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Review

Cellulose Nanofibers and Other Biopolymers for Biomedical Applications. A Review

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Abstract: Biopolymers are materials synthesised or derived from natural sources, such as plants, animals, microorganisms or any other living organism. The use of these polymers has grown significantly in recent years as industry shifts away from unsustainable fossil fuel resources and looks towards a softer and more sustainable environmental approach. This review article covers the main classes of biopolymers: Polysaccharides, proteins, microbial-derived and lignin. In addition, an overview of the leading biomedical applications of biopolymers is also provided, which includes tissue engineering, medical implants, wound dressings, and the delivery of bioactive molecules. The future clinical applications of biopolymers are vast, due to their inherent biocompatibility, biodegradability and low immunogenicity. All properties which their synthetic counterparts do not share.

Keywords: biopolymers; cellulose nanofibers; polysaccharides; proteins; tissue engineering; medical implants; wound healing; biocompatibility

1. Introduction

Due to the pressures of a growing population and rapid growth in materials research, manufacturing demands have resulted in unsustainable use of petrochemical resources which in turn have contributed greatly to global pollution and climate change. In today's more eco-conscious society, environmentally friendly biopolymers present a sustainable alternative to fossil-based resources. In this regard, cellulose is the main component of lignocellulosic biomass and is the most abundant biopolymer on earth [1]. Cellulose is a β(1-4)-linked chain of glucose molecules, accounting for around 50% of the carbon content in the lignocellulosic biomass [2,3]. Therefore, this macromolecule, as a sustainable and renewable source, has garnered significant interest and encouraged researchers to develop cellulose-based materials with novel functions [4]. Nanomaterials derived from cellulose are referred to as nanocellulose. Nanocellulose can be mainly categorised into nanocrystalline cellulose (NCC), nanofibrillated cellulose (NFC), and bacterial nanocellulose (BNC). These types of nanocellulose, although very similar in composition, could present some differences in morphology, particle size or crystallinity, due to the different sources and extraction methods [5–7].

NCC is also known as cellulose nanocrystals (CNC) or cellulose nanowhiskers (CNW), and it is one of the most studied types of nanocellulose. Its preparation requires an acid hydrolysis process

to dissolve the amorphous regions of the cellulose chains and release the crystalline domains [8]. To prepare NCC, the cellulose fibers must undergo a purification process. In this process, the extraction with organic solvents is first carried out in order to eliminate the extractables from the biomass followed by an alkaline treatment with sodium hydroxide (NaOH) or potassium hydroxide (KOH) to purify the cellulose, and finally a bleaching process with sodium chlorite ($NaClO_2$). This process is key to ensure the effectiveness of acid hydrolysis and facilitate the attack on amorphous regions. The alkaline treatment aims to solubilise most of the pectin and hemicellulose, and the bleaching process, decompose the phenolic compounds or molecules with chromophore groups in the lignin, and eliminate the by-products of such decomposition [9]. Once the cellulose has been purified, acid hydrolysis is carried out using different acids. The most commonly used acid is sulphuric acid (H_2SO_4), due to the esterification process of the anionic sulphate ester groups ($-OSO_3^-$) with the hydroxyl groups on the surface of the cellulose, which induces the formation of a negative electrostatic layer on the surface of the nanocrystals, and facilitates their dispersion in water. During acid hydrolysis, the hydronium ions (H_3O^-) penetrate the cellulose chain by hydrolysing the glycosidic bonds of the amorphous regions releasing the individual crystalline regions [10]. The resulting product is dialysed against distilled water and then dispersed with a sonication stage. These nanocrystals have a short rod shape between 2–20 nm in diameter and 100–500 nm in length, being composed of 100% cellulose with a high crystallinity (54–88%) [6,11].

Cellulose nanofibers (CNF), also known as nanofibrillated cellulose, are a type of flexible, elongated, cross-linked nanocellulose that can be extracted from cellulose fibers by mechanical treatment [12]. In order to obtain cellulose nanofibers, it is necessary to subject the raw material to a purification process similar to that which is carried out to obtain CNC. NFC is generally produced by mechanical delamination of cellulose fibers after a previous treatment (pre-treatment) that facilitates disintegration. These pre-treatments avoid the occlusion of the equipment where the disintegration process takes place, and reduces the number of passes or treatment time, with the reduction of energy that this entails. CNF was first isolated in 1983 by Turbak et al. using bleached softwood fiber and subjected to a high-pressure homogenisation process [13]. However, the high energy consumption necessary for its production limited the scientific and industrial interest. In order to reduce energy consumption and make disintegration more efficient, different pretreatments, such as enzymatic hydrolysis [14], partial carboxymethylation [15], catalytic oxidation using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) [16], and mechanical refining [17] were employed. After pre-treatment, the fiber is subjected to a mechanical disintegration process, including high pressure, microfluidisation, friction grinding, extrusion, cryopressure and high intensity ultrasonication [18].

Bacterial nanocellulose (BNC) is another type of nanocellulose that has special features compared to the other types. While CNC and CNF are isolated from lignocellulosic biomass using top-down deconstruction methods of cellulose fiber, BNC is produced by a down-top process, which consists of the assembly of low molecular weight sugars by bacteria [19]. The main microorganism producing BNC is *Gluconacetobacter xylinus*; however, several species are able to synthesise this biopolymer. The production of nanocellulose by bacteria consists of three main stages: (i) Polymerisation of glucose residues in β-1-4 glucan, (ii) extracellular secretion of linear chains, and (iii) organisation and crystallisation of glucan chains through hydrogen bonds and van der Walls forces, arranged in a hierarchy in fibers [20]. Unlike the other types of nanocellulose mentioned above, BNC is produced in a pure way, without lignin, hemicellulose, pectin or any other component of the lignocellulosic matrix of biomass. Its high purity, mechanical resistance, insolubility, biodegradability and non-toxic properties make it an ideal starting material for the production of high value-added products in the biomedical field [21].

Therefore, cellulose materials possess properties of biodegradability and renewability. These materials also have low environmental, animal/human health, and safety risks [22]. Indeed, they have already been incorporated in the daily life for thousands of years, for instance, in the textile, food and pharmaceutical industries, among others [22,23]. In addition, nanomaterials derived from cellulose

possess high specific strength and modulus, high specific surface area, and the extensive ability for chemical modification [22]. Due to all the above, nanocellulose is an excellent candidate to be used in biomedical applications.

Biopolymers are polymers produced from natural sources either chemically synthesised from biological material or entirely biosynthesised by living organisms. These include: Polysaccharides, such as cellulose, hemicellulose, starch, or chitosan; protein animal-based polymers, such as silk and collagen; and lignin, a high-molecular weight biopolymer present in the cell walls of vascular plants, which has complex and variable structures. Lignin is formed by randomly cross-linked networks of methoxylated and hydroxylated phenylpropane [24–26]. 'Biopolymer' can be considered an umbrella term as it covers polymers which can be characterised as one or more of the following [27]:

- Biobased: Polymers composed entirely or in some part from biomass (natural sources).
- Biodegradable: Polymers capable of being composed by bacteria or other organisms.
- Biocompatible: Polymers which are compatible with human or animal tissue, i.e., does not harm the body whilst fulfilling its function.

This review will focus on common biopolymers and their biomedical applications. Naturally derived polymers possess huge potential for medical use due to their inherent biocompatibility, biodegradability, non-toxicity and ability to deliver bioactive molecules [28].

