Electrospun Polylactide/Silk Fibroin–Gelatin Composite Tubular Scaffolds for Small-Diameter Tissue Engineering Blood Vessels

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ABSTRACT: Many synthetic scaffolds have been used as vascular substitutes for clinical use. However, many of these scaffolds may not show suitable properties when they are exposed to physiologic vascular environments, and they may fail eventually because of some unexpected conditions. Electrospinning technology offers the potential for controlling the composition, structure, and mechanical properties of scaffolds. In this study, a tubular scaffold (inner diameter = 4.5 mm) composed of a polylactide (PLA) fiber outside layer and a silk fibroin (SF)–gelatin fiber inner layer (PLA/SF–gelatin) was fabricated by electrospinning. The morphological, biomechanical, and biological properties of the composite scaffold were examined. The PLA/SF–gelatin composite tubular scaffold possessed a porous structure; the porosity of the scaffold reached 82 ± 2%. The composite scaffold achieved the appropriate breaking strength (1.28 ± 0.21 MPa) and adequate pliability (elasticity up to 41.11 ± 2.17% strain) and possessed a fine suture retention strength (1.07 ± 0.07 N). The burst pressure of the composite scaffold was 111.4 ± 2.6 kPa, which was much higher than the native vessels. A mitochondrial metabolic assay and scanning electron microscopy observations indicated that both 3T3 mouse fibroblasts and human umbilical vein endothelial cells grew and proliferated well on the composite scaffold *in vitro* after they were cultured for some days. The PLA/SF–gelatin composite tubular scaffolds presented appropriate characteristics to be considered as candidate scaffolds for blood vessel tissue engineering. J Appl Polym Sci 113: 2675–2682, 2009

Key words: electrospinning; biocompatibility; composites; gelation; polylactide

INTRODUCTION

Large numbers of patients suffer from cardiovascular diseases, and most need proper vascular grafts.[1] Presently, autologous vascular grafts and allografts are widely used for the reconstruction of small-diameter vessels. However, their clinical utility is limited by potential immunogenic responses and the origin of suitable vessels.[2] Recently, synthetic grafts, such as woven poly(ethylene terephthalate) (Dacron) and extended polytetrafluoroethylene, have also been used for vascular reconstruction, but these grafts tend to fail when they are applied to small-diameter vessels because of their poor biocompatibility.[3,4]

With the development of tissue engineering, blood vessel tissue engineering has emerged as a promising approach for addressing the shortage of current therapies.[5] Blood vessel tissue engineering attempts to fabricate functional small-diameter grafts by combining cells with scaffold materials under suitable culturing conditions, which results in a tubular scaffold that can be used *in vivo*.[6] A tissue-engineered blood-vessel scaffold should be biocompatible, have appropriate mechanical properties, and be readily available in a variety of sizes for grafting applications.[5]

Numerous fabrication techniques have been used to produce vascular scaffolds.[7–9] Recently, electrospinning technology has gained much attention because it can provide a biomimetic environment with nanoscale to microscale diameter fibers, and it may be easy to form a tubular scaffold with a desirable diameter. Scaffolds fabricated by the electrospinning of crude and synthetic polymers, such as polylactide (PLA), poly(lactide-*co*-glycolide), poly (ε-caprolactone), collagen, silk fibroin (SF), and

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gelatin, have been applied widely to biomedical areas (e.g., drug release, wound dressing and bone regeneration).[10–13] However, fewer studies have concentrated on vascular scaffolds that were fabricated by these polymers via electrospinning.

SF is a typical fibrous protein, which shows unique physical and chemical properties. Many researchers have recently investigated SF as one candidate material for biomedical applications because it has several useful properties, including good biocompatibility, good oxygen and water vapor permeability, and minimal inflammatory reaction.[14,15] Gelatin is a highly biodegradable polymer derived from collagens, which has almost identical compositions and biological properties as those of collagens.[16] PLA is a synthetic polymer, which has good biological, biodegradable, and biomechanical properties.[17] The objective of this study was to fabricate a PLA/SF–gelatin composite tubular scaffold with good biomechanical and biological properties via electrospinning. The testing results show that the composite scaffold is an appropriate candidate for blood vessel tissue engineering.

