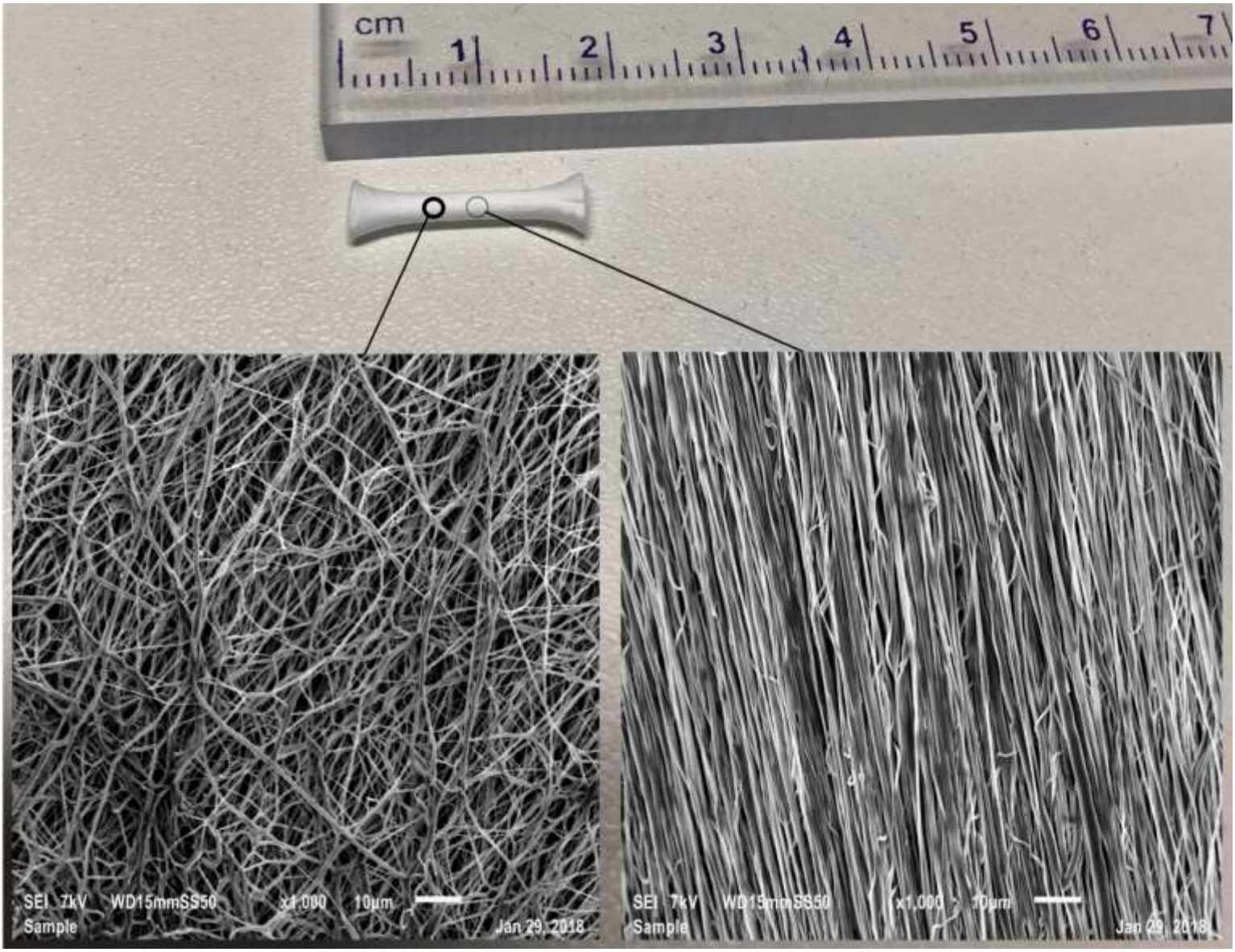

Picture 1: Caption 1: Figure1. Tubular electrospun PCL/Gelatin/Chitosan nanofiber structure with SEM images of inside (right) and outside (left) layers

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Poster presentation

320 Robust supramolecular incorporation of anti-fouling additives in electrospun scaffolds

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INTRODUCTION:

Control over adsorption of proteins and cells at the biomaterial surface is important to tune the response to the tissue a biomaterial is exposed to. Functionalization of biomaterials based on oligo(ethylene glycol) (OEG) for anti-fouling properties has been well established. In most studies, the biomaterial is functionalized through covalent immobilization of OEG molecules. In this study, three designs of supramolecular OEG-based additives are investigated to gain insight in the best way to incorporate anti-fouling properties in a non-covalent fashion in a supramolecular base polymer.

METHODS:

Primary screening of the OEG-additives was performed on solution cast films, by assessing surface properties with atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS) and water contact angle measurements. Additionally, myofibroblast cell adhesion was studied as a functional read-out. Based on these results, a selection of material mixtures was further processed into fibrous meshes with electrospinning. Functional anti-fouling properties were determined on these scaffolds *in vitro* using cell culture and protein adsorption experiments.

RESULTS AND DISCUSSION:

AFM revealed characteristic nanofibrous surfaces for the pristine material.[1] Fibrous morphologies are still observed with incorporation of the different OEG-additives, indicative of proper supramolecular incorporation. XPS measurements on solution-cast films confirmed an increased OEG-content at the surface of films that completely resisted cell adhesion. Electrospun scaffolds show a significant decrease in cell adhesion, indicating that processing of the supramolecular mixtures into functional constructs is possible. Cell adhesion was negligible on electrospun scaffolds with incorporation of one of the selected OEG-additives in cultures up to 7 days. Moreover, adsorption of the first three proteins from the Vroman series[2], which are the first to adsorb from blood, is greatly reduced upon incorporation of an OEG-additive in the scaffold.

CONCLUSION:

Cell adhesive properties of supramolecular mixtures with OEG-based additives were tuned in both solution-cast films and electrospun scaffolds. Importantly, incorporation of one OEG-additive lead to robust non-cell adhesive properties, which shows that molecular design of additives is important for non-cell adhesive supramolecular functionalization strategies.

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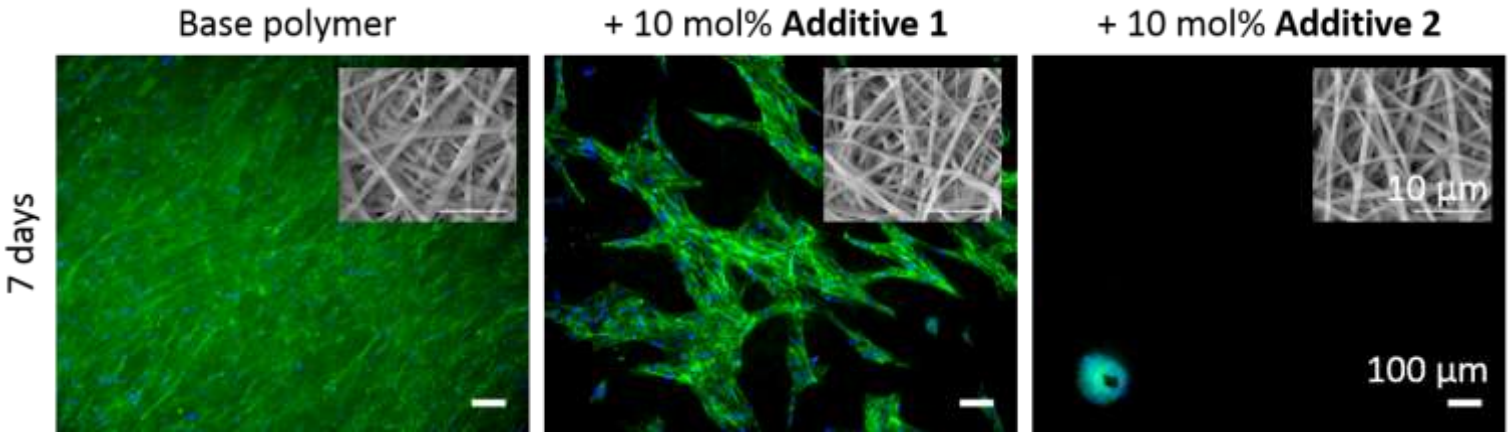
ACKNOWLEDGMENTS:

This project is funded by ZonMW within the Life Science and Health 2Treat programme with additional support from the Dutch Kidney Foundation. Project number: 436001003



Picture 1: Caption 1: Cell adhesion after 7 days on electrospun scaffolds. Actin cytoskeleton in green, nuclei in blue. Insets show scanning electron microscopy images of t

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Poster presentation

328 Development of Collagen Substrates to Study Antimicrobial Peptide Biopolymer Tethering

Terri Anne Camesano, Lindsay Lozeau, Marsha Rolle, Errando Berwin Jayapurna

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INTRODUCTION:

Collagen tethering of antimicrobial peptides (AMPs) using collagen-binding domains (CBDs) is being developed as a method to deliver non-cytotoxic, antimicrobial and pro-healing therapy to chronic wounds. AMPs, such as human LL37, demonstrate broad antimicrobial activity and many immunomodulatory functions using unique physical mechanisms that have a lower likelihood of bacterial resistance. Previously, we demonstrated that tethering LL37 onto collagen-based wound dressings using CBDs from collagenase (*c*CBD-LL37) and fibronectin (*f*CBD-LL37) allowed for maintained peptide antimicrobial activity while reducing cytotoxicity toward mammalian fibroblasts. Peptide loading concentrations onto the scaffold were calculated based on the assumption of a 1:1 binding ratio of collagen monomers in the dressing to collagenase binding domains, but we wanted to optimize this loading further. We hypothesize that the peptide loading concentration needed to create an effective therapy will depend on CBD affinity for collagen, peptide mechanism, scaffold composition, and available binding sites.

METHODS:

We developed a method to study the binding of CBD-LL37 onto collagen via quartz crystal microbalance with dissipation (QCM-D), which characterizes nanogram-level mass and rigidity changes of deposited films. Concentration- and substrate-dependent deposition of collagen type I on silica and gold sensors were studied. We characterized the density and thickness of each film using a viscoelastic model. The binding of *c*CBDLL37, *f*CBDLL37, and unmodified LL37 was characterized at four concentrations (0.5, 1, 5, and 10 µM).

RESULTS AND DISCUSSION:

Thickness and density of collagen substrates were characterized on gold and SiO_2 sensors. For example, at 0.1 mg/mL on gold, collagen thickness and density were 104 ± 8.43 nm and 2227 ± 72.7 kg/m^3 on gold. Significant

amounts of peptide adsorption were observed in a concentration- and peptide-dependent manner. For example, at 5 μM we found that LL37, *c*CBD-LL37 and *f*CBD-LL37 demonstrated frequency changes of -10.96 ± 1.23, -12.49 ± 1.63 and -12.48 ± 0.76 Hz, which correlated with peptide mass adsorption. Qualitatively, it was observed that *f*CBD-LL37 demonstrated the largest initial adsorption profile while *c*CBD-LL37 demonstrated the highest overall retention on collagen, suggesting CBD-dependent binding interactions and affinities between CBD-LL37 and collagen. The viscoelastic model will be used to relate peptide adsorption to peptide layer thickness and binding orientation on collagen substrates. Finally, we developed an *ex situ* method to study the antimicrobial effectiveness of the bound peptides.

CONCLUSION:

This QCM-D method provides a powerful platform to study CBD-LL37 tethering and loading concentrations for the effective delivery of AMPs on collagen-based biopolymer scaffolds.

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Poster presentation

331 Bioprinting of cardiomyocytes in collagen-based hydrogel scaffolds for the development of a cardiac tissue patch

Rabeil Sakina, Aida Llucià-Valldeperas, Carlos Mota, Paul Wieringa, Lorenzo Moroni

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INTRODUCTION:

Cardiovascular diseases are the leading cause of mortality worldwide. Limited regenerative capacity of the heart and donor shortage has driven research in cardiac tissue engineering to provide alternative therapies. However, insufficient vascularization and poor myocardium architecture limits scaling-up of tissue constructs for *in vitro* and *in vivo* studies as well as eventual clinical translation.

Despite bioprinting being able achieve a defined spatial cell distribution (Duan B, 2017); the ideal hydrogel and cell type to be printed remain elusive. Our hypothesis is that bioprinting will enable the development of a physiologically functional myocardial construct for regenerative medicine applications. Using a bioink comprised of a collagen solution laden with cardiomyocytes, we report on a hydrogel scaffold with an incorporated cardiac cell population.

METHODS:

Immortalized mouse HL-1 cardiomyocytes were mixed with a 2 mg/mL collagen solution and printed. The collagen bioink was prepared according to manufacturer protocol for gelation. The bioprinting was executed with a Bioscaffolder platform. The bioink was extruded through a 25G needle at 12 kPa and 27 mm/s. Cell viability was evaluated with Live/Dead assay. Cardiac immunostainings were performed by using specific antibodies against MEF2, SERCA2, α-actinin and cardiac troponin T. Bioprinted scaffolds were fixed with 4% paraformaldehyde, permeabilized, incubated for 1 hour at 21^{o}C with primary antibodies, and incubated at 21^{o}C for 1 hour with fluorophore conjugated secondary antibodies. Nuclei were counterstained with DAPI. Constructs were evaluated via microscopy and quantification (i.e. construct thickness, immunofluorescence intensities) performed with ImageJ.

RESULTS AND DISCUSSION:

Hydrogel cytotoxicity test evidenced good viability of HL-1 cells embedded in 2 mg/mL collagen gels. Bioprinted collagen scaffolds composed of 4 layers showed fiber size of 208.20 ± 27.92 μm, while after 9 days in culture, fiber size was 695.6 ± 59.80 μm. Bioprinted HL-1 cells proliferated within the collagen scaffold forming aggregates. 87% of the HL-1 cells remained viable in 2 mg/mL collagen printed scaffolds after 1 week. HL-1 cells maintained the

expression of cardiac transcription factors (i.e. MEF2), calcium-handling genes (i.e. SERCA2), and structural genes (α-actinin and cardiac troponin T) after being bioprinted within collagen.

CONCLUSION:

HL-1 cells were successfully bioprinted within a 2 mg/mL collagen solution and remained viable. The cardiac phenotype was preserved, as evidenced by the cardiac markers expression. Further hydrogel formulations, namely fibrin and decellularized extracellular matrix, may enable further optimization of the bioink composition for cardiac tissue engineering applications.

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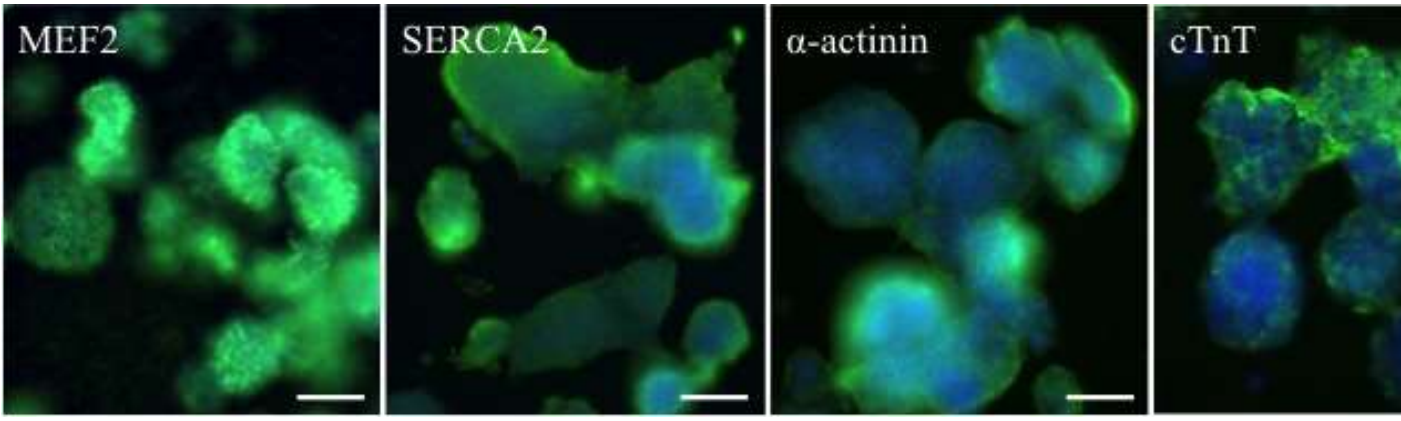
Picture 1:



Caption 1: Cardiac marker expression in HL-1 printed cells on collagen after 1 week. Scale bars = 100 μm

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Poster presentation

333 Study of metal-ceramic bond strength using different techniques for the production of the metal substrates

Christina Mouchtaridi[1], Marta-Muñoz Hernandez[2], Konstantin Spiropoulos[3], Triantafillos Papadopoulos[1]

[1]National and Kapodistrian University of Athens, Athens, Greece

[2]University Rey Juan Carlos, Madrid, Spain

[3]Technological and Educational Institution of Athens, Greece

INTRODUCTION:

A clear knowledge is vital for using (CAD/CAM)[1] and (SLM)[2] techniques, especially for the construction of the metal ceramic dental works[3].

METHODS:

56 metallic substrates were constructed using Co-Cr dental alloys applying Casting (group 1), CAM/ Milling (group 2), SLM (group 3) and CAM/SM (group 4) techniques equally divided in the four groups. 10 substrates of each group used for the fabrication of metal ceramic specimens and four for recording the modulus of elasticity. Dimensions were adjusted according to ISO 9693 (0.5 ± 0.05 mm, width 3 ± 0.1 mm, length 25 ± 1 mm). They were radiographically checked for porosity and the ceramic mass was placed in dimensions 8x3x1mm, using a special matrix. XRD analysis was conducted for every group to record the crystallographic microcrostuctural changes. The specimens were tested using 3 point bending test to record the bond strength. The fractured specimens were observed in an optical microscope to record mode of failure. EDS examination was held in a SEM for detecting the different phases. Statistical analysis was made by one –way ANOVA where $p < .05$ was considered significant.

RESULTS AND DISCUSSION:

Three bending test gave 43,0± 7,9 MPa for group 1, 54,5± 4,5 MPa for group 2, 44,5± 8,7 MPa for group 3, 44,1 ±9,4 MPa for group 4. No statistically significant difference was recorded among the groups ($p > .05$). All receved values reflect the values reported between Co-Cr alloys and feldspathic porcelains by many other researches. A result supporting satisfactory metal ceramic bond strength was that in all groups mode of failure was of cohesive type. XRD results revealed a variety of different phases after employing the individual techniques of production and after porcelain firing.

CONCLUSION:

From the results it is concluded that new technologies can offer alternative solutions for reliable, faster, ecological and efficient metal ceramic dental restorations.

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ACKNOWLEDGMENTS:

Authors would like to thank ACH Dental Laboratory for the contribution of the specimen's fabrication.

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Poster presentation

342 Functionalized alginate-based hybrid hydrogels with enhanced mechanical properties and potential for cell transplantation

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[2]Geneva University Hospitals, Switzerland

INTRODUCTION:

The increasing number of patients suffering from metabolic diseases (such as diabetes)[1] or the uncommon but highly life-threatening acute liver failure[2], calls for urgent and sustainable therapeutic solutions. Cell based therapies, especially transplantation of microencapsulated cells can potentially overcome the limitations of whole organ transplantation, which is the main treatment for end-stage diseased patients at the moment. The success of a cell transplantation highly depends on the quality of the encapsulating biomaterial thus posing a big challenge to develop adequate materials.

METHODS:

Sodium alginate (Na-alg) stands as one of the most utilized encapsulation material. However, its mechanical and permselectivity properties are not optimal.[3] To improve these, we developed a variety of hybrid hydrogels combining the alginate backbone with poly(ethylene glycol) (PEG) derivatives allowing for the formation of covalent crosslinking and electrostatic interactions in the resulting microcapsules. We worked out a straightforward method for grafting the PEG derivatives on the alginate hydroxyl groups through stable carbamate linkage.[4] Thanks to different PEG reactive functionalities, the physical and mechanical properties of the microcapsules can be tuned and adjusted to the intended biomedical application.

RESULTS AND DISCUSSION:

The developed polymers were tested for their potential to form 3D microspheres for cell immobilization. They were assessed for their mechanical resistance, elasticity and permselectivity. We demonstrated that the nature of the covalent crosslinking has a significant impact on the physical properties of the microspheres, with major improvement compared to Ca-alginate beads. In vitro tests encapsulating hepatocytes, MIN6 cells and islets from human and porcine origin showed good viability and functionality. Empty beads and encapsulated MIN6 cells were transplanted into mice and remained stable up to 35 days in vivo.

CONCLUSION:

We established a strategy for functionalizing Na-alg on the hydroxyl moieties with different types of PEGs, which are able to form hybrid electrostatic-chemical hydrogels. Based on in vitro and in vivo results, the developed polymers show promising potential for cell immobilization and transplantation, offering the perspective for the treatment of several diseases using easily available and relatively inexpensive materials.

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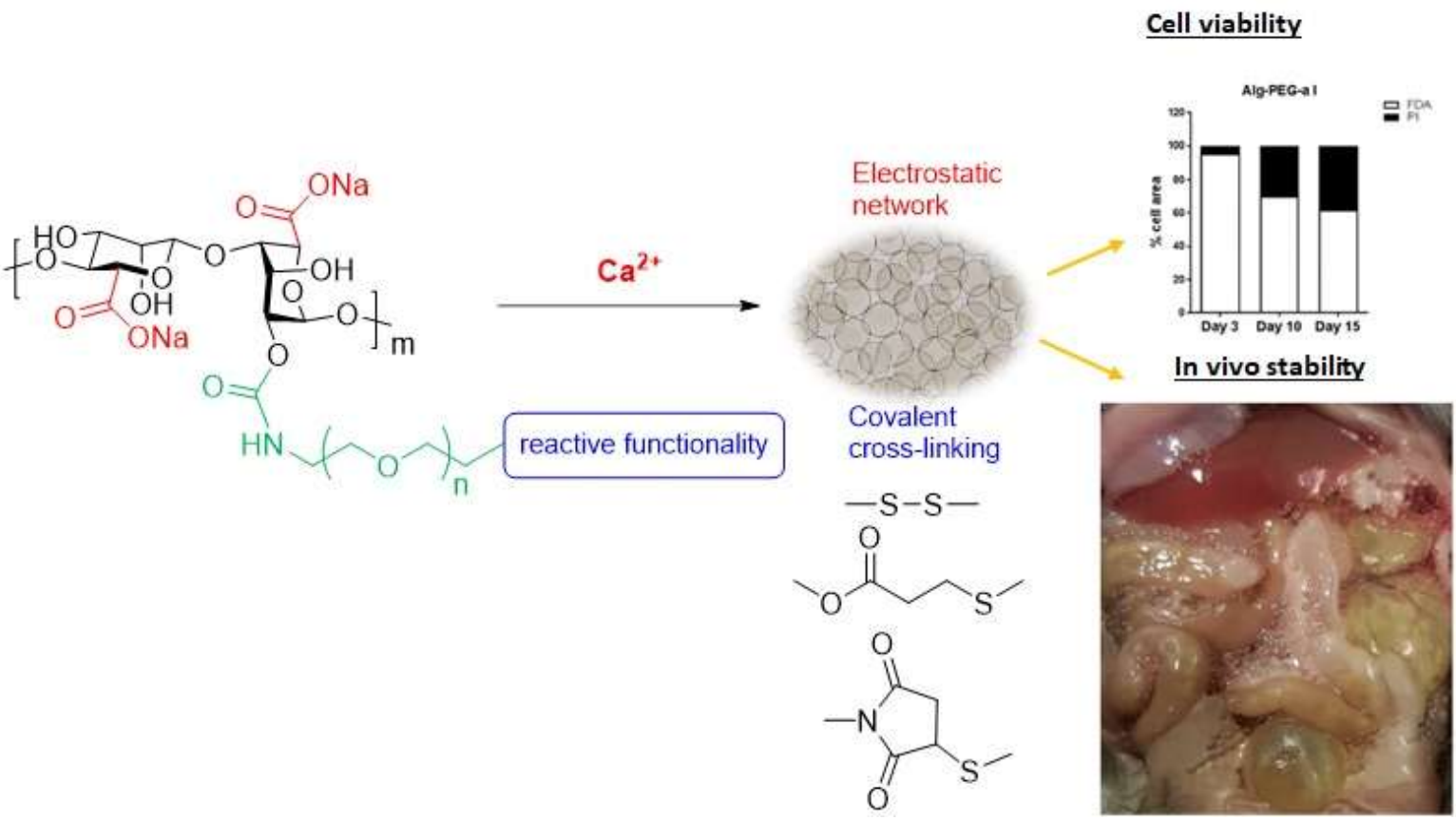
ACKNOWLEDGMENTS:

We thank the Swiss National Science Foundation (grant N° CR23I2_152974 and 310030E-164250) and the Foundation Insuleman for financial support. We are grateful to Dr Christine Wandrey for helpful scientific discussions.



Picture 1: Caption 1: PEG functionalized Na-alg and their application

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Poster presentation

345 Nanosized apatite coating by ultrasonic spraying for orthopedic applications

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INTRODUCTION:

Calcium-phosphate (CaP)-coated metallic implants have been widely used in orthopedic and dental surgery with the aim to improve osteointegration of the implant into the surrounding bone tissue. So far, many methods have been developed to deposit CaP metallic implants, including plasma spraying, dip coating and biomimetic deposition from aqueous solutions [1]. The role of the thin CaP apatite layer is to be a bonding interface that stimulates collagen production and mineralization [2]. It is suggested that nanostructure of the implant surface affects osteoblast adhesion and differentiation, and tissue deposition [3]. The deposition of CaP on metal substrates by ultrasonic spraying can be considered one of the methods for overcoming the drawbacks of plasma-spraying related to poor control of the stoichiometry and composition of the deposited material. In this work, an ultrasonic spraying was developed to deposit thin nanosized hydroxyapatite (HA) onto titanium surfaces

METHODS:

Nanosized HA particles with a size of ~50-200 nm were fabricated by Xpand Biotechnology BV, the Netherlands. Sandblasted titanium plates (20x20x1mm) were used as coating substrates. The coating process was carried out using an ultrasonic spray Nozzle (Sinosonic, China) with a DP30-UG90 generator. The flowrate of the nanosized apatite solution (2wt% HA in a 0.1wt% of methylcellulose aqueous solution) was set at 0.1ml/min using a syringe pump, and the spraying time was set at 1 minute.

RESULTS AND DISCUSSION:

Upon optimized coating process, scanning electron microscopy images exhibited the presence of a nanocrystalline coating with controllable thickness (Fig.1). The coating was homogenous, both in terms of substrate coverage and chemical composition. In vitro degradation studies in a saline physiological solution (pH7.4) showed the release of calcium and inorganic phosphate ions, which is an important factor in the process of osteointegration with bone

CONCLUSION:

The thin nanosize HA coating was successfully coated on the titanium substrate by ultrasonic spraying system at room temperature. The degradation showed a release of calcium and phosphate ions, which may be important factors for bone formation. Furthermore, this technique, which takes place and environmental conditions, enables the incorporation of biomolecules and bioinorganics into the HA coating to further enhance the bioactivity that is required for use in biomedical applications.

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ACKNOWLEDGMENTS:

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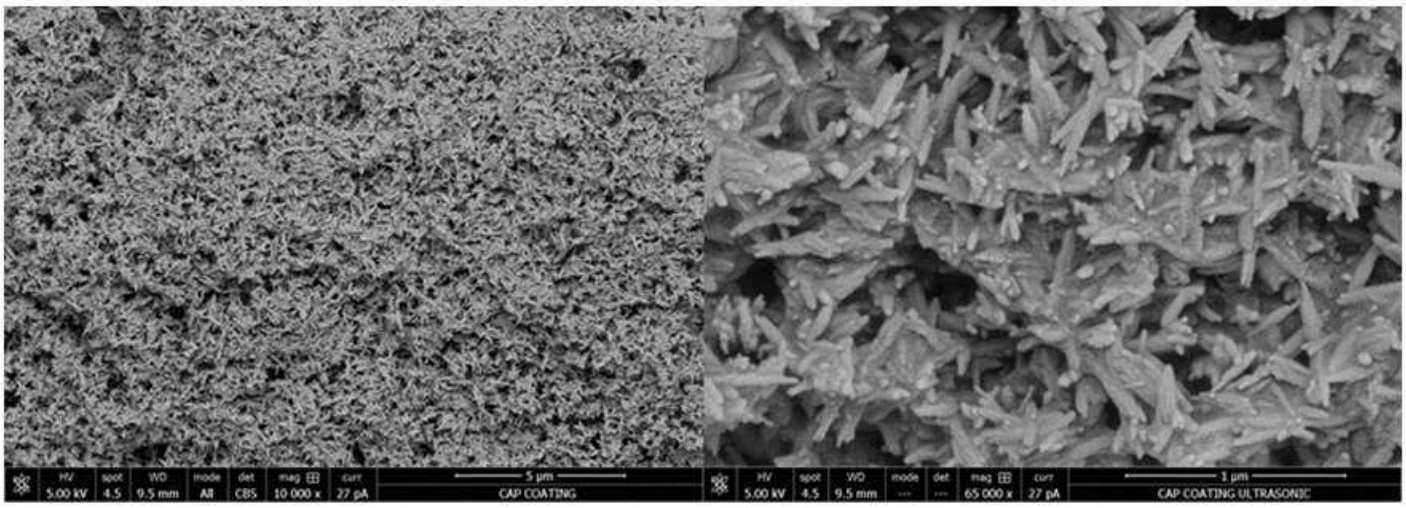

Picture 1: Caption 1: Fig.1 Nano apatite coating on the titanium substrate with low and high magnification

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Poster presentation

361 3D bioactive composite scaffold by fused deposition modelling

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INTRODUCTION:

Recently, additive manufacturing (AM) technologies have gained popularity for scaffold fabrication, especially for bone tissue engineering due to reproducibility, reliability and capability to design porous architecture[1]. Fused deposition modeling (FDM) is a popular 3D printing technology taking thermoplastic filament as printing material. The filament is melt at a hot nozzle and extruded to build 3D models. It is low cost, high reliability and simple operation. Recently, more scientists are interested in developing new material to make scaffold by FDM [2]. In our

lab, in order to implement FDM to our scaffold process, Composite filament were developed to fabricate 3D composite scaffold by FDM.

METHODS:

Composite filament preparation: 50/50 wt% of TriCalcium phosphate (TCP, 1μm, Xpand. NL) and Polyactice 1000/70/30 (Polyvation BV. Netherlands) was mixed and extruded to fabricate composites filament by twin-screw extruder (HAAKE MiniCTW**,** Germany) at 150± 1^0C and speed of 100±1 rpm of mixing speed for 5±0.5 min.

FDM 3D scaffold: The filament is loaded on 3D printing machine (Hy-rel , US). The melted composite fiber from heated nozzle at 180°C is plotted on platform, and then the scaffold is fabricated by layering a 0°-90° pattern of fibers.

RESULTS AND DISCUSSION:

The relationship between the filaments' density and temperature was studied to control the quality of filament. Meanwhile, the screw speed of 30 rpm was set to extrude filament smoothly (Fig.1A Filament of composite).

During FDM printing process, the temperature was adjusted to melt composite filament and extrude out the nozzle, the feed speed is optimized to the speed which fiber could attached each other and keep constant fiber diameter. Therefore, temperature of 180°C and 5 mm/s of feed speed were set for printing process. The resulting scaffold exhibited a fully interconnected porous network with highly controllable porosity and pore size (Fig.1B and 1C shows the scaffold from FDM).

CONCLUSION:

3D composite scaffold was successfully produced by FDM. To meet practical application needs, the pore size and porosity can be changed by design, and final strength of 3D composite scaffold can be adjusted by controlling of the porosity of 3D composite scaffold. With the AM, scaffold, not only mimic the structures and properties of bone, but also design 3D scaffolds with biomaterial to better understand cell-material interactions.

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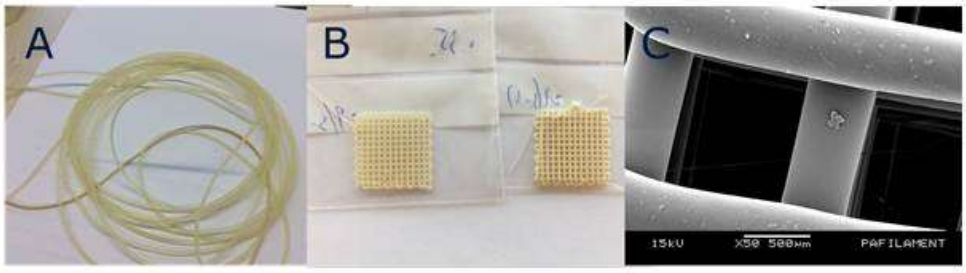

Picture 1: Caption 1: Fig.1 Composite filament (1A: Filament) and resulting 3D composite scaffold(Fig.1B: 3Composite scaffold; 1C: SEM of scaffold)

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Poster presentation

362 Characterization of the microstructures of hydrazone crosslinked polysaccharide-based hydrogels

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INTRODUCTION:

Microstructure controls different properties of hydrogels, therefore it also determines how suitable they are as biomaterials, especially when drugs or cells are encapsulated inside the hydrogel[1]. Structural parameters, such as mesh size and crosslinking density, dictate the mechanical and diffusional properties of hydrogels[2-4]. Indeed, the mesh size has been used to correlate the diffusivity of molecules inside the hydrogels[5]. The aim of this study was to characterize the microstructures of hydrazone crosslinked polysaccharide (hyaluronan (HA), gellan gum (GG) and alginate (AL)) -based hydrogels using rheology- and diffusion-based methods.

METHODS:

The microstructures of hydrazone crosslinked HA-polyvinyl alcohol (PVA)-, AL-PVA-, GG-HA- and HA-HA(-collagen I)-based hydrogels[6-8] were evaluated using rheology- and diffusion (fluorescence recovery after photobleaching, FRAP)-based methods. The effect of the gel parameters (degree of substitution and molecular weight of gel components, ratio of gel components, polymer concentration of hydrogel) on the viscoelastic and diffusion properties of hydrogels, and further to their structural parameters (mesh size, crosslinking density and average molecular weight of the polymer chain between neighboring crosslinks) were studied.

RESULTS AND DISCUSSION:

Results showed that diffusivity decreased when larger dextran sizes (500-2000 kDa) were used. Further, those molecule sizes were equivalent to the mesh sizes of hydrogels determined by the rheological method. The evaluated mesh sizes were comparable with many other hydrogels. This size range allows the transportation of smaller molecules, peptides and most of the proteins. The results also showed a proportionality between the structural parameters and storage moduli (and second order elastic constants we determined earlier[6-8]).

CONCLUSION:

To conclude, the results showed that the microstructures of hydrogels can be evaluated using rheology- and FRAP-based methods. In addition, the results showed that hydrazone crosslinking offers an easy way to produce polysaccharide-based hydrogels with variable microstructures, as well as viscoelastic and diffusion properties, by altering different gel parameters.

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Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

372 Coating of vascular stents with simvastatin loaded nanofibers by electrospinning for the prevention of restenosis and thrombosis

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INTRODUCTION:

Cardiovascular diseases are the first cause of death in developed countries. These diseases are mainly due to atherosclerosis, a systemic arterial pathology responsible for stenosis and thrombosis[1]. The angioplasty stenting is often applied to treat this pathology but unfortunately, restenosis occurs in 30% of cases. The objective of this work is to coat stents by electrospinning technique with 2 layers of nanofibers based on biopolymers: a first layer with antithrombotic properties to prevent blood coagulation intra lumen and a second drug layer to deliver simvastatin (SV) to the vessel wall, for preventing restenosis.

METHODS:

Electrospinning was used to coat NiTiNOL stents with nanofibers (NFs). The first NFs intraluminal layer is based on an anticoagulant sulfonated chitosan (CHTs) and the second one is based on chitosan (CHT) and cyclodextrin polymer (PCD)[2] forming a polyelectrolyte complex CHT/PCD loaded with SV (CHT/PCD-SV NFs). The electrospinning of both types of NFs was optimized in terms of precursor polymers solutions and process parameters. Different thicknesses of the outer layer were obtained by varying the electrospinning time. The physico-chemical properties of the NFs were characterized (SEM, TGA …). The *in vitro* SV release study in PBS (Phosphate Buffered Saline, pH 7.4) from the NFs was followed in dynamic conditions (35 ml/min).

RESULTS AND DISCUSSION:

The deposition of NFs on the stent was successfully achieved as presented in Figure 1.

CHT/PCD NFs were thicker than CHT ones. The quantity of SV loaded on the stent could be controlled by the thickness of the CHT/PCD-SV itself dependent on the electrospinning treatment. The release time of SV was correlated with the presence of PCD in the NFs and also on the thickness of CHT/PCD-SV NFs. The hemocompatibility and antithrombotic CHTs properties of the intraluminal layer were studied by hemolysis and adequate coagulation tests respectively.

CONCLUSION:

Two types of bioactive NFs were consecutively coated with success on NiTiNOL stents. After the promising preliminary tests described here, the in vitro biocompatibility of the coated stents has still to be assessed. Finally, experiments will be carried out using a rabbit model to investigate the *in vivo* efficiency of the coatings.

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ACKNOWLEDGMENTS:

The authors thank University of Lille, Interreg 2 Seas IMODE program and Institut Chevreul for their financial support.

Picture 1: 

Caption 1: Figure 1. (a) Stent coated with nanofibers and (b) SEM picture of nanofibers obtained

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Poster presentation

374 Designing copolymeric scaffolds for bone tissue engineering

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INTRODUCTION:

The main focus of this study was to develop a graft polymer, comprising chitosan (CS) and poly(L-lactide) (PLLA), to be used as a biomaterial with tunable chemical, mechanical and biological properties for the fabrication of scaffolds in bone tissue engineering. CS, a natural polysaccharide, is a biocompatible polymer, slightly soluble in the aqueous culture medium with poor mechanical properties[1]. On the other hand, PLLA, is a biocompatible synthetic polymer with excellent mechanical properties[2]. Therefore, grafting the synthetic polymer along the CS backbone is an attractive way to regulate the physical and mechanical properties of the latter. In this work, we present the preparation of two CS-*g*-PLLA graft copolymers, CS-*g*-PLLA(20) and CS-*g*-PLLA(50), with 20 wt% and 50 wt% PLLA content, respectively, their mechanical properties and biological evaluation.

METHODS:

The CS-*g*-PLLA copolymers were synthesized by grafting PLLA chains carrying a terminal carboxylic acid group along CS backbones[3], and characterized physicochemically and nanomechanically. Discs and surfaces of the CS-*g*-PLLA(20) and CS-*g*-PLLA(50) copolymers were used to study the degradation profile, the nanomechanical properties, the morphology, viability, proliferation and osteogenic response of MC3T3-E1 pre-osteoblastic cells.

RESULTS AND DISCUSSION:

We prepared CS-*g*-PLLA graft copolymers with PLLA content varying from 20 wt% to 50 wt%. Analysis of the ^{1}H NMR data allowed to calculate the copolymer grafting density, which was found to be one PLLA chain every 180 CS monomer repeat units for CS-*g*-PLLA(20) and one PLLA chain every 25 CS monomer repeat units for CS-*g*-PLLA(50). Degradation studies showed a total weight loss of 9% after 21 days in culture for both copolymers. Nanomechanical investigation indicates significant increased Young modulus and hardness values for the CS-*g*-PLLA(20) compared to CS-*g*-PLLA(50) which may be attributed to the higher viscoelasticity of the samples with a

higher CS concentration. Biological experiments showed that both CS-*g*-PLLA copolymers promote pre-osteoblastic cell adhesion, viability and proliferation, and increase the levels of osteogenic markers, with the CS-*g*-PLLA(50) material showing a significant increase in the cell proliferation.

CONCLUSION:

We have synthesized CS-*g*-PLLA copolymers by grafting end-functionalized PLLA chains onto the hydroxyl groups of CS, varying the PLLA content from 20 to 50 wt% and controlling their nanomechanical properties. The biological assessment of the copolymers shows a strong cell adhesion, increased proliferation and osteogenic response of pre-osteoblastic cells on the CS-*g*-PLLA scaffolds.

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Poster presentation

385 Biocompatible hydrogels as traps for invasive glioma cells for novel therapeutic applications

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INTRODUCTION:

Glioblastoma multiforme (GBM) is the most deadly and aggressive malignant brain tumour of the central nervous system in adults[1]. Current conventional methods of treatment include surgical resection combined with radiotherapy or chemotherapy, are mainly unsuccessful. For these reasons, there is an urgent need to develop new methods and understand the complex behaviour and microenvironment of GBM tumours. Therefore, we here investigated the use of 3D biomaterials such as hydrogels loaded with a chemo-attract in order to create an artificial scaffold that can mimic the tumour microenvironment *in vitro*[2] and can be used for attracting and trapping brain cancer cells.

METHODS:

In this work, HA hydrogels with different crosslinking degrees were prepared and their mechanical properties were studied. The effect of the degree of crosslinking on the microstructure and porosity of the hydrogels was evaluated by Scanning Electron Microscopy (SEM). The swelling behavior of the hydrogels in PBS at 37°C was studied and *in vitro* degradation of hydrogels was performed in order to ensure the integrity of the scaffold upon exposure to enzymes found *in vivo*. Moreover, oscillatory rheology was used to characterize the viscoelastic properties of the hydrogels. Doxorubicin (DOX) and temozolomide (TMZ) were loaded on hydrogels and their release profiles were studied. The efficacy of DOX and TMZ was investigated against glioma cells. Furthermore, hydrogels were loaded with a chemokine and biological experiments were performed in order to study further the behaviour and migration of glioma cells in the 3D network.

RESULTS AND DISCUSSION:

The SEM images indicated that the structure and the porosity were mainly correlated with the crosslinking density of the hydrogels. The degradation profile of the hydrogels is consistent with the results obtained from the swelling studies. Oscillatory rheology confirmed that the viscoelastic properties of the hydrogels were close to those of the CNS. Drug release varies between the different crosslinking densities. Preliminary biological data confirmed the adhesion and migration of cells into the scaffold.

CONCLUSION:

HA hydrogels were prepared at different crosslinking densities in order to identify the optimal mechanical properties that promote adhesion and migration of glioma cells. Preliminary biological results demonstrated that glioma cells can migrate in the 3D network.

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ACKNOWLEDGMENTS:

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Poster presentation

396 Functionalized polymer-derived bioceramics for bone-tissue engineering.

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INTRODUCTION:

Bone autografts are the gold standard in bone regeneration[1], however, their limitated availability and donor site morbidity, necessitates development of novel biocompatible, osteoconductive synthetic substitutes allowing early implant loading[2]. Bio-ceramic foams comprising hardystonite (HT) enriched with Strontium and Magnesium, are obtained from preceramic polymers (silicone resins) containing micro-sized filler powders[3]. This process presents advantages; limited processing temperature, microstructural homogeneity resulting in foams that mimic porous internal structure of human cancellous bone[3]. Recent discovery of small peptides able to drive cell functions are opening new avenues toward the manufacture of osteoinductive materials. We demonstrated that the nona-peptide (HVP) derived from the Vitronectin sequence is able to enhance human osteoblast adhesion through an osteoblast-specific mechanism[4]. In the present study, polymer-derived silicate foams were covalently and selectively functionalized with a retro-inverted dimeric analogue of HVP (D-2HVP) aiming to increase ionic interactions with cellular Glico-Amino-Glycans (GAG) and avoid enzymatic degradation of HVP peptide in serum-containing media[5].

Functionalized bio-ceramic scaffolds were subsequently functionalised with ionic-complementary self-assembly peptides (SAP)[6] to promote cell viability over foam-scaffolds' surface.

METHODS:

Hardystonite foams enriched with Sr and Mg, were prepared dissolving pre-ceramic polymer and micro-sized filler powders in isopropanol, and thermally treated to achieve the porous structure and a ceramization step. Foams were selectively and covalently functionalized with D-2HVP[5], and treated with SAP solution.

RESULTS AND DISCUSSION:

Preliminary *in vitro* cell proliferation on non-functionalized HT foams, enriched with Sr and Mg, showed an increase in cells numbers when compared to the controls (TCP and pure HT). Mechanical characterization demonstrated that functionalisation did not alter mechanical properties of HT foams derived scaffolds. Further studies on osteoblast response (viability, proliferation, morphology, mechano-sensing) to functionalised scaffolds (compared with non-functionalised ones) are currently under investigation. Furthermore, the molecular YAP/TAZ transcription co-activators in relation to mechanosensing and osteoblast differentiation) are also ongoing.

CONCLUSION:

The synergistic effect between bioceramic structure/composition and specific covalent conjugation of a protease-resistant osteoblast-specific adhesive peptide, combined with the properties of SAP is a potentially useful approach for the production of next generation scaffold for bone tissue engineering.

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Poster presentation

399 Hybrid electrospun wound dressings for skin regeneration

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INTRODUCTION:

Electrospun nanofibers wound dressing presents exceptional properties compared to the conventional dressing but its characteristic small pore size and the lack of specific groups limits the cellular migration into the scaffold[1-3]. To overcome these limitations the present work explores different electrospinning approaches (multimaterial, copolymer, coated and single) to develop hybrid structures through the combination of synthetic and natural polymers.

METHODS:

Polycaprolactone and Gelatine have been widely used on regenerative medicine due its biodegradability, biocompatibility and cost-effective, additionally the good mechanical properties and many integrin-binding sites for cells, respectively, are key properties[1,2,4]. Different characterisations (thermal, mechanical, biological and morphological) were performed to evaluate the hybrid structures properties.

RESULTS AND DISCUSSION:

The current study demonstrates the impact of using different strategies to combine two widely studied materials into hybrid structures on several properties. The hybrid electrospun meshes obtained show different properties and performances being the most promisors the Multilayer and Blend structures. Both have shown to have a great potential as wound dressings due to their ideal water vapor permeability rate, adequate water uptake, hydrophilicity, non-toxicity and capability to promote fibroblasts attachment, proliferation and ECM production (fibronectin). The multilayer structure has the particularity of being easier to handle due to its multiscale fibers and, therefore, ideal to be used as wound dressing.

CONCLUSION:

Hybrid structures were successfully developed combining the major advantages from individual PCL and Gelatin electrospun meshes make possible to obtain constructs with good mechanical and biological performance, fitting the requirements to promote an optimal wound healing process.

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Poster presentation

401 Magnetic stimulated cryogels to enhance stem cell differentiation

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INTRODUCTION:

Magnetic nanoparticles are used in several fields like electronics, catalysis technology as well as fundamental medicine and biomedical. Their potential use have also investigated in applications such as controlled release, cellular hyperthermia and magnetic resonance imaging. However, their applications in tissue engineering and regenerative medicine are still limited. Magnetic nanoparticles can be used, as a novel and efficient way, for making such a mechanotransductive effect on cells to promote growth or differentiation.

METHODS:

Super-paramagnetic particles were synthesized by co-precipitation method[1]. Magnetic cryogels were prepared by slight modification of previous report[2]. Chemical and structural characterizations were performed with X-RD, VSM, TEM and SEM. Mesenchymal stem cells were seeded on magnetic cryogels ($1x10^5$ cell/well) and were cultured in osteogenic and chondrogenic media up to 28days under three conditions: (I) Non-magnetic field (II) 1000 Gauss static magnetic field (III) 4000 Gauss static magnetic field. Stem cell behavior changes and metabolic activities through osteogenic and chondrogenic ways were examined by, proliferation and doubling time calculations, morphological observations, gene expression profiling and quantitative histochemical staining.

RESULTS AND DISCUSSION:

According to TEM and VSM results, these particles have 10nm average diameter and have 55emu/g magnetic strength which is well-enough for cellular applications and cryogels have proper pore formation (Figure 1).

According to results demonstrated here revealed that batch magnetic field by force of our magnetic cryogels have significant influence on stem cell differentiation behavior. Cells seeded on magnetic cryogels exposed to certain magnetic field and showed significantly better osteo or chondro-like behavior with respect to cells that were seeded on non-magnetic cryogels.

CONCLUSION:

Within the lights of the results, these magnetic cryogels are promising material for hard tissue regeneration as they promote cell proliferation and growth as well as differentiation with the ability to generate proper physical stimuli throughout the cells.

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ACKNOWLEDGMENTS:

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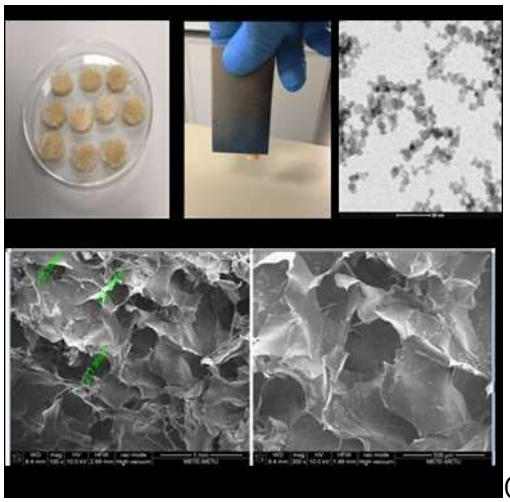
Picture 1:  Caption 1: Figure 1 (a-b) Magnetic cryogels (c) TEM analysis of synthesized magnetic nanoparticles (d-e) SEM images of magnetic cryogels

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Poster presentation

405 XS-Graft: electrospun grafts for hemodialysis access

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INTRODUCTION:

In end-stage renal diseased patients (ESRD), renal functionality is below 10% of the normal capacity and therefore require hemodialysis (HD). A proper vascular access is necessary to provide sufficient blood flow for HD. In some patients, the connection of autologous arteries to a vein (arteriovenous fistula, AVF) is not feasible (1). For these patients, a polymeric, non-biodegradable construct is inserted between an artery and a vein, creating an artificial fistula and increasing blood flow for HD cannulation. Currently, grafts are made of expanded polytetrafluoroethylene (ePTFE), but thrombosis and neo-intimal hyperplasia are the most common cause of graft failure. In the XS-Graft project, we are developing a non-biodegradable, electrospun polyurethane-based graft for hemodialysis access.

METHODS:

Grafts with varying fiber diameters (polyurethane1: 1.5 µm and polyurethane2: 4 µm fibers) were produced by electrospinning. Grafts were analyzed by Scanning Electron Microscopy (SEM) and mechanical properties were analyzed by biaxial tensile tester. In case the fibers were doped with additives, cytotoxicity was tested on endothelial cells (Lonza) and antimicrobial activity was investigated on E. coli. Electrospun grafts were implanted in a rat aorta interposition model.

RESULTS AND DISCUSSION:

1.5 fiber diameter grafts provided a higher Young's modulus and showed a reduced dilation of the graft diameter after 1 month implantation. Also, mechanical properties seemed to increase during implantation. Endothelial cells showed an elongated morphology and formed a confluent monolayer on the 1.5 µm fiber group. These results lead to conclude that a 1.5 fiber diameter was preferred over the larger fiber diameter. New recipes were developed to achieve similar fibers diameter, by using different polyurethanes. Doping with additives was sometimes necessary to improve the spinnability of the material and obtain homogeneous meshes. Additives exerted limited to no cytotoxic effect and a variable antimicrobial activity at the highest concentration used.

CONCLUSION:

These results indicated a preferred fiber size of 1.5 µm diameter and new recipes were developed to obtain electrospun meshes of different polymers with the target fiber size. Those new recipes are currently under evaluation in a new rat aorta interposition model. Additives addition proved to be useful both for ameliorating the spinnability of the material as well to include additional antimicrobial functionality in the electrospun graft.

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Poster presentation

412 New strategies for novel cell-adhesive and protein-repellent surface functionalizations

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INTRODUCTION:

The successful incorporation of dental or orthopedic biomaterials in the human body depends strongly on the integration of the implant in the surrounding tissue. In this process, the cell adhesion is governed both by the physicochemical properties of the substrate as well as by the initially adsorbed protein layer[1, 2]. The surface functionalizations are known to affect further the cell physiology such as signaling events[3].

Thus, this study focuses on the interplay of physicochemical surface properties, protein adsorption and cell physiology on novel surface modifications.

METHODS:

Silicon- and titanium-based model substrates were functionalized with the following organic molecules with terminal amino groups but different structure (Fig. 1): a linear poly(ethylene imine) (PEI) polymer (a), N,N'-bis(3-aminopropyl)-1,3-propanediamine (APD), an oligo(propylene imine) (b), fourth-generation poly(propylene imine) dendrimer (PPI) (c) and fifth-generation polyamidoamine (PAMAM) dendrimers with terminal amino or carboxylic acid groups (d). Physicochemical properties were studied by IR spectroscopy, an amino group detecting assay, water contact angle measurements and zeta potential analysis. Protein adsorption was investigated with the bicinchoninic acid (BCA) assay and tensiometry, studying adsorption of human serum albumin (HSA, pI 4.7) and lysozyme (pI 10.9) as well as from human whole saliva and fetal bovine serum (FBS). To analyze the cell physiology human osteoblasts MG-63 were cultured within 24 h on the functionalized substrates. The cell adhesion, morphology, cellular organization and function, like proliferation or cell signaling were determined by scanning electron microscopy, confocal laser scanning microscopy and flow cytometry.

RESULTS AND DISCUSSION:

All investigated surfaces exhibited hydrophilic properties (< 60°), but strongly differing amino group densities and electrophoretic properties. In the protein adsorption experiments, the APD and PEI polymer coated surfaces showed protein-repelling properties, whereas large amounts of protein adsorbed on PPI-G4 substrates from HSA solution and FBS. The hydrophilic surface properties improved the cellular adhesion and spreading.

CONCLUSION:

In summary, APD and PEI polymer functionalized surfaces are thus promising surface coatings for further application regarding their non-fouling properties. The novel surface functionalizations control the cellular behavior such as cell-adhesive properties.

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ACKNOWLEDGMENTS:

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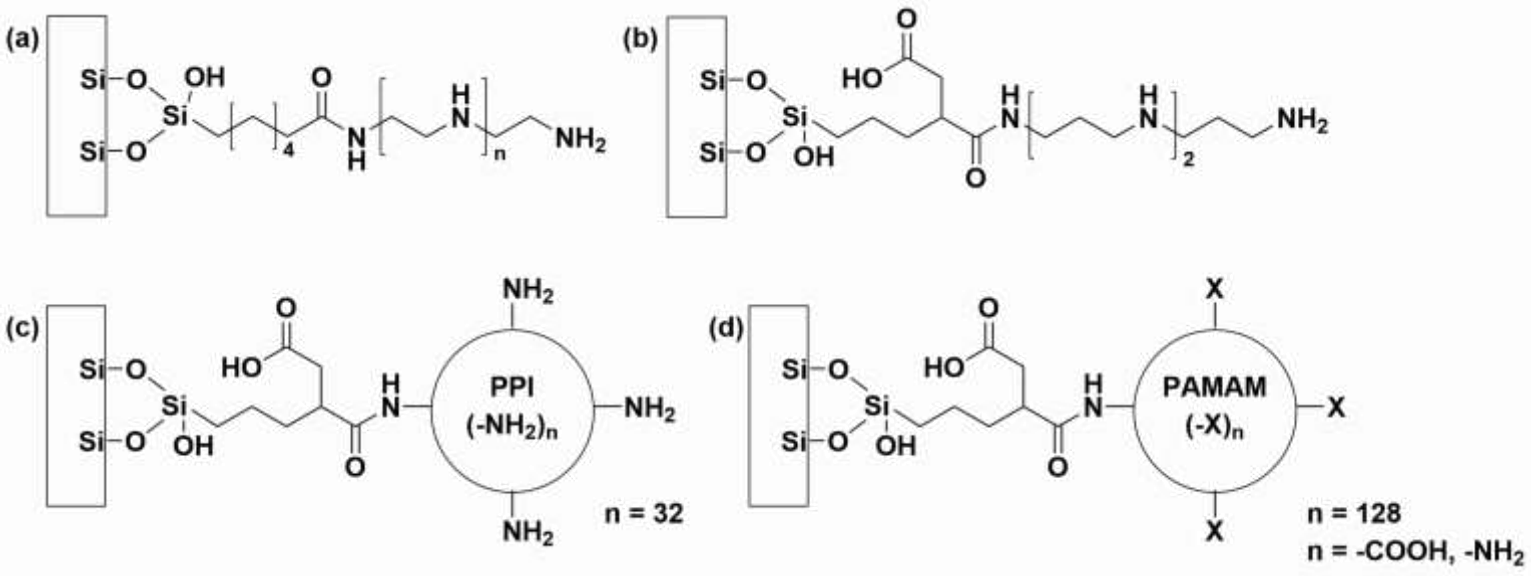

Picture 1: Caption 1: Figure 1. Overview of the surface modifications.

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Poster presentation

414 Candidates for biomedical applications based from castor oil polyurethanes with polycaprolactone diol and chitosan

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INTRODUCTION:

Polyurethanes are widely used in the development of medical devices due to their biocompatibility, degradability, non-toxicity and chemical versatility[1]. The different properties of polyurethanes usually depend on the type of the polyol, the diisocyanate and additives used in the synthesis. Some medical devices of polyurethanes are: catheters, artificial heart valves, orthopedic prostheses, wound closure mechanisms,…[2]. The objective of this research was to evaluate the effect of the incorporation of polycaprolactone-diol (PCL) and chitosan (Ch) on the mechanical and biological properties of the polyurethanes to identify the optimal ones for applications such as wound dressings or tissue engineering.

METHODS:

Polyurethanes were obtained from castor oil (*Ricinus communis*) (PU), and isophorone diisocyanate, in a 1:1 ratio of NCO/OH, with incorporation of PCL (15%w/w) and Ch (3%w/w). Polyurethanes were characterized by stress-strain (ASTM-D638-10), contact angle by sessile drop method, thermogravimetric analysis (ASTM-D6370), differential scanning calorimetry, water uptake and *in vitro* degradation by PBS and enzymatic processes. *In vitro* biological

properties were evaluated by a 24h cytotoxicity test using the colorimetric assay MTT with cell line L-929 (ATCC® CCL-1, mouse embryonic fibroblasts).

RESULTS AND DISCUSSION:

The addition of Ch increases the tensile strength of polyurethanes by 30%. The contact angle decreases 10 and 17 degrees with the addition of PCL and Ch, which seem to confer more hydrophilicity to polyurethane. The percentage of water uptake after 24h improves with the addition of Ch (1.2%). The highest value of the percentage of enzymatic degradation with an esterase from porcine liver was presented by the materials with addition of Ch (0.9%) after 7 days of evaluation (Figure 1). The *in vitro* biological properties test showed values higher than 80% of cell viability, indicating that the materials are not toxic for the L-929 fibroblasts. Polyurethanes loaded with Ch showed improved mechanical and biological properties of interest for a wide range of applications[3].

