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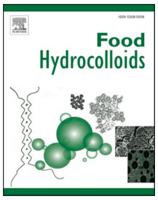

Review

The versatility of collagen and chitosan: From food to biomedical applications

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ABSTRACT

Biodegradable polymers obtained from renewable resources, such as chitosan and collagen, are sustainable alternatives to develop environmentally friendly materials. Due to their abundance, biocompatibility and antimicrobial properties, chitosan and collagen could become a suitable source for food and biomedical applications. In particular, chitosan formulations are used for food packaging purposes to develop intelligent packaging with the aim of providing information about the quality of the packaged product or to prepare active packaging and extend food shelf life. In this regard, chitosan nanoparticles can be used to provide a sustained release of active substances. Regarding collagen, denatured collagen or gelatin is prevalently used in food industry as a food additive, microencapsulating agent or biodegradable packaging material due to its rheological properties and physical versatility. In turn, collagen-derived peptides have revealed antioxidant and antihypertensive activity, among other health beneficial effects for cosmetic and nutraceutical applications. Additionally, collagen is widely used in tissue engineering, also combined with chitosan, to achieve the functional properties required for specific applications in the biomedical field. In this sense, collagen/chitosan scaffolds have been used for bone, cartilage and skin regeneration. This research in the design and processing of materials based on proteins and polysaccharides is leading to great advances in food and biomedical fields.

1. Introduction

Sustainable production has become a critical challenge due to social and environmental concerns together with the growing global population. In particular, valorisation of materials may be a promising solution not only to minimize waste, but also to produce high value-added products (Xiong et al., 2019). In this context, the design and development of innovative products for food, cosmetics and biomedical applications have attracted an increasing interest.

The United Nations defined food waste as the end products of food processing industries that have not been recycled or used for other purposes. These are the non-product flows of raw materials whose economic value is less than the cost of collection and recovery for reuse and, thus, they are discarded as waste (United Nations, 2015). Several initiatives have been implemented worldwide to promote the prevention of food waste in all the life stages. In 2015, the United Nations defined the Sustainable Development Goal (SDG) 12 to ensure sustainable consumption and production patterns with the aim of reducing by half the global per capita food waste at the retail and consumption levels by 2030 and to reduce food losses along production and supply chains, including post-harvest losses (United Nations, 2015). Moreover, the European Commission committed to achieving this objective in the European Action Plan for the Circular Economy (European Commission, 2015), in which industrial ecology concepts, such as “from cradle to cradle” and “zero waste” have been promoted. Therefore, the exploitation of food waste as a source of biopolymers and bioadditives for the creation of a new generation of products has attracted the attention of researchers and industry, promoting the conversion of food industry

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wastes into value-added raw materials (Ong, Kaur, Pensupa, Uisan, & Lin, 2018).

In this context, some opportunities can be generated from by-products from the slaughter of livestock and the processing of fish. In this regard, industrial processing of livestock generates waste viscera, fat, skin, bones and feet, while industrial fishing waste is mainly composed of muscle, skin, fins, bones, viscera and scales, making these by-products rich sources of various valuable materials, such as proteins, polysaccharides, lipids, minerals, nutrients and flavours (Lee, Patel, Sung, & Kalia, 2020; Martínez-Alvarez, Chamorro, & Brenes, 2015; Meena, Banu, Kannah, Yogalakshmi, & Kumar, 2020). In this sense, natural polymers represent a challenging opportunity for the development of sustainable materials due to their structural and physical characteristics, as well as their safety, availability, biocompatibility and biodegradability (Nasrollahzadeh, Sajjadi, Iravani, & Varma, 2021).

Polysaccharides and proteins provide many possibilities for food and biomedical purposes. Regarding food applications, they can be used as encapsulating agents of flavours, texturizing agents or food thickeners, as well as for food packaging or delivery of bioactives compounds. When employed for biomaterials, they can be used for tissue regeneration or perform as drug carriers; regarding food industry, they show good barrier and mechanical properties for packaging materials (Bealer et al., 2020; Haghighi, Licciardello, Fava, Siesler, & Pulvirenti, 2020). Concerning polysaccharides, chitosan is a copolymer of D-glucosamine and N-acetyl-D-glucosamine (Fig. 1), obtained by the deacetylation of chitin (Rasente et al., 2016). Although it can be obtained from crustaceans, insects, molluscs, fungi or shrimps, the main extraction sources are shrimp and crab exoskeleton residues, producing approximately 2000 tons of chitosan annually (Santos et al., 2020; Sharma & Tiwari, 2020). Chitosan is nontoxic, biocompatible and biodegradable, and it can provide antimicrobial and antioxidant activities (Casadidio et al., 2019).

With regard to proteins, collagen (Fig. 1) can be extracted from skin and bones of animals (Senadheera, Dave, & Shahidi, 2020). The primary sources of commercial collagen are bovine and porcine; however, production has decreased due to bovine spongiform encephalopathy (BSE) and other prions diseases outbreak (Senadheera et al., 2020) and religious considerations for the development of kosher and halal products (Pal & Suresh, 2016). In this sense, seafood by-products are a promising alternative as a collagen source to avoid health, religious and social restrictions (Coppola et al., 2020). The main difference among marine and mammalian collagen is related to the content of hydroxyproline and proline amino acids (Yousefi, Ariffin, & Huda, 2017). Regarding collagen applications, due to its biocompatibility, low antigenicity, high biodegradability and cell growth potential properties, collagen has been widely used in food industry, tissue engineering, pharmaceutical and biomedical industries (Subhan, Ikram, Shehzad, & Ghafoor, 2015). In this context, this work aims to review the latest studies concerning chitosan and collagen biopolymers for food and biomedical applications (Fig. 2).