2. Nanocellulose and Other Cellulose Derivatives

Cellulose is the most abundant polymer on the planet—40% of all organic matter is cellulose with 75–100 billion [1] tonnes produced each year through photosynthesis (wood and cotton being the primary industrial sources) [29]. Cellulose was first discovered in 1838 and is the principle constituent of plant cell walls. Structurally, cellulose is a carbohydrate with similar chemical composition to starch $(C_6H_{10}O_5)_n$. Although the chemical composition is similar, and both are formed by glucose as a monomer with (1,4) glyosidic bonds, there is a difference in the type of bond, since the cellulose monomers are β-1,4 bonds, and the starch bonds are α-1,4. However, unlike starch, cellulose is insoluble in water solvents. Starch (formed by amylose and amylopectin) dissolves in water because of the α-1,4 glycosidic causes the formation of a helical structure that does not form inter-strand hydrogen bonds, producing easily-disruptable non-polar interactions between glucopyranose rings. In the case of the cellulose, this presents a structure that is tightly bound in a set of hydrogen bonds between individual chains, resulting in an inter-strand hydrogen bond (Figure 1A) that can easily expand and flex to allow the penetration of water, which together with its higher degree of polymerisation, mean that it is insoluble.

Currently, nanomaterials derived from cellulose have garnered significant interest, due to their excellent inherent and physical properties, such as high tensile strength and elastic modulus (130–150 GPa), high specific surface area (up to several hundreds of m^2/g), low density (1.6 g/cm^3), reactive surfaces combined with biodegradability and renewability [9]. Figure 1B–E shows different examples of the structure of nanocellulose obtained using transmission electron microscopy.

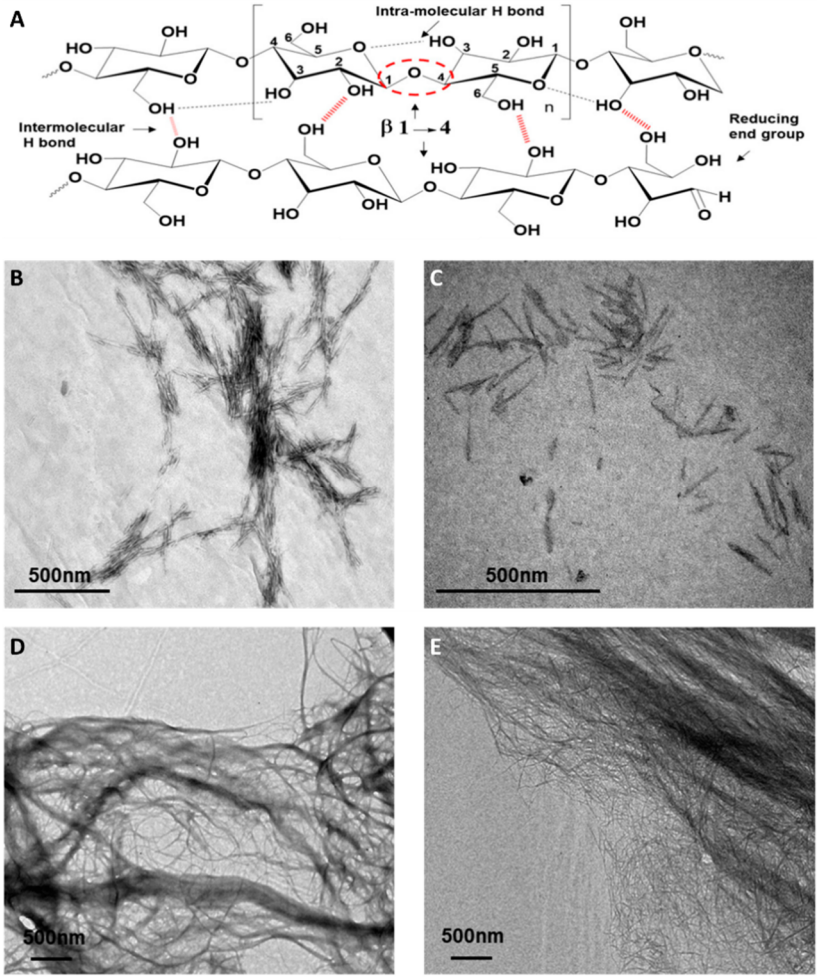

Figure 1. Cellulose structure and intra/intermolecular hydrogen bonding pattern (**A**). Morphological and structural characteristics of the unmodified and oxidised nanocellulose samples: Transmission electron micrographs of cellulose nanocrystals (**B**), oxidised cellulose nanocrystals (**C**), cellulose nanofibers (**D**) and oxidised cellulose nanofibers (**E**). Reprinted with permission from Tayeb et al. [30] and Siqueira et al. [31].

Nanocellulose has been widely used for biomedical applications, due to its attractive properties of biocompatibility, mechanical strength, chemical and morphological controllability [1,32]. CNC and CNF have been used in hydrogel preparation for several applications, such as wound dressing [1], drug delivery [33] and tissue engineering scaffolding [34]. Also, BNC is believed to be a promising and cost-efficient natural nanomaterial for biomedical applications, due to the high mechanical properties and its high purity and non-toxic properties. In this case, there are two major limitations to the use of BNC in biomedical uses. One of them is its limited production because of the high capital investments, production cost and lack of well-organised scale-up processes. Another problem is that the pore size of the BNC matrix is not sufficiently big to house the cells, limiting the cell penetration and hindering their proliferation and differentiation. In order to reduce the production costs of the BNC, several lines of action are being carried out, such as the study of the influence of static and agitated modes to optimise the production process [35], the discovery of new and more effective strains to produce BNC [36], and the use of novel substrates rich in sugars, such as agri-food industry wastewater, wood extracts, etc. [37,38].

Its rheology and shear thinning behaviour make nanocellulose suitable for use in the formulation of bioinks for 3D bioprinting. This technology can be used for several applications, including wound dressings, drug delivery and as the basis for scaffolds for regenerative medicine. The formulation of

nanocellulose-based bioinks can be based purely on nanocellulose [39] or by combining it with different biopolymers, such as polyethylene glycol [40], gelatin methacrylate [41] or alginate [42]. The use of nanocellulose in biomedical applications requires a full evaluation of its biocompatibility, including parameters, such as cytotoxicity, skin irritation, skin sensitisation and genotoxicity. Endes et al. [43] reported extensively on the biological impact of nanocellulose to analyse the potential hazard of nanocellulose. In this review, the cytotoxicity, inflammation, oxidative stress and genotoxicity were evaluated. The conclusion obtained was that there is a disparity between the different studies, as some authors manifest the benign nature of nanocellulose, and others emphasise the potential for adverse effects. These differences can be attributed to the variation of different studies in cell systems, starting material, treatment, characteristics and concentration of nanocellulose. Several authors reported the low toxicity potential and the benign nature of these materials, and therefore, their suitability for biomedical application, such as tissue engineering [44–48]. On the other hand, other authors have reported the toxic nature of nanocellulose when present at high concentration (>250 µg/mL) or when it is chemically modified [49–51].

Therefore, it is concluded that several types of nanocellulose (BNC and various types of CNF and CNC) do not present cytotoxicity or genotoxicity against a series of cell lines. However, chemically modified nanocellulose (e.g., carboxymethylation and periodate oxidation) can induce inflammation and may cause chronic complications [52]. The cytotoxicity of these nanocelluloses can be reduced by subjecting the cellulose fibers to an alkaline autoclave treatment to obtain an ultrapure nanocellulose with an endotoxin level of < 50 endotoxin units/g [53]. The initial inflammatory and coagulation responses of ultrapure nanocellulose have been evaluated using a lepirudin-based human whole blood model showing a coagulation, complement and cytokines activation improving the selection possibilities in several treatments' situation of wound healings.

