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TERM

Electrospun Fibrous Silk Fibroin/Poly(L-Lactic Acid) Scaffold for Cartilage Tissue Engineering

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For successful tissue engineering of articular cartilage, a scaffold with mechanical properties that match those of natural cartilage as closely as possible is needed. In the present study, we prepared a fibrous silk fibroin (SF)/poly(L-lactic acid) (PLLA) scaffold via electrospinning and investigated the morphological, mechanical, and degradation properties of the scaffolds fabricated using different electrospinning conditions, including collection distance, working voltage, and the SF:PLLA mass ratio. In addition, *in vitro* cell-scaffold interactions were evaluated in terms of chondrocyte adhesion to the scaffolds as well as the cytotoxicity and cytocompatibility of the scaffolds. The optimum electrospinning conditions for generating a fibrous SF/PLLA scaffold with the best surface morphology (ordered alignment and suitable diameter) and tensile strength (~1.5 MPa) were a collection distance of 20 cm, a working voltage of 15 kV, and a SF:PLLA mass ratio of S50P50. The degradation rate of the SF/PLLA scaffolds was found to be determined by the SF:PLLA mass ratio, and it could be increased by reducing the PLLA proportion. Furthermore, chondrocytes spread well on the fibrous SF/PLLA scaffolds and secreted extracellular matrix, indicating good adhesion to the scaffold. The cytotoxicity of SF/PLLA scaffold extract to chondrocytes over 24 and 48 h in culture was low, indicating that the SF/PLLA scaffolds are biocompatible. Chondrocytes grew well on the SF/PLLA scaffold after 1, 3, 5, and 7 days of direct contact, indicating the good cytocompatibility of the scaffold. These results demonstrate that the fibrous SF/PLLA scaffold represents a promising composite material for use in cartilage tissue engineering. Tissue Eng Regen Med 2016;13(5):516-526

Key Words: Electrospinning; Silk fibroin; Poly(L-lactic acid); Cartilage tissue engineering; Scaffold

INTRODUCTION

The low frictional property of the articular cartilage covering the outer surface of the hip and knee joints is critical for the proper functioning of these joints. Damage to the articular cartilage (e.g., the occurrence of osteoarthritis) greatly impairs patients' quality of life [1,2]. Total joint replacement has been applied in orthopedic surgery for more than 50 years, and major progress has been achieved to gain insight into the detailed mechanisms by which the human body responds to endosseous implants *in vivo* [3-6]. However, wear on the implants inevitably occurs, eventually resulting in failure of total joint replacement, and the overall survivorship of implants has been unsatisfactory with revision as an endpoint [7-10]. Cartilage tissue engineering is considered as an innovative and promising approach by which a supporting scaffold, seeded cells, and delivered growth factors are integrated to facilitate cartilage regeneration [11]. The scaffold, as one of the most important factors in cartilage tissue engineering, should provide a microenvironment that supports cell attachment, proliferation, and differentiation and promotes the generation of new tissues [12]. The scaffold actually serves as a temporary extracellular matrix (ECM) for regenerative cells, and therefore, it is necessary for the scaffold to emulate advantageous features of the natural ECM, such as a similar composition, structure, biocompatibility, biodegradability, and mechanical properties [13].

Poly(L-lactic acid) (PLLA), an aliphatic thermoplastic polyester, has been widely investigated in tissue engineering applications and is produced by either poly-condensation of lactic acid or ring opening polymerization of lactide [14]. PLLA is widely considered a useful biodegradable synthetic polymer due to its relatively strong mechanical properties and good biocom-

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patibility, with carbon dioxide and water as the final products of PLLA degradation [15]. PLLA was approved for clinical use by the American Food and Drug Administration as one of the first degradable and absorbable materials. Silkworm silk fibroin (SF) is another attractive biomaterial widely used for tissue regeneration because it possesses good biocompatibility, excellent breathability, and controllable biodegradability [16-18]. However, PLLA and SF both have inherent shortcomings. Because cells are more likely to interact with and adhere to a hydrophilic surface, only limited cell adhesion occurs on the hydrophobic surface of PLLA [19]. Additionally, PLLA degrades slowly in physiologic conditions, and this slow degradation rate does not match perfectly with the formation of new tissues. As a natural protein, pure SF has relatively weak mechanical properties, which makes it less suitable for use as a scaffold material in tissue engineering applications [20].

The concept of a composite material may solve the deficiencies of pure biomaterials, offering new properties of the composite due to a synergistic effect between pure materials while also still maintaining the original desirable properties of the pure biomaterials. Recently, much attention has been paid to electrospinning, a simple, cheap, and effective method for fabricating continuous and ultra-fine fibers, as a method for preparing tissue engineering scaffolds that is achieved by forcing a polymer solution through a spinneret under a high voltage electric field [21,22]. The fibers, with a diameter ranging from tens of nanometers to several micrometers, form an aligned or unordered three-dimensional (3D) structure similar to that of natural ECM. In addition, scaffolds fabricated by electrospinning possess porous structures with extensive pore interconnectivity, high surface area and porosity, as well as good continuity of fibers, and such scaffolds have been applied in the regeneration of various tissues, including bone, nerve, cartilage, and blood vessels [23-26]. In the present study, we fabricated fibrous SF/PLLA scaffolds via electrospinning and evaluated the surface morphology, tensile strength, and degradation properties of scaffolds prepared using different electrospinning conditions. The *in vitro* interactions between cells and fibrous SF/PLLA scaffolds obtained using the optimized electrospinning conditions in terms of cell adhesion and scaffold cytotoxicity also were investigated using chondrocytes isolated from rabbits.

