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REVIEW ARTICLE

Electrospun Collagen Nanofibers and Their Applications in Skin Tissue Engineering

Jia Xian Law[1] · Ling Ling Liau[2] · Aminuddin Saim[3] · Ying Yang[4] · Ruszymah Idrus[2]

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Abstract Electrospinning is a simple and versatile technique to fabricate continuous fibers with diameter ranging from micrometers to a few nanometers. To date, the number of polymers that have been electrospun has exceeded 200. In recent years, electrospinning has become one of the most popular scaffold fabrication techniques to prepare nanofiber mesh for tissue engineering applications. Collagen, the most abundant extracellular matrix protein in the human body, has been electrospun to fabricate biomimetic scaffolds that imitate the architecture of native human tissues. As collagen nanofibers are mechanically weak in nature, it is commonly cross-linked or blended with synthetic polymers to improve the mechanical strength without compromising the biological activity. Electrospun collagen nanofiber mesh has high surface area to volume ratio, tunable diameter and porosity, and excellent biological activity to regulate cell function and tissue formation. Due to these advantages, collagen nanofibers have been tested for the regeneration of a myriad of tissues and organs. In this review, we gave an overview of electrospinning, encompassing the history, the instrument settings, the spinning process and the parameters that affect fiber formation, with emphasis given to collagen nanofibers' fabrication and application, especially the use of collagen nanofibers in skin tissue engineering.

Keywords Electrospinning · Collagen · Skin · Tissue engineering · Nanofiber · Scaffold

1 Introduction

Skin injury can cause serious morbidity or even mortality if not treated promptly. Extensive skin injury affecting large surface area is commonly treated with autologous skin grafts [1]. The harvesting of autologous split skin graft from the donor site creates secondary wound and will definitely cause extra pain to the patient. Thus, tissue-engineered skin was developed as an alternative to skin graft. According to Langer and Vacanti, tissue engineering is an interdisciplinary field that applies the principles of engineering and life science towards the goal of achieving tissue regeneration [2]. Tissue engineering has three core components: cells, signaling molecules and biomaterials that often referred as the tissue engineering triad [3]. These components can be applied individually or in combination to restore, maintain, or improve the tissue or organ function. The classic tissue engineering strategy is highlighted in Fig. 1.

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✉ Ruszymah Idrus
ruszyidrus@gmail.com; ruszymah@ppukm.ukm.edu.my

[1] Tissue Engineering Centre, Universiti Kebangsaan Malaysia Medical Centre, 56000 Kuala Lumpur, Malaysia

[2] Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, Cheras, 56000 Kuala Lumpur, Malaysia

[3] Ear, Nose and Throat Consultant Clinic, Ampang Puteri Specialist Hospital, 68000 Ampang, Selangor, Malaysia

[4] Institute for Science and Technology in Medicine, School of Medicine, Keele University, Stoke-on-Trent ST4 7QB, UK

The application of biomaterials in tissue engineering is predominately in the form of scaffolds which act as the temporal shape or physical guidance of the tissue to be formed. Scaffold is very important in the fabrication of engineered tissues. Scaffold not only provides physical supports to cells but its structure and micropattern also give important biochemical and biomechanical cues that guide the fundamental cellular processes (e.g. attachment, migration, proliferation and differentiation) that lead to tissue regeneration instead of fibrosis and scarring. A good scaffold for tissue engineering applications should serve the following functions. Firstly, it should provide a physical environment and bioactive cues that support the cell biofunctionality and tissue formation. For tissue-engineered skin, the scaffold must be able to support keratinocytes and fibroblasts [4–7]. Secondly, it should give mechanical strength (e.g. stiffness and elasticity) similar to the tissue it replaces. Thirdly, the scaffold should act as a reservoir of soluble factors which include growth factors, chemokines and cytokines. Fourthly, it should allow remodeling by cells to restore the tissue architecture. An ideal scaffold should have the architecture, mechanical and biological features that similar to the native tissue. However, this is very difficult to achieve due to the complexity of extracellular matrix (ECM). Nowadays, decellularized tissue is probably the scaffold that most closely resembles the native tissue [8]. Although technically challenging, it is now possible to remove all the cellular components without causing significant lost of the growth factors trapped within the ECM and damage to the tissue architecture [9, 10]. Nonetheless, shortage of allogeneic donors, difficulty in removing all the immunogenic cellular components, and the risk of illicit host immune responses and pathogen transmission render this technique less favorable for clinical applications.

Selection of appropriate biomaterials is critical in order to produce a scaffold that recapitulates the architecture and functionality of the native tissue ECM. Biocompatibility is probably the single most important requirement when selecting the right biomaterials for scaffold fabrication. An appropriate material must be able to perform with an appropriate host response without inflicting excessive inflammation or rejected by the immune system. Other important criteria include biodegradable, bioresorbable, non-toxic, low (preferably no) risk of disease transmission, support cell growth and tissue remodeling, have similar mechanical and physical properties to the tissue is replaces, low cost, readily available and with harmless degradation products [11, 12]. By fulfilling the majority of the requirements mentioned above, collagen is an ideal biomaterial for tissue engineering applications. In fact, collagen is one of the most useful biomaterials available and is now commonly used in tissue engineering [13]. Collagen has been used as the scaffolding material of tissue-engineered skin, cartilage, tendon, cornea, kidney, trachea, nerve, blood vessel, skeletal muscle, cardiac muscle and many more.

Preparation of nanofiber scaffolds that structurally mimic the ECM of body tissue has become one of the fastest growing areas in tissue engineering. Several

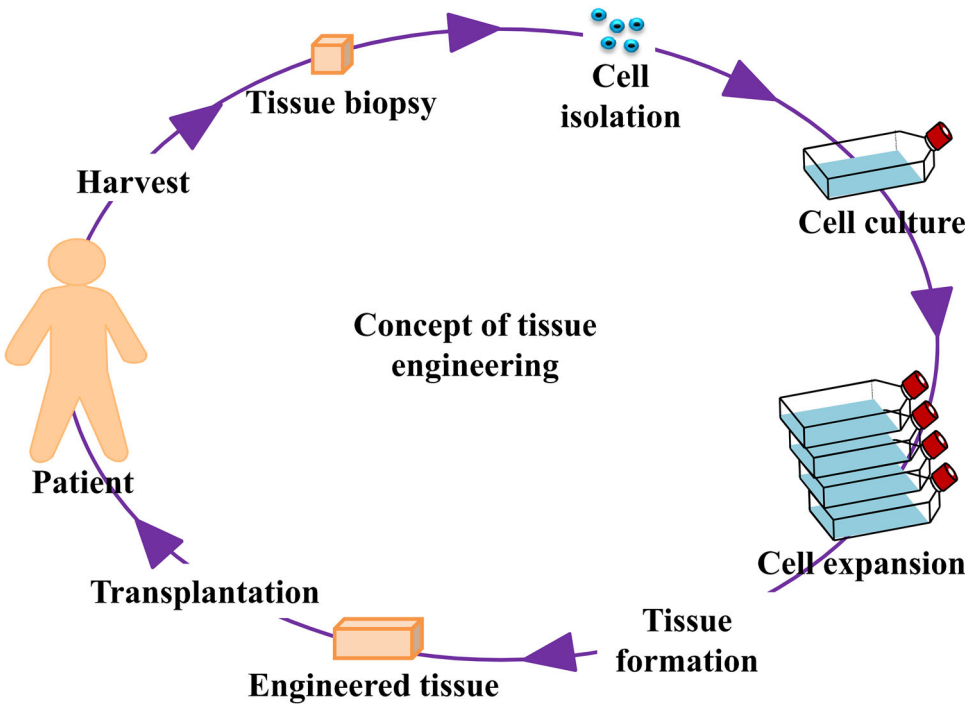

Fig. 1 Classic concept of tissue engineering. Generally tissue engineering strategy involves the isolation of stem cells from a piece of healthy tissue. Harvested stem cells were cultured and expanded *in vitro* until the desirable cell number was achieved. Subsequently, the stem cells were seeded in 3-D constructs to form an engineered tissue which will be transplanted back to the patient. However, have to bear in mind that there are a number of different tissue engineering approaches which may skip or add additional steps to achieve the goal of tissue regeneration. For examples, the expanded stem cells can be injected directly back to the patient without a scaffold

methods have been developed to produce nanofibers, including nanolithography [14], self-assembly [15] and electrospinning [16]. Compared to other techniques, electrospinning has the advantages of relatively low cost and comparatively higher yield. Electrospinning can produce non-woven fibers of various diameters, ranging from several micrometers to a few nanometers. This technique can produce nanofibers from almost all of the soluble natural and synthetic polymers with sufficient molecular weight [17]. Electrospinning is a relatively simple system as it only consists of 4 major components: syringe pump, high voltage power supply, grounded collector and charged polymeric solution. Electrospinning utilizes the electric potential to form fibers. This process involves the application of a high electric potential to a polymeric solution which forms a fiber jets that dry up as it accelerating towards a collector, forming solid non-woven fibers. Characteristics of the fabricated fibers can be controlled by manipulating the solution composition and processing parameters [18].