CONCLUSION:

The incorporation of PCL and Ch to polyurethanes did not generate a cytotoxic effect on the evaluated cell line. The assessed polyurethanes are suggested as possible candidate biomaterials for wound dressings due to their improved mechanical properties and biocompatibility.

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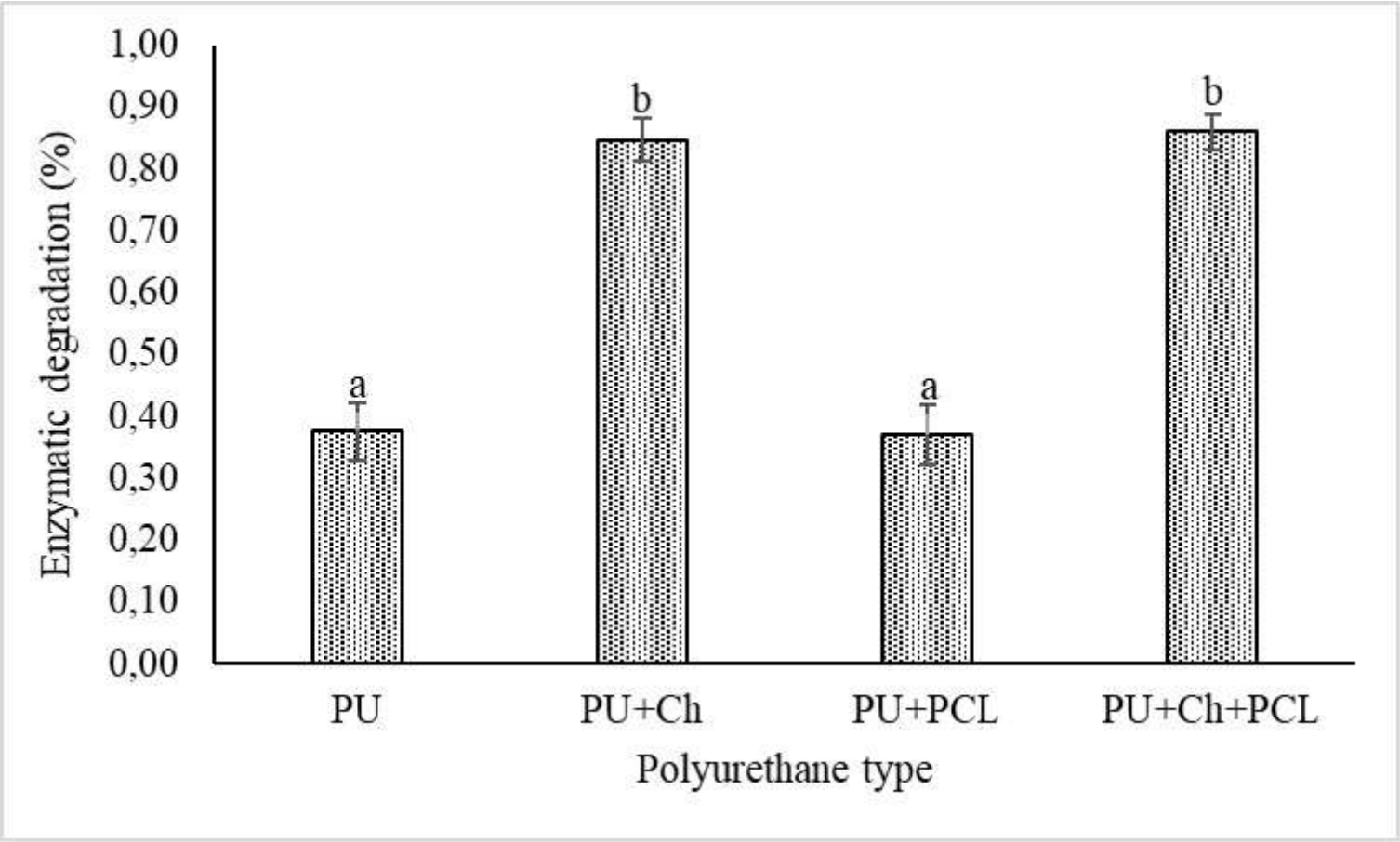
ACKNOWLEDGMENTS:

The authors thank the Universidad de La Sabana, Colombia, for financing research project ING-176-2016, Colciencias for the doctoral scholarship under grant 617-2 of 2014, and the Universitat Politècnica de València, Spain, for assistance and advice with the equipments.



Picture 1: Caption 1: Figure 1. Enzymatic degradation of polyurethanes by esterase at 7 days: mean±SD (n=4). Letters indicate significant differences.

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Poster presentation

416 Sodium alendronate loaded degradable microparticles immobilized on titanium dioxide scaffolds for the treatment of bone defects

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INTRODUCTION:

Three-dimensional scaffolds are beneficial to support tissue healing in bone defects that fail to heal properly. This process can be enhanced with drugs that augment bone turnover, e.g. sodium alendronate (Aln). Aln, a popular antiosteoporosis drug, exhibits strong inhibitory effect on bone resorption performed by osteoclasts[1]. TiO_2 scaffolds, which are characterised by microstructure and mechanical properties similar to those of healthy trabecular bone, were found biocompatible *in vitro* and *in vivo*[2].

The aim of this study was to extend functionality of TiO_2 scaffolds by providing them with therapeutic function. Thus, on the pore walls of TiO_2 scaffolds Aln-loaded resorbable microparticles (MPs) were co-immobilised with collagen type I in order to achieve a system, which supports osteoblast adhesion and proliferation but inhibits osteoclast activity by providing required doses of Aln.

METHODS:

TiO_2 scaffolds were obtained by sponge replication method and sintered as described earlier[2]. Poly(L-latide-*co*-glycolide) (85:15, Mn=100 kDa, d=1.9) MPs loaded with Aln were obtained by solid-in-water emulsification. MPs were attached to the scaffolds pore walls by coating with human recombinant collagen solution (hrColl, 40 µg/mL). Aln release kinetics to PBS up to 60 days and cytocompatibility on MG-63 osteoblast-like cells in contact with extracts from the scaffolds were studied. The influence of the scaffolds' extracts on model bone resorbing cells, i.e. peripheral blood mononuclear cells (PBMCs) differentiated with M-CSF and RANKL, was investigated.

RESULTS AND DISCUSSION:

The MPs were effectively immobilized on the surface of the scaffolds' pore walls by hrColl as shown by SEM observations. Drug release from the scaffolds was characterised by initial burst of 20% dose followed by a sustained release phase. AlamarBlue and live-dead tests on days 1, 3, 6 showed that Aln in concentrations of 5 µg/mL and 2.5 µg/mL was not cytotoxic for MG-63 osteoblast-like cells. Nevertheless, those Aln concentrations prevented RANKL-induced formation of osteoclast-like cells from PBMCs, as shown by reduced fusion capability and decreased activity of TRAP 5b related to total DNA content.

CONCLUSION:

Developed system provided sufficient doses of Aln inhibiting osteoclastogenesis, reducing osteoclast activity, but at the same time not affecting osteoblast functions, which is beneficial in the treatment of bone tissue defects.

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ACKNOWLEDGMENTS:

The authors thank National Science Center, Poland (Grants No.2013/09/N/ST8/00309 and No.2014/14/M/ST5/00649), BMBF, Germany (Grant GoBone, No.01DS16010A) and Norwegian Research Council (Grant No.228415) for financial support.

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Poster presentation

422 Anticoagulant electrospun nanofibers based on sulfonated chitosan

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INTRODUCTION:

In recent years, significant efforts have been devoted to develop biomaterials with anti-thrombogenic and anti-calcification properties for cardiovascular applications. In this work, we aimed to synthesize two N-sulfonated Chitosans (CHTS) through a reductive amination reaction using formylbenzene mono- and di-sulfonates reactants under mild conditions and then to process both polymers in the shape of nanofibers (NFs) by electrospinning in order to develop scaffolds with anticoagulant properties.

METHODS:

Synthesis of CHTS: raw CHT was dissolved in aqueous acetic acid 1% (v/v). Two reagents, 2-formylbenzenesulfonic acid, sodium salt (BZ1S) or 2,4-formyl-1,3-benzenedisulfonic acid, disodium salt (BZ2S) dissolved in methanol were added to the CHT solution in order to prepare N-mono-sulfonated (CHT1S) and N-di-sulfonated (CHT2S) CHT derivatives, respectively. After 5 minutes of reaction at room temperature, the reductive reagent, sodium cyanoborohydride, was added. Finally, the resulting precipitate was filtered, washed with water and freeze-dried. *Electrospinning*: Both CHT1S and CHT2S dissolved in NaOH (0.05M) were blended with Poly (ethylene oxide) (PEO) using a ratio of CHTS/ PEO (8/2). The resulting NFs have been crosslinked with genipin before physico-chemical and biological characterization.

RESULTS AND DISCUSSION:

The sulfonate groups introduction in raw CHT skeleton was confirmed by ^{1}H-NMR, FT-IR and elemental analysis. It has been observed that the degree of substitution (DS) was dependant on the initial ratio CHT/BZS introduced. Contrarily to CHT, CHTSs were soluble over a wide range of pH, highlighting its amphoteric character. Indeed, zeta potential measurement showed that, in contrast with raw CHT that has neutral electric charge around the physiological pH, all sulfonated derivatives presented negative ZP. Viability assay (alamar blue method) using fibroblasts NIH/3T3 evidenced the cytocompatibility of synthesized CHTS. Besides, the anticoagulation assay revealed that the clotting time significantly increased with the DS and the concentration of CHTS.it also reveals that the anticoagulant activity of CHT2S was greater than CHT1S due to the higher quantity of sulfonate groups in CHT2S. The optimal electrospinning conditions were determined for CHT2S and homogeneous nanofibers were obtained.

CONCLUSION:

A novel sulfonated-CHT derivative was successfully synthesized and exhibited an excellent anticoagulant activity. Stable and defect-free nanofibres of CHT2S were obtained by electrospinning. More physico-chemical (stability of Nanofibers, …) and haemocompatibility (haemolysis and coagulation assays, platelet adhesion and activation, etc.) studies are in progress.

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ACKNOWLEDGMENTS:

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Poster presentation

431 Copper nanoparticles obtained by laser ablation in liquids for dental applications

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INTRODUCTION:

Noble metal nanoparticles are having great attention due to their remarkable optical, electrical and antimicrobial properties[1]. In particular, the antibacterial activity of copper nanoparticles is potentially effective against different bacterial pathogens[2]. But not only the size is important, also shape of the particles which depends on the fabrication method. Laser ablation of solids in liquids (LASL) is a key technique for controlling size and shape of nanoparticles, making possible obtaining pure nanoparticles with no need of chemical precursors or chemical reactions which can contaminate the obtained material, of special importance in the case of biomedical applications.

METHODS:

Copper foils with 99.99% of purity were used as laser ablation targets. The targets were submerged in two different solvents (pure deionized water and propyl alcohol) and ablated by two different laser sources: a nanosecond laser (532nm) and a picosecond laser emitting radiation at 1064nm.

Size, morphology, crystalline phases and optical properties of the obtained nanoparticles were studied by means of transmission electron microscopy (TEM), high resolution transmission electron microscopy (HRTEM), energy dispersive X-ray spectroscopy (EDS) and UV/VIS absorption spectroscopy. The antibacterial properties of the Cu nanoparticles were evaluated in dynamic conditions using a Gram negative bacterial strain *Porphyromona gingivalis* one of the main pathogens responsible for inducing periimplatitis.

RESULTS AND DISCUSSION:

The obtained colloidal solutions consisted of Cu nanoparticles showing rounded shape with diameters ranging from few nanometers to 50nm. The nanoparticles obtained in propyl alcohol are smaller and more dispersed as shown in Figure 1. The EDS analysis reports low presence of oxidation, being lower in the case of those obtained in propyl alcohol. The HRTEM confirms that all nanoparticles obtained are crystalline.

Microbiology tests confirm the strong activity of Cu nanoparticles against *P. Gingivalis*

CONCLUSION:

Feasibility of LASL to produce copper nanoparticles without any additional chemical compound is demonstrated. The type of solvent has more influence than the laser source, and determines to a large extent the characteristic of the nanoparticles obtained. Bactericidal activity of produced Cu nanoparticles against P. gingivalis encourages their use as anti- periimplantitis agent in oral implants.

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Picture 1:

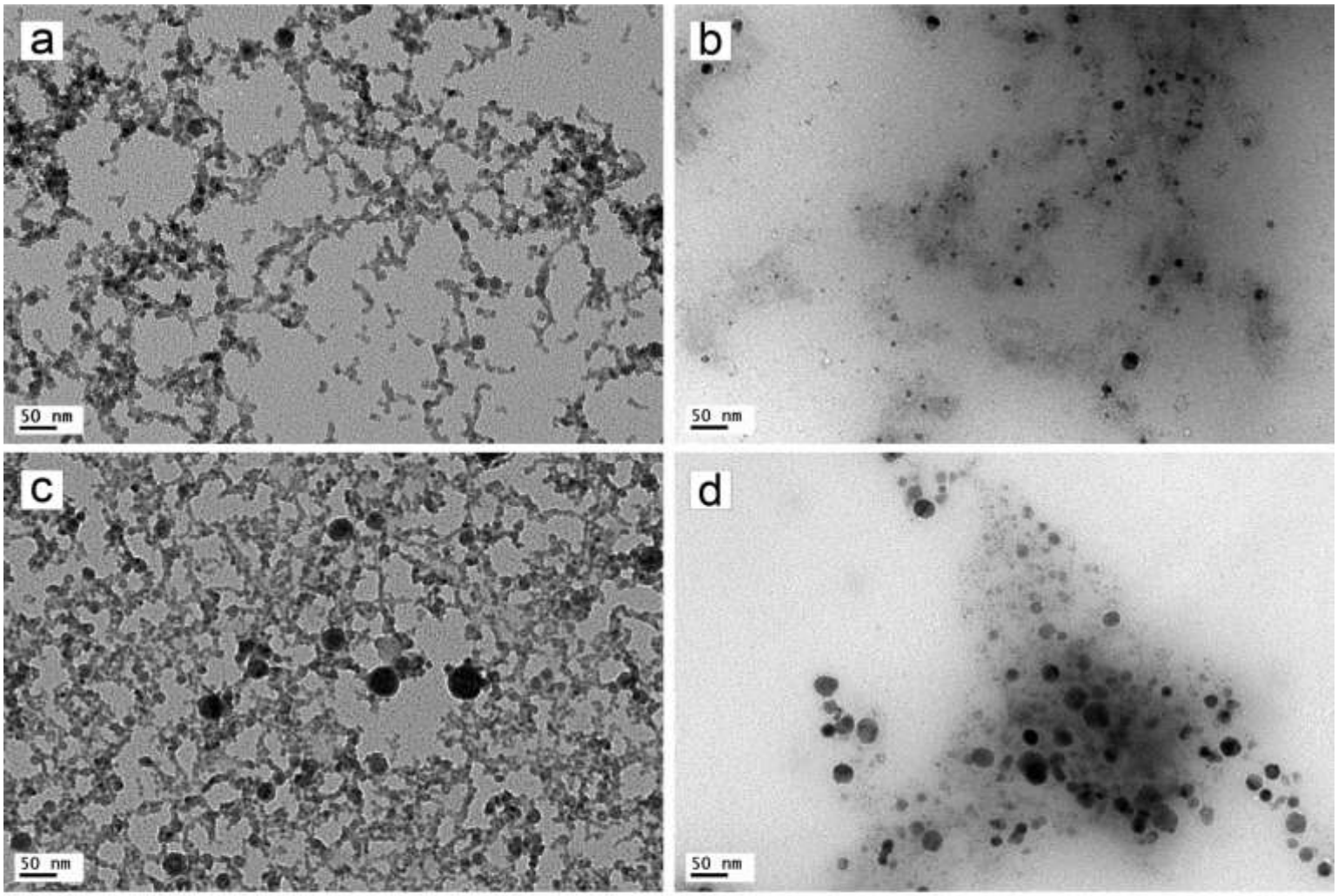

Fig.1 TEM micrographs of Cu nanoparticles obtained by laser ablation using (a) pulse nanosecond laser in water, (b) pulse nanosecond laser in propyl alcohol, (c) pulse picosecond laser in water, (d) pulse picosecond laser in propylacohol.

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Poster presentation

451 Surface Chemistries Influence Fibroblast Differentiation into Myofibroblasts: a High Throughput Approach

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INTRODUCTION:

The implantation of foreign material into a host body, as used in medical devices, is known to induce an immune response. This immune response, known as the foreign body response, dictates whether biomaterial implantation leads to successful material integration and healing or towards encapsulation of material leading to fibrosis and rejection[1].

Fibroblasts have been shown to play an important role in both healing process and fibrotic capsule formation depending on the nature of biochemical stimuli, and the physicochemical characteristics of the material. Conversion of fibroblasts to myofibroblasts is associated with enhanced ECM secretion and could lead to fibrotic capsule formation. Regulation of fibroblast to myofibroblast differentiation using biomaterial surface characteristics such as surface chemistry to control fibrosis is the goal of this work. To identify such a material high throughput screening was used to identify polymers that modulate fibroblast differentiation into myofibroblasts.

METHODS:

Human fibroblasts (MRC-5 cell line) were seeded onto a polymer microarray consists of approximately 300 different homo-polymers from the acrylate and methacrylate family[3]. After overnight culture to allow cell attachment, cells were stimulated with different concentration of Transforming Growth Factor Beta-1 (TGF-β1). After 72 hours of culture, the cells were stained for alpha – smooth muscle actin (alpha-SMA), F-actin and nuclei to assess myofibroblast differentiation and cell morphology.

RESULTS AND DISCUSSION:

High throughput screening enables assessment of biological responses to a large number of conditions in a time and cost efficient manner. In this study we assessed the ability of 300 homo-polymers in modulating fibrotic responses in human fibroblasts which identified a number of polymers with the ability to modulate fibroblasts to myofibroblast differentiation in response to TGF- β, figure 1. Future work will co-polymerise these hit homopolymers to explore synergies that may result in improved materials for biomaterials with application in wound healing.

CONCLUSION:

Changing the surface chemistry of materials could provide a means for modulating deleterious fibrotic responses by directly influencing fibroblast differentiation and proliferation. Future work will focus on fuller characterization of fibroblast response to the identified polymers.

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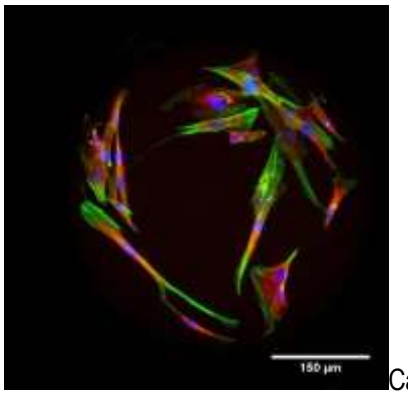
Picture 1: 

Caption 1: Adhered hFibroblasts on polymer 79 having elongated, spindle like morphology and expression of alpha - SMA.

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Poster presentation

452 Smart Carbohydrate Nanonets: a sweet trap to sour the cancers' progression

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INTRODUCTION:

Biocatalytic self-assembly(BSA) has emerged as an effective and selective therapy aganist tumor progression.[1-3]It relys on the use of so-called pre-gelators: amphiphilic molecules(usually peptide amphiphiles, PA) with incorporated enzyme-sensitive units. Upon enzymatic action, the amphiphilicity of these molecules is rebalanced and they become gelators, i.e. able to self-assemble into nanofibers and gels. Among the enzymes, phosphatases overexpress by different cancer cells(e.g. alkaline phosphatase, ALP) are the most used ones in BSA cancer treatment. Recently, we suggested a simple carbohydrate amphiphile(CA) as an alternative of peptide pre-gelators and demonstrated its potential for treatment of osteosarcoma using BSA approach.[3]Given the similarity of the used amphiphile with glucose and the energetic dependence of cancer cells on an accelerated glycose metabolism, we investigated the mechanism(s) by which the amphiphile influence the apoptosis of cancer cells that have a glycolytic phenotype.

METHODS:

We have investigated four type of cancer cells that differ by the expression of ALP and glucose transporters(GLUTs): Bone(SaOs2) and liver cancer cell lines(HepG2) overexpress ALP and GLUTs while a breast cancer cell line(MDAMB468) overexpress GLUTs but not ALP. Chondrocyte cell line(ATDC5) was used as a control that does not express either ALP or GLUTs. The effect of three simple glucose amphiphiles that differ by the incorportated enzymatic-sensitive unit(phosphate, sulphate or none) on the glucose metabolism, activity and apopotosis of these cell lines was studied.

RESULTS AND DISCUSSION:

We observed formation of nanofibers/hydrogel in the pericellular space of the cells overexpressing ALP when these were exposed to the phosphated glucose amphiphile. The expression of GLUTs by the tested cancer cell lines increased in the presence of the CA but the uptake of glucose was reduced. These results suggest possible blocking of GLUTs and glycolysis by the used glucose analogues. The observed by us mechanism for CA is different that the reported for the PA: peptide analogues are usually internalized by the cells and self-assemble/gelate in the intracellular space while CA act only in the pericellular space.

CONCLUSION:

The results suggested that two synergistic mechanisms induce the selective apoptosis of cancer cells: formation of pericellular net that traps selectively the ALP overexpressing cells and blockage of glucose transport in these cells.

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Poster presentation

473 Recovery kinetics of a 3D printed poly(D,L-lactide) based shape memory polymer

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INTRODUCTION:

Shape memory polymers (SMPs) are materials able to retain a deformed state until specific stimuli trigger recovery to the original geometry[1]. Achieving good predictability over the shape recovery is fundamental for the application of biocompatible SMPs in medical practice. In this study we fabricate SMPs from a poly(D,L-lactide) (PDLLA) photocurable resin and empirically assess the range of times over which the original shape is recovered.

METHODS:

Specimens were obtained through photopolymerisation of 3 kg/mol dimethacrylate terminated PDLLA resin on an Autodesk Ember stereolithography apparatus. Their shape memory behaviour was characterised by means of dynamic mechanical thermal analysis (DMA Q800, TA Instruments) in tension mode. All specimens were strained by 10% after equilibrating at 80 °C for one hour. The deformed specimens were vitrified at 0 °C for one more hour, then unloaded and the shape recovery was monitored at 37, 45 or 50 °C. We characterised the specimens' glass

transition temperature (T_g) by means of the same DMA apparatus. The same photocurable resin was used to 3D print objects with simple geometry.

RESULTS AND DISCUSSION:

All specimens recovered 90% or more of the original shape and were characterised by high values of shape fixity[2], i.e. the percentage of deformation recovered right after unloading was negligible. When heated up to 45 and 50 °C the specimens recovered 90% of deformation in 35 and 11 minutes, respectively. These temperatures are close to the measured T_g of 58 °C and therefore result in high chain mobility and fast relaxation. On the other hand, when equilibrated at 37 °C the specimens recovered in about 600 minutes, as the network is mostly glassy and segmental relaxation is hindered. Furthermore, 3D printing of simple objects was successful.

CONCLUSION:

We studied the recovery kinetics of PDLLA based SMPs and empirically assessed recovery time at 50, 45 and 37 °C. The material would be suitable for body temperature applications requiring recovery within a day, biodegradability and high stiffness. We will move towards networks with longer recovery time at body temperature, while retaining biodegradability and mechanical properties.

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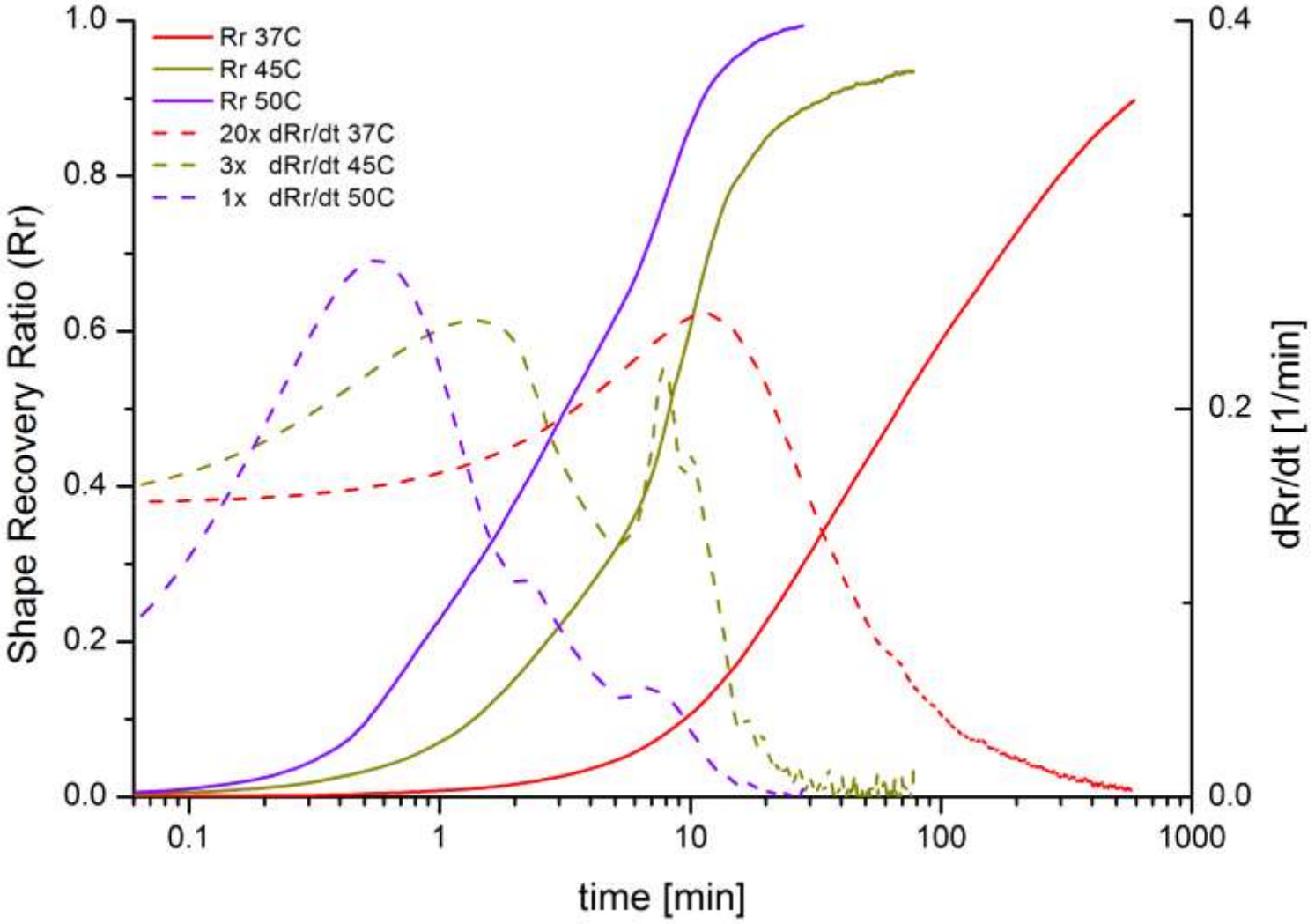

Picture 1: Caption 1: Figure 1 Shape recovery ratio (left, solid lines) and recovery rate (right, dashed lines, smoothed) of PDLLA specimens.

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Poster presentation

474 Combinatorial design of mechanically tough bone-like biocomposites

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INTRODUCTION:

Strategies in designing bone substitutes include creating a 3D-matrix which mimic the extracellular matrix, allowing for attachment of osteoprogenitor cells to proliferate and differentiate into osteoblasts whilst simultaneously providing requisite mechanical integrity. We previously reported a calcium metaphosphate (CMP) scaffold with porous architecture [1] that showed new bone formation within eight weeks in critical defects in a rabbit model with little evidence of any remaining scaffold. However, these scaffolds are brittle and limit manipulation to fit the defect shape and are mechanically weak. Based on the excellent in vivo performance of CMP, a combinatorial approach was used to design a hydrogel composite with CMP as filler particles and poly(vinyl alcohol) (PVA) as the matrix. PVA is biocompatible and has been widely used in biomedical and pharmaceutical industries; it comprises a simple structure, easily tailored and can form crosslinked structures without the incorporation of toxic additives [2]. Furthermore, a hydrogel nature enabling the incorporation of biomolecules to enhance bone formation.

METHODS:

PVA solutions were homogenously mixed with CMP powder in different ratios, then subjected to one or two freeze-thawing cycles. The parameters varied were the number of freeze-thawing cycles and PVA concentration, with the inclusion of particulate gelatin as porogen. The scaffolds were characterized using X-ray diffraction, infrared and Raman spectroscopy, mechanical tests, water uptake, differential scanning calorimetry, scanning electron microscopy, μCT, and cytotoxicity evaluation.

RESULTS AND DISCUSSION:

A set of elastomeric composites were obtained that had rigid sponge-like properties yet allowed it to be shaped to enable surgical manipulation. Results showed that the physical properties could be tailored by controlling the number of freeze-thawing cycles, concentration of CMP fillers and PVA. CMP particles distributed well in PVA matrix and the FTIR spectra showed evidence of a weak interaction between CMP-PVA, and PVA-gelatin. The EWC of the scaffolds showed a relationship with PVA concentration and the environment. Cytotoxicity assays demonstrated that the scaffolds were non-cytotoxic to human osteoblast-like cells.

CONCLUSION:

A new design of tough and tailorable hydrogel composites made of relatively affordable materials with simple and green chemistry have been developed. The hydrogel matrix would also facilitate incorporation of orthobiologics and drugs. These scaffolds are suitable as bone plugs for use in maxillofacial and other bone defects.

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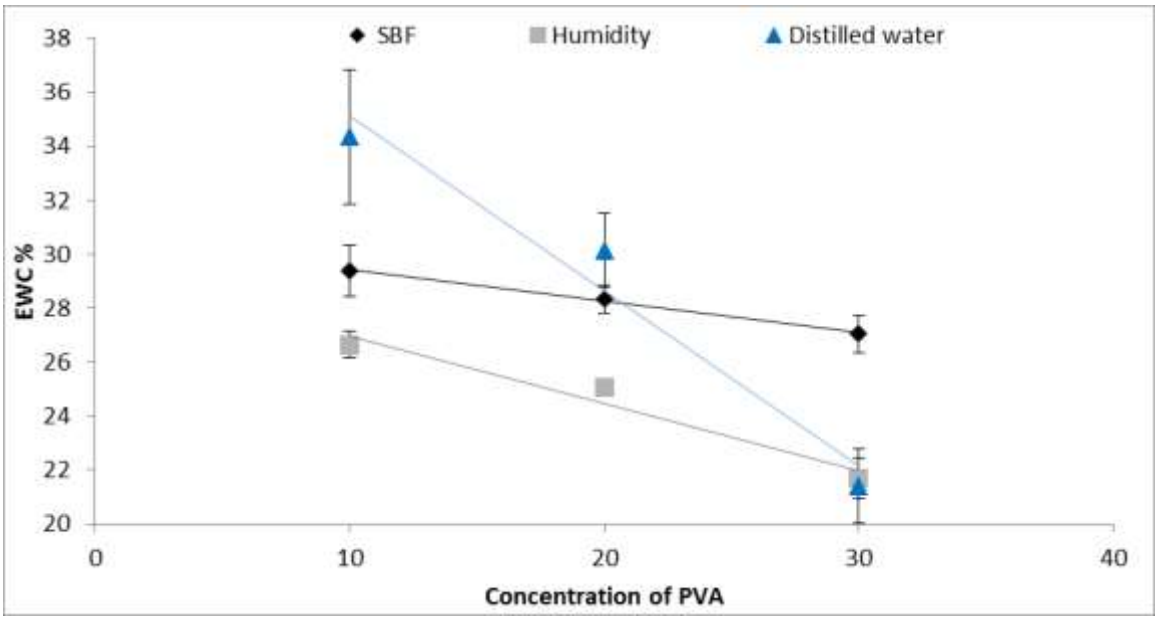
ACKNOWLEDGMENTS:

Authors acknowledge Indonesian Endowment Fund for Education and University of Botswana for funding LI and SN.

Picture 1:



Caption 1: Equilibrium water content (EWC) of PVA-CMP composites with varying PVA concentration in simulated body fluid (SBF), 100% humidity and distilled water

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Poster presentation

486 3D bioplotting together with calcium phosphate cements

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INTRODUCTION:

Calcium phosphates are among others the most promising biomaterials for bone regeneration, due to their property to resemble natural bone matrix. Calcium phosphate cements (CPC) with a retarded setting reaction offer the possibility of being processed by low temperature extrusion-based manufacturing, namely 3D plotting. We developed scaffolds based on CPC, which were co-extruded with cell-laden bioinks in order to achieve biphasic constructs for tissue engineering of bone and its interfaces.

METHODS:

CPC was manufactured by INNOTERE GmbH (Radebeul, Germany) and γ-sterilized. The bioinks used were 3 % alginate-9% methylcellulose (alg-mc) or 3 % Laponite-3 % alginate-3 % methylcellulose (Lap-alg-mc). These bioinks were developed previously [1,2]. Biphasic scaffolds were manufactured using the BioScaffolder 3.1 (GeSiM mbH, Radeberg, Germany) and characterized microscopically, mechanically and by microcomputed tomography.

RESULTS AND DISCUSSION:

Biphasic constructs consisting of CPC and cell-laden bioinks were plotted successfully. Previously, the setting reaction of CPC scaffolds was proven to be optimal in humid atmosphere, as microcracks are prevented, which impair mechanical strength [3]. It was investigated, that an initial incubation for 20 min in humid atmosphere was enough to prevent formation of microcracks. Cell viability of hMSC inside an alg/mc bioink was not affected in humidity up to a time of 30 min, defining a process window for 3D plotting of CPC-bioink scaffolds. Cell viability inside the bioink was not affected by CPC setting process. However, cell viability decreased in direct contact of CPC and bioink. CLSM micrographs revealed, that cells started to migrate from the bioink onto CPC between 7 days and 21 days of cell culture; there they attached and proliferated at the surface, compensating the initial drop of cell viability at the interface. The greatest advantage of bioprinting is the possibility to spatially define cell distribution. We developed a bioprinted osteochondral model with a highly porous CPC part mimicking subchondral bone and a dense, cell-laden layer, which resembles the chondral part. Moreover, by defined distribution of a cell-laden Lap-alg-mc bioink, a perfusable blood vessel was plotted together with the bone resembling CPC.

CONCLUSION:

CPC are novel stabilizing and shape-defining materials, that can be utilized in a bioplotting process.

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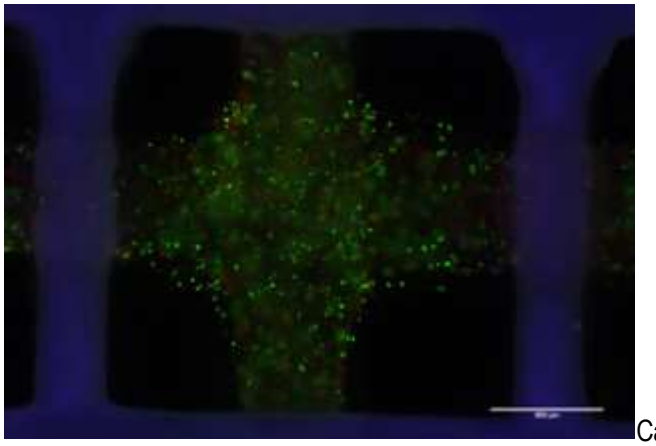
ACKNOWLEDGMENTS:

This project was supported by the German Federal Ministry for Economic Affairs and Energy (BMWi) in collaboration with the German Federation of Industrial Research Associations (AiF; ZIM project, contract No. KF3359301AK4).

Picture 1:  Caption 1: Live/dead image of biphasic constructs comprised of CPC (blue) and embedded cells (green, red).

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Poster presentation

487 CT and MR Imaging of 3D Printed Gelatin Methacrylate (GelMA) Scaffolds for Longitudinal Bone Tissue Engineering Applications

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[2]Radboud University Medical Center, Netherlands

INTRODUCTION:

Gelatin methacrylate (GelMA) is an inexpensive, cell-responsive hydrogel. 3D printing of GelMA has been previously demonstrated to promote cell penetration and the diffusion of nutrients.

METHODS:

Here, a 3D printed GelMA hydrogel is designed and tested for in vivo bone tissue engineering (BTE) applications. Furthermore, two imaging strategies for the non-invasive monitoring of the scaffold degradation are investigated in vivo: i.e. 1) combination of GelMA with gold nanoparticles (AuNPs) for computed tomography (CT) imaging; 2) T1-weighted and ultrashort echo time (UTE) magnetic resonance imaging (MRI). To this end, 3D printed GelMA hydrogels are implanted in cylindrical defects created into the condyle of rat model. Longitudinal follow-up is performed up to 8 weeks post-surgery.

RESULTS AND DISCUSSION:

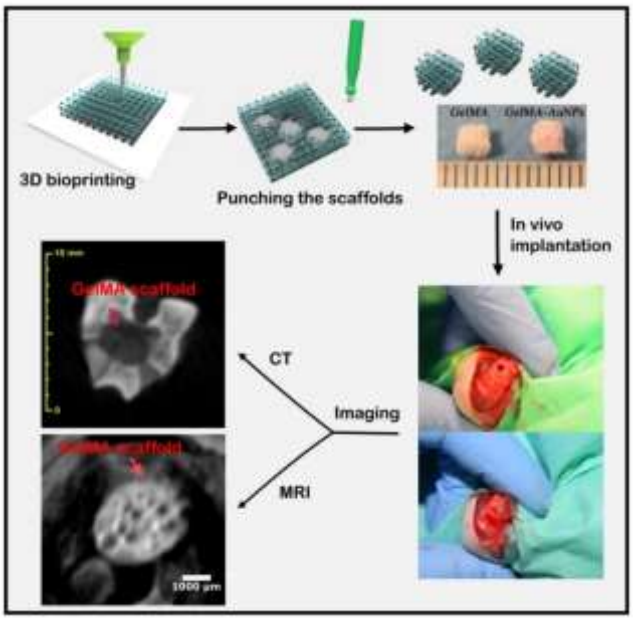
The in vivo assay proves excellent biological properties of the 3D printed GelMA scaffold and no signs of fibrotic encapsulation or inhibition of the bone formation as estimated via histology and micro-CT. Imaging assessment shows T1-weighted and UTE MRI can give morphological information of both GelMA and bone tissue during regeneration

CONCLUSION:

In conclusion, the herein designed 3D printed GelMA is proven to be a good candidate scaffold material for BTE purposes, while MRI is suggested as the optimal technique for its non-invasive longitudinal monitoring.

ACKNOWLEDGMENTS:

The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grants agreement No. 607868 (iTERM).



Picture 1:
Caption 1: Study design of 3D printinted gelatin methacrylate hydrogels in critical bone defect regeneration

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Poster presentation

488 Elaboration and characterization of an innovative composite based on polymer/bioactive glass for bone tissue engineering

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INTRODUCTION:

The use of bioactive glass has gained interests in the past years pertaining to their ability to release ions enabling osteoinduction[1]. The 13-93 glass is one such bioactive glass[2,3], however, the brittleness and hardness of the glass remain a challenge. To solve this problem, here we present the processing and characterization of two PLA/bioactive glass composites.

METHODS:

The composites, obtained by twin-screw extrusion, contained 30 wt% of either glass 13-93 or 13-93B20 (where 20% of the Si was replaced with B). An in vitro study was conducted in Simulated Body Fluid and TRIS solution. The physical and mechanical properties were characterized as a function of immersion time. Ion leaching was quantified using ICP-OES. Change in polymer molecular weight was determined by GPC to study polymer degradation induced by the materials' processing and/or the dissolution. Moreover, muscle myoblastic cells C2C12 and osteoblastic cells MC3T3-E1 were used to see cell osteogenic commitment. The release of mineral matter was observed thanks to the Red Alizarin Stain and confirmed by SEM-EDX.

RESULTS AND DISCUSSION:

Despite the molecular weight lost, PLA alone did not induce any change in the solution pH, whereas the bioactive glass led to a moderate increase in pH. This is due to the preferential leaching of Ca^{2+} and Na^{+} ions. Over the immersion period, the ion release is constant. SBF, however, contain Ca^{2+} and $PO4^{3-}$ and is employed to prove the ability of the bioactive glass to favor precipitation of HA. From mechanical properties point of view the presence of glass leads to increase in the rigidity. Upon immersion all composite maintained their mechanical properties even up to 10 weeks of immersion.The cell culture experiment has allowed relating the cell count, morphology and ability to differentiate to the concentration of ion leached out from each glass type in the culture medium.

CONCLUSION:

We demonstrate that the choice of the bioactive glass can lead to tailored release of ions. The change in the mechanical properties with increasing glass content and immersion time is discussed in light of the co-degradation of the polymer and the bioactive glass. The role of bioactive glass, in the cell activity and osteogenic potential will be discussed based on dissolution rate. These materials are promising, particularly in bone applications.

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ACKNOWLEDGMENTS:

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Poster presentation

490 A rabbit iliac artery model for anti in-stent restenosis devices evaluation

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INTRODUCTION:

In-stent restenosis, due to neointimal hyperplasia, remains a major issue after arterial stenting (1). New drug eluting stent are currently in progress but there is no gold standard for in vivo evaluation (2). This work aims at optimizing a double-injury rabbit iliac artery model for future in vivo evaluation of a simvastatin loaded electrospun membrane covered stent.

METHODS:

NiTiNOL stents (ZILVER FLEX, Cook®) were covered with electrospun membrane of chitosan (106000g/mol, 2.97% w/v), polymer of cyclodextrin (64650 g/mol, 3.3% w/v), polyethylene oxide (PEO, 900000 g/mol, 0.33% w/v) and simvastatin (5 wt-%). *In vitro* SV release study was studied in PBS/SDS 0,7 wt-% (pH 7.4, 37°C) using a fully automated flow-through cell dissolution apparatus coupled with UV spectrophotometer in a dynamic system (35 mL/min). Double injury rabbit iliac artery model was conducted with white New Zealand rabbits fed with 1% cholesterol and 4.9% coco oil during 7 weeks, following by 6 weeks of normal diet. At the end of the first week, bilateral iliac injury using a balloon 2,75 x 18 mm at 9 atm was performed, through left carotid access. Stents were implanted 8 weeks later using bilateral femoral access. Euthanasia was performed at the end of the 13th week. Angiograms were performed before and after balloon injury and stent implantation and before euthanasia to control arteries patency.

RESULTS AND DISCUSSION:

In vitro release study of SV from the nanofibers showed 0.34 ± 0.02 µg/mm2 (95.2 ± 5.3%) of loaded SV was released after 24 hours. Validation of rabbit model before *in vivo* evaluation of electrospun covered stent required 24 rabbits. After the first surgery, 4 animals died of unexplained death and 1 due to spinal cord ischemia. One rabbit died of iliac rupture after stenting and 5 of diet complications. We observed 2 failure of stenting. Histomorphologic analysis of tissues are in progress.

CONCLUSION:

Electrospinning of CHT/PCD/SV nanofibers was successfully performed on NiTiNOL stents and allows a sustained release of SV. Double-injury rabbit iliac artery model seems to be a good model for in-stent restenosis studies but require a learning curve. Further *in vitro* evaluation of nanofibers covered stents and *in vivo* experiments on this rabbit model will follow to assess efficiency and safety of the device.

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ACKNOWLEDGMENTS:

The authors thank University of Lille, Interreg 2 Seas IMODE program and the SCVE for their financial support.

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Poster presentation

496 Aerosol assisted atmospheric pressure plasma deposition of amine/amide-rich biocompatible coatings

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[2]Ghent University, Belgium

INTRODUCTION:

Amine/amide-rich coatings possessing good biocompatibility are of high interest in the medical field. A new type of coatings with high content of NH groups has been prepared in a single step plasma process. Deposition is

performed by atmospheric pressure plasma jet (APPJ) which is coupled to aerosol injection of liquid precursor[1]. The method is applied for the polymerization and deposition of N,N'-methylenebisacrylamide (MBA)[2].

METHODS:

The deposition was carried out for 1 min on PE substrate and He was used as process gas. The plasma is excited by a 10 kHz bipolar high voltage signal of 15 kV amplitude. The MBA precursor is injected in aerosol form into the APPJ. The surface chemistry of the coatings was determined by XPS and FTIR spectroscopy while biocompatibility of the coatings was tested qualitatively by an assay with STEM cells.

RESULTS AND DISCUSSION:

FTIR spectrum reveals precursor polymerization and formation of NH-rich coatings. Furthemore, although it is known that MBA is highly soluble in water based solutions, the plasma polymerized film has shown high stability since all of its characteristic FTIR peaks are detected even after immersion. The chemical composition of the coatings' surface has been studied by XPS analysis. The coating contains around 75 at.% of C, 14.7 at.% of O and 8 at.% of N (a significant drop with respect to the 15.2 at.% of N in MBA precursor). Additionally the deconvolution of high resolution N_{1s} peak reveals an high amount of amide groups, which can be indicative for a good biocompatibility of the coatings. A live/dead cell staining was used to evaluate cell viability by fluorescence imaging. MBA plasma coated samples are characterized by cells having extended shape and spreading over the surface of the film and the absence of dead cells, indicating good cell-substrate interaction and biocompatibility.

CONCLUSION:

For the first time an APPJ was used for depositing coatings with MBA as precursor. Up to 8 at.% of N containing bonds is determined in the coatings by XPS analysis. The deposited films are water stable and characterized by high biocompatibility.

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Poster presentation

497 Approximation of a surface solution for prothesis applications for young patients

Cristina Díaz, Gonzalo García

Asociation of Navarra Industry, Cordovilla-pamplona, Spain

INTRODUCTION:

The demand for reliable articular joints is relatively high for young and active patients to maintain their work and life style comfortably[1]. Titanium alloys are well known for prosthesis applications, specially for corrosion protection in contact with biological fluids. However, when wear and friction effects are relevants in articular joints, some abrasive wear particles and ion releasing might well appear, what might generate a premature fail of these prosthesis, specially in these kind of patients[2]. In the market, ceramic coatings such as coatings based on Diamond-like-Carbon (DLC) and other nitride coatings (CrCN, CrN, TiN) some used for friction coefficient and wear rate reduction and

alternatively, some oxides layers (zirconium oxides, Titanium oxides) might reduce tribological effects and improve corrosion problems[3].

METHODS:

In this context, some coatings based on a bilayer made of Ti and another metals such as Nb, Ta, Hf and Zr were developed by PVD (Physical Vapor Deposition) technology on Ti, TiAlV and CoCrMo substrates.

For characterization of the coatings and substrates, 10 samples for each bilayer and substrate were made. Then, hardness, wettabilitty, friction coefficient, wear rate and corrosion resistant characteristics were studied. Tribological and corrosion tests, were carried out at 37±2°C and using Hank's solution as electrolite.

RESULTS AND DISCUSSION:

Results of the different analysis showed excellent tribological behaviour of the coatings due to a significant reduction of friction coefficients and wear behaviour in comparison to all substrates in study. Therefore, corrosion tests results showed an improvement on all substrates in study, included Ti alloys.

After this work, it has been observed that it is possible to reduce friction and wear effects and moreover, introduce some improvements in corrosion resistant in the alloys in study, what provides a interesting step in the market of articular joints.

CONCLUSION:

In the context of this work, using bilayers based on Ti and another metal such as Nb, Ta, Hf and Zr might introduce an improvement in wear and corrosion resistant on the surface of Ti and CoCrMo alloys, what might will increase the longevity of the implant.

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ACKNOWLEDGMENTS:

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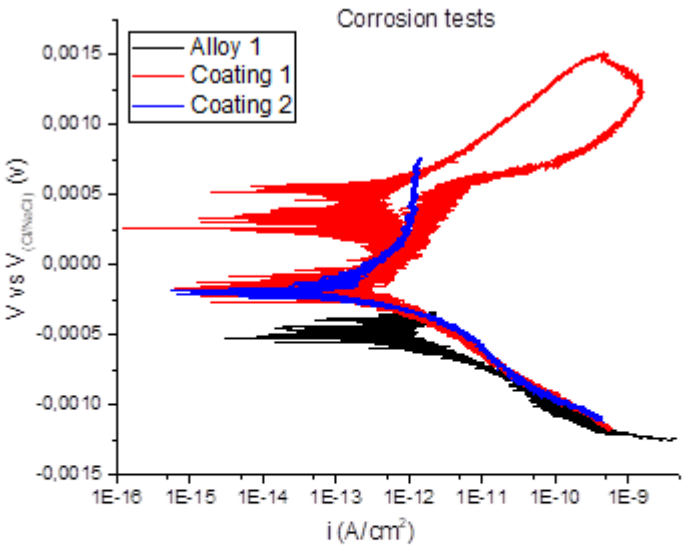
Picture 1:



Caption 1: Potentiodinamic tests of Ti alloys.

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Poster presentation

498 Application of spin-coated cellulose nanowhiskers to engineer skeletal muscle

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University of Manchester, Manchester, United Kingdom

INTRODUCTION:

Skeletal muscle has a high capacity for self-regeneration yet it has limitations. Accidents and disease can lead to muscle loss or the formation of scar tissue which restricts the original function of the tissue.[1] The application of a substrate designed to promote myogenic differentiation could aid the repair of the muscle's functionality.

It is proposed that aligned cellulose nanowhiskers (CNWs) could be used to direct broad area alignment of myotubes.[2] The combination of the aligned topography and adjustable mechanical properties in the polyelectrolyte layers could act as physical stimuli for myogenic expression.

METHODS:

CNWs with dimensions of around 5nm in diameter and lengths ranging from nanometres to microns can be produced through the partial acid hydrolysis of tunicin cellulose with sulphuric acid. The sulphuric acid gives a slight negative charge which enables the building of polyelectrolyte layers through dip coating with positively charged chitosan, resulting in a layered substrate. Orientation of the top layer CNWs was controlled through spin coating which produced a radially aligned pattern. Myoblasts, C2C12s, were cultured on a range of CNW substrates with different numbers of polyelectrolyte layers. Alignment and myogenic expression was investigated using bright field imaging and immunofluorescence staining respectively.

RESULTS AND DISCUSSION:

Orientation of the CNWs was confirmed using atomic force microscopy. Cells were observed to align end-to-end and elongate along the topography of the oriented CNWs and begin to differentiate in to myotubes. Immunofluorescence staining for the presence of myogenin, a muscle specific transcription factor, indicated that there is potential for myogenic differentiation to occur. Image stitching showed myoblast alignment and myotube formation on oriented substrates over a larger area (Figure 1).

CONCLUSION:

Oriented CNWs are functional in inducing myogenic differentiation and promoting broad myotube alignment, although the effect of the number of polyelectrolyte layers still needs to be determined. The application of the CNW substrate gives a basis for further investigation in to establishing optimal conditions for myogenic expression in human skeletal muscle cells and also stem cells in vitro.

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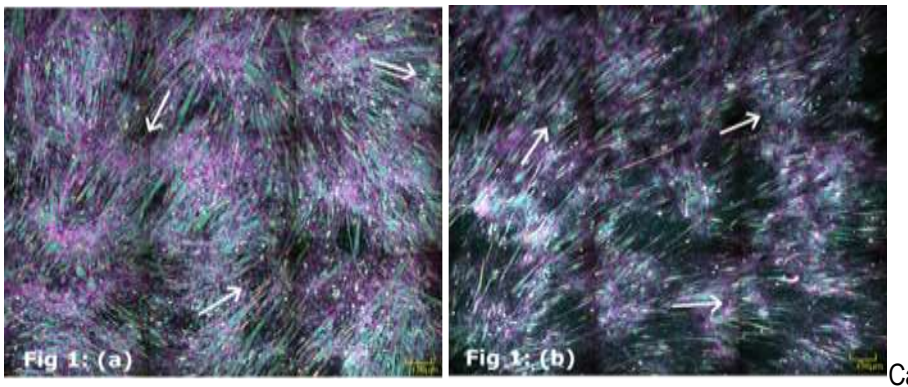
ACKNOWLEDGMENTS:

The authors would like to thank EPSRC for providing financial support for this project.

Picture 1: 

Caption 1: Figure 1: Fluorescent microscope images at 20 mag. (a) Myoblasts undergo local alignment on the un-oriented surface. (b) The radial pattern produced f

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Poster presentation

501 Osteogenesis and inflammation interaction on Chitosan-based 3D matrices bioactivated by two different signals

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[2]IPCB-CNR, Italy

INTRODUCTION:

In recent years several studies are aimed at investigating the *in vitro* effects of proinflammatory cytokines, on mesenchymal progenitor populations in order to determine how an inflammatory reaction might influence bone formation. The main goal is to promote tissue regeneration in areas of the body where there is significant damage due to an inflammatory reaction. However, pro-inflammatory cytokines combined with osteogenesis promoters are involved in several bone regeneration processes. Here, we propose a biomimetic chitosan-based scaffold as 3D matrix with two different osteoinductive signals that can promote bone tissue regeneration and treat bone damage related inflammation.

METHODS:

Biological evaluations by using hMSC cells to estimate the effect of Chitosan-based scaffold with the two bioactive signals (inorganic and inorganic) on cellular behaviour in terms of osteogenic differentiation related to inflammatory response were performed. In order to study antinflammatory and osteoinductive properties of bioactivated Chitosan-based scaffold, the modulation of several markers of inflammation involved in osteogenesis process such TGF-β was measured on a co-culture model consisting of osteoblasts and macrophages stimulated by lipopolysaccharide (LPS) for better mimicking damaged bone. Oxidative stress metabolites, interleukins and COX-2 expression related to osteogenic markers production were analyzed in order to understand the correlation between bone focal inflammation and bone regeneration.

RESULTS AND DISCUSSION:

Chitosan-based scaffolds bioactivated by using inorganic signals inhibit pro-inflammatory mediators production (IL-1β and IL-6), induce antinflammatory cytokynes generation (IL-10) and reduce nitric monoxide metabolites (nitrites) both on MSC and on co-cultures stimulated by LPS. Conversely, scaffold bioactivated by using organic signals are able to decrease pro-inflammatory markers without any effect on antinflammatory cytokines levels and on nitrites. However these scaffolds are able to maintain high levels of TGF-β that, combined with BMP-2, promotes osteogenesis [2]. In addition bioactivation of the surface of chitosan-based scaffold by using two different bioactive signals induces an osteoinductive effect on hMSC by promoting the expression of early (ALP) and later (OCN) signals of osteogenic differentiation than the no-biomimetic scaffold.

CONCLUSION:

Both beneficial properties of bioactivated chitosan-based scaffolds to inhibit inflammation and to promote an osteogenic biological response suggest the opportunity to develop multi-target devices able to regenerate damaged bone and to treat bone related inflammation.

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ACKNOWLEDGMENTS:

This study has received funding from the PNR-CNR Aging Program 2012-2018. The authors also thank Mrs. Cristina Del Barone Mrs Stefania Zeppetelli for supporting of our investigations.

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

511 Bioelectric testing on cutaneous wound model in vitro

Jerome Hunckler, Peter Butler, Brian Cousins

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INTRODUCTION:

Efficacious healing of chronic wounds is still a clinical challenge and the complications associated with impairment in wound healing carry a great financial burden, as well as, a negative impact on patient lifestyle. Wounded skin possesses a specific pattern of electrical activity called current of injury[1]. Several studies and reviews have concluded that bioelectricity is essential to healing and that applying electrical Stimulation (ES) may mimic the natural current of injury and enhance regeneration. The difference in methods and study design render the comparison between studies impossible[2]. The overall aim of this study is to set up a model of a cutaneous injury in vitro, optimise the ES parameters on this model using human dermal fibroblasts (HDF), and investigate the behaviour of HDF and cells derived from patient with specific diseases under optimised direct current and Naturepulse ® clinical device (Globe Microsystems Ltd, UK).

METHODS:

Adult HDF were cultured in low glucose Dulbecco's Modified Eagle Medium supplemented with 10% Foetal Bovine Serum and 1% Penicillin Streptomycin at a density of 20000 Cell per well in a 6 well plate. The cells were given 24 to 48hours to attach and create a confluent layer. The confluent cell layers were then scratched using a 200µL sterile pipette tip perpendicular to the bottom of the dish, generating a wound of around 750µm average width. Then, sterilised made platinum electrodes were carefully placed into the wells. A direct current density of 0.1 to 10µA/cm^2 was applied for 20min. Using time-lapse images, AlamarBlue fluorescence and DAPI/Phallotoxin immunostaining, we are observing the effects of electrical stimulation on cellular behaviour, metabolic activity and morphology over a period of 12 to 24 hours.

RESULTS AND DISCUSSION:

Herein we will present and discuss recent progress in this project toward choosing the right electrical stimulation parameters (direct and pulsed current) that can significantly enhance the healing behaviour of Human Dermal fibroblasts and cells derived from patient with specific diseases.

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Poster presentation

512 A new approach based on dendrimeric aptamers-modified magnetic nanoparticles for early detection and treatment of Alzheimer's disease.

Valeria Perugini[1], Manuel Gonzalez[2], Yolanda Piñeiro[2], Jose Rivas[2], Matteo Santin[1]

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[2]Departamento de Física Aplicada, Santiago de Compostela, Spain

INTRODUCTION:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder mainly characterised by the production and deposition of the β-amyloid peptide (Aβ) within the brain. To date, the diagnosis of AD is based on clinical examinations and identification of typical symptoms such as memory loss and disorientation[1]. The use of biological markers through MRI, PET scans and cerebrospinal fluid (CSF) analyses has also been used, but still face limitations in detecting Aβ at the earliest signs of AD impairment[2]. Recently, short peptides [KLVFF] have demonstrated to overcome these problems by inhibiting Aβ formation and aggregation both *in vivo* and *in vitro*[3]. However, their efficacy in the treatment of AD is restricted by their lack of stability. This work aims to validate the ability of dendrimeric KLVFF aptamers to interact with Aβ once covalently grafted on the surfaces of silica magnetic nanoparticles.

METHODS:

Poly (epsilon lysine) dendrons [gen3K] were designed to expose on the uppermost branching generation sixteen linear KLVFF peptides and to have an arginine root [R]. Rgen3K$[KLVFF]_{16}$ were synthesised using a modified microwave based solid phase peptide (Biotage, UK) on TentaGel S (-NH_2) resin. After being isolated and characterised by mass spectrometry and HPLC, they were grafted onto 1 mg/mL silica magnetic nanoparticles (SiO_2-MNP) using carbodiimide chemistry. A solution of 1mg/mL Aβ (Sigma, UK) was then added and incubated overnight. FTIR, Bradford assay and dynamic light spectroscopy were used to assess the functionalisation of SiO_2-MNP at every stage of their preparation.

The known linear sequence [GGKLVFF] was used as positive control.