MATERIALS AND METHODS

Materials and solvents

SF and PLLA (MW: 300k Da) were purchased from Huzhou Heavens Silk Biological Technology Co., Ltd., China and Shandong Institute of Medical Appliances, China, respectively. 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) and trifluoroacetic acid (TFA), which were used as the solvents for SF and PLLA, were obtained from Sigma-Aldrich, St. Louis, MO, USA, and Tianjin Fuchen Chemical Reagents Factory, China, respectively. Additionally, trypsin-EDTA, collagenase II, and fetal bovine serum (FBS) were purchased from Gibco Life Technologies, Carlsbad, CA, USA. Phosphate-buffered saline (PBS), Dulbecco's Modified Eagles Medium (DMEM), penicillin, and streptomycin were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Fabrication of fibrous SF/PLLA scaffolds

SF and PLLA at specific mass ratios were dissolved completely in TFA and HFIP (a widely used solvent in electrospinning [27]), respectively, and then mixed together. The polymer solution was prepared in a 5-mL syringe with a steel needle (inner diameter: 0.4 mm) attached. The aligned fibrous SF/PLLA scaffolds were fabricated through electrospinning at a working voltage supplied by a high voltage power system. A flat copper wire drum receiver was used to collect the fibrous SF/PLLA scaffolds at a specified distance from the needle. Three electrospinning conditions were varied in order to determine the optimum parameters for preparing fibrous SF/PLLA scaffolds with the most suitable properties: 1) collection distance: 17 cm, 20 cm, and 23 cm; 2) working voltage: 12 kV, 15 kV, and 18 kV; and 3) SF:PLLA mass ratio: S40P60, S50P50, and S60P40.

Characterization of fibrous SF/PLLA scaffolds

Surface morphology

The fibrous SF/PLLA scaffolds were sputter-coated with a thin layer of gold to enhance conductivity, and then the scaffold surface morphology was examined by scanning electron microscopy (SEM, SHIMADZU SSX-550, Kyoto, Japan) at an accelerated voltage of 15 kV. In addition, Image-Pro Plus software (Media Cybernetics, Rockville, MD, USA) was used to quantitatively evaluate the diameter of fibers and the distribution of diameters. Approximately 100 fibers in SEM images were analyzed from selected SEM images for scaffold type.

Tensile strength

The mechanical properties of the fibrous SF/PLLA scaffolds were evaluated via tensile testing using a universal material testing machine (Shanghai Instrument Inc., Shanghai, China). Each SF/PLLA scaffold was fabricated as a dumbbell strip sample (25×4 mm) and fixed firmly by the material testing machine. A tensile load was applied at a crosshead speed of 10 mm/min, and the tensile stress-strain curve was recorded until failure occurred. At least three samples were tested for fibrous SF/PLLA scaffolds fabricated using each set of electrospinning conditions.

Degradation

PBS was employed to simulate body fluid in the degradation test according to International Standard 10993-13. The degradation of fibrous SF/PLLA scaffolds fabricated using different SF:PLLA mass ratios was evaluated by immersing the scaffolds in PBS (pH=7.4) in a constant temperature container maintained at 37°C for a period of 12 weeks. The sampling time points were 1, 2, 3, 4, 6, 8, and 12 weeks. The degradation rate was obtained by calculating the weight loss of the SF/PLLA scaffolds between sampling times.

In vitro cell-scaffold interactions

The following procedures for investigating *in vitro* cell-scaffold interactions were approved by the Animal Care and Use Committee of Jilin University. Fibrous SF/PLLA scaffolds with the best surface morphology, mechanical properties, and degradation profile as determined during the SEM evaluation, tensile testing, and degradation study were used to assess cell adhesion, cytotoxicity, and cytocompatibility.

Cell isolation and culture

Fresh articular cartilage was harvested from the knee joints of 2-week-old Japanese rabbits under aseptic conditions, digested employing 0.25% trypsin plus 0.02% EDTA at 37°C for 30 minutes, and then cut into approximately 1-mm^3 pieces. The cartilage pieces were washed with PBS (supplemented with 100 U/mL penicillin and 100 mg/mL streptomycin) three times and digested using 0.1% collagenase II in serum-free DMEM at 37°C overnight. The digested tissue was filtered through a 200-mm nylon mesh and centrifuged at 1000 rpm for 5 minutes to obtain a chondrocyte pellet after removal of the supernatant. The pellet was resuspended in DMEM supplemented with 15% FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin and seeded in a culture flask at a density of 5×10^5 cells/mL. The culture flask was incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. The culture medium was refreshed every 2 days, and chondrocytes were subcultured when they reached 80−90% confluence. Chondrocytes at passage 2 were used for the following experiments.

