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Structure and Properties of Natural Biopolymers

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Abstract: Biopolymers are derived from polymers in which the prefix 'bio' refers to the living matter. They are produced by living organisms such as nucleic acids, polysaccharides, and amino acids and can be extracted from plants, or chemically synthesized from basic biological systems. Biopolymers are biocompatible (non-toxic), renewable, biodegradable because of the oxygen and nitrogen atoms found in their structural backbone, sustainable, eco-friendly, ease of handling, long-term stability, abundance, and simplicity of functionalization, Therefore, they are used in food, pharmaceutical, medical, and environmental applications. However, they have high production cost, ineffectiveness caused by the synthesis, development, and downstream processing processes.

Keywords: *natural biopolymers; biodegradation; collagen; chitosan.*

Definitions:

Degradable polymers are a broad term applied to polymers that disintegrate by physical, chemical, and biological mechanisms.

Biodegradable means that polymers will degrade under the enzymatic action of microorganisms into carbon dioxide and water which in turn are recycled in the nature.

Bio-based polymers are polymers derived from renewable resources such as plant sources[1].

Classification of biopolymers:

a) **According to the source of the raw materials and the biodegradability of the biopolymer [1]:**

1) **Biopolymers made from renewable raw materials (bio-based) and being biodegradable:** Such as poly (lactic acid) (PLA), Poly hydroxy alkanoates (PHAs), starch or proteins.
2) **Biopolymers made from renewable raw materials (bio-based), and not being biodegradable:** Such as polyamides from castor oil, and natural rubber.

3) **Biopolymers made from fossil fuels and being biodegradable:** Such as polycaprolactone (PCL).

b) According to the origin of the biopolymer:

1) Natural biopolymers:

- Polysaccharides such as cellulose, starch, and chitosan.
- Protein based biopolymers include albumin, gelatin, and legumin.

2) Microbial biopolymers: Poly hydroxy alkanoates (PHAs)

3) Chemically synthesized biopolymers: Poly lactic acid (PLA) and Polycaprolactone (PCL)[2].

Natural Biopolymers	Synthetic Biopolymers
• Biologically renewable. • Biocompatible (non-toxic). • Bio-adhesive material.	• Higher reproducibility. • Better mechanical and chemical stability. • Flexible in design and properties.
• Less stable. • Low melting point. • Structurally more complex.	• Less biocompatibility. • Expensive synthesis procedure. 6,7

Structure of Natural Biopolymers:

a) Polysaccharides:

- **Chitosan**: Chitosan is a straight chain polysaccharide that occurs naturally or can be obtained by deacetylation of chitin. Chitosan has reactive functional groups: amino and hydroxyl groups therefore, they have strong affinity with water and dilute acids, such as acetic acid, lactic acid, and inorganic acids. The solubility of chitosan depends on the concentration of amino groups. The amino groups of chitosan are highly reactive due to presence of free electron pair of nitrogen in the amino groups[3, 4]. The negatively charged surface of bacterial cells interacts with the positively charged amino groups in chitosan may cause damage to the cell wall and leakage of cell contents[3, 5]. Chitosan causes stimulation of inflammatory cells such as macrophages, fibroblasts and PMN neutrophils. Therefore, it could be used treatment of inflammation in the periodontium[3].

- **Hyaluronic Acid**

It is a linear mucoadhesive polysaccharide used in the preparation of gels for drug delivery and in wound healing by extracellular and epithelial regeneration. HA is characterized by ease of chemical functionalization, biodegradability, and hydrophilicity; therefore, it can maintain a humid environment and bond water molecules[6, 7].

- **Sodium Alginate:**

Alginate is a linear polysaccharide, a derivative of alginic acid. The main chain of sodium alginate contains many $–COO^-$ reactive groups. Therefore, it is used in drug and gene delivery, tissue engineering, wound healing, and taking oral impressions. It is characterized by biocompatibility, biodegradability, muco-adhesion, and low-cost production. However, it has poor mechanical properties and high degradation rate, therefore it could be combined with other polymers such as cellulose, chitosan and hyaluronic acid[8, 9].

- **Agarose:**

Agarose is a nondegradable polysaccharide. It is used in biomimetic remineralization. Agarose hydrogel can control the size and form of the hydroxyapatite crystal. It acts as a durable matrix for enamel rebuilding through interaction between the hydroxyl group of agarose and calcium, as well as providing a mineral reservoir for further remineralization. Moreover, agarose gel may be used as a scaffold for bone regeneration, especially when combined with hydroxyapatite (HA) or calcium carbonate ($CaCO_3$)[10, 11].

