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Short review

Electrospun ECM macromolecules as biomimetic scaffold for regenerative medicine: challenges for preserving conformation and bioactivity

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Abstract: The extracellular matrix (ECM), the physiological scaffold for cells *in vivo*, provides structural support to cells and guaranties tissue integrity. At the same time, however, it represents an extremely complex and finely tuned signaling environment that contributes in regulating tissue homeostasis and repair. ECM can bind, release and activate signaling molecules and also modulate cell reaction to soluble factors. Cell-ECM interactions, as a result, are recognized to be critical for physiological wound healing, and consequently in guiding regeneration. Due to its complexity, mimicking ECM chemistry and architecture appears a straightforward strategy to exploit the benefits of a biologically recognizable and cell-instructive environment. As ECM consists primarily of sub-micrometric fibers, electrospinning, a simple and versatile technique, has attracted the majority efforts aimed at reprocessing of biologically occurring molecules. However, the ability to trigger specific cellular behavior is likely to depend on both the chemical and conformational properties of biological molecules. As a consequence, when ECM macromolecules are electrospun, investigating the effect of processing on their structure, and the extent to which their potential in directing cellular behavior is preserved, appears crucial. In this perspective, this review explores the electrospinning of ECM molecules specifically focusing on the effect of processing on polymer structure and on *in vitro* or *in vivo* experiments designed to confirm the maintenance of their instructive role.

Keywords: electrospinning; synthetic ECM; biomimicry; bioactivity; regenerative medicine scaffold; collagen; gelatin; elastin; hyaluronic acid; fibrinogen

Abbreviations

AA	Acetic Acid
CD	Circular Dichroism
DMF	Dimethyl-formamide
DMSO	Dimethyl-sulfoxide
DSC	Differential Scanning Calorimetry
DSS	Disuccinimidyl suberate
ECM	Extra-Cellular Matrix
EDC	N-3-Dimethylaminopropyl-N'-ethylcarbodiimide hydrochloride
ES	Electrospinning
GAGs	Glycosaminoglycans
HA	Hyaluronic Acid
HEPM	Human Embryonic Palatal Mesenchymal
HMDI	4,4'-Diisocyanato-methylenedicyclohexane
FTIR	Fourier Transform Infrared Spectroscopy
HFIP	1-1-1,3-3-3-hexafluoro-2-isopropanol
HFP	1-1-1,3-3-3-hexafluoro-2-propanol
MEM	Minimum Essential Medium
NHS	N-hydroxysuccinimide
PBS	Phosphate Buffered Saline
PCL	Poly-ε-caprolactone
PHB	Poly-hydroxybutyrate
PLA	Polylactic acid
PLGA	Poly-lactide-co-glycolide
SHG	Second Harmonic Generation
TCPS	Tissue-Culture polystyrene
TEM	Transmission Electron Microscopy
TFE	Tetrafluoroethylene
XRD	X-ray diffraction

1. Introduction

In regenerative medicine, the possibility to generate a functional tissue strongly relies on the ability to fabricate scaffolds capable to direct cell organization, elicit specific cellular responses and orderly guide proliferation and differentiation for effective tissue repair. Unfortunately, addressing cell behavior is far from being a simple objective.

Cell fate is influenced by a variety of signals, that originates from soluble factors, neighboring cells and interactions with the extra-cellular matrix [1,2].

The ECM, in particular, not only provides physical support to cells and guaranties integrity to tissues, but also affects cellular processes, including migration, proliferation, differentiation and synthesis, through a complex variety of pathways (Figure 1) [3–6]. The most thoroughly studied mechanism is the direct pathway, where signaling is originated by interactions between cell surface receptors and ECM ligands. However, ECM also participates, indirectly, in regulating cell function by acting as substrate for other molecules (e.g., by binding, protecting, releasing and/or activating growth factors). Furthermore, it plays a role in modulating cellular reaction to soluble factors as interaction with ECM components can be required for an efficient response.

The composition and morphology of ECM are tissue specific and substantial variations can also be observed within the same tissue. However, common components and architecture can be identified for ECM, and for interstitial matrix in particular [4]. In general terms, interstitial matrix can be described as a three dimensional network of fibrous proteins, mostly collagens (fibrillar and non-fibrillar), glycoproteins, glycosaminoglycans, such as HA, and proteoglycans.

Collagens are the main structural components, primarily account for tissue strength and limit tissue deformation. Glycoproteins, such as laminin, elastin and fibronectin, also contribute to ECM architecture and properties, and elastin, in particular, is responsible for elastic recoil. Proteoglycans and HA, on the other hand, are interspersed among collagen fibrils and control tissue hydration by sequestering water molecules.

Given the complexity of this environment, designing scaffolds that mimic, at least to some extent, the ECM in composition and structure appears a straightforward strategy.

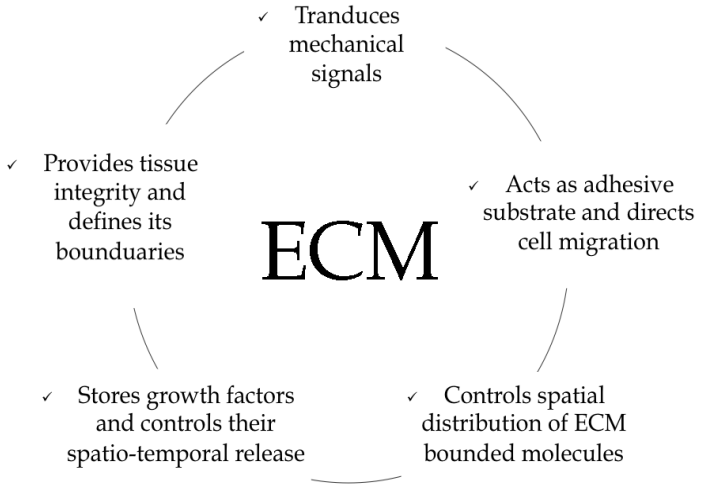

Figure 1. Main functions of ECM [7].

ECM macromolecules support non-structural functions principally through their ability to bind other biologically active molecules, which, in turn, are mediated by multiple domains in their structure [4,7]. Accordingly, reconstituted ECM polymers and decellularized ECM are excellent substrates for studying cell behavior and have also potential for selected regenerative applications.

However, they might be inadequate in terms of mechanical performance, porosity or medium term stability. Decellularized ECM structure, in particular, might not always be an ideal scaffold for tissue regeneration, as mature tissue matrix often does not possess the highly interconnected porosity required to support cell ingrowth and vascularization.

For the abovementioned reasons, techniques enabling to design and control scaffold features (such as, pore size, interpore connectivity, etc.) are subject of intensive research efforts. In this perspective, natural occurring polymers should, at least partially, preserve their signaling capabilities, and scaffold with tunable composition, architecture, mechanical performance and degradation profiles can be fabricated.

As ECM primarily consists of nanometer fibrils and fibers between 50 and 500 nm in diameter, techniques to fabricate nano-scaled structures from ECM occurring polymers are particularly attractive for creating ECM inspired scaffolds, as topographical cues can also be included and their role in guiding cell migration and behavior preserved.

Although other techniques capable to process these biomolecules into fibrous scaffold are also encountered (e.g., molecular self-assembly or thermally induced phase separation) [8,9], electrospinning has attracted significant interest mostly for being a relatively simple, but extremely versatile technique [10]. Self-assembly relies on the intrinsic capability of molecules involved to spontaneously aggregate into fibers under appropriate conditions [11,12] and the possibilities to control the process, and its outcome, are therefore limited. Thermally-induced phase separation, on the other hand, is generally successful for synthetic crystalline polymers and effective for preparing microporous structures, while obtaining a nanofibrous structure is more challenging [8].

On the contrary, electrospinning allows to obtain well defined and controlled nanofibers morphologies from virtually any soluble polymer. Accordingly, a large variety of synthetic and natural polymers were successfully electrospun for the preparation of artificial ECM in many regenerative applications, such as skin substitutes, vascular grafts, scaffolds for bone, neural and cartilage tissue engineering, wound healing dressings [13–18].

Despite the favorable results published, including excellent *in vitro* viabilities for both immortalized and primary cells [13,19] and some indication of superior performance for natural polymers *in vivo* [20], the experimental evidence supporting the extent to which using ECM molecules is beneficial is more rarely discussed.

The use of ECM polymers, in fact, does not per se guarantees that signaling capabilities will be preserved and that the electrospun scaffold will have a favorable instructing role. In many cases, reprocessing biopolymers into an electrospun scaffold can compromise many of their structural and biological properties. Moreover, in some cases even the procedures required to isolate and purify the polymers can also decrease their intrinsic bioactive properties, as can the crosslinking methods, generally required to achieve the necessary stability.

When materials are processed by electrospinning, the two major menaces to molecule conformation and structure are represented by the solvent and the intense electrostatic field. The former is generally considered as the major threat for biological molecules proper folding and systematic studies on their denaturation by solvent can be found [21]. However, the high voltage biomolecules are subjected to might also interfere with their structure. Although folding and unfolding of proteins under intense electric fields is receiving attention as an autonomous

phenomenon [22], this is usually overlooked in electrospinning and the effect of solvent and field are regarded as a whole.

The preparation of electrospun scaffolds fabricated from ECM-polymers and their application in different regenerative medicine application are reviewed in previous papers [19,23] and in many of them the terms *bioactive* and *biomimetic* are used to support the rationale and the specific benefits for electrospun natural polymers. The specific objective for this work was, therefore, to review the experimental evidence supporting the preservation of polymer bioactivity. For this reasons, only papers with experimental design enabling to extract information related to the effect of the electrospun material itself were primarily searched for. In addition, based on the hypothesis that bioactivity strongly depends on macromolecule conformation, papers focusing on the effect of processing on their structure were also included.

2. Electrospinning

Electrospinning is a well-established technique to produce sub-micron non-woven fibers from polymer solutions or more rarely, melts and emulsions.