Chondrocyte adhesion

Fibrous SF/PLLA scaffolds were prepared as round samples with a diameter that fit within the wells of 24-well culture plates. Before cell seeding, the samples were sterilized under ultraviolet light, soaked in 70% ethanol, and then washed with Hank's salt solution, each for 30 minutes. Then, 100 μL of chondrocyte suspension was seeded onto the SF/PLLA scaffolds at a density of 1×10^5 cells/mL. The culture plate was gently placed in an incubator (FORMA 311, Thermo scientific, Waltham, MA, USA), and the chondrocytes were cultured in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. After 48 h, each scaffold was rinsed thoroughly with PBS to remove unattached cells, and the attached cells were fixed by placing the scaffolds in 2% glutaraldehyde in PBS overnight at 4°C. Subsequently, the scaffolds were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, 95%, and 100%) and freeze-dried for 8 h. Finally, the scaffold was sputter-coated with a thin layer of gold and examined by SEM [28,29].

Scaffold cytotoxicity and cytocompatibility

The *in vitro* cytotoxicity of the fibrous SF/PLLA scaffolds was evaluated employing the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method according to International Standard 10993-5. Initially, SF/PLLA scaffold extract was prepared by soaking a sterile scaffold in DMEM supplemented with 15% FBS at 37°C for 24 h. After the extraction was complete, degerming was conducted using a filter membrane, and the scaffold extract was used as the culture medium in the experimental group. Additionally, DMEM containing 15% FBS and DMEM containing 15% FBS plus 0.64% phenol were utilized as the culture medium of the negative control and positive control groups, respectively. Chondrocytes were seeded into the wells of a 96-well culture plate at a density of 7×10^3 cells/well, and then 200 μL culture media for the negative control group, positive control group, or experimental group were added (n=8 per group). The culture plate was transferred to the incubator, and chondrocytes were incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. After culturing for 24 h and 48 h, 20 μL MTT solution (5 mg/mL in PBS, AMRESCO, Albany, NY, USA) was added to each well and incubated for 4 h. Finally, the culture medium was removed, and 150 μL dimethyl sulfoxide was added to each well. The optical density was measured using an enzyme labeling instrument (RT-6000, Lei Du Life Science and Technology Co. Ltd., Shenzhen, China) at an excitation wavelength of 490 nm. The relative growth rate (RGR) was calculated as the optical density of the experimental group divided by that of the negative control group, and the cell toxicity scale was determined as described previously [30].

The cytocompatibility of the scaffolds was also evaluated based on the viability of chondrocytes in direct contact between with the scaffolds employing the MTT method. The detailed steps were as follows. First, the scaffolds were cut into a circular shape with a diameter that fit the inside diameter of 96-well culture plates. Then the scaffolds were sterilized under UV light for 1 h on each side, soaked in 75% ethanol for 30 min, and finally washed with PBS three times, each for 5 minutes. The scaffolds were immersed in culture medium (DMEM/F12, 15% FBS, 100

units/mL penicillin, and 100 mg/mL streptomycin) over night for pre-wetting. A specific volume of cell suspension was added to the scaffolds in the 96-well plates to achieve a density of 4×10^3/scaffold, and samples were then incubated at 37 °C in the humidified incubator for 2 h. Subsequently, an additional 1−2 mL culture medium was added to completely cover the cell/scaffold construct. The culture medium was exchanged every 2−3 days. Finally, the absorbance values were measured using the MTT method as mentioned above at 1, 3, 5, and 7 days. We used cells cultured on a tissue culture plate surface (TCPS) treated to provide a more hydrophilic surface to promote cell attachment as a positive control group [31] and cells on pure PLLA