EXPERIMENTAL

Materials

Raw silk fibers were degummed twice with a 0.5% (w/w) $NaHCO_3$ solution at 100°C for 30 min and then rinsed with warm distilled water. Degummed silk (SF) was dissolved in a ternary solvent system of $CaCl_2/CH_3CH_2OH/H_2O$ (molar ratio = 1 : 2 : 8) at 70°C for 6 h. After dialysis with a cellulose tubular membrane (Sigma Co., St. Louis, MO, 250–257 μm) in distilled water for 3 days, the SF solution was filtered and lyophilized to obtain regenerated SF sponges. The SF and gelatin (molecular weight = 300, Bloom, XinTa Co., Shanghai, China) were dissolved in formic acid (98 wt %, Tianjin Organic Synthesize Co., Tianjin, China) to prepare them for the spinning solution. The concentration of SF and gelatin in the formic acid solution was 13 wt %. PLA (molecular weight = 1.0×10^5 kg/mol, Natureworks Co., Minnetonka, MN) was dissolved in a mixed solvent of chloroform and acetone (Shanghai Chemistry Reagent Co., Shanghai, China; 2 : 1 volume ratio) to achieve a concentration of 5 wt %.

Electrospinning

A composite tubular scaffold of PLA (the outer layer) and SF–gelatin (the inner layer) was fabricated by electrospinning with a rotating mandrel-type collector (Tianjin Dongwen Co., Tianjin, China); that is, first SF–gelatin and then PLA were sequentially electrospun (PLA/SF–gelatin). Figure 1 shows the schematic illustration of the electrospinning setup. The spinning conditions for each polymer were set as follows (listed in the order of voltage, polar distance, flow rate, and mandrel rotating speed): (1) SF–gelatin: 30 kV, 13 cm, 0.2 mL/h, and 1000 rpm and (2) PLA: 25 kV, 15 cm, 0.1 mL/h, and 2000 rpm. For comparison, the PLA and SF–gelatin tubular scaffolds were fabricated with the same procedure.

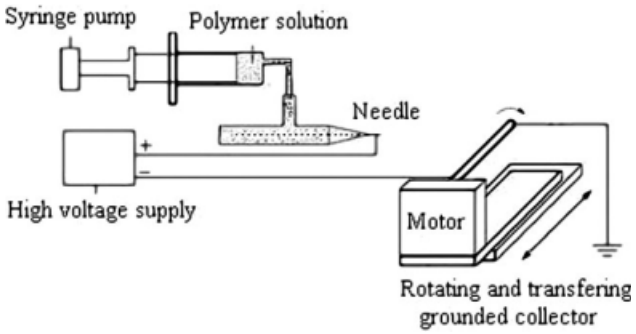

Figure 1 Schematic illustration of the electrospinning setup.

Morphological characterizations

Scanning electron microscopy (SEM) observation

The morphology of the electrospun PLA/SF–gelatin composite tubular scaffold was observed by a Hitachi S-4700 (Tokyo, Japan) scanning electron microscope operated at an acceleration voltage of 15 kV with magnifications of 10,000× (inner layer) and 1000× (outside layer), respectively. On the basis of the SEM images, the fiber diameter and standard deviation (SD) were analyzed with an image analysis program (Adobe Photoshop 7.0).

Porosity and pore diameter

The porosity was calculated according to the method described by Vaz et al.[18] The porosity (ε) was calculated with the measured average density (ρ) of the samples and the standard density of the scaffold ($\rho_0 = 1.2$ g/cm^3), as shown in eq. (1). ρ_0 is the density of air-dried PLA/SF–gelatin membrane (gained from the PLA and SF–gelatin solution), and the membrane does not have any pores:

$$\varepsilon(\%) = \left(1 - \frac{\rho}{\rho_0}\right) \times 100 \quad (1)$$

According to the SEM image, the area of the pore was calculated with Adobe Photoshop 7.0, and then, the area of the pore was converted into the area of a circle. The circular diameter could be obtained according to the area of the circle, which was regarded as the pore diameter.[19]

