1 Electrospinning: A versatile processing technology for producing nanofibrous materials for biomedical and tissue-engineering applications

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1.1 Introduction

Design and development of functional materials and tailoring their functionalities in biomedical applications is a great challenge [1]. Electrospinning technique has proved to be a relatively simple and versatile method for producing functional nanofibers and nanofibrous membrane. The fundamentals of electrospinning have been known for a long time, and the effects of various processing parameters on the fibers morphology have been extensively studied [2,3]. The exceptional characteristics of electrospun nanofibrous membrane including high surface area, high porosity, flexibility, and adjustable pore-size distribution make them more suitable candidates in a wide range of applications including biomedical and tissue-engineering applications [3–7].

The designing of hierarchical architecture that mimics the extracellular matrices (ECM) of the native tissues is important for rapid and regeneration of tissues [8]. The electrospinning process gives an opportunity to tailor the scaffold topography [5]. Moreover, surface modification of electrospun nanofibers with bioactive molecules improves the interaction between the scaffolds and cells. Generally, scaffolds provide a temporal support for the regeneration/repair of the tissues. Therefore, it could be highly expected that the scaffolds must fulfill the several requirements. The biocompatibility of the material is considered to be an essential factor for preventing the inflammation and toxicity. The biodegradability of the scaffolds is also a very significant fact because the degradation of the scaffolds and regeneration of the tissues should happen in a timely manner [4].

The diameter and morphology of the nanofibers can be effectively tuned by the number of processing parameters. The collector design has led to tune the orientation of fibers. The uniaxially aligned nanofiber scaffolds can only promote the cell migration along one specific direction and are thus not useful as dural substitutes. In order to promote cell migration from the surrounding tissue to the center of a dural defect and

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shorten the time for healing and regeneration of dura mater, a surface patterned with a radially aligned, nanoscale features would be highly desired for an artificial dural substitute. More specifically, scaffolds constructed with radially aligned nanofibers could meet such a demand by guiding and enhancing cell migration from the edge of a dural defect to the center [9]. The dynamic rotating collector is the most widely accepted approach for fabricating aligned fibers from different polymers. The promising use of nanofibers in drug delivery system might result in the salient features such a high loading capacity and concurrent release of diverse therapies. A variety of different drugs ranging from antibiotics and anticancer agents to proteins, aptamer, DNA, and RNA have been successfully incorporated into electrospun fibers [10,11].

The performance of the incorporated biomolecules often loses their activity due to conformational changes in the harsh solvent environment. The modification of the nozzle configuration paves a way to prepare the core-shell morphology. Such a promising system provides a protective environment and sustained drug release because the prepared core-shell fibers have immense potential to preserve the drugs during the electrospinning process. Even though there were a huge number of research reports on the development of electrospun nanofibrous scaffold, researchers are still gaining new insights and developing new ways of utilizing this technique for producing biomedical materials [7].

This chapter highlights the significance of electrospinning approach in fabricating advanced functional nanofibrous scaffolds for biomedical applications including tissue engineering, drug release, wound dressing, and antimicrobial. We do not make any effort to provide library of literature for specific application rather provide a brief view on the advancement of electrospinning approach to prepare a functional nanofibrous biomaterials.

1.2 Biomedical application of electrospun nanofibers

1.2.1 Drug delivery

An ideal drug delivery system should possess the characteristic to enable the controlled release of drugs toward alleviating medical conditions at a defined rate over a definite time period. Electrospun nanofibers are recognized as an advantageous material in drug delivery owing to their higher surface area with interconnected pores, which offer better dissolution and high therapeutics loading capacity. More importantly, the rate of drug release can be tailored by ease tuning of electrospun nanofiber properties including fiber diameter, porosity, and drug-binding mechanism. Until now, a wide variety of drugs and biomolecules have been successfully loaded into electrospun nanofibers using various approaches mainly by coating, embedding, and encapsulating [7,12,13]. The possible three different modes of physical drug-loading method on electrospun nanofiber surface for drug delivery application are given in Fig. 1.1.

A one-step, single-nozzle electrospinning technique was used to fabricate electrospun composite nanofibers containing nanoparticles for the programmable release of dual drugs by Wang et al. [14]. Briefly, dual drug-loaded chitosan (CS)

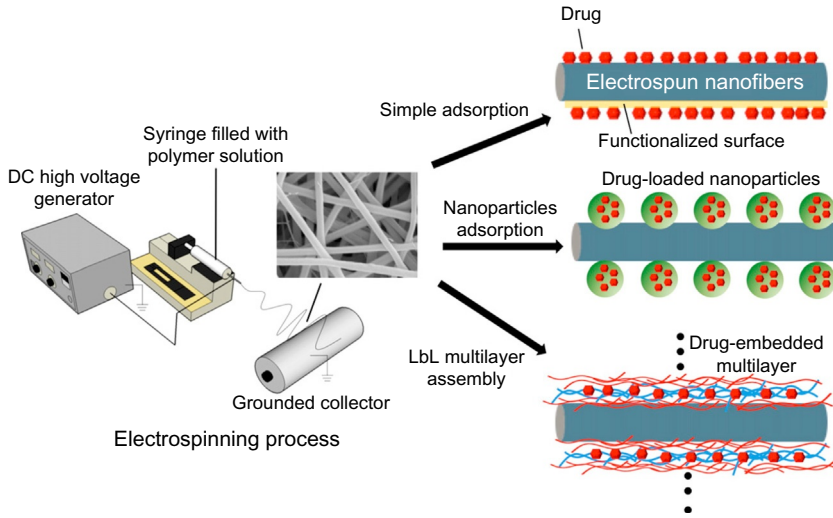

Fig. 1.1 Three modes of physical drug loading on the surface of electrospun nanofibers. Reprinted with permission from Elsevier (Yoo HS, Kim TG, Park TG. Surface-functionalized electrospun nanofibers for tissue engineering and drug delivery. Adv Drug Delivery Rev 2009;61:1033–42).

nanoparticle-containing composite nanofibers were prepared for topical drug delivery applications. Fig. 1.2 illustrates the electrospinning setup and the preparation of nanoparticle-containing composite nanofibers. The controlled and programmable release of two drugs was achieved through their different distributions in the shell and core regions of the composite nanofibers, respectively. Fig. 1.3 shows the optical and fluorescence images of the nanoparticles encapsulated by the electrospun composite nanofibers. The clear distribution of the rhodamine B-loaded and fluoresce in isothiocyanate (FITC)-labeled CS nanoparticles was observed. The laser scanning confocal microscopy (LSCM) imaging revealed the core-shell structure of the composite nanofibers, which consisted of a polycaprolactone (PCL) shell and an array of CS nanoparticles as the core. The release behaviors of the drugs indicated that a controlled release pattern for dual drugs was achieved by adjusting the process used to prepare the electrospinning solution.

In order to overcome the limitations of single-nozzle electrospinning, coaxial electrospinning technique has been attracted much attention for encapsulating fragile, water-soluble bioactive agents and their controlled release. The coaxial electrospinning also controls initial burst release with their core-sheath nanofibers with a blank (drug-free) sheath. In addition, coaxial electrospinning offers the advantage over more sustained release of the encapsulated agents and single-step coencapsulation of multiple drugs with different soluble nature [15]. A study reported by Mickova et al. [16] showed that the liposomes encapsulated with horseradish peroxidase embedded polyvinyl alcohol-core/poly-ε-caprolactone-shell nanofibers prepared by

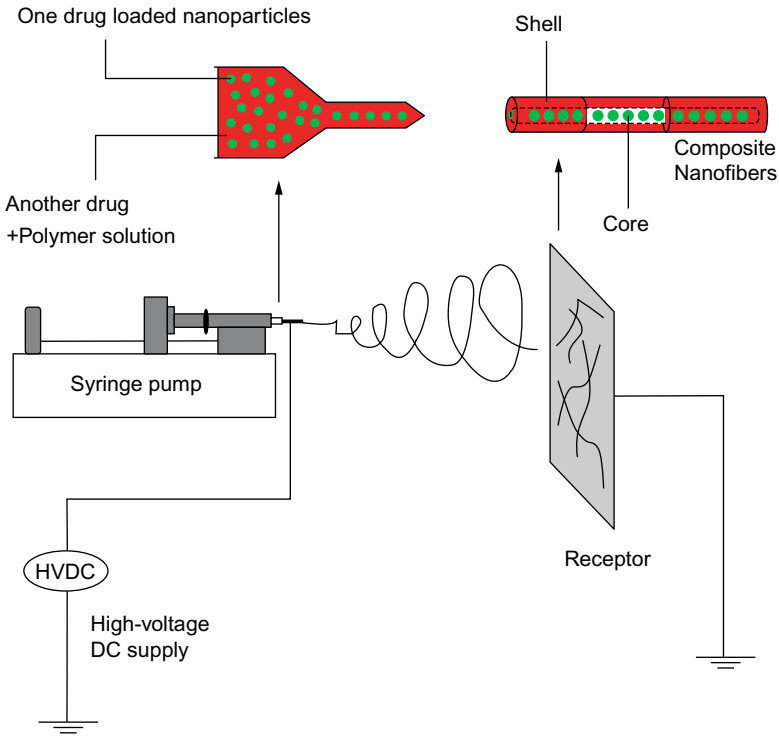

Fig. 1.2 Schematic illustration of the electrospinning setup and the preparation of nanoparticle-containing composite nanofibers.
Reprinted with permission from Nature Publishing Group (Wang Y, Qiao W, Wang B, Zhang Y, Shao P, Yin T. Electrospun composite nanofibers containing nanoparticles for the programmable release of dual drugs. Polym J 2011;43:478–83).

coaxial electrospinning revealed enhanced performance than the fibers prepared by blend electrospinning.

Man et al. [17] developed a codelivery system using a polyvinyl pyrrolidone/bovine serum albumin/recombinant human transforming growth factor-β1 (rhTGF-β1) composite solution as the core fluid and PCL solution as the sheath fluid. Subsequently, bone marrow-derived stem cell (BMSC)-affinity peptide E7 was conjugated to the coaxial electrospun fibers to develop a codelivery system of rhTGF-β1 and E7. Fig. 1.4 shows the schematic illustration of the preparation process and working hypothesis for coaxial electrospun fiber scaffolds. The bioactive assay demonstrated that the rhTGF-β1 released from the peptide-modified scaffolds was at least partially bioactive. Based on the outcome of the study, it is concluded that the prepared rhTGF-β1 in the core and E7 in the PCL shell of the fibers (CBrhTE) scaffold has several properties that can not only enhance BMSC adhesion and growth but also promote their chondrogenic differentiation in vitro.

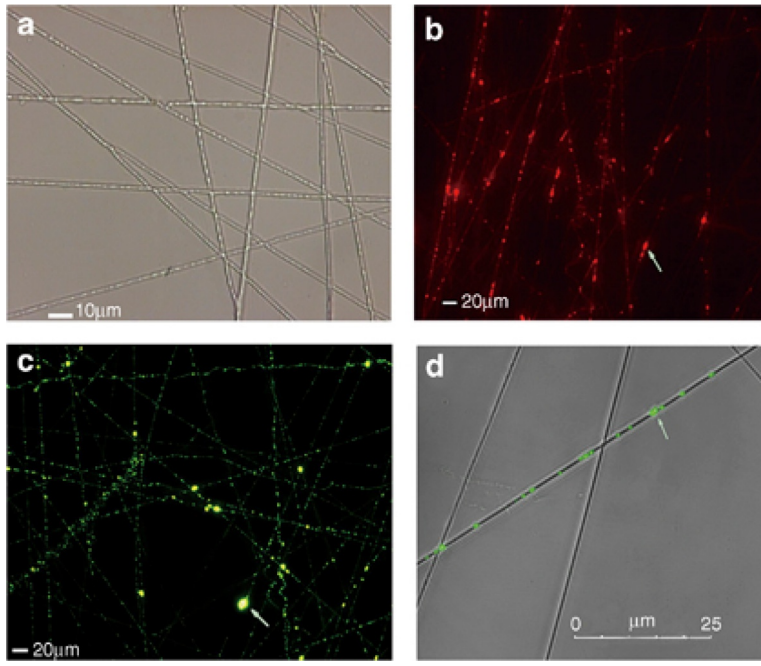

Fig. 1.3 Electrospun composite nanofibers containing chitosan nanoparticles: (A) optical image; (B) and (C) fluorescence images of composite nanofibers containing rhodamine B- and fluorescein isothiocyanate (FITC)-labeled nanoparticles, respectively; and (D) laser scanning confocal microscopy image.
Reprinted with permission from Nature Publishing Group (Wang Y, Qiao W, Wang B, Zhang Y, Shao P, Yin T. Electrospun composite nanofibers containing nanoparticles for the programmable release of dual drugs. Polym J 2011;43:478–83).