- **Cellulose:**

Cellulose is a high molecular weight (long chain) polysaccharide that is present in plant cell walls and could be produced by microorganisms. Each linear chain contains 1000-1500 glucose units. There are three hydroxyl groups in each unit. These hydroxyl groups interact with each other by intra and inter hydrogen bonding to form crystalline (ordered) regions. Cellulose is used in drug delivery systems because they are porous materials, which can facilitate the liquid uptake. Cellulose can interact strongly with water, thus swelling readily in water leading to its dissolution[12].

- **Gum Arabic:**

Gum Arabic is a dried, and gummy exudate from the stems and branches of Acacia Senegal. Gum Arabic has antibacterial activity *P. gingivalis* and *P. intermedia*. GA could improve dental remineralization and inhibit the formation of plaque, acting as a potential preventive agent in the formation of caries. Such effects are attributed to the high salt content of Ca^{+2}, Mg^{+2} and K^{+}. Incorporation of Gum Arabic improves the hardness of gypsum products. Small amount of Gum Arabic plus calcium carbonate added to the hemihydrate can reduce the amount of water required for mixing of both plaster and stone[6].

b) Protein based biopolymers:

- **Collagen:**

Collagen is one of the most abundant cellular matrix proteins. The basic structural unit of collagen is composed three polypeptide chain arranged in the form of a triple helix with two identical chains and the third differs in its chemical composition. Collagen presents in the gingival epithelium. It is used mainly to facilitate the process of healing[13]. Collagen membranes are also very useful for periodontal procedures of guided tissue regeneration. Collagen stimulates the differentiation and proliferation of osteoblasts and increases expression of bone morphogenic proteins which influences the regeneration of peri-implant and periodontal and improves the overall mechanical strength and stability of the regenerated tissue[14].

- **Fibrin:**

Glycoprotein present in human blood plasma characterized by porosity, deformability, elasticity and biodegradability, fibrin has great potential for application as a scaffold in tissue engineering. A fibrin matrix with entrapped cytokines, growth factors and cells, called platelet-rich fibrin (PRF) could be used in oral and maxillofacial surgery to improve bone healing, enhance new bone formation, and bone regeneration, and reduce pocket depth[15].

- **Gelatin:**

Biocompatible polymer with adhesive properties and high hemostatic activity used to improve the process of healing/regenerating damaged tissues, including post-extraction wounds. As a polymer

that promotes cellular attachment and growth but suffers from poor mechanical and antimicrobial properties, it could be used to create ideal wound dressing only after crosslinking with other polymers and the incorporation of antimicrobial agents e.g., gelatin-hyaluronic acid (HA) hybrid hydrogels[16].

- **Bone Morphogenetic Proteins (BMPs):**

Bone morphogenetic proteins (BMPs) are signaling molecules that are obtained from non-mineralized bone matrix. BMPs play an important role in regeneration and bone remodeling. They also increase bone response to alloplastic materials[7].

- **Silk:**

The raw silk thread produced by a silkworm is composed of core silk fibroin, and a glue-like coating consisting of sericin proteins. Silk fibroins possess high mechanical resistance. This is mainly due to strong hydrogen bonds (inter- and intra-chain) and van der Waals interactions generate a structure that is thermodynamically stable. Silk fibroin is in controlled drug delivery and in the production of three-dimensional porous scaffolds[17]. Combining PMMA with silk fibroins shows increased elastic modulus, tensile stress, and melting temperature[18].

Structure of Microbial Biopolymers:

- **Polyhydroxyalkanoates (PHAs):**

PHA polymers composed of short-chain-length monomers. Depending on the number of carbon atoms, PHAs are classified as short-chain-length PHAs that contain three to five carbon atoms and medium-chain-length PHAs that contain 6–14 carbon atoms. The PHAs are commercially produced by several bacteria as intercellular carbon and energy storage materials. PHAs can be processed by traditional polymer techniques for use in medical products such as surgical sutures, wound dressings, blood vessels, tissue scaffolds, surgical implants, and bone fracture fixation plates[1, 5, 19].

Properties of biopolymers:

1) **Density:**

Most biopolymers have higher densities than conventional polymers. The higher density values of biopolymers indicate higher energy and cost required for production[20].

2) Water sorption and Solubility:

Most biopolymers are more susceptible to the water sorption due to their natural source and hydrophilicity. For solubility, high crystalline biopolymers offer more resistance to dissolution in comparison to lower crystallinity biopolymers[21]. Water sorption is an essential property in rehydration and exudate absorption during wound-healing. Adequate moisture is mandatory for rapid treatment, preventing dehydration, bacterial proliferation, and infection[22].