3. Alternative Biopolymers

3.1. Polysaccharides

Polysaccharides are carbohydrates composed of many monosaccharides held together by glycosidic bonds. They serve may physiological functions, such as: Energy storage, e.g., glycogen; structural components of cells, e.g., cellulose in plant cell walls; and cell signalling pathways, to name a few. This article will be focused on macromolecules. Accordingly, relevant oligosaccharides in the field of drug delivery, such as cyclodextrins [54,55] will not be covered in this review.

3.1.1. Starch

Starch is a polysaccharide composed of many glucose monomers and acts as an energy store within plants. After photosynthesis, glucose is stored as starch granules within the chloroplasts of plant cells. Depending on the type of plant, these starch granules are composed of varying percentages of amylose and amylopectin. Amylose is a linear polymer of glucose monomers bonded together by (1,4) glycosidic bonds. Amylopectin also contains these (1,4) glycosidic bonds, but is also a branched molecule containing multiple side chains found every 24–30 glucose monomers on average and held together by (1,6) glycosidic bonds (Figure 2).

Starch is one of the most abundant polysaccharides on the planet, sourced primarily from corn, rice, potato and wheat crops. It is also inexpensive. It is easy to obtain and modify to improve its mechanical properties via processes, such as cross-linking or blending with synthetic polymers. As such, starch is an attractive biopolymer for development within biomedicine and other fields. For example, starch-based biomaterials have been developed for use in producing scaffolds in tissue engineering [56] and wound healing [57]. However, limitations, such as high water sensitivity (very hydrophilic), poor barrier properties at high moisture concentrations, and mechanical difficulties, remain major obstacles in the biomedical development of starch [27].

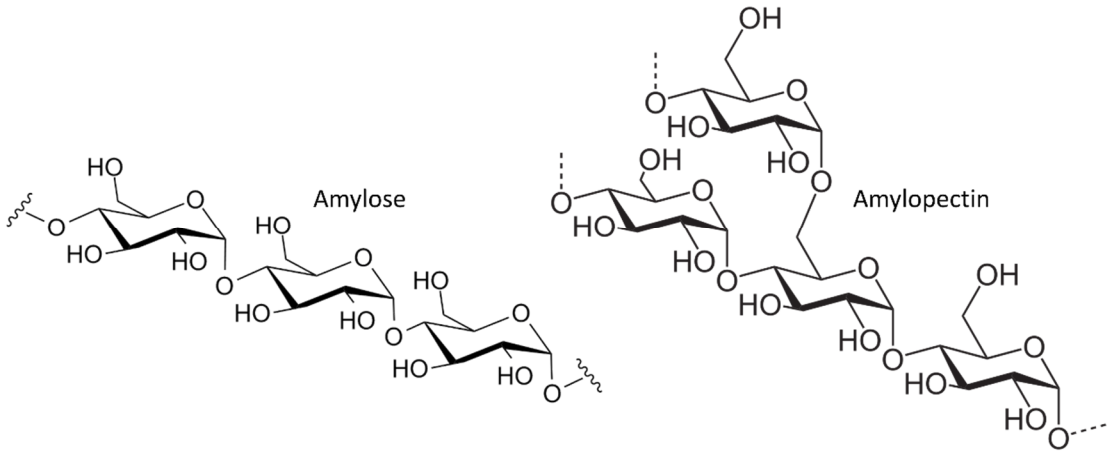

Figure 2. Chemical structure of starch detailing both amylose and amylopectin subunits.

3.1.2. Hyaluronic Acid

Hyaluronic Acid (HA) is a non-sulfated, linear natural polysaccharide composed of d-glucuronic acid and d-n-acetylglucosamine monomers linked by alternating (1,4) and (1,3) glycosidic bonds (Figure 3).

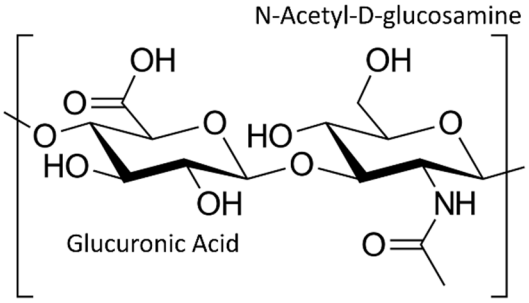

Figure 3. An image of the repeating unit of hyaluronic acid (HA).

HA was first isolated from the vitreous humour of bovine eyes in 1934 [58,59]. HA is a major component of the extracellular matrix (ECM) of skin and cartilage, playing a role in cell proliferation, differentiation and tissue repair [60]. HA's molecular weight has been shown to affect its biological functions, half-life and rheological properties, e.g., only high molecular weight HA possesses muco-adherence and anti-inflammatory properties [61]. HA has been used in the production of tissue scaffolds [62] and conjugated as hydrogels [63,64].

3.1.3. Chitin and Chitosan

Chitin is a naturally occurring polysaccharide that is found as a structural constituent in fungi and yeast, as well as in the exoskeletons of insects and crustaceans. Chitin is a polysaccharide consisting of p-(1→4) 2-acetamido-2-deoxy-ɒ-glucose repeat units, some of which are deacetylated (Figure 4). Depending on the origin of chitin, once deacetylation reaches 50% chitin is converted into chitosan. Chitin and chitosan are highly basic due to their high nitrogen concentration, making them useful chelating agents [60]. Other uses of chitin/chitosan include wound healing, due to its biodegradability, water sorptivity, oxygen permeability, blood coagulation and cytokine inductive properties [65,66] and hydrogel controlled drug delivery [67].

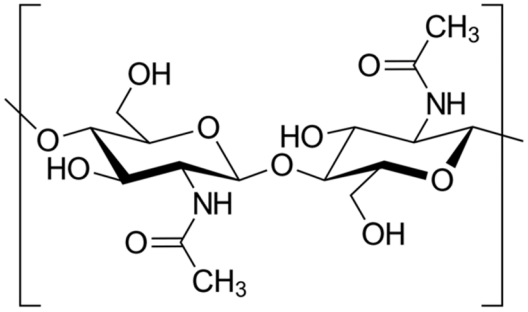

Figure 4. An image of the chemical structure of chitin.

3.1.4. Hemicellulose

Hemicelluloses are another major component of lignocellulosic materials. It is defined as a complex branched heteropolymer of lower molecular weight than cellulose, which acts as a binding agent between lignin and cellulose through covalent and non-covalent interactions (Figure 5). Among the monosaccharides that form hemicelluloses, we can find C6 sugars called hexoses (D-glucose, D-mannose and D-galactose), C5 sugars called pentoses (mainly D-xylose and L-arabinose), uronic acids (D-glucuronic, D-galacturonic or D-4-0-methylgalacturonic) and deoxy-hexoses (L-ramnose and L-fucose) that present a partial acetylation of their hydroxyl groups [68]. The content and structure of hemicelluloses differ in the different vegetable species. Research on the chemical structure of hemicelluloses has been mainly based on the determination of the main chain and branched glucan chains. The main chain may consist of one or more types of monosaccharides, and the connections between these monosaccharides may also be different. In order to study their chemical composition, it is necessary to classify the different polysaccharides that may present, which include xylans, glucuronoxylanes, arabinoxylanes, mannan, glucomannan and galactoglucomannan [69]. This complex mixture of polysaccharides can represent 25–30% of the total weight of plant biomass. Herbaceous and hardwoods are composed mainly of glucuronoxylans, and softwood of glucomannan and galactoglucomannan [70]. In general, the different units of the main chain of these polysaccharides are linked by links β-1,4. However, there may also be links β-1,3 forming β-1,3-1,4 glucans which are found mainly in the cell well of *Poaceae* family, and are restricted to members of this family, being particularly abundant in the cell wall of the endosperm of cereals, such as wheat, rice, barley, oats and rye [71].

