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REVIEW ARTICLE

Current research on electrospinning of silk fibroin and its blends with natural and synthetic biodegradable polymers

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ABSTRACT: Silk fibroin (SF) is a kind of natural polymers with a great potential in biomedical application. Due to its good biocompatibility, biodegradability and minimal inflammatory reaction, SF is an excellent candidate for generating tissue engineering scaffolds. Electrospinning is a simple and effective method to fabricate nanofibers, which has several amazing characteristics such as very large surface area to volume ratio, flexibility in surface functionalities, and superior mechanical performance. The electrospun nanofibers from SF and its blends have been used for varied tissue engineering. This paper will give a brief review about the structure, properties and applications of SF and blend nanofibers via electrospinning.

KEYWORDS: electrospinning; nanofiber; silk fibroin (SF); biodegradable polymer

Contents

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1 Introduction

Tissue engineering is a new subject which aims at rehabilitating of the damaged tissues. One of the key points for tissue engineering is to fabricate an ideal scaffold that has porous structure, good biodegradability and can be implanted into patients' bodies for functional tissue regeneration [1]. While, scaffolds with nanoscale architectures have larger surface areas to adsorb proteins, present many more binding sites to cell membrane receptors and increase cell attachment, proliferation, and expression of matrix components [2].

Electrospinning is a new technique which has been used for the fabrication of nanofibrous scaffolds for tissue engineering. Compared with the traditional technology, it is

a simple and efficient method to produce a scaffold with suitable micro-environment for cell hostage and proliferation [3]. Furthermore, the diameter of electrospun nanofibers can be modulated in the size to mimic the structural aspects of native extracellular matrices (ECMs) [4].

1.1 Electrospinning process

A typical electrospinning setup consists of a syringe with a needle, a high-voltage supplier and a grounded collector (Fig. 1). The flat collector will result in a fiber mat with randomly distribution structure, while a rotating collector will generate mat with aligned fibers. For example, aluminum foil can be used to collect a sheet, while rotating mandrel can be used to construct a tubular conduit [5]. During the electrospinning process, the pendant droplet develops into a Taylor cone which is ejected from the tip of the syringe. When the applied voltage is increased, the electrostatic repulsion of the surface charges overcome surface tension, the fiber jet will be emitted from the apex of the Taylor cone and fly towards the grounded collector, on the way the solvent evaporated and fiber formed. That is why electrospinning can be used to generate ultrafine fibers as thin as several nanometers [6].

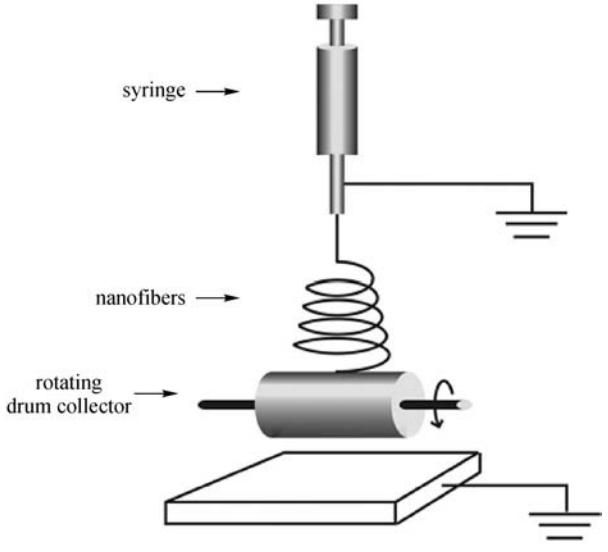

Fig. 1 A schematic of the electrospinning system with a rotating collector. (Reproduced with permission from Ref. [5], Copyright 2008 Elsevier Ltd.)

1.2 Processing parameters affecting electrospinning

Electrospinning can be used to form nanofibers depending on many important parameters which must be optimized. These parameters include not only applied voltage, needle-to-collector distance, and spinning flow rate, but also polymer and solution properties like polymer concentration, solvent volatility, and solution conductivity. Factors affecting the electrospinning process and fiber morphology are summarized in Table 1 [6].

1.3 Electrospinning of polymers

The electrospinning technique can be used to fabricate continuous nanofibers from a range of materials including synthetic and natural polymers. In order to obtain better applications in tissue engineering, apart from mechanical properties and biocompatibility, polymers also should have degradability. Many synthetic polymers (such as polyglycolic acid (PGA), polylactic acid (PLA), and their copolymers with ε-caprolactone) and natural polymers (such as collagen, chitosan (CS), silk fibroin (SF) and polysaccharide) are widely used to fabricate scaffolds for tissue engineering applications.Natural polymers are usually biocompatible whereas synthetic polymers have good mechanical properties and thermal stability. In order to get the materials with both good biocompatibility and good mechanical properties, most researchers started the research based on blend synthetic polymers with natural ones during the last three decades. Blends of synthetic polymers with natural polymers do improve the mechanical properties and biocompatibility comparing with single components [7].

2 Electrospinning of SF

SF is a natural biopolymer that derives from silkworm cocoons. It is selected as an attractive natural polymer for biomedical applications due to its good biocompatibility, good mechanical properties and minimal inflammatory reaction [8].

SF has been used as biomaterial for decades. Zarkoob et al. [9] first reported that SF was able to be dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) to prepare nanofibers. The average diameter of such generated fibers ranged from 6.5 to 200 nm, smaller than that of natural silk fibers. SF has been fabricated into nanofibrous scaffolds via electrospinning to improve tissue regeneration in skin, nerve, and cartilage [10–12]. Jin et al. [13] found that electrospun silk matrices have supported human bone marrow stromal cell attachment, spreading and growth *in vitro*. Min et al. [14] demonstrated that electrospun SF

Table 1 Effect of electrospinning parameters on fiber morphology [6]

Parameter	Effect on fiber morphology
Applied voltage ↑	Fiber diameter ↓ initially, then ↑ (not monotonic)
Flow rate ↑	Fiber diameter ↑ (beaded morphologies occur if the flow rate is too high)
Distance between capillary and collector ↑	Fiber diameter ↓ (beaded morphologies occur if the distance between the capillary and collector is too short)
Polymer concentration (viscosity) ↑	Fiber diameter ↑ (within optimal range)
Solution conductivity ↑	Fiber diameter ↓ (broad diameter distribution)
Solvent volatility ↑	Fiber exhibit microtexture (pores on their surfaces, which increase surface area)

nanofibers promote adhesion and spreading of normal human keratinocytes and fibroblasts *in vitro*. The results indicated that the SF nanofibers may be a good candidate for biomedical applications, e.g. wound dressing.

SF has been electrospun with some organic solvents, such as HFIP, formic acid, which are toxic and unfriendly to humans. Since SF is water soluble, SF aqueous solutions can be used for electrospinning nanofibers, which is good for mass production. In our lab, SF nanofibers were successfully electrospun from their aqueous solution in 2009 [15]. The results demonstrated that the electrospun SF scaffolds from aqueous solutions are beneficial to cell proliferation. The proliferation of pig iliac endothelial cells (PIECs) on SF nanofibers, cast film and coverslip are shown in Fig. 2, from which it can be seen that the cell proliferation speed on SF nanofibers was higher than that on cast film or on coverslip. It is also found that the surfaces of the electrospun SF fibers treated with methanol are characteristic with grooves (Fig. 3), which was possibly due to the change of SF conformation from random coil to the β-sheet structure. Furthermore, Singhvi et al. [16] had demonstrated that this groove was good for cell adhesion.