Electrospinning has been used to produce collagen nanofiber mesh that closely resembles native tissue architecture. Nonetheless, collagen nanofibers are commonly cross-linked or blended with synthetic polymers to improve its physical properties for tissue engineering applications. In this review, we will consider the use of electrospun collagen nanofibers in tissue engineering with emphasis given to engineered skin.

2 Electrospinning

2.1 History

Electrospinning is an very old technique which can be dated back to 1882 when Rayleigh studied the maximum amount of charge that can be held by a drop of liquid before the electrical forces overcome the surface tension of the drop [19]. In 1914, Zeleny [20] studied the electrical discharge from liquid points. Formhals patented the electrospinning process and apparatus for the fabrication of textile yarns in 1934 and he was granted more than 30 US patents for his work on electrospinning [21–23]. Later in 1936, Norton [24] patented the electrospinning of melts based on air-blast mechanism.

In 1969, Taylor [25] observed that polymeric solution at the tip of the capillary formed a cone (Taylor cone) when the surface tension was balanced by the applied electric potential. He also found that fiber jet was emitted at the tip of the cone which explained why fibers of smaller diameter than the diameter of the capillary were formed during the electrospinning process. Using acrylonitrile/dimethylformamide solution, Baumgarten [26] found that only a single fiber jet was emitted from the Taylor cone. He also found a direct relationship in solution viscosity with fiber diameter, whereby fibers with larger diameter were formed when the more viscous solution was used. However, in the same study, he observed that fiber diameter decreased in response to increase in electric potential until a minimum, whereby a further increase in electric potential produced fibers with a larger diameter. Larrondo and John Manley [27] found that fibers with relatively large diameter were formed when a melt was electrospun compared to a solution and the diameter of electrospun fibers is inversely proportional to the melt temperature. More recently, the Bending Instability theory [28, 29] and Electrically Forced Jet-Stability theory [30] were introduced to explain the spiraling of the jets during the travelling toward the collector.

The capacity of electrospinning to produce fibers at biological size scale in submicron range created interest of utilizing it for the preparation of scaffolds for tissue engineering usage. The use of electrospinning in tissue engineering began as early as 1978 when Annis et al. [31] examined the potential of electrospun polyurethane mat as vascular prosthesis. Later on, Fisher et al. [32] evaluated the long-term performance of electrospun arterial prosthesis transplanted *in vivo* using a canine model [32]. However, usage of electrospinning technique in tissue engineering did not receive much attention until the 1990th and not until the 2000th to see an encouraging increase in the number of publications investigating the potential of electrospun fiber mats as a scaffold to promote tissue regeneration.

Nowadays, electrospun fibers have been used in the field of nanocatalysis, protecting clothing, filtration, biomedical, biotechnology, sensors and optical electronics [33]. The simplicity, robustness and cost effectiveness of electrospinning are the main reasons for it to gain popularity in these fields. In the biomedical field, it has been explored for potential use in medical prostheses fabrication, as the wound dressing, as the scaffold for tissue engineering and as drug/pharmaceutical delivery vehicle [34]. New innovations in electrospinning technology lead to the production of core–shell, hollow and porous nanofibers which will broaden the use of electrospinning in new fields and areas.

2.2 Electrospinning process

Electrospinning has a relatively simple instrument setup as it only needs a syringe pump that pushes the polymeric solution or molten polymer out from the capillary, a high voltage power supply and a grounded collector to operate (Fig. 2). Polymeric solution is more commonly electrospun compared to the molten polymer as the solution preparation is easier and it does not need a heating system to maintain

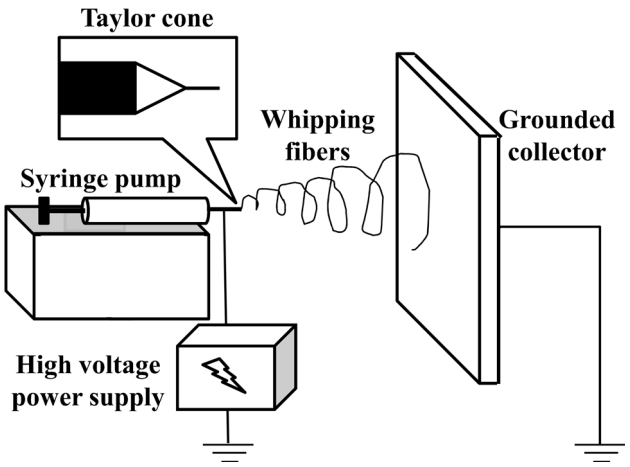

Fig. 2 Schematic diagram of a typical electrospinning system. A Taylor cone is formed when polymeric solution collected at tip of needle is subjected to high electric potential. The polymeric solution whips across the gap between tip of capillary and grounded collector once electrical potential reached the critical value. Solvent is evaporated during traveling resulted in formation of solid non-woven fibers at grounded collector

the temperature. Electrospinning can be used to prepare fibers from almost all soluble polymers with sufficient molecular weight. Thus far, electrospinning has been used to fabricate nanofibers from more than 200 natural and synthetic polymers. Composite nanofibrous scaffold can be produced by spinning different polymeric solutions together. For example, poly(glycolic acid) (PGA) and poly(lactic acid) (PLA) can be dissolved together and electrospun to form composite poly(lactic-co-glycolic acid) (PLGA) nanofibers [35].

During the spinning process, the polymeric solution is delivered to the capillary tip at a constant rate and subjected to an electric potential. Increased electric potential led to the formation of Taylor cone at the tip of the capillary. Once electric potential reaches the critical value, it overcomes the surface tension forces, resulted in the formation of a charged jet ejected from the apex of Taylor cone. The charged jet whips across space between capillary tip and collector, becoming thinner as the solvent evaporates, forming solid fibers at grounded collector that is oppositely charged [17].

Even though electrospinning system is easy to assemble and simple to operate, a number of processing parameters must be optimized in order to get the desired fibers. Insufficient optimization of electrospinning system may cause reduced fiber formation and the formation of droplets or beads, discontinued fibers and fiber with irregular diameter. Fiber diameter can be regulated by adjusting the processing parameters such as the distance between capillary and collector, the solution flow rate, and the applied voltage. Furthermore, changes in solution parameters, including viscosity, surface tension, conductivity, and polymer molecular weight and environmental parameters such as temperature, humidity, and air velocity also will influence the morphology and diameter of fabricated fibers. Salts, e.g. tetrabutylammonium bromide, sodium chloride and calcium chloride, can be added to the polymeric solutions or melts to increase the conductivity [36, 37]. A summary of the impact of each parameter on electrospun fibers was listed in Table 1. It is difficult to isolate the impact of individual solution parameter as changes in one solution parameter can generate an effect on another solution parameter (e.g. a change in polymer molecular weight also change the solution viscosity).

The fiber arrangement is largely dependent on the geometry of the collectors. Electrospinning can produce both random and aligned fibers. Aligned nanofibers provide topographic guidance to regulate cell alignment and subsequently tissue formation which is important for ligament, tendon, cardiac and skeletal muscle, blood vessel, and nerve that contain cells in a highly aligned arrangement. Aligned fibers are commonly collected using rotating mandrel and the degree of anisotropy is greatly affected by the rotating speed. In addition, aligned fibers also can be collected using a pair of parallel steel wires connected to the electrode, whereby the fibers deposited across the gap between the electrodes [38, 39]. Random fibers are collected when a plate collector or a ring-shaped collector are used. Generally, aligned fiber mats have smaller pore size compared to the random fiber mats as the fibers are more closely packed. Nevertheless, pore size is also affected by fiber diameter. Increased fiber diameter creates larger pore size.

Apart of collector geometry, the collector material also affects the morphology of deposited fibers. Kim et al. [40] found that PLLA and PLGA collected using metal collectors, a water reservoir and a methanol collector yielded smooth fibers, shrink fibers and swell fibers, respectively. Liu and Hsieh [41] showed that electrospun cellulose acetate collected on collectors with better conductivity was more closely packed, probably due to the faster dissipation of fibers' charge. In addition, they also found that highly porous fiber mesh was collected when porous collectors were used.

