Chapter 3

Electrospinning of Collagen and Its Derivatives for Biomedical Applications

Wei Peng Lu and Yanchuan Guo

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73581

Abstract

Collagen, gelatin and their derived polypeptides can act as multifunctional natural polymers with excellent physicochemical properties for biomedical applications. The use of electrospinning technology can convert collagen materials into nanofibrous materials that exhibit porous micro-nanostructures with good mechanical properties and excellent biocompatibility profiles. In this chapter, a systematic review of collagen electrospinning is presented and related applications are introduced including tissue engineering (e.g., artificial skin, artificial vasculature, cartilage repair, etc.), drug delivery, hemostatic dressings, periodontal restoration, biofilms, and wound dressings will now be discussed.

Keywords: collagen, gelatin, electrospinning, fiber

1. Introduction

Electrospinning is an easy and inexpensive process that can be used to prepare nanofibers from almost any soluble or fusible polymer under the action of a high electrostatic field. These electrospun nanofibers often possess extremely high surface areas, high porosities, tunable pore structures, and superior mechanical properties, which means they can be processed into materials with a wide variety of structure and function design [1–6]. Due to these advantages, electrospun nanofibers have been used for a broad range of biomedical and industrial applications, such as protective clothing, wound dressings, drug delivery applications, and tissue engineering [7–10].

The emergence of electrospinning (also known as electrostatic spinning) originated more than 100 years ago, with Zeleny first reporting on a variant of the electrospinning process in

1917 [11]. The first patent that described the process of electrospinning appeared in 1934, with Formalas describing apparatus to prepare polymer filaments, using an approach that relied on electrostatic repulsions between surface charges [12]. In 1966, Simons invented an electrospinning device that could be used to produce ultra-thin nonwoven fibers [13]. In 1981, Manley and Larrondo described how fabricated continuous fibers could be produced using melt-electrospinning polyethylene (PE)/polypropylene (PP) blends [14]. Then, Rutledge et al described new techniques for the production of polymer fibers by electrospinning in 1995 [15]. These breakthroughs resulted in a large increase in the number of reports describing electrospinning processes, with hundreds of different electrospinning polymers having been described for ultrafine fiber material applications. These include widely used synthetic polymers, such as polylactic acid, polyglycolide, polyethylene oxide, polycaprolactone, as well as natural polymers such as silk fibroin, fibrous protein, collagen, chitosan, hyaluronic acid, and gelatin. Natural biorenewable polymers often exhibit advantages in terms of their biocompatibility and biodegradability, making them a popular choice for a wide range of biomedical applications.

Among these natural polymers, collagen is one of the major extracellular matrix proteins that are present in many tissues and organs [16–18]. For instance, collagen in human skin accounts for approximately 70% of the extracellular matrix, where it functions as a network of elongated fibers to provide structural stability [19]. So far, more than 29 different types of collagen have been documented, with different species employing 46 different types of polypeptide chain for their assembly [20]. Collagen has many functional characteristics that are favorable for cell and tissue growth, and as a consequence it has been widely used as a biomaterial for medical and biotechnological applications [21–23].

Gelatin is a denatured protein that is obtained by acid, alkaline, and enzyme processing of collagen, which can exhibit similar physical and biological properties to those of collagen [24–28]. Due to its excellent biocompatibility, biodegradability, and immunogenicity profiles, gelatin is one of the most common biopolymers used for biomaterial applications [29–31]. The polypeptides derived from collagen play an important role in tissue remodeling [26]. Furthermore, collagen-derived peptides are also known to express biological activities, such as antioxidant, anti-osteoporosis, anti-photoaging properties, as well as acting as inhibitors of angiotensin-I converting enzyme [32, 33].

However, conventional polymeric products derived from collagen and gelatin (and their derived polypeptides) do not exhibit well-defined nanostructures, meaning that their mechanical, adhesion, and hydrophilic properties are not ideally suited for many biomedical applications. It has been shown that electrospinning is a useful technique to transform collagen, gelatin, or polypeptide into nanostructured fibers materials that can display small-size effects, high specific surface areas, and high porosities [21]. Furthermore, it also has been demonstrated that nonwoven electrospun collagen, gelatin, or polypeptide nanofibers can be used as ideal models to mimic the biochemical and ultrastructural properties of the extracellular matrix of tissue [34, 35]. In terms of performance, nanofibers materials also possess strong adsorbent powers, good filtration qualities, excellent obstruction performance, good binding affinities, and desirable moisturizing properties. Therefore, this chapter will now review the electrospinning techniques that can be used to transform collagen, gelatin, or derived polypeptides into nanofibers materials for biomedical applications.

2. Devices used for collagen electrospinning

Electrospinning is carried out using a Taylor cone that is generated by applying a high voltage to a polymer or melt solution, which results in formation of a liquid jet that is formed from interaction of a continuously increasing electric field with the surface tension of a droplet surface. During this process, a liquid jet starts oscillating to generate an irregular high-frequency spiral motion that leads to stretching of a fiber that is accompanied by fast volatilization of the solvent. This results in nano-scale fibers either being formed in a random manner on a collecting device, or being cross-linked into a membrane when a move and rotate collecting device is employed for processing [36, 37]. The electrospinning apparatus is comprised of three parts: a high-voltage direct-current power supply, a liquid supply unit, and a collecting device [38]. Depending on the nature of the liquid supply unit, the collagen electrospinning apparatus can be classified into two types: needle electrospinning or needleless electrospinning. The liquid supply unit of a needle electrospinning unit normally consists of a microinjection pump, a syringe, a single spinneret (or spinneret array), and a metal conducting wire that connects the spinneret to a high-voltage power source. The collecting device is comprised of a carrier, such as a metal plate, metal roller, metal disk, metal drum, or a ground metal conducting wire. The key component of any needle electrospinning assembly is the spinneret, which serves to prevent the monomer solution from becoming too viscous and solidifying under the spinning conditions, thus preventing blockage of the needle and potential damage to the equipment. However, the limited throughput of the needle spinneret means that the efficiency of polymer production using this technique is generally low [39]. Needleless electrospinning techniques represent a better way for high efficiency of polymer nanofiber production that can solve many of the limitations associated with traditional needle electrospinning techniques [40–42]. There are many methods for generating the surface disturbance of spinning solutions that is required for needless electrospinning, including ultrasonic disturbance, agitation, and acoustic bubble disturbance [43]. The stability of these surface disturbance processes is related to the quality (diameter distribution and L/D ratio distribution) of the needleless electrospinning membrane materials, resonance factors, liquid levels, solution concentrations, and solution viscosities. Needleless electrospinning equipment employs various types of electric field distribution, which can sometimes lead to membranes being produced with uneven thickness. Therefore, it is important that new collecting devices are designed to solve these important performance problems.

3. Introduction of composite nanofibers electrospinning technology

To date, electrospinning is the common method available to prepare nanofibers directly, quickly, and continuously under mild conditions in a low-cost, fast, and efficient manner [44, 45]. Depending on their applications, electrospinning nanofibers can be divided into two categories: single-component nanofibers and composite nanofibers [46]. Early reports described nanofibers that were prepared by electrospinning homogeneous polymers, with changes of starting materials, solution conditions, and electrospinning parameters used to prepare single-component nanofibers with different morphologies and properties. However, many of these

single-component nanofibers such as collagen [47–49], gelatin [50, 51], elastin [52], and fibrinogen [53, 54], have some limitations including weak mechanical properties, poor processability, poor moisture resistance, rapid degradation rate, and potential immunogenic properties [55, 56]. Thus, composite or hybrid nanofibers with different compositions (e.g., organic/organic, organic/inorganic) have been proposed as promising materials that exhibit physicochemical properties arising from both the host and guest materials [57]. For example, Chen et al. used electrospinning to prepare collagen/chitosan nanofiber membranes, which could promote the growth of dermal and epidermal layers [58]. Gu et al. used electrospinning to prepare porous biocompatible nanofibers mats from poly(L-lactide)/gelatin, which exhibited controlled evaporative water losses and promote fluid drainage, which made them potentially useful materials for wound dressing applications [59]. For the preparation of composite nanofibers, electrospinning technics can be divided into three fundamental types: (1) blend electrospinning; (2) mixing electrospinning; and (3) coaxial electrospinning. Blend electrospinning is the most commonly used method, which involves a process whereby a spinning solution is generated by mixing different polymers in a defined ratio. Mixing electrospinning refers to an electrospinning process that employs two or more separate liquid feeding devices containing different solutions. The electrostatic field results in each polymer being stretched into nanofibers which then overlap with each other to form composite nanofiber membranes. Coaxial electrospinning involves the use of a spinneret consisting of two or more capillary tubes with different inner diameters which results in a defined gap between the two capillary tubes. The same (or different) electrostatic field is applied to the inner and outer layers of electrospun solutions which results in solutions of the core and surface polymers being expelled from each coaxial nozzle to generate a concentric stratified flow. Because each of the electrospinning solutions has a short confluence time and low diffusion coefficient, they are stretched into coaxial composite nanofibers by the presence of the electric field force. In a comparative study, Chen et al. prepared a range of composite nanofiber membranes using blend electrospinning, mixing electrospinning, and coaxial electrospinning. They found that the composite nanofibers membranes prepared by coaxial electrospinning had high regularity, the membranes produced by blend electrospinning had good moisture resistance, while nanofiber membranes fabricated by using mixing electrospinning exhibited the highest mechanical strength [60].

4. Technical factors of collagenous electrospinning

There are various factors that can influence the morphology and structural properties of nanofibers produced in the electrospinning process. These include: (1) the properties of the electrospinning solution, such as concentration, viscosity, electrical conductivity, surface tension, and distribution of polymer molecular weight; (2) process parameters, such as electric voltage, spinning temperature, spinning speed, collection speed, and spinning distance; and (3) environmental parameters, such as temperature, moisture, air velocity, and atmospheric composition.

Kazanci investigated the role of temperature, solvent, and pH on the properties of collagen-type I nanofibers that were prepared under electrospinning conditions. They found that decreasing the temperature by 10°C, the PP-II (folded) fraction ratio of the resultant fibers

increased from 37 to 52.5%. Moreover, nanofibers obtained from acidic solutions contained 59% of PP-II, suggesting that the collagen structure was well preserved [61]. Dulnik et al. prepared electrospun polycaprolactone/gelatin and polycaprolactone/collagen nanofibers using various solvents (hexafluoroisopropanol and a mixture of acetic acid and formic acid). The result showed that electrospun PCL/gelatin and PCL/collagen nanofibers obtained by various solvents had similar morphologies, although there were some differences in their internal structures that affected their susceptibility toward biodegradation [62].

Lu et al. used water as a solvent to electrospin pure gelatin solution, finding that low concentrations of gelatin had low viscosity which were insufficient to produce continuous nanofibers, resulted in the formation of unwanted microbeads. When the concentration of gelatin used was >25%, the spinning solution became too viscous, which inhibited efficient electrospinning, resulted in a few nanofibers [63].

Wang et al. investigated the influence of solution concentration, salt concentration, solvent type, ambient temperature, and environmental humidity on the electrospinning of gelatin solutions. Their results showed that using polymers with high dielectric constants and solvents with low volatility solvents resulted in formation of nanofibers with small diameters. The diameters of the nanofibers were found to increase as the temperature rose. Low temperatures were not conducive to effective volatilization of solvent, leading to prolonged solidification time and the production of superfine nanofibers. The morphology of nanofibers was also found to be affected by ambient humidity. When relative humidity levels were increased to 45%, the resultant nanofiber membranes were shown to contain small areas of reticular formation and 'beads-on-a-string' structures. As the relative humidity was increased to 60%, the nanofiber membranes produced were contained uneven nanometer-diameter distributions with associated micro-bead structures. This study also revealed that the average diameter of nanofibers decreased with an increase in ambient humidity [64] (**Figure 1**).