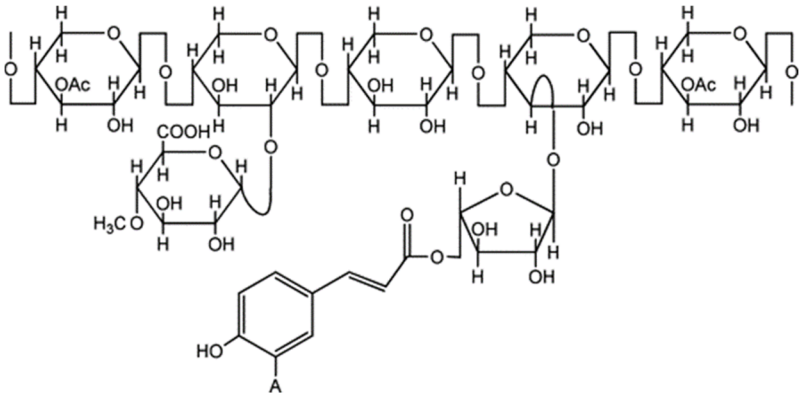

Figure 5. A representative structure of hemicellulose. Reproduced with permission from Terzopoulou et al. [72].

3.2. Proteins

Proteins are polymers composed of one or more polypeptide chains of amino acids and are essentially the 'building blocks of life'. Proteins have essential roles within organisms, whether it be as structural components, e.g., collagen and skin, or regulating physiological processes as enzymes.

3.2.1. Albumin

Serum albumin is one of the most abundant proteins in human plasma [73,74] (Figure 6). This protein presents a molecular weight of 66.5 kDa, high stability at relatively high temperatures (lower than 60 °C) and good stability at pHs ranging from 4 to 9 [73]. It is biodegradable, biocompatible, non-immunogenic and suitable for blood circulation (half-life of 19 days) [73]. Finally, albumin is a natural transport protein [74] that is involved in the delivery of nutrients in the body. Accordingly, all these properties make albumin an ideal candidate for drug delivery applications [75].

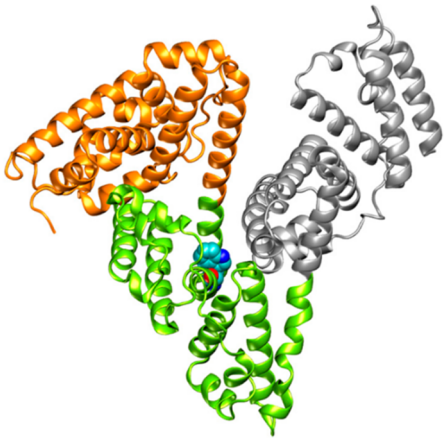

Figure 6. Crystallographic structure of HSA (PDB: 1N5U), presenting the three structurally similar domains: I (Brown), II (green), and III (grey). Reproduced with permission from Chaves et al. [76].

Due to its transport function, albumin presents a wide variety of binding sites that can be used for drug delivery applications [74]. There are a wide variety of products in the market that use albumin-drug conjugates for drug delivery. The drug-albumin conjugate types include simple systems, where the drug is attached to the protein by non-covalent interactions and more complex approaches that require the formulation of drug containing nanoparticles or albumin-drug covalent bonds [74].

3.2.2. Collagen and Gelatin

Collagen is found in the connective tissues of animals and possesses huge potential in tissue engineering application and drug delivery, due to its high biocompatibility and mechanical properties. Collagen molecules form a triple helix structure, held together by inter and intra chain hydrogen bonding (Figure 7).

There are 20 genetically distinct members of the collagen family. The major ones are type I (skin, tendon and bone), type II (cartilage), and type III (skin and vasculature) [77]. Cross-linked collagen-based materials can be used to produce tissue scaffolds or matrices for wound healing. Properties of collagen-based materials are dependent on the source, age of animal and type of collagen used.

Gelatin is a soluble protein obtained from the partial hydrolysis of collagen, and its properties are wholly dependent on the collagen source used [60]. Traditionally gelatin has been used in the pharmaceutical industry in capsule dosage forms, however, in more recent times cross-linking has led to developments in tissue scaffolding and transdermal drug delivery by way of microneedles [78].

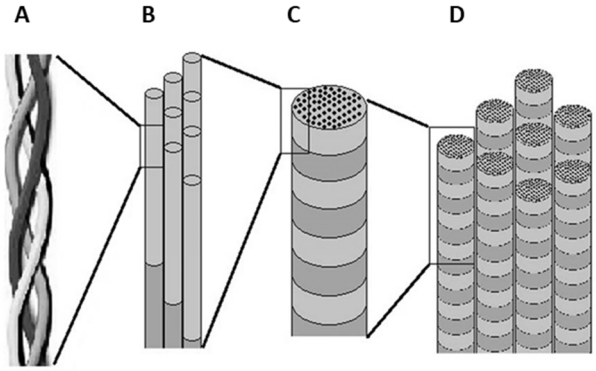

Figure 7. Collagen molecules form a triple helix structure (**A**). Assembled tropocollagen molecules (**B**). Collagen fibril ranging from 10 to 300 nm in diameter (**C**). Collagen fibrils forming a collagen fiber ranging from 0.5 to 3 μm in diameter (**D**). Reproduced with permission from Parenteau-Bareil et al. [79].

3.2.3. Silk Fibroin

Silk is a naturally occurring protein biopolymer that is spun into fibers by certain *Lepidoptera larvae* (such as silkworms), some arachnids (spiders, mites, and some scorpions), and a few flies [80]. Polymer chains of a silk fiber are organised parallel to its axis resulting in strong and inextensible fibers (Figure 8), lending itself to use as surgical sutures in the past [60]. Recent applications of silk have been in scaffolds for bone tissue engineering [80]. Silk is an easy-to-process biopolymer; this allows silk-based biomaterials to be moulded into diverse forms and architectures, which further affects the degradability and suitability for use in medical micro-devices. This makes silk-based scaffolds suitable for treating a variety of bone reconstruction and regeneration objectives [81].

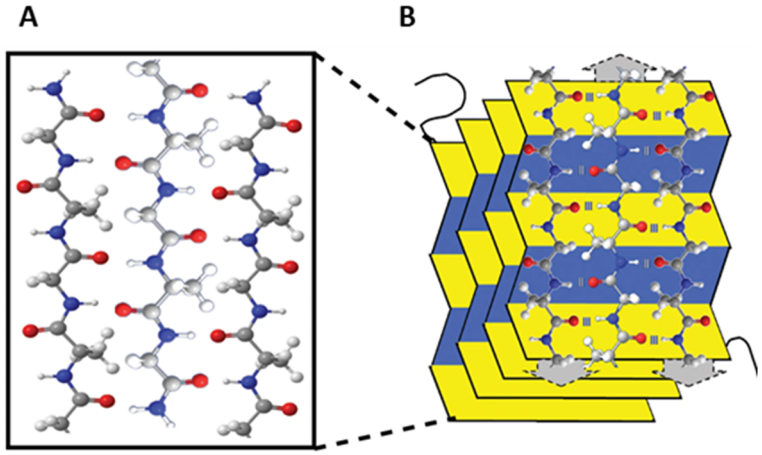

Figure 8. An image of a three-chain silk sequence composed of carbon (grey), nitrogen (blue), oxygen (red) and hydrogen (white) (**A**), and a 3-dimensional β-sheet of silk (**B**). Reproduced with permission from Cebe et al. [82] and Stewart et al. [83].