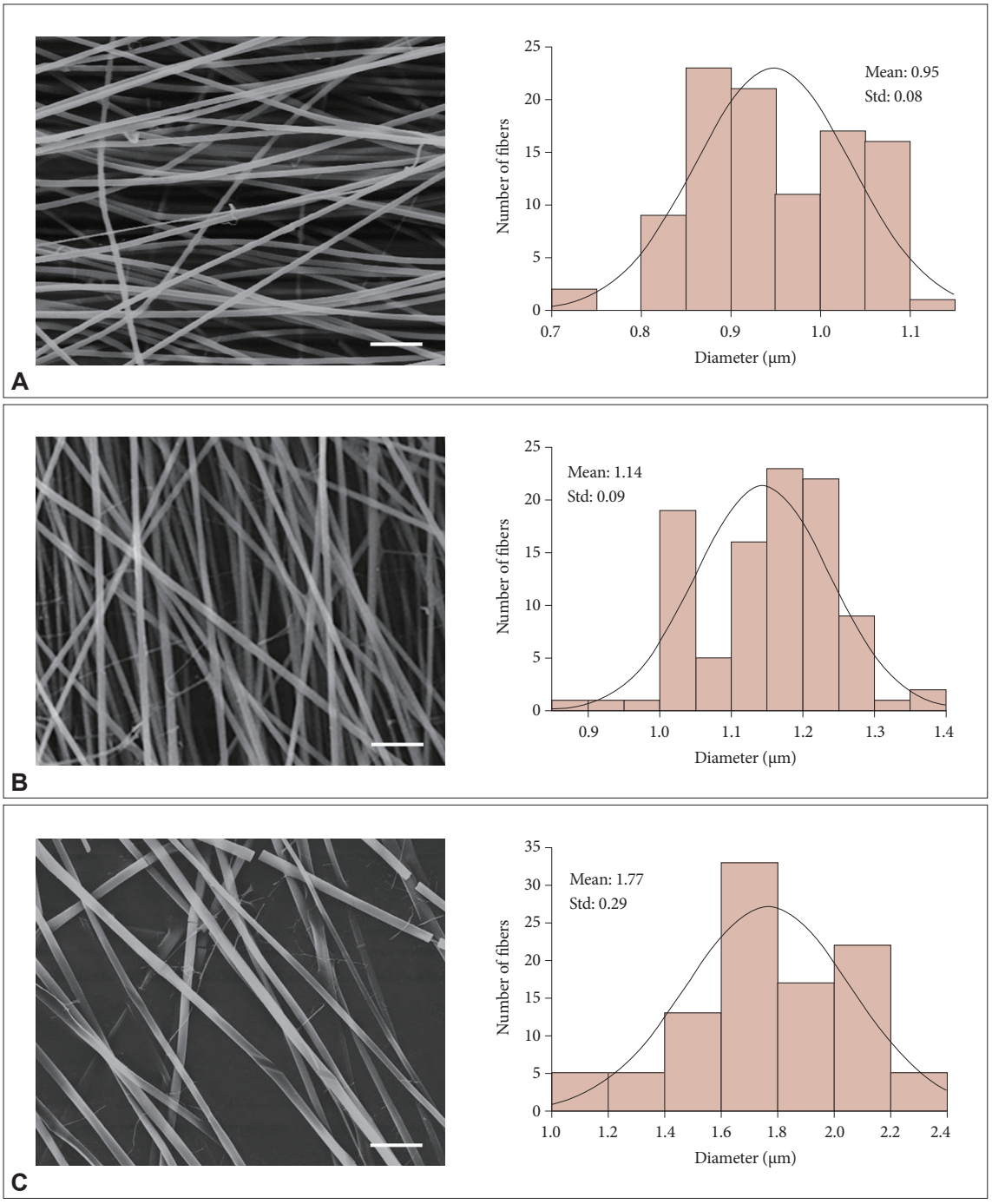

Figure 1. SEM micrographs of fibrous SF/PLLA scaffolds prepared with different collection distances and the corresponding diameter distributions: (A) 17 cm, (B) 20 cm, and (C) 23 cm. The working voltage and SF:PLLA mass ratio were 15 kV and S50P50, respectively. Scale bar: 10 μm. SEM: scanning electron microscopy, SF: silk fibroin, PLLA: poly(L-lactic acid).

scaffolds as a negative control group (n=6 per group).

Statistical analysis

All quantitative data were presented as mean values±standard deviation and analyzed using Statistical Product and Service Solutions 16.0 software (SPSS, Chicago, IL, USA). The significance of differences was evaluated by one-way analysis of variance, with statistical significance set at $p<0.05$.

