Abstract

The development of new strategies for wound healing has resulted in the design of biomedical devices using polymers of natural origin. Hydrogels are biomaterials formed by three-dimensional polymeric networks that can retain large amounts of water or biological fluids, and smooth texture similar to living tissue. Chitosan is a linear polysaccharide, (1-4)-2-amino-2-deoxy-β-D-glucan, which has desirable features such as biocompatibility, non-toxicity, hemostasis and antibacterial character. Xyloglucans have different applications in tissue engineering for their physicochemical properties, biocompatibility and control of cell expansion. Hydrogels had been made of homogeneous mixtures prepared of chitosan and purified xyloglucan, followed by a freeze-drying process to develop a flexible and porous structure. Additionally, their mechanical properties such as porosity, solubility, biodegradation, and the antibacterial activity of the hydrogels are studied. The results suggest that the incorporation of xyloglucan favors the characteristics from chitosan-based hydrogels, providing a promising alternative for application in biomaterials with antimicrobial activity.

Keywords: chitosan, chitin, xyloglucan, hemicellulose, hydrogels, biocomposites, biomaterials, polysaccharide

1. Chitosan

1.1. Chemical properties and production

Chitosan is an amino-polysaccharide of natural origin. This polymer consists of a linear chain of repeating monomers of D-glucosamine and N-acetyl-D-glucosamine, whose contents and
sequence are variable [1]. The amino groups allow specific chemical reactions and confer very important functional properties [2]. It is usually, produced by the partial deacetylation of chitin, a linear polymer of N-acetyl-2-amino-2-deoxy-D-glucopyranose linked with β-(1-4) bonds [3].

It is estimated that approximately 10 million tons of chitin, can be synthesized in nature every year [4]. Chitin and its derivatives are renewable, biocompatible, non-toxic, biodegradable and have biological properties such as anti-cancer, antioxidant, antimicrobial and anticoagulant [5]. It is mainly found in the exoskeleton of crustaceans such as shrimp and crab with contents from 58 to 85%, which are the most important source of chitin for commercial use due to their availability as waste produced during its industrial processing. These residues in turn constitute to one of the main problems of these industries for society because of its negative impact on the environment [5, 6]. Also, chitin is a structural component of the cell wall of fungi such as Aspergillus niger and Mucor rouxii with up to 45% [7]. Some marine invertebrates contain from 3 to 28% of chitin. In squid pens, 31–49% of chitin has been reported [8]. Moreover, in a recent investigation it has been reported that insect larvae and imagos are an alternative source of isomorphic α-chitin, in a range of 20–60% [4, 9], authors reported 14% of α-chitin [10] in grasshoppers (Dociostaurus maroccanus).

Chitin exists in three major polymorphic forms, α, β and γ-chitin. These differ in the arrangement of the chains within the crystalline regions [11]. α-chitin is the most stable and abundant form [5]. It possess a compact rhombic structure, due to the antiparallel chain that favors the formation of interlaminar hydrogen bonds [12] between the hydroxyl and carbonyl groups [13]. Conversely, the β-chitin structure is monoclinic with a parallel arrangement that inhibits the formation of interlaminar hydrogen bonds. In some studies, it have been reported that β-chitin has a higher solubility, reactivity and affinity to polar solvents than α-chitin [14, 15]. γ-chitin, is a combination of the α- and β-chitin configurations, has been found in the stomach of squid and in the buds of beetles. Squid pens, extracellular fibers of diatoms and spines of annelids are sources of β-chitin [5], while α-chitin is isolated from the exoskeletons of crustaceans, particularly shrimp and crab [15].

Traditionally, the extraction of chitin from exoskeletons of crustaceans consists of a treatment with hydrochloric acid in order to remove inorganic components such as calcium carbonate and calcium phosphate. This is followed by an alkaline treatment with NaOH to solubilize the proteins and remove some pigments such as melanin and carotenoids [16] temperature control and concentration of NaOH are crucial to achieve a satisfactory result. In addition to the chemical methods for obtaining chitin, biological methods involving the use of microorganisms [17] and enzymatic hydrolysis [18] have been reported.

One of the limitations in the use of chitin on a large scale is its insolubility in water, due to which water-soluble derivatives are produced, and chitosan being the most important of them [5]. Once the decalcification and deproteinization steps are completed, chitosan is obtained by alkaline deacetylation of chitin using a saturated solution of NaOH 45% [19, 20]. The deacetylated form of chitosan in acidic solutions offers the advantage to be efficiently processed as powder, pastes, gel, membranes, sponges, beads, microparticles, nanoparticles and nanofibers [21]. Some methodologies for the production of chitosan by chitin deacetylation are presented in Table 1.
Chemical method for the preparation of chitosan provides a degree of deacetylation of 85–93%, products with a wide range of molecular weight are obtained [22]. Studies have shown that the enzymatic conversion offers a degree of deacetylation up to 97%, and could generate new polymers with different characteristics. Steam explosion is a hydrothermal method to deacetylate chitin, where the biomass is treated with saturated steam at high pressure and temperature for minutes, followed by an explosive decompression; during the process, molecular interactions are broken by thermo-mechanical forces [12]. It has been proposed to differ between chitin and chitosan based on their solubility in acid solutions, that is, if chitosan is soluble and chitin is insoluble [16].

Table 1. Chemical and biological methods for the chitin deacetylation.

| Reference | Sources of chitin | Conditions/chemical method | Properties |
|-----------|-------------------|----------------------------|------------|
| [11]      | Crab shells       | NaOH 12 M, Under nitrogen atmosphere 110°C (2–3 h) | — |
| [25]      | Shrimp shells     | NaOH 50%, ratio 1/50 g mL⁻¹, Under nitrogen atmosphere, 100°C (9 h) | DD = 80% |
| [26]      | Shellfish         | NaOH 47%, ratio 1/10 g mL⁻¹, Under nitrogen atmosphere 110°C (30 min) | DA = 26.9 ± 0.8% |
| [13]      | Prawn shells      | NaOH 40%, high intensity ultrasound irradiation | DA = < 32%<br>1 × 10⁵ g mol⁻¹ < Mₐ < 2 × 10⁵ g mol⁻¹ |
| [27]      | Shrimp shells     | NaOH 50%, ratio 1/20 g mL⁻¹, Intensity 350 W (8 min) | DD = 82.73%,<br>Mw = 2.3 × 10⁻¹⁸ ± 137 g mol⁻¹ |
| [28]      | Squid pens        | NaOH 40%, ratio 1/10 g mL⁻¹, Ultrasound irradiation, 60°C (50 min) | DA = 36.7%,<br>Mₐ = 10.3 ± 0.3 × 10⁵ g mol⁻¹,<br>Mw = 12.6 ± 0.4 × 10⁵ g mol⁻¹ |
| [29]      | Lobster by-products | NaOH 50%, ratio 1/10 g mL⁻¹, 120°C (4 h) | DD = 71.59% |
| [30]      | —                 | Deacetylase chitin from Colletotrichum lindemuthianum, Reaction performed at 45°C | — |
| [31]      | Shrimp shells     | Deacetylase chitin from Mucor rouxii, Reaction performed at 50°C | Fₐ = 0.582, 0.400 y 0.188 |
| [32]      | Shrimp shells     | Deacetylase chitin from Pichia pastoris, Reaction performed at 50°C (60 min) | DA = 33% |
| [33]      | Crab and shrimp shells | Chitinase isolated from the stomach of Parapristipoma trilineatum, Reaction performed at 37°C (2 h) | — |
| [34]      | —                 | Deacetylase chitin from Absidia orchidis vela coerulea, Reaction performed at 50°C (250 h) | — |
| [35]      | —                 | Steam explosion (SE), High pressure (1 Mpa), at 180°C | DA = 3.7%<br>Reduction of crystallinity index 11.28% |
| [12]      | Shrimp shells and squid pens | Steam explosion (SE), 9 Kg/cm², at 179°C | DD = 42.9% (α-chitin)<br>DD = 43.7% (β-chitin) |