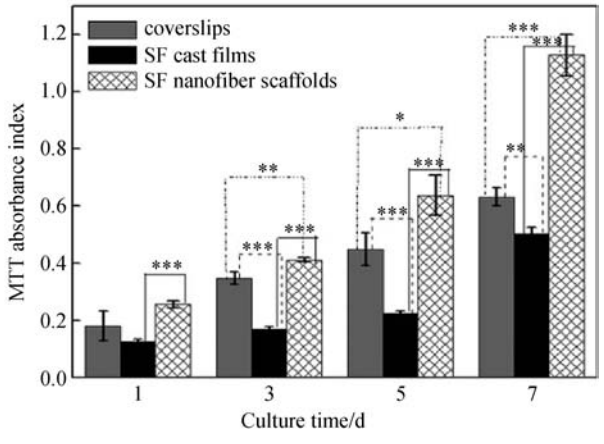

Fig. 2 Proliferation of PIECs cultured on SF nanofibers, SF cast film, and coverslip for 1, 3, 5, and 7 d. (Reproduced with permission from Ref. [15], Copyright 2009 Wiley Periodicals, Inc.)

Wang et al. [17] revealed that the electrospun SF nanofiber scaffolds modified with heparin increase the anticoagulation and biocompatibility. In their study, L929 fibroblasts were used to evaluate the cell growth and proliferation on the SF nanofibrous scaffolds before and after heparin modification *in vitro* (Fig. 4). The results indicated that the heparin-modified SF nanofibers were good for cell adhesion, growth and proliferation. On the other hand, SF nanofibers can be loaded with vitamins or growth factors for skin care and wound healing applications [18–19].

3 Electrospinning of SF blends

Because SF nanofibers can biomimic the structure and function of the native ECM, provide mechanical support and regulate cell activities, recently many scaffolds have been made from the blends of SF with natural or synthetic polymers, and it has been found that such blended materials could provide desired properties such as strength, biodegradability and biocompatibility.

3.1 SF blends with natural polymers

Electrospinning nanofibers can mimic the nanofibrous structure of native ECM, while the components of the native ECM are mainly collagens and glycosaminoglycans, which remind people to use the blends of protein and polysaccharides electrospun into nanofibers for biomimicing the native ECM both in components and in structure. SF as a protein has also been investigated to blend with natural polymers, such as CS, collagen and gelatin for those purposes.

3.1.1 SF blends with CS and CS derivative

CS is a cationic copolymer of β-(1→4)-glucosamine and N-acetyl-D-glucosamine, which is obtained from chitin. CS is a biodegradable natural polymer that has been used

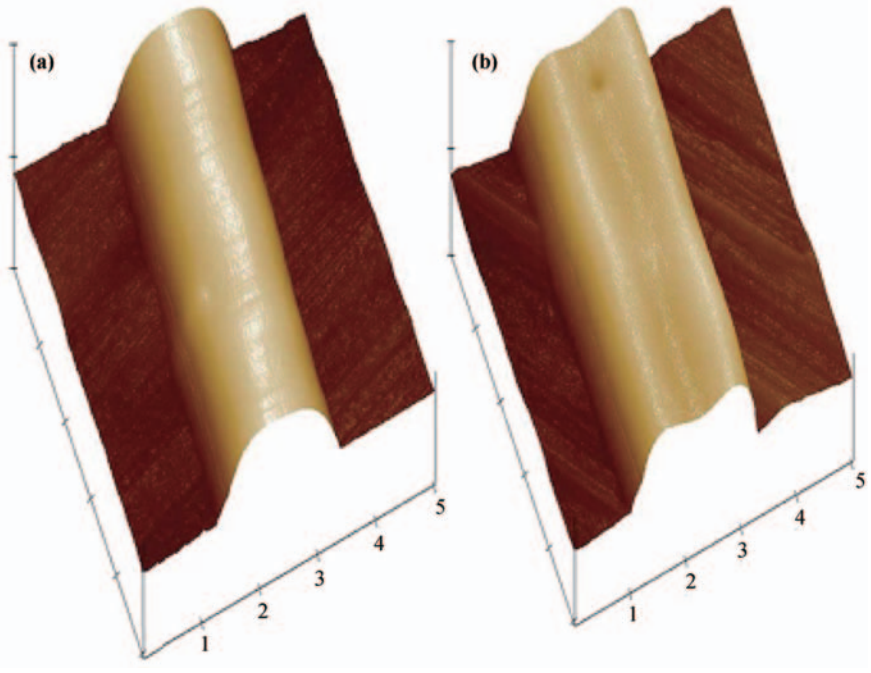

Fig. 3 Atomic force microscopy (AFM) images represented by height mode: **(a)** electrospun SF fiber; **(b)** electrospun SF fiber treated with methanol. (Reproduced with permission from Ref. [15], Copyright 2009 Wiley Periodicals, Inc.)