2. Chitosan for food applications

Chitosan is a suitable material for applications such as food packaging due to its good mechanical properties and capacity of selective permeability to O_2 and CO_2 (Cazón & Vázquez, 2020), which play an important role for protecting food quality during transportation, storage and distribution, when food can be spoiled by chemical and microbiological processes (Sahraee, Milani, Regenstein, & Kafil, 2019). Owing to its antimicrobial activity, chitosan can preserve foods from foodborne pathogens (Shin, Kim, & Shin, 2019). In this regard, the most accepted mechanism of action is the electrostatic interaction between the protonated amine of chitosan and the anionic charges on the microbial surface, which results in a leakage of the cell components and, thus, in the cell necrosis (Amato et al., 2018). Therefore, chitosan is able to prevent microbial spoilage of foods, a major factor that affects shelf life and food quality. With this aim, chitosan can be applied as films or coatings (Fig. 3). Films are preformed layers that can be wrapped around the food (Gudjónsdóttir, Gacutan Jr., Mendes, Chronakis, Jespersen, & Karlsson, 2015) or used as a pouch for foodstuff (Zhang, Liu, et al., 2020), while coatings are thin layers directly formed on food surface by immersing the product in a solution (Yu, Jiang, Xu, & Xia, 2017) or by spraying the solution (Jiang et al., 2020). In this regard, Alemán et al. (2016) studied the shelf life of fish sausages packaged with chitosan-based films and coatings. Results showed that chitosan coatings were imperceptible and able to extend sausage shelf life by 15 days, while sausages packaged with films showed a pickled appearance with lower pH values and water content, and harder texture than coated sausages.

Packaging designed to extend shelf life or provide information about the product's quality is defined as active and/or intelligent packaging (Fig. 4). Active packaging prevents food from deterioration and foodborne pathogens; therefore, it preserves food quality, extends food shelf life, and enhances food safety (Jha, 2020). In this sense, the oxidation of foodstuff is considered a food spoilage factor, which causes discoloration and rancidity affecting food quality negatively (Charles et al., 2021). In food industry, special attention is being paid to natural antioxidant compounds (Talón et al., 2017), such as those containing polyphenols (Zhang, Liang, Li, & Kang, 2020). In this regard, rainbow trout fillets coated with chitosan-*Ferulago angulata* essential oil reduced the increase of thiobarbituric acid reactive substances (TBARS) during storage at 4 °C, improving fish shelf life up to 16 days (Shokri, Parastouei, Taghdir, & Abbaszadeh, 2020). Bioactive compounds, in spite of enhancing the antioxidant capacity, can also improve other properties of chitosan films. *Eucalyptus globulus* essential oil incorporated into chitosan films increased the film water resistance and the 2,2-diphenyl-1-picrylhydrazil (DPPH) free radical scavenging capacity up to 43% (Hafsa et al., 2016). Additionally, other natural antioxidants extracted from fruits discards, such as banana peel extracts, reduced water solubility, moisture content and water vapour permeability while increased the antioxidant capacity of chitosan films (Zhang, Li, & Jiang, 2020).

Besides antioxidant capacity, the safety of food is impaired by microbiological activity (Ebadi, Khodanazary, Hosseini, & Zanguee, 2019). Like antioxidants, synthetic antimicrobial compounds, such as sodium nitrite, can generate side effects and health risks for the consumer (Chang, Chen, & Tsai, 2020; De Mey, De Maere, Paelinck, & Fraeye, 2017). Therefore, the food industry seeks substitutes for these compounds from natural sources that do not compromise the sensory properties of the food (Ozaki et al., 2020). In addition to antioxidant activity, essential oils exhibit antimicrobial activity when added to chitosan formulations. For instance, rosemary essential oil inhibited foodborne pathogens, both gram-positive (*Bacillus cereus*, *Straphylococcus aureus* and *Listeria monocytogenes*) and gram-negative bacteria (*Escherichia coli*, *Salmonella enterica* and *Pseudomonas aeruginosa*), mainly due to its high content of phenolic compounds (Souza et al.,

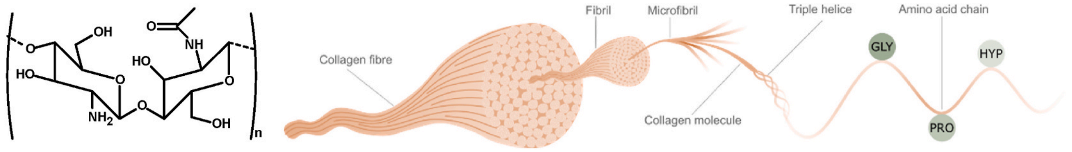

Fig. 1. Structure of chitosan (A) and collagen (B).

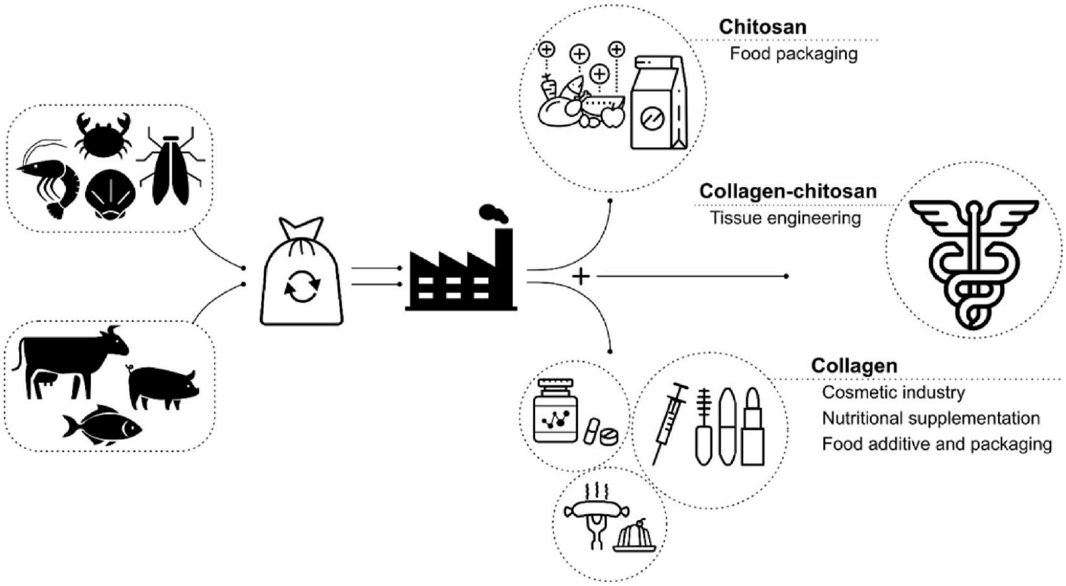

Fig. 2. Food and biomedical applications of chitosan, collagen and their combination.