DA, degree of acetylation; DD, deacetylation degree; Mw, weight average molecular weight; Mv, viscosity average molecular weight; FA, fraction of acetylated units.
Chitosan oligomers are short fragments of chitosan composed by the same units and glycosidic bonds, commonly oligomers are obtained by chemical or enzymatic methods [23]. They are named according to the number of sugar rings in their chemical structure (dimer, tetramer and hexamer). Compared with conventional chitosan and its derivatives, chitosan oligomers have relatively lower molecular weight and attributed remarkable characteristics of water solubility [24].

The confection of chitosan with respect to the degree of polymerization, polydispersity, degree of acetylation, molecular weight and acetyl group distribution provides tools to manipulate functions and properties in regard to their biological effects and/or applications [18, 23]. These parameters are important to examine the relation of structural units between N-acetyl glucosamine and glucosamine, for example, in the case of N-acetylation degree, the molecular weight depends on the source of obtention and the deacetylation conditions [16] during the conversion process, that is, temperature, time of exposure and alkali concentration.

1.2. Biological properties

Chitosan is one of the most widely used natural biopolymers due to its high biocompatibility, biodegradability, non-toxicity, bioadhesivity, antigenic capacity and hemostasis [23, 36–38]. In the materials science, biocompatibility is defined as the absence of cytotoxicity of a biomaterial and its biofunctionality that allows it to support cell-biomaterial interactions [39]. The evaluation of the biocompatibility of the implantable systems requires an understanding of the inflammatory and curative responses of each material. Inflammation, scarring and response to foreign bodies are tissue responses to injury [40]. Bioadhesivity refers to the ability of the polymer to adhere to hard or soft tissues [41]. It adheres to epithelial tissues and the mucous coating present on the surface of tissues [37]. Clinically, when the chitosan biocomposites come in contact with a wound, it adheres to covering the site of the lesion and attracts the red blood cells, forming a seal that prevents further bleeding [42]. Chitosan hemostatic mechanism involves agglutination of blood cells, possibly due to its intrinsic polycationic properties and non-specific binding to cell membrane [43]. Research has led the addition of new formulations to adapt the biocomposites to the needs of the injury. Researchers studied the gelatin-chitosan interaction for sponge formulation as a hemostatic agent [4, 44]. Chitosan-based hydrogel sheets with honey and gelatin have been made as a coating for burn wounds [45]. Authors reported the development of chitosan-agarose hydrogels for tissue engineering application [46]. Chitosan biocomposites treated with sodium hydroxide (NaOH) and sodium tripolyphosphate (Na5P3O10) for hemostatic use have been developed [42]. Phosphate incorporation as a precoagulant and silver nanoparticles as antimicrobial agent into biocomposite-based chitosan has resulted in the blood clotting acceleration, platelet adhesion and significantly absorb more blood than chitosan biocomposites [47].

Researchers defined biodegradation as an event that takes place through the action of enzymes and/or chemical decomposition associated with living organisms and their secretion products [48]. The final result is a loss of structural integrity and radical decrease of molecular weight [4, 49].
The biodegradable chitosan effect is attributed to lysozyme, an existing enzyme in several plants and in the human body [4, 21, 50], which is produced by macrophages during wound healing [51]. It is known as a glycoside hydrolase that possess the ability to slowly hydrolyze the β-(1-4) N-acetylmuramic acid and N-acetyl-D-glucosamine bonds or between N-acetyl-D-glucosamine residues [52, 53] of chitosan membranes. Moreover, promotes tissue granulation, increases the expression of collagen among other components of the extracellular matrix and accelerates wound healing [54, 55]. Additionally, it acts by enhancing the proliferation and migration functions of inflammatory cells such as polymorphonuclear leukocytes (PMN), macrophages and fibroblasts [56] at the site of injury [6, 57]. The N-acetylglucosamine of chitin and chitosan are the major components of dermal tissue, and is essential for the wound repair; in addition, its positive surface charge allows it to be a support for cellular development and promotes blood clotting [58, 59].

1.3. Antimicrobial activity

An ideal antimicrobial polymer must be economically and simply synthesized, stable in long term, soluble in water or neutral medium, should not be decomposed or emit toxic products, must possess bactericidal activity against to a broad spectrum of pathogenic microorganisms in brief times of contact [16]. Chitosan has been shown to have advantages over other disinfectants, due its high antimicrobial capacity, a broad spectrum of activity and higher mortality rate [14, 60]. In medicine, wounds caused by burns are highly susceptible to infection by skin deterioration that acts as a barrier against microorganisms [54]. Researchers report that biocomposites with more than 0.025% of chitosan inhibits the growth of *Escherichia coli*, *Fusarium*, *Alternaria* and *Helminthosporium* [21]. Studies about the antimicrobial activity of chitosan, honey and gelatin hydrogels as possible coatings for burn injuries reported antibacterial efficiency against *S. aureus* and *E. coli* [45]. Chitosan-gelatin composites have presented similar inhibitory activity against Gram-positive and Gram-negative microorganisms [61]. PVA addition to chitosan solutions for nanofibers productions with multiple applications reported bacteriostatic activity against *E. coli* [62]. According to investigations, the use of chitosan sponges for diabetic foot ulcers treatment prevents polymicrobial infection and decrease the risk of amputation [63].

Chitosan it is a potent antimicrobial agent of cationic nature at pH below 6.3 [62]. An antimicrobial agent is one that eliminates microorganisms or inhibits its growth [21]. Some hypotheses indicate that chitosan could interact with anionic groups on the cell surface of microorganisms increasing the permeability of the membranes, facilitating the scape of proteins and other intracellular constituents of the microorganisms [61]. Another mechanism involves the formation of chitosan, chelates with trace elements or nutrients, resulting in the enzymatic activity inhibition [64] due to the chitosan-DNA interaction that modifies the synthesis of RNA messenger [7]. The antimicrobial effects are regulated by intrinsic factors including the type of chitosan, degree of polymerization, the source, chemical composition of substrates (e.g., moisture and/or water activity) and environmental conditions [21]. Research on antimicrobial properties of chitosan films with different deacetylation degree (DD) and molecular weight, against Gram-positive and Gram-negative bacteria demonstrated that the inactivation step of the bacteria increases with the increase in deacetylation degree of the biopolymer; however, the bacteriostatic and bactericidal mechanism of action is not fully known [65].
1.4. Structural properties

At neutral or basic pH, chitosan contains free amino groups and is insoluble in water, while in acidic pH it is soluble in water, due to the protonation of its amino groups [21]. Chitosan exhibits unique polycationic characteristics, chelating properties and film forming abilities, due to the presence of amino and hydroxyl active groups [64]. It is widely used to prepare natural hydrogels, however, they generally lack mechanical stability unless they are cross-linked and/or reinforced by suitable compounds [66]. By definition, hydrogels are polymer networks having hydrophilic properties [67, 68]. They have been used in the pharmaceutical and biomedical area for wound care, as drug releasers, organ and tissue transplantation [69]. The incorporation of therapeutic agents into the hydrogel formulations has been used in order to facilitate many healing processes, particularly in burn wounds [70].

