Advances in Biotechnology

Chapter 6

Concepts and Recent Advances on Biopolymers for Biomedical applications: Special Mention to the PHAs Family

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Abstract

Not only in the biomedical field but also in other applications, synthetic polymers are gradually being replaced by biodegradable materials, especially by those derived from natural resources. In this regard, many types of natural polymer or biopolymers have been developed to satisfy the ever-increasing application requirements. Since the demand for biomedical materials grows, significant attention is being given to tailor the structure, properties, and function of biopolymers to fulfill the requirements for applying them in biomedicine. Due to their inherent material properties, biopolymers are an appealing alternative to the synthetic polymers in the biomedical field. So far, a considerable number of natural polymers have been studied in detail regarding their suitability for applications in tissue engineering, wound-healing, bone regeneration, and drug delivery. Most of these biopolymers can be classified in the polyester, protein, polysaccharide, lipid and polyphenol families. In this chapter, the importance of biopolymers in the biomedicine is evidenced, and the main and most recent advances of the principal natural polymers used in this field are briefly reviewed, paying special attention to the natural biopolyesters, the PHAs family.

1. Introduction

Biomedicine is the theoretical branch of medicine that applies the principles of biology, biochemistry, and biophysics for the understanding of medical research and its practice. On one hand, an emerging area in biomedicine is that of biomimetic materials and systems. On the other hand, there is an imminent need for developing new materials for specific purposes in particular medical fields.

The main objective of implantable devices and biomedical structures is to mimic a body's system and/or to replace a damaged organ in order to maintain normal body functions. The three main families of materials, metals, ceramics, and polymers, have been applied to this purpose. However, they may present some disadvantages like immunological rejection by the body [1]. Especially, synthetic polymers may present concerns about their biodegradation products since they can lead to an undesirable immunogenic response [2, 3]. In general, it is difficult to mimic living systems and satisfy the growing biomedical needs with conventional synthetic materials alone. In some cases, the combination of both synthetic and natural materials can be a solution [4-7]. Nevertheless, biopolymers have been highlighted among the traditionally used materials and have been established as a promising class of biomaterials with a wide range of applications in biomedicine. Since they are produced by living organisms, biopolymers show unique properties such as degradability and biocompatibility, which provide them with advantages over other material families. They represent a solution for many biomedical applications due to the combination of their inherent properties, including great versatility and processability, biocompatibility, biodegradability, bio/absorbability and absence of cytotoxicity, all of which are essential properties that a material used for medical applications should possess. Thus, several studies using biopolymers as biomimetic materials are frequently found in the literature. For instance, Ochetta et al. [8] mimicked a fibrosis-like environment by embedded cardiac fibroblasts in a 3D fibrin-hydrogels. Bazrafshn et al. [9] recently reviewed the use of chitosan to mimic some body fibrous assemblies. Another recently used biopolymer to mimic the carbohydrate moieties of mammalian glycosaminoglycans is a sulfated polysaccharide found in the cell walls of the brown algae [10]. Examples of how biopolymers can be used in specific situations in biomedicine are the preparation of natural biocomposites. With the aim of reducing drug consumption, Ye et al. [11], prepared a biocomposite based on porous chitosan with silver nanoparticles that promoted wound healing and showed good antimicrobial activity and biocompatibility. Sharabi et al. [12] recently dressed one of the challenges of future research for the replacement or repair of the degenerated intervertebral disc. They developed a complex 3D biocomposite of long collagen fibers embedded in alginate hydrogel, which mimics the form of annulus fibrous lamellar. The mechanical behavior was found to reproduce the natural stress-strain behavior.

Since there is a continuing development and design of new systems involving biopolymers

for biomedical applications, the focus of this chapter is to provide a brief overview of the more recent advances in the application of the main biopolymers used in medicine, with emphasis on the of natural biopolyesters, the PHAs.

2. Biopolymer Recent Market and Environmental Aspects

We are now well aware of the environmental problems related to the huge quantities of wastes produced by human activity, especially in regards to plastic. Fossil-based polymers correspond, in general, to non-biodegradable materials, which leads to two principal problems: the accumulation of waste in natural environments, including the sea with negative effects on marine fauna through plastic ingestion, and the leaching of plastic products with the potential to transfer chemicals to human beings and wildlife [13]. Despite of that, the global production of plastics is increasing every year (according to Consumer News and Business Channel, more than 9 billion tons of plastic have been produced worldwide since the 1950s, of which 9% was recycled, 12% was incinerated and 79% was built up in landfills or disposed indiscriminately). As a result, there is a growing realization that organic matter from biological origins, with mainly a polymeric structure, can be a solution. Thus, there is a need to continue developing biotechnological processes to achieve large scale production of these natural occurring polymers. However, one of the major remaining concerns is the high production costs that present biopolymers from being economically competitive. Nonetheless, this market is continuously growing, and sophisticated biopolymers are emerging along with innovative applications in different fields, including that of biomedicine, and other new products. According to the latest market data collected by European Bioplastics in cooperation with the research institute Nova-Institute, global bioplastics production capacity is set to increase from around 2.11 million tonnes in 2018 to approximately 2.62 million tonnes in 2023. However, the annual capacity growth rate for bio-based polymers has been slowed down sharply since 2015 (reduced more than half) . This lower annual growth rate is mainly caused by the decrease of oil prices, low political support, a slower than expected growth of the capacity utilization rate and the populist debates about using food crops for industry use [14]. It is believed that, during the next few decades, the demand for these products will rapidly increase and they will be widely used in a broader range of applications.