Recently, Dubey and Gopinath [18] fabricated electrospun poly(ethylene oxide)-poly(capro lactone) composite nanofibers for codelivery of niclosamide (nic) and silver nanoparticles (Ag NPs). Initially, the drugs (niclosamide (nic)) and Ag NPs were loaded separately and together (nic@Ag NPs) into the nanofiber, and their in vitro release was investigated. The drug release profile and kinetic studies have been shown in Fig. 1.5. There was a distinct release profile for the drug and nanoparticles released from nic, Ag NPs, and nic@Ag NPs composite nanofibers once brought into contact with the hydrophilic environment. The drug and nanoparticle both showed initial burst release around 15% and 18%, respectively, which could be due to surface adhered particles and initial rapid dissolution of the PEO polymer as shown in Fig. 1.5. The initial rapid phase was followed by slow controlled release of nanofibers, which could be around 43% for the drug and 50% for the nanoparticle in 20 days and 100 h, respectively (Fig. 1.5A and B). Water intrusion into the scaffold is of noteworthy importance for the study of release and degradation kinetics. Thus, diffusion and dissolution are the key players of the drug release mechanism, and the schematic presentation of drug

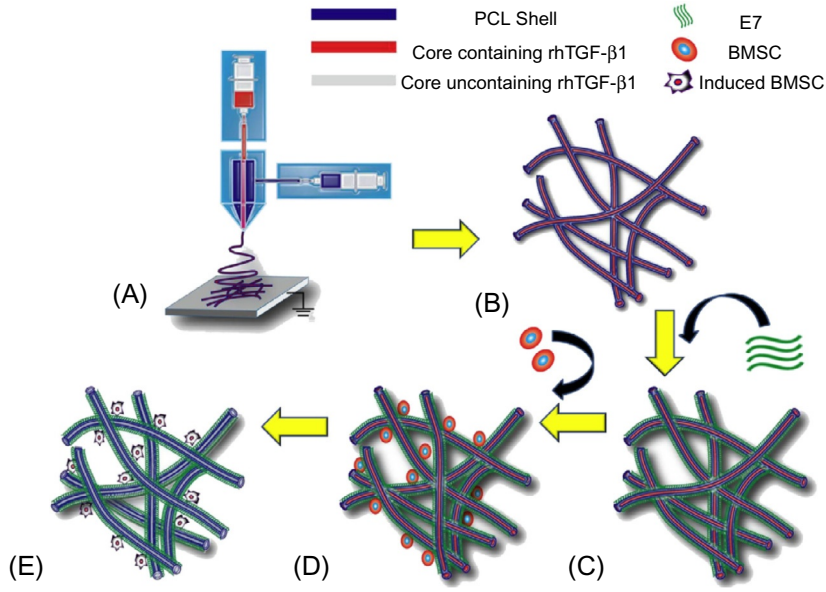

Fig. 1.4 Schematic illustration of the preparation process and working hypothesis for coaxial electrospun fiber scaffolds. (A) The setup of coaxial electrospinning: the spinneret is composed of two concentric needles; the outer needle is used to deliver the shell solution (blue), while the inner needle is used to eject the core solution (red). (B) Scaffold composed of electrospun coaxial fibers, with core (red) and shell (blue) structure. (C) Scaffold conjugated with the BMSC-specific affinity peptide (E7) (green). (D) E7-modified scaffold promoting adhesion of BMSCs onto the scaffold. (E) The rhTGF-β1 encapsulated in the core of the coaxial fibers could be released in a sustained manner (the core changing from red in D to gray in E) to promote chondrogenic differentiation of BMSCs adhered on the scaffolds.
Reprinted with permission from Elsevier (Man Z, Yin L, Shao Z, Zhang X, Hu X, Zhu J, et al. The effects of co-delivery of BMSC-affinity peptide and rhTGF-ß1 from coaxial electrospun scaffolds on chondrogenic differentiation. Biomaterials 2014;35:5250–60).

release from various composite nanofibers has been shown in Fig. 1.5C. It was found that the drug showed sustained and controlled release followed by initial burst release from both drug alone loaded and nic@Ag NPs loaded nanofibers. The codelivery of anticancer drugs nic@Ag NPs from nanofibers displayed superior anticancer potential in vitro when compared with nic alone or Ag NPs composite nanofibers. Additionally, nic@Ag NPs showed better therapeutic efficacy against MCF-7 cells.

A new strategy for creating functional trilayer nanofibers through triaxial electrospinning was demonstrated by Yu et al. [19] in which each layer was based on the same polymer matrix with different functional components or compositions. Ethyl cellulose (EC) was used as the filament-forming matrix in the outer, middle, and inner working solutions and was combined with varied contents of the model active ingredient ketoprofen (KET) in the three fluids, and their dissolution was determined in accordance with the Chinese Pharmacopoeia. A diagram illustrating the

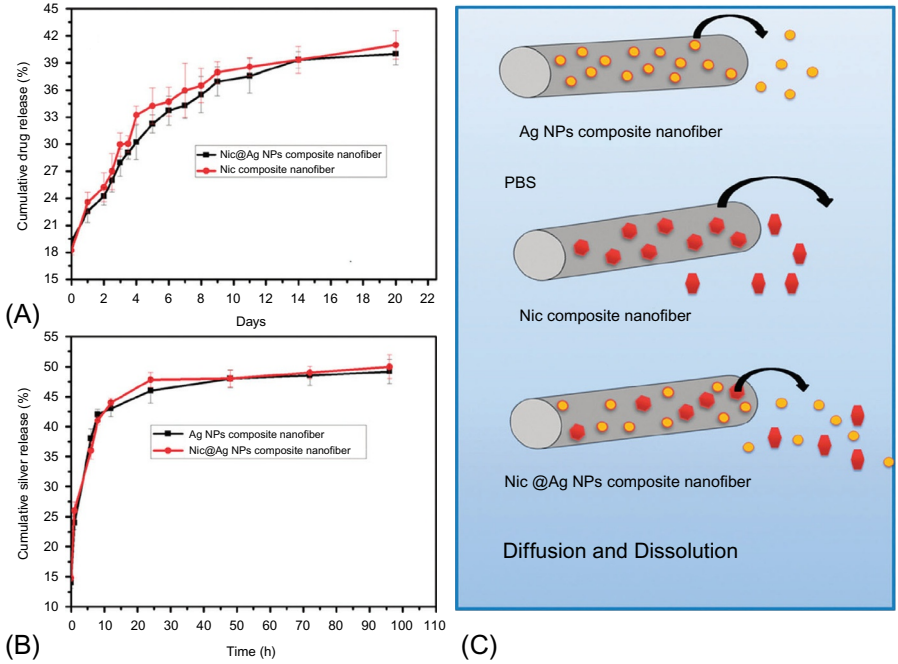

Fig. 1.5 (A) Cumulative drug release profile of the drug released from the drug alone and the nic@Ag NP composite nanofiber. (B) Ag NP release profile from Ag NPs alone and nic@Ag NP composite nanofibers. (C) Schematic illustration of drug released from various composite nanofibers.
Reprinted with permission from The Royal Society of Chemistry (Dubey P, Gopinath P. Fabrication of electrospun poly(ethylene oxide)–poly(capro lactone) composite nanofibers for co-delivery of niclosamide and silver nanoparticles exhibits enhanced anti-cancer effects *in vitro*. J Mater Chem B 2016;4:726–42).

triaxial electrospinning process is shown in Fig.1.6A, and a digital photograph of the triaxial electrospinning process is presented in Fig. 1.6B. Monolithic nanofibers prepared individually from each of the three working solutions using single-fluid electrospinning have similar drug release profiles. An initial burst release is followed by a slowing in the release rate. In sharp contrast, the three-layer nanofibers were able to provide zero-order KET release for over 20 h with a best-fit equation of $Q = 1.73 + 4.24\ t$ ($R^2 = 0.9972$), where Q is the drug release percentage, t is the release time, and R is the correlation coefficient. The initial burst release and the slow down at the end of the dissolution process are negative phenomena that inevitably arise with monolithic drug-loaded nanofibers. No burst release was observed from the triaxial nanofibers, and the drug was released at a constant rate for nearly 1 day. This study developed an advanced functional nanofibrous materials using a triaxial electrospinning process and solutions of the same filament-forming polymer matrix for all three working

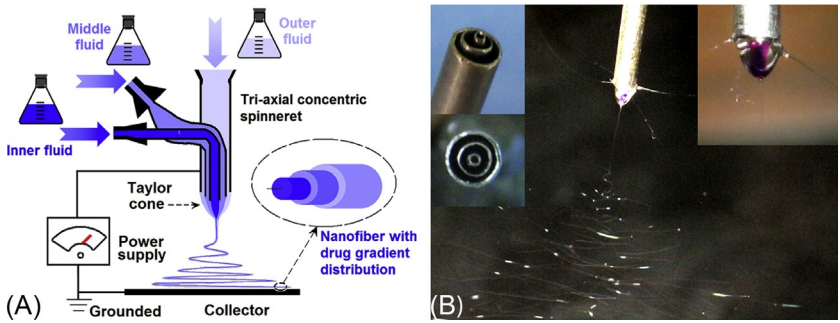

Fig. 1.6 (A) Schematic of the triaxial electrospinning process. (B) Digital photographs of the triaxial electrospinning process. The upper-left insets show the structure of the homemade spinneret, and the upper-right inset show a typical compound Taylor cone.
Reprinted with permission from American Chemical Society (Yu DG, Li XY, Wang X, Yang JH, Bligh SWA, Williams GR. Nanofibers fabricated using triaxial electrospinning as zero order drug delivery systems. ACS Appl Mater Interfaces 2015;7:18891–7).

fluids. A range of different types of functional nanofibrous materials can be generated from this electrospinning process.

1.2.2 Tissue engineering

The theme of the tissue engineering is dedicated to improve/regenerate the damaged tissues. The exceptional characteristics of electrospun nanofibers including flexibility, highly porous, and interconnected three-dimensional structures mimic the extracellular matrix (ECM) that effectively support the cell adhesion and deliver growth factors. The fibrous structure, pore geometry, and interconnecting pores are significant for cell attachment, migration, growth, and nutrient flow. The biocompatibility and biodegradability of the selected scaffold is an essential parameter in regulating the cell growth rate and preventing the inflammation and toxicity. The architecture of the fibrous scaffolds affects the proliferation and differentiation of the cellular activities. And further, the efficiency of the fibrous scaffold is improved by modifying the fiber surface chemically and physically with bioactive molecules and ligands [5,20,21]. The surface modification not only changes the chemical composition of the surface but also tunes the wetting characteristics of the membrane that can provide the more favorable environment for cellular adhesion. The various surface modification technique for tuning the characteristics of the fiber surface is demonstrated in Fig. 1.7. The plasma treatment is a convenient and cost-effective method that enables the introduction of the various active groups such as carboxyl groups and amine groups on fiber surface. The prepared hydrophilized nanofibers are generally shown to enhance the fibroblast adhesion and proliferation [13]. For example, various kinds of electrospun nanofibers were successfully modified and demonstrated their enhanced performance [20,22,23].

Tian et al. [23] demonstrated the fabrication of poly(lactic acid) (PLA)/silk fibroin/nerve growth factor (PS/N) by encapsulating nerve growth factor (NGF) along with

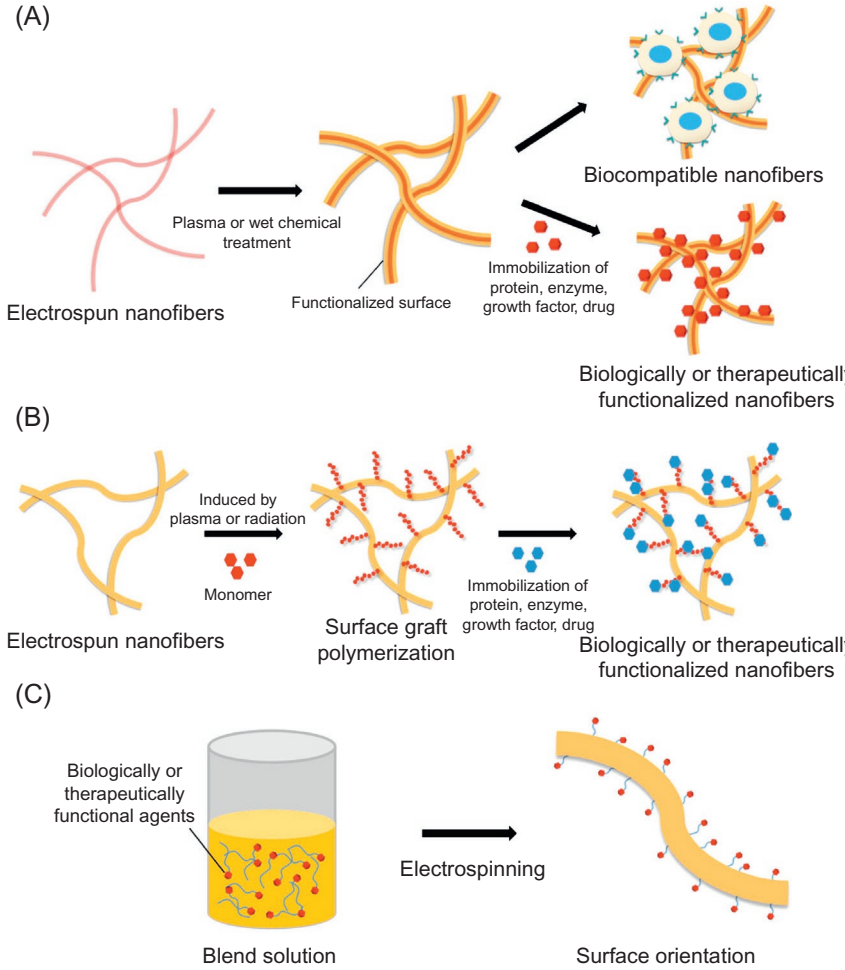

Fig. 1.7 Surface modification techniques of electrospun nanofibers. (A) Plasma treatment or wet-chemical method. (B) Surface graft polymerization. (C) Coelectrospinning. Reprinted with permission from Elsevier (Yoo HS, Kim TG, Park TG. Surface-functionalized electrospun nanofibers for tissue engineering and drug delivery. Adv Drug Delivery Rev 2009;61:1033–42).

silk fibroin (SF) as the core of the scaffold. Air plasma treatment was adopted to improve the hydrophilicity of the PS/N surface without causing any damage to the nanofibers. The fibrous scaffold was prepared by using coaxial electrospinning method, and the preparation procedure of p-PS/N scaffold is schematically demonstrated in Fig. 1.8. Initially, pure PLA scaffold was exposed to plasma at various time periods (60, 90, 120, 150, and 180 s). The observed morphological and wetting characteristics suggested that the optimized time period for air plasma treatment was evaluated as 120 s.

