Expanding the repertoire of electrospinning: new and emerging biopolymers, techniques, and applications

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ABSTRACT

Electrospinning has emerged as a versatile and accessible technology for fabricating polymer fibers, particularly for biological applications. Natural polymers or biopolymers (including synthetically derivatized natural polymers) represent a promising alternative to synthetic polymers, as materials for electrospinning. Many biopolymers are obtained from abundant renewable sources, are biodegradable, and possess inherent biological functions. This review surveys recent literature reporting new fibers produced from emerging biopolymers, highlighting recent developments in the use of sulfated polymers (including carrageenans and glycosaminoglycans), tannin derivatives (condensed and hydrolyzed tannins, tannic acid), modified collagen, and extracellular matrix extracts. We also discuss the proposed advantages of these biopolymer-based fibers, focusing on their biomedical applications, to highlight the use of new and emerging biopolymers (or new modifications to well-established ones) to enhance or achieve new properties for electrospun fiber materials.

Keywords: Biopolymers, electrospinning, sulfated polymers, tannin derivatives, collagen, nanofibers.

1. INTRODUCTION

The selection of a starting material is one of the most critical aspects in material development. Materials that are abundant, or widely available, and which can be easily and reliably processed into value-added products make attractive raw materials [1–4]. Several techniques are commonly used for processing and engineering new biomaterials. However, due to its remarkable simplicity, robustness, and versatility, electrospinning has emerged as one of the most widely used techniques for the formation of porous fiber materials for tissue engineering and other biomedical applications [5–7]. Electrospinning can generate fibers with diameters down to the nanoscale, and with properties like an extremely high surface area-to-weight ratio, low density, high porosity, variable small-pore size distribution, superior stiffness and high tensile strength compared to conventional fibers [8,9].

Biopolymers and their derivatives by chemical modifications are promising candidates for electrospun fibers [10]. Polysaccharides are renewable, biodegradable, and abundant natural polymers, and include the most abundant polymers on Earth (cellulose and chitin) [11,12]. Polysaccharides have, on average, three hydroxyls in each repeating unit, providing a large number of sites for chemical modifications [13,14]. Because of these characteristics, they have been extensively used in biomaterial formation to achieve biodegradable products from renewable sources, with important physicochemical properties [15–17]. Also, due to their solubility in different solvents and different structural behavior in solution, biopolymers are extensively used for scaffolds and fiber formation by electrospinning, and have shown promising biological properties, such as cytocompatibility for different cell phenotypes, enhanced blood coagulation for applications as wound dressings, enhanced antibacterial activity, and absorption capacity for dyes and other molecules [1,18,19].

In this review, we focus on the recent developments in electrospinning technology. In particular, we describe new fibers produced from emerging biopolymers with features that impart useful biochemical function, including sulfated polysaccharides (carrageenans and glycosaminoglycans), tannins and their derivatives (condensed and hydrolyzed tannins, tannic acid), modified collagens, and extracellular matrix (ECM) extracts. Examples of each of these materials are shown in **Figure 1**. Moreover, applications of these electrospun fibers mainly in biomedical and environmental fields are highlighted.

2. BIOPOLYMERS

Biopolymers are polymeric biomolecules derived from cellular or extracellular matter, synthesized by living organisms [20,21]. Commercially relevant biopolymers include a diverse set of polysaccharides, proteins, lipids, polyphenols, and specialty polymers produced by bacteria, fungi, plants, and animals. Mainly, they are divided into polynucleotides (such as the nucleic acids DNA and RNA), polypeptides (proteins) and polysaccharides (polymeric carbohydrates), consisting of long chains made of repeating, covalently bonded units, such as nucleotides, amino acids, or monosaccharides [22]. Other biopolymers include those that are primarily composed of polyphenolic, aliphatic, and heterocyclic groups, such as suberin, lignin, sporopollenin, melanin, and tannin derivatives. Some of these polymers are highly branched, durable, multifunctional, and difficult to process by solution-based techniques.

Naturally occurring polymers perform a diverse set of functions in their native biological settings. Polysaccharides can be found in the composition of the cell wall structures of many organisms, as capsular layers or protective barriers around cells. Polysaccharides participate in recognition events at the cell surface, act in membranes, and regulate cellular communication. Proteins function as structural materials, catalysts, regulators of cell signaling, adhesives, and recognition elements. Lipids function as energy stores, emulsifiers, and barriers in cell and organelle membrane structures [23].

Biopolymers offer great choices for material manufacturing applications as they are readily biodegradable, renewable, abundant, and contain many inherent biochemical functions. Furthermore, their production and application may be less hazardous compared to synthetic polymers, which are mostly derived from non-renewable sources, like petrochemicals [24,25]. Also, due to their similarity to biomolecules found in the human body, and their ability to participate in biochemical processes, they are great candidates for medical materials, for applications including tissue engineering [26,27]. Polypeptides and polysaccharides present hydroxyl, amine, sulfate, carboxylic acid and other functional groups, imparting reactivity and ability to undergo chemical modifications. Functional group derivatization is an important route to enhance, develop, or change properties of these biopolymers [28,29]. Biopolymers modified by the inclusion of a single group can enhance or completely alter biological and physical properties. For example, cellulose is completely insoluble in water; carboxymethylation of cellulose imparts water solubility [30]. A wide range of naturally occurring polymers derived from renewable resources is available for material applications and many of them are used in commercial products. These include starch, chitin and cellulose [10,13,33]. However, many others with potentially revolutionary properties remain underutilized.

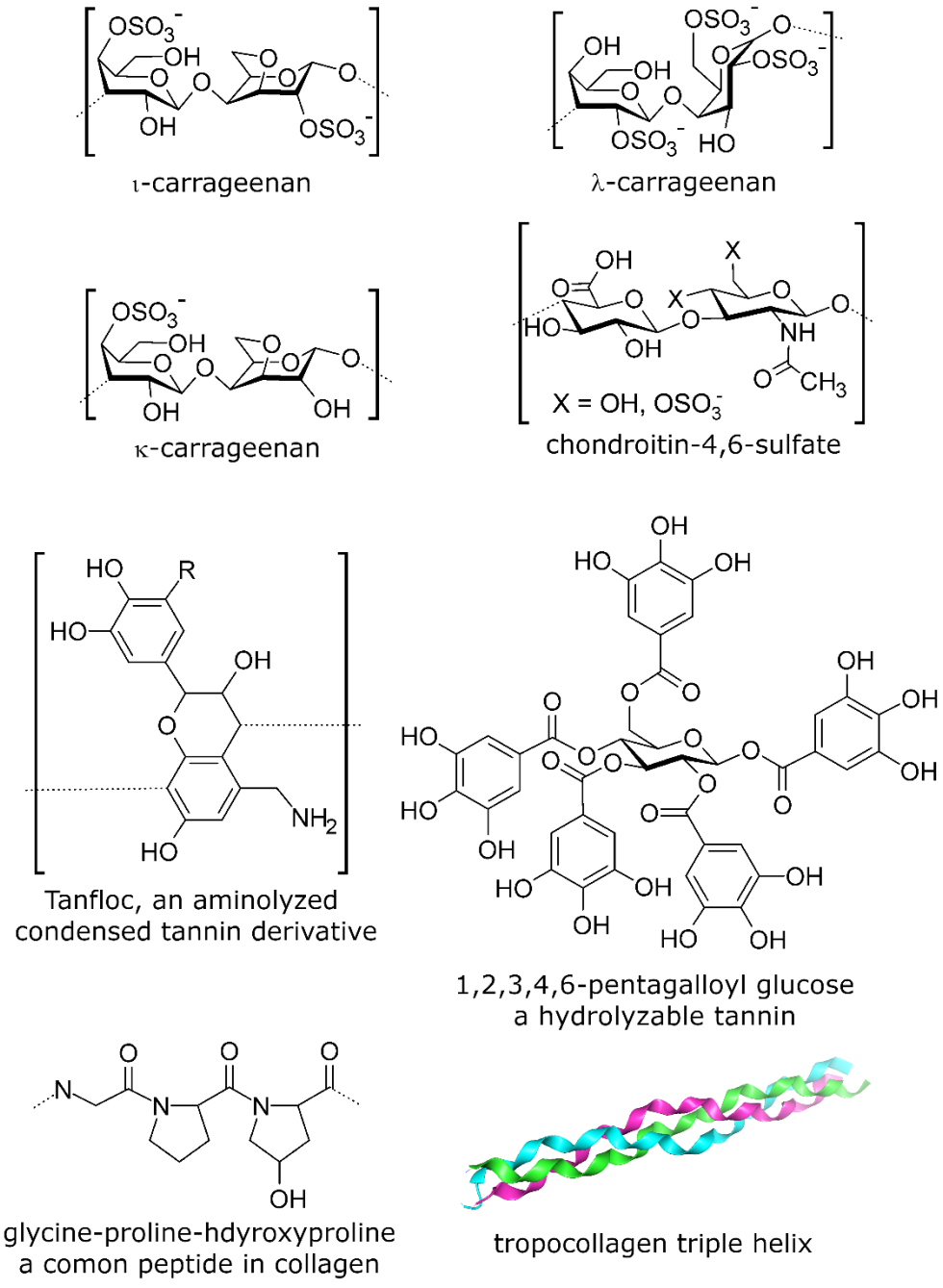

Figure 1. Biologically-derived materials discussed in this review for forming electrospun fibers. (first and second rows) Four different biologically-derived sulfated polysaccharides. (third row) Two types of tannins, an aminolyzed condensed tannin polymer (tanfloc), and a simple hydrolysable tannin, 1,2,3,4,6-pentagalloyl glucose. (fourth row) A common tripeptide sequence found in collagen (glycine-proline-hydroxyproline) and the crystal structure of the tropocollagen triple helix (generated using PyMOL, using PDB ID: 1WZB).

2.1 Sulfated Biopolymers

Sulfated biopolymers have attracted recent research attention. These polymers can be naturally sulfated or obtained by sulfation of natural polymers. The glycosaminoglycans (GAGs) represent an important class of sulfated polysaccharides, found in almost all animal tissues. The GAGs include chondroitin sulfate (CS), heparin, heparan sulfate, keratan sulfate, dermatan sulfate, and hyaluronan (the only non-sulfated GAG) [34]. The glycosaminoglycans participate in many cytokine signaling pathways, by binding and stabilizing cytokines and growth factors, and also binding their respective cell surface receptors [35,36]. GAGs also impart mechanical properties to the extracellular matrix (ECM), and they help to organize and stabilize the nanoscale assembly of other ECM components [37]. Because of their many beneficial biochemical functions, non-natural or semisynthetic sulfated polysaccharides have also been proposed as biomaterials, such as sulfated dextran and sulfated chitosan.

Another important class of the natural sulfated polymers are the carrageenans, which are anionic heteropolysaccharides extracted from red algae, and commercially found as kappa (κ), iota (ι) and lambda (λ) carrageenan, (**Figure 1**) [38]. These three carrageenans are differentiated by the location and number of sulfate groups per disaccharide repeat unit, where kappa carrageenan (KC) has only one sulfate group, iota-carrageenan (IC) has two, and lambda-carrageenan (LC) has three. KC has been proposed as a natural material for use in drilling fluids [38], regenerative medicine [27], and for the development of edible films and coatings [39]. KC can impart immunomodulatory and antitumor activities, and improve the hemocompatibility and non-cytotoxicity of materials [40,41]. Carrageenan oligosaccharides show antitumor and free radical scavenger activity, act as antioxidants, participate in biochemical signaling, and prevent oxidative damage in living organisms [42]. Commercial carrageenans such as IC and LC show higher coagulant activity and inflammatory potential than KC [43]. Antiviral activity for herpesvirus type 1 and 3 have been reported for LC, which also absorbs methylene blue dye in aqueous solutions [44–46].

Other seaweeds also produce sulfated polysaccharides with potentially useful biological properties. Fucose-containing sulfated polysaccharides and other sulfated polymers present in brown algal cell walls have a broad range of biological properties such as anti-infectious and anticancer actions, regulation of the immune response, promotion of hemostasis and anti-inflammatory responses, antioxidant activity, and free radical scavenging activity [47]. Sulfated polysaccharides isolated from *Gracilaria rubra*, a genus of red algae, presented no cytotoxicity, and strong antioxidant activities, exhibiting strong protective effect on H_2O_2-induced oxidative injury in cells. They also behave as immunostimulants, enhancing phagocytic activity, acid phosphatase activity and NO production, due to their higher sulfuric radical content [48]. Sulfated polysaccharides from pollen provide one example of the immunomodulatory effects of sulfated biopolymers. Sulfated pollen polysaccharides promote murine macrophage proliferation and immune activation functions in macrophages, mainly through the toll-like receptor 4- (TLR4-) mediated activation, accompanied by an increased intracellular calcium ion concentration [49].

2.2 Tannins and Tannin Derivatives

As new and emerging biopolymers, tannins and tannin derivatives have gained attention due to their inherent biological properties. Tannins are plant-derived, water-soluble polyphenols and can be found in wood, bark, fruit, fruit pods, leaves, roots and plant galls [50]. These natural biopolymers can protect plants against pathogens and are responsible for the astringent flavor in some wines and teas [51]. Tannins are classified as hydrolysable and condensed. Hydrolysable tannins originate from esterification of gallic acid with glucose and products of their oxidative reactions, in which gallic acid is the most basic constituent, and the molecules of D-glucose are the central core (**Figure 1**). Condensed tannins are composed of polyhydroxy-flavan-3-ol oligomers by C–C linkages between flavanol units. They are far more common than hydrolysable tannins in plants, with more complicated structures [52–54]. Tannin-

based polymers have recently been proposed for several applications. Tannin-inspired gelatin bioadhesives were produced by the modification of gelatin with tannic acid, showing excellent cytocompatibility, as well as antibacterial and antifungal properties. The polyphenol-based gelatin-tannin tissue adhesives utilized tannic acid, as an abundant and low-cost raw material. In comparison to mussel-inspired bioadhesives, the tannin-based adhesive is lower cost and eliminates the concerns of potential neurological effects, since there is no use of dopamine or 3,4-dihydroxyphenylalanine (DOPA). The tannin-inspired gelatin bioadhesives exhibit antimicrobial activity, and may be used as a tissue sealant for wound healing [55]. The biological activity and nutritional value of tannin-rich diets can be complementary in grass-based ruminant diets, improving diet quality and providing additional ruminant feed during the dry season [56,57]. Condensed tannins protect poplar against methyl viologen-induced oxidative stress in high concentrations, by a mechanism for mitigating the reactive oxygen species [58]. The same class of tannins, in special (–)-epigallocatechin-3-gallate, exhibited remarkable combinational effects with other anticancer drugs, including antagonizing, chemosensitive and chemoprotective effects. Therefore, tannins are a promising class of natural compounds for cancer treatment and prevention, either used alone or in combination with other chemotherapeutic agents [52].

A hydrophilic, cationic, and condensed amino-functionalized tannin derivative synthesized from a Mannich-type reaction using tannins from the black wattle, and sold commercially as tanfloc, has recently attracted some interest because of its promising cytocompatibility, biodegradability, and antimicrobial properties (**Figure 1**) [59,60]. Tanfloc has been largely used as a natural coagulant and flocculant for treatment of domestic wastewater to enhance the performance of clarification in biological treatment units [61–63]. Improvement in the nitrification process was observed, by increasing the percentage of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria as a consequence of the favorable environment due to the application of tanfloc [63]. In recent work proposing the use of tanfloc as a biomaterial, tanfloc-based polyelectrolyte multilayers were shown to promote stem cell adhesion and proliferation,

to improve hemocompatibility through interactions with platelets and serum proteins, and to possess antibacterial activity, as well [59,64,65].