RESULTS AND DISCUSSION:

The higher HPLC purity of Rgen3K$[KLVFF]_{16}$ (80%) led to the successful functionalisation of SiO2-MNP as confirmed by FTIR. Except for non-modified SiO_2-MNP, increase protein concentrations correlated well with the excess of free amine-specific functional groups exposed on Rgen3K$[KLVFF]_{16}$ – modified SiO2-MNP which led Rgen3K$[KLVFF]_{16}$ to efficiently bind Aβ. Thus, the higher affinity between Aβ and Rgen3K$[KLVFF]_{16}$ –modified SiO2-MNP induced formation of larger aggregates, often big enough to be detected with the naked eye when compared to the linear form.

CONCLUSION:

The use of Rgen3K[KLVFF]$_{16}$ – modified SiO2_MNP may provide a robust and evidence-based method for the detection of AD and indeed to be utilised in the context of clinical diagnoses.

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Poster presentation

514 Selective Targeting of Cancer Cells with Phenylboronic Acid Nanogels

Guangyue Zu, Olga Mergel, Philipp T. Kühn, Dirk Grijpma, Inge Zuhorn, Patrick van Rijn

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INTRODUCTION:

Selective targeting of cancer cells is highly desirable for developing better diagnostics and treatments. Particularly, inexpensive approaches that are combined with interesting nanotechnologies such as inorganic nanoparticles, polymersomes, and polymeric nanogels. Ligand-mediated active targeting could be achieved in the past by using phenylboronic acid (PBA) which have high affinity toward sialic acid (SA) overexpressed by carcinoma cells. SA is an anionic monosaccharide that is generally present in tumor associated carbohydrate antigens and the overexpression of sialylated glycans is important for the formation of metastases. Here we aim the integrate PBA with nanohydrogel particles which are known to be interesting responsive nanostructures for delivery, sensing, catalysis, and many more interesting applications[2]

METHODS:

The PBA-functionalized poly(N-isopropylacrylamide) (pNIPAM) nanogels with core-shell structure were synthesized via precipitation polymerization using 3-acryamidophenyl-boronic acid (APBA) as a comonomer. The copolymer shell was prepared by adding comonomer mixture after 30 min polymerization of pNIPAM core. The pNIPAM nanogel was prepared as a control. The morphological and physical properties of the nanogels were characterized by transmission electron microscope (TEM) and dynamic light scattering (DLS). The temperature dependent swelling/deswelling behavior and pH dependent zeta potential was determined. All nanogels were fluorescently labelled by copolymerization of fluorescein o-acrylate monomer, which are used to evaluate the SA-mediated targeting ability of nanogels.

RESULTS AND DISCUSSION:

The DLS results show similar hydrodynamic radius of pNIPAM and core-shell nanogels, with a polydispersity index below 20%. Both nanogels are temperature responsive and almost completely deswollen at physiological conditions. Compared to pure pNIPAM nanogel, MCF7 cells (breast cancer cell line) incubated with core-shell nanogel show

higher fluorescence intensity at all concentration, revealing that the PBA-functionalized core-shell nanogel has better targeting ability of MCF7 cells.

CONCLUSION:

PBA-functionalized nanogels enhanced the tumor targeting ability by specific interaction with SA overexpressed in tumor cells. The developed nanogel offers a highly translational approach for clinical diagnosis and therapy of solid tumors.

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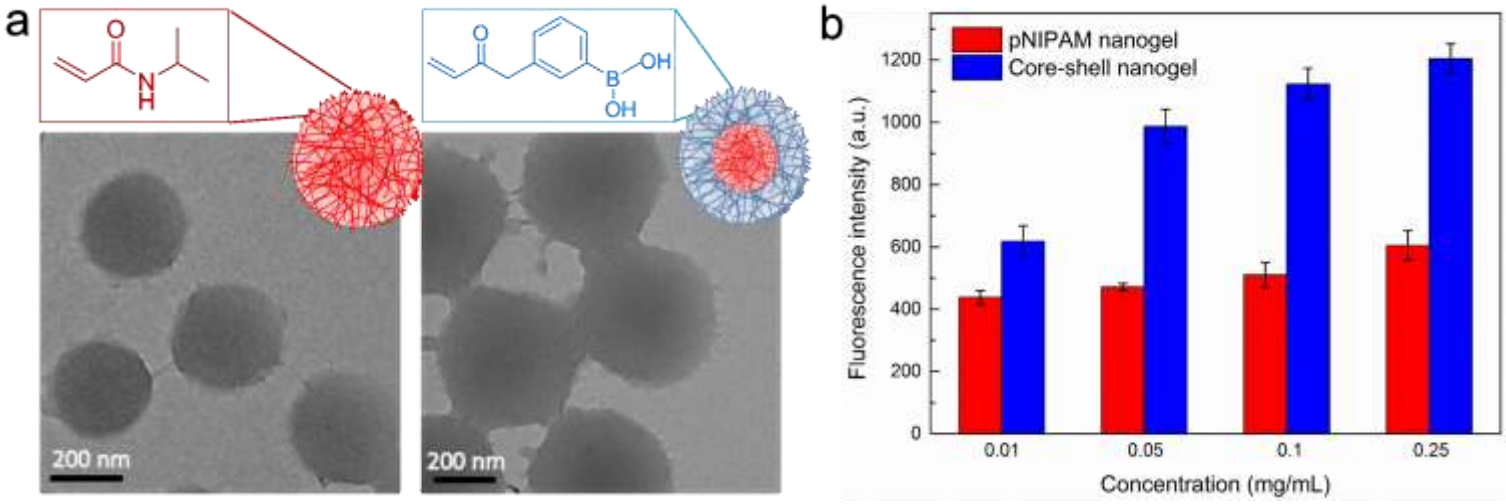

Picture 1: Caption 1: TEM images (a) and fluorescence intensity (b) of MCF7 cells incubated with pNIPAM and core-shell nanogels.

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Poster presentation

519 Superparamagnetic Iron Oxide Nanoparticles as MRI Contrast Agent in Articular Cartilage Imaging

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INTRODUCTION:

Identification of early pathological changes (biochemical, biomechanical, or structural) in avascular tissues, such as articular cartilage[1], through magnetic resonance imaging (MRI) prior to morphological changes has clinical

significance. recent research has revealed nano-sized T1 contrast agents that possess positive contrast properties similar to Gd^{3+} complexes and have higher relaxivity and longer circulation lifetime[3].

In this work, we designed an ultrasmall superparamagnetic iron oxide nanoparticles (SPION) as T1 and T2 Dual-Mode MRI contrast agents, capped with poly(ethylene glycol) (PEG) and having a hydrodynamic diameter of 6 nm.

METHODS:

Ultrasmall SPIONs were synthesized by high temperature decomposition of Iron(III) acetylacetonate, and transferred into aqueous phase with the help of citric acid and dopamine modified PEG.

Ultrasmall SPIONs were injected intra-articularly into the knee of normal rabbit, T1WI MR images were acquired under SE sequence: TR = 600, 800, 1000, 1400, 1800, 2200, 2600, 3000 ms, TE = 12 ms using a keen coil on a 3T MRI scanner (Siemens, MAGNETOM Skyro, in West China Hospital) before and after the injection. We can get T1 map from these eight T1WI images by nonlinear fitting based on Matlab. Similarly, T2 map was obtained from T2WI images under SE sequence: TR = 2800 ms, TE = 13, 25, 38, 41ms by linear fitting.

RESULTS AND DISCUSSION:

Ultrasmall SPIONs have good solubility and are well dispersed in hexane, and they also have good water solubility after ligand exchange with dopamine modified PEG. PEG-SPION presented a diameter of 5.9±1.1 nm in dynamic light scattering. Ultrasmall SPION showed higher r1 value (4.8 mM^{-1} s^{-1}) than GdDOTA (3.3 mM^{-1} s^{-1}) at 3.0T and can effectively enhance the contrast of cartilage. We found that T1 value and T2 value of cartilage significantly decreased after injection. The mean T1 and T2 has 52% and 21% decline from 859 ms to 415 ms and 38 ms to 30 ms respectively.

CONCLUSION:

PEGylation SPIONs with excellent water solubility and size distribution were developed in this study. Intra- articular injection of SPIONs led to decrease T1 and T2 value of cartilage under clinical MRI observation.

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ACKNOWLEDGMENTS:

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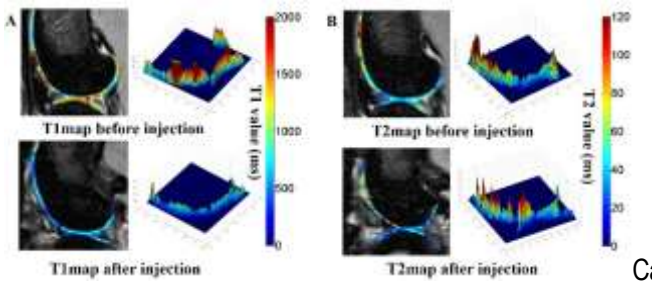

Picture 1: Caption 1: (A) T1 map MR image of pseudo-color articular cartilage (region of interest, ROI) and T1 mean value decline from 859 ms to 415 ms before and after inj

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Poster presentation

520 Polyurethane foams for cartillage tissues engineering

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INTRODUCTION:

Scaffolds made of synthetic polymers offers a promising approach to allow cells culture in a tridimensional environment that can mimic the extracellular matrix (ECM). Herein, we propose a "sphere templating"[1] route to build polyurethane foam with controlled pores and interconnections size, that promote cell survival and proliferation. The resulting scaffold allows cells to survive for at least 3 weeks and taking a three-dimensional organization.

METHODS:

Scaffold were produced using the so-called "sphere template" method described elsewhere[1]. Briefly, we produced paraffin spherical particules via an emulsion dispersion, and we picked out desired size range by sieving. Particules were packed in a mold and heat up to coalesce creating the interconnections. The polyurethane was then cast on the top and let to polymerize for 72h. Paraffin was finally dissolved to leave the porous structure. 3T3 Cells mouse fibroblasts (ATCC) cells were seeded at passage in 6 onto cylindrical samples at a density of 2.10^6 cells per scaffold. Cells were observed in confocal microscope after 21 days of culture using DAPI/Phalloidin staining to visualized F-actin and the nucleus.

RESULTS AND DISCUSSION:

Figure 1 on the left shows our resulting foam from "sphere templating approache", pores size is between 125 and 200µm and interconnections measure 57±10 µm.Moreover, scaffolds are well interconnected to allow nutrients and waste diffusion[2], and to promote cells migration across the whole structure, which is indeed the case on **figure 1**. Fibroblast proliferation was assessed with rezazurin (data not shown) and indicate an increase in cell proliferation between day 14 and 21. The right and middle pictures on **figure 1** shows that cells invaded the pores of the foam after 21 days. Fibroblasts tend to fill the pores but leave the edges almost empty. It has been demonstrated that cells prefer concave surfaces[3], which is also the case in our foam. Beside, cells aggregate together creating cell-cell contacts and organize in a 3D manner.

CONCLUSION:

Foams were generated with well controlled pore sizes and interconnections using "sphere tempating" approache. We used NIH 3T3 murine fibroblasts to evaluate scaffold ability to promote cell survival, and proliferation. Cells aggregated within the spherical shaped pores and took a three-dimensional organization.

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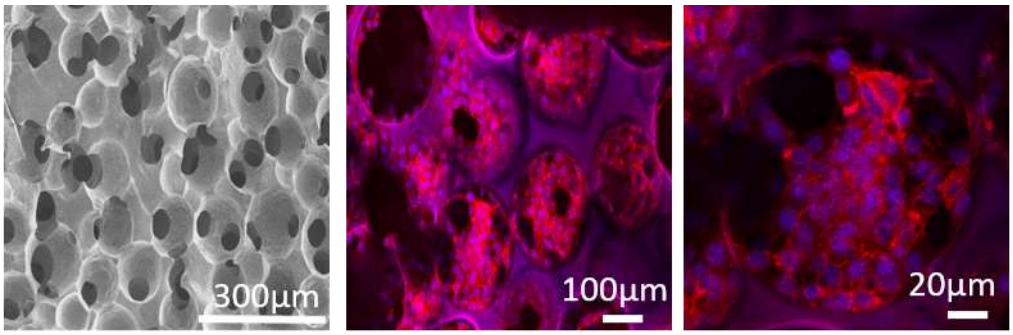

Picture 1:
Caption 1: figure 1) left: Scanning electron microscopy (SEM) of foam, middle and right, stained cells with f-actin (red) and nucleus (blue) within the scaffold

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Poster presentation

521 'Nucleo-mechanosensing' crosstalk between 3-D pillar topography and cyto-nucleoskeleton

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INTRODUCTION:

During migration through narrow constrictions a metastatic cell has to squeeze, deform and alter its morphology to pass through different tissues. To comprehend the mechanisms governing these deformations we employ 3D microtopography on which we had previously demonstrated that human osteosarcoma SaOs-2 cells disfigure their nucleus extensively[1,2].

METHODS:

Square micropillars measuring 7x7x7µm (height/length/space) were prepared by hot embossing cell-attractive poly-L-lactic acid. To control cell adhesion, 11x11x3 pillars were microfabricated in cell-attractive poly (n-butylacrylate) and chemically modified for making the top or interpillar-space cell-repellent or attractive with poly (dimethylacrylamide) and poly (n-butylacrylate) respectively. To decipher the main players in nucleus deformation of SaOs-2, cytoskeletal inhibition by drugs and gene-silencing of nucleoskeleton proteins was performed before inoculation on 7x7x7µm pillars. Nuclear deformation and focal adhesions (FAs) spatio-temporal distribution on pillars was quantified using confocal microscopy. The dynamics of cell deformation between pillars was analyzed via live imaging.

RESULTS AND DISCUSSION:

Our results demonstrated that acto-myosin contractility and vimentin with the aid from focal adhesions pull the nucleus down in between interpillar space, independent of microtubules. Disintegrating the link between cyto-nucleoskeleton completely reduces deformation suggesting the importance of connection between nucleoskeleton and cytoskeleton in channeling forces to deform nucleus. Furthermore advancement of nuclear deformation increases with time until it reaches a steady state, facilitated by remodeling of chromatin architecture Spatio-temporal kinetic results on cell adhesion revealed focal adhesions localization on top of pillars at early times with a displacement of it towards lateral sides of pillars at later times. Hence we presumed that the pulling down forces in between the pillars might be more potent than the pushing down forces. This hypothesis was verified by topography differing in chemistry, which proved the dominance of pulling down forces in steering nuclear distortion.

CONCLUSION:

Thus, micropillar topology provides a brilliant approach in perceiving the mechanisms involving the nuclear distortion in metastatic cells. To summarize, we illustrated that nucleus deformation is guided by focal adhesions via actomyosin pulling down forces, independent of microtubules and requires connection of cyto-nucleoskeleton to channel these forces.

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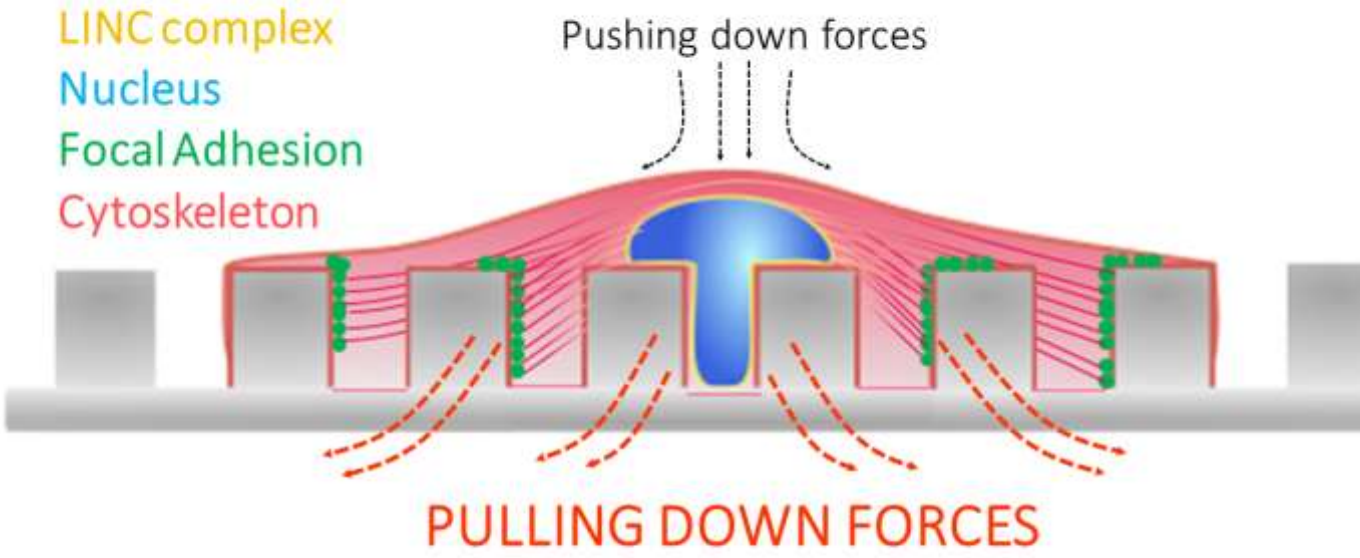
Picture 1:



Caption 1: Nuclear deformation mechanism between pillars

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Poster presentation

524 Promoting osseointegration of Ti implants through micro-nano scaled hierarchical hybrid coating

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INTRODUCTION:

Ti-based implants have been widely used in orthopaedics and dental surgery due to their excellent mechanical properties, chemical stability and biocompatibility. However, the lack of initial osseointergration often leads to implant failure [1-2]. In order increase the surgery success rate and prolong the life span of the implants, modifying the Ti implant surface while retaining the excellent bulk properties is normally required [3]. In this work, hybrid coatings with unique bioactive surface chemistry (titanium phosphate and titanium dioxide) and micro-nanoscaled hierarchical structures have been created for Ti implants. In vitro and in vivo evaluation of the implant performance confirmed the new coatings with tailored structures and chemistry is effective in promoting osteointegration.

METHODS:

Ti plate was machined into 5mm×1mm discs and '1mm×10mm rods. All Ti samples were polished to mirror finishing by sandpaper and cleaned by sonication in DI water for 20 min. The samples were then subjected to hydrothermal reaction in a mixture of aqueous phosphoric acid and hydrogen (at different composition) in a Teflon-lined autoclave under 0.15MPa pressure and 120^{o}C for 24 h.

RESULTS AND DISCUSSION:

As show in Fig. 1, three types of surface coatings, namely P-C-Ti, P-G-Ti and P-R-Ti, have been produced under different hydrothermal reaction conditions. Each of these coatings exhibits a unique micro-nanoscaled hierarchical structure and varied surface chemistry as compared to the polished controlled Ti sample (cp-Ti). In vitro studies show that allimplant surface with hybrid coatings have improved cell viability, adhension and spreading, proliferation and differention as compared to cp-Ti, with P-G-Ti giving the best results In vivo testing (12 weeks post-sugery) shows that the modified implants have significantly boosted histomorphometrical parameters and P-G-Ti surface demonstrated the strongest bone healing effects and the greatest mechanical stability.

CONCLUSION:

Unique hierarchical micro/nano scaled hybrid coating have been created for Ti implant using modified HPT treatment. The novel surface chemistry and topography of the hybrid coatings have led to improved cell proliferation, adhesion and differentiation, which subsequently promoted the implant osseointegration.

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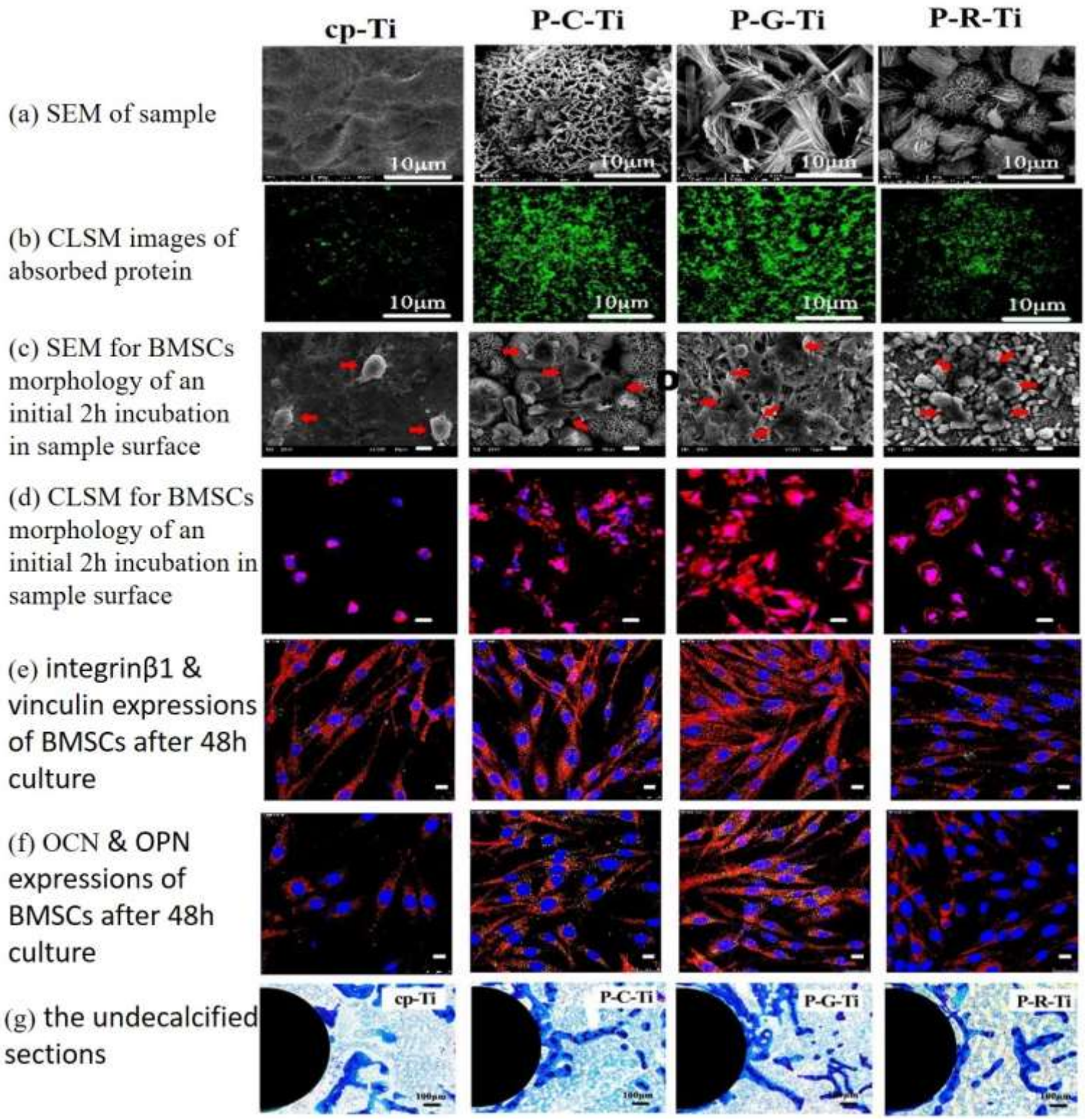

Picture 1: Caption 1: The reults of samples both in vitro and vivo studies

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Poster presentation

527 Biomimetic skin adhesives from glycosaminoglycans

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INTRODUCTION:

Medical adhesives and sealants that form *in situ* offer minimally invasive alternatives to surgical procedures such as suturing and stapling. Synthetic (e.g. cyanoacrylates) and protein (e.g. collagen and fibrin) sealants are commonly used. However, synthetic adhesives often exhibit cytotoxicity, while allergic reactions and poor mechanical properties are concerns related to proteins. Since medical adhesives must work in physiological environments, inspiration was found in the glue produced by the sandcastle worm, consisting of oppositely charged phosphorylated and sulfated proteins[1]. We propose glycosaminoglycans (GAGs) as an alternative class of biomolecules for medical adhesives. We hypothesized that the high affinity of GAGs to different extracellular matrix proteins would similarly originate bonding agents with high adhesive properties.

METHODS:

The sulfated GAGs heparin (HEP) and chondroitin sulfate (CS), and non-sulfated hyaluronic acid (HA) were complexed with poly-L-lysine (PLL). The optimal weight ratio of PLL/GAG for the formation of stable polyelectrolyte complexes (PECs) was determined by zeta-potential titration measurements. The PECs were isolated by centrifugation (14000*g*, 30 min, 37 ºC) and their rheological properties were assessed by rotational shear tests. Each adhesive was applied to rabbit skin specimens with an overlap area of 12.7×6.35 mm^2. Their ultimate strain was determined based on modified ASTM 2255-05 lap shear test standards. Data are means with n=3.

RESULTS AND DISCUSSION:

PLL/HEP, PLL/CS and PLL/HA PECs were prepared using 1.47:1, 1.10:1, and 1.13:1 weight ratios, respectively. Their corresponding shear elastic moduli (G') were 269±46.6 kPa, 30±1.4 kPa and 15±7.4 Pa. The low G' of PLL/HA adhesives is consistent with the non-sulfated nature of HA and the described high hydration state of the carboxyl groups as compared with the sulfates ones[2]. Rabbit skin specimens bonded with PLL/HEP, PLL/CS, and PLL/HA exhibited ultimate strains of 44%±10.3, 47%±10.0 and 57%±14.3, respectively. These values represent more than half of the elongation of intact skin (71%±11.7). Particularly, HA-based PECs provided the sturdiest adhesion thanks to its highly hydrated nature in improving the bonding between wet tissues.

CONCLUSION:

PLL/GAG complexes generated promising adhesives for damaged skin, leading to the recovery of a great deal of resistance to deformation. In the future, they can be scaled to *in vivo* models and other soft tissues such as cartilage and cardiac muscle.

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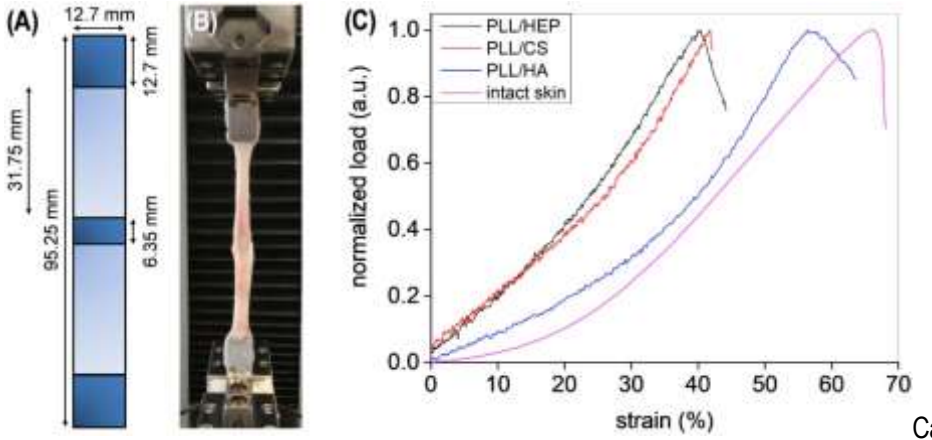

Picture 1: Caption 1: A) Dimensions of rabbit skin specimens. B) Repaired specimens under high tensile strain. C) Representative strain/stress curves of bonded specimens.

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Poster presentation

531 Analysing microtopography in electrospun scaffolds: a comparative study of fibre alignment analysis

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University of Sheffield, Sheffield, United Kingdom

INTRODUCTION:

Electrospun scaffolds are utilised in the manufacture of medical devices and *in vitro* tissue models. Electrospinning conditions determine fibre diameter and orientation, and both of these characteristics are known to influence cell behaviour, via mechanotransductive ques[1]. In order to investigate the effects of scaffold alignment on cell behaviour, it is essential that this property can be measured accurately and with a high degree of confidence. The aim of this project was therefore to perform a comparative study of two methods used in the determination of alignment.

METHODS:

The method for manufacturing polycaprolactone (PCL) scaffolds with random and aligned fibre orientations was reported last year[2] (see Figure 1). PCL from Sigma (UK) was used for the purposes of comparing fibre orientation in a preliminary study, whereas PCL from Corbion (The Netherlands) was later utilised to produce optimised scaffolds. Scaffolds were imaged using scanning electron microscopy (SEM), and fibre characterisation was initially performed using two methods: Fibermetric software analysis (Lamda Photometics, UK), or alternatively a manual method based on ImageJ (Fiji[3]) was utilised. ANOVA was used to demonstrate the statistical significance of the findings.

RESULTS AND DISCUSSION:

Fibermetric software was capable of analysing a large number of fibres in a very short time (typically 5 to 10 seconds), whereas the manual method produced comparable data using a smaller number of fibres but a longer

time period (typically 15 minutes). Both methods showed a statistical difference between random and aligned fibres ($p<0.0001$), and both were effective in determining this property. The Fibermetric approach is recommended as the most rapid, but if access to this system is not possible then the manual method is equally effective. Research on the response of cultured stem cells (alpha-tanycytes of the vertebrate hypothalamus) to random versus aligned mats as substrates is continuing.

CONCLUSION:

Although the Fibermetric method was more rapid, Fiji offered a reliable and low cost alternative approach. Fiji characterisation for analysing alignment was optimised here to provide consistent evaluation of manufactured scaffolds. Fully characterised scaffolds are valuable research tools to investigate the effect of fibre orientation on the behaviour of cultured cells.

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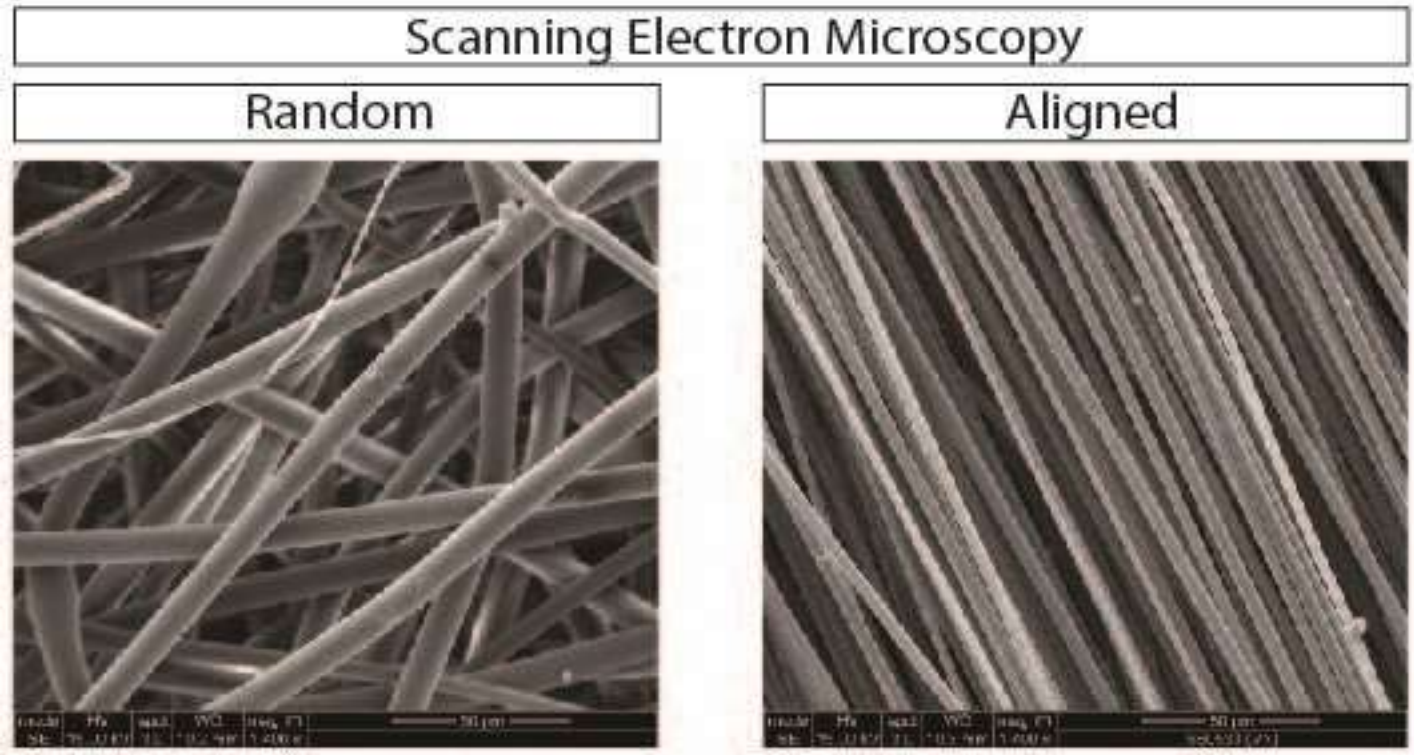

Picture 1: Caption 1: Figure 1: PCL (Corbion, Netherlands) scaffolds.

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Poster presentation

532 Design of Scaffold for Bone Bioengineering by Dual Approach

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INTRODUCTION:

A major focus in the field of tissue engineering is to build new functional structures looking to repair missed damaged tissues using three-dimensional (3D) systems; designed to mimic the *in vivo* environment[1]. Cells lack the ability to grow in functional 3D orientations by themselves, thus porous scaffolds may contribute by defining the anatomical shape of the tissue required[2]. By combining core macroscopic reproducible structures obtained by 3D-printing with outer layers of nanofibers many critical functions can be achieved[3]. The aim of the present work was to analyze the fabrication of a 3D cylindrical scaffold, by combining 3D printing and Air Jet Spinning techniques.

METHODS:

Cylindrical scaffolds were digitally design and constructed by 3D printing, using PLA polymer. Each 9 mm scaffold was then covered with PLA nanofibers by using air jet spinning technique. Thermodynamic and physical properties were characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and microporosity analysis by profilometry. Biological properties of adhesion and proliferation of osteoblasts were determined.

RESULTS AND DISCUSSION:

Microphotographs showed that PLA nanofibers layer was well adapted on the 3D scaffold. The thickness and the distribution of PLA nanofibers were homogeneous in all the areas analyzed. TGA showed that the polymer does not undergo changes on its properties in any of the groups. Melting points of the pure polymer and the printed scaffold were similar. The porosity of the nanofibers surface was increased when compared with pure 3D scaffold. Adhesion and proliferation assays showed statistically differences between scaffolds, showing a higher biological response on the functionalized coated nanofiber 3D cylinder ($p<0.05$).

CONCLUSION:

It was possible to manufacture and characterize a cylindrical 3D nanofibrillar scaffold. The nanofibrillar coating improves significantly cell biocompatibility providing a biological support to enhance cellular colonization by osteoblasts cells. 3D-nanofibrillar cylinder scaffold open a possibility for future use in bone tissue regeneration.

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Poster presentation

534 Design of bone tissue engineering tissue scaffold-based bio-ink for 3d-printing

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INTRODUCTION:

Advances in tissue engineer focus on the development of tridimensional scaffold that mimic the extracellular matrix has evolved to improve predesigning with specific cells onto the device, that in the moment of being implanted, reorganizes and recapitulate the original function of tissue[1]. 3D Bioprinting has emerged as an exciting technology that through of bio-inks allow to be created different morphologies of scaffolds that could mimicking the natural environment with a specific geometric control that allows the cellular aggregation and nutrient diffusion. The aim of this study was to develop a bio-ink that could be used for designing a 3D scaffold with osteoblasts for bone tissue engineering strategy[2]

METHODS:

The Bio-ink was synthesized by using a solution of sodium alginate, gelatin type A and nanoparticles of ZrO_2 in a proportion (3:1:1). The 3D scaffold was fabricated with the bio-ink and evaluated *in vitro* biocompatibility with osteoblast cells and differentiation to bone tissue. Characterization of the properties of the 3D bio-ink scaffold was undertaken by fourier transform infrared spectroscopy (FITR), confocal microscopy and biomechanical testing.

RESULTS AND DISCUSSION:

We obtain a printable porous scaffold with the bio-ink solution. Moreover, the printable 3D scaffold maintains its architecture during the biological assays, showing a good cell viability and proliferation of the cells until 21 days with the presence of calcium slats visualized by alizarin red S statin. FITR showed the bands corresponding to groups of carbonates CO_3 and phosphates PO_4 and hydroxyproline as signal of the presence of mineralized tissue.

CONCLUSION:

3D printing strategies allowed to print scaffolds with complex architecture and the combination of alginate, gelatin, and ZrO_2 nanoparticles showed high printability and cell survival. This investigation might provide highlighted the applicability for 3D bioprinting as a scaffold potential for bone tissue engineering.

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Poster presentation

539 The effect of catalyst addition over mechanical properties and biocompatibility of castor oil/cellulose nanocrystals polyurethane composites.

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INTRODUCTION:

The incorporation of fillers into polymeric matrices has been lately pursued to improve their properties and to achieve completely new ones. The design of polyurethane/cellulose composites is an emerging field of study, since cellulose high elasticity modulus, low density and cost, and the opportunity to incorporate a renewable carbon source make it an interesting reinforcement material. The purpose of this study was to evaluate the effect of using an organotin catalyst over the physicochemical and biological properties of materials synthesized from cellulose nanocrystals (CNC), castor oil (CO) and isophorone diisocyanate (IPDI).

METHODS:

CNC were obtained by acid hydrolysis of microcrystalline cellulose in sulfuric acid (64% v/v) aqueous medium, next changed for acetone to obtain cellulose-acetone gels. CO with a hydroxyl value (OHV) of 160 mg KOH/g, and IPDI where used for the synthesis of neat polyurethane (COPU), and composites where *in situ* polymerized adding 2% of CNC (COPU/CNC). Another set of samples where synthesized using dibutyltin dilaurate as catalyst (COPU/Cat. and COPU/CNC/Cat.). Materials were characterized by elongation mechanical tests, FTIR spectra and MTT cell viability assay.

RESULTS AND DISCUSSION:

COPU/CNC, having the same tensile strength as neat PUs, did not show improved mechanical properties, which can be attributed to agglomeration of filler. Indeed, synthesis of the polyurethane matrix needs long curing times, due to CO low OHV, leading to the formation of aggregates that act as defects in the composites[1]. COPU/CNC/Cat. showed an evident increase in its tensile strength and elongation at break, of 112% and 79% respectively compared to PU/Cat. The reinforcement effect of cellulose fillers was herein attributed to the formation of hydrogen bonds between a CNC 3D network and PU hard segments.

The addition of CNC had a negative impact over viability of L-929 mouse fibroblasts cultured in samples extracts. This could be attributed to the release of solvents used in the synthesis of the material. The use of catalyst also seems to have a negative effect over cell viability.

CONCLUSION:

The use of catalyst in the synthesis of polyurethanes/CNC composites, based on low OHV polyols, diminishes the formation of agglomerates and enhances mechanical properties. Nevertheless, it would be necessary to assess if other catalysts has a lower biological impact.

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ACKNOWLEDGMENTS:

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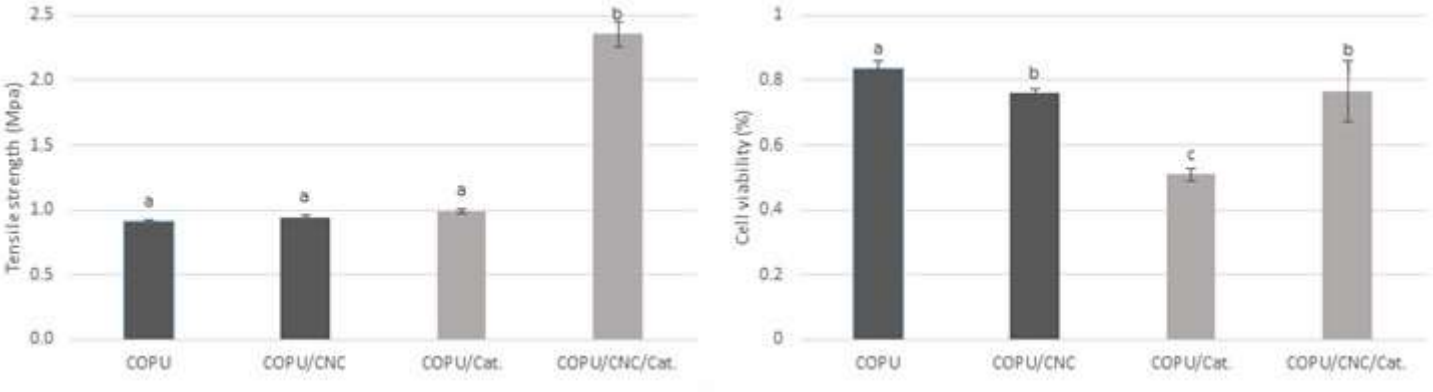

Picture 1: Caption 1: Tensile strength and cell viability (compared with a positive control). Bars with different letters indicate statistically significant difference (p<0

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

543 Osteogenesis of biomimetic calcium phosphate with nano/micro- hierarchical structure

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INTRODUCTION:

Biomimetic mineralization is a promising method to develop bone grafts and tissue engineering scaffolds. The remarkable biological functions of the biomineral are related to its hierarchical structures. In the present study, we fabricated calcium phosphate scaffolds with different morphology and nano/micro- hierarchical structures through biomimetic mineralization and calcination/sintering at different temperatures. The osteogenesis effect of the different scaffolds was investigated with in vitro cell culture and in vivo rat critical-sized calvarial defect model.

METHODS:

The calcium phosphate powders were prepared by using citrates in the process of the hydrothermal treatment to selectively induce the nucleation and assembly of inorganic materials. Implant was prepared by isostatically pressing powders in a steel cylindrical mould at room temperature (thickness about 0.4 mm). Thereafter the plates were calcinated at different temperature to obtain various crystallinity, micro-/nano hierarchical structure and roughness. Rat adipose-derived stem cells (ADSCs) viability assay and attachment on the samples was determined. The

osteogenesis effect in vivo was determined in a rat critical-sized calvarial defect model by micro-computed tomography (micro-CT), histological processing and analysis, and mechanical properties analysis.

RESULTS AND DISCUSSION:

The scaffolds have complex hierarchical architectures, with characteristic dimensions spanning from the nano- to the macroscale. The biomimetic bioceramic scaffolds enhanced rat adipose-derived stem cells (ADSCs) attachment, viability, proliferation and alkaline phosphatase (ALP) activity. The in vivo bone regeneration results of rat critical-sized calvarial defect models indicated the nano/micro- hierarchical structures did facilitate osteogenesis. Besides, the scaffolds had excellent performance on angiogenesis. After twelve weeks, the calvarial defects were fully repaired, and the regenerated new bone had sound mechanical properties. Detailed analysis further indicated subtle difference of the osteogenesis effect between different nano/micro- hierarchical structure.

CONCLUSION:

The biomimetic CaP bioceramic scaffolds with nano/micro- hierarchical structures can be a promising bone tissue engineering material.

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ACKNOWLEDGMENTS:

This work was supported by The National Key Research and Development Program of China (2017YFC1105000), National Natural Science Foundation of China (51572087), the 111 Project (B13039), and Program for Changjiang Scholars and Innovative Research Team in University (IRT 0919).

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Poster presentation

548 Biocompatibility and Osteoconductive Capacity of Devitalized Coral

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INTRODUCTION:

The gold standard in bone trauma repair is autograft, a procedure largely in decline due to the requirement for a secondary invasive procedure required to harvest bone from the patient. Alternative allograft material may be sourced, but carries with it the risks associated with any allogenic transplantation. The demand for new materials to serve as bone void fillers is therefore driving innovation to meet clinical demand. Coral skeletons are excellent bone grafting materials due to their biocompatibility and osteoconductive properties. The coral's native architecture is designed to host life, influence cellular behaviour and encourage cellular adhesion, qualities retained after scaffold

devitalization and preparation as a biomaterial. Their porous structure and mineral composition are similar to that of human bone[1]; they are biologically inert and are resorbed at the same rate as the reparative host bone is generated. With newly developed controlled, environmentally responsible cultivation techniques, the GMP production of coral scaffolds for orthopaedic, dental and veterinary use is possible. Here the composition, structure and biocompatibility of cultivated coral was investigated.

METHODS:

Using scaffolds derived from four species of devitalized coral samples, the structure and composition were characterized through scanning electron microscopy (SEM), energy dispersive X-ray analysis and micro X-ray computed tomography. LIVE/DEAD cell viability, MTS, lactate dehydrogenase (LDH) and proliferation assays were performed to assess the biocompatibility of the coral in co-culture with adult human bone marrow-derived mesenchymal stromal cells (MSCs). Finally, MSC adhesion to the coral was visualized with SEM and confocal microscopy.

RESULTS AND DISCUSSION:

All coral samples were composed primarily of calcium carbonate ranging in density from 1,753-1,903mg/cm^3. Visualization of the naturally occurring pores demonstrated interconnectivity. The porosity changed with species, ranging from 3.7%-41.5%. LIVE/DEAD staining viability, MTS and proliferation assays demonstrated maintained, high levels of MSC viability, metabolism and cellular proliferation indicating biocompatibility. MSCs were directly seeded onto coral scaffolds and cultured for 14 days before SEM visualization. A thick, confluent, organized layer of fibroblast-shaped MSCs was observed on the external surface of the coral skeleton and both covering and lining the calice (external pore-like) structures (Figure 1).

CONCLUSION:

The four coral species evaluated in this study were deemed biocompatible, supporting bone marrow derived MSC viability, metabolism, adhesion and growth. They are therefore prime candidates for further investigation as a replacement for auto- or allograft.

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ACKNOWLEDGMENTS:

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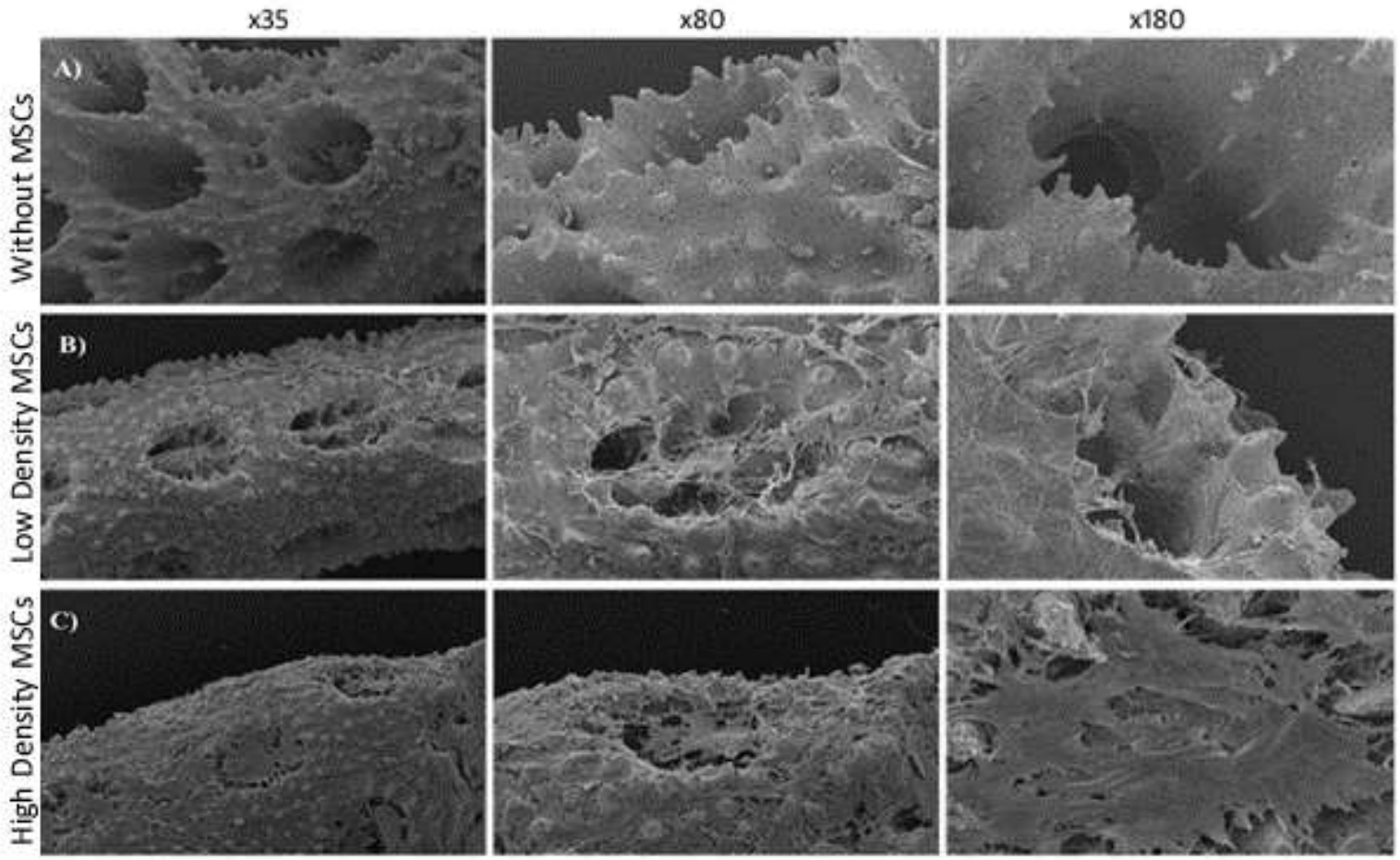

Picture 1: Caption 1: Coral cultured (A) without MSCs or with a (B) low density or (C) high density of MSCs..

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Poster presentation

550 Biodegradable nanoparticles for active tumor targeting: hyaluronic acid decoration to promote cell internalization

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INTRODUCTION:

Hyaluronic acid (HA) has attracted significant research attention for tumor-targeted delivery due to its ability to specifically bind CD44 and RHAMM receptors, which are overexpressed in many tumor tissues. Here we have designed, produced and characterized nanoparticles (NPs) based on poly(lactic-co-glycolic acid) (PLGA), externally

decorated with 200, 800 and 1600 kDa HA, (named HA2, HA8 and HA16). The effect of HA molecular weight on Irinotecan (IRIN)-loaded NP uptake kinetics and cytotoxicity have been assessed on CD44-overexpressing breast carcinoma cells (HS578T) using fibroblasts originating from mouse connective tissue (L929) as control [1].

METHODS:

NPs were produced by nanoprecipitation. Briefly, the organic phase (solutions of PLGA and poloxamers in acetone, possibly containing Irinotecan) was precipitated in an aqueous phase containing different amounts of HA and poloxamers. The concentrations of different molecular weight HA were set to ensure equal viscosities of the external water phase. NP morphology were studied by TEM and AFM, while their size and zeta potential by PCS. *In vitro* drug release profile in PBS were quantified by spectrophotometric assay. The uptake kinetics of fluorescent NPs (loaded with Nile Red) were obtained through spectrofluorimetric assay and cell lysis at predetermined time points. Alamar Blue assay was used to assess NP cytotoxicity.

RESULTS AND DISCUSSION:

NPs were spherical and their average size ranged from 95 to 120 nm. AFM images showed the presence of HA on NP surface (Fig.1 top). The *in vitro* release profiles showed a sustained IRIN release up to 7 days. Uptake analyses revealed that NP internalization was strongly enhanced in HS578T cells compared to L929 (Fig.1 bottom), therefore pointing at the major role of HA tropism NPs toward tumor cells overexpressing CD44 receptor. Furthermore, a significant influence of HA molecular weight was found. Consistently, cytotoxicity tests showed that HA-decorated NPs were more toxic than bare PLGA NPs against CD44-overexpressing cells, therefore indicating their ability to target CD44 receptor.

CONCLUSION:

The results here collected strongly corroborate the hypothesis of the pivotal role played by HA in determining a preferential NP uptake in tumor cells lines overexpressing CD44.

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ACKNOWLEDGMENTS:

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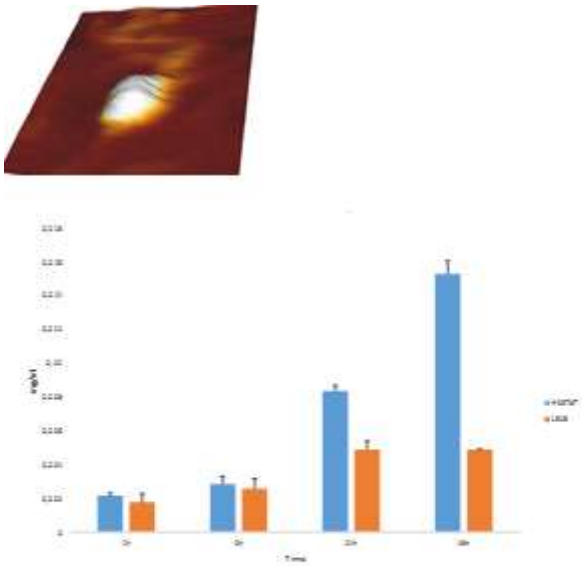

Picture 1: Caption 1: Figure 1. top: AFM micrograph of HA8 NPs, bottom: HA8 NPs uptake kinetics in HS578S and L929 cells .

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Poster presentation

555 Development of dynamic culture system with thermo-responsive polymer

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[2]Doshisha University, Japan

INTRODUCTION:

Dynamic compressive stimulation was available to improve extracellular matrix (ECM) synthesis for cultured cartilage[1]. Since compressive deformation was applied to cultured cartilage with an indenter directly, it may cause damage and contamination for cultured cartilage. We focused on thermo-responsive polymer gel to apply dynamic compression to cultured cartilage with its volume change. The purpose of this study was to develop the dynamic culture system with thermo-responsive polymer under temperature control of culture medium.

METHODS:

The developed culture chamber consisted of a polycarbonate well, an ITO heater glass as a chamber bottom and two peltier elements for cooler. The temperature of culture medium was controlled with a thermocouple set on the ITO heater glass and a PID controller, and temperature of culture medium was monitored with another thermocouple.

The polymer gel was prepared with *N*-isopropylacrylamide (NIPAAm) and *N*,*N*-dimethylacrylamide (DMAM) as a monomer, *N*,*N*'-methylenebisacrylamide (MBA) as a cross-linker. [NIPAAm]:[DMAM]:[MBA] were 75:25:1, 75:25:3 and 75:25:5 to examine effect of cross-linking density for the expansion/contraction behavior. The polymer gel expands and contracts upon cooling and heating. The polymer rings were cut out from the synthesized polymer gel sheet in culture medium. Expansion/contraction behavior of the polymer ring that 1% agarose gel as scaffold was installed inside was measured in culture medium under alternately repeating 36.0 and 38.0 ºC.

Cultured cartilages were prepared with porcine chondrocytes seeded in agarose gel (1.0×10^7 cells/ml). The cultured cartilages installed in the polymer ring were cultured for one week with and without stimulation, and glycosaminoglycan amount was measured.

RESULTS AND DISCUSSION:

The diameter change ratios of the agarose gel were about 9.2, 7.2 and 5.9% for the polymer ring of [NIPAAm]:[DMAM]:[MBA]=75:25:1, 75:25:3 and 75:25:5 respectively. The diameter change ratio decreased with increase of MBA linker because a mobility of polymer chains was regulated with increasing cross-linking points. Figure 1 shows expansion/contraction behavior of the polymer ring of [NIPAAm]:[DMAM]:[MBA]=75:25:1. It took over 80 minutes until the polymer ring was completed to expand at 36.0 ºC, and it took over 60 minutes until the polymer ring was completed to contract at 38.0 ºC. Glycosaminoglycan amount in the cultured cartilage increased due to dynamic compression under alternately repeating 36.0 ºC for 80 minutes and 38.0 ºC for 60 minutes.

CONCLUSION:

The developed dynamic culture system with thermo-responsive polymer was effective to improve ECM synthesis for cultured cartilage.

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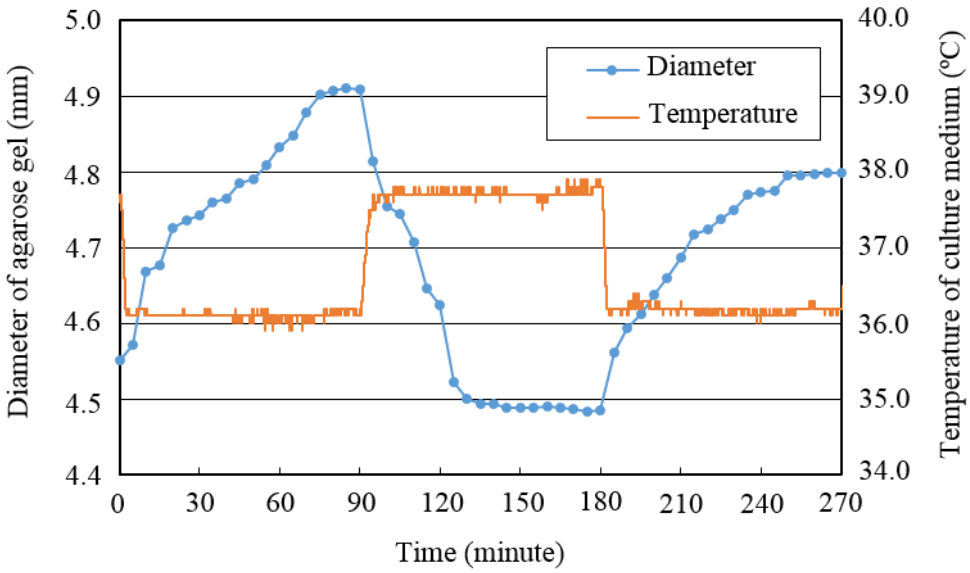
Picture 1:



Caption 1: Fig.1 Expansion/contraction behavior of the polymer ring of [NIPAAm]:[DMAM]:[MBA]=75:25:1

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Poster presentation

558 Atmospheric plasma assisted additive manufacturing of 3D scaffolds for tissue regeneration applications

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[2]Fraunhofer Institute for Surface Engineering and Thin Films IST, Germany

[3]Department of Physics and Astronomy, Padova University, Italy

INTRODUCTION:

3D scaffolds with optimum physicochemical properties are able to elicit specific cellular behaviours and guide tissue formation. However, cell-material interactions are limited in synthetic polymer scaffolds manufactured by additive manufacturing (AM). Plasma treatment is one of the conventional methods used to render the surface of 3D scaffolds more functional. Nevertheless, post-fabrication plasma treatments may result in limited control over the functionalization in the internal architecture of scaffolds. To overcome this limitation, here, we developed a new hybrid AM technology, which combines a standard melt extrusion technique with an atmospheric plasma jet. This new design enabled the layer-by-layer chemical vapour deposition of functional groups on scaffold filaments while printing for tuning cell adhesion, proliferation and ultimately cell differentiation.

METHODS:

Initially, hot pressed 2D PEOT/PBT films were fabricated in order to evaluate the effect of different coatings on cell adhesion and proliferation. Nucleophilic and electrophilic layers were deposited with the plasma jet on the films with (3-aminopropyl)trimethoxysilane (APTMS) and a mixture of vinyltrimethoxysilane (VTMOS) and maleic anhydride (MAA), respectively. Hexamethyldisiloxane (HDMSO) and tetramethylsilane (TMS) precursors were used to render the films hydrophobic. The stability of the coatings was evaluated by storing coated substrates in water and air. Next, 3D scaffolds were fabricated and functionalized ex-situ with the plasma jet. The penetration depth of the treatment was analysed using ATR FTIR and colorimetric dyes.