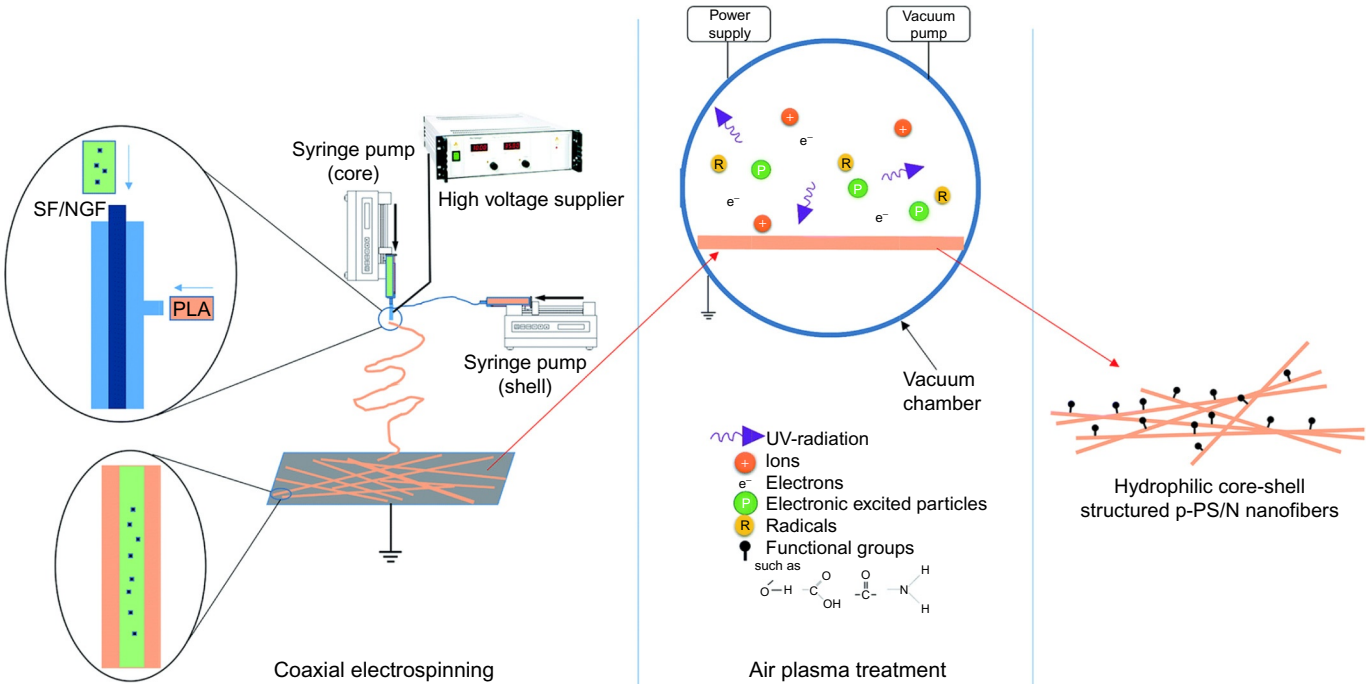

Fig. 1.8 Schematic illustration of the electrospinning and air plasma treatment toward the formation of p-PS/N scaffolds.
Reprinted with permission from Royal Society of Chemistry (Tian L, Prabhakaran MP, Hu J, Chen M, Besenbacher F, Ramakrishna S. Coaxial electrospun poly(lactic acid)/silk fibroin nanofibers incorporated with nerve growth factor support the differentiation of neuronal stem cells. RSC Adv 2015;5:49838–48).

The change in the fiber morphology, surface roughness, and hydrophilicity of PS/N nanofibers before and after plasma treatment was studied, and the results were shown in Fig. 1.9. The observed results suggested that there is no significant change in the fiber diameter. The measured diameters of PS/N and p-PS/N were to be 221 ± 49 and 228 ± 39 nm, respectively. The dramatic change in the water contact angle from 133.60° to 0° proves that the plasma treatment alters the hydrophilicity of the scaffolds significantly ($P \leq 0.05$), by introducing functional groups on the surfaces. But, the plasma treatment does not have any terrible impact on the fibers morphology. The values of average roughness (Ra) for PS/N and p-PS/N were measured to be

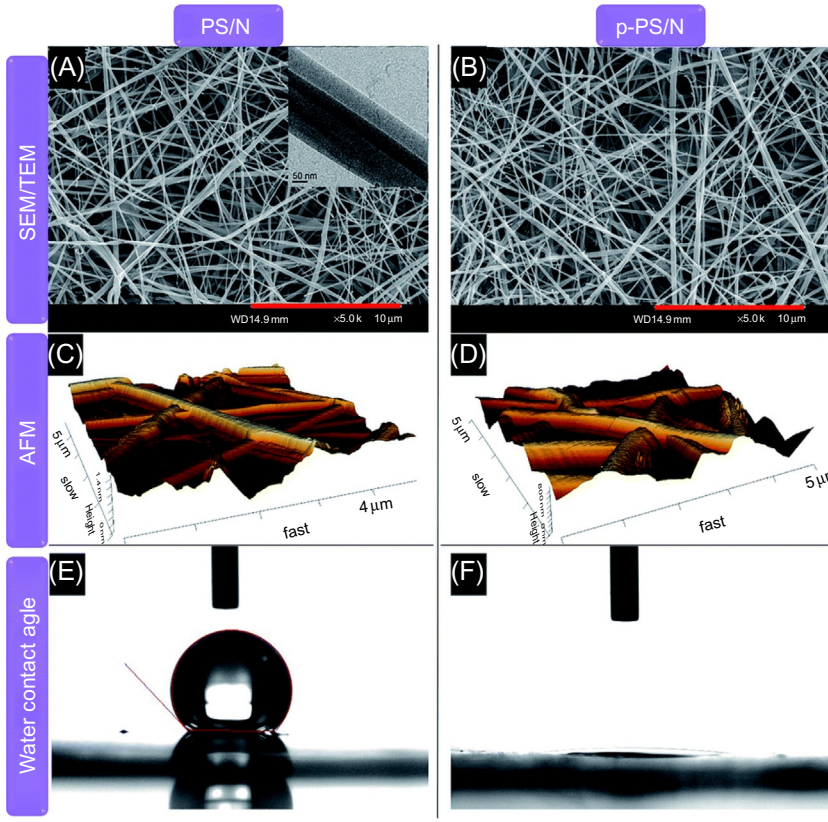

Fig. 1.9 Fiber morphologies, surface roughness and hydrophilicity of PS/N and p-PS/N scaffolds. SEM images of (A) PS/N and (B) p-PS/N scaffolds. The in situ picture shown within (A) is the TEM image, which shows the core-shell structure of the nanofibers. The AFM images of (C) PS/N and (D) p-PS/N scaffolds and the water contact angle of (E) PS/N and (F) p-PS/N scaffolds; the scale bar for (A) and (B) is 10 mm.
Reprinted with permission from Royal Society of Chemistry (Tian L, Prabhakaran MP, Hu J, Chen M, Besenbacher F, Ramakrishna S. Coaxial electrospun poly(lactic acid)/silk fibroin nanofibers incorporated with nerve growth factor support the differentiation of neuronal stem cells. RSC Adv 2015;5:49838–48).

217.97 ± 38.53 and 171.63 ± 32.50 nm, respectively. The surface of the nanofibers became smoother after plasma treatment. Further, MTS assay has been studied to evaluate the PC12 cell proliferation. The gradual increase in the cell proliferation has been noted on tissue culture plate (TCP) for all electrospun nanofibers from day 2 to day 8. After growing the cells for a period of 6 days, the cells were able to contact each other and facilitated the growth of the neighboring cells. NGF is a factor that is supportive toward PC12 cells differentiation, and PC12 stops dividing and proliferating once they are treated with certain concentration of NGF.

The plasma treatment sometime cannot be effectively modified the buried nanofibers surface due to the limited penetration depth of plasma in the nanopores. It is believed that wet-chemical etching approach can offer the flexibility for surface modification of the thick nanofibrous meshes. The concentration of the hydrolyzing agent and hydrolysis time is an important parameter to produce the optimal functional groups on the fiber surface [13,24]. In general, most of the synthetic biodegradable polymers retain their hydrophobic surface nature, but the creation of hydrophilic surface is highly required for ideal cellular responses. Not only surface graft polymerization has been applied to create the surface hydrophilicity, but also it can be used to introduce the various functional groups on the surface of the fibers for enhanced cell adhesion, proliferation, and differentiation [13,25].

In the recent past, much effort has been made to optimize the physiological behavior of cells by designing the suitable three-dimensional (3-D) geometry, chemical composition, and topography. The diameter of the electrospun fibers, alignment of the fibers, can have a great impact on cell adhesion, proliferation, and cell morphology. The aligned fibers are very attractive topographical cues to guide the cell growth with the desired morphology than randomly oriented fibers [26–28]. In addition to that, it has been found that the cellular activities such as cell proliferation and differentiation are modulated through electric stimulation [29]. The recent research reports have been demonstrated that the possible preparation of conductive electrospun fibers is by incorporating the various conductive materials such as polyaniline, carbon nanotubes, and polypyrrole [26,27,29,30]. The study by Chen et al. [26] prepared a nanofibrous scaffold that could simultaneously provide the two types of guidance cues, electric and topographical, to cells.

The prepared scaffold was composed of electrically conductive nanofibers with highly oriented structures and was fabricated by electrospinning a blended solution of poly(ε-caprolactone) (PCL) and PANi. PCL has been selected as a core material because of its good biodegradability, biocompatibility, and mechanical properties. The well-aligned electrospun nanofibers are prepared by using two parallel block magnets collector in electrospinning setup. The electrospinning conditions and PANi content on the morphology and alignment of PCL/PANi composite nanofibers were well studied. The SEM images and histograms of the orientation distribution of the PCL/PANi-3 nanofibers at various solution flow rates are shown in Fig. 1.10. The observed results have been well proved that the alignment fibers are increased with decreasing solution flow rate. The optimum fiber alignment was achieved at a flow rate of 0.1 ml h^{-1}, and more than 90% of the fibers were found to be aligned within $\pm 10^{\circ}$ of the preferred direction (Fig. 1.10D).

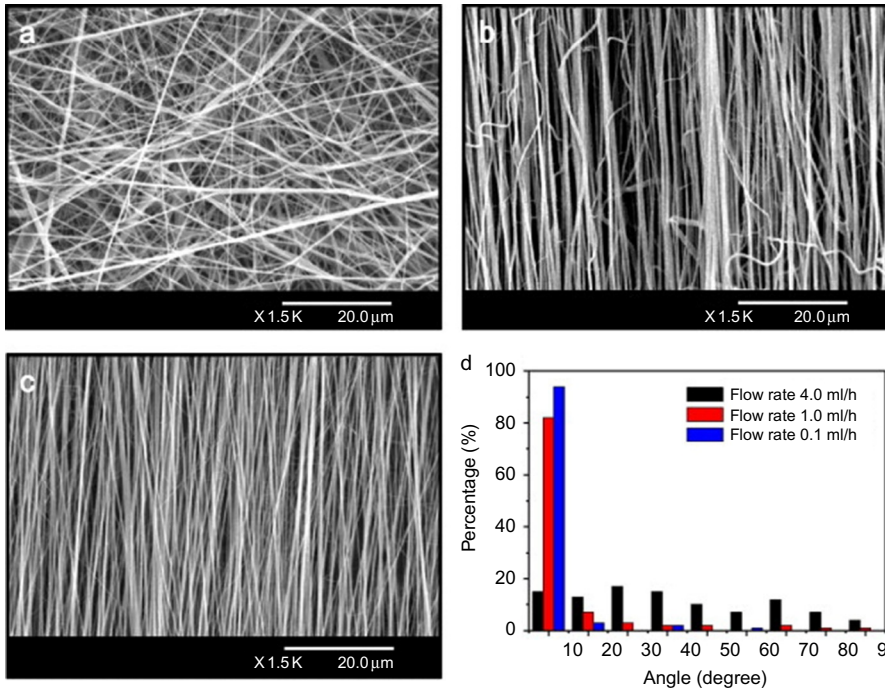

Fig. 1.10 (A–C) The representative SEM micrographs and (D) histograms of the orientation distribution of the PCL/PANi-3 nanofibers fabricated using the MFAES method at solution flow rates of (a) 4.0, (b) 1.0, and (c) 0.1 ml h^{-1}.
Reprinted with permission from Elsevier (Chen MC, Sun YC, Chen YH. Electrically conductive nanofibers with highly oriented structures and their potential application in skeletal muscle tissue engineering. Acta Biomater 2013;9:5562–72).

The designing of scaffolds for treating the skeletal muscle defects requires highly aligned fibrous membrane because skeletal muscle has a highly aligned architecture that consists of long, parallel multinucleated myotubes that are formed through the differentiation and fusion of myoblasts [31]. To overcome this problem, the highly aligned PCL/PANi nanofibers are prepared to provide functional scaffolds for engineering the parallel aligned myoblasts and myotubes. Murine skeletal muscle cells (C2C12 myoblasts) have been taken as a model to explore the effects of the electric cues and aligned topography of nanofibrous scaffolds on cell viability and alignment. The representative immunofluorescent images of myotubes differentiated for 5 days on the fibrous scaffolds and immunostained for MHC (green) and DAPI (blue) are demonstrated in Fig. 1.11. The aligned nanofibrous scaffolds (A-PCL and A-PCL/PANi) induced muscle cell alignment and promoted myotube formation compared with the randomly oriented nanofibers (R-PCL). In addition, the incorporation of electrically conductive PANi into the PCL fibers (R-PCL/PANi and A-PCL/PANi) also enhanced myoblast differentiation compared with the R-PCL nanofibers.