In the late 1980s and early 1990s, innovative biopolymers were introduced to the market for the first time, and were mainly based on starch and polyhydroxyalkanoates (PHAs) produced by fermentation. These biodegradable first-generation biopolymers did not successfully become established in the market, mostly due to their yet unknown material properties, unfavorable political and economic circumstances, and a lack of political will [15]. In recent years, improved second-generation biopolymers have been developed almost exclusively as degradable and compostable materials for the packaging, agriculture and gardening sectors [15]. The trend among the third-generation biopolymer materials is away from degradability and instead

towards resistance (15). In parallel, the use of biopolymers from different origins has been investigated for many years for pharmaceutical and biomedical applications. This has resulted in a multitude of healthcare products on the market that is biopolymer-based. Nowadays, biopolymer production for biomedical applications only corresponds to approximately 1% of the annual polymer production. However, an increase of 19% is expected for 2020 compared to 2017. Among other natural polymers such as dextran, xanthan gum, and pullulanin, polylactic acid (PLA) and PHAs are the most recognizes ones for contributing to this increase [16].

3. Biopolymer Definition, Main Properties and Classification

Natural polymers or biopolymers may be defined as naturally-occurring polymeric macromolecules synthesized during the life cycles of plants, animals, bacteria or fungi [17, 18]. Since they are generated from renewable sources and their structural backbone is composed of oxygen and nitrogen atoms, they are easily biodegradable [19]. Biodegradation converts them into CO_2, water, biomass, humid matter, and other natural substances [20], making them harmless and non-toxic for the human body. As a result of their suitable properties such as good biocompatibility, biodegradability, and non-toxicity combined with versatile mechanical properties, there has been a growing demand for biomedical biopolymers in the last years, as well as an increase of their number and class [17]. Therefore, there are several classifications for biopolymeric materials. Usually, they are divided according to their repeating monomeric units in polynucleotides (DNA and RNA which are formed by nucleotide monomers), polypeptides (amino acids are their monomeric units) and polysaccharides (different carbohydrates structures) [17, 21]. They can be classified by their origin, depending on the synthesis and on the sources: from biomass (polysaccharides, protein and lipids, from animal or plants), from microbial production (PHA), from chemical synthesis using monomers obtained from agro-resources (such as PLA), and polymers whose monomers and polymers are both obtained by chemical synthesis from fossil resources (such as polycaprolactones, polyesteramides, aliphatic and aromatic co-polyesters). Biopolymers obtained from non-renewable resources are also included [22, 23]. The United States Congress Office of Technology Assessment classifies them into nucleic acids, proteins, polysaccharides, polyhydroxyalkanoates and polyphenols [17]. Their source origin classifies them in natural or semi-synthetic and based on their applications they can be bioplastics, biosurfactant, biodetergent, bioadhesive, or biofloculant [18]. In this chapter, a biopolymer classification based on the backbone of the polymer chain is presented (**Figure 1**). Special attention must be given to poly(lactic acid) (PLA), which in several cases is considered as a synthetic polymer. The production of PLA is based on the production of the lactide monomer from lactic acid, which is produced by the fermentation of agricultural source corn [24]. Then, high molecular mass PLA is produced by ring-opening polymerization of the lactide. In our classification, we consider PLA as a biopolymer since it is made from renewable resources.
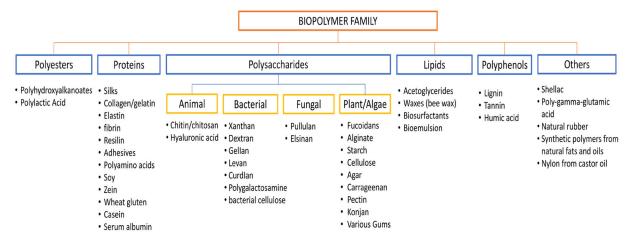
Figure 1: Biopolymer backbone-based classifications.

4. Most Recent Advances of the Main Biopolymer used in Biomedicine

4.1. Polyhydroxyalkanoates (PHAs)

PHAs represent a family of biopolyesters synthesized by several microorganisms. They are intracellular storages of carbon and energy, accumulated in the shape of granules [25]. They are produced when nutrients such as nitrogen, phosphate or oxygen are depleted, and there is an excess of carbon source. Under these conditions, microorganisms can divert the usual carbon flux (conversion of acetyl-CoA in the tricarboxylic acid cycle to create energy and metabolites for biomass formation) towards the synthesis of PHA [26,27]. PHAs can be produced by biotechnological processes *via* bacterial fermentation. *Cupriavidus necator* is the most extensively studied bacterial strain for PHA production on an industrial scale. *Azahydromonas lata* (formerly known as *Alcaligenes latus*), *Azotobacter sp.* and recombinant *Escherichia coli*, are among the PHA-producer bacteria, but to a lesser extent [28,29]. Especially, extremophile bacteria such as halophiles or thermophiles are of great interest in the production of PHAs [30]. Gram-negative halophile PHA-producers such as *Haloferax mediterannei*, *Halomonas campaniensis LS21*, *Haloarcula marismortui*, *Halomonas TD0*, *Bacillus megaterium* uyuni S29 have been reported for their high PHAs production. The strain *Chelatococcus sp*. is an example of a thermophile bacteria that is also studied for its ability to synthesis biopolymer [30]. Several microorganisms secrete extracellular PHA-depolymerases to degrade the biopolymer into oligomers and monomers, so that they can consumed these degradation products as nutrients [31]. This is the reason why these kinds of polymers has the inherent property of being biodegradable.

Chemically, PHAs are linear biopolymers composed of hydroxyalkanoate units (HA) as the basic structure (**Figure 2**). PHAs are biocompatible, biodegradable and non-toxic, and their members differ in their structure and mechanical properties, depending on the producing microorganism, the conditions of biosynthesis, and the type of carbon source used in the production process [32-34]. Poly-3-hydroxybutyrate (PHB) is the simplest and most commonly

produced PHA. It is a linear, unbranched homopolymer consisting of (R)-3-hydroxybutyric acid (HB) units [35]. Because of its competing thermoplastic and mechanical properties, which are similar to those of petroleum-derived plastics such as polypropylene, it is gaining interest as a substitute for these synthetic polymers [30,35]. Besides HB, microorganisms can incorporate up to 60 different types of monomers in their inner storage (**Figure 2**). For instance, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), a PHB copolymer, can be produced by adding valeric acid to the fermentation medium [36]. PHBV is characterized as less crystalline and more flexible than the PHB itself [37]. These properties vary according to the hydroxyvalerate content in the structure: a higher hydroxyvalerate monomer number leads to a lower crystallinity and to greater flexibility, strength, and elongation at break [34]. Another member of the PHA family is poly-4-hydroxybutyrate (P4HB), a resorbable, thermoplastic homopolyester with a linear chain structure of 4-hydroxybutyrate (4HB) monomers. It can be produced by using sodium 4HB as a precursor for its synthesis [38,39].