RESULTS AND DISCUSSION:

An increase on cell adhesion and proliferation was observed on films coated with APTMS and VTMOS-MAA compared to cells cultured on untreated PEOT/PBT films or films coated with HDMSO and TMS. The activity of the coatings was found to be stable for several days in water (>6 days for APTMS and >1 day for VTMOS-MAA). The atmospheric plasma treatment penetrated around 2.5 mm within a 3D scaffold with 250 µm filament diameter and 500 µm pore size, when treated ex-situ. Ultimately, 3D scaffolds will be plasma treated during the printing process.

CONCLUSION:

The new hybrid technology, which combines an AM melt-extrusion based technique with atmospheric plasma, allows a selective gas treatment during scaffolds manufacturing process. This enables to tune the surface functionality of 3D polymeric scaffolds for a broad range of tissue engineering applications.

ACKNOWLEDGMENTS:

We are grateful to H2020-NMP-PILOTS-2015 (GA n. 685825) for financial support.

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Poster presentation

566 Mechanical conditioning of bone-ligament-bone constructs

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INTRODUCTION:

The limitations of the current methods for reconstructing the anterior cruciate ligament (ACL) have led to ongoing research in tissue engineering to create an engineered ligament construct that could behave in a similar way of the native tissue.[1,2,3] In this project, we aim to incorporate a mechanical stimulus into a tissue engineering strategy that when applied to the ligament construct, the collagen production and organization and the cell alignment will be optimized thereby increasing the strength of the construct. This will be done through the development of the thickness of the hydrogel substrate using cost effective materials, creating a sinew structure, and the manufacture of a bioreactor.

METHODS:

Fibroblasts were sourced from sheep by excising the cruciate ligaments, cleaning thoroughly, digesting the tissue, and culturing the fibroblasts at low passages. A hydrogel substrate was created using chitosan, gelatin and proanthocyanidin. Anchors were cast using $CaCO_3$ and $Ca_3(PO_4)_2$ into a cylindrical mold. Fibroblasts were seeded onto the hydrogel with anchors roughly 20mm apart. The fibroblasts were then allowed to proliferate on the gel, causing the gel to contract into a sinew shape around the anchors. Cell proliferation was measured by alamar blue assay.

RESULTS AND DISCUSSION:

Increasing thickness of hydrogel was designed to determine the most efficient gel. The fibroblasts proliferated greatest on the thinnest gel, seen in Figure 1. The 1.0mm thick gel was chosen for future work, as there was much inconsistency in creating gels 0.5mm thick. In order to allow the contraction of the hydrogel into the sinew shape, the adhesion of the gel to the well plate was prevented using a silicone elastomer that was cut to the shape of the well. The bioreactor was designed to maintain a sterile environment and minimize debris from moving parts without compromising the ability to apply tension.

CONCLUSION:

The primary ovine fibroblasts show promise as they maintain their fibroblast characteristics, and because they are from the equivalent ligament of the ACL should have similar intrinsic characteristics. The 1mm thick gel was chosen as a result of consistency in creation, appropriate proliferation results, and satisfactory substrate stiffness. The silicone elastomer successfully inhibits the adhesion of the hydrogel to the well plate. The modifications to the bioreactor effectively reduce contamination and debris.

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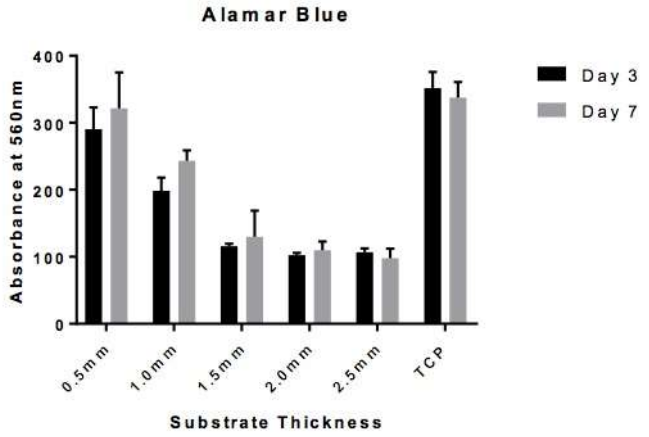

Picture 1: Caption 1: Figure 1: Proliferation of cruciate ligament fibroblasts using the Alamar Blue Assay.

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Poster presentation

567 3D printing of methylcelluose-based hydrogels as substrates for cell sheet engineering

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INTRODUCTION:

Cell sheet engineering (CSE) consists in the development of smart culture surfaces that allow obtaining intact cell sheets[1]. Methylcellulose (MC) can be used to produce thermo-responsive hydrogels, as substrates for CSE[2,3]. This work aims at exploiting the possibility to use MC-based hydrogels in 3D printing process, via an extrusion-based printing. Furthermore, the substrates were *in vitro* tested with two cellular phenotypes, and the obtained cell sheets (CS) were characterized.

METHODS:

Hydrogels were prepared via MC powder dispersion in saline solutions of Na_2SO_4 and PBS[4]. A Kiwi 4D printer (Sharebot, Nibionno, LC, IT) was used to extrude the MC-based hydrogels. MC-based hydrogels were characterized using a rotational rheometer, to investigate possible structural hydrogel modifications induced by the extrusion process. The stability of printed and non-printed (bulk) MC-based hydrogels was evaluated in a swelling test, using distilled water as physiological-like environment. For *in vitro* cell tests bulk and ring-shaped printed samples were considered. Cell viability tests and immunofluorescence analysis were performed on CS after culturing for 48 h

murine embryonic fibroblasts (NIH/3T3) and endothelial murine cells (MS1) on the MC-based hydrogels. Image elaboration (ImageJ, NIH) was performed to quantify cell orientation in the CS[5].

RESULTS AND DISCUSSION:

The printing process reduces the LCST of MC-based hydrogels of 7°C. Moreover, after extrusion, the hydrogels show a higher degree of swelling in water compared to bulk hydrogels (+60% and +95% for Na_2SO_4 and PBS hydrogels, respectively). These results can be related to the shear stress induced by the needle on the gel during the extrusion process. With respect to the cell tests, CS obtained from bulk hydrogels appear compact, while assume the expected ring shape in the case of printed hydrogels. Considering cell orientation, the cells orientation on ring-shaped CS seems influenced by the topography of MC-based hydrogels. In fact, nuclei appeared more elongated compared to CS obtained on bulk samples.

CONCLUSION:

Extrusion-based printing was shown to be an effective strategy for CS having a desired shape using MC-based hydrogels as ink, with the ultimate goal of the regeneration of complex tissues.

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ACKNOWLEDGMENTS:

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Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

569 Using (bis)phosphonate groups to tailor properties of poly(β-amino ester) network polymers for potential biomedical applications

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INTRODUCTION:

The subject of biodegradable polymers has been an extensively studied area of research for biomedical applications such as tissue engineering, drug delivery and gene delivery. Among the biodegradable polymers, poly(β-amino ester)s (PBAE)s that were firstly developed by Langer are advantageous due to their pH sensitivity, biodegradability and low cytotoxicity. In this study, novel phosphonate- and bisphosphonate-functionalized PBAE macromers were synthesized to investigate the effects of the (bis)phosphonate functionality on degradability and binding to tissues of the polymers.

METHODS:

The macromers were prepared from Michael addition of (bis)phosphonate-functionalized primary or secondary (di)amines to diacrylates such as 1,6-hexane diol diacrylate (HDDA) and poly(ethylene glycol) diacrylate (PEGDA) efficiently without any catalyst and they were photopolymerized to give gels. Some of the macromers were also incorporated into hydrogels by copolymerization with PEGDA or 2-hydroxyethyl methacrylate (HEMA) at different ratios to control degradabilities and swelling properties of the hydrogels. The degradation of the PBAE networks was investigated in PBS (pH = 7.4) at 37^0C. The morphology of PBAE network polymers was observed by scanning electron microscopy (SEM). Cytotoxicity of the degradation products was tested on NIH 3T3 or SaOS-2 cells using MTT assay. The effect of gel composition on cellular interactions was investigated using SaOS-2 cells seeded on polymer films formed from the various macromers.

RESULTS AND DISCUSSION:

Phosphonated or bisphosphonated amines were used for synthesis of novel phosphonate- or bisphosphonate-functional PBAEs with higher degradation rates than non-phosphonated ones, although the degradation is mostly governed by the hydrophilicity of the starting diacrylate. The in vitro studies indicate that phosphonate incorporation into the PBAE films provide a more favorable matrix for cell interaction and the structure of the macromer has a dramatic effect on the cell attachment and spread. PEGDA-containing films failed as a cell-attachment matrix. According to the cytotoxicity studies degradation products of the films do not reduce the viability of SaOS-2 or NIH 3T3 cells under tested conditions.

CONCLUSION:

These materials have potential to be used as nontoxic degradable biomaterials for tissue engineering and drug delivery systems.

ACKNOWLEDGMENTS:

This work was supported by a grant from TÜBİTAK (114Z926).

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Poster presentation

577 Sacrificial phosphate based glass coatings for in vivo biofilm prevention

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INTRODUCTION:

Biofilm infections affect 1-4% of orthopaedic implants. Until 6 h post-surgery normal tissue immune response is supressed, allowing the biofilm to develop after bacteria attach to the implant surface. These individual bacteria become surrounded by a self-derived matrix, providing protection from antibiotics and the immune system [1].

Degradable coatings could prevent biofilm formation by reducing or eliminating bacterial attachment via surface instability. The added potential to release antibacterial agents into the wound could offer complimentary protection. However, any coating must be cytocompatible to minimise damage to surrounding tissues and allow wound healing.

Phosphate based glasses (PBGs) are a candidate coating material as they degrade fully in aqueous environments into their ionic constituents. Magnetron Sputtering is a strong candidate coating method as it allows the deposition of thin-film coatings of PBGs in the nano- to micrometer range and have been shown to deliver strong interfacial adhesion (78.6 MPa exceeding FDA guidelines [2]). Gallium has been reported to both osteogenic and antibacterial at certain concentrations [3,4].

Here we present work on unique gallium doped PBG coatings via magnetron sputtering with the potential to be degradable, antibacterial and cytocompatible.

METHODS:

Three gallium-doped PBG coatings and a non-doped control were coated onto Ti6Al4V using RF magnetron sputtering with multiple targets. Chemical compositions were elucidated (EDX and XPS analysis) and degradation behaviour characterised in biological media. Cytocompatibility studies used MG63 human osteocytes and bacterial growth assays used *S. aureus.*

RESULTS AND DISCUSSION:

Coating thicknesses ranged from 105 – 415 nm. A blending sputtering process (2 targets) produced a glass coating containing 47 mole% Ga_2O_3, which is impossible to produce via standard commercial methods.

Coatings were shown to be cytocompatible, although rapid degradation rates limit adhesion and proliferation. Many ions (Ca^{2+}, PO_4^{3-}, Mg^{2+}) released are suggested to stimulate growth and differentiation of osteocytes, potentially offering additional wound healing benefits. Initial biofilm growth assays suggest these degradation rates are sufficient to prevent bacterial attachment.

CONCLUSION:

Magnetron sputtering allows for the deposition of thin-film PBG coatings containing higher concentrations of gallium ions than can be achieved through other methods such as sol-gel processing. These results indicate strong potential for cytocompatible coatings which can prevent biofilm formation on implant surfaces.

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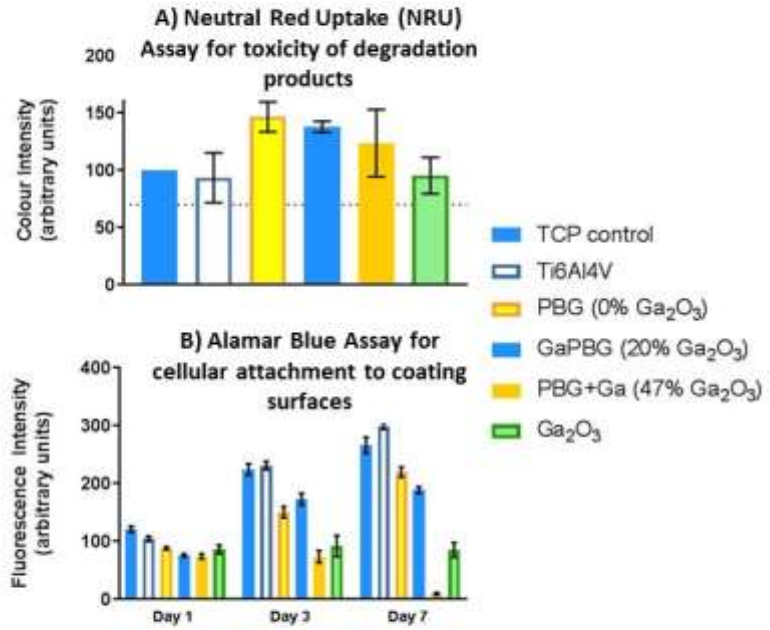

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Poster presentation

583 Nanopatterned micro-scaffolds for bone tissue engineering applications

Isabel Bjørge, Clara Correia, João Mano

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INTRODUCTION:

Regenerative medicine strategies in orthopaedics often resort to conventional porous scaffolds with fixed geometry, which require invasive implantation and prevent cellular self-organisation. Thus, bottom-up approaches have been investigated for the assembly of smaller units such as microparticles (MP). MP support cell expansion and cell-MP aggregation forms 3D structures that can be injected, independently or encapsulated, through minimally invasive procedures. Yet, with growing evidence that surface topography, such as grooves, impact cell expansion and direct the differentiation pathway of mesenchymal stem cells towards osteogenesis[1], the question arises whether spherical MP with no surface topographical cues are the best option.

We have developed a new class of MP with (1) disc-like shape with enhanced surface/volume ratio to maximise cell-adhesion sites; and (2) grooved patterning on the surface to control cell orientation or adhesion and direct osteogenic differentiation. Patterned disc-like micro-scaffolds (PDSS) act as cell carriers while delivering topographical cues via surface patterning.

METHODS:

PDSS were produced by nanoimprinting in a straight-forward manner, without requiring advanced equipment. Water-soluble polyvinyl alcohol moulds were prepared using compact discs as nanopatterned templates. Moulds were used to nanoimprint spherical polycaprolactone MP (diameter range: 25-40µm) at an optimised time, temperature, and load. Upon collection, PDSS size, grooves, and patterning homogeneity were characterised by scanning electron microscopy (SEM). To compare cellular response to PDSS versus spherical particles, pre-osteoblastic MC3T3-E1 cell line was cultured with both types of plasma-treated MP. Cell morphology analysis via immunofluorescence assays (F-actin visualisation by phalloidin staining counterstained with DAPI for nuclei) and SEM were used to study cellular interaction with particles.

RESULTS AND DISCUSSION:

PDSS (ca. 70x30x10 µm) were successfully produced and presented a marked grooved surface patterning (figure 1A). Fluorescence assays showed cell attachment and elongation along the nanopattern of PDSS, demonstrating the viability of PDSS for cell expansion (figure 1B).

CONCLUSION:

The proposed PDSS represent a new type of microparticle that can act as physical support for cell proliferation while delivering topographical cues, which are expected to favour osteogenic differentiation in our system. Thus, studying the potential of this unique microplatform for stem cell osteogenic differentiation would be the next step. Ultimately, 3D structures formed by cell-mediated assembly may be applied as injectable cell carriers for bone regeneration.

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ACKNOWLEDGMENTS:

I. Bjørge acknowledges financial support by the Portuguese Foundation for Science and Technology through doctoral grant (SFRH/BD/129224/2017). This work was supported by European Research Council grant agreement ERC-2014-ADG-669858 for project “ATLAS”.

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Caption 1: Figure 1.A: Representative SEM image of PDSS (scale bar 7.5 µm). B: DAPI/Phalloidin staining of MC3T3-E1 cells seeded on PDSS (scale bar 50 µm).

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Poster presentation

586 The development of a three-dimensional osteoprogenitor culture model for investigating future osteoporosis therapies

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INTRODUCTION:

Osteoporosis represents an increasing burden to healthcare systems worldwide, and their associated fractures are expected to double in incidence by 2050.[1] Current therapies have proven inadequate, and new, targeted therapies are required.[2] A consistent and reliable three-dimensional (3D) model is required to perform high throughput drug screening, in order to successfully develop improved therapies. Multicellular spheroids have long been established in research fields such as cancer, yet there remains a paucity of information on 3D spheroid cultures for osteoprogenitor cells, which are vital to musculoskeletal research.[3] This study will analyse 3 spheroid-forming techniques with human osteoprogenitor cells to identify the optimal culture conditions.

METHODS:

Human bone marrow aspirates, taken at time of total hip replacement, were utilised. The adherent cells were cultured, which gave rise to the osteoprogenitor population. Three spheroid techniques were assessed; hanging drop (HD), ultra-low attachment (ULA) and magnetic levitation techniques. Spheroids were generated using several cell seeding densities and cultured up to 21 days; analysis included cell viability, morphology (light microscopy) and scanning/transmission electron microscopy at time-points 1,7 and 21 days.

RESULTS AND DISCUSSION:

The HD and ULA techniques consistently produced circular spheroids, with viable cells, which increased in diameter with increasing cell number. However, the ULA method formed stable spheroids faster and more efficiently. The magnetic levitation technique formed spheroids of a maximum diameter, regardless of cell density, with clear internalisation of the nanoparticles visible on electron microscopy, but suffered areas of cell death within the spheroid. Electron microscopy demonstrated cell-cell interactions within all models.

CONCLUSION:

The ULA technique rapidly produces stable and uniform osteoprogenitor spheroids which remain viable in long term culture. The ULA model can be adopted as a standard 3D model to allow comparison of both osteoprogenitor cells from healthy and osteoporotic patients. This will contribute to the development of targeted therapies.

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ACKNOWLEDGMENTS:

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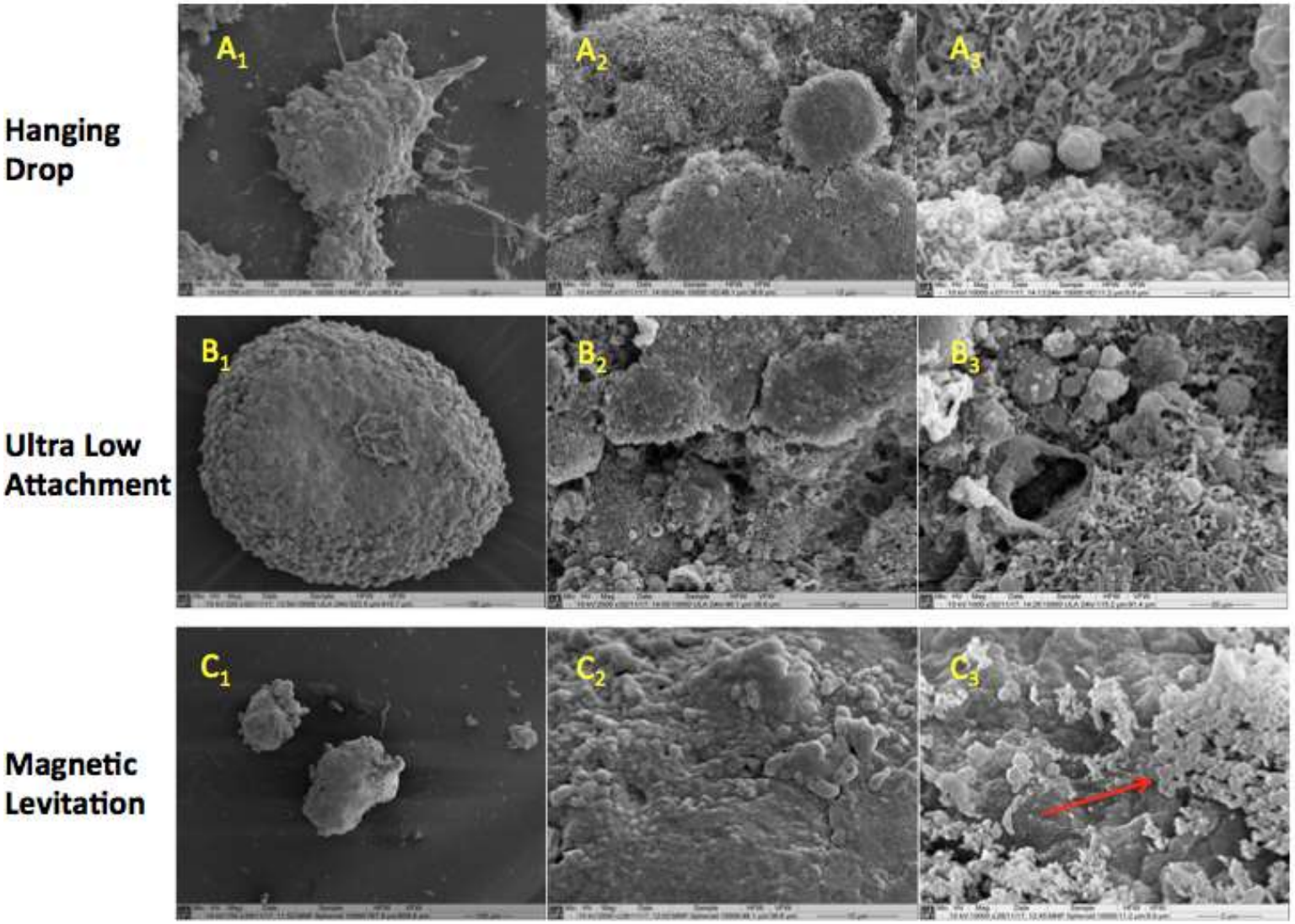

Picture 1: Caption 1: Figure 1: Scanning electron microscopy images of the three spheroid forming techniques, demonstrating the produced morphology after 24 hours.

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Poster presentation

590 Sophisticated cell microfactories co-encapsulating osteoblastic and adipose stem cells for bone regeneration

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INTRODUCTION:

The successful regeneration of large bone defects remains a significant challenge in orthopedic research. Along with tissue engineering and regenerative medicine (TERM) field evolution, new technologies aiming tissue repair have been emerged. Stem cells are the focus of many TERM applications and adipose derived stem cells (ASCs) quickly

became attractive for bone tissue engineering. However, controlling stem cell multipontency and engineering bone *in vivo* remains a challenge, as it often leads to heterotypic and inferior osseous tissues.

METHODS:

Inspired by the multiphenotypic cellular environment of bone, we hypothesized that self-regulated liquefied and multilayered capsules [1], loaded with ASCs and human osteoblasts (hOBs) cells are a promising attempt. The multilayered membrane ensures permeability to essential molecules for cell survival. Furthermore, the surface functionalized poly(ε-caprolactone) microparticles loaded into the liquefied core act as cell adhesion sites, allowing cells to construct their own 3D cell culture assembly system. For that, by using the electrospraying technique, liquified capsules encapsulating only ASCs or a co-culture with hOBs were cultured with or without osteogenic differentiation factors. Here, we aim to promote a well-orchestrated cell-to-cell interaction, evaluating the osteogenic potential of hOBs on ASCs. Moreover, the proposed capsules were tested using a rotary cell culture system to better mimic the dynamic environment of native tissues. Static culture conditions were tested as control.

RESULTS AND DISCUSSION:

Results show the successful development of microtissues inside the compartmentalized and controlled environment of capsules with an appropriate diffusion of essential molecules for the long survival of encapsulated cells. The distribution and structural organization of encapsulated cells within the cross-linked core was assessed by DAPI-phalloidin fluorescence assay (Figure 1). We also observed that the bioreactor developed larger aggregates of cells and microparticles, providing biophysical stimulation and robust improvements in bone formation over static culture. Moreover, the system allowed to develop microtissues inside the capsules, even in the absence of osteogenic differentiation factors.

CONCLUSION:

Microtissues were successfully developed inside the controlled environment of capsules, even in the absence of osteogenic differentiation factors. Accordingly, we intend to use the proposed system as hybrid devices implantable by minimally invasive procedures for TERM applications.

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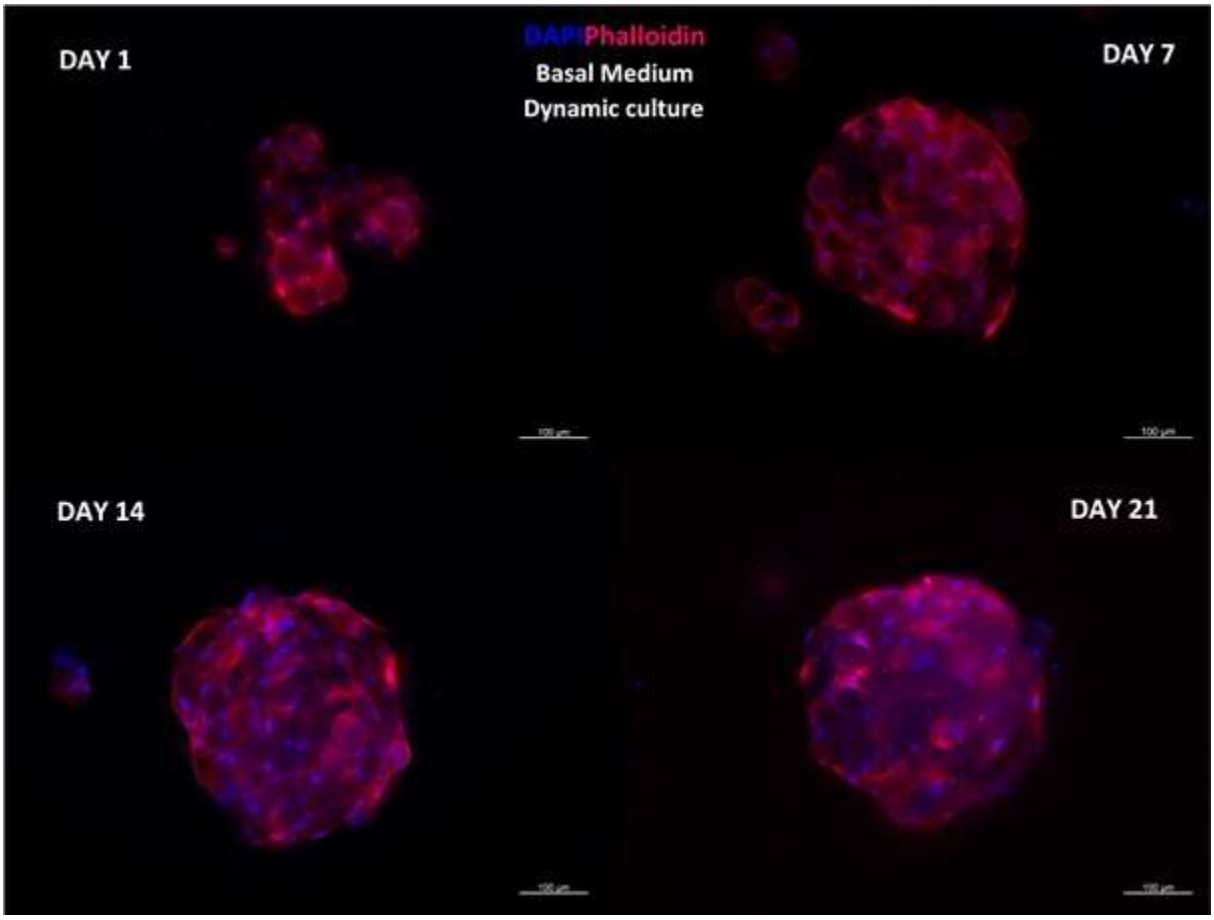

Caption 1: Figure 1. Capsules encapsulating cells at different time-points, assessed by DAPI-phalloidin.

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Poster presentation

592 Assessment of the Host Response to a Porous Polymer Cell Encapsulation Device Following Subcutaneous Implantation

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INTRODUCTION:

Islet cell transplantation, in which insulin producing pancreatic islet cells are isolated from donor organs and implanted in recipients, is a promising treatment for diabetes mellitus. Various encapsulation techniques have been described to improve cell retention and eliminate the need for chronic immunosuppression[1]. However, these cell

encapsulation strategies have been plagued by poor islet cell viability due to the high metabolic demands of islet cells and the hypoxic environment of the implant site. To overcome these hurdles, we have designed a novel porous polymer macroencapsulation device to serve as a vector for islet cell delivery to the host transplant site. In this study, we characterized the host response to an empty encapsulation device (β-shell) following implantation in the subcutaneous tissue of a rat.

METHODS:

Empty β-shell devices were implanted in the subcutaneous space of male Sprague Dawley rats (N=4) for 28 days, then removed en block with surrounding tissue. Tissue samples were fixed, prepared for histology, then stained with H&E and Mason's Trichrome to assess fibrous capsule formation. CD31 and CD68 staining was used to evaluate angiogenesis and macrophage infiltration, respectively. Samples were transected obliquely, processed and imaged with scanning electron microscopy (SEM) at the tissue/device interface. Samples sectioned into smaller blocks were dehydrated in an ethanol gradient, stained in 1% iodine w/v in absolute ethanol, then imaged using microCT.

RESULTS AND DISCUSSION:

β-shell implants were well tolerated over a 28 day time course without any adverse effects. H&E and Trichrome stain reveals a fibrous capsule of variable thickness surrounding the device that is dependent on the contoured surface. CD31 staining indicates the presence of blood vessels within the fibrous capsule, while CD68 staining shows macrophages at the tissue/device interface. SEM imaging demonstrates integration with surrounding tissue, with clear identification of a fibrous capsule of varying thickness surrounding the device. These findings were confirmed with microCT imaging of the β-shells.

CONCLUSION:

Preliminary results demonstrate that the β-shell is well tolerated *in vivo* and produces a favorable host response to the implant. Ongoing work will evaluate effectiveness of the β-shell as cell delivery vehicle.

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ACKNOWLEDGMENTS:

Funding for this project was provided by DRIVE (EU Horizon 2020 grant # 645991), and in part by EDGE (EU Horizon 2020 Marie Sklodowski-Curie grant # 713567) and the SFI research center AMBER.

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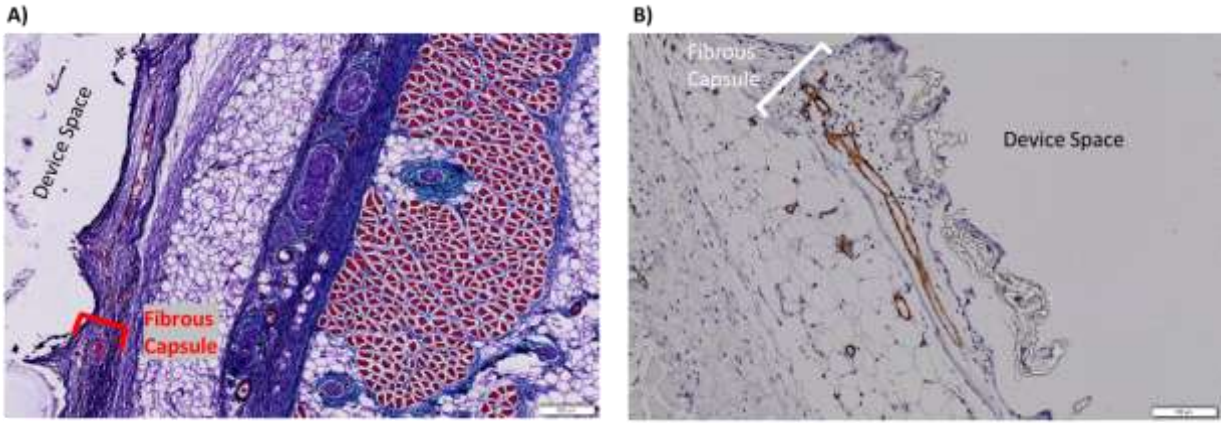

Caption 1: A) Trichrome stain and B) CD31 stain of empty β-shell 28 days after implantation in the dorsal subcutaneous space of a rat

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Poster presentation

597 Novel design of 3D-printed cage structures as basis for an in vivo bone model

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INTRODUCTION:

Much research has been conducted concerning the design of an 'ideal' scaffold to induce and support the development of bone tissue, including 3D-printing of cell-laden gels[1], freeze drying or laser sintering.[2] However, these scaffolds often do not represent the complex architecture of bone and fall short regarding either their mechanical or biological properties or both. Our group works towards alleviating this problem by developing techniques to structure hydrogel, mineral and cell based scaffolds through multiple length scales to develop improved mimics of bone tissue, primarily for *in vitro* testing. Here, we introduce a novel cage-like design which aims to facilitate the monitoring of bone tissue formation within our hierarchically structured mineralized hydrogel.

METHODS:

Single scaffold layers (SSL) with an integrated locking mechanism were 3D-printed in poly lactic acid. SSLs were chemically treated to render their hydrophilicity prior to loading with mineralized alginate microbeads produced as described previously.[3]

RESULTS AND DISCUSSION:

The resulting SSLs enabled straightforward loading of discrete hydrogel microbeads, which could be monitored over time. Assembly of multiple layers resulted in channel like structures that ensure exchange of fluids (figure 1) and the potential for vascular ingrowth, which is an ongoing focus of our research. The locking mechanism provides mechanical stability of the construct, but also means the scaffold layers can be separated for interrogation by a variety of materials characterization techniques.

CONCLUSION:

Our design represents a convenient platform with which to house and monitor cell and mineral containing hydrogel scaffolds. Using a modular design principle, we will be able to modify parameters independently and deconstruct the layered scaffold easily to probe the construct with a wide variety of characterization techniques. Future work will focus on the patterning of cells and collagen within the hydrogel matrix and stimulating blood vessel growth within the resulting interconnected pores.

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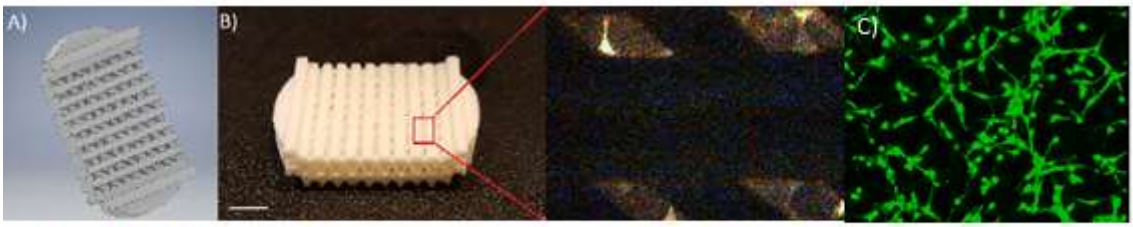

Figure 1 A) CAD design of SSL. B) 3D-printed version of 2 interlocked SSL loaded with mineralized alginate beads. C) Osteoblast cells spreading within alginate-collagen hybrid gel. Scale bar: 5 mm

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Poster presentation

598 Interplay between composition, mechanical properties and biological properties in a fibrin-based bio-intelligent scaffold for skin tissue engineering

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INTRODUCTION:

A biomaterial scaffold must exhibit appropriate biological and structural behaviours to support cell ingress and tissue regeneration. In chronic wounds, scaffolds must withstand degradation by elevated levels of neutrophil-secreted enzymes such as elastase and matrix metalloproteinases, as well as the mechanical demands of patients' movement [1,2].

Here, we examined the interplay of manufacturing parameters by the effect on structural, mechanical and biological behaviours of the resultant scaffold properties. Understanding this is critical for controlled manufacture of scaffolds at clinical translation scale.

METHODS:

Porous fibrin scaffolds were manufactured (method confidential), varying material composition and cross-linking to obtain differential properties. The effect of cross-linking and addition of a polymer ("Px") alone and in combination were examined. Microstructure was evaluated by scanning EM. Coagulometry (Stago STartMax) was used to determine the effect of Px on the fibrin formation time. Instron UTM was used to examine the effect of microstructure, cross-linking and presence of Px on the tensile strength of the fibrin scaffolds (lyophilised and rehydrated).

CCK-8 and in-situ imaging were used to assess biocompatibility. Enzymatic degradation was assessed following incubation with 0.25% trypsin or plasmin.

RESULTS AND DISCUSSION:

Increasing P*x* concentration (0.2%-2%) shows a marked change in microstructure. At low concentrations, the structure is hierarchical and fibrous. At higher concentrations, P*x* adheres to the fibrin framework, effectively binding and closing up the nano-scale fibrous structure.

Cross-linking fibrin increases mechanical strength and resistance to enzymatic degradation but can reduce elasticity and flexibility. Addition of P*x* increased elasticity, even at very low P*x* concentrations despite no visible change to microstructure.

Low Px concentrations gave an insignificant reduction in fibrin formation time, and are therefore unlikely to interfere with fabrication by extending the mixing and casting time.

CCK-8 indicated no cytotoxic effect, with reduced activity at 24h compared to plate control (while cells adhered to the 3D matrix, confirmed by microscopy), but scaffold populations recovering to close to the control after 120h.

CONCLUSION:

Porous biocompatible scaffolds have a complex microstructure, which affects mechanical and biological properties. The understanding gained of the interplay of manufacturing parameters allows fabrication of a scaffold with a specific set of properties.

Optimising Px content and cross-linking produces a strong, flexible biocompatible scaffold with low shrinkage and suitable biodegradation.

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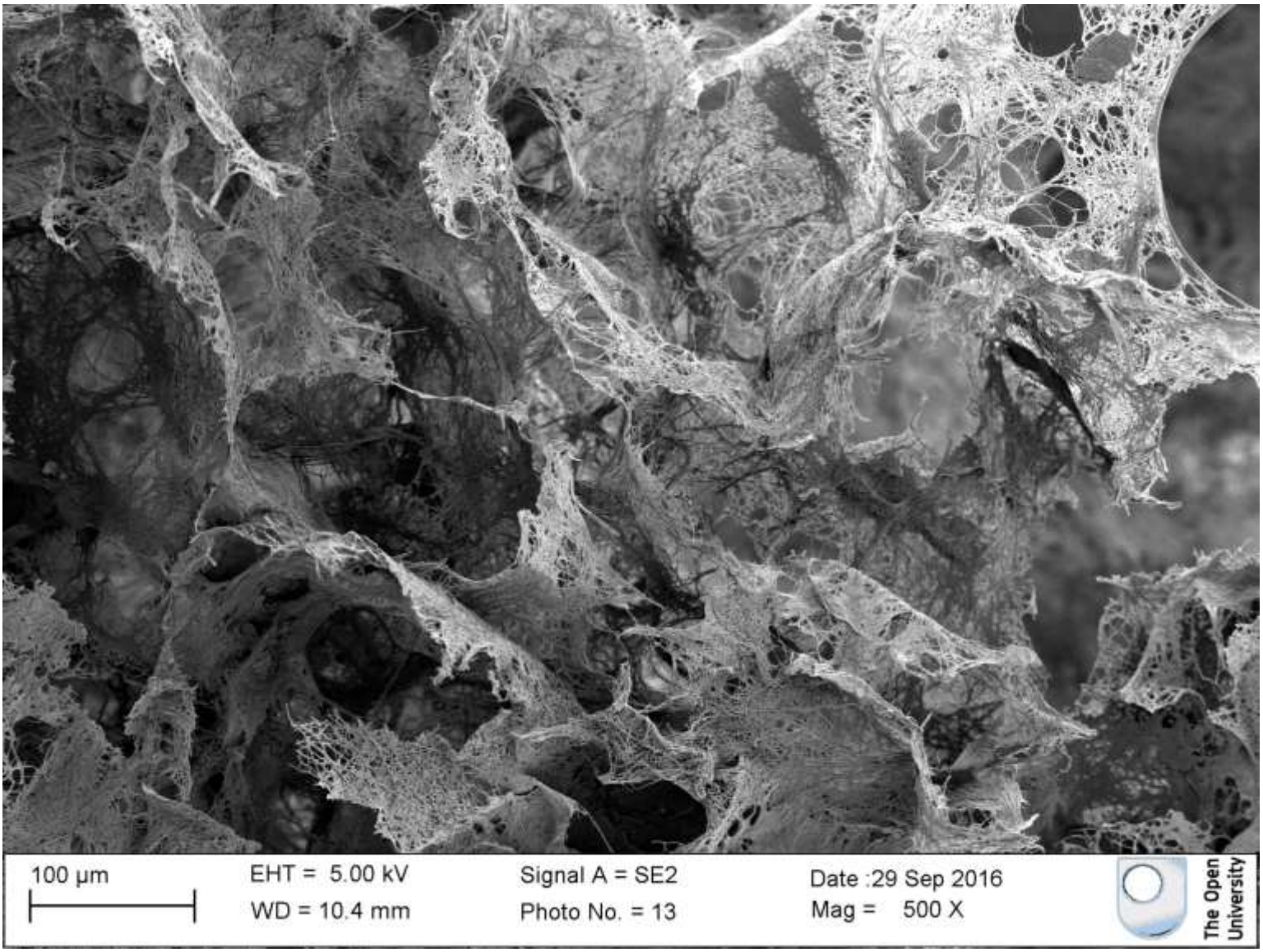

Picture 1: Caption 1: SEM cross-sectional micrograph of a lyophilised fibrin-based scaffold, containing 0.2% Px, showing rounded, highly interconnected pores.

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Poster presentation

601 Design and manufacture of biocompatible scaffolds with fused deposition model for cell culturing

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INTRODUCTION:

Traditionally, cell culture has been done in 2D, nevertheless physiological behavior and metabolism could be affected[1,2,6]. Employing 3D-Printing technologies allow obtaining scaffolds with 3D geometries designed under desired requirements. Literature have shown that the 3D models are similar to physiological model[4,5]. However, researchers have not study deeply 3D culture in different period conditions.

This study aims to compare different scaffolds geometries and materials when MCF-7 breast cancer cells are cultured. Impact of geometry and material on the cell proliferation had been analyzed.

METHODS:

Fused Deposition Model machine has been used to manufacture scaffolds. Three different scaffolds configurations have been manufactured with different angle between layers (45º, 90º and 135º) and two different materials: Polycaprolactone (PCL) and Polylactide Acid (PLA) have been assayed. Scaffolds were cultivated with MCF-7 cells for a long time (28 days; Figure 1).

Adhesion and cell proliferation were analyzed using a colorimetric assay (MTT).

RESULTS AND DISCUSSION:

Fig.1 3D MCF-7 cell adhesion and proliferation in different scaffold geometries and materials (PLA and PCL) *versus* 2D after 28 days. Each bar represent n=3 samples.

After 28 days of cell culture, it was observed more cell proliferation in PLA *versus* PCL scaffolds in a significant manner. Regarding the geometries, it was not seen differences between 45º, 90º and 135º in the assayed conditions.

CONCLUSION:

Scaffold material manufactured with FDM affects MCF-7 cancer cell proliferation in long-term period culture. However, geometries do not affect MCF-7 cancer cell proliferation.

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ACKNOWLEDGMENTS:

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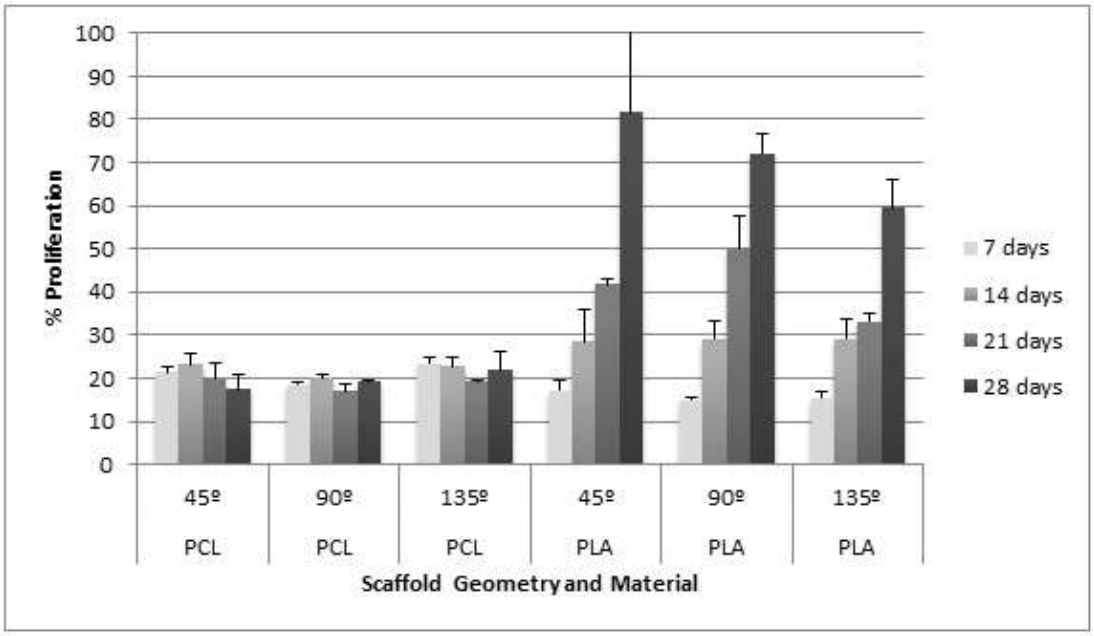

Fig.1 3D MCF-7 cell adhesion and proliferation in different scaffold geometries materials (PLA and PCL) *versus* 2D after 28 days. Each bar represent n=3 sam

Caption 1: Fig.1

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Poster presentation

604 Bioprinted thiol-ene photocrosslinkable bioinks guide morphogenesis in tissue-engineered constructs for skin repair

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[2]University of Manchester, Manchester, United Kingdom

[3]3B´s Research Group, University of Minho, Portugal

INTRODUCTION:

Skin is a multilayered organ responsible for vital functions in human body. Bioprinting is emerging as a viable alternative to automatically create tissue-engineered constructs for skin repair. One main drawback is the lack of suitable materials that can be printed with cells, providing an instructive microenvironment that guides cell function and morphogenesis. This study aimed at developing printable and chemically-defined pectin bioinks allowing rapidly photocrosslinking through thiol-ene click chemistry.

METHODS:

Purified low-methoxyl citrus pectin was modified through reaction with methacrylic anhydride (24h, PBS, pH 8) or carbic anhydride (1h, DMSO). Prior modification in DMSO, pectin was converted to its tetrabutylammonium salt[2]. Bioinks were designed using a cell-adhesive (CGGGGRGDSP) and a matrix metalloproteinase (MMP)-sensitive peptide (CGPQG↓IWGQC) as crosslinker. Rheological properties were characterized by oscillation rheometry. Dermal fibroblasts were suspended in bioinks, printed into 3D-constructs (Regemat3D bioprinter), and photocrosslinked (365nm, 7mW/cm^2). Cell morphology and extracellular-matrix (ECM) deposition were analyzed by immunofluorescence.

RESULTS AND DISCUSSION:

A major challenge in bioprinting is the design of bioinks exhibiting suitable printability and cell-responsive properties. Herein, chemically-defined pectin bioinks were designed with controlled density of cell-adhesive and MMP-cleavable moieties, providing higher control over hydrogel composition, compared to protein-derived materials. Pectin was firstly modified with methacrylate or norbornene groups, yielding vinyl-functionalized macromers. Thiol-ene bioinks were developed by exploring the reactivity of a bis-cysteine-containing MMP-cleavable peptide towards methacrylates and norbornenes. Protease-degradable bioinks at different concentrations (1.5, 2.5%wt) were printed into cell-laden constructs and rapidly achieved mechanical stability (40s) through free radical UV polymerization. Rheological properties were adjusted by calcium-mediated ionic-crosslinking prior cell suspension. Bioinks showed shear-thinning behavior and allowed extrusion bioprinting of 3D constructs with high levels of shape fidelity. Mechanical properties (0.1–3kPa) were easily tailored by changing MMP-cleavable peptide content (0.5–5mM), while *in vitro* degradation was confirmed by enzymatic assay. After 14 days of culture, printed constructs supported extensive cell spreading, proliferation and deposition of new ECM rich in fibronectin and collagen type-I, main components of the dermis.

CONCLUSION:

Thiol-ene bioinks with tunable properties were synthetized and printed into functional tissue-engineered constructs that guide fibroblasts to secret endogenous ECM with potential for dermal repair.

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Poster presentation

606 Pressureless spark plasma sintering of 3D-plotted titanium scaffolds

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[2]Centro de Investigación e Innovación Tecnológica, Instituto Politécnico Naciona, Mexico

[3]Instituto de Investigaciones en Materiales, UNAM, Mexico

[4]Facultad de Química, UNAM, Mexico

INTRODUCTION:

Pressureless spark plasma sintering (PL-SPS) consist in the modification of the conventional SPS graphite die in such way that the mechanical pressure applied by the electrodes is supported by the rigid die instead the sample[1]. Similar than SPS, the electric current flow warm the die but sample is heated due to radiation heat transfer, keeping advantages such as fast heating/cooling rates and a short sintering time. The aim of this work was to pioneer the use of PL-SPS for the fast consolidation of 3D-plotted titanium scaffolds.

METHODS:

Titanium inks were obtained by mixing spherical titanium powder with an organic binder. The inks were deposited following an orthogonal mesh pattern with a robotic dispensing device. Afterwards, the binder was removed at 230°C in air and PL-SPS was carried out in vacuum at temperatures between 1100 and 1600 °C, varying sintering time between 0 and 15 min and heating rates between 100 and 500 °C/min. Sintered samples were chemically, microstructurally and mechanically characterized.

RESULTS AND DISCUSSION:

Fig. shows the process overview of a titanium scaffold sintered at 1600°C for 21min, including heating and dwell time. Only titanium with hexagonal close-packed crystal structure was observed by XRD. However, EDX analysis revealed a slight oxidation and carburization on the surface of the scaffold's strands. Scarce sintering necks were observed at temperature equal or less than 1300°C, while between 1400 and 1500 °C clear unions were observed between the well distinguishable spherical particles. The largest strand densification was observed at 1600°C, leading on sample shrinkage of 12%, while retaining the orthogonal pattern with overall porosity of 75%. The effective compressive strength and elastic modulus in compression for this sample were 52.5 ± 4.8 MPa and 0.80 ± 0.04 GPa, respectively. It is expected that the open-porous structure of the scaffold provides the conditions for fast bone regeneration, while maintaining mechanical stability during bone grafting or as scaffold for bone tissue engineering applications.

CONCLUSION:

PL-SPS considerably reduces the production time of titanium scaffolds in comparison to conventional powder metallurgy processes. In addition, the flexible additive manufacturing of green scaffolds opens the opportunity to design custom implants.

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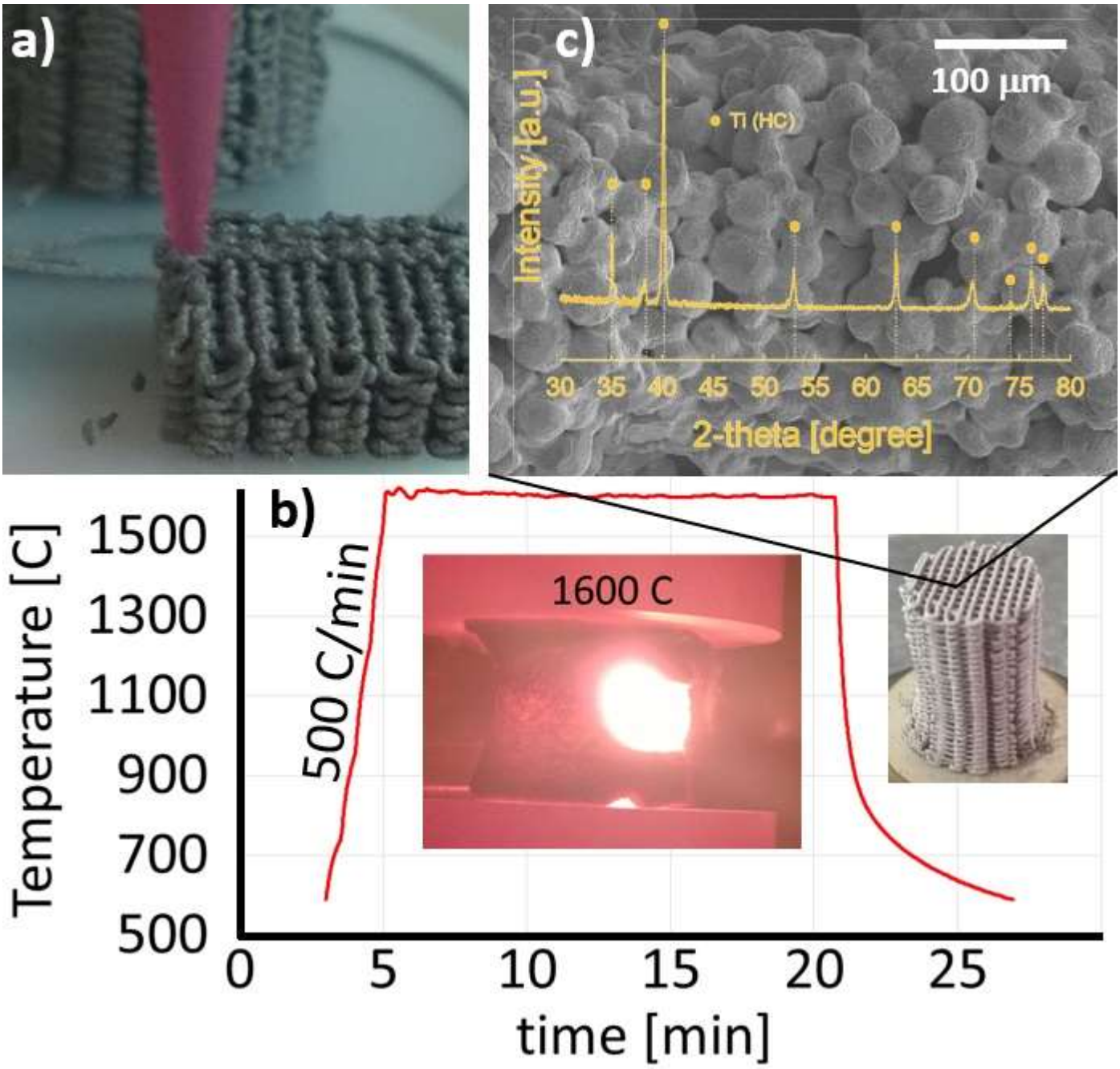
ACKNOWLEDGMENTS:

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie and it is co-financed by the South Moravian Region under grant agreement no.665860.



Picture 1: Caption 1: Process overview, a) 3D plotting of scaffolds, b) temperature profile and die aspect during PL-SPS, c) microstructure and crystalline composition

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Poster presentation

609 Preparation of bioactive and antibacterial raw silk fabrics by metal doping

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INTRODUCTION:

Raw silk is expected to be useful as a biomaterial because of its high content of hydrophilic amino acids in sericin, outer layer of raw silk. Also, sericin has a high affinity for metallic ions, such as zinc, calcium and iron ions[1], and hence metal-doped raw silk can show specific functions due to the doped metal. In this study, we attempted to enhance apatite-forming ability, which is important for osteoconduction (i.e. bioactivity), and to induce antibacterial activity of the raw silk fabrics by incorporating calcium, copper or zinc into the fabric surface.

METHODS:

Commercially available raw silk fabric (R-Silk) was cut into 1.0 cm square. Then, R-Silk was soaked in 30 mL of 1.0 M $CaCl_2$, $CuCl_2$ or $ZnCl_2$ solution for 24 hours. The sample names were R-Ca, R-Cu and R-Zn, respectively. The metal content at the surface of sample was measured by X-ray photoelectron spectroscopy (XPS). In order to estimate bone-bonding ability, the samples were soaked in simulated body fluid (SBF) for 7 days and their surfaces were analyzed using scanning electron microscope (SEM) and X-ray diffractometer (XRD). Further, antibacterial activity of the sample against *Escherichia coli* (*E. coli*) was evaluated by qualitative and quantitative method based on JIS L 1902:2015.

RESULTS AND DISCUSSION:

From the result of XPS, calcium, copper and zinc was detected for R-Ca, R-Cu and R-Zn, respectively. According to SEM and XRD results, R-Ca formed apatite on its surface in SBF, but R-Cu and R-Zn did not. This indicates that calcium ions released from R-Ca increase a degree of supersaturation of SBF with respect to apatite and accelerate the apatite formation. Thus, R-Ca has a potential to bond to living bone. On the other hand, R-Ca, R-Cu and R-Zn showed antibacterial activity against *E. coli* (see Fig. 1). The bacteria were killed by released copper and zinc ions which are known as antibacterial elements[2] for R-Cu and R-Zn. Interestingly, R-Ca showed strong antibacterial activity even though calcium ion is not toxic to bacteria. This might be because the release of calcium ions causes environmental changes, such as pH increase, where the bacteria hardly survive.

CONCLUSION:

Raw silk fabrics doped with calcium showed both apatite-forming ability in SBF and antibacterial activity against *E. coli*. We can expect that calcium-doped raw silk fabrics are useful as bone-bonding and antibacterial materials.

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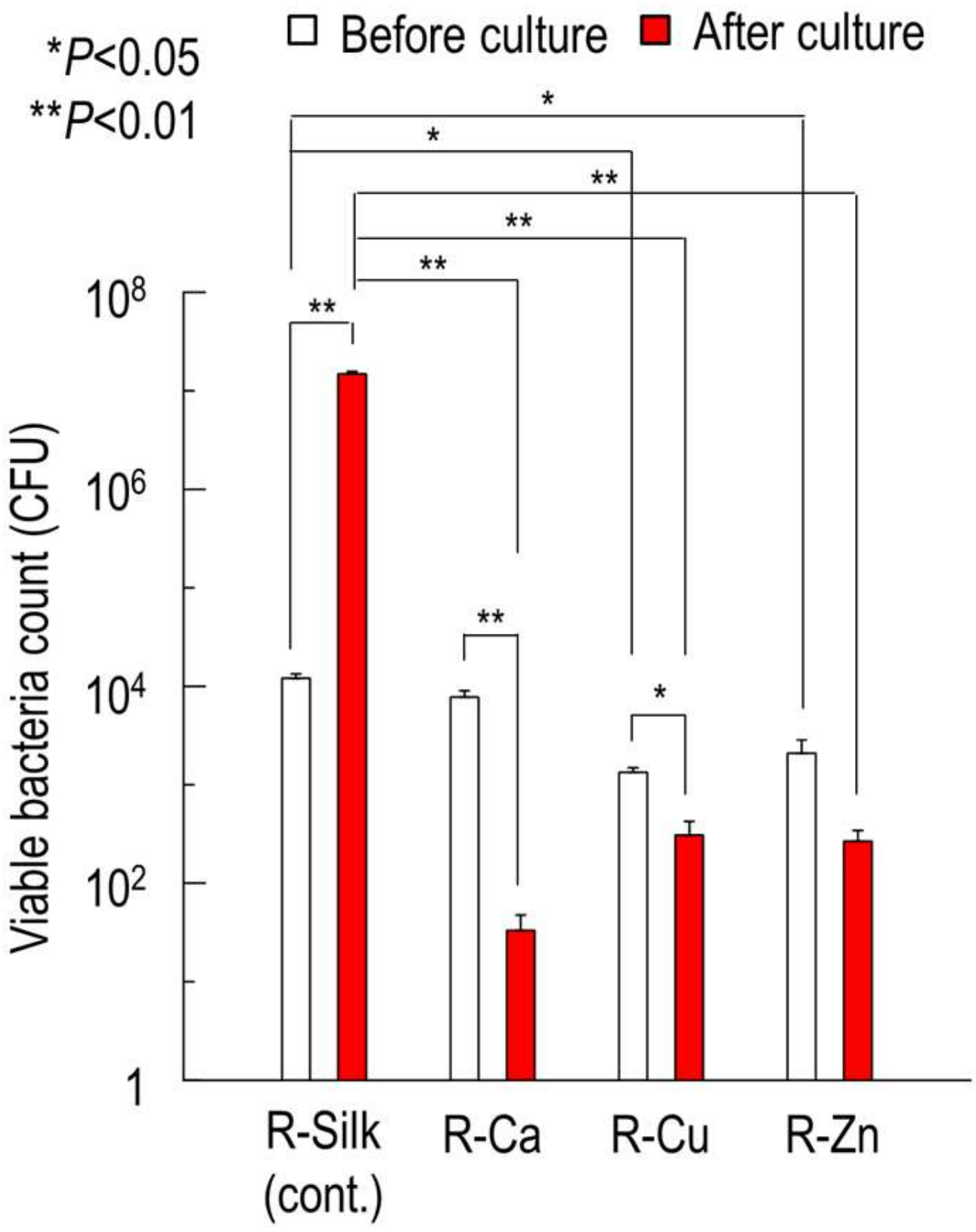
Picture 1:



Caption 1: Fig.1 Viable bacteria count before and after culture.