2.3 Collagen and Collagen Derivatives

Collagen is a very well-studied natural macromolecule that has been receiving renewed attention in recent years, due in part to the development of chemically modified collagen derivatives [66,67]. Collagen is the major component of the extracellular matrix and is abundant in mammalian tissues. Since it can be extracted in large quantity, collagen and its derivatives, formulated as fibers, membranes, gels or sponges have been widely used as natural materials in the field of tissue engineering, as wound dressings and for drug delivery [68–71]. Twenty-eight different types of collagen have been identified in the literature [72]. However, type I collagen occupies the dominant position in collagen applications because of its relatively abundant availability. Collagen consists of two homologous chains (α1) and one supplementary chain (α2) that vary slightly in chemical composition, which assemble into characteristic triple-helical tropocollagen motifs (**Figure 1**) [73].

Collagen chemical modification can impart many new properties. For example, the free amino group is prone to acylation with maleic anhydride, to introduce double bonds. This process was performed by Li and coworkers to form injectable hydrogels with modified collagen and hyaluronan that proved to be biocompatible and provided a good environment for cell growth and proliferation [68]. A similar procedure was achieved by using maleilated collagen and 2-hydroxyethyl methacrylate hydrogels, showing potential applications on tissue engineering [74]. Collagen hydrogels grafted with dodecenylsuccinic anhydride by covalent bonds to hydroxyl or amine groups, and loaded with simvastatin showed antimicrobial and anti-inflammatory properties, for cutaneous wound healing [66]. Mineralized collagen-modified polymethylmethacrylate cement enhances bone integration and reduces fibrous encapsulation in the treatment of lumbar degenerative disc disease, with improved hydrophilicity, dynamic

mechanical performance, and capacity to hinder the proliferation and fusion of macrophages [75]. Plasma-modified collagen coatings applied to wounds in an immuno-compromised rabbit wound model showed significantly lowered volume fraction of inflammatory cells and produced some indications of improved wound healing [76]. In the same way, a modified bovine collagen-based wound dressing induced transition of wound macrophages from an inflammatory to a reparative phenotype [77]. Modification of collagen gels with biomimetically mineralized collagen was shown to prevent cell-laden gels from shrinking and suppressing alkaline phosphatase gene expression, improving osteocytic differentiation of human osteoblasts [78]. Also, a naturally derived, environmentally friendly, strong elastic macromolecule film was prepared based on leather collagen fibers from mammalian skin and enhanced natural rubber, significantly improving dispersion and interfacial compatibility in the matrix, leading to effective interfacial stress transfer [79].

2.4 Decellularized Extracellular Matrix (dECM)

The extracellular matrix (ECM) has a complex structure composed primarily of collagen and other proteins, such as elastin, laminin, fibronectin and glycosaminoglycans. The ECM serves many functions, such as regulating intercellular signaling and providing mechanical support [80]. Decellularized extracellular matrices (dECMs) have been used in tissue engineering to support the regeneration of tissues and organs, due to their ability to preserve the complex composition of the ECM and maintain the functionality of the natural ECM [81]. The use of dECM for biomaterial manufacture is hypothesized to enhance the regeneration of a specific organ or tissue, since the structure and composition of the manufactured material will be similar to the natural ECM. Human adipose-derived tissue dECM scaffolds injected subcutaneously in mice promote host cell infiltration without any sign of immunogenic response, showing promising results for allograft tissue engineering [82]. Human cardiac progenitor cells in dECM bioinks were used to 3D print a complex tissue construct for cardiac repair. The printed structure

promoted strong vascularization and tissue matrix formation in vivo (mice), enhancing cardiac functions, such as reduced cardiac hypertrophy and fibrosis, neo-muscle formation, and capillary formation [83]. Liver dECM has been shown to enhance the viability of hepatocytes, when used as a substrate for culture or as a hydrogel to support the hepatocyte growth. The presence of key molecules found in the native liver enhanced hepatocyte functions, including albumin and urea synthesis, when compared with collagen type I [84,85]. In addition, dECM from cancerous tissues has been shown to be a more suitable option for studying the role of cancerous cell behavior when compared to the normal tissues, contributing to the evaluation of cancer progression and cancer therapy [86].

From the information presented above, biopolymers like sulfated polymers, tannin derivatives, modified collagen, and dECM can impart beneficial biological properties to materials, including regulating immune responses. Their multiple inherent biological properties may make them excellent alternatives to synthetic polymers for biomaterials development [87–91]. Based on these excellent properties, these classes of materials should be further explored as candidates for electrospun biomaterials.

3. ELECTROSPINNING

3.1 Electrospinning overview

In the last twenty years, electrospinning has gained popularity as a straightforward and versatile method for generating fibrous materials with different morphologies from the nano- to the micro-scale [5,18,92–94]. This technique is preferred over several other methods (including melt-spinning and 3D printing) due to the high surface area-to-volume ratio that can be achieved and the relatively large number of inter- and intra-fiber pores obtained in the fibers [95–98].

The electrospinning process employs electrostatic forces to produce polymeric fibers, in which various parameters such as applied voltage, polymer concentration, solvent, tip-to-target distance and spinneret gauge size influence the quality of the fibers [18]. In practice, a

polymeric solution in a volatile solvent is fed at a controlled rate to a needle that serves as the spinneret, via a syringe pump. A high voltage (10 - 30 kV) is then applied to the solution, and a grounded or oppositely charged target (collector) is placed at a certain distance (5 to 30 cm) from the needle to collect the fibers produced. As the polymer solution is pumped out of the spinneret a small droplet of polymer solution forms and is maintained by its surface tension at the tip of the needle; when the electric field is applied, charges accumulate on the surface of the solution, where the repulsion of the mutual charge causes a force opposite to the surface tension. As the electric field increases, the surface of the solution drop elongates to form a conical shape, called a Taylor cone (the result of a combination of charge repulsion and surface tension). When the electric field reaches a critical value, the electrostatic force overcomes the surface tension; a jet charged with the solution is then formed. This jet, from the Taylor cone, travels through the region of instability, where the evaporation of the solvent alters the mechanical properties of the fiber, resulting in flexing and whiping movements that cause the jet to thin as the fiber is drawn. The bending movement becomes faster and faster as the jet travels, making it appear as sprayed drops or separate fibers to the human eyes, even if only a single fiber is ejected. Finally, continuous fibers are deposited in the collector, forming an interconnected non-woven material on the collector. The process, illustrated in **Figure 2**, can be conducted at room temperature under atmospheric conditions [8,99,100].

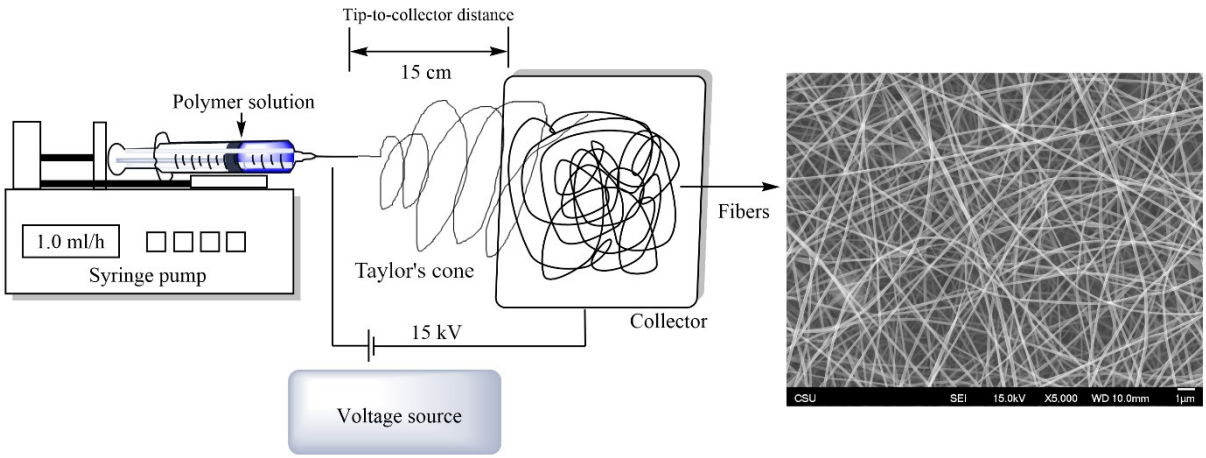

Figure 2. Schematic of electrospinning technique and SEM image of poly(vinyl alcohol) electrospun fibers, obtained from a 5% (wt./vol.) aqueous solution.

Properties such as viscosity, electrical conductivity and solution concentration are important for the electrospinning process [96]. The viscosity of the solution can influence the fiber size and morphology. For solutions with low viscosity, surface tension is the dominant force, and the continuous formation of smooth fibers is not possible; rather, the fibers are dominated by beads or polymeric agglomerates in the fiber structure. However, for very high viscosity values, it is difficult to eject the solution drop from the tip of the needle. Therefore, the ideal viscosity is an intermediate value, which depends upon the polymer-solvent combination [101,102]. The conductivity of the solution is mainly related to the type of polymer, the solvent used, and the presence of ionizable salts or groups on the polymer. If the conductivity of the solution is very low, the jet is not sufficiently elongated by the electrical force to produce fibers, and beads are obtained. However, highly conductive solutions result in unstable fibers when a strong electric field is applied, forming fibers with a wide diameter distribution. Increasing the conductivity of the solution decreases the fiber diameter. Natural polymers are generally polyelectrolytic, and the ions increase the charge capacity of the jet, subjecting it to a higher force with the applied electric field [103,104]. At low concentrations of polymer in solution, a mixture of beads and fibers is obtained and, as the solution concentration increases, the shape of the beads changes from spherical to spindle shaped and finally uniform fibers with increased diameters are formed due to the viscosity. At high concentrations, the stability of the process is hampered by the impossibility of maintaining a continuous flow of the solution at the needle tip, mostly due to increased viscosity [101,105].

Once a suitable polymer-solvent system is selected, the flow rate, and the intensity of the electric field can be used to regulate the fiber diameter. The electric field strength is adjusted by modulating the applied voltage and tip-to-collector distance. The flow rate is a fundamental parameter, as it influences the jet speed and the polymer transfer rate. Low flow rates are most desired so that the solvent has enough time to evaporate as the fiber travels from the tip to the

collector. Higher flow rates generally increase the diameter of the fiber, which can result in the formation of beads and solvent residue in the final obtained fibers [106]. The electric field strength must be controlled since the formation of a fiber occurs only if the voltage applied to the solution is above a limiting value, which induces the necessary loads in the solution via the electric field and starts the electrospinning process. Within a certain range, an increase in voltage increases the repulsive electrostatic forces, which causes a decrease in the dimensions of the fiber. In most cases, a higher voltage causes a greater stretching of the solution due to the greater Coulomb forces in the jet, which leads to a reduction in the fiber diameter and to the rapid evaporation of the solvent. However, higher voltage values may increase the formation of beads [107,108]. If the needle and the collector are too close or too far apart, beads are formed. Typically it is desirable for the resulting fibers to have no residue of the solvent used; thus, an adequate distance between the tip and the collector is necessary to obtain complete evaporation of the solvent [109].

Electrospinning is an excellent and widely used technique for preparing new biomaterials, because it can generate fibers and nanofibers with 3D structure, and offers flexibility in terms of choice of material, scaffold geometry, and fiber orientation. The fibers can be composed of natural (of vegetable, microbial, or animal origin) or synthetic polymers. For regenerative medicine scaffolds or tissue engineering, some desired characteristics are that the material used to form the fiber is biocompatible, non-toxic, hemocompatible, biodegradable and has antibacterial activity. Fibers with the same range of dimensions (50-500 nm) as the ECM can be obtained using natural polymers, thus mimicking both the structure and the chemical composition of the physiological matrix [105,110]. The sulfated polymers, tannin-based materials, and collagen (including chemically modified collagens) are natural polymers with many favorable properties, that provide opportunities to expand the list of materials for electrospinning. These are each reviewed in the following section.

3.2 Overcoming Aqueous Instability of Bipolymers

Many biological macromolecules tend to be readily soluble in aqueous solutions, in aqueous solutions of organic acids, and in other polar solvents, all of which can be suitable for electrospinning [111,112]. However, one frequently encountered difficulty is that the final electrospun fibers can be soluble in aqueous solutions at neutral pH, even if the starting materials were not originally soluble under these conditions [113]. This solubility makes them unsuitable for use in biological applications, where they will be exposed to cell culture media or biological fluids. A variety of physical and chemical crosslinking methods have been implemented to overcome this important problem.

Crosslinking fibers can be achieved by two general classes of techniques. The first is by introduction of a reactive small molecule; the second is by supplying energy (*e.g.* via heat or light) to overcome an activation energy barrier that generates a reactive group on the constituent polymer. Introducing a small molecule reactant is challenging because it requires that a crosslinking agent penetrate into the fibers. Often the crosslinking agent is prepared in a dilute solution, which must swell the fibers (to ensure that the crosslinking agent penetrates to the fiber interior) without dissolving the fibers on the time scale of the crosslinking. For example, when a carbodiimide crosslinker is used to stabilize demineralized bone matrix fibers, polar solvents for the crosslinker (chloroform, dichloromethane, and isopropanol) do not enable the crosslinker to sufficiently penetrate the fiber to achieve crosslinking, and the fiber structure is lost upon subsequent exposure to phosphate buffered saline (**Figure 3**) [110]. The loss of porosity of the fibers hinders their application, espcially for tissue engineering, because the 3D porous environment of the fiber is essential for cell attachment, spreading and growth, and also for nutrient and waste transport [114–116]. Therefore, methods of crosslinking that do not require exposing the fibers to a solvent to introduce the crosslinker have also been developed, such as exposure to a crosslinker in the vapor phase (*e.g.*, glutaraldehyde vapor) (**Figure 3**) [110]. Supplying energy to generate a reactive group is also a solvent-free method, and can be

implemented by introduction of a photo-crosslinkable group or by thermal crosslinking (relying on reactions between functional groups that already exist within the polymer structures) [117,118]. When supplying energy, the source of power should not be strong enough to degrade the polymer [119,120].