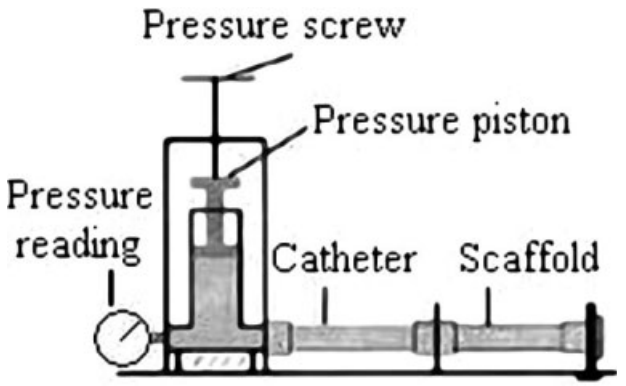

Figure 2 Schematic illustration of the pressure pump setup.

Biomechanical evaluations

Tensile properties

The tensile properties of the scaffolds were characterized with an Instron tensile tester (model 3365, Issaquah, WA) in ambient conditions. The strips ($40 \times 2 \times 0.5$ mm^3), which were cut from the tubes, were measured in tension with a crosshead speed of 10 mm/min by the Instron tensile tester. The gauge length was set at 15 mm, and a load cell of 100 N was used. The tensile strength per cross-sectional area (kg/mm^2) and the ratio of the relative elongation to the initial sample length at break (%) were determined from observation of the stress–strain curves. The ambient conditions were controlled at 20°C and 70% humidity. All samples were stored under ambient conditions before testing. Each test was performed three times.

Suture retention strength

The suture retention strength of the scaffolds was measured as described by Schaner et al.[20] One end of the scaffold was fixed to the stage clamp of the Instron tensile tester (model 3365), and the opposite end was connected to another clamp by a polypropylene suture (5-0, Ethicon Inc., Piscataway, NJ). The suture was placed in the four corners 2 mm from the edge of the scaffolds. The distance between the clamps was 15 mm. The scaffolds were pulled at a crosshead speed of 10 mm/min until the suture pulled through the scaffolds. The suture retention strength, which was defined as the fracture strength, was obtained. Each test was performed three times.

Burst pressure strength

We measured the burst pressure for the composite scaffolds by increasing the pressure within the scaffolds until failure occurred. A pressure pump (Merit Medical Systems Inc., South Jordan, UT) was introduced to test the burst pressure strength. Figure 2 shows the schematic illustration of the pressure pump setup. The pressure was increased until failure or leakage occurred, and the pressure change was recorded. Each test was performed three times.

Biological property characterizations

Cell growth and proliferation

3T3 mouse fibroblasts (Chinese Academic of Science, Shanghai, China) were cultured in Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum at 37°C with 5% CO_2. The medium was changed three times a week. After about 80% confluence was reached, the cells were detached by 0.25% trypsin. The scaffolds were cut out and sterilized by γ radiation and placed in 24-well plates. The 3T3 mouse fibroblasts were cultured at a density of 5×10^4 per well in three different types of scaffolds: (1) a culture dish, (2) PLA/SF, and (3) PLA/SF–gelatin. The growth and proliferation of the cells on the scaffolds were determined by mitochondrial metabolic activity assay. After removal of medium, the cell layers were rinsed twice with PBS. Mitochondrial metabolic solution (100 μL) and phenol red-free RPMI1640 medium (1 mL; Sigma) were added to each well, and the wells were incubated for 4 h. Thereafter, 1 mL/well dimethyl sulfoxide was added

