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# Electrospinning in Tissue Engineering

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## Abstract

Electrospinning employs a strong electric field to draw charged polymer fluids or melts into fibers with diameter in the range from tens of nanometers to microns. The relatively simple experimental setup, a wide range of suitable materials, and the possibility of incorporating bioactive molecules into the fibers make electrospinning a versatile process in creating scaffolds for tissue engineering applications. This chapter reviews the electrospinning process and discusses how solution and processing parameters affect the electrospun fiber structure and function. A brief overview of various surface modification methods used for enhancing the cell adhesion, proliferation, and differentiation on the fibrous scaffolds is provided. Commonly used methods include physical entrapment, chemical treatment, and coelectrospinning. The application of electrospun fibrous scaffolds in tissue engineering is reviewed, focusing on recent progress in the regeneration of skin, vasculature, bone, ligaments, and tendons.

**Keywords:** electrospinning process, electrospun fibers, scaffolds, surface modification, tissue engineering

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## 1. Introduction

Electrospinning is closely related to the more established technology of electrospraying, a process in which electrostatic forces are used to control the formation of fluid droplets. The earliest description of electrospraying can be traced back to the early seventeenth century, when William Gilbert observed that a drop of water deformed in a cone in the presence of

charged amber [1]. John Zeleny's work on the effect of an electric field on a liquid meniscus in the early twentieth century was considered as the beginning to the development of electro-spraying and electrospinning technologies [2, 3]. In the mid-1960s, Sir Geoffrey Ingram Taylor published a series of articles and established the theoretical framework to understand the behavior of electrified fluids [4, 5]. The theory has been further developed and refined by more recent literature [6–9] that helps to better understand and guide the electro-spraying/electrospinning process.

Electro-spraying has been widely applied to develop commercial technologies such as ionization source for mass spectrometry, liquid metal ion source for ion implantation, focused ion beam instruments, and electrostatic precipitation of nanoparticles. Interest in electrospinning grew more slowly until mid-1990s, when work led by Reneker and coworker demonstrated the production of continuous nanofibers using electrospinning and predicted their potential applications in filtration, biology, energy conversion, and agriculture [10]. The past decade has seen exponential growth in electrospinning-related literature, totaling over 3000 articles and 1000 issued patents from 2001 to 2015 [11].

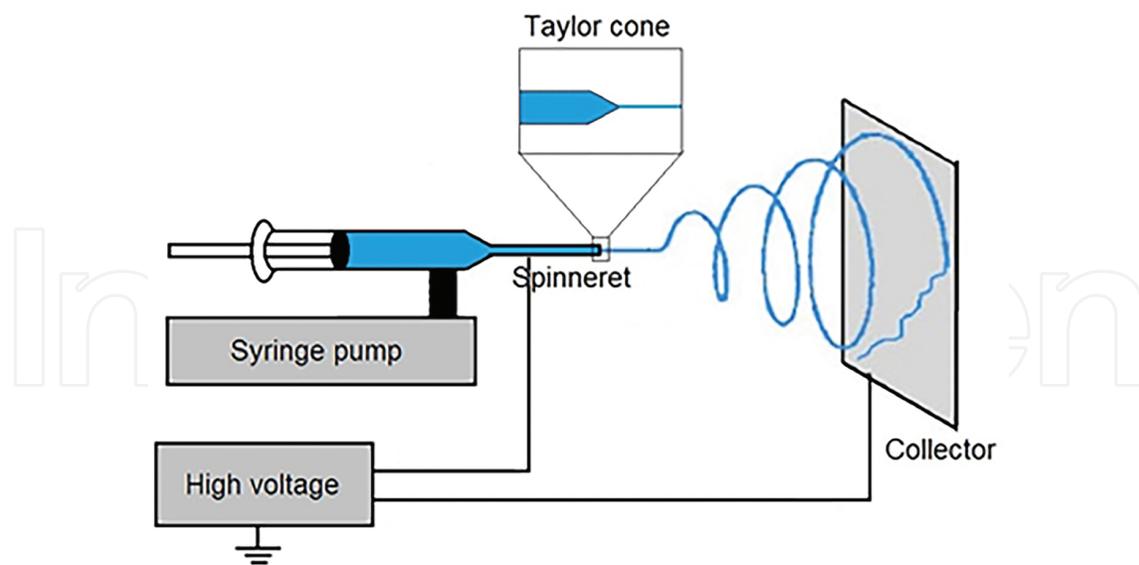
Tissue engineering is an emerging multidisciplinary field that integrates engineering principles with biology and medicine with the goal of restoring or enhancing tissue or organ functions [12]. One key component of a tissue-engineered construct is a porous biodegradable scaffold to provide structural support for the cells. The relatively simple and inexpensive setup makes electrospinning a versatile process with high production capability to form submicron sized nonwoven fibrous scaffolds. The possibility of incorporating one or multiple bioactive factors is another advantage of the process.

This chapter will review the electrospinning process, and discuss how solution and process parameters affect the morphology of different types of polymer fibers. The inherent hydrophobicity of many synthetic polymers leads to suboptimal cell adhesion and functions on electrospun fibrous scaffolds. We will discuss different surface modification methods to enhance cell adhesion, proliferation, and differentiation. Finally, we will provide some case studies to illustrate the application of electrospun fibers as scaffolds to regenerate a variety of tissues.

## 2. Electrospinning process control

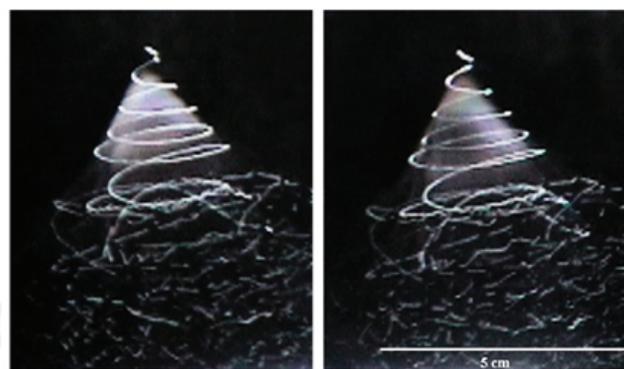
### 2.1. Process overview

Electrospinning uses electrostatic forces to produce fibers from polymer solutions. One attractive feature of this process is the relatively simple and inexpensive experimental setup. A typical electrospinning apparatus (as shown in **Figure 1**) consists of three components: a spinneret (usually a metal hollow needle), a high voltage source, and a collector (grounded or negatively biased). A syringe pump is commonly used to drive a polymer solution or polymer melt out of the spinneret.