Electrospinning can produce coaxial and hollow fibers when a coaxial spinneret is used (Fig. 3). Coaxial spinneret allows 2 polymeric solutions to be spun simultaneously with one as 'core', surrounded by the 'shell' without mixing to produce the coaxial fibers [42]. This technique allows less spinnable polymers (e.g. polymer with limited solubility, low molecular weight and compact molecular conformation) to form fibers by using a readily spinnable polymer as supporting shell. For an instant, Li et al. [43] successfully electrospun poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV) in chloroform

Table 1 Summary of the effects of electrospinning parameters on fiber formation

Parameters	Effects on fiber formation	References
Processing parameters		
Distance between capillary and collector	Formation of beads when distance too short or too long, optimum distance needed for fiber formation	[58, 137, 138]
Solution flow rate	Larger fiber diameter with increase in flow rate, fiber with beads when flow rate too high	[58, 139, 140]
Applied voltage	Ambiguous[a]	
	No effect on fiber diameter	[141, 142]
	Larger fiber diameter with increase in voltage	[139, 143]
	Smaller fiber diameter with increase in voltage	[144, 145]
Solution parameters		
Surface tension	Less beads with decrease in surface tension	[146, 147]
Conductivity	Smaller fiber diameter and less beads with increase in conductivity	[148–150]
Polymer molecular weight	Larger fiber diameter and less beads with increase in molecular weight	[151–153]
Polymer concentration/viscosity	Larger fiber diameter and less beads with increase in concentration/viscosity	[154–156]
Dipole moment	Solvent with high dipole moment have better chance of producing spinnable polymeric solutions and give higher yield of fibers[b]	[157, 158]
Dielectric constant	Smaller fiber diameter and less beads with increase in dielectric constant	[159–161]
Environmental parameters		
Temperature	Smaller fiber diameter with increase in temperature	[162–164]
Humidity	High humidity resulted in pore formation on fiber	[165–168]

[a] Applied voltage affects the fiber diameter, but the effect is greatly influenced by other parameters

[b] Very few data

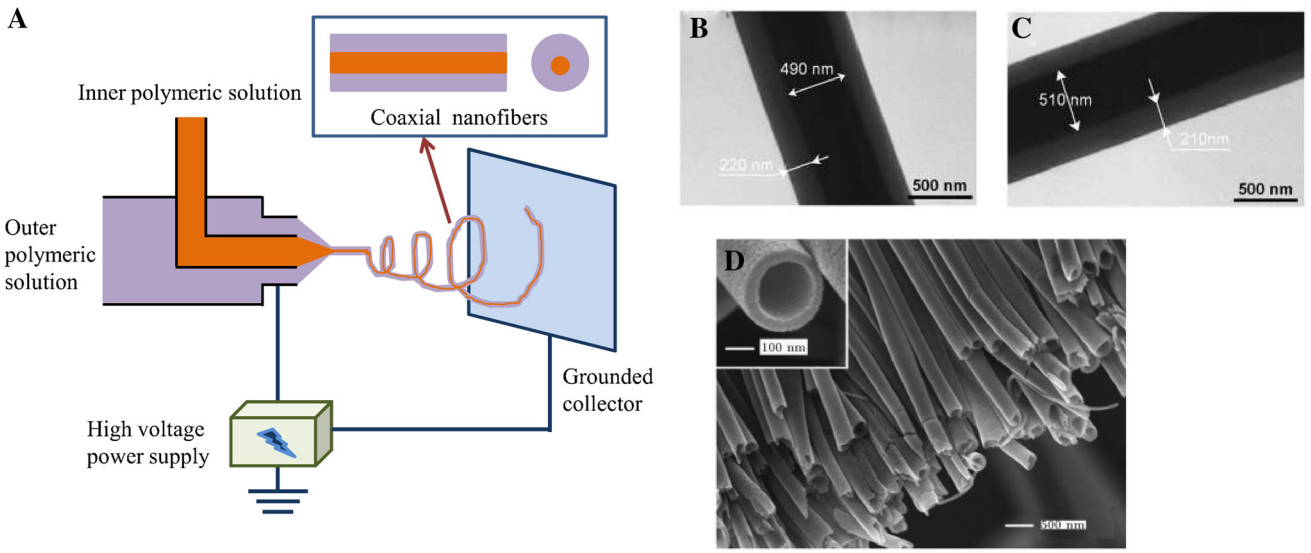

Fig. 3 Schematic diagram of a coaxial electrospinning system **A**. Two different polymeric solutions were extruded through the inner needle and outer needle of a coaxial spinneret. The spinneret was subjected to high electrical potential for the formation of a charged jet that will whip across the distance towards the collector to form the coaxial nanofibers. Hollow nanofibers were produced when the core was removed. **B** and **C** Transmission electron microscopy image of coaxial nanofibers [170] (Reproduced with permission from MDPI). **D** Scanning electron microscopy image of hollow nanofibers (Reprinted with permission from Ref. [171]. Copyright © 2004 American Chemical Society)

with poly(vinyl pyrrolidone) (PVP) in ethanol as the shell to stabilize the core solution. The PVP were later on removed by ethanol extraction. Wang et al. [44] produced silk nanofibers by extracting the poly(ethylene oxide) (PEO) shell with water after spinning. Yu et al. [45] used coaxial electrospinning to produce fibers from highly concentrated polymeric solution which was impossible using the conventional electrospinning system. They

successfully electrospun 35% (w/v) PVP into fibers using *N*,*N*-dimethylacetamide (DMAc) as the sheath fluid. They postulated that DMAc functioned as a lubricant that prevents polymer clogging at the needle tip and slows down the solvent evaporation, thus allowing the formation of Taylor cone and keeping the jet in fluidic condition for a longer period of time to form continuous fibers. Apart from coaxial fibers, triaxial fibers also have been produced. For example, Han and Steckl [46] and Yu et al. [47] produced triaxial nanofibers to control drug delivery.

Hollow fibers were produced when the core of the coaxial fibers was removed using specific solvent. For example, hollow TiO_2/PVP nanofibers were produced by removing the mineral oil core with octane [48]. Hollow chitosan nanofibers were formed by extracting the PEO of the core–shell structured PEO-chitosan nanofibers with water [49]. Zhang et al. [50] fabricated TiO_2 hollow nanofibers by calcined the PEO core-PVP/TiO_2 shell at 450 °C. PEO and PVP were decomposed during the calcination process.

The jet stability and fiber morphology of coaxial electrospinning are not only affected by the processing, solvent and environmental parameters but the solvent miscibility as well. However, the role of solvent miscibility is still no clearly elucidated as contradict results were reported. McCann et al. [51] claimed that immiscibility of solvents is critical for the formation of coaxial nanofibers. However, several research groups have described the fabrication of coaxial nanofibers using a miscible or the same solvent for the core and shell [52–54]. They postulated the core–shell morphology was maintained as the short time period of spinning process prevented the solvents from mixing.

In life science, coaxial electrospinning has been used to produce coaxial fibers with the synthetic polymer as core and natural polymer as shell, taking advantage of the polymer excellent mechanical strength and biological activity, respectively, to produce fiber scaffolds for tissue engineering applications. Furthermore, coaxial fibers have been tested for controlled release of bioactive molecules [55, 56]. The rate of molecule delivery can be altered by regulating the degradation rate of the outer shell, allowing sustained release of trapped molecules for a long period of time. Different molecules can be loaded into the core and shell, respectively, permitting controlled release of molecules at different time period (Fig. 4).

Porous fibers were fabricated when electrospinning was performed under humidity using a highly volatile liquid as the solvent (Fig. 5). For example, Lee et al. [57] produced highly porous polycarbonate (PC) hollow nanofibers via coaxial electrospinning under humidity using PC dissolved in highly volatile methylene chloride as the shell and silicon oil as the core. Pores were formed on the surface of the fibers due to rapid evaporation of the volatile solvents. Pore formation is a complex process and several pore forming

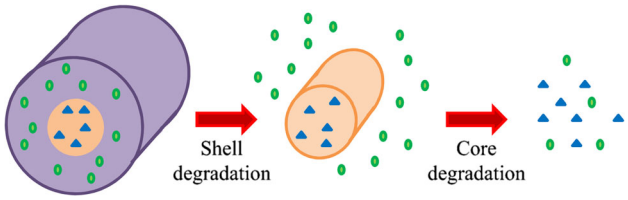

Fig. 4 Schematic diagram of a bi-biofunctionalized coaxial nanofiber. Two different biomolecules were incorporated to the core and shell comprised of different polymers respectively. The shell will degrade first to release molecule A (*round*) followed by the degradation of the core to release molecule B (*triangle*) to achieve dual-stage molecule release

mechanisms had been suggested. One of the possible mechanisms for pore formation is the breath figures. Water droplets formed at the fiber surface as the air condenses due to the cooling of fiber surface following the solvent evaporation. As the electrospinning jet dries, the water droplets leave a mark on the fiber surface in the form of pores. The pore formation also can be due to the vapor-induced phase separation (VIPS) as the water accumulated on fiber surface can act as a non-solvent. In addition, solvent evaporation during the spinning process causes solution instability that leads to phase separation into polymer rich and polymer poor regions. The polymer-rich region solidifies into the matrix and the polymer poor region forms pores. This technique was known as the thermally induced phase separation (TIPS) [58, 59].