An et al. studied the influence of formic acid concentration, gelatin concentration, and electric voltage on the electrospinning process used to prepare gelatin/polylactide composite nanofibers. They found that the use of low formic acid concentrations (50%) resulted in nanofibers, along with the formation of significant amounts of microbeads. Increasing formic acid concentration led to the formation of randomly arrayed ultrafine nanofibers, without any microbeads. For example, increasing the concentration of formic acid from 70 to 98%, resulted in an increase in the average diameter of gelatin nanofibers from 208 to 312 nm. Moreover, it was found that the concentration of gelatin affected the surface tension and viscosity of the spinning solution, with high surface tension favoring formation of microbeads, and high viscosity minimizing their formation. For example, when gelatin concentration was too low, discrete microbeads or beaded nanofibers were formed, while an increase in concentration of gelatin to >10% resulted in exclusive formation of smooth nanofibers. It was also found that the average diameter of nanofibers increased from 260 to 335 nm as the gelatin concentration rose from 10 to 23% [65]. Huang et al. had also reported that elastin-mimetic peptide polymers could be electrospun into nanofibers, who investigated the effect of polypeptide content and solution flow rate on the morphology and mechanical properties of the resultant nanofibers [66].

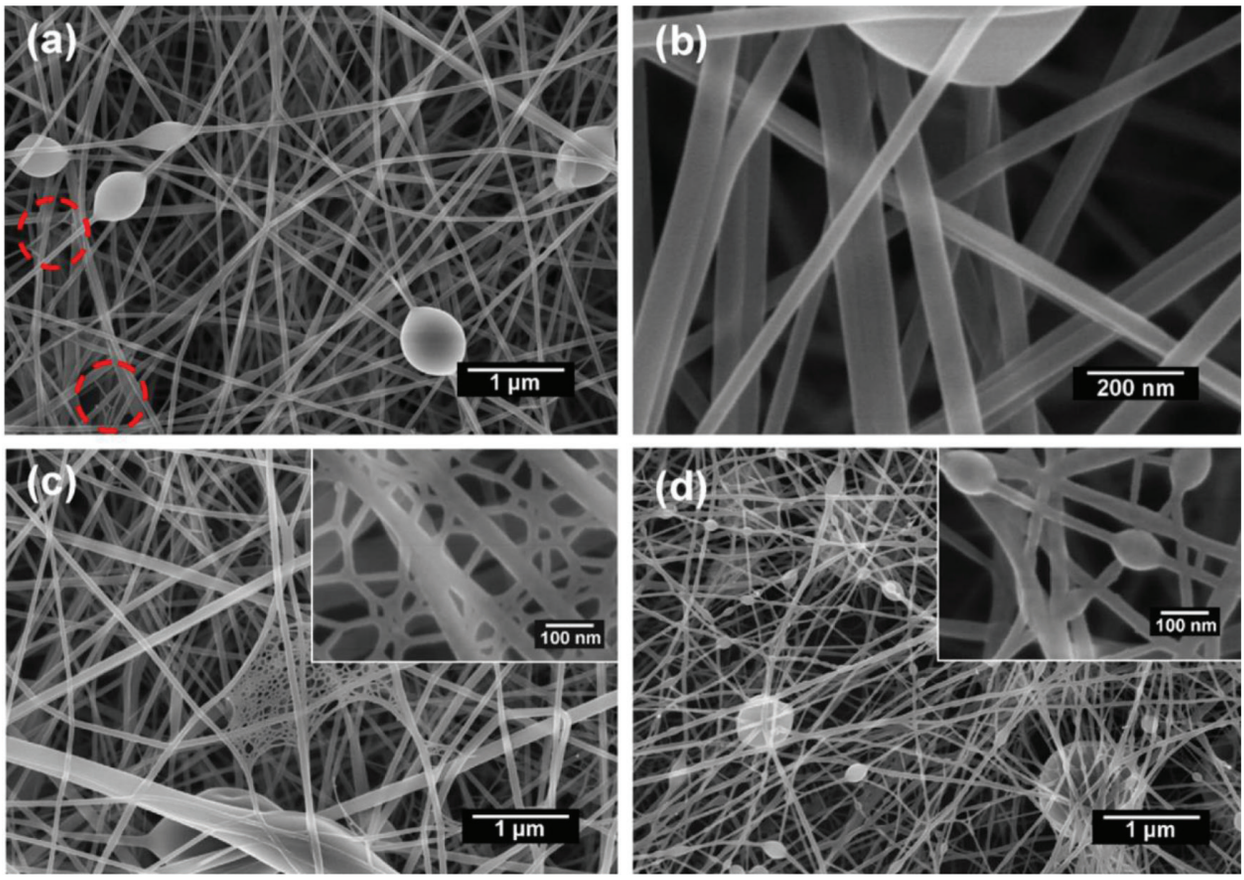


Figure 1. FE-SEM images of as-spun fibers using 10 wt.% gelatin/FA solution containing NaCl (0.1 wt.%) at temperature of 15°C (a and b) (voltage of 30 kV, and RH of 25%) and different RH: (c) 45% and (d) 60% (voltage of 30 kV, temperature of 24°C). The insets show the corresponding higher magnification images [64].

It is now clear that various factors in the electrospinning process play a crucial role in determining the 'spinnability' of a polymer solution and the physical and chemical properties of the resultant nanofibers. However, adjustment of these parameters can result in improvements to the electrospinning process, thus enabling the properties of functional nanofibers to be tuned to meet the needs for biomedical applications.

5. Applications of electrospun collagen

Electrospinning technology can be used to convert collagen materials into nanofibers materials that exhibit porous micro-nanostructures with good mechanical properties and excellent biocompatibility profiles. Potential uses of these nanofibers for biomedical applications include tissue engineering (e.g., artificial skin, artificial vasculature, cartilage repair, etc.), drug delivery, hemostatic dressings, periodontal restoration, biofilm, and wound dressings. Some applications of polymers derived from composite collagen and gelatin nanofibers, along with information on their preparation (type and solvent) and fiber diameter are provided in **Table 1**.

Composition	Solvent	Fiber diameter (nm)	Targeted applications	Ref.
Collagen/PLGA	HFP	50–500	Bone tissue scaffolds	[67]
Collagen/PHBV	HIFP	300–600	Scaffold for tissue engineering	[68]
Collagen/PCL	HFP	210–225	Vascular tissue engineering	[69]
Collagen/PLLA	HFIP	1290–1560	Tissue engineering	[70]
Collagen/TPU	HFP	700–800	Tissue engineering and functional biomaterials	[49]
Collagen/alginate/ chitosan/hydroxyapatite	Ethanol/ glycerol	300–800	Scaffold for regenerating bone tissue	[71]
Collagen/PCL	HFP	300	Implantable functional muscle tissues for patients with large muscle defects	[72]
Collagen/PCL	HFP	520	Autologous nerve grafts or proximal nerve stumps	[73]
Collagen/PLGA	HFIP	185–314	Long-term drug delivery of various pharmaceuticals	[74]
Collagen/PHBV/GO	TFE	400–500	Wound coverage material	[75]
Collagen/chitosan	HFP	434–691	Vascular and nerve tissue engineering	[76]
Collagen/collagen	HFP	210–540	Tissue engineering	[77]
Collagen/elastin	HFP	110–1120	Cardiovascular tissue engineering	[78]
Collagen/PLC	HEP	520	Vascular tissue engineering	[79]
Collagen/PLC	HEP	600–900	Human skin tissue engineering	[80]
Collagen/PEO	Aqueous	100–150	Wound dressings and tissue engineering	[81]
Collagen/PLC	HFP	500–600	Peripheral nerve regeneration	[82]
Collagen/PLLA-CL	HFP	100–200	Vascular tissue engineering	[83]
Collagen/PLLA-CL	HFP	120–520	Vascular tissue engineering	[84]
Collagen, elastin/PLGA, PCL, PLLA, or PLLA-CL	HFP	470–770	Cardiovascular tissue engineering	[85, 86]
Gelatin/PLLA-CL	TFE	50–500	Human skin tissue engineering	[87]
Gelatin/PCL	HFP TFE	640–880 50–1000	Cardiovascular tissue engineering	[78, 88]
Gelatin/PCL	TFE	2790–4630	Tissue engineering	[89]
Gelatin/PCL	TFE	160–232	Neural tissue engineering	[90]
Gelatin/zein	Acetic acid	380.3–695.5	Bioactive delivery in food industry	[91]
Gelatin/tannic Gelatin/gallic Gelatin/caffeic Gelatin/ferulic	Acetic acid	280	Biomaterials and tissue engineering Delivery system in medicinal or food industry	[92]

Composition	Solvent	Fiber diameter (nm)	Targeted applications	Ref.
Gelatin/GO	Acetic acid	200 ± 50–270 ± 50	Tissue engineering and wound dressing	[93]
Gelatin/PLC/QAS	TFE	180 ± 40–220 ± 80	Antibacterial wound dressing	[94]
Gelatin/chitosan	Acetic acid	202 ± 13.4–223.1 ± 69.8	Drug release	[95]
Gelatin/PCL	Acetic acid	250–400	Tissue engineering	[96]
Gelatin/PCL/CeNP	HFIP	616 ± 216	Wound dressing material	[97]
Gelatin/PLLA	Aqueous acetic acid solution	86–148	Wound dressing	[59]
Gelatin/PCL	TFE	800–2660	Various medical applications	[49]
Gelatin/PCL or collagen/PCL	HFIP or AA/FA	\	Wound healing, scaffolds, drugs delivery	[51]
Gelatin/NaCl	Formic acid	37–90	Filtration, tissue engineering, energy storage, sensors, and catalysis	[53]
Gelatin/PLLA	Formic acid	–	Tissue engineering scaffolds	[54]
Gelatin/PCL	TFE	312 ± 146	Guided bone regeneration	[98]
Gelatin/siloxane	Formic acid	–	Bone tissue engineering	[99]
Gelatin/PANi	HFP	61 ± 13–803 ± 121	Biocompatible scaffolds for tissue engineering	[100]
Gelatin/PLGA/FBF	TFE	310 ± 24	Drug delivery	[102]
Gelatin/PLA/PA	Formic acid	412	Healing material	[103]
Gelatin/$AgNO_3$	Glacial acetic acid/distilled water	280 ± 40	Wound dressing materials	[105]
Gelatin/PLCL/PLA	HFP	540 ± 230	Periodontal regeneration	[107]
Gelatin/PLA/MET		960 ± 560		
Gelatin/PLA/HAP		650 ± 440		
Gelatin/PCL/ZnO	HFP	56–1180	Periodontal regeneration	[108]

Table 1. Examples of collagen and gelatin electrospun nanofibers.

5.1. Tissue engineering applications

The three-dimensional microstructure of materials prepared from electrospinning collagen-derived materials can be used to effectively stimulate tissue regeneration. The network structure of these materials serves to promote the integration and recruitment of new tissue into their fibers scaffolds, thus accelerating the growth of new tissue. The main function of the nanofiber scaffold is to provide a suitable three-dimensional environment that cells can adhere to and proliferate.

Yu et al. used of electrospinning to prepare composite nanofiber scaffolds made of alginate, chitosan, hydroxyapatite, and collagen, whose porous structure was beneficial for cell infiltration and growth. What is more, the release of collagen from the scaffolds was, respectively, 17 and 2% of that from the collagen film after immersing in SBF and collagenase solution for 10 days, which indicated that the disintegration of scaffold for bone tissue engineering can be reduced comparing to conventional collagen scaffold. Therefore such a composite mat would be applicable as a scaffold for regenerating bone tissue [71] (**Figure 2**).

Choi et al. used electrospinning techniques to fabricate aligned polycaprolactone/collagen nanofiber meshes that were used to guide morphogenesis of skeletal muscle cells and enhance their cellular organization. Comparison with randomly oriented nanofibers, the results revealed that unidirectionally oriented nanofibers were better at inducing muscle cell alignment and myotubule formation [72] (**Figure 3**).