**Figure 1.** Schematic of a typical electrospinning system. A high voltage applied between the spinneret tip and the collector creates an electrified fluid jet, which is accelerated toward the grounded collector, forming continuous fibers.

In electrospinning, polymer fibers are formed by the generation and elongation of an electrified fluid jet. Under the application of a high voltage, when the electrostatic force from the repulsion of like charges in the fluid overcomes the surface tension, the fluid droplet coming out of the spinneret deforms to a conical shape called a Taylor cone, named after Sir Geoffrey Ingram Taylor with his pioneering work on electrified fluids [5].



**Figure 2.** A pair of stereographic stroboscopic picture of a jet of polyethylene oxide, molecular weight 400,000, dissolved at a concentration of 6% in a mixture of 75% water and 25% ethanol, recorded during electrospinning. Reprinted with permission from Reneker and Yarin [13].

Taylor also proposed a “leaky dielectric” model for the moving electrified fluid that behaves like neither a perfect dielectric nor a perfect conductor [4]. The flow of charge through the fluid leads to elongation and thinning of the jet. Repulsive interactions between like charges in the fluid cause bending instability [9]. Theoretical modeling and video observation using high speed CCD cameras both showed that the fluid jet bent and stretched in a conical envelop, creating a highly spiral jet path, as shown in **Figure 2**. When the jet reaches the collector,

continuous fibers are produced. The diameter of electrospun fibers usually falls in the range between tens of nm and 1 mm.

Despite the simple experimental setup, the electrospinning process is affected by many variables such as the polymer type and molecular weight, solution concentration, viscosity, surface tension and conductivity, solvent type, voltage, flow rate, distance between needle tip to collector, and ambient parameters (temperature, humidity). Several theoretical models have been developed with limited success in predicting the electrospun fiber properties [14, 15]. Obtaining desirable fiber size and distribution remains largely dependent on empirical observations. On the other hand, while a universal model is not available to predict the electrospun fiber morphology for different polymer/solvent systems under different processing conditions, some general relationships can be drawn to guide the electrospinning process, as shown in **Table 1**. The sections below discuss some of these key parameters that affect the electrospinning process.

Parameters	Effect on fiber morphology
<b>Solution parameters</b>	
Solvent vapor pressure	Higher porosity with higher volatility
Polymer concentration	Increasing fiber diameter with higher concentration (within optimal range)
Solution viscosity	Increasing fiber diameter with higher viscosity (within optimal range)
Solution surface tension	No conclusive link
Solution conductivity	Decreasing diameter with higher conductivity
<b>Processing parameters</b>	
Voltage	No conclusive link between fiber diameter and voltage; higher probability of bead formation with higher voltage
Flow rate	Increasing fiber diameter and bead formation with higher rate (above minimum rate)
Needle-collector distance	Decreasing fiber diameter with larger distance (within optimal range)
<b>Ambient parameters</b>	
Temperature	Decreasing fiber diameter with higher temperature
Humidity	Higher humidity induces circular pores

**Table 1.** General relationships between electrospinning parameters on fiber morphology, adapted with permission from Bhardwaj, Sill and Pham [16–18].

## 2.2. Solution parameters

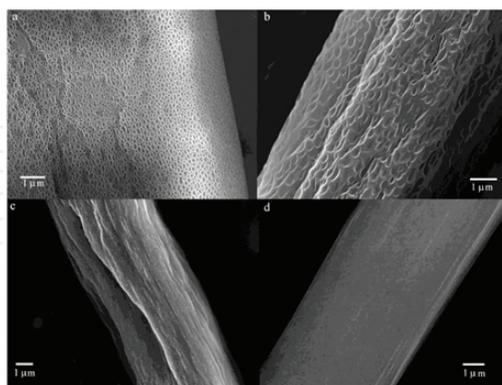
A number of solution parameters (such as solvent type, polymer concentration, solution viscosity, surface tension, and conductivity) play an important role in the electrospun fiber formation and morphology. These parameters are usually correlated to each other. For example, a higher polymer concentration corresponds to a higher solution viscosity. The surface tension and conductivity are both directly related to the type of solvent used.

### 2.2.1. Solvent properties

The first step in electrospinning is to dissolve the polymer in a suitable solvent. As the electrified fluid jet travels toward the collector, the solvent evaporates and phase separation occurs, leading to the formation of fibers. **Table 2** lists some commonly used solvents and their property data.

	Density (g cm <sup>-3</sup> )	Vapor pressure (kPa at 25°C)	Boiling point (°C)	Dielectric constant	Viscosity (cP at 25°C)
Acetone	0.785	30.6	56.1	20.7	0.31
Acetic acid	1.049	2.1	118–119	6.2	1.16
Chloroform	1.49	25.9	61.2	4.8	0.53
Carbon disulfide	1.266	48.1	46.2	2.6	0.36
Cyclohexane	0.778	13.1	80.7	2.02	0.98
Dimethyl formaldehyde	0.948	0.52	152–154	36.7	0.80
Ethanol	0.789	7.87	78.4	24.5	1.07
Hexafluoro-2-propanol	1.596	16	58.2	16.7	1.02
Methanol	0.792	17.0	64.7	32.7	0.544
Tetrahydrofuran	0.889	15.2	66	7.6	0.48
Toluene	0.87	3.8	111	2.4	0.56
Water	1	3.17	100	80.1	0.89

**Table 2.** Properties of commonly used solvents in electrospinning.



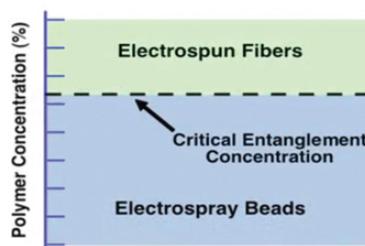
**Figure 3.** Field emission scanning electron microscopy images of electrospun PS fibers as a function of solution volatility: (a) 100% THF (15 kV, WD 8.9 mm); (b) 75/25% THF/DMF (15 kV, WD 9.3 mm); (c) 50/50% THF/DMF (15 kV, WD 8.8 mm); (d) 100% DMF (15 kV, WD 9.0 mm). Reprinted with permission from Megelski et al. [18].

