Chapter 1

Decellularized ECM-Derived Hydrogels: Modification and Properties

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Abstract

Extracellular matrix (ECM) hydrogels are water-swollen fibrillary three-dimensional (3D) networks where collagen type I is the major component. The hierarchical network formed by the polymerization of tropocollagen molecules with enhanced properties is an attractive template for generating biomaterials. The mammalian tissue source from which collagen is extracted and its consequent modification are variables that impact the physicochemical and biological properties of the collagen network. This chapter has the purpose to provide a review of the research of different strategies to modify and characterize the properties of decellularized ECM-derived hydrogels in the context of safe biomaterials with immunomodulatory properties.

Keywords: ECM, collagen, hydrogel, cross-linking, properties

1. Introduction

Hydrogels are water-swollen polymeric materials with specific three-dimensional (3D) structure. During the last years, hydrogels have been investigated for enhancing biomedical applications. These biomaterials offer a moist environment that can be enriched to provide protection against infections, regulate the inflammation process, promote tissue regeneration, and remove wound exudates [1]. ECM-based hydrogels are promising materials for tissue engineering and regenerative medicine application due to the balance of biochemical and physical characteristics that can be achieved by their modification. Collagen is the main structural protein of the mammalian ECM. It has a favorable impact on blood coagulation, promoting the aggregation

of platelets, and the absorption of fluid, and regulating the deposition of other ECM's proteins such as fibrin, laminin, elastin, and fibronectin [2]. Besides, collagen can induce processes of the cell signalization involved in the growth, proliferation, migration, and differentiation of cells. Low inflammatory and cytotoxic responses and high biodegradability are other attractive properties of collagen [3]. The collagen can be extracted from diverse ECMs using acid hydrolysis assisted by proteolytic enzymes. The extracted collagen can be subsequently polymerized under physiological conditions (pH 7, 37°C) to generate a highly hydrated 3D network [4].

The ECM-based hydrogels maintain the biocompatibility and biodegradability associated with the collagen. Diverse authors use to refer ECM hydrogels like collagen hydrogels, as the collagen is the major component inside ECM. However, these biomaterials have poor mechanical properties and fast degradation rate, limiting the range of use in applications such as the loading, encapsulating and controlled delivery of cells or drugs, or as wound care dressings [5]. The structure and mechanics of the ECM hydrogels can be modified by chemical cross-linking (using glutaraldehyde, genipin, carbodiimides, acrylates, oligourethanes, and among others); and/or by physical cross-linking (using freeze-drying cycles, forming interpenetrated networks (IPN) with other proteins or polymers). The selection of the cross-linking strategy has to consider the impact upon the structure-property relationships. After modification, several advances have been reported in the design of ECM-based hydrogels. The delivery of cells and biomolecules, the enhancing of the stiffness, the regulation of the cell-material interactions, the control of the cell fate and function, and the modulation of the environment of both normal and injured/diseased tissues are among them [6]. As shown in **Figure 1**, ECM hydrogels have been studied as substrates for ophthalmology, sponges for burns/wounds, systems for controlled delivery of functional molecules or

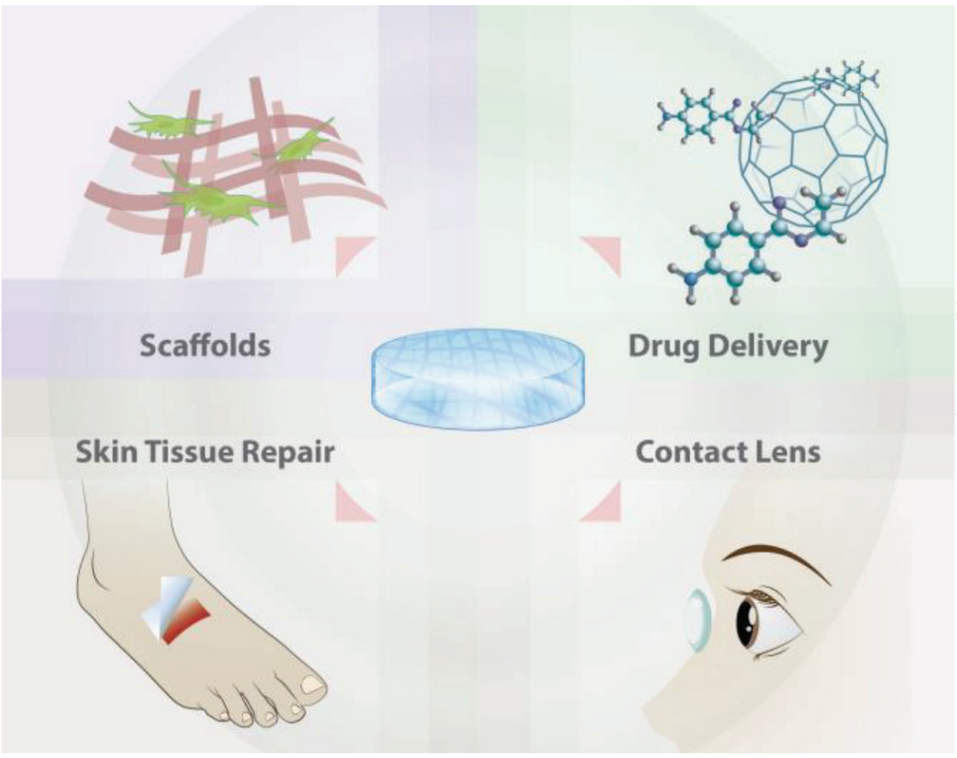

Figure 1. Schematic representation of the biomedical applications of collagen-based hydrogels.

nanoparticles, and matrices for 3D cell culture. They are also investigated for tissue engineering including skin replacement, bone substitutes, and artificial blood vessels and heart valves [7].