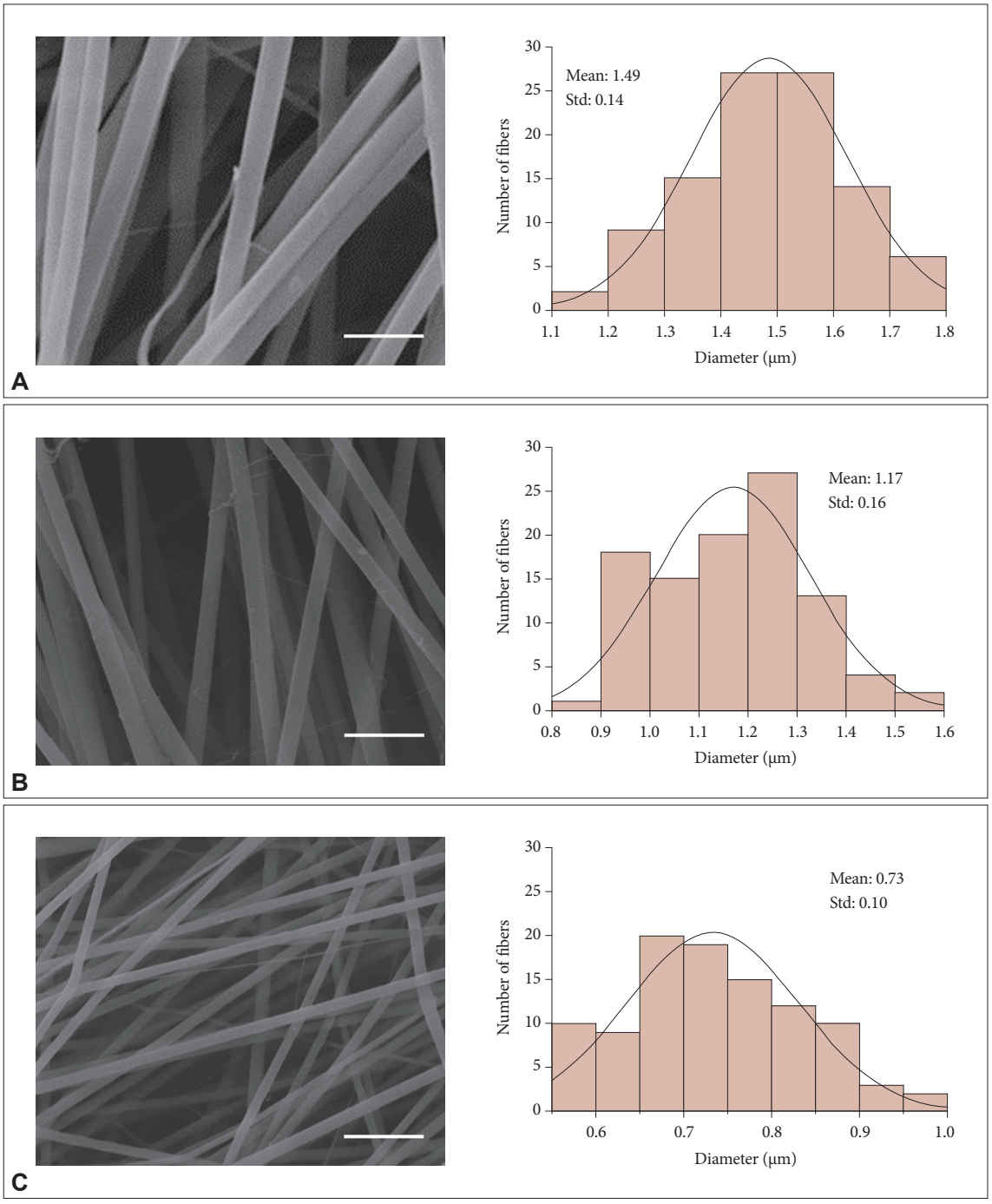

Figure 2. SEM micrographs of fibrous SF/PLLA scaffolds prepared with different working voltages and the corresponding diameter distributions: (A) 12 kV, (B) 15 kV, and (C) 18 kV. The collection distance and SF:PLLA mass ratio were 20 cm and S50P50, respectively. Scale bar: 5 μm. SEM: scanning electron microscopy, SF: silk fibroin, PLLA: poly(L-lactic acid).

RESULTS

Surface morphology

The effect of collection distance (17 cm, 20 cm, or 23 cm) on the surface morphology of fibrous SF/PLLA scaffolds is shown in Figure 1 (working voltage 15 kV, SF:PLLA mass ratio S50P50). The SEM micrographs clearly showed that an aligned surface morphology was obtained with all three collection distances, and the diameter became larger, from 0.95 μm to 1.77 μm, as the collection distance increased. Additionally, the fibers were slightly curved and flat when the collection distance was 17 cm, and the alignment was not as good as that achieved at a collec-