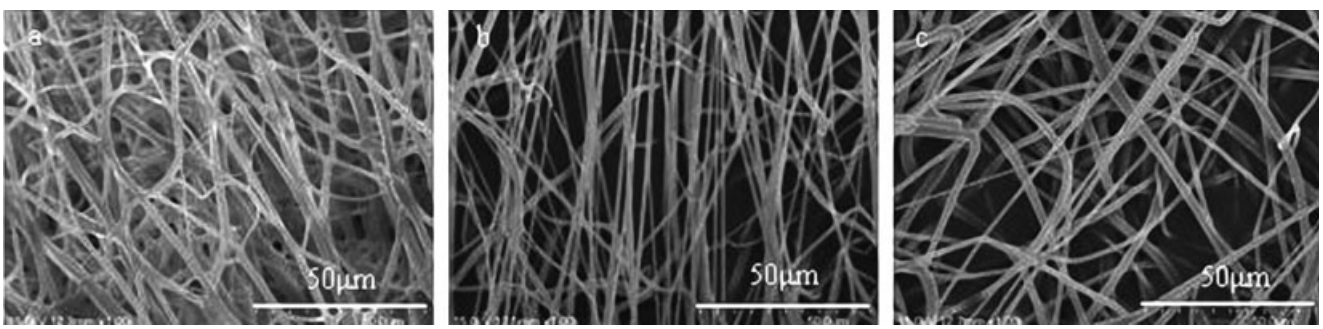

Figure 3 SEM images of the PLA fibers (outside layer) gained at different mandrel rotating speeds: (a) 1000, (b) 2000, and (c) 3000 rpm.

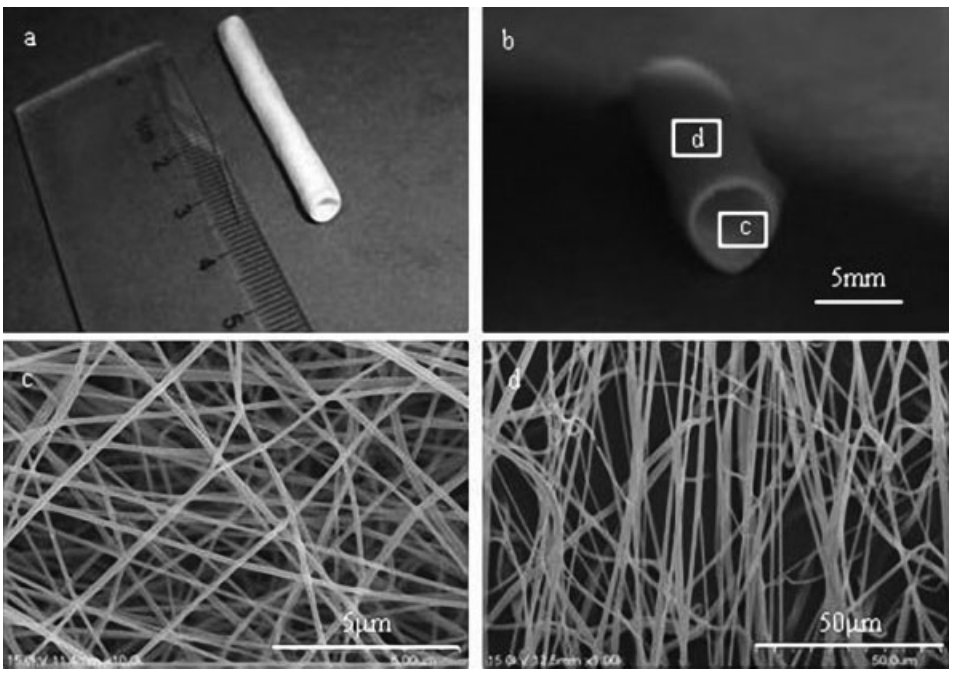

Figure 4 Macroscopic and microscopic views of the PLA/SF–gelatin composite tubular scaffold: (a,b) whole body of the composite scaffold, (c) SEM of the SF–gelatin layer (10,000×), and (d) SEM of the PLA layer (1000×).

and shaken for 10 min. Subsequently, the absorbance at 540 nm was measured with a spectrophotometer.

Cell morphology

The PLA/SF–gelatin composite scaffolds were cut out and sterilized by γ radiation and then put onto 24-well plates. Human umbilical vein endothelial cells (HUVECs; Chinese Academic of Science) were cultured at a density of 5×10^4 well in RPMI1640 medium (Gibco Co., Carlsbad, CA) containing 10% fetal bovine serum, 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cell culture was maintained at 37°C in a humidified 5% CO_2 incubator. SEM was used to determine the morphology of the cells seeded on the scaffolds. HUVECs were observed under SEM after 7, 14, and 21 days. After harvesting, the seeded PLA/SF–gelatin scaffolds were immediately rinsed in 0.2*M* sodium cacodylate buffer and then fixed in Karnovsky fixative (2.5% glutaraldehyde in 0.1*M* sodium cacodylate) overnight at 4°C. Fixed samples were dehydrated through exposure to a gradient of alcohol followed by Freon (1,1,2-trichlorotrifluoroethane, Aldrich, Milwaukee, WI) and allowed to air dry in a fume hood.