3.2.4. Casein

The main milk protein component is casein. There are mainly four types of casein proteins: αS1-, αS2-, β-, and κ-casein [84]. These proteins have molecular weights ranging from 19 to 25 kDa, and their average isoelectric point ranges from 4.6 to 4.8 [84]. These proteins are amphiphilic, and accordingly, have the capacity of self-assemble-via intermolecular interactions (mainly hydrophobic) (Figure 9A). Casein can be used in its acid form (low aqueous solubility) or in its sodium caseinate form. The latter is freely soluble in water except when the pH is close to its isoelectric point [84]. Another interesting property of casein is that this family of proteins have a relatively low percentage of secondary or tertiary structure [84]. Due to this, caseins are relatively heat stable, and they are easily accessible for

proteolytic cleavage. This property makes casein a good candidate for oral drug delivery, due to its facility to release drug cargos in the stomach upon proteolytic degradation.

Due to its capacity to self-assemble into micelles/nanoparticles and its safety (casein is one of the main components of milk), casein has been extensively used for drug delivery applications. The main application of this family of proteins has been the development of drug nanocarriers for oral drug delivery [73,85,86] or nutraceutics [87,88] (Figure 9B). In addition to nano/microparticles casein has been used for other types of drug delivery applications, such as hydrogels [89] and film coatings [90,91].

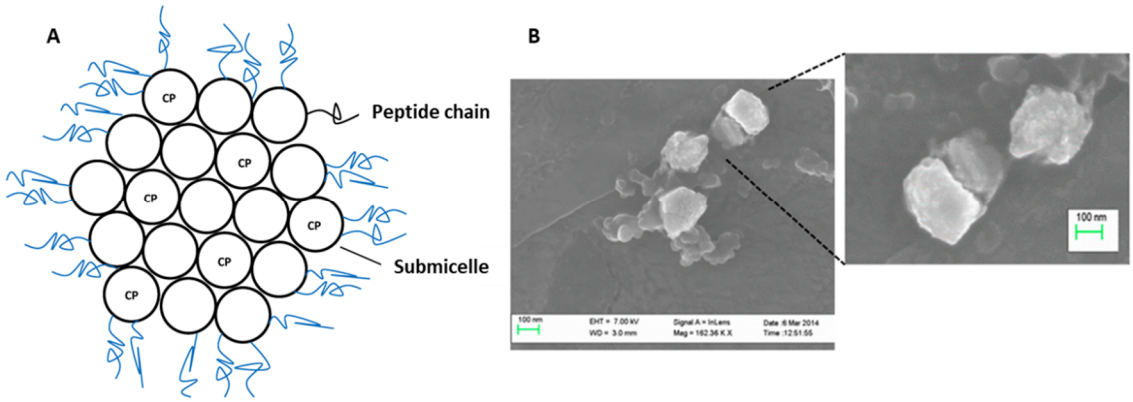

Figure 9. The structural arrangement of casein micelles. CP-Calcium phosphate (**A**). Reproduced with permission from Pereira et al. [92]. Scanning Electron Microscopy microphotographs of casein nanoparticles (**B**). Reproduced with permission from Peñalva et al. [87].

3.2.5. Zein

Zein is a prolamin protein that is obtained from corn [93,94]. It presents a molecular weight of about 40 kDa [93]. Zein has been classified into four different classes: α-, β-, γ-, and δ-zeins [93]. Zein is mainly a hydrophobic protein that is insoluble in water, but soluble in organic solvents, such as ethanol or acetone [93,94]. This is due to the high concentration of non-polar amino acids within its structure [93] (Figure 10). The structure of this protein is still not 100% reported. Due to its hydrophobicity and its excellent water barrier properties, the use of zein for coating applications has been extensively described [93].

The use of zein has been extensively reported for drug delivery applications in a wide variety of formulations. The main types of applications involve the use of films and fibers [95–97]. An alternative application of zein has been the formulation of micro- and nanoparticles for drug delivery [98–100]. Finally, other drug delivery systems have been described using zein, such as composites or gels [93].

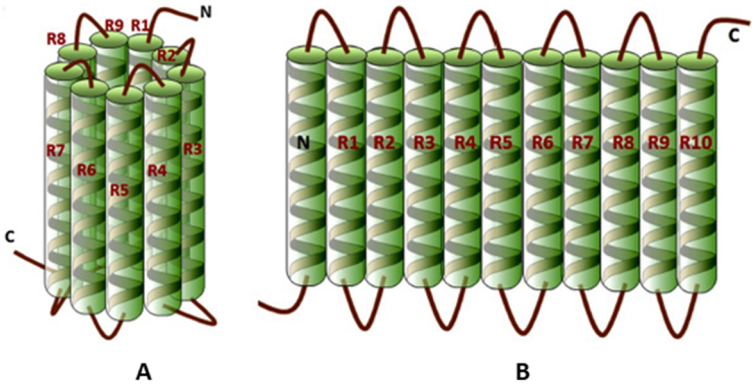

Figure 10. Two proposed three-dimensional structures of α-zein: Cylindrical model (**A**) and ribbon-like model (**B**). Reproduced with permission from Zhang et al. [101].

3.3. Lignin

Lignin is a highly biocompatible biopolymer, which is found in most terrestrial plants in the approximate range of 15 to 40% dry weight, conferring its rigid property to the cell walls [102,103]. Lignin is a complex aromatic heteropolymer synthesised mainly from three primary monolignols, *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. During the lignification, each of these monolignols gives rise to a different type of lignin subunit called *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, respectively [104,105] (Figure 11). This biosynthesis process creates a unique and distinctive lignin polymer depending on the plant species and even, depending on the type of tissue of the same specimen [106]. Thus, the structure and composition of lignins could vary depending on the plant origin, cell type, and even with the climate conditions. Lignin is produced in large quantities as a by-product in paper manufacturing and new biorefineries that convert cellulosic biomass into liquid transportation fuels [103]. In most cases, it is simply used as an energy source or just discarded as waste, and less than 2% of the total amount of lignin produced is reused to transform it to value-added products [24,103]. Therefore, extensive efforts in different fields of knowledge are being made to find alternative uses for lignin [107–113]. Moreover, lignin possesses antimicrobial and antioxidant properties. Because of this, and due to its high availability, lignin has a potential for biomedical applications.

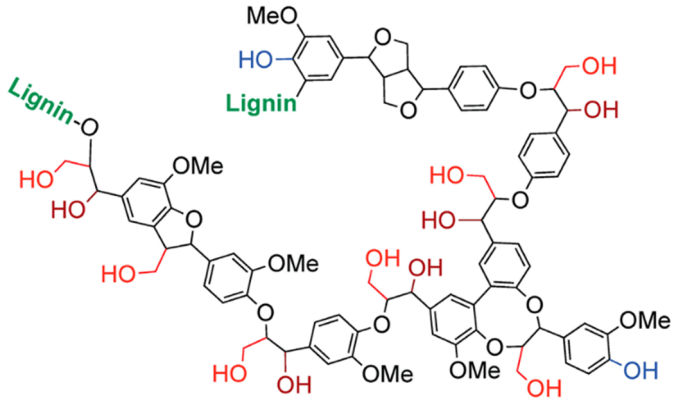

Figure 11. The proposed structure of lignin. Reproduced with permission of Dabral et al. [114].