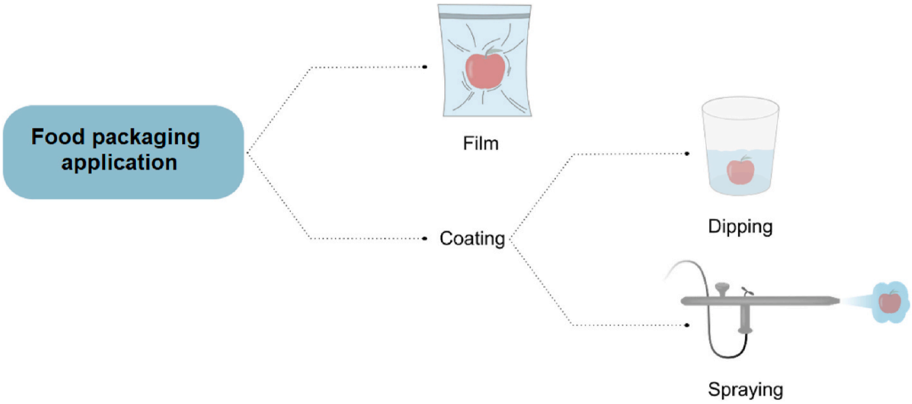

Fig. 3. Schematic representation of chitosan applications for food packaging.

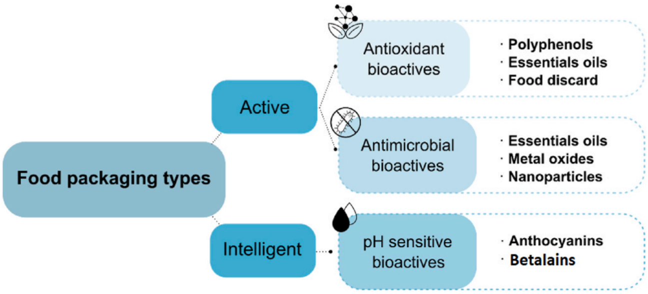

Fig. 4. Schematic representation of different types of chitosan food packaging.

2019). On the other hand, inorganic antimicrobial materials are represented by metal oxides that have higher thermal resistance and broader biocidal spectrum than organic antimicrobials (Al-Tayyar, Youssef, & Al-hindi, 2020). In this regard, zinc oxide (ZnO) nanoparticles decreased initial numbers of *Escherichia coli* by 2.8 log CFU/g and 2.1 log CFU/g in white brined cheese stored at 4 °C and 10 °C, respectively (Al-Nabulsi et al., 2020). In particular, Zn^{2+} ions can attack cell wall, leading to a leakage and, finally, to bacteria death (Yadav, Mehrotra, & Dutta, 2021).

In recent years, chitosan nanoparticles are gaining more attention, since they can improve functional properties due to their bigger contact surface, which make them more reactive and increase the relative surface of the mass (Istúriz-Zapata, Hernández-López, Correa-Pacheco, & Barrere-Necha, 2020), resulting in the enhancement of antimicrobial activity (Badawy, Lotfy, & Shawir, 2020). Chitosan nanoparticles can be integrated into food formulations, such as those incorporated with cinnamon essential oil for retarding beef patties deterioration during refrigeration storage (Ghaderi-Ghahfarokhi, Barzegar, Sahari, Gavlighi, & Gardini, 2017) or those incorporated with gallic acid and used for Atlantic horse mackerel fillets' coatings (Zarandona et al., 2021). Furthermore, chitosan nanoparticles can be used to provide a sustained release of active substances (Kuai, Liu, Ma, Goff, & Zhong, 2020).

Also recently and due to consumer concerns about the safety and quality of food products, smart packaging has been developed to provide information on food quality of packaged products (Wu, Luo, Liu, Jiang, & Mu, 2019). In general, most smart food packaging has been used to check the freshness of seafood, fish, meat and fruits, using quality indicators such as pH-sensitive colour change films (Merz et al., 2020). The most commonly compounds used as pH indicators are anthocyanins extracted from different food waste. Anthocyanins extracted from purple corn were used in chitosan-silver nanoparticles films, showing pH sensitive properties (Qin, Liu, Yuan, Yong, & Liu, 2019). Additionally, anthocyanins extracted from purple and black eggplant peels were incorporated to chitosan films, resulting effective for the spoilage control of milk (Yong et al., 2019).

3. Collagen for food and cosmetics industries

Collagen, the main component of the extracellular matrix, is largely used in food and cosmetic industries (Fig. 5) in its native fibrillary form, as well as after denaturation, due to its multiple functional properties and physical versatility to be processed in a variety of products, including gels, porous scaffolds, fibers, films, meshes and micro/nanoparticles (Oliveira et al., 2021). In this context, gelatin, thermally denatured collagen with molecular weight (MW) from 15 to 250 kDa, shows unique rheological properties and is prevalently used in food industry as a food additive, microencapsulating agent, and biodegradable packaging material (Bello, Kim, Kim, Park, & Lee, 2020). Additionally, peptides with biological activity and MW between 300 and 8000 Da can be obtained by several chemical hydrolysis, enzymatic treatment or/and proteolytic fermentation of collagen or gelatin (León-López, Fuentes-Jiménez, Hernández-Fuentes, Camps-Montiel, & Aguirre-Álvarez, 2019). The studies about collagen-derived peptides have revealed their antioxidant and antihypertensive activity, as well as other promising health beneficial effects for cosmetic and nutraceutical applications (Nguyen, Heimann, & Zhang, 2020).