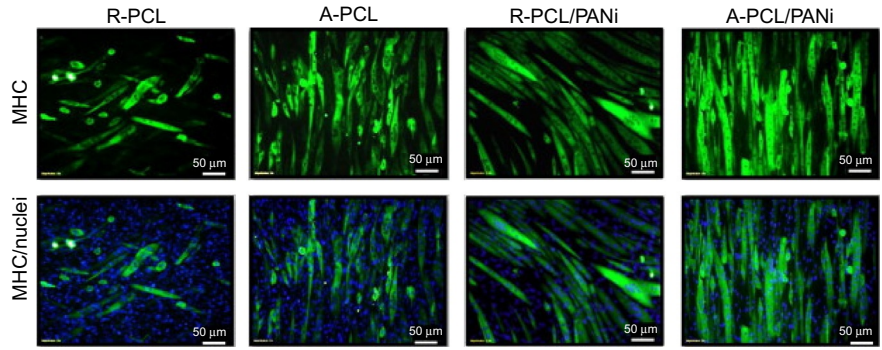

Fig. 1.11 Representative immunofluorescent images of myotubes differentiated for 5 days on random PCL (R-PCL), aligned PCL (A-PCL), random PCL/PANi-3 (R-PCL/PANi), and aligned PCL/PANi-3 (A-PCL/PANi) nanofibers and immunostained for MHC (green) and nucleus (blue). Reprinted with permission from Elsevier (Chen MC, Sun YC, Chen YH. Electrically conductive nanofibers with highly oriented structures and their potential application in skeletal muscle tissue engineering. Acta Biomater 2013;9:5562–72).

So far, it has been understood that the electrospun nanofibrous membrane acts as an excellent scaffold for various kinds of tissue engineering. However, the shrinkage and distortion of the electrospun scaffolds restrict the initial cell adhesion and further growth. Ru et al. [32] developed and demonstrated the suspended, shrinkage-free, electrospun. Poly(lactic-co-glycolic acid, PLGA) nanofibrous scaffold for skin tissue engineering. The obtained electrospun PLGA mat is able to maintain its initial shape and size with the auxiliary support from a polypropylene (PP) ring. The used polypropylene (PP) in this study is mechanically strong, chemically stable, and biocompatible. It has a density of 0.91 g/cm^3 enabling the scaffold to suspend in cell culture medium. The use of a PP ring as auxiliary support serves two purposes: (1) to make the overall nanofiber scaffold suspend in liquid, which is necessary for skin cells to be exposed to air during growth, and (2) to prevent nanofiber assemblies from shrinking and maintain long-term membrane integrity. The procedure for constructing the suspending nanofiber scaffolds is schematic illustrated in Fig. 1.12. Initially, the nanofiber assemblies were opened longitudinally and cut into circular membranes with a diameter of 20 mm. And then, an auxiliary plastic ring made of PP paper was heat sealed with the circular PLGA nanofiber membrane. The PLGA membrane fuses onto the PP ring to form a complete scaffold since the melting points of PLGA and PP are approximately 60°C and 170°C, respectively.

The applied high-electric field during the electrospinning process induces the inner stress in the nanofibrous system. The macromolecular chains can rapidly relax when fibrous membrane got immerged into solvent at a temperature higher than 35°C. The result of which induces the shrinkage in the PLGA fibrous assembly. The original and shrunken fibrous assemblies after incubating in PBS at 37°C for 24 h are shown in Fig. 1.13A and B. In contrast, for a PLGA scaffold consisting of a nanofiber assembly fused on a PP ring, no observable shrinkage occurred because of the enhanced

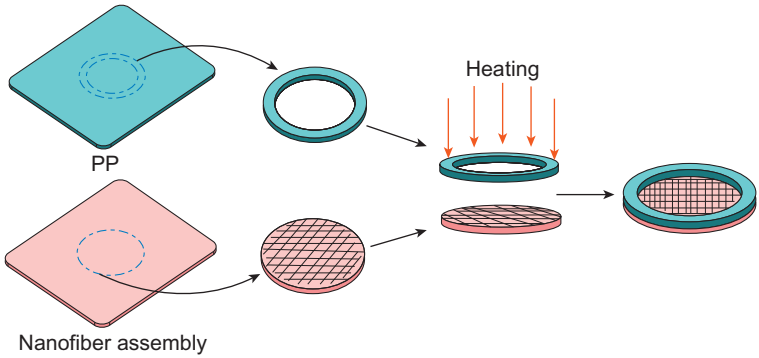

Fig. 1.12 Schematic illustrating steps for constructing suspending nanofiber scaffolds. Reprinted with permission from American Chemical Society (Ru C, Wang F, Pang M, Sun L, Chen R, Sun Y. Suspended, shrinkage-free, electrospun PLGA nanofibrous scaffold for skin tissue engineering. ACS Appl Mater Interfaces 2015;7:10872–7).

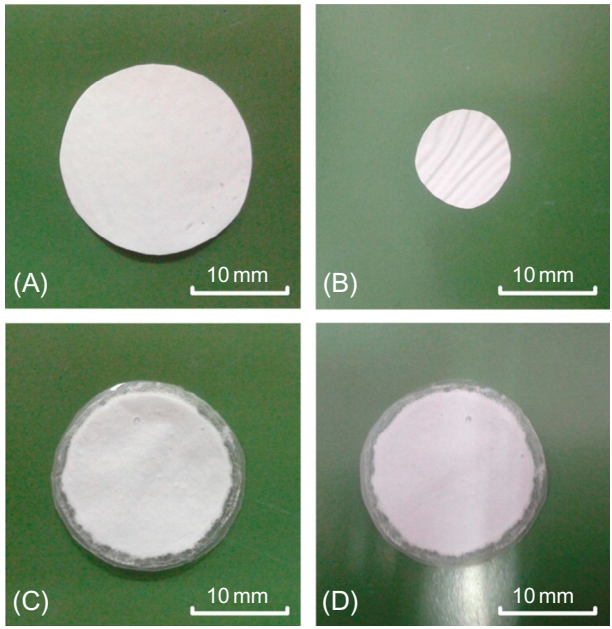

Fig. 1.13 Shrinkage test of fibrous membranes. (A, B) Nanofibrous assembly before and after incubation in PBS at 37°C for 24 h. (C) Scaffold formed by fusing a nanofibrous assembly on a PP ring. (D) The same scaffold after incubation in PBS at 37°C for 24 h. Reprinted with permission from American chemical Society (Ru C, Wang F, Pang M, Sun L, Chen R, Sun Y. Suspended, shrinkage-free, electrospun PLGA nanofibrous scaffold for skin tissue engineering. ACS Appl Mater Interfaces 2015;7:10872–7).

mechanical property. Fig. 1.13C and D shows an original scaffold and the same scaffold after incubation in PBS at 37°C for 24 h.

The inadequate mechanical properties and low pore-structure controllability of electrospun nanofibrous membrane limit further their performance in tissue engineering. To overcome these limitations, the electrospinning approach has been coupled with various methods including melt-plotting method. The study by Yang et al. [33] demonstrated the fabrication of hybrid scaffold composed of microsized struts by using direct electrospinning writing (DE-writing) coupled with melt-plotting method. The obtained scaffold shows the good mechanical strength, and the width of the electrospun fibrous mat is controllable. Another main part of this study focuses on the creation of pore structure that is believed to have a high impact on cellular behavior. The schematic illustration of preparation method for hybrid scaffolds (HS) is shown in Fig. 1.14. The obtained hybrid scaffolds are comprised of melt-plotted struts and an electrospun fibrous layer.

The morphology of the scaffold structure also severely affects the cell attachment, proliferation, and infiltration in addition to the factors including pore size, porosity, and pore interconnectivity. The morphology of the three different pure melt-plotted polycaprolactone (PCL) scaffold (MC), conventional hybrid scaffold (HS1) consisting of a completely interlayered fibrous mat in the multilayered PCL struts (HS1), and novel hybrid scaffold (HS2) using DE-writing are presented in Fig. 1.15. The observed results clearly show that each scaffold type is composed of melt-plotted PCL layer with a strut diameter and pore size of 701 ± 63 μm and 1.2 ± 0.2 mm, respectively.

The hybrid scaffold is composed of additional layer, which is generated by DE-writing. The melt-plotted struts and electrospun fibers produced two different pores

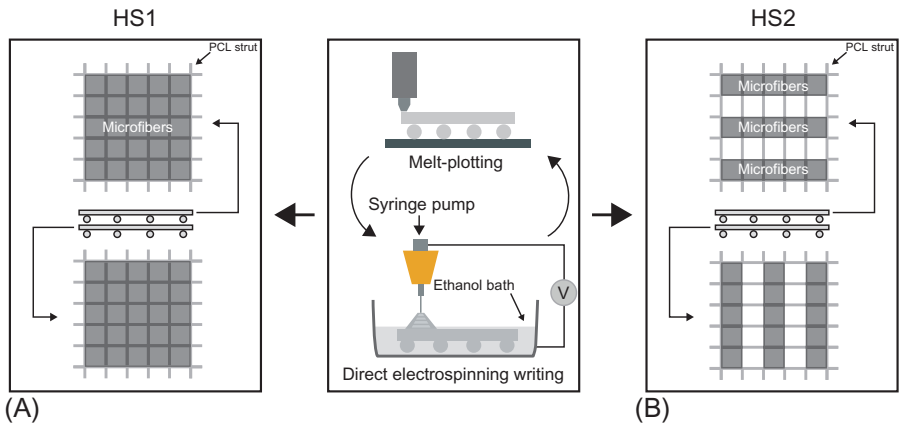

Fig. 1.14 Schematic illustrating generation of the hybrid scaffolds using melt-plotting method and direct electrospinning writing. (A) Fibrous mat covering the whole layer. (B) Fibrous mat covering three lines in the first and second layers horizontally and vertically, respectively. Reprinted with permission from Elsevier (Yang GH, Mun F, Kim G. Direct electrospinning writing for producing 3D hybrid constructs consisting of microfibers and macro-struts for tissue engineering. Chem Eng J 2016;288:648–58).

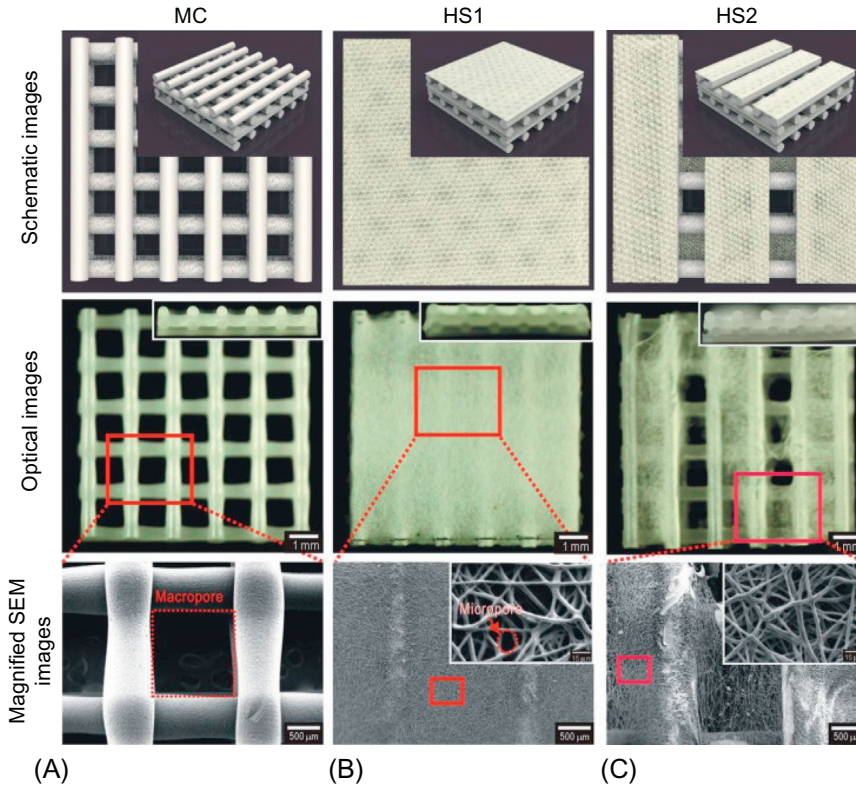

Fig. 1.15 Schematic, optical, and SEM images of (A) the MC scaffold fabricated using the melt-plotting method and two hybrid scaffolds, (B) HS1 and (C) HS2, fabricated using the melt-plotting/direct electrospinning writing.
Reprinted with permission from Elsevier (Yang GH, Mun F, Kim G. Direct electrospinning writing for producing 3D hybrid constructs consisting of microfibers and macro-struts for tissue engineering. Chem Eng J 2016;288:648–58).

(macropores and micropores) in HS2 scaffolds. The created macropores in the HS2 scaffold could influence the cellular infiltration, nutrient transport, and metabolism, and micropores could enhance the cell attachment and proliferation. Thus, the prepared HS2 scaffold can overcome the limitations found in the HS1 because the insufficient pore size of the interlayered electrospun fibrous mat does not allow the easy migration and proliferation. The in vitro cellular activities suggest that the hybrid scaffold (HS2) shows a promise for regenerating hard tissues than conventional hybrid scaffold (HS1).

1.2.3 Wound dressing

The wound-care management is considered to be a serious health-care issue since the skin is the main organ of the body and plays vital role in multiple functions [34]. Depending on the healing time, the wounds are categorized as acute and chronic in

which the chronic wounds are highly exposed to the risk of bacterial infection. Acute wounds are generally caused by traumas, and the wounds are usually healable within 8–12 weeks. This kind of wounds can happen by mechanical damage, stabbing action of hard objects, exposure of extreme heat, irradiation, and so on. But the chronic wounds are formed as a result of chronic diseases such as diabetes, tumors, and severe physiological contaminations. The time period for healing the chronic wound needs more than 12 weeks [35]. According to the appearance, the wounds are further classified in different four types: epithelializing (clean, medium-to-high exudates), granulating (clean and exudating), slough-covered, and necrotic (dry) wounds as seen in Fig. 1.16.