Bacterial cellulose (BC) has a complex molecular structure, with water molecules bonded through hydrogen bonds. The BC fibers are composed of linear chains of glucan units linked through inter- and intramolecular hydrogen bonds, allowing BC to be mechanically robust while maintaining elasticity. Therefore, the free water (unbonded) can penetrate and exit the BC molecular structure[23].

3) Barrier properties:

Membranes or films are devices that promote the separation of a structure and the environment in which the structure is located such as wound dressing. Their objective is selective mass transport between both sides such as oxygen, water vapor, carbon dioxide and microorganisms[22]. Biopolymer films could be impermeable to both water vapor and oxygen. impermeability to water vapor but highly permeable to oxygen, or impermeable to oxygen and greatly permeable to moisture[21].

A wound dressing should be capable of free flow of oxygen to the wound which is essential for cell growth making the healing process faster. Increasing the hydrophilic nature of a polymer membrane increases water vapor permeation. Moreover, wound dressing material must have adequate bacterial barrier property to protect the wound from bacterial infection[24]. ***For example,*** polyvinylpyrrolidone (PVP)/ sodium-carboxymethylcellulose (CMC) hydrogel wound dressing has high rate of water vapor and oxygen permeability and performs as a good barrier for bacterial penetration[24].

4) Thermal properties:

One of the chief flaws of biopolymers is their tendency to easily deform under high temperatures[21]. Thermally instable biopolymer such as lignin should be incorporated with other polymers as blends, composites and copolymers thereby modifying the thermal properties[2].

5) Mechanical properties:

The mechanical properties depend upon the application of biopolymer. Applications, such as wound-healing dressings or tissue engineering require appropriate mechanical properties that can be achieved with plasticizer and crosslinking methods. In the case of dressings, flexibility is an important requisite in biopolymer films to improve the patient compliance and adaptation to the oral mucosa which is a very sensitive tissue (chitosan and pectin films). Addition of plasticizer increases the flexibility of biopolymer films. For tissue engineering, the mechanical properties of biopolymers films must be like the tissue to be repaired, crosslinking can be a useful tool to improve the tensile strength of biopolymers [22, 25].

Natural biopolymers are not suitable for applications that require high mechanical stress or load-bearing capacity. In contrast, synthetic biopolymers show better structural properties for load-bearing medical applications than natural polymers[26]. The modulus of elasticity and tensile strength is increased with a greater value of molecular weight. For example, PHAs are brittle, have low percentage elongation and tear strength because they are formed of short length chains[21].

6) Optical properties:

Some biopolymers are colorless and transparent such as starch-chitosan composites [2]. Bacterial cellulose composites containing silver nanoparticles that are used as wound dressing are transparent, allowing uninterrupted visualization of the wound without having to remove the dressing[23]. The crystalline structured materials showed more opacity compared to the amorphous structured materials. Transparency could be achieved by incorporating a transparent nucleating additive in the biopolymer matrix which tends to crystallize the polymer into a tremendously large number of crystals while maintaining a miniscule size lesser than the wavelength of visible light[21].

7) Biodegradability of biopolymers:

Biodegradability of biopolymers is a biochemical process, which involves hydrolytic cleavage of chemical bonds under specific ecological conditions by microorganisms or enzymes into their constituents and it might be aerobic or anaerobic[27]. In general, the biodegradation process could be biotic or abiotic. **Biotic degradation** occurs through enzymatic actions produced by organisms. While **Abiotic degradation** is the chemical and physical break down of the material, e.g., photodegradation, mechanical degradation, thermal degradation, and chemical hydrolysis under acidic or basic conditions. Biotic and abiotic degradation processes influence each other, mechanical degradation can for example lead to increased susceptibility of the polymer to enzymatic degradation, accelerating biodegradation[22, 28].

Biodegradation of biopolymers has many advantages and applications in tissue engineering and drug/gene delivery. Biodegradability of biopolymers makes them eco-friendly because they can maintain the environmental aspects in the presence of active compounds in the environment[27]. Conventional synthetic polymers might collect in the body, resulting in the degradation of toxic products[29].

- **Stages of biodegradation of biopolymers:**

1) Biodeterioration: breakdown the biodegradable materials into small pieces.
2) Depolymerization: the polymeric molecules are converted into lower-molecular-weight polymers.
3) Assimilation: Transported molecules are used as energy and carbon sources.
4) Mineralization: Metabolites may be excreted and reach the outside of the cellular medium[30].

