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BIOMATERIALS BASED ON COLLAGEN AND POLYSACCHARIDES INVOLVED IN TISSUE REGENERATION - MINIREVIEW

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Abstract. *This article aims to review the specialized literature regarding biomaterials based on collagen and polysaccharides useful in tissue regeneration. Collagen is the predominant protein in the animal body. The rather large spread of this protein, its physico-chemical and biological properties allow its use in the creation of materials that can come into direct contact with animal tissues, including human ones. Biomaterials based on collagen play an important role in tissue engineering. These can be spongy matrices, membranes or hydrogels. Combining collagen with different polysaccharides (cellulose, chitosan, alginate, hyaluronic acid) leads to the improvement of the physical-chemical, mechanical and biological properties of the resulting biomaterials. Studies have shown that they can be used in the regeneration of epidermal tissue, bone tissue, neural tissue, eye tissues. Collagen combined with chitosan can be used in bioprinting. The studies carried out on cell cultures demonstrated that the biomaterials resulting from the combination of collagen with different polysaccharides have a low degree of cytotoxicity. In various articles it was shown that these biomaterials have the physico-chemical properties (degree of biodegradability, degree of swelling, degree of porosity) necessary for tissue regenerative engineering.*

Keywords: collagen, polysaccharides, biomaterial, biopolymer, properties
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1. Introduction

Biomaterials based on collagen are intensively used in tissue regeneration due to its biological and physico-chemical properties such as biodegradability, lack of immunogenicity. There is a rather small difference between the structure of human collagen and the structure of animal collagen.

Collagen is the main protein in the living organism. In the meat processing industry, there is waste (animal remains) rich in collagen, so an easily accessible and cheap source. The sources can be aquatic or terrestrial, vertebrate or invertebrate [15, 16].

Collagen protein is a natural polymer, obtained by the polymerization of 20 amino acids, the main amino acid being glycine which is found in a proportion of up to 33%. The amino acids proline and hydroxyproline are found in a proportion of up to 35%, hydroxyproline being the amino acid specific to collagen. The collagen molecule consists of 3 helical polypeptide chains, twisted to the right, and the resulting triple helix is twisted to the left. This structure is stabilized due to the hydrogen bonds present. The physical, chemical and biological properties of collagen are due both to the composition of collagen in amino acids and to its triple helix structure.

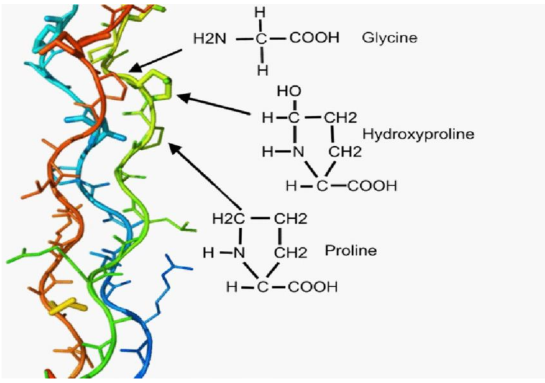

Fig. 1. Collagen triple helix formation (Berillis, 2015) [3].

In contact with human tissues, the properties of collagen-based biomaterials can change under the action of temperature or enzymes. Different physical and chemical methods improve the mechanical properties of collagen by forming intra- and intermolecular chemical bonds.

The properties can be improved by combining with other biopolymers, including polysaccharides. Different intermolecular interactions take place between proteins and polysaccharides, such as electrostatic or hydrogen bonds, ionic or dipole-

dipole bonds. The biomaterials resulting from the combination of collagen with different polysaccharides can be in the form of spongy matrices, biofilms, membranes or hydrogels.