3.1. Gelatin for food industry

Collagen and gelatin have also found wide application in food industry as food additives or packaging materials. Gelatin is incorporated in foods during the food processing to modify colour, texture, flavour and consistence, among others food properties. However, the prevalent applications of gelatin are as food stabilizers and consistence enhancers to form stable gels, emulsions or foaming (Mardani et al., 2019; Yang, Li, Li, Sun, & Guo, 2020). The most popular single use of gelatin as gelling agent may be in water gel desserts, due to its unique melt-in-the-mouth property, but it is also commonly used to form insoluble cross-linked hydrogels that maintain their shape after swelling equilibrium (Huang et al., 2019; Li et al., 2021). Additionally, heat treated collagen fibres have been used as emulsifiers, as natural alternatives to synthetic emulsifiers, especially in acidic products. For example, the incorporation of collagen improves the rheological properties and avoid the fat cap of the oil-in-water emulsions of sausages (Fustier et al., 2015; Huang et al., 2020). In this context, thanks to their antioxidant activity, collagen hydrolysates have been frequently used to inhibit the peroxidation of lipids whose reaction products are dangerous for human health (Bolognesi, Spier, & Rocha Garcia, 2020). This antioxidant activity is generally associated to the radical scavenging capacity of the imidazole group of histidine (Pan et al., 2020).

In recent years, collagen-based films and coatings have played an important role in the development of sustainable packaging materials to protect, maintain, and extend the shelf life of foods (Moreno, Atarés, Chiralt, Cruz-Romero, & Kerry, 2018; Pellá et al., 2020). Generally, food-packaging materials are required to act as a barrier against the migration of oxygen and moisture, as well as to preserve the sensory qualities and prevent fat oxidation, discoloration, and microbial activity (Regubalan, Pandit, Maiti, Nadathur, & Mallick, 2018). In this context, gelatin has been widely studied thanks to its film-forming ability, biodegradability and good gas barrier properties. However, it has poor mechanical strength, and due to its high hygroscopic nature, it tends to swell and dissolve when it is in contact with food with high moisture content, limiting its direct application in food packaging (Liu et al., 2020; Jiang et al., 2020). Therefore, chemical or physical methods, such as crosslinking (Beghetto, Gatto, Conca, Bardella, & Scrivanti, 2019; Tonndorf, Aibibu, & Cherif, 2020; Uranga, Nguyen, Si, Guerrero, & De la Caba, 2020; Wu, Luo, et al., 2019) or combination with other biopolymers (Bhuimbar, Bhagwat, & Dandge, 2019; Hou et al., 2020; Zhuang, Tao, & Cui, 2017), are carried out to improve those properties. Crosslinking reduces the mobility of gelatin chains, improving dimensional stability, water and heat resistance, barrier and mechanical properties (Huang et al., 2019).

The production of sausage casings using coextrusion process has been the best-known industrial application of collagen and gelatin films. However, the strong sensitivity of gelatin to moisture reduces the barrier and mechanical properties of the casings (Chen et al., 2019; Tosati et al., 2017) and multilayered structures can be a strategy to overcome this kind of weakness, where layers with different moisture and oxygen barrier properties are combined in order to comply the required specific package conditions (Figueroa-Lopez, Castro-Mayorga, Andrade-Mahecha, Cabedo, & Lagaron, 2018; Nilsuwan, Guerrero, de la Caba, Benjakul, & Prodpran, 2020; Wang et al., 2020).

In recent years, gelatin has been reported to be one of the first materials used as a carrier of bioactive substances. Gelatin films and coatings can be functionalized with the incorporation of natural antioxidants and antimicrobial components, obtaining active packaging. Many bioactive components have been reported for active packaging but, in the last years, there is a growing interest in using plant extracts like

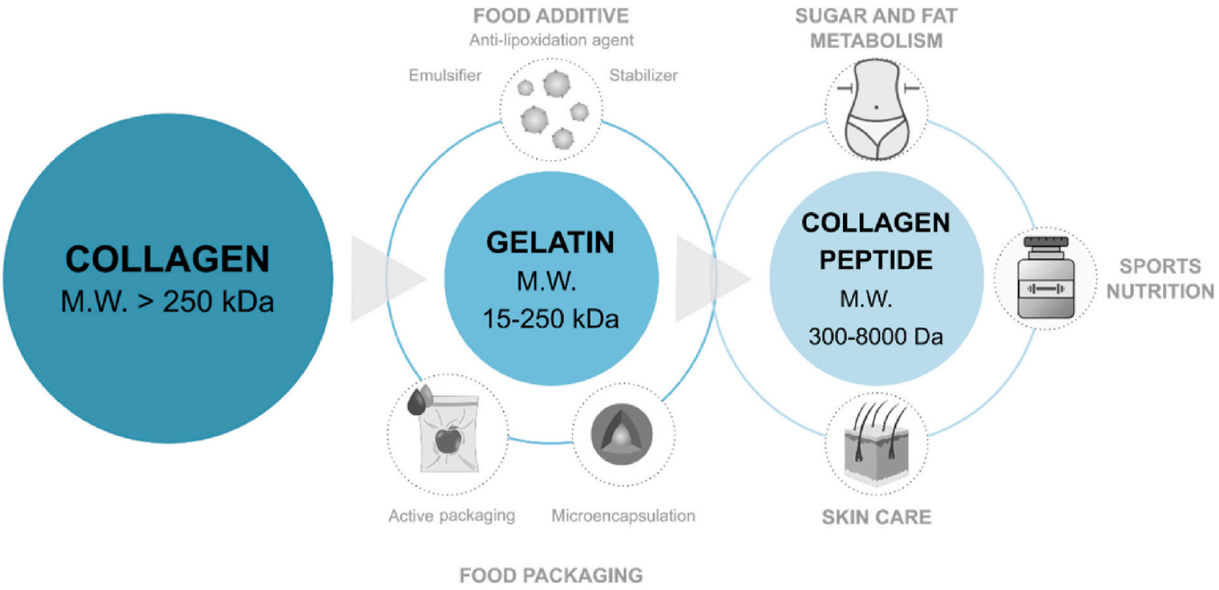

Fig. 5. Collagen application in food and cosmetics industries.