- **Factors affecting biodegradation of biopolymers:**

1) The environment: The situations in which biodegradation processes happen are divided into two types of aerobic and anaerobic.
2) Other factors: chemical bonds, presence of microorganisms, temperature, wetness, oxygen accessibility, chemical conditions such as pH, and the molecular weight distribution of the polymer[27].

➢ **Aerobic biodegradation:** Occurs in the presence of oxygen in which the chemistry of the environment, system, or organism is characterized by oxidative conditions. For example, aerobic

biodegradation of lignin is an oxidative process carried out by lignin peroxidase as an extracellular enzyme in the presence of H_2O_2. Aerobic processes, unlike anaerobic processes, do not produce potent gases[27].

$$C_{Biopolymer} + O_2 \rightarrow CO_2 + H_2O + C_{Residue} + C_{Biomass}$$

- **Anaerobic biodegradation:**

Anaerobic process is a process in which there is no oxygen. Some biodegradable biopolymers are destroyed by the anaerobic process in the absence of oxygen. For example, cellulose degradation[27].

$$C_{Biopolymer} \rightarrow CO_2 + CH_4 + H_2O + C_{Residue} + C_{Biomass}$$

- **Enzymes for biopolymers biodegradation:**

These processes are the biotic side of the total decomposition of a compound. Several enzymes such as proteases, esterases, and glycoside hydrolases are involved, depending on the type of the bond to be hydrolyzed.

1) **Proteases:** Proteolytic enzymes (proteases) catalyze the hydrolysis of peptide (amide) bonds[31]. The amino acids glycine, proline and hydroxyproline base are hydrolyzed because of collagenase[32].
2) **Esterases:** Esterases are the most widely found enzymes in the nature. They help split of ester linkages by the addition of water[31]. The hydrolysis of ester bonds liberates the monomers which are transported and further digested through endoenzymes[28].
3) **Glycosidases:** Glycoside hydrolases cleave the glycosidic bond in polysaccharides such as starch (amylase), cellulose (cellulase), chitosan (chitosanase), and hyaluronic acid (hyaluronidases)[32].

8) Muco-adhesion property:

It is a multifactorial and complex property, essential for the success of orally administered pharmaceutical forms. Chemical (mucin adsorption) and mechanical (muco-adhesion strength) techniques are important to understand the muco-adhesion ability of biopolymers nanofilms used

as drug delivery systems for the oral mucosa. Chitosan and pectin biopolymers exhibit invitro muco-adhesion[25].Chitosan is the gold standard mucoadhesive biopolymer due to electrostatic interaction between its positively charged amine groups and the available carboxyl groups of mucins[25]. Pectin is another mucoadhesive biopolymer. The interaction between Pectin and mucin is driven by the formation of hydrogen bonds between their free carboxylic acid groups[33].

Modifications of Biopolymers structure and Properties:

1) **Crosslinking:**

Crosslinking reactions provide higher mechanical strength and improved stability by interconnecting molecules; however, crosslinking could cause reduced degradability and lower availability of functional groups in biopolymer[34].

Types of crosslinking:

Ionic Crosslinking through addition of divalent cations (e.g., Ca^{2+}) to the solution of biopolymer (e.g., alginate and pectin) enables the rapid formation of hydrogel. ***For example***, preparation of Interpenetrating polymer network (IPN) hydrogel, which relied on Ca^{2+} cross-linking between alginate chains and hydrogen bonding interaction between polyvinyl alcohol (PVA) chains[9].

Hydrophobic interactions through heat-induced gelation of proteins (e.g., milk protein) which is based on the denaturation and coagulation[35].

Hydrogen bonding that is formed upon cooling of a heated aqueous solution of biopolymer (e.g., agarose) below its gelation temperature. This approach is used in the encapsulation of biomolecules/cells without the necessity of chemical crosslinkers or initiators that can lead to undesired interactions with the bioactive agents[35].

For example, Hydroxyapatite become uniformly distributed throughout the agarose upon cooling, the agarose solution sets into a gel in which chain segments are stabilized by hydrogen bonding, unlike the cross-linking of alginate which is limited by the chemical crosslinkers concentration[36].

Chemical crosslinking reactions through covalent crosslinking of reactive functional groups of biopolymers. Covalent bonds produce strong and permanent networks compared to physical interactions, covalently crosslinked biopolymers exhibit enhanced mechanical properties, colloidal stability in vivo conditions, few by-products and high specificity and selectivity[35].