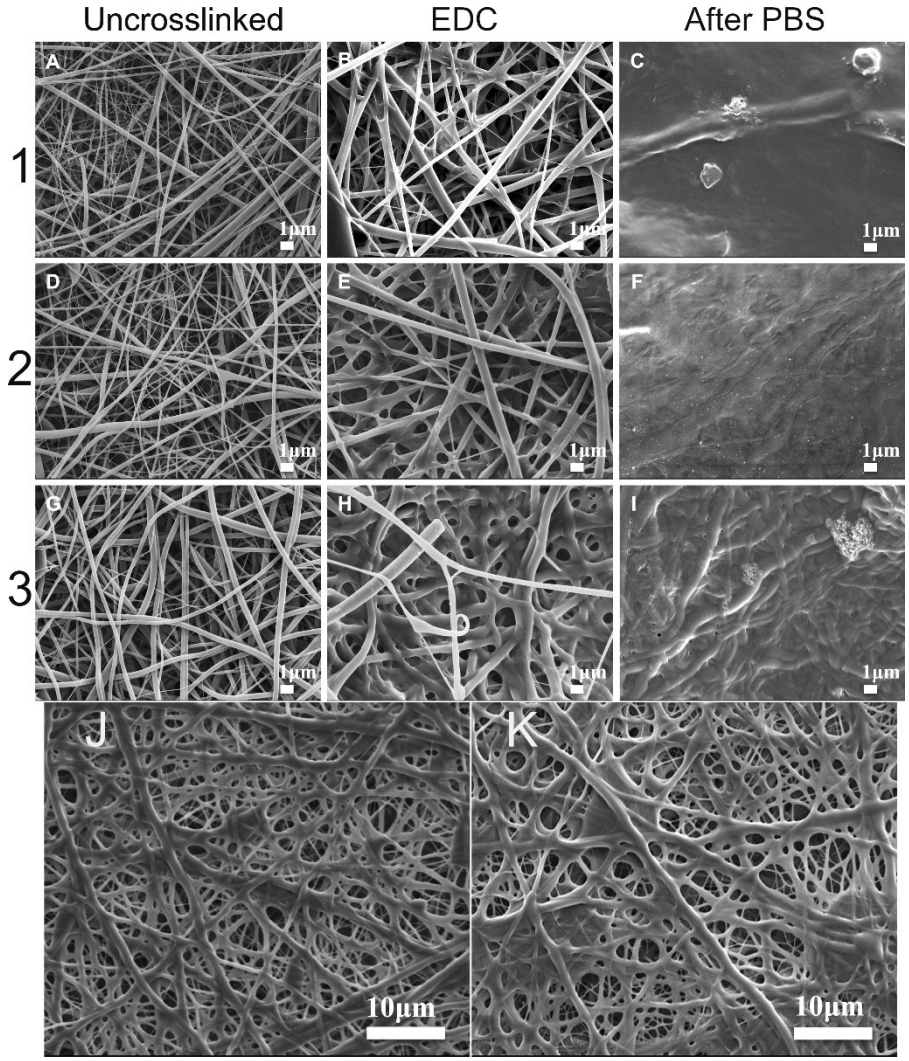

Figure 3. SEM micrographs of electrospun fibers of demineralized bone matrix. Solvents for EDC crosslinking are chloroform (1) (a, b and c), dichloromethane (2) (d, e, and f) and isopropanol (3) (g, h and i). Fibers crosslinked with glutaraldehyde vapor before (j) and after exposure to PBS (k). Reprinted (adapted) with permission from [110], copyright 2014, American Chemical Society.

In some cases, trace amounts of residual electrospinning solvent may also be retained in the resulting fibers. This could be particularly problematic for organic acid solvents that can form salts with positively charged functional groups in the polymer (*e.g.*, amines), and for

halogenated solvents that have strong dipoles. Removal of trace residual solvents can help mitigate the solubility problem, and may be essential to ensure that trace residual solvent does not cause cytotoxicity. Removal of residual solvent may not be achieved by heat or vacuum if the solvent interacts strongly with the polymer. Other methods of solvent removal include neutralizing acid solvents using a base and extraction using a non-solvent for the fiber polymer [121–125].

4. ELECTROSPUN BIOPOLYMERS

4.1. Sulfated biopolymers

Sulfated polysaccharides play important roles in the regulation of the immune system, stimulating immune cell proliferation and production of immune-related molecules. Sulfated polysaccharides from marine origin have shown the ability to bind to receptors on macrophages and lymphocytes resulting in signal transduction and thereby stimulating immune functions [49,89]. Due to the great variety of properties, biomaterials based on sulfated biopolymers have been extensively studied for several applications, and, in particular, for regenerative medicine and tissue engineering. Sulfated polymers are also readily soluble in many polar and aqueous solvents, suggesting that they may be excellent candidates for solution processing such as electrospinning. However, sulfated polymers also may exhibit strong electrostatic repulsion, causing the polymer chains to adopt extended confirmations in solution and reducing entanglement formation. This phenomenon may inhibit the formation of stable fibers. As reviewed below, many examples of electrospinning sulfated polymers found in the literature overcome this challenge by blending with other more readily electrospun materials. Blends containing poly(vinyl alcohol) (PVA), poly(ε-caprolactone) (PCL), gelatin (GE), and polyethyelene oxide (PEO) combined with a sulfated biopolymer have all been reported.

4.1.1 Tissue engineering applications

Table 1 summarizes the fibers produced by electrospinning using different sulfated biopolymers for diverse biomedical applications.

Table 1. Electrospun fibers based on sulfated biopolymers.

Fiber composition[a]	Distance / voltage / flow rate	Solvents and crosslinkers[b]	Desired application	References
GE / CS (13% w/v)	10 cm / 15 – 25 kV / 0.6 – 1 mL.h^{-1}	TFE / water (100:0 - 50:50)	Scaffold for skin, cartilage, and cornea tissue engineering	[126]
CS / PVA (15% w/w) (7:3 / 5:5)	25 cm / 20 kV / 1.5 mL.h^{-1}	Distilled water	Biocompatibility and controlled drug release.	[127]
PCL (13% w/v) CHI / SCHI (2% w/v – 90/10)	10 cm / 11 kV / 0.4 mL.h^{-1}	FA / AA (3:1) 0.5% AA EDC / NHS crosslinking	Scaffolds for cell proliferation and spreading.	[128]
PCL (17% w/v) CS (2% w/v)	20 cm / 12 kV / 1.0 mL.h^{-1}	Chloroform / DMF (70:30) EDC / NHS crosslinking	Scaffolds for cartilage tissue engineering.	[129]
PVA (10% w/v) SA (4-10% w/v)	12 – 15 cm / 20 – 28 kV / 0.2 – 5 mL.h^{-1}	Deionized water	Development of new single-nuzzle electrospinning methodology.	[130]
PVA / CS (9% w/v / 15% of PVA wt.) GE (5:5 w/w)	17 cm / 20 – 26 kV / 1 – 1.4 mL.h^{-1}	Water:AA (7:3, 6:4, 5:5, 4:6, 3:7) GA crosslinking	Scaffolds for tissue engineering.	[131]
CS/PEO (2% / 0.2% w/v)	22 – 24 cm / 60 – 75 kV	30% AA Deionized water	Bilayer matrix for tissue engineering.	[132]
GE (4.5% w/v) PVA (4.5% w/v) CS (0 – 20% w/v)	17 cm / 20 – 26 kV / 1 – 1.4 mL.h^{-1}	Water:AA (50:50) GA crosslinking	Scaffolds for tissue engineering.	[133]

PCL (12.5% w/v) CS (5 mg/L)	15 cm / 25 kV / 1 mL.h^{-1}	FA:AA (9:1)	Scaffolds for cartilage tissue engineering.	[134]
PVA (5% w/v) CMKC (1.25 – 3% w/v)	15 cm / 15 kV / 1.0 mL.h^{-1}	Deionized water Thermal crosslinking	Scaffolds to support cell differentiation. Wound dressings for wound healing.	[135] [136]
PVA (9% w/v) SA (0.9 – 2.7% w/v)	10 cm / 15 kV / 0.4 mL.h^{-1}	Deionized water GA crosslinking	Scaffolds for neural tissue engineering.	[137]
PCL (12.5% w/v) GE / CS (13% - 85:15)	12 cm / 19 kV / 0.6 – 0.9 mL.h^{-1}	FA:AA (9:1) TFE:water (50:50)	Scaffolds for cartilage tissue engineering.	[138]
PCL (7% w/v) GAS (5 and 10% w/v)	18 kV / 1 mL.h^{-1}	HFIP	Scaffolds for cartilage regeneration.	[139]

[a] carboxymethyl kappa carrageenan (CMKC); chitosan (CHI); sulfonated chitosan (SCHI); chondroitin sulfate (CS); D-glucosamine sulfate (GAS); poly(galacturonic acid) (PGA); gelatin (GE); polycaprolactone (PCL); poly(ethylene oxide) (PEO); poly(vinyl alcohol) (PVA); poly(vinyl pyrrolidone) (PVP); sulfated alginate (SA).

[b] acetic acid (AA); dimethylformamide (DMF); 1-ehthyl-3-(3-dimethylaminopropyl)carbodiimide (EDC); ethanol (EtOH); formic acid (FA); glutaraldehyde (GA); 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP); *N*-hydroxysuccinimide (NHS); trifluoroethanol (TFE).

Recent studies have shown that electrospun sulfated fibers demonstrate promising applications in tissue engineering due to their versatility and compatibility with different tissues and cell lineages, such as fibroblasts, osteoblasts, and chondrocytes. Some materials have also demonstrated the capacity as scaffolds to support multipotent mesenchymal stem cell (MSC) growth and differentiation (**Table 1**).

Tissue engineering aims to maintain tissues and their functions in culture or to repair diseased or damaged tissues, by combining cells and biodegradable scaffold materials under appropriate (static or dynamic) conditions. The development of tissue engineering methods relies heavily on culturing cells *in vitro* on or in biodegradable scaffold materials [140].

Electrospun fibers based on sulfated polymers are a suitable environment for 3D soft-tissue regeneration. Gelatin (GE)/chondroitin sulfate (CS) blend fibers have mean fiber diameter ranging from 187 to 248 nm, which is in the range of the fibers in the ECM. Culturing human dermal fibroblast cells (HDF) on these fibers up to 7 days and later imaging by scanning electron microscopy (SEM) showed that HDF cells can attach, grow and spread on the fabricated scaffold surface [126]. Polyvinyl alcohol/gelatin/chondroitin sulfate (PVA/GE/CS) fibrous scaffolds prepared using water and acetic acid (with 9% w/v polymer) as a non-carcinogenic solvent system and crosslinked by glutaraldehyde (GA) vapor showed potential application for soft tissue engineering. The fibers showed no cytotoxicity to L929 mouse fibroblast cells, and SEM results demonstrated that the cells attached and proliferated well on the fibers after 24 and 48 h [131]. PVA/GE/CS fibers with 15% of CS in the composition had a tensile strength of 4 MPa in dry state and elongation at break of 200% in the wet state, demonstrating excellent mechanical properties for skin tissue engineering. Culturing human dermal fibroblast-green fluorescent protein-positive (HDF-GFP+) cells on PVA/GE/CS fibers up to 14 days showed that a higher concentration of CS on the composition accelerated HDF-GFP+ cell adhesion, proliferation and attachment, illustrating the biological significance of using sulfated polymers in electrospun tissue scaffolds [133].

MSCs can self-reproduce and can differentiate into numerous tissue cell types including fat, bone, muscle, cartilage, ligament and tendon. Their differentiation is highly dependent upon signals provided from their surrounding matrix [36]. The addition of induction hormones and soluble growth factors to cell culture media may achieve differentiation in culture. However, this strategy may not be easily translated to a wound healing environment *in vivo*. Instead, the design of tissue engineering scaffolds can rely on other differentiation cues that the MSCs may respond to, such as the local surface chemistry, mechanical properties, and receptor ligands bound to the surface of a scaffold. 3D tissue engineered scaffolds, especially electrospun nanofibrous scaffolds, provide a porous architecture with very high surface area, similar to the

native extracellular matrix, and with the ability to present many differentiation signals [141]. Electrospun fibers can also support cell attachment, growth, and differentiation, and can provide space for native ECM production as the supported stem cells differentiate into mature tissue cell types [142].

Electrospinning strong polyelectrolytes, such as sulfated polymers can be challenging. An alternative to blending the sulfated biopolymer with a more easily electrospun polymer is to first prepare an electrospun polymer from another material, then coat the fibers with the sulfated polymer after electrospinning. A simple method for coating fibers with sulfated polymers is to use the layer-by-layer assembly of polyelectrolyte multilayers. This method was used to prepare heparin-coated chitosan fibers [143]. This method was later expanded to demonstrate that the growth factor binding capacity of heparin-based nanomaterials could be used to preserve fibroblast growth factor 2 (FGF-2) bioactivity, for delivery of FGF-2 to mesenchymal stem cells [144]. Heparin-coated fibers were also used deliver growth factors (FGF-2 and TGF-β) and stem cells *in vivo* on the surfaces of bone allografts, to improve bone allograft healing. Despite promising *in vitro* results, the fiber coatings containing growth factors and stem cells did not improve bone matrix deposition or healing of the allografts *in vivo*, but the growth factor delivery did significantly reduce inflammation scores [124]. This work is an example demonstrating the necessity of *in vivo* evaluation for more fully characterizing the biological responses to electrospun materials.

An innovative modification to the standard electrospinning method that has been demonstrated for sulfated biopolymers is “needleless” electrospinning. An electrospun bilayer nonwoven material was fabricated from chondroitin sulfate (CS) and hyaluronan (HA) by needleless electrospinning. Biocompatibility tests with MSCs revealed the formation of MSC spheroids on the surface of the fibers. Compared to a CS matrix control, the CS–HA fibers improved cytocompatibility by increasing the viability of the MSCs and the observed cell adhesion on the surface of the material [132]. On the other hand, electrospun polyvinyl

alcohol/sulfated alginate (PVA/SA) nanofibrous scaffolds with 30 wt-% SA is a suitable substrate for MSC growth and is capable of inducing neuronal differentiation of human bone marrow mesenchymal stem cells (hBMSCs). The fibers support hBMSCs proliferation and neurogenesis during two weeks of cell culture, without using any growth factors. Immunocytochemistry results showed the neuroinductive effect of SA on the differentiation of hMSCs to neural-like cells with no differentiation agents added. The expression of microtubule-associated protein 2 (MAP-2) confirmed neural differentiation for up to 14 days [137]. Poly(vinyl alcohol)/carboxymethyl-kappa-carrageenan (PVA/CMKC) fibers produced through electrospinning of an aqueous blend solution of both polymers, followed by thermal crosslinking, support mesenchymal stem cells and favor osteogenic differentiation, with no generation of hazardous waste in the process. The incorporation of CMKC in the fibers modulated the phenotype of human adipose-derived stem cells (ADSCs), increased biodegradability, and improved cell growth, adhesion, biocompatibility and promoted osteogenic differentiation of adipose-derived stem cells in osteogenic induction media. The addition of a sulfated polymer to the fibers enhanced ADSC response to osteogenic differentiation signals showing that sulfated polymers are great candidates for application in tissue engineering to support stem cells (**Figure 4**) [135]. PVA/CMKC fibers also showed potential application as wound dressings for wound healing due to their increased procoagulant and antibacterial activities when compared to the pure PVA fibers. CMKC contributed to higher platelet adhesion and activation on the fibers, coagulation in contact with human whole blood and bactericidal activity to *P. aeruginosa* and *S. aureus* bacteria [136].

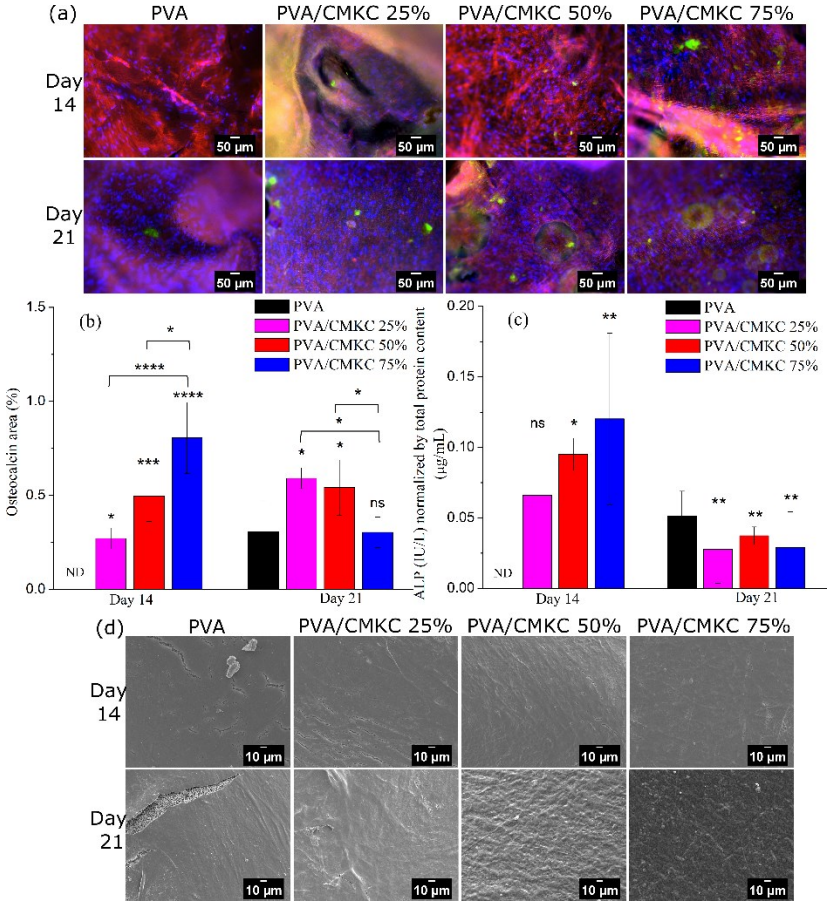

Figure 4. Immunofluorescent microscopy of CMKC-containing fibers seeded with ADSC. The cell cytoskeleton is represented by red, nuclei by blue, and osteocalcin by green (a). Osteocalcin area (b) and ALP content on the fibers (c). SEM micrographs of ADSCs after 14 and 21 days of culture on the fibers (d). PVA fibers was used as control samples. Reproduced with permission from [135], copyright 2020, John Wiley and Sons.