rosemary (Yeddes et al., 2020), grape (Rodrigues, Bertolo, Marangon, Martins, & Plepis, 2020), lemon (Jiang et al., 2020), and oregano (Hernández-Nava, López-Malo, Palou, Ramírez-Corona, & Jiménez-Munguía, 2020). Since those extracts may be readily inactivated by exposure to light, heat, and oxygen (Molino, Casanova, Rufián Henares, & Fernandez Miyakawa, 2020), several researchers have tried to embed solid, liquid, or gaseous materials in microcapsules to entrap functional components in a carrier, protect them, and control their release (Mohseni et al., 2019). Moreover, encapsulation may be a useful solution to minimize the taste and odor of some vegetable extracts. Thus, gelatin encapsulation technology could be used to manufacture functional food formulations (Kuai et al., 2020; Paula et al., 2019; Peanparkdee, Yamauchi & Iwamoto, 2018).

3.2. *Peptides for cosmetics and nutraceutical industry*

Based on the above-mentioned bioactive properties, collagen has also been increasingly utilized for the development of cosmeceutical products, such as skin anti-aging formulations with moisturizing, softening and glowing, antioxidant and UV protective properties (Abuine, Rathnayake, & Byun, 2019; Zamorano-Apodaca et al., 2020). It should be noted that hydrolysed collagen is mainly applied in cosmetic formulations because it offers better effects than native collagen thanks to its superior solubility at neutral pH, water-binding properties, and easy dermis penetration (Alves, Marques, Martins, Silva, & Reis, 2017; Skov, Oxfeldt, Thøgersen, Hansen, & Bertram, 2019). Although hydrolysed collagen can slightly penetrate the epidermis, it cannot replace the lost collagen of the skin (Venkatesan et al., 2017). In this regard, cosmetics industries are interested in subcutaneous injections and oral supplementations. Collagen injections have been popularly used for the repair of dermatological defects, as well as subcutaneous disorders, such as acne scars, and aging symptoms, thanks to collagen biodegradability, price and facility to be produced (Cockerham & Hsu, 2009). When collagen is injected into the dermis, it seems that collagen promotes the biosynthetic capacity of fibroblasts and the formation of an optimal physiologic environment and, thus, increases cell activity, hydration, and the synthesis of collagen (Ganceviciene, Liakou, Theodoridis, Makrantonaki, & Zouboulis, 2012). Alternatively, the positive effect of oral collagen-based supplements on skin appearance has been recently observed (Wang et al., 2018). The fast digestibility of bioactive collagen peptides in the form of liquids, pills or functional foods seems to contribute to the increase of fibroblast density and, hence, to the production of collagen. In that way, several studies have shown the efficacy of the daily bioactive collagen peptides supplementation in the skin hydration, wrinkling, elasticity and density (Genovese, Corbo, & Sibilla, 2017; Ito, Seki, & Ueda, 2019; Kim, Chung, Choi, Sakai, & Lee, 2018).

Besides cosmetics applications, several recent studies have reported the potential of collagen in functional foods and health care applications in form of pills and beverages (Bilek & Bayram, 2015; Guo et al., 2015; Pal & Suresh, 2016). On the one hand, collagen peptides were found to increase the fatty acid metabolism and fats burn, and reduce hypertension and hyperlipidemia. Additionally, they can inhibit the fatty acid synthesis of the liver, decreasing hepatic fat accumulation (Ishak & Sarbon, 2017). On the other hand, they enhanced insulin sensibility and reduced blood sugar levels, which could be attributed to their antioxidant property (Lauritano & Ianora, 2016). Therefore, bioactive collagen peptides are used to prevent and treat obesity and type 2 diabetes, inducing the weight loss and reestablishing lipid and blood glucose level (Astre et al., 2018). In this regard, other studies reported that the ingestion of food or drinks enriched with hydrolysed collagen can help on wound healing, bone formation, mineral density and osteoarthritis (Sato, 2017; Suresh, Sugihara, Suzuki, Inoue, & Venkateswarathirukumara, 2015). Collagen is also in demand within the sport nutrition field, nutraceutical industry offers dietary supplements intended to sport nutrition field boost, since it can increase lean muscle, decrease recovery time, reconstruct damaged joint and improve cardiovascular performance (Oertzen-Hagemann et al., 2019).

4. Chitosan and collagen as materials for biomedical use

Tissue engineering is a multidisciplinary field that combines the knowledge of engineering and biology in order to develop tissue substitutes capable of replace or improve damaged tissues or organs. The methodology of this science is based on the integration of cells, biologically active molecules and materials, recreating in that way the native structure (Langer & Vacanti, 1999). In that context, the material acts as a template to provide structural integrity, define a potential space, guide the restructuring while regeneration, permit diffusion of the nutrients and gas, and provide mechanical characteristics needed for the cell proliferation (Hollister, 2009). In other words, the material attempts to shape the cell microenvironment.

4.1. *Characteristics of materials for use in biomedicine*

Regardless of the tissue type, a number of key considerations are important when choosing a material for use in tissue engineering (Fig. 6). Therefore, the achievement of these features will determine the suitability of the scaffolds to have the most promising results for the biomaterials that best imitate cells' native physiological environment.