In its more basic and commonly encountered configuration, high voltage (few to tens kV) is applied to the polymer solution using a set up as the one schematized in Figure 2. The polymer solution, generally, fed through a syringe pump, it is charged in a metal capillary (spinneret) and a grounded, or opposite charged, target is placed to set distance. When the electric field produces a force strong enough to overcome the surface tension of the polymer solution, a Taylor cone forms at the tip of the spinneret. From the Taylor cone the polymer solution is stretched and attracted to the grounded or oppositely charged collecting target. As the jet travels through the field and solvent gradually evaporates, bending instability will cause the jet to whip, stretch and elongate and almost dried, ultra-fine fibers will be collected on the target.

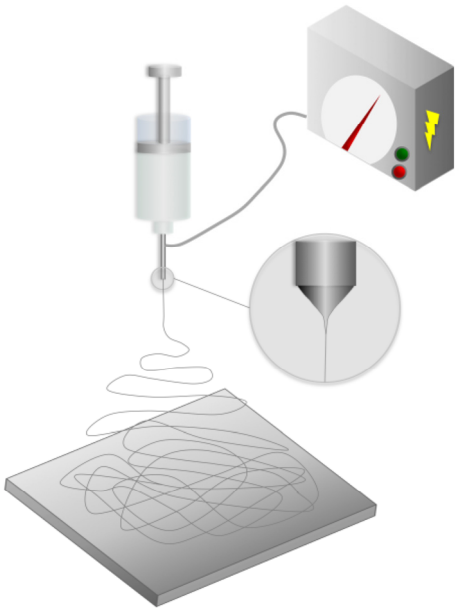

Figure 2. Scheme of a typical laboratory-scale electrospinning setup.

Together with being a constructively simple process, the success of electrospinning is related to its high versatility. Most soluble polymers with reasonable molecular weight are likely to be processable and a large variety have, in fact, been successfully electrospun into micro- and nano-fibers with diameter down to 20 nanometers [24].

Furthermore, many modifications of the set up are easily implemented as to obtain coaxial fibers [25], aligned fibers [26], composite mats [27], or 3D shaped structures [28], and to simultaneously spin multiple polymers to obtain mats with mixed fibers.

Obtaining fibers instead of beads or beaded fibers, however, requires the management of a large number of parameters that include polymer and solution properties (polymer molecular weight, solution viscosity, solvent surface and vapor tension), processing parameters (voltage, distance, and flow-rate) and environmental conditions (temperature, relative humidity).

All these parameters were shown to affect the morphology of the electrospun fibers and on their homogeneity and diameter in particular (a detailed analysis of parameter influence can be found in [29]). Within the range of parameters in which defect free fibers are obtained, fiber diameter can be controlled mainly by reducing polymer concentration or increasing voltage, distance from target and electrical conductivity [30,31,32].

3. Challenges for ECM Analogous Fabrication

Because of the morphological resemblance that electrospun fibers show with the fibrous structure of the ECM (Figure 3), electrospinning has attracted significant attention for cell culture and tissue regeneration applications [33].

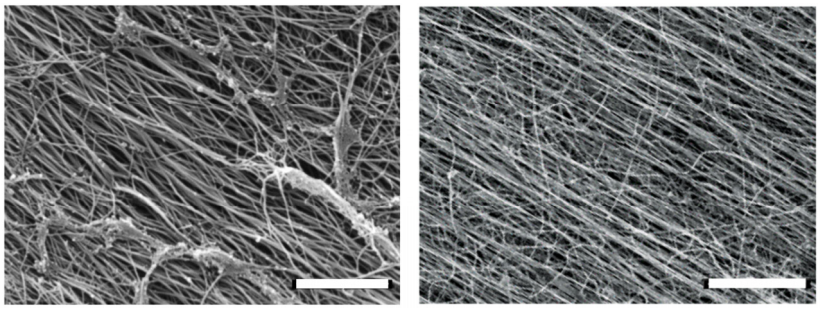

Figure 3. ECM collagen fibers in articular cartilage (left, scale bar = 2 μm, reprinted with permission from [34]), and electrospun fibrinogen fibers from HFIP (right, scale bar = 100 μm).

Electrospun matrices are well suited as they offer a large surface area for cell migration and thin-fiber topography can actively support cell migration and affect cell behavior by contact guidance [35].

For biomedical applications synthetic and biodegradable aliphatic polyesters (PLA, PLGA, PCL, PHB) are often preferred for their good processability, tunable and controllable properties and mechanical performance. However, in the context of a biomimetic approach, synthetic polymers can, at best, only reproduce the physical architecture of ECM fibrous components, but they lack all the

other properties that ECM can offer, including recognition sites. Moreover, their degradation products can locally lower pH and have a detrimental effect on regeneration [36]. For the abovementioned reasons, blending with ECM polymers is a common solution to combine the favorable properties of synthetic polymers and cell recognition signals [19].

However, electrospinning appears particularly attractive when ECM molecules are processed, as scaffold with both morphological and chemical resemblance to the ECM structure can be designed. Multiple biological polymers can be spun together, and active molecules, as growth factors or drugs, can be loaded within the electrospun fibers, as they were shown to represent excellent matrices for controlled release under different profiles [37].

Unfortunately, processing biological polymers via electrospinning is significantly more challenging than processing synthetic materials. In fact, ECM polymers show poor solubility and only very few solvent systems with suitable characteristics (volatility, surface tension, dielectric constant) are suitable for their dissolution at the concentrations required for electrospinning [29]. In addition, the viscoelastic properties of ECM-polymer solution are frequently inadequate for guaranteeing a stable process [38]. Moreover, although this is generally barely discussed, stable interactions created between hydrophobic sites exposed by solvent can cause gelification of the ES polymer solution at the tip of the spinneret and consequently give discontinuity to the process [39].

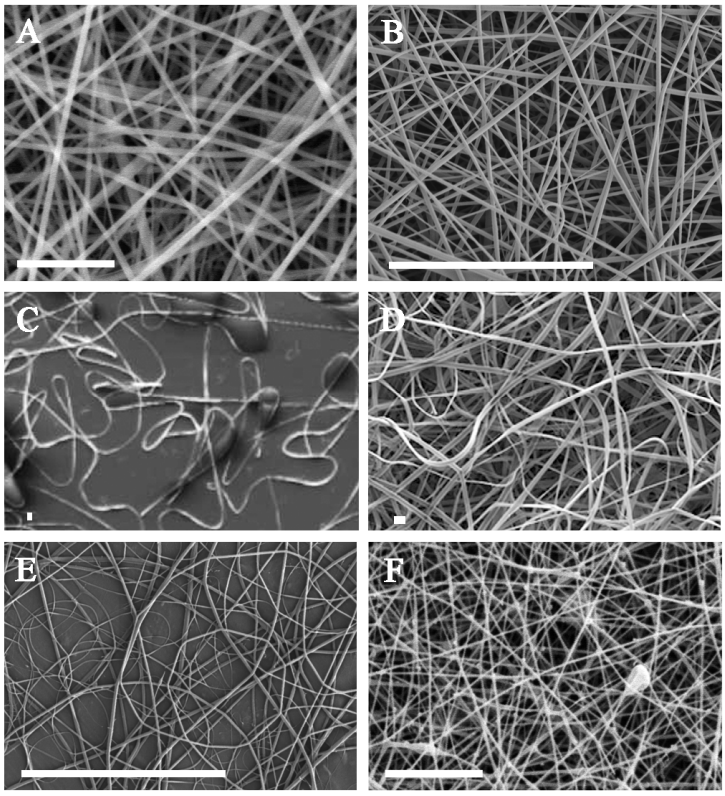

Figure 4. Scanning electron micrographs of different electrospun ECM macromolecules: type I collagen spun from HFP (A), type A gelatin from acetic acid/distilled water (9:1) (B), α-elastin from HFP (C), reprinted from [40], tropoelastin from HFP (D), reprinted from [41], hyaluronic acid from NH_4OH/DMF mixture (E) and fibrinogen from formic/acetic acid (F). Scale bar = 5 μm.

4. Collagen(s)

Collagens are a family of proteins that counts almost 30 members with a common motif of helical fibrils formed by three polypeptide subunits. The fibril-forming collagens (types I, II and III) are the primary proteins in interstitial ECM and represent the main structural component in many tissues [42,43].

Because of their predominance in the ECM, collagens are considered as ideal scaffold materials and type I, in particular, has been thoroughly investigated for regenerative medicine [44]. As it is isolated mainly from animal tissues, concerns have raised about its antigenicity. However, a variety of sources is available and refined purification techniques have been developed so that collagen can now be recognized as a biomaterial with low antigenicity [43].

Successful electrospinning of collagen can be achieved from fluorinated solvents (e.g., HFIP, TFE) (Figure 4A, Table 1) [45,46,47]. However, electrospinning of water-insoluble collagen results in water-soluble scaffolds; this behavior raised some concerns about the possible protein denaturation during the process.

Although the D-banding pattern characteristic of the native arrangement of collagen fibrils is sometimes observed in TEM analysis of HFIP electrospun collagen [45,48] the majority of analysis investigating the effect of processing failed in finding evidence of structure preservation.

The extensive characterization of HFIP electrospun nanofibers and HFIP-recovered collagen performed in [49] suggests that electrospinning in HFIP can degrade collagen into gelatin. In fact, the characteristic cross-striation pattern of collagen was not appreciable in TEM images, no signal was recorded in SHG analysis, indicating that the native crystalline structure of collagen was compromised, and massive loss of triple-helical structure (up to 45% according to Yang et al. [50]) was demonstrated with CD spectroscopy. Moreover, denaturation enthalpies measured by DSC were similar or even inferior to those of gelatin. Denaturation of collagen α-chains using HFIP as ES solvent was confirmed by SDS-PAGE even on samples where the 67 nm pattern was shown by TEM images [48].