The volatility of the solvent plays an important role in determining the fiber morphology. For example, polystyrene (PS) fibers electrospun from 100% tetrahydrofuran (THF) (more volatile)

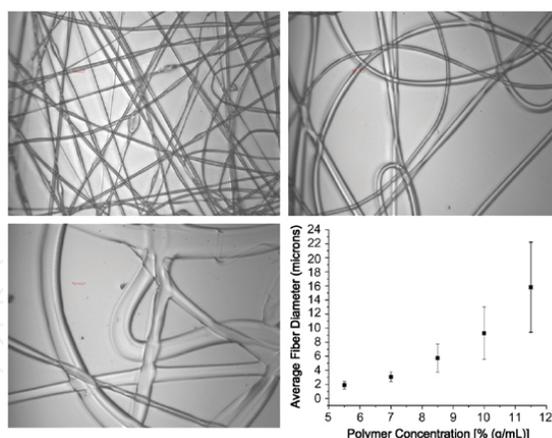
are highly porous (**Figure 3a**), whereas using 100% dimethyl formaldehyde (DMF) (less volatile) gives very smooth fibers and complete disappearance of microtexture and nanopores (**Figure 3d**). When a mixture of THF and DMF is used as the solvent, the fibers show increased pore size and decreased porosity as the solvent volatility decreases [19].

### 2.2.2. Solution concentration

The polymer concentration influences both the viscosity and surface tension of the solution, both of which can affect the electrospinning process. The critical entanglement concentration (**Figure 4**) is defined as the minimum concentration below which electrospay beads instead of electrospun fibers will form [20]. On the other hand, if the solution is too concentrated, the high viscosity will also inhibit the flow of fluid to the needle tip and consequently slows the electrospinning process.



**Figure 4.** Critical entanglement concentration for electrospinning. Reprinted with permission from Leach et al. [20].



**Figure 5.** Effect of polymer concentration on fiber diameter. Fibers were electrospun from solutions containing varying concentrations of poly(ethylene-co-vinyl alcohol) in 70:30 (v:v) 2-propanol: DI water. Top left: fibers electrospun from a 5.5% (g/mL) solution. Top right: fibers electrospun from an 8.5% (g/mL) solution. Bottom left: fibers electrospun from an 11.5% (g/mL) solution. The following processing parameters were used for all experiments: applied voltage: 20 kV, flow rate: 3 mL/h, capillary-collector distance: approximately 25 cm. In the bottom right panel the relationship between the average fiber diameter and the polymer concentration is given. Note that the mean fiber diameter increases monotonically with increasing polymer concentration. Additionally, it is evident that ribbon-like fibers are formed at higher concentrations (11.5%), which indicates incomplete polymer drying. (Error bars represent the standard deviation.). Reprinted with permission from Sill and von Recum [18].

The optimal range of solution concentration varies for different polymer/solvent systems. For example, for an aqueous solution of polyethylene oxide (MW 400,000), fibers can be produced when the solution concentration is in the range of 4–10 wt% [21]. Within the optimal range, the fiber diameter usually increases with the increasing concentration, as shown in **Figure 5**.

### 2.3. Processing parameters

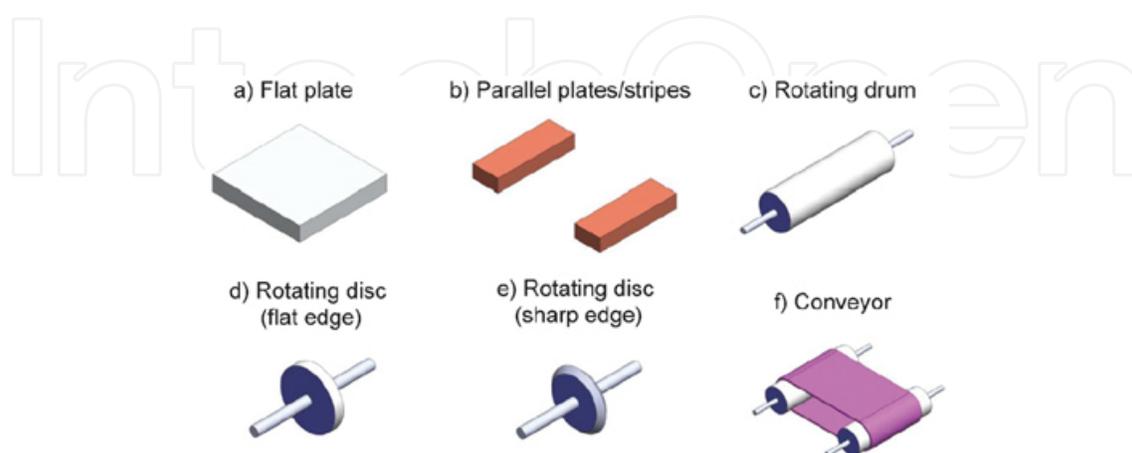
For a specific polymer/solvent system, there is usually an optimal range of processing parameters to obtain electrospun fibers with desirable size and distribution, as summarized in **Table 1**.

#### 2.3.1. Applied voltage

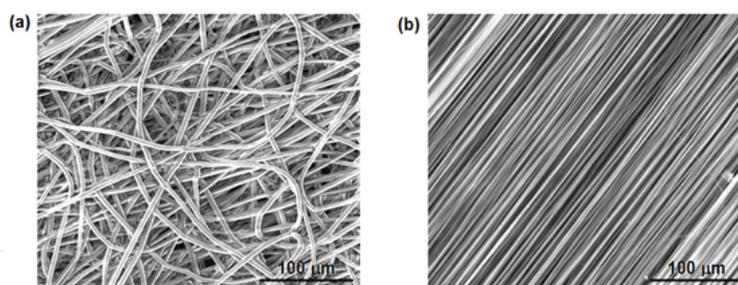
The applied voltage is an important parameter in the electrospinning process. A threshold voltage needs to be reached to initiate the fiber formation process. The relationship between the applied voltage and fiber diameter is not clear. Some studies reported a reduction in the fiber diameter with increasing voltage, presumably due to greater stretching of the fluid jet by the stronger electric field [22]. Other studies showed that higher voltage led to increase in fiber diameter [23]. In addition, higher voltage has also been found to facilitate the bead formation [21].

#### 2.3.2. Collector configuration

Earlier electrospinning experiments generally used a stationary collector to obtain randomly oriented fibers. As aligned electrospun fibers were demonstrated to be more important for many applications ranging from tissue engineered scaffold construction to photonics and fuel cells, new collectors have been developed to better control the fiber orientation, as illustrated in **Figure 6**. **Figure 7** shows randomly oriented fiber mat and aligned fibers by changing the collector configuration.