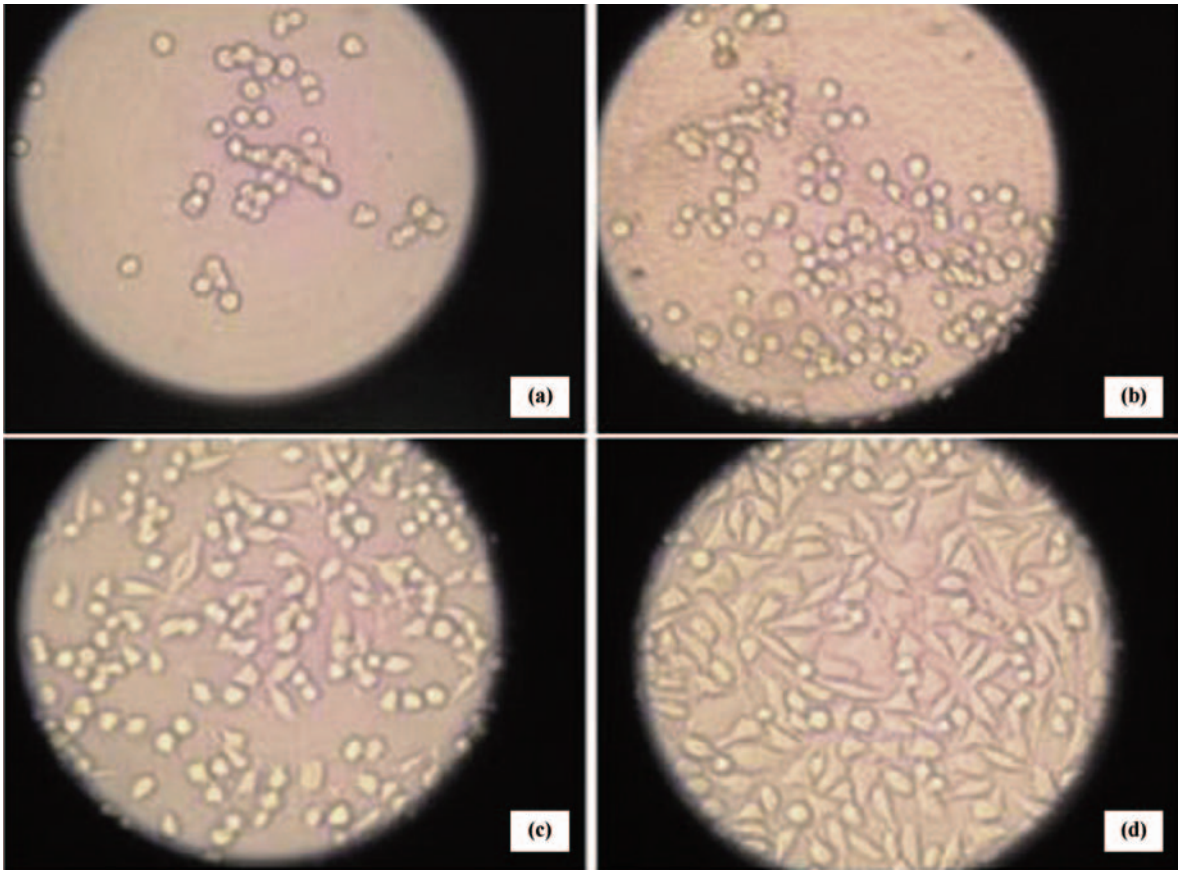

Fig. 4 Light micrographs of L929 fibroblast cells cultured on various scaffolds: **(a)** culture dish (24 h); **(b)** pure SF nanofibers (24 h); **(c)** heparin grafting SF nanofibers (24 h); **(d)** heparin grafting SF nanofibers (48 h). (Reproduced with permission from Ref. [17], Copyright 2011 Elsevier B.V.)

for cosmetic and pharmaceutical applications due to its biocompatibility, good biodegradability and excellent antibacterial properties [20]. Due to its structural resemblance with glycosaminoglycan, it has been blended with SF for nanofiber fabrication to biomimic the native ECM. In the CS and SF blends (CS/SF), the addition of SF enhanced the mechanical properties of CS/SF nanofibers (Table 2) (Fig. 5), the tensile strength of the cross-linked nanofibrous membranes increased from 1.3 to 10.3 MPa and the elongation at break of the cross-linked nanofibrous membranes showed an increased trend with the increasing of SF content [21].

Table 2 Mechanical properties of cross-linked CS/SF nanofiber membranes [21]

Cross-linked CS/SF (wt/wt)	Tensile stress /MPa	Ultimate strain /%
0:10	10.3±0.24	2.8±0.22
2:8	1.2±0.13	3.8±0.21
5:5	1.1±0.22	2.5±0.25
8:2	1.0±0.21	1.3±0.22

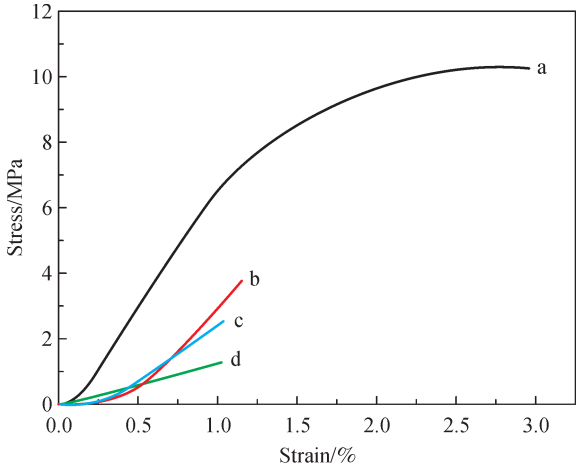

Fig. 5 Tensile stress–strain curves of cross-linked CS/SF composite membranes with various CS contents: 0% (a); 20% (b); 50% (c); 80% (d). (Reproduced with permission from Ref. [21], Copyright 2010 MDPI AG)

However, CS has poor solubility in common organic solvents, which gives a challenge during the electrospinning process. Many laboratories have attempted to modify the properties of CS. Hydroxybutyl chitosan (HBC) is fabricated by conjugation of hydroxybutyl groups to the hydroxyl and amino reactive sites of CS. This modification could increase the solubility of CS in water or organic solution and enhance the electrospinability of CS solutions [22]. SF and HBC blend nanofibers were fabricated by electrospinning using HFIP and trifluoroacetic acid mixture as solvents to biomimic the native ECM in our lab [23]. Scanning electron microscopy (SEM) revealed that the average nanofiber diameter increased when the content of HBC in SF/HBC blends was raised from 20% to 100%. Fourier transform infrared spectroscopy (FTIR) and ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectroscopy clarified that hydrogen bonding interactions exist in SF/HBC blend nanofibers and HBC did not induce conformation of SF transforming from random coil form to the β-sheet structure. Differential thermogravimetric analyses demonstrated that the thermal stability of SF/HBC blend nanofibrous scaffolds was improved. The mechanical properties were tested for the blend nanofibers with different SF/HBC blend weight ratios (100:0, 80:20, 50:50, 20:80, 0:100). Pure SF nanofibrous scaffolds showed a typical brittle fracture; the elongation at break was only (3.85±0.30)% and the tensile strength was (2.72±0.60) MPa. At the SF/HBC blend ratio of 20:80, the elongation at break and the tensile strength of SF/HBC blend nanofibers increased up to (11.42±0.88)% and (4.35±0.45) MPa, respectively [24]. The electrospun SF/HBC nanofiber scaffolds could provide a preferable matrix for cell adhesion and proliferation by the method of mimicking the native ECM in the body (Fig. 6). It was revealed that all the nanofibrous scaffolds had good cell viability in comparison with coverslip. On Day 3, Day 5 and Day 7, the proliferation on pure SF, SF/HBC (80:20) and SF/HBC (50:50) nanofiber scaffolds was increased significantly compared to HBC nanofibers, tissue culture

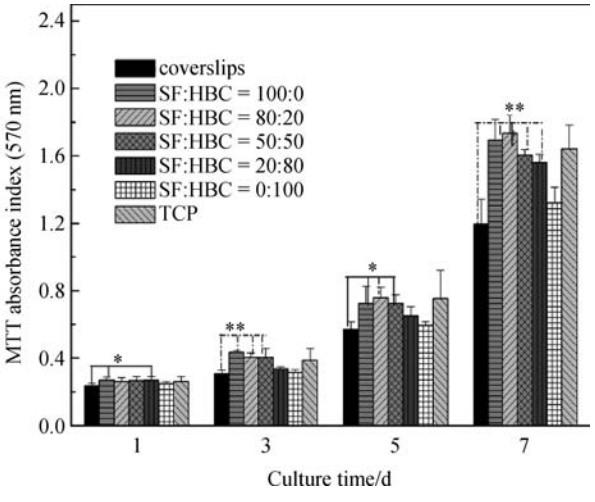

Fig. 6 Proliferation of PIECs cultured on SF/HBC nanofibrous scaffolds, coverslip and TCP for 1, 3, 5 and 7 d. Data are expressed as mean±SD (n = 4). Statistical difference between groups is indicated (*$p < 0.05$; **$p < 0.01$). (Reproduced with permission from Ref. [24], Copyright 2011 Taylor & Francis)

plate (TCP) and coverslip. So, scaffolds fabricated by pure SF and SF/HBC exerted positive effects on cell growth and proliferation compared to TCP.