Globally, PHAs have gained considerable commercial interest in fields such as pharmaceuticals, veterinary scince, food packaging, agriculture, industry and especially in medicine because of their unique material properties [40]. Besides their non-toxic nature, biodegradability, and biocompatibility, they have antioxidant properties, optical activities, piezoelectric property, impermeability to gas, good resistance to ultraviolet, resistance to hydrolytic degradation, thermoprocessability, and stereospecificity [41]. In biomedicine, PHAs have been exploited in numerous forms when performing tissue engineering to repair the liver, bone, cartilage, heart tissues, cardiovascular tissues, bone marrow, and nerve conduits [34,38, 42,43]. Tissue engineering is an interdisciplinary field of research focused on the creation of vital tissues by a combination of biomaterials, cells, and bioactive molecules, aiming to repair damaged or diseased tissues and organs [44]. In this regard, P4HB has acquired importance in this field due to its unique set of properties and advantages: lower modulus, higher elongation and flexibility, and the ability to be oriented so as provide tensile strengths comparable or superior to existing resorbable synthetic polymers, such as poly(glycolic acid) (PGA) or PLA. kai *et al.* [38] review a list of applications where P4HB is used as heart valves, stents, and cardiovascular and pulmonary patches.

The degradation rate of a polymeric material is important for its exploitation as a biomaterial. Because PHAs have a biodegradable nature, they are used for absorbable sutures, surgical pins and staples, delayed drug release, and as drug carriers. Ali and Jamil [45] reported that PHB degrades more *in vitro* and in living mammalian cells than the other synthetic polymeric materials like PLA or poly(lactic-co-glycolic acid) (PLGA). The importance of degradability is reflected in the application of drug delivery systems. The latter consist of a material with an encapsulated active principle that is introduced inside the body to reach a located point for healing. It is by means of their biodegradation in the tissues of the host organism, that the

materials provide the liberation of bioactive substances. Drug discharges can be regulated over a determinate period of time, depending on the rate and degradation process of the material used. Recently, the use of PHAs in the form of coatings and micro- and nano-particles as resorbable matrices for controlled drug release has been reported [46-48]. **Figure 2 d** and **e** show Field Emission Scanning Electron Microscope (FESEM) images of these studies. These recent publications discuss the objective of overcoming implant-related infection and bacterial load on the implant surface. This promising strategy consists of using PHA biopolymers as drug carriers to control the release of antibiotic by the biopolymers degradation [41,48].

The versatile structure of PHA can be modified simply through physical blending and chemical alteration to improve its efficacy for medicinal use. PHAs have therefore been used in combination with other materials for fine-tuning their mechanical properties, and increasing their range of applications [49,50]. Recently, more sophisticated and complex PHAs have been developed. Examples include the production of PHBVHHx (consisting of a copolymer of HB, HV, and HHx) microspheres to serve as a carrier or scaffold to support cell growth for injectable purposes [51], and the poly(3-hydroxyhexanoate-co-3-hydroxyoctanoate-co-3-hydroxydecanoate-co-3-hydroxydodecanoate) being used as a scaffold for tissue engineering [52].

Although the major obstacle for the broad commercial utilization of PHAs is that their production costs are still higher than synthetic plastics, PHAs show several advantages when compared with other synthetic polyesters such as PLA, PGA, and their copolymers, polycaprolactone (PCL), and PLGA, all of which are examples of biodegradable polymers used in biomedicine [53,54]. The first advantage is that the biopolymer production *via* fermentation prevents the presence of toxic products in the synthetic polymerization process. Second, the hydrolytic degradation of PHB leads to obtaining monomer D-3-hydroxybutyric acid, which is a common blood constituent (a ketone produced by the liver from fatty acids, ketogenesis). Third, the use of extremophile bacteria for PHAs production not only enables their cultivation under drastically reduced or even absent sterility precautions, but also reduce factors affecting the production cost of these biopolymers, the sterilization process [30,48]. Fourth, they can be produced from renewable resources, low cost raw and/or waste materials and this allows their production to be pollution-free and independent of the oil industry [28,29,52,55].
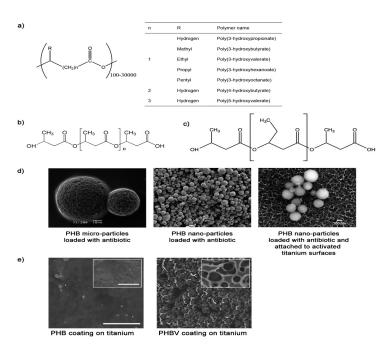
Figure 2: PHAs Chemical Structure and Application as Drug Delivery Systems: (a) PHAs are biopolyesters with hydroxyl and the carboxylic groups of the hydroxyalkanoic acids linked together via oxoester bonds. (b) PHB chemical structure. (c) PHVB chemical structure. (d) FESEM images of antibiotic-loaded PHB micro- and nano-particles for drug delivery systems [47]. FESEM images of antibiotic-loaded PHB nano-particles covalent attached on activated titanium surface [48]. (e) FESEM images of PHB and PHBV coatings loaded with antibiotic. The scale bar corresponds to 300 μm. The inset images correspond to higher magnifications micrographs. Scale bar corresponds to 40 μm [41].

4.2. Proteins: Collagen

A variety of proteins and protein-derived products (polypeptides) have been used and characterized for their use in medical and pharmaceutical applications. Protein-based matrices such as gelatin, albumin, elastin, casein, collagen, corn protein, and whey protein have been applied in biomedicine to form micro- or nano-spheres, hydrogels, films, and scaffolds [56, 57]. Among all these proteins, collagen is highlighted as one of the most abundant biopolymers within biomimetic materials and it is widely used in different areas of biomedicine.