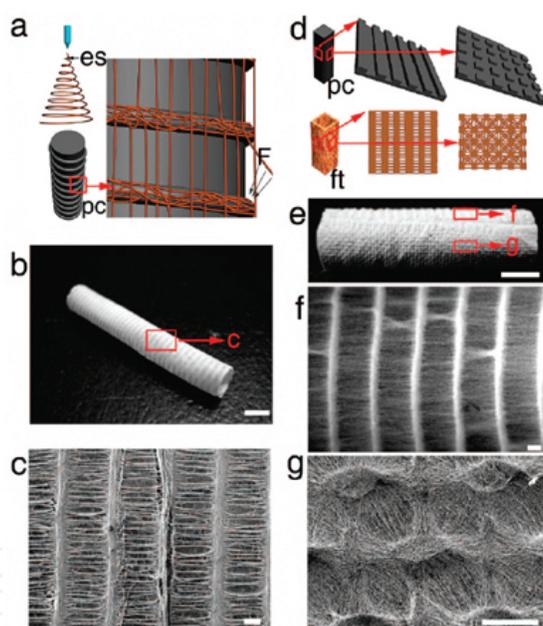


**Figure 6.** Sketches of electrospinning collectors (a) is used to collect randomly oriented fibers; (b)–(f) are used to collect aligned fibers. Reprinted with permission from Persano et al. [25].



**Figure 7.** Electrospun PCL fibers (20% PCL, 25 kV) collected as (a) a randomly oriented fiber mat using a stationary collector; (b) aligned fibers using a rotating disc with sharp edge.

More recent collector development has made it possible to create complex bi- and tri-dimensional architectures in a single run. Careful design of the 3D collectors with static collection allows one-step production of micro- and macro patterned tubes (diameter 500–5 mm) with different shapes and architectures, and T-shaped interconnections [24]. Such structures are attractive for the regeneration of many tubular fibrous tissues (**Figure 8**).

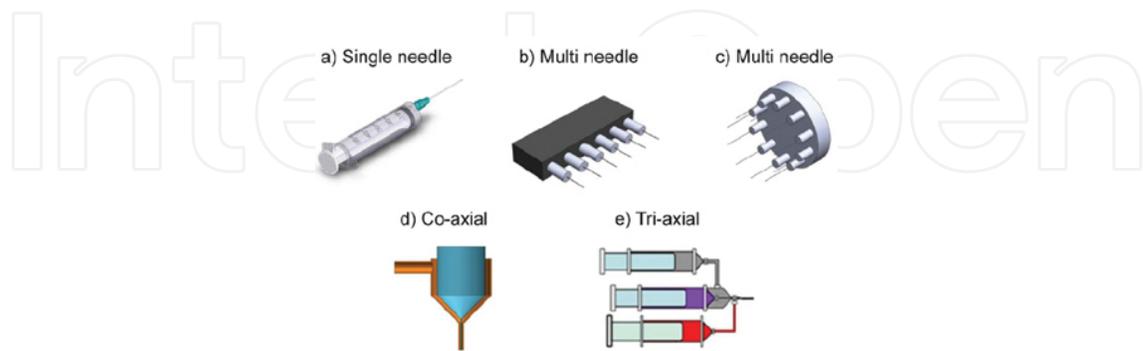


**Figure 8.** (a) Schematic illustration of collecting process using a cylindrical collector with equally spaced circular protrusions (es, electrospinning process; pc, patterned collector). (b) A fibrous tube with patterned architectures (scale bar, 5 mm). (c) Magnified image of panel b (scale bar, 200  $\mu\text{m}$ ). (d) Schematic illustration of collectors with two different patterns and relevant fibrous tube (pc, patterned collector; ft, fibrous tube). (e) A fibrous tube with two different patterns (scale bar, 5 mm). (f) and (g) Magnified images of two different patterns of panel e (scale bar 200  $\mu\text{m}$ ). Reprinted with permission from Zhang and Chang [23].

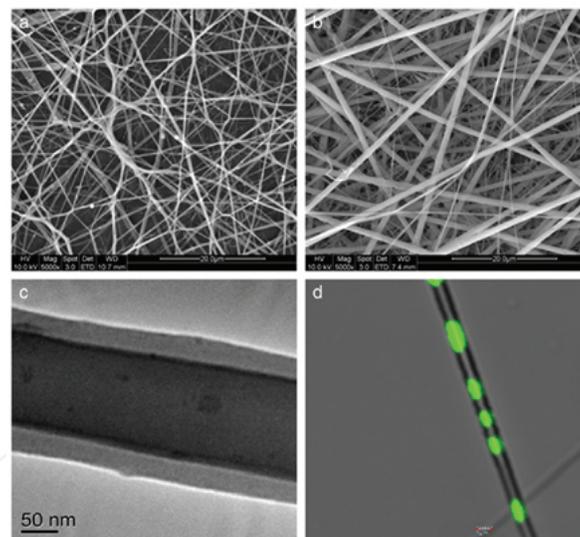
### 2.3.3. Spinneret design

A single needle attached to a syringe pump is the most commonly used spinneret design in electrospinning. Other designs have been developed to either create a core/shell fiber struc-

ture (Figure 9d and e) or to increase the throughput and thickness of the fiber production (Figure 9b and c). The core/shell structure (Figure 10) can be utilized to synthesize a composite electrospun fibrous scaffold [27, 28]. Therapeutic agents and signaling molecules can also be incorporated into the core or shell of electrospun fibers with tailored release profile to modulate the cell behavior.



**Figure 9.** Sketches of different spinneret designs used in electrospinning. (a) single needle; (b) Multi needle with linear configuration; (c) Multi needle with circular configuration; (d) Co-axial needle; and (e) tri-axial needle. Reprinted with permission from Persano [3].



**Figure 10.** FESEM images showing the fiber morphology of (a) fibrinogen nanofibers, (b) PGS/fibrinogen core/shell fibers, (c) PGS/fibrinogen core/shell morphology (higher magnification), and (d) PGS/fibrinogen core/shell (FITC dye in core). Reprinted with permission from Ravichandran et al. [26].