Statistical analysis

Means and SDs were calculated for all of the quantitative data (and are reported as mean ± SD). The differences between the PLA/SF–gelatin composite scaffolds and the PLA and SF–gelatin scaffolds specific to suture retention strength and burst pressure strength were analyzed by SPSS13.0 (Microsoft Co., Redmond, WA). Differences were considered as significant at $p < 0.05$.

RESULTS AND DISCUSSION

Composite scaffold morphological characterizations

The rotating speed of the mandrel affects the orientation of fibers remarkably. Moreover, the orientation of the fibers has an important influence on their mechanical properties.[21,22] In this study, the fibers'

TABLE I
Morphological Characterizations of the PLA/SF–Gelatin Composite Tubular Scaffold

Scaffold		Fiber diameter (nm)	Average pore diameter (nm)	Porosity (%)
PLA/SF–gelatin composite scaffold	SF–gelatin layer	143 ± 36	161 ± 55	82 ± 2
	PLA layer	1337 ± 427	—	

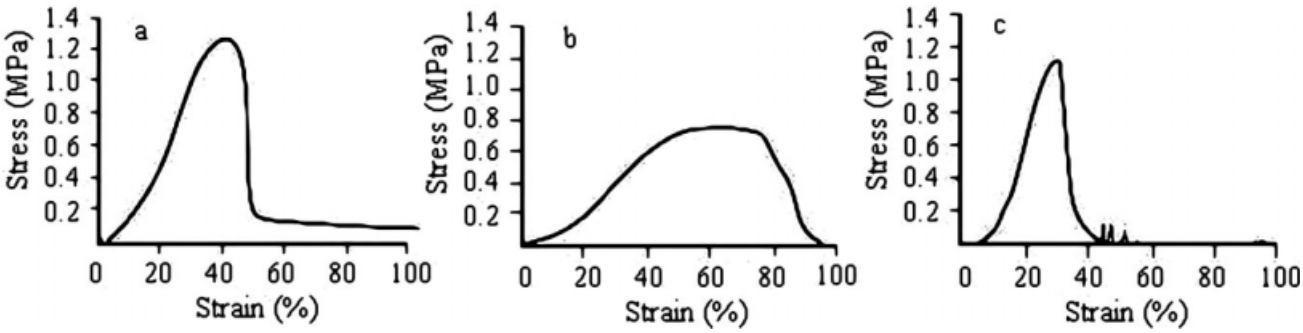

Figure 5 Stress–strain curves of the scaffolds: (a) PLA/SF–gelatin composite scaffold, (b) PLA scaffold, and (c) SF–gelatin scaffold.

orientation in the PLA layer was investigated. Different orientations of PLA fibers were obtained with the rotating speed of the mandrel, which ranged from 1000 to 3000 rpm. Figure 3 shows the SEM images of the PLA fibers gained at different mandrel rotating speeds.

As shown in the SEM images, aligned fibers were obtained when the rotating speed was 2000 rpm [Fig. 3(b)], speeds lower or higher than this did not produce aligned fibers [Fig. 3(a,c)]. The mechanism of this technique is given as follows.[23] When the linear speed of the rotating mandrel surface, which served as a fiber take-up device, matched that of the evaporated jet depositions, the fibers were taken up on the surface of the mandrel tightly in a circumferential manner, which resulted in a fair alignment. Such a speed can be called an *alignment speed.* If the surface speed of the mandrel was slower than the alignment speed, randomly deposited fibers were collected, as it was the fast chaos motions of the jets that determined the final deposition manner. On the other hand, there must have been a limit rotating speed, above which continuous fibers could not be collected because the overfast take-up speed would break the fiber jet. So, in this study, the rotating speed of the mandrel was set at 2000 rpm when the PLA fibers were collected.