Mixed fibers and multilayered fibers can be produced by electrospinning multiple spinnerets concurrently and sequentially, respectively (Fig. 6). Simultaneous electrospinning of multiple polymeric solutions produces hybrid scaffolds which permit the fine tune of the scaffold mechanical, chemical and biological properties. Multilayering electrospinning enables the control of composition, structure and mechanical properties of the layers within the scaffold. This technique is extremely useful for the tissue engineering of tissues with multilayer structure such as blood vessel. Vaz et al. [60] produced a bilayered tubular scaffold consists of circumferentially orientated outer PLA layer with randomly aligned inner polycaprolactone (PCL) layer for blood vessel tissue engineering. The outer layer is important in giving the blood vessels strength and resilient to withstand pressure and to guide smooth muscle cell orientation, whereas the inner layer aids endothelium formation.

3 Collagen

3.1 Family of collagen

Collagen is the major fibrous protein of ECM [61]. It constitutes 20–30% of total body protein and plays an

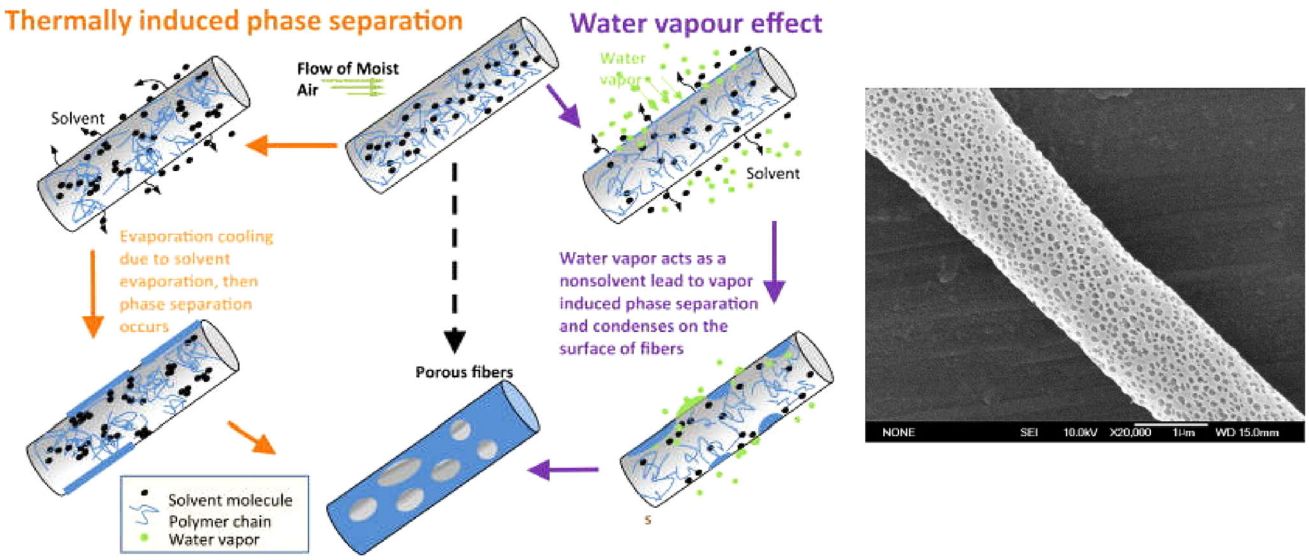

Fig. 5 Schematic diagram illustrating the mechanisms of pore formation on surface of nanofibers during the electrospinning process [172] (Reproduced with permission from Elsevier). Scanning electron microscopy image of porous nanofibers [173] (Reproduced with permission from Elsevier)

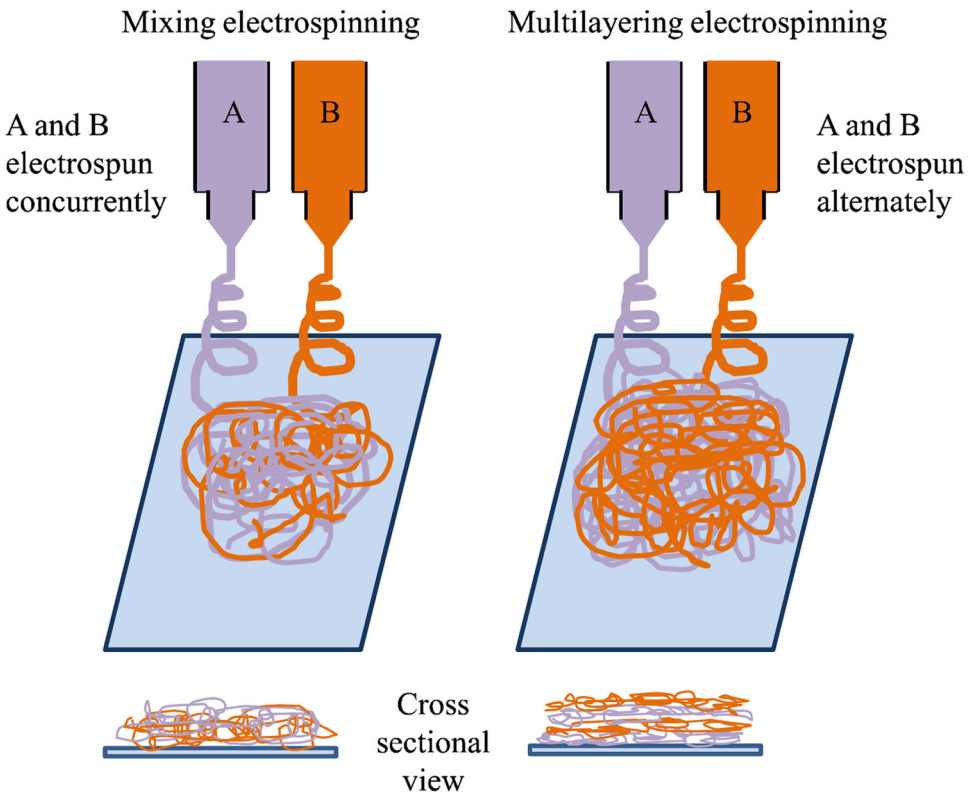

Fig. 6 Schematic diagram illustrating mixing and multilayering electrospinning system to produce mixed and multilayered nanofiber scaffolds

important role in the regulation of cell function and providing the structural support to tissues and organs. So far, 29 types of collagen composed of at least 46 distinct polypeptide chains have been identified [62, 63]. Collagen composed of 3 polypeptide α-chains, each possessing the left-handed conformation, and polyproline II-type (PPII) helix about 300 nm long and 1.5 nm in diameter [63, 64]. Collagen can be found in almost all parts of the body, but the types of collagen presence differ between different organs. For example, skin predominantly consists of

collagen type I and III, whereas collagen type II is the major constituent of cartilage. Abnormality in collagen has been linked to a number of human diseases (Supplement 1).

According to Myllyharju and Kivirikko [65], collagen superfamily can be subdivided into 8 families based on the supramolecular assemblies and other features of its members. The 8 families are: (1) fibril-forming collagens—types I, II, III, V, XI, XXIV and XXVII; (2) fibril-associated collagens with interrupted triple helices and structurally related collagens—types IX, XII, XIV, XVI, XIX, XX, XXI, XXII and XXVI; (3) collagens forming hexagonal networks—types VIII and X; (4) the family of type IV collagens that forms the basement membrane; (5) type VI collagen that forms beaded filaments; (6) type VII collagen that forms anchoring fibrils for basement membrane; (7) collagens with transmembrane domains—types XIII, XVII, XXIII and XXV; (8) the family of type XV and XVIII collagens.

Collagen is synthesized by a number of cells, including fibroblasts, chondroblasts and osteoblasts. In brief, newly synthesized polypeptide precursors of α chains with a special amino acid sequence at their N-terminal ends are processed in rough endoplasmic reticulum to form the pro-α chains by removing the special sequence. Then, the pro-α chains undergo hydroxylation and glycosylation to form procollagen before transported to Golgi apparatus, whereby they are packaged in secretory vesicles that fuse with the plasma membrane and release its content into the extracellular region. In the extracellular region, procollagens are cleaved by *N*- and *C*-procollagen peptidases to form triple-helical tropocollagen molecules that spontaneously join together to form collagen fibrils. Oxidative deamination of part of the lysyl and hydrolysyl residues in collagen fibrils resulted in the formation of reactive aldehydes which condense with lysyl or hydroxylysyl residues of neighboring collagen to form cross-linked collagen fibers [66]. Collagen is relatively stable within the body but remodel constantly. It can be digested by enzymes such as collagenase [67].

3.2 Characteristics of collagen for tissue engineering applications

Majority of the cells in human body are in direct contact with ECM proteins in nanometer scale [68]. This interaction gives both direct and indirect signaling cues to the cells, regulating cellular activities such as migration, proliferation, differentiation, gene expression and protein secretion. Collagen is widely used for the fabrication tissue engineering scaffolds as it gives structural support to cells and provides both biological and mechanical cues that define cell behavior and tissue formation. Pros and cons of collagen for it use in tissue engineering is listed in Table 2.