## 2.4. Ambient parameters

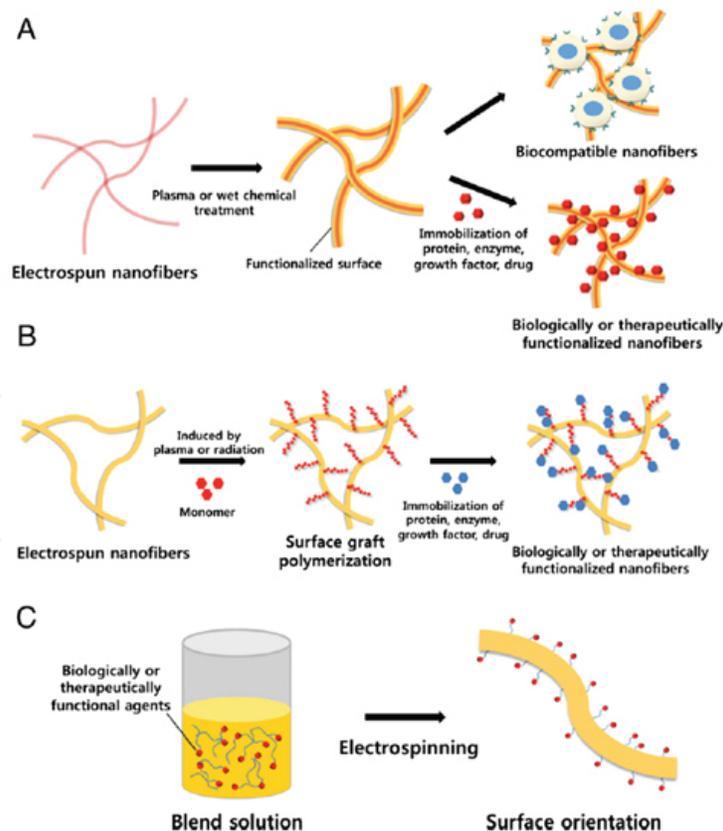
Ambient parameters such as temperature and humidity have also been found to affect the fiber morphology. As most solution viscosity changes inversely with temperature, higher temperature generally leads to thinner fibers, as has been supported by Mit-upathum's study [29].

Higher humidity has been found to induce the appearance of circular pores on electrospun fibers. When the humidity is too low, the fast drying of solvent may cause clogging of the needle tip.

### 3. Surface modification of electrospun fibers

#### 3.1. Electrospun fibers as biomimetic tissue engineering scaffolds

Tissue engineering represents a revolutionary approach to the repair and regeneration of diseased tissues and organs through a combination of biomaterial scaffolds, cells, and regulators. A biomimetic approach is often used in tissue engineering scaffold design that aims to mimic certain advantageous features of the extracellular matrix (ECM), such as the materials' composition, surface chemistry, mechanical properties, structural features, and growth factor delivery strategies [30]. Electrospinning is an attractive method to produce biomimetic tissue engineering scaffolds due to the simple and low-cost experimental setup, the wide variety of materials that can be electrospun, and inherent nanostructure feature with large surface area and high porosity.



**Figure 11.** Surface modification methods for electrospun polymer fibers. (A) Plasma or wet chemical treatment; (B) surface graft polymerization; and (C) co-electrospinning. Reprinted with permission from [5].

Electrospun fibers from natural polymers (such as collagen, silk, chitin, elastin, and fibrinogen) usually exhibit similar structural features as the ECM, making it easy to guide or direct cellular response during the tissue regeneration process. Major limitations of these natural polymers are the difficulty to create fibers reproducibly due to large variations in the structure and properties of the source polymers, and their generally insufficient mechanical properties. Electrospun synthetic polymers usually have higher mechanical strength than natural ones. Their structure and properties are also more reproducible and tailorable. On the other hand, many electrospun synthetic polymer fibers are hydrophobic and lack bioactive components on the surface to modulate cell response.

Surface modification is a cost effective approach to change the surface properties of the material without significantly altering its bulk properties. A common strategy in tissue engineering scaffold design is to incorporate bioactive molecules onto the fiber surface to improve biocompatibility or induce specific cellular response such as cell adhesion, proliferation, antigen presentation, and activation of explicit pathways to promote specific cell functions.

In general, any method used for surface modification of bulk polymers can be applied for electrospun fiber functionalization as long as it does not alter the fibrous structure. As illustrated in **Figure 11** and **Table 3**, the electrospun polymer fiber surface can be modified through physical entrapment, plasma treatment, chemical immobilization, or coelectrospinning of bioactive molecules.

Method	Electrospun fibers	Immobilized agents	TE applications
Physical entrapment	PCL	Gelatin, calcium phosphate, soluble eggshell membrane protein	Bone, blood vessel, general
	P(LLA-CL)	Collagen	Blood vessel
	PLLA	Laminin	Neural
	Silk	Fibrin	Cartilage
Wet chemical (NaOH hydrolysis)	PLLA	HAp	Bone
	PCL	–	Bone, ligament
Chemical immobilization	PET/PMAA	Gelatin	Blood vessel
	PLLA (PGA, PLGA) /PAA		General
	PCL	RGD	Ligament
Co-electrospinning	PLLA	HAp	Bone
	PLGA-PEG-NH <sub>2</sub>	RGD	General

Abbreviations: PCL, poly( $\epsilon$ -caprolactone); P(LLA-CL), poly(L-lactic acid)-co-poly( $\epsilon$ -caprolactone); PLLA, poly(L-lactic acid); PLLC, poly(L-lactide-co-caprolactone) copolymer; PET, polyethylene terephthalate; PGA, poly(glycolic acid); PLGA, poly(lactic-co-glycolic acid); PMAA, poly(methacrylic acid); PAA, poly(acrylic acid); PVP, poly(4-vinylpyridine); HAp, hydroxyapatite; PEG, poly(ethylene glycol); RGD, Arg-Gly-Asp.

**Table 3.** Surface modification techniques for electrospun polymer fibers in tissue engineering applications, adapted with permission from Yoo et al. [31].

### 3.2. Physical entrapment

Soaking the electrospun fibers in a solution that contains a high concentration of the bioactive components is the simplest surface modification approach. The high porosity of the nanostructure facilitates the adsorption of bioactive molecules through hydrogen bonding, van der Waals force, or electrostatic forces. For example, electrospun chitosan nanofibers were surface modified with the fibronectin, an adhesion molecule, to enhance the attachment of rat cardiomyocytes onto the 3-D cardiac tissue scaffold [32].

During the last decade our knowledge about amphiphilic proteins like hydrophobias increased tremendously and these small proteins were used to modify the surface of biosensors to render them more hydrophilic proteins [33]. The mechanism of their action as described by several investigators is that they self-assemble into amphiphilic membranes leading to increase wettability of hydrophobic surfaces of materials [19, 34]. This specific nature attracted more investigators to use them to incorporate growth factors and other proteins to improve cellular attachment to materials with good mechanical properties but with poor biological activity. Zhao et al. demonstrated that the hydrophilicity of electrospun PCL fibers can be improved by using a fusion protein consisting of HGFI and vascular endothelial growth factor (VEGF). In this study, the self-assembled layer of VEGF-HGFI effectively enhanced the adhesion, migration, and proliferation of human umbilical vein endothelial cells [35]. Another polymer that is particularly useful for coating and modification of various surfaces is Polydopamine (PDA) formed by the oxidation of dopamine. This polymer was used to modify the surface of PLA nanofibers in order to promote the adhesion and proliferation of human adipose-derived stem cells (hADSCs), cell attachment was significantly enhanced on PDA/PLA modified surfaces relative to control [28].