As we look back upon history, the first records of wound care can be found in ancient Sumerians who used to apply poultices of mud, milk, and plants to wound, and Egyptians prepared plasters of honey, plant fibers, and animal fats as bandages for the wounds [36]. The outstanding characteristics of electrospun nanofibrous

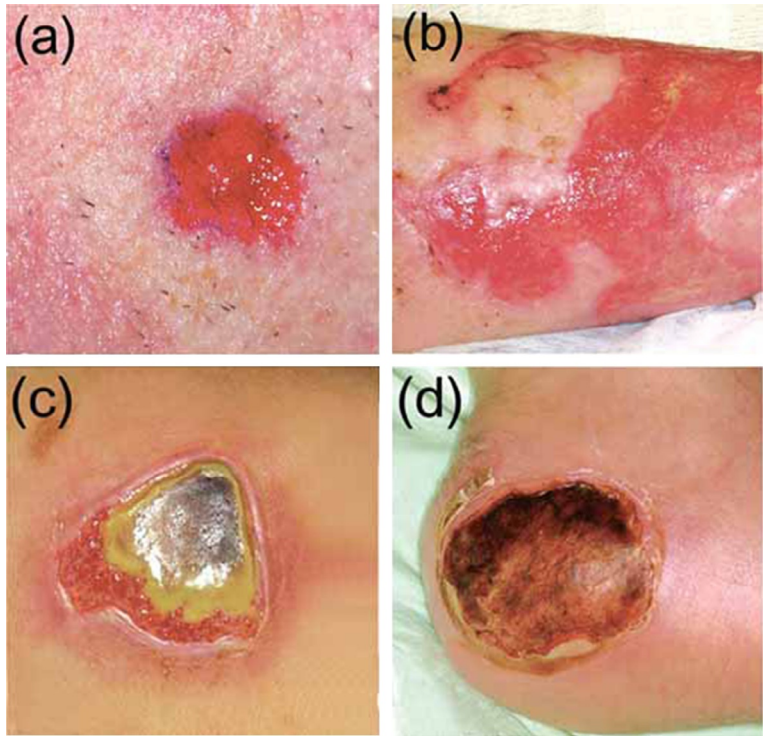

Fig. 1.16 Types of wounds based on their appearances: (A) epithelializing (clean, medium-to-high exudates), (B) granulating (clean and exudating), (C) slough-covered, and (D) necrotic (dry).
Reprinted with permission from John Wiley Sons, Ltd. (Zahedi P, Rezaeian I, Ranaei-Siadat SO, Jafari SH, Supaphol P. A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages. Polym Adv Technol 2010;21:77–95).

scaffolds including flexibility, high porosity with excellent pore interconnectivity, high surface area, and gas permeation demonstrate their capability as an excellent dressing material for wound healing. The ideal fibrous scaffold should actively initiate the healing process and might reduce the bacterial contamination [36]. Until now, there are a variety of synthetic and natural polymers; chitosan, hyaluronic acid, poly(ethylene oxide) (PEO), poly(lactide) (PLA), poly(caprolactone) (PCL), poly(vinyl alcohol) (PVA), and silk were electrospun due to their nontoxic and biodegradable nature and used for developing a wound-healing scaffolds that can actively support the deposition of healthy tissues [37,38].

The healing process happened through three different stages: (i) inflammation, (ii) new tissue formation, and (iii) remodeling. The first stage in wound repair process happens immediately after tissue damage and multiple biological pathways, that is, coagulation cascade, inflammatory pathways, and immune system that are greatly necessary to prevent the blood and fluid losses, to remove dead and devitalized (dying) tissues, and to prevent infection. The new tissue formation is the second stage of the wound repair and occurs 2–10 days after injury and characterized by cellular proliferation and migration of different cell types. And the third stage of the wound repair is remodeling [39].

The strategy to incorporate the active agents inside/outside the fibers through different approaches is demonstrated in Fig. 1.17. The blending core/shell approach enables the homogeneous distribution of active agents throughout the fiber surface. In such a case, most of the active agents are presented inside the fibers. Typically, modification of electrospun fibers allows the active agents to be present on the outer surface of the fibers. Occasionally, the active agents are very sensitive to the harsh environment [40].

The core/shell fiber scheme provided an efficient platform in which the active agents are fed through the inner channel, and outer shell acts as a protective barrier. The fibrous scaffolds prepared by emulsion approach eliminate the initial burst release and showed the stable release rate. The surface modification of nanofibers facilitates the existence of more active agents on the surface of the fibers. To date, there are different approaches such as dip coating, layer-by-layer assembly, and electrostatic attachment that are adopted to functionalize the fiber surface as demonstrated in Fig. 1.18.

The selected synthetic polymers in making wound-dressing material are mostly because of their desirable mechanical, cytocompatibility properties, and low cost. However, most of the polymeric system lacks to prevent the nonspecific adsorption of proteins resulted in nonspecific cell attachment and bacterial adhesion. The result of which turns to encourage the bacterial infections and pain upon removing the wound-dressing material. In this regard, Unnithan et al. [34] prepared poly (carboxybetaine-co-methyl methacrylate) copolymer (CBMA) for active nonadherent wound-dressing application. They have investigated cell and platelet adhesion behavior in detail. The prepared wound-dressing material can be applied as easy removable, no-pain wound-dressing bandages. The wound-healing ability of the prepared nanofibrous membrane was tested with Wistar rats. The photographs of the in vivo wound-healing study are presented in Fig. 1.19. The results demonstrated that the fibrous mats showed excellent healing capability in the first and second weeks

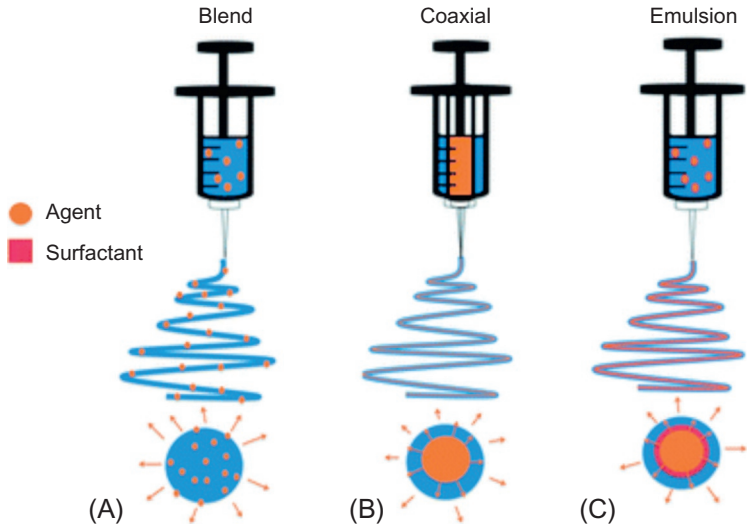

Fig. 1.17 Schematic displays the spinneret loaded with a bioactive agent for (A) blend, (B) coaxial, and (C) emulsion electrospinning. Coaxial electrospinning requires the use of a concentric spinneret configuration. (A) Blend electrospinning often yields fibers that contain the active agent dispersed throughout the fibers, whereas (B) coaxial and (C) emulsion electrospinning lend well to the synthesis of a core/shell morphology. The cross section of an individual fiber produced via the three methods is displayed.
Reprinted with permission from Royal Society of Chemistry (Rieger KA, Birch NP, Schiffman JD. Designing electrospun nanofiber mats to promote wound healing—a review. J Mater Chem B 2013;1:4531–41).

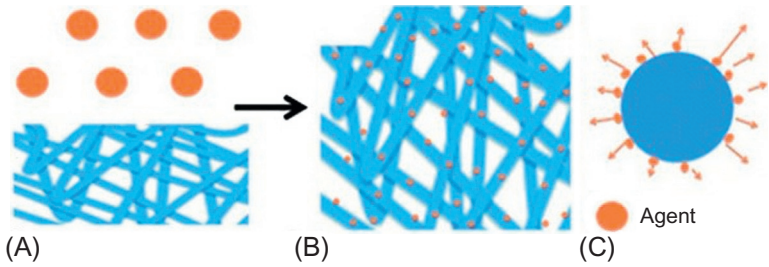

Fig. 1.18 Postproduction, (A) as-spun mats can be modified with functional agents (e.g., polymers, drugs, and biomolecules) to (B) alter their surface chemistry and functionality. (C) A cross section of an individual fiber postmodification displays that the new functional units are located on the surface of the fiber.
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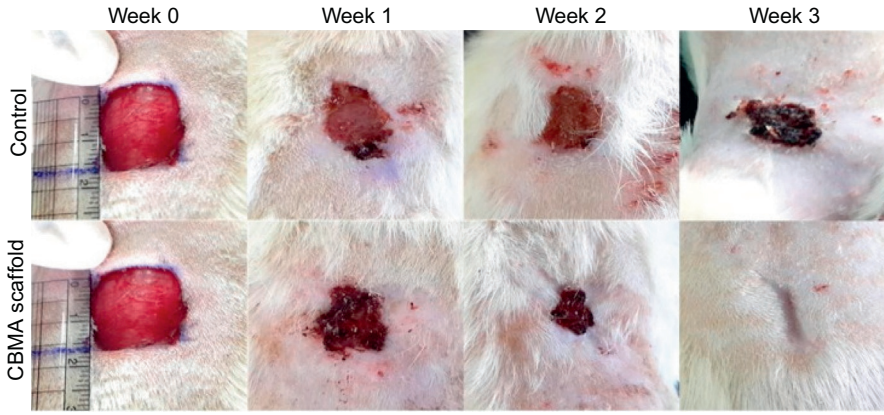

Fig. 1.19 Photographs of the in vivo wound-healing study.
Reprinted with permission from Elsevier (Unnithan AR, Nejad AG, Sasikalaa ARK, Thomas RG, Jeong YY, Murugesan P, et al. Electrospun zwitterionic nanofibers with in situ decelerated epithelialization property for non-adherent and easy removable wound dressing application. Chem Eng J 2016;287:640–8).

relative to the bare wound. After 3 weeks, the amount of wound closure was observed and evaluated. The obtained results showed that CBMA mat significantly improved the closure at $\sim$92% when compared with bare wounds that showed a wound closure of $\sim$70%. After 3 weeks of contact with the CBMA composite mat, the thickness of the granulation layer was similar to that of unwounded skin, indicating optimal healing. They suggested that prepared membranes do not make any pain upon frequent removal. Furthermore, the healing tissue will not be damaged since the newly formed layer of the skin is not disturbed.

It has been well reported that the composite fibers have been widely studied for wound healing owing to the enhanced properties as compared with pristine nanofibrous mat. Later, the interest has been drawn toward to the design of multilayered wound dressing, in which each layer exhibits different functionalities. Tan et al. [41] demonstrated the bilayered nanofibrous mats (BNFs) through sequential electrospinning of polyurethane (PU) and gelatin. Amoxicillin was successfully incorporated into the PU layer to attain the antibacterial function. The morphology and cross-sectional view of the fibrous layer is shown in Fig. 1.20. The prepared BNFs show dual wetting characteristics, that is, the gelatin nanofibrous mats exhibit relative hydrophilicity with WCA of $84.43 \pm 2.76°$, while PUa layer shows hydrophobicity with WCA of $123.07 \pm 8.12°$. The obtained tunable BNFs could be highly recommended as potential wound dressings.

Hajiali et al. [42] combined the effect of two naturally derived compounds, namely, sodium alginate and lavender essential oil, for the development of bioactive nanofibrous dressing. The loaded lavender oil shows an admirable antimicrobial efficiency and also acted to control the induced inflammation. The obtained materials show their excellent efficacy for the treatment of UVB-induced skin injuries. The

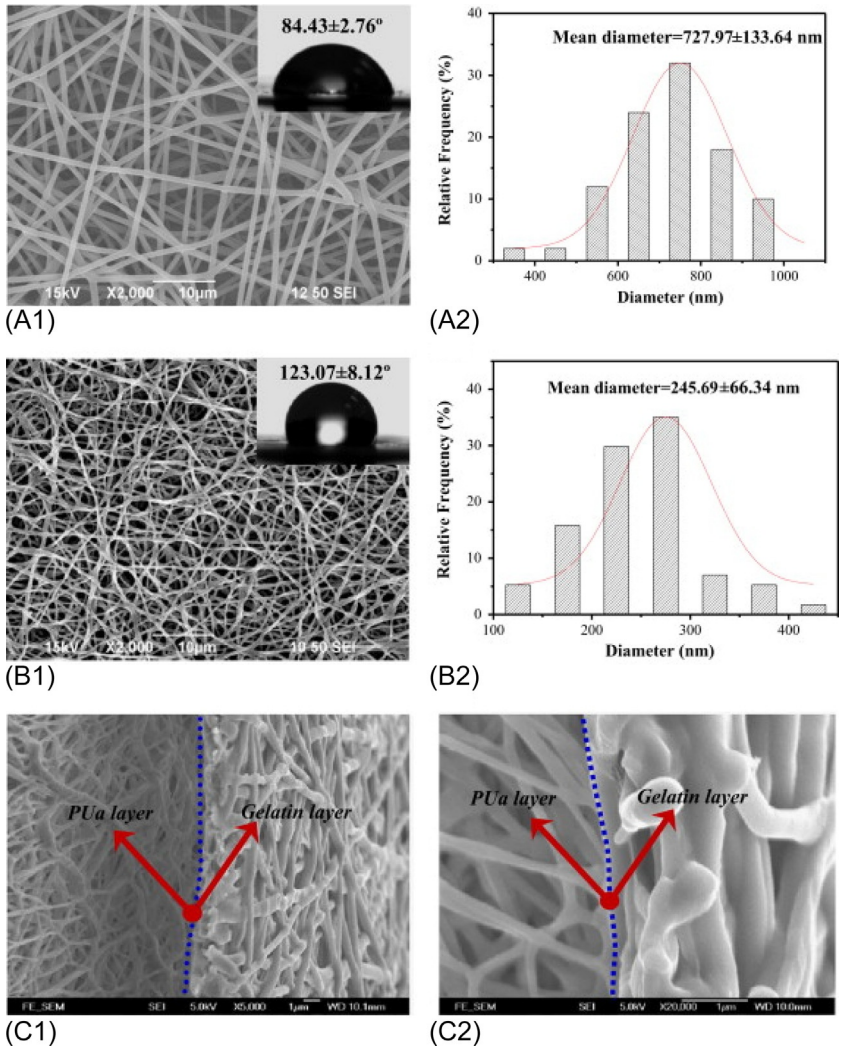

Fig. 1.20 SEM images and diameters distribution of gelatin nanofibers (A1 and A2) and polyurethane/amoxicillin nanofibers (B1, B2) and the cross-sectional views of bilayered nanofibrous mats (C1 and C2).
Reprinted with permission from Elsevier (Tan L, Hu J, Zhao H. Design of bilayered nanofibrous mats for wound dressing using an electrospinning technique. Mater Lett 2015;156:46–9).