As the most abundant protein on earth, collagen can be harvested from almost all living animals. Bovine skin and tendon, rat tail and porcine skin are the most common sources of collagen used in tissue engineering [13]. Collagen from different species has slightly different properties [69, 70]. Recombinant collagen is a newer source of collagen which is produced in the recombinant system, including plants, bacteria, insect cells and yeast [71]. Recombinant human collagen eliminates the risk of pathogen transmission and batch-to-batch variation but is more costly [72].

Collagen is extremely pliable and can be processed into various forms for biomedical applications. Collagen has been used in the form of sheets, tubes, foams, sponges, nanofibers, fleeces, powders, injectable viscous solutions and dispersions [73]. Scaffolds that provide microenvironment as closely as possible to the nature ECM will have a better chance of achieving tissue regeneration. Thus, collagen fiber in nanometer scale is highly desirable for tissue engineering applications. Electrospinning is one of the most promising techniques to produce collagen nanofibers.

4 Electrospun collagen nanofibers

Collagen electrospinning has been reported in many studies (Supplement 2). Currently, only collagen type I, II, III and IV has been electrospun. Electrospinning can produce both aligned and random collagen nanofibers for various purposes. In tissue engineering, fiber alignment is important as it affects the mechanical property and cell activity.

Electrospun collagen nanofibers have been found to promote wound healing. Powell et al. [74] compared the wound healing potential of freeze-dried and electrospun collagen nanofibers and found that both scaffolds supported the formation of keratinized epidermal layer and stratified dermal layer *in vitro* when cultured with human epidermal keratinocytes and dermal fibroblasts at the air–liquid interface. A layer of the basement membrane was detected at the dermal–epidermal junction. Grafting of these cultured skin substitutes *in vivo* showed that electrospun collagen nanofibers have higher take rate and lower wound contraction.

The electrospun collagen nanofibers are superior to other polymer nanofibers in several aspects. Firstly, collagen is the main ECM protein of many tissues in the body. Thus, collagen nanofibers are most closely mimicking the ultrastructure of native tissues. Secondly, collagen has poor immunogenicity. Implantation of collagen nanofibers is not likely to activate the host immune response. Thirdly, collagen has excellent biocompatibility. Almost all cells like collagen, thus collagen nanofibrous scaffold is suitable for

Table 2 Pros and cons of collagen as biomaterial in tissue engineering (modified from Lee et al.) [169]

Pros	Cons
Biocompatible	High cost of purification
Abundant and easily available	Batch to batch variation
Non-antigenic	Risk of disease transmission (e.g. bovine spongeform encephalopathy)
Non-toxic	
Biodegradable (degradation can be regulated via cross-linking)	Variability in enzymatic degradation rate (depending on enzyme concentration)
	Difficult to maintain its dimension *in vivo* due to swelling
Compatible with synthetic polymers	
Malleable into various forms (e.g. sheets, tubes, sponges, foams, nanofibers)	Poor mechanical strength *in vivo* (not suitable for load bearing tissues, e.g. bone)
Promote blood coagulation	
Synergic with bioactive components	
Easily modifiable	
Bioreabsorbable	

the engineering of most body tissues. The most obvious shortcoming of collagen nanofibers is the poor mechanical properties. The mechanical properties of collagen nanofibers can be improved via cross-linking or modified with natural/synthetic polymers or inorganic molecules.

4.1 Effects of collagen sources on electrospun collagen nanofibers

Collagen that is suitable for electrospinning can be collected from many sources. Thus far, most of the studies used type I bovine skin collagen to produce electrospun collagen nanofibers. Nonetheless, other sources of collagen also have been tested. Hofman et al. [75] electrospun fish collagen in multiple molecular formats and found that low molecular weight gelatin and atelocollagen failed to form fibers. Denatured collagen dissolved in 10% acetic acid formed fibers but acid soluble collagen with intact triple helical structure must be denatured by dissolving in 40% acetic acid or HFP to form a spinnable solution. Interestingly, they also found that higher collagen concentration (at least 20%) was needed when acetic acid was used as the solvent to prepare the spinning solution. Fish collagen electrospinning also has been reported by other groups and the produced nanofibers were found to support cell biological activities [76–78].

Recently, recombinant human collagen has been tested for electrospinning. The use of recombinant collagen carries no risk of disease transmission and possesses the biocompatibility of human proteins. Plant-derived human collagen has been successfully electrospun and showed excellent biocompatibility with human keratinocytes, fibroblasts and endothelial cells [79]. The keratinocytes and fibroblasts cultured on electrospun plant-derived human collagen scaffolds formed stratified epidermal and dermal layers *in vitro*. We believed that recombinant human collagen will be the key research area in the use of collagen for medical, cosmetic and pharmaceutical sectors as they devoid of the geological, political, ethical and religion issues.

Source of collagen has been found to affect the properties of resulting fibers. According to Matthews et al. [80], electrospinning of collagen type I isolated from human placenta using parameters optimized for calf skin collagen type I resulted in the formation of less uniform fibers with a larger range of diameter. In the same study, they also found that collagen isotype (collagen type I and collagen type III) also influences the fiber structural properties.

Zeugolis et al. [61] electrospun an in-house prepared collagen and collagens obtained from 5 different companies and found that not all collagens can form fibers. For those who form fibers, the fiber properties vary from one to another. Interestingly, they found that even collagen isolated from the same source also will give rise to fibers with different properties whereby electrospinning of purified type I bovine dermal atelocollagen from 2 different companies, Koken (Tokyo, Japan) and Symatese Biomateriaux (Chaponost, France) yielded fibers with a mean diameter of 665 ± 150 and 495 ± 78 mm, respectively. They speculated that this discrepancy may be due to differences in glycosylation or glycation which was possibly related to the race and age, respectively, of the animal used. In the same study, they also found that collagens that are more readily dissolve in acetic acid give rise to fibers with better quality with HFP as the solvent.

4.2 Effects of solvents on electrospun collagen nanofibers

Solvent selection is a very important aspect of collagen electrospinning. An ideal solvent should facilitate fiber formation without denatures or compromises the collagen

integrity. HFP (1,1,1,3,3,3-hexafluoro-2-propanol), TFE (2,2,2-trifluoroethanol), acetic acid and PBS/EtOH (phosphate buffered saline/ethanol) have been used for collagen electrospinning with HFP being the most popular choice (Supplement 2). HFP is a highly volatile organic solvent with low boiling point (59 °C). Due to its low boiling point, HFP is an ideal solvent for electrospinning as it evaporates very fast when fibers travel across the gap between the tip of capillary and collector. Besides that, HFP is also a stronger solvent for collagen compared to acetic acid. Similar to HFP, TFE is a highly volatile fluoroalcohol that can facilitate collagen fiber formation.

Although HFP promotes collagen fiber formation, however, it denatures the collagen and is toxic to cells. Zeugolis et al. [81] suggested that electrospinning of collagen from HFP may lead to the formation of gelatin fibers. Gelatin is the denatured form of collagen, acquired by denaturing the triple helical structure. However, it has been shown that electrospinning of collagen with HFP only partially denatures the protein, indicated by the 45% loss of native triple helical structure [82]. A separate study by Liu et al. [83] found that collagen electrospun from acetic acid contained more triple helical structure compared to collagen electrospun from HFP. Comparison between electrospun collagen and gelatin revealed that the fibers are structurally different. In addition, the biological activity also differs whereby collagen nanofibers demonstrated more favorable cellular responses [84, 85]. These results suggested that it might be necessary for electrospun collagen to fully recapitulate the structure of native fibers to initiate proper tissue regeneration.

HFP and TFE are excellent solvents for collagen and for the fabrication of electrospun collagen nanofibers. However, these fluoroalcohols are expensive and toxic to the environment. Furthermore, fluoroalcohols cause collagen denaturation. The possibility of substituting fluoroalcohol with the benign solvent for collagen electrospinning has been reported in several studies. Elamparithi et al. [77] used glacial acetic acid/DMSO at a ratio of 93/7 as the solvent and successfully produced collagen nanofibers that retain the characteristics of native collagen. Dong et al. [86] used a mixture of PBS and ethanol at 1:1 ratio for the preparation of collagen nanofibers that maintain its triple-helical structure. Baek et al. [87] showed that collagen dissolved in PBS and ethanol at 1:1 ratio can produce both random and aligned nanofibers.

4.3 Cross-linking of collagen nanofibers

Electrospun collagen nanofibers are mechanically weak in nature and readily soluble in water [84, 88, 89]. Rapid degradation is not ideal for tissue engineering application as the scaffold will disappear before the cells lay out their own ECM. Thus, collagen fibers have to be cross-linked to reduce the water solubility, to improve the resistant to enzymatic degradation and to enhance the mechanical strength (Fig. 7). Collagen can be chemically (e.g. glutaraldehyde, genipin, carbodiimides) [90–92], enzymatically (e.g. transglutaminase, tyrosinase, laccase) [93, 94] or physically (e.g. UV radiation, gamma radiation, dehydrothermal treatment) [95, 96] cross-linked. Every cross-linking method has its limitations. Chemical treatment introduces toxic compounds to the material. Thus, it may be unfavorable for biomedical applications [97]. Physical treatment produces a low degree of cross-linking as the reaction only happens at the material surface [98]. Enzymatic treatment only targets specific amino acids and the cross-linking process is more difficult to control [99].