Originally, biomaterials used in the field of biomedicine tended to be inert, such as metals and ceramics, in order to avoid any eventual immune response. However, with the course of time and a better awareness of cellular biology, the use of new materials such as polymers, both natural and artificial, emerged. These materials not only comply with the function of a scaffold, but they are able to interact with the organism, contributing to an active regeneration. A promising approach has been the use of natural polymers, as they have an inherent interaction with the organism, facilitating the development of new tissues while enhancing regeneration. The most widely used among them is collagen, as the major structural component of the native extracellular matrix (O`Brien, 2011). Thus, collagen presents high biocompatibility, biodegradability and malleability. However, collagen by its own forms fiber-like structure scaffolds that exhibit poor mechanical properties, making necessary the modification of the material for its optimal effectiveness (Benayahu et al, 2018, 2020; Pawelec, Best, & Cameron, 2016). One of the strategies employed is the combination of collagen with other natural polymers. The combination of collagen and chitosan

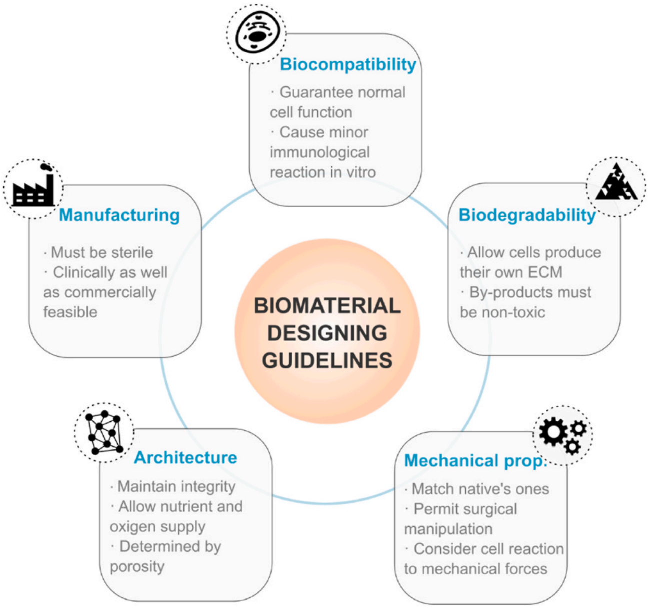

Fig. 6. Criteria to be considered when choosing or designing biomaterials.

is largely employed due to its great potential in tissue engineering applications (Shah, Stodulka, Skopalova, & Saha, 2019). Chitosan offers high biocompatibility, moderate degradation, and antimicrobial effect, among other characteristics. By the addition of chitosan, sheet-like structures with enhanced integrity, mechanical properties and better viability are obtained (Hayashi, Yamada, Guchi, Koyama, & Ikeda, 2012; Hollister, 2009; McBane et al., 2013). According to the literature, the collagen-chitosan construct acquires suitable characteristics when higher concentrations of collagen (>50%) are employed, although a small proportion of chitosan is sufficient to achieve the desired mechanical properties while maintaining cell adhesion and proliferation (Pezeshki-Modaress, Zandi, & Rajabi, 2018).

4.2. Scaffold preparation techniques

The combination of collagen and chitosan is characterized by its high malleability. This feature enables the processing of the materials with a great variety of techniques, allowing a range of forms and structures that can be adapted to the product requirement in each case (Fig. 7).

4.2.1. Films

They are developed by solution casting. The polymer is dissolved in an organic solvent and the solution is poured into a mould. After the casting process, the solvent evaporates. The resulting product shows relatively poor mechanical properties (Shah et al., 2019).

4.2.2. Sponges/porous scaffolds

3D porous structures are mainly obtained by lyophilisation, which produces an interconnected porous structure through the elimination of the ice crystals from the frozen solution. Sponges tend to have poor mechanical properties and low stability in aqueous solutions (Kafi, Aktar, Phanny, & Todo, 2019; Zhang, Zhang, & Wu, 2013).

4.2.3. Hydrogels

These are polymeric networks that absorb and retain large amounts of water. Such formation gives rise to a great capacity of dissolution, so crosslinks are needed to avoid the breakdown of hydrophilic polymer segments in the aqueous phase (Hennink & van Nostrum, 2012).

4.2.4. Fibres

They are based on interconnected structures that resemble the ultrafine fibrous network of the extracellular matrix, thus, they can better promote cell adhesion and proliferation. However, they have poor stability in water due to their fine structure and high surface area. Electrospinning and phase separation are the options for developing this type of material. The latter also provides randomly oriented fibres, which are closer to the 3D structure of the extracellular matrix (Guo et al., 2020).

4.3. Modulation of material properties

Recently, physical and chemical strategies have been implemented in order to ensure that the above-mentioned characteristics are satisfied. On the one hand, mechanical features can be tuned by means of physical or chemical techniques. Those are based on the creation of non-covalent interactions through the use of UV light or temperature, among other agents (Guan et al., 2017). These are inexpensive and easy-to-perform methods that prevent the introduction of possible cytotoxic chemical substances. However, the process is often hard to monitor and fails to achieve a sufficiently high level of cross-linking to meet mechanical requirements (Perez-Puyana, Jiménez-Rosado, Romero, & Guerrero, 2019). This is why the most common approach to overcome the limitations of biomaterials, such as collagen-chitosan scaffolds, is chemical crosslinking. By this method, a chemical compound is added to the material and irreversible covalent bonds are created to interconnect molecules. This approach offers enhanced mechanical properties and an improvement in stability. However, the unreacted crosslinker inside the scaffold can lead to an increase in cytotoxicity together with subsequent processing difficulties caused by the reaction itself (Hennink & van Nostrum, 2012; Reddy, Reddy, & Jiang, 2015; Shah et al., 2019).