Irrespectively of the effect on collagen structure, HFIP can persist in non-negligible quantities and vacuum or heat treatments are required to lower the values below 100 ppm [51].

In the search for more benign solvents, water based acidic solutions with or without addition of ethanol were also found to be effective for protein solubilization and spinning. However, the results in terms of the effect on collagen structure are conflicting.

In some cases, results of CD spectroscopy and FT-IR analyses on electrospun collagen fibers appeared to indicate that, compared to HFIP, the native helical structure can be better preserved using 40% acetic acid [52], or mixture with acid/EtOH (1:1) [38]. For this latter, triple helical fraction was found preserved up to 85% [53].

In similar studies, however, the opposite conclusions were drawn. For collagen electrospun from 40% acetic acid solution, for example, no characteristic 67 nm banding pattern was observed, evidence for β-sheet formations resulted from Raman spectroscopy and negligible increase in the folded ratio was observed with respect to fluorinated solvents [54]. Similarly, compared to native structure, retained fraction of triple helix was found to be 18% for diluted acidic solutions versus 16% for fluorinated solvents [55].

In terms of structure preservations, interesting results are reported in [56] where successful collagen spinning was obtained using a PBS and ethanol mixture as a solvent system, that is potentially less harmful to the protein than other more common options.

Irrespectively of the extent of structure preservation, the conclusive question to be answered remains if electrospun collagen retains some of its biological activity. The response should be searched among *in vivo* and *in vitro* experiments that compare collagen to other materials with known biological activity.

In vitro studies, in fact, generally show excellent cell viability, adhesion and proliferation, but frequently polystyrene multiwell plates are used as control, and this makes the comparison unfair (at the very least for the surface area available for cell adhesion, but also for topographical cues offered by sub-micron scaled fibers) and does not enable extracting the searched information. Similarly, studies indicating exciting results in term of tissue regeneration of electrospun collagen scaffolds for tissue regeneration can be found [57,58] but frequently controls are very different surgical strategies (e.g., no treatment, simple suture, etc.), and, again, it is difficult to isolate the effect of material itself.

Very few studies so far have investigated the behavior of electrospun collagen scaffolds in experiments specifically designed to actually contribution of collagen structure, for example by comparison with reconstituted collagen, electrospun gelatin or synthetic materials.

Interesting results in this direction were obtained by Jha and coworkers in a comprehensive research comparing the biocompatibility of electrospun collagen and electrospun gelatin [48]. Basing, among other, on the capacity to induce osteoblast differentiation and hydroxyapatite deposition, not observed for electrospun gelatin and reconstituted collagen films, they concluded that electrospun collagen still contains important instructive motifs. After specific investigations, they suggested this might be related to the presence of intact α-chains that preserve strong biological activity independently from their arrangement, as long as the degree of crosslinking is contained. Furthermore, improved healing was observed for electrospun collagen compare to electrospun gelatin for both *in vivo* dermal and muscle reconstruction, to further suggest that the exact collagen native structure might not be necessarily replicated to take advantage of its biological properties.

Another significant indication can be found in the work of Liu and colleagues [59]. In their researches, aiming at the development of conduits for spinal cord injuries treatment, extensive cell penetration into the electrospun collagen was observed in constructs used to repair acute spinal cord injury in a rat hemi-section model, where non similarly encouraging results were obtained on electrospun synthetic materials tested in previous experiments.

Table 1. Main characteristics of electrospun collagen fibers.

Polymer	Solvent	Polymer concentration	Voltage applied	Flow rate	Tip to collector distance	Fiber dimensions	Porosity	Reference
Collagen type I and III	HFIP	0.083 g/ml	25 kV	5 ml/h	12.5 cm	250 ± 150 nm 100–730 nm	-	[45]
Collagen type II	HFIP	0.1 g/ml 0.04 g/ml	22 kV	2 ml/h	10 cm	1.75 ± 0.9 μm 0.11 ± 0.09 μm	-	[46]
Collagen type II	HFIP	-	30 kV	-	-	70 nm–2.74 μm (496 nm)	Average pore area: 6.94 μm^2	[47]
Collagen type I	HFIP	1–7%	7–30 kV	1–5 ml/h	5–30 cm	0.2–2 μm	-	[60]
Collagen type I	HFIP	80 mg/ml	20 kV	-	12.5 cm	500–900 nm	2000–6000 μm^2	[61]
Collagen type I	HFIP	8.3%	10 kV	1–10 ml/h	15 cm	400–600 nm	-	[41]
Collagen type I	HFIP	8%	15–20 kV	0.02 ml/min	8 cm	100–1200 nm, average: 460 nm	-	[58]
Collagen type I	HFIP	0.083 g/ml	15 kV	1 ml/h	15 cm	250 nm	-	[62]
Collagen type I	Acetic acid/HFIP (1:1)	18%	14 kV	9 ml/h	22 cm	3–6 μm	-	[63]
Collagen type II	HFIP	60 mg/ml	22 kV	2 ml/h	12.7 cm	180 ± 69 nm	-	[64]
Collagen type I	Weak aqueous acetic acid solution (0.3%)	1%	18 kV	0.01 ml/min	18 cm	Hundreds nanometers	-	[65]
Collagen type I	PBS/EtOH (1:1)	16%	20 kV	1 ml/h	10 cm	0.54 ± 0.21 or 0.21 ± 0.06 depending on salts concentration	-	[66]
Collagen type I	HFIP–40% acetic acid	8% 25%	10–12 kV 15–16 kV	1 ml/h 0.3 ml/h	6–8 cm	569.1 ± 124.6 nm 149.1 ± 20.6 nm	-	[52]
Collagen	HFIP/acetic acid (1:1)	7%	15 kV	0.2 ml/h	20 cm	150–200 nm	-	[67]

Collagen type I	HFIP	8%	7 kV	0.5 ml/h	10 cm	785 ± 177 nm	84.63%	[68]
Collagen	HFIP	55 mg/ml	22 kV	3–7 ml/h	25 cm	500–1000 nm	-	[48]
Collagen type I	PBS/EtOH (3:2)	150 mg/ml	16 kV	0.3 ml/h	12 cm	200–400 nm	-	[56]
Collagen type I	EtOH/PBS (1:1) + EDC/NHS	16%	20 kV	0.5 ml/h	12 cm	0.42 ± 0.11 μm	-	[69]
Tropocollagen type I	HFIP–TFE	10%	20–22 kV	0.2–0.5 μl/min	15 cm	150–200 nm	-	[38]
Collagen type I	acetic acid	7.5%	6 kV	0.15 ml/h	4 cm	272 ± 183 nm	-	[57]
Tropocollagen type I	50% EtOH at pH = 2.3	15%	20 kV	1 ml/h	15 cm	600 nm	-	[53]
Collagen type I	HFIP–acetic acid	10% 40%	16 kV	0.5 μl/min	15 cm	150–200 nm	-	[54]
Collagen type I	TFE–dilute acetic acid	10% 30%	18 kV 24 kV	0.015 ml/min 0.001 ml/min	-	320 ± 80 nm 150 ± 30 nm	-	[55]

5. Gelatin

Gelatin is obtained by controlled hydrolysis of insoluble collagen and is derived from sources rich in Type I collagen [70], frequently bovine or porcine skin. The resulting product can have different molecular weights (MWs) and isoelectric points according to the source and method of preparation. In particular, from acid and alkaline processing of collagen, gelatin with different isoelectric points, type A and type B respectively, are derived [43].

Because of its origin, gelatin has similar composition and maintains some of the favorable properties of collagen, including biodegradability and adhesive sequences to promote cell adhesion and migration [19,71]. Moreover, by virtue of processing and purification, gelatin is a non-immunogenic product [70], has high commercial availability and low cost. However, as gelatin rapidly dissolves in water at temperatures above 37 °C, a crosslinking treatment is generally required for its use as scaffold. Both physical (e.g., dehydrothermal treatment, UV radiation or plasmo-chemical treatment) [72,73] and chemical (e.g., glutaraldehyde, genipin, EDC) [72,74,75] methods can be chosen among, where these latter generally appear to be more efficient in term of mechanical stability, but also have more potential drawbacks in term of biocompatibility [76].

For the abovementioned reasons, gelatin found a number of applications in the biomedical area, including carrier for drug delivery [19], sealants for vascular prostheses [77] and dressings for wound healing [29].

Unlike collagen, gelatin solubility does not represent a problem for its electrospinning, as it even dissolves in water at mild temperature (30–40 °C). However, ionizable side chains and strong hydrogen bonding can cause considerable aggregation and hinder fiber formation. For these reasons, polar organic solvents, such as HFIP and TFE, as well as formic and acetic acid are also frequently encountered (Figure 4B, Table 2) [71,78,79,80].

As gelatin is per se a denatured material, very few works are dedicated to the assessment of structural changes of gelatin molecules caused by electrospinning.

Among them, Ki and colleagues investigated the potential denaturing effect of electrospinning in formic acid solution [78] and found smaller intensities in CD spectra compared to native gelatin, indicating large contents of random coil structure in the electrospun samples. The loss of biopolymer crystallinity was also confirmed by XRD and DSC analyses as gelatin nanofibers were found to be mostly amorphous.

Similar results were obtained from Panzavolta and colleagues, that performed FTIR and XRD analyses to investigate structural modifications of gelatin imputable to electrospinning [75]. They established that both formic and acetic acid prevent the partial renaturation of gelatin that generally occurs in gelation from aqueous solution, and this accounts for the observed decrease in crystallinity.

As for collagen, although very exciting results can be found for both *in vitro* and *in vivo* applications of gelatin electrospun membranes, the extent of the effectiveness of the biological molecule in scaffold is less inspected.