Glutaraldehyde (GTA) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) are the most popular cross-linkers used to cross-link electrospun collagen fibers (Supplement 2). GTA is a popular protein cross-linking agent as it is less costly, reacting relative fast, cross-linking over various distances and producing extensive cross-linking [100]. However, use of GTA compromises biocompatibility as it is cytotoxic and has been linked with tissue calcification [101–103]. Proper washing to remove GTA residue can reduce the cytotoxicity [104]. EDC is a zero-length cross-linking agent which does not incorporate into the macromolecule and is not cytotoxic [105]. However, it has been reported that collagen mats cross-linked with EDC lost it porous structure due to fiber swelling and pore occlusion, whereas cross-linking with 1,4-butanediol diglycidyl ether (BDDGE) better preserved the porous structure, although a certain degree of fiber swelling and pore size reduction was observed [106]. Kidoaki et al. [107] irradiate the collagen fibers with UV light (0.5 mW/cm^2 at 365 nm) for 60 min to induce cross-linking and found that the fibers become water insoluble. Huang et al. [108] tried 4 different cross-linkers, GTA, EDC, EDC with NHS (*N*-hydroxysulfosuccinimide) and genipin to cross-link the electrospun collagen nanofibers. It was found that the fiber physical and biological properties were different when different cross-linkers were used. Electrospun collagen nanofibers lost its fiber morphology after treated with GTA and genipin cross-linking only can maintain the nanofiber architecture for a short term. Cross-linking with EDC and EDC-NHS showed the best results whereby it retain the fiber morphology, slow down the fiber degradation and improve the fiber biocompatibility. Similarly, Torres-Giner et al. [109] found that EDC-NHS and transglutaminase (TG) were superior cross-linkers for electrospun collagen nanofibers for electrospun collagen nanofibers compared to UV and genipin. Electrospun collagen nanofibers cross-linked with UV underwent dissolution in culture medium whilst those cross-linked with

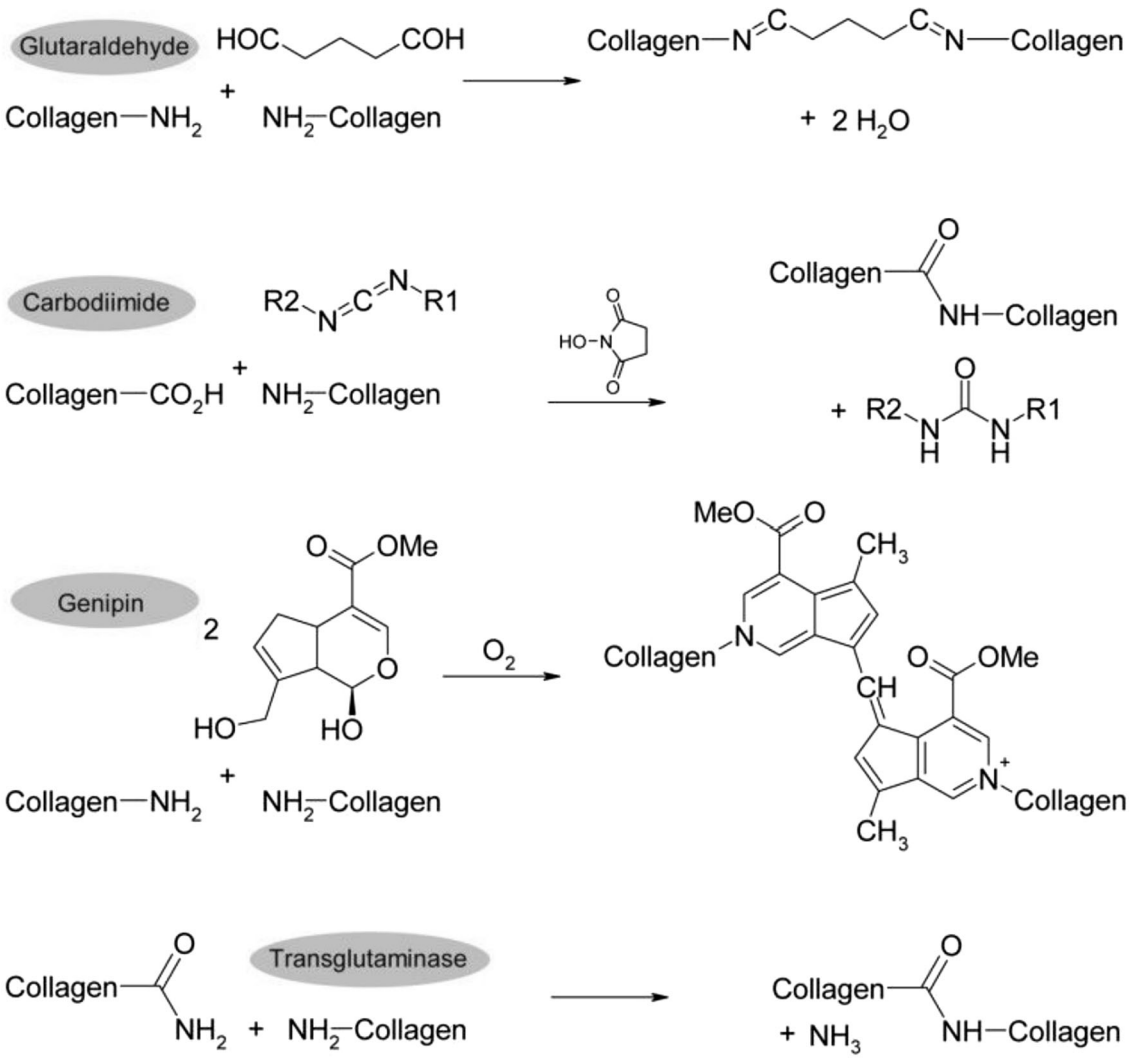

Fig. 7 Schematic diagram of commonly used cross-linking methods for collagen [174] (Reproduced with permission from BioMed Central)

genipin reduced cell viability. Nonetheless, cellular response to genipin cross-linking varied with cells whereby Subbiah et al. [110] demonstrated that genipin cross-linking reduced the proliferation of H9c2 cells (rat cardiomyoblasts) but not the human umbilical vein endothelial cells and human mesenchymal stem cells.

4.4 Composite collagen nanofibers

Pure collagen nanofibers have poor mechanical strength. Thus, collagen was normally mixed with synthetic polymers during the spinning solution preparation to produce composite nanofibers. The addition of other polymers helps to modify the morphology, mechanical and biological properties of the composite nanofibers. Hydroxyapatite, PCL, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and PGA are examples of synthetic polymers that have been blended with collagen to tweak the nanofibers properties for engineering of different tissues [111–114]. Preparation of composite collagen nanofibers with natural polymers is less common. Nonetheless, collagen has been blended with elastin and chitosan to prepare composite nanofibers in the previous studies [115, 116].

Another strategy to produce composite collagen fiber mesh is via multilayering electrospinning whereby different polymeric solutions were spun sequentially. Multilayering electrospinning permits the production of multilayered scaffolds. Each layer of the multilayered scaffold has distinct structural features, biological activities and physical characteristics. The multilayered scaffold can be used for tissue engineering of layered tissues such as blood vessels and skin. Kidoaki et al. [107] produced multilayer sheets via layer-by-layer electrospinning of segmented poly(urethane), styrenated gelatin and collagen type I and bilayered tubular constructs via sequential electrospinning of collagen type I and segmented polyurethane (PU). Apart from layer-by-

layer electrospinning, mixing electrospinning that involves simultaneous electrospinning of 2 different polymeric solutions for 2 separate spinnerets also can be used to produce composite collagen fiber mesh.

Coaxial electrospinning that produces fibers with core–shell structure is another method to produce composite collagen fibers. Collagen can be electrospun as the inner (core) or outer (sheath) layer. Nonetheless, due to its excellent biological activity, collagen is commonly electrospun as the outer layer with a synthetic polymer as the inner layer to improve the mechanical strength [117, 118]. To produce the coaxial collagen nanofibers, collagen and synthetic polymer dissolved in the right solvent were extruded through the inner needle and outer needle respectively at appropriate flow rate. The needles were applied with suitable voltage for the formation of a charged jet that will whip across the distance to form coaxial nanofibers on the collector.