4. Outline of Biomedical Applications

4.1. Tissue Engineering

Through tissue engineering, synthetic substitutes can be created which act to replace or repair damaged organs and tissues. The biocompatible and biodegradable nature of biopolymers makes them ideal materials for development in this field. Many studies have been undertaken in developing biopolymer composites for tissue scaffolds [115]. 3D Scaffolds are used in tissue engineering as an artificial ECM for cell seeding, proliferation, and new tissue formation. One of the advantages of biopolymers (nanocellulose, collagen, xylans, etc.) for scaffolds is the biological recognition that may positively support cell adhesion and function. The scaffold should biodegrade naturally in the body at the same rate as new tissue grows, and subsequently, be safely eliminated [62]. The chemical structure of nanocellulose allows it to easily form a hydrogen bond network which has high mechanical strength, good hydrophilicity and excellent biocompatibility. These properties make nanocellulose a perfect candidate for being used in tissue engineering applications. The nanocellulose scaffolds present all the requirements of tissue engineering technology, such as superior water absorption, water retention, biocompatibility and mechanical properties. The scaffolds for tissue engineering can be prepared in several ways, such as electrospinning, freeze-drying, 3D printing, solvent casting, etc. Nanocellulose has been used as a major component or as reinforcement in many formulations for

tissue engineering that covers its application in the repair of skin, vascular, neural, muscular, hepatic and ophthalmological tissue [116].

For skin tissue engineering CNC and CNF have been combined with several polymers to produce structures to obtain features similar to native skin improving the biocompatibility, mechanical, thermal and swelling properties. CNC was incorporated to polylactide-polyglycolide (PLGA) to produce PLGA/CNC membranes by electrospinning improving the cell compatibility, adhesion, spreading and proliferation of fibroblast in comparison with neat PLGA to skin tissue engineering [117]. PVA, GelMa, collagen, gelatin and chitosan has been used in combination with nanocellulose to produce structures to create the optimum microenvironment for cells to adhere, grow and metabolise in a similar way that natural skin does [118,119]. CNC and CNF improve the fiber morphology, tensile strength, elastic modulus, indentation modulus and biocompatibility of the scaffolds created for vascular tissue engineering. The endothelialisation is a key parameter in the production of artificial vessels in this application. CNC and CNF have been combined with poly(lactic acid) (PLA) or poly(butylene succinate) (PBS) to create scaffolds with multi-scaled structure with interconnected porous morphology and high surface area that are essential to cell growth and nutrient exchanges [120]. Nanocellulose can act as control elements for the porosity, mechanical strength, orientation and flexibility of scaffolds for neural tissue engineering. Neural tissue presents a limited capacity to be self-repaired and regenerated after some damage. Nanocellulose scaffolds alone or in combination with carbon nanotubes guide and enhance the development of neural cells, maintaining the axon channel and promoting the neural stimulation and activity [121]. For skeletal muscle, CNC was used to produce tissue engineering scaffolds with the ability to contract and posses' similar functionalities to native muscle. CNC was used to produce spin-coated structures with oriented direction. The oriented surface of CNC promotes the fusion degree of myoblasts being capable of ameliorating the myotube orientation [122]. Scaffolds for cardiac tissue engineering should have stability, stretchability, flexibility, along with cytocompatibility. CNF obtained by TEMPO-mediated oxidation or acetylation process exhibited favourable fidelity under wet and dry cases, remarkable stability, and good biocompatibility with cardiac myoblast cells [123]. Hepatic tissue engineering aims to construct functional hepatic organs for organ transplantation. CNF hydrogels promote the 3D hepatocyte culture and induce the formation of hepatocyte HepaRG and HepG2 cells. Bioinks of CNC/Alginate were used to mimic hepatic structure by three-dimensional bioprinting, obtaining great shape fidelity with minimal cell mortality [124,125]. The excellent optical, mechanical, barrier and compatibility properties of nanocellulose allow to use it in ophthalmic tissue engineering. CNC and CNF were used as a matrix to produce hyperelastic hydrogels in combination with PVA matrix obtaining exceptionally high-water content and similar mechanical properties to native tissues [126].

In addition, other biopolymers have been used to produce materials for tissue engineering. A silk/collagen/hyaluronic acid composite scaffold was produced incorporating pilose antler polypeptides. It was shown to have repaired cartilage defects in rabbit studies in 13 weeks [127]. Similarly, silk fibroin and melanin scaffolds seeded with human neuroblastoma cells (SH-SY5Y) have demonstrated the potential for nerve regeneration as they successfully promoted neuronal growth along the axis of the scaffold [128]. These scaffolds can also be applied to stimulate bone growth. Figure 12 shows an example of scaffolds prepared using silk fibroin via electrospinning. Porous hydroxyapatite scaffolds for bone tissue engineering were successfully prepared using 3D gel-printing technology [129]. Tissue engineered heart valves are also being developed using biopolymers to eventually replace mechanical counterparts as they hold advantages in terms of durability, the abilities to grow, repair and remodel cardiac tissue [130].

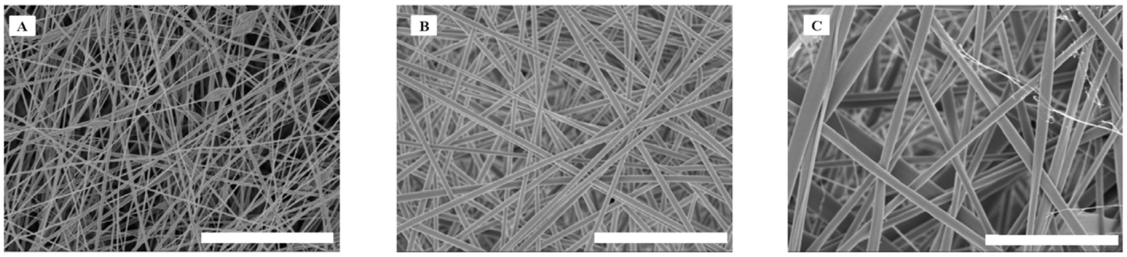

Figure 12. Scanning Electron Microscopy images of silk fibroin scaffolds obtained using different electrospinning concentrations: 7 wt % (**A**), 8 wt % (**B**) and 9 wt % (**C**). Scale bars: 10 µm. Reproduced with permission from Chen et al. [131].

Xylan/poly(vinyl alcohol) (PVA) based nanofibrous scaffolds cross-linked with glutaraldehyde vapours have been investigated as a support for cell adhesion and proliferation in cardiac tissue engineering [132]. Similarly, Xlyan-based hydrogels have been synthesized and tested for use for cell encapsulation and in vivo delivery. Mesenchymal stem cells were encapsulated in the hydrogel for one week and were shown to have retained their adipogenic differentiation capacity within the gel [133]. The addition of lignin and xylan to cellulose-based hydrogels has been shown to improve the mechanical strength and stiffness, whilst lignin imparted antimicrobial properties to the film [134]. Alginate/lignin aerogels have been shown to be non-cytotoxic and feature good cell adhesion [110]. Lignin derived nanoparticles have been reported to show gram-negative and gram-positive biocidal activity at significantly lower silver concentrations compared to conventional reagents, such as silver nanoparticles or silver nitrate, while leaving an inactive biodegradable particle after the release, and thus, having a smaller environmental impact [111].