### 3.3. Plasma treatment

Synthetic polymers are generally hydrophobic. Plasma treatment using oxygen or air can introduce hydroxyl groups onto the fiber surface and effectively decrease its hydrophobicity. Several studies have reported increased hydrophilicity and enhanced attachment of fibroblasts on electrospun PCL or poly(butylene carbonate) fibers [36]. Plasma treatment using oxygen, ammonia, or air generates carboxyl groups, or amine groups on the surface, thus serving as a precursor to other surface modification methods. For example, oxygen plasma treatment of PLLA nanofibers followed by cross-linking with cationized gelatin allowed better chondrocyte attachment to the functionalized PLLA nanofibers [11].

### 3.4. Wet chemical treatment

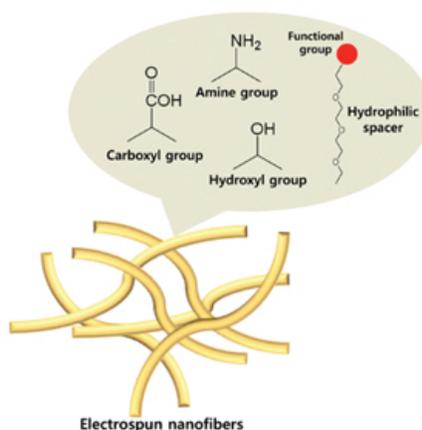
Acidic or alkaline solutions can be used to induce partial surface hydrolysis of electrospun polyester fibers to modify the surface wettability or to create nanotopography.

The random chemical scission of ester links leads to generation of carboxylic and hydroxyl groups. For example, NaOH-treated PLLA nanofibrous mesh showed greatly enhanced nucleation and growth of hydroxyapatite minerals on the fiber surface, likely due to the chelation of calcium ions by carboxylic acids after the NaOH treatment. When electrospun

PCL nanofibers were treated with 5 M NaOH, the fiber wettability was dramatically enhanced with almost zero water contact angle due to the capillary action on the highly rough surface. The nanotopography also led to more favorable cell adhesion.

### 3.5. Chemical immobilization

Compared to physical entrapment, chemical immobilization provides a stronger covalent attachment of bioactive molecules on the electrospun fiber surface. The fibers need to be pretreated to generate reactive functional groups before bioactive molecules can be immobilized, as illustrated in **Figure 12**.



**Figure 12.** Common surface functional groups for immobilization of bioactive molecules on electrospun fibers. Reprinted with permission from Yoo et al. [31].

A most commonly used chemical immobilization approach involves the use of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) to activate carboxylic acid groups on the fiber surface, which are then conjugated to primary amine groups of bioactive molecules. In the case of the PCL, this step can be accomplished by alkaline hydrolysis using sodium hydroxide surface treatment [37]. A study by Cheng showed that covalent immobilization via EDC coupling of collagen onto electrospun nanofiber matrices of PCL-chitosan (CS) blends significantly improved rat bone marrow derived stromal cells (rBMSCs) adhesion, spreading, proliferation, and osteogenic differentiation as compared to control groups [38]. Other cross-linking agents like aldehydes like glutaraldehyde are used where maintenance of structural rigidity of protein is required. This method was used to cross-link hydroxyethyl cellulose nanofibrous mats to improve their cellular adhesion characteristics and stability for potential scaffold for skin tissue engineering [39]. Glutaraldehyde was also used by Krishnan and colleagues for cross-linking Xylan, a natural polysaccharide and polyvinyl alcohol (PVA) to produce Xylan/PVA nanofibers for skin tissue engineering [40].

### 3.6. Co-electrospinning

The surface modification methods discussed above all use posttreatment on electrospun fibers. Co-electrospinning the bioactive agents with polymers provides an alternative approach to

biofunctionalize the fiber *in situ*. The bioactive agents can be directly mixed with the polymer solution before electrospinning. The core/shell structure discussed in Section 2.3.3 can also be utilized to directly incorporate bioactive molecules on the fiber surface.

#### 4. Application of electrospun fibers in tissue engineering

The ease of producing nanofibers and the variety of biocompatible polymers that can be formed by electrospinning have uncovered many of their potential applications in emerging fields such as tissue engineering. One of the many advantages of electrospun nanofiber scaffolds is that their surface can be modified by controlling the electrospinning parameters to obtain the topography that best fits the application. Another advantage is that nanofiber sheets or matrices can be formed into almost any shape (patches, mats, tubes, fibers, multilayered matrices) based on the site of desired implantation. As shown in **Table 4**, electrospun fibrous scaffolds have been used to regenerate a variety of tissues such as the skin, vasculature, neural, bone, ligament, and tendon.

No.	Polymer	Solvent	Fiber diameter	Nozzle configuration	Application (cell type/drug)
<b>General tissue engineering (T.E.)</b>					
1	Poly( $\epsilon$ -caprolactone)	(a) Chloroform and methanol (b) Chloroform and DMF	2–10 $\mu$ m ~600 nm	Single nozzle	General T.E. (rat marrow stromal cells)
2	(a) Poly( $\epsilon$ -caprolactone) (core) (b) Zein (shell)	(a) Chloroform and DMF (b) DMF	500–900 nm	Coaxial	General T.E. (none used)
3	(a) Poly( $\epsilon$ -caprolactone) (core) (b) Collagen (shell)	(a) 2,2,2-Trifluoroethanol (b) 2,2,2-Trifluoroethanol	~513 nm	Coaxial	General T.E. (human dermal fibroblasts)
4	Poly(D,L-lactic-co-glycolic acid) and PLGA-b-PEG-NH <sub>2</sub>	DMF and THF	449–1312 nm	Single nozzle	General T.E. (NIH3T3 fibroblasts)
5	Poly(D,L-lactide-co-glycolide)	DMF AND THF	500–800 nm	Single nozzle	General T.E. (human mesenchymal stem cells and BALB/c C7 mouse fibroblasts)
6	Poly(ethylene glycol-co-lactide)	DMF and acetone	1.25–4.25 $\mu$ m	Single nozzle	General T.E. (none used)