4.5 Biofunctionalization of collagen nanofibers

The purpose of biofunctionalization is to provide extra biological guidance to the cells on top of the physical and biological signals given by the fibers to enhance cell biological activity and tissue formation by regulating the activities of various skin cells. Electrospinning allows the incorporation of fragile biomolecules such as growth factors, cytokines, DNA, RNA and drugs into the fibers as it is a mild nanofiber fabrication technique. For example, Tang et al. [119] prepared PLGA/HA (core)-collagen/amoxicillin (shell) nanofibers via coaxial electrospinning. Amoxicillin, an antibiotic, trapped in the collagen was gradually released as the collagen degraded. In addition, biomolecules also can be introduced to the electrospun fibers. The biggest different between in situ and post-electrospinning biofunctionalization is biomolecule distribution whereby post-electrospinning biofunctionalization only introduces the biomolecules to the fiber surface (Fig. 8). Since biomolecule distribution is limited to the fiber surface, the signals provided by post-electrospinning biofunctionalization last for a shorter period of time compared to in situ biofunctionalized counterpart that supports local delivery of biomolecules for a longer period of time (can up to several months depending on fiber degradation). By producing coaxial nanofibers, two different biomolecules can be released in stages by incorporated one biomolecule in the shell and another in the core.

In situ biofunctionalization involves the direct addition of biomolecules into the spinning solution, whereas introduction of biomolecules to the fiber surface can be done via physical or non-covalent adsorption and chemical or covalent conjugation. Physical adsorption involves the attachment of biomolecules to the fiber surface via electrostatic and van der Waals interactions. Physical adsorption is not suitable for small biomolecules due to weak individual interactions between biomolecule and polymer surface. Covalent conjugation involves the modification of fiber surface or molecules to introduce new functional groups (such as amines, carboxylic acids, aldehydes and thiols) that will be used for biomolecule conjugation. Covalent conjugation offers the advantages of slower release for a sustained period of time and slower degradation [120]. However, special care is needed when designing the conjugation protocol as it may lead to biomolecule inactivity due to disappearance or damage of the functional groups and masking of the active site.

4.6 Improving cell penetration into collagen fiber mesh

Studies have proven that cells can migrate into the electrospun collagen fiber mesh to a certain extent [74, 80, 88], probably by pushing the nanofibers aside through the amoeboid movement. In spite of this, cell ingrowth is still a concern as the low porosity (especially the aligned fiber mesh) hinders cell penetration. The usefulness of collagen

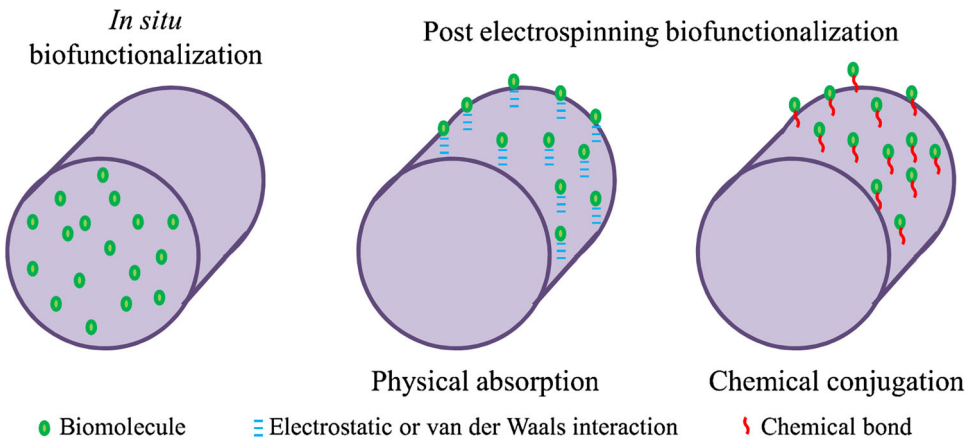

Fig. 8 Schematic diagram showing in situ and post-electrospinning biofunctionalization of electrospun nanofibers

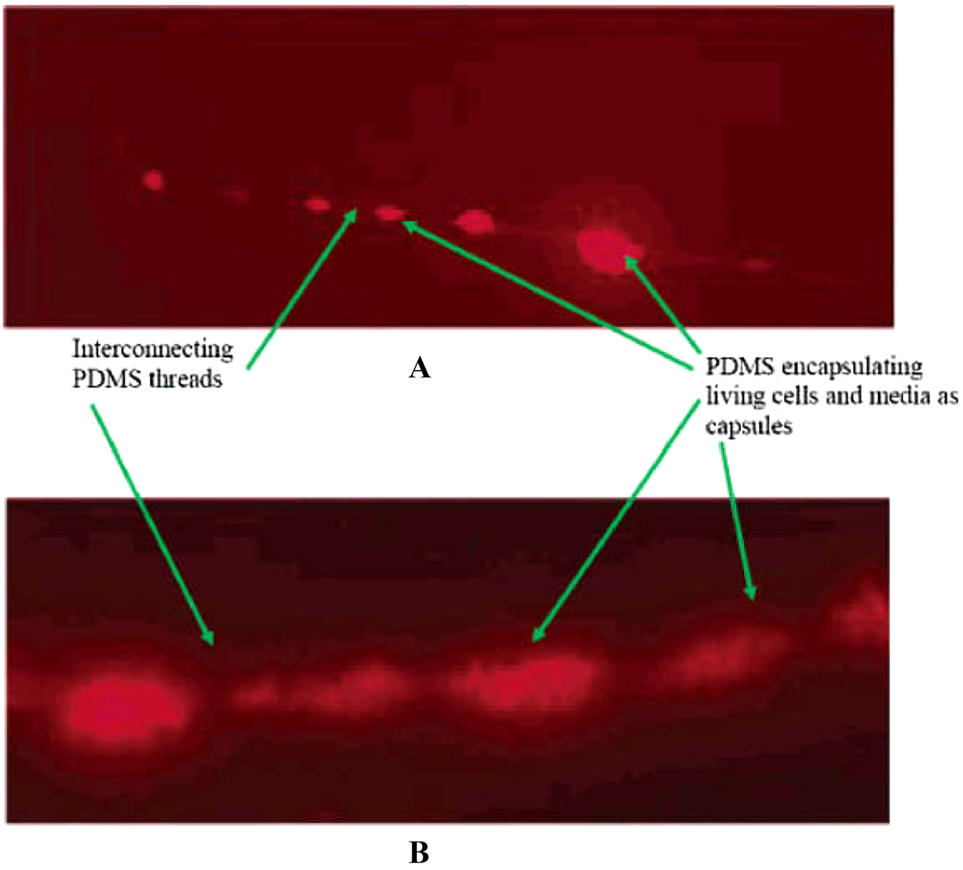

Fig. 9 Encapsulation of living cells within the electrospun nanofibers (Reprinted with permission from Ref. [121]. Copyright© 2006 American Chemical Society)

nanofibers in tissue engineering will be very limited if it only supports the formation of the single cell layer.

One of the solutions to overcome limited cell infiltration is via cell electrospinning by which live cells were added to the spinning solution or by using coaxial electrospinning to produce cells-encapsulated nanofibers. These techniques deliver cells directly into the 3-D nanofiber mesh. However, the viability of electrospun cells is of major concern as they were exposed to toxic solvent, suboptimal environment condition during electrospinning and high shear stress created by the narrow nozzle. Using the coaxial electrospinning, Townsend-Nicholson and Jayasinghe [121] successfully electrospun living cells suspended in culture medium with polydimethylsiloxane (PDMS) shell into fibers (Fig. 9).

Electrospinning and electrospraying have been combined to deliver cells into the fiber scaffolds. In this setting, simultaneous electrospinning of nanofibers and electrospraying of cell-encapsulated nanospheres allows the dispersion of cells throughout the fiber mesh. Stankus et al. [122] electrosprayed smooth muscle cells concurrently with electrospinning of poly(ester urethane)urea (PEUU) and found that the electrosprayed cells have excellent viability and growth rate. Layer-by-layer assembly is another technique to distribute cells inside the scaffolds. In this approach, cells were seeded on electrospun fiber mesh before new fiber mesh were electrospun on top of the cells. New cells were seeded on the newly spun fiber mesh and this process was repeated until the desirable thickness was achieved. The biggest advantage of this approach is different cell types can be seeded to form functional tissue. For example, Yang et al. [123] produced skin-like tissue by preparing 18 layers of fiber-fibroblast with 2 layers of fiber-keratinocyte on top using PCL/collagen.

Cell penetration into fiber mesh can be enhanced by increasing the pore size. One of the easiest ways to increase pore size is by increasing the fiber size. As an alternative, bigger pore size can be achieved by removal of one of the polymer from composite fiber mesh prepared via mixing electrospinning. Cross-linking prior fiber removal is vital as the reduced mechanical strength after polymer removal may cause the scaffold to collapse and end up with lower porosity. In addition, pores also can be mechanically introduced to electrospun fiber mesh. For example, Bonvallet et al. [124, 125] used acupuncture needle to create pores of size 160 nm on electrospun PCL/collagen mat and found that the pores-induced scaffolds accelerated wound healing, in relative to the nonporous scaffolds.

Apart from direct loading of cells into the fiber mesh and increasing pore size to facilitate cell infiltration, bioreactor also can aid cell infiltration by 'pushing' the cell into the scaffolds using the hydrodynamic forces. In addition, bioreactor provides the extra advantage of improving nutrient and waste exchange which is important to maintain cell viability.