commercially available alginate-based dressing (Tegadermt) was tested for comparison, and the results show that the inhibitory effect of the electrospun nanofibers on cytokine production was higher than that of Tegadermt. Even though there were a huge number of studies that have been evaluated and found electrospun membrane as an efficient wound-dressing material, it remains challenging to prepare a practical

and comfortable nanofibrous dressing. Dong et al. [43] developed an in situ deposition of a personalized nanofibrous dressing by a novel handy e-spinning device and evaluated their skin wound-care properties. Figs. 1.21 and 1.22 demonstrated the probable mechanism for the preparation of the e-spun personalized nanofibrous dressing. The incorporation of appropriate drug into electrospun nanofibrous membrane is a key parameter to produce an active wound-dressing material. They have incorporated the most known mesoporous silica nanoparticles decorated with silver nanoparticles (Ag-MSNs) as additives to the biopolymeric e-spun membranes.

The added Ag-MSNs could release Ag ions continuously and had a long-lasting antibacterial activity. In contrast, the gauze and control groups showed either the remaining scab or an inflamed and unclosed wound. Meanwhile, the wounds covered with e-spun Ag-MSN/PCL nanofibrous membranes presented better fluid retention when compared with the ones covered with gauze. We also found that the nanofibrous membranes were comfortable, flexible, and easier to be handled than the gauze. They

Fig. 1.21 Schematic illustration of the e-spun personalized nanofibrous dressing via a handy e-spinning device for wound healing.
Reprinted with permission from Royal Society of Chemistry (Dong RH, Jia YX, Qin CC, Zhan L, Yan X, Cui L, et al. In situ deposition of a personalized nanofibrous dressing via a handy electrospinning device for skin wound care. Nanoscale 2016;8:3482–8).

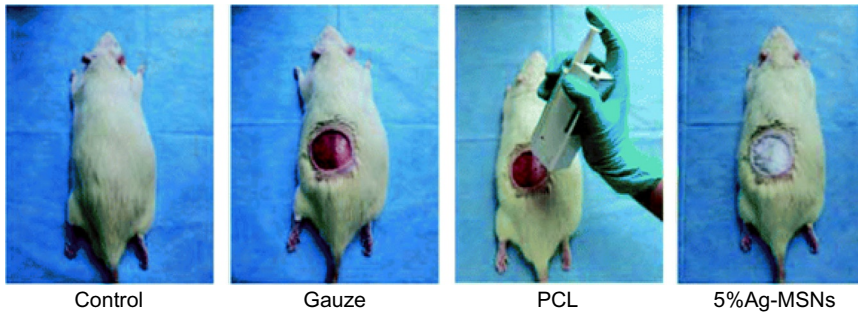

Fig. 1.22 Skin wound healing accelerated by Ag-MSN/PCL nanofibrous membranes. (A) Exhibition of the entire skin injury model and treatment process in a Wistar rat. Reprinted with permission from Royal Society of Chemistry (Dong RH, Jia YX, Qin CC, Zhan L, Yan X, Cui L, et al. In situ deposition of a personalized nanofibrous dressing via a handy electrospinning device for skin wound care. Nanoscale 2016;8:3482–8).

have studied and compared the performance of conventional gauze, virgin PCL nanofibrous membranes, and Ag-MSN/PCL nanofibrous membranes on the surface of the entire back skin wounds of different groups of Wistar rats. The gross appearance of the skin injury was observed at definite time intervals at 1, 2, 3, 4, and 5 weeks after immediate treatment. The observed results demonstrated that gauze and control groups show either the remaining scab or an inflamed and unclosed wound. At the same time, wounds covered with e-spun Ag-MSN/PCL nanofibrous membranes presented better fluid retention.

In most of the reported studies, the materials and bioactive molecular guidance are concerned to be primary factors to determine the functionality and healing properties of the mat. Despite, the impact of surface morphological properties of nanofibrous to wound dressing has not been considered seriously. The study by Kim et al. [44] developed an advanced electrospinning method that is capable of introducing a specific morphology into the nanofibrous mat. The proposed electrospinning method is mainly for the alteration of the surface morphological properties of electrospun mats. In this approach, the nanofibers are deposited onto a conductive mold with a human skin pattern. Fig. 1.23A and B shows a photograph and SEM image of a fabricated human-skin-patterned mat. The prepared fibers provided morphological guidance for cell growth, which could play a crucial role in the esthetic outcomes of skin tissue regeneration and wound healing. The in vitro cell tests (14 days) using a mouse embryonic fibroblast cell line (NIH-3T3) were carried out. The fabricated mat provided excellent morphological guidance of cells along the skin pattern, and as expected, cells cultured thereon exhibited a level of viability equivalent to those cultured on conventional electrospun fibers.

1.2.4 Antimicrobial

The infectious diseases caused by microbes are most significant rationale for human deaths worldwide than any other single cause. A microbe that is capable of causing

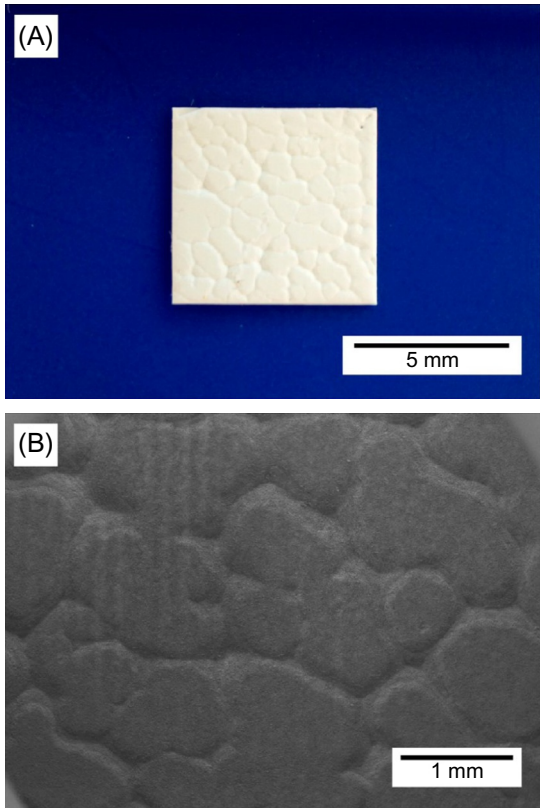

Fig. 1.23 Photograph (A) and SEM image (B) of a human-skin patterned nanofibrous mat. Reprinted with permission from American Chemical Society (Kim JH, Jang J, Jeong YH, Ko TJ, Cho DW. Fabrication of a nanofibrous mat with a human skin pattern. Langmuir 2015;31:424–31).

any infectious diseases to the organism is referred as pathogen. The health risks owing to these pathogenic microbial agents remain a challenge, and thus, preparation of effective antimicrobial agents using different approaches is always a great concern. Electrospinning is a cost-effective versatile technique that is used for generation of continuous, one-dimensional nanofibers from a wide variety of materials especially from the polymers and proved to possess a wide range of applications. In recent years, there have been numerous efforts directed toward fabrication of electrospun nanofibers with antimicrobial properties using various approaches, namely, encapsulation of antimicrobial agents, functionalization, and surface modification owing to their unique features. The properties of electrospun nanofibers such as fiber diameter, specific surface area, composition of the polymer, and porosity significantly play vital role in the release profile of encapsulated agent. An antimicrobial agent is a molecule of natural, semisynthetic or synthetic origin, which inhibits the growth of the microbes or kills with minimal or no adverse effect to the host. There is particularly a significant focus on the incorporation of drugs, nanoparticles, and plant-derived compounds in nanofibers, which exhibit antimicrobial property. To date, numerous active antimicrobial agents have been successfully encapsulated into nanofibrous membranes and demonstrated their potency in controlling the microbial growth including

antibiotics, triclosan, essential oils, chlorhexidine, silver nanoparticles, and metal oxide nanoparticles.

In an overview, encapsulation of active agents in electrospun nanofibers can be achieved by blending in polymer solution before electrospinning, confining in core of the nanofiber using coaxial electrospinning, dispersing the nanostructures in electrospinning solution, converting a precursor into active agent through post-treatment process, and attaching on the nanofiber surface as indicated by Gao et al. [45]. A graphic illustration on different methods of incorporating biocides into electrospun nanofibers is presented in Fig. 1.24. However, during encapsulation of antibiotics, the close relationship between the polymer solution and hydrophilicity/hydrophobicity nature of the antibiotics should be taken into account to result in successful encapsulation of antibiotics in nanofibers. For instance, if a hydrophilic polymer is chosen to encapsulate hydrophilic drug, which might result in complete encapsulation, then a hydrophobic drug might not be fully encapsulated.

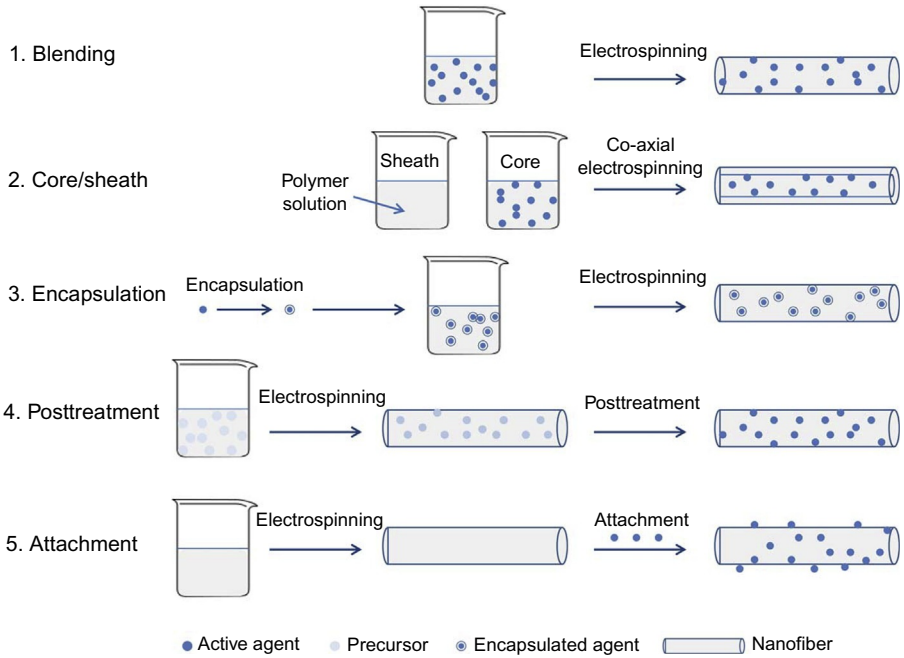

Fig. 1.24 Various methods of incorporating biocides into electrospun nanofibers. (1) Blending/dispersion of the active agent in the polymer solution prior to electrospinning, (2) confinement of the active agent in the core of the fiber through coaxial electrospinning, (3) encapsulation/adsorption of the active agent in nanostructures before dispersion in the electrospinning solution, (4) conversion of a precursor to active agent in the nanofibers after electrospinning, and (5) attachment of the active agent onto the nanofibers after electrospinning.
Reprinted with permission from Wiley Periodicals, Inc. (Gao Y, Truong YB, Zhu Y, Kyratzis IL. Electrospun antibacterial nanofibers: Production, activity, and *in vivo* applications. J Appl Polym Sci 2014;131:40797).

Recently, Yang et al. [46] prepared agar/polymer electrospun hybrid to couple the advantage of both agar and electrospun fibers to achieve a novel composite with enhanced, comprehensive properties and a high potential for drug delivery. Ampicillin (AMC)-loaded, agar-doped polyacrylonitrile (PAN) composite nanofibers were prepared, and their performance in bioactivity assays against *Escherichia coli* bacteria was studied using disc diffusion method. The preparation of electrospun nanofiber using agar as an additive is illustrated in Fig. 1.25. In addition, the relationship between the spinnability and agar concentration in order to determine the critical factors in achieving a successful electrospinning.

The antimicrobial properties of silver have been noted since ancient times. Silver nanoparticles (AgNPs) are well known as a broad spectrum of antibiotic with strong antibacterial properties against many potential pathogens. Due to their excellent antimicrobial activities, preparation of silver nanoparticles containing electrospun nanofibers attracted intensive research interest. Xu et al. [47] demonstrated the preparation of biodegradable poly(l-lactide) (PLA) ultrafine fibers containing nanosilver particles via electrospinning technique. The in vitro antibacterial activities of PLA fibers with silver nanoparticles were studied. The observation shows that the silver is released steadily, and thus, the antibacterial activity is durable.