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Poster presentation

615 Nanostructured titanium surfaces for the improvement of human gingival cell differentiation.

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[2]Balearic Islands Health Research Institute (IdISBa), Spain

INTRODUCTION:

In dental implants, a good sealing between the patient's tissues and implant abutment is key for the implant success and for the prevention of periimplantitis, the main cause of implant failure. Implant surface is directly exposed to the patient's tissues; thus, its modification is being explored in order to improve tissue integration. One strategy is the formation of nanostructures, such as ordered nanotube structures or nanopores on the implant surface.

The main objective of this research was the development of nanostructured titanium (Ti) surfaces that promote soft tissue integration with potential application in dental implants.

METHODS:

Mirror polished titanium discs, c.p. grade IV, 6.2 mm diameter and 2 mm height were used in this study. Nanostructured Ti surfaces were developed by electrochemical anodization and characterized by atomic force microscopy, scanning electronic microscope and contact angle analysis. Primary human gingival fibroblasts (HGFs) were used to test cell adhesion, cytotoxicity, metabolic activity and collagen synthesis. In addition, an exudate test was performed to test nanoparticle release and apoptosis activation on HGFs.

RESULTS AND DISCUSSION:

Using different anodization parameters, two different nanostructures were produced: nanopores (NP) and nanonets (NN), which differed in roughness and pore diameter. HGFs adhesion and metabolic activity at 48h and 7 days was lower on NP surfaces compared to Ti and NN. Metabolic activity and collagen synthesis at 14 days were higher on NN surfaces compared to Ti and NP. On the other hand, only NP surfaces showed nanoparticles release on the exudate test, which increased apoptosis in HGF measured by caspase 3/7 activity.

CONCLUSION:

Our results suggest that NN structuration of Ti surfaces has a great potential to be used for dental implant abutments to improve soft tissue integration.

ACKNOWLEDGMENTS:

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Poster presentation

618 Thiol coating of poly-caprolactone nanofibers by inductively coupled plasma (ICP) polymerization

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[2]University of Mons, Belgium

INTRODUCTION:

The application of nanofibers (NFs) in tissue engineering to restore damaged tissues is strongly increasing as a result of their morphological similarity to the extracellular matrix[1]. Unfortunately, a lot of NFs exhibit poor cell-material interactions as a result of their hydrophobic surface properties[2]. To resolve this issue, NF surface modification through immobilizing of biomolecules is considered as one of the most effective approaches. A possible immobilization strategy is to incorporate in a first step thiol (-SH)[3] groups on the NF's surfaces by means of plasma polymerization after which peptides and/or proteins are covalently linked through these thiol groups to the surface of NFs.

METHODS:

In this study, poly-caprolactone (PCL) nanofibrous mats (Ø: 107 ± 20 nm) were prepared by electrospinning and subsequently subjected to an ICP plasma polymerization treatment using propanethiol as monomer. The surface properties of the PCL NFs before and after the plasma polymerization step were characterized by high-resolution X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM). Biocompatibility of the NFs before and after plasma deposition was also evaluated by seeding human foreskin fibroblasts (HFFs) on differently prepared NFs. 1 and 7 days after cell seeding, cellular behaviour was examined making use of live/dead fluorescence imaging and MTT assays. In addition, the morphology of the cells on the NFs was also examined after cell fixation making use of SEM.

RESULTS AND DISCUSSION:

The obtained results revealed that the total amount of sulphur on the surface of PCL NFs increased from approximately 0 to 32% after plasma polymerization and this without causing any significant morphological changes to the NFs as only a very thin coating is deposited (60 nm) on the NFs by selecting a short deposition time of 1 min. Moreover, the performed surface modification step was found to increase cell proliferation, even without subsequent protein immobilization.

CONCLUSION:

The incorporation of thiol functional groups via plasma polymerization can improve the biocompatibility of NFs and at the same time provide active sites for subsequent biomolecule conjugation.

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Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

627 Bioresorbable and gelatin-enriched bacterial cellulose membrane for periodontal application

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Institute of Engineering Materials and Design, Maribor, Slovenia

INTRODUCTION:

As chronic inflammatory disease, the periodontitis affects the tooth supporting structures, destroying the alveolar bone to extent of eventual tooth loss. The predictable and effective guided tissue regeneration (GTR) principle involves acellular (bio)materials [1] aimed to *ad integrum* regenerate the periodontal tissues and re-establish the periodontium as an inter-tissues´ link. Bacterial cellulose as ultra- fine, pure, gelatinous, network membrane offer numerous assets as biomaterial, yet, demonstrating very limited bioresorption, and ¨inertness˝ towards human cells. Presented research involves post-synthetic BC oxidation and further processing with carbodiimide- stabilised gelatine (GEL) into microstructurally diverse composites. Mineralization screen-up was performed as well as cellular functional testing, as relevant to potential application.

METHODS:

BC pellicle from *Gluconacetobacter xylinum* (Fzmb GmbH, Germany) were treated with 1-2% w/v $NaIO_5$ at dark for 24h, followed by rigorous washing and further evaluation by FTIR/UV-vis spectroscopy and SEM. The specific volumes of 5-10% w/v GEL solution were casted over BC, placed onto bottom of Teflon liners and further freeze/thawed. Specific volumes of EDC/NHS solutions were added to GEL right before casting. Microstructure of BC-GEL composites was examined by CFM using the FITC-labelled GEL. The swelling and physiological stability were examined by immersion test (PBS; 37°C). Mineralization procedure was performed by modified procedure as described [2] and evaluated by FTIR, SEM/EDX and XRD. Cell testing (direct test, MTS assay) was performed on selected composites.

RESULTS AND DISCUSSION:

The BC oxidation was demonstrated by membrane shrinkage, and identified within FTIR spectral band ~ 1720 cm^{-1} (C=O). Oxidation introduce cleavage sites, fostering the physiological degradation process, as evidenced by 3-66% weight loss in 2 weeks immersion. Diverse micro structuring patterns and pore size/wall distributions were identified (**Fig. 1**). Rapid mineralization results identify formulation-dependent capacity for apatite formation.

Fig. 1. CFM (a: membranes´ top, bottom and cross-section) and SEM (b) microgrpahs of native and oxidised BC, as single and composite material with 5(10) % GEL solution.

CONCLUSION:

Native and by periodate-oxidised BC followed by GEL coating with freeze/thaw processing is demonstrated to be feasible method for constructing highly integrated and morphologically graded biocompatible and physiologically degradable composite. Rapid mineralization procedure was successfully applied for semi quantitative screening of capacity to adapt apatite minerals.

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ACKNOWLEDGMENTS:

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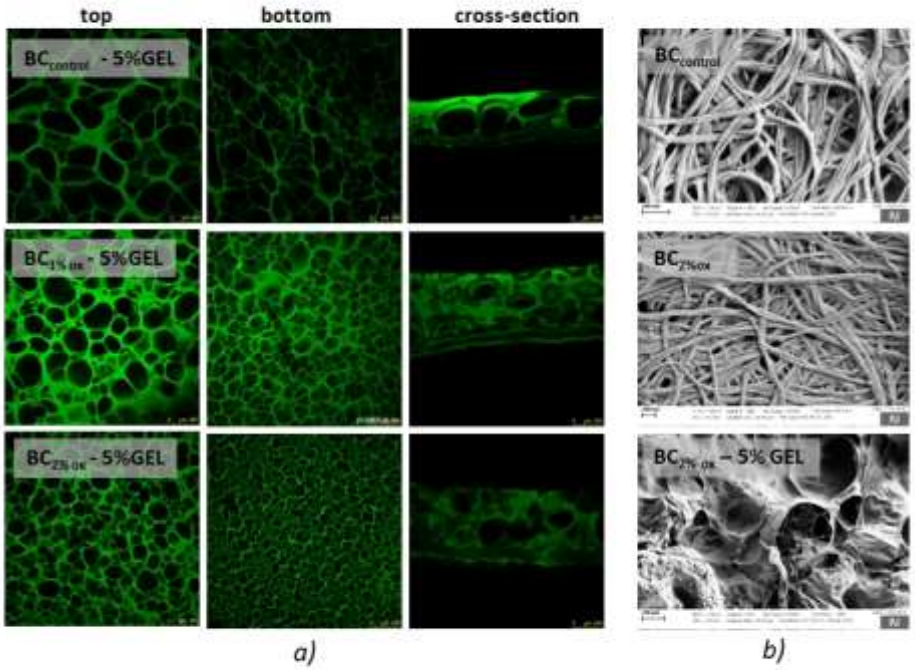
Picture 1:



Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

644 Astaxanthin-loaded Nanostructured Lipid Carriers, a galenic formulation with exceptional antioxidant capacities

Violeta Rodriguez-Ruiz[1], José Ángel Salatti-Dorado[2], Amel Houaoui[3], Emmanuel Pauthe[3], Soledad Rubio[2], Virginie Gueguen[4], Graciela Pavon-Djavid[4]

[1]University of Cergy Pontoise, ERRMECe laboratory, Neuville sur oise, France

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INTRODUCTION:

Astaxanthin is a xanthophyll carotenoid mainly extracted from microalgae *Haematococcus pluvialis*. Astaxanthin possesses powerful natural antioxidant properties that make it a good candidate to be used in antioxidant therapy for

medical, cosmetic and nutritional applications. However, its use, as for many lipophilic antioxidants, is limited by its poor water solubility, chemical instability and its low biovailability, which compromise their pharmacological efficacy. To overcome these drawbacks we propose herein the use of Nanostructured lipid carriers as delivery system to preserve astaxanthin antioxidant capacity.

METHODS:

The aim of the study is to design and develop a green chemistry-made formulation of NLC containing astaxanthin as antioxidant active ingredient. Physicochemical and morphological properties as well as stability of astaxanthin-loaded NLC formulations were comprehensively investigated.

RESULTS AND DISCUSSION:

Spherical and surface negative charged astaxanthin-loaded NLC with a z-average and polydispersity values of ~150 nm and ~0.3 respectively were obtained and characterized by dynamic light scattering (DLS), atomic force microscopy (AFM) and scattering electron microscopy (SEM) techniques. Astaxanthin loading was also investigated showing an astaxanthin recovery of more than 90% after synthesis of NLC suspensions assessed by spectrophotometric measurements. Lipophilic antioxidant test was performed to confirm the protective properties of systems.

CONCLUSION:

Our results demonstrated a novel galenic formulation with exceptional antioxidant capacities that protects astaxanthin and enhances its solubility, improving thereby the astaxanthin antioxidant capacity (more than 20 times).

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ACKNOWLEDGMENTS:

The authors are grateful to Pigmentos Naturales S.A. Chile for providing *Haematococcus pluvialis* powder and to Dr. Sébastien Peralta (AFM) LPPI (EA2528) and Rémy Agniel (MEB) ERRMECe (EA1391) both from Institut des matériaux, I-MAT (FD4122), Université de Cergy-Pontoise, F-95000 Cergy-Pontoise, France

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

649 Comparison of 3D Composite Scaffold made by 3D Fiber Deposition(3DFD) and Fused Deposition Modeling (FDM)

Jiaping Li, Pamela Habibovic, Lorenzo Moroni

MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, Netherlands

INTRODUCTION:

Design of open-cell porous scaffold to match the mechanical properties of bone is of importance for long term stability of orthopaedic implant (1). Additive manufacturing (AM) techniques, which combine computer aided design and manufacture, give the ability to define and control individual process parameters and enable the creation of highly accurate and consistent pore morphologies. Recently, we developed porous Polyactive/β-TCP scaffold by two AM techniques 3DFD and FDM (2).

METHODS:

3DFD Scaffold: A composite particle with 50 wt% of Tri Calcium phosphate in Polyactive 1000/70/30 is loaded in stainless syringe and melted at 190°C and forced through the syringe nozzle by using 3D-bioplotter machine. The melted composite is plotted on a stage as a fiber, which rapidly solidified, and then the scaffold is fabricated by layering a 0°-90° pattern of fibres from a steel nozzle layer-by-layer..

FDM 3D scaffold: A filament of composite scaffold (50/50, same composition as 3DFD scaffold) was made by using twin extruder (HAAKE MiniCTW**,** Germany). This filament is loaded on 3D printing machine(Hy-rel , US). The melted composite fiber from heated nozzle at 180°C is plotted on a stage as a fiber, which rapidly solidified, and then the scaffold is fabricated by layering a 0°-90° pattern of fibers.

RESULTS AND DISCUSSION:

Both technologies are similar, the main difference is raw materials. Composite particle was used in 3DFD, and composite filament is applied in FDM. The layer thickness, shape and fiber diameter of 3DFD and FDM are dependent on the nozzle size. Due to the difference of extruding, the nozzle size of 3DFD could be smaller than that of FDM. However, FDM is low cost, high productivity and easy operation.

CONCLUSION:

In conclusion, 3D composite scaffold can be made by both 3DFD and FDM, which is of interest in orthopaedic implant fabrication and tissue engineering. In view of process of FDM, in the future this technology offers the low cost, high reliability and simple operation for scaffold design and fabrication.

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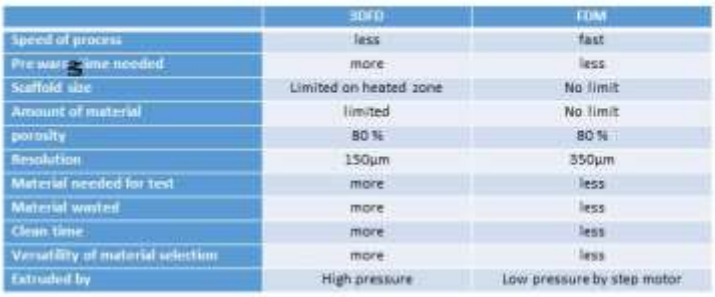
ACKNOWLEDGMENTS:

This research has been in part made possible with the support of the Dutch Province of Limburg.

Picture 1:



	3DFD	FDM
Speed of process	less	fast
Pre warm time needed	more	less
Scaffold size	Limited on heated zone	No limit
Amount of material	limited	No limit
porosity	80 %	80 %
Resolution	150µm	350µm
Material needed for test	more	less
Material wasted	more	less
Clean time	more	less
Versatility of material selection	more	less
Extruded by	High pressure	Low pressure by step motor

Caption 1: Table 1 Comparison of 3DFD and FDM

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Poster presentation

651 Characterisation of hydroxyapatite thin film coatings on magnesium alloys for orthopaedic applications

Stephen Mckillop, Jonathan Acheson, Patrick Lemoine, Brian Meenan, Adrian Boyd

Ulster University, Belfast, Northern Ireland

INTRODUCTION:

Magnesium (Mg) alloys offer significant benefits for the fabrication of orthopaedic implants[1]. In addition to exhibiting high strength and toughness, Mg alloys undergo complete resorption in vivo, meaning, once implanted, devices employing Mg alloys do not need a second surgery for their subsequent removal. The key to creating a suitable Mg based alloy implant is the control of the alloy's dissolution rate[2]. One approach is to apply a barrier coating, such as hydroxyapatite (HA), which has the added benefit of being bioactive, as well as being stable in physiological solution. Work reported here investigates the coating of Mg alloy using hydroxyapatite via radio frequency (RF) magnetron sputtering, providing a high degree of process control during deposition. Once applied the physical, mechanical and chemical properties of the coatings will be fully investigated.

METHODS:

Commercial magnesium alloy AZ31 (Goodfellow) was abraded using SiC papers beginning at 800grade rising to 1200grade. The HA coating was applied using RF magnetron sputtering (150W,10hrs,3.3W/cm^2,0.00005Pa). The target used was pressed using commercial HA, (Plasma Biotal, UK). Characterisation of the surfaces produced included the use of X-ray Photoelectron Spectroscopy (XPS), Scanning Electron Microscopy (SEM), Time of Flight Secondary Ion Mass Spectroscopy (ToF-SIMS) and Atomic Force Microscopy (AFM).

RESULTS AND DISCUSSION:

XPS results show the presence of strong Ca2p(347.5 eV) and P2p(133.2 eV) peaks with a Ca/P ratio close to 1.67, highlighting that the deposition of hydroxyapatite has occurred. No Mg was detected in the HA sputtered coating, indicating the surfaces were continuous and homogeneous. These results were confirmed by ToFSIMS , with CaOH+ and Mg+ peaks (m/z-56.9 and 23.9, respectively) being used to map the surface and to undertake depth profiling, as highlighted in figure 1. Similarly, ToFSIMS, in combination with stylus profilometry , was used to determine that the coating thickness, with a nominal thickness of up to 700 nm.

CONCLUSION:

Results show how a HA coating can be easily sputter deposited onto Mg based alloys in order to produce a dense, coherent and homogenous HA surface. Such a surface will provide a potential barrier to resist dissolution of the alloy, and provide a bioactive layer in order to promote Osseointegration.

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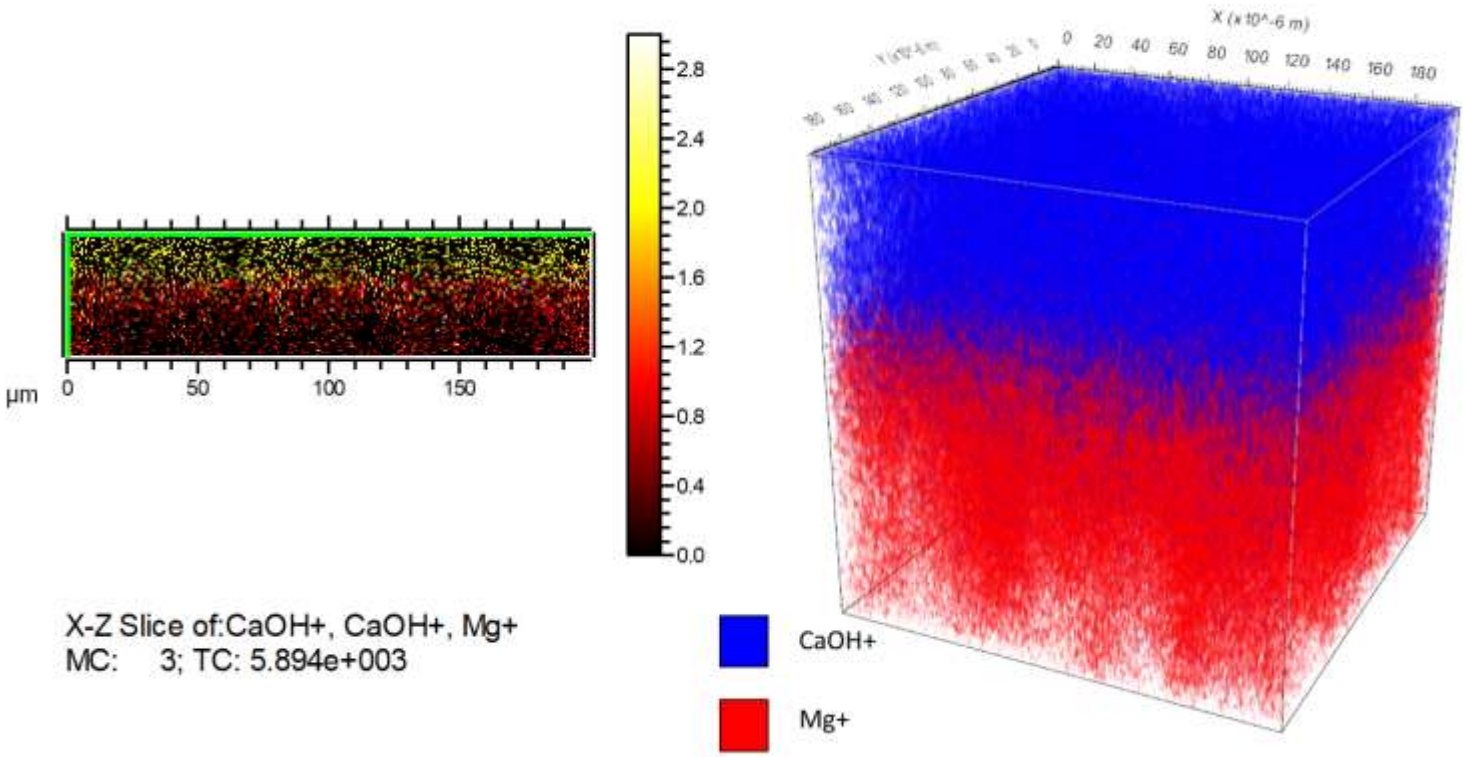
Liu X *et al.*Applied Surface Science,434,1101–1111,2018

ACKNOWLEDGMENTS:

The authors would like to acknowledge the PhD funding provided by the Department for the Economy (Northern Ireland) to support this work.



Picture 1: Caption 1: Figure1. ToFSIMS 3D depth profiling reconstruction using CaOH+ and Mg+ ions for coating and substrate respectively.

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

655 Curvotaxis directs cell migration through cell-scale natural landscapes

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[6]Federal University Rio de Janeiro, Brazil

INTRODUCTION:

In vivo, cells are in contact with various topographical features that span several orders of size and organization. The cellular integration of these topographical traits affects multiple aspects of the physiology of the cell. A large body of

studies have highlighted that cells are sensitive to nanotopographies or geometrical cell-scale structures[1,2]. Natural biotopes also exhibit much larger topographical cues that are often curved and smooth, such as walls of blood vessels, bone cell cavities, acini, or other cell bodies. Very little is known about how cells read and integrate cell-scale curvatures, and the mechanisms leading to the integration of such physical cues.

METHODS:

The model surfaces that are usually used present sharp edges and angles that strongly limiting their relevance for mimicking the *in vivo* topographical context. Herein we develop a two-step fabrication method to produce a series of edge-free cell-scale sinusoidal landscapes with minimized anisotropy and very low micro roughness. We employ these new model surfaces to investigate specifically the mesenchymal stem cell response to cell-scale curvature variations. We combine live imaging, biochemistry and modeling approaches to decipher the cellular integration mechanism.

RESULTS AND DISCUSSION:

We report a new cellular sense which we term “curvotaxis” that enables the cells to react to cell-scale curvature variations, a ubiquitous trait of cellular biotopes. We show that cells avoid convex regions during their migration and position themselves in concave valleys. Computational modeling, pharmacological assays and live imaging show that curvotaxis relies on a dynamic interplay between the nucleus and the cytoskeleton - the nucleus acting as a curvature sensor that guides cell migration towards concave curvatures[3]. Further analysis show that substratum curvature affects nuclear shape, intracellular tensions and gene expression. Differentiation of stem cells is limited when they are in concave curvatures.

CONCLUSION:

Altogether, this work identifies curvotaxis as a new guiding mechanism and promotes cell-scale curvature as an essential physical cue fully integrated by the cells. Moreover, our results suggest that cell-scale curvature might be a true component of the stem cell niche.

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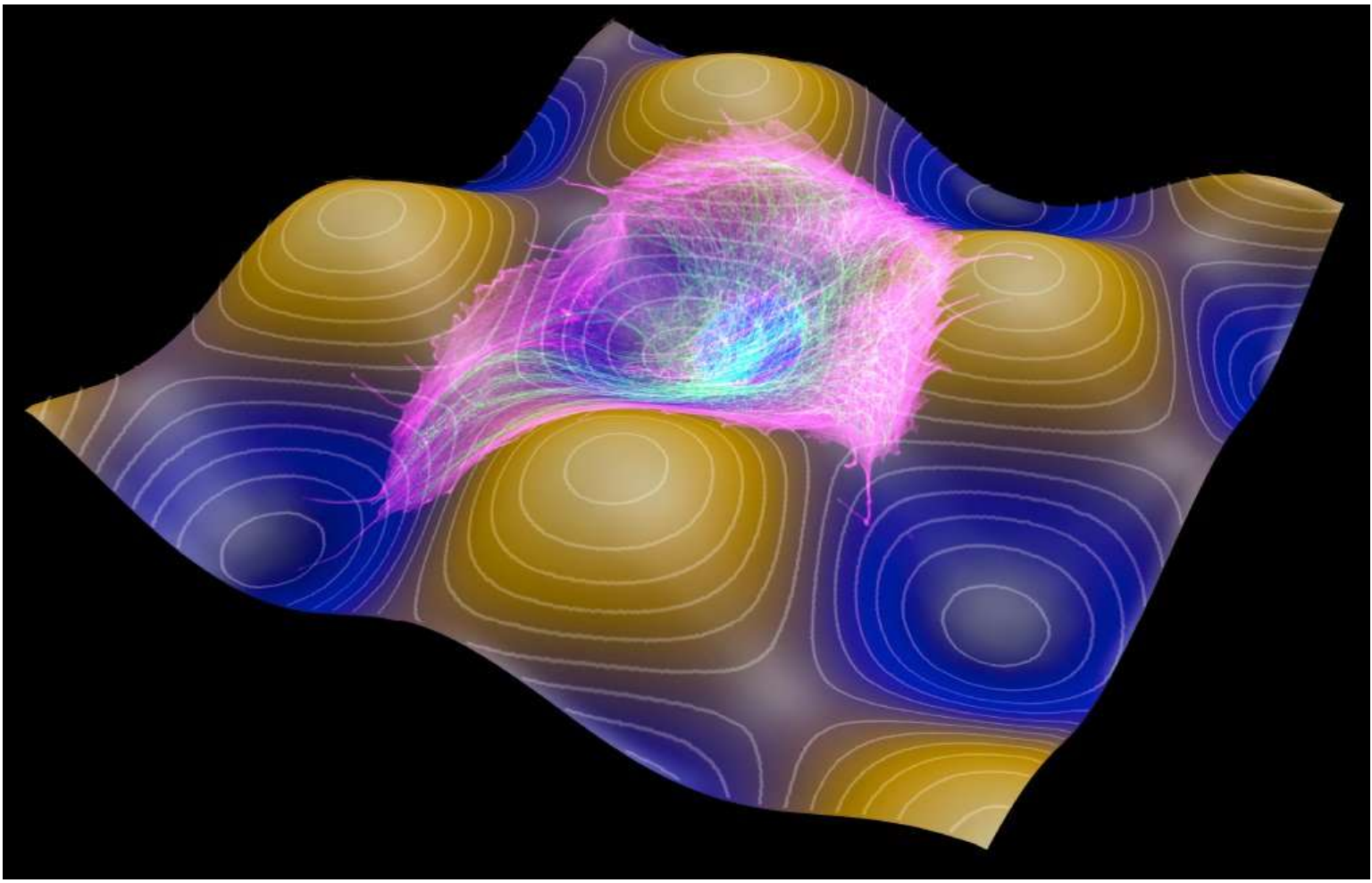
ACKNOWLEDGMENTS:

The authors thank the Agence Nationale de la Recherche (Grant No. ANR-12-BSV5-0010-02) for funding the research.



Picture 1: Caption 1: Mesenchymal stem cell surfing a 3D sinusoid

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

658 Continuous Flow Synthesis of Gold Nanoparticles for Biomedical Applications

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[2]University College London / Department of Chemical Engineering, United Kingdom

INTRODUCTION:

Gold nanoparticles (Au NPs) have many potential applications as biomaterials e.g. in cancer diagnostics, biomedical imaging, for antimicrobial surfaces and as carriers for therapeutic agents. The properties of the gold nanoparticles and their applicability as biomaterials strongly depend on their particle size and surface functionality. Special attention thus has to be centered to the precise engineering of nanomaterial surfaces, as they make up the interface to the biological environment.

METHODS:

Gold nanoparticles were synthesized from gold (III) chloride trihydrate and trisodium citrate in a capillary-based continuous flow system, as it allows for much better control of particle size and surface functionality as compared to the conventional batch process. Surface-to-volume ratio, average residence time and temperature effects were investigated, but the material and surface properties of the capillaries were found to crucially affect the characteristics of small nanoparticles.

Since surface charge determines the electrostatic interaction between the solid surface and nanoparticles in the liquid phase, it is a key parameter that affects continuous flow synthesis. Different tubing materials, i.e. polymeric and fused silica, were tested by means of zeta potential analysis using the streaming potential method. The zeta potential studies of the inner surface of the capillaries were performed in a proprietary measuring cell at defined pH and ionic strength.

RESULTS AND DISCUSSION:

Fast nucleation is the key for the formation of monodispersed and small nanoparticles. The nucleation rate during gold nanoparticle synthesis was found to be affected by the surface interaction between the reactants and the walls of the capillary reactor. The zeta potential of the tubing wall was found to have a direct influence on the concentration of gold precursors, whereas opposite surface charge of the tubing walls and the gold precursors were found to be beneficial for decreasing particle size. The surface charge of different tubing material was tested, among them PTFE, FEP, PEEK and fused silica, whereas the latter was found to favor the formation of the smallest-sized gold nanoparticles.

CONCLUSION:

During continuous flow synthesis of gold nanoparticles, special attention has to be paid to the surface charge of both, the nanoparticles but also the capillary surfaces. In combination with the charge of the nanoparticles, the zeta potential of the capillaries is thus a key parameter for controlling gold nanoparticle synthesis.

ACKNOWLEDGMENTS:

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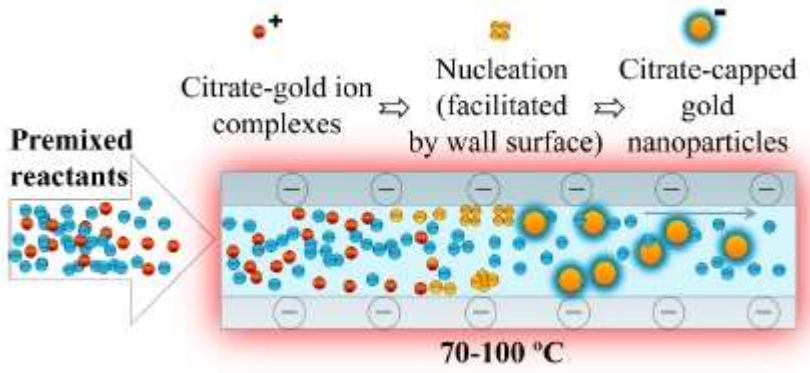

Picture 1:

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Poster presentation

667 A comparative in vitro study of cell growth on polylactide textile scaffolds for tissue engineering applications

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INTRODUCTION:

Biodegradable textiles have potential for tissue engineering scaffolds due to their porosity and possibility to manufacture high number of devices in decent time period. For successful production of textiles, good enough mechanical properties are required from the fibers/yarns. Long enough retention of properties *in vitro* and biocompatibility are required from fibers and textiles. Porosity, pore sizes and pore shapes affect cell coverage and distribution.[1]

The aims of this study were to evaluate the strength retention of fibers and compare cell viability and distribution on textile scaffolds.

METHODS:

Poly-L/D-lactide 96/4 (PLDLA 96/4) fiber bundles were melt-spun and used to manufacture braided, knitted and woven textiles. They were processed into multi-layered scaffolds using heat sealing and compression at elevated temperature, packed and gamma-irradiated for sterility.

A 36-week hydrolytic degradation experiment in cell culture medium was performed to gamma-irradiated fiber bundles to demonstrate changes in mechanical properties, crystallinity and thermal properties.

Human urothelial cells (hUCs) and human foreskin fibroblasts (hFFs) were used in the cell culture. Live/Dead analysis (days 1, 7 and 14) and crystal violet staining (day 4) were used to assess cell viability and distribution (2 parallel samples).

RESULTS AND DISCUSSION:

Tensile strength and strain at load (max) of the fibers decreased during degradation with 33 % and 9 % in 36 weeks. Crystallinity increased few percent. Glass transition temperature slightly decreased as expected from the literature [2,3]. Melting temperature increased with 2 %. Average pore size in the scaffolds varied from 0 mm^2 to 0.350 mm^2.

The cell cultures demonstrated good biocompatibility of the textile scaffolds. After 2 weeks, the dense braided and woven scaffolds had the highest number of attached cells. The large pores of knitted scaffolds remained mostly cell free with both cell types. In addition, the cells preferred to attach to pits instead of ridges.

CONCLUSION:

The experiments demonstrated that polylactide fiber bundles have suitable properties to be processed into biodegradable braided, knitted and woven textile scaffolds that have different porosities. These structures support viability of cells *in vitro*. The number of attached cells was higher in structures with smallest pores and pits were preferred to ridges.

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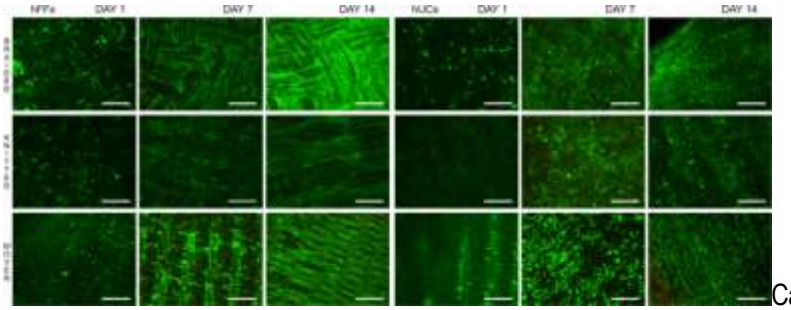
ACKNOWLEDGMENTS:

This study was supported by the Human Spare Parts project of Business Finland.

Picture 1: 

Caption 1: Fluorescence images of hFFs and hUCs on PLDLA 96/4 scaffolds. Scale bars 500 µm.

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Poster presentation

672 Micropetrosis - magnesium whitlockite single-crystal formation in bisphosphonate-exposed human bone

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INTRODUCTION:

In bone, osteocytes (Ot) reside within lacunae (Lc) and play a key role in bone remodelling. Bisphosphonates are administered frequently to "prevent osteoporotic fractures; they suppress Ot apoptosis, may be localised" within Ot.Lc[1], reduce osteoclast" viability and thus impair damaged tissue repair[2]. Following apoptosis, the Ot.Lc undergoes mineralisation – a phenomenon called "micropetrosis". The prevalence of such hypermineralised Ot.Lc increases bone fragility[3].

METHODS:

Using nanoanalytical electron microscopy with complementary" spectroscopic, crystallographic, and nanoindentation experiments, we investigate Ot.Lc mineralisation in bisphosphonate-exposed human alveolar bone from women who had received bisphosphonates (*BP*) and from healthy, non-osteoporotic women (*Ctrl*).

RESULTS AND DISCUSSION:

Hypermineralised Ot.Lc in *BP* bone contained equiaxed, ~80 nm to ~3 µm wide, spherical to rhomboidal mineral nodules, as observed using backscattered electron scanning electron microscopy. In comparison to the surrounding bone matrix, these mineral nodules had ~120% higher hardness and ~50% higher reduced elastic modulus. Raman spectroscopy revealed a whitlockite-like structure of these nodules. The v_1 PO_4^{3-} and the v_2 PO_4^{3-} bands appeared at ~970–972 cm^{-1} and ~407 cm^{-1}, respectively. For bone apatite, the v_1 PO_4^{3-} and v_2 PO_4^{3-} bands typically occur at 958–960 cm^{-1} and ~432 cm^{-1}, respectively. High-angle annular dark field scanning transmission electron microscopy also showed spherical and strikingly facetted rhomboidal nodules, and occasionally spherical nodules having a fuzzy exterior, a core-shell structure and nanoporosities throughout the bulk. Selected area electron diffraction showed that the rhomboidal nodules were almost exclusively single-crystals of magnesium whitlockite. Spherical nodules were also highly crystalline. However, spherical nodules having a fuzzy exterior appeared polycrystalline. Elemental analysis using energy dispersive X-ray spectroscopy showed higher Mg, P, and O content of the mineral nodules, and higher Mg/Ca but lower Ca/P ratios than bone matrix. The lower Ca and higher O content of mineral nodules than bone matrix was confirmed using electron energy loss spectroscopy. In *Ctrl* bone, hypermineralised Ot.Lc where individual mineral nodules could not be readily identified mainly contained heterogeneously oriented acicular apatite nanocrystallites. Compared to bone matrix, Mg-enrichment of these nanocrystallites was almost negligible. Occasionally, these nanocrystallites stacked together to form dense aggregations.

CONCLUSION:

In *BP* bone, Ot.Lc mineralisation involves the formation of ~80 nm to ~3 µm wide, facetted, magnesium whitlockite crystals and altered local mechanical properties as a consequence.

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Poster presentation

676 Tissue engineering a model of bisphosphonate-related osteonecrosis of the jaw

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INTRODUCTION:

Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is a disease found in patients taking bisphosphonates (BPs), a group of drugs widely used to treat osteoporosis and bone metastases. BRONJ often follows dental surgery, and presents as exposed, necrotic sections of the jaw where the overlying soft tissue fails to heal. This study aims to model the soft tissue effects of BRONJ in vitro using a 3D oral mucosa model which can be used to develop future treatment strategies.

METHODS:

Human oral fibroblasts and keratinocytes were cultured onto de-cellularised dermis for 3 days before models were lifted to air liquid interface (ALI)[1]. Models were then cultured at ALI for up to 14 days to allow keratinocytes to form a stratified epithelium. Different wounding methods were tested to optimise the model to more represent the clinical scenario. After growth at ALI, the models were dosed with pamidronic or zoledronic acid, two BPs most likely to cause BRONJ. Resazurin assays were used to assess the viability of cells within the oral mucosa, with histology used to monitor wound healing. The effect of BPs on these three dimensional models as well as in 2D cell monolayers was measured to determine the effect of BPs on soft tissue viability.

RESULTS AND DISCUSSION:

Tissue comparable to native oral mucosa was cultured, with histology showing a stratified squamous epithelium. Wounding caused a drop in cell viability which was recovered over time. Biopsy punch wounds to the epithelium healed completely over 10 days. Oral mucosa cell viability was lowered in the presence of physiologically relevant concentrations of BPs in a dose dependent response, in both 2D and 3D.

CONCLUSION:

Here we have demonstrated that a tissue engineered model of the soft tissue component of BRONJ can be cultured *in vitro*, containing both dermal and epithelial cells, a healing wound which represents those found in BRONJ patients and relevant BP concentrations. This model will allow for more detailed study of the development of the disease and also present a method by which to study potential treatments.

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ACKNOWLEDGMENTS:

The authors would like to thank the EPSRC for funding this work.

Picture 1: Caption 1: H&E stained sections of oral mucosa model grown for 7 days then wounded with a biopsy punch, fixed (A) immediately and (B) 10 days after wounding.

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Poster presentation

678 Degradable polymers with time-dependent mechanical characteristics or influenced by deformation strain contribute to improved tissue regeneration

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INTRODUCTION:

Cells are continuously influenced by a range of mechanical strains, for example fluid-flow-induced shear stress, cyclic stretching, compression and undirected deformation. The acting physical force is subsequently communicated through extracellular matrix to the cytoskeleton via physical mechanotransduction. Recognizing such influences of mechanical stimuli on cell phenotype regulation has allowed important advances in tissue engineering. Herein, we have evaluated mechanical strain stimulation from two different perspectives 1) preclinical evaluation using material with time-dependent mechanical characteristics[1] 2) in vitro evaluation of degradable polymers influenced by deformation strain.[2,3]

METHODS:

TIGRâ Matrix Surgical Mesh is a macroporous multifilament surgical mesh, knitted from two different synthetic resorbable fibers, possessing different degradation characteristics. In 14 female sheep, a medial incision of the abdominal skin was performed. Four full-thickness, 3*3 cm defects were created within the abdominal musculature, a mesh was placed in an onlay position.

3D poly(L-lactide-co-e-caprolactone) porous scaffolds of 10.5 mm in diameter and 12 mm in length were exposed, in a bioreactor, to uniaxial compression and unloading. Media perfusion was robustly ensured thorough flow control.

RESULTS AND DISCUSSION:

The mesh gradually degraded and the formed granulation tissue was at the beginning rich in collagen type III, during the wound healing process it was replaced by collagen type I. When compared to a synthetic non-degradable mesh, the ratio collagen type I:collagen type III was higher in the mesh with time dependent characteristics. The degradable mesh was well integrated into fibrous connective tissue and early neovascularization was observed.

Compared with a constant flow control conditions, the level of calcification did not increase when the scaffold was deformed at 2 Hz or when the deformation amplitude varied but the level of calcification increased significantly when the scaffold was deformed at 1 Hz. The results also revealed that deformation strain of degradable 3-dimensional scaffolds was the predominant stimulus for skeletal precursors to undergo osteogenesis in earlier stages of osteogenic cell maturation.

CONCLUSION:

The results demonstrate the importance of mechanical stimulation in tissue engineering. Mechanical strain using time dependent mechanical characteristics stimulate remodeling of collagen towards a strong connective tissue and deformation strain stimulate early osteogenic cell maturation.

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Poster presentation

679 Molecular weight of Hyaluronic acid modulates the morphology of gastric cancer cell

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INTRODUCTION:

Hyaluronic acid (HA) is a glycosaminoglycan and one of the main ligands of the transmembrane receptor CD44, usually overexpressed by gastric cancer cells.[1]CD44-HA interactions activate different signaling pathways that trigger the potential of malignant cells to migrate, leading to the formation of metastasis.[2] Epithelial-mesenchymal transition (EMT) is associated with cancer progression and thought to be a key event in cancer invasion and metastasis of epithelial cancer cells.[3] Herein, we studied the effect of the HA molecular weight on the adhesion and morphology of gastric epithelial cell line AGS and its relationship with a mesenchymal phenotype. We immobilized HA with different M_w in a Layer-by-Layer (LbL) fashion and studied the interactions of the substrates with AGS. In these substrates, HA is presented in an ECM-relevant manner with restricted mobility but able to reorganize and bind to other biomolecules, such CD44.

METHODS:

The adsorption (10 layers) of HA (6.4 kDa, 752 kDa or 1500 kDa) and PLL (30-70 kDa), was followed by QCM-D. HA was the last layer of all LbL constructs. SurPASS electrokinetic analyzer, was used to determine the zeta potentials of the films (n=3). The adherent cells were fixed and stained for actin (phaloidin) and nuclei (DAPI).

RESULTS AND DISCUSSION:

On the QCM-D, the adsorption of PLL and HA was confirmed by a decrease of F and an increase of D (Fig. 1A). The presence of HA as the end layer of the substrates', was verified by electrokinetic analysis (Fig. 1B). The diffusion between the positive (PLL) and negative (HA) layers is more pronounced in the surfaces with 6.4 kDa, being reflected on a lower zeta potential. AGS cells seeded on $(PLL\text{-}HA)_5$ (Fig. 1C), show a spindle-like morphology (white arrow), predominant on the surface presenting HA 6.4 kDa. The acquisition of this morphology is associated with a mesenchymal phenotype [4], which decreases as the Mw of HA increases. The occurrence of this phenotype is

characteristic of EMT, in which epithelial cells loses their polarity and intercellular adhesion and gain characteristics of mesenchymal cells.[3]

CONCLUSION:

We demonstrate the LbL deposition is a feasible approach for presenting HA in a ECM relevant way. The morphology of AGS cells is affected by the Mw of the immobilized HA.

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ACKNOWLEDGMENTS:

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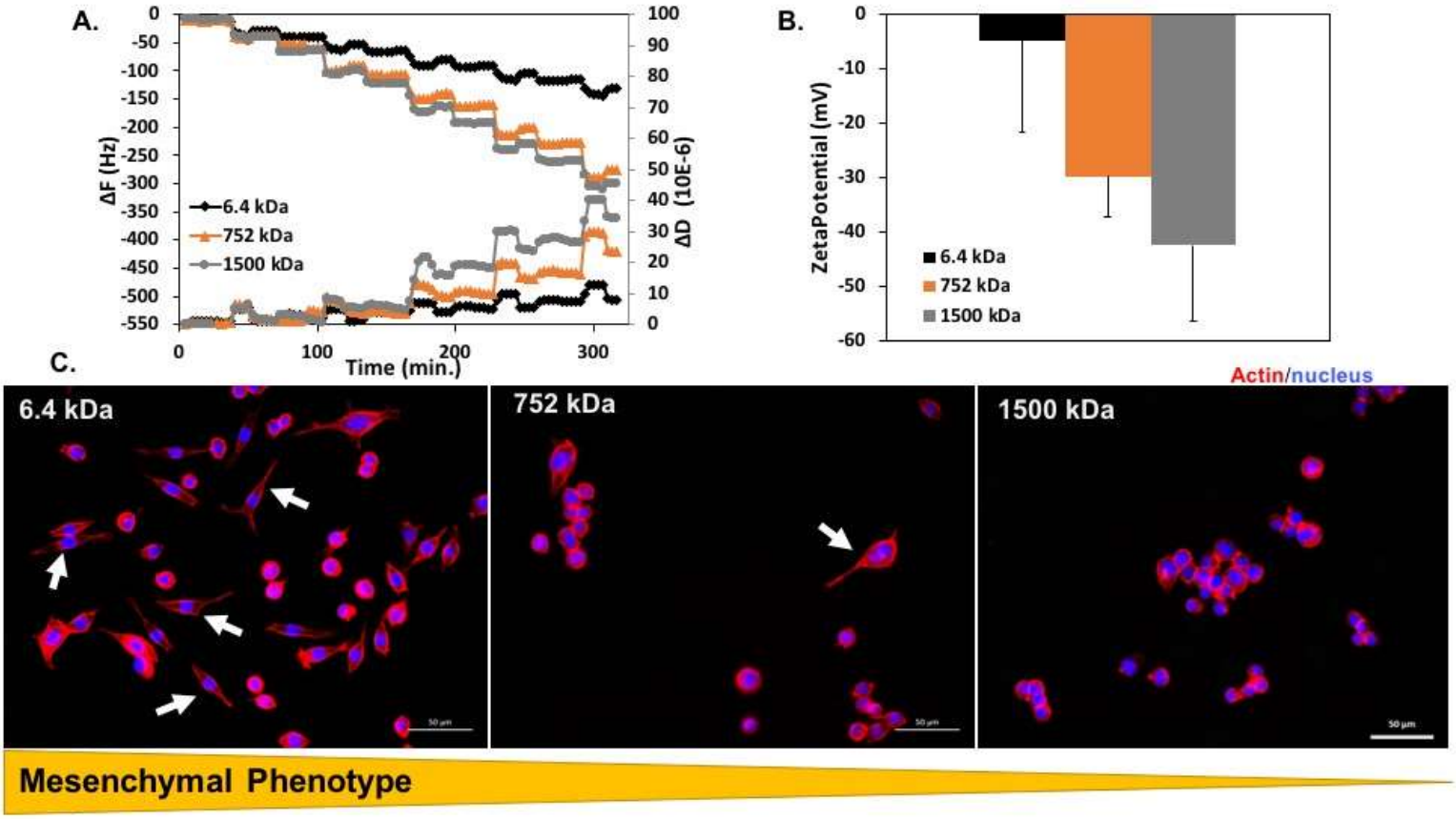

Picture 1: Caption 1: LBL adsorption of PLL-HA followed by QCM-D (A.); Zeta potential of (PLL-HA)5 with different HA Mw (B.); AGS cells seeded on (PLL-HA)5 surfaces (C.)

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Poster presentation

681 Silicon oxynitride breath figures patterned coatings for enhanced bone cells interaction.?

Cristiano Carlomagno[1], Claudio Migliaresi[1], Antonella Motta[1], Pranesh Aswath[2], Gian Domenico Sorarù[1], Devid Maniglio[1]

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INTRODUCTION:

Titanium alloys are the material of choice for bone prostheses. Their osteointegration can be improved using bioactive coatings with specific morphology, extending, consequently, the duration of the implant[1]. Recently, silicon oxynitride (SiON) has been proposed as titanium implant coating, due to its osteoinductive ability, the capacity to bind titanium, and the possibility to impress specific patterned features.

In this work we propose a new method for realizing SiON patterned coatings suitable for titanium alloys, using a fast surface-assembly technique called Breath-Figure (BF) method, thus improving the properties of titanium alloys.

METHODS:

A medical grade photo-crosslinkable silicone (Loctite® 5248™, Germany, Henkel) in ethyl acetate solutions (3%, 5% and 7%) was used as precursor for the BF method. BFs were realized pouring the precursor solutions onto Ti6Al4V disks exposed to moist air flow in a flow chamber with a UV-light transparent window to allow photo-curing during the assembly process. After the BF formation, the pre-ceramic material underwent pyrolysis in furnace (Thermo Scientific Heraeus) in ammonia atmosphere to induce ceramic conversion (Figure). Samples were tested by SEM, XRD and XPS. ICP was used to evaluate the Si^{4+} ion released from the surface after 6 weeks of immersion in alpha-MEM.

hMSC adhesion, distribution and morphology on the samples were analyzed by confocal microscopy and by AlamarBlue, Pico-Green, Alkaline-Phosphatase assays.

RESULTS AND DISCUSSION:

The process variable influencing the BF formation on the silicone film were studied and selected to realize specific geometrical patterns. Four different surface patterns, with single or bimodal pore size distribution were obtained by varying the precursor concentrations: 13.1±0.6 µm (7%), 34.4±0.2 µm and 58.8±0.6 µm (5%), 71.3±1.3 µm and 155.0±1.6 µm (3%). Si^{4+} ions release from the samples was measured and increased inversely with the precursor concentration.

The effect of topography and Si ions release on cells in terms of adhesion, proliferation and viability was studied using hMSC in contact with the samples. Samples having porosity range of 34.3±0.2 µm and 58.8±0.6 µm showed a high initial adhesion and proliferation rate, with respect of all the other ceramic materials. Moreover materials with the highest ions release, show the most significant ALP activity.

CONCLUSION:

The ceramic coatings produced by ammonia pyrolysis after BF patterning showed a direct impact on cell proliferation and activity. The release of Si^{4+} ions positively modulated ALP activity.

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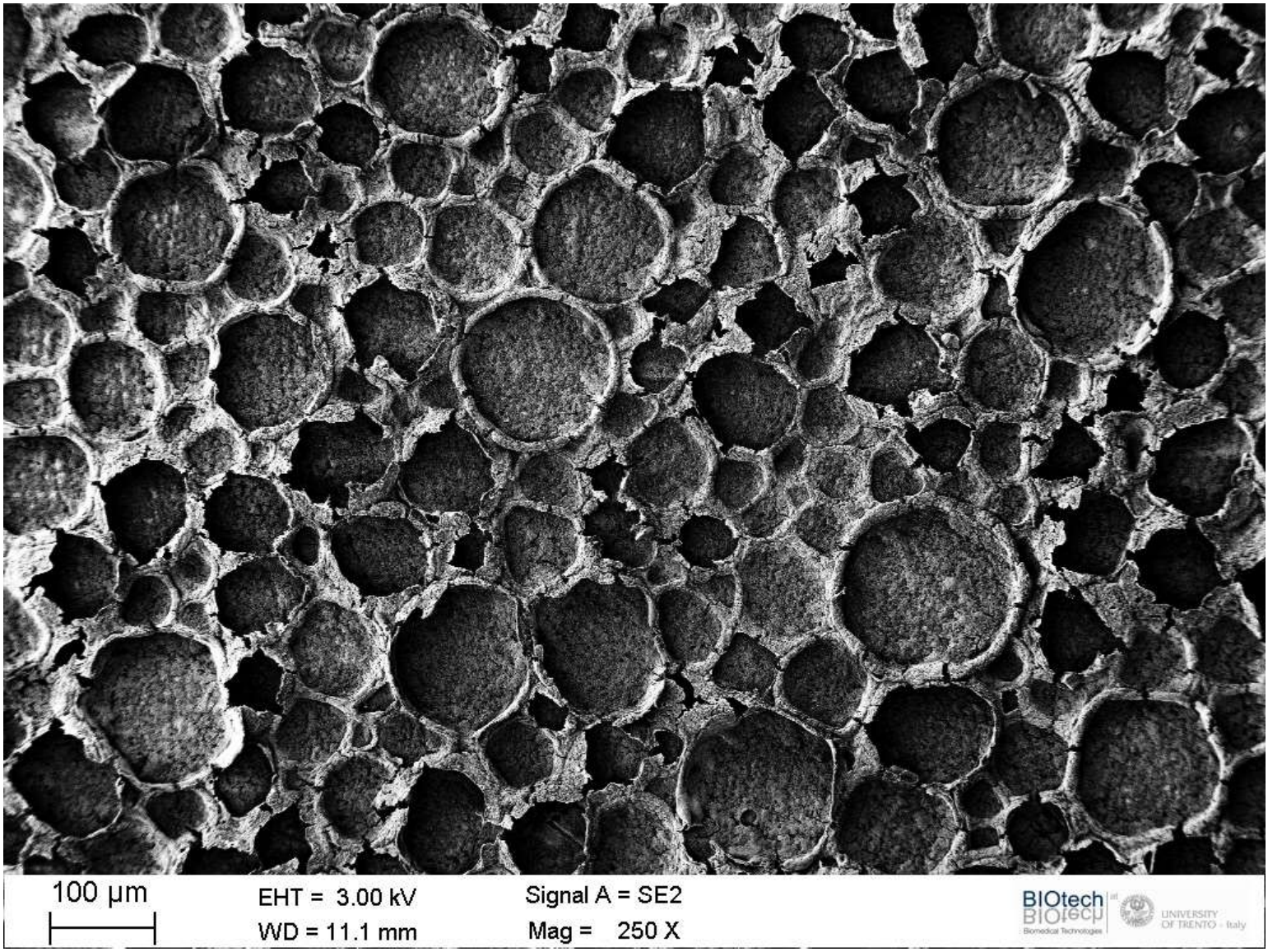

Picture 1: Caption 1: SiON coating obtained starting from alcoxy-silicone film patterned using Breath Figures method, followed by pyrolysis in reactive NH3 atmosphere.

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Poster presentation

687 Synthesis of iron nitride for magnetic hyperthermia of cancer

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Tohoku University, Sendai, Japan

INTRODUCTION:

There has been a strong demand for minimally invasive treatments of cancer. One of them is the magnetic hyperthermia, where magnetic particles generate heat due to the magnetic loss under alternating current (AC) field and thus give hyperthermal effect locally. Iron oxide, specifically magnetite and maghemite have been primarily studied as thermoseeds. Iron nitride with specific compositions is another candidate material since it has a higher saturation magnetization (*Ms*) than iron oxide. In this study, iron nitride was synthesized from magnetite nanoparticles (MNPs) with several sizes. Its structure and magnetic properties were investigated, and heat generation was also estimated from the hysteresis loops.

METHODS:

MNPs with various sizes (8-400 nm) were synthesized following the procedures established in previous studies[1]. Synthesis of iron nitride nanoparticles was attempted by reducing and nitriding them in H_2 and NH_3 gas flow. The structural characteristics were investigated using powder X-ray diffraction and a transmission electron microscope, and the magnetic properties were measured using a vibrating sample magnetometer. The heat generated by the samples due to the magnetic hysteresis loss under AC field (100 kHz, 300 Oe) was estimated by calculating the area of the hysteresis loops measured in direct current (DC) field up to 300 Oe.

RESULTS AND DISCUSSION:

Iron nitride with the composition of $Fe_{16}N_2$ was successfully synthesized from MNPs with several sizes by modifying conditions of reduction and nitriding. Especially, the MNPs of 50 nm in size (M50) showed *Ms* of 82 emu/g and coercive force (*Hc*) of 160 Oe, and the sample synthesized from M50 after nitriding for 30 hours (M50-30h) showed higher *Ms* of 120 emu/g and higher *Hc* of 1000 Oe. Moreover, under DC field up to 300 Oe, M50-30h gave a hysteresis loop with larger area than M50 (see Fig. 1), and it was estimated from the loops that heat generation of M50-30h and M50 were 23 and 11 W/g, respectively. The higher heat generation is mainly due to the higher *Ms* and *Hc* of $Fe_{16}N_2$ in M50-30h. These results suggest that $Fe_{16}N_2$ can be used as thermoseeds with high heating efficiency for hyperthermia.

CONCLUSION:

Iron nitride ($Fe_{16}N_2$) was successfully synthesized from MNPs with modified reduction and nitriding. The estimated heat generation of the obtained samples was greater than that of MNPs. Hyperthermia with higher heating efficiency would be realized when $Fe_{16}N_2$ is used as thermoseeds.

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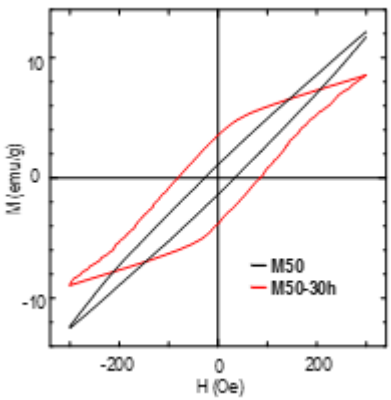

Picture 1:

Caption 1: Fig. 1 Magnetization curves of M50 and M50-30h under DC fields up to 300 Oe.

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Poster presentation

692 Synthesis and characterization of graphene-graft-poly(trimethylene carbonate) for nerve regeneration

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University of Twente, Enschede, Netherlands

INTRODUCTION:

Nerve regeneration in case of long-gap peripheral nerve damage remains a challenge. Graphene/polymer composites for biomedical applications attracted a lot of attention in recent research due to the extraordinary properties of graphene. In our previous study [1], graphene/poly(trimethylene carbonate) (PTMC) composites showed electrical conductivity and facilitated the growth of neuronal cells. However, at relatively high graphene concentrations, the graphene/PTMC composites tended to become inhomogeneous. The aim of this study was to solve this problem by synthesizing graphene-graft-PTMC, by using graphene as initiator for the ring-opening polymerization of TMC.