In this chapter, a review of the state of the art of different strategies to modify and characterize the properties of natural ECM hydrogels in the context of the biomedical applications is presented. The chapter emphasizes the chemical and physical methods intended to enhance physicochemical properties and immunomodulatory applications of the ECM hydrogels.

2. Methods of preparation of natural ECM-based hydrogels

The versatility of collagen to generate biomedical hydrogels is primarily associated with its complex hierarchical structure originated from its amino acid molecular sequence and the formation of triple helical structures [8]. The collagen polymerization is a self-assembly process of long fibrillar structures, regulated by both electrostatic and hydrophobic interactions that promote the collagen fibers cross-linking [9]. This process is influenced by parameters such as temperature, pH, collagen concentration, and the presence of other biomolecules or polymers [10]. The macroscopic result of the in vitro collagen fibrillogenesis is a 3D water-swollen network.

ECM hydrogels are very suitable materials for biomedical applications due to their good interaction with living tissues, biocompatibility, soft and elastic consistency, high water content, and ECM remaining composition [11]. The swelling in liquid medium gives them the capacity to absorb, retain and release under controlled conditions amounts of water; regulating their structural conformation [12]. The ECM residual composition and the methods of modification of the hydrogels determinate the water uptake capacity and influence their biological and physicochemical properties. This section is dedicated to the discussion of the main characteristics of strategies for the modification of collagen in hydrogel state.

2.1. Importance of the tissue source in the natural ECM-based hydrogels

The ECM is the noncellular component present within all tissues and organs that provides not only essential physical scaffolding for the cellular constituents but also initiates crucial biochemical and biomechanical cues, which are required for tissue morphogenesis, differentiation, and homeostasis [13]. As shown in **Figure 2**, this matrix is composed of a variety of proteins and polysaccharides that are locally secreted and assembled into an organized network in close association with the surface of the cells that produced them [14].

Collagen is the main component of the ECM [15]. The collagen is extracted from diverse ECM by multistep processes including the tissue decellularization and acid hydrolysis assisted with proteases. Among others, collagen has been extracted from porcine dermis [16], bovine pericardium [17], porcine urinary bladder [18], porcine small intestine submucosa [19], bovine Achilles tendon [20], and rat tail tendon [21]. The polymerization of the extracted collagen under physiological conditions (37°C, pH 7) has allowed to develop biomedical hydrogels mimicking the structure and function of the ECM in vitro. The polymerization kinetics and the structural characteristics of the fibrillar collagen gel network are influenced by the residual composition of the ECM. Consequently, the swelling, mechanics, degradation, and biological

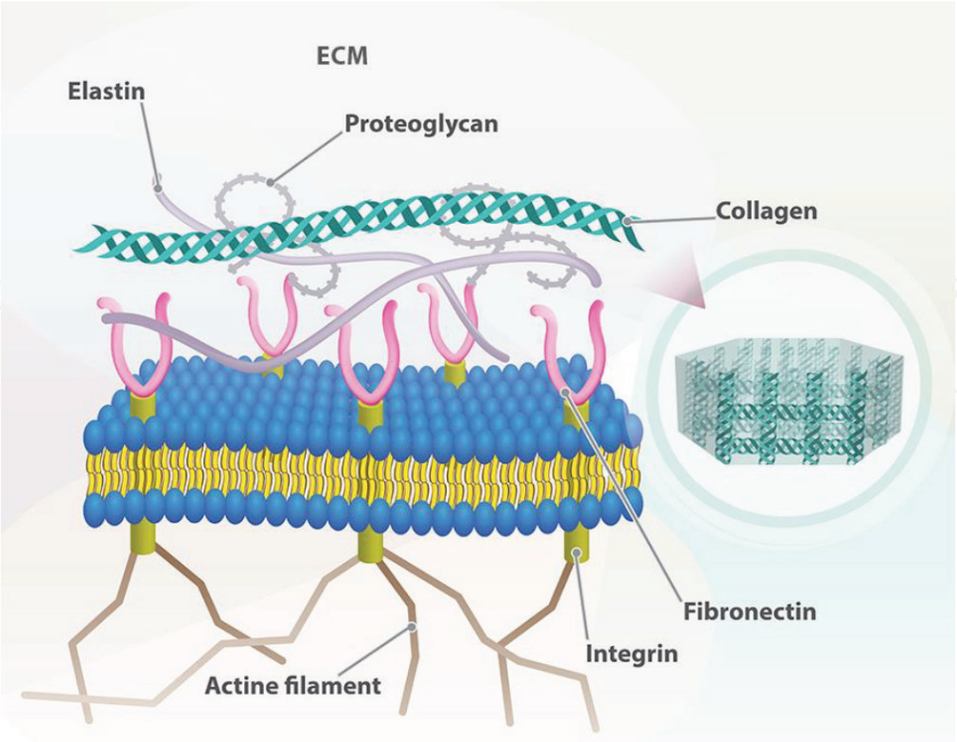

Figure 2. The ECM composition as source for the preparation of collagen-based hydrogels.