3.1.2 SF blends with collagen and gelatin

Collagen is extracted from mammal organizations and has been widely used for tissue engineering scaffolds due to its excellent biocompatibility and biodegradability [25]. Collagen is better than SF for cell adhesion and proliferation [26]. Hence, the structural and biological properties of pure SF scaffolds were often needed to be improved by blending with collagen or gelatin. Zhou et al. [27] reported a SF/collagen tubular scaffold via electrospinning and found that the addition of collagen could induce SF conformation from the α-helix to the β-sheet that is good for cell adhesion.

Gelatin is a natural biopolymer derived from collagen which was found in animal tissues. Okhawilai et al. [28] developed the electrospun fiber from Thai SF and type B gelatin (GB) blend for long-term drug release application. The diameter and morphology of the obtained SF/GB nanofiber were investigated. It has been found that the average diameter of SF/GB nanofibers tended to increase as the SF content in the blends increased (Table 3), and the fiber morphology turned to ribbon-like shape when the SF content was more than 60 wt.% (Fig. 7).

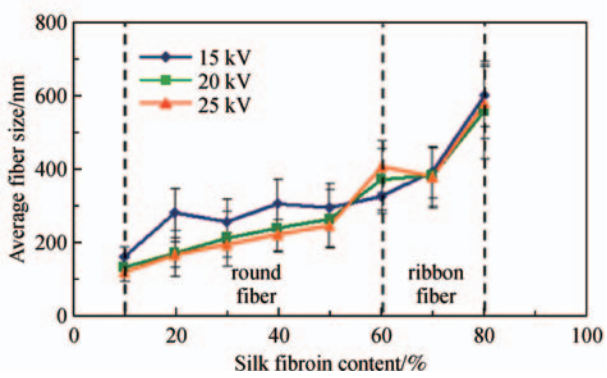

Fig. 7 Average fiber size and the standard deviation of the size of Thai SF/type B gelatin (SF/GB) electrospun fiber mats obtained from various applied voltages. (Reproduced with permission from Ref. [28], Copyright 2010 Elsevier B.V.)

3.1.3 SF blends with other polysaccharides and proteins

Hyaluronic acid (HA) is a linear polysaccharide consisting of alternating, disaccharide units of (1, 4)-linked α-D-gluconic acid and (1, 3)-linked β-N-acetyl-D-glucosamine. As one of the main components of the ECM, HA is conducive to cells growth, adhesion and proliferation. HA and its derivatives have been widely used in biomedical areas, such as wound dressing, drug delivery systems and implanted materials. Garcia-Fuentes et al. [29] prepared the HA and SF blend scaffold by freeze drying and found that HA improved the cell adhesion and proliferation when culturing human mesenchymal stem cells on the blended scaffolds.

Our lab fabricated nanofiber scaffolds from the aqueous solutions of SF and HA blends (SF/HA) to biomimic the natural ECM by electrospinning [30]. Figure 8 shows the SEM images of electrospinning nanofibers and the average width of nanofibers at different volume ratios when blending 30 wt.% SF water solution with 4 wt.% HA water solution. From the SEM images it can be observed that electrospun SF/HA nanofibers were ribbon-shaped and their average width obviously decreased with the increase of HA content. Furthermore, there is no fiber that can be spun out when the HA content increased up to 60%, indicating that pure HA solution was hard in fabricating nanofibers. Figure 9 shows the cell proliferation speed on SF/HA nanofibers with different blend ratios. It is seen that SF/HA blend nanofibers are more suitable for the cell proliferation than coverslips.

Keratin is one of the most abundant proteins in animal population. Blends of SF with wool keratose have been

Table 3 Thai SF/type B gelatin (SF/GB) fiber size at various weight blending ratios and applied voltages [28]

SF/GB blend ratio	The size of SF/GB fiber /nm					
	15 kV		20 kV		25 kV	
	Min–Max	Average	Min–Max	Average	Min–Max	Average
10/90	117–206	162±24	58–248	133±36	79–174	118±21
20/80	157–404	280±66	64–343	172±61	109–238	167±35
30/70	153–373	258±60	56–456	211±75	140–267	195±32
40/60	194–437	304±65	57–444	240±67	137–322	221±43
50/50	195–427	296±63	58–414	265±78	148–373	245±56
60/40	210–408	325±44	136–526	371±82	277–529	406±71
70/30	245–505	389±68	181–512	380±80	220–572	376±81
80/20	395–735	598±83	102–891	557±133	391–765	577±98