The positive effect of using gelatin to improve cell compatibility of scaffolds can be presumed from the increased compatibility that blends with synthetic polymers generally show when compared to these latter alone, although a direct correlation among gelatin content and cell compatibility is not

always observed [81]. More rarely, direct comparison among electrospun gelatin and other polymers similarly processed can be found. In a comparative study on gelatin and PCL aligned fibers for peripheral nerve repair, for example, contrasting results were found on two different models, as superior differentiation of immortalized neuronal cell line was observed on gelatin but no difference was found when using primary cells in dorsal root ganglion model [82].

Although different solvents were used, a direct comparison between gelatin and electrospun poly-caprolactone behavior can be found in [83] where significantly faster regeneration was observed for gelatin mats in a *in vivo* wound healing model, where *in vitro* tests on cell proliferation were not conclusive.

Table 2. Main characteristics of electrospun gelatin fibers.

Polymer	Solvent	Polymer concentration	Voltage applied	Flow rate	Tip to collector distance	Fiber dimensions	Porosity	Reference
Gelatin type A	TFE	5–12.5%	10–16 kV	0.8 ml/h	12 cm	50–4800 nm	-	[84]
Gelatin type A	TFE	2.5–12.5%	0.5 kV/cm	-	-	-	-	[85]
Gelatin	Formic acid	7–12%	1 kV/cm	-	10 cm	from 74 ± 16 nm to 169 ± 33 nm	-	[78]
Gelatin	HFIP	100 mg/ml	20 kV	-	12.5 cm	100–700 nm	2000–6000 μm^2	[61]
Gelatin type B	HFIP	8.3%	10 kV	1–10 ml/h	15 cm	200–500 nm	-	[41]
Gelatin	HFIP	15%	15 kV	1 ml/min	22 cm	2–6 µm	-	[63]
Gelatin type B	TFE	10–16%	26–28 kV	8–12 ml/h	-	from 0.57 ± 0.01 µm to 3.01 ± 0.06 µm	from 93.5% ± 0.2% to 89.3% ± 0.4%	[79]
Gelatin type B	Acetic acid; AA/TFE; AA/DMSO; AA/ethylene glycol; AA/formamide	15–29% 19%	7.5 kV	-	7.5 cm	70–839 nm	-	[86]
Gelatin type B	Water/acetic acid/ethyl acetate	10%	12 kV	0.06 ml/h	8 cm	47–145 nm	-	[87]
Gelatin	water, 35–50 °C	30–40%	22 kV	-	12 cm	258–169 nm depending on the temperature	-	[88]
Gelatin type B	HFP	5–10%	25 kV	-	15 cm	0.59 ± 0.09 µm or 0.66 ± 0.25 µm	Pore size: 50.45 ± 10.34 or 35.01 ± 8.13 μm^2	[71]

Gelatin type B	20% EtOH/2% formic acid/water	20%	20 kV	1 ml/h	10 cm	from 85 ± 42 nm to 169 ± 125 nm	-	[89]
Gelatin	Acetic acid/ethyl acetate/water (50:30:20) (60:10:30)	10–25%	12 kV	0.5 ml/h	-	110 ± 40 nm or 600 ± 110 nm	1.00 ± 0.61 μm^2, 10.7 ± 5.7 μm^2	[90]
Gelatin	HFP	110 mg/ml	22 kV	3–7 ml/h	25 cm	250–3000 nm	-	[48]
Gelatin type A	60% acetic acid	30%	15 kV	0.005 ml/min	15 cm	440 ± 50 nm	-	[75]
Gelatin type B	20% acetic acid	20%	35 kV	0.1 ml/h	10 cm	45–88 nm	-	[80]

6. Elastin

Elastin is a highly insoluble protein that represents a major structural component of the ECM and is largely responsible for mechanical performance of tissues where elastic properties are essential (e.g., walls of arteries, or lungs).

Elastin fibers consist of covalently cross-linked molecules of its precursor, tropoelastin, a soluble, non-glycosylated and highly hydrophobic protein [42]. Tropoelastin, per se, is a highly bioactive molecule and several cell-interaction sites were identified on its sequence [91].

Elastin can be isolated by hydrolization of animal derived elastic fibers using different methods, that, in turn, result in different end-products [92]. Among them, solubilization with oxalic acid or potassium hydroxide produce, α-elastin or κ-elastin respectively. However, water soluble tropoelastin is generally used for electrospinning (Table 3) [19].

Alpha-elastin and tropoelastin were successfully electrospun using HFIP as solvent (Figure 4C and 4D). Fibers with diameters ranging from 170 nm to 7 μm were reported, and frequently flattened fibers with a ribbon-like shape were observed [93]. Similarly to collagen, electrospun elastin is water soluble [94] and possesses poor mechanical properties; therefore, crosslinking agents such as glutaraldehyde, EDC, HMDI or DSS, are used to stabilize electrospun membranes [42].

According to the results in [94], electrospinning does not appear to significantly affect tropoelastin structure. In fact, not only no change in protein molecular weight could be observed, but also CD spectroscopy confirmed the conservation of the secondary structure.

Electrospun elastin appears a very promising scaffold material as extremely encouraging results are generally reported from *in vitro* and *in vivo* studies.

Interestingly, in a comparative experiment evaluating cell compatibility of electrospun collagen, gelatin, alpha-elastin and human recombinant tropoelastin, both alpha-elastin and tropoelastin scaffolds supported cell attachment, migration and proliferation better than TCPS control, and cell proliferation of HEPM on electrospun elastin was found to be higher than all the other proteins [41].

Electrospun elastin has attracted interest for different applications, and in particular for skin regeneration and vascular tissue engineering. To support its relevance for skin regeneration applications, very fast adhesion and spreading of dermal fibroblasts on electrospun elastin membranes was reported by Rnjak-Kovacina *et al.* and, when processing parameters were adjusted to increase membrane porosity, colonization through membrane thickness and progressive increase in newly deposited ECM were also observed [95]. The ability of cells to remodel highly porous electrospun elastin scaffold was confirmed by *in vivo* tests, as moderate degradation and evidence of collagen deposition were observed 6 weeks after implantation in mice [95].

For vascular graft applications, collagen and elastin are often electrospun together as they represent the two main constituents of native blood vessels [96]. In particular, tropoelastin and its biocompatibility have been evaluated seeding endothelial vascular cells [97] and smooth muscle cells [40,98].

Table 3. Main characteristics of electrospun elastin fibers.

Polymer	Solvent	Polymer concentration	Voltage applied	Flow rate	Tip to collector distance	Fiber dimensions	Porosity	Reference
Tropoelastin	HFP	9%, 15%, 20%	18.5 kV	2 ml/h	12.5 cm	167 ± 32, 735 ± 270 nm	Pore sizes: 0.4–4.9 μm	[98]
Tropoelastin	HFP	9%, 15%, 20%	18.5 kV	2 ml/h	12.5 cm	580 ± 94 nm	-	[97]
Tropoelastin	HFP	20%	20 kV	1–3 ml/h	20 cm	1–4 μm	14.5 ± 0.8 %, 34.4 ± 1.3 %	[95]
Tropoelastin	HFP	20%	20 kV	1 ml/h	20 cm	1.8 ± 0.4 μm	-	[94]
Tropoelastin	HFP	20%	20 kV	1 ml/h	20 cm	1.76 ± 0.37 μm	-	[40]
Tropoelastin	HFP	20%	20 kV	1–5 ml/h	15 cm	2.3 ± 0.5 μm	-	[93]
Alpha-elastin and Tropoelastin	HFP	20%	10 kV	1–10 ml/h	15 cm	0.6–3,6 μm 1.4–7.4 μm	-	[41]

7. Hyaluronic Acid

Hyaluronic acid (HA) is a linear polysaccharide, and it is the structurally simplest member in the GAGs family (the only not found as proteoglycan, for not being covalently associated to a core protein). HA is a main component of the ECM of connective tissues and skin, and actively participates in regulating cell proliferation, differentiation, and tissue repair [29,43,99].

The extremely high molar mass of HA (up to millions Dalton) is associated to its unique viscoelastic and rheological properties that, in turn, play important physiological roles [100].

Commercially available HA can be extracted from different sources (e.g., umbilical cord, synovial fluid or vitreous humor) or more easily and controllably produced through microbial fermentation [43].

Its excellent biocompatibility and biodegradability have made HA gels an extensively used material in many biomedical applications including ophthalmology, dermatology, tissue engineering, wound dressings, and drug delivery [99].

Unfortunately, despite its solubility in water, HA is not well suited for electrospinning. This is mainly related to its polyanionic nature [101] together with the unusually high viscosity and surface tension of its aqueous solutions. Furthermore, the strong water retention capability of HA generally impairs adequate solvent evaporation and causes the deposition of fused wet electrospun fibers on the collector [99,100].

However, the fabrication of HA into nanofibrous membranes from aqueous solution was successfully achieved using air-flow-assisted systems with cold or hot airflows introduced in the electrospinning set up to facilitate solvent evaporation [102].

As alternative to aqueous solutions, solvent mixtures are used to enhance fiber formation (Table 4). Among them DMF/water mixture was widely used for the capability of DMF to significantly decrease the surface tension [103,104,105]. Solvent systems capable to break intra-molecular H-bonds and increase molecule flexibility and chain entanglements, such as NaOH/DMF or water/formic acid/DMF mixtures, also proved to be beneficial for fiber formation (Figure 4E) [104,105].

As most proteins from ECM, electrospun HA is generally cross-linked to increase its *in vivo* stability and proposed cross-linkers include EDC; in some cases, HA membranes can also be stabilized by means of aqueous acidic solutions or vapors [106,107,108].

As the relationship between structure and function for polysaccharides is not as important as for proteins, the only works investigating the effect of solvents on HA structure are mainly aimed to control solution properties and, therefore, their spinnability. Results of rheological measurements performed on HA solution in DMF/formic acid/water mixtures indicates, for example, that formic acid partially disrupts inter- and intra-molecular H-bonds providing better chain flexibility and entanglements formation, and therefore superior processability by ES [105]. However, since H-bonds stabilize α-helix structure of HA molecule, their disruption causes a transition of HA chain conformation to a coil structure.