4.2. Wound Healing

Wound dressings act as barriers, permeable to oxygen and moisture, which are not only essential to the healing process, but also protect the area from further harm-mainly the risk of infection from microorganisms. Biopolymers, such as cellulose, have been used in wound dressings as they have exhibited useful properties. They protect the skin outside the wound, maintain suitable moisture at the wound level, prevent and control microbial biofilms, cleanse the injured tissues, eliminate/minimise pain, remove dead spaces and non-viable tissues and control odours [135]. Biopolymer hydrogel dressings are used widely because of their ability to hold moisture and closeness to the ECM, helping to induce wound healing. In recent years, nanocellulose has been investigated to be used as potential material for wound healing application, due to its high surface to volume ratio that can enhance the proliferation, migration and attachment of cells. The high concentration of -OH groups in the surface of the nanocellulose offers a hydrated layer over the wounds. The application of nanocellulose to wound healing was recently reported in several studies [40,66,136–139]. Nanocellulose, in combination with antibiotic agents (polyhexamethylene biguanide and tetracycline hydrochloride), was used to obtain bioactive wound dressing to prevent the colonisation of bacterial strips, especially *Staphylococcus aureus* and *Escherichia coli* [138,140]. The controlled release of drugs contained in wound dressings allowed the healing process in burn victims to be completed in the period of 10–16 days [139,141]. Regarding the utilisation of nanocellulose for this application, no cytotoxicity of CNF obtained by TEMPO-mediated oxidation against 3T3 cell lines and human skin cells has been reported by several authors [53,142,143]. In addition, the matrix of nanocellulose and other polymers has been used successfully in wound dressings. Chitosan/PVP/Nanocellulose composite dressing in combination with stearic acid was used as temporary biological wound healing material on albino rats, revealing a healing process faster than the control wounds [144]. Hydrogels of nanocellulose and polydopamine crosslinked physically with Ca^{2+} resulted in an excellent drug release for wound healing process, showing good antibacterial properties against different bacteria [140]. The addition of PEG as plasticiser into nanocellulose films improved the mechanical properties of nanocellulose wound dressings and increased the swelling of

the wound dressing to 600% and 1000% in PBS and water, respectively [143]. Moreover, no effect in the metabolic activity was reported by the addition of PEG when fibroblasts were incubated when amounts of 10 and 25% were used; however, it was detected in the presence of 40% [143]. Additionally, no significant cell death was detected in any amount of PEG.

Several biopolymers can be used for this application. For instance, chitosan has been investigated and deemed suitable for wound dressings by the US Food and Drug Administration (FDA). Low molecular weight Chitosan has been shown to have antimicrobial properties through testing against several bacteria present in human wounds. Chitosan also induces wound healing through fibroblast activation by way of releasing *N*-acetyl-beta-d-glucosamine into the wound site. Haemostatic and analgesic effects have also been observed [145,146]. Figure 13 shows an example of a chitosan-alginate wound dressing. Biopolymer wound dressings can also be impregnated with antimicrobials, such as antibiotics or silver to help manage chronic wounds—these are termed 'active dressings'. Commercially available silver products include Aquacel Ag®, DynaGinate™ AG Silver Calcium Alginate dressing and Biatain® Alginate Ag [147].

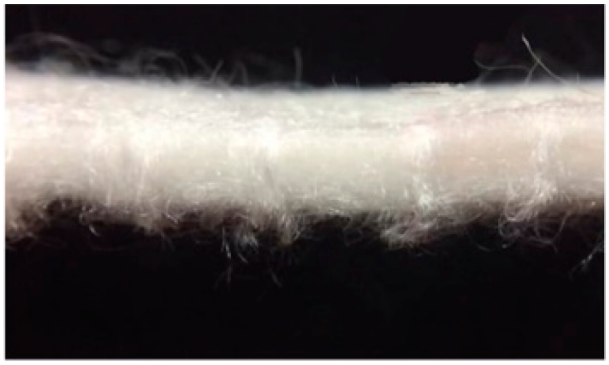

Figure 13. Chitosan-alginate nonwovens Composite Wound Dressings. Reproduced with permission from Gao et al. [148].

Cross-linked xyloglucan/PVA based hydrogel films investigated for wound healing were found to form a homogeneous, flexible and easy to handle film which showed partial cell adhesiveness and could be applied to the wound and removed without pain. They were fully cyto-compatible, blood-compatible and did not activate an immunogenic response and inhibited *E. coli* infiltration [149]. Thin xyloglucan-based hydrogel films have been synthesised and characterised with the prospect of producing wound dressings. PVA and glycerol were added to give an optimal combination of softness, conformability and resilience. Physical hydrogels were transformed into permanent covalent hydrogels by reaction with glutaraldehyde. The chemically cross-linked films displayed good cyto-compatibility, and no toxic components were released by the films. Moderate cytotoxicity was observed for the physical hydrogel films. Further investigations on both in vitro and in vivo biocompatibility need to be performed in order to transform this interesting material into a medical device for wound management [150]. Hydrogel-based smart wound dressings that combine the traditional favourable properties of hydrogels as skincare materials with sensing functions of relevant biological parameters for the remote monitoring of wound healing have been investigated. Lightweight, ultra-high frequency radiofrequency identification (UHF RFID) sensors have been combined with xyloglucan/PVA hydrogel films to allow battery-less monitoring of moisture levels [151]. Lignin-PLA combinations have been investigated as wound dressings. The inclusion of lignin yielded materials with lower resistance to fracture and higher wettability and resulted in a material with antioxidant capabilities which would be beneficial for a wound dressing [152]. Biocompatible hydrogels were prepared from chitosan, and alkali lignin were found to be non-toxic to Mesenchymal stem cells, in vitro, and to zebrafish up to 100 μg/mL, in vivo. These gels provided a conducive surface for cell attachment and proliferation, making them suitable for application as scaffolds in tissue engineering. In the presence of the hydrogel,

NIH 3T3 mouse fibroblast cells showed good cell migration characteristics suggesting that the gel might be suitable for wound healing applications [113].

Lignin/chitosan/PVA composite hydrogels were prepared as wound dressings. The introduction of lignin effectively improved the mechanical strength, protein adsorption capacity, and wound environmental regulation ability of the hydrogel. In a murine wound model, the lignin/chitosan/PVA composite hydrogel significantly accelerated wound healing. The novel hydrogel developed also exhibits potential for a wide range of medical applications, such as packaging of expensive drugs [153].

4.3. Medical Implants

Medical implants are designed to replace a damaged organ or structure within the body in order to sustain normal function, e.g., hip replacements. Traditionally materials like metals, ceramics and synthetic polymers have been used in the manufacturing of these devices. Despite being suitably functional as implants, these materials have been shown to be inflammatory and immunogenic [154]. Biopolymers offer a potential solution to resolving these issues as they are biocompatible, biodegradable and non-immunogenic. Bacterial nanocellulose shows promising results for engineering and non-biodegradable ear implants with suitable mechanical properties and patient-specific shapes via 3D bioprinting [155]. Some biopolymers, such as lignin have antimicrobial properties, offering potential as antimicrobial coatings of implants, such as catheters by minimising colonisation of these devices by bacteria, reducing the risk of infection within the body [156]. In terms of stent manufacturing, use of metallic stents can prevent acute occlusion; however, they carry risks of thrombosis and restenosis later on, and patients are required to be on anti-platelet medications to reduce this risk. Bioresorbable stents (BRS) have been designed from biopolymers, such as Poly-L-Lactic acid (PLLA) to overcome these challenges [157]. These stents can treat the coronary lesion and also restore endothelial function, while being reabsorbed months after being implanted, as opposed to metallic stents which remain intact. Powerstent® is an example of a BRS currently in use; and the stent is designed by co-formulating amorphous calcium phosphate (ACP) nanoparticles with PLLA and 2% Paclitaxel. Early results in animal studies have shown lower rates of restenosis, lower stent recoil, and lower inflammation [158]. It should be noted that the evidence base for BRS is still very limited and mechanical properties can still be improved; therefore, adoption of BRS over more established metallic stents cannot be advocated. Nevertheless, future applications of biopolymers in medical implants are promising. Moreover, PLLA interference screws were tested in patients with a unilateral anterior cruciate ligament rupture, obtaining promising results [159].