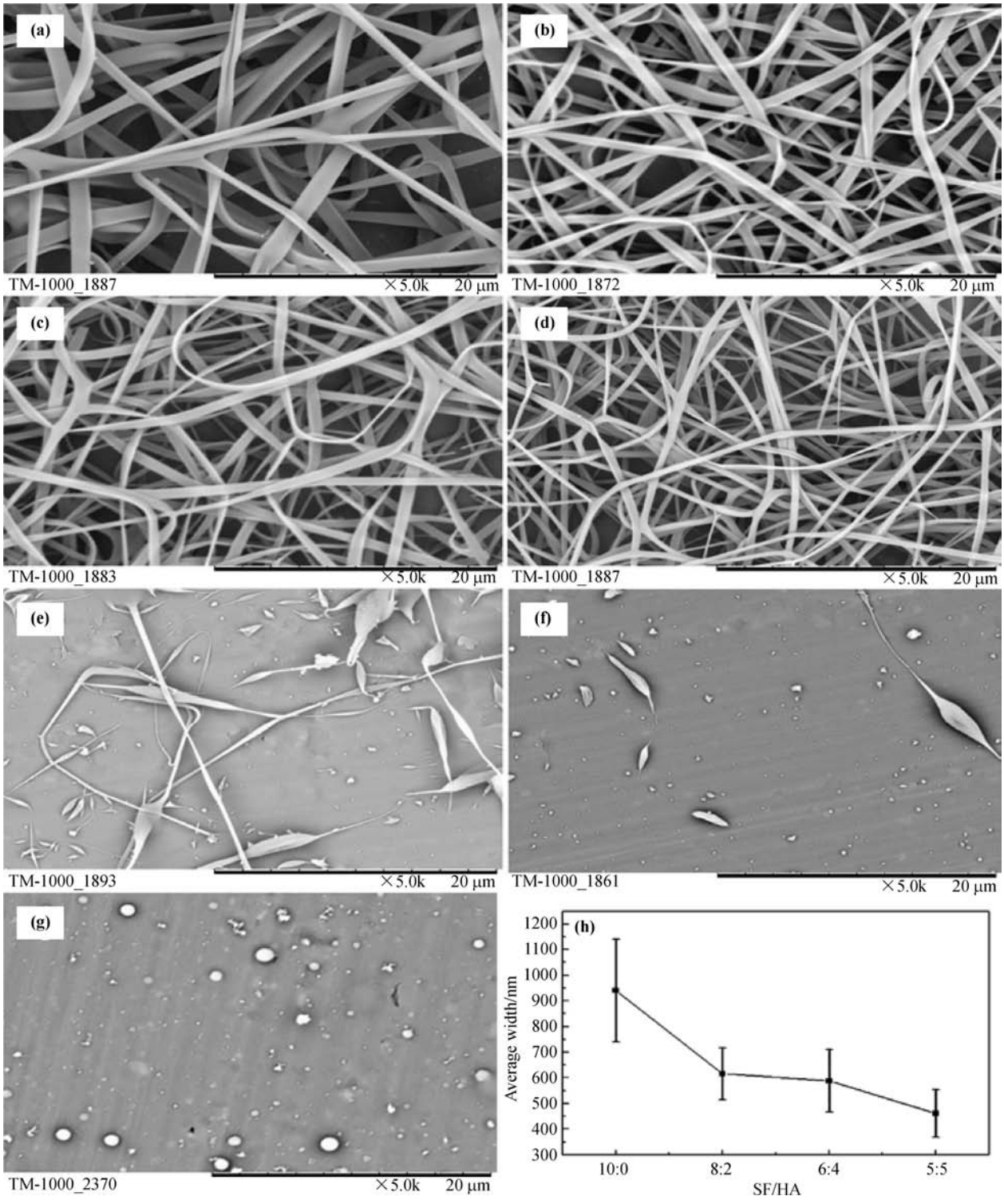

Fig. 8 SEM images of blends with the volume ratio of 30 wt.% SF solution to 4 wt.% HA solution at **(a)** 10:0, **(b)** 8:2, **(c)** 6:4, **(d)** 5:5, **(e)** 4:6, **(f)** 2:8, and **(g)** 0:10. **(h)** Variation of width of SF/HA blended nanofibers with volume ratio. (Reproduced with permission from Ref. [30], Copyright 2012 Taylor & Francis)

electrospun into nanofibers from their formic acid solution, and the as-spun fibers are smooth with diameters between 160 and 900 nm, which fibers can absorb the heavy mental ion for water purification application [31].

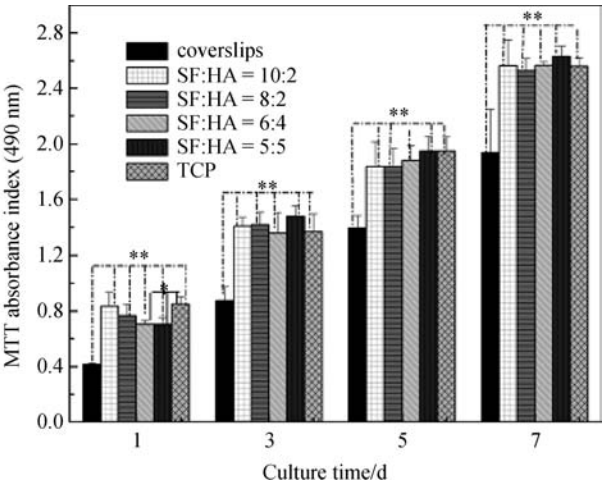

Fig. 9 Proliferation of PIECs cultured on SF/HA nanofibrous scaffolds, coverslips and TCP for 1, 3, 5, 7 d. Data are expressed as mean±SD (n = 3). Statistical difference between groups is indicated (*$p < 0.05$; **$p < 0.01$). (Reproduced with permission from Ref. [30], Copyright 2012 Taylor & Francis)

3.2 SF blends with synthetic biodegradable polymers

Generally, the scaffold should also have mechanically supportiveness for tissue regeneration. Although natural polymers exhibit good biocompatibility and biodegradability, they do not possess strong mechanical properties. In order to improve mechanical properties, synthetic biodegradable polymers blended with SF are widely studied for tissue engineering application, which are poly (ε-caprolactone) (PCL), PLA, PGA, and their copolymers.

3.2.1 SF blends with PCL

PCL was proven to be able to degrade by microorganisms in 1985 [32]. It was also reported as a biodegradable nanofiber matrix for bone regeneration in 2004, as the electrospun PCL scaffolds provided an environment for tissue formation [33].

Li et al. [34] generated nanofibers with a core–shell

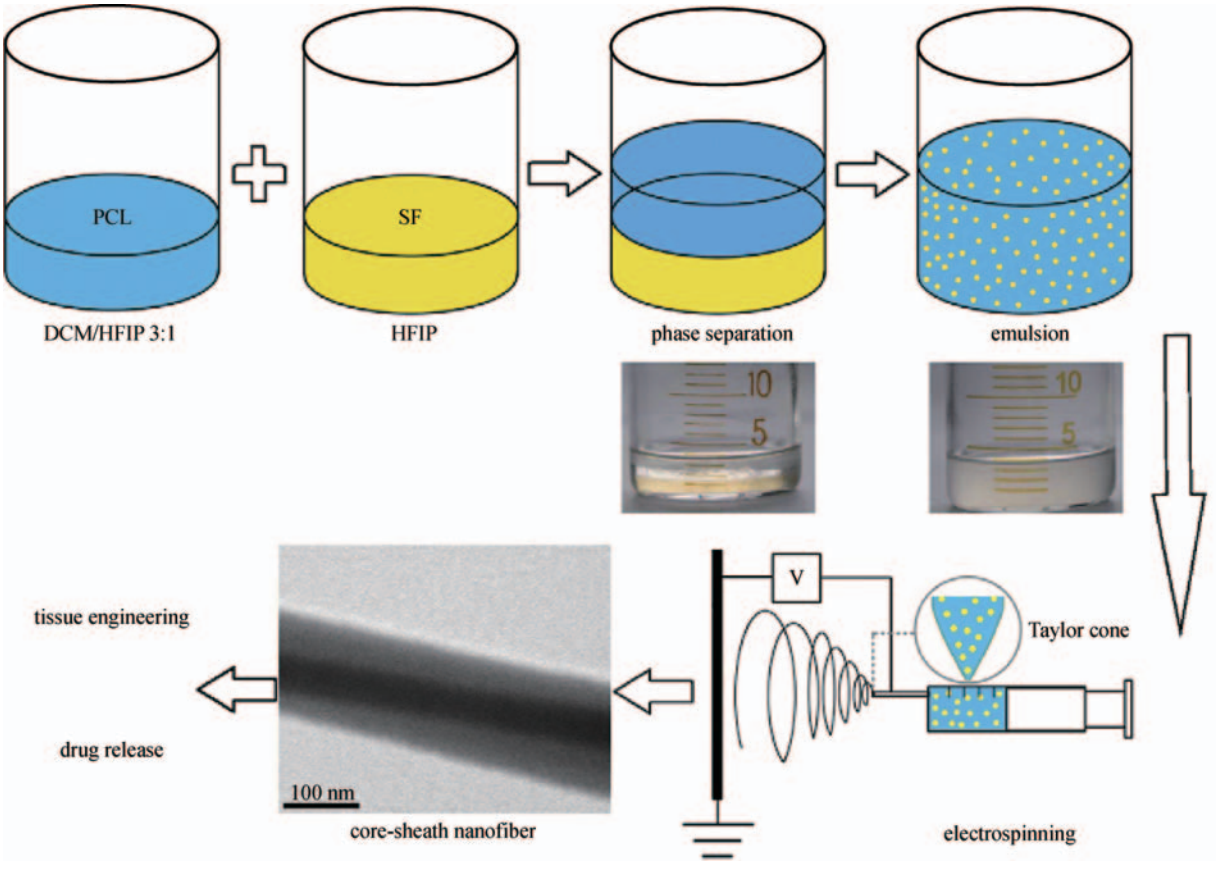

Fig. 10 The schematic illustration of the process for electrospun PCL/SF core–shell nanofibers. (Reproduced with permission from Ref. [34], Copyright 2011 Elsevier B.V.)

structure that was composed of PCL as shell and SF as core via emulsion electrospinning (Fig. 10). The tensile strength of PCL/SF nanofibers increased, but the elongation at break decreased when the SF content in blends increased. MeOH-treated PCL/SF (7:3) nanofibers give higher tensile strength ((2.25±0.6) MPa) and larger elongation at break (100%) comparing with untreated PCL/SF (7:3) nanofibers (Fig. 11). Furthermore, the cell proliferation speed was higher on PCL/SF nanofiber scaffolds than that on PCL nanofiber scaffolds (Fig. 12), suggesting that the incorporation of SF into PCL was beneficial for cell proliferation.