Hydrogels are defined as three-dimensional, hydrophilic networks capable of swelling and absorbing large amounts of water or biological fluids [71], when deposited in aqueous solutions. Hydrogels containing more than 95% water are called as “superabsorbents” and have a high biocompatibility due to their high degree of water retention [72], which is due to its high water content, porosity and soft consistency that are very similar to the natural living tissues [73].

Physical hydrogels are the result of environmental changes (temperature, pH, molecular arrangements and supramolecular interactions), which have the advantage of forming gels under mild conditions, without the use of organic solvents, while chemical hydrogels can be produced by radical polymerization, chemical reactions and/or enzymatic reticulation that also possess better mechanical properties; however, they need solvents and/or toxic crosslinkers [74].

The cross-linking of chitosan can be achieved with the implementation of chemicals products such as epichlorohydrin or glutaraldehyde to enhance their stability in acid solutions [75]. Researchers studied the combination of two cross-linking methods, ionic with CaSO4 and covalent with genipin for the preparation of chitosan-based hydrogel films [76]. They found that ionic and covalent cross-linking exhibit differences in mechanical characterization (strength and maximum load). Investigators designed thermosensitive hydrogel-based chitosan and its derivatives, without the addition of chemical cross-linking agents [77], showed that it may be a viable alternative as a vehicle for the release of injectable drugs. Further studies on the behavior of chitosan-agarose hydrogels as a biocomposite for tissue regeneration [78], determined that the hydrogel provides an adequate environment for healing, that is, meets the criteria for an ideal wound dressing. Chitosan cross-linking with poly(alginic acid) for the manufacture of nanohydrogels (30–80 nm) with ability to remove aqueous metals, reported excellent absorption abilities for Cr (IV) removal [79].

The structures formed in the chitosan hydrogels are: (a) chitosan cross-linked with itself, that is, without the need for any additives, the process that is based on the neutralization of the amino groups of chitosan and thus the inhibition of repulsion between the chains of the polymer [73, 80]. (b) Hybrid polymer network consists of the mixing of two polymer solutions, which commonly use the same solvent, with or without the addition of cross-linking agents [81]. (c) Semi-interpenetrating polymer network is formed when a linear, biological or synthetic polymer is trapped within a polymer matrix [71] and (d) ionic cross-linking are considered as biocompatible have a non-permanent network formed by reversible bonds and have a...
greater sensitivity to swelling in pH changes [50]. Depending on the nature of the cross-linker, the main interactions that form the network are ionic or covalent bonds [21].

1.5. Other uses

In addition to the biomedical and pharmaceutical area, the multiple properties of chitosan have made it a polymer of interest for food preservation, agriculture and water treatment, among others (Figure 1).

It has been used in water treatment as a flocculating polymer [66]. Chitosan has high efficiency in the removal of organic pollutants, suspended solids and metal ions in comparison with commercial chemical flocculants [82, 83]. In addition, its potential use as a natural coagulant in the hybrid membrane coagulation-nanofiltration process for water treatment has been studied [84], also the manufacture of hollow fiber nanocomposites in the removal of chemical compounds from water [85]. Chitosan even has been applied as a simple films for the selective removal of mercury in multimetal solutions [86].

Figure 1. Potential applications of chitin and chitosan.
During food production, the packaging is an important part to ensure its integrity, in this sense, the food industry has put special interest in the application of materials with antimicrobial capacity. Edible coatings can be applied in liquid form while edible films are made as solid sheets and can be used to wrap food products, its application improves the quality and extends the shelf life of slightly elaborated products [87–89].

Panczyk et al. [65] evaluated the antimicrobial properties of chitosan-gelatin films, and found that *Pseudomonas fluorescens* and *Listeria innocua* were more sensitive to chitosan than *Escherichia coli* and *Staphylococcus aureus*. Studies have reported effective antimicrobial properties against *Listeria monocytogenes* in chitosan films added with plasticizers [19] (Sorbitol and glycerol) for active packaging use. Similar results were reported by Coma *et al.* [90] in chitosan solutions for the production of edible films. Leceta *et al.* [91] reported a bacteriostatic behavior of high and low molecular weight chitosan films against *E. coli* and *L. plantarum*, which cause the decomposition of food. Bourtoom *et al.* [92] investigated the mixture of chitosan and rice starch for the elaboration of edible films as an alternative to commercial packaging materials. Its applications have been studied as a cholesterol-lowering agent and its application as an agent for weight reduction [16].

Chitosan has been shown to stimulate plant growth and promote tolerance to abiotic and biotic stress in various horticultural products [93]. It has been studied that the application of chitosan, controlled the release of agrochemicals and genetic materials, and they function as a reservoir of protection for active ingredients [94].

In the cosmetic industry, it has been incorporated in the elaboration of shampoos, conditioners and hair coloring agents. Additionally, in deodorants, and for moisturizing the skin, it could compete with hyaluronic acid [18]. It can also be used for the formulation sunscreens, minimize acne problems, and reduce static electricity of hair, among others [37].

The textile industry has made use of biodegradable polymers for the manufacture of towels, filters and geotextiles for the control of erosion and landscaping [48]. The lack of commercial chitosan-based products could be attributed to the several challenges when working with it [95].

2. **Xyloglucan**

2.1. Chemical structure and sources

Hemicelluloses are branched polymers, and the main monomers found are D-glucose, D-mannose, D-xylose, D-glucuronic acid, 4-O-methyl-D-glucuronic acid and D-galacturonic acid [96]. Together with lignin, they form the microfibrils surrounding cellulose [95].

Xyloglucans (XG) are structural polysaccharides and the major components of hemicellulose [97]. They are localized in the middle lamellar and gelatinous layer of the primary cell walls of the superior plants [98, 99]. XG are structurally related to cellulose, as it is associated in a non-covalently form within the cell walls of plants [100].
Xyloglucans or generally called galactoxyloglucans, possess a main chain identical to cellulose, is a glucose polymer linked by $\beta-(1\rightarrow4)$ bonds, with side residues of xylose linked by bonds 1–6 along the backbone. The major structural differences of the polysaccharide occur when galactosyl and fucosyl-galactosyl residues are added to the xylose residues in dicotyledons, as well as less residues of xylosyl in monocotyledons [96, 99].

Xyloglucans can be extracted from different species as *Copaifera langsdorffii*, *Hymenaea courbaril* and *Tamarindus indica*. Some investigations indicate that they are composed by the same monomers, nonetheless, their proportion and distribution result in a fine structure, which varies according to the species and even within the same species [101]. The XG commercialized on a large scale is extracted from the tamarind seeds [102]. Table 2 describes the morphological characteristics of plants that produce seeds as a source of xyloglucan as well as their potential applications.