4.1.2. Cartilage regeneration

Cartilage is aneural and has far fewer blood vessels than other connective and skeletal tissues. In joints, cartilage protects the ends of the bones from damage caused by both shear and compressive stresses. Nutrient supply and waste removal are accomplished primarily by the diffusion of synovial fluid, enhanced by joint motion and compression. Consequently, cartilage has very limited capacity to heal, in adults [145]. Therefore, repeated stress on joints can lead to cartilage injury, resulting in inflammation and degradation of the cartilage matrix. Matrix degradation leads to further injury that may go undetected until the injury is so severe that the tissue cannot heal on its own. Osteoarthritis can ultimately result in debilitating joint injury, requiring joint replacement [146].

Cartilage tissue engineering has a goal to produce scaffolds that can mimic the mechanical properties of cartilage extracellular matrix (ECM) and provide a 3D environment for the adhesion and proliferation of stem cells that can differentiate into chondrocytes [147]. The ability to support chondrocyte differentiation and the chondrocyte phenotype is also important, and is often evaluated by expression of chondro-inductive genes (*e.g.*, SOX9), and by measuring the production of cartilage ECM components, such as sulfated glycosaminoglycans and collagen type II. Electrospun matrices presenting sulfated polymers have been shown to support proliferation and differentiation of stem cells toward a chondrogenic lineage, and to support the chondrocyte phenotype. Chondroitin sulfate immobilized (CS-immobilized) at the surface of poly(caprolactone) electrospun fibers (PCL) represent an efficient substrate for the maintenance of human articular chondrocyte (hAC) phenotype. hACs cultured on the CS-immobilized PCL fibers over 28 days proliferated, maintaining the typical round shape and cellular agglomeration/clustering phenotype of the cells and over-expressed cartilage-related genes including those for aggrecan, collagen type II, cartilage oligomeric matrix protein, and the transcription factor SOX-9 (**Figure 5**) [129]. CS-immobilized PCL fibers also supported chondrogenic differentiation during 21-days of MSC culture in a medium and with no exogenous chondrogenic differentiation signals. After 14 days of culture on the fibers, the cells passed the last phase of chondrogenesis and the expression of SOX-9, collagen type 10 and collagen type II showed that the presence of the sulfated polymer favors chondrogenic differentiation [134]. Composite scaffolds of PCL and gelatin-chondroitin sulfate blend (GE-CS) fibers fabricated by co-electrospinning induced chondrogenesis of hMSCs with no addition of differentiation agents to the culture media. The scaffolds containing the highest GE-CS content (2/1) exhibit better chondrogenic differentiation, showed by gene expression (COL2a1 and SOX9) and ECM secretion (sulfated glycosaminoglycan and collagen type II) [138]. While CS is a component of the native cartilage ECM, similar effects can be achieved using other sulfated biopolymers. PCL fibers containing glucosamine sulfate (GAS), a hydrolyzed chitin derivative,

by coaxial electrospinning technology for maintenance and sustained release of GAS promote the growth of rat articular chondrocytes (rACs). After 48 h the total release amount of GAS was less than 50% and the cell viabilities of rACs were higher than 130%, indicating that the release of the sulfated moiety was beneficial to cell growth and proliferation. The composition with 5 wt.-% of GAS expressed the best stress-strain behavior and chondrocyte-specific gene expression, being more suitable for repairing articular cartilage [139].

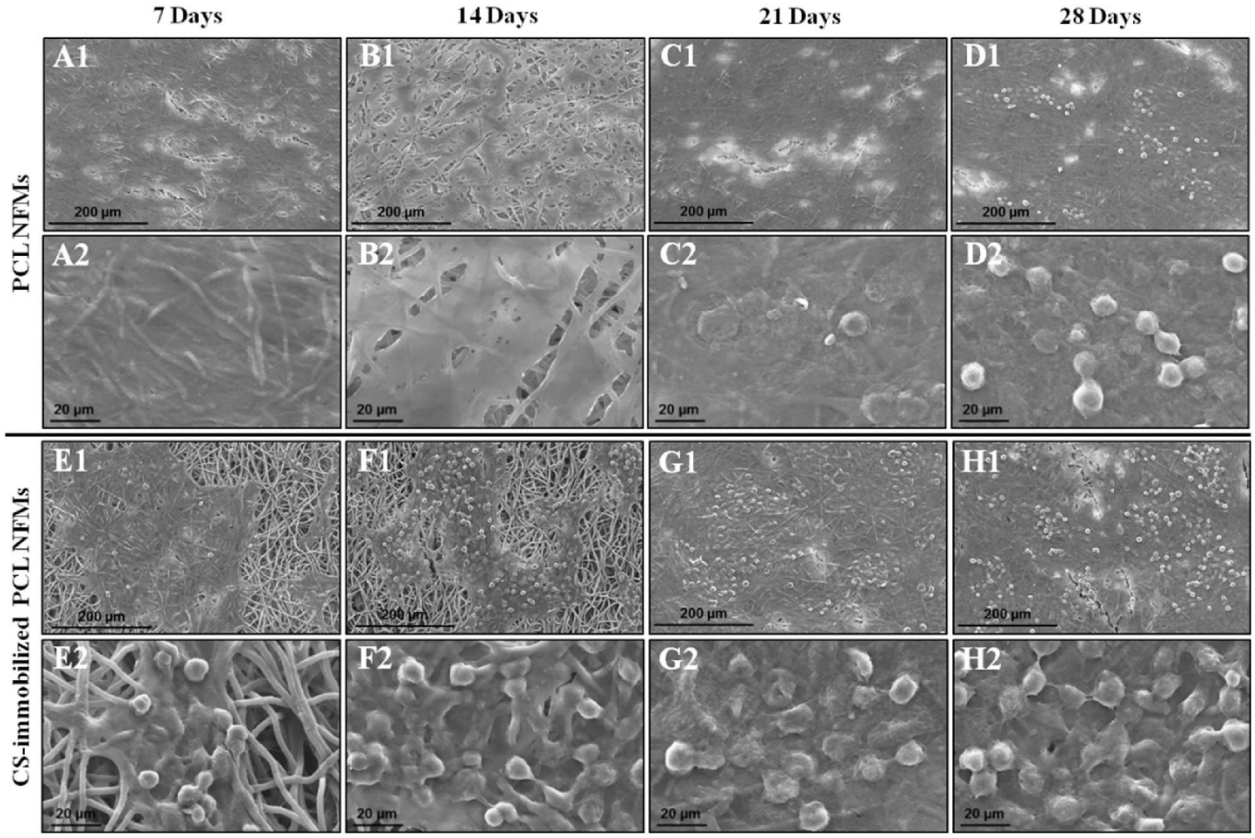

Figure 5. SEM micrographs of human articular chondrocytes on PCL fibers (a-d) and CS-immobilized PCL fibers (e-h) for 7 to 28 days. Reproduced with permission from [129], copyright 2016, Elsevier.

4.1.3. Drug delivery

For drug delivery applications the chemical composition and the morphology should be optimized to achieve ideal and controled drug delivery. Drug carrier can also be prepared to be stimuli-response, so that external conditions, such as pH, temperature, and ionic strength can be used to modulate the drug release. The use of naturally derived macromolecules like

polysachharides has been extensively studied for controlled delivery resulting in versatile production of different scaffolds with stimulus-response characteristics [148]. Using electrospinning to fabricate naturally-derived polymer fibers for drug delivery applications can combine the stimulus-response properties with the porous morphology necessary to obtain controled release. The electrospun fibers possess high surface area-to-volume ratio which can imporve the transport of the drug from the matrix to the sourroundings, enhancing the efficiency of the drug. Electrospun fibers can achieve drug loading with higher drug encapsulation efficiency, improved drug stability, and improved control over drug release kinetics compared to other controlled release formulations [120]. The sizes of the pores of the fibers are also critical for regulating controlled release. Depending on the drug size and interaction with the matrix, release of the drug can be accelerated when the pores are larger. Smaller porous can lead to a burst release followed by a slow controlled release [149]. However, fibers fabricated using natural macromolecules generraly tend to have low mechanical and elastic properties, which could hinder the drug release. Combining biologically-derived polymers with synthetic polymers is a simple way to improve mechanical properties of these scaffolds [150]. PVA-CS electrospun fibers loaded with combretastatin A-4 phosphate (CA4P) were used to evaluate a model for testing drug release. Assays of *in vitro* drug release revealed a higher release rate of CA4P at the beginning of 10 h followed by a slow release rate, showing that the prepared fibers can control drug delivery for hours [127].

4.1.4. Future challenges

Even though it is clear by the recent studies that electrospun sulfated polymer fibers have excellent characteristics and enhancements to biological properties that enable their use as scaffolds for engineering diverse types of tissues, and for supporting and differentiating several cell types, many outstanding questions remain. Since it is known that some sulfated polymers can modulate immune responses and can act as antioxidant, anti-inflammatory, and

antiviral agents, fibers containing them can be designed to exploit these properties. Future research should prioritize immune responses to a material that is proposed as a scaffold for cell growth and tissue repair. Future studies should also focus on demonstrating whether these fibers would obviate inflammatory reactions or macrophage activation, understanding the mechanical properties to mimic specific tissues, such as cartilage and bones, and demonstrating protection from the oxidative effects on tissues. For example, for cartilage tissues, if the fibers could provide or be modified to promote chondroprotection, protecting from cartilage diseases, fibers from sulfated polysaccharides would represent significant technological advances in biomaterials.

4.2. Tannins and Tannin Derivatives

Tannins and tannin derivatives have been studied extensively as potential defenses against pests and pathogens, and for their beneficial effects to human health. Due to their low-cost, natural abundance, antibacterial, antioxidant, anti-mutagenic and anti-carcinogenic properties, they are great candidates as biologically active components for biomaterials. Some tannin derivatives demonstrate powerful scavenging abilities for hazardous molecules, metals, and free radicals, thereby protecting cells against oxidative damage [151]. **Table 2** shows the composition and parameters used to obtain fibers produced by electrospinning using different tannin derivatives for diverse biomedical applications.

Table 2. Electrospun fibers based on tannin and derivatives.

Fiber composition[a]	Distance / voltage / flow rate	Solvents[b]	Desired application	References
Zein / Tannin (20% w/v – 9:1; 8.5:1.5; 8:2)	12 cm / 20 kV / 1.2 $mL.h^{-1}$	Water / EtOH (8:2)	Tannin incorporation into fiber biomaterials.	[152]
Cellulose acetate (17% w/v)	20 cm / 17 kV	Acetone / DMAc (2:1 w/w)	Catalytic hybrid fibers.	[153]

+ Bilayer of lysozyme / TA (each 1 $mg.ml^{-1}$)		0.01 M PBS pH 7.4		
PVA (6% w/v) TA / $FeCl_3 \cdot H_2O$ (0.2 / 0.05 $mg.mL^{-1}$)	15 cm / 15 kV / 0.5 $mL.h^{-1}$	Water pH 5.5	Antioxidant fibers with high functionality reinforced with Fe^{3+}.	[154]
PVCL (33.3 – 21.5% wt.) TA (5% - 15.5% wt.)	10 cm / 15 kV / 0.8 $mL.h^{-1}$	DMF	Crosslinking of PVCL fibers by hydrogen bonding with TA.	[155]
PCL (14% w/v) TA / Fe^{3+} metal-phenolic networks	12 cm / 13 kV / 1.0 $mL.h^{-1}$	DCM/DMF (3:1)	Promoting the adhesion and spreading of endothelial cells.	[156]
PCL (6.6 – 14% w/v) / Tanfloc (5 – 22% wt.)	12.5 cm / 15 kV / 1.0 $mL.h^{-1}$	AA / FA (70:30)	Scaffolds for tissue engineering.	[157]
PVA (4.8 % w/v) GE (7.2 % w/v) BT	20 kV / 0.3 $mL.h^{-1}$	Water AA	Uranium (VI) extraction from seawater.	[158]
TA / partially hydrolyzed PAN (0:9, 1:9, 3:9, 5:9)	15 cm / 20 kV / 0.5 $mL.h^{-1}$	DMF	Removal of trace metal ions in organic complexes from wastewater.	[159]

[a] bayberry tannin (BT); gelatin (GE); polyacrylonitrile (PAN); polycaprolactone (PCL); poly(vinyl alcohol) (PVA); poly(*N*-vinylcaprolactam) (PVCL); tannic acid (TA).
[b] acetic acid (AA); dichloromethane (DCM); dimethylacetamide (DMAc); dimethylformamide (DMF); formic acid (FA); phosphate buffered saline (PBS).

Incorporating natural and renewably sourced polymers with favorable biochemical activities, such as anti-inflammatory, antibacterial, antiseptic and antimicrobial activity, like tannin and tannin derivatives, to a biomaterial composition is highly desirable for biomedical applications. Bark tannins extracted from barbatimao species incorporated into electrospun zein fibers exhibited improvements of thermal properties. The addition of tannin increases the glass transition temperature of the fibers, exhibiting an antiplasticizer effect of the tannin on the zein

matrix and changing the morphology from a cylindrical to a ribbon-like shape. Zein-bark tannin fibers can combine the improved thermal properties with biochemical activities of tannin for development and enhancement of different materials, however biological studies need to be performed on these fibers for confirmation of the combined properties. [152]. Fibers produced following the principles of "green chemistry" are prepared by methods that reduce the use of toxic chemicals and the generation of toxic waste. Using tannic acid (TA) as a natural molecule soluble in non-toxic solvents, electrospun TA fibers were successfully fabricated due to favorable hydrogen bonding between the galloyl groups of tannic acid molecules in the solvent, and were crosslinked. The crosslinking was achieved by the oxidation of the galloyl groups into quinone with sodium periodate ($NaIO_4$), and by oxidation of galloyl using Fe^{3+} under acidic pH, creating permanent covalent bonds. The TA fibers formed Fe^{3+}–TA coordination bonds at basic pH mimicking the mussel adhesive threads with the ability to coordinate with a wide variety of metals. These materials were proposed as as "smart nanowebs" for catalytic and biomedical applications [160].