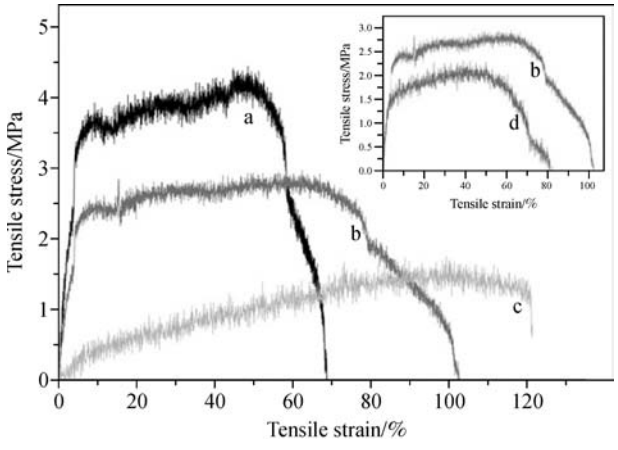

Fig. 11 Strain–stress curve of MeOH-treated PCL/SF 5:5 (a), MeOH-treated PCL/SF7:3 (b), PCL (c) and untreated PCL/SF 7:3 (d). (Reproduced with permission from Ref. [34], Copyright 2011 Elsevier B.V.)

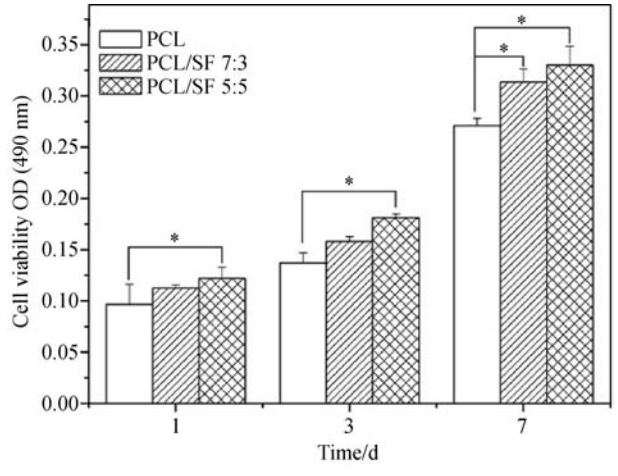

Fig. 12 MTS assay for FEK4 cells proliferation on electrospun scaffolds after 1, 3, and 7 d. Bar represents mean standard deviation. Data are expressed as mean±SD (n = 3). Statistical difference between groups is indicated. (Reproduced with permission from Ref. [34], Copyright 2011 Elsevier B.V.)

3.2.2 SF blends with PLA

PLA is another biodegradable polymer which is biocompatible and undergoes scission in the body to lactic acids. Lactic acid is a chiral molecule existing in two steroisomeric forms that are L-lactic acid and D-lactic acid. Two stereoregular polymers, poly (D-lactide) (PDLA) and poly (L-lactide) (PLLA), could be polymerized separately from D-lactic acid and L-lactic acid. Usually, PLLA is more frequently used than PDLA, since the hydrolysis of PLLA yield L-lactic acid, which is the naturally occurring stereoisomer of lactic acid in the body [35]. The big disadvantage of PLA used in body is that it can induce an inflammatory response. SF blended with PLA could reduce the inflammatory response. It has been reported that the PLLA/SF hybrid scaffold promoted the hepatocyte proliferation and decreased the macrophage responses [36].

Wang et al. [37] fabricated a bi-layer tube scaffold by electrospinning. The PLA layer (outside layer) is responsible for the mechanical properties and the SF–gelatin layer (inner layer) supports cell growth (Figs. 13 and 14). The PLA/SF–gelatin tubular scaffolds, with the porosity of approximately (82±2)%, possessed appropriate breaking strength ((2.21±0.18) MPa), pliability (60.58±1.23)% and suture retention strength ((4.58±0.62) N). The burst pressure strength of the composite scaffolds reached (1596±20) mmHg (1 mmHg = 0.133 kPa), which is similar to that of the native vessels.

3.2.3 SF blends with poly(L-lactic acid-*co*-ε-caprolactone) (P(LLA-CL))

P(LLA-CL) is a copolymer of L-lactic acid and ε-caprolactone. It can show different mechanical properties and biodegradability by changing the weight ratio of L-lactic acid to ε-caprolactone in the copolymer. Although P(LLA-CL) has been electrospun into nanofibers for different tissue engineering applications [38–39], it is still week in cell attachment because of the lacking of cell recognition sites. Blending SF with P(LLA-CL) will lead to an ideal scaffold with both good mechanical properties and biocompatibility.

Our lab has done a lot of work about the SF and P(LLA-CL) blending nanofibers via electrospinning. We have fabricated SF and P(LLA-CL) blend nanofibers with different weight ratios and investigated the morphology, structure and properties of SF/P(LLA-CL) nanofiber scaffolds [40]. The mechanical properties of SF/P(LLA-CL) nanofibers were shown in Table 4. Pure SF nanofibers

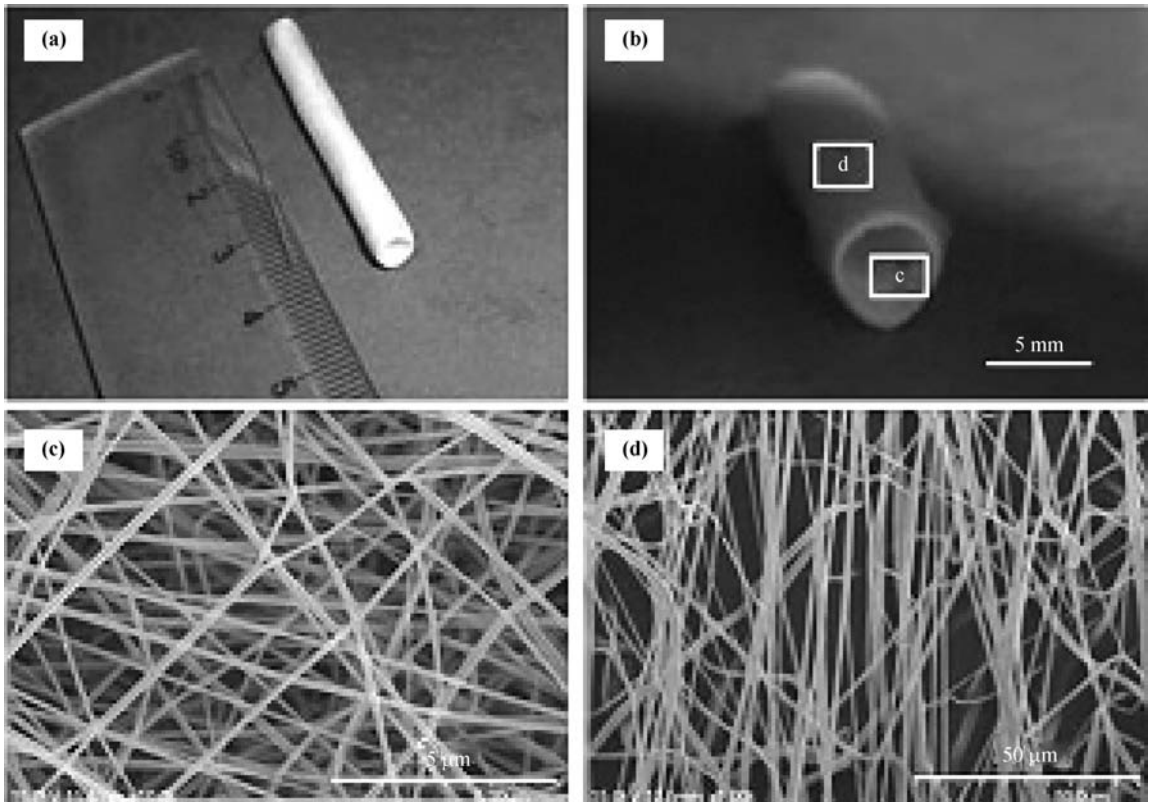

Fig. 13 Macroscopic and microscopic views of the PLA/SF–gelatin composite tubular scaffold: **(a)(b)** whole body of the composite scaffold; **(c)** SEM image of SF–gelatin layer (× 10.0 k); **(d)** SEM image of PLA layer (× 1.0 k). (Reproduced with permission from Ref. [37], Copyright 2009 American Chemical Society)

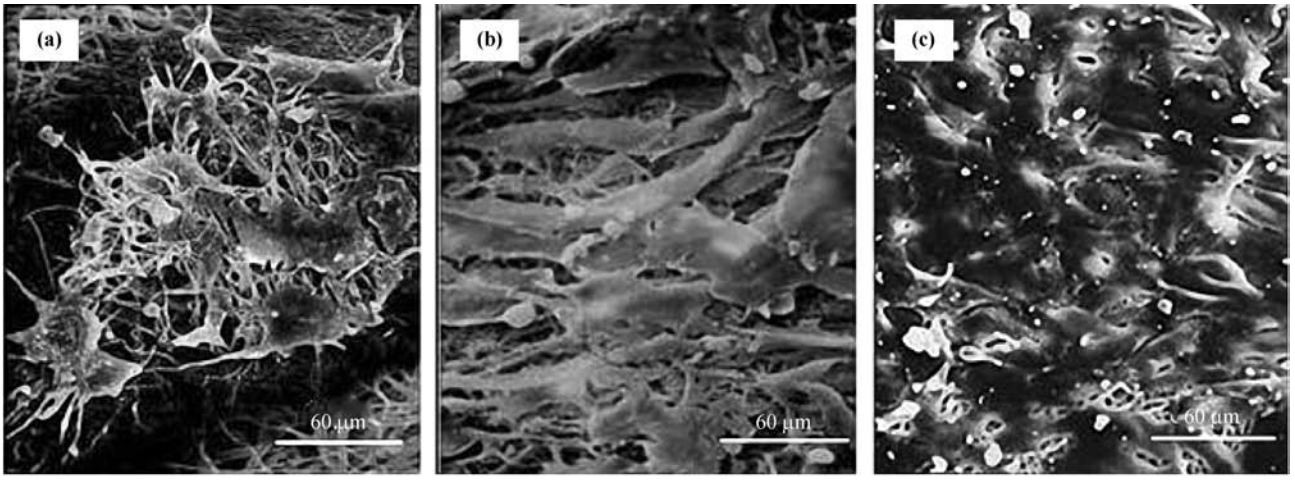

Fig. 14 SEM images of human umbilical vein endothelial cells seeded on the PLA/SF–gelatin composite tubular scaffolds: **(a)** after 7 d of culture; **(b)** after 14 d of culture; **(c)** after 21 d of culture. (Reproduced with permission from Ref. [37], Copyright 2009 American Chemical Society)

were week and brittle in mechanical properties with the tensile strength of 2.72 MPa and the elongation at break of 3.85%, while, pure P(LLA-CL) nanofibers are elastic materials with the tensile strength of 6.96 MPa and the elongation at break of 458%. Interestingly, with a small amount of SF blended into P(LLA-CL), the SF/P(LLA-CL) nanofibers with the ratio of 25:75 showed the highest tensile strength of 10.6 MPa, even though its elongation at

Table 4 Mechanical properties of SF/P(LLA-CL) nanofiber scaffolds with various blend ratios [40]

SF/P(PLLA-CL) weight ratio	Specimen thickness /mm (n = 6)	Elongation at break /%	Tensile strength /MPa
0:100	0.088±0.005	458.20±52.35	6.96±3.13
25:75	0.078±0.008	279.67±34.98	10.60±2.45
50:50	0.075±0.004	168.75±29.70	5.62±1.61
75:25	0.082±0.006	98.86±16.98	4.00±0.44
100:0	0.050±0.005	3.85±0.30	2.72±0.60

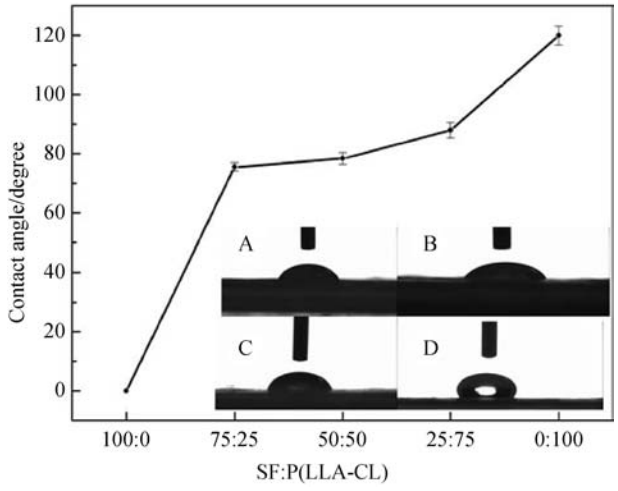

Fig. 15 Water contact angle of SF/P(LLA-CL)-blended nanofibrous scaffolds [Inset this figure shows the variation shapes of contact angle on different scaffolds (A, 75:25; B, 50:50; C, 25:75; D, 0:100)]. Date are mean±SD (n = 3). (Reproduced with permission from Ref. [40], Copyright 2009 Wiley Periodicals, Inc.)

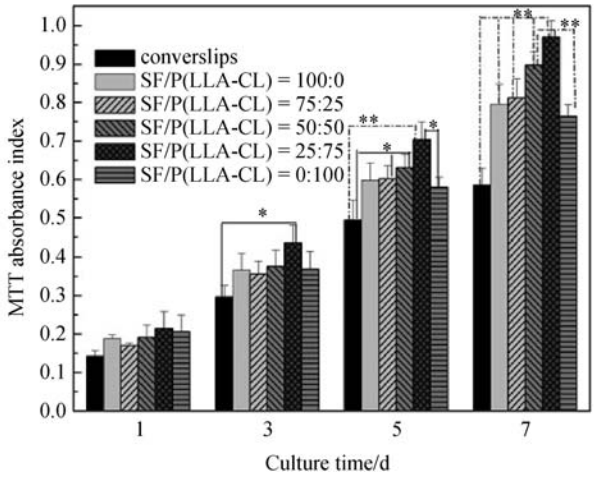

Fig. 16 Proliferation of PIECs cultured on SF/P(LLA-CL) nanofiber scaffolds and coverslips for 1, 3, 5, 7 d. Data are expressed as mean + SD (n = 3). Statistical difference between groups is indicated (*$p < 0.05$; **$p < 0.01$). (Reproduced with permission from Ref. [41], Copyright 2011 Acta Materialia Inc.)

break reduced to 279%. However, with further increasing the SF content in the SF/P(LLA-CL) nanofibers, both the tensile strength and the elongation at break went to decrease. So, SF/P(LLA-CL) (25:75) blended nanofiber scaffolds exhibited best mechanical properties, which can be considered for tissue engineering application.

Water contact angle is usually used for testing the wet ability of a material surface, which can be used to estimate the biocompatibity of a biomaterial. The pure P(LLA-CL) nanofibers showed an water contact angle at around 120°, indicating that P(LLA-CL) nanofibrous scaffolds were hydrophobic. If increasing the ratio of SF in the blended nanofibers, the contact angle of the nanofibrous scaffolds decreased continuously from 120° to 0° (Fig. 15). Blending SF in P(LLA-CL) improved the biocompatibity of P(LLA-CL) [40]. The cells proliferated much faster on SF/P(LLA-CL) nanofibers comparing with those on P(LLA-CL) and SF nanofibers, especially for SF/P(LLA-CL) (25:75) (Fig. 16).

The SF/P(LLA-CL) nanofiber scaffolds were further used for peripheral nerve regeneration *in vivo* [41]. Aligned SF/P(LLA-CL) nanofibers were fabricated by electrospinning and then reeled into nerve guidance conduits (NGCs) with the fiber alignment along the axial direction (inner diameter of 1.5 mm). Both aligned P(LLA-CL) and SF/P(LLA-CL) (25:75) NGCs were used to bridge the 10 mm defect in the sciatic nerve of rats and the defected nerves regenerated at 4 weeks. Electrophysiological examination showed that the functional recovery of regenerated nerve in the SF/P(LLA-CL) NGC was superior to that in the P(LLA-CL) NGC. The results revealed that the SF promoted peripheral nerve regeneration significantly.

We have also studied the *in vitro* degradation of pure SF, P(LLA-CL) and SF/P(LLA-CL) blended nanofibers in phosphate-buffered saline (PBS; pH (7.4±0.1)) at 37°C for 6 months [42]. All results indicated that the pure SF nanofibrous scaffolds were almost not degradable in PBS, while pure P(LLA-CL) nanofibrous scaffolds had the fastest degradation rate in PBS (Fig. 17). The degradation speeds of SF/P(LLA-CL) blended nanofibers are decreased with the increasing of the SF content in the blended nanofibers. This was probably due to the inter molecular

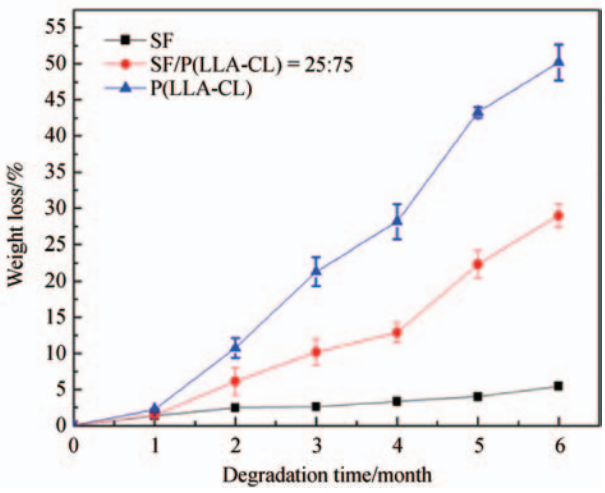

Fig. 17 Weight loss of SF/P(LLA-CL) nanofiber scaffolds after degradation in PBS for different time. (Reproduced with permission from Ref. [42], Copyright 2011 Elsevier Ltd.)

interactions between SF and P(LLA-CL), which hindered the movement of P(LLA-CL) molecular chains. Moreover, the degradation of P(LLA-CL) released the acid which caused lower pH value for the release system, while the addition of SF in SF/P(LLA-CL) nanofibers reduced the acid concentration in the release system.

Electrospinning three dimensional nanofiber scaffolds is a challenge in tissue engineering. To face the challenge, we fabricated a nano-yarn scaffold by dynamic liquid electrospinning method from P(LLA-CL) and SF blends and investigated the potential application for bone tissue engineering [43]. Because SF is an amphiprotic macromolecule composed of hydrophobic side chain and hydrophilic blocks with charged amino acids [44], SF/P(LLA-CL) solution will have higher conductivity than P(LLA-CL) solution, which caused the diameter of electrospun nanofibers in SF/P(LLA-CL) nano-yarns much smaller than that in P(LLA-CL) nano-yarns.

4 Conclusions and prospects

SF as a natural biomaterial has good biocompatibility, which is extracted from silkworm cocoons and much cheaper than other natural biomaterials like collagen. When SF was electrospun from its water solution, the formed fibers are in ribbon-like shape. SF has been blended with many different natural materials for nanofiber fabrication, which includes CS and its derivative and HA as polysaccharides, collagen and gelatin as protein. SF blended with polysaccharide forming the nanofibers can biomimic the structure and components of native ECM and can give good mechanical properties and cell biocompatibility. For instance, SF/HBC blend nanofibers show higher tensile strength and elongation at break than the pure SF nanofibers, and the cell proliferation speed on SF/HBC nanofibers is faster than that on pure HBC nanofibers.

Pure natural materials and its blend nanofibers can not give enough mechanical properties as scaffolds like blood vessel and nerve conduit yet, while blending synthetic polymers with SF can improve mechanical properties significantly. Furthermore with small amount of synthetic polymer blending with SF the cell proliferation ability on the nanofibers can also be improved. PLLA, PCL and P(LLA-CL) are all tried to be blended with SF for the nanofiber fabrication. For SF/P(LLA-CL) nanofibers when the P(LLA-CL) content is 25%, it gives the highest tensile strength of 10.6 MPa, which is higher than that of pure P(LLA-CL) nanofiber and SF nanofibers. Meantime these nanofibers show the highest cell proliferation rate.

Therefore, combining SF with natural or synthetic polymers to make complex nanofibers is an effective way to get a new kind of materials with good mechanical properties and biocompatibility. Electrospun SF blended nanofibers with either natural or synthetic biomaterials have shown great potential for different tissue regeneration, such as nerve, blood vessel, skin, tendon, bone and cartilage.

Abbreviations

AFM	atomic force microscopy
CS	chitosan
ECM	extracellular matrix
FTIR	Fourier transform infrared spectroscopy
GB	type B gelatin
HA	hyaluronic acid
HBC	hydroxybutyl chitosan
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
NGC	nerve guidance conduit
NMR	nuclear magnetic resonance
PBS	phosphate-buffered saline
PCL	poly (ε-caprolactone)
PDLA	poly (D-lactide)
PGA	polyglycolic acid
PIEC	pig iliac endothelial cell
PLA	polylactic acid
PLLA	poly (L-lactide)
P(LLA-CL)	poly(L-lactic acid-*co*-ε-caprolactone)
SEM	scanning electron microscopy
SF	silk fibroin
TCP	tissue culture plate

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