Additionally, temperature-sensitive hemicellulose-based hydrogels were synthesised using hemicellulose obtained from acetic acid pulping of eucalyptus and N-isopropylacrylamide (NIPAAm) through UV photo-crosslinking. This material is promising for use as a smart material for biomedical applications [160].

4.4. Delivery of Bioactive Molecules

Drug delivery systems (DDS) are used to achieve a higher therapeutic effect of a pharmaceutical drug or natural compound in a specific diseased site. Uses of biopolymers in nanotechnology DDS have better biocompatibility, biodegradability and lower immunogenicity than synthetic counterparts. Biopolymers, such as silk, collagen, gelatin, starch, cellulose and chitosan can be easily made into suspensions that serve as delivery vehicles for drug molecules [29]. CNFs have been used as a matrix former for sustained drug delivery and as a film for the immediate release of poorly soluble drugs [161]. In addition to the work carried out on the controlled release of drugs to obtain wound healing dressings produced from nanocellulose others work investigated the potential for drug delivery have been reported. Amin et al. studied the possibility of using BNC-acrylic acid (AA) hydrogels for drug delivery [162]. AA was grafted onto the surface of BNC by electron-beam irradiation obtaining hydrogels with thermo and pH-responsive properties [162]. BNC was also evaluated as a matrix for controlled drug release for other drugs, such as famotidine and tizanidine [163]. CNC was also utilised

as a vehicle for drug-delivery systems. CNC was used to stabiliser the alginate matrix to produce higher encapsulation and control the release rate of theophylline in the treatment of respiratory-related illness, as well as to control the release rate of bovine serum albumin (BSA) [164,165]. Jackson et al. [166] extensively studied the use of CNC as a drug carrier element. In this work, the negatively charged surface of CNC, due to the sulphate groups of the acid hydrolysis using sulfuric acids interact with the positive charge of doxorubicin hydrochloride (DOX) and tetracycline hydrochloride (TET). Approximately 80% of DOX and TET was released within the first 4 h. CNC was also functionalised with cetyl trimethylammonium bromide (CTAB) to change the hydrophilic nature of the surface into hydrophobic. The functionalised CNC was used to encapsulate hydrophobic anticancer compounds, such as etoposide (ETOP), docetaxel (DTX) and paclitaxel (PTX) showing a slower and controlled release of the drugs. Oxidised CNC by TEMPO-mediated oxidation was also tested in this study presenting a loading efficacy of 14% w/w for procaine hydrochloride showing 80% of drug released in an hour. Cellulose nanofibers (CNF) was also reported to be used for drug delivery by several authors [166–169]. CNF was used as a pharmaceutical excipient of indomethacin, metoprolol tartrate, verapamil hydrochloride, nadolol, ibuprofen and atenolol. Except for the indomethacin, all the drugs were embodied prevalently in the CNF network, showing a quick discharge rate in the period of 10–14 days and a much slower discharge rate over time up to 60 days [168,169]. CNF films were used for controlled drug delivery containing water-insoluble drugs, such as indomethacin, itraconazole or beclomethasone dipropionate. The results showed that indomethacin was released in a period of 15–30 days; however, beclomethasone dipropionate and itraconazole were released more gradually over a period of 90 days [169]. Ariga et al. [170] developed a thermoresponsive framework by the addition of multilayers of CNF and N-isopropyl acrylamide. This structure presents a controlled porosity and release by temperature adjustment. CNF was also used to placed chitosan-benzalkonium chloride (BAC) complex showing antimicrobial action against *S. aureus and E. coli*, being able to be used as new drug-delivery frameworks [171].

Chitosan nanoparticles have also been used to deliver medications, such as methotrexate and 5-fluorouracil over a sustained period of time in the treatment of cancer [172]. Designer biopolymers (DBPs) are genetically engineered biomacromolecules designed to condense plasmid DNA and overcome intra- and extra-cellular barriers for successful gene delivery [173]. Tumour molecular targeting peptide-1 (TMTP-1) was delivered via DBPs and shown to successfully target metastatic prostate cancer cells which express TMTP-1 receptors on their surface and exhibited low toxicity based on cell recovery post-transfection [174].

Hemicellulose/chitosan films cross-linked with epichlorohydrin were shown to be biocompatible, non-toxic and could be loaded with up to 18% of a model drug (ciprofloxacin). This material shows promise in both wound healing and drug delivery applications [175]. Xylan-based temperature and pH sensitive hydrogels were prepared, which showed a drug encapsulation efficiency of up to 97.6% and cumulative release of acetylsalicylic acid of 90.12% and 26.35% in intestinal and gastric fluids, respectively. The hydrogels were shown to be biocompatible and could be promising candidates for drug carriers for intestinal targeted oral drug delivery [176]. pH-responsive prodrug nanoparticles based on xylan-curcumin conjugates were developed to enhance the therapeutic efficacy of curcumin in cancer therapy. The cytotoxic activity of the prodrug nanoparticles against human colon cancer cells (HT-29, HCT-15) showed a greater cytotoxic effect than curcumin alone. Therefore, these results reveal xylan-curcumin nanoparticles could be a promising candidate for improving the intracellular delivery of curcumin in cancer therapy [177]. Lignin-based hydrogels were prepared with lignin contents ranging from 24% to 40% and water uptake capabilities up to 500%. The hydrophobic nature of lignin facilitated loading of a model hydrophobic drug (curcumin). The hydrogels were capable of sustaining the delivery of this compound for up to four days, and the inclusion of lignin also provided the material with significant resistance to bacterial adherence when compared to similar hydrogels without lignin [156]. Lignin was combined with microcrystalline cellulose and investigated as an excipient in the production of directly compressed tablets containing tetracycline. The presence of

lignin in the tablets significantly modified the release profile and the maximum amount of tetracycline released, and lignin provided antioxidant properties to the formulations. Accordingly, this showed the potential of lignin to be used as a pharmaceutical excipient [112].

5. Conclusions

Environmentally friendly biopolymers and their applications are being developed at an increasing rate to meet the needs of a more environmentally conscious society. The potential of these materials for use within biomedicine is vast, due to their advantageous properties of biocompatibility, biodegradability and low immunogenicity-properties that synthetic polymers do not share. Significant progress has been made with successes in tissue engineering, medical implants, wound healing/dressings and drug delivery systems. However, production methods need to be optimised to overcome the variability in mechanical properties, structure and performance of the products. This is largely due to the chemical heterogeneity and high dispersity of these materials. Furthermore, the clinical applications of biopolymers, such as bioresorbable stents need to be studied further so that their performance can be accurately predicted before they crossover into human use.

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