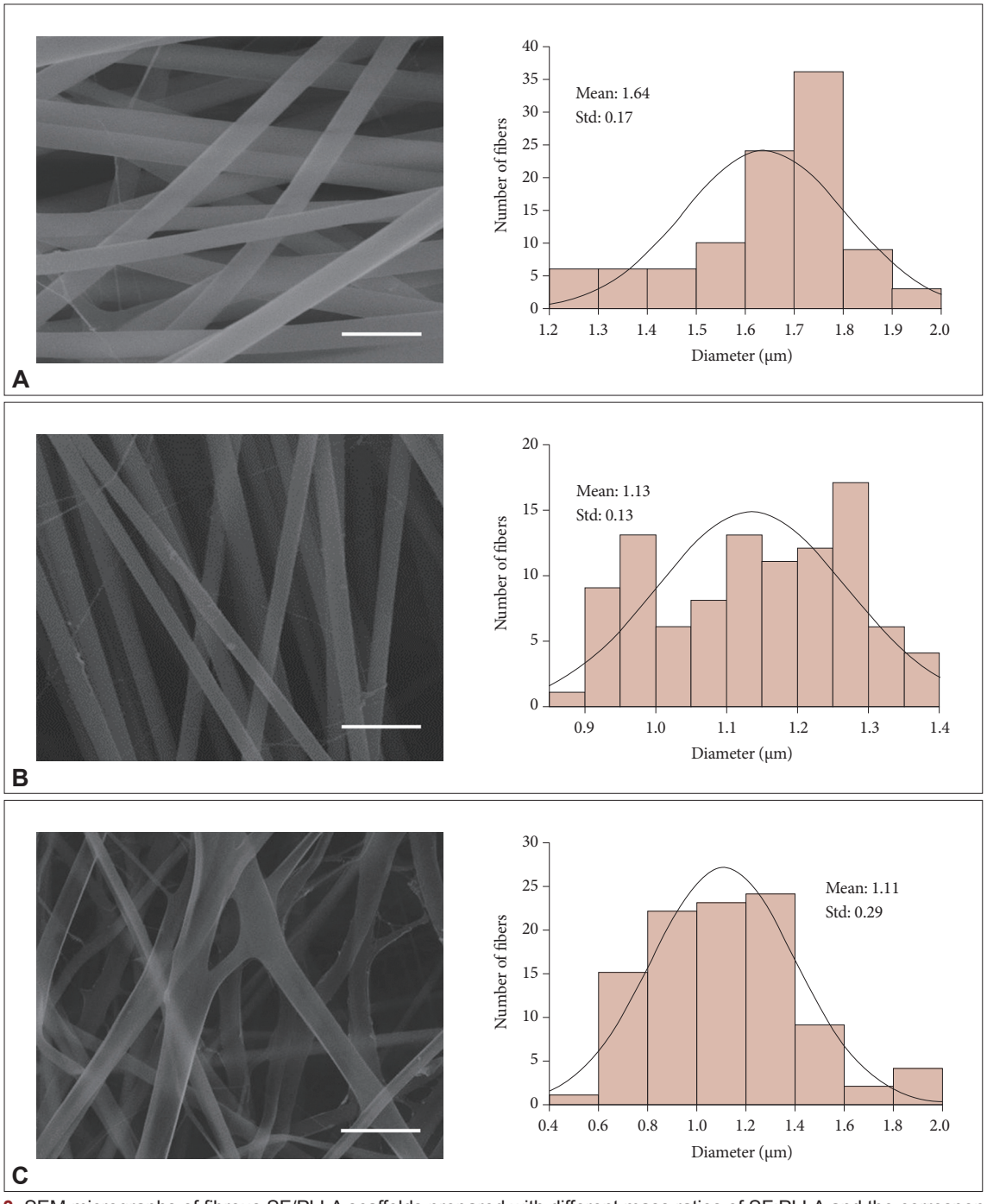

Figure 3. SEM micrographs of fibrous SF/PLLA scaffolds prepared with different mass ratios of SF:PLLA and the corresponding diameter distributions: (A) S40P60, (B) S50P50, and (C) S60P40. The collection distance and working voltage were 20 cm and 15 kV, respectively. Scale bar: 5 μm. SEM: scanning electron microscopy, SF: silk fibroin, PLLA: poly(L-lactic acid).

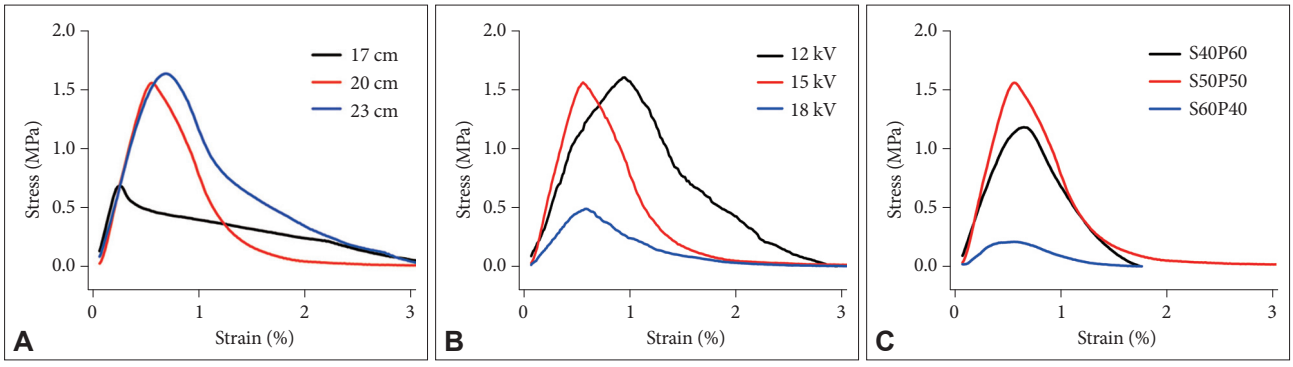

Figure 4. Tensile stress-strain curves for fibrous SF/PLLA scaffolds prepared with different: (A) collection distances, (B) working voltages, and (C) SF:PLLA mass ratios. SF: silk fibroin, PLLA: poly(L-lactic acid).

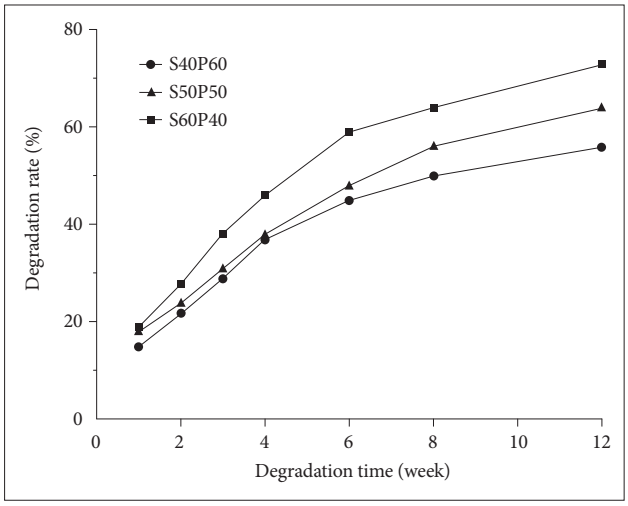

Figure 5. Degradation rate of fibrous SF/PLLA scaffolds prepared with different SF:PLLA mass ratios after immersing in PBS. The collection distance and working voltage were 20 cm and 15 kV, respectively. SF: silk fibroin, PLLA: poly(L-lactic acid), PBS: phosphate-buffered saline.