response of the ECM hydrogels have shown a direct relationship with the ECM remaining composition [11]. The understanding of the formation process of collagen gels is of relevant importance for the development of strategies capable to synthesize them successfully. The next subsections are focused on those strategies.

2.2. Collagen gel formation in response to change of pH and temperature

The physical methods for the modification of the ECM hydrogels are related to the physical cross-linking of collagen fibers caused by pH, temperature, electrical fields or other physical stimuli, as schematized in **Figure 3** [22]. The advantages of this type of process are the relatively easy manufacture, and the absence of exogenous cross-linking agents, which could reduce the toxicity risks [23]. The variation of pH and temperature of the collagen solution during the in vitro fibrillogenesis produces the collagen cross-linking and increases the fiber size [11, 24]. The temperature-dependent process is reversible [25]. Commonly, the physical methods are not associated with a significant improvement of the mechanical properties of ECM hydrogels, limiting the use of these methods in the preparation of biomedical hydrogels [26].

An interesting physical method to improve the mechanical properties of ECM hydrogels is to apply lyophilization cycles. In this methodology, extracted collagen is incubated at 37°C during 24 h to induce the collagen polymerization, later the hydrogel is frozen at −20°C for 3 h, −80°C for 3 h, and in liquid nitrogen, and then lyophilized. The resulting collagen network demonstrated highly aligned fibrillar features along the scaffold surface, decreased pore size, and increased mechanical properties [27]. However, a major disadvantage related to the

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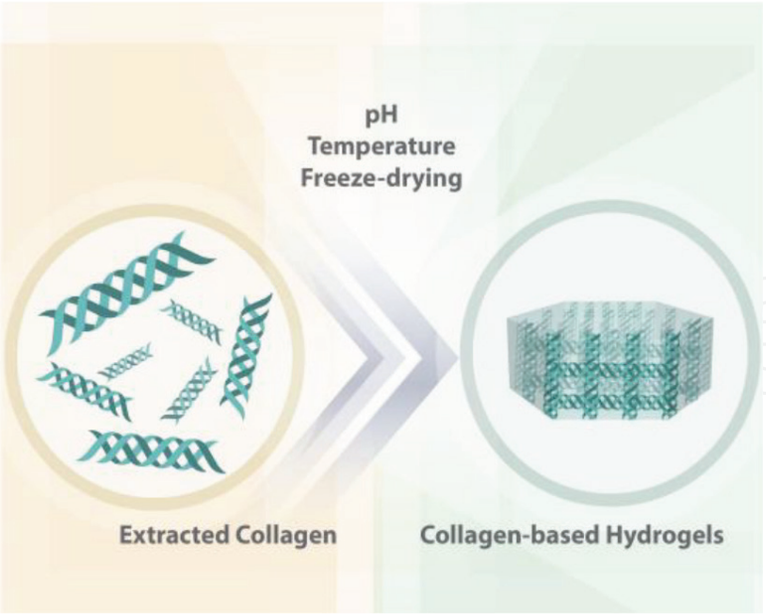

Figure 3. Physical methods for preparation of collagen-based hydrogels.

execution of freeze-drying cycles is the decrease in water uptake of the collagen scaffold [11]. Physical methods do not allow controlling the rate of degradation of collagen-based hydrogels [28]. Therefore, it is still necessary to investigate methods to regulate the characteristics and to expand the use of collagen scaffolds and hydrogels in the medical biotechnology field.

2.3. Interpenetrated networks (IPN) based on collagen and other polymers

IPN hydrogels are based on the physicochemical interactions between the collagen polymeric chains and chains of another type of polymer, as shown in **Figure 4**. The hydrophobic, ionic or hydrogen bonding inside the IPN is responsible for the improved mechanics and degradation behavior. Two examples are the IPNs formed between collagen and chitosan [29], and collagen and polyethylene oxide (PEG) [30]. In these approaches, the ECM extracted collagen is combined with different mass concentrations of polymers, and later this mixture is incubated at 37°C to induce the collagen polymerization. The polymerization process is influenced by the presence of the exogenous polymeric chains altering the collagen fiber size and the physical cross-linking. The IPN hydrogels show poor stability with the change of the temperature and pH [31]; but the enhanced mechanical properties of these biomaterials are adequate for the cell and drug encapsulation [32].