Lee et al. utilized electrospinning techniques to prepare PCL/collagen nerve conduits for complex peripheral motor nerve regeneration studies using end-to-side neurorrhaphy techniques. Results revealed that axonal continuity was normally recovered 8 weeks after surgery, with the muscle function recovery occurring after 1–20 weeks, and recovery of donor nerve function occurring after 20 weeks. Therefore, the use of electrospun PCL/collagen nerve conduits appeared to have great potential as materials for complex peripheral motor nerve repair [73] (**Figure 4**).

Composite nanofiber scaffolds have been prepared by electrospinning polycaprolactone and gelatin, which were then modified using cell-derived factor-1α. Experiments revealed that these scaffolds not only had good biocompatibility profiles, but also accelerated the healing of skull injuries in mice [98]. Ren et al. used gelatin and silicone to prepare composite nanofiber scaffolds as bone repairing material incorporating Ca^{2+} ions. Their results indicated that these composite materials could promote accumulation of bone apatite and the differentiation and

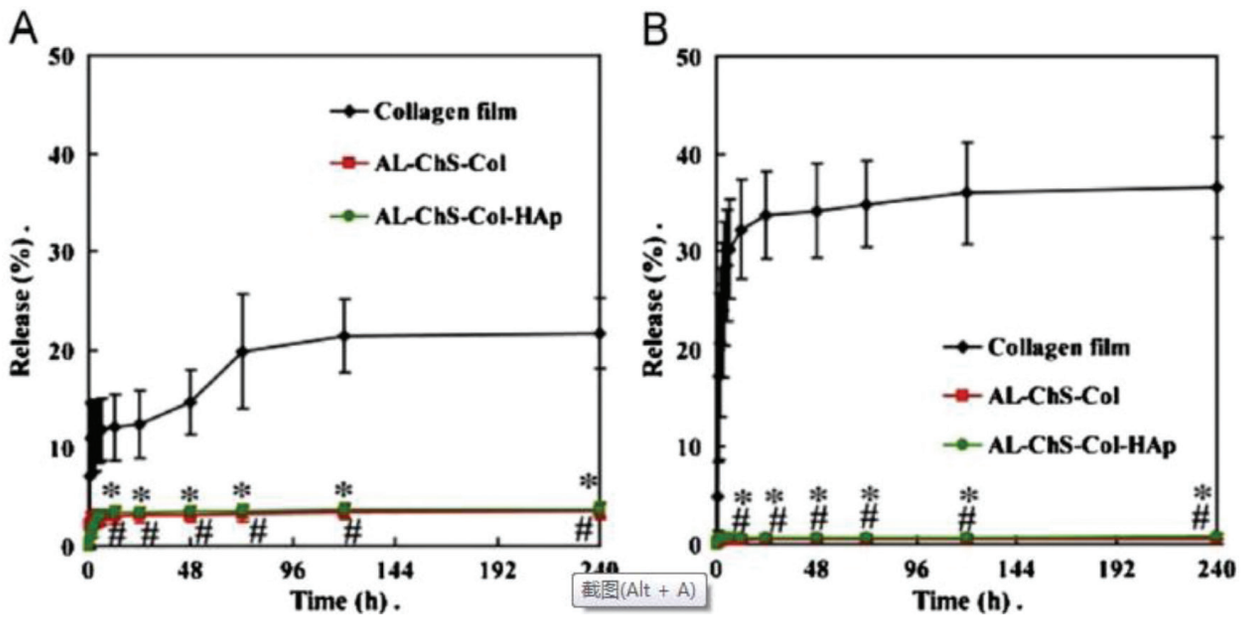


Figure 2. (A) Release of collagen in SBF solution for 10 days and (B) release of collagen in collagenase solution 10 days [71].

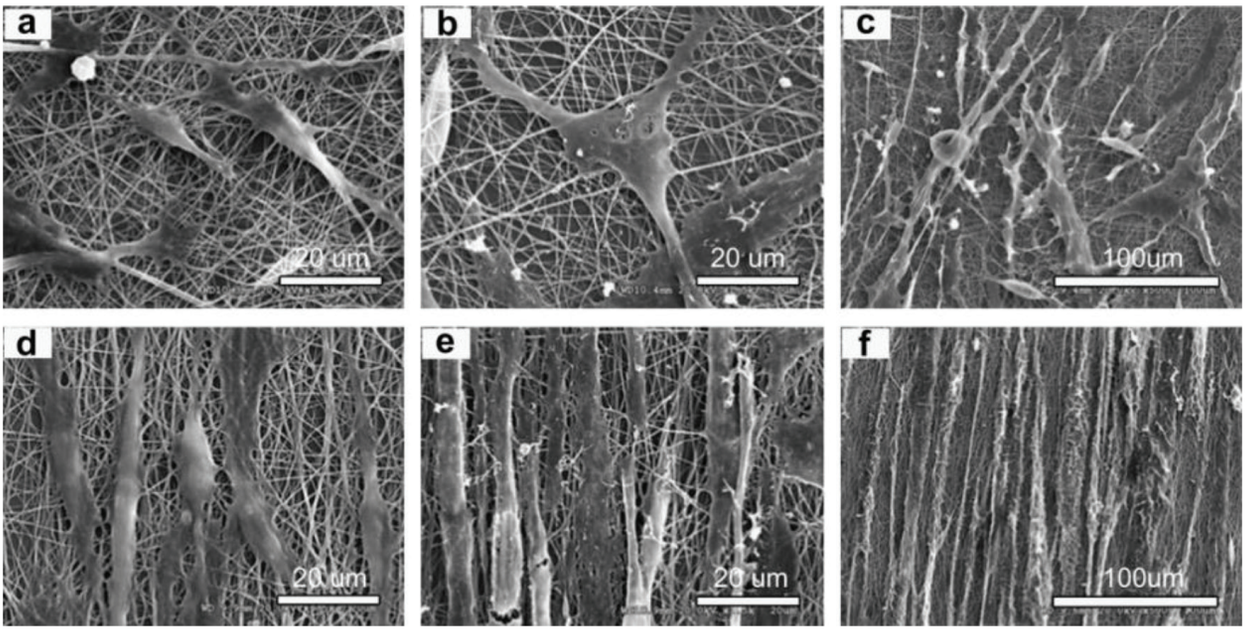


Figure 3. SEM images of human skeletal muscle cells on electrospun PCL/collagen nanofiber membranes: (a–c) randomly oriented and (d–f) aligned electrospun membranes, (a and d) 1 day and (b and e) 3 days after cell seeding and (c and f) 7 days after cell differentiation [72].

proliferation of osteoblasts [99] (**Figure 5**). Li et al. used electrospinning to prepare composite nanofiber scaffolds from polyaniline and gelatin, whose properties were dependent on the amount of polyaniline present. For example, when the amount of polyaniline was increased from 0 to 5% (w/w), the average diameter of the fibers decreased from 803 ± 121 to 61 ± 13 nm, and its elastic coefficient increased from 499 ± 207 to 1384 ± 105 MPa. These composite nanofiber scaffolds not only had excellent mechanical strength, but also had excellent biocompatibility in cell culturing experiments [100].

Blit et al. utilized the electrospinning to fabricate fibrous scaffolds which were subsequently surface modified with polypeptide and used them as membrane supports to culture smooth muscle cells (SMCs). Results showed that SMCs seeded onto these elastin-like polypeptide-4 membranes exhibited a spindle-like morphology, with good actin filament organization and smooth muscle myosin heavy chain expression. Therefore, these electrospun nanocomposite

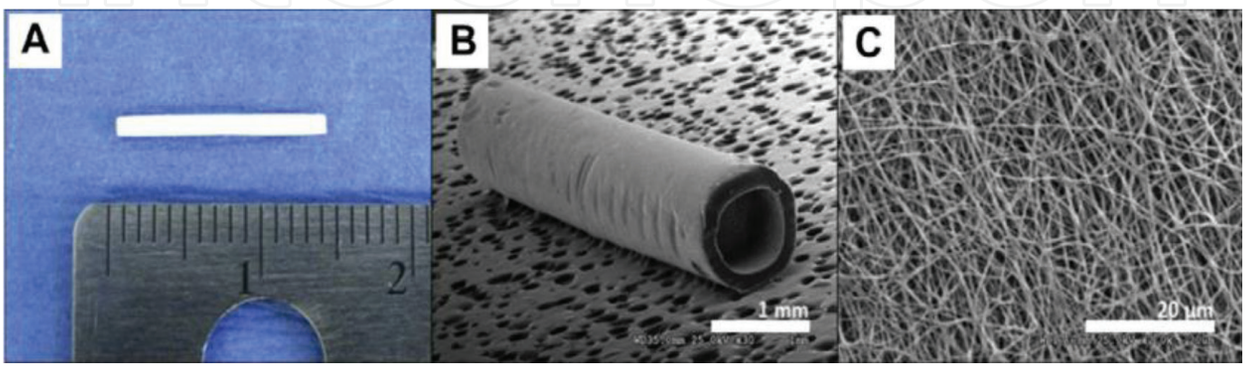


Figure 4. (A) Gross appearance and SEM images of electrospun PCL/collagen conduits: (B) entire (30×) and (C) surface (2.0× K) [73].

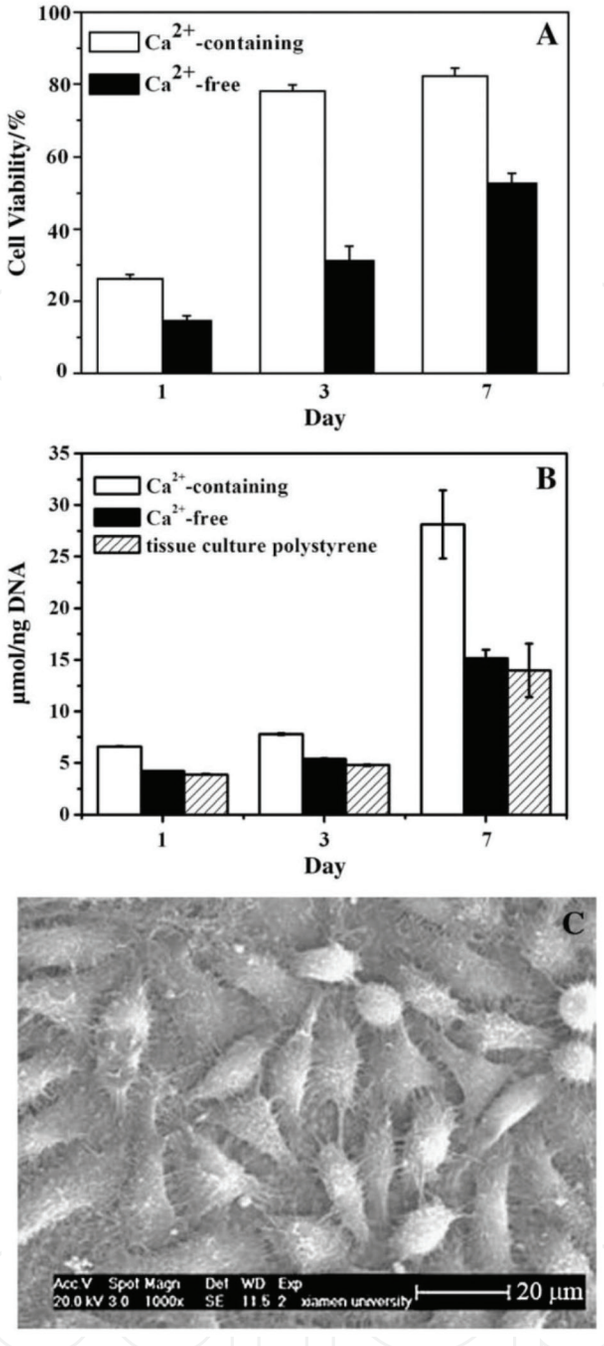


Figure 5. (A) MTT assay for proliferation of bone marrow-derived mesenchymal stem cells (BMSCs); (B) changes in ALP activity of BMSCs cultured on Ca^{2+}-containing gelatin/siloxane (GS) fiber mats and Ca^{2+}-free GS fiber mats at 1, 3, and 7 days, respectively. (C) SEM image of the Ca^{2+}-containing GS fiber mat seeded with BMSCs for 7 days [99].

membranes were promising candidates for the fabrication of contractile tissue engineered SMC-rich vascular medial layers [101] (**Figure 6**).