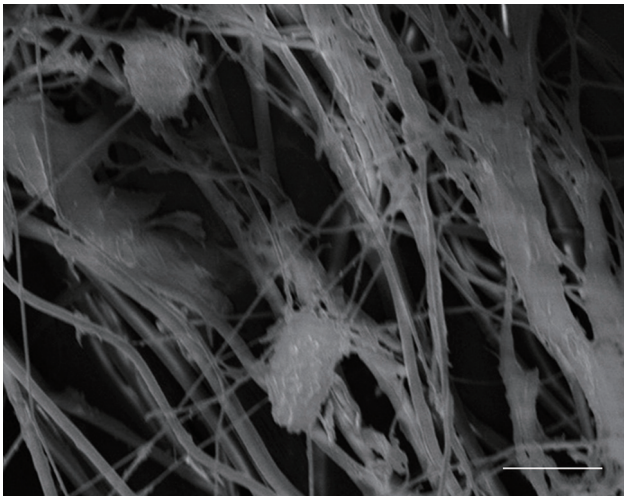

Figure 6. SEM micrograph showing chondrocyte morphology on a fibrous SF/PLLA scaffold after 48 h of incubation. Scale bar: 10 μm. SEM: scanning electron microscopy, SF: silk fibroin, PLLA: poly(L-lactic acid).

tion distance of 20 cm. The fibers were fractured and loosely arranged when the collection distance was 23 cm, with a broad diameter distribution. Therefore, a collection distance of 20 cm was considered to generate fibrous SF/PLLA scaffolds with the best surface morphology.

Figure 2 presents the effect of working voltage (12 kV, 15 kV, or 18 kV) on the surface morphology of the fibrous SF/PLLA scaffolds (collection distance 20 cm, SF:PLLA mass ratio S50P50). In the SEM micrographs, a fracture was not observed in scaffolds prepared under all the working voltages, and the diameter of the fibers decreased gradually, from 1.49 μm to 0.73 μm, as the working voltage increased. In addition, the diameter distribution of the fibers was good in scaffolds prepared using all of the tested working voltages, but the alignment was worse when the working voltage was 18 kV. Consequently, a working voltage of 15 kV was considered to be the suitable choice for the fabrication of fibrous SF/PLLA scaffolds.

The effect of the SF:PLLA mass ratio (S40P60, S50P50, or S60P40) on the surface morphology of the fibrous SF/PLLA scaffolds is illustrated in Figure 3 (collection distance 20 cm, working voltage 15 kV). The SEM micrographs showed that the diameter of the fibers decreased gradually, from 1.64 μm to 1.11 μm, as the proportion of PLLA decreased. The best diameter distribution was obtained when a SF:PLLA mass ratio of S50P50 was used, and adhesion between the fibers was detected when the SF:PLLA mass ratio was S60P40. Considering these experimental results together, the optimal SF:PLLA mass ratio for the fabrication of the fibrous SF/PLLA scaffolds was determined to be S50P50.

Tensile strength

The tensile stress-strain curves for fibrous SF/PLLA scaffolds prepared using different electrospinning conditions, i.e., collection distance, working voltage, and SF:PLLA mass ratio, are demonstrated in Figure 4. The tensile strength clearly increased

Table 1. Evaluation of cytotoxicity of the fibrous SF/PLLA scaffold according to the RGR and CTS after incubation of chondrocytes in scaffold extract for 24 h and 48 h

Group	Optical density		RGR		CTS	
	24 h	48 h	24 h	48 h	24 h	48 h
Positive control group	0.074±0.004	0.086±0.005	6	5	IV	IV
Negative control group	0.533±0.006	0.815±0.009	100	100	0	0
Experimental group	0.529±0.004*	0.809±0.013*	99	99	I	I

*p<0.05 compared with positive control group. SF: silk fibroin, PLLA: poly(L-lactic acid), RGR: relative growth rate, CTS: cell toxicity scale

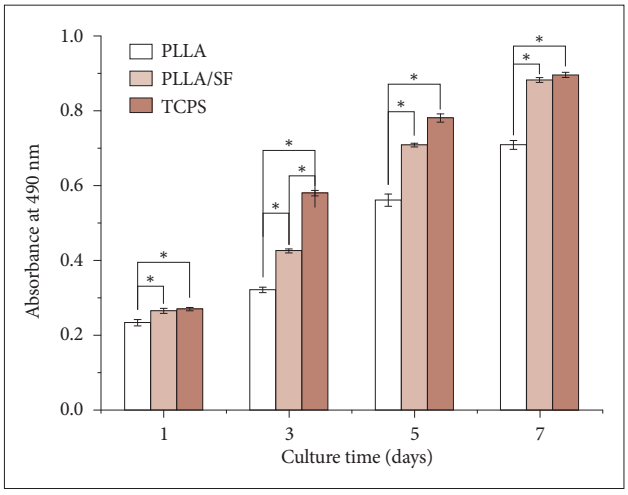

Figure 7. MTT results for viability of chondrocytes cultured on different substrates for 1, 3, 5, and 7 days. n=6. Absorbance readings at wavelength 490 nm. *p<0.05 between two groups. MTT: (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), PLLA: poly(L-lactic acid), SF: silk fibroin, TCPS: tissue culture plate surface.

with a greater collection distance, and similar values were obtained with collection distances of 20 cm and 23 cm (working voltage 15 kV, SF:PLLA mass ratio S50P50) (Fig. 4A). The tensile strength decreased gradually with the increase in working voltage, and there seemed to be no significant difference between scaffolds prepared at 12 kV and those prepared at 15 kV (collection distance 20 cm, SF:PLLA mass ratio S50P50) (Fig. 4B). In addition, the greatest tensile strength of the fibrous SF/PLLA scaffold was obtained when the SF:PLLA mass ratio was S50P50 (collection distance 20 cm, working voltage 15 kV) (Fig. 4C).