There is a great diversity of chemical cross-linking agents according to the degree of improvement desired. In the case of artificial tissues

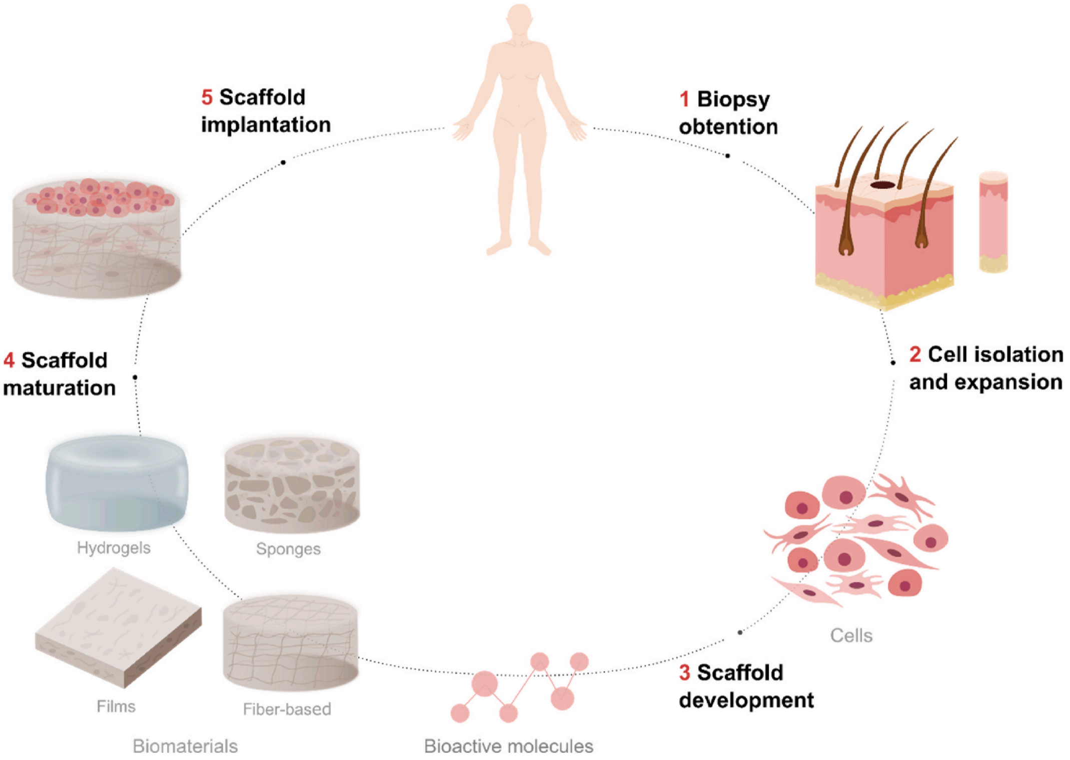

Fig. 7. Schematic representation of the workflow in tissue engineering.

based on collagen and chitosan, one of the most widely used chemical compounds is glutaraldehyde due to its great effectiveness but, even at concentrations higher than 10%, produces cytotoxicity (Liu, Ma, & Gao, 2012; Reddy et al., 2015; Reyna-Urrutia, Mata-Haro, Cauich-Rodriguez, Herrera-Kao, & Cervantes-Uc, 2019). Additionally, tannic acid stands out for its antimicrobial, anti-inflammatory and antioxidant properties (Shah et al., 2019; Sionkowska, Kaczmarek, & Lewandowska, 2014), the combination of N-ethyl-N'-(3-dimethylamino propyl) carbodiimide with N-hydroxysuccinimide (EDC-NHS) enables the maintenance of the porous structure (Martínez, Blanco, Davidenko, & Cameron, 2015; Reddy et al., 2015), and citric acid permits the availability of the binding sites of both biomaterials making feasible further bioconjugations (Reddy et al., 2015; Uranga et al., 2019). Finally, genipin is an aglycone present in the *Gardenia jasminoides*. This natural compound is effective at low concentrations, creating both intramolecular and intermolecular unions with primary amines of collagen and chitosan, and achieving a totally stable structure that maintains its original porosity (Perez-Puyana et al., 2019; Yan et al., 2010). The properties of collagen-chitosan scaffolds using the abovementioned crosslinking agents are summarized in Table 1.

On the other hand, a major remaining challenge in the design of scaffolds, which directly affect regeneration, is the inadequate interaction between the polymer and the cells, which *in vivo* may lead to foreign body reactions such as inflammation, infection and implant encapsulation. This problem is correlated with the material surface and the cell recognition motifs that control the interaction between the cells and the substrate. When necessary, cell-binding peptides derived from extracellular matrix proteins can be used to facilitate such interactions between the material and the cell receptors. In this regard, the arginine-glycine-aspartic acid (RGD) sequence is currently the most effective and commonly used peptide sequence due to its extensive distribution in the organism, its ability to target more than one cell adhesion receptor, and its biological impact on cell anchorage, behaviour and survival (Hansson et al., 2012; Hersel, Dahmen, & Kessler, 2003). The RGD sequence has been reported to allow the rearrangement of the cellular cytoskeleton, thus facilitating the adhesion and propagation of cells along the collagen-chitosan scaffolds. *In vivo*, it was demonstrated that a small concentration of RGD is necessary to effectively enhance the cell-material interaction, improving the maturation of the artificial tissue (Hansson et al., 2012; Miklas et al., 2013; Xiao et al., 2013). In many cases these sequences are not accessible in the native conformation of collagen, but collagen denaturalization can lead to conformational changes and to the accessibility of RGD sequences (Pawelec et al., 2016). Along with surface motifs, it has been reported that polarity, surface charge, and surface roughness play a relevant role in the functionality of cells attached to biomaterials.