5.2. Drug delivery applications

Materials for drug delivery are often employed to release drug molecules into body tissues over extended periods of time. However, traditional drug delivery materials often do not

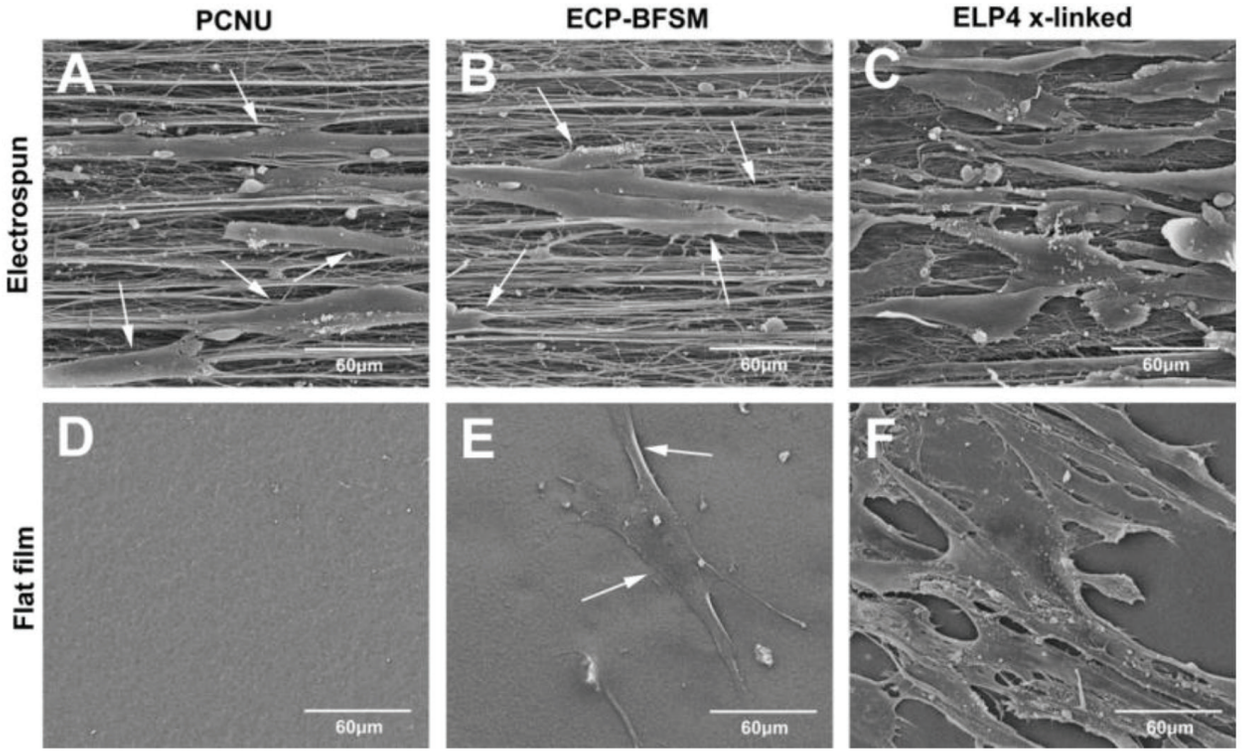


Figure 6. Scanning electron micrographs of SMC seeded materials after 1 week of culture (500× magnification). (A and D) polycarbonate-urethane (PCNU), (B and E) elastin cross-linking peptide bioactive fluorinated surface modifiers (ECP-BFSM) modified PCNU, and (C and F) elastin-like polypeptide-4 (ELP4) cross-linked aligned electrospun and flat film materials, respectively. Arrows indicate the edges of SMCs on the PCNU and ECP-BFSM modified materials [101].

control the rate of release of drugs effectively, while their poor drug absorption properties mean that they can only be used to deliver relatively low loadings of drug. Nanofibers are well suited as drug delivery vehicles, because their fine nanostructures are capable of absorbing significant amounts of drug molecules in a uniform manner. Gradual degradation of these nanofibers in the body then allows for gradual release of the drug in a controlled manner.

Polylactide-polyglycolide (PLGA) and collagen have been electrospun into sandwich structured drug-loaded membranes, with PLGA/collagen used for the surface layers, and PLGA/drugs contained in their core layer. The ability of these sandwich structured membranes to release vancomycin, gentamicin, and lidocaine in vitro was investigated. These results showed that these membranes could deliver therapeutic concentrations of vancomycin and gentamicin to human fibroblasts, ranging from 37 to 100% and 30 to 100% over 3 and 4 week periods, respectively [74] (**Figure 7**).

Meng et al. used electrospinning to uniformly distribute the drug Fenbufen throughout the structure of a nanofiber membrane prepared from gelatin and polylactide. The effect of gelatin concentration, fiber orientation, cross-linking time, and buffer pH on the sustained release of Fenbufen was successfully determined [102] (**Figure 8**). Huang et al. used proanthocyanidins as a cross-linking agent to prepare nanofibers as drug delivery vectors to deliver vitamin C magnesium phosphate. This study showed that the presence of the proanthocyanidin resulted in an increase in drug loading, which was important in maintaining a consistent rate of drug release. In addition, gelatin nanofibers that were cross-linked by proanthocyanidins

were also seen to promote the proliferation of L929 cells. Therefore, this type of drug-loaded nanofibers material could potentially be used for controlled drug delivery, as well as for the promotion of wound healing [103] (**Figure 9**).

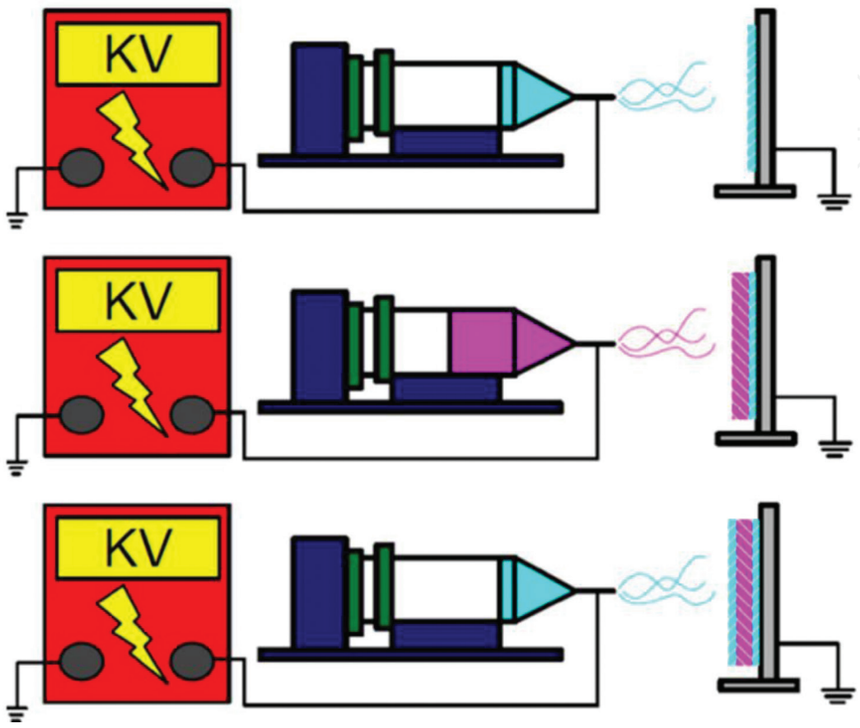


Figure 7. Apparatus used for electrospinning of sandwich structured membranes [74].

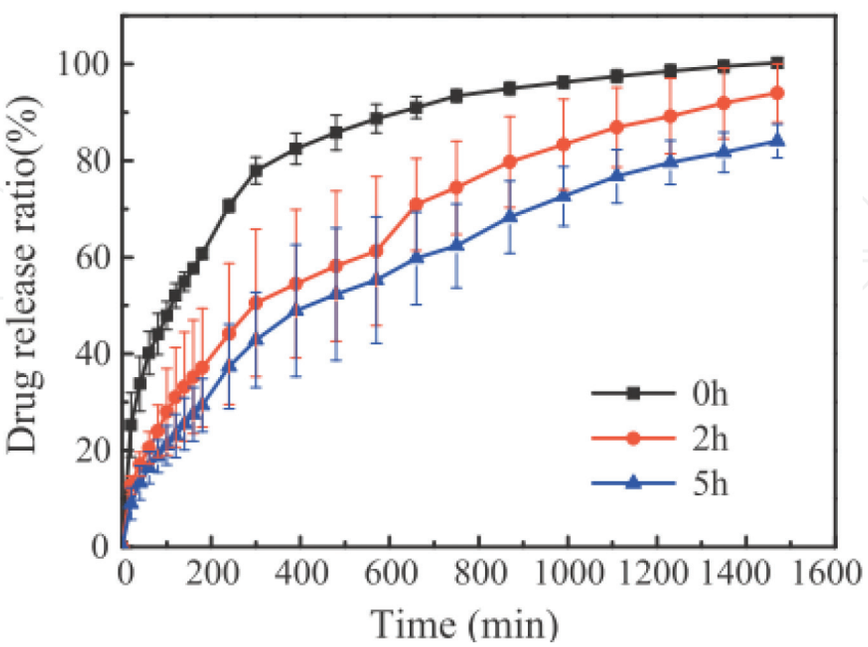


Figure 8. Release curves of FBF from PLGA/gelatin (9/1) electrospun nanofibers with different cross-linking times (0, 2, and 5 h) [102].