The word collagen has a Greek origin and can be divided into "kola" and "gen", which means gum and producing [58]. According to the Protein Data Bank, it is the most abundant fibrillar protein, and is available in the extracellular matrices of many connective tissues of mammals including skin, joints, cartilage, teeth (collagen joined to mineral crystals), tendon, bones, and others [58]. This structural protein comprises about 25-35% of the whole-body protein content, and its main function is to provide mechanical stability, strength, and elasticity to native tissue [9]. Since the discovery of collagen II by Miller and Matukas (1969) [59], 29 new collagen types have been found [60]. Various types of collagen, their tissue distribution and functions are widely described in the literature. Recently Muthukumar *et al.* [61], Lin *et al.* [60] and others [58] summarized this information. Among all collagen types, type I forms over 90% of the collagen of the body [9]. The structure of the collagen types can be grouped into fibrils, networks, beaded filaments, anchoring fibrils, and fibril-associated collagen with interrupted triple helices. These types of fibrils are the most common form, distributed in

most connective tissues [60]. **Figure 3** from Lin *et al.* (2019) show the fibrillar structure of collagen, from the proteins to the collagen fibers.

Collagen sources include bovine, porcine, and human origin, with bovine and porcine being the most commercialized. However, these together with other collagen sources used such as chicken neck (type I, II, III and V), kangaroo tail, rat-tail tendon, bird's beak, equine skin, cartilage and flexor (type I and type II), alligator bones and skins, sheepskin, frog skin, and so on, they are associated with the risk of transference of zoonotic diseases [58,62]. Recently, marine collagen, extracted from various marine sources (predominantly scale and skin fish) has emerged as the most appropriate alternative [63,64].

Due to its excellent properties, such as low immunogenicity, biodegradable, biocompatibility, hydrophilicity, easy processing, and weak antigenicity, collagen has become the primary resource of protein in medical applications [65,66]. However, collagen suffers from poor physical and chemical properties such as mechanical strength, thermostability, and resistance to enzymes [66]. Due to the extraction process, its mechanical properties and stability are lesser than those in its natural state. This seriously limits its potential in biotechnological applications. Consequently, crosslinking is a wide recognised solution for the improvement of its properties [67]. Exogenous crosslinks have been used to modify the molecular structure of collagen to minimize degradation and enhance mechanical stability [67]. There are different crosslinking methods, including physical, chemical and biochemical modifications. Physical crosslinking is carried out *via* UV or gamma radiation. For chemical modification - the most effective and most widely used crosslinking method for collagen - glutaraldehyde, isocyanates, hexamethylene diisocyanate, polyepoxy compounds, as well as plant extracts or inorganic crosslinking agents are the most utilized. Of recent, low toxic chemical crosslinking agents based on traditional biomasses such as dialdehyde cellulose or oxidized starch, are also employed. Enzymatic modification with oxidoreductases, transferases, and hydrolases is known as biochemical crosslinking [66, 67]. In this regard, there are however new studies that discuss the improvement of the mechanical properties of collagen by other alternatives. For instance, Rieu *et al.* [68] by novel process for collagen production, developed a collagen-only, non-cross-linked scaffolds with uncommon mechanical properties which they applied to 3D cell culture. As well, the blend of collagen with other biomaterials and biopolymers is another alternative to prepare collagen-based biocomposites with more suitable physical and mechanical properties [69].

The most relevant and advanced applications of collagen in biomedicine are: (1) Shields in ophthalmology [70-72], (2) sponges for burns and wounds [73-76], (3) mini-pellets for protein and drug delivery [56], (4) controlling material for transdermal delivery [77,78], (5) nanoparticles for gene delivery [79], (6) drug/gene delivery formulations for tissue healing, used in the form of film [80,81], sheet [82], disc or scaffolds [83], (7) 3D scaffold or gels

for cell embedding (68, 84-88), (8) organoids or neo-organs for gene therapy [89], (9) tissue engineering including skin replacement, bone substitutes, and as artificial blood vessels and valves [61,67].
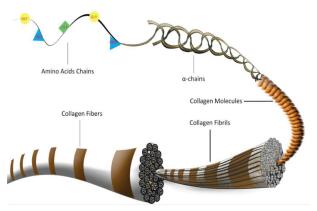

Figure 3: Collagen Structure [60]: Collagen is composed of specific amino acids including glycine, proline, hydroxyproline as the smallest units. According to particular alignment with other amino acids, it becomes *peptide chains* (α1, α 2, β chain). Three of the same or different peptide chains tangle together form triple helices. This is called *collagen molecule*. Many triple helices crosslinked together form *collagen fibrils*. Several of collagen fibrils crosslink together to become *collagen fibers*.

4.3. Polysaccharides

Natural polysaccharides have been recognized and applied as viable candidates for various biomedical, pharmacological and biotechnological applications. Within these fields, saccharides, oligosaccharides, and polysaccharides are used for bioactive therapies, diagnosis, controlled drug release, gene therapy, cell-encapsulation, tissue engineering, and medical devices [90]. They are of special interest due to their high abundance, good biological performance, structural similarity to the extracellular matrix, and degradability by enzymes present in the body [91]. Polysaccharides can be obtained from a variety of sources including human and animal, bacterial, fungal or vegetal origins (**Figure 1**).