Degradation rate

The degradation of the fibrous SF/PLLA scaffolds fabricated with different SF:PLLA mass ratios (collection distance 20 cm, working voltage 15 kV) is shown in Figure 5. The results indicated that the degradation rate of all scaffolds followed a similar trend during the 12-week degradation period. Generally, the degradation rate was relatively faster during the first 4 weeks and then slowed from week 4 to week 8, followed by a further decrease from week 8 to week 12. Finally, the degradation rate was found to increase as the proportion of PLLA was decreased in the fibrous SF/PLLA scaffold.

Chondrocyte adhesion

The results obtained from the characterization of fibrous SF/PLLA scaffolds via SEM evaluation, tensile testing, and degradation study indicated that the electrospinning conditions that produced SF/PLLA scaffolds with optimized properties were as follows: collection distance of 20 cm, working voltage of 15 kV, and SF:PLLA mass ratio of S50P50. Chondrocyte adhesion on fibrous SF/PLLA scaffolds prepared under these conditions after 48 h in culture is presented in Figure 6. The cells exhibited an ellipsoid shape and spread well on the surface of the scaffolds, and ECM secretion was observed.

Scaffold cytotoxicity and cytocompatibility

The data in Table 1 demonstrate the cytotoxicity of the fibrous SF/PLLA scaffolds prepared using the optimized electrospinning condition after 24 h and 48 h in culture. The optical density values of the negative control group and experimental group greatly increased when the incubation time was extended from 24 h to 48 h. Additionally, after both incubation periods, the optical density values for the experimental group were significantly higher than those of the positive control group (p< 0.05), whereas the difference between the experimental group and negative control group was not significant (p>0.05). The RGR of the experimental group was 99% indicating that the SF/PLLA scaffold showed good biosafety. The proliferation of chondrocytes on PLLA, SF/PLLA scaffolds, and TCPS is shown in Figure 7. The growth of chondrocytes on TCPS was the fastest, whereas that on PLLA scaffolds was the slowest at the four time points, with statistical differences in the growth rates between the two groups. At day 3, the chondrocytes on TCPS proliferated faster than those on the SF/PLLA scaffold. However, the proliferation rate on the SF/PLLA scaffold caught up with that on TCPS at days 5 and 7 with no statistical differences between the two groups. The data demonstrated that chondrocytes could attach and proliferate well on the SF/PLLA scaffold, indicating that the SF/PLLA scaffold offers good cytocompatibility.

DISCUSSION

The rapid development of tissue engineering and the troublesome wear-related problems associated with total joint replacement have stimulated considerable concentration of research efforts on tissue regeneration [32-35]. Cartilage tissue engineering requires further intensive investigation because the mechanism of cartilage regeneration remains elusive [36]. Electrospinning has been advocated as a promising technology for manufacturing tissue engineering scaffolds [23,26]. In the present study, we successfully prepared fibrous SF/PLLA scaffolds via electrospinning and investigated in detail the influence of various electrospinning conditions (collection distance, working voltage, and SF:PLLA mass ratio) on the properties of the scaffold, including the surface morphology, tensile strength, and degradation rate. In addition, fibrous SF/PLLA scaffolds manufactured using the optimized electrospinning conditions demonstrated greater biocompatibility based on the assessments of chondrocyte adhesion, cytotoxicity tests and MTT assay, indicating that this material could potentially be used in cartilage tissue engineering applications.

A previous study reported that various electrospinning conditions result in different surface morphologies of fibrous SF/PLLA scaffolds [37]. For example, the collection distance determines the electric field intensity and the length of time that the fibers spend in the electric field. When the electric field intensity dominates the electrospinning process, the diameter of the fibers becomes larger with an increase in the collection distance as a result of the reduced electrostatic repulsive force (i.e., the fibers cannot be fully stretched). On the other hand, if the electric field intensity is sufficient to stretch the fibers, the diameter will decrease with a greater collection distance as the solvent is able to evaporate completely, avoiding the occurrence of adhesion and bending of the fibers. However, it is relatively difficult for the fibers to overcome gravity when the collection distance is too large, resulting in a disordered arrangement of the fibers and even breakage. In the present study, we found that the diameters of the fibers became larger with an increase in the collection distance, and the fibers were fractured at a collection distance of 23 cm, which was in agreement with the above analysis. Working voltage is another important factor influencing the surface morphology of the fibrous SF/PLLA scaffolds [38]. Basically, a higher working voltage promotes a decreased diameter of the fibers because it accelerates the injection of the solution, which is consistent with our findings in the present study. Furthermore, we observed that the diameter of the fibers became smaller as the proportion of PLLA decreased, and this can be attributed to the hydrophobic behavior of the PLLA material. The fluid viscosity of the solution is considered to be smaller at a lower PLLA proportion, which facilitates the volatilization of the solvent and