Examples, Glutaraldehyde can react with functional groups in both proteins and carbohydrates and shows improvement in tensile properties. Although glutaraldehyde provides good improvement in mechanical properties, contradictory evidence has been provided on the cytotoxicity of glutaraldehyde-crosslinked materials. Cytotoxicity of glutaraldehyde is dependent on the concentration used, and up to 8% glutaraldehyde was shown to be non-cytotoxic[34].

Carboxylic acids such as citric acid could be used to crosslink and improve the mechanical properties and stability without compromising the cytocompatibility. Crosslinking biomaterials with citric acid provide pendant functionality and allows formation of ester bonds leading to increased availability of binding sites for bioconjugation[34].

Photo-crosslinking and enzymatic crosslinking approaches are used for in situ gelation of biopolymer due to the rapid gelation (normally no more than 10 min) via strong covalent bonding at ambient temperature[35]. To avoid the undesirable changes and possible side effects of chemical crosslinkers, photo-crosslinking have been used. Collagen films are crosslinked with a combination of glucose and UV irradiation which generates free radicals that form reactive, linear glucose molecules and enhance crosslinking[34].

Regarding the reaction catalyzed by enzymes, an oxidation reaction catalyzed by tyrosinase, or peroxidase is one of the main enzyme-mediated cross-linking methods. These enzymes oxidize substrates to reactive forms, which have the potential to make covalent bonds. Also, transglutaminase or sortase make bonds between specific amino acids, are examples of enzyme-mediated reactions used in hydrogel fabrication[37].

Natural crosslinkers such as proanthocyanidin (PA) found in grape seeds increases the thermal resistance and resistance to enzymatic degradation of collagen films after crosslinking, without affecting their cytocompatibility. Several weeks after subcutaneous implantation, the PA-crosslinked membranes showed considerably higher penetration of fibroblasts without any disintegration of tissue[38].

2) Composites:

Material Composite material consists of two or more materials that behave together to get the better properties. Biopolymer/ceramics composite scaffolds are imitations of natural bone.

Hydroxyapatite, as the mineral part (osteoconductive function) is used in the formation of composites with collagen, gelatin, chitosan, chitin, elastin, PCL, PLLA, and PGA can be the matrix phase for the bone replacement[39].

3) Blending:

Blends composed of a mixture of two or more biopolymers and a mixture of biopolymers with synthetic polymers to modify their properties. Blending is a cost-effective technique in which the components can be combined in a molten state or can be solubilized in the same solvent to form a homogeneous material through hydrogen, ion, and dipole bonds. Miscibility of the components has a key impact on the properties of the blend such as single glass transition temperature and show intermediate mechanical properties between those of the two constituents[40]. Blending synthetic and natural polymers provides a control of the degradation rate of the system as the degradation kinetics of a polymeric blend increases on increasing the amount of the natural polymer, the blend composition can be adjusted to make the scaffold degradation rate match the growth rate of the regenerating tissue[39].

Examples:

- A blend between PCL and starch has good mechanical properties and enzymatic degradation[39].
- Chitosan-gelatin (CS/G) coatings to a Titanium surface supports osteoblasts attachment, migration, and proliferation. Moreover, new bone formation around CS/G implants occurs at 8 and 12 weeks[41].
- Nano sized cellulose imparts higher stiffness to the nanocomposites even at low concentration when incorporated in polymer matrices due to their ability to form interconnected network structures through hydrogen bonding. thus, its incorporation in chitosan matrix can improve mechanical properties of chitosan[42]. Chitosan blended with cellulose nanofiber showed better physical characters and stability which made them useful in pharmaceutical applications[20, 42].

4) Graft copolymerization:

Grafting is the process of incorporation of new and desired properties biopolymers without affecting the basic properties of the polymeric backbone. Reactive functional groups in the chemical structure of biopolymers, including hydroxyl, carboxylic acid and amino groups act as sites for grafting to which monomers are covalently reinforced onto the polymer chain[43, 44].

Methods of grafting:

- Grafting initiated by chemical method (free radical uniting and ionic uniting).
- Grafting initiated through the radiation method
- Photochemical grafting method
- Enzymatic grafting method (e.g., tyrosinase in protein biopolymers)
- Plasma radiation-induced grafting[44].

Example:

Graft copolymerization of cellulose with ethyl acrylate monomer (chemical free radical grafting) reduces the water absorptivity and improves the heat stability, moisture, chemical, and thermal resistance[43].

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