4.3.1. Polysaccharides from Animal Source: Chitosan

The exploitation of the sea as a renewable source of biocompounds provides a positive step in the development of new systems and devices for biomedical applications. Marine polysaccharides are among the most abundant materials in the seas. While alginate, carrageenan and fucoidan polysaccharides are extracted from algae, chitosan and hyaluronan can be obtained from marine animal sources. They show important biological properties like biocompatibility, biodegradability, and anti-inflammatory activity, as well as adhesive and antimicrobial actions. [92]. Among them, chitosan and its oligosaccharides have received considerable attention due to their biological activities and properties in commercial applications [93]. Chitosan is a molecule with a carbohydrate backbone structure similar to cellulose, consisting of two types of repeating units: N-acetyl-D-glucosamine and D-glucosamine monosaccharides, bonded together with a (1-4)-β-glycosidic linkage (**Figure 4**) [94-96]. It is a biopolyaminosaccharide natural polymer that is obtained by treating the chitin *via* alkaline deacetylation [96]. Chitin

was first isolated and characterized from mushrooms by the French chemist Henri Braconnot in 1811 [97]. Except for celluloses, chitosan is the most abundant polysaccharide in nature. It is the main component of the exoskeleton of crustaceans and insects, and also occurs in nematodes and in the cell wall of yeast and fungi (**Figure 4**) [17] [92]. Until recently, only marine sources (shrimp, prawn, crab) have been used to provide the starting chitin. Lately, new commercial chitosans, better characterized by manufacturers and with enhanced safety characteristics for certain pharmaceutical, cosmetic, and biomedical applications have been produced at lower costs [98]. Chitosan is one of the marine polysaccharides most widely used and studied for biomedical applications, not only because it has revealed some therapeutic activity such as lowering of cholesterol, wound healing, antiulcer, and antimicrobial effects [96], but also due to its non-toxicity (it has been approved by the US Food and Drug Administration), its biodegradability, and bacteriostatic and fungicidal characters [94,96,99]. Furthermore, it shows advantages in regards to its special used as drug carrier, and thus it has been extensively exploited in the preparation of micro-/nano-particles, beads, and capsules for controlled drug delivery systems [96,100-102]. Ahmed *et al.* [96] describe some of its advantages that make chitosan the appealing biopolymer for the development of polymeric particles: its mucoadhesive nature (which increases the time of attachment at the absorption site), the easy availability of free amino groups (for cross-linking),the ease of fabrication of polymeric particles without using hazardous solvents, the cationic nature that permits ionic cross-linking with multivalent anions, and its ability to control the release of an administered drug. Also, membranes, films and scaffolds **[**94,103**]** of chitosan have benn developed for tissue engineering, regenerative medicine and therapy [100]. Recently, Bazrafshan *et al.* [9] reviewed the use of chitosan to mimic fibrous assemblies. Chitosan can be also mixed with other synthetic or natural polymers in order to help its processability and fine-tuning its properties [94].
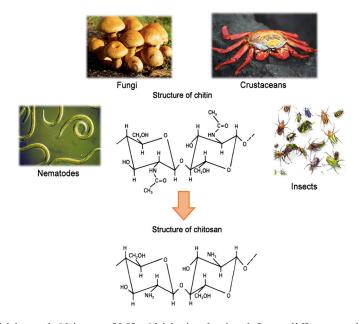

Figure 4: Structures of Chitin and Chitosan [95]: Chitin is obtained from different animal sources (nematodes, fungi, crustacean and insects) , especially from the demineralization and deproteinization crustacean shells and insect exo-squeleton. Then, chitosan is obtained by removing the acetyl groups (CH3-CO-) of chitin. This process, called deacety-lation, releases amine groups (NH2) and gives chitosan its cationic characteristic.

4.3.2. Polysaccharides from Bacteria Source

Most microorganisms are able to secrete exopolysaccharides (EPS's) naturally into the extracellular environment. They are high molecular mass biopolymers, showing extreme diversity in terms of chemical structure and composition. EPS's tend to be bioactive, depending on their backbones, chain length, and substitution [104]. The use of these bacterial EPS's in medical applications started with the first clinical trials on dextran solutions as plasma expanders in the middle of the 20th century [105]. Later, other bacterial EPS's such as xanthan or pullulan were used in medicine as pharmaceutical excipients (as suspension stabilizers and in capsules and oral care products, respectively). A number of other EPS's has been added to the list, counting with alginates used as anti-reflux, dental impressions, or as matrixes for tablets, hyaluronic acid (also called hyaluronan) and derivatives used in surgery, arthritis treatment, or wound healing, and bacterial cellulose applied in wound dressings or scaffolds for tissue engineering [105,106]. The following table summarises EPS's naturally produced by different microorganisms, and the most recent advances where they are applied in the biomedical field.

The use of microbes to produce EPS's shows several advantages over plant- or macro algae-derived products that make them more suitable for industrial and commercial use. The production time (the obtaining of EPS's from bacteria takes only days compared to months from plant-based products), the surface required (there is no land needed for cultivation), the controlled production with defined and reproducible parameters, and the high quality of the final product are some of these advantages [105,106]. However, the production cost is still one of bacterial EPS disadvantages, since the expenses are directly related to the cost of the substrate, required for microbial growth, as well as the cost of bioreactors to grow microorganisms in large quantities [106]. Even so, the possibility of finding new bacterial polysaccharides with bioactive properties and potential applications in the fields of pharmaceutics, cosmetics, and in biomedicine is still being investigated [107,108].

4.3.3. Polysaccharides from Fungal Source

There is growing interests in polysaccharides being isolated from mushrooms, which are recognized as safe and effective natural antioxidants. For a long time, mushrooms have attracted significant interest as traditional food and medicine. They are also used as functional health promotors because of they are biochemically composed of significant amounts of carbohydrates, lipids, proteins, enzymes, minerals, and vitamins. Polysaccharides such as pullulan, elsinan, and yeast glucans, which are among the most important active components of mushrooms, have been reported to possess broad-ranging and potentially valuable pharmacological properties in biochemical and medicinal areas, including anti-tumor, anti-inflammatory, immunomodulatory, and in particular, antioxidant activities.