Figure 4(a,b) shows the photograph of the whole body of the PLA/SF–gelatin composite tubular scaffold, which had a length of 40 mm and a inner diameter of 4.5 mm with a thickness of 0.5 mm. A randomly oriented fibrous SF–gelatin layer (diameters = 143 ± 36 nm and average pore diameters = 161 ± 55 nm) and an aligned fibrous PLA layer (diameters = 1337 ± 427 nm) were electrospun on the mandrel; these are illustrated in Figure 4(c,d). The preferred porosity of scaffolds used for cellular penetration should generally be within the range 60–90%.[24–26] The porosity of the PLA/SF–gelatin composite tubular scaffold reached 82 ± 2% (Table I), which was shown to be desirable for the scaffolds and facilitated the spreading and proliferation of cells.

Biomechanical property evaluations

Tensile properties

Figure 5 presents the typical stress–strain curves of the electrospun PLA/SF–gelatin composite tubular scaffold and the PLA and SF–gelatin tubular scaffolds (used for comparison, thickness = 0.5 mm), and the detailed data are shown in Table II.

As shown in Figure 5 and Table II, the PLA tubular scaffold had a lower breaking strength (0.75 ± 0.04 MPa) and a higher elongation (58.89 ± 3.36%), which showed a pliable character, whereas the SF–gelatin tubular scaffold showed a rigid character with a higher breaking strength (1.12 ± 0.11 MPa) and a lower elongation (30.55 ± 3.46%). The breaking strength of the PLA/SF–gelatin composite tubular scaffold reached 1.28 ± 0.21 MPa, and it had an elongation of 41.11 ± 2.17%. The PLA/SF–gelatin composite tubular scaffold had a higher breaking strength and elongation at the same time. The PLA/SF–gelatin composite tubular scaffold had desirable tensile properties for vascular grafts, which are generally accepted to be 1.0 MPa (breaking strength) and 40.0% (elongation).[27]

Suture retention strength

Suture retention strength is a crucial factor in the fabrication of vascular scaffolds, as it relates directly

TABLE II
Tensile Properties of the Tubular Scaffolds

Sample	Breaking strength (MPa)	Elongation (%)
PLA	0.75 ± 0.04	58.89 ± 3.36
SF–gelatin	1.12 ± 0.11	30.55 ± 3.46
PLA/SF–gelatin	1.28 ± 0.21	41.11 ± 2.17

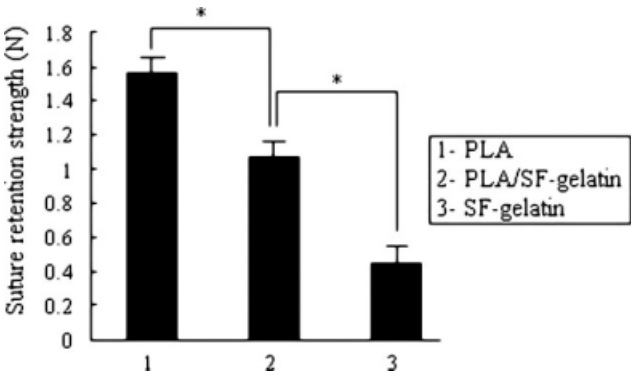

Figure 6 Suture retention strength of the PLA/SF–gelatin composite scaffold compared to the comparison (*$p < 0.05$).

to the success of the graft implantation procedure. The suture retention strengths of the PLA/SF–gelatin composite scaffold and PLA and SF–gelatin scaffolds (used for comparison, thickness = 0.5 mm) are shown in Figure 6. The PLA/SF–gelatin composite scaffold (1.07 ± 0.07 N) had a lower suture retention strength than the PLA scaffold (1.56 ± 0.20 N) and a higher suture retention strength than the SF–gelatin scaffold (0.45 ± 0.02 N), as shown in Figure 6. This was because the SF–gelatin scaffold had a rigid character. However, the PLA scaffold showed a pliable character. The composite scaffold had adequate suture retention strength for suturing during implantation, which is generally accepted to be 1.0 N.[28]