4.2.1. Tissue engineering applications

To obtain materials for tissue engineering applications, properties like antioxidant activity, antimicrobial activity, cell compatibility, and hemocompatibility are desired. Metal-phenolic networks (MPNs) developed by coating TA and Fe^{3+} ions on PCL fibers modulated human umbilical vein endothelial cell (HUVEC) morphology and function, showing promising results. The HUVECs cultured on TA–Fe^{3+} coated networks presented higher and more stable focal adhesion density than the ones cultured on pure PCL fibers. The cells remained active on the coated fibers for over 14 days of culture, exhibiting a stable angiogenic phenotype and function in vitro, which shows that the TA coating enhances pre-vascularization and may promote effective, functioning vasculature after implantation [156]. Electrospun fibers from blends of PCL with a new hydrophilic amino-functionalized tannin (tanfloc) improved adhesion

and proliferation of ADSC cells (**Figure 6**), as well as antibacterial activity against *P. aeruginosa*, when compared to PCL fibers. Higher tanfloc content in the fibers improved the hydrophilicity, due to $-NH_2$/$-NH_3^+$ and -OH functional groups, and the cytocompatibility and bactericidal activity. In comparison to chitosan-based materials, tanfloc has a superior potential as a material for tissue engineering applications, due to its ease of production from natural condensed tannins and its enhanced biological properties [157].

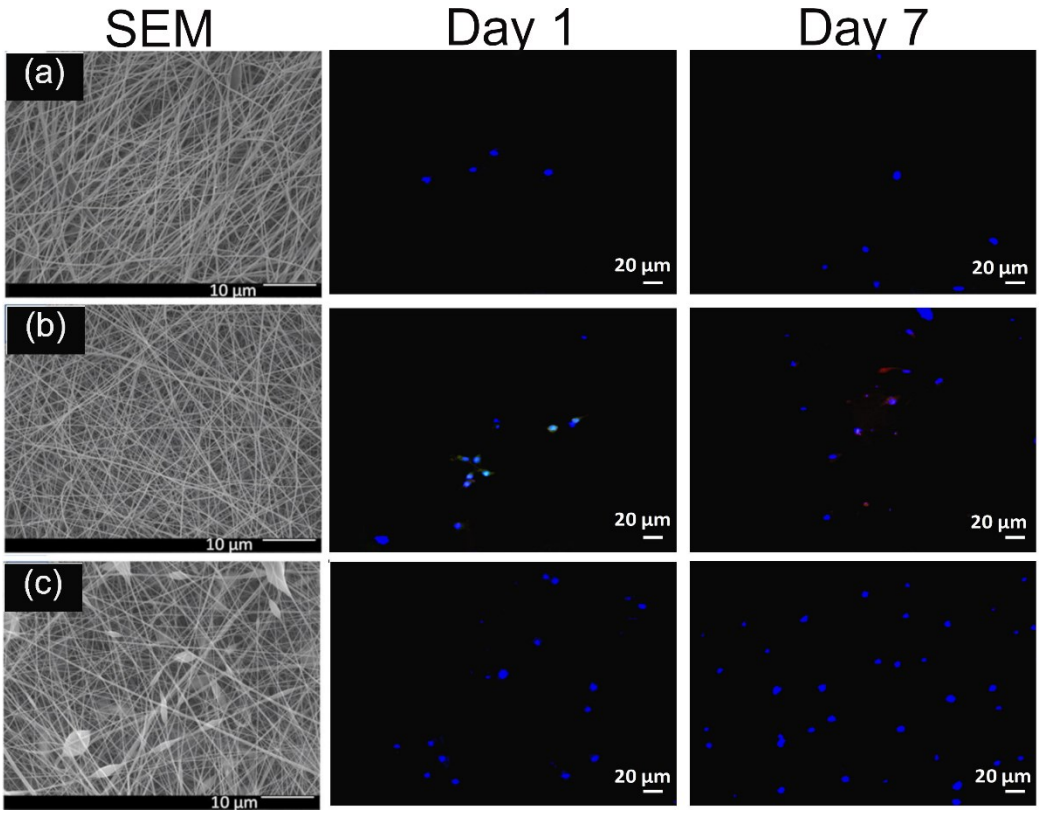

Figure 6. SEM micrographs of PCL (a), PCL-tanfloc (5 wt.-%) (b), and PCL-tanfloc (22 wt.-%) fibers. Fluorescence microscope images of adipose derived stem cells on the respective fibers cultured for 1 and 7 days. Reproduced with permission from [157], copyright 2018, Elsevier.

4.2.2. Wastewater treatment

Removal and recovery of metals from wastewater is a focus of attention in industries such as dyeing and finishing, the electroplating industry, and battery manufacturing. Metals in wastewater effluents can lead to severe environmental and health problems. GE/PVA composite fibers loaded with bayberry tannin (GPN-BT) demonstrated the capacity of adsorption of uranium (VI) from simulated seawater. The fibers showed a maximum adsorption capacity of

254.8 mg g^{-1} at a pH of 5.5, adsorbent dosage of 0.02 g, contact time of 12 h, and room temperature. GPN-BT fibers resulted in more than 90% removal efficiency and adsorption capacity of 7.2 mg g^{-1} for uranium after 24 h, even at an extremely low initial concentration of 3 mg L^{-1} in simulated seawater, which is attributed to the high density of adjacent phenolic hydroxyl groups and the specific surface area of the fibers (**Figure 7**) [158]. Tannery wastewater is generated by the preparation and tanning processes of wet operations employed in leather production [161]. This waste contains high contents of organic, inorganic and nitrogenous compounds, chromium, sulfides, suspended solids and dissolved solids, and its treatment is of high concern [162,163]. Electrospun polyacrylonitrile (PAN) fibers modified with tannic acid (TA) demonstrated good removal of trace Cr (III) in an organic complex, showing good adsorption toward Cr (III)-collagen complexes and effective reduction of total organic carbon in tannery wastewater. PAN-TA fibers presented a maximal adsorption capacity of Cr (III) as 79.48 mg g^{-1}, at pH 7.0 and initial Cr (III) concentration of 50 mg g^{-1}, due to the high content of polyphenol groups present on TA. The PAN-TA fibers are an effective adsorbent candidate for tannery wastewater, because of their facile complexation of Cr (III) and removal of the hydrolyzed collagen, which is largely stable in water and difficult to remove by common chemical precipitation [159].

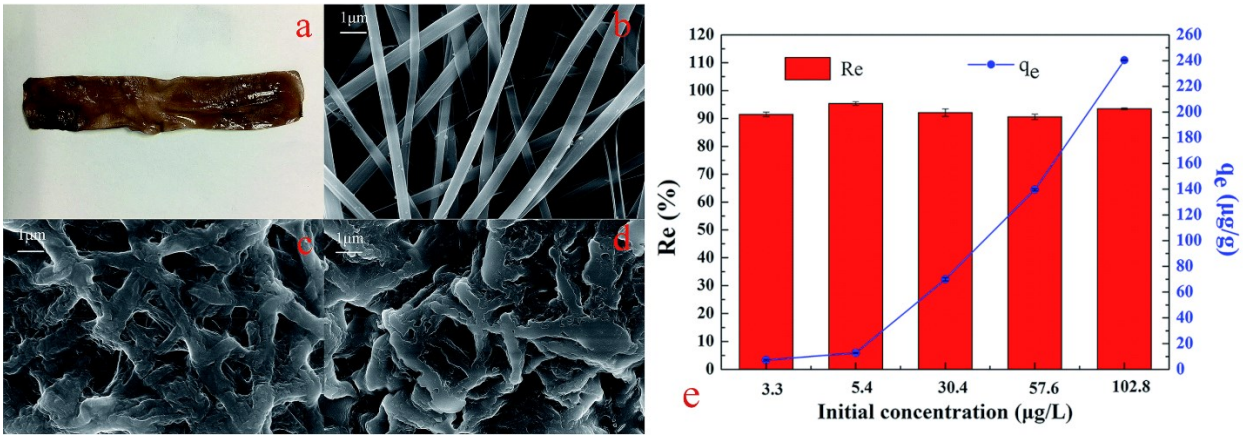

Figure 7. Digital photo of gelatin/poly(vinyl alcohol) composite fiber band (GPNB) loaded with bayberry tannin (BT) (a), SEM micrographs of the fibers (b), before (c) and after the adsorption of uranium on GPNB (d). Simulation of adsorption of uranium on the fibers using

seawater (e). Reproduced with permission from [158], copyright 2019, Royal Society of Chemistry.

4.2.3. Crosslinking

Fabricating fibers that are insoluble in aqueous environments can be challenging, and may require use of several crosslinking agents; crosslinking can increase the cost of the final material and increase the difficulty of the process. Therefore, if the addition of a simple molecule such as TA in the fiber composition could lead to improvement or stabilization of the mechanical properties, the process would be cheaper and simpler. PVA electrospun fibers reinforced with TA and Fe^{3+} assemblies had improved mechanical properties, such as tensile strength and elongation-at-break, and a fully entangled morphology by only adding TA to the composition, when compared to PVA fibers. The fibers maintained the antioxidant activity even with the Fe^{3+} inclusion [154]. Also, poly(*N*-vinyl caprolactam) (PVCL) electrospun fibers were, for the first time, made water-insoluble using TA as a crosslinker due to the hydrogen bonding interaction of PVCL with TA, imparting an easy crosslinking method [155].

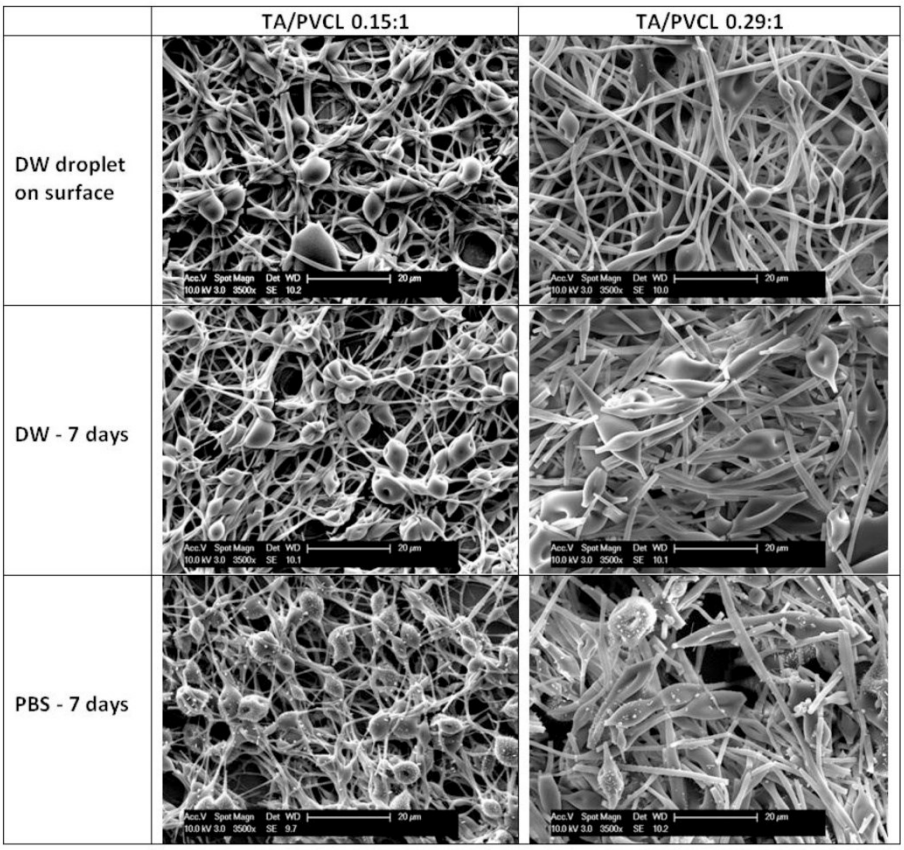

Figure 8. SEM micrographs of tannic acid/ poly(*N*-vinylcaprolactam) (TA/PVCL) fibers after immersion in deionized water (DW) or PBS for 7 days. Reproduced with permission from [155], copyright 2016, Elsevier.

4.2.4. Future challenges

Tannin and derivatives have well established functionalities for the treatment of wastewater. However, the biomedical applications of tannin-based materials remains under-investigated. Therefore, this application area is ripe for future development. Prospective research should take advantage of the inherent biological activities of tannin and derivatives to create and evaluate new and already produced tannin-based fibers, regardless of antipathogenic activity against bacteria, viruses, fungus, parasites, and anticarcinogenic activity against different cancer cell lines. Future studies should evaluate the ability to support and differentiate different cell lineages, and should determine immune and inflammatory responses that these fibers might activate or suppress. Regarding the new and underexplored chemical modifications

of tannin, tanfloc is a polymer with promising properties and could be applied to new fibers or blended with different polymers to achieve synergic properties.

4.3. Collagen

Collagen is a widely used biomacromolecule for biomedical applications, due to the broad number of successful clinical studies employing it, and its acceptance as a biocompatible material for many applications. For use in commercial medical products, collagen is typically a purified product from animal sources, reconstituted, often by blending with other materials, and then processed into materials for applications ranging from wound dressings to bioprosthetic heart valves. Collagen fibers promote wound healing through hemostasis and support stem cell homing and tissue regeneration [164]. However, there are several challenges for collagen electrospinning, such as the poor mechanical strength, reduced elasticity, the high hydrophilicity of the obtained fibers, and difficulty in solubilizing collagen while preserving its native tertiary structure. To improve the mechanical properties of electrospun collagen fibers, some approaches like blending collagen with other biopolymers (*e.g.*, chitosan) or synthetic polymers (*e.g.*, PCL and poly(glycolic acid)) successfully increased the tensile strength, elongation at break, and Young's modulus [165–168]. Crosslinking of collagen fibers is an effective method for making the fibers water resistant, decreasing the hydrophilicity and boosting the mechanical properties in some cases. However, using aldehyde-based crosslinking, such as glutaraldehyde vapor, for collagen fibers has proven challenging due to reducing the uniformity, swelling, and decreasing the porosity of the fibers (**Figure 9**) [169–171]. A common crosslinking agent used to overcome this is the combination of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxy succinimide (NHS), which stabilizes the fibers by forming covalent amide bonds between carboxyl groups and amino groups on the fibers [172,173].

Strong organic and fluorinated solvents commonly used in electrospinning like hexafluoro-2-propanol (HFIP), trifluoroethanol (TFE) and tri-fluoro acetic acid (TFA) can lead to the loss of the tertiary structure of collagen, forming mostly gelatin fibers [174–176]. Some studies show that dissolving collagen in weak organic acids, such as acetic acid, or dissolving collagen in high concentrations (above 20%) can preserve a larger fraction of native collagen chains [177–179]. New methods for processing collagen-based fibers continue to be developed. **Table 3** shows the composition and parameters of fibers produced by electrospinning using modified collagen for various biomedical applications.

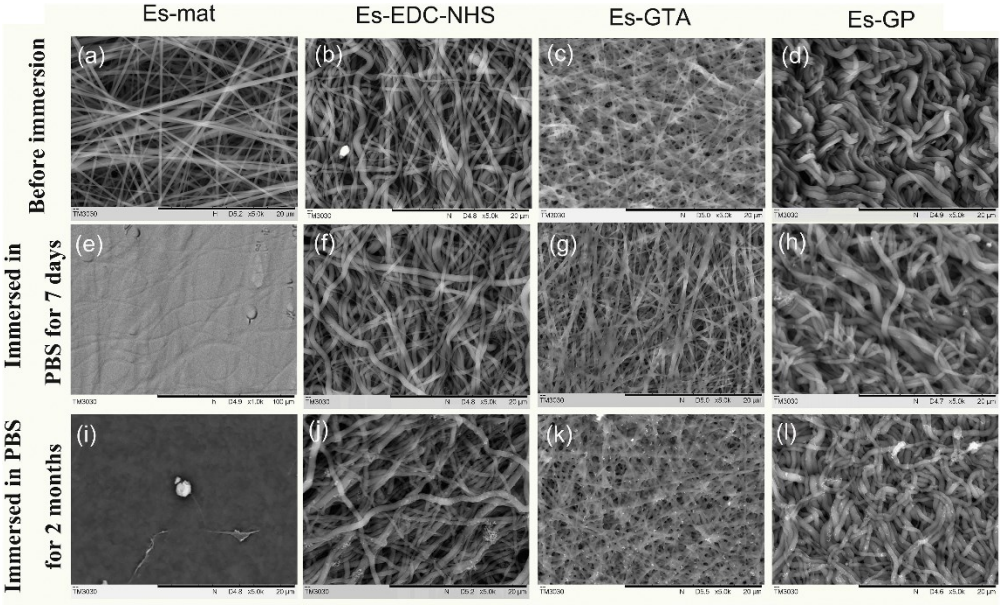

Figure 9. SEM micrographs of collagen fibers uncrosslinked (Es-mat), crosslinked with EDC/NHS (Es-EDC/NHS), glutaraldehyde vapor (Es-GTA), and genipin (Es-GP) before (a-d) and after 7 days (e-h) or 2 months (i-l) of immersion in PBS. Reproduced (adapted) with permission from [180], copyright 2018, Elsevier.