An alternative source of xyloglucan is chia seed (*Salvia hispanica L.*). This ancient seed is native from the central-west Mexico region to the north of Guatemala, where it was consumed by the Aztecs; however, at present it has spread to other regions. The seed has essential fatty acids such as linoleic acid ($\omega$-6) and $\alpha$-linoleic acid ($\omega$-3), additionally, it is a source of phenolic compounds that provide various effects as antioxidant, antitumor, antithrombotic and anti-inflammatory [103]. It possess high fiber content formed of natural sugars [104].

| Source | Plant description/cultivation area | Xyloglucan content | Applications |
|--------|-----------------------------------|--------------------|--------------|
| Chia seed (*Salvia hispanica* L.) | Seeds are 2 mm long, with a diameter 1.5 mm/from the region center-west of Mexico to north of Guatemala | 4–6% [105] | Edible films [106]; pharmacology industry and nanocomposites [107]; drug delivery excipients for site-specific release and transdermal drug delivery agents [105] |
| Seed kernel of tamarind (*Tamarindus indica* L.) | Adult tree (20–30 m, with a trunk diameter (1.5–2 m), Seeds are 1.6 cm long/India, Africa, Pakistan, Bangladesh, Nigeria and most of the tropical countries. | Abundant deposits of xyloglucan [108] | Drug delivery [98]; stabilizer, binder and gelling agent [109]; food thickener, sizing agent in textile, paper and jute industries [110] |
| Seeds of *Copaifera langsdorffii* | Adult tree (up to 35 m), seeds are 1.5 cm long/forest and savanna populations | 40% [111] | Potential use in the pharmaceutical, food or cosmetic industries [112] |
| Seed of *Hymenaea courbaril* L. (Jatobá) | Adult tree produces an average of 10 kg of seed. Seeds are 1.5 cm long with a diameter of 2.5 cm/Neotropical region of the world | 40–50% [113, 114] | Useful as partial substitute for agar in culture media for micropropagation of apples [115] |
| Seed of *Guibourtia hymenaefolia* | Adult tree (10–18 m, with diameters 40–70 cm) produce approximately 1400 seeds per kg/Congo, Equatorial Africa, Nigeria, Cameroon Gabon and other tropical regions | 54.20% | Food technologies and biotechnological processes, pharmaceutical and medical industries [116] |
| Seed of *Detarium senegalense* | Small tree (5–7 m high)/mainly found in West Africa, Chand and Sudan | Abundant deposits of xyloglucan [108] | Pharmaceutical (for controlling drug release) and food industries [117] |

Table 2. Description of plants as xyloglucan sources and its applications.
When the seed is placed in contact with water, the gum or mucilage is secreted by chia seed (Figure 2), covering it with a transparent halo [118]. This mucilage represents 5–6% of the total weight of the seed, and is described as an anionic heteropolysaccharide with a high molecular weight (800–2000 kDa) [106]. Structurally xyloglucan is constituted by xylose monomers linked to glucose monomers in a 2:1 ratio [105]. A tentative polysaccharide structure is a tetrasaccharide with the main chain composed by (1-4)-β-D-xylopyranosyl-(1-4)-α-D-glucopyranosyl-(1-4)-β-D-xylopyranosyl units with 4-O-methyl-α-D-glucuronic acid ramifications in the O-2 position of β-D-xylopyranosyl main chain [119].

_Hymenaea courbaril L._ (legume tree) seeds store xyloglucan, which has a major chain of β-(1-4) glucose units, with some ramifications α-(1-6) xylanopiranosyl or β-(1-2)-D-galactopyranosyl-α-(1-6)-D-xylaropiranosyl [113].

Aspen wood, is a source of xylan composed by a linear chain β-(1-4), linked to a D-xylose with a 4-O-methyl-α-D-glucuronic acid replacing the 2-position of approximately every 8 xylose units [120]. Various sources of hemicellulose and the monomers that compose them are presented in Table 3. Aspen wood hemicellulose fractions contain mainly xylose monomers and in a less proportion arabinose and glucose, in comparison with abedul wood commercial xylan [121].

The monosaccharides composition from three different biomasses studied by liquid chromatography, reported a composition of arabinose: galactose: glucose: mannose: xylose (2.2: 1.4: 1.3: 4.7:13.5) for pine wood, arabinose: galactose: glucose: mannose: xylose (3.1: 0.8: 1.0: 0.2: 21.8) for switchgrass (_Panicum virgatum_) and for coastal bermudagrass, the composition was arabinose: galactose: glucose: xylose with a 4.4: 1.9: 0.8: 22.0 proportion, respectively [66].

Glucose, galacturonic acid and arabinose are the principal monomers found in quinoa (_Chenopodium quinoa W._) and amaranth (_Amaranthus caudatus L._) identified by gas chromatography [100]. In almond gum, the main hemicellulose monomers identify by gas chromatography are galactose, arabinose, xylose, mannose, rhamnose and glucuronic acid with 45:26:7:10:1:11 molar ratio, respectively [123].

The composition of hemicellulose monomers studied by acid hydrolysis and HPLC analysis in three fractions (apical, middle and basal) of _Neolamarckia Cadamba_ [125], reported that xylose is
the main monomer on the three fractions. The mannose glucuronic acid, rhamnose, galactose and fucose content is in the range 22–28%.

2.2. Extraction methodologies

Recent research has shown interest in the xyloglucans extraction from several vegetal sources. The methodologies involve the hydration of matter in water, the methods major differences are based on the raw material/water (w/v) proportion, temperature (°C), drying methods (vacuum drying or lyophilization) and the process of seeds separation (filtration or sieving).

Specifically, for the mucilage extraction from chia seed, the treatments involve hydration with distilled water (1:40, w/v) at 80°C for 2 h. Finally, the gum drying process is carried out at 50°C and the seed detachment is performed by sieving [119]. Another method involves soak the seed into water (1:30 w/v, ratio) at 25°C for 2 h to moisturize the chia seed, afterwards the mucilage is separated by centrifugation and vacuum filtration to remove the solid waste, subsequently the gum is stored as lyophilized material [106].

Capitani et al. [118] discuss two methods for chia seed mucilage extraction, in the first, it is proposed to submerge the seeds in distilled water (1:10, w/v) for 4 h, followed by a lyophilization process, the mucilage separation occurs by friction in a sieve. The second method involves the same water repose and vacuum filtration separation, followed by a pre-concentration on a rotary evaporator and complemented by lyophilization. After evaluating its rheological properties, it was concluded that the second extraction method provides a greater consistency to the mucilage.

Simi et al. [97] studied the xyloglucan physicochemical properties extracted from tamarind seeds. The procedure involves a pulverized seed deproteinization step with protease (at 30 ± 2°C, pH 6). For grease extraction, they used hexane as solvent, subsequently a 95% (v/v)

| Authors | Source | Monomers |
|---------|--------|----------|
| [98, 122] | Seed kernel of *Tamarindus indica* | Xylose-glucose (3:1) |
| [105] | Chia seed (*Salvia hispanica L.* | Xylose-glucose (2:1) |
| [118, 119] | Chia seed (*Salvia hispanica L.* | Xylose, glucose and glucuronic acids (2:1:1) |
| [120] | Aspen wood (*Populus tremula*) | Xylose: glucuronic acids |
| [66] | Pine wood | Arabinose, galactose, glucose, mannose and xylose |
| | Switchgrass (*Panicum virgatum*) | Arabinose, galactose, glucose, mannose and xylose |
| | Coastal bermudagrass | Arabinose, galactose, glucose and xylose |
| [101] | *Hymenaea courbaril* seed | Glucose: xylose: galactose (4:3:2) |
| [100] | Quinoa (*Chenopodium quinoa W.*) | Glucose: galacturonic acid: arabinose: galactose: mannose: xylose |
| | *Amaranth (Amaranthus caudatus L.*) | Glucose: galacturonic acid: arabinose: galactose: mannose: xylose |
| [123, 124] | Almond Gum | Arabinose: galactotose: xylose: glucose: rhamnose: glucuronic acid |
| [125] | *Neolamarckia cadamba* (Rubiaceae) | Xylose: galactose: rhamnose: glucuronic acid: mannose: fucose |

Table 3. Monomers and hemicellulose extraction sources.
ethanol solution was used to precipitate the XG, once extracted it is lyophilized and sprayed before use.