2.4. Chemical cross-linking methods

The search for an ideal procedure to stabilize the structure of collagen maintaining its physical integrity and natural conformation has led to the evaluation of diverse strategies to form covalent bonds. As shown in **Figure 5**, this takes advantage of the conjugation of reactive groups of collagen molecule such as carboxylate (—COO—) and amine (—NH_2) with reactive cross-linkers. Among the most studied processes are the glutaraldehyde (a pentadialdehyde) cross-linking,

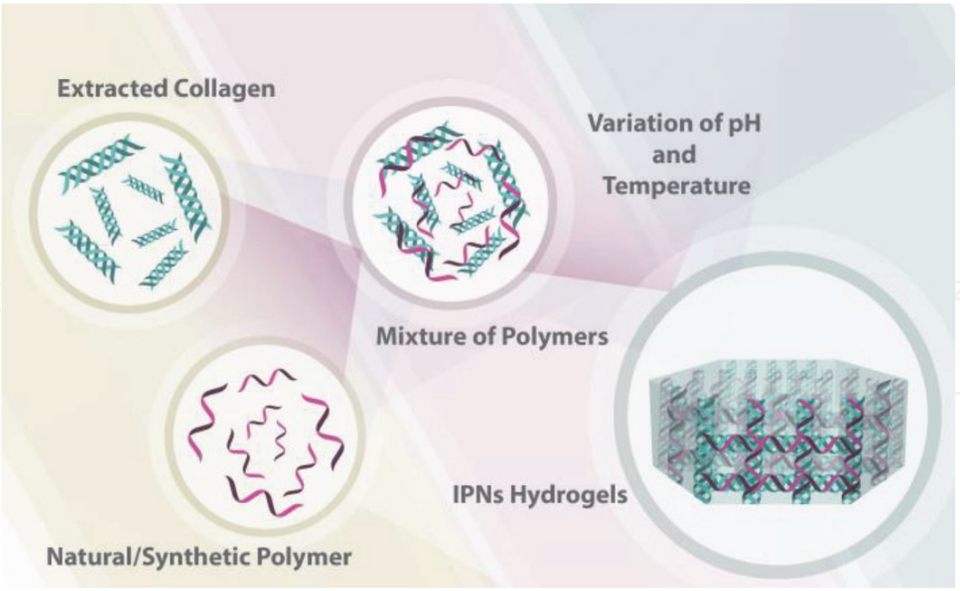

Figure 4. Preparation of hydrogels derived from polymeric IPNs.

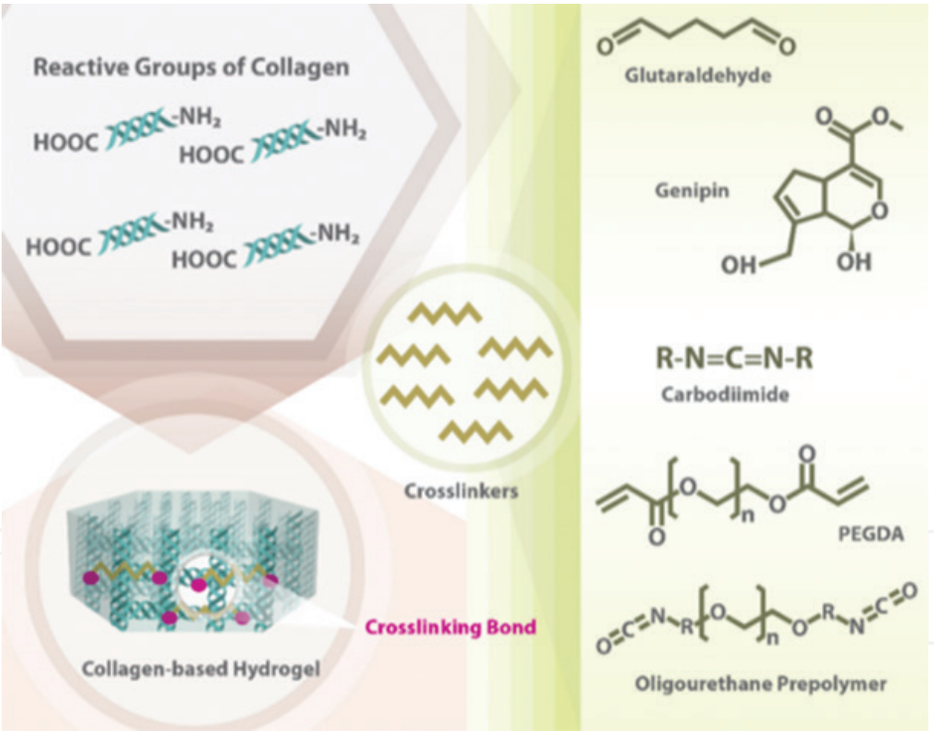

Figure 5. Chemical cross-linking to generate biomedical collagen-based hydrogels.

and the use of carbodiimide 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, a water-soluble carbodiimide), genipin, polyethylene glycol diacrylate (PEGDA), and aqueous polyurethane prepolymers. These methods increase the resistance of the hydrogel toward both chemical and enzymatic degradation, reduce its immunogenicity, sterilize and improve its mechanical