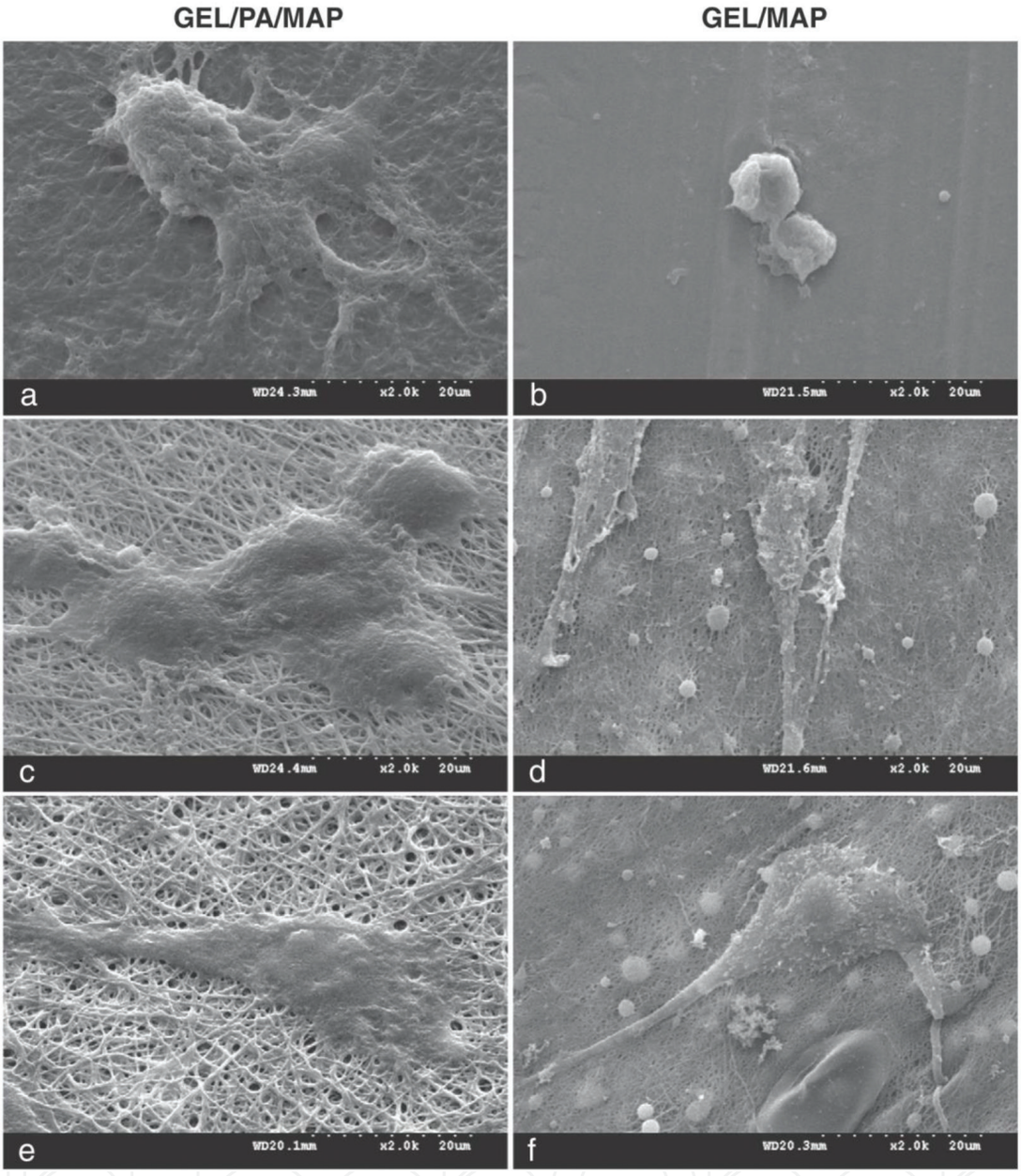


Figure 9. SEM photographs of the L929 cells cultured with gelatin (GEL)/magnesium ascorbyl phosphate (MAP) and GEL/proanthocyanidin (PA)/MAP membranes cross-linked by 50 wt.% glutaraldehyde vapor for 0, 15, and 45 min. (a) GEL/PA/MAP, 0 min. (b) GEL/MAP, 0 min. (c) GEL/PA/MAP, 15 min. (d) GEL/MAP, 15 min. (e) GEL/PA/MAP, 45 min. (f) GEL/MAP, 45 min [103].

Khadka et al. prepared electrospun composite nanofibers derived from a recombinant elastin-like peptide (ELP), or from a mixture of ELP with synthetic polypeptide and co-poly(L-glutamic $acid_4$, L-$tyrosine_1$) (PLEY). These materials contained numerous pores on their nanofibers surfaces, which ranged in diameter from <1 to 0.5 μm. Consequently, drugs were doped into the pores of these nanofibers materials and their use as potential drug delivery systems explored [104] (**Figure 10**).

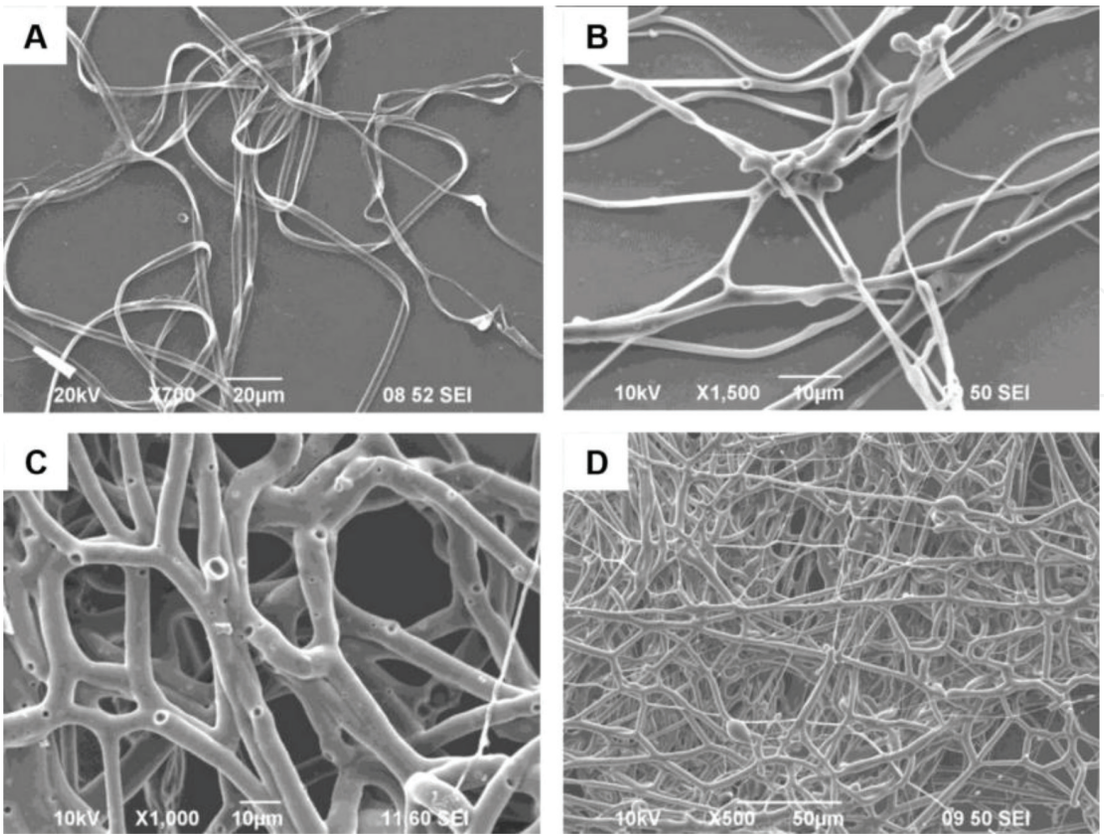


Figure 10. Scanning electron microscope image of a nonwoven fibers electrospun from a blended feedstock. In each case the nominal final polymer concentration was 48% (w/v) (A) (ELP)-C2:PLEY::2:1, 20 μm scale bar, (B) ELP-C2:PLEY::1:1, 10 μm scale bar, (C) ELP-C2:PLEY::2:3, 10 μm scale bar, (D) ELP-C2:PLEY::1:2, 50 μm scale bar. Note that the mean fiber diameter was −5 μm in panels A, B and D, but −10 μm in panel C [104].

5.3. Wound dressing applications

Collagen, gelatin, or polypeptide nanofiber membranes exhibit high porosities, small pore diameters, large surface areas, and fine microstructures that affords them good biocompatibility, biodegradability, biological adhesiveness, and moisture absorption properties. Therefore, these materials can be used to prepare dressings that keep wounds moist and which help prevent bacterial infection. Composite nanofiber materials prepared from gelatin and other materials such as fungicides, inflammatory drugs, and growth factors, can improve the performance of wound dressings. This is because they effectively improve the speed of wound hemostasis and healing by reducing its exposure to the external environment and protecting it from exposure to air.

Zine et al. prepared composite nanofibers by electrospinning a mixture of poly3-hydroxybutyric acid-co-3-hydroxyvaleric acid (PHBV), graphene oxide (GO), and collagen. GO was used to prepare these nanofibers to increase their mechanical strength and convey antibacterial activity against pathogenic bacteria such as *Escherichia coli* and *Staphylococcu aureus*. Collagen was included with the aim of enhancing cell proliferation, without affecting the composite materials mechanical strength or porosity. Subsequent biological results revealed that these nanofiber membranes generally had good wound healing properties [75] (**Figure 11**).

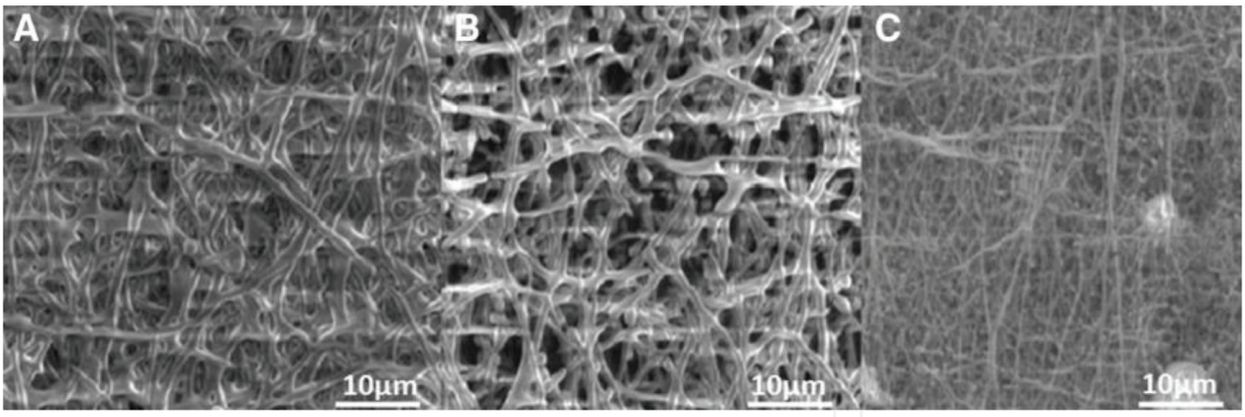


Figure 11. Scanning electron microscopy images of polymer (A) PHBV, (B) PHBV + GO, and (C) PHBV + GO + collagen [75].

Rujitanaroj et al. dissolved 22% (w/v) gelatin and 2.5 wt.% nitrate silver in 70 vol% of acetic acid to provide a stock solution for electrospinning nanofibers wound dressings with an average diameter of 280 nm. Glutaraldehyde was used as a cross-linking agent to improve the stability of the composite material in a moisture-rich environment. This nanofibers material was shown to have good sustained-release properties for Ag^{+}, resulting in these materials displaying good antimicrobial properties against *Pseudomonas aeruginosa* and *S. aureus* [105] (**Figure 12**).

Dubsky et al. used gelatin and polycaprolactone to prepare composite electrospun nanofibers, with subsequent cell culture experiments showing that these nanofibers can promoted cell adhesion and proliferation effectively. These composite nanofibers and medical gauze were applied to wounds of injured mice, with control experiments showing that inclusion of the nanofibers resulted in faster healing rates [106].

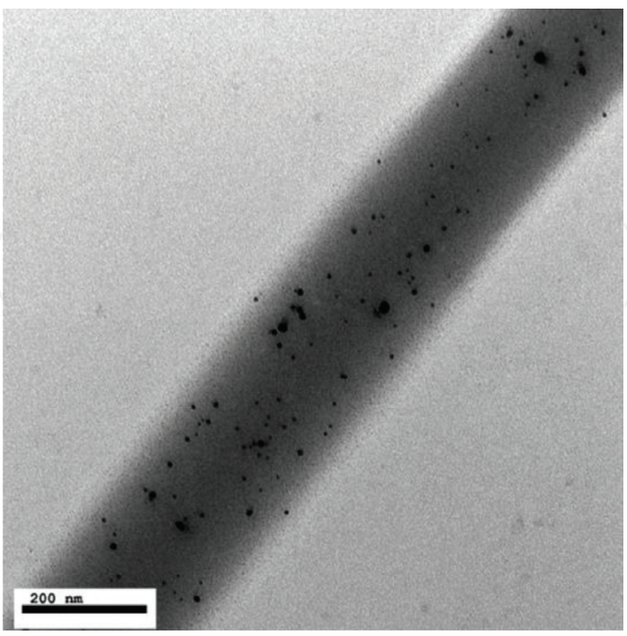


Figure 12. Selected TEM image of an electrospun fiber from the $AgNO_3$-containing gelatin solution that had been aged for 12 h [105].

5.4. Restorative materials for periodontal applications

The application of nanofiber membrane as periodontal restorative materials is an interdisciplinary field that spans the areas of tissue engineering and wound dressings. Periodontal repair requires membrane materials that prevent epithelial cells and connective tissue from growing into defect areas that can result in the creation of space for the co-migration and proliferation of periodontal ligament cells. Materials used for this dental application must be mechanically robust, exhibit good biodegradability profiles, and present robust three-dimensional nanostructures that are biocompatible with cell tissues. Therefore, collagen, gelatin, or polypeptide-derived nanofibers materials are potentially good choices for periodontal restorative applications.