Because of significant HA spinnability issues, biological properties of the electrospun matrices have not yet been extensively investigated. However, some work was done in the direction of demonstrating the important functional properties of the polysaccharide in wound healing

applications. Electrospun collagen/HA blends gave, for example, better results as wound dressing in terms of scarless skin regeneration than pure collagen matrix [109].

More recently, results of an *in vivo* characterization demonstrated that electrospun HA membranes were found to allow a faster healing of skin wounds in pig compared to solid HA dressing, to evidence once again the importance of the electrospun morphology [110].

8. **Fibrinogen**

Although it cannot be considered strictly an ECM component, fibrinogen is the precursor of fibrin that, is the major structural element of clot and constitutes a highly instructive provisional structural matrix for cell migration and organization in wound repair. For this reason, it appears an extremely interesting material for tissue engineering scaffolds and wound dressings, and was shown to promote cellular migration [111] and to be non-immunogenic [112].

Fibrinogen is a 340 kDa glycoprotein comprised of a pair of three polypeptide chains (2Aα, 2Bβ and 2γ) and it contains cell binding sites and also has the capacity to bind a wide array of molecules that play a role in tissue regeneration (e.g., growth factors and cytokines) [113].

To date, the only solvent reported for fibrinogen electrospinning is HFIP with 10× MEM in 9:1 ratio [114]. Using this system 50 to 1000 nm fibers were produced (Table 5 and Figure 4F). Likewise other electrospun polymers, as solution viscosity increases with polymer concentration, the fiber diameter increases linearly [115].

Despite HFIP effectiveness for electrospinning of fibrinogen, that appears mainly related to its capacity to break hydrophobic interactions [42], this solvent can induce changes in protein secondary structure and, in turn, affect fibrinogen bioactivity. In Carlisle *et al.*, CD spectroscopy results demonstrated an increase in α-helical content close to 70% for fibrinogen dissolved in HFIP (from about 34% in PBS to 57% in HFIP) [116]. In a different study, the electrophoretic profile of HFIP treated fibrinogen was acquired and no substantial variation compared to native material was observed [39]. However, clear evidence of protein denaturation can be found in the fact that electrospun fibrinogen becomes water insoluble, although this behavior can even be considered advantageous, as eliminates or at least reduces the need for crosslinking. In fact, not only the protein becomes insoluble, but fibrinogen-fibrinogen homotypic interactions also increases nanofibers resistance to enzymatic degradation [117]. Unfortunately, the formation of intermolecular non-covalent bonds can cause premature solution gelification, thus leading to discontinuities in the electrospinning process.

Electrospun fibrinogen, however, appeared an excellent substrate for cells. In *in vitro* studies a rapid attachment and migration together with progressive collagen deposition and matrix remodeling were observed for different cell types, including human bladder smooth muscle cells [117] and neonatal rat cardiac fibroblasts [118].

Interestingly, with these latter cells, seeded electrospun mats appeared comparable to fibrinogen hydrogel in term of cell migration and scaffold remodeling [118]. In addition, up to two times faster adhesion was observed on fibrinogen nanofibers compared to fibrinogen-coated flat surfaces, to further confirm the instructive role of nanometric geometries resembling the native ECM spatial organization, that can also activate specific adhesion mechanisms (fibrillar adhesion) [39].

It was suggested that the capacity of electrospun fibrinogen to support cellular interactions might be related to the exposure of a hidden molecule region (epitope β15-42) that is typical of thrombin cross-linked fibrin matrix and plays an important role in supporting cell spreading and proliferation [117,118,119].

9. Hybrid Strategies

To combine the favorable processing and tunability of synthetic polymers with the biological advantages of ECM macromolecules, different hybrid approaches were proposed as alternatives to the electrospinning of purified ECM molecules.

The most frequently adopted strategy is blending [120,121,122]. Synthetic polymers (PLA, PGA, etc.) are mixed in solution and spun together with ECM polymers (collagen, gelatin, fibronectin or laminin) to improve the interaction of resulting fibers with cells. Generally superior adhesion, migration and survival/proliferation are reported, although not always results are univocal [83,121]. As previously mentioned, from the perspective of this review, however, blending is also an interesting model to appraise the biological relevance of electrospun ECM polymers.

Coating is also a common strategy to improve material compatibility and support cell adhesion [123,124,125]. Fibronectin and laminin are frequently chosen to this end but also collagen, gelatin and hyaluronic acid are employed. Coating is obtained by physical adsorption or covalent coupling (e.g., by glutaraldehyde [124] or EDC [125]). In this latter case, higher amounts of biopolymers stably immobilized were reported and improved adhesion, spreading, proliferation and differentiation were observed for rat bone marrow-derived mesenchymal stem cells.

ECM polymers can also be blended with natural occurring polymers not found in ECM [126,127], but possessing a history of successful application in tissue engineering as silk fibroin [128,129] or chitosan [130]. These polymers do not generally share the processability or tunability of synthetic polymers, but possess other favorable properties (e.g., long term stability or mechanical properties) and excellent cell compatibility at the same time.

Another possible strategy involves the electrospinning of synthetic polymers with decellularized ECM fragments instead of purified ECM components. In [131], for example, cauda equina fragments were mixed to a PLGA solution in HFIP and spun. In *in vitro* test, axons from dorsal root ganglia were found to outspread to a greater extent when ECM fragments were included.

Table 4. Main characteristics of electrospun HA fibers.

Polymer	Solvent	Polymer concentration	Voltage applied	Flow rate	Tip to collector distance	Fiber dimensions	Porosity	Reference
HA	Acidic aqueous solution (pH = 1.5)	1.3–1.5%	40 kV	5–10 µl/min	9.5 cm	-	-	[102]
HA	Acidic aqueous solution (pH = 1.5)	2–3%	25–40 kV	40 µl/min	9.5 cm	40–100 nm	-	[108]
HA	DMF/water/EtOH	1.3–1.5%	22 kV	60 µl/min	15 cm	200–250 nm	-	[100]
HA	DMF/H_2O (1:1)	1.5%	22 kV	60 µl/min	15 cm	-	-	[106]
HA	H_2O with cocamidopropyl betaine as surfactant	1%	15 kV	0.5 ml/h	2.5 cm	from 58 ± 20 to 645 ± 269 nm	-	[110]
HA	H_2O/formic acid/DMF (25:50:25)	0.8–1.2 %	20 kV	0.3 ml/h	15 cm	20–90 nm	-	[105]
HA	NaOH/DMF (4:1)	3%	10 kV	15 µl/min	5 cm	224 ± 81 nm	-	[104]
	NH_4OH/DMF (2:1)	1.5%	20 kV	0.01 µl/min	6 cm	39 ± 12 nm		
HA	H_2O/DMF (1:1) with phosphate salts	1.5%	15 kV	0.01 µl/min 0.008 µl/min	6 cm	143 ± 34 nm or 88 ± 17 nm depending on the salt	-	[103]
HA	DMF/H_2O (0:1, 0.25:1, 0.5:1, 1:1)	0.75%	8–30 kV	1.2 ml/h	10 cm	from 33 ± 5 to 113 ± 19 nm	-	[107]

Table 5. Main characteristics of electrospun fibrinogen fibers.

Polymer	Solvent	Polymer concentration	Voltage applied	Flow rate	Tip to collector distance	Fiber dimensions	Porosity	Reference
Fibrinogen	HFIP/MEM (9:1)	167 mg/ml	22 kV	1.85 ml/h	12.5 cm	80–700 nm	-	[114]
Fibrinogen	HFIP/MEM (9:1)	80–140 mg/ml	22 kV	1.8 ml/h	10 cm	120–610 nm	-	[132]
Fibrinogen	HFIP/MEM (9:1)	100 mg/ml	22 kV	-	12.5–20 cm	208 ± 18 nm	1.3–13 μm^2	[116]
Fibrinogen	HFIP/MEM (9:1)	110 mg/ml	22 kV	1.8 ml/h	10 cm	320 ± 110 nm	-	[118]
Fibrinogen	HFIP/MEM (9:1)	110 mg/ml	22 kV	1.8 ml/h	12 cm	-	-	[117]
Fibrinogen	HFIP/MEM (9:1)	120 mg/ml	25 kV	3,5 ml/h	12 cm	710 ± 120 nm	-	[113]
Fibrinogen	HFIP/MEM (9:1)	100–150 mg/ml	25 kV	4 ml/h	12 cm	500–1040 nm	0.57–3.7 μm	[115]
Fibrinogen	HFIP/DMEM (9:1)	100 mg/ml	20–25 kV	0.3 ml/h	12.5 cm	192 ± 46 nm	-	[39]
Fibrinogen	HFIP/MEM (9:1)	100 mg/ml	22 kV	2 ml/h	16 cm	30–200 nm	-	[133]

10. Conclusions and Future Directions

The physiological scaffold for cells is a very complex environment that displays a finely tuned, highly specific and dynamic set of instructive cues. Years of research have provided a great insight in the mechanisms by which native ECM regulates tissue development, and many signals initiated by interactions between cell surface-receptors and ECM-ligands have been identified.

Due to the complexity of these interactions, using naturally occurring biomolecules for fabricating fibrous scaffolds appears a shortcut strategy to mimic the natural ECM in composition and structure.

Provided that *in vitro* and *in vivo* results are extremely promising, additional work appears to be required for understanding the extent to which the functions that ECM performs persist after reprocessing to achieve adequate morphological and structural properties.

Only a minority of the studies dealing with the electrospinning of ECM molecules actually focuses on the effect of processing on polymer structure. With few exceptions, however, the majority of works looking into this aspect found evidence of substantial loss of secondary and tertiary structures in scaffolds. Furthermore, due to the large number of scaffold parameters involved in regulating the cellular functions (including fiber diameter, mechanical properties, porosity, degradability, water absorption) extrapolating the sole information deriving from molecular structure is difficult.