METHODS:

Graphene oxide (GO) was synthesized by a modified Hummer's method as described in our previous work [1]. The method to prepare graphene initiator was followed from Gao's work [2]. GO was dissolved in N-methyl-2-pyrrolidone (NMP) by 2h sonication. Azido ethanol was reacted with GO at 160^{o}C under nitrogen atmosphere. The product was washed with acetone and dried in a vacuum oven. The graphene initiator was dispersed in toluene by sonication for 1h. The ring-opening polymerization of TMC monomer was carried out in toluene at 110 ^{o}C under argon atmosphere with stannous octoate as catalyst. The graphene-graft-PTMC was obtained by repeated suspending and centrifugation in dichloromethane and characterized by FTIR and TGA.

RESULTS AND DISCUSSION:

As shown in Figure 1a, –OH groups (2900-3700 cm^{-1}) of GO were removed during azido ethanol reaction with GO in NMP at high temperature. The FTIR spectrum of graphene-graft-PTMC shows C-O (1230 cm^{-1}), C=O (1700 cm^{-1}) and C-H (2900 cm^{-1}) peaks due to PTMC on the surface of the graphene. From the content of graphene initiator as determined by TGA and the weight of the obtained graphene-graft-PTMC, the molecular weight of the PTMC was calculated. This amounted to 200 - 6000 g/mol, depending on the amount of TMC used for the polymerization.

CONCLUSION:

Graphene-graft-PTMC with different PTMC chain lengths was successfully synthesized by ring-opening polymerization of TMC using a graphene initiator.

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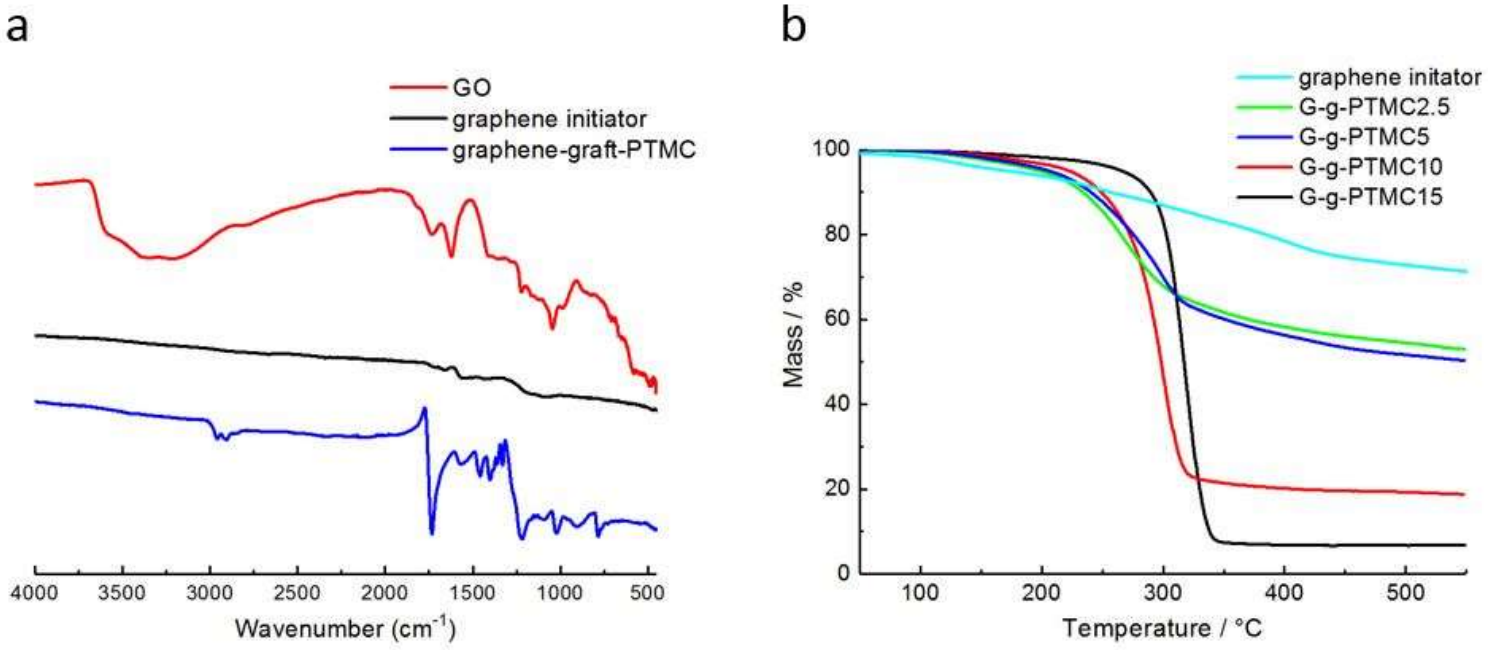
ACKNOWLEDGMENTS:

The authors would like to thank Foryou Medical Company for kindly providing TMC monomer and the Chinese Scholarship Council for financial support.



Picture 1: Caption 1: Figure1 a: FTIR of GO, graphene initiator and graphene-graft-PTMC; b: TGA of graphene initiator and graphene-graft-PTMC with different amounts of PTMC

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Poster presentation

697 Nanoformulations of Injectable PolyNIPAM Hydrogels for the Intervertebral Disc

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INTRODUCTION:

Injectable hydrogels offer benefits to the healthcare system, minimising patient discomfort, risk of infection, scar formation and the cost of treatment. Degradation of the intervertebral disc (IVD) currently has no preventative treatments; an injectable hydrogel material to provide mechanical support and promote tissue regeneration could transform healthcare practices. The ideal material for this application would allow addition of cells and biomolecules, be injectable at a biocompatible temperature through a small gauge needle, solidify *in situ*, allow cell migration and promote endogenous tissue regeneration. We present a range of synthetic thermosensitive hydrogels based on Laponite® associated poly(N-isopropylacrylamide)-co-poly(dimethylacrylamide) which have previously demonstrated *in-vitro* efficacy for nucleus pulposus regeneration [1].

METHODS:

The hydrogels were synthesised using methods described in [1], and summarised in figure 1. An Anton Paar Rheometer was used to determine gelation kinetics, viscosity and mechanical properties with a range of dynamic measurement modes. SEM, FTIR and swelling was used to characterise freeze dried material properties. The influence of composition variables were assessed using central composite designs.

RESULTS AND DISCUSSION:

A significant increase in the lower critical solution temperature (LCST) of the hydrogels was observed with increased ratios of co-monomer N,N-dimethylacrylamide (DMAc), however this had a critical impact on the injectability with increased viscosity at clinically viable delivery temperatures (38-40^{o}C). Rheology demonstrated analogous final mechanical with the DMAc:NIPAM range properties at 25^{o}C, however at 37^{o}C gelation rates and mechanical properties were increased with greater ratios of pDMAc. Swelling of the hydrogels showed significant differences between water and phosphate buffered saline incubated samples.

CONCLUSION:

The results demonstrate the importance of testing samples in physiological conditions. We also show that this system allows for the optimisation of resultant hydrogel mechanical properties whilst maintaining injectability through small gauge needles. We also identified key material variables to design a flexible assortment of nanoformulations suitable for the treatment of a range of tissues including the intervertebral disc.

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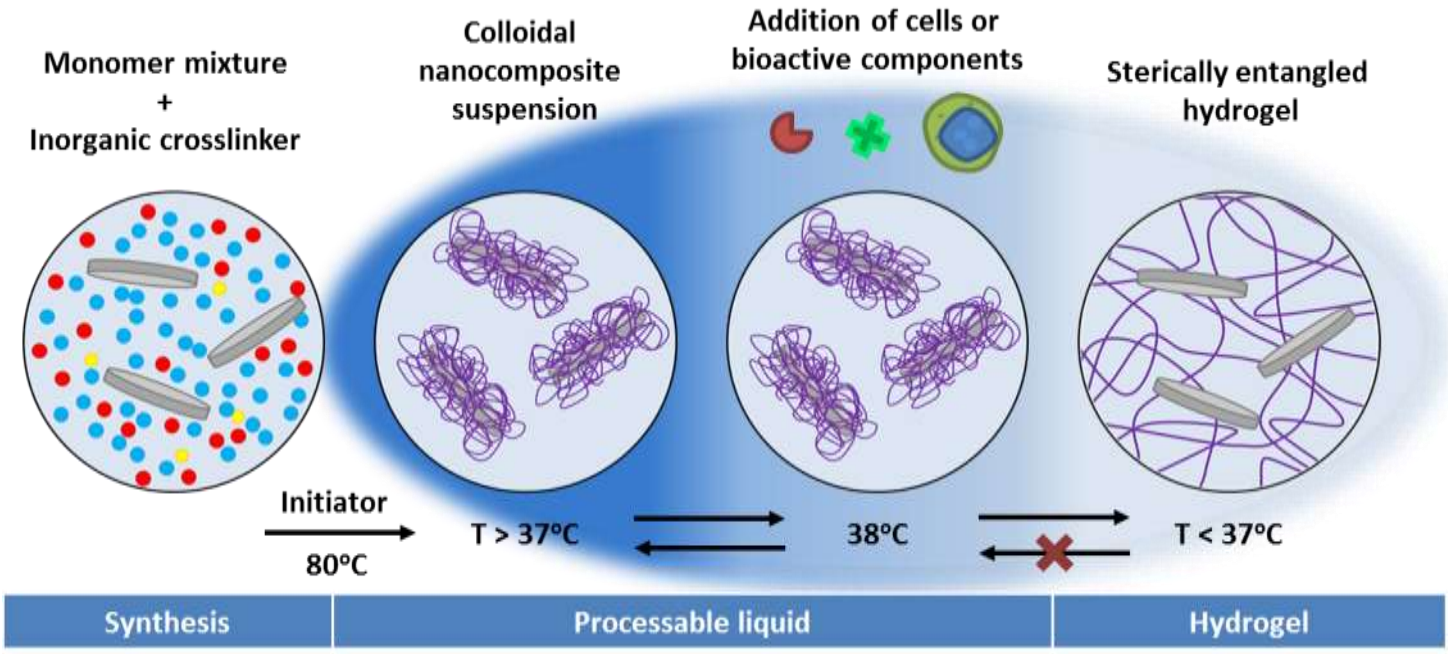
ACKNOWLEDGMENTS:

We would like to thank Arthritis Research UK grant number *21497* for supporting this research.



Picture 1: Caption 1: Figure 1: Laponite® pNIPAM-co-pDMAC hydrogel synthesis and processing route.

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

701 Pellet Additive Manufacturing for tissue engineering applications

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INTRODUCTION:

Bioabsorbable polymers are widely used in tissue engineering but are complicated to process by conventional polymer processing techniques due to their thermal and moisture sensitive behavior. Moreover, the manufacturing of complex 3D structure and the control of their porosity is difficult with conventional processing (1) but they can be obtained by Fused Deposition Modelling (FDM) (2). Unfortunately, high processing temperatures required to first create filament and then to print it, affect the polymer properties (3,4). In our study, we have developed 3D porous scaffold for tissue engineering by Pellet Additive Manufacturing (PAM), a one-step method compared to FDM. We have characterized two absorbable polymers for 3D printing by PAM technology by evaluating their physicochemical properties before and after processing including sterilization.

METHODS:

PAM printer from POLLEN was used to generate 3D scaffolds. Two medical-grade absorbable poly(lactic-co-glycolic) acid pellets LA:GA ratio 85:15 (Mw: 2,194E+05 g/mol) and 82:18 (Mw: 1,711E+05 g/mol) were used. The printing parameters were optimized to obtain 3D scaffold via PAM. The impact of different processing step (printing, EtO sterilization, degradation) were evaluated by HPLC-GPC (Molecular weight), DSC (crystallinity) and weight loss (degradation). Degradation evaluation was performed in PBS (pH 7.4, 37°C, 80rpm) according to ASTM F1635-16 Standard); finally, mechanical strength of the scaffolds was evaluated by uniaxial compression test.

RESULTS AND DISCUSSION:

The general trend for both polymers show a significant drop in molecular weight after 3D printing process which are respectively 47% for PLGA 82:18 and 35% for PLGA 85:15. After EtO sterilization the molecular weight of PLGA 8218 remained unchanged whereas it shows a 42% decrease for PLGA 85:15. The drop of molecular weight after 3D printing is quite predictable due to high processing temperatures required but EtO sterilization should not affect molecular weight as observed with PLGA 85:15.

CONCLUSION:

Our objective was to evaluate the printability of PLGA 85:15 and 82:18 via PAM and the effect of printing and sterilization process on the molecular weight. According to GPC results, PLGA 82:18 seems to be more appropriate because it is less affected by EtO sterilization than PLGA 85:15. An in vitro degradation study will be performed comparing the two polymers in order to evaluate the long-term effects of the processing procedures.

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Poster presentation

706 Functionally graded scaffolds manufactured using a novel 3D printing process

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INTRODUCTION:

Functionally-graded lattice structures are promising in the design and engineering of biomedical implants, for example, as tissue scaffolds used in the treatment of bone defects, where such structures would facilitate a reduction in shear stress between the implant and the adjacent tissue[1]. To fabricate functionally graded structures, the main focus in the previous works has been either on porosity, adjusting the spacing between filaments[2], or on the combination of filaments with different properties, achieved with modified 3D printers[3]. The present research, however, focuses on functionally graded regular lattice structures fabricated using fused deposition modelling (FDM) 3D printing techniques with a single material. The feasibility of the new approach developed is demonstrated by the production of continuously graded lattices using a standard commercial 3D printer.

METHODS:

A series of functionally-graded lattice structures which varied in the lattice spacing were produced using an Ultimaker 3 Extended 3D printer and a poly(lactide) filament. Mechanical buckling tests were performed on the samples using a Universal Tensile Tester to demonstrate the asymmetry of bending under load. A mathematical model has been derived to describe the buckling of thin, graded lattices and the results of which are compared with mechanical tests.

RESULTS AND DISCUSSION:

The analytical response obtained depends only on the parameter that describes the gradation of the structure. Furthermore, the response is symmetrical when modelling a non-graded sample. Overall, a good correlation was found between the observed mechanical response and mathematical model, in particular at small displacements and for large spacing between filaments. Figure 1 shows the asymmetric response under buckling conditions for functionally-graded lattice samples.

CONCLUSION:

Samples with the desired continuous gradation have been successfully obtained through in-situ control of the FDM 3D printing process. In parallel, a mathematical model has been developed which describes the asymmetry of the mechanical behaviour of graded structures. Predictions were accurate and were found to be in line with buckling tests on printed samples.

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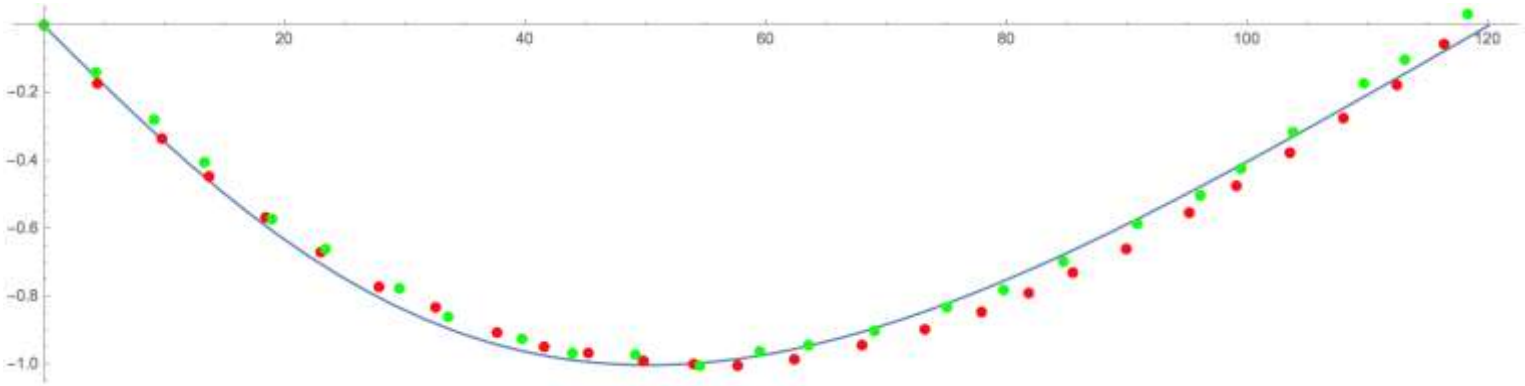
ACKNOWLEDGMENTS:

This work has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie Grant Agreement No. 643050.



Picture 1: Caption 1: Figure 1: Asymmetric response of 3D-printed samples - dots - obtained from buckling experiments. The blue solid line is the mathematical model.

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Poster presentation

722 Bioactive hybrid materials for soft and hard tissue engineering

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INTRODUCTION:

Bioactive glasses can be defined as amorphous materials with good biocompatibility[1]. They have gained a great interest as biomaterials for hard and soft tissue engineering[2,3].

This work comprises two parts: we first want to investigate the bioactivity properties that fluorinated phosphate-based bioactive glasses (PBGs) will exhibit once implanted in the body for dental repair applications. We are then designing patches containing 45S5 bioactive glasses for tissue engineering.

METHODS:

Bioactivity and structure are closely related[4,5] so we study the effect of integrating fluorine into the structure of PBGs using classical molecular dynamics simulations.We developed an empirical force field including polarisation

effects.The molecular dynamics simulations were performed on the following systems $(P_2O_5)(50-(x/2))$ − $(CaO)(50-(x/2))$ − $(CaF_2)(x)$ with x= 0,2,5.

Tissue engineering aims at mimicking the extra cellular matrix using natural or synthetic polymeric materials[6]. To engineer innovative bioresorbable and biocompatible patches we combine 45S5 bioactive glasses with biocompatible polymeric scaffolds. These cardiac patches are engineered using 3D electrospinning techniques.

RESULTS AND DISCUSSION:

For all the compositions simulated, the g_{P-O} radial distribution function (rdf) indicated the presence of double and single bonds. In the composition with 2% and 5% of CaF_2, the g_{P-F} rdf shows no bonding between the phosphorus and fluorine atoms. Analysis of the Ca-O and Ca-F rdfs and coordination numbers show that the fluorine atom prefers to bond to the calcium atom. The atomistic visualisation shows the presence of Ca/F clusters(Figure 1).

For the cardiac patches we used solutions of biopolymers. The morphological assessment of the scaffold done through scanning electron microscopy demonstrates that the fibres had continuous morphology without any observed beads. The different scaffolds will now be mechanically tested, as well as tested for biocompatibility, angiogenic properties and cellular regeneration.

CONCLUSION:

The main effect of fluoride addition is its bonding to the calcium atom. This bonding leads to a re-polymerization of the network and the formation of F-rich/poor regions which, overall, decrease the bioactivity of the glass.

The electrospun scaffold gave good results with no apparent bend which represents interesting properties for patches.

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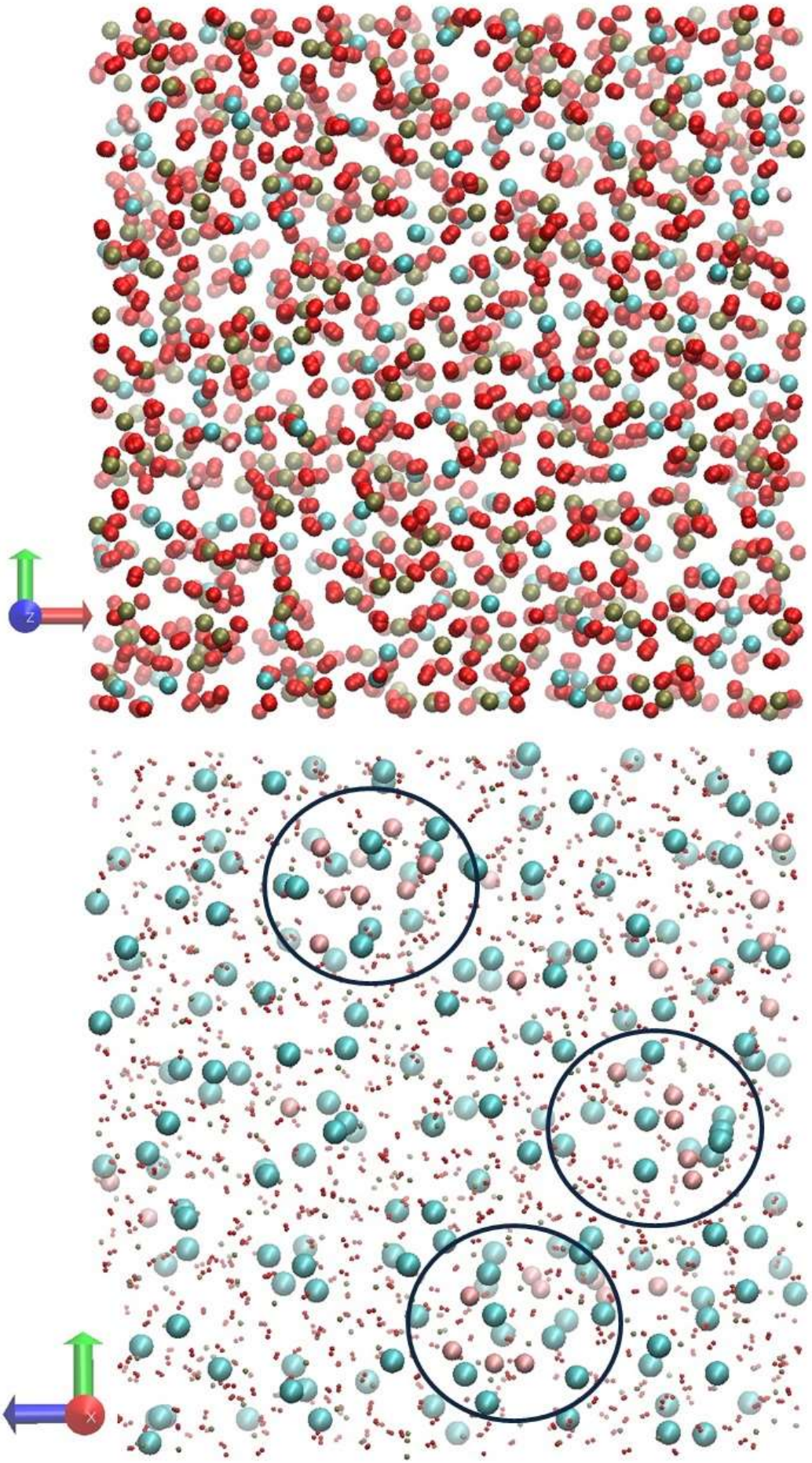
ACKNOWLEDGMENTS:

The authors would like to thank the Loughborough University and HPC facilities. We also thank Gavin Mountjoy, Laura Swansbury and Steven Kenny for useful discussions and Oliver Scaife and Sandra Dreßler for their help.



Picture 1:

Caption 1: View of the composition with 5% of CaF2 The colours are: phosphorus (green)oxygen (red), calcium (blue) and fluorine (pink).

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Poster presentation

730 Nanostructured surfaces with high bactericidal effect against Helicobacter pylori

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INTRODUCTION:

Helicobacter pylori chronic infection leads to several gastric disorders, such as gastric cancer [1]. Success rate of available therapy, based on antibiotics, has dramatically decreased due to bacterial resistance [2]. Antimicrobial peptides (AMPs) are low molecular weight peptides that target bacterial cell membranes and cause disintegration of the lipid bilayer structure, having low propensity to induce resistance [3]. The AMP MSI-78A (GIGKFLKKAKKFAKAFVKILKK), also known as PexigananA, was effective against *H. pylori in vivo* but in dosages close to toxic level [4], which is related to AMPs loss of activity due to susceptibility to extreme pH and proteases [5]. AMP immobilization may allow overcoming these drawbacks and boosting its activity [5].

METHODS:

MSI-78A synthesized with an extra cysteine (C-MSI-78A) was immobilized onto biotinylated model surfaces (self-assembled monolayers; SAMs), using neutravidin as a protein bridge and a biotin-polyethylene glycol $(PEG)_n$-maleimide spacer. Spacers with different PEG_n arm length (n=2; n=11) were tested. Immobilization was characterized with Quartz Crystal Microbalance with Dissipation. Functionalized surfaces were tested against a human highly pathogenic *H. pylori* strain (*H. pylori* J99).

RESULTS AND DISCUSSION:

The PEG11 spacer yelded higher AMP surface coverage, 30.2 ± 1.2 ng/cm^2, as determined by the Sauerbrey equation. Functionalized surfaces were tested against a human highly pathogenic *H. pylori* strain (*H. pylori* J99). *H. pylori* J99 viability was reduced in 75% for surface adherent bacterial cells and, more outstandingly, viability of planktonic bacteria was reduced in 99% after 2h of exposure to these surfaces. Also, no bacterial recovery occurred when transferred to optimal growth conditions, stressing the bactericidal effect.

CONCLUSION:

These results highlight the potential of AMP surface immobilization for development of non-antibiotic therapies against the gastric pathogen while demonstrating, for the first time, the activity of an immobilized AMP against planktonic *H. pylori.*

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ACKNOWLEDGMENTS:

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Poster presentation

737 Marine Fish Collagen and its Potential for Biomedical and Cosmetic Application

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INTRODUCTION:

Collagen is the major structural protein in the extracellular matrix of the connective tissues as skins, tendons, ligaments, and bones. It is known for having an excellent biocompatibility and weak antigenicity, making it a primary resource in biomedical applications. So far, the common sources for the extraction of this biopolymer in industrial context have been mainly bovine and porcine origin by-products, but industry is looking for other sources such as recombinant production - technologically challenging and expensive - or the extraction from marine organisms, namely by-products. Once fishery industries generate tons of fish skins as by-products from fish processing for food, their use for the production of collagen promises the establishment of a sustainable source with high economic and environmental benefits, namely when considering its biomedical relevance for tissue engineering, as well as with potential contribution as age-reversing product or moisturizer in cosmetic formulations.

METHODS:

Collagen was extracted from Atlantic cod (*Gadus morhua*) skin and further characterized to assess amino acid profile, denaturation temperature and structural characteristics, as well as the purity of the extract regarding collagen contents and eventual contaminants as heavy metals. Its potential in the biomedical and cosmetic contexts was also addressed by evaluating respectively its cytocompatibility as a main component in biomaterials, and its capacity to uptake humidity or to provoke any irritant reaction.

RESULTS AND DISCUSSION:

Results showed the successful isolation of type I collagen with high purity (Fig.1A). The cytocompatibility evaluation showed a concentration-dependent effect in metabolism and cell adhesion of lung fibroblast MRC-5 cells (Fig. 1C). Moreover, codfish collagen demonstrated to have a good ability to retain water, while its exposure to a human reconstructed dermis confirmed that the produced collagen did not induce the expression of molecular markers for irritation and inflammation (Fig. 1B).

CONCLUSION:

This work showed the potential of codfish skin collagen as a sustainable and low-cost platform for biotechnological valorization of codfish by-products towards biomedical and dermo-cosmetic applications.

ACKNOWLEDGMENTS:

Funding from the European Union Seventh Framework Programme through grant agreement ERC-2012-ADG 20120216-321266 (ERC Advanced Grant ComplexiTE), from Interreg España-Portugal 2014/2020 through project 0245_IBEROS_1_E and FCT Doctoral Programme Do*Mar (ALA scholarship: PD/BD/127995/2016).

Picture 1:

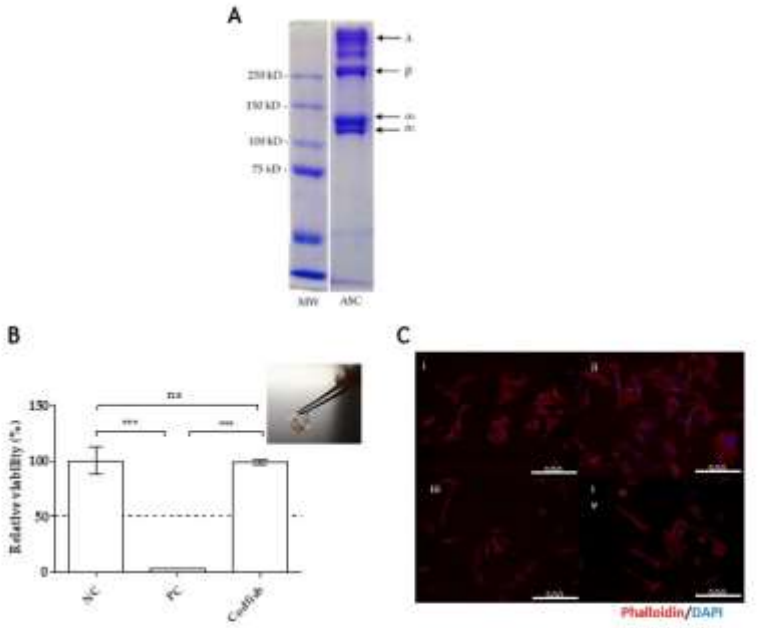

Figure 1 - A) SDS-PAGE of collagen showing it purity. B) Topical exposure to a skin reconstructed model and MTT cytotoxicity assay to assess skin irritation. C) Live & dead assay for viable (green) and dead cells (red); i) mg/mm2; ii) 0.5 mg/mm2; iii) 1.0 mg/mm2; and iv) 1.5 mg/mm2.

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Poster presentation

742 Effect of loading nano hydroxyapatite on in situ crosslinking alginate-based hydrogels for bone fracture repair

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[3]Dublin City University; Queen's University, Belfast, UK; Trinity College Dubli, Ireland

INTRODUCTION:

Hydrogels offer an interesting platform for bone tissue engineering, due to their potential for use in minimally invasive procedures, to provide excellent biocompatibility, and to allow an adequate environment for loading nano particulate cargos. This study investigates the potential for two *in situ* crosslinking hydrogels, an ionic crosslinked hydrogel and a thermoresponsive hydrogel, to be loaded with ceramic nanoparticles for the treatment of non-union fractures.

METHODS:

An optimal ionic crosslinked hydrogel was synthesised based on alginate concentration (3wt./vol.), calcium carbonate molarity (70 mM) and gluconic acid δ-lactone molarity (140 mM). The thermoresponsive alginate-based hydrogel was produced via a one-step free radical co-polymerisation method[1]. Briefly, 10 mM NIPAAm was added to 10 wt.% w.r.t alginate in aqueous solution and purged using dry nitrogen. The initiator APS and the accelerator TEMED were added until copolymerisation was achieved. The solution was then dialysed (MWCO:10kDa) in double distilled water for 120 h at room temperature and recovered by freeze-drying. Subsequently hydroxyapatite nanoparticles (nHA)[2] were loaded into the hydrogels in varying concentrations (1 to 10%wt./vol.). Hydrogels were characterised in terms of their physiochemical, setting, mechanical, viscoelastic, swelling and degradation properties pre- and post-incorporation of nHA cargo.

RESULTS AND DISCUSSION:

The ionic crosslinked hydrogel demonstrated a setting time of 30 min, compressive modulus of 60 kPa, G' of 2 kPa and the ability to offer 100% release of a small molecule steadily over a 15-day period (Figure 1). The thermoresponsive hydrogel exhibited instantaneous gelation at a temperature over 32 ^{o}C, a G' of 827 Pa, approx. 70% of small molecule (NaF) released within 4 weeks, and the ability to degrade up to 60% of its original mass within 8 weeks (Figure 1). nHA particles of varying concentrations were successfully loaded into the alginate-based hydrogels. The optimal concentration of nHA cargo will be identified through *in vitro* assessment.

CONCLUSION:

Alginate-based hydrogels represent a promising tool for bone tissue engineering. This study highlights the effect of loading nHA on the properties of two *in situ* crosslinking hydrogels, thus providing an insight on the possibility to tailor the alginate-based hydrogels for treatment on non-union fractures.

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ACKNOWLEDGMENTS:

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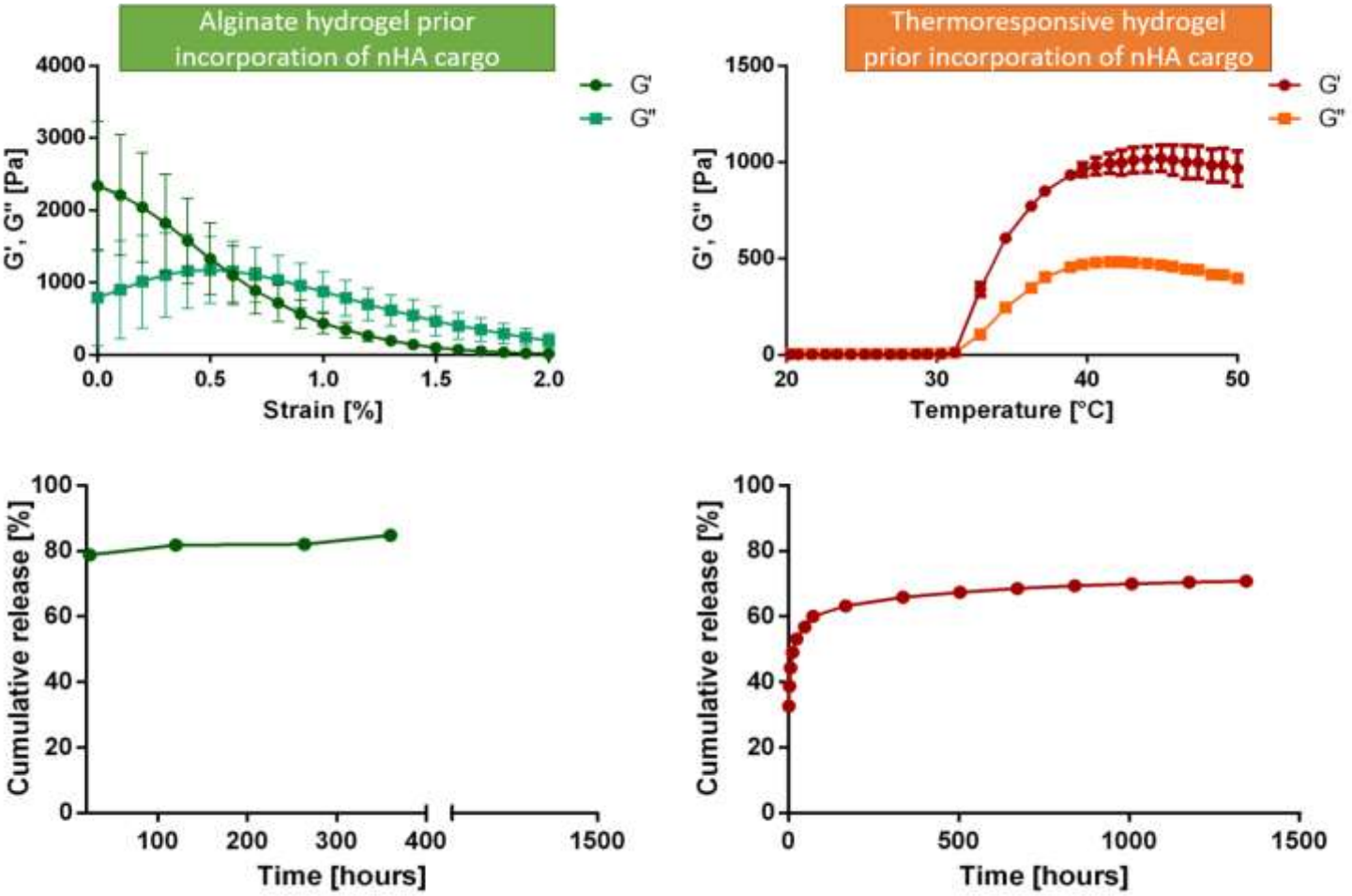

Picture 1: Caption 1: Rheological characterisation for a) alginate and b) thermoresponsive hydrogels; c) NaF release profile from alginate and d) thermoresponsive hydrogels

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Poster presentation

749 Dissolution studies of dentine and enamel after treatment with a toothpaste containing NaF and bioactive glass toothpaste using an in-situ liquid TEM cell

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INTRODUCTION:

An in-situ liquid TEM1 cell has been used to study the acid etching/dissolution of enamel and dentine treated with a toothpaste formulation containing both NaF and 45S5 bioactive glass. To aid understanding the dissolution kinetics of the formed fluorapatite and the fluorine-substituted hydroxyapatite analysis techniques such as wavelength dispersive spectroscopy to measure the ingress of fluorine and serial block microtoming, for 3D characterisation, were also undertaken to provide information across a range of length scales.

METHODS:

Citric acid etched dentine and enamel discs were brushed with a dentifrice containing both NaF and bioactive glass and then immersed in artificial saliva for 12 hrs. TEM slices, FIB-prepared or microtomed, were placed onto the nitride membranes of the liquid cell or onto TEM carbon grids for EELS, EDS and diffraction analysis. The etching within the liquid cell was performed using 0.05% citric acid flowing at 5 µl min-1 and by the radiolysis of the water. The treated discs were subjected to an acid challenge etch using 5% citric acid for 2 minutes. Different analysis methods, such as digital image correlation were used to try and quantify the rate of etching and these will be discussed.

RESULTS AND DISCUSSION:

The near surface regions of both the toothpaste treated enamel and dentine slices etched slower than that of the central areas beneath. This can partly be attributed to the formed needle-shaped fluorapatite crystals and fluorine substitution in the hydroxyapatite. The needle-shaped fluorapatite crystals decorated both the enamel and the dentine surfaces, the dentinal tubules and bridged between the etch enamel prisms. WDS/EDS showed that the fluorine signal could be measured to a depth of 1 micrometres beneath the surface in the treated samples. The 3D analysis enabled the effect of the acid challenge to be quantified and correlated with the in-situ etching. A chief difficulty with using the in-situ cell was the placing of the lamellae onto the cell's nitride membranes and the changes in the volume of the etching solution during imaging and these will be discussed in detail.

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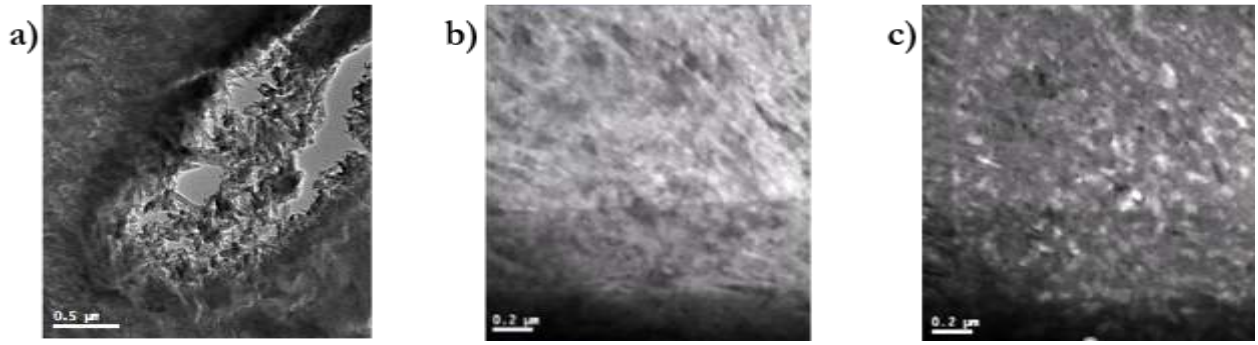
ACKNOWLEDGMENTS:

The authors would like to thank the Engineering and Physical Sciences Research Council (EPSRC) and Glaxo Smith Kline (GSK) for the funding provided to execute this project.

Picture 1:



Caption 1: Figure 1: (a) STEM images of fluorapatite and (b) and (c) BF TEM stills taken of enamel being acid etched within the insitu TEM holder.

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Poster presentation

756 Conductive Elastomers for Flexible Bioelectronics

Josef Goding, Rylie Green

Imperial College London, London, United Kingdom

INTRODUCTION:

Bioelectronic devices use metallic materials such gold and platinum to conduct electricity and inject electrical charge inside the body. However, the mechanical properties of metals limit their applications in flexible electronics. Conducting polymers (CP) are a promising alternative to metals for use in bioelectronics, however they are limited by their substrate-dependent nature and poor mechanical stability. This study aimed to overcome the limitations of CPs in flexible electronics by combining them with elastomeric polymers to create soft and flexible conductive elastomers (CE).

METHODS:

Polyurethane (PU) was dissolved in dimethylsulfoxide (DMSO). Poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS) was dispersed in the PU solution at a range of weight percents (8, 16 and 24 wt%). Films of CE were fabricated by solvent casting. The electrochemical performance of CE films was assessed using cyclic voltammetry and electrochemical impedance spectroscopy. Mechanical properties of CE films were assessed using tensile testing.

RESULTS AND DISCUSSION:

Increased loading of PEDOT:PSS resulted in increased electrical activity in the resulting CE films. PU with 8 wt% PEDOT was found to have a charge storage capacity (CSC) of 33 $mC.cm^{-2}$ (Fig 1). Increasing PEDOT loading to 24 wt% resulted in a significant increase in charge storage capacity to 224 $mC.cm^{-2}$. Similarly, increasing PEDOT loading resulted in a decrease in impedance from 41.8 $\Omega.cm^2$ at 8 wt%, down to 10.6 $\Omega.cm^2$ at 24 wt%. Tensile testing of CE films demonstrated that they retain their elastomeric nature and are capable of considerable extension. At a loading of 8 wt%, CE films had a fracture strain of 485% at 9.1 MPa. Increasing the loading of PEDOT:PSS acted to reduce the viscoelastic nature of the composite with a loading of 24 wt% resulting in a fracture strain of 97% at 1.5 MPa. Overall, increasing PEDOT:PSS loading in the PU resulted in a trade-off between electrochemical and mechanical properties.

CONCLUSION:

Dispersing a CP throughout an elastomeric network resulted in a soft, flexible elastomer capable of conducting and injecting electrical charge. CEs hold great promise for use in flexible bioelectronics due to their unique combination of electrochemical and mechanical properties. Future studies will focus on improving the electrochemical properties of CEs via electrochemical deposition of CP throughout a CE network. Subsequent electrodeposition has been found to increase conductivity of hydrogel-based materials and will be explored in application to elastomers[1].

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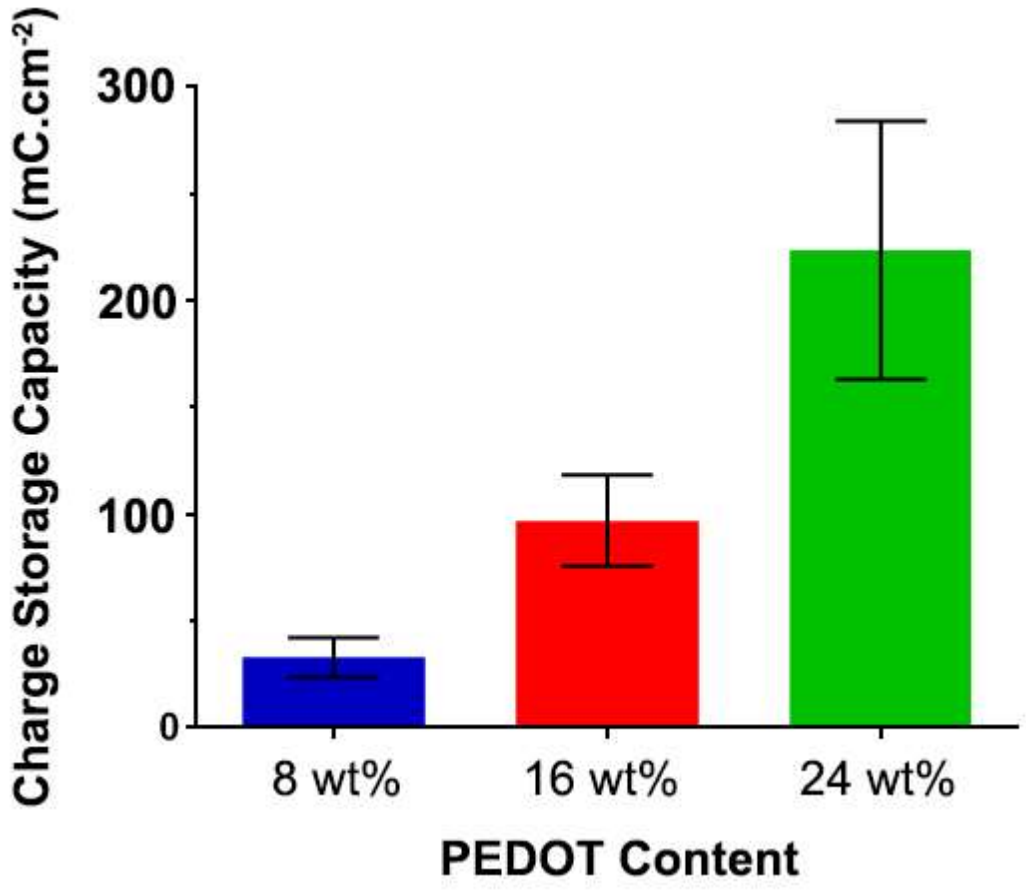

Picture 1:
Caption 1: Fig 1 - Charge storage capacity of CE films as a function of PEDOT:PSS loading.

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Poster presentation

762 Enzymatically crosslinked natural polymers for cartilage repair

Bram Zoetebier, Pieter Dijkstra, Sanne Both, Rong Wang, Yao Fu, Jan Hendriks, Marcel Karperien

University of Twente, Enschede, Netherlands

INTRODUCTION:

Osteoarthritis is the most common chronic condition of the joints. It is characterized by the degeneration of articular cartilage, formation of osteophytes and alterations in the synovium. This process has a severe impact on the quality of life of the patients and the currently available treatments are unsatisfactory and often merely focused on pain relief.[1]

In our group we are working on the development of in situ crosslinkable hydrogel platforms that could be used for resurfacing the damaged articular cartilage using a minimally invasive arthroscopic procedure.[2-4] Stable fixation of the gel at the joint surface, facilitating the ingrowth of local stem and progenitor cell populations and supporting

intrinsic repair mechanisms are considered minimal design parameters. To achieve this we are exploring the use of enzymatically crosslinkable natural polymer-tyramine conjugates.

METHODS:

Dextran-tyramine conjugates were prepared by activation of dextran-OH and subsequent reaction with tyramine. Hyaluronic acid-tyramine and gelatin-tyramine conjugates were prepared using DMTMM coupling. In situ crosslinking is achieved by mixing the polymer conjugates with the enzyme HRP and minute, non-toxic amounts of H_2O_2 as initiator. Bonding to cartilage and bone was measured in a custom designed set up. Support of cartilage formation was studied after mixing of the polymer conjugates with mesenchymal stem cells, chondrocytes or combinations of both prior to crosslinking. Cell ingrowth was studied by cell-seeding on top of preformed gels.

RESULTS AND DISCUSSION:

We prepared dextran-tyramine conjugates with a degree of substitution of 12 tyramine residues per 100 monosaccharide units. The conjugated hyaluronic acid-tyramine and gelatin-tyramine had a degree of substitution of 10% of the carboxylic acid groups.

Dextran-tyramine and hyaluronic acid-tyramine gels are binding 10 times stronger to damaged cartilage than clinically applied fibrin glue.[4]

While culturing chondrocytes in these hydrogels, the secretion of extracellular matrix increased the storage modulus of the gels, indicating that the ECM could take over the slowly degrading hydrogel matrix (supported by histology and degradation studies).

Cell ingrowth, spreading and attachment was improved by co-enzymatic crosslinking of gelatin-tyramine into the hydrogel network.

CONCLUSION:

Enzymatically crosslinked hydrogels, based on dextran and hyaluronic acid, with the addition of co-crosslinkable gelatin show excellent properties for application in the regeneration of damaged cartilage.

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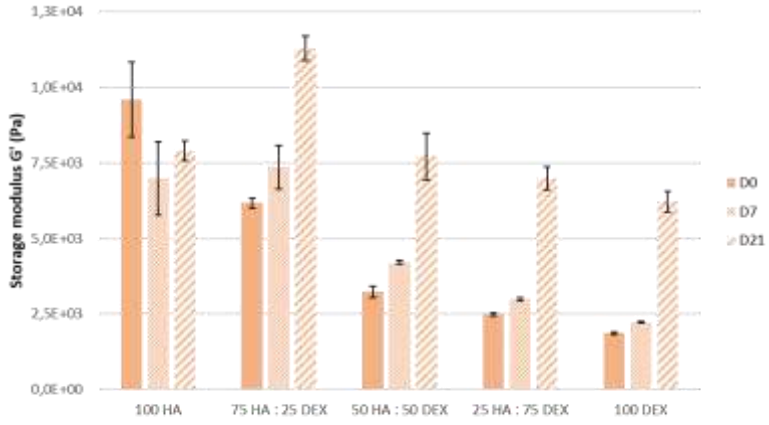
ACKNOWLEDGMENTS:

The authors would like to thank NWO P15-23 (Project 1) for providing financial support to this project.

Picture 1:



The change in storage modulus of cell-laden hydrogels consisting of dextran-tyramine and hyaluronic acid-tyramine co-enzymatically crosslinked in different ratios cultured in chondrogenic differentiation medium. The polymer concentration was 5 % (w/v) and the cell concentration was $10^6 ml^{-1}$.

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

765 Evaluation of a surgical wound closure system

Rita Peixoto[1], Dimitrios Zeugolis[1], Dimitrios Tsiapalis[2]

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INTRODUCTION:

A suitable wound closure is an indispensable requirement for an uncomplicated and expedient recovery after an abdominal surgery. The closure technique will have a great impact on the healing process of the wound. Surgical complications, such as wound dehiscence (sometimes associated with evisceration), infection, hernia, nerve injury and incisional pain are very common in the postoperative period of an abdominal surgery. Besides, although their development can be promoted by other risk factors like age, sex, lifestyle, diet, health condition, the closure method can also influence the emergence of these undesirable complications [1]. For this reason, and having the wellbeing and quality of life of the patients in mind, particularly high-risk patients, a closure system consisting of anchors applied on either side of the wound aims to reduce the tension caused on the surrounding tissues of a wound and, consequently, decrease the risk of tissue strangulation, subsequent herniation, tissue breakdown and nerve entrapment.

METHODS:

A midline incision was made in the rat abdomen. The incision was then closed with one of the following closure methods – interrupted suture, continuous suture, anchors under the fascia and anchors over the fascia. Three different tissue bites, i.e. distance between the stitch and the wound edge, were used – 0.5, 1.0 and 1.5 cm. After the abdomen was excised, the tissue was subjected to mechanical testing. The tissue was placed horizontally and the suture pulled perpendicularly.

RESULTS AND DISCUSSION:

No significant differences were seen between the different techniques (interrupted suture, continuous suture, anchors under the fascia and anchors over the fascia) within the same tissue bite group. However, with the exception of interrupted suture, a smaller bite showed higher tensile strength.

CONCLUSION:

Fascia approximation via anchors on either side of the wound represent a good alternative to close surgical wounds.

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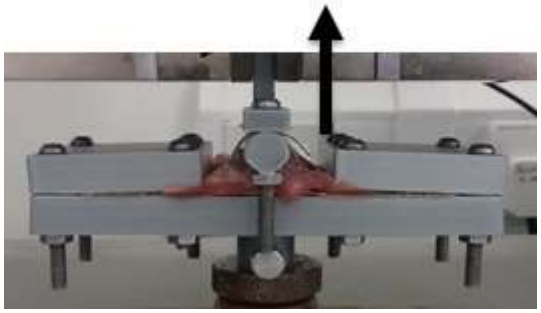
ACKNOWLEDGMENTS:

The authors would like to thank Science Foundation Ireland and the European Regional Development Fund (Grant Agreement Number: 13/RC/2073) for providing financial support to this project.

Picture 1: 

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

766 Natural-derived composite scaffolds envisioning regeneration in cartilage lesions

Gabriela s. Diogo[1], Rogério p. Pirraco[1], Ricardo i. Pérez-Martín[2], Carmen g. Sotelo[2], Tiago h. Silva[1], Rui l. Reis[1], Ana Catarina Oliveira[1]

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INTRODUCTION:

Cartilage lesions are very problematic pathological conditions due to the deprived capacity of this tissue to regenerate. One of the major challenges to develop a suitable tissue engineering approach to treat cartilage lesions is to mimic the physiological environment of the native tissue in which cells have the ability to migrate, grow and differentiate into a chondrogenic line avoiding fibrocartilage tissue formation. In that sense, it is proposed in the present study the preparation of blue shark collagen and collagen:hyaluronic scaffolds by a freeze-drying technique

using EDC/NHS as crosslinking agent as biomaterials aiming to treat cartilage lesions. The ability of the scaffolds to promote chondrogenic differentiation was the main goal of the study.

METHODS:

Blue shark collagen extracted from *Prionace glauca* skins was solubilised in hydrochloric acid and combined with high molecular hyaluronic acid in a desired ratio. After that, 3 hours of crosslink reaction with EDC/NHS at negative temperatures was carried out to increase scaffolds stability followed by freeze-drying technique. The potential of the developed structures for application as scaffolds for cartilage tissue regeneration was firstly assessed by *in vitro* biocompatibility assays using human derived adipose stem cells (ASC) previously isolated from lipoaspirates.

RESULTS AND DISCUSSION:

The blending and further crosslinking of both biopolymers resulted in cohesive hydrogels supporting manipulation and cell culture over several days. From the cytotoxicity results assessed with ASC through a metabolic activity assay (MTS), we can observe the non-cytotoxic character of the scaffolds. By quantitative Live/Dead assay images, the significant abundance of adhered cells (green) one day after seeding was demonstrated. Moreover, it was possible to observe by immunohistochemistry analysis, through haematoxylin & eosin staining, the potential of the structures to ensure the migration and survival of the cells within the entire scaffold. In addition, Sox9 expression confirmed cell differentiation into chondrogenic lineage.

CONCLUSION:

The potentiality of the proposed marine collagen:hyaluronic acid scaffolds to be used on cartilage tissue engineering was demonstrated not only by the morphological features of the developed hydrogels, but also for its ability to support the proliferation of ASCs and promote their chondrogenic differentiation.

ACKNOWLEDGMENTS:

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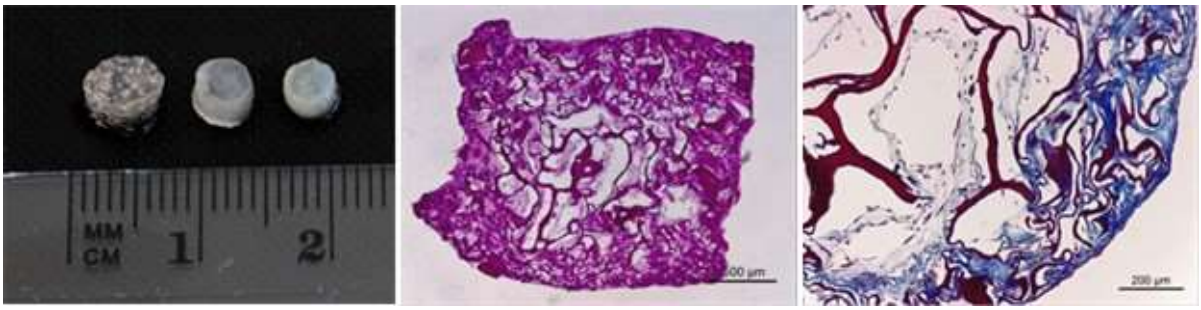

Caption 1: a) Effect of cell seeding on scaffolds contraction; b and c) Haematoxylin & eosin and Masson's trichrome staining.

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

771 Silk fibroin bio-sintering as a new route to produce solid bulk components

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INTRODUCTION:

The possibility to realize solid macroscopic objects with silk fibroin could open to the production of shape-defined biodegradable implants. Despite the large amount of protocols available to produce materials starting from silk fibroin, so far, only one method based on high concentrated solution casting has been published. However, since the entire process takes days, or even weeks to be completed, it can be hardly exploited for manufacts realization [1]. A sintering procedure to produce bulk fibroin material in severe forming conditions (200°C and 100MPa pressure) was also recently proposed, however these conditions are not compatible with the molecular stability of fibroin, together with the possibility to incorporate thermolabile molecules, such as enzymes or drugs [2].

In this work we report a method (named cold bio-sintering) to produce large monoliths made of silk-fibroin powder sintered at low temperature (60°C) and applying a fast pressure ramp.

The process was also optimized for allowing sintering at higher temperature (120°C) together with low pressure (40MPa). Finally, exploiting our findings, we report the possibility to realize in one step complex shape objects by compression molding or, even more complex shapes, by laser cutting.

METHODS:

Samples were prepared applying pressure on a cylinder mold, using a hydraulic universal testing machine. The procedure was optimized using a full factorial design-of-experiment with 16 samples prepared in different conditions. Secondary structure analysis was conducted by FTIR. SEM was used to study the microstructure.

RESULTS AND DISCUSSION:

The optimization showed that the compression rate is high enough to take the fibroin chain closer prior to the β-sheets transition, allowing the viscous flow, while β-sheets is able to oppose a larger resistance to compression. At low temperature the process is more complex because the crystalline transition is primarily due to compression. In figure we report the first attempt to produce a compact cylinder shape pin obtained by fibroin powder cold-sintering process and SEM micrographs that compare the pre-sinter (left) and post-sinter microstructure (right).

CONCLUSION:

In this work we reported a method to obtain a compact material form fibroin powder in a single compression mold step at low temperature. Large object can be produced in few minutes with a high reproducibility. The mild forming conditions allow the possibility to incorporate temperature degradable bioactive additives.

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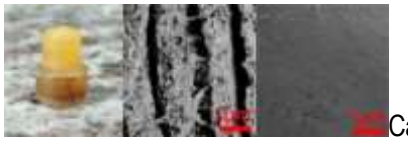
Picture 1:  Caption 1: silk fibroin pin obtained by sintering, and microstructural analysis

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

774 Biocompatible silicone rubber mechanical reinforcement with graphene-nanoplatelets

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[1]Eindhoven University of Technology, Eindhoven, Netherlands

[2]UWEB - University of Washington Engineered Biomaterials, Seattle, WA, United States of America

[3]LEPABE, Faculty of Engineering, University of Porto, Portugal

[4]INEB (Instituto de Engenharia Biomédica) - i3S, Universidade do Porto, Portugal

INTRODUCTION:

There is a need to improve silicone rubber (SR) mechanical properties for some of its common applications, namely biomedical uses. A new made available class of nano-fillers, graphene-based materials (GBMs) is promising for that purpose [1].