Collagen cross-linker	Main characteristic of the process	Advantages	Disadvantages	Ref.
Glutaraldehyde (GA)	The ε-amine groups of collagen yield an imine bond (so-called as Schiff base), when they react with a GA molecule.	The cross-linking reaction is relatively fast; reacting with most ε-amine groups, improving both mechanics and degradation resistance.	Drastic reduction of the biocompatibility.	[29]
1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC)	Effective catalyst in the condensation of collagen carboxylic acids with alcohols and amines, without presence of the carbodiimide (so-called zero-length).	The degradation products of these biomaterials do not show cytotoxic character.	The cross-linking reaction is not taken out at physiological conditions.	[33–35]
Genipin	Spontaneous cross-linking by formation of Schiff base is produced. A Michael reaction is involved in this process.	The structure and properties of hydrogels show a direct relationship with the genipin concentration.	Generation of blue residues during the preparation of biomaterials, limiting their transparence and use as 3D culture systems.	[36–38]
Poly(ethylene glycol) diacrylate (PEGDA)	Photo cross-linking based on the formation of covalent linkages among the functional groups acrylamide with the collagen-amines.	Hydrogels show enhanced hydrolytic stability, susceptibility to collagen enzymatic degradation. Mechanical properties depend on time of UV irradiation.	Limitations of use of UV irradiation for applications related to gelation in situ or cell encapsulation.	[39]
Polyurethane prepolymers (Pp)	Pp based on PEG and aliphatic diisocyanates cross-links the collagen chains. The process involves the formation of urea linkages between end-blocked isocyanate of Pp and collagen-amines.	The cross-linking process is taken out at physiological conditions. The structure and properties of collagen hydrogels show a direct relationship with the chemical structure of Pp. Pp accelerates the polymerization.	Higher concentrations of Pp inhibit the collagen polymerization, and decrease its biocompatibility.	[40]

Table 1. Chemical cross-linkers for the preparation of collagen-based hydrogels.

properties. **Table 1** summarizes the main characteristics, advantages and disadvantages of the covalent chemical cross-linking methods.

The elucidation of the impact of the modification upon the structure and properties of the hydrogels derived from decellularized ECM requires the use of a combination of distinct techniques. A forthright correlation between modification and properties is key to balance stability and bioactivity. This chapter thus discusses some aspects of the methods used to discern the characteristics of collagen hydrogels and scaffolds and the implications of their use as safe biomaterials with immunomodulatory properties.

3. Methods for physicochemical characterization of collagen-based hydrogels

A collagen scaffold is a hierarchical, protein-based fibrillary network: after the triple helix formation that conforms tropocollagen, it forms fibrils that align themselves in microfibers and finally, in collagen fibers of a tissue [41]. To correlate the different physicochemical properties observed in a collagen derivative scaffold with its hierarchical network is an attractive challenge partially explored. It is important to notice that the chemical modification of plain tropocollagen is a current practice to tune some properties as the mechanical ones. In general, the characterization methods for the collagen-based materials do not vary when it is chemically modified as we will see in this chapter. On the other hand, for composites that contain collagen, it is intuitive to imagine that the methods to determine their properties could be different depending on the other components of the materials.

3.1. Spectroscopy techniques

Distinct kinds of techniques have been used to characterize the physical and chemical structure of the collagen. Confocal microscopy using the second-harmonic generation [42] and Raman effect [43] have proved to be valuable techniques to determine the presence of the plain collagen in different tissues. The generation of the second harmonic signal in collagen scaffolds is due to the fiber alignment, and it is poor in ECM hydrogels, but it can be enhanced once the collagen is aligned (in natural collagen tissues) or stained. Staining, however, is not recommended because can affect the conformation and interaction among the different components of the scaffold. On the other hand, Raman spectroscopy does not have this limitation and can be used to determine and map collagen in dry and wet samples. In addition, it is sensible to the relative composition of amino acids that confirm the collagen and as a consequence, can be used to determine different kinds of collagen or collagen degradation in time during a disease as cancer for instance [43]. Infrared spectroscopy (IR) can be seen as a complement of the Raman spectroscopy because they are sensitive to the same organic groups. The technical difference is that in Raman, we observe the energy of photons scattered from the sample after excitation using a single wavelength, and in IR, we observe the absorption of photons in a range of wavelengths [44]. In general, IR is used when the sample is not extremely complicated, and signals in the spectra can be assigned to specific interactions in the gel as a chemical modifier as a cross-linker for instance [45, 46]. In such a way, another classical technique that determines chemical interactions among distinct parts of a composite, as ^{1}H-NMR can be used also in ECMs [47].

3.2. Microscopy techniques

Scanning and transmission electronic microscopy (SEM and TEM) are also important techniques for collagen characterization and a first easy access to get the pore size [48], length and width of the fibers as well as shape [49], amount and location of nano- and microstructures of different materials added to the scaffold as can be inorganic salts [50] or nanoparticles [51]. SEM and TEM are excellent characterization techniques for lyophilized scaffolds [52], although

in the case of TEM is essential a good handle of the available staining techniques to avoid "artifacts" in the images. However, when those measurements are the reference for wet properties of the scaffold it can be taken only as a guide and other techniques on wet materials are needed to confirm what is being observed.