Table 3. Electrospun fibers based on modified collagen.

Fiber composition[a]	Distance / voltage / flow rate	Solvents[b]	Desired application	References
Type I collagen from calfskin (7% w/v) HAP (30 wt.-%)	12 cm / 15 kV / 0.2 mL.h^{-1}	HFIP	Collagen/hydroxyapatite composite fiber fabrication.	[181]
PAN (10% w/v)	20 cm / 10 kV / 0.2 mL.h^{-1}	DMF	Scaffolds for tissue engineering.	[182]

Modified collagens				
PLGA (20% w/v) Collagen (2 mg/mL)	15 cm / 28 kV / 0.8 mL.h^{-1}	DMF / THF (1:3)	Scaffolds for bioengineered skin applications.	[183]
PLGA / PCL (7:3 w/w) Collagen I (250 µL)	13-18 cm / 16 kV / 0.6 mL.h^{-1}	TFE	Biomimetic architecture for osteogenic cell proliferation and differentiation.	[184]
PLGA / Collagen (4:1 – 20% w/v)	17 cm / 28 kV / 1.0 mL.h^{-1}	HFIP	Composite scaffolds as skin substitute.	[185]
PLA / Collagen (8% / 1% w/v)	10 – 12 cm / 10 – 18 kV / 0.5 – 1.5 mL.h^{-1}	Chloroform / DMF (9:1)	Scaffolds for tissue engineering applications.	[186]
Modified collagen (5% w/v)	15 cm / 20 kV / 0.8 mL.h^{-1}	HFIP	UV-crosslinked fibers for controlled drug release.	[187]
PLGA (8% w/v) Collagen / fibronectin (25 – 100 µg/mL)	20 kV / 0.5 mL.h^{-1}	Chloroform / DMF (7:3)	Scaffolds for drug toxicity studies and tissue engineering applications	[188]
Collagen / HAP Collagen / oHA (8% w/v)	14 cm / 20 kV / 0.8 mL.h^{-1}	HFIP	Scaffolds for bone tissue engineering approaches.	[189]
oHA / Collagen HA / Collagen (8% w/v)	-	HFIP / TFA (95 / 5 v/v)	Vascular tissue-engineered scaffold for promoting endothelial cell proliferation.	[190]
PLGA / PCL (7:3 w/w) 2% collagen	13 – 18 cm / 16 kV / 0.6 mL.h^{-1}	TFE	Scaffolds for orofacial tissue regeneration.	[191]
Modified collagen (5 – 10% w/v)	15 cm / 10 kV / 1.0 mL.h^{-1}	FA	Fabrication of graft polymerized collagen fibers.	[192]

[a] hyaluronan (HA); hyaluronan oligosaccharides (oHA); hydroxyapatite (HAP); poly(acrylonitrile) (PAN); polycaprolactone (PCL); poly(lactic acid) (PLA); poly(lactic-*co*-glycolic acid) (PLGA).

[b] dimethylformamide (DMF); formic acid (FA); 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP); 2,2,2-trifluoroethanol (TFE); tetrahydrofuran (THF); trifluoroacetic acid (TFA).

4.3.1. Tissue engineering applications

Skin tissue engineering has emerged as an important strategy for treating chronic and extensive skin wounds, including burns and ulcers. Materials for wound dressings, adhesion barriers or tissue mimicking are highly desired for these applications. Several collagen modifications, such as deamidation, succinylation, maleylation, and citraconylation were made, and the resulting modified collagens were used to coat polyacrylonitrile and poly(D,L-lactide-co-glycolide) fibers by a layer-by-layer technique with alternating layers of triple-helical anionic and cationic collagens. The collagen-coated fibers provided a good substrate for L-929 fibroblast cell adhesion and spreading while maintaining dimensional stability **(Figure 10)** [182]. Coating poly(lactic-*co*-glycolic acid) (PLGA) fibers with collagen solution and crosslinking significantly increased human dermal fibroblast (HDF) and keratinocyte attachment and cell proliferation, showing that stable collagen-coated fiber surfaces increase the bioactivity remarkably, which can be promising for bioengineered skin applications [183]. In addition, co-dissolving collagen and PLGA in the electrospinning solution achieved improvements in the hydrophilicity, mechanical properties, and biocompatibility outcomes compared to the coated PLGA fibers. However, the degradation rate of the materials prepared by blending was nearly five times more than the degradation rate of the samples prepared by the coating method, which could lead to cytotoxicity [185].

Bone healing and regeneration is a multi-step process, involving the recruitment of osteoprogenitor cells and their differentiation into osteoblasts, which subsequently produce a series of ECM components and mineralization-inducing enzymes, and finally the remodeling of matrix and mineral deposits into a mature bone matrix. To improve bone healing, bone tissue engineering scaffolds can be designed with osteoconductive (promoting deposition of bone matrix) and osteoinductive (promoting recruitment and differentiation of osteoprogenitor cells) properties [193]. Osteoinduction and osteoconduction can be promoted by the porous and high-surface area structures and surface chemistries of fibers. Collagen I incorporated PLGA/PCL

(7:3 w/w) fibers presented favorable results for promoting mouse preosteoblast (MC3T3-E1) cell adhesion and spreading compared to a solvent-cast film of the same composition. Analysis of β1 integrin expression by immunofluorescence showed that the biomimetic architecture of the collagen I-incorporated fibers supports mouse parietal bone cell (MC3T3-E1) adhesion and proliferation, with promising results for improving cell responses needed to accelerate bone healing [184].

The maturation of healthy bone tissue requires blood supply. Therefore, both endothelialization and osteogenic differentiation are desirable for bone tissue engineering applications. Biomimetic fibers based on modified collagen with hyaluronic acid oligosaccharides (Col/oHAs) and mineralized Col/oHAs, achieved by self-assembly methods (using $CaCl_2$ and NaH_2PO_4 solutions to form and deposit $Ca_3(PO_4)_2$ on the modified collagen), were cultured with arterial endothelial cells (PIEC) and the mouse parietal bone cells (MC3T3-E1), separately. Both cells infiltrated the materials, forming an interconnected cell community, and the fibers with Col/oHAs noticeably enhanced cell adhesion, proliferation, upregulate alkaline phosphatase activity (ALP), and osteocalcin (OCN), compared to pure collagen and pure hyaluronic acid [189]. Silver-modified/collagen-coated electrospun PLGA/PCL fibers (PP-pDA-Ag-COL) fabricated with silver nanoparticle (AgNPs) impregnation via *in situ* reduction, polydopamine (pDA) coating, and collagen I coating exhibited antimicrobial and osteogenic properties. The fibers showed controlled release of Ag ions and inhibitory functions in infection and inflammation. The incorporation of collagen enhanced cell attachment and osteogenic differentiation of MC3T3 cells compared to PLGA/PCL, and PLGA/PCL with addition of pDA and Ag, suggesting promising application of the collagen based fiber for bone regeneration [191].

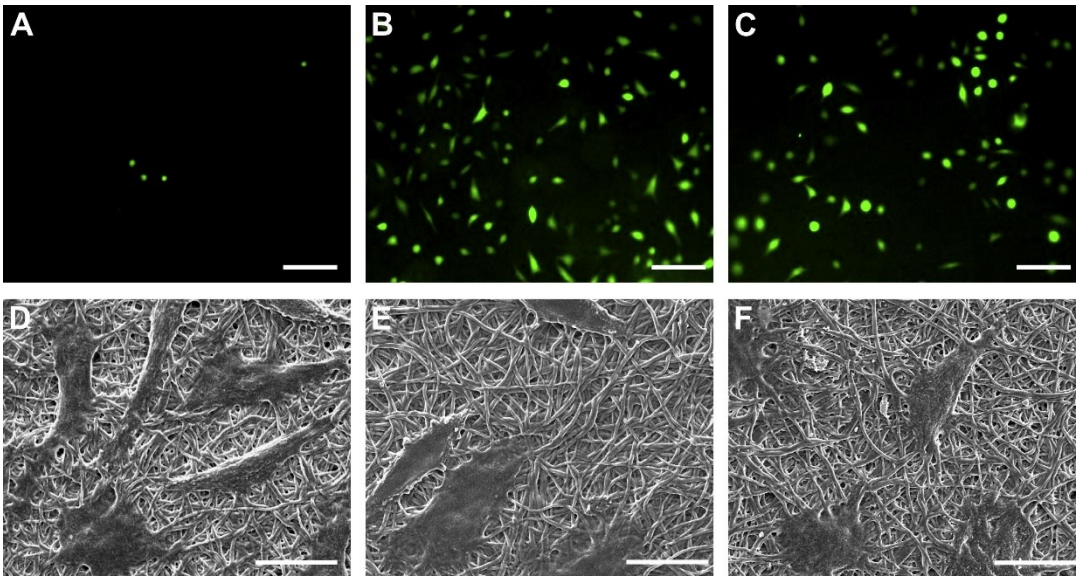

Figure 10. Live/dead staining of L-929 fibroblast cells on PLGA fibers (a), PLGA fibers coated with type I collagen and succinylated type I collagen (b), and PLGA fibers coated with type I collagen and citraconylated type I collagen. SEM micrographs of the cells on PLGA fibers coated with unmodified and succinylated type I collagen (d), unmodified and citraconylated type I collagen (e), and unmodified and maleylated type I collagen. Bars (A-C) are 100 mm. Bars (D-F) are 20 mm. Reproduced with permission from [182], copyright 2012, Elsevier.

4.3.2. Drug delivery

Medical implants, including tissue scaffolds that support tissue healing and regeneration, can provide favorable environments for bacterial growth, leading to infections. Implant-associated infections are a major contributor to the failure of implants, often requiring costly revision surgeries. Systemically delivered antibiotics can be ineffective at treating infections associated with orthopedic implants. Therefore, the incorporation of antimicrobial activity to a tissue scaffold can obviate the need for systemic antibiotic delivery, reduce the risk of implant associated infections, and improve healing outcomes. Collagen I and PLA electrospun fibers loaded with triclosan (an antibacterial agent) or levofloxacin (LEVO, a broad-spectrum quinolone antibiotic) showed high growth inhibition against *E. coli* and *S. aureus* for over 48 h compared to PLA fibers. The collagen introduction on the fibers helped control the release of both drugs to sustain the antibacterial activity for longer times, showing promising applications as tissue engineered scaffolds to treat bacterial infections [186]. Modified collagen-based fibers loaded with 5-fluorouracil (5-FU) were studied as a model for controlled release system on the

treatment of colorectal cancer. The collagen was methacrylated to insert double bonds and facilitate the loading process of (5-FU) on the fibers using photo-initiated polymerization under UV irradiation as a crosslinking method. Unmodified collagen fibers exhibited a burst release of almost all the loaded drug within 2 h, due to the rapid dissolution of the drug from the fiber surface. However, the UV-crosslinked modified collagen fibers released only 60% of the loaded drug in 2 h and continued to release for 10 h, showing sustained-release [187].

4.3.3. Chemical modifications

Chemically modifying collagen is one good way to enhance biological properties. More recent studies on modified collagens, such as collagen grafted with methyl methacrylate (MMA) and ethyl acrylate (EA), demonstrated enhanced spinnability of the fibers. Short-chain branching of P(MMA-co-EA) influences the fiber morphology and the thermal stability of the collagen graft copolymer, and significantly reduces the degradation rate of the collagen chains during processing. By contrast, long-chain branching of collagen graft copolymers can provide a higher entanglement density, leading to the higher uniformity of fibers [192]. Glycosylating collagen by conjugation of oligosaccharides (oHAs) on collagen by reductive amination was used to fabricate oHA-collagen fibers as a vascular tissue-engineered scaffold for promoting endothelial cell proliferation. The collagen fibers modified with oligosaccharides promoted the proliferation of endothelial cells. In contrast, the collagen fibers modified by HA with high molecular weight inhibited the proliferation of the cells (**Figure 11**). In addition, the oHA-collagen fibers had no detectable degree of hemolysis and coagulation, suggesting potential for use in promoting angiogenesis and vascular regeneration [190].

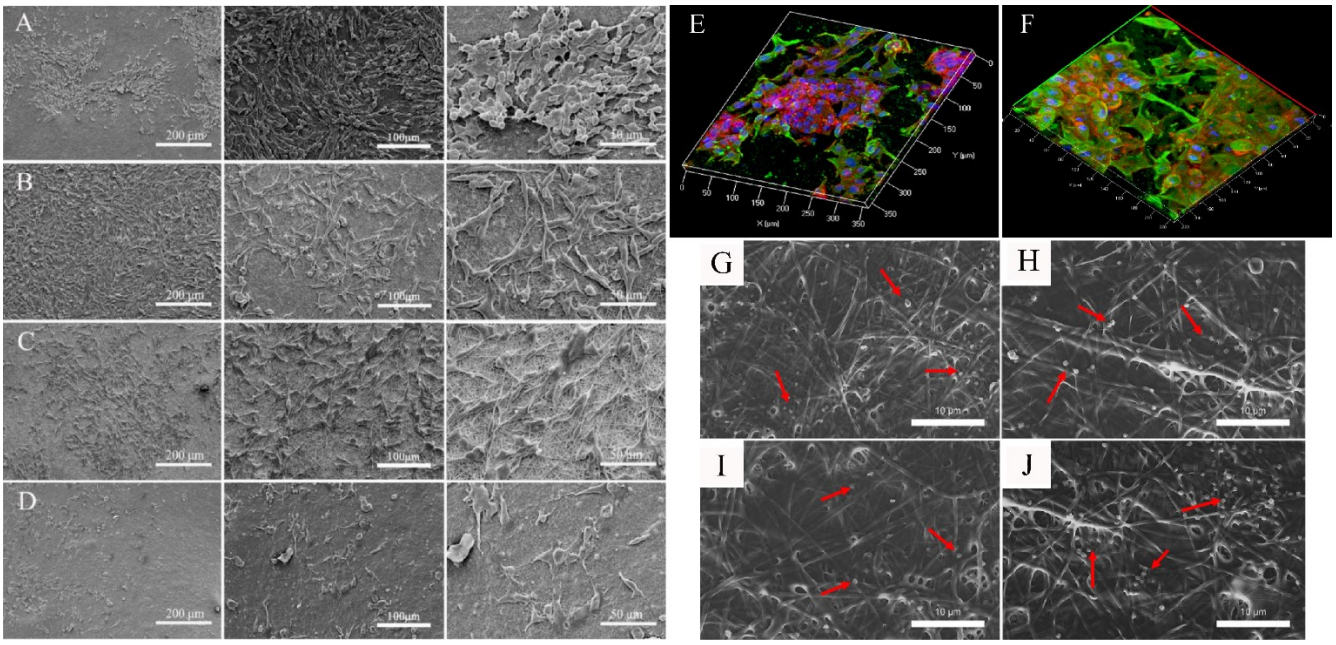

Figure 11. SEM micrographs of (A) collagen, (B) hyaluronic acid oligosaccharide/collagen, (C) 5 kDa hyaluronic acid/collagen, (D) 870 kDa hyaluronic acid oligosaccharide/collagen fibers, (E) and (F) confocal images of porcine iliac artery endothelial cells on the hyaluronic acid oligosaccharide/collagen fibers along the Z-axis (nucleus is blue, F-actin is red, intracellular adhesion molecule is green). Representative SEM images of adhered platelets onto (G) collagen, (H) hyaluronic acid oligosaccharide/collagen, (I) 5 kDa hyaluronic acid/collagen, (J) 870 kDa hyaluronic acid oligosaccharide/collagen fibers. Reproduced (adapted) with permission from [190], copyright 2019, Elsevier.