Burst pressure strength

The burst pressure strength is one of the most important indices for examining the mechanical properties of scaffolds. To determine whether the composite scaffolds possessed adequate strength to endure physiological forces, burst pressure testing was performed to identify the maximum pressure that the scaffolds could endure before failure. The burst pressure strength of the PLA/SF–gelatin composite tubular scaffold, compared to the control (thickness = 0.5 mm), are illustrated in Figure 7. The PLA/SF–gelatin composite scaffold had a higher burst pressure strength (111.4 ± 2.6 kPa) than PLA (91.2 ± 1.8 kPa) and lower burst pressure strength than the SF–gelatin scaffold (121.6 ± 3.4 kPa). This was because the PLA scaffold had a large porosity (86%), the SF–gelatin scaffold had a small porosity (80%), and the PLA/SF–gelatin composite scaffold had a porosity intermediate between the PLA and SF–gelatin scaffold porosities (82%).

Because the normal blood pressure in the human body is 12.0–18.7 kPa,[29] the results demonstrate that the PLA/SF–gelatin composite tubular scaffolds possessed adequate physical strength and could be developed as substitutes for native blood vessels.

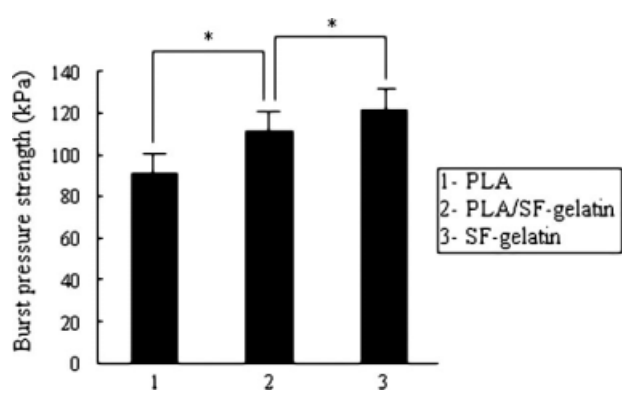

Figure 7 Burst pressure strength of the PLA/SF–gelatin composite scaffold compared to the comparison (*$p < 0.05$).

TABLE III
Biomechanical Properties of the PLA/SF–Gelatin Composite Scaffolds with Different Wall Thicknesses

Wall thickness (mm)	Porosity (%)	Suture retention strength (N)	Burst pressure strength (kPa)
0.3	84	0.47 ± 0.06	60.8 ± 3.4
0.5	82	1.07 ± 0.12	111.4 ± 2.6
0.7	80	1.53 ± 0.08	172.2 ± 1.8

Influence of the wall thickness on the biomechanical properties

It is well known that the biomechanical properties of scaffolds are influenced remarkably by their wall thicknesses.[30] In this study, the suture retention strength and burst pressure strength of the PLA/SF–gelatin composite scaffolds with different wall thicknesses (0.3, 0.5, and 0.7 mm) were measured, and the detailed data are shown in Table III.

As shown in Table III, the suture retention strength increased from 0.47 ± 0.06 to 1.53 ± 0.08 N,

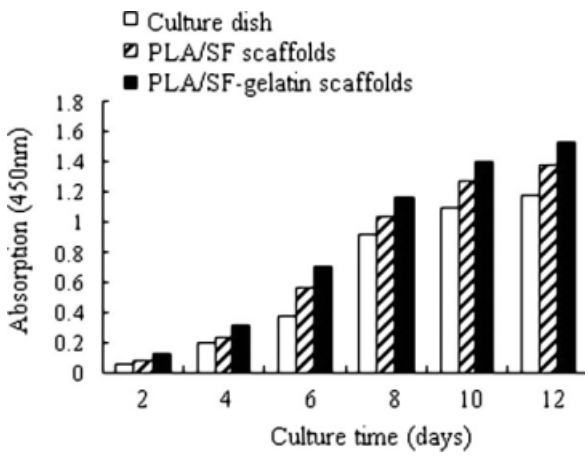

Figure 8 Growth and proliferation of the 3T3 mouse fibroblast on the scaffolds.