To minimize the aggregation of silver nanoparticles in polymeric solution and avoid environmentally hazardous chemicals, an effort was made by reducing silver through atmospheric plasma treatment. Thus, silver/polyacrylonitrile (Ag/PAN) hybrid nanofibers were prepared by coupling atmospheric plasma treatment and electrospinning. Further, the antibacterial efficiency was investigated against a gram-negative enteric pathogen *E. coli* O157:H7 (B179) and a spore-forming gram-positive pathogen *Bacillus cereus* (B002). As a result, PAN nanofibers without silver or silver compounds showed no significant antibacterial activity. Conversely,

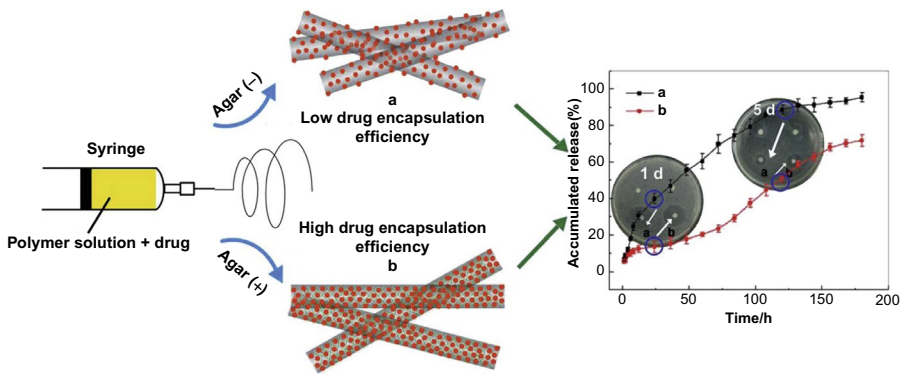

Fig. 1.25 Schematic illustration for the drug delivery systems by means of the agar doped electrospun fibers.
Reprinted with permission from Royal Society of Chemistry (Yang H, Gao PF, Wu WB, Yang XX, Zeng QL, Li C, et al. Antibacterials loaded electrospun composite nanofibers: release profile and sustained antibacterial efficacy. Polym Chem 2014;5:1965–75).

Ag/PAN hybrid nanofibers showed complete inhibition of both gram-negative and gram-positive microorganisms, indicating that the nanofibers are endowed with excellent antibacterial properties due to the introduction of Ag nanoparticles [48].

The incorporation of silver nanoparticles in electrospun chitosan nanofibers has been shown to improve their antibacterial performance. This modification has been achieved by loading prefabricated silver nanoparticles into the electrospinning chitosan solution or by adding a silver precursor to the formulation and then performing the thermally induced synthesis of silver nanoparticles. However, the antibacterial performance of these silver nanoparticles is often restricted because they are uniformly distributed and mostly embedded below the nanofiber surface. To ensure the maximum exposure of silver nanoparticles, the reduction reaction of the precursor should be directed or confined to the nanofiber surfaces. Annur et al. [49] recently reported the argon-plasma synthesis of silver nanoparticles for the surface immobilization of silver nanoparticles without the use of additional chemical reagents. Argon plasma etching and nanofiber thinning also promoted the further accumulation and exposure of silver nanoparticles on the nanofiber surfaces.

The antibacterial performances of chitosan nanofibers with and without surface-immobilized silver nanoparticles were investigated by the disk diffusion method using *E. coli* as the model bacteria. Inhibition zone improvements were observed in the silver nanoparticle-immobilized C10Ag (silver nitrate ($AgNO_3$) 1.0 wt%) and C20Ag ($AgNO_3$ 2.0 wt%) nanofibers after the argon-plasma bombardment. For the 0.5 and 1 min plasma exposure time, both samples demonstrated increased inhibition zone sizes to 0.36 and 0.48 mm, respectively. When treated with 1.5 min of argon plasma, the C10Ag and C20Ag samples had the maximum antibacterial activity and inhibited bacterial growth up to a distance of 0.78 mm. These results for the C0, C10Ag, and C20Ag samples are summarized in Fig. 1.26 as a function of the plasma dosage.

Among other metal oxides, zinc oxide (ZnO) and titanium dioxide (TiO_2) have been considered as versatile and eco-friendly materials owing to their exceptional physicochemical properties. The ZnO nanostructures were extensively studied for their antimicrobial activities; a considerable attention was paid on the preparation of ZnO-incorporated nanofiber via electrospinning process. In addition, ZnO is listed as a generally regarded as safe (GRAS) by the US Food and Drug Administration, which has been used in many personal and health-care products. Anitha et al. [50] reported the in situ generation of ZnO nanoparticles in cellulose acetate (CA) fibrous membrane and their excellent antibacterial activity.

Lee et al. [51] developed a facile two-step fabrication method to enhance the surface area of electrospun TiO_2 nanofibers by subsequent hydrothermal synthesis of TiO_2 nanowires. Furthermore, the uniform deposition of a large quantity of silver nanoparticles on the surface of the TiO_2 nanofibers ensured a significant enhancement of the antibacterial performance, even under dark conditions. The schematic illustration of the preparation procedure is shown in Fig. 1.27.

The hierarchical TiO_2 nanofibers were prepared through a hydrothermal synthesis of TiO2 nanowires on pure TiO_2 nanofibers prepared via electrospinning, followed by calcination. The morphology of pure and hierarchical TiO_2 nanofibers is shown in

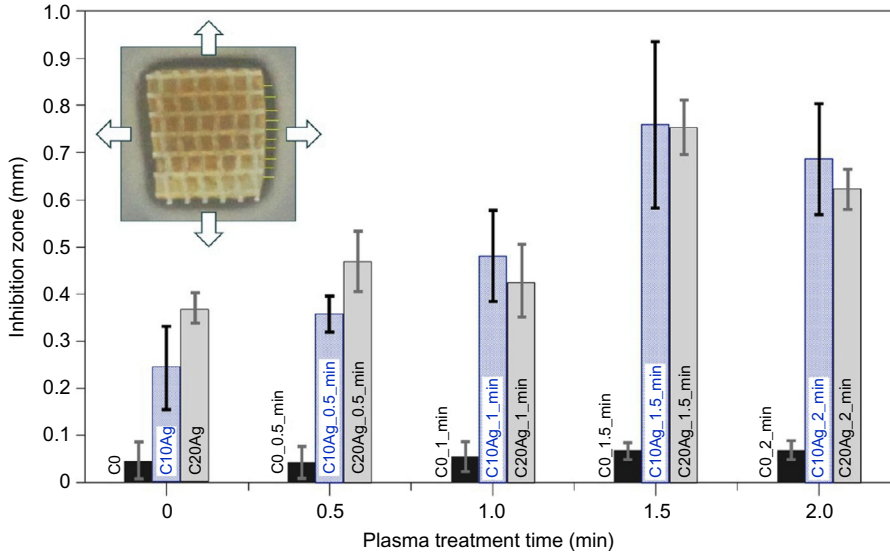

Fig. 1.26 Antibacterial inhibition zone measurements for C0, C10Ag, and C20Ag samples versus the argon-plasma dosages. The inset shows an example of the inhibited-growth zones surrounding the nanofiber sample.
Reprinted with permission from American Chemical Society (Annur D, Wang ZK, Liao JD, Kuo C. Plasma-synthesized silver nanoparticles on electrospun chitosan nanofiber surfaces for antibacterial applications. Biomacromolecules 2015;16:3248–55).

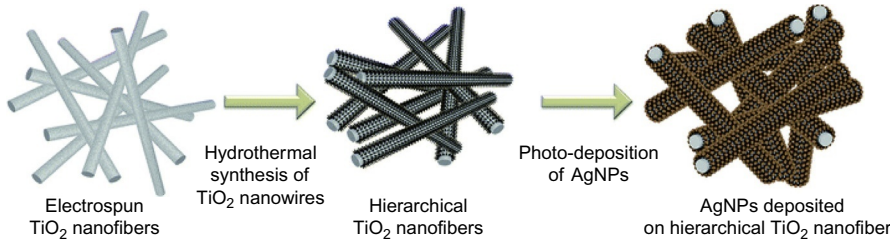

Fig. 1.27 Schematic illustration of the approaches used to achieve enhanced antibacterial activity of TiO_2 NFs. Hierarchical structures prepared by electrospinning, pyrolysis, hydrothermal synthesis of nanowires, and deposition of silver nanoparticles.
Reprinted with permission from Royal Society of Chemistry (Lee WS, Park YS, Cho YK. Significantly enhanced antibacterial activity of TiO2 nanofibers with hierarchical nanostructures and controlled crystallinity. Analyst 2015;140:616–22).

Fig. 1.28. The prepared structures exhibited a highly enhanced photocatalytic effect owing to an increased surface area and an improved crystallinity of the surface. This enhanced photocatalytic effect led to a highly enhanced antibacterial activity of $89.90 \pm 2.02\%$ in the presence of UV light, even for a very short irradiation time

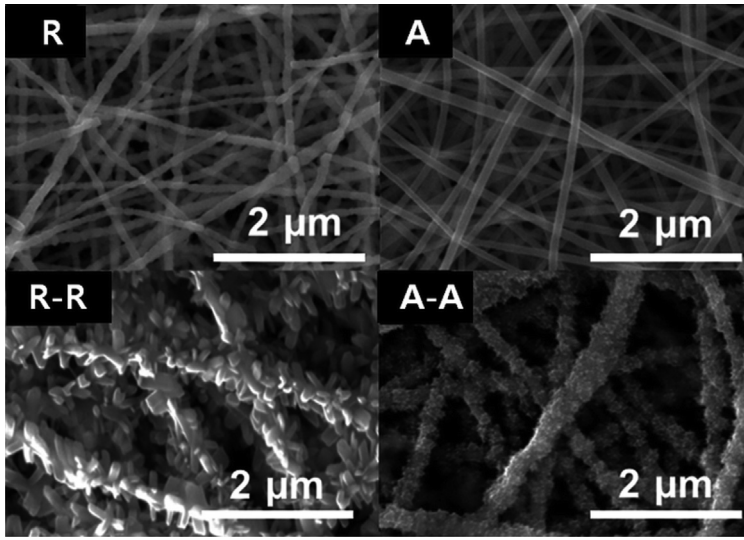

Fig. 1.28 SEM images of TiO_2 nanofibers with various crystalline phases and nanostructures: (R) pure TiO_2 nanofibers with rutile phase, (A) pure TiO2 nanofibers with anatase phase, (R–R) hierarchical TiO_2 rutile nanofibers with rutile–TiO_2 nanowires, and (A–A) hierarchical TiO_2 anatase nanofibers with anatase TiO_2 nanowires.
Reprinted with permission from Royal Society of Chemistry (Lee WS, Park YS, Cho YK. Significantly enhanced antibacterial activity of TiO2 nanofibers with hierarchical nanostructures and controlled crystallinity. Analyst 2015;140:616–22).

(1.5 min). Furthermore, Ag/TiO_2 nanofiber composites were prepared via photoreduction synthesis of Ag NPs that were deposited on the hierarchical anatase TiO_2 nanofibers under UV illumination.

Airborne fine particulates raise severe health and safety issues as they can be inhaled and may also cause explosions. It is vital to improve the indoor air quality by controlling these particulates under a certain concentration both in industry and in our daily life. Zhong et al. [52] prepared an air filter by growing one-dimensional ZnO nanorods on the three-dimensional porous networks of expanded polytetrafluoroethylene (ePTFE). The schematic for the preparation process and the morphologies of the filter at different stages are shown in Fig. 1.29. The preparation of the filters contains two simple steps: seeding of ZnO nanoparticles on the ePTFE networks and hydrothermal growth of ZnO nanorods on the seeding layer. They have adopted atomic layer deposition (ALD) to uniformly seed ZnO on the surface of ePTFE matrix and then synthesize well-aligned ZnO nanorods with tunable widths and lengths from the seeds under hydrothermal conditions. The resultant filters show ultrahigh efficiency and an interesting antibacterial functionality because of the conjunction of ZnO NRs on the network of ePTFE.

A recent report by Song et al. [53] has shown a route to fabricate low-cost porous carbon nanofibers (CNFs) using biomass tar, polyacrylonitrile (PAN), and silver

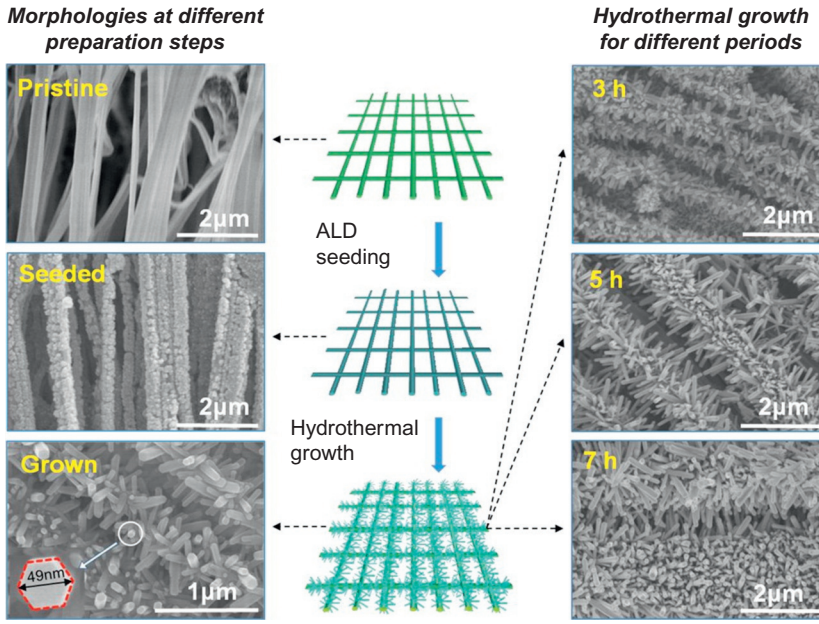

Fig. 1.29 Schematic of the preparation process of the ZnO-functionalized ePTFE filters and the SEM images of the filters at different stages. The inset shows the magnified top-view SEM image of an individual ZnO NR prepared with a hydrothermal growth period for 3 h. Reprinted with permission from American Chemical Society (Zhong Z, Xu Z, Sheng T, Yao J, Xing W, Wang Y. Unusual air filters with ultrahigh efficiency and antibacterial functionality enabled by ZnO nanorods. ACS Appl Mater Interfaces 2015;7:21538–44).

nanoparticles by coupling electrospinning technique, subsequent stabilization, and carbonization processes. The resultant membrane exhibited high specific surface area (>400 m^2/g) and microporosity. A schematic illustration for fabricating the porous tar-derived CNFs through electrospinning is presented in Fig. 1.30. The porous characteristics of nanofibers increased the exposures and contacts of silver nanoparticles to the bacteria, leading to excellent antimicrobial performances.