No.	Polymer	Solvent	Fiber diameter	Nozzle configuration	Application (cell type/drug)
7	Poly(ethylene-co-vinyl alcohol)	2-Propanol and water	0.2–8.0 $\mu\text{m}$	Single nozzle	General T.E. (human aortic smooth muscle cells and human dermal fibroblasts)
8	Collagen	HFP	180–250 nm	Single nozzle	General T.E. (rabbit conjunctiva fibroblasts)
9	Collagen	HFP	100–730 nm	Single nozzle	General T.E. (aortic smooth muscle cells)
10	(a) Collagen	(a) HFP and acetic acid	3–6 $\mu\text{m}$	Single nozzle	General T.E. (human osteosarcoma cells)
	(b) Gelatin	(b) HFP	2–6 $\mu\text{m}$		
11	Gelatin	2,2,2-Trifluoroethanol	0.29–9.10 $\mu\text{m}$	Single nozzle	General T.E. (none used)
12	Fibrinogen	HFP and 10 $\times$ minimal essential medium	0.12–0.61 $\mu\text{m}$	Single nozzle	General T.E. (neonatal rat cardiac fibroblasts)
13	Poly(glycolic acid) and chitin	HFP	130–380 nm	Single nozzle	General T.E. (normal human epidermal fibroblasts)
14	Collagen and Poly(ethylene oxide)	10 mM HCl (pH 2.0)	100–150 nm	Single nozzle	General T.E. (none used)
15	Poly(DTE carbonate)	DCM	1.9–5.8 $\mu\text{m}$	Single nozzle	General T.E. (NIH3T3) (mouse embryo fibroblasts), MCF-7 (human mammary carcinoma), PC-12 (rat adrenal pheochromocytoma) and KB (KB/HeLa; human cervical carcinoma))
<b>Vascular T.E.</b>					
1	Poly( $\epsilon$ -caprolactone)	Chloroform and DMF	0.2–1 $\mu\text{m}$	Single nozzle	Vascular T.E. (human coronary artery endothelial cells)
2	Poly(D,L-lactide-co-glycolide), collagen, and elastin	HFP	720 $\pm$ 350 nm	Single nozzle	Vascular T.E. (bovine endothelial and smooth muscle cells)
3	Poly(L-lactide-co- $\epsilon$ -caprolactone)	Acetone	200–800 nm	Single nozzle	Vascular T.E. (human coronary artery smooth muscle cells)
4	Poly(L-lactide-co- $\epsilon$ -caprolactone)	HFP	799–820 nm	Single nozzle	Vascular T.E. (human umbilical vein endothelial cells (HUVECs))

No.	Polymer	Solvent	Fiber diameter	Nozzle configuration	Application (cell type/drug)
5	Poly(L-lactide-co- $\epsilon$ -caprolactone)	HFP	700–800 nm	Single nozzle	Vascular T.E. (none used)
6	Poly(L-lactide-co- $\epsilon$ -caprolactone)	HFP	100–300 nm	Single nozzle	Vascular T.E. (human coronary artery endothelial cells (HCAECs))
7	Poly(propylene carbonate)	Chloroform	$\sim 5 \mu\text{m}$	Single nozzle	Vascular T.E. (rat bone marrow mesenchymal stem cells)
<b>Neural T.E.</b>					
1	(a) Poly( $\epsilon$ -caprolactone)	(a) Chloroform and methanol	$559 \pm 300 \text{ nm}$	Single nozzle	Neural T.E. (DRG explants, dissociated DRG, Schwann cells, olfactory ensheathing cells, and fibroblasts)
2	Poly(L-lactic acid)	DMF and DCM	300–3500 nm	Single nozzle	Neural T.E. (mouse neural stem cells)
<b>Bone T.E.</b>					
1	Poly(L-lactic acid) and hydroxylapatite	DCM and 1,4-dioxane	$\sim 313 \text{ nm}$	Single nozzle	Bone T.E. (MG-63 osteoblasts)
<b>Skin T.E.</b>					
1	Chitin	HFP	$0.163\text{--}8.77 \mu\text{m}$	Single nozzle	Skin T.E. (normal human oral keratinocytes, normal human epidermal keratinocytes, and normal human gingival fibroblasts)

**Table 4.** Example electrospun polymer fiber scaffolds used in tissue engineering, adapted with permission from Sill and von Recum [18].

#### 4.1. Skin tissue regeneration

Flat and flexible nanofiber sheets are preferred to promote skin healing, regeneration, and substitution with the advantage of providing coverage of the exposed dermis in most situations. One study describes the potential use of polycaprolactone (PCL) and collagen nanofiber matrices as a dermal substitute. They performed *in vitro* testing that showed matrices supported the attachment and proliferation of human dermal fibroblasts as compared to the control [41]. Another study by Veleirinho and colleagues evaluated hybrid nanofibrous mats prepared by electrospinning consisting of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and chitosan for wound healing using a full thickness wound healing model. They showed that these scaffolds promoted wound healing in the rat [42]. The positive feedback from the *in vitro* and the animal studies stimulated the movement toward human clinical trials

including the use of nanofibers for drug release to promote healing. A double blind, randomized, placebo controlled clinical trial was performed in 2007 and completed in 2011, to evaluate the effectiveness and safety of a novel nitric oxide (NO) releasing wound dressing patch (PATHON) for the treatment of diabetic foot ulcers. The patch consists of electrospun nanofiber mesh that encapsulates a NO donor, permitting a constant release of NO for a 12 h period. Up to now, there are no available results [43]. Some preliminary results are published by Demircan and colleagues on the clinical usage of the collagen-elastin matrix in 15 children with facial burns. They show encouraging early results: the graft quality was close to normal skin in terms of vascularity, elasticity, pliability, texture, and color [44].

#### 4.2. Cardiovascular tissue engineering

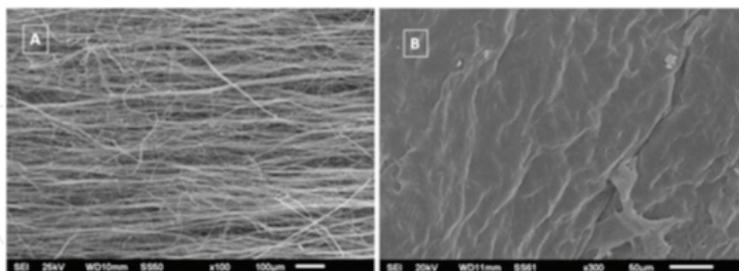
The feasibility of forming various constructs from electrospun nanofiber allowed the use of sheets or tubes for cardiovascular tissue engineering applications. In an experiment to study the effect of nanofibrous collagen/elastin/polycaprolactone patch loaded with cardiac nature protein (NP) on cardiac repair after myocardial infarction, it was found that bone-marrow cells seeded sheets improved the cardiac function in MI mice after 4 weeks of transplantation [45]. Due to the ability to modify the mechanical and chemical properties of nanofiber material, and in order to improve the patency rate following small-diameter vascular grafting, several groups are investigating the potential use of tubular nanofibers for small blood vessel substitution. One group was testing heparin-bonded P(LLA-CL) vascular scaffolds seeded with autologous endothelial cells EC and implanted into canine arterial model to promote graft patency rate. The scaffold inner layer was fabricated by heparin-bonded P(LLA-CL) nanofibers through coaxial electrospinning, while the outer layer was woven by pure P(LLA-CL) nanofibers. In this *in vivo* canine femoral artery replacement study the authors were able to demonstrate that the proposed biomaterial significantly promoted the 24 weeks patency rate (88.9%) in the experiment group as compared to control (12.5%) [46].