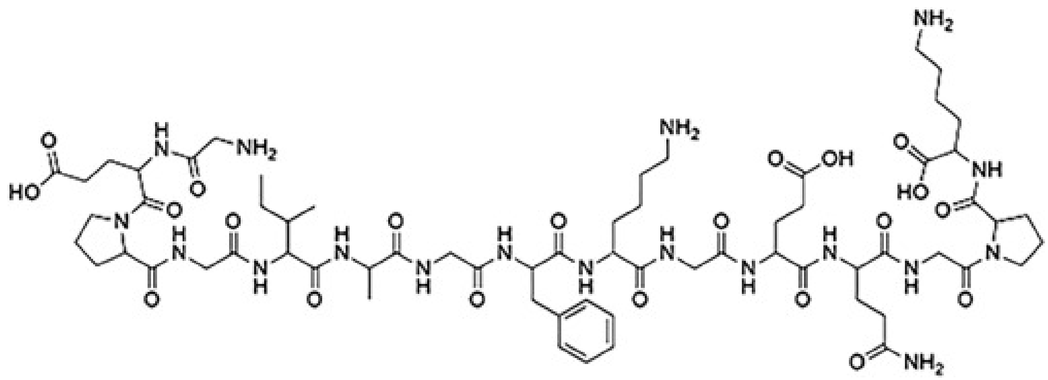

Fig. 2. Structura chimica a colagenului (Biswal, 2021) [4].

The biopolymers used in tissue engineering are used in the form of matrices, films or membranes. They control and regulate cell adhesion. The matrix used must be porous, and the pore size must be adequate for cell diffusion, nutrition and proliferation. The biodegradation of the biomaterial used for tissue regeneration must be proportional to the tissue regeneration [4]. It is necessary for the biomaterial to be homogeneous and the spatial distribution of cells and nutrients to be uniform. Biomaterials are used in the repair of various tissues in the body with a high degree of damage.

Collagen is an intense biopolymer used in the case of tissue engineering, in particular, in the repair of damage to the skin, bone, cartilage, etc. It can be used as a membrane, matrix or film. It presents a high biocompatibility with the cells of the human body and has the necessary porosity, high permeability, hydrophobicity, regenerative properties. It allows the seeding of cells, stimulating their growth once introduced into the body.

Collagen blended with chitosan

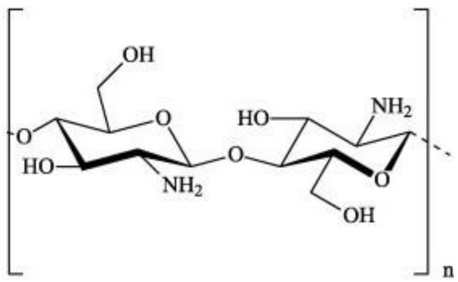

Fig. 3. Chitosan structure

Chitosan is a polysaccharide derived from chitin. This is a biodegradable polymer, biocompatible with the human body, with a high potential to be used in medicine, pharmaceutical and cosmetic industry. It can be extracted from aquatic (crustaceans) or terrestrial (mushrooms) environments [8].

The polysaccharide can be positively charged, due to the amino group, and can interact with proteins, due to the positive charge it holds [1]. The properties of the biomaterial depend on the concentration of each component. Rfatery et al in 2016, studied the effect of adding chitosan in collagen matrix. It has been observed that it improved the mechanical properties in the case of compression, in the case of swelling and the degradation rate is proportional to the amount of chitosan contained. The matrix containing chitosan stimulated chondrogenesis and osteogenesis of mesenchymal stem cells in a greater proportion compared to the simple collagen matrix [13].

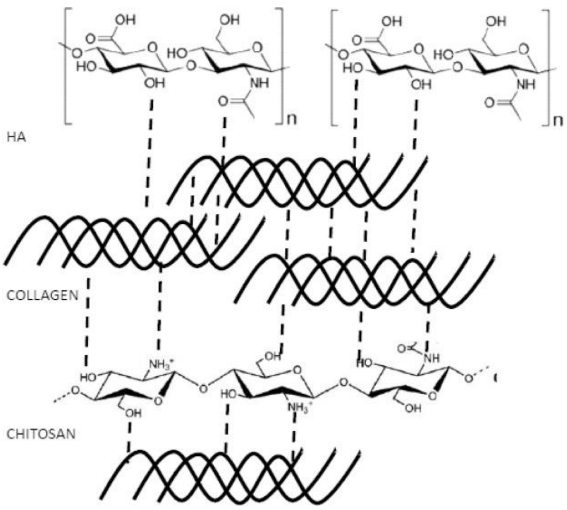

Fig. 4. Bonds between collagen and chitosan (Sionkowska, 2021) [14].

In the matrix based on bovine collagen and chitosan, a decrease in pore size was observed proportional to the increase in chitosan content compared to the porous matrix made of fish collagen, in which increasing the concentration of chitosan produces an increase in pore size. So, the concentration of chitosan acts differently depending on the origin of the collagen.