Table 1: Application of some ESP in biomedicine

ESP	MICROORGANISM	APPLICATION	REFERENCE
Xanthan Gum	bacterium *Xanthomonas campestris*	Intra-abdominal adhesion, high thickening capacity, emulsifying, film forming, release control agent	[109, 110]
Gellan	*Bacterium Sphingomonas paucimobilis* (formerly *Pseudomonas elodea*)	Excipient in oral, ophthalmic and nasal drug formulations, gelling/thickening agents, drug release, scaffolds for bone tissue engineering applications, cell encapsulation.	[105, 111-115]
Dextran	Bacterium *Leuconostoc mesenteroides*	Molecule-carrier or drug delivery system, plasma volume expander, peripheral flow enhancer, antithrombolytic agent and for the rheological improvement for artificial tears	[105, 116, 117]
Alginate	Several bacteria strains *Azotobacter vinelandii, Pseudomonas aeruginosa*	Controlled drug release, encapsulation, scaffolds in ligaments, tissue engineering and in dentistry for the preparation of forms in the presence of slow-release calcium salt, cell microencapsulation	[118-120]
Hyaluronic acid/ hyaluronan	Bacteria *Streptococcus equisimilis/zooepidemicus, Bacillus subtilis*	Gelling/thickening agents, skin regenerating, collagen and elastin stimulating efficacy, drug release for treating tumor cells, skin regenerating and collagen stimulating efficacy	[112, 120-123]
Bacterial cellulose	Aerobic bacteria, belonging to the genus *Acetobacter* (primarily by *Gluconacetobacter xylinum)*	Artificial skin, artificial blood vessels and microvessels, wound dressing, implants and scaffolds for tissue engineering, carriers for drug delivery, wound-dressing materials	[124-127]
Levan	Different bacteria, *Bacillus polymyxa* PTCC1020, *Bacillus subtilis, Aerobacter levanicum, Erwinia herbicola, Streptococcus salivarius* and *Zymomonas mobilis*	Thickeners and encapsulating agents. film agent, a carrier for drug delivery systems, an anti-inflammatory compound or its potential use for functional food as prebiotic.	[128-130]
Polygalactosamine	Bacterium *Paecilomyces sp*	Growth inhibitor of some tumor cells. With Chitosan microspheres for drug delivery system	[131]
CurdIan	*Agrobacterium* species	Inhibit tumors, anti-HIV effect, tablets and gels for drug delivery	[132]

Among these bipolymers, pullulan is a natural linear homo-polysaccharide obtained from the polymorphic fungus *Aureobasidium pullulans*. It consists of three glucose units attached by $\alpha(1\rightarrow4)$ glycosidic linkages, which are attached to each other by $\alpha(1\rightarrow6)$ glycosidic linkages [16,133,134]. There is extensive work to improve the production of pullulan as well as its yield by changing fermentation parameters or the substrate used in order to improve their economic viability [16,135-138]. Pullulan exhibits unique physicochemical properties such as high water solubility and biodegradability. This is due to the coexistence of different glycosidic bonds [133,138]. Pullulan is used as a stabilizer, an adhesive, and a coating or packaging material in the food industry. Because of its inherent non-toxic, non-immunogenic, and biodegradable characters [133], it also offers a wide range of potential applications in biomedicine such as targeted drug/gene imaging and tissue engineering. In particular, pullulan has been used as hydrogels for tissue engineering. Wong *et al.* [139] demonstrated that pullulan hydrogels are an effective cell delivery system, and improve mesenchymal stem cell survival and engraftment in high-oxidative-stress environments. From pullulan, Autissier *et al.* [140]

prepared and evaluated a novel biomaterial for vascular engineering, consisting of pullulan gels with water-content higher than 90%. Pullulan-collagen composite hydrogel matrices were fabricated by Wong *et al.* [141], resulting in a structured yet soft scaffold for skin engineering. More recently, the novel topical film prepared with vernicifula extract-loaded pullulan hydrogel was synthesized for atopic dermatitis treatment [142]. Similarly, Zhang *et al.* [133] developed a gelatin hydrogel with oxidized-pullulan. which gave extraordinarily high strength and mechanical enhancement to the hydrogel. Hydrogels-based scaffolds [143], microbeads [144] and composites [141,145] were also created with this fungal polysaccharide.

4.3.4. Polysaccharides from Plant Source: Starch

A substantial amount of research indicates that polysaccharides derived from herbs can be effectively used in many applications and have diverse therapeutic properties such as antioxidant, antitumor and immunostimulatory activities, and the effect of promoting wound healing [146]. Starch is one of the most abundant polysaccharides from plant origins, and has been used in food applications such as a thickening, binding, sweetening, and also as emulsifying agents [147]. It is mainly obtained from cereals and tubers. Chemically, starch is a polymeric carbohydrate composed of glucose units linked together, comprising two types of α-glucan: linear amylose (poly-α-1,4-D-glucopyranoside) and branched amylopectin (poly-α-1,4-D-glucopyranoside and a-1,6-D-glucopyranoside). Therefore, it is stablished as a heterogeneous material. This polysaccharide is produced from agricultural plants, mainly potatoes, rice, maize, and wheat. Depending on the botanical source, the percentage of each glucan type varies, as well as the whole morphology and molecular structure. Starch is a water-soluble biopolymer that produces viscous dispersions, solutions, or gels at low concentrations [13]. Structurally, native starch occurs mostly in the form of semicrystalline granules, with a complex hierarchical structure. These granules are generally composed of an amorphous bulk core surrounded by altered concentric semicrystalline and amorphous growth rings. Its availability of hydroxyl groups makes it tremendously hydrophilic and easy to chemically react (esterification, oxidation, etherification, and cross-linking) [148]. **Figure 2a** shows a scheme from Wang *et al.* [149] of the starch structure.