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of α(1,4)-linked glucopyranose units having a truncated cone-shaped molecular structure. Due to their unique molecular structure, CDs can form intriguing supramolecular assemblies by forming noncovalent host-guest inclusion complexes with a variety of small molecules and macromolecules. Hence, CDs are applicable in many areas including antibacterial application [54,55]. Celebioglu and Uyar [56] established the first studies on electrospinning of CD nanofibers by itself without the use of a carrier polymer matrix. Essential oils (EOs) are volatile compounds and well known for their antimicrobial and antioxidant properties. Owing to their volatile nature, encapsulation/inclusion is considered very necessary for their effective utilization in different applications. A recent study by Aytac et al. [57] reported that geraniol (which is a

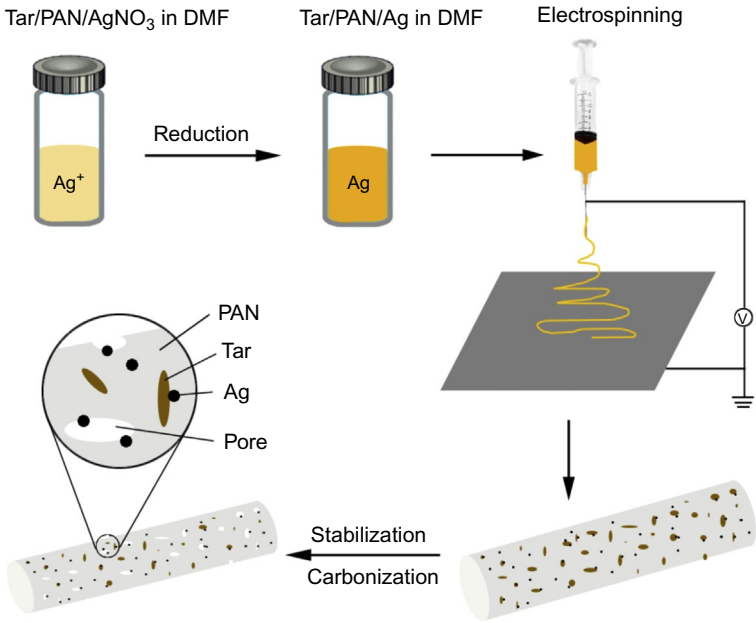

Fig. 1.30 A schematic illustrating the fabrication of the porous tar-derived CNFs through electrospinning followed by stabilization and carbonization processes.
Reprinted with permission from American Chemical Society (Song K, Wu Q, Zhang Z, Ren S, Lei T, Negulescu II, et al. Porous carbon nanofibers from electrospun biomass tar/polyacrylonitrile/silver hybrids as antimicrobial materials. ACS Appl Mater Interfaces 2015;7:15108–16).

well-known volatile essential oil compound) was effectively encapsulated into three different cyclodextrins (CDs), namely, hydroxypropyl-beta-cyclodextrin (HPβCD), methylated-beta-cyclodextrin (MβCD), and hydroxypropyl-gamma-cyclodextrin (HPγCD), which is a cyclic oligosaccharide. The schematic representation of the experimental procedure is shown in Fig. 1.31. The cyclodextrin/geraniol-inclusion complex (CD/geraniol-IC) furthers electrospun as a free-standing nanofibrous webs CD/geraniol-IC-NF. The antibacterial studies indicated that prepared CD/geraniol-IC-NFs possessed strong antibacterial activity.

1.3 Commercialization prospectus

So far, the electrospun nanofibers have been well demonstrated for their potentiality in a variety of biomedical applications. To date, numerous electrospinning-based technologies were already transferred out of the laboratories, and products are commercialized in a wide range of industrial sectors including filtration, energy storage, and tissue engineering. Persano et al. [3] reviewed the status of electrospinning in industrialization

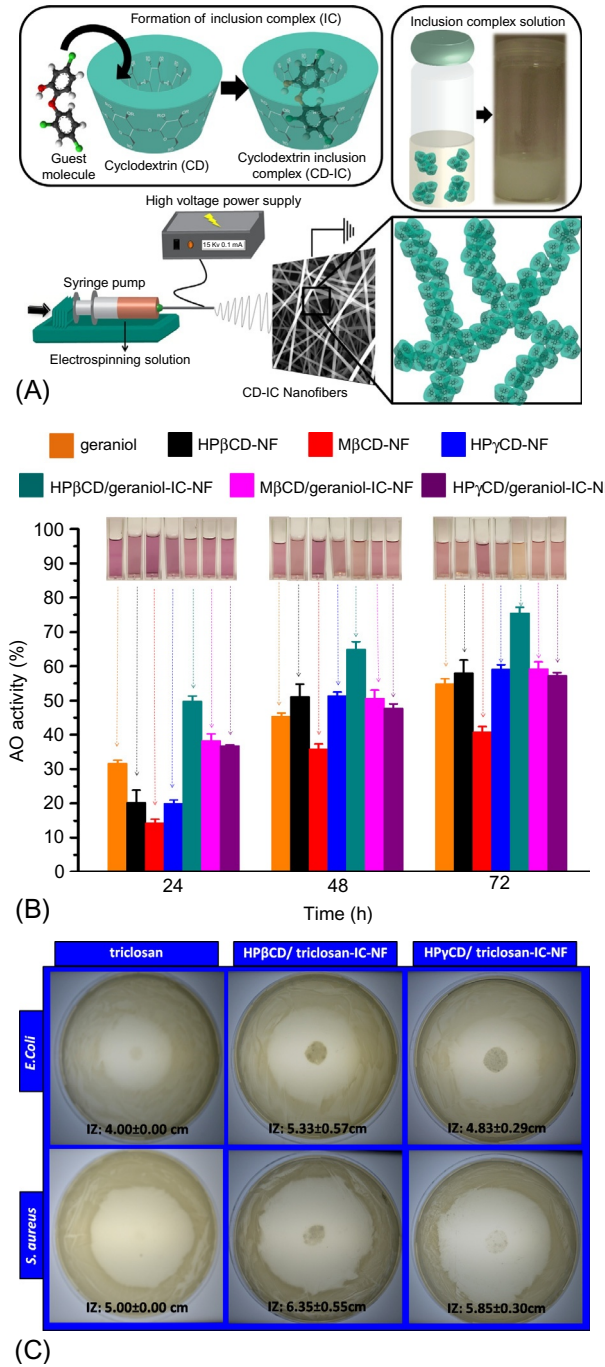

Fig. 1.31 (A) Schematic representation of IC formation and IC solution; illustration of electrospinning of CD-IC nanofibers. (B) The antioxidant (AO) activity (%) of geraniol, HPβCD-NF, MβCD-NF, HPγCD-NF, HPβCD/geraniol-IC-NF, MβCD/geraniol-IC-NF, and HPγCD/geraniol-IC-NF and the photographs of DPPH solutions in which geraniol, HPβCD-NF, MβCD-NF, HPγCD-NF, HPβCD/geraniol-IC-NF, MβCD/geraniol-IC-NF, HPγCD/geraniol-IC-NF were immersed, respectively. (C) The representative photographs of antibacterial test plates with the average inhibition zone (IZ) and standard deviation calculations for *E. coli* and *S. aureus* treated with pure triclosan, HPβCD/triclosan-IC-NF, and HPγCD/triclosan-IC-NF.

perspectives and listed the companies involved in supplying industrial-scale electrospinning equipment and nanofiber-based products. A list of representative companies providing industrial-scale electrospinning equipment includes Elmarco (www.elmarco.com), Holmarc Opto-Mechatronics (www.holmarc.com), NaBond (www.electro-spinning.com), E-Spin Nanotech (www.espinnanotech.com), Electrospinz (www.electrospinz.co.nz), Electrospunra (www.electrospunra.com), Linari Engineering (www.linaribiomedical.com), Mecc Co. (www.mecc.co.jp), Kato Tech (www.keskato.co.jp), Inovenso (www.inovenso.com), Toptec (www.toptec.co.kr), IME Technologies (www.imetechnologies.nl), and Yflow (www.yflow.com). And also, a list of companies involved in supplying electrospun products for different fields of application includes Ahlstrom Corporation (www.ahlstrom.com), Donaldson (www.donaldson.com), DuPont (www.dupont.com), eSpin Technologies (www.espintechnologies.com), Esfil Tehno AS (www.esfiltehno.ee), HemCon Medical Technologies, Inc (www.hemcon.com), Johns Manville (www.jm.com), Finetex Technology (www.finetextech.com), Hollingsworth and Vose Company (www.hollingsworthvose.com), Japan Vilene Company (www.vilene.co.jp), Nanofiber Solutions (www.nanofibersolutions.com), Kertak Nanotechnology (www.kertaknanotechnology.com), Nano109 (www.nano109.com), NanoSpun (www.nanospuntech.com), Soft Materials and Technologies S.r.l (www.smtnano.com), Polynanotec (www.polynanotec.com),) Yflow (www.yflow.com, and SNS Nano-Fiber Technology (www.snsnano.com). In addition, the ElectrospinTech listed variety of companies involved in electrospinning-based products/service suppliers [58]. The list of selected companies involved in tissue-engineering products services is presented in Table 1.1.

Even though the technical specification and requirements in instruments have been solved to produce nanofibers in industrial scale, it is more important that the produced material should possess the biological compatibility to further apply in tissue-engineering applications. Another key challenge is quality control; most importantly, these materials need to be produced by following the good manufacturing practice (GMP) conditions to meet FDA and other regulatory standards. Therefore, the innovations at laboratory scale related to biomedical applications must travel through in line with industrial requirements to avoid failure at later stage.

1.4 Conclusion

In this chapter, we highlighted the advancement of nanofibrous membrane in biomedical applications. The outstanding uniqueness of electrospun nanofibrous membrane provides a wide range of opportunities for their use in many different biomedical applications. The easy functionalization of nanofibrous assembly yielding significant advancements in the development of functional nanofibrous scaffolds. An obvious advantage of core-shell fibrous assembly has led the burst and sustained release of bioactive molecules. Besides controlled nanofibrous structures, hydrophilicity of the fibrous membrane also provides the more favorable environment for cellular adhesion. The wetting characteristics of the nanofibrous scaffold are also efficiently

Table 1.1 List of companies involved in tissue-engineering products service

S. No.	Company name	Product/service	Country	Website
1	Ahlstrom Corporation	Nanofiber-based products for medical care, life science and diagnostics	Finland	www.ahlstrom.com
2	3-D Biotek	Cell culture device	The United States	www.3dbiotek.com
3	BioSurfaces Inc.	Drug-loaded electrospun fibers for implantable and nonimplantable devices	The United States	www.biosurfaces.us
4	Biotronik	Covered Stent	Germany	www.biotronik.com
5	Espin Technologies	Nanofiber-based products for regenerative medicine and drug delivery	The United States	www.espintechnologies.com
6	HemCon Medical Technologies, Inc	Nanofiber-based products for wound care	The United States	www.hemcon.com
7	Nanofiber Solutions	3-D cell culture consumables and scaffolds for tissue engineering	The United States	www.nanofibersolutions.com
8	Neotherix	Nanofiber scaffold for tissue regeneration	The United Kingdom	www.neotherix.com
9	Nicast Ltd	Nanofiber implants	Israel	www.nicast.com
10	Sigma-Aldrich	Nanofiber culture plates	The United States	www.sigmaaldrich.com
11	Soft Materials and Technologies S.r.l.	Fibrous tissues	Italy	www.smtnano.com
12	Stellenbosch Nanofiber Company	Nanofiber-based materials for medical applications	South Africa	www.sncfibers.com
13	The Electrospinning Company	Nanofiber scaffold for tissue regeneration	The United Kingdom	www.electrospinning.co.uk

modified via plasma treatment and chemical-etching treatment. The shrinkage and distortion of the electrospun scaffolds restrict the initial cell adhesion and further growth, and therefore, suspended, shrinkage-free electrospun scaffold has been developed. A hybrid scaffold has also been well demonstrated to enhance the cellular infiltration and nutrient transport. The practical and comfortable wound dressing is a critical challenge even though there were a huge number of studies that have been evaluated and found electrospun membrane as an efficient wound-dressing material. The novel handy e-spinning device has also been demonstrated to develop a personalized nanofibrous dressing. The research may extent to incorporate the sensor to monitor the wound management. It may give necessary information on infection feedback, which may help to change the dressing material if necessary. The successful clinical translation of such demonstrated nanofibrous scaffold may provide their effectiveness in biomedical field. Therefore, the toxic profile of nanofibrous membrane should be carefully studied. The potential of nanofibrous scaffold provides the improved performances in biomedical applications, suggesting that their participant remains promising.

Acknowledgments

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