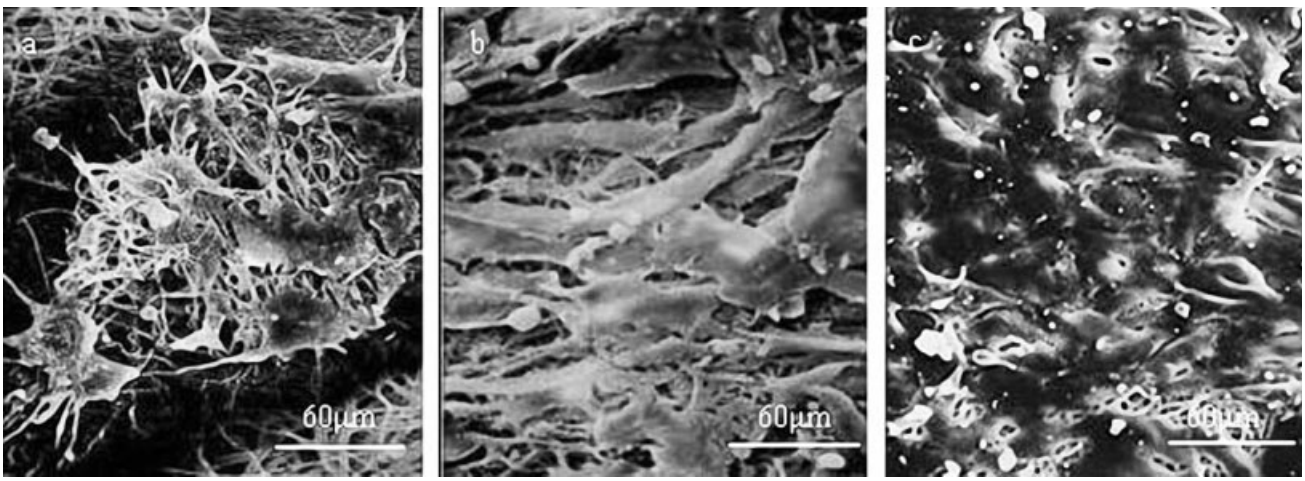

Figure 9 SEM images of the HUVECs seeded on the PLA/SF–gelatin composite tubular scaffolds after (a) 7, (b) 14, and (c) 21 days of culturing.

and the burst pressure strength increased from 60.8 ± 3.4 to 172.2 ± 1.8 kPa with increasing wall thickness from 0.3 to 0.7 mm. This was probably due to the decrease in the porosity of the scaffolds because a small porosity results in a compact structure.

Biological property evaluations

Cell growth and proliferation

As shown in Figure 8, both the PLA/SF and PLA/SF–gelatin composite scaffolds could support 3T3 mouse fibroblast growth and proliferation, which suggested that the scaffolds had no cytotoxicity. Cell proliferation on the composite scaffolds was higher than on the controls, which indicated that the three-dimensional scaffolds, with a high surface-area-to-volume ratio and porosity, were more suitable for cell growth than the culture dish, whose surface was smooth. In addition, the proliferation of 3T3 mouse fibroblasts on the PLA/SF–gelatin scaffolds was higher than on the PLA/SF scaffolds; this was because gelatin is a biocompatible polymer, which can promote cell proliferation.

Cell morphology

The cell morphology and the interaction between cells and scaffolds were studied by SEM for 21 days. Figure 9 shows the HUVEC attachment and growth on the PLA/SF–gelatin composite scaffolds (inner layer) separately on days 7, 14, and 21 after seeding. The HUVECs adhered and spread on the surface of the scaffolds from day 7 to day 21. On day 7, only a few cells were observed on the scaffolds. On day 14, there was an increase in the cell density compared to day 7; the cells were also observed to migrate and proliferate in certain patterns and form a continuous monolayer. On day 21, the cells significantly increased in number and almost reached confluence on the scaffolds.

CONCLUSIONS

We developed PLA/SF–gelatin composite tubular scaffolds using electrospinning techniques that could withstand physiological vascular conditions. The PLA/SF–gelatin composite tubular scaffolds possessed excellent biomechanical and biological properties. All of the investigations demonstrated that the electrospun PLA/SF–gelatin composite tubular scaffolds are appropriate candidates for blood vessel tissue engineering.

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