Alternative methods of hemicellulose extraction involve alkaline treatments with NaOH combined with an ultrafiltration process. The previous has been applied to aspen wood (Populus tremula), the final product is obtained by spray drying [120].

Xyloglucan extraction from Hymenaea courbaril L. is carried out with 80% ethanol (80°C, for 10 min). Water is then added and maintained at 80°C for 3 h. The insoluble material is extracted with 4 M KOH. The polymer extracted with alkali is neutralized with acetic acid, followed by a dialysis process and lyophilization [113].

Researchers performed a hemicellulose alkaline extraction from sugarcane bagasse with NaOH (1:25, w/v), to precipitate the hemicellulose, four different ethanol solutions were tested and the pellet was dried at 40°C for 24 h [126] the extract was used to prepare biodegradable films.

Arruda et al. [101] studied the biological activities of xyloglucan extracted from courbaril seeds (Hymenaea courbaril). The extraction procedure included enzymes inactivation, as well as treatments with NaCl and ethanol at 46% (1:3, v/v) to precipitate the gum.

In another trial, alkaline extraction and delignification with toluene-ethanol (2:1, v/v) is proposed, for obtaining Neolamarckia cadamba (Rubiaceae) hemicellulose monomers. The ethanol precipitate was finally lyophilized [125].

2.3. Properties

Xyloglucans possess important applications, especially in pharmaceutical formulations for the gel production [122]. Furthermore, they participate in the control of cellular expansion, own an effect on cell growth, and act as a seeds carbon reserve of many dicotyledons. XG are neutral, non-mutagenic, non-irritating, non-toxic and blood compatible [127, 128]. Additionally, increase the viscosity, have wide pH tolerance, high temperature regimes resistance and salt, also possess adhesiveness, non-carcinogenicity and biocompatibility properties [101, 102]. Considering its attributes, xyloglucans have promising biotechnological purposes [129].

This polysaccharide is considered a hydrocolloid, as a result of its viscosity and ability to retain water, particularly when highly viscous solutions are formed. Currently they are used in a wide range of industries for different applications [118]. Other investigators have attributed properties such as thickener, binder, as a controlled release, texture modifiers, gelling agent, emulsion stabilizers and syneresis control [106, 119]. In Japan xyloglucan is widely used as a food additive, provide texture and can be used in combination or replacement of starch [108]. The previous attributes are due to the xyloglucan possess a high viscosity degree and stability in acid pH and resistance to high temperatures [130].

Researchers evaluated the solubility and water vapor transmission rate (WVTR) effect in xyloglucan films from sugarcane bagasse [126] concluded that the purification process intervenes directly in the micro-structural properties of biodegradable films. In agreement with
other authors, xyloglucan films can be industrially used as coatings in ready-to-eat foods and
with health benefits because of their high content of soluble fiber [106].

Biosorption is the property possessed by some biomolecules to bind and concentrate selected
ions and other molecules in aqueous solutions [131]. Hemicelluloses mainly conformed by
xylloses have application field in the ecology. Thus their combinations with biopolymers such
as chitosan have been investigated to produce biosorbent materials in the desalination and
heavy metals (Ni, Cu & Pb) removal from water [66]. Other research suggests its application
for the development of flocculant-adsorbents for remove several types of dyes from textile
wastewater [132].

Bioadhesion can be defined as the state in which two materials, being at least one of them from
biological nature, are maintained together by interfacial forces for long periods of time [133].
Natural polymers have been widely used as bioadhesives because of their biocompatibility,
specifically the xyloglucan extracted from tamarind seeds has been studied as a mucoadhesive
polysaccharide for the transport of medicament administered through the oral route [109].

3. Chitosan-xyloglucan hydrogels

3.1. Preparation

The use of natural polymers with different mechanical, physical and biological properties is
frequent in the design and development of biomedical matrices [134]. Biopolymers, which
include polysaccharides such as cellulose, chitosan, wool, silk, gelatin and collagen, have been
found promising for multitudinal applications in different forms [21].

Chitosan is compatible with a wide variety of biologically active components [18]. The inclu-
sion of carbohydrates such as glucose, cellulose and hemicellulose in chitosan particles gener-
ate changes in their structure and by consequence in the biomaterials properties [135].
However, the addition of biopolymers such as xyloglucan (hemicellulose) for the formulation
of hydrogels confers resistance properties that increase the value and suitability of the poly-
mer. The main component of chitosan is glucosamine, being a natural substance produced by
the body from glucose and it is related to the production of glycosaminoglycans (GAG) that
form cartilage tissue in the body and that is also present in ligaments and tendons. It is a
biocompatible material that slowly decomposes into harmless products that are completely
absorbed in the body [21].

The antimicrobial effect of chitosan has been shown to be beneficial for its application as
implants and drug liberators [23]. Hydrogels have attracted attention in various investigations
because of their great ability to absorb liquids and their swelling-deswelling capacities sensi-
tive to stimuli without disintegration, what makes it of interest in biomedical and pharmaceu-
tical applications [36, 67]. The swelling is associated by itself with the bioadhesiveness, this
depends on the concentration of the polymer, ionic strength, as well as the presence of water,
during the dynamic process of bioadhesion, the maximum bioadhesion in vitro occurs with a
optimal water content [41]. The addition of xyloglucan for the formulation of chitosan-xyloglucan hydrogels increases the swelling capacity, because it increases the amount of hydrogen bonds and facilitates the absorption of water [135]. Investigations on the formulation of films from chitosan-hemicellulose demonstrated the capacity and biocompatibility for the application of coatings for wounds, because both polymers are of natural origin and the native properties of chitosan and xyloglucan are beneficial for cell growth [2].

During the healing process, it is indispensable to count on wound coatings for regenerate and repair dermal and epidermal tissue. For a passive coating is essential to stimulate wound healing and to preserve a humidifying environment [70, 136]. In addition, it should prevent the loss of body fluid, prevents accumulation of exudate and protects the lesions from external contamination [21].

Hydrogels have attracted attention because their three-dimensional polymer networks [74] have high capacity to absorb and retain large amounts of water, saline or physiological solutions [137] and present stimulus of response to the swelling-deswelling without disintegration [121]. They owe their mechanical stability to the cross-links introduced between the macromolecular chains that allow flexibility and sufficient resistance [66]. From the science of materials point of view, biological tissues, the essentially moist and soft materials have an elastic modulus of \(10^4 - 10^7\) Pa and a water content of 50–85% [138].