Due to its extensive availability, low cost and total composability without generating any hazardous residues, starch is used for a number of biomedical applications such as tissue engineering, wound healing, bone regeneration, and drug delivery, and it has also been used for adhesion, proliferation, differentiation, and regeneration of cells [13,44]. The employment of starch for biomedical functions is also appealing due to its similarity to the native cellular environment [44]. In order to enable applications in tissue engineering, starch has been manipulated to improve some of its properties such as its mechanical properties and moisture sensitivity [44]. Starch alone is inadequate to develop scaffolds. However, its mechanical stability can be improved to convert the material to an appropriate option. For

instance, Waghmare *et al.* [44] developed starch-based nanofibrous scaffolds using polyvinyl alcohol (a non-toxic, water-soluble, biocompatible, synthetic polymer) as the plasticizer and glutaraldehyde as a crosslinking agent for application in wound healing. The evaluation of the nanofibrous scaffolds in cellular assays demonstrated their non-toxicity and their ability to promote cellular proliferation. The strategy of employing starch as matrix not only reduced production costs, but also endowed the products with the features of biodegradation, biocompatibility and specific interactions with biological systems. Among other biopolymers such as alginate, gelatin, and collagen, starch is also used for bone substitution to fabricate scaffolds for bone tissue engineering. Aidun *et al.* [148] recently reported the fabrication of a bioactive porous scaffold of starch-siloxane for bone regeneration by cross-linking with 3-glycidoxypropyltrimethoxysilane as a biocompatible and hydrophobic material. The ability of the growth and proliferation of bone marrow mesenchyme stem cells on the constructs confirmed the suitability of these scaffolds for bone tissue engineering applications. **Figure 5** shows the surface topography of the starch-siloxane scaffolds. In regards to drug delivery system, starch has been used as particles and hydrogels. The group of Shi *et al.* [150] synthesized starch-based fluorescent organic nanoparticles for biomedical applications, while Gholamali *et al.* [151] recently developed a novel type of nanocomposite by combining copper oxide nanoparticles with oxidized starch hydrogels as a controlled drug delivery system (**Figure 5d**).
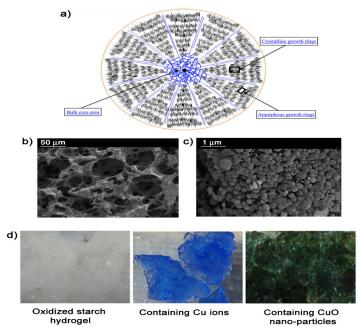

Figure 5: Inner Starch Structure: (a) Stylized model representing the distribution of amylose and amylopectin molecules. The blue lines in represent amylose molecules, and the black lines represent amylopectin molecules (149). FE-SEM images of (b) freeze-drying starch-siloxane scaffold, and (c) mineralized hydroxyapatite on the scaffolds [148]. (d) Photography's of oxidized starch hydrogels [151]. Copper ion (Cu) and copper oxide (CuO) nano-particles were incorporated into the hydrogel matrix.

4.4. Lipids: Biosurfactants and Bioemulsions

Microorganisms produce a variety of surface-active compounds (SAC), classified as biosurfactants and bioemulsifiers. The terms "Bioemulsifiers" (BE) and "Biosurfactants" (BS) are not interchangeable, and their definitions are based on their physico-chemical properties and physiological roles [152]. The natural SAC have become important products of biotechnology for industrial, pharmaceutical and biomedical applications [153,154]. As they are mostly produced on microbial cell surfaces or excreted extracellularly, they can be produced *via* fermentation using cheap agro-based substrates and other waste materials; unlike the synthetic surfactants, which are petroleum-derived. In general, they are amphiphilic compounds composed by both hydrophobic and hydrophilic groups that confer the ability to accumulate between fluid phases and reduce their interfacial tension [154,155]. These microbial SAC have different chemical structures, and surface properties, and are mainly classified according to their chemical composition, microbial origin, mode of action, molecular mass, and general physico-chemical properties [155].

4.4.1. Biosurfactants (BS)

BS are low-molecular-mass molecules microbial products, generally glycolipids, lipopeptides, and proteins with a lower surface and interfacial tensions between different phases [155]. The glycolipids (rhamnolipids, sophorolipids, trehalose lipids) consist of different sugars linked to β-hydroxy fatty acids, while lipopeptides (surfactin, iturin, fengycin) consist of cycloheptapeptides with amino acids linked to fatty acids of different chain lengths. These amphiphilic molecules are soluble in both polar and non-polar solvents [152]. The important features that biosurfactants have as compared to chemically synthesized surfactants are their biodegradability, bioavailability, lower toxicity, higher foaming, and high specific activity at extreme pH, temperature and salinity [156].

The best-studied glycolipids BS are rhamnolipids synthetized by several species including *Pseudomonas aeruginosa*. They are usually produced as a mixture of two or four species, by natural fermentation. They differ by the length of hydrophobic chains (from C8 to C12) some of which are unsaturated with one double bond (**Figure 6**) [157]. Rhamnolipids are biodegradable low toxic BS, with antimicrobial and anti-biofilm-formation properties [158, 159]. Therefore, rhamnolipids are used as a biofilm control agent to prevent medical device-related infections and to inhibit biofilm formation. They are also an anticancer agent, which inhibits the growth of many f the human cancer cell lines [160]. An example of the biomedical application of these biosurfactants is described in the recently published work of Jovanovic *et al.* (159) who used rhamnolipids to prevent adhesion and biofilm formation of *Candida albican.*
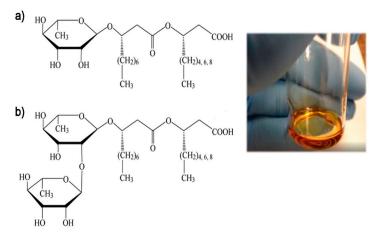
Figure 6: Structure of Rhamnolipids: Under typical growth conditions with Pseudomonas aeruginosa, two main rhamnolipids homologues are obtained: (a) monorhamnolipid (RL-1) and (b)dirham-nolipid (RL-2) [157]. Image of Rhamnolipids extract [3].