Bottino et al. reported that a novel functionally graded membrane (FGM) could be prepared via sequential multilayer electrospinning. This FGM consisted of five different component layers, with each layer comprised of PLA:GEL+10 wt.% n-HAp, PLCL:PLA:GEL, pure PLCL, PLCL:PLA:GEL, and PLA:GEL+25 wt.% MET, respectively. Gelatin was used to enhance the bioactivity of this FGM, with poly-(DL-lactide-co-e-caprolactone) used to strengthen its

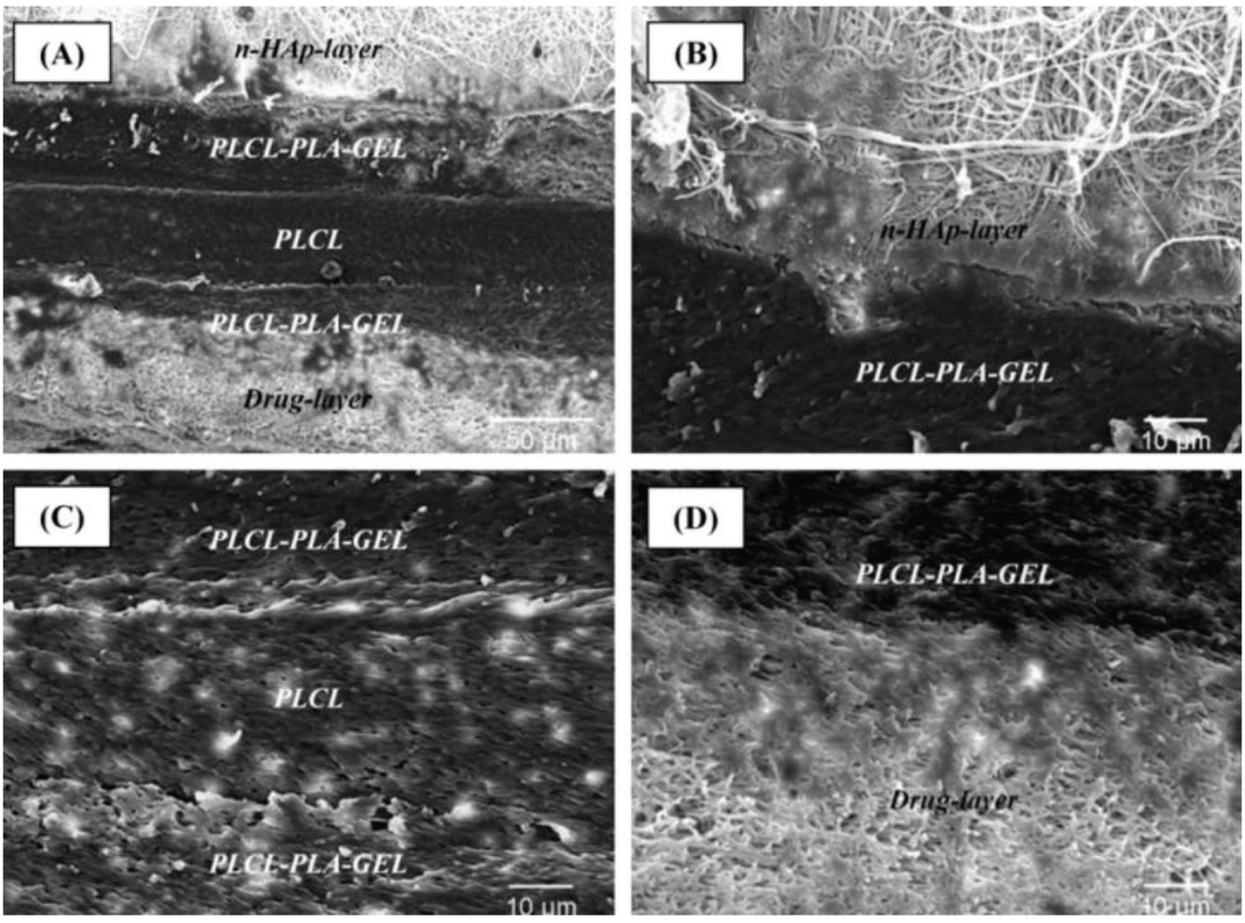
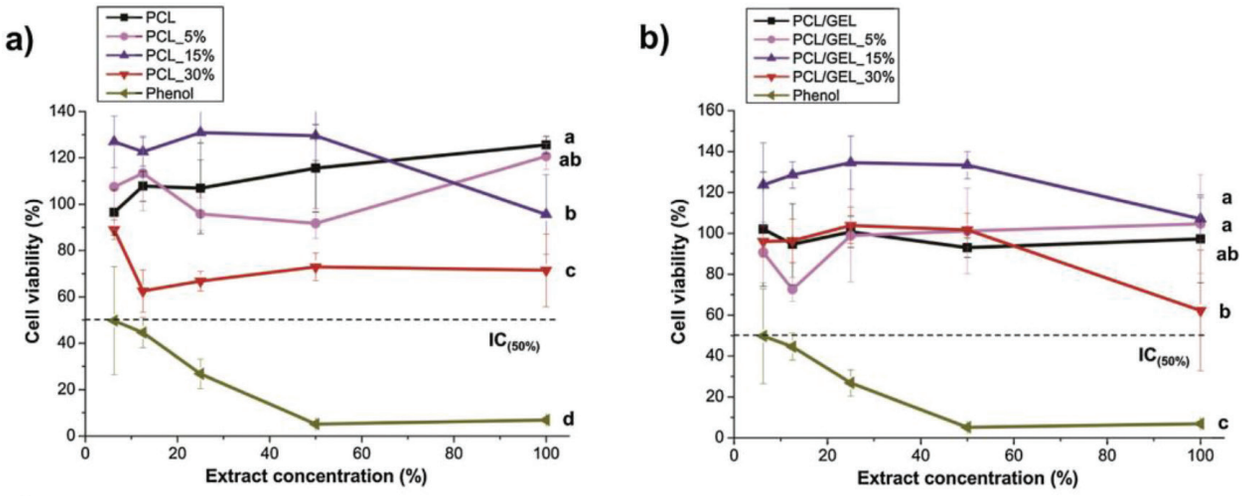


Figure 13. Cross-section SEM micrographs of the FGM processed via multilayering electrospinning. (A) General view of the FGM; (B) n-HAp-containing layer/PLCL:PLA:GEL interface; (C) CL structure; (D) MET-loaded layer/PLCL:PLA:GEL interface [107].



c)

Membranes	ZnO content (wt.%)			
	0	5	15	30
PCL	A 125.5 (±3.8) a	A 120.6 (±5.7) a	A 95.6 (±17.2) ab	A 71.5 (±15.7) b
PCL/GEL	B 97.3 (±21.4) ab	A 104.7 (±24.1) a	A 107.1 (±10.4) a	A 62.3 (±29.5) b

Figure 14. Cytotoxicity assays results on PCL (a) and PCL/GEL-based (b) membranes and cell viability [means (%) and standard deviations (±SD)] after exposure to concentrated (100%) extracts (c) [108].

mechanical properties and metronidazole benzoate (MET) included to prevent bacterial infection. n-HAp was incorporated into the PLA:GEL membrane to mimic the collagen-HAp matrix that was present in bone and enhance the composites osteoconductive behavior. This formulation afforded an electrospun FGM with excellent mechanical integrity, biodegradability, and good cell-membrane interactions that was explored as a periodontal restorative material [107] (**Figure 13**).

Mixtures of polycaprolactone and gelatin and ZnO in hexafluoropropanol had also been electrospun into nanofibers to afford ZnO-loaded electrospun membranes that possessed good biocompatibility, stretching ability, antibacterial activity, as potentially useful materials for periodontal regeneration [108] (**Figure 14**).

6. Outlook

Collagen and gelatin (and their derived polypeptides) are natural biopolymers that exhibit good biocompatibility, biodegradability, and low immunogenicity, as well as being excellent materials as hosts for cell and tissue growth. With the development of collagen in medical application, many processing methods are required to ensure that their application can be fully realized. The rapid evolution of electrospinning techniques is well suited to meet this need, enabling nanofiber membranes with three-dimensional pore structures, which imitated the microstructure of the extracellular matrix. Consequently, these electrospinning techniques have attracted increasing attention for applications in the fields of tissue engineering, drug delivery,

wound dressing, nerve regeneration, periodontal regeneration, and vascular reconstruction. The organic solvent used in electrospinning processes has potential toxicity issues, so the development of approaches that allow electrospinning to be carried out under aqueous conditions is highly desirable. Collagen, gelatin, or polypeptide nanofibers have been used to prepare biopolymers for applications in many different biomedical fields, including wound repair, artificial skin, and drug delivery. In order to satisfy a large number of medical applications, it will be necessary to develop more efficient electrospinning technics that produce large amounts of material with optimal microscopic structures. Current problems need to be resolved, include low efficiency, low mechanical strength, and poor spinning reproducibility. In addition, a lot of effort should be done in spinneret design, collecting device optimization, and solution delivery techniques. If these issues can be overcome, it is anticipated that collagen-derived nanofiber materials will have a major role for biomedical and biotechnological applications.

Author details

Wei Peng Lu[1] and Yanchuan Guo[1,2]*

*Address all correspondence to: yanchuanguo@mail.ipc.ac.cn

1 Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Science, Beijing, China

2 University of Chinese Academy of Sciences, Beijing, PR China

References

[1] Chen Z, Mo X, Qing F. Electrospinning of collagen-chitosan complex. Materials Letters. 2007;**61**(16):3490-3494

[2] Chakrapani VY, Gnanamani A, Giridev VR, Madhusoothanan M, Sekaran G. Electrospinning of type I collagen and PCL nanofibers using acetic acid. Journal of Applied Polymer Science. 2012;**125**(4):3221-3227

[3] Zhang M, Wang J, Xu W, Luan J, Li X, Zhang Y, Dong H, Sun D. The mechanical property of *Rana chensinensis* skin collagen/poly(L-lactide) fibers membrane. Materials Letters. 2015;**139**:467-470

[4] Homaeigohar SS, Mahdavi H, Elbahri M. Extraordinarily water permeable sol-gel formed nanocomposite nanofibers membranes. Journal of Colloid and Interface Science. 2012;**366**(1):51-56

[5] Chang B, Guan D, Tian Y, Yang Z, Dong X. Convenient synthesis of porous carbon nanospheres with tunable pore structure and excellent adsorption capacity. Journal of Hazardous Materials. 2013;**262**:256-264

[6] Liang D, Hsiao BS, Chu B. Functional electrospun nanofibers scaffolds for biomedical applications. Advanced Drug Delivery Reviews. 2007;**59**(14):1392-1412

[7] Maheshwari S, Chang H-C. Assembly of multi-stranded nanofiber threads through AC electrospinning. Advanced Materials. 2009;**21**(3):349-354

[8] Forward KM, Flores A, Rutledge GC. Production of core/shell fibers by electrospinning from a free surface. Chemical Engineering Science. 2013;**104**(0):250-259

[9] Zhou T, Wang N, Xue Y, Ding T, Liu X, Mo X, Sun J. Electrospun tilapia collagen nanofibers accelerating wound healing via inducing keratinocytes proliferation and differentiation. Colloids and Surfaces B: Biointerfaces. 2016;**143**:415-422

[10] Jing X, Mi H-Y, Cordie TM, Salick MR, Peng X-F, Turng L-S. Fabrication of shish–kebab structured poly(ε-caprolactone) electrospun nanofibers that mimic collagen fibrils: Effect of solvents and matrigel functionalization. Polymer. 2014;**55**(21):5396-5406

[11] Hiraoka K. Fundamentals of electrospray. In: Hiraoka K, editor. Fundamentals of Mass Spectrometry. New York, NY: Springer New York; 2013. pp. 145-171