In Table 4, some biocomposites formulated with mixtures of chitosan, xyloglucan, crosslinkers and other materials are described. Some hydrogels experience continuous and discontinuous changes in swelling, these are regulated by external stimuli such as changes in pH, temperature, ionic strength, solvent type, electric and magnetic field, light and the presence of chelating agents [129].

| Reference | Components | Application |
|-----------|------------|-------------|
| [139]     | Chitosan: cellulose | Films |
| [110]     | Plasticized xyloglucan | Films |
| [122]     | Bacterial cellulose/xyloglucan (10, 20, 30 wt%) | Films |
| [106]     | Xyloglucan (1%) - glycerol as plasticizer (25, 50 and 75% w/w), based on XG weight. | Films |
| [2]       | Chitosan (0.1%): Hemicellulose, (1:1) | Films |
| [121]     | Chitosan 1%: hemicellulose 1% (70:30, 30:70) | Hydrogels |
| [128]     | Oxidized xyloglucan-chitosan (1:0.5, 1:1, 1:2, 1:3, 1:4) in acetic acid solution | Transparent hydrogels |
| [140]     | Chitosan: xylan hemicellulose, (3:1) | Hydrogels for bone tissue regeneration |
| [141]     | Pharmaceuticals coated with xyloglucan (0.5% or 3%) | Nanocomposites for drug delivery |
| [96]      | Hemicellulose citrate: chitosan, (1:1 w/w) | Aerogel foams |
| [142]     | Chitosan: cellulose | Sponges |

Table 4. Biocomposites for medical applications.
The films formation by mixtures of cellulose and chitosan in the presence of ionic liquid has shown a successful miscibility in the solid state [139].

The characterization of bacterial cellulose compounds added with xyloglucan (10, 20 and 30 wt %) by means of drying techniques, show that the inclusion of XG promotes better fiber adhesion and orientation [122].

The addition of plasticizers such as sorbitol, glycerol, urea and polyethylene glycol has been studied in order to facilitate thermal processes and to improve the mechanical properties of xyloglucan films. Thus, the results have shown considerable thermal stability as well as strength and hardness for the xyloglucan and sorbitol (20–30%) combination [110].

Results on the films characterization from xyloglucan extracted from *Salvia Hispanica L.* seeds added with glycerol (25, 50, 75% w/w) indicate that the moisture content, water vapor permeability and solubility of the film in water increased with increasing plasticizer. A high solubility could be advantageous in various applications such as transporter of bioactive compounds [106].

The addition of cross-linking agents such as epichlorohydrin (ECH) for the films preparation of hemicellulose and chitosan (0.1%) involves the reaction of ECH with the chitosan hydroxyl groups. As a result, the smooth, homogeneous and porous surfaces of the obtained films can be beneficial for the breathing of the skin [2].

In the studies on the hydrogels preparation for various applications have proposed the integration of oxidized xyloglucan in combination with chitosan solutions 1% (w/v), the results have evidenced that the strength of the gel depends on the concentration of both polymers [128].

Some researchers have used hemicellulose extracted from woods in combination with chitosan and cross-linked with glutaraldehyde for the hydrogels formation, in consequence the results showed a high response to swelling with increasing hemicellulose content [121].

In the hydrogels preparation studies for the regeneration of osseous tissue, xylan hemicellulose has been incorporated to improve the chitosan properties and was found improvement in the healing of tibia fractures caused by blows, the films preparation was achieved with chitosan: xylan (3:1) mixtures [140].

Researchers have proposed the use of xyloglucan as a protective agent for controlled release of drugs that are limited by the pH of the stomach (pH~1.2), for this, mixtures of xyloglucan (0.5% and 3%) and Enalaprilat (Enal) as pharmaceutical component have been implemented. The results suggest that formulations with 3% xyloglucan can be used as slow drug releaser, especially when needed in other parts of the intestinal tract [141].

The incorporation of carboxylic acid groups by the reaction with citric acid, followed by chitosan addition, has shown an improvement in the properties of sponge-like products, with regard to its elasticity, softness, durability and high porosity [96].

The use of chitosan microparticles to reinforce cellulose biocomposites prepared by lyophilization has been studied, researchers found a more uniform pore size distribution, additionally increasing the chitosan concentration from 0.0 to 1.0% improves the sponge’s resistance to breakage, also an antibacterial behavior against *S. aureus* and *E. coli* is exhibited [142].
Xyloglucans show no activity as a bacterial growth inhibitor. In contrast, chitosan exhibits activity against a broad spectrum of microorganisms with absence and absence of hemolytic activity, so it can be potentially applied in the health industry [101].

Assessments of antimicrobial activity of xyloglucan hydrogels from Tamarindus indica seeds and chitosan did not show growth of microorganisms on nutrient agar plates exposed to air pollution [128].

Studies on the biological properties of hemicellulose obtained Prunus amygdalus showed that it could be a promising component to replace synthetic antioxidants [123].

Some alternatives for the conjugates preparation involve the xyloglucan dissolution in chitosan solutions in oil bath for the heat reaction [60]. Other published methods involve the dissolution of hemicellulose xylan, chitooligomers and glucosamine hydrochloride in distilled water, after adjusting the pH with NaOH (1 M) in an oil bath at 100°C for 4 h [143]. The previous oxidation of xyloglucan with sodium periodate has been proposed for the preparation of complex with chitosan to form hydrogels [128]. In other investigations, the addition of hemicellulose to chitosan solutions is carried out in a water bath at 60°C during the preparation of conjugates [2].

Although it is known that cellulose by itself does not possess antimicrobial activity to prevent infection in wounds [81]. Cellulose and its derivatives have been used extensively in combination with chitosan for the preparation of new materials with antimicrobial activity. Some studies report that cellulose and chitosan can be bound by intermolecular inclusion interaction and also based on their antimicrobial capacity against Escherichia coli (Gram−) and Staphylococcus aureus (Gram+), such materials can be used as wound coverings, due to their potential to prevent excessive dehydration and wound infection [95]. Similar studies on the antimicrobial activity of xyloglucan-chitosan hydrogels exposed to atmospheric contamination showed no growth of microorganisms on nutritive agar [128]. Studies on the characterization of chitosan microparticles to reinforce cellulose biocomposites showed antimicrobial activity against Escherichia coli and Staphylococcus aureus with an average zone of inhibition>2 mm and an inhibition rate greater than 80% [142].

3.2. Interaction between functional groups

In hydrogels based on polysaccharides the term “cruising zone” is used to describe crosslinking, because each aggregate involves molecular chains in the form of helices. Generally, the helices are united by non-covalent bonds such as hydrogen bonds, hydrophobic interactions, ionic bonds, etc. [128].

The molecular interaction of both polymers, chitosan and xyloglucan, results in the improvement of hydrogel properties. It is known that cellulose derivatives can act as reinforcement to improve the mechanical and thermal barrier properties. Chitosan by itself has no mechanical and barrier properties [95]. Some factors that influence the combination of both polymers for the formulation of hydrogels is the order of the addition, the concentration and molecular weight of both polymers, temperature, pH and ionic strength of the medium in which they are immersed.
The process for the formation of hydrogels from chitosan and xyloglucan involves the previous dissolution of chitosan in acid solutions, and xyloglucan in distilled water at elevated temperatures and under continuous agitation to prepare the mixtures of both solutions [121]. Other proposed methods involve the oxidation of xyloglucan with periodate prior to the mixing of both polymers [128].