4.7 Pros and cons of electrospun collagen nanofibers

Replication of native ECM by engineered scaffolds is a crucial aspect of tissue engineering as scaffold give mechanical and structural supports, provide cell attachment sites, support cell–cell and cell–ECM interactions, regulate cell orientation and arrangement, control cell biological activity and differentiation, guide tissue formation and remodeling, provide provisional matrices to enhance and regulate tissue development, and create microenvironments that favor healing. The diameter of collagen fibrils in native tissues is in the range between 30 and 300 nm [126]. Electrospinning has been reported to produce collagen fibers in the range from 50 to 1200 nm [82, 88, 127]. Shih et al. [127] electrospun 4, 8 and 12% w/v collagen and yielded fibers with the diameter ranging from 50 to 200, 200 to 500 and 500 to 1000 nm, respectively. This proved that electrospinning has the capacity to produce collagen fiber of various diameters in nanometer scale that identical to the native collagen fibrils.

In comparison to other fabrication techniques, electrospinning tremendously increases the scaffold surface area. This feature improves the cell–scaffold interaction which plays an important role in regulating cell activity and tissue regeneration. In addition, it also can be engineered to deliver drugs, growth factors, genes and other bioactive molecules that promote tissue regeneration. Electrospun collagen nanofibers can be collected in multiple geometries. For example, it can be collected as a sheet for skin tissue engineering and in a tubular shape for vascular tissue engineering. Furthermore, fibers alignment, pore size and porosity also can be tailored.

The biggest shortcoming of electrospun collagen nanofibers is probably the collagen denaturation when HFP was used as the solvent. Nevertheless, HFP remained to be the most popularly used solvent for collagen electrospinning as it is difficult to control fiber formation when other more benign solvents (e.g. acetic acid) was used. Other limitations with electrospun collagen nanofibers are the rapid degradation, weak mechanical strength and shrinkage. Shrinkage reduces the porosity, cell penetration and diffusional capacity of the fiber mesh. However, these problems can be overcome via cross-linking and blending of collagen with other biomaterials with better mechanical properties. Cross-linking with chemicals has potential toxicity. However, the toxicity can be reduced via the use of natural cross-linker such as genipin. Preparation of composite collagen nanofibers reinforced with synthetic polymers can improve the physical property without compromising the biological activity.

5 Application of electrospun collagen nanofibers in skin tissue engineering

Electrospun collagen nanofibrous scaffold is widely used in the preparation of engineered skin due to its excellent biocompatibility. Nonetheless, other biomaterial is often added to the collagen nanofibrous scaffold to improve the mechanical property. PCL is a biodegradable synthetic polymer that has been widely electrospun with collagen due to its excellent mechanical strength and biocompatibility. Powell and Boyce [128] prepared engineered skin using electrospun collagen/PCL nanofibers as the scaffold and found that blending a small amount of PCL to collagen nanofibers significantly improved the mechanical property without compromising the biocompatibility. Collagen/PCL fiber scaffolds cultured with human epidermal keratinocytes and dermal fibroblasts supported the formation of the well stratified epidermis and dermis with continuous basal cell layers and basement membrane. In a more recent study, Suganya et al. [114] electrospun collagen/PCL nanofibers as a scaffold for skin tissue regeneration and found that the fibers mesh supports mice dermal fibroblast proliferation and collagen secretion. Electrospun collagen/PCL nanofibers also have been tested with human adipose stromal cells to promote wound healing [129].

PGA is another synthetic biomaterial that has been electrospun together with collagen. Sekiya et al. [113] compared electrospun collagen/PGA nanofibers with commercial collagen matrix on wound healing and found that the collagen/PGA nanofibers promote cell migration and neovascularization. They speculated that it may be due the nanostructure effect which provides more space for cell infiltration and the presence of PGA that was known to enhance angiogenesis. Clearly, combination of collagen and PGA in this study merged the excellent biocompatibility and proangiogenic property of respectively material to produce a scaffold with superior characteristic for skin tissue engineering applications.

Mixing of collagen and natural biomaterials for electrospinning is less common. Rnjak-Kovacina et al. [116] mixed collagen and elastin for electrospinning and found that GTA-cross-linked electrospun collagen/elastin nanofibers supported human dermal fibroblast attachment, migration and proliferation. *In vivo* implantation for 6 weeks showed that the scaffold performance was comparable to Integra, by which both persisted over 6 weeks

with moderate degradation and remodeling, promoted fibroblast migration and collagen deposition, allowed vascular infiltration, and stimulated a mild immune response.

In some of the studies, the composite collagen nanofibers were loaded with biomolecules to enhance it wound healing properties. Chong et al. [130] tested the feasibility of ASC-J9, an androgen receptor inhibitor loaded electrospun collagen/PCL nanofibers to expedite wound healing and found that the scaffold promotes fibroblast attachment and proliferation, and facilitates keratinocyte migration and wound closure. In a separate study, electrospun collagen/PCL nanofibers were covalently conjugated with EGF to promote skin dermis regeneration [131]. Lai et al. [132] utilized dual-needle electrospinning system by which one needle extrudes hyaluronic acid loaded with bFGF and VEGF-loaded gelatin nanoparticles and the another needle extrudes collagen loaded with EGF and PDGF-loaded gelatin nanoparticles to produce composite fiber mesh that allow staggered release of growth factors. This design allowed the rapid release of bFGF and EGF to accelerate re-epithelialization and angiogenesis at the initial stage, followed by the slower and sustained release of VEGF and PDGF at the later stage to stimulate vascular maturation. Diabetic rats implanted with the scaffold demonstrated accelerated wound closure, collagen deposition and vascularization.

Lin et al. [133] electrospun collagen/zein nanofibers with berberine, an antimicrobial drug. Implantation of this scaffold promoted the healing of full-thickness wounds. Uzunalan et al. [134] proposed the use of silver nanoparticles loaded collagen nanofibers as a scaffold to promote wound healing. In the study, silver nanoparticles were sprayed to the surface of nanofibers and were found to inhibit the growth of *Escherichia coli*. Nonetheless, the cytocompatibility of the nanofibers was not evaluated. Specifically to treat diabetic wounds, Lee et al. [135] loaded glucophage (an anti-diabetic drug) into electrospun collagen/PLGA nanofibers. The scaffold permit sustained drug delivery and *in vivo* application of this scaffold expedited the wound healing of diabetic rats. Wound analysis found a greater accumulation of collagen as a result of MMP-9 inhibition by glucophage.

Wei et al. [136] used coaxial electrospinning to produce PCL/vitamin A palmitate core and collagen/silver nanoparticles shell nanofibers. Vitamin A palmitate was encapsulated as healing-promoting drug whilst silver nanoparticles as anti-bacterial agent. Thus, by incorporating both molecules, the coaxial nanofibers gained the antibacterial and wound healing promoting properties. Their results showed that the coaxial nanofibers inhibited the growth of *Staphylococcus aureus* and vitamin A palmitate gradually released up to 72 h with initial burst release on the first 5 h. In addition, the coaxial nanofibers demonstrated excellent biocompatibility to help cell attachment.

6 Conclusion

Electrospinning is a simple and versatile technique for the formation of nanofibers that morphologically similar to natural ECM comprising of ultrafine fibers with high porosity and large surface area. Improvement in the basic knowledge of electrospinning has led to the introduction of many new electrospinning systems with more control on the nanofiber structural feature and functionality, resulted in the production of core–shell, hollow and porous nanofibers. Future research of electrospinning may be focused on the 3-D arrangement of electrospun nanofibers.

Collagen has excellent biological activity required for the regulation of cellular function. However, collagen nanofibers might be denatured during the electrospinning process. Denatured collagen, also known as gelatin, has different physiochemical and biological characteristics compared to native collagen. These changes will affect the scaffold properties and cellular response which lead to improper tissue regeneration. Thus, it is very important to develop an electrospinning system that can produce nanofiber consistently without causing collagen denaturation. To improve the mechanical strength, electrospun collagen nanofibers can be strengthened via cross-linking or prepared as composite nanofibers via reinforcement with synthetic polymers. Recognizing that collagen nanofibers may not provide sufficient biological cues to regulate cell function and tissue formation, biomolecules such as growth factors can be incorporated to the nanofibers. We are expecting that in the near future, collagen nanofiber-based engineered skin will be commercialized and used clinically. However, this can only be achieved when the challenges such as mass production and reproducible fiber formation are overcome. Together, electrospinning and collagen may hold the key to achieve the holy grail of tissue engineering, the regeneration of lost tissues and organs.

Authors' contributions All the authors participate in drafting the article and revising it critically for important intellectual content. All the authors give final approval of the version to be published.

Compliance with ethical standards

Conflict of interest The authors have no financial conflicts of interest.

Ethical statement There is no animal experimental carried out for this article.

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