Using atomic force microscopy (AFM) is possible to observe collagen fibers with different shape and structures on different surfaces [48, 54, 60] as well as to determine micromechanical properties of a collagen hydrogel; both in wet and dry formulations. AFM is microscopy based on the movement of a microtip, in the range of micro- and nanometers, that interacts with the surface at the microscopic level and sense its shape and roughness. The movements of the tip are followed by a laser on it; forming an image of deflection laser intensity. This image, if the deflection force of the tip is known, forms also a map of micromechanical properties.

3.3. X-ray techniques

Another important set of characterization techniques are the X-ray techniques; although their availability depends strongly on the level of development of each particular scientific community in specific countries due to the extensive facilities and economic resources that they need. The more accessible are the X-ray photoelectron spectroscopy (XPS) and the small angle X-ray scattering (SAXS). XPS is able to measure the carbonyl, C═O, and C—C interaction on a collagen surface [53], as well as traces of silicon commonly used now in collagen-based scaffolds [11], for instance. It is in general, the right technique to obtain the gross chemical composition on the collagen surface and a definitive indication for traces of impurities of other elements [54]. Depending on the instrumentation available, wet samples can be measured, since X-rays must work under light vacuum. In addition, it is not expected to see under the surface because the X-ray source is weak and cannot penetrate the sample. On the other hand, scattering X-ray techniques can be used to get the shape, length and width, pore size, and fiber orientation directly from the sample, wet or dry, without further manipulation [55–57]. Depending on the distance to determine, normal X-ray diffraction equipment can also be used [58]. Those techniques are based on the scattering of the X-rays from the sample: a scattering vector is an inverse function of the X-ray wavelength and proportional to the sine of the half the scattering angle. A characteristic distance in the material scatters X-rays of a specific wavelength proportional to the scattering vector and with a different scattering intensity. This intensity is a function of the scattering angle, and it is from where the different properties of the material can be extracted. In general, using smaller scattering angles, it is possible to obtain information about larger distances, as those observed in the collagen when X-rays are used [59].

3.4. Mechanical tests

Mechanical properties (determined at microscopic or macroscopic level) of the collagen are those of a gel or an entangled polymer: basically, oscillatory rheology shows a plateau of the storage modulus (G′) in a frequency ranging between hertz and kilohertz, which can be considered as its Young's modulus, and three times this value can be determined by extensional and compressional experiments of strain versus stress [52]. The convergence of micro- and macromechanical moduli values are not common in the literature [42, 61], although it is

expected a similar trend of increment or decrement of the properties toward the same change in a particular variable as can be the cross-linking degree. The Young's modulus can easily vary between cross-linked and uncross-linked collagen one order of magnitude [62, 63]. An important physical parameter directly correlated to the value of the storage modulus is the pore size of the collagen network: the size of the pore is simply the cubic root of the thermal energy (3kT) over the Young's modulus [64]. Alternatively, the pore size distribution of a scaffold can be obtained by analyzing images of thin sections of paraffin-embedded samples obtained by optical microscopy [42] or using electron microscopy as previously explained [48].

Shear flow experiments are useful to obtain the viscosity of the collagen hydrogel precursors, the concentration of the proto-collagen present in a solution, and an estimation of the molecular weight of the minimal structured collagen in solution [65]. It has been also suggested that collagen denaturation can be determined by viscosimetric measurements [66]. Those experiments become important in the case of development of injectable systems because parameters as viscosity [47, 67] and compressibility [52] are important during extrusion. Rheological methods described previously are also convenient to measure the formation of the gel in time: storage (G′) (colloquially speaking, how much the viscoelastic material looks like a solid) and loss modulus (G′′) (how much the viscoelastic material looks like a liquid) can be determined in an oscillatory rheological measurement to get the gel formation point: where the storage modulus becomes higher than the loss modulus (G′ > G′′) [68].

3.5. Thermal stability test

The denaturation heat and denaturation temperature of a collagen scaffold are obtained from calorimetric experiments commonly using a differential scanning calorimeter (DSC) [67]. Since the technique is based on calorimetric differences sensed by an extremely sensitive electronic device, it is important to consider that minimal differences in the medium concentration (buffer concentration, conductivity of the water used as a solvent, etc.) or during the preparation of the samples (pH, size and shape of the particles, etc.), are observed [48, 69]. Thermal denaturation peak of wet collagen occurs around 50°C, although the heat absorption peak is broad and could start under 20°C before the peak; a straightforward evidence that the collagen has distinct levels of structure. The integral under this endothermal process, that is, energy versus temperature, gives the denaturation heat of the collagen. In general, it has been reported that both denaturation heat and temperature are higher for cross-linked collagen that for uncross-linked collagen [50].

4. Perspectives of the decellularized ECM-based materials in immunomodulation

Biomaterials with immunomodulatory activity are being studied in the context of the repair/regeneration of soft tissues, such as diabetic chronic wounds. Evidences indicate the effect of the characteristics of biomaterials and their (released/biodegraded) by-products over promoting of required immunological responses that could support the wound healing. Moreover, the residual components remaining the animal source as well as the modification of ECM-based materials can elucidate an undesirable response.