Most *Bacillus* species were found to be able to produce lipopeptides BS such as *Bacillus pumilus*, *Bacillus cereus*, *Bacillus thuringiensis*, and *Bacillus licheniformis* [161]. Studies show that most of these *bacilli* can produce one type of lipopeptide, and just a few of them can produce two or the three types together [161]. In the case of surfactin, *Bacillus subtilis* K1 and *Bacillus siamensis* are known to extracellularly secrete it [152]. Surfactin is a cyclic lipopeptide that exhibits very good emulsification activity as well as excellent emulsion stability, and it has been found to be a better surface active agent in comparison to iturin and fengycin [156]. This BS is used in the biomedicine field as an antibacterial, antiviral [162], anti-tumoral, anti-coagulant agent, and shows broad-spectrum inhibition activities [152]. In a very recent study, the surfactin antibacterial activity against various Gram-positive and Gram-negative bacteria was confirmed [163]. An application of surfactin in biomedicine is seen in the recent work of Wang *et al.* [164]. They developed a novel "mosaic-type" nanoparticle system for selective drug release targeting hypoxic cancer cells, by assembling nanoparticles with surfactin. Another example is the study of Xing *et al.* [165], who used iturin together with surfactin in the form of enteric-coated insulin micro-particles for the oral drug delivery.

4.4.2. Bioemulsifiers (BE)

Several bacterial species from different genera produce extracellular polymeric emulsifiers composed of polysaccharides, proteins, lipopolysaccharides, lipo-proteins or complex mixtures of these biopolymers [152,153]. They are high molecular mass BE, which bind tightly to hydrocarbon surfaces and form stable emulsions by increasing kinetic stability in very low concentrations [152,155]. The first well-studied BE is Emulsan RAG-1 (1000 KDa) which is an extracellular poly-anionic BE produced by Acinetobacter calcoaceticus RAG-1 [155,166]. Yi et al. [167], recently used Emulsan RAG-1 to create oil in water-type nanoparticles loaded with pheophorbide to create a drug delivery system for treating tumor tissue. Another well-studied BE with potential environmental and biomedical applications is Alasan (45-230 KDa). It is produced by Acinetobacter radioresistens and is a complex union

between anionic polysaccharide and protein. In the case of Alasan, if the protein portion is damaged (by being digested by proteolytic enzymes), the BE turns into a thick polysaccharide, losing its emulsifying properties [155,166].

4.5. Polyphenols: Tannins and Lignins

Natural phenol-based polymers are widely represented in nature, and they include a variety of classes like tannins and lignins, which are the most prominent. Polyphenols are especially found in highly consumed foods: grape skin and seeds, seaweeds, wood and agro-wastes, primarily grape pomace and other by-products of fruit and coffee processing [168, 169]. Several phenolic polymers have been evaluated as biomaterial additives to favor cell growth and differentiation. Thanks to their antioxidant and antimicrobial properties [170], polyphenols have been found to stimulate bone formation, and mineralization, as well as stimulate the proliferation, differentiation, and the survival of osteoblasts [171]. They are able to counteract the inhibitory effects of reactive oxygen species (ROS) during the process of bone formation by osteoblastic cells [169]. Furthermore, polyphenols improve the performance of biomedical devices used in cardiovascular systems by improving the mechanical properties of grafted heart valves, enhancing microcirculation through the relaxation of the arterial walls, and improving capillary blood flow and pressure resistance [171].

The recently discovered phlorotannins are a peculiar class of tannins that are produced exclusively by marine brown seaweeds [172,173]. They show very especial properties such as antimicrobial, antioxidant, anticancer, radiation protection, anti-coagulant and other pharmacological activities [169,172]. Especially, they are effective in enhancing osteoblast differentiation and promote intracellular calcification [169]. In an application of these tannins, Douglas *et al.* [174] enriched mineralized gellan gum hydrogels with phlorotannins to endow antibacterial properties and promote mineralization with calcium phosphate uptake. More recently, Park *et al.* [6] fabricated a poly(vinyl alcohol) hydrogel for wound healing application, which showed an increase in cell attachment and proliferation when phlorotannins were added to the system. The study for hard tissue regeneration of Im *et al.* [175] demonstrated that their polycaprolactone scaffolds supplemented with collagen extracted from fish skin and phlorotannins exhibited marked calcium deposition and osteogenesis abilities compared to the ones without polyphenols supplements.

Lignocellulosic biomass is the most promising renewable carbon-containing source on Earth. Depending on the origin and species of the biomass, lignin consists of 20–35% of the lignocellulosic biomass. After it has been extracted, lignin can be modified through diverse chemical reactions [176]. The interest in lignin for biomedical applications lies in its specific antioxidant and antimicrobial activities. Lignin is utilized as a renewable macromolecular building block for the preparation of polymeric drug encapsulation and scaffold materials

[177,178]. For example, Kai *et al.* [179] created nanofibers of PLA-lignin copolymers further blended with poly(L-lactide) (PLLA) and demonstrated that the addition of lignin protects cells from oxidative stress conditions. Among the recent studies with lignin, Vinardell *et al.* [178]] reviewed some of their pharmacological activities for the treatment of diabetes and obesity control, along with other properties such as its antiviral, anti-coagulant and anti-emphysema, activities, and their application as nanoparticles for drug delivery. Figueiredo *et al.* [176] reviewed recent developments in the design and fabrication of lignin-based nanostructures for biomedical applications.

5. Conclusions

There is an increasing awareness of the danger of synthetic materials, and the negative environmental consequences that come with their excessive use. As a result, there is a growing motivation to use more natural resources or substitute synthetic materials by other ones with less environmental impact. Since synthetic polymers are now known to be a threat to the environment, natural polymers have come to play an important role in different areas of application. In the field of biomedicine, biopolymers show many advantages precisely because of their natural origin. Many biopolymers show common properties such as biocompatibility, biodegradability, and non-toxicity, which make them very appealing for their application not only in biomedicine but also in other fields like pharmacology and biotechnology. Due to their similarity to the native natural environment, their biopolymer functions show good biological performance, adaptability and adequate body reaction. Their mechanical properties are proving to be very versatile in many biopolymer families. Furthermore, there are now technological advances which can vary and tune their mechanical properties by chemical and physical treatment to make them exploitable in a greater range of applications. In most cases, the production cost is still one of the drawbacks that make biopolymers not yet competitive with other synthetic materials, making it relevant to continue investigating the production processes in order to economically optimize their efficiency.

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