The molecular structure from chitosan and the main chain of xyloglucans are very similar, the difference is the functional group bonded to carbon two in both carbohydrate. Recent research has reported that the addition of hemicelluloses in chitosan polymer biomaterials increases crystallinity and water retention capacity, especially at low pH [135].

Crystallinity occurs because the hemicellulose is capable of interacting with the bonds formed by the chitosan molecules. The ability to retain water is improved by increasing the concentration of hemicellulose increases the amount of hydrogen bonds, which favors the absorption of water. A graphical representation of the physical interaction between chitosan and xyloglucan is shown in Figure 3.

Studies on the characterization of hemicellulose and chitosan hydrogels report that the FTIR analyzes showed deformation in the amide II bands and stretching between the OH and NH groups. This is due to the intermolecular interactions between hemicellulose and chitosan, this could be attributed to events such as hydrogen bonds and hydrophobic attractions. Some ionic
interactions could take place between carboxyl groups in hemicelluloses and free amino groups in chitosan, although the presence of carboxyl groups in hemicellulose is relatively low [121, 139, 144].

In research on the properties of hemicellulose hydrogels from aspen wood and their interactions with chitosan solutions, it is concluded that the stability of films and hydrogels formation is attributed to the crystalline arrangements and electrostatic interactions of the acidic groups in the hemicellulose and the amino groups in the chitosan [120].

Hydrogels are capable of transforming into a variety of physical forms including slabs, membranes, waists, microspheres, microgels, nanoparticles and porous materials once they have been lyophilized [81]. The biological properties of chitosan and xyloglucan allow the formation of conjugates that transformed into sponges by the process of lyophilization allow obtaining flexible, porous materials and with ability to absorb large amounts of physiological fluids. Properties that make them suitable for biomedical applications in the preparation of coatings [150].

3.3. Mechanical stability

A biocomposite for the restoration of biological tissue based on repair and/or regeneration strategies must meet criteria such as: (a) force to resist application manipulation, (b) biocompatibility with natural polymers, (c) defined structure at the micro-molecular and macromolecular levels and (d) deformation recovery capacity without fracture [74].

Researchers have proposed numerous strategies to promote the chitosan stability and the materials based on this compound. The stability of the chitosan mixtures depends on specific interactions such as hydrogen bonds, ionic bonds, dipole interference; finally, the final properties depend on the miscibility of its components [95]. The addition of plasticizers is necessary to improve the mechanical and permeability properties of some polymer matrices. Such properties could be attributed to the lubricating action to reduce the frictional forces between the chains of the polymers [135].

Plasticizers are usually small molecules that have been employed to increase flexibility and improve the handling of polymeric films. Among plasticizers, glycerol is one of the most widely applied in the films elaboration. It has been successfully introduced in the production of films based on polysaccharides. Features such as water solubility, polarity, non-volatility, and low molecular weight have converted glycerol into a plasticizer compatible with water-soluble polymers (Dick et al. 2015).

The addition of 20% of glycerol to the chitosan films causes the reduction in the tension modulus and the increase in the values of elongation at break [19]. The addition of sorbitol (20–30%) in combination with XG results in a considerable thermal stability superior than that of other plastics, which have disadvantages by evaporation or decomposition at high temperatures [110].

Researchers implemented a methodology for the hydrogel elaboration with a high degree of resistance, through the combination of extracts of bamboo hemicellulose (Phyllostachys pubescens), polyvinyl alcohol (PVA), and chitin nano-cylinders, this methodology allows to associate their
molecular chains through physical cross-linking. The polymer segments guarantee the connectivity around the porous membrane, while the water fills the pores and acts as a swelling agent forming hydrogen bonds between the hydroxyl groups of the three polymers [145].

Investigations suggest that the mechanical properties of microporous membranes based on chitosan and hemicellulose are related to the concentration of the cross-linking agent and the reaction temperature, because the increase in the concentration of epichlorohydrin (ECH) during the preparation of membranes results in an increase in tensile strength [2].

3.4. **In vivo and in vitro assays**

Skin lesions are a common problem affecting the world’s population. Traditionally, the normal healing process is divided into several systematic, and coordinated [2] but overlapping phases: hemostasis/coagulation, inflammation, proliferation (granulation tissue formation), re-epithelialization and remodeling [146, 147]. Usually wounds are classified as wounds from trauma, abrasion or secondary events, wounds without tissue loss, and wounds with tissue loss such as burns [148].

Burn wounds are one of the most complex and painful conditions to treat and handle [56]. These lesions are highly susceptible to infection mainly due to the deterioration of the skin, which acts as a protective barrier against microorganisms [54].

The safety and effectiveness of medical devices made of resorbable biomaterials depends largely on its complete biocompatibility [149]. That is, the tissues and the human body accept the implantable material completely and do not provoke a massive immune response as a result of a threatening material.

3.4.1. **Mixture of both polymers**

Guan *et al.* [2] carried out tests on cell viability with films made of hemicellulose—chitosan, and found to exhibit drug-loading capability, non-toxicity, and good compatibility for wound healing application. In addition, the films showed good mechanical properties, uniform porous structure and adequate optical transparency. The trial demonstrates the potential use of natural polymers for the production of films with future perspectives in the biomedical area.

Bush *et al.* [140] proposed the addition of xylan to chitosan hydrogels to improve restoration of bone fractures in mice. The hydrogels were injected into the site of the lesion. The differences between treatments of chitosan and chitosan with xylan were presented between the 3rd and 4th week. Chitosan hydrogels added with xylan revealed improvement in healing and reduction in fracture size. The untreated fracture was maintained without union after 6 weeks. The above demonstrates that the composite is capable of accelerating the normal healing process in severe wounds, without the need to add growth factors.

3.4.2. **Without xyloglucan**

One of the essential features that is considered for medical devices is the biocompatibility with the host cells, the anterior is to reduce collateral damage. Biomaterials must accomplish specific requirements in relation to their interactions with the blood elements, thus the materials should
not induce coagulation or thrombus formation. Studies on hemocompatibility have established that the lower the value of the hemolysis ratio, the better the biomaterial compatibility with blood. Some authors have reported that a value of up to 5% hemolysis is permissible for biomaterials. Investigations on the in vitro evaluation of chitosan-based nano-materials have shown hemolysis values at 1.14% after 60 min of the material contact with the blood [58].

Investigations in relation to the homeostatic mechanism of biomaterials, through the reaction with erythrocytes, have compared and tested the conventional dressing gauze, polyurethane sponges, chitosan absorbents and aqueous chitosan solution [150]. Experimental studies showed a change in the shape of erythrocytes in contact with conventional gauze and polyurethane sponges, this can be attributed to the morphological changes of the red blood cells by external stimuli; however, no aggregation was observed. Nevertheless, when the erythrocytes were in contact with the porous chitosan sponges showed deformation and its incorporation on the surface of the chitosan sponges, as a result of a hemostatic interaction. The coagulation of blood cells by the chitosan action could be the result of the interaction of the polymer positive charges with the receptors on the cell surface of the erythrocytes.