Chitosan, in this study, promoted the proliferation of osteoblasts and the mineralization of the biomaterial based on bovine collagen. The biomaterial resulting from the mixture of collagen and chitosan had the necessary properties to be applied in cartilage and bone tissue engineering [13]. Mixing the collagen, chitosan and calcium results a biomaterial that can be useful in restoring bone tissue [14].

In other studies, was observed that collagen and chitosan fibers had an antithrombogenic effect, being able to be used in the engineering of the vascular system [14].

Chitosan increased the stability of the film resulting from mixing it with collagen. [18].

The hydrogel based on chitosan and collagen increased cell attachment and proliferation.

Membranes based on the mixture of collagen and chitosan can be used to regenerate the skin, retina or cornea [14]. This mixture can also had antibacterial properties, especially by including silver nanoparticles. The latter demonstrating antiseptic properties against Staphylococcus aureus [14].

The biomaterial based on collagen and chitosan, due to its texture, can be used in bioprinting. Through this procedure, based on such a biomaterial, a support was obtained for restoring the spinal cord, obtaining results on rats in this sense [14].

Vitamins or different extracts can be included in biomaterials based on collagen and chitosan [14].

Adding chitosan to a collagen solution leads to a reduction of its viscosity [18].

Andonegi et al analyzed films based on collagen and chitosan from the point of view of their biomedical potential. By adding chitosan, the swelling degree of the film decreased, the denaturation temperature increased and the enzymatic biodegradability decreased. The cellular biocompatibility being over 70% means that this film has quite a high biomedical potential [2].

Collagen blended with cellulose

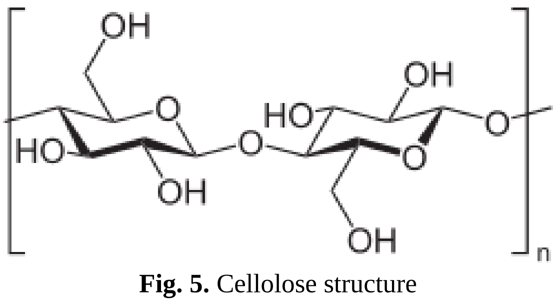

Fig. 5. Cellolose structure

Cellulose is the main plants cell wall constituent, giving it rigidity and elasticity. This being the main source, but it can also be produced by bacteria.

Bacterial cellulose is a biopolymer with unique properties such as mechanical resistance, high absorption capacity, porous structure and biocompatibility**.**

Through the FTIR analysis of the biomaterial resulting from the combination of collagen with bacterial cellulose, the appearance of strong intermolecular bonds was observed, which are not present in either cellulose or collagen. Also, the resulting material has a higher degree of absorption, greater resistance to mechanical factors or the degradative action of enzymes. Following in vitro experiments, it was observed that the presence of cellulose in the collagen matrix stimulated both cell proliferation and osteogenesis. The in vivo experiment demonstrated that this biomaterial improved neovascularization [11].

The cellulose fiber from softwood in combination with collagen forms hydrogen bonds, leaded to an increase in the resistance of the resulting biomaterial in the case of mechanical factors. The appearance of hydrogen bonds was observed following FTIR analysis. The addition of cellulose increases the degree of water absorption. These were observed by Xu et al., who analyzed the influence of physical properties in the case of mixing collagen with cellulose fibers of resinous origin and chitosan, in the case of a film [18].

Chitosan and cellulose influenced differently the viscosity of the collagen solution, in the sense that the first reduced viscosity and the second increased it [18].

Collagen blended with hyaluronic acid

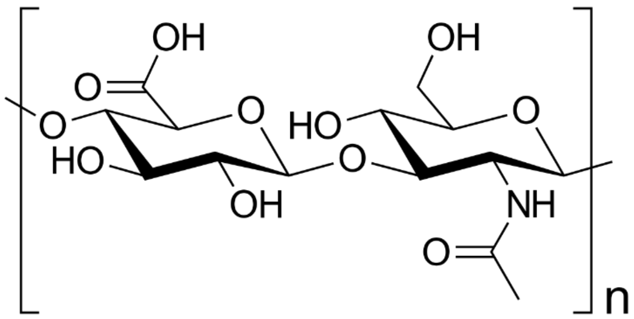

Fig. 6. Hyaluronic acid structure

Hyaluronic acid is a polysaccharide, more precisely a glycosaminoglycan that is part of connective, epithelial and nervous tissues.