METHODS:

GNP-M15, and C750 (XG-Sciences,US) were oxidized by modified Hummers method [2], being designated as GNP-M-ox and C-ox. GNPs were dispersed in different amounts (0.25-5wt.%) by mechanical mixing into SilasticMDX4-4210 elastomer and curing agent, cast and cured (RT,3d). DowCorning360 Medical-Fluid (Fluid) was used in a 1:1ratio to decrease SR viscosity to assist GNP-M 5wt.% dispersion. Some samples were extracted (ex) 24h with hexanes, acetone, methanol, ethanol, and transferred to water. Tensile properties of the samples were measured using an Instrom test frame, (water,37 °C,20 mm min−1). Cytotoxicity was tested by direct contact and performing the elution method (ISO10993-5). Metabolic activity was evaluated performing the resazurin assay. Endotoxins were tested according to USP-chapter85, using Lonza's plus-pyrogent kit.

RESULTS AND DISCUSSION:

The mechanical properties of SR/GNP with lower loadings (0.25-1wt.%) were similar to the ones of unfilled SR. For SR/GNP-M 2, and 5 wt.% Young's modulus increased by 55, and 73%, respectively. For SR/GNP-C-ox, it increased by 44%, while for SR/M Fluid 5 wt.%, it decreased by 50%. Cell morphology was normal for cells in direct contact with all the materials at 12-72h. Also, metabolic activity was always above the toxicity limit. In the live/dead assay few dead cells were observed for all materials at 48, and 72h. For cells in contact with 24h extracts of the materials, cell morphology was also normal at 12-48h. All the materials were above the toxicity limit at 24, and 48h, presenting few cells stained red in the live/dead assay (Figure 1). The extracts of all the materials presented endotoxin levels below 0.06 EU limit.

CONCLUSION:

The incorporation of 2, and 5wt.% GNP-M increased SR Young's modulus by 55, and 73%. Incorporation of 5wt.% GNP-C-ox in SR or reducing SR/GNP 5 wt.% viscosity with a 1:1 ratio of DowCorning360 Medical-Fluid decreases composites mechanical properties. The fact that some materials were extracted with solvents resulted in no differences in terms of mechanical properties, and *in vitro* biocompatibility. All the materials shown to be biocompatible *in vitro*.

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[2] Pinto, A. et al., Carbon 99 (2016) 318.

ACKNOWLEDGMENTS:

Projects POCI-01-0145-FEDER-007274, POCI-01-0145-FEDER-006939 and PTDC/CTM-Bio/4033/2014, funded by FEDER through COMPETE2020 and FCT.

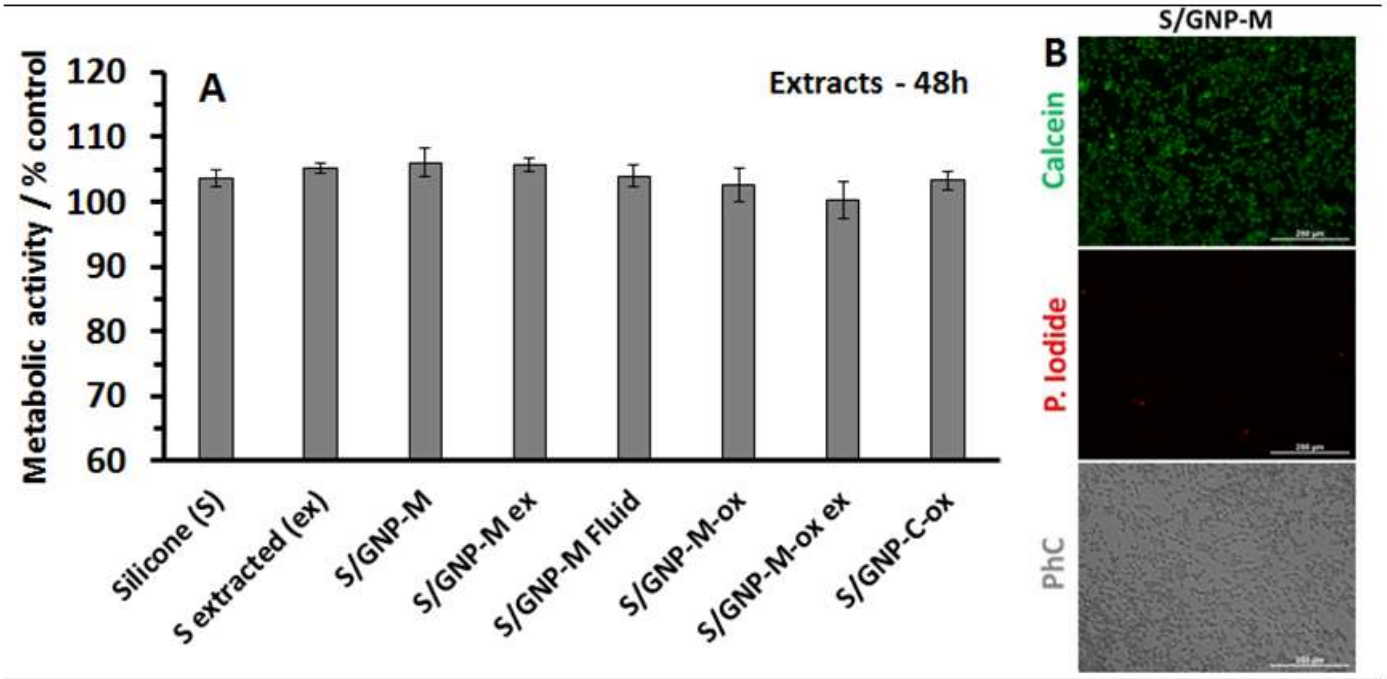

Figure 1: A - Metabolic activity at 48h of 3T3 mouse embryo fibroblasts exposed to 24h extracts of the materials. TCPS control was considered 100%, and latex was close to 0%. B – Live/dead staining for SR/GNP-M 5 wt.%, similar images were obtained for all materials tested. Calcein is staining live, and propidium iodide dead cells.

Picture 1: Caption 1: A - Metabolic activity at 48h of fibroblasts exposed to 24h extracts. B - Live/dead staining for SR/GNP-M 5 wt.%.

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Poster presentation

784 Monitoring of polyglycerol sebacate synthesis and correlation with the resultant network parameters

Rubén Martín-Cabezuelo, Raquel Sancho-Sanmartín, Guillermo Vilariño-Feltrer, Jose A. Gómez-Tejedor, Ana Vallés-Lluch

Center for Biomaterials and Tissue Engineering, Valencia, Spain

INTRODUCTION:

Polyglycerol sebacate (PGS), a tough elastomer, was introduced a decade ago as a potential biomaterial for soft tissue repair. Obtained from a two-stage polycondensation reaction between sebacic acid and glycerol, it provides a broad range of interesting chemical and physical properties in terms of its micro-architecture and its potential combination with other compounds. This, together with the fact that both reagents are non-toxic, and FDA approved, confers to PGS a great ability to adapt for several biomedical applications[1].

The main purpose of this work was to follow PGS viscosity with time, as it polymerizes from the mixture of reactants, at different temperatures and environmental controlled conditions, and to correlate the results with the physicochemical characteristics of the PGS networks obtained.

METHODS:

Monomers of PGS were mixed at an equimolar ratio of sebacic acid and glycerol rising the temperature up to 140ºC for 10 min under N_2 environment. The resulting mixture was used for viscosity measurements by means of a rheometer with 25 mm-diameter parallel plate geometry at 1.5 $rad \cdot s^{-1}$ of rotational speed. Viscosity of monomers was followed at different temperatures between 110ºC and 180ºC for as long as needed for the achievement of maximum viscosity. For synthesis under other environments (compressed air, nitrogen, argon, oxygen), melted mixture was polymerized at 130ºC for 24h at a gas flow rate such that a slight overpressure was achieved. To obtain Fourier-transform infrared spectroscopy (FTIR) scans, samples were previously vacuum-dried for 48h.

RESULTS AND DISCUSSION:

The rheological behavior of pPGS as it condenses, and cross-links shows that the higher the synthesis temperature, the lower the maximum viscosity achieved during curing and the lower time of curing needed (Figure 1). By changing the atmosphere, the resultant effect is similar. FTIR spectra of samples treated under different atmospheres showed differences in the peaks characteristic of OH, C=O and C-O groups, which can be attributed to a different cross-linking efficiency.

CONCLUSION:

Viscosity measurements conducted as PGS polymerizes from its reactants showed how curing time and the maximum viscosity achieved (and thus, the crosslinking density of the resultant network) strongly depend on the curing temperature and atmosphere.

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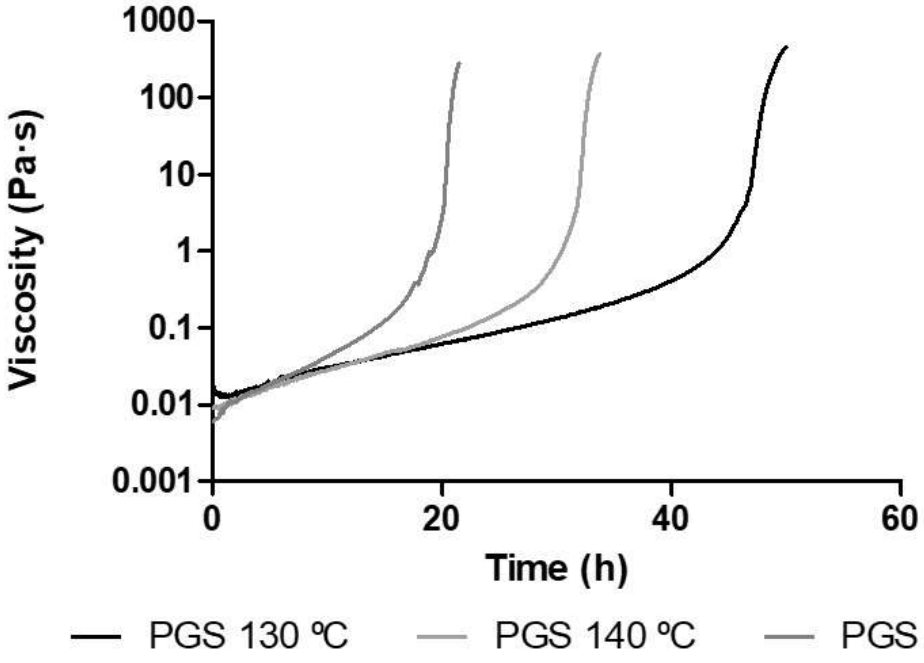
ACKNOWLEDGMENTS:

This work was funded by the Spanish Ministerio de Economía y Competitividad through DPI2015-65401-C3-2-R project.

Picture 1:



	PGS 130 ºC	PGS 140 ºC	PGS 150 ºC
Mc[1] (g/mol)	1822.7±98.6	2297.4±57.2	1787.2±381.3
E (kPa)[1]	4638,84±243,54	3688,17±83,07	4744,30±1007,54
tmax (h)	50	33,8	21,5

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Poster presentation

787 Surface immobilized N-acetyl cysteine avoids bacteria adhesion without preventing cell adhesion and proliferation

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[2]UCIBIO-REQUIMTE, Portugal

[3]I3S/INEB/ICBAS, Portugal

INTRODUCTION:

N-acetyl cysteine (NAC) is an FDA-approved drug clinically applied on a broad range of pathologies[1]. Further research has been done to explore its antimicrobial activity potential[2]. However, NAC has a very short half-life and thus strategies to promote high local concentrations would be beneficial. In this study, covalent immobilization of NAC was performed onto a chitosan(Ch)-film in order to obtain high density NAC-chitosan(Ch)-derived coating. Water-based carbodiimide chemistry was applied for the development of NAC-functionalized Ch films, avoiding toxic

organic solvents use. Here we report the optimization steps performed to immobilize NAC onto the surface of pre-prepared Ch coatings, to ensure full exposure of NAC, and the assessment of its biological performance against Methicillin-resistant Staphylococcus aureus (MRSA), and cytotoxicity towards Osteoblast MC3T3-E1 cell line[3].

METHODS:

Increasing NAC concentrations were grafted onto spincoated chitosan films (Ch), through carbodiimide chemistry. Surface characterization included Infrared Reflection Absorption Spectroscopy, ellipsometry, X-ray Photoelectron Spectroscopy (XPS), Quartz Crystal Microbalance with Dissipation (QCM-D) and contact angle measurements (CA). Also, film stability was studied after surfaces immersion on PBS for 7 at 37ºC, using XPS. Biological performance towards (i) MRSA adherence (even in the presence of plasma proteins), viability, proliferation and biofilm production was assessed by Fluorescence Microscopy, CFU counts, Scanning Electron Microscopy imaging, and crystal violet; (ii) MC3T3-E1 cell line metabolic activity and morphology was assessed by resazurin and DAPI/Phalloidin staining.

RESULTS AND DISCUSSION:

Surface characterization demonstrated the success of NAC grafting using 4 mg/mL concentration.This surface was stable for 7 days in PBS at 37ºC. QCM-D demonstrated that NAC functionalization decreases plasma protein adsorption to Ch coatings. Biological studies confirmed that immobilized NAC avoids MRSA adhesion to Ch coating (without being bactericidal), impairing biofilm formation, while allowing osteoblast adhesion and proliferation.

CONCLUSION:

Ch_NAC is a promising material as it avoids bacterial adhesion, impairs biofilm formation, while allowing osteoblast adhesion and proliferation. This can be further explored as a prevention coating or as coadjuvant in systemic antibiotic therapies.

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ACKNOWLEDGMENTS:

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Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

794 A Highly Programmable and Non-invasive Biomaterial to Aid Wound Healing

Virginia Llopis-Hernandez, Manuel Salmeron-Sanchez, Matthew Dalby

University of Glasgow, Glasgow, United Kingdom

INTRODUCTION:

We are working towards a safe therapeutic solution in the management of complex (partial and full thickness) wounds. Non-healing ulcers, among other problems, can lead to infection and disability and current wound

management protocols are not satisfactory. Wound healing is promoted by a number of growth factors (GFs) including PDGF-BB. Current GF products have a large associated cost and potential adverse effects (e.g. PDGF containing Regranex® warns not to use more than 3 times due to health warnings such as death secondary to malignancy).

METHODS:

PEA samples and proteins coatings were prepared as in 1. ELISAs were used to study GF adsoption and release. AFM was used to image GFs bound to fibronetin. Cell lines L929 (mouse) and hTERT (human) and human adult keratinocytes were used to study wound clousure and individual cell migration during 48 hours (EVOS microscope). Fluorecent microscopy, in-cell western and flow citometry was used to analyse keratinocyte maduration.

RESULTS AND DISCUSSION:

Appropriate interactions of GFs with the extracellular matrix are critical to regulation of signalling and effectiveness. Our approach utilizes poly(ethyl acrylate) (PEA) that promotes self-organization of fibronectin (FN) into biological nanonetworks, unravelling the FN molecule to reveal cell adhesive and GF binding domains. We have previously shown successfully effectiveness of this system to deliver BMP2 for bone regeneration (1). Here, we use PEA to facilitate exposure of FNIII12-14 to bind PDGF-BB in close apposition to the integrin binding FNIII9-10 domain. The FN nanonetworks sequester PDGF-BB at very low concentration (100 ng/ml) compared to GF containing products (approx. 300 fold lower). A newly approach is the introduction of cytokines, making use of the FNI1-5 domain that has been demonstrated to bind and efficiently present cytokines/chemokines (2). The biological activity of the system with the GF PDGF-BB and chemokines CXCL11 and CXCL12 has been evaluated in vitro using fibroblasts to correlate enhanced cellular migration to the wound site with the synergistic presentation of GFs and chemokines using wound healing assays. The future implantation of the system in clinics in a bandage form has been studied. Further experiments using keratinocytes have been performed to confirm their maturation.

CONCLUSION:

We have been able to develop a biomaterial coating providing accelerated cell migration coupled to low dose PDGF-BB delivery will provide cost-effective, safe, enhanced wound closure.

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ACKNOWLEDGMENTS:

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Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

795 How to print simultaneously cells and thermoplastics and frequently forgotten parameters of 3D printing that affect the stability, mechanical properties and biological activity of thermoplastic scaffolds.

Nieves Cubo Mateo, Daniela Sanchez Tellez, Luis Rodriguez Lorenzo

ICTP-CSIC, Madrid, Spain

INTRODUCTION:

3D Printing allow to build personalized scaffolds through different techniques, materials and parameters to configure the printed structures in advance (infill percentage, layer height, temperature, speed, etc.). However, this versatility also comes with a huge change in the module and other physical properties of the used material. The mechanical properties of these scaffolds will determine the short and long-term stability and will be a key point in the regeneration of the tissue. In this work we present some of the most “forgotten/not studied” parameters that must be taken in account when using 3D printing of thermoplastics[1] and new building techniques to print thermoplastics and cells at the same time.

METHODS:

For these studies a commercial printer (Witbox 2,BQ). PCL was purchased from 3D4Makers (Mw: 84500±1000). Main studied parameters were: (1) Different cool down ratios/speeds; (2) Layer alternating space to improve isotropic properties; (3) Deposition speed, that affects to the micro and nano-porosity as higher speeds can create random fibers and niches. For simultaneous deposition pores have been filled with fibrinogen and hyaluronic based hydrogels[2]. For mechanical characterization a QTest 1/L universal test machine in compression mode was used. Pore morphology was determined by imaging cut samples using a PHILIPS XL30 microcopy operating at 20kV and coated with Au-Pd using a Polaron SC7640 sputter coater. Biological assays include histological analysis and cell survive studies.

RESULTS AND DISCUSSION:

The temperature of the deposition environment has revealed as an important factor for scaffold microstructure. Controlled cool down of the ink yield lower surface microcracks, which induce poor mechanical properties. Compression module can be modified from 2.13MPa to 4.25MP by modifying the printing protocol and geometry of the pattern. In addition, new simultaneous deposition techniques have demonstrated to improve cell survival when using a combination of thermoplastics and hydrogels (biological studies on going.). Higher speeds resulted in more chaotic microstructures, but increasing the range of microstructures for cell development.

CONCLUSION:

3D printing approaches needs to be further studied in order to use it along bioprinting. New deposition strategies can achieve cell survival when printing simultaneously. Frequently forgotten parameters matters when doing a final bioprinted assembly.

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ACKNOWLEDGMENTS:

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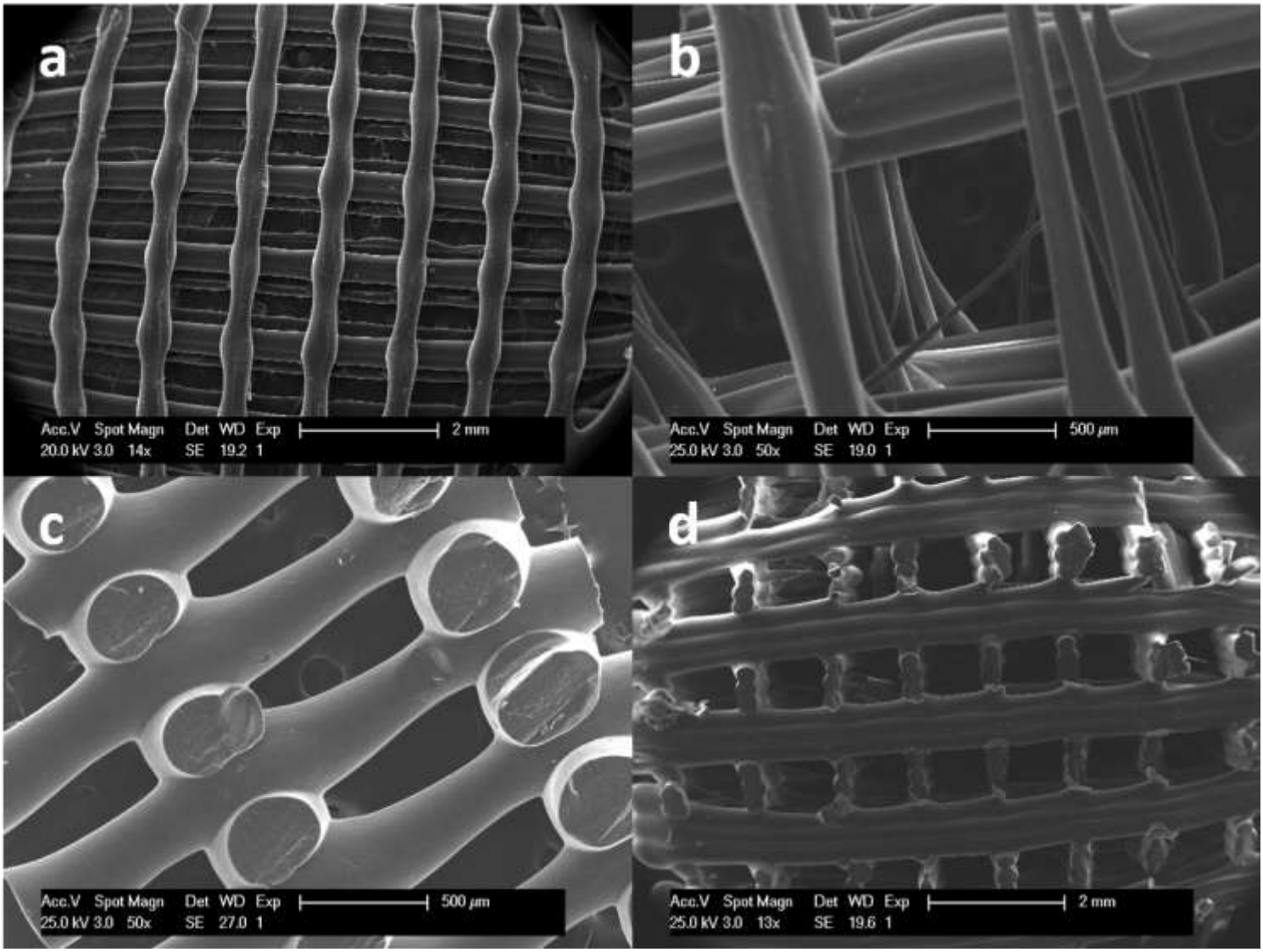

Picture 1: Caption 1: (a) Top view of normal scaffold. (b) Side view of normal (anisotropic) (c) Top view of isotropic scaffold. (d) Side view of isotropic scaffold.

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

805 Hybrid biomimetic chitosan-titanium interfaces tuned by plasma-induced activation to enhance soft-hard tissue regeneration

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[2]University of illinois at urbana, United States of America

INTRODUCTION:

To address the full regeneration of wound tissue, hybrid materials that aid in the reconstruction of soft (skin, muscle and cartilage) and hard tissues are needed. Titanium (Ti) has been used as an implant device in biomedical field for bone reconstruction. Despite its good biocompatibility, Ti has shown poor properties regarding soft tissue interactions [1].

Chitosan(CS) is a biocompatible and non-toxic biopolymer, with antimicrobial properties. CS-Ti composites have been produced but they require processing under strong chemical and aggressive conditions (sulfuric acid [2], silane-coupling agents [3], acetone [4], and piranha solution [5]) which leave toxic compounds reducing the cell cytocompatibility.

METHODS:

A new methodology to achieve a highly specific crosslinking between chitosan and Ti surfaces is studied. Plasma irradiation is used to activate the Ti surface prior to adding chitosan to produce CS-Ti scaffolds. Irradiations in atmospheric and low pressure were carried out, with process parameters such as fluence, time and gas species systematically studied. Coating composition, thickness, topography and roughness were studied using EDS, SEM and AFM. In vitro testing was performed using cell cultures to elucidate the cytocompatibility and stability of these scaffolds.

RESULTS AND DISCUSSION:

The topography of CS-Ti scaffolds highly depended on the irradiation parameters and varied from uniform surfaces to agglomerate-like structures (shown un figure).

The surface morphology influenced the cell viability. Tunning the irradiation parameters allows to control the surface morphology cell of the scaffolds.

CONCLUSION:

Chitosan membranes were produced on irradiated titanium.

By producing this type of hybrids, the mechanical properties of the CS and Ti can be used to improve soft-hard tissue regeneration.

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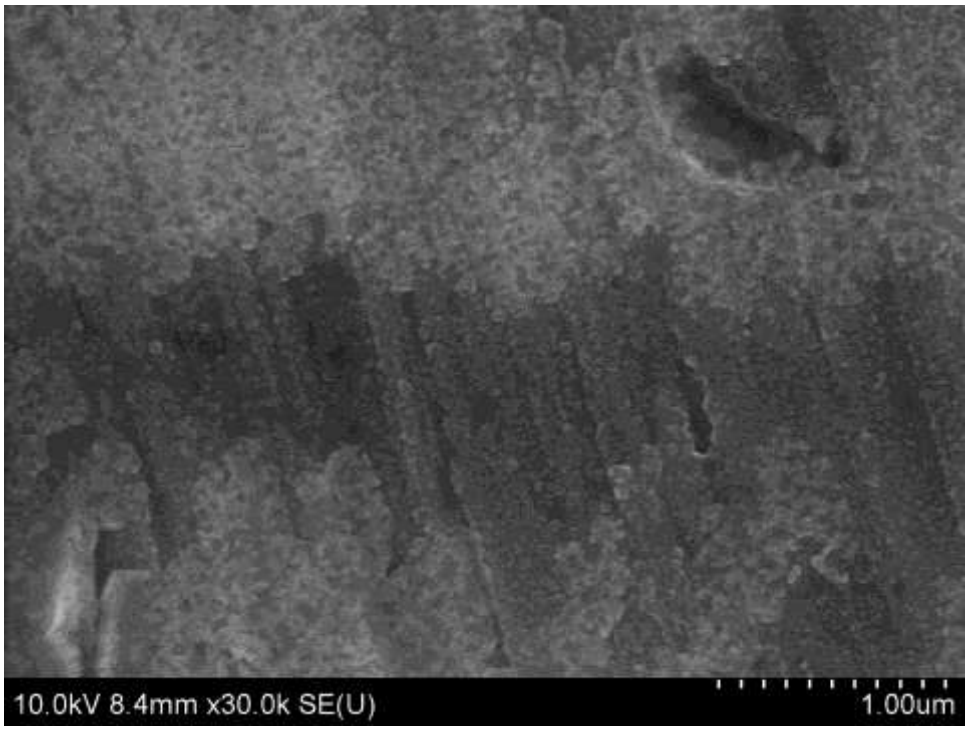
Picture 1: 

Caption 1: CS-Ti irradiated with atmospheric plasma

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Poster presentation

810 Combinatorial coating development of Si-N-Fe-C coatings for joint implants

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INTRODUCTION:

Joint replacements of hip and knee are generally considered successful procedures, with a survival rate of approximately 95% after 10 years. However, the increasing, more active elderly population puts higher demands on implants, which need to last longer. Some of the main limiting factors for the longevity of these implants are the generation of wear debris and release of metal ions. These wear particles and ions could be reduced with the use of ceramic coatings e.g. silicon nitride. Silicon nitride coatings have in laboratory investigations been shown to reduce the wear rate and act as a barrier for metal ions and therefore warrant further investigation for use in joint implants. An addition of the biocompatible elements Fe and C could be used to tune the dissolution rate and increase the deposition rate.

METHODS:

Coatings were deposited on silicon wafers using magnetron co-sputtering. The targets used were Si (99.99%purity) powered by pulsed DC at 200 W, 200 kHz and 2µs. The C and Fe targets were powered by DC at 65 W and 25 W respectively. During deposition N_2 was introduced. Elemental gradients were obtained by angling of the targets and the use of no rotation.

The coatings were investigated using elastic recoil detection analysis (ERDA), atomic force microscopy (AFM), scanning electron microscopy (SEM) and nanoindentation in five different points on the sample. The different points were chosen at coordinates (0,0), (0,40), (40,0), (40,40) and (20,20) based on a coordinate system with origin in the lower left corner.

The cytotoxicity of the coatings was evaluated *in vitro* with mouse osteoprogenitor cells (MC3T3).

RESULTS AND DISCUSSION:

Clear elemental gradients could be obtained with 26 wt.% < Si < 34 wt.%, 10 wt.% < Fe < 20 wt.%, 8 wt.% < C < 14 wt.% and 40 wt.% < N < 47 wt.% (figure 1). The coatings appeared dense in SEM, with a smooth surface for all investigated points (Ra ~ 2 nm, AFM). The cross-sectional morphology was slightly columnar with broader columns for higher Fe content. The modulus (202 GPa < M < 221 GPa) correlated positively to the Si content and negatively to the Fe content while for the hardness (14 GPa < H < 18 GPa) no statistically significant correlations were found. This can be compared to earlier coatings, only containing Si and N, which have showed a Young's modulus of 170-240 GPa and a hardness of 12-26 GPa[1], as well as the currently used metals such as CoCrMo, showing a Young's modulus of 293 GPa and a hardness of 6 GPa[1] .

The *in vitro* evaluation indicated biocompatibility with viable cells that adhered and spread across the surface.

CONCLUSION:

Si-N-Fe-C coatings show promise for applications exposed to wear with their low surface roughness, high hardness, high modulus and biocompatibility. These combined merit further investigations into the suitability of Si-N-Fe-C coatings for joint implants.

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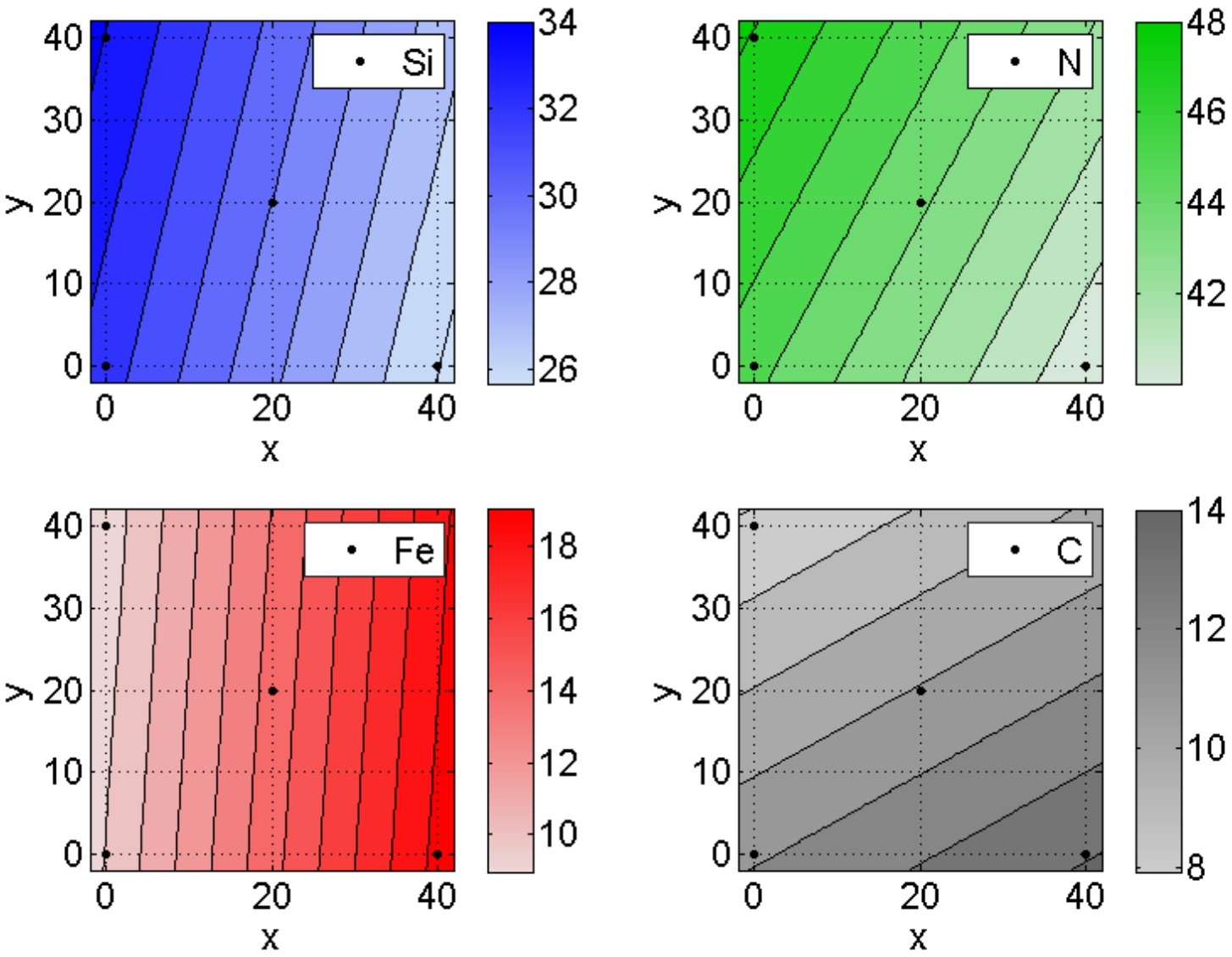
ACKNOWLEDGMENTS:

The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under the LifeLongJoints Project, Grant Agreement no. GA-310477.



Picture 1: Caption 1: Si, Fe, N and C composition over the substrate.

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Poster presentation

829 CXCL12-loaded poly(lactic-co-glycolic) acid microspheres for the chemotactic recruitment of glioblastoma stem cells

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INTRODUCTION:

Glioblastoma (GBM) stem cells (GSC) have been found to specifically migrate in response to a gradient of CXCL12 in a CXCR4-dependent manner[1]. This enables the escape of GSCs from the tumor mass, thereby potentially evading treatment and initiating metastases. We seek to take advantage of this mechanism for GBM therapy. By encapsulating CXCL12 in poly(lactic-co-glycolic) acid (PLGA) microspheres, we aim to create a polymeric platform capable of recruiting and directing migratory GSCs in order to influence GBM progression and metastasis formation.

METHODS:

Human CXCL12 was initially complexated with heparin and poloxamine (Tetronic 1107)[2]. Resulting nanocomplexes were encapsulated in PLGA via emulsion solvent evaporation/extraction to form microspheres. Microspheres were characterized for morphology, encapsulation efficiency, and *in vitro* release characteristics. To verify promigratory activity, media preconditioned with blank and CXCL12-loaded microspheres were evaluated for chemotactic activity on U87MG GBM cells using a transwell migration assay.

RESULTS AND DISCUSSION:

CXCL12-heparin nanocomplexes were successfully encapsulated in PLGA microspheres with diameter of 81.9±58.3 μm. The formulation exhibited low initial burst release *in vitro* at <8%, and released >40% of the payload over a period of up to 90 days. Furthermore, media preconditioned with CXCL12-loaded microspheres for 1 to 8 weeks displayed promigratory activity towards GSCs. The number of migrating cells were 1.8- to 2.8-fold higher as compared to media preconditioned with blank microspheres. Treatment with AMD3100, a CXCR4 antagonist, abrogated this promigratory effect, indicating CXCR4 involvement.

CONCLUSION:

The obtained results point to the potential of CXCL12-loaded microspheres for long-term recruitment of GSCs. These chemotactic microspheres, in combination with conventional and/or novel therapies, present a promising strategy for tackling GBM and its recurrence.

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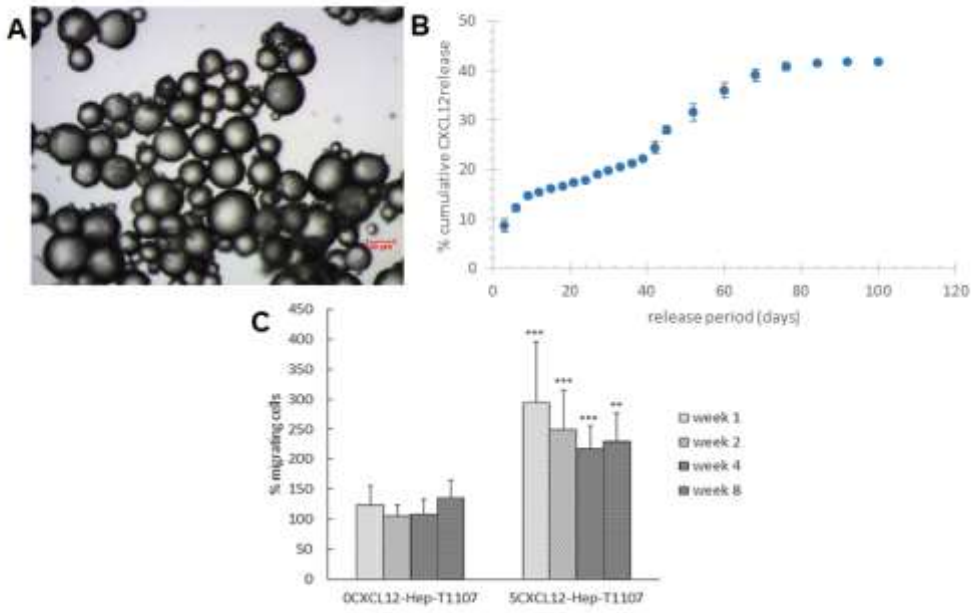
ACKNOWLEDGMENTS:

This project has received funding from NanoFar, Erasmus Mundus Joint Doctorate in Nanomedicine and Pharmaceutical Innovation under the European Union's Horizon 2020.

Picture 1:



Caption 1: Image of CXCL12-loaded PLGA microspheres (A), its in vitro release profile (B); and, effect on GSC migration (C)

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Poster presentation

847 Composite of borate bioactive glass and PVA /SA hydrogel used for wound healing

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INTRODUCTION:

The composite of PVA/BG hydrogel has showed good biological properties [1]. Since sodium alginate (SA) hydrogel promotes chondrocyte proliferation both in vivo and in vitro [2], SA gel can be used as an effective hemostatic agent with good histocompatibility for the healing of the wound [3]. The aims of this work are to prepare PVA/ BG/ SA hydrogels with different compositions, to explore the effect of SA hygrogel content on the PVA/ BG/ SA hydrogel properties, and to find the best composition of PVA/ BG/ SA hydrogel for wound healing treatment.

METHODS:

The cytotoxicity of hydrogls were assessed by CCK-8 assay, in which two kinds of cells EA.hy926 cells and hFBs (human fibroblasts, hFBs) were employed. The wound healing ability were evaluated from: 1) The tubular formation activity of hydrogels was estimated by calculating the number of complete capillaries counted under light microscopy; 2) The bioactivity of hydrogel in angiogenic-related gene expression (fibroblasts of VEGF, bFGF and PDGF) was used assesed by qRT-PCR.

RESULTS AND DISCUSSION:

CCK-8 assays of PVA/ BG/ SA hydrogels showed that the 20% SA hydrogel samples were a little bit better than others. The gene expressions of VEGF, PDGF and bFGF were increased with the SA content in all incubation time (3 and 7 days). Meanwhile, the 20% SA samples showed much better gene expression than others, seeing the attached figure. The strong wound healing ability of PVA/ BG/ SA hydrogels is come from both SA and BG. When SA content reaches too high, for example, up to 50%, the BG content would be decreased, and the function of BG also be decreased, thus the bioactivity for SA 50% samples is lower than that for 20% samples.

CONCLUSION:

The PVA/BG/SA hydrogels can be prepared by BG as a function of cross-linking agent, and these hydrogels exhibited good physical properties of porous structure and mechanical strength. With the increase of the SA content in the PVA/BG/SA hydrogels, the wound healing ability would be increased. When the SA content reached to 20% in the PVA/BG/SA hydrogel samples, they had the highest wound healing ability.

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ACKNOWLEDGMENTS:

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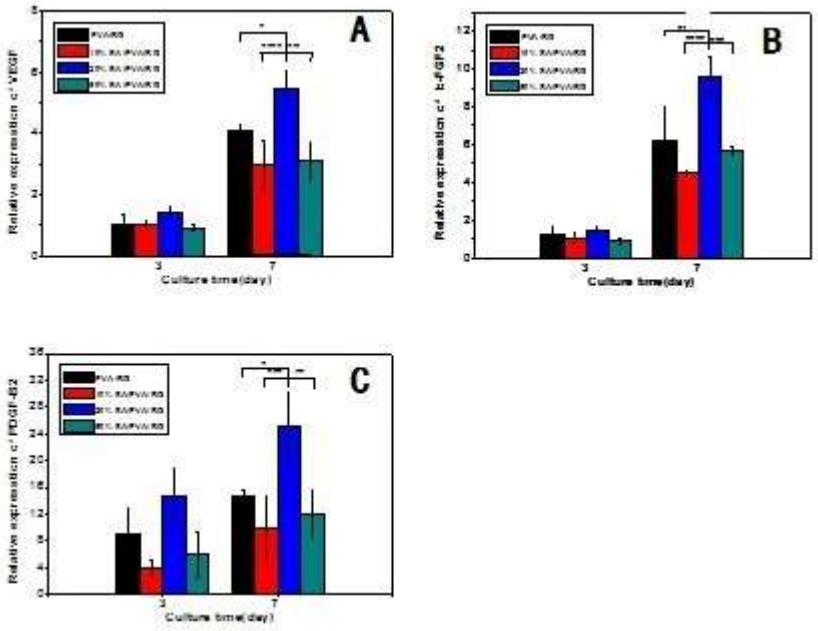

Picture 1: Caption 1: Gene expression VEGF(A), PDGF(B) and bFGF(C) of fibroblasts 3 and 7 days on the four groups of hydrogel samples were shown. Mean ± SD; n = 5. Signific

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Poster presentation

855 Magnesium-Tricalcium Phosphate biodegradable composites fabricated by additive manufacturing and metal infiltration

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INTRODUCTION:

In this work robocasting and liquid metal infiltration are combined to fabricate magnesium-tricalcium phosphate (Mg-TCP) biodegradable composites, hypothetically with properties suitable for temporal and load-bearing orthopaedic applications.While Mg and its alloys are lightweight, structural and biodegradable materials with elastic modulus quite similar to that of bones, TCP is an osteoconductive and biodegradable ceramic, extensively used in clinic to regenerate bone tissue.

METHODS:

TCP porous preforms were manufactured by robocasting. The bodies were sintered at 1200 °C for 5 h. For infiltration the preform was placed in a crucible and one metal coupon was located on it. The temperature was increased until 750 °C to melt the metal in high purity argon atmosphere (3.4 KPa). Both, commercial pure Mg and in house produced Mg-2Zn-3Ag alloy were studied. The obtained Mg-TCP composites were characterized in terms of chemical composition, microstructure, mechanical properties and degradation rate under simulated physiological conditions using methods such as DRX, SEM, EDX, micro-CT, compression test, indentation test, mass loss, hydrogen evolution and electrochemical tests.

RESULTS AND DISCUSSION:

The preform produced by robocasting had an orthogonal grid structure with pore size and rod size of 500 and 400 μm, respectivelly. Fig. 1a shows a micro-CT reconstruction of the Mg-TCP composite with 96 % of pores filed with Mg. While XRD confirmed the retention of the TCP crystalline structure during infiltration, metallography analysis revealed the precipitation of a second phase in the grain boundaries of the pure Mg (Fig. 1b). In the case of Mg-2Zn-3Ag alloy, the metallic phase was formed by three components, an almost pure Mg solid solution, Mg-Zn-Ag solid solution and Mg-Zn-Ag eutectic (Fig. 1c). Indentation test showed an intermediate hardness value at the Mg/TCP interface, indicating interfacial bounding between phases. In vitro test showed fast degradation of the composite that may be reduced by the deposit of a biodegradable coating.

CONCLUSION:

Combination of robocasting and Mg infiltration allowed on demand fabrication of Mg-TCP interpenetrated composites. This represent a significant step forward respect composites reinforced either with random distributed particles or fibres. Although more research is required to control the degradation rate of the composite, this material has the potential to function as temporal load-bearing implant for the fixation of bone fractures.

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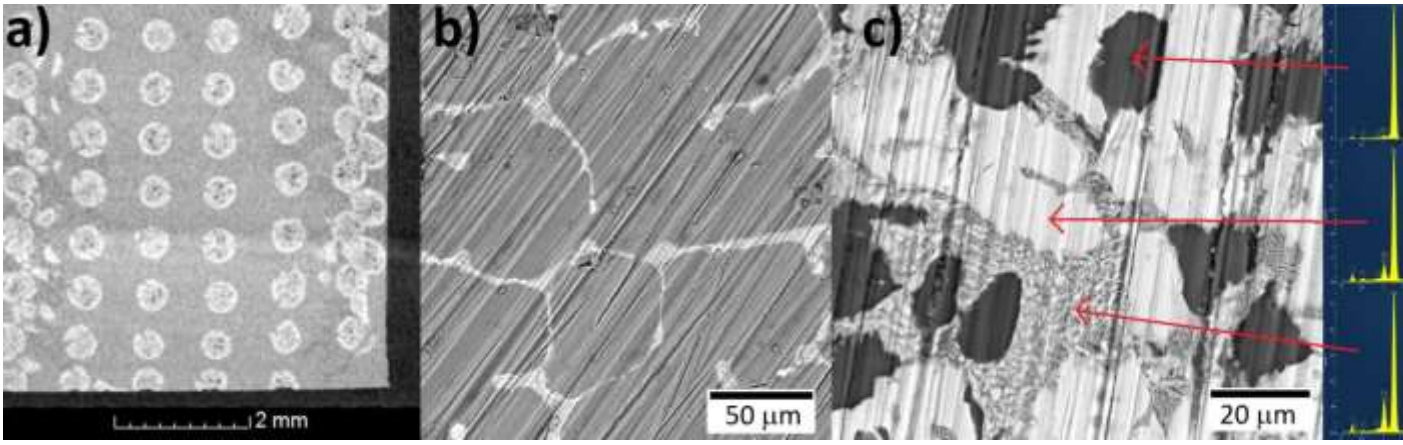

Caption 1: Fig.1. Microstructure of Mg-TCP composites. a) CT reconstruction, metallography of the metallic component for sample infiltrated with pure Mg (b) and

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Poster presentation

860 Biocompatibility of calcium carbonate particles synthesized at controlled pH

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INTRODUCTION:

Calcium carbonate ($CaCO_3$) particles have been used in various applications, including drug delivery and orthopedics, due to their solubility, dispersability and biocompatible nature.[1] $CaCO_3$ is generally found in its anhydrous polymorphs, *i.e.* vaterite (unstable), aragonite (metastable) and calcite (stable).[2] Because of the stability difference of anhydrous polymorphs, control on $CaCO_3$ particles is challenging. Herein, the effect of pH on polymorph, morphology and size of synthesized $CaCO_3$ particles was investigated. By altering precursor solution pH and molarity ratios, spherical, ellipsoidal and spheroidal vaterite particles along with cuboidal and flower-like calcite particles were synthesized in different sizes. In addition, the effects of $CaCO_3$ particle morphology and properties on bone cell functions were assessed.

METHODS:

Aqueous calcium acetate ($Ca(CH_3COO)_2$) and sodium bicarbonate ($NaHCO_3$) solutions at different molarity ratios (5:1 to 1:3) were used to synthesize $CaCO_3$ particles. 4 ml of these solutions along with 20 ml ethylene glycol were mixed under magnetic stirring for 15 minutes at different pH values (8, 9, 10, 11, 12, 13). After 1 hour without magnetic stirring, synthesized $CaCO_3$ particles were washed with ethanol and water, collected by centrifugation and dried at 50 °C. To investigate biocompatibility of the particles, cell culture media (DMEM) having different concentrations of $CaCO_3$ particles were incorporated onto osteoblast (ATCC CRL-11372) and MTT assay was completed to assess cell viability up to 5 days of culture.

RESULTS AND DISCUSSION:

At low pH values (8-10) spherical (Figure 1a) and ellipsoidal (Figure 1b) vaterite particles were obtained using 5:1 and 1:2 ratios, respectively. When pH values were increased to 11-13, cuboidal (Figure 1c) and flower-like (Figure 1d) calcite particles were obtained at 5:1 ratio. Preliminary results showed that when the synthesized $CaCO_3$ particles interacted with osteoblasts, they did not exhibit any toxic effect depending on the particle geometry and polymorph.

CONCLUSION:

By altering precursor solution pH and molarity ratios, $CaCO_3$ particles having different morphologies and sizes were obtained in vaterite and calcite polymorphs. Osteoblast viability did not change with particle morphology and polymorph of the $CaCO_3$ particles.

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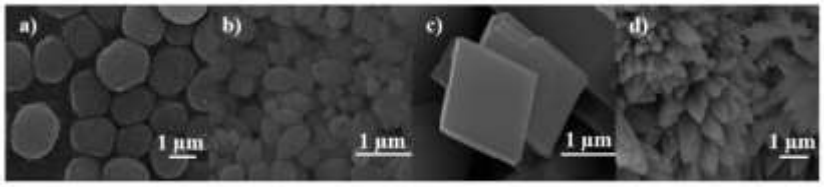
ACKNOWLEDGMENTS:

The authors would like to thank Middle East Technical University (Grant No: 03-08-2016-009) for providing financial support.

Picture 1: 

Caption 1: Synthesized CaCO3 particles: a) spherical and b) ellipsoidal vaterite, c) cuboidal and d) flower-like calcite.

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Poster presentation

862 Preparation of hyaluronate-alginate hybrid microgels for cartilage regeneration

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INTRODUCTION:

In tissue engineering, proper delivery of tissue-specific cells is important for the formation of new tissue. Scaffold structures significantly regulate cellular behavior in matrix-associated cell transplantation.[1] Hyaluronate is one of the major extracellular matrix components and has been widely used for cartilage regeneration. Alginate has also been frequently used for gel formation. We previously reported a hybrid polymer of hyaluronate and alginate can form gels via ionic cross-linking.[2] In this study, we hypothesized that the mechanical stiffness of hyaluronate-alginate hybrid (HAH) gels could be controlled by varying the type of linkers. HAH gels were used to prepare microspheres, which may be potentially useful for cartilage regeneration.

METHODS:

Sodium hyaluronate and sodium alginate were purchased from Humedix (Korea) and FMC Biopolymer, respectively. Histidine-alanine-valine (HAV) peptide and arginine-glycine-aspartate (RGD) peptide were purchased from Anygen (Korea). Various linkers such as ethylenediamine, 1,4-diaminobutane, and 1,8-diaminooctane were used to synthesize HAH. In brief, hyaluronate was reacted with bi-functional linkers prior to being reacted with alginate that was modified with HAV and RGD peptide.[2] A HAH solution formed hydrogels with calcium ions, and their mechanical properties were measured using a rotational viscometer (Malvern). HAH microspheres were prepared using the water-in-oil emulsion method and cross-linked with calcium chloride.

RESULTS AND DISCUSSION:

In the presence of calcium ions, a simple mixture of hyaluronate and alginate remained as a solution, but a hybrid solution formed hydrogels. As the length of a spacer arm between hyaluronate and alginate increased, the storage shear modulus (G') of HAH hydrogels increased. The mechanical stiffness of HAH gels was dependent on the type of linkers used. All HAH polymers did not indicate significant cytotoxicity. Microspheres with the average diameter of 20µm, similar to the size of cells, were prepared (Fig. 1). HAH microgels modified with HAV and RGD peptide formed an aggregate with chondrocytes.

CONCLUSION:

We demonstrated that the type of linkers used for the preparation of HAH could influence the mechanical stiffness of HAH gels. HAH microspheres formed the aggregated structure with chondrocytes. This cell/microsphere aggregate can be practically useful for the cell delivery in tissue engineering.

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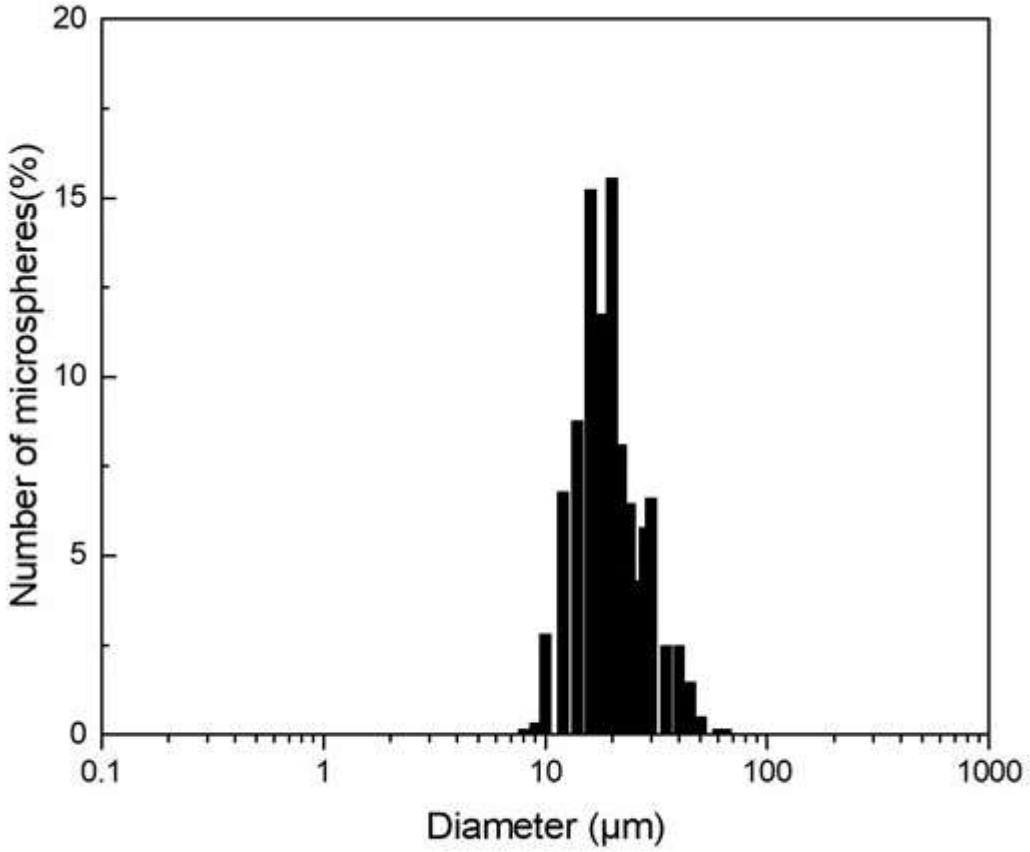

Fig. 1. Size distribution of HAH microspheres.

Picture 1:
Caption 1: Fig. 1. Size distribution of HAH microspheres.

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Poster presentation

867 Skelatal muscle regeneration in 3D bioprinted hydrogels

Nehar Celikkin[1], Tiziana Fischetti[2], Marco Costantini[3], Chiara Rinoldi[3], Nicola Contessi[2], Silvia Fare[2], Feng-Huei Lei[4], Wojciech Swieszkowski[1]

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INTRODUCTION:

The increasing number of extensive injuries of skeletal muscles due to accidents, cancer resection, combined with the shortcomings of the conventional treatment procedures, creates demand for advanced solutions. Muscle tissue engineering (TE) is a promising strategy for fabrication of tissue substitutes from biomaterials and cells. Scaffolds are designed to mimic the native extracellular matrix (ECM), guide cell growth through a defined geometry, surface properties, porosity and mechanical properties. 3D bioprinting is an emerging tissue engineering technology that holds promise for fabricating skeletal muscle. The advantage of this technology is the ability to encapsulate muscle cells into bioinks and precisely print aligned cell-laden fibers in a 3D design.

METHODS:

In this study, we have used C2C12 laden chitosan-gelatin and GelMA based bioinks for 3D bioprinting process. Firstly we studied the stability of the 3D printed pristine scaffolds, swelling characteristic, rheological and mechanical properties. Thereafter, 10^7 cell / mL was encapsulated separately in 5% GelMA and in 2% Chitosan – 6% Gelatin bioinks and 3D printed on 4°C printing plate.

RESULTS AND DISCUSSION:

Cytocompatibility of the pH dependent crosslinking of chitosan-gelatin and thermal/UV crosslinking of GelMA hydrogels were compared to attain a cell friendly bioprinting conditions. Furthermore, the cell alignment, ECM deposition through growth and differentiation phases was evaluated with immunostaining.

ACKNOWLEDGMENTS:

The research leading to these results has received funding from National Center for Research and Development (PL-TWIII/5/2016, project 3DMUSCLE).

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Poster presentation

868 Reduced inflammatory response and bacterial growth on ZnO decorated nanochannels formed on TiZr alloy

Raluca Ion[1], Marcela Popa[1], Cristina Dumitriu[2], Cristian Pirvu[2], Luminita Marutescu[1], Carmen Chifiriuc[1], Madalina Necula[1], Valentina Mitran[1], Anisoara Cimpean[1]

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INTRODUCTION:

Bacterial infection and tissue inflammation are the main causes of early bone implant failure. To address these problems functional nano-topographic surfaces incorporating various antimicrobial agents have been developed and showed great promise both *in vitro* and *in vivo*. Here, we combined the immunomodulatory traits of nanochannelar surfaces formed on TiZr substrates, which were previously shown to reduce the foreign body reaction,[1] with the antimicrobial properties of ZnO nanoparticles[2] in an attempt to regulate macrophage activity and reduce bacterial adhesion and growth on Ti-based medical devices, without the use of antibiotics.

METHODS:

Nanochannels were formed on TiZr alloy by anodization in hot glycerol-phosphate electrolytes. ZnO nanoparticle decoration was achieved by spin coating. Samples were characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM) and contact angle measurements (CA). The *in vitro* experiment examined the behavior of RAW 264.7 macrophages on nano- and flat TiZr 24 h post-seeding in standard and pro-inflammatory (lipopolysaccharide treatment) conditions, in terms of cell viability, proliferation and cytokine secretion. A Live/Dead Viability/Cytotoxicity Assay was used to image and assess cellular viability on the tested substrates while cell proliferation was quantified by CCK8 colorimetric assay. Commercially available enzyme-linked immunosorbent assay kits were used to determine the concentrations of tumor necrosis factor alpha, interleukin-6 and monocyte chemotactic protein - 1. Time-kill studies for evaluation of antibacterial activity were performed with 10^5 UFC/ml of *Staphylococcus aureus* ATCC 25923 at baseline. Bacterial growth was quantified after 0, 2, 3, 4, 5, 6, and 24 h incubation at 37 ^{0} C by plating 2-fold dilutions on plate count agar.

RESULTS AND DISCUSSION:

The SEM, AFM and CA results demonstrated that the nanostructured ZnO-decorated surface was successfully fabricated. ZnO-incorporated nanochannelar surfaces restricted macrophage proliferation and reduced pro-inflammatory cytokine secretion (Fig. 1a). Furthermore, the results showed that the nanochannelar samples decorated with ZnO nanoparticles inhibited the growth of *Staphylococcus aureus* compared to unmodified nanochannels and flat TiZr surface, albeit the ZnO nanoparticles concentration still needs to be optimized for maximal effect (Fig. 1b).

CONCLUSION:

Overall, we demonstrated that ZnO decorated nanochannels display antibacterial and anti-inflammatory properties that are promising for improving the performance of titanium-based implants.