[12] Kong CS, Lee TH, Lee SH, Kim HS. Nano-web formation by the electrospinning at various electric fields. Journal of Materials Science. 2007;**42**(19):8106-8112

[13] Goh Y-F, Shakir I, Hussain R. Electrospun fibers for tissue engineering, drug delivery, and wound dressing. Journal of Materials Science. 2013;**48**(8):3027-3054

[14] Mohammadzadehmoghadam S, Dong Y, Jeffery Davies I. Recent progress in electrospun nanofibers: Reinforcement effect and mechanical performance. Journal of Polymer Science Part B: Polymer Physics. 2015;**53**(17):1171-1212

[15] Cipitria A, Skelton A, Dargaville TR, Dalton PD, Hutmacher DW. Design, fabrication and characterization of PCL electrospun scaffolds-a review. Journal of Materials Chemistry. 2011;**21**(26):9419-9453

[16] Choi DJ, Choi SM, Kang HY, Min H-J, Lee R, Ikram M, Subhan F, Jin SW, Jeong YH, Kwak J-Y, Yoon S. Bioactive fish collagen/polycaprolactone composite nanofibers scaffolds fabricated by electrospinning for 3D cell culture. Journal of Biotechnology. 2015;**205**:47-58

[17] Arafat MT, Tronci G, Yin J, Wood DJ, Russell SJ. Biomimetic wet-stable fibres via wet spinning and diacid-based crosslinking of collagen triple helices. Polymer. 2015;**77**:102-112

[18] Law JX, Musa F, Ruszymah BHI, El Haj AJ, Yang Y. A comparative study of skin cell activities in collagen and fibrin constructs. Medical Engineering & Physics. 2016;**38**(9):854-861

[19] Gelse K, Pöschl E, Aigner T. Collagens—Structure, function, and biosynthesis. Advanced Drug Delivery Reviews. 2003;**55**(12):1531-1546

[20] Shoulders MD, Raines RT. Collagen structure and stability. Annual Review of Biochemistry. 2009;**78**(1):929-958

[21] Arslan YE, Sezgin Arslan T, Derkus B, Emregul E, Emregul KC. Fabrication of human hair keratin/jellyfish collagen/eggshell-derived hydroxyapatite osteoinductive biocomposite scaffolds for bone tissue engineering: From waste to regenerative medicine products. Colloids and Surfaces B: Biointerfaces. 2017;**154**:160-170

[22] Wei S, David CM, Sunxi W, Tong S, Guangzhao M, Weiping R. Electrospun polyvinyl alcohol-collagen-hydroxyapatite nanofibers: A biomimetic extracellular matrix for osteoblastic cells. Nanotechnology. 2012;**23**(11):115101

[23] Zulkifli FH, Jahir Hussain FS, Abdull Rasad MSB, Mohd Yusoff M. In vitro degradation study of novel HEC/PVA/collagen nanofibers scaffold for skin tissue engineering applications. Polymer Degradation and Stability. 2014;**110**:473-481

[24] Kuttappan S, Mathew D, Nair MB. Biomimetic composite scaffolds containing bioceramics and collagen/gelatin for bone tissue engineering—A mini review. International Journal of Biological Macromolecules. 2016;**93**:1390-1401

[25] Johnson BJ. Synthesis, structure, and biological properties of sequential polypeptides. Journal of Pharmaceutical Sciences. 1974;**63**(3):313-327

[26] Fontaine-Vive F, Merzel F, Johnson MR, Kearley GJ. Collagen and component polypeptides: Low frequency and amide vibrations. Chemical Physics. 2009;**355**(2):141-148

[27] Tabata Y, Ikada Y. Protein release from gelatin matrices. Advanced Drug Delivery Reviews. 1998;**31**(3):287-301

[28] Wang Y, Zhang W, Yuan J, Shen J. Differences in cytocompatibility between collagen, gelatin and keratin. Materials Science and Engineering: C. 2016;**59**:30-34

[29] Nieto-Suárez M, López-Quintela MA, Lazzari M. Preparation and characterization of crosslinked chitosan/gelatin scaffolds by ice segregation induced self-assembly. Carbohydrate Polymers. 2016;**141**:175-183

[30] Huang X, Zhang Y, Zhang X, Xu L, Chen X, Wei S. Influence of radiation crosslinked carboxymethyl-chitosan/gelatin hydrogel on cutaneous wound healing. Materials Science and Engineering: C. 2013;**33**(8):4816-4824

[31] Sarika PR, Cinthya K, Jayakrishnan A, Anilkumar PR, James NR. Modified gum arabic cross-linked gelatin scaffold for biomedical applications. Materials Science and Engineering: C. 2014;**43**:272-279

[32] Hou H, Li B, Zhang Z, Xue C, Yu G, Wang J, Bao Y, Bu L, Sun J, Peng Z, Su S. Moisture absorption and retention properties, and activity in alleviating skin photodamage of collagen polypeptide from marine fish skin. Food Chemistry. 2012;**135**(3):1432-1439

[33] Chen T, Hou H. Protective effect of gelatin polypeptides from Pacific cod (*Gadus macrocephalus*) against UV irradiation-induced damages by inhibiting inflammation and improving transforming growth factor-β/Smad signaling pathway. Journal of Photochemistry and Photobiology B: Biology. 2016;**162**:633-640

[34] Rizvi MS, Kumar P, Katti DS, Pal A. Mathematical model of mechanical behavior of micro/nanofibers materials designed for extracellular matrix substitutes. Acta Biomaterialia. 2012;**8**(11):4111-4122

[35] Hong Y, Huber A, Takanari K, Amoroso NJ, Hashizume R, Badylak SF, Wagner WR. Mechanical properties and in vivo behavior of a biodegradable synthetic polymer microfiber–extracellular matrix hydrogel biohybrid scaffold. Biomaterials. 2011;**32**(13): 3387-3394

[36] Arnal-Pastor M, Ramos CM, Garnés MP, Pradas MM, Lluch AV. Corrigendum to electrospun adherent-antiadherent bilayered membranes based on crosslinked hyaluronic acid for advanced tissue engineering applications. Materials Science and Engineering: C. 2014;**37**:405

[37] Arnal-Pastor M, Martínez Ramos C, Pérez Garnés M, Monleón Pradas M, Vallés Lluch A. Electrospun adherent–antiadherent bilayered membranes based on cross-linked hyaluronic acid for advanced tissue engineering applications. Materials Science and Engineering: C. 2013;**33**(7):4086-4093

[38] Soukup K, Petráš D, Topka P, Slobodian P, Šolcová O. Preparation and characterization of electrospun poly(p-phenylene oxide) membranes. Catalysis Today. 2012;**193**(1):165-171

[39] Kostakova E, Meszaros L, Gregr J. Composite nanofibers produced by modified needleless electrospinning. Materials Letters. 2009;**63**(28):2419-2422

[40] Vysloužilová L, Buzgo M, Pokorný P, Chvojka J, Míčková A, Rampichová M, Kula J, Pejchar K, Bílek M, Lukáš D, Amler E. Needleless coaxial electrospinning: A novel approach to mass production of coaxial nanofibers. International Journal of Pharmaceutics. 2017;**516**(1):293-300

[41] Hapidin DA, Saleh I, Munir MM, Khairurrijal. Design and development of a series-configuration mazzilli zero voltage switching flyback converter as a high-voltage power supply for needleless electrospinning. Procedia Engineering. 2017;**170**:509-515

[42] Li D, Wu T, He N, Wang J, Chen W, He L, Huang C, Ei-Hamshary HA, Al-Deyab SS, Ke Q, Mo X. Three-dimensional polycaprolactone scaffold via needleless electrospinning promotes cell proliferation and infiltration. Colloids and Surfaces B: Biointerfaces. 2014;**121**:432-443

[43] He JH, Liu Y, Xu L, Yu JY, Sun G. BioMimic fabrication of electrospun nanofibers with high-throughput. Chaos, Solitons & Fractals. 2008;**37**(3):643-651

[44] Huang ZM, Zhang YZ, Kotaki M, Ramakrishna S. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. Composites Science and Technology. 2003;**63**(15):2223-2253

[45] Khamforoush M, Mahjob M. Modification of the rotating jet method to generate highly aligned electrospun nanofibers. Materials Letters. 2011;**65**(3):453-455

[46] Yan H, Liu L, Zhang Z. Continually fabricating staple yarns with aligned electrospun polyacrylonitrile nanofibers. Materials Letters. 2011;**65**(15):2419-2421

[47] Matthews JA, Wnek GE, Simpson DG, Bowlin GL. Electrospinning of collagen nanofibers. Biomacromolecules. 2002;**3**(2):232-238

[48] Chen R, Li XQ, Ke QF, He CL, Mo XM. Fabrication and characterization of collagen (shell) /thermoplastic polyurethane (core) composite nanofibers by coaxial electrospinning. e-Polymers. 2010;**10**(1):67-74

[49] Chen R, Huang C, Ke QF, He CL, Wang HS, Mo XM. Preparation and characterization of coaxial electrospun thermoplastic polyurethane/collagen compound nanofibers for tissue engineering applications. Colloids and Surfaces B: Biointerfaces. 2010;**79**(2):315-325

[50] Huang ZM, Zhang YZ, Ramakrishna S, Lim CT. Electrospinning and mechanical characterization of gelatin nanofibers. Polymer. 2004;**45**(15):5361-5368

[51] Su Y, Li XQ, Liu SP, Wang HS, He CL. Fabrication and properties of PLLA-gelatin nanofibers by electrospinning. Journal of Applied Polymer Science. 2010;**117**(1):542-547

[52] Li MY, Mondrinos MJ, Gandhi MR, Ko FK, Weiss AS, Lelkes PI. Electrospun protein fibers as matrices for tissue engineering. Biomaterials. 2005;**26**(30):5999-6008

[53] Wnek GE, Carr ME, Simpson DG, Bowlin GL. Electrospinning of nanofiber fibrinogen structures. Nano Letters. 2007;**3**(2):213-216

[54] Gugutkov D, Gustavsson J, Ginebra MP, Altankov G. Fibrinogen nanofibers for guiding endothelial cell behavior. Biomaterials. 2013;**1**(10):1065-1073

[55] Gunn J, Zhang MQ. Polyblend nanofibers for biomedical applications: Perspectives and challenges. Trends in Biotechnology. 2010;**28**(4):189-197

[56] He C, Nie W, Feng W. Engineering of biomimetic nanofibers matrices for drug delivery and tissue engineering. Journal of Materials Chemistry B. 2014;**2**(45):7828-7848

[57] Vohra V, Calzaferri G, Destri S, Pasini M, Porzio W, Botta C. Toward white light emission through efficient two-step energy transfer in hybrid nanofibers. ACS Nano. 2010;**4**(3):1409-1416

[58] Chen JP, Chang GY, Chen JK. Electrospun collagen/chitosan nanofibers membrane as wound dressing. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2008;**313**:183-188

[59] Gu SY, Wang ZM, Ren J, Zhang C-Y. Electrospinning of gelatin and gelatin/poly(L-lactide) blend and its characteristics for wound dressing. Materials Science and Engineering: C. 2009;**29**(6):1822-1828

[60] Chen Z, Cao L, Wang L, Zhu H, Jiang H. Effect of fiber structure on the properties of the electrospun hybrid membranes composed of poly(ε-caprolactone) and gelatin. Journal of Applied Polymer Science. 2013;**127**(6):4225-4232

[61] Kazanci M. Solvent and temperature effects on folding of electrospun collagen nanofibers. Materials Letters. 2014;**130**:223-226

[62] Dulnik J, Denis P, Sajkiewicz P, Kołbuk D, Choińska E. Biodegradation of bicomponent PCL/gelatin and PCL/collagen nanofibers electrospun from alternative solvent system. Polymer Degradation and Stability. 2016;**130**:10-21

[63] Lu W, Xu H, Zhang B, Ma M, Guo Y. The preparation of chitosan oligosaccharide/alginate sodium/gelatin nanofibers by spiral-electrospinning. Journal of Nanoscience and Nanotechnology. 2015;**15**:1-5