4.3.4. Future challenges

Collagen is a well-established biopolymer that has been researched extensively, resulting in established extraction and blending methods with different polymers, to achieve improvement in the biological properties of several materials. However, chemically modified collagen-based materials are gaining more attention in recent years. Chemical modification of collagen fibers facilitates the crosslinking and stabilization, improves the drug delivery capacity, and can be designed to improve cell proliferation and osteoconductive properties. Despite the large body of literature on collagen and modified collagen fibers, opportunities remain in the development of blood-contacting materials. Methods to control inflammatory responses to collagen-based fibers should also be further investigated. These future studies are essential for establishing the hemocompatibility and immune response profiles of materials that are intended to support and grow various cell types. For wound healing, tissue engineering, and vascular

applications of collagen and modified collagen-based fibers, the validation of their hemocompatibility, immune system compatibility, antibacterial, and antiviral activities are highly desired. With respect to chemistry, developing new modifications to collagen that maintain the native structure of the triple helix during solubilization, increase the mechanical strength of the final material, and improve the inherent properties of collagen-based fibers should be targets for future research and development.

4.4. Decellularized extracellular matrix (dECM)

The development of materials that can mimic biological properties, chemical composition, and morphology of the extracellular matrix (ECM) is challenging for tissue engineering applications [26,167]. Electropsinning offers the capability to control the formation of 3D fibers from biopolymers and biopolymer-containing blends to achieve chemical, morphological, and biological properties similar to the ECM. Decellularized extracellular matrices (dECM) from different tissues are emerging as a class of biomaterials that contains the native chemical and biological properties of the ECM, preserving composition of the native cellular microenvironment [85,194]. Decellularization methods to extract dECM include physical, chemical and biological processes. The goals of these methods are to maximize the removal of nuclear material, minimize the loss of ECM components and retain components that give rise to the tissue bioactivity [84,195]. The use of dECM to form electrospun fibers represents a promising application in tissue engineering, due to the possibility of forming a biomaterial with the morphology and bioactivity of the native tissue. However, applying dECM in electrospinning is challenging, due to the difficulty of finding compatible volatile solvents to solubilize the dECM without denaturing the components, the necessity of a high concentration to form stable fibers, and the development of crosslinking methods for making these fibers water-stable. **Table 4** summarizes the composition and parameters of electrospun fibers using different dECM recently proposed for diverse biomedical applications.

1

2 **Table 4.** Electrospun fibers based on dECM.

Fiber composition[a]	Distance / voltage / flow rate	Solvents[b]	Desired application	References
hAt-ECM (60 – 140 mg.mL^{-1}) hAt-ECM / PDO (10:90 v/v)	12 cm / 20 kV / 2.5 mL.h^{-1}	HFIP TFA	Scaffolds for tissue engineering.	[196]
PCL (12% w/v) hUBE	21 cm / 10 kV / 1.8 mL.h^{-1}	HFIP	Scaffolds for liver tissue engineering.	[197]
pLu-ECM / PLLA (46.7/400 mg.mL^{-1})	27 cm / 27 kV / 4.5 mL.h^{-1}	HFIP	Scaffolds for airway smooth muscle culture.	[198]
pCa-ECM / PEO (1:14 0.1 wt.-%)	10 cm / 9 kV / 1.0 mL.h^{-1}	HFIP	Scaffolds for cardiac tissue regeneration.	[199]
PHB / PHBV (1:1 6 wt.-%) hNSC-ECM (5 mg.mL^{-1})	20 cm / 16 kV / 1.5 mL.h^{-1}	DMF	Scaffolds for cartilage regeneration.	[200]
pM-ECM / PCL (6 / 8% w/w) (0:5 to 5:0 ratios)	10 cm / 15 kV / 1.8 mL.h^{-1}	HFIP	Scaffolds for meniscus regeneration.	[201]
Ht-ECM / PLCL (5-12.5 wt.-% / 9% w/v/)	10 cm / 12 kV / 0.4 mL.h^{-1}	AA / HFIP	Wound dressings for reducing scarring in wound healing.	[202]
PLLA (10% w/v) hUBE	14 cm / 13 kV / 0.5 mL.h^{-1}	HFIP	Scaffolds for liver tissue engineering.	[203]
pLi-ECM / GE / PCL (5-10% v/v / 5-10% w/v / 2.5-5% w/v)	15 cm / 15 kV / 2.0 mL.h^{-1}	HFIP 0.1 N HCl	Scaffolds for liver tissue engineering.	[204]
PCL (11% w/v) hMSC / hUVEC (0.025% w/v)	21 cm / 20 kV / 3.0 mL.h^{-1}	HFIP	Scaffolds for bone tissue engineering.	[205]

PLGA (8% w/v) hADSC-ECM	10 cm / 16 kV / 4.0 mL.h^{-1}	HFIP	Wound dressings for wound healing.	[206]
bSM-ECM (10% w/v) PCL / bSM-ECM (5/5% w/v)	20 cm / 20 – 25 kV / 0.75 – 4.0 mL.h^{-1}	HFIP	Scaffolds for muscle regeneration.	[207]
bA-ECM / PCL (0.25 or 1% / 8% w/v)	12 cm / 10 kV / 0.8 mL.h^{-1}	HFIP	Scaffolds for tissue engineering.	[208]
rSM-ECM (10% w/v)	10 – 15 cm / 5 – 15 kV / 1.0 – 2.5 mL.h^{-1}	HFIP	Scaffolds for tissue engineering.	[209]
hLi-ECM / PLLA (25 µg.mL^{-1} / 22% w/v)	23 cm / 13 kV / 2.5 mL.h^{-1}	0.25 M AA HFIP	Scaffolds for liver tissue engineering.	[210]
pK-ECM / PCL (100 mg.mL^{-1} / 15% w/v) (7:3 to 3:7 ratios)	15 cm / 18 kV / 0.5 mL.h^{-1}	HFIP	Membranes for kidney tissue engineering.	[211]
pCo-ECM / PCL (1% / 9% w/v)	12 cm / 15 kV / 2.0 mL.h^{-1}	HFIP	Scaffolds for corneal stroma regeneration.	[212]
GE / PLGA (16% / 8% w/v) (10:3 ratio v/v) cSC-ECM	15 cm / 16 kV / 3.0 mL.h^{-1}	HFIP	Scaffolds for cartilage regeneration.	[213]
PCL (10% w/v) rA-ECM	10 cm / 20 kV / 0.5 mL.h^{-1}	Chloroform:methanol (5:1)	Scaffolds for cardiac tissue regeneration.	[214]

[a] Bovine aorta-ECM (bA-ECM); bovine skeletal muscle-ECM (bSM-ECM); cow scapular cartilage-ECM (cSC-ECM); gelatin (GE); heart tissue-ECM (Ht-ECM); human adipose derived stem cells-ECM (hADSC-ECM); human adipose tissue-ECM (hAt-ECM); human liver-ECM (hLi-ECM); human mesenchymal stem/stromal cells (hMSC); human nasal septum cartilage-ECM (hNSC-ECM); human umbilical vein endothelial cells (hUVEC); human urinary bladder epithelium (hUBE); pig lung-ECM (pLu-ECM); polycaprolactone (PCL); poly(ethylene oxide) (PEO); poly-L-lactic acid (PLLA); poly(lactic-co-glycolic acid) (PLGA); poly(l-lactide-co-caprolactone) (PLCL); polydioxanone (PDO); porcine cardiac-ECM (pCa-ECM); porcine cornea-ECM (pCo-ECM); porcine kidney-ECM (pK-ECM); porcine liver-ECM (pLi-ECM); porcine meniscus-ECM (pM-ECM); rabbit skeletal muscle-ECM (rSM-ECM); rat aorta-ECM (rA-ECM).

[b] acetic acid (AA); dimethylformamide (DMF); 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP); trifluoroacetic acid (TFA).

4.4.1. Tissue engineering applications

Using a dECM from a specific tissue to form biomaterials can provide an environment similar to that native tissue, and becoming a valuable tool for tissue-specific regeneration and disease investigation. Decellularized pig lung ECM (pLu-ECM) and PLLA electrospun fibers showed promising results as an *in vitro* airway model. Human bronchial smooth muscle cells (hBSMCs) cultured on the pLu-ECM fibers for one week resulted in organized arrangement of actin filaments, increased production of collagen type 1 and expression of contractile protein calponin 1, when compared to PLLA fibers [198]. Combined electrospun fibers of porcine meniscus dECM (pM-ECM) and PCL showed tensile moduli in the same range as that of human meniscus (132 to 331 MPa) and higher yield stresses than PCL fibers. In addition, pM-ECM/PCL fibers are biocompatible and support rabbit meniscus fibrochondrocyte cell adhesion and proliferation for over 10 days, increasing the gene expressions of aggrecan, SOX 9, and collagen I and II, showing potential in meniscus tissue engineering [201]. To achieve renal filtration barrier mimicking biomaterials, electrospun fiber blends of decellularized porcine kidney ECM (pK-ECM) and PCL were fabricated and tested with a human kidney cortex/proximal tubule established cell line (HK-2) for over 7 days. The cells cultured on the fibers showed increased metabolic activity, adhesion, proliferation and protein content, as the pK-ECM content increased on the fibers (**Figure 12**). Tight junction protein-1 expression was observed on the ECM fibers, but not on the PCL fibers, demonstrating promising application for regenerative nephrology [211]. Porcine cornea ECM (pCo-ECM) and PCL electrospun fibers with different fiber alignments cultured with human corneal stromal cells (hCSCs) for over 7 days showed preliminary results as a scaffold for corneal stroma regeneration. The addition of pCo-ECM to the random and aligned fibers showed no changes on the Young's modulus, and

increased keratocyte markers, such as ALDH3A1, ACTA2 and collagen I, when compared to tissue culture polystyrene [212].

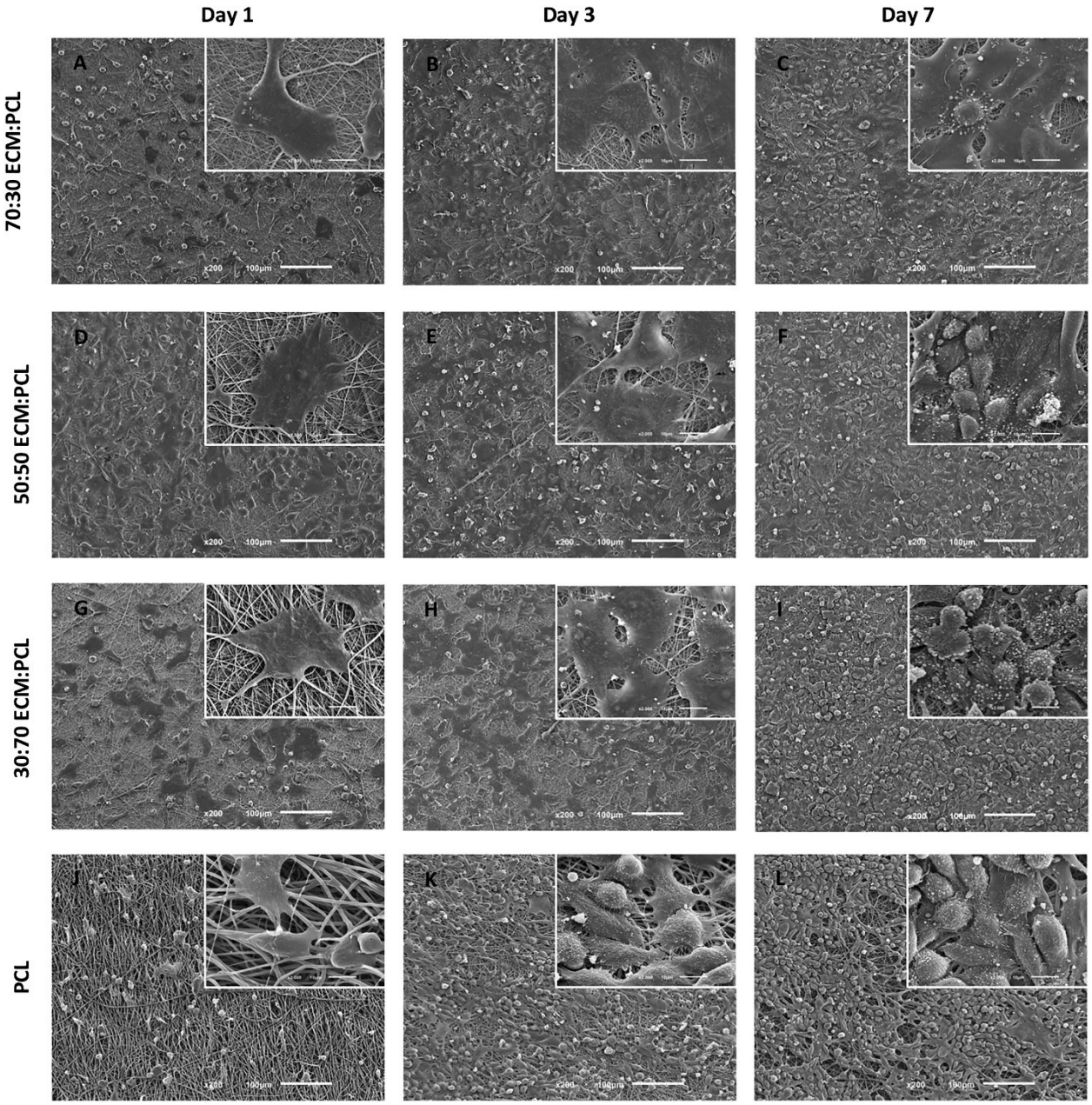

Figure 12. SEM images of human kidney cells (HK-2) cultured on PCL and pCo-ECM/PCL (at different ratios) fibers for 1, 3 and 7 days. Reproduced with permission from [211], copyright 2019, John Wiley and Sons.