4.4. *Applications of collagen-chitosan scaffolds*

Because of their excellent characteristics, particularly their malleability, the combination of collagen and chitosan has been used for the regeneration of a wide variety of tissues. One of the widest applications has been the regeneration of bone. It has been demonstrated that collagen-chitosan membranes have a higher elongation to fracture and lower rate of degradability, critical factors for bone regeneration, than those scaffolds formed only by collagen (Guo et al., 2020). *In vitro*, the collagen-chitosan combination presents osteoid differentiation capacity, even in the absence of a specific differentiation medium, indicating the capacity of induction to self-differentiation (Georgopoulou et al., 2018; Wang, Wang, Liu, & Zhang, 2016). *In vivo*, the collagen-chitosan constructs have also demonstrated optimal properties for guided bone regeneration. In this case, electrospun collagen-chitosan membranes allow the formation of new bone in models of calvarial bone defect. By week eight, the cranial defect was completely restored by massive and mature bone tissue (Guo et al., 2020; Lotfi et al., 2016). Similarly, collagen-chitosan scaffolds, crosslinked with carbodiimide, were shown to promote cartilage regeneration in rabbit articular cartilage defects. New cartilage formation was observed as early as at 1 month, and by 6.5 months the cell number, collagen quantity, as well as compressive and storage moduli, approached to the normal cartilage (Whu et al., 2013). Recently, a porous collagen-chitosan scaffold enriched with hydroxyapatite, as a bioactive component, was developed (Campos et al., 2020).

Chitosan-collagen scaffolds approximate very closely the structural hierarchy, organization, biochemical composition, and functional features of native skin extracellular matrix. Furthermore, the combination presents hemostasis and antibacterial properties along with the potential to accelerate the synthesis of extracellular matrix compounds by fibroblast induction, thus, its potential use in skin tissue engineering. The microfiber structures may favour these properties since it has been shown that cross-linked collagen-chitosan microfiber scaffolds can control water loss by evaporation. In addition, the re-epithelization of wounds after 14 days of application of the scaffold, through guided infiltration of fibroblasts and remodelling of collagen in synchrony with the degradation of the scaffold, has been demonstrated (Sarkar, Farrugia, Dargaville, & Dhara, 2013). In skin tissue engineering, the thickness of the graft is crucial, as this influences the effectiveness of the construct implantation. Thinner scaffolds, especially 0.5 mm thick, have been shown to promote ordered fibroblast infiltration and better collagen remodelling. By week 16 after implantation in skin defects, such implants showed total degradation together with complete replacement with new well-arranged host tissue, newly formed vessels, and 75.9% of the tensile strength of native skin (Haifei et al., 2014).

Recently, some attempts have been made to use collagen-chitosan combination in diseases related to the neural tissue such as spinal cord injury. In this case, 3D printed scaffolds could have significant therapeutic effects by bridging axons across the fracture and allowing the mobility of cells, partially re-establishing a microenvironment for axonal regeneration. Once implanted in rat models, nerve-fibre regeneration as well as neurological and locomotor recovery were achieved (Sun et al., 2019).

5. Challenges and new opportunities

Concerning food applications, new opportunities are opening up in the field of nano and intelligent food packaging. The latest studies related to chitosan nanoparticles in the food industry indicate that, due to their bigger contact surface, chitosan nanoparticles can enhance functional properties compared to classical chitosan coatings and,

Table 1
Assessment of different crosslinking agents used in collagen-chitosan scaffolds based on the crosslinking effectivity or level, cytotoxicity, improvement of the biomechanical properties and morphology of the resulting structure. Differences between techniques are shown on a scale of −/+++, with higher crosslinking level, cytotoxicity and biomechanical improvement corresponding to +++.

Agent		Crosslinking level	Cytotoxicity	Biomechanical properties	Remaining structure
Physical	Temperature	–	–	++	No change
Chemical	Glutaraldehyde	+++	+	+	Smaller pores
	Tannic acid	+	–	+	High porosity
	EDC-NHS	+	–	+	No change
	Citric acid	+	–	+	Rougher surface
	Genipin	++	–	+	No change

hence, new methods for food coating are being sought to obtain a greater surface coverage due to a greater penetration of nanoparticles. In this regard, a recent study employed aerosolisation of chitosan nanoparticles for hake fillet coating treatment, which showed that a good coating coverage was achieved with small volumes of solution and the coating had minimal impact on physicochemical parameters (Sullivan et al., 2020). Regarding intelligent food packaging, in addition to anthocyanins, the isolation of new indicators from other natural sources is being analysed. In this sense, betalains have been extracted from vegetable amaranth for application in monitoring of shrimp freshness (Hu, Yao, Qin, Yong, & Liu, 2020). Results suggested that films containing betalains showed good response to the volatile ammonia produced by the shrimp's metamorphism, changing colour when the total volatile basic nitrogen slightly exceeded the limit of the standard (Hu et al., 2020).

In the biomedical field, major efforts are being focused on adapting the properties of collagen and chitosan constructs to clinical demands. In this sense, the development of additive manufacturing techniques has meant a great advance in the field, as they allow the rapid and reproducible manufacture of complex 3D shapes. Recently, the emergence of a novel and innovative 4D printing has allowed the combination of traditional and "smart" materials. These possess features that allow to respond to external stimuli (heat, moisture, light, magnetic field or pH), adapting their properties to the microenvironment (change shape or colour, produce an electrical current, become bioactive, or perform an intended function). 4D printing benefits from the property of smart materials to have dynamic responses and to control the construct spatially and temporally. Moreover, 4D printing eliminates the need for external devices or methods for post-processing. Therefore, 4D printing can cause a disruptive effect in the medical field, since it represents a great potential for non-invasive and remoted-control therapies, such as drug delivery, biosensors or regenerative medicine. Considering that each model in medicine varies from patient to patient, 4D printing could allow the achievement of effective personalized medicine (Lui et al., 2019; Mantha et al., 2019; Piedade, 2019; Shie et al., 2019; Tamay et al., 2019).

Author contributions

Conceptualization, K.C and P.G.; resources, K.C and P.G.; investigation, A.I., I.Z. and M.A.; supervision, K.C and P.G.; writing—original draft preparation, A.I., I.Z. and M.A.; writing—review and editing, K.C and P.G.; funding acquisition, K.C.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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