4.1. Macrophage polarization in decellularized ECM-based materials

Macrophages are cells of the innate immune system with a dominant effector activity in the injury site after biomaterial implantation. Cross-talk between immune cells activates macrophages after which, they release a variety of signaling molecules. Signaling molecules secreted by macrophages such as cytokines (as interleukins), growth factors as the basic fibroblast growth factor, the vascular endothelial growth factor and the transforming growth factor-beta 1 (bFGF, VEGF and TGF-β1 respectively); and tumor necrosis factor (TNF-α) influence the development of other cell types [70]. In fact, the profile of signaling molecules secretion is commonly evaluated to study the polarization of the macrophage response from an inflammation and tissue injury process to a repair process [71, 72] or to study angiogenesis and scaffold vascularization [73]. Macrophages mediate the healing responses to implanted biomaterials, fundamentally by two outcomes: scar tissue formation (M1M pathway) or regeneration (M2M pathway) [70]. The modulation of the inflammatory response by the physical and chemical properties of biomaterials represents a hypothesis currently assessed in the design of biomaterials intended to the repair/regeneration of soft tissue.

4.2. Impact of the residual composition on the immune response

The goal of the decellularization process of mammalian tissues is to remove its cellular and nuclear components. This aim must be balanced with retention of both the extracellular composition and microstructural characteristics, as much as possible. As result, an incomplete removal of nuclear components has been reported in diverse ECM biomaterials, even in commercial biological implants [74]. The intensity of the host immune response after implantation is heavily influenced by the residual material, which acts as like cell signals [74, 75]. For instance, a decrement in the DNA amount in small intestine submucosa tissue provoked a shift of the M1M proinflammatory macrophage phenotype to the M2M anti-inflammatory one [76]. On the other hand, the tissue regeneration induced by the ECM-based biomaterials has been associated with extracellular residual components such as collagen type I, polysaccharides or basal membrane complex components [77]. Glycosaminoglycans such as hyaluronic acid extracted from brain and urinary bladder have been associated with an up-regulated secretion of anti-inflammatory factors and suppressed secretion of proinflammatory factors, consistent with M2M phenotype macrophages [76]. Moreover, studies revealed that the anionic detergent sodium dodecyl sulfate and nonionic detergent TritonX-100 produce a different impact over the stability of ligands and proteins in the basal membrane complex [80]. The decellularization method and tissue source thus influence the retention of the basal membrane complex components within ECM materials and the bioactivity of them. The bioactivity of ECM-based materials was also evidenced by the differentiation of human monocytes differentiated to macrophages. The higher amounts of interleukin-6 (IL-6), interleukin-8 (IL-8), and monocyte chemoattractant protein-1, but lower amounts of interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1ra) were detected on decellularized pericardium matrix, in comparison with polydimethylsiloxane or polystyrene surfaces [81]. Cellular residual components such as damage-associated molecular patterns (DAMPs, proteins that are retained within the ECM scaffolds) have been considered as bioinductive molecules with a key role in the macrophage polarization [78]. High-mobility group box 1 (a DAMP that functions intracellularly as a DNA binding nuclear protein), detected in ECM biomaterials derived from small intestinal submucosal, and urinary bladder matrix, was correlated with differences in cell

proliferation, death, secretion of the immunomodulatory factors [78]. Altogether, reports suggest that decellularization, as the first step in the development of ECM-based hydrogels, and scaffolds, impact the cellular and extracellular components within biomaterials. Consequently, these components become in a key player in the mechanisms of tissue regeneration observed when decellularized ECM materials are used. The ability to support the proliferation and migration of different cells [82], to allow the differentiation of mesenchymal cells [83, 84], and to transit from the inflammatory first steps to a regenerative action [75, 85] are among the mechanisms by which the ECM biomaterials participate. Once animal tissues are decellularized, they are cross-linked to increase their stability, reduce degradation, and immunogenicity. However, the reconstruction of functional tissue appears to be compromised after cross-linking as discussed below.