4.4.2. Soft tissue regeneration

Collagen is commonly used for soft tissue regeneration and replacement applications, presenting good results in many forms, including 3D fibers. Since collagen is the major component of the ECM, recent studies employed soft tissue dECM, like adipose, muscle and

cartilage, for the production of electrospun fibers to evaluate as soft tissue regeneration scaffolds [215]. Poly(3-hydroxybutyrate)/poly(3-hydroxybutyrate-co-3-hydroxy-valerate) fibers with immobilized human nasal septum cartilage ECM (hNSC-ECM) showed high collagen II production, and aggrecan and SOX 9 expression for human primary chondrocytes (hPChs) and ADSCs, after 21 days of culture (separately), when compared to the PHB/PHBV fibers, indicating tissue reconstruction [200]. Rabbit hind leg skeletal muscle ECM (rSM-ECM) electrospun fibers were produced without a blending polymer, and the highest tensile modulus was achieved by aligned, crosslinked fibers (glutaraldehyde 25%), however no cell study was made to support the application as scaffolds for muscle tissue engineering [209]. On the other hand, primary satellite cells isolated from rat hind limb muscle cultured for over 4 days on bovine skeletal muscle ECM (bS-ECM) and PCL/bS-ECM fibers, were proposed as a viable scaffold for muscle tissue engineering. The PCL/bS-ECM showed the best results of tensile mechanical tests. The incorporation of the bS-ECM increased cell adhesion and proliferation, myogenic protein expression (higher myoblast determination protein 1) and myokine production, such as vascular endothelial growth factor (VEGF) and interleukin 6 (IL-6), demonstrating potential for muscle loss regeneration [207]. Pure human adipose tissue ECM (hAt-ECM) and blend electrospun fibers with polydioxanone (PDO) supported ADSC growth and adhesion for over 14 days. The presence of collagen I and IV on the ECM fibers was essential for the cell growth, and the addition of the PDO increased the integration of the cells after 14 days, being of interest to soft tissue engineering applications (**Figure 13**) [196].

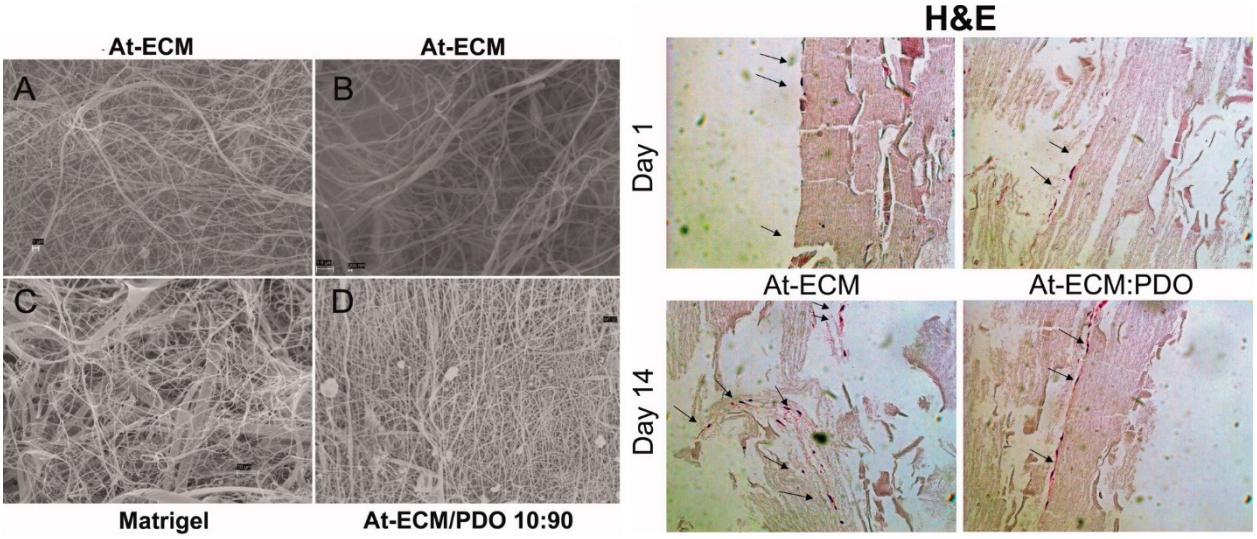

Figure 13. SEM micrographs of human adipose tissue ECM fibers (At-ECM) at concentration of 80 (a) and 100 mg mL^{-1} (b), matrigel fibers (c) and blend fibers of PDO/ECM (d). ADSCs seeded on the fibers for 14 days and stained with hematoxylin and eosin (H&E) images. Reproduced (adapted) with permission from [196], copyright 2012, John Wiley and Sons.

4.4.3. Liver tissue engineering

Liver tissue engineering is one of the most challenging tissue engineering applications due to the difficulty of creating support materials that sustain a functional environment for hepatocytes. Natural, synthetic polymer and protein blended 2D coatings have been used as candidate materials to mimic the liver ECM and to provide a viable and functional niche for hepatocytes. None of these approaches has proven to be sufficient to maintain hepatocyte phenotypes long term [194,216,217]. Liver ECM blended with polymers to produce electrospun fibers can promote a viable environment to maintain hepatocyte functionality. Electrospun blend fibers of PCL and GE with incorporations of 5-10% (v/v) porcine liver ECM (pLi-ECM) improved primary human hepatocytes (PHH) adhesion and liver functions. Porcine liver ECM (pLi-ECM) incorporated fibers had slightly lower tensile strength and higher hydrophilicity. Over 7 days of PHH culture on the fibers a tissue-like structure was formed and increased CYP1A1 activity and albumin secretion were detected, demonstrating that incorporation of

liver ECM may improve the capacity of fibers to support hepatocytes [204]. Culturing THLE-3 cells for over 16 days on PLLA electrospun fibers (22% w/v) containing human liver ECM (hLi-ECM) (25 μg mL^{-1}) provides a mechanically and biologically viable niche for the cells. The incorporation of hLi-ECM in the fibers promoted an increment to the Young's modulus, maintained cell growth, albumin production and expression of hepatic genes, such as fibronectin, collagen I and IV, alpha-1 antitrypsin, hepatocyte nuclear factor 4, CYP1A1, CYP1A2 and CYP3A4 [210]. A different ECM was also used for liver tissue engineering applications. Human urinary bladder epithelial derived ECM (hUBE-ECM) coating on PCL electrospun fibers were used to evaluate the viability for HepG2 hepatocytes cultured on the fibers for over 5 days. When compared to the PCL fibers, the ECM-coated fibers induced higher expression of albumin, CYP1A1, CYP1A2, CYO3A4, collagen I and IV, and fibronectin showing potential for liver tissue engineering applications [197]. Also using hUBE-ECM coated PLLA fibers showed similar improvements for HepG2 cells on the fibers for over 5 days, suggesting that hUBE-ECM has interesting potential for use in biomaterials for liver tissue engineering and regeneration [203].

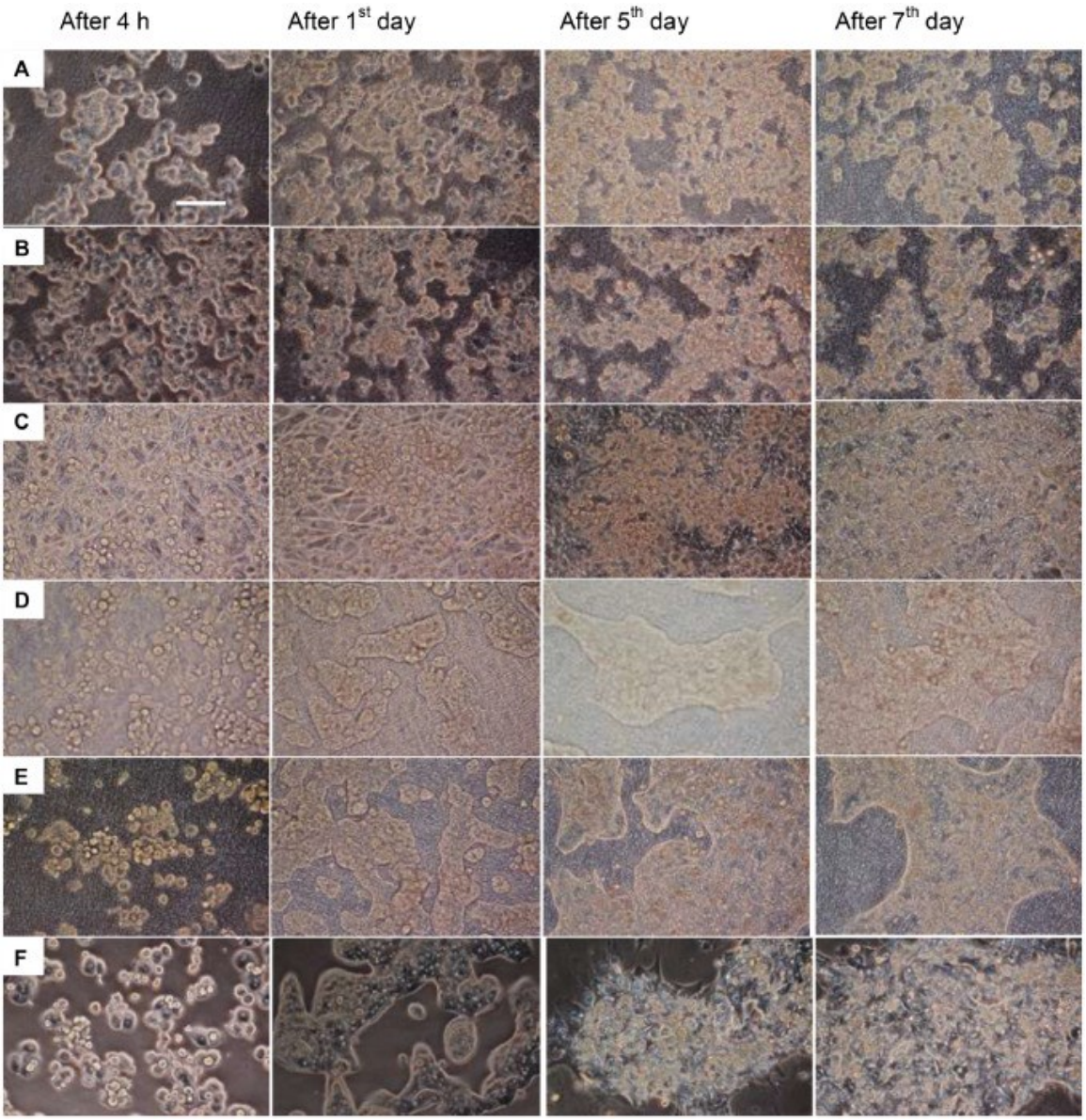

Figure 14. Phase-contrast microscope images of hepatocytes seeded on fibers for 7 days: GE/PCL (5/ 2.5 % w/v) (a), 5% (v/v) liver ECM containing GE/PCL (5/ 2.5 % w/v) (b), GE/PCL (10/ 5 % w/v) (c), 5% (v/v) liver ECM containing GE/PCL (10/ 5 % w/v) (d), 10% (v/v) liver ECM containing GE/PCL (10/ 5 % w/v) (e) and collagen film control (f). Reproduced with permission from [204], copyright 2018, Elsevier.

4.4.4. Future challenges

Electrospun fibers from several tissue ECM from both human and animal sources have been studied in recent years. The studies reviewed here have demonstrated promising properties and applications. However, while ECM-derived materials are supposed to be non-immunogenic, in theory, due to decellularization processes removing the primary immunogenic factors (such as DNA), few works have tested new ECM-derived fibers to prove their non-immunogenicity. Blending ECM with different polymers seems to be the first alternative to most studies presented, instead of using pure ECM fibers, which could be correlated to the difficulty of electrospinning pure ECM. This difficulty arises due to the need for appropriate solvent conditions. Improvements in processing methods for ECM fibers should also consider the extraction processes used to obtain materials that would facilitate dissolution. The extraction from some tissues may result in a heterogenous and poorly defined mixtures of proteins, lipids, and polysaccharides, that may hamper development of suitable electrospinning solvent condiations and processing conditions. Also, few studies using tissue ECM fibers have advanced to *in vivo* studies, to demonstrate efficacy for the desired application, in part because of the lack of information on immune response of the materials. This is a therefore a very important area for future research that will enable growth of ECM-derived electrospun fiber applications.

5. CONCLUSIONS

Biopolymers are readily biodegradable, renewable, abundant, and non-hazardous compared to synthetic polymers. Their similarity to biomolecules found in the human body, and their ability to participate in biochemical processes make them excellent candidate materials for biomaterials, particularly for tissue engineering. Here, new fibers and nanofibers produced from emerging biopolymers with excellent biological properties including sulfated polymers (carrageenans, chondroitin sulfate, among others), tannin and tannin derivatives

(condensed and hydrolyzable tannins, tannic acid), modified collagen, and ECM extracts for electrospun fiber fabrication are reviewed.

Electrospinning is commonly employed in biomaterials fabrication, since it can generate fibers with 3D structure, with great flexibility in terms of choice of material, scaffold geometry, fiber orientation, and properties of the biopolymer used in the manufacture. As emerging biopolymers with increasing research and attention, sulfated polysaccharides possess many favorable biological properties, including biocompatibility, antioxidant, antiviral, antibacterial, immunomodulatory, and antitumor activities. The incorporation of sulfated biopolymers in electrospun fibers can improve the compatibility and proliferation for fibroblast cells, chondrocytes, and adipose-derived stem cells. Sulfated biopolymers can also promote osteogenic and chondrogenic differentiation, and enhance the efficiency and control the release of drugs. As new and emerging biopolymers, tannins and tannin derivatives have gained more visibility due to their inherent biological properties, such as biocompatibility, antifungal, antibacterial, and antioxidant properties. Their incorporation in electrospun fibers can improve the compatibility and proliferation for adipose-derived stem cells and endothelial cells, and show antimicrobial activity. Tannins also promote high adsorption of toxic metals, such as chromium and uranium, maintain antioxidant activity and can also be used as cross-linking agents. Modified derivatives of collagen are also receiving recent attention due to their ability to enhance cell responses, leading to improving bone integration, reducing inflammatory cell activation, and accelerating wound healing. Collagen or modified collagen-based electrospun fibers can improve the compatibility, proliferation, and differentiation of fibroblasts, preosteoblasts, artery endothelial cells, and mouse parietal bone cells. They can also promote controlled and sustainable drug release and can improve the stability of fibers. These benefits make collagen-based fibers excellent candidate materials for promoting angiogenesis and vascular regeneration, with improved antithrombotic properties. Electrospun fibers containing ECM-derived components provide a similar environment to native ECM of the tissue from

which they are derived, improving the biological and mechanical properties of electrospun fibers and making them suitable for tissue-specific engineering and regeneration. Many of the examples reviewed here report favorable cytocompatibility and lack of cytotoxicity. However, inflammatory responses, immune reactions, and other indications of potential toxicity *in vivo* are not as frequently interrogated in the existing literature.

Future research with these emerging biopolymers should address issues specific to each of these types of bipolymer-derived fibers and nanofibers. Some of the answers that should be pursued to advance the use of these materials are common for all of the fibers discussed. These questions include the evaluation of immunogenicity of the material, and application in *in vivo* studies to evaluate inflammation and tissue healing. Sulfated polymers and tannin derivatives modulate immune responses and collagen/ECM materials are supposed to be non-immunogenic, however, changing the morphology to obtain a 3D structure like a fiber can lead to activation of immune responses, which makes the question of immunogenicity an important topic for future research. While demonstrations of cytocompatibility, hemocompatibility, and non-immunogenicity are excellent preliminary results to support the development of materials for tissue engineering or regeneration applications, *in vivo* studies can result in divergent results, by uncovering previously unforeseen biological responses. The review presented here suggests that *in vivo* studies of the electrospun fibers composed of biopolymers and ECM should be pursued.

Acknowledgements
The authors gratefully acknowledge the financial support from the National Science Foundation (award number 1933552).

Conflict of Interest
The authors declare that there are no financial or commercial conflicts of interest.

Received: ((will be filled in by the editorial staff))
Revised: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

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This review summarizes the recent developments in electrospinning technology. In particular, we describe new fibers produced from emerging biopolymers with features that impart useful biochemical function, including sulfated polysaccharides (carrageenans and glycosaminoglycans), tannins and their derivatives (condensed and hydrolyzed tannins, tannic acid), and modified collagen structures. Moreover, applications of these electrospun fibers mainly in biomedical and environmental fields are highlighted.

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Expanding the repertoire of electrospinning: new and emerging biopolymers, techniques, and applications