However, although native conformation is likely to provide a more recognizable environment compared to a denatured molecule, using ECM molecule for scaffold fabrication can still be advantageous. After all, gelatin is considered to possess excellent bioactivity [134,135,136] and according to evidence, there is no need to have the exact copy of protein to have biological activity.

The presence of recognition sequences involved in integrin-mediated cell adhesion does not justify, per se, the complexity of extracting and processing ECM molecules, as small peptide sequences can be grafted on tunable and easy processable synthetic polymers [137,138]. However, there is evidence that short integrin-binding fragments by themselves can be less active than the native molecule or than the larger sequence they are included in [139]. Moreover, biopolymers might still be sensitive to matrix-degrading enzymes. Misfolded proteins, in particular, can be marked for proteolytic degradation [140]. This would allow a cell-mediated scaffold degradation that can follow more accurately new matrix deposition and tissue neo-formation rates, compared, for example, to hydrolytic route. Finally, denaturation in some cases might even be beneficial, as exposure of hidden sites can sometimes activate favorable signaling, as in the case of fibrinogen [117].

Future work on the biological side will have to fully clarify the effect of electrospinning on molecular conformation and, in cascade, on biological activity of ECM polymers. In the meantime, regenerative applications can surely benefit from additional investigations and further refinements of ECM processing. In particular, spinning methods that facilitate jet formation (e.g., bubble spinning or other needle-less spinning methods) could enable the use of less aggressive solvents. This not only would allow to better preserve the structure of processed ECM polymers, but also of smaller signaling molecules, as cytokines and growth factors, that can be blended in the solution. Electrospinning can, in fact, be detrimental to the activity of many bioactive compounds that can be

advantageously incorporated in fibers [141]. Similarly, further research on the electrospinning of more controlled and less batch-sensitive artificial analogs of ECM proteins synthetized recombinant DNA technology can enable to more precisely govern the process. Finally, investigation on new crosslinking methods has the potential to further reduce the contribution of this step in alteration of polymer native structure.

Research efforts in all these directions can result in the capacity to better preserve biochemical attributes of the reprocessed ECM polymers and fully exploit the beneficial effects of biological signaling by creating an instructive electrospun scaffold.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

References

1. Flaumenhaft R, Rifkin DB (1991) Extracellular matrix regulation of growth factor and protease activity. *Curr Opin Cell Biol* 3: 817–823.
2. Frantz C, Stewart KM, Weaver VM (2010) The extracellular matrix at a glance. *J Cell Sci* 123: 4195–4200.
3. Bonnans C, Chou J, Werb Z (2014) Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Bio* 15: 786–801.
4. Kim SH, Turnbull J, Guimond S (2011) Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. *J Endocrinol* 209: 139–151.
5. Schultz GS, Wysocki A (2009) Interactions between extracellular matrix and growth factors in wound healing. *Wound Repair Regen* 17: 153–162.
6. Roskelley CD, Srebrow A, Bissell MJ (1995) A Hierarchy of ECM-Mediated Signaling Regulates Tissue-Specific Gene-Expression. *Curr Opin Cell Bio* 7: 736–747.
7. Altınay S (2016) Is extracellular matrix a castle against to invasion of cancer cells? In: Xu K, *Tumor Metastasis*, InTech.
8. Laurencin CT, Nair LS (2014) *Nanotechnology and regenerative engineering: the scaffold*, CRC Press.
9. Smith LA, Ma PX (2004) Nano-fibrous scaffolds for tissue engineering. *Colloid Surface B* 39: 125–131.
10. Khajavi R, Abbasipour M (2012) Electrospinning as a versatile method for fabricating coreshell, hollow and porous nanofibers. *Sci Iran* 19: 2029–2034.
11. Fessler JH (1974) Self-assembly of collagen. *J Supramol Struct* 2: 99–102.
12. Bellingham CM, Keeley FW (2004) Self-ordered polymerization of elastin-based biomaterials. *Curr Opin Solid ST M* 8: 135–139.
13. Zhu X, Cui W, Li X, et al. (2008) Electrospun fibrous mats with high porosity as potential scaffolds for skin tissue engineering. *Biomacromolecules* 9: 1795–1801.

14. Lee SJ, Yoo JJ, Lim GJ, et al. (2007) In vitro evaluation of electrospun nanofiber scaffolds for vascular graft application. *J Biomed Mater Res A* 83: 999–1008.
15. Holzwarth JM, Ma PX (2011) Biomimetic nanofibrous scaffolds for bone tissue engineering. *Biomaterials* 32: 9622–9629.
16. Xie J, MacEwan MR, Schwartz AG, et al. (2010) Electrospun nanofibers for neural tissue engineering. *Nanoscale* 2: 35–44.
17. Li WJ, Tuli R, Okafor C, et al. (2005) A three-dimensional nanofibrous scaffold for cartilage tissue engineering using human mesenchymal stem cells. *Biomaterials* 26: 599–609.
18. Mogoşanu GD, Grumezescu AM (2014) Natural and synthetic polymers for wounds and burns dressing. *Int J Pharm* 463: 127–136.
19. Sell SA, Wolfe PS, Garg K, et al. (2010) The use of natural polymers in tissue engineering: a focus on electrospun extracellular matrix analogues. *Polymers* 2: 522–553.
20. Gomes SR, Rodrigues G, Martins GG, et al. (2015) In vitro and in vivo evaluation of electrospun nanofibers of PCL, chitosan and gelatin: A comparative study. *Mater Sci Eng C* 46: 348–358.
21. Herskovits TT, Gadegbeku B, Jaillet H (1970) On the structural stability and solvent denaturation of proteins I. Denaturation by the alcohols and glycols. *J Biol Chem* 245: 2588–2598.
22. Freedman KJ, Haq SR, Edel JB, et al. (2013) Single molecule unfolding and stretching of protein domains inside a solid-state nanopore by electric field. *Sci Rep-UK* 3: 1638.
23. Ingavle GC, Leach JK (2013) Advancements in electrospinning of polymeric nanofibrous scaffolds for tissue engineering. *Tissue Eng B* 20: 277–293.
24. Tan SH, Inai R, Kotaki M, et al. (2005) Systematic parameter study for ultra-fine fiber fabrication via electrospinning process. *Polymer* 46: 6128–6134.
25. Yarin A (2011) Coaxial electrospinning and emulsion electrospinning of core-shell fibers. *Polym Advan Technol* 22: 310–317.
26. Xu C, Inai R, Kotaki M, et al. (2004) Aligned biodegradable nanofibrous structure: a potential scaffold for blood vessel engineering. *Biomaterials* 25: 877–886.
27. Ding B, Kimura E, Sato T, et al. (2004) Fabrication of blend biodegradable nanofibrous nonwoven mats via multi-jet electrospinning. *Polymer* 45: 1895–1902.
28. Del Gaudio C, Bianco A, Grigioni M (2007) Electrospun bioresorbable trileaflet heart valve prosthesis for tissue engineering: in vitro functional assessment of a pulmonary cardiac valve design. *Annali dell'Istituto superiore di sanita* 44: 178–186.
29. Bhardwaj N, Kundu SC (2010) Electrospinning: a fascinating fiber fabrication technique. *Biotechnol Adv* 28: 325–347.
30. Pham QP, Sharma U, Mikos AG (2006) Electrospinning of polymeric nanofibers for tissue engineering applications: a review. *Tissue Eng* 12: 1197–1211.
31. Ferreira JL, Gomes S, Henriques C, et al. (2014) Electrospinning polycaprolactone dissolved in glacial acetic acid: Fiber production, nonwoven characterization, and in vitro evaluation. *J Appl Polym Sci* 131.
32. Gupta RK, Kennel E, Kim KJ (2009) *Polymer nanocomposites handbook*, CRC press.

33. Patel H, Bonde M, Srinivasan G (2011) Biodegradable polymer scaffold for tissue engineering. *Trends Biomater Artif Organs* 25: 20–29.
34. Powell S (2010) Langevin Dynamics Study of Water Diffusion in Model Articular Cartilage [Master's Thesis], Queensland University of Technology Brisbane, Brisbane, Australia.
35. Chew SY, Mi R, Hoke A, et al. (2008) The effect of the alignment of electrospun fibrous scaffolds on Schwann cell maturation. *Biomaterials* 29: 653–661.
36. Gunatillake PA, Adhikari R (2003) Biodegradable synthetic polymers for tissue engineering. *Eur Cells Mater* 5: 1–16.
37. Hu X, Liu S, Zhou G, et al. (2014) Electrospinning of polymeric nanofibers for drug delivery applications. *J Control Release* 185: 12–21.
38. Bürck J, Heissler S, Geckle U, et al. (2013) Resemblance of electrospun collagen nanofibers to their native structure. *Langmuir* 29: 1562–1572.
39. Gugutkov D, Gustavsson J, Ginebra MP, et al. (2013) Fibrinogen nanofibers for guiding endothelial cell behavior. *Biomater Sci-UK* 1: 1065–1073.
40. Rnjak J, Li Z, Maitz PK, et al. (2009) Primary human dermal fibroblast interactions with open weave three-dimensional scaffolds prepared from synthetic human elastin. *Biomaterials* 30: 6469–6477.
41. Li M, Mondrinos MJ, Gandhi MR, et al. (2005) Electrospun protein fibers as matrices for tissue engineering. *Biomaterials* 26: 5999–6008.
42. Khadka DB, Haynie DT (2012) Protein-and peptide-based electrospun nanofibers in medical biomaterials. *Nanomed-Nanotechnol* 8: 1242–1262.
43. Malafaya PB, Silva GA, Reis RL (2007) Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Adv Drug Deliver Rev* 59: 207–233.
44. Badylak SF (2005) Regenerative medicine and developmental biology: the role of the extracellular matrix. *Anat Rec Part B* 287: 36–41.
45. Matthews JA, Wnek GE, Simpson DG, et al. (2002) Electrospinning of collagen nanofibers. *Biomacromolecules* 3: 232–238.
46. Matthews JA, Boland ED, Wnek GE, et al. (2003) Electrospinning of collagen type II: a feasibility study. *J Bioact Compat Pol* 18: 125–134.
47. Shields KJ, Beckman MJ, Bowlin GL, et al. (2004) Mechanical properties and cellular proliferation of electrospun collagen type II. *Tissue Eng* 10: 1510–1517.
48. Jha BS, Ayres CE, Bowman JR, et al. (2011) Electrospun collagen: a tissue engineering scaffold with unique functional properties in a wide variety of applications. *J Nanomater* 2011: 7.
49. Zeugolis DI, Khew ST, Yew ES, et al. (2008) Electro-spinning of pure collagen nano-fibres—just an expensive way to make gelatin? *Biomaterials* 29: 2293–2305.
50. Yang L, Fitie CF, van der Werf KO, et al. (2008) Mechanical properties of single electrospun collagen type I fibers. *Biomaterials* 29: 955–962.
51. Lannutti J, Reneker D, Ma T, et al. (2007) Electrospinning for tissue engineering scaffolds. *Mater Sci Eng C* 27: 504–509.