#### 4.3. Bone tissue engineering

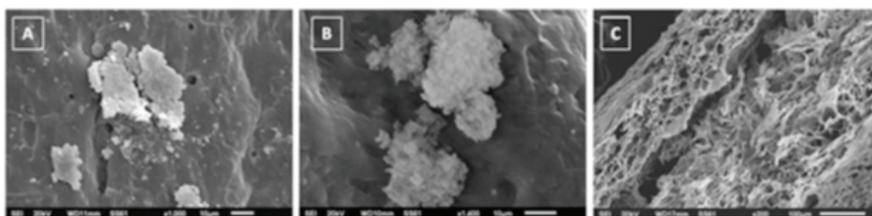
Tissue engineered bone materials are attractive alternative to synthetic grafts since they are biocompatible, bioactive, and designed to degrade after the appropriate cells start making their own matrix. Collagen is extensively involved in the process of adhesion and proliferation of many cell types and its physicochemical properties can be readily modified by cross-linking with many reagents since it has amino, carboxyl and hydroxyl groups that can serve as cross-linking sites. On the other hand stem cells are becoming more involved in tissue regeneration. Thus, the combination of collagen nanofibers and stem cells make them suitable for the engineering of various tissues including bone. Cheng et al. reported that PCL electrospun nanofiber matrices blended with chitosan and functionalized with type I collagen regulated the differentiation of rat bone marrow derived stromal cells (rBMSCs) into osteogenic lineage [47].

In our laboratories we fabricated aligned electrospun collagen nanofibers, we cross-linked them with EDC and we seeded them with rBMSCs (**Figure 13**). In this work, we tested the differentiation capability of these cells on this matrix without the use of any differentiation

factors. After 5 weeks of culture, the BMSC differentiated into osteoblasts and were able to deposit calcium minerals on the material surface (**Figure 14**) [48].



**Figure 13.** (A) SEM image showing the morphology of collagen nanofibers before seeding. (B) SEM image of seeded nanofibers after 5 weeks of culture showing complete covering of the material by differentiating cells.

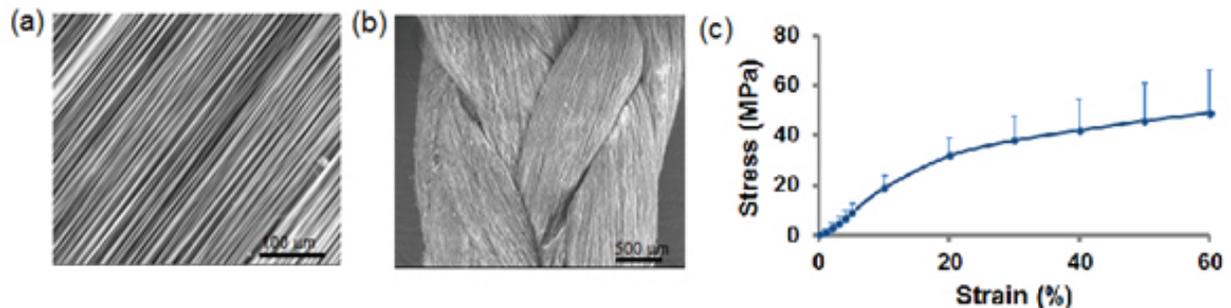


**Figure 14.** SEM images of nanoscaffolds after 5 weeks of culture with BMSCs showing different morphology of the mineral deposition on the surface of the material, (A and B) and a cross-section view showing the porous structure of the material (C).

#### 4.4. Ligament and tendon tissue engineering

The ability to regenerate tendon or ligament tissues is a major goal of tissue engineering. The specific structure and alignment of collagen fibers within a tendon plays a significant role in their tensile behavior. They preferentially align to the applied stresses. Many of the used biomaterials so far support the cellular growth and function but lack the initial mechanical strengths needed before the new tissue takes place. A recent work by Wang et al. explored the effect of aligned nanofibers on inducing tenogenic phenotype of human dermal fibroblasts (HDFs) *in vitro* and on inducing tendon regeneration *in vivo*. They showed that the aligned nanofibers induced tenogenic phenotype and Achilles tendon regeneration in a rat model [49]. An interesting application of PCL nanofibers was introduced by Martin and colleagues that involves the modification of PCL with radio opaque particles of zirconia to enhance the visualization of the implant by X-ray. The modified material was seeded with bovine MSCs and implanted *in vivo* in a model of total disc replacement in the rat coccygeal spine for 4 weeks. In this study the authors showed that radio opaque nanoparticles can be included into nanofibrous scaffolds and thus providing a biocompatible template for *in vivo* visualization, future image-guided implantation and long term evaluation of scaffold location and performance [50].

Our lab has developed a braiding technique for electrospun PCL fibers to mimic the hierarchical architecture of collagen seen in native anterior cruciate ligament (ACL). The braided scaffolds also showed comparable mechanical properties as the native ACL (**Figure 15**, unpublished). Ongoing work is focused on evaluating the biocompatibility of the braided scaffolds.



**Figure 15.** (a) ESEM image of electrospun PCL nanofibers; (b) a braided structure using nine fiber strips by first braiding three fiber strips into a triple helix, then braiding three such subunits into a second-order triple helix structure; and (c) stress-strain plot of 11 samples from 6 rounds of braids giving an elastic modulus ( $228 \pm 50$  MPa), ultimate tensile strength ( $52 \pm 17$  MPa) and toe region ( $3.3 \pm 1.6\%$ ).

## 5. Summary

The recent interest in electrospinning as a tissue engineering scaffold fabrication method is attributed to its relatively simple and inexpensive experimental setup, the wide variety of applicable materials, and the inherent nanoscale nature of the fibers closely mimicking that of the ECM. Further study of the electrospinning process and new development in the spinneret and needle configuration, along with effective utilization of surface modification methods, will help to create more biomimetic scaffolds tailored to the specific tissue regeneration applications.

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