Hydrogels based on collagen in combination with hyaluronic acid can be used to restore the corneal tissue. Chen et al analyzed a hydrogel based on collagen and hyaluronic acid hydrogel, a possible biomaterial for corneal defects repair. This was observed to have a very high degree of transparency, a low refractive index, a very high cytocompatibility in the case of corneal epithelial cells. The degree of epithelialization was very high both in vivo and in vitro. These results indicate the possibility of using this hydrogel in corneal tissue repair , but also the possibility of its use in obtaining a synthetic cornea [6].

The membrane-type biomaterial based on collagen and hyaluronic acid is biocompatible in human cell culture. A characteristic of these membranes is the hydrolytic stability in water, PBS or the culture medium, that is, the lack of swelling of the biomaterial regardless of the contact time with the liquid. One use of these membranes would be to increase the compatibility of the allograft type tissue, by covering it with this type of membrane before being introduced into the body. Various growth factors or other necessary molecules can be inserted into the membranes resulting from the combination of collagen and hyaluronic acid [7].

The combination of hyaluronic acid-collagen or hyaluronic acid-chondroitinsulfate-collagen can be ideal for tissue engineering of the brain, due to the porosity and stimulation of neurogenesis. Lee et al. obtained a biomaterial by mixing hyaluronic acid, collagen and chondroitin sulfate or by mixing collagen with hyaluronic acid. These matrices were seeded with mouse neural stem cells. It was observed that the percentage of neuronal microtubules formed is higher in the case of the biomaterial composed of collagen and chondritin sulfate, or collagen and hyaluronic acid or collagen, hyaluronic acid and chondroitin sulfate compared to the collagen matrix [9].

Collagen blended with alginic acid

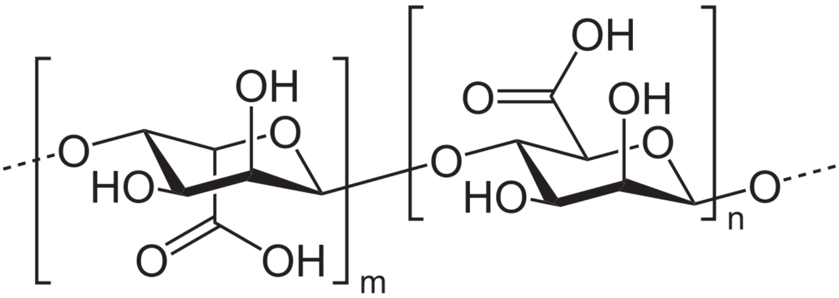

Fig. 7. Alginic acid structure (mannuronic acid and guluronic acid residues)

Alginic acid is a linear polysaccharide produced by brown algae. It has a structure similar to hyaluronic acid, it has a negative charge.

By mixing collagen with alginate in different proportions, the gelling mechanism can be adjusted, resulting in a hydrogel with controllable mechanical properties. The resulting hydrogel is uniform, the gelation being uniform. This is a heterogeneous network of reticulated collagen and alginate fibers, a biomaterial that facilitated neural cell attachment, proliferation and maturation. Thus, the neurons encapsulated in this hydrogel interact with the collagen fibers present in the matrix, generating a neural network. These alginate and collagen gels can be used as substrates for studying neural responses to the action of different physico-chemical and mechanical factors [10].

Xie et al formed a biomaterial after mixing collagen with alginate and chitosan. It had a porosity and a fairly high degree of swelling. It supported the proliferation of fibroblasts and endothelial cells, restoring the damaged tissue. It does not show cytotoxicity and has a fairly good hemocompatibility. All this recommends it to be a biomaterial conducive to healing wounds [17].

The combination of collagen (extracted from jellyfish) and chitosan results in a porous biomaterial that stimulates chondrogenic differentiation (by observing the appearance of the specific shape of the cells, the appearance of markers) of stem cells in a higher percentage than the 2 polymers separately. Alginate has weak mechanical properties, and the combination with collagen supplements this [12].

The biomaterial resulting from the combination of fish collagen with alginate had a very high absorption capacity, and its improvement with oligochitosan reduced its degree of absorption and increases its resistance to enzymatic degradation [5].

Conclusions

(1). By combining collagen with different polysaccharides, results biomaterials with improved properties compared to the component polymers result, biomaterials with potential for use in tissue engineering.

(2). Their properties differ depending on the polysaccharide used and its ratio with the collagen in the resulting biomaterial.

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