Lysozyme is present in the human body as body fluid and in tissues, and hydrolyzes the glycosidic bonds (1–4) of chitin and some peptidoglycans. Studies on the in vitro biodegradation of chitosan: gelatin dressings with lysozyme, suggested a total incubation time of 8 days at 37°C [151]. Researchers observed biodegradation of the prototype by 28% after day 8, through the evident destruction of the structure. Studies on the enzymatic activity in lyophilized chitosan-collagen hydrogels exhibited a dynamic degradation from day 14 [152], the anterior could be attributed to the hydrophilicity in sponges that consequently causes the biodegradation and cleavage of the bonds after the swelling of the matrix.

Burns can be classified according to their degree of severity as wounds with loss of tissue and wounds without loss of tissue. To evaluate the curative activity in second degree burns, chitosan coatings were used in rat assays. The coating was replaced every 2 days in the inflammatory phase, 3–4 days in the proliferative phase and every 4–7 days during the maturation phase [153]. It was observed that 90% of the burn healing was reached between the 9th and 12th day. Similar results were obtained in a study where chitosan dressings were loaded with antibiotics. A reduction in wound size was observed between 80 and 90%, with no significant difference on the 15th day after wound induction [154].

To evaluate the efficiency in the quality of cicatrization, female pigs were used for the application of chitosan hydrogel as a coating for third degree burns. Dermal-epidermal reconstruction and re-epithelialization of the affected area were observed without irritation or noxious effects. After 10 months of the cutaneous condition [155], it was observed that the quality of healing, especially in thickness, was better with chitosan hydrogels than with commercial gauze.

Histological observations on the effect of chitosan, heparin and mixtures of both on partial depth burns in adult rats, indicate that burns with local application of chitosan powder are much less severe than control wounds [156]. It also was observed that the mixture of chitosan and heparin inhibited the inflammatory reaction.

Studies on the speed and effectiveness of second degree burns healing in rabbits using chitosan gel, microscopically confirmed the acceleration of wound healing on the 20th day,
as a consequence of the epidermal cells proliferation with complete re-epithelialization of the affected area [157].

Clinical studies reported on the healing of human wounds with chitosan-based materials are the result of previous observations in animals. The use of chitosan membranes to cover fresh wounds as a result of a skin graft donor site [53], reported that the wound adherence was uniform, which is a requirement for a successful biomaterial. It was concluded that chitosan membranes promote hemostasis, healing and rapid re-epithelialization of the affected area, transforming it into new, healthy and esthetically acceptable tissue.

Studies on the development and characterization of chitosan-based microparticles added with Aloe Vera and vitamin E, incorporated into a gel for the burns treatment showed mucoadhesive properties influenced by the presence of chitosan. A high degree of re-epithelialization was found after 14 days of treatment [56].

Researchers proposed combining sodium alginate, compatible biopolymer, with chitosan for the films production, this with the aim of improving the skin burn healing in rats as a research model. They concluded that the combination of low level laser therapy with a film based on chitosan and alginate improves the healing process, specifically with respect to re-epithelialization and supply of blood vessels [134].

Chitosan hydrogels with gelatin and honey have been used as coatings for wounds caused by second degree burns in rabbits as an experimental unit. A positive synergistic effect was found with the chitosan and honey mixture in terms of antibacterial activity. Thus, the primary objective in the treatment of burns is achieved, which involves preventing infection and acting as an effective promoter of wound healing caused by burns [45].

Magnesium meets various characteristics to be used as a candidate for applications in biodegradable implants. However, some studies have shown that the rate of magnesium corrosion is too rapid in body fluid to meet medical application characteristics. To counteract the effect, researchers have applied chitosan as an effective corrosion resistant coating, reducing the hydrogen released by the corrosion of the magnesium matrix [158].

Researchers developed nanoparticles of encapsulated insulin as an oral delivery system, which were administered in diabetic rats. The study concluded that oral administration of nanoparticles could be a promising tool to counteract adverse reactions, which are associated with subcutaneous insulin application [159].

3.4.3. Without chitosan

The effectiveness of a polymeric biomaterial for cell restoration depends on the binding of the cells to their surface. In evaluations of in vitro cell adhesion of animal fibroblasts on films with xyloglucan, values of 83% in cell adhesion to membranes have been reported [160]. Due to the anterior, the cell adhesion of the coatings determines its potential biotechnological application in biocompatible wounds.

Recent investigations have focused on the incorporation of growth factors (FGF-18) into injectable xyloglucan gels to promote cartilage reconstruction [161]. These authors report that the porosity and mechanical properties of the gels are highly dependent on the concentration of
the polymer, in addition, they exhibit a weight loss between 15 and 20%, followed by a slow disintegration, with increase in rheological properties and porosity. Also, they demonstrated that growth factors are not released by the gel, so that the uncontrolled growth of cartilage in healthy areas is avoided. The cell viability of chondrocytes in xyloglucans with growth promoters involved a suitable environment to grow and proliferate.

In vitro studies with 3% (w/v) xyloglucan covered nanocomposites for the transport of drugs (Enalaprilate) through the gastrointestinal tract, report that these formulations could be used for slow release of drugs when needed in other regions of the gastrointestinal tract [141].

Researchers developed and evaluated xyloglucan-based ocular films as a possible antibiotic-releaser agent such as ciprofloxacin. They performed an eye irritation test on rabbits, in order to determine their ability to cause damage to the cornea, and an acceptable tolerance was reported, with no redness or inflammation. Therefore, ocular administration of xyloglucan is suggested because of the potential absence of irritation [162].

In studies on the biological characterization of xyloglucan extracted from seeds of Hymenaea courbaril var., an evaluation was made on its hemolytic activity. In the essay, the red blood cells were diluted with saline solution to a 1% (v/v) suspension, then was mixed with a xyloglucan solution. The researchers concluded that the polysaccharide has no hemolytic activity, verifying its potential application in the health industry [101].

Tests on the application of cellulose membranes in patients with burn injuries showed that cellulose offers advantages over conventional treatments such as the use of gauze with Vaseline. Total re-epithelialization occurred in about 7 days [163]. The rate of re-epithelialization is closely related to the age of the patient. The healing process slows down with aging.

Some other important properties of xyloglucan include non-carcinogenicity, mucoadhesivity, biocompatibility and high thermal stability. Therefore, researchers have used it for the preparation of microspheres and the encapsulation of anti-asthmatic agents. *In vitro* pulmonary pharmacokinetic evaluation indicated the potential use of xyloglucan as a release of anti-asthmatic agents through the pulmonary route [164].

### 4. Conclusion

The review presented in this chapter shows studies on the effectiveness of biomaterials for medical application based on chitosan and xyloglucan, promising results for application in tissue restoration are reported because of their multiple biological properties. Xyloglucans of various sources are defined as a promising biopolymer for biomedical purposes because of their ability to form gels, biocompatibility, adhesiveness, non-carcinogenicity and compatibility with blood. On the other hand, chitosan has remarkable characteristics such as biocompatibility, biodegradability, non-toxicity, bioadhesivity, antigenic capacity and hemostasis. According to the published *in vivo* and *in vitro* tests, both polymers could be used for the hydrogels preparation and their application for the wounds with a high level of dehydration to improve their ability to re-epithelialize. In addition, the interaction of xyloglucan with chitosan confers on these biomaterials the antimicrobial capacity over a wide range of pathogenic microorganisms that cause
infection. Additionally, the introduction of xyloglucan favors the characteristics of fluid absorption and mechanical resistance in chitosan hydrogels.

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