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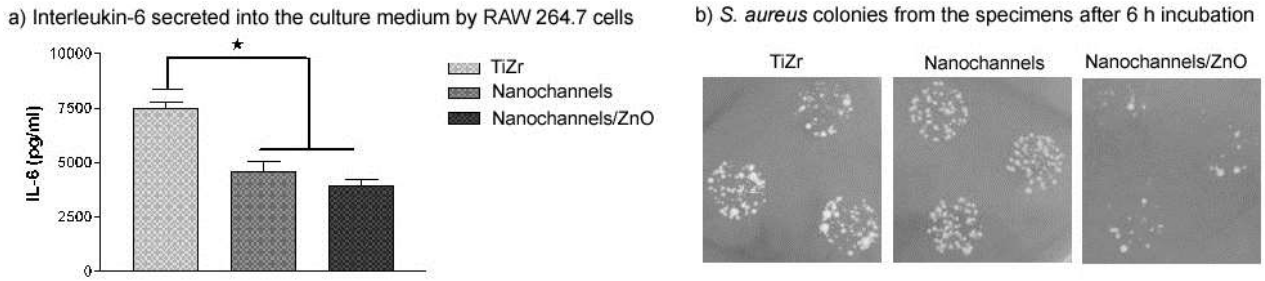

Caption 1: Fig. 1. Biological response to modified TiZr surfaces.

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Poster presentation

869 The effect of Xeno-free media for optimisation human mesenchymal stem cells osteogenesis in 3D Poly(?-Caprolactone) scaffolds

Dirar Qassim, Gwendolen Reilly, Helen Colley

University Of Sheffield, SHEFFIELD, United Kingdom

INTRODUCTION:

Animal sera are routinely used as a supplement in the culture media to support mesenchymal stem cell (MSC) proliferation and differentiation. However, severe drawbacks with its use exist such as contamination, poorly undefined components and diseases transmission[1-2]. Recently xeno-free media have been introduced to overcome some of these issues and have been shown to support MSC proliferation, and to a lesser extent, osteogenic induction. The ultimate aim of this work was to evaluate the effectiveness of xeno-free media on proliferation and differentiation of human bone marrow mesenchymal stem cells (hBMSCs), with the long-term intention of defining the optimal xeno-free formula for clinical applications particularly in maxillofacial surgery such as cleft palate.

METHODS:

Polycaprolactone (PCL) was electrospun to generate three-dimensional (3D), non-aligned scaffolds. hBMSCs were seeded onto the scaffolds at a density of 2 x 10^5 cell/cm^2 in two commercially available media 'Human Mesenchymal-XF Expansion Medium' (XF-2; Millipore), 'Stemulate® TM Pooled Human Platelet Lysate' (5% HPL; Cook Regents) compared with standard medium 10% foetal bovine serum (FBS), as a control. Metabolic activity was monitored using resazurin reduction assay and cell number by measuring total DNA content over a 14 days culture period. Scanning electron microscope was employed to assess a hard bony layer on the 3D PCL scaffolds.

RESULTS AND DISCUSSION:

hBMSC viability and growth in 3D PCL scaffold were enhanced in both xeno-free media compared to standard medium at day 4 and day 7 culture (Fig. 1). DNA content was higher in XF2 after day 14. Entirely bone regeneration was created on 3D PCL Scaffolds with higher of osteoinduction capacity in XF2 osteogenic supplements (Fig. 2).

These results suggest that the human supplements (serum and platelets) are better defined to support hBMSCs culture compared to animal serum.

CONCLUSION:

Our results indicated that xeno-free media with human extracts are supportive of hBMSC growth and osteoinduction and may be used as a pre-clinic medium for cell proliferation and differentiation in bone tissue-engineering applications.

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ACKNOWLEDGMENTS:

Funding for this project was provided by the Republic of Iraq, Ministry of Higher Education and Scientific Research, Iraqi Culture Attaché-London. University of Mosul/ College of Dentistry.

Picture 1:

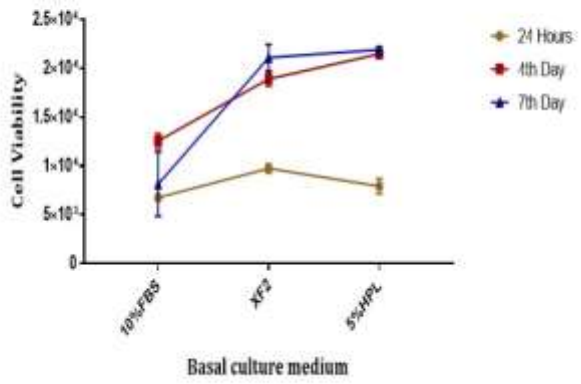

Figure 1: Mean±SD Resazurin Reduction in 3D Electrospun PCL Scaffolds at the different time. Xeno-Free Media are Substantially different from Animal Component Medium

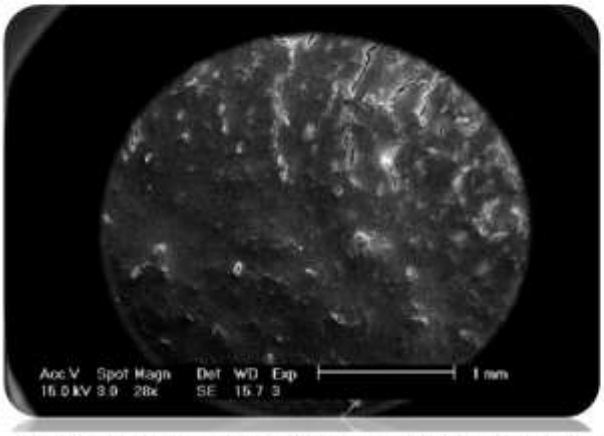

Figure 2: Bone formation on 3D PCL scaffolds in XF2 media imaged using scanning electron microscope.

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

870 Molecular transport of signaling molecules in glycosaminoglycan-based hydrogels

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INTRODUCTION:

Understanding the transport processes of signaling molecules within cell-instructive polymer networks is critically important for the rational design of engineered living matter[1]. Glycosaminoglycans (GAGs), including heparan sulfate, are major components of the extracellular matrix (ECM) and known to control the activity of many soluble signaling molecules[2]. Therefore, the transport of selected signaling molecules of different sizes and affinity towards heparin has been studied in starPEG-heparin hydrogels[3].

METHODS:

Hydrogels with varying mesh size and sulfate content were prepared as described elsewhere[4]. Recombinant VEGF 165, VEGF 121, SDF1α, and EGF were labelled with Alexa 488, incorporated into the hydrogels and subsequently fluorescent recovery after photobleaching (FRAP) measurements were performed to determine the diffusion coefficient. FRAP was determined in three independent experiments for each condition.

RESULTS AND DISCUSSION:

FRAP analysis revealed that the mobility of heparin-affine proteins (SDF1a & VEGF165) within starPEG-heparin hydrogels is independent of the molecular size while the mobility of non-heparin-affine proteins (EGF & VEGF121) decreases with increasing molecular size. Similarly, a reduced mesh size of the hydrogels decreases the mobility of the non-affine proteins. On the other hand, the hydrogel's mesh size does not significantly influence the diffusion of the highly affine proteins independent of their size. As expected, the sulfate content, and therefore the overall charge of the hydrogels, was found to be inversely proportional to the mobility of the highly affine proteins. Thus, the mobility of the proteins within a given hydrogel matrix is largely dependent on their size and charge and can be further controlled by adjusting the hydrogel network properties, namely mesh size and sulfation degree.

CONCLUSION:

Our systematic study of the mobility of signaling molecules in heparin-based hydrogels has revealed key parameters that govern the mobility of the proteins. The obtained results allow for the customization of transport phenomena in GAG-based biohybrid hydrogels.

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ACKNOWLEDGMENTS:

This work was supported by the Deutsche Forschungsgemeinschaft through ZI 1238/4-1 (Y.L. and R.Z.), CRC TR 67 (P.A., C.W. and U.F.), and FR 3367/2-1 (U.F.). Furthermore, the authors would like to thank Dr. Lars D. Renner (MBC Dresden) for the introduction with the FRAP experiments.

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Poster presentation

872 Screening Biomaterials Using 3-D Cell Culture

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INTRODUCTION:

Currently, *in vitro* methods for screening candidate biomaterials for biocompatibility rely on 2-dimensional culture of cells in contact with films of the biomaterial.[1,2] However, 2-dimensional culture suffers from limitations due to the inherent differences between culture of cells in 2-D and the 3-dimensional organization of cells in organs.[3,4] The culture of cell spheroids is a step towards bridging the gap between 2-dimensional culture *in vitro* and experiments in living organisms[5], but to date has not been used for screening candidate biomaterials.

Therefore, we are developing a method to culture cell spheroids containing finely ground biomaterials. This should give results that are more similar to those that would be obtained *in vivo*.

METHODS:

MG-63 osteosarcoma cells are cultured in standard 24-well plates on polycarbonate microwell chips fabricated by microthermoforming.[6] Physical confinement in the microwells induces the cells to form 3-dimensional aggregates (spheroids) which are imaged by fluorescence microscopy.

The cells are also cultured and aggregated with microscale (40-50 μm) samples of various materials of interest ,[7] including metals, polymers and ceramics. Cell viability and proliferation are quantified, and the aggregates are imaged using fluorescence microscopy to determine any morphological changes due to the presence of materials.

RESULTS AND DISCUSSION:

As of the submission date of this abstract materials have not yet been added to the spheroids. However, initial proof-of-concept studies have shown that it is possible to produce MG-63 spheroids on microthermoformed chips, a more controllable and reliable method for spheroid production than the previously used[8] hanging drop method. These spheroids could also be imaged by fluorescence microscopy, including in the 24-well plates, giving the potential to follow aggregate formation with materials in real time.

CONCLUSION:

In this work, we have developed a method to test materials for biocompatibility in a system which more faithfully replicates tissues *in vivo*, while requiring only a small sample of material that does not need to be fabricated into a specific shape.

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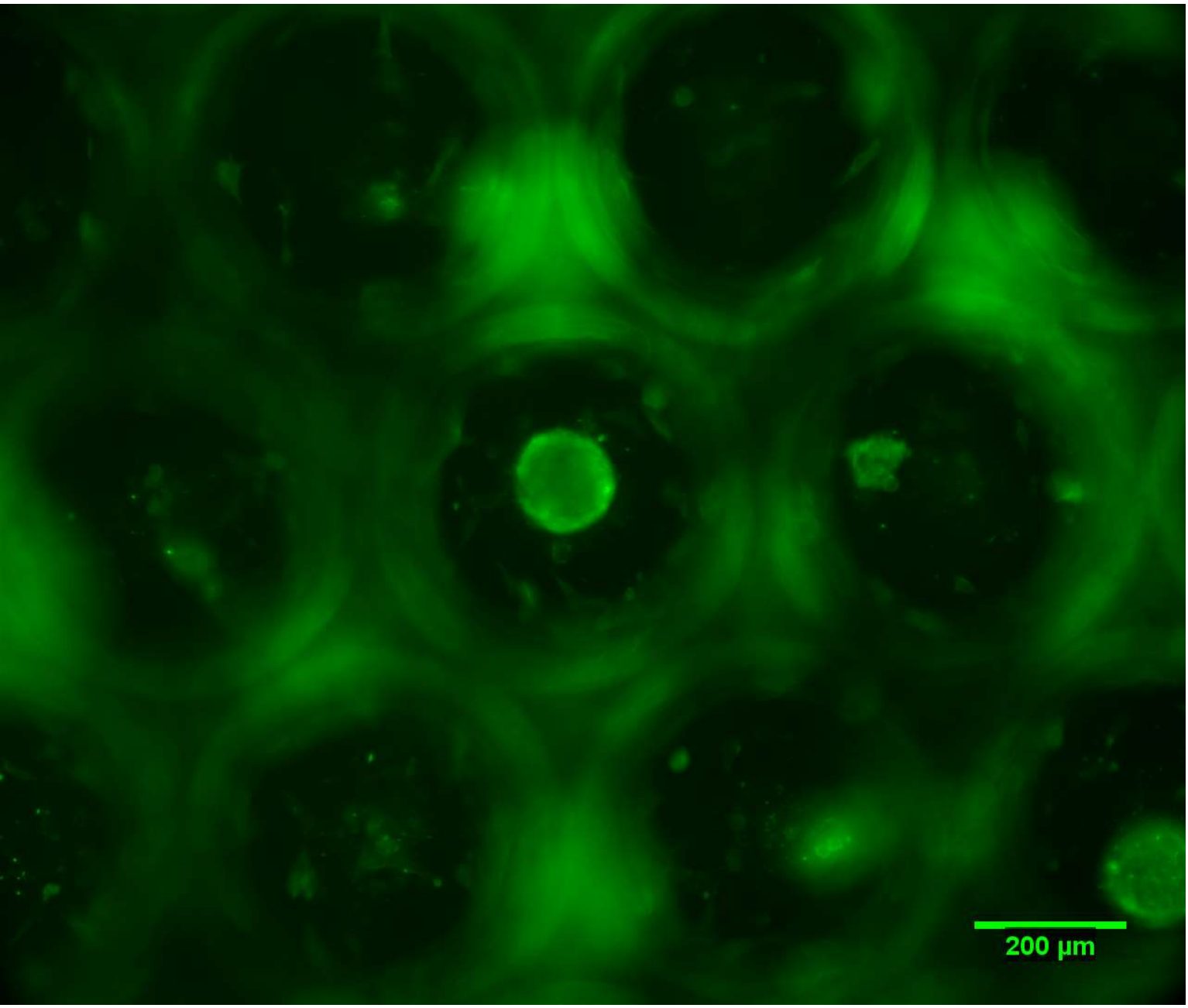
ACKNOWLEDGMENTS:

The authors thank Interreg Vlaanderen-Nederland for financial support.

Picture 1: Caption 1: MG-63 aggregates stained with phalloidin



Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

874 3D printing electroactive biomaterial based on composites of Polycaprolactone with Thermally Reduced Graphene Oxide for antibacterial applications in tissue engineering

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[2]Department of Pathology, Oral Microbiology Laboratory, Chile

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[4]School of Materials, The University of Manchester, MANCHESTER, United Kingdom

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INTRODUCTION:

Electroactive biomaterials allow electrical stimulation to all types of cells [1,2,3,4]. Several studies have shown the effects of direct current (DC) on cell proliferation and differentiation which accelerate the process of regeneration of muscles, organs and bones. In addition, DC has a bactericidal effect on biomaterials [5,6,7,8]. In this research, several scaffolds obtained by 3D printing that allow electric conduction are presented. The electroactive scaffolds (PCL/TrGO) obtained bactericidal and cytocompatibility characteristics under DC conditions

METHODS:

Graphene Oxide (GO) was synthesized by the Hummers & Offeman method and then a thermal reduction in an inert atmosphere at 700 and 1000 °C was used to obtain TrGO. Using melt mixing technique, electrically conductive PCL/TrGO films were obtained after the scaffolds were fabricated with a 3D-Bioplotter (Developer-Series-EnvisionTEC). Material characterization was carried out by means of XRD, XPS, TEM, RAMAN and TGA. Studies of antibacterial properties of scaffolds were performed using bacterial biofilms under DC. The cytotoxicity of PCL/TrGO was evaluated by Resazurin assay with stem cells derived from human bone marrow under DC.

RESULTS AND DISCUSSION:

The spectra high-resolution XPS of the GO and TrGO samples evidence that the epoxy and carbonyl functional groups of the GO with a portion of oxygen species of 53%. The spectra of TrGO samples resulted that most of the chemically attached functional groups were removed by thermal reduction and the portion of C Sp2 increased from ~46% (GO) to ~55% (TrGO-700) and ~81% (TrGO-1000), which is favourable for electric conduction in the composites as was demonstrated by electrical resistance measurements ~0,4 MΩ obtained with an insulation multimeter (U1461-Keysight).

CONCLUSION:

Electroactive biomaterials PCL/TrGO were synthesized and used to fabricate scaffolds by 3D-Bioplotting technology. Thermal reduction of GO allowed increasing the fraction of C Sp2 leading to conductive composites with cytocompatibility and bactericidal effects against *S. aureus*. These scaffolds can be used in electric stimulation for differentiation and proliferation of stem cells and tissue engineering

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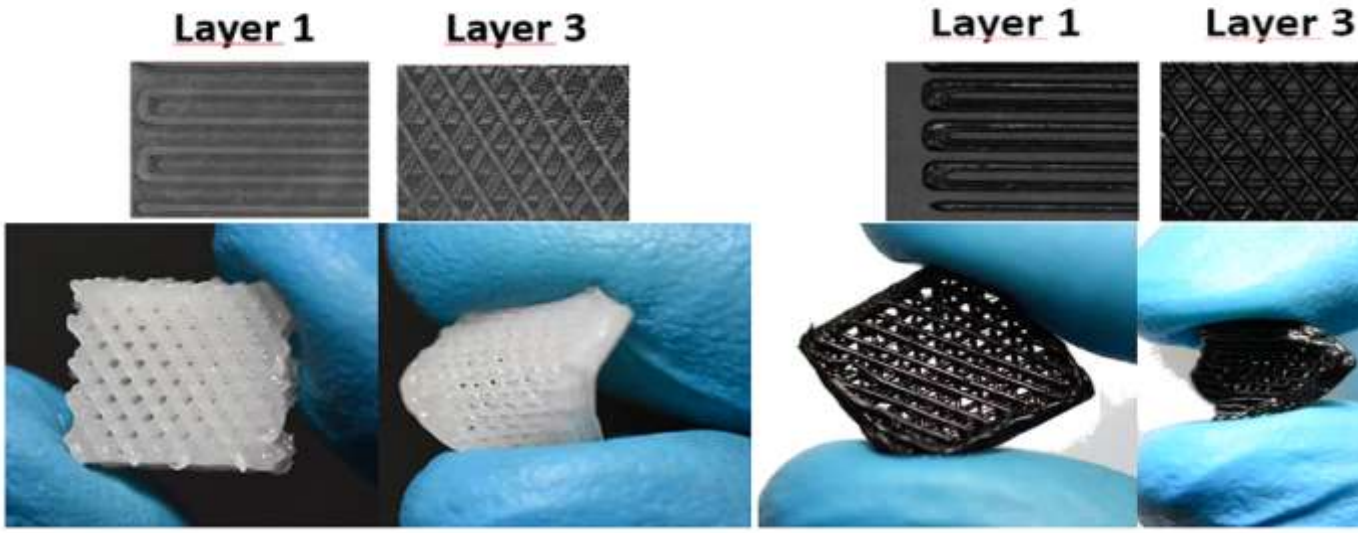
ACKNOWLEDGMENTS:

The authors gratefully acknowledge CONICYT for the doctoral fellowship 21150921 and project FODECYT 1150130.

Picture 1:



Caption 1: Photographs of PCL scaffolds (left) and PCL/TrGO scaffolds (right)

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Poster presentation

875 Effect of poly(glycerol succinate) addition on properties of PLA electrospun fibres

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[2]Faculty of Chemistry, Warsaw University of Technology, WARSAW, Poland

INTRODUCTION:

Polylactic acid (PLA) is a biobased polymer, produced from renewable resources [1]. High tensile strength, biodegradability and non-toxicity of PLA, allows it to be used in medical applications. However, the problem of pure PLA is high brittleness. Therefore it is copolymerized or blended to improve elasticity, e.g: with polycaprolactone [2]. “Green” substitute for currently used components could be poly(glycerol) succinate (PGS), which is synthesized via polycondensation from bio-sourced monomers - glycerol and succinic acid [1]. It was shown that PGS and its modifications with maleic anhydride are effective enhancers elasticity of extruded PLA [1,3].

For the first time we show electrospun blends of PLA and PGS. Morphology, structure and mechanical properties of PLA/PGS electrospun fibers were evaluated.

METHODS:

PLA/PGS fibers with different PGS gel content (2-40 wt%) were obtained via electrospinning.Morphology was characterized using scanning electron microscope (JSM-6010PLUS/LV InTouchScope™Jeol). Structure and thermal properties were evaluated by X-ray diffraction (XRD, D8 Discover, Bruker) and differential scanning calorimetry (DSC, Pyris-1, Perkin-Elmer). Mechanical properties of 10x50 mm samples were measured by uniaxial testing machine (Lloyd EZ-50).

RESULTS AND DISCUSSION:

Addition of PGS did not affect fibers morphology significantly. Even in the case of 40 wt% content of PGS fibers are free of beads and uniform. XRD results indicated amorphous nature of obtained materials. Temperature of cold crystallization increased with the PGS addition, which can be caused by lower mobility of PLA chains in blends. Elongation at break was raised from 100% for pure PLA to 200% and 240% for PLA with 10% and 20% of PGS, respectively. Interestingly, also enhancement of stress at break and Young modulus was observed for sample with the highest amount of PGS.

As it was discussed in the literature, the phenomenon of enhancement of PLA elasticity could be related to molecular bonding interactions and entanglement between PLA and PGS chains [1].

CONCLUSION:

It was shown that it is feasible to obtain uniform electrospun PLA/PGS fibers. Investigations confirmed that PGS is effective enhancer of PLA elasticity, without changing amorphous nature of PLA. Due to suitable biomimetic structure of PLA/PGS electrospun fibers, the next step will be investigation of cytotoxicity.

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Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

876 Agarose based nanocomposites for pancreatic tissue engineering

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BITS-Pilani, KK Birla Goa campus, GOA, India

INTRODUCTION:

With a global prevalence of 422 million people, diabetes is the eighth leading cause of death among both the sexes. As the current available treatment options have serious implications, research have focussed on new possibilities including tissue engineering and stem cell therapy. In the past few decades, mesenchymal stem cells (MSCs) have shown promising results for treatment of various diseases. Tissue engineering has also shown great advancements in construction of bio-artificial organs.

METHODS:

The current study demonstrates the differentiation of mesenchymal stem cells into insulin producing cells using pre-constructed agarose based nanocomposites (agarose and chitosan coated silver nanoparticle based scaffold, AG-CHNp). Furthermore, these scaffolds were also tested for bio-compatiblity against mice pancreatic cells. AG-CHNp scaffolds were synthesized using freeze drying technique with glutaraldehyde as cross linker and were characterized for various physical, chemical, mechanical and biological parameters. Primary cells isolated from human umbilical cord were seeded onto the scaffolds and were supplemented with differentiation media. After differentiation, the presence of insulin producing cells were confirmed using gene expression profiles for pancreatic markers.

RESULTS AND DISCUSSION:

The scaffolds were found to be bio-compatible with cell lines and primary cells and showed sustained growth of the cells for prolonged periods which was confirmed by MTT reduction and microscopic analysis using SEM and DAPI staining. Primary cells from umbilical cord were positive for CD-73, CD-105 and CD-90 and negative for CD-45 and CD-34 confirming the presence of MSCs. Differentiated cells were characterized by reverse transcriptase PCR, western blotting and Immunofluorescence. They showed positive results for pancreatic markers.Lastly, mice pancreatic cells also showed sustained growth for a period of 30 days.

CONCLUSION:

The present study highlights the use of stem cells and AG-CHNp scaffolds as a prospective candidate for tissue engineering of pancreas.

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Khorsandi L, Nejad-Dehbashi F, Ahangarpour A, Hashemitabar M. Three-dimensional differentiation of bone marrow-derived mesenchymal stem cells into insulin-producing cells. *Tissue & Cell* 47(1):66-72. DOI: 10.1016/j.tice.2014.11.005.

ACKNOWLEDGMENTS:

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Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

877 Cryogel system development for study and treatment of Parkinson's disease

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University of Geneva, GENEVA, Switzerland

INTRODUCTION:

There is a vast potential in using porous cryogel scaffolds in study and treatment of neural diseases, such as Parkinson's disease or stroke. Cryogels are a relatively new form of culture system that combines the three dimensions, provided by the classical hydrogels, the permeability necessary for media and nutrient exchange, as well as porosity. They allow minimally invasive delivery of cryogel-based microparticles (1, 2). Previous studies showed the possibility for short-term proliferation of a neuronal cell line (2). However, proper culture conditions and analysis tools need to be developped in order for such system to be attractive for cell transplantation studies.

Our aim with this study was to develop a carboxymethyl cellulose-based 3D culturing system, which, on a large (milimeter) scale, would be a useful tool for neuronal culture and characterization, while on a smaller (micrometer) scale, could act as a microcarrier system for neuronal replacement therapy for Parkinson's disease.

METHODS:

Carboxymethyl cellulose (CMC) cryogels were produced using carbodiimide-based crosslinking (2).

Coating molecules were covalently bound to the cryogels using carbodiimide chemistry and labeled by rhodamine isothiocyanate.

Luhmes cell were seeded onto the scaffolds and differentiated according to the published protocol (3).

RESULTS AND DISCUSSION:

Four adhesion molecules (collagen IV, fibronectin and laminin), as well as a coating mix, Matrigel, were compared for induction of neurite spread along the biomaterial walls. Among the one-component coatings, laminin has shown the most neurite adhesion. Best coating conditions (pH and EDC content) were adjusted for more efficient laminin adhesion.

Similar differentiation pattern was observed on 3D cryogels as in 2D for the Luhmes cell line.

In further steps, post-transplantational survival and integration of mature neurons differentiated on CMC cryogels will be evaluated in the murine brain.

CONCLUSION:

Cryogel coating has been developped with regard to future clinical trials. The 3D cryogel culture system has been proven successful for long-term culture and differentiation of neural precursor cells. Three dimentional neurite extension of dopaminergic fibers was obtained. In vivo evaluation of cell survival on the micron-scale cryogel carriers will be evaluated next.

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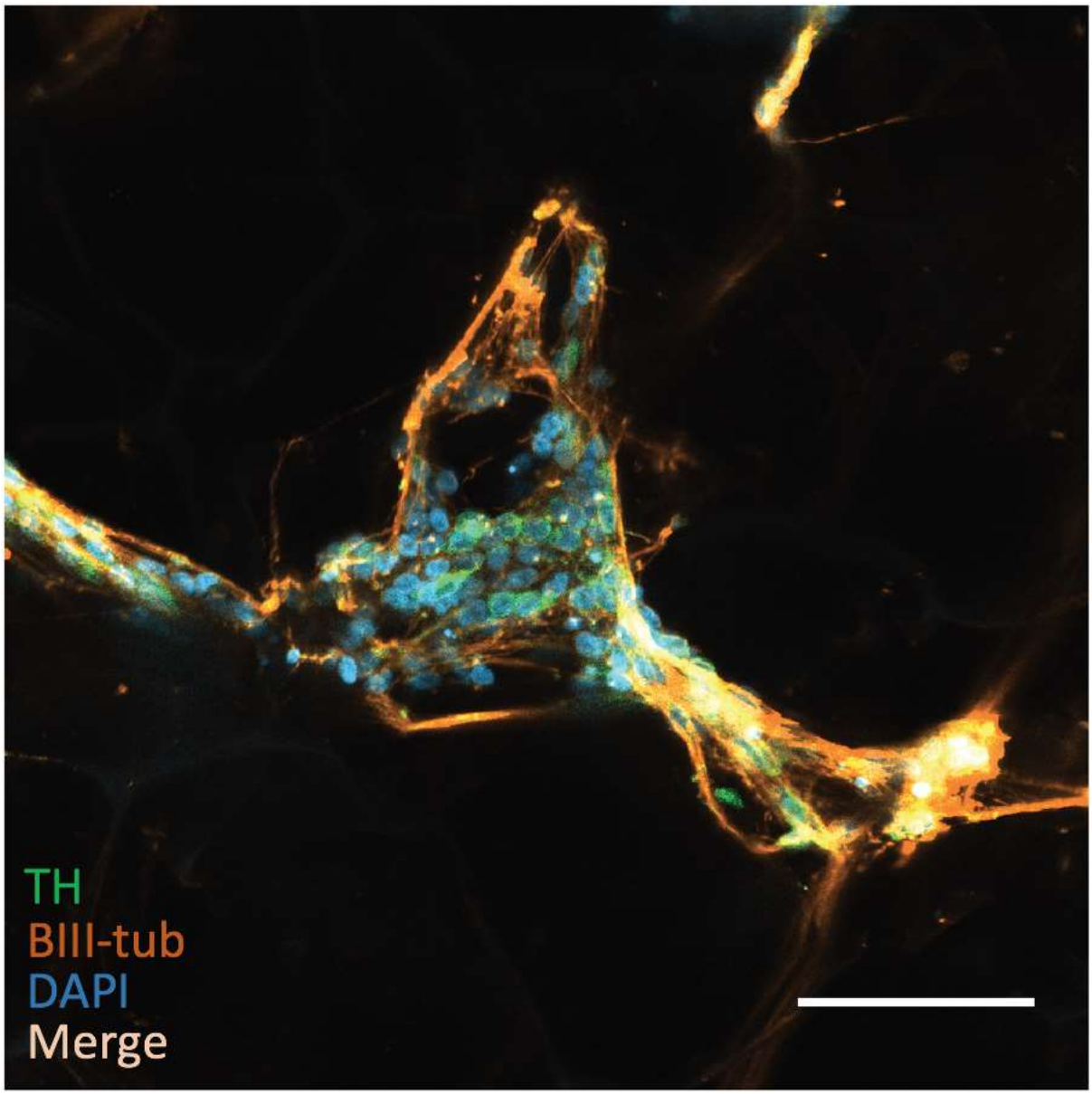
Picture 1:



Caption 1: Luhmes cells, differentiation day 7

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

878 Myoblast Alignment Enhance Electrical Conductivity of Polypyrrole-Incorporated Hydrogel Microfibers

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[2]TOBB University of Economics and Technology, ANKARA, Turkey

INTRODUCTION:

Engineering functional contractile skeletal muscle is a global clinical need. Biomimetic strategies to recapitulate the anisotropic, conductive and dynamic nature of the tissue is of utmost importance[1]. In addition, electrical stimulation is required in the development of contractile function of muscle cells to mimic the signals generated by the nerves[2]. In this study, we investigated the effects of myoblast alignment and electrical stimulation on the conductivity and functionality of aligned polypyrrole (PPy)-incorporated alginate/fibrin (AF) microfibers.

METHODS:

C2C12 mouse myoblasts were encapsulated at 2000 cells/µl within wet spun AF microfibers with and without PPy. Fibers were aligned by wrapping around frames to obtain constructs of 1 mm diameter. Acellular microfibers were also produced as a control group. Electrical stimulation was applied with parameters: Frequency: 1 kHz, Amplitude: 1 V/m, L: 200µm, 10 min/day between days 1 and 7 using a Keysight waveform generator. Cell proliferation, viability and the variation in electrical conductivity were assessed. Electrical conductivity was measured according to Ohm's law through an electrical circuit where 4 Vpp sinusoidal signals were supplied. Data was recorded using an oscilloscope at 10 kHz to analyze the capacitive effects, and were compared to the conductivity of natural tissue excised from rabbit (Figure 1a).

RESULTS AND DISCUSSION:

Myoblasts proliferated well within AF and AF:PPy fibers, where the number of viable cells were higher on electrically stimulated samples at day 7. Cells grow randomly within AF fibers while they compacted, aligned and fused more within AF:PPy fibers especially when electrically stimulated (Figure 1c). The conductivity of the acellular microfibers was lower than natural skeletal muscle at day 1, while the values increase in culture for 7 days (Figure 1b). The conductivity of the cell-laden samples were significantly higher than natural skeletal muscle at days 1 and 7. PPy contributed significantly to the conductivity of hydrogel microfibers.

CONCLUSION:

The electrical conductivity of PPy-incorporated hydrogel fibers enhance with myoblast growth within them during culture. Electrical stimulation enhanced myoblast fusion indicating better functionality.

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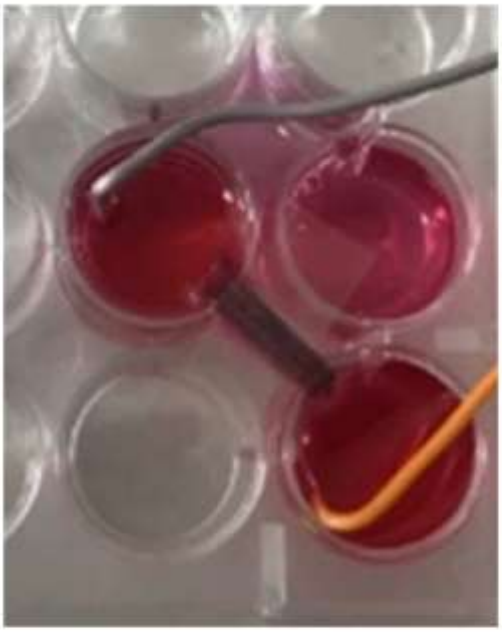
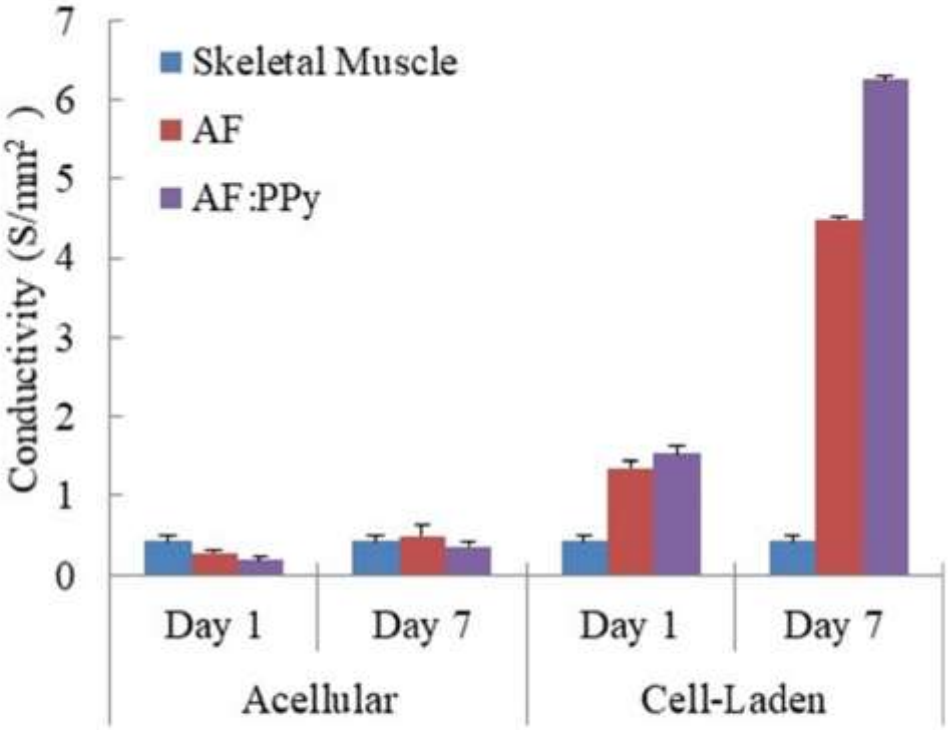
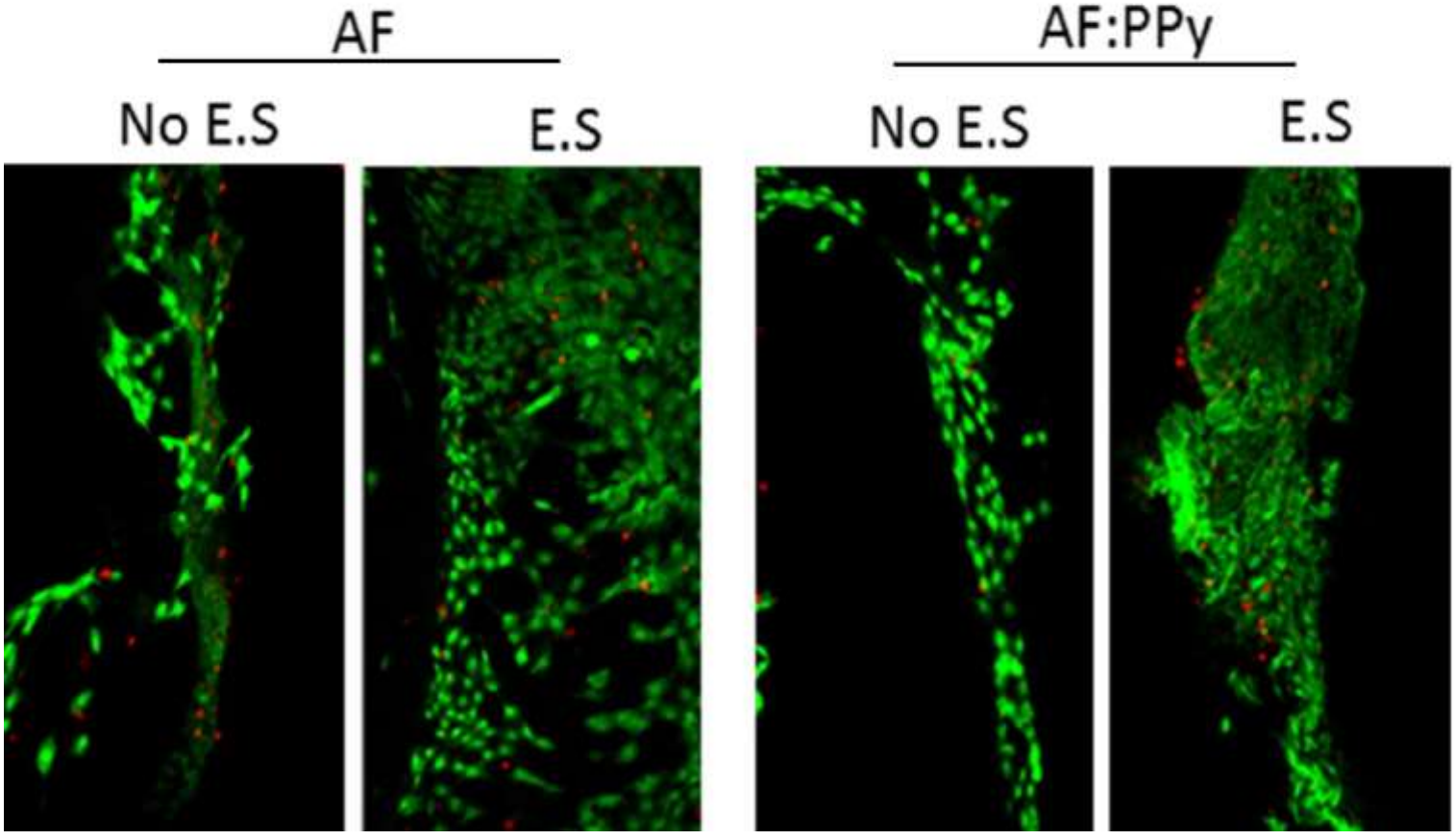
ACKNOWLEDGMENTS:

The authors would like to thank Turkish Academy of Sciences (Grant no: TUBA-GEBIP 2016) for providing financial support to this project.

Picture 1: Caption 1: Figure 1. a) Conductivity measurement setup. b) Electrical conductivity of native tissue and acellular/cell-laden AF and AF:PPy microfibers on Days 1







Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

879 Safety and performance of polydioxanone medical devices

Joana Martins, Hayley Morris, Antonina Lach, Pierre-Alexis Mouthuy, Andrew Carr

Botnar Research Centre, OXFORD, United Kingdom

INTRODUCTION:

Medical device failure can lead to device recall and patient harm. Wherever possible, the risk to the end user must be minimised. It is necessary to assess the biological effects of an implantable biomaterial before its use.

Polydioxanone (PDO) is a synthetic polymer commonly used in medical devices.[1] PDO is nonantigenic and is found to induce minimal tissue reaction during absorption. In this paper we review safety and performance outcomes of PDO medical devices in patients. With this work we can ascertain PDO suitability for the development of new medical devices within our research group.

METHODS:

PDO medical devices were searched in the U.S. Food and Drug Administration (FDA)'s 510(k) and premarket approval databases. Additionally, we searched through two clinical trials platforms and four research databases to find published papers reporting the use of PDO in humans.

Based on a scoring system, PDO devices were evaluated and compared to non-PDO devices. This assessment was based on clinical outcomes such as surgical-site infection rates, foreign body reaction, and device performance.

RESULTS AND DISCUSSION:

43 PDO submissions were reported from the 510(k) and the premarket approval databases. 12 Clinical trials with published results and 32 papers were found, including 8 case reports. More than 20 devices approved by the FDA do not have available clinical results from their implantation in patients despite the increase of the number of PDO devices since 1981 (Figure 1). However, scientific papers reporting their application in humans only started being published ten years later. Since then, this number has been increasing, revealing a higher interest for this polymer and its performance.

Analysis based on the scoring system showed that 6 papers reported that PDO devices had less negative outcomes than non-PDO devices, 9 papers reported similar outcomes and 1 paper noted that the PDO device implanted had more negative outcomes than the comparative device. When analysing case reports or papers that assessed only PDO devices, general results showed good performance and low rates of negative outcomes, with the exception of PDO clips that had the lowest score.

CONCLUSION:

There is an increasing number of PDO medical devices and these were found to be clinically safe. PDO is a safe polymer to use in future medical devices.

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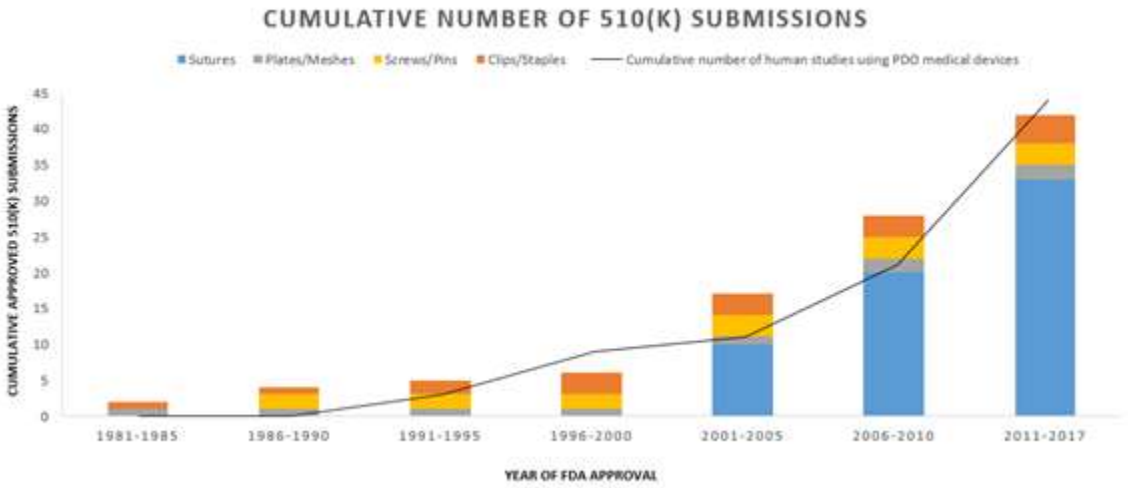
We would like to thank Wellcome Trust and National Institute for Health Research for supporting this work.

Picture 1:



Caption 1: Figure 1. Evolution of approved PDO medical devices and number of human studies with PDO implants through time.

Poster presentation session C 11:15 - 12:15 12/09/2018

Poster presentation

880 Low pressure cold spray: a novel approach for obtaining bioactive coatings of hydroxyapatite using design of experiments

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INTRODUCTION:

Recently, the bonding mechanisms during coating formation by Cold spray (CS) techniques have attracted scientific interest due to the versatility for coating deposition at low working temperatures, as an alternative to avoid the material decomposition [1,2]. Within the CS techniques, Low Pressure CS (LPCS) is a novel and low-cost methodology for deposition of ductile materials by the mechanism of mechanical interlocking. However, it has been already demonstrated, that an adequate combination of variables allows the deposition of synthetic hydroxyapatite (HAp) despite its low ductility [3, 4]. The aim of this work is to study the feasibility of using LPCS to deposit well bonded coatings of bovine-derived-HAp (B-HAp) powder by applying design of experiments (DoE) to optimize the bonding mechanism and understand the correlation between process parameters and coating formation.

METHODS:

Coatings were obtained with a LPCS equipment (DYMET-423). Pressure, stand-off distance and traverse velocity were chosen as inputs for a 2^3 factorial design. Particle morphology, trace width and covered area after 1 pass (quantified with Image-J®) and coating adhesion by pull-out testing were statistically analyzed and simultaneous optimized using MiniTab®. Coatings bioactivity was assessed with the morphological and structural changes, before

and after different immersion times in Hank´s solution. Lattice parameters, Ca/P ratio and crystallite size were calculated by Rietveld refinements of the XRD patterns. Coatings stability was measured by quantifying degradation products.

RESULTS AND DISCUSSION:

The study of the first particles deposited showed irregular agglomerates with small surrounding fragments possibly associated with the rebound of some fractured particles. Feedstock powder and coatings were identified with a single crystalline HAp phase (JCPDS 9-432). Morphological and structural variations on coatings surface observed during the bioactivity test provided clear evidence of bone-like apatite layer formation. Finally, coatings´ degradation products were related to deposition parameters.

CONCLUSION:

The adequate combination of parameters in LPCS leads to deposition of well bonded, bioactive and stable B-HAp coatings with a high cost/benefit ratio. Coatings properties and deposition efficiency show LPCS as a promising technique for biomedical implants coatings.

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Poster presentation

884 Design and preparation of Chitosan Nanoparticles - GelMA Hydrogel formulation with antimicrobial properties for 3D Printing technologies

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INTRODUCTION:

More than 4 million European patients acquire nosocomial infections every year. At least half of these infections are related to medical device use, such as medical implants and catheters. Around 80% of all these infections are related to microbial biofilms. A strategy to overcome this problem is to modify the surface of the devices via deposition of microbicidal or anti-adhesive molecules. However, one of the limitations of these methods is the

difficulty in controlling the bacterial growth on the device as biofilm, as well as in the tissue surrounding the implant, which are the major causes of infection associated with inserted implant devices.

RESULTS AND DISCUSSION:

We propose a novel approach, the usage of high-precision 3D printing technology as a fabrication method of bactericidal coatings for medical devices. Our main goals were to design, develop and characterize new formulations for 3D printing technologies. Two different formulations based on two different antibiotics encapsulated in chitosan nanoparticles-gelatin methacrylate hydrogel were developed. The drug release profile and degradation process were studied. The physico-chemical properties of chitosan nanoparticles were characterized by Scanning Electron Microscopy, Dynamic Light Scattering and Fourier-Transform Infrared Spectroscopy. Furthermore, the antimicrobial activity of the formulation was analysed against two different *Staphylococcus aureus* strains, one resistant to antibiotic 1 and the other resistant to antibiotic 2.

CONCLUSION:

We developed a new formulation based on chitosan nanoparticles - GelMA hydrogel printed by using 3D-Bioplotter (Envisiontec). The samples were printed layer by layer, alternating the formulation in each layer. The result was a hybrid antibacterial nanoparticle-hydrogel structure loaded with two different antibiotics. The structure showed a sustained released of both antibiotics, being effective against both antibiotic resistant *Staphylococcus aureus* strains.

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Design and preparation of Chitosan Nanoparticles - GelMA Hydrogel formulation with antimicrobial properties for 3D Printing technologies

Picture 1:

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Poster presentation

886 Synthesis of antibacterial hydroxyapatite by a simple sol-gel method

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INTRODUCTION:

Hydroxyapatite (HA) is known for its *in vivo* osteoconductive and osteoinductive properties [1,2]. However, the physico-chemical characteristics that support osteoblast attachment and differentiation onto HA surfaces are also responsible for its proneness to bacterial colonization [3,4]. This study aims to take advantage of the antibacterial properties of silver and zinc ions to synthesize HA powders immune to colonization by Gram- and Gram+ microorganisms [5].

METHODS:

Samples of HA co-doped with Ag^{+} and Zn^{2+} were synthesized by a simple sol-gel method: M1 (2 mol% Ag and 0.5 mol% Zn), M2 (1.5 mol% Ag and 1 mol% Zn) and M3 (1.3 mol% Ag and 1.3 mol% Zn). Calcium nitrate, phosphorus pentoxide, silver nitrate and zinc nitrate hexahydrate were used as precursors. The synthesized powders, after sinterization, were analyzed for their structure (XRD), chemical composition (FTIR), cytotoxicity and antibacterial activity.

RESULTS AND DISCUSSION:

XRD analysis showed that the addition of the dopants does not change the crystalline structure of hydroxyapatite. Small changes of the degree of crystallinity and lattice parameters *(a, c)* were observed in doped samples, resultant from the ion insertion in the apatitic structure. FTIR spectra evidenced the presence of characteristic bands of phosphate and hydroxil groups, representative of an apatitic phase.

Cell culture studies performed on all samples, using Vero cells, showed that cell viability was higher than 95% revealing that the presence of dopants doesn't induce citotoxicity.

As demonstrated in figure 1, antibacterial assays revealed a synergistic effect between Ag^{+} and Zn^{2+} ions, resulting in antibacterial activity both for Gram- and Gram+ microorganisms.

CONCLUSION:

HA co-doped with Ag^{+} and Zn^{2+} ions was synthesized by a simple sol-gel method. The co-doped powders revealed antibacterial properties for both Gram+ and Gram- microorganisms.

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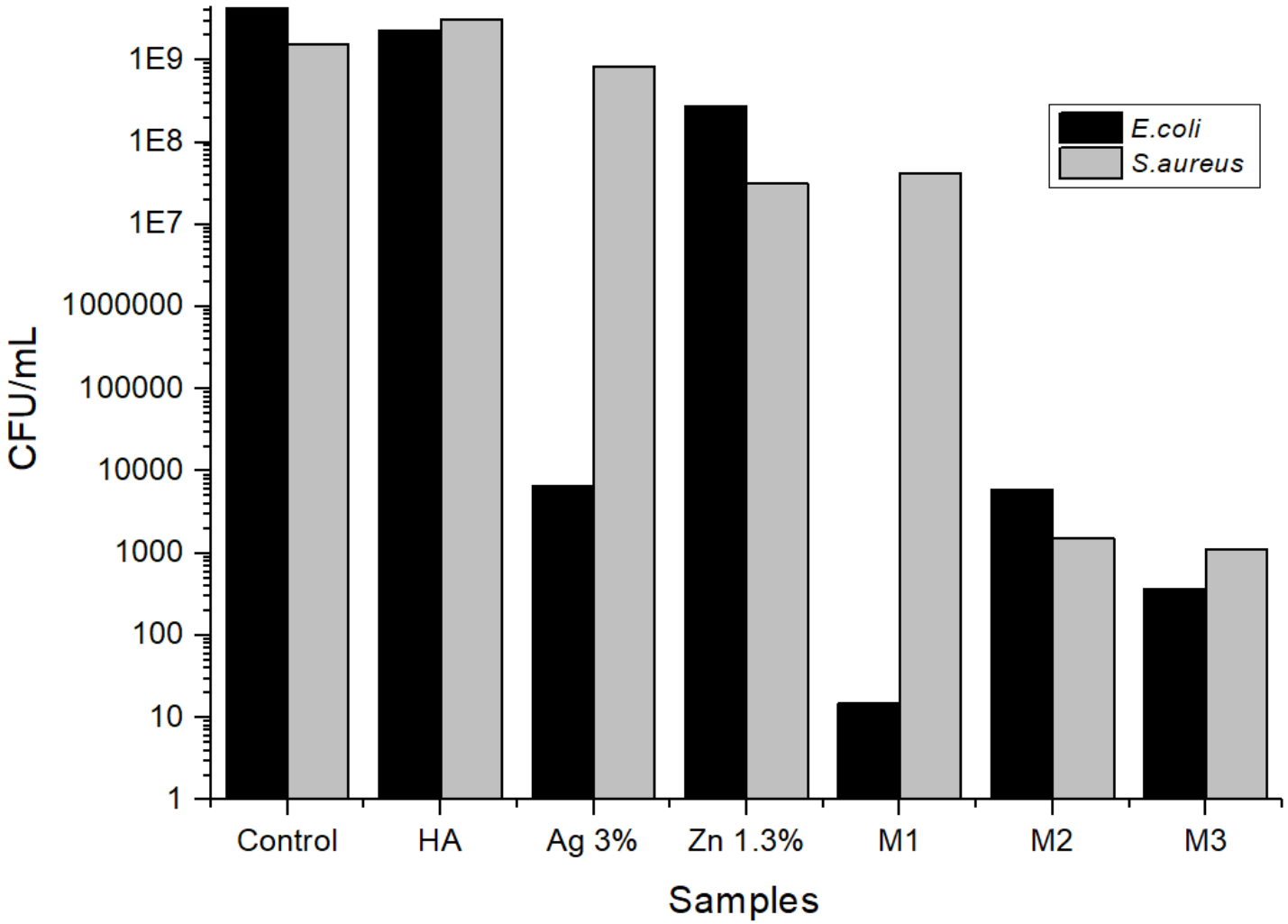
Picture 1: Caption 1: Antibacterial activity assays of synthesized powders.



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Poster presentation

888 CU/Se-modified PVC/PU for nitric oxide generation

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INTRODUCTION:

The most commonly used polymers for blood-contacting applications are poly(vinyl chloride) (PVC), polyurethane (PU), poly(tetrafluoroethylene). The foremost important requirement for these polymers is to acquire the anticlotting activity because a surface-induced thrombosis is the main complication which associated with medical device failure. It was discovered that nitric oxide (NO) inhibits platelet activation/aggregation[1]. Another authors also reported

that polymers with immobilized catalyst such as transition metals (iron, copper) and selenium compounds decompose S-nitrosothiols (GSNO) with NO release[2]. In this work we modified PVC and PU with Cu and Se species to improve thromboresistance of these polymers.

METHODS:

PVC and PU surfaces were modified with polydopamine (PDA) and poly(norepinephrine) (PNE). Selenium species were immobilized onto PDA/PNE functionalized PVC/PU polymers via EDC/NHS coupling. Cu-loaded PVC/PU samples obtained using redox and metal ions chelating activity of PDA/PNE coating. The chemical composition of coating was analysed using XPS and FT-IR Spectroscopy. The amount of Se/Copper attached to the surface were measured using an ICP-OES. NO release was analyzed using ion-selective microelectrodes (Lazar Res. Lab., USA). Platelet adhesion on samples was observed by SEM.

RESULTS AND DISCUSSION:

The attachment of Cu/Se species to the surface of polymers and their amount was measured by FTIR spectroscopy, XPS, ICP-OES. The Cu content for samples varies from 16-27 nmole/cm^2, whereas and selenium content lies in the range from 0.037-0.67 µmole/cm^2. Amount of NO generated by Cu/Se-modified polymers after their incubation in 100 µM GSNO/GSH for 1h was in the wide nanomolar range from 0.07 to 0.5 nanomole/cm2. The Cu-PNE samples showed the lowest generation ability in comparison to Cu-PDA samples. It was reported before that 13 nmole/cm^2 of Cu content[1] and 0.036 µmole/cm^2 of Se content is sufficient to produce physiological relevant level of NO. The amount of NO generated by our modified polymers get into this range.

CONCLUSION:

PVC and PU were successfully modified with Cu/Se via PDA/PNE. The measured amount of Se and Cu species on the surface of modified polymers was in a wide range of concentration which depends on the immobilization method and what allow us to regulate the amount of NO produced during incubation at physiological conditions.

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Poster presentation

889 Acellular and solubilized fetal membranes: a natural source of bioactive factors for regeneration

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INTRODUCTION:

The human fetal membranes (FM) are composed of amnion and chorion, and have been extensively studied for they appeal in tissue engineering (TE), due particularly to their high levels of bioactive factors[1]. However, the big challenge concerning its use remains on the decellularization of the chorion without loosing its physical integrity[2]. Thus, this work aimed to develop and optimize a decellularization protocol for FM and to physical and chemical characterize the resulting chorionic and amniotic membranes.

METHODS:

A total of 8 eligible parturient agreed to participate in the study by signing an informed consent and the study was approved by Ethic Comittee. Decellularization was optimized based on the combination of Triton-X and DMSO reagents. Solubilization was performed through pepsin digestion. Histological evaluation, and immunohistochemical analysis were used to confirm the absence of nuclei and to assess dECM integrity. Membranes were chemically characterized after and before decellularization by Confocal Raman microscopy. Soluble bioative factors were quantified using a human cytokine antibody array kit. Native membranes were used as controls.

RESULTS AND DISCUSSION:

The absence of nuclei in both chorionic and amniotic membranes confirmed the effectiveness of the decellularization procedure. It was observed that dECM kept its structural integrity when compared to the native ECM, presenting a very similar morphology. The Raman spectra of the membranes before and after decellularization revealed similarities in peaks, intensities and shapes being the prominent Raman bands associated with collagen. Moreover, glycosaminoglycans, which are present in native FM, were still found in FM dECM. Angiogenic regenerative and immune modulating factors were detected on the solubilized FM dECM.

CONCLUSION:

The developed protocol was able to successfully decellularized both chorion and amnion, while maintaining their structure and matrix composition. The use of the same protocol for decellularization of both membranes unravels a new way of FM engineering.

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Poster presentation

890 Development of a hydrogel flowable dressing for the prevention of corneal scarring

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INTRODUCTION:

Blindness can result from corneal scarring and vascularisation that develops after infectious diseases, inflammation and corneal trauma. The 'gold standard' of treatment is amniotic membrane (AM) transplant to the ocular surface that induces the healing process and reduces scarring, through the release of anti-fibrotic/anti-inflammatory factors. However, the reproducibility and repeatability of the clinical effects of AM transplants is limited by biological variability of the harvested membranes. Consequently, a plethora of alternative anti-scarring treatment strategies have been investigated, with nothing yet adopted to replace AM transplants. We are developing a transparent gel dressing that can be applied as an eye drop to the damaged ocular surface, which is capable of protecting the injured eye, providing sufficient lubrication as well as delivering the anti-scarring agent, Decorin, in a sustained manner to the cornea.

METHODS:

Gel dressings were produced by heating the hydrocolloid to melting point with controlled temperature processing to form a fluid gel. *In vitro* Decorin release from the fluid gel was measured using a Decorin ELISA. For the *ex vivo* studies, the Decorin fluid gel was applied as a single dose to a rat corneal abrasion model to observe the effects on wound healing and re-epithelialisation using fluorescein. An *in vivo* mouse model of bacterial keratitis was also used to assess the anti-scarring effects of the Decorin fluid gel when used in combination with the standard clinical treatments of gentamicin and steroid eye drops.

RESULTS AND DISCUSSION:

Thickening of the gel occurred immediately *in vivo* when placed on the rat cornea as an eye drop. Decorin gels released a sustained dose of Decorin over 4h (0.13mg/ml/4hr). Complete corneal re-epithelialisation occurred within 48h in the presence of the Decorin gel in the *ex vivo* models when compared to a Decorin-PBS drop which showed limited wound closure over the same time. Additionally, Decorin gel reduced corneal opacity compared to controls in the bacterial keratitis model when administered together with the gold standard steroid and gentamicin treatment, compared with steroid and gentamicin treatment alone.

CONCLUSION:

We have successfully demonstrated the utility of a fluid gel 'eye drop' therapy for the attenuation of corneal scarring. The properties of the fluid gel allowed a sustained, effective dose of anti-fibrotic Decorin to be released onto the corneal surface over 4h after application and showed efficacy in reducing the corneal opacity associated with bacterial keratitis.