[64] Wang X, Ding B, Yu J, Yang J. Large-scale fabrication of two-dimensional spider-web-like gelatin nano-nets via electro-netting. Colloids and Surfaces B: Biointerfaces. 2011;**86**(2):345-352

[65] An K, Liu H, Guo S, Kumar DNT, Wang Q. Preparation of fish gelatin and fish gelatin/poly(L-lactide) nanofibers by electrospinning. International Journal of Biological Macromolecules. 2010;**47**(3):380-388

[66] Huang L, McMillan RA, Apkarian RP, Pourdeyhimi B, Conticello VP, Chaikof EL. Generation of synthetic elastin-mimetic small diameter fibers and fiber networks. Macromolecules. 2000;**33**(8):2989-2997

[67] Jose MV, Thomas V, Dean DR, Nyairo E. Fabrication and characterization of aligned nanofibers PLGA/collagen blends as bone tissue scaffolds. Polymer. 2009;**50**(15):3778-3785

[68] Meng W, Kim SY, Yuan J, Kim JC, Kwon OH, Kawazoe N, Chen GP, Ito Y, Kang IK. Electrospun PHBV/collagen composite nanofibers scaffolds for tissue engineering. Journal of Biomaterials Science Polymer Edition. 2007;**18**(1):81

[69] Venugopal J, Zhang YZ, Ramakrishna S. Fabrication of modified and functionalized polycaprolactone nanofibre scaffolds for vascular tissue engineering. Nanotechnology. 2005;**16**(10):2138-2142

[70] Chiu JB, Liu C, Hsiao BS, Chu B, Hadjiargyrou M. Functionalization of poly(L-lactide) nanofibers scaffolds with bioactive collagen molecules. Journal of Biomedical Materials Research Part A. 2010;**83A**(4):1117-1127

[71] Yu CC, Chang JJ, Lee YH, Lin YC, Wu MH, Yang MC, Chien CT. Electrospun scaffolds composing of alginate, chitosan, collagen and hydroxyapatite for applying in bone tissue engineering. Materials Letters. 2013;**93**:133-136

[72] Choi JS, Lee SJ, Christ GJ, Atala A, Yoo JJ. The influence of electrospun aligned poly(ε-caprolactone)/collagen nanofiber meshes on the formation of self-aligned skeletal muscle myotubes. Biomaterials. 2008;**29**(19):2899-2906

[73] Lee BK, Ju YM, Cho JG, Jackson JD, Lee SJ, Atala A, Yoo JJ. End-to-side neurorrhaphy using an electrospun PCL/collagen nerve conduit for complex peripheral motor nerve regeneration. Biomaterials. 2012;**33**(35):9027-9036

[74] Chen DW, Hsu Y-H, Liao J-Y, Liu S-J, Chen J-K, Ueng SW-N. Sustainable release of vancomycin, gentamicin and lidocaine from novel electrospun sandwich-structured PLGA/collagen nanofibers membranes. International Journal of Pharmaceutics. 2012; **430**(1):335-341

[75] Zine R, Sinha M. Nanofibers poly (3-hydroxybutyrate-co-3-hydroxyvaletate)/collagen/grapheme oxide scaffolds for wound coverage. Materials Science and Engineering: C. 2017;**80**:129-134

[76] Chen ZG, Wang PW, Wei B, Mo XM, Cui FZ. Electrospun collagen-chitosan nanofiber: A biomimetic extracellular matrix for endothelial cell and smooth muscle cell. Acta Biomaterialia. 2010;**6**(2):372-382

[77] Dong B, Arnoult O, Smith ME, Wnek GE. Electrospinning of collagen nanofiber scaffolds from benign solvents. Macromolecular Rapid Communications. 2009;**30**(7):539-542

[78] Heydarkhan-Hagvall S, Schenke-Layland K, Dhanasopon AP, Rofail F, Smith H, Wu BM, Shemin R, Beygui RE, MacLellan WR. Three-dimensional electrospun ECM-based hybrid scaffolds for cardiovascular tissue engineering. Biomaterials. 2008;**29**(19):2907-2914

[79] Lee SJ, Liu J, Oh SH, Soker S, Atala A, Yoo JJ. Development of a composite vascular scaffolding system that withstands physiological vascular conditions. Biomaterials. 2008;**29**(19):2891-2898

[80] Powell HM, Boyce ST. Engineered human skin fabricated using electrospun collagen-PCL blends: Morphogenesis and mechanical properties. Tissue Engineering. Part A. 2009;**15**(8):2177-2187

[81] Huang L, Nagapudi K, Apkarian RP, Chaikof EL. Engineered collagen-PEO nanofibers and fabrics. Journal of Biomaterials Science Polymer Edition. 2001;**12**(9):979-993

[82] Schnell E, Klinkhammer K, Balzer S, Brook G, Klee D, Dalton P, Mey J. Guidance of glial cell migration and axonal growth on electrospun nanofibers of poly-epsilon-caprolactone and a collagen/poly-epsilon-caprolactone blend. Biomaterials. 2007;**28**(19):3012-3025

[83] He W, Yong T, Teo WE, Ma ZW, Ramakrishna S. Fabrication and endothelialization of collagen-blended biodegradable polymer nanofibers: Potential vascular graft for blood vessel tissue engineering. Tissue Engineering Part A. 2005;**11**(10):1574-1588

[84] Kwon IK, Matsuda T. Co-electrospun nanofiber fabrics of poly(L-lactide-co-epsilon-caprolactone) with type I collagen or heparin. Biomacromolecules. 2005;**6**(4):2096-2105

[85] Stitzel J, Liu L, Lee SJ, Komura M, Berry J, Soker S, Lim G, Van Dyke M, Czerw R, Yoo JJ, Atala A. Controlled fabrication of a biological vascular substitute. Biomaterials. 2006; **27**(7):1088-1094

[86] Lee SJ, Yoo JJ, Lim GJ, Atala A, Stitze J. In vitro evaluation of electrospun nanofiber scaffolds for vascular graft application. Journal of Biomedical Materials Research Part A. 2007;**83**(4):999-1008

[87] Jeong SI, Lee AY, Lee YM, Shin H. Electrospun gelatin/poly(L-lactide-co-epsilon-caprolactone) nanofibers for mechanically functional tissue-engineering scaffolds. Journal of Biomaterials Science Polymer Edition. 2008;**19**(3):339-357

[88] Zhang YZ, Ouyang HW, Lim CT, Ramakrishna S, Huang ZM. Electrospinning of gelatin fibers and gelatin/PCL composite fibers scaffolds. Journal of Biomedical Materials Research Part B Applied Biomaterials. 2005;**72B**(1):156-165

[89] Zhao PC, Jiang HL, Pan H, Zhu KJ, Chen W. Biodegradable fibers scaffolds composed of gelatin coated poly(epsilon-caprolactone) prepared by coaxial electrospinning. Journal of Biomedical Materials Research Part A. 2007;**83**(2):372-382

[90] Gupta D, Venugopal J, Prabhakaran MP, Dev VRG, Low S, Choon AT, Ramakrishna S. Aligned and random nanofibers substrate for the in vitro culture of Schwann cells for neural tissue engineering. Acta Biomaterialia. 2009;**5**(7):2560-2569

[91] Deng LL, Zhang X, Li Y, Que F, Kang XF, Liu YY, Feng FQ, Zhang H. Characterization of gelatin/zein nanofibers by hybrid electrospinning. Food Hydrocolloids. 2018;**75**:72-80

[92] Tavassoli-Kafrani E, Goli SAH, Fathi M. Fabrication and characterization of electrospun gelatin nanofibers crosslinked with oxidized phenolic compounds. International Journal of Biological Macromolecules. 2017;**103**:1062-1068

[93] Jalaja K, Sreehari VS, Kumar PRA, Nirmala RJ. Graphene oxide decorated electrospun gelatin nanofibers: Fabrication, properties and applications. Materials Science & Engineering C Materials for Biological Applications. 2016;**64**:11-19

[94] Shi R, Geng H, Gong M, Ye JJ, Wu CG, Hu XH, Zhang LQ. Long-acting and broad-spectrum antimicrobial electrospun poly (ε-caprolactone)/gelatin micro/nanofibers for wound dressing. Journal of Colloid & Interface Science. 2017;**509**:275-284

[95] Talebian A, Mansourian A. Release of Vancomycin from electrospun gelatin/chitosan nanofibers. Materials Today Proceedings. 2017;**4**(7):7065-7069

[96] KarbalaeiMandi A, Shahrousvand M, Javadi HR, Ghollasi M, Norouz F, Kamali M, Salimi A. Neural differentiation of human induced pluripotent stem cells on polycaprolactone/gelatin bi-electrospun nanofibers. Materials Science & Engineering C. 2017;**78**:1195-1202

[97] Rather HA, Thakore R, Singh R, Jhala D, Singh S, Vasita R. Antioxidative study of cerium oxide nanoparticle functionalised PCL-Gelatin electrospun fibers for wound healing application. Bioactive Materials. 2017:1-11

[98] Ji W, Yang F, Ma J, Bouma MJ, Boerman OC, Chen Z, van den Beucken JJJP, Jansen JA. Incorporation of stromal cell-derived factor-1α in PCL/gelatin electrospun membranes for guided bone regeneration. Biomaterials. 2013;**34**(3):735-745

[99] Ren L, Wang J, Yang FY, Wang L, Wang D, Wang TX, Tian MM. Fabrication of gelatin–siloxane fibers mats via sol–gel and electrospinning procedure and its application for bone tissue engineering. Materials Science and Engineering: C. 2010;**30**(3):437-444

[100] Li M, Guo Y, Wei Y, MacDiarmid AG, Lelkes PI. Electrospinning polyaniline-contained gelatin nanofibers for tissue engineering applications. Biomaterials. 2006; **27**(13):2705-2715

[101] Blit PH, Battiston KG, Yang M, Paul Santerre J, Woodhouse KA. Electrospun elastin-like polypeptide enriched polyurethanes and their interactions with vascular smooth muscle cells. Acta Biomaterialia. 2012;**8**(7):2493-2503

[102] Meng ZX, Xu XX, Zheng W, Zhou HM, Li L, Zheng YF, Lou X. Preparation and characterization of electrospun PLGA/gelatin nanofibers as a potential drug delivery system. Colloids and Surfaces B: Biointerfaces. 2011;**84**(1):97-102

[103] Huang CH, Chi CY, Chen YS, Chen KY, Chen PL, Yao CH. Evaluation of proanthocyanidin-crosslinked electrospun gelatin nanofibers for drug delivering system. Materials Science and Engineering: C. 2012;**32**(8):2476-2483

[104] Khadka DB, Niesen MI, Devkota J, Koria P, Haynie DT. Unique electrospun fiber properties obtained by blending elastin-like peptides and highly-ionized peptides. Polymer. 2014;**55**(9):2163-2169

[105] Rujitanaroj P-O, Pimpha N, Supaphol P. Wound-dressing materials with antibacterial activity from electrospun gelatin fiber mats containing silver nanoparticles. Polymer. 2008;**49**(21):4723-4732

[106] Dubský M, Kubinová Š, Širc J, Voska L, Zajíček R, Zajícová A, Lesný P, Jirkovská A, Michálek J, Munzarová M, Holáň V, Syková E. Nanofibers prepared by needleless electrospinning technology as scaffolds for wound healing. Journal of Materials Science: Materials in Medicine. 2012;**23**(4):931-941

[107] Bottino MC, Thomas V, Janowski GM. A novel spatially designed and functionally graded electrospun membrane for periodontal regeneration. Acta Biomaterialia. 2011; **7**(1):216-224

[108] Münchow EA, Albuquerque MTP, Zero B, Kamocki K, Piva E, Gregory RL, Bottino MC. Development and characterization of novel ZnO-loaded electrospun membranes for periodontal regeneration. Dental Materials. 2015;**31**(9):1038-1051

IntechOpen