52. Liu T, Teng WK, Chan BP, et al. (2010) Photochemical crosslinked electrospun collagen nanofibers: synthesis, characterization and neural stem cell interactions. *J Biomed Mater Res A* 95: 276–282.
53. Jiang Q, Reddy N, Zhang S, et al. (2013) Water-stable electrospun collagen fibers from a non-toxic solvent and crosslinking system. *J Biomed Mater Res A* 101: 1237–1247.
54. Kazanci M (2014) Solvent and temperature effects on folding of electrospun collagen nanofibers. *Mater Lett* 130: 223–226.
55. Fiorani A, Gualandi C, Panseri S, et al. (2014) Comparative performance of collagen nanofibers electrospun from different solvents and stabilized by different crosslinkers. *J Mater Sci-Mater M* 25: 2313–2321.
56. Dong Z, Wu Y, Clark RL (2011) Thermodynamic modeling and investigation of the formation of electrospun collagen fibers. *Langmuir* 27: 12417–12422.
57. Meimandi-Parizi A, Oryan A, Moshiri A (2013) Role of tissue engineered collagen based tridimensional implant on the healing response of the experimentally induced large Achilles tendon defect model in rabbits: a long term study with high clinical relevance. *J Biomed Sci* 20: 28.
58. Rho KS, Jeong L, Lee G, et al. (2006) Electrospinning of collagen nanofibers: effects on the behavior of normal human keratinocytes and early-stage wound healing. *Biomaterials* 27: 1452–1461.
59. Liu T, Houle JD, Xu J, et al. (2012) Nanofibrous collagen nerve conduits for spinal cord repair. *Tissue Eng A* 18: 1057–1066.
60. Kidoaki S, Kwon IK, Matsuda T (2005) Mesoscopic spatial designs of nano-and microfiber meshes for tissue-engineering matrix and scaffold based on newly devised multilayering and mixing electrospinning techniques. *Biomaterials* 26: 37–46.
61. Telemeco T, Ayres C, Bowlin G, et al. (2005) Regulation of cellular infiltration into tissue engineering scaffolds composed of submicron diameter fibrils produced by electrospinning. *Acta Biomater* 1: 377–385.
62. Zhong SP, Teo WE, Zhu X, et al. (2007) Development of a novel collagen–GAG nanofibrous scaffold via electrospinning. *Mater Sci Eng C* 27: 262–266.
63. Casper CL, Yang W, Farach-Carson MC, et al. (2007) Coating electrospun collagen and gelatin fibers with perlecan domain I for increased growth factor binding. *Biomacromolecules* 8: 1116–1123.
64. Barnes CP, Pemble IV CW, Brand DD, et al. (2007) Cross-linking electrospun type II collagen tissue engineering scaffolds with carbodiimide in ethanol. *Tissue Eng* 13: 1593–1605.
65. Foltran I, Foresti E, Parma B, et al. (2008) Novel biologically inspired collagen nanofibers reconstituted by electrospinning method. *Macromol Symp* 269: 111–118.
66. Dong B, Arnoult O, Smith ME, et al. (2009) Electrospinning of collagen nanofiber scaffolds from benign solvents. *Macromol Rapid Comm* 30: 539–542.
67. Timnak A, Gharebaghi FY, Shariati RP, et al. (2011) Fabrication of nano-structured electrospun collagen scaffold intended for nerve tissue engineering. *J Mater Sci-Mater M* 22: 1555–1567.

68. Wang Y, Yao M, Zhou J, et al. (2011) The promotion of neural progenitor cells proliferation by aligned and randomly oriented collagen nanofibers through β1 integrin/MAPK signaling pathway. *Biomaterials* 32: 6737–6744.
69. Meng L, Arnoult O, Smith M, et al. (2012) Electrospinning of in situ crosslinked collagen nanofibers. *J Mater Chem* 22: 19412–19417.
70. Gorgieva S, Kokol V (2011) *Collagen-vs. gelatine-based biomaterials and their biocompatibility: review and perspectives*, INTECH open access publisher Croatia.
71. Heydarkhan-Hagvall S, Schenke-Layland K, Dhanasopon AP, et al. (2008) Three-dimensional electrospun ECM-based hybrid scaffolds for cardiovascular tissue engineering. *Biomaterials* 29: 2907–2914.
72. Ratanavaraporn J, Rangkupan R, Jeeratawatchai H, et al. (2010) Influences of physical and chemical crosslinking techniques on electrospun type A and B gelatin fiber mats. *Int J Biol Macromol* 47: 431–438.
73. Masutani EM, Kinoshita CK, Tanaka TT, et al. (2014) Increasing thermal stability of gelatin by UV-induced cross-linking with glucose. *Int J Biomater* 2014.
74. Zhang Y, Venugopal J, Huang ZM, et al. (2006) Crosslinking of the electrospun gelatin nanofibers. *Polymer* 47: 2911–2917.
75. Panzavolta S, Gioffrè M, Focarete ML, et al. (2011) Electrospun gelatin nanofibers: optimization of genipin cross-linking to preserve fiber morphology after exposure to water. *Acta Biomater* 7: 1702–1709.
76. Sisson K, Zhang C, Farach-Carson MC, et al. (2009) Evaluation of cross-linking methods for electrospun gelatin on cell growth and viability. *Biomacromolecules* 10: 1675–1680.
77. Madaghiele M, Piccinno A, Saponaro M, et al. (2009) Collagen-and gelatine-based films sealing vascular prostheses: evaluation of the degree of crosslinking for optimal blood impermeability. *J Mater Sci-Mater M* 20: 1979–1989.
78. Ki CS, Baek DH, Gang KD, et al. (2005) Characterization of gelatin nanofiber prepared from gelatin–formic acid solution. *Polymer* 46: 5094–5102.
79. Powell H, Boyce S (2008) Fiber density of electrospun gelatin scaffolds regulates morphogenesis of dermal–epidermal skin substitutes. *J Biomed Mater Res A* 84: 1078–1086.
80. Okutan N, Terzi P, Altay F (2014) Affecting parameters on electrospinning process and characterization of electrospun gelatin nanofibers. *Food Hydrocolloid* 39: 19–26.
81. Yao R, He J, Meng G, et al. (2016) Electrospun PCL/Gelatin composite fibrous scaffolds: mechanical properties and cellular responses. *J Biomat Sci-Polym E* 27: 824–838.
82. Ghasemi-Mobarakeh L, Prabhakaran MP, Morshed M, et al. (2008) Electrospun poly (ε-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering. *Biomaterials* 29: 4532–4539.
83. Dubský M, Kubinová Š, Širc J, et al. (2012) Nanofibers prepared by needleless electrospinning technology as scaffolds for wound healing. *J Mater Sci-Mater M* 23: 931–941.
84. Huang ZM, Zhang Y, Ramakrishna S, et al. (2004) Electrospinning and mechanical characterization of gelatin nanofibers. *Polymer* 45: 5361–5368.
85. Zhang Y, Ouyang H, Lim CT, et al. (2005) Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds. *J Biomed Mater Res B* 72: 156–165.