4.3. Impact of the collagen cross-linking on the immune response

The cross-linking process of ECM-based biomaterials is commonly associated with a detrimental effect on the ultrastructure and composition of the ECM and consequently the biological response [78]. The ability of the decellularized ECM materials to interact with cells is modified by the altered surface chemistry after cross-linking. As discussed above, distinct methods for cross-linking collagen biomaterials have been studied. The understanding and control of cell fate in modified chemically collagen materials is a matter of study. For instance, the cell membrane morphology, cell adhesion and enzymatic activity of the acid phosphatase and esterase of U937 macrophage-like cells have shown to be differentially influenced by the glutaraldehyde cross-linking, and EDAC coupling methods. Glutaraldehyde cross-linking induced an increase in the release of the proinflammatory cytokines IL-1ra, IL-6, IL-10, and TNF-α, unlike to EDAC-cross-linked materials and uncross-linked tissues [86]. Differences in the microenvironment of ECM-based implants cross-linked with glutaraldehyde and diisocyanate (aliphatic) cross-linking methods modified the infiltration of neutrophils and the function of macrophages [87]. A strong proinflammatory milieu was observed in glutaraldehyde-cross-linked materials, while in diisocyanate-cross-linked materials, an anti-inflammatory milieu was seen. The proliferation of immune cell subpopulations was found stronger on both porcine nondecellularized and decellularized materials than on the glutaraldehyde-cross-linked ones [79]. This observation has been reported in the case of cross-linking of tissue-derived heart valves [88]. The integration of this implant type has been associated with a reduced antigenicity by masked immunogenicity [88]. A lack of acute inflammation in dermis-derived implants (fixed with glutaraldehyde at low concentration) both in animal models and humans was observed. Thus, the high concentrations of aldehyde employed in the processing of ECM biomaterials appear to induce a more pronounced and sustained inflammatory response [89]. The dermis-derived implants cross-linked with diisocyanates showed a low chronic inflammatory response after a 20-week period of implantation with both limited collagen degradation and vascular ingrowth [89]. Non–cross-linked ECM materials showed earlier cell infiltration, extracellular matrix (ECM) deposition, scaffold degradation, and neovascularization compared with cross-linked materials, after a 1-month period of implantation. However, after 6 and 12 months, diisocyanate-cross-linked materials showed comparable results compared with the non–cross-linked materials [90]. The cross-linked collagen-derived implants showing an acceptable performance in diverse applications would seem to suggest a degree of tolerance to these materials [90]. However, the tissue remodeling associated with the ECM constituents is yet a challenge to be addressed in the development of new cross-linked ECM biomaterials.

4.4. Immunomodulation with decellularized ECM-based hydrogels

The performance of hernia standard surgical grafts, manufactured from polypropylene, has been improved by the coating of them with decellularized ECM-based hydrogels. This was attributed to the polarization of alternatively-activated and constructive M2Ms macrophages induced by the degradation products from ECM materials, which in turn facilitates migration and myogenesis of skeletal muscle progenitor cells [84]. The migration and proliferation of perivascular stem cells are influenced by the structural components (include a number of partially digested proteoglycans and proteins such as collagens, elastin, laminin, fibronectin, hyaluronan, and heparan) as well as soluble components of hydrogels derived from urinary bladder matrix (include cryptic peptide fragments generated from partial proteolysis of scaffold resident growth factors, and matricellular proteins, e.g., tenascin, osteopontin, and thrombospondin) [76]. The mechanism through the soluble and structural components of ECM-based hydrogels contribute to the host response appears to be different. Both components altered the macrophage behavior but with different fingerprints according to the cytokines secretion profiles [76]. A hernia rodent model study revealed that the implantation of polypropylene meshes coated with ECM hydrogel for a period of 14 days decreased the inflammatory response, which was characterized by the number and distribution of M1Ms around polypropylene fibers, compared to the uncoated devices. After a period of 180 days, the density of mature type I collagen deposited between mesh synthetic fibers was decreased with the coating of ECM hydrogel was used [76]. The coating based on ECM-based hydrogel suggested a low scar tissue deposition on the synthetic mesh, which can be associated with a mitigated chronic inflammatory response, an attenuated M1M response, and an increased M2M/M1M ratio to abdominal defect polypropylene standard grafts [91]. The use of decellularized amniotic membrane tissue combined with poly (urethane-ester) showed a better biocompatibility compared to polypropylene meshes when implanted into abdomen of rabbits over a period of 10 months [92]. Results of in vitro cytocompatibility tests demonstrated that this composite can support primary smooth muscle cells to grow and differentiate, with high proliferation, mitochondrial activity, and special protein expression (α-smooth muscle actin).

5. Final remarks

Mammalian tissues from various sources can be used as biomaterials after modification by decellularization and cross-linking processes. Among these materials, the ECM-based hydrogels seem promising alternatives to modulate the required properties in applications related to biomedicine and tissue engineering. Current approaches usually affect the network structure, physicochemical properties, and biocompatibility of natural ECM-based scaffolds. Thus, a balance between the mechanical and degradation properties and immunology response is a present challenge. In this respect, methodologies based on the combination of the ECM with natural and synthetic polymers, minimizing the removal of the characteristics of the natural ECM, seem to be the best alternatives for this purpose. The structural modification of the natural ECM is related to the variation of its properties; this process can be monitored by a variety of physicochemical techniques, which could provide direct evidence of the structure-property relationship in ECM-based biomaterials. A direct evidence of the ECM properties is definitely a challenge, because some of the most common

techniques give only approximations to them. A sample preparation that could include denaturation, drying, staining, etc., can completely change a parameter as the pore size or the fiber alignment. In such a way, new or revised techniques that can be used on undamaged and functional ECM are desirable [93, 94]. Those new techniques where the cellular function is not compromised, will give not only more reliable information about the way ECM interacts in the body, but will open new perspectives on the way to study and prepare ECMs for future applications.

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Conflict of interest

The authors declare no conflict of interest.

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