86. Choktaweesap N, Arayanarakul K, Aht-Ong D, et al. (2007) Electrospun gelatin fibers: effect of solvent system on morphology and fiber diameters. *Polym J* 39: 622.
87. Song JH, Kim HE, Kim HW (2008) Production of electrospun gelatin nanofiber by water-based co-solvent approach. *J Mater Sci-Mater M* 19: 95–102.
88. Zhang S, Huang Y, Yang X, et al. (2009) Gelatin nanofibrous membrane fabricated by electrospinning of aqueous gelatin solution for guided tissue regeneration. *J Biomed Mater Res A* 90: 671–679.
89. Chen HC, Jao WC, Yang MC (2009) Characterization of gelatin nanofibers electrospun using ethanol/formic acid/water as a solvent. *Polym Advan Technol* 20: 98–103.
90. Sisson K, Zhang C, Farach-Carson MC, et al. (2010) Fiber diameters control osteoblastic cell migration and differentiation in electrospun gelatin. *J Biomed Mater Res A* 94: 1312–1320.
91. Martin SL, Vrhovski B, Weiss AS (1995) Total synthesis and expression in Escherichia coli of a gene encoding human tropoelastin. *Gene* 154: 159–166.
92. Yeo GC, Aghaei-Ghareh-Bolagh B, Brackenreg EP, et al. (2015) Fabricated Elastin. *Adv Healthc Mater* 4: 2530–2556.
93. Nivison-Smith L, Weiss AS (2012) Alignment of human vascular smooth muscle cells on parallel electrospun synthetic elastin fibers. *J Biomed Mater Res A* 100: 155–161.
94. Nivison-Smith L, Rnjak J, Weiss AS (2010) Synthetic human elastin microfibers: stable cross-linked tropoelastin and cell interactive constructs for tissue engineering applications. *Acta Biomater* 6: 354–359.
95. Rnjak-Kovacina J, Wise SG, Li Z, et al. (2011) Tailoring the porosity and pore size of electrospun synthetic human elastin scaffolds for dermal tissue engineering. *Biomaterials* 32: 6729–6736.
96. Boland ED, Matthews JA, Pawlowski KJ, et al. (2004) Electrospinning collagen and elastin: preliminary vascular tissue engineering. *Front Biosci* 9: e32.
97. McKenna KA, Hinds MT, Sarao RC, et al. (2012) Mechanical property characterization of electrospun recombinant human tropoelastin for vascular graft biomaterials. *Acta Biomater* 8: 225–233.
98. McKenna KA, Gregory KW, Sarao RC, et al. (2012) Structural and cellular characterization of electrospun recombinant human tropoelastin biomaterials. *J Biomater Appl* 27: 219–230.
99. Lee KY, Jeong L, Kang YO, et al. (2009) Electrospinning of polysaccharides for regenerative medicine. *Adv Drug Deliver Rev* 61: 1020–1032.
100. Li J, He A, Han CC, et al. (2006) Electrospinning of hyaluronic acid (HA) and HA/gelatin blends. *Macromol Rapid Comm* 27: 114–120.
101. Schiffman JD, Schauer CL (2008) A review: electrospinning of biopolymer nanofibers and their applications. *Polym Rev* 48: 317–352.
102. Um IC, Fang D, Hsiao BS, et al. (2004) Electro-spinning and electro-blowing of hyaluronic acid. *Biomacromolecules* 5: 1428–1436.
103. Brenner EK, Schiffman JD, Toth LJ, et al. (2013) Phosphate salts facilitate the electrospinning of hyaluronic acid fiber mats. *J Mater Sci* 48: 7805–7811.
104. Brenner EK, Schiffman JD, Thompson EA, et al. (2012) Electrospinning of hyaluronic acid nanofibers from aqueous ammonium solutions. *Carbohyd Polym* 87: 926–929.

105. Liu Y, Ma G, Fang D, et al. (2011) Effects of solution properties and electric field on the electrospinning of hyaluronic acid. *Carbohyd Polym* 83: 1011–1015.
106. Xu S, Li J, He A, et al. (2009) Chemical crosslinking and biophysical properties of electrospun hyaluronic acid based ultra-thin fibrous membranes. *Polymer* 50: 3762–3769.
107. Yao S, Wang X, Liu X, et al. (2013) Effects of ambient relative humidity and solvent properties on the electrospinning of pure hyaluronic acid nanofibers. *J Nanosci Nanotechno* 13: 4752–4758.
108. Wang X, Um IC, Fang D, et al. (2005) Formation of water-resistant hyaluronic acid nanofibers by blowing-assisted electro-spinning and non-toxic post treatments. *Polymer* 46: 4853–4867.
109. Hsu FY, Hung YS, Liou HM, et al. (2010) Electrospun hyaluronate–collagen nanofibrous matrix and the effects of varying the concentration of hyaluronate on the characteristics of foreskin fibroblast cells. *Acta Biomater* 6: 2140–2147.
110. Uppal R, Ramaswamy GN, Arnold C, et al. (2011) Hyaluronic acid nanofiber wound dressing—production, characterization, and in vivo behavior. *J Biomed Mater Res B* 97: 20–29.
111. Ahmed Z, Underwood S, Brown R (2000) Low concentrations of fibrinogen increase cell migration speed on fibronectin/fibrinogen composite cables. *Cytoskeleton* 46: 6–16.
112. Ye Q, Zünd G, Benedikt P, et al. (2000) Fibrin gel as a three dimensional matrix in cardiovascular tissue engineering. *Eur J Cardio-Thorac* 17: 587–591.
113. Sell SA, Francis MP, Garg K, et al. (2008) Cross-linking methods of electrospun fibrinogen scaffolds for tissue engineering applications. *Biomed Mater* 3: 045001.
114. Wnek GE, Carr ME, Simpson DG, et al. (2003) Electrospinning of nanofiber fibrinogen structures. *Nano Lett* 3: 213–216.
115. Sell S, Barnes C, Simpson D, et al. (2008) Scaffold permeability as a means to determine fiber diameter and pore size of electrospun fibrinogen. *J Biomed Mater Res A* 85: 115–126.
116. Carlisle CR, Coulais C, Namboothiry M, et al. (2009) The mechanical properties of individual, electrospun fibrinogen fibers. *Biomaterials* 30: 1205–1213.
117. McManus M, Boland E, Sell S, et al. (2007) Electrospun nanofibre fibrinogen for urinary tract tissue reconstruction. *Biomed Mater* 2: 257.
118. McManus MC, Boland ED, Simpson DG, et al. (2007) Electrospun fibrinogen: feasibility as a tissue engineering scaffold in a rat cell culture model. *J Biomed Mater Res A* 81: 299–309.
119. Guadiz G, Sporn LA, Simpson-Haidaris PJ (1997) Thrombin cleavage-independent deposition of fibrinogen in extracellular matrices. *Blood* 90: 2644–2653.
120. Stitzel J, Liu J, Lee SJ, et al. (2006) Controlled fabrication of a biological vascular substitute. *Biomaterials* 27: 1088–1094.
121. Schnell E, Klinkhammer K, Balzer S, et al. (2007) Guidance of glial cell migration and axonal growth on electrospun nanofibers of poly-ε-caprolactone and a collagen/poly-ε-caprolactone blend. *Biomaterials* 28: 3012–3025.
122. López-Calzada G, Hernandez-Martínez AR, Cruz-Soto M, et al. (2016) Development of meniscus substitutes using a mixture of biocompatible polymers and extra cellular matrix components by electrospinning. *Mater Sci Eng C* 61: 893–905.
123. Koh HS, Yong T, Chan CK, et al. (2008) Enhancement of neurite outgrowth using nano-structured scaffolds coupled with laminin. *Biomaterials* 29: 3574–3582.

124. Zhu Y, Leong MF, Ong WF, et al. (2007) Esophageal epithelium regeneration on fibronectin grafted poly(L-lactide-co-caprolactone) (PLLC) nanofiber scaffold. *Biomaterials* 28: 861–868.
125. Cheng Y, Ramos D, Lee P, et al. (2014) Collagen functionalized bioactive nanofiber matrices for osteogenic differentiation of mesenchymal stem cells: bone tissue engineering. *J Biomed Nanotechnol* 10: 287–298.
126. Yeo IS, Oh JE, Jeong L, et al. (2008) Collagen-based biomimetic nanofibrous scaffolds: preparation and characterization of collagen/silk fibroin bicomponent nanofibrous structures. *Biomacromolecules* 9: 1106–1116.
127. Dhandayuthapani B, Krishnan UM, Sethuraman S (2010) Fabrication and characterization of chitosan–gelatin blend nanofibers for skin tissue engineering. *J Biomed Mater Res B* 94: 264–272.
128. Altman GH, Diaz F, Jakuba C, et al. (2003) Silk-based biomaterials. *Biomaterials* 24: 401–416.
129. Plowman JE, Deb-Choudhury S, Dyer JM (2013) Fibrous protein nanofibers, In: Gerrard JA, *Protein Nanotechnology: Protocols, Instrumentation, and Applications*, 2nd Eds, Humana Press, 61–76.
130. Kim IY, Seo SJ, Moon HS, et al. (2008) Chitosan and its derivatives for tissue engineering applications. *Biotechnol Adv* 26: 1–21.
131. Wen X, Wang Y, Guo Z, et al. (2014) Cauda Equina-Derived Extracellular Matrix for Fabrication of Nanostructured Hybrid Scaffolds Applied to Neural Tissue Engineering. *Tissue Eng A* 21: 1095–1105.
132. McManus MC, Boland ED, Koo HP, et al. (2006) Mechanical properties of electrospun fibrinogen structures. *Acta Biomater* 2: 19–28.
133. Baker S, Sigley J, Helms CC, et al. (2012) The mechanical properties of dry, electrospun fibrinogen fibers. *Mater Sci Eng C* 32: 215–221.
134. Wan C, Frydrych M, Chen B (2011) Strong and bioactive gelatin–graphene oxide nanocomposites. *Soft Matter* 7: 6159–6166.
135. Nadeem D, Kiamehr M, Yang X, et al. (2013) Fabrication and in vitro evaluation of a sponge-like bioactive-glass/gelatin composite scaffold for bone tissue engineering. *Mater Sci Eng C* 33: 2669–2678.
136. Merkle VM, Zeng L, Slepian MJ, et al. (2014) Core-shell nanofibers: Integrating the bioactivity of gelatin and the mechanical property of polyvinyl alcohol. *Biopolymers* 101: 336–346.
137. Hersel U, Dahmen C, Kessler H (2003) RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. *Biomaterials* 24: 4385–4415.
138. Paletta JRJ, Bockelmann S, Walz A, et al. (2010) RGD-functionalisation of PLLA nanofibers by surface coupling using plasma treatment: influence on stem cell differentiation. *J Mater Sci-Mater M* 21: 1363–1369.
139. Nomizu M, Utani A, Shiraishi N, et al. (1992) The all-D-configuration segment containing the IKVAV sequence of laminin A chain has similar activities to the all-L-peptide in vitro and in vivo. *J Biol Chem* 267: 14118–14121.
140. Lodish H, Zipursky SL (2001) Molecular cell biology. *Biochem Mol Biol Edu* 29: 126–133.

141. Peh P, Lim NSJ, Blocki A, et al. (2015) Simultaneous delivery of highly diverse bioactive compounds from blend electrospun fibers for skin wound healing. *Bioconjugate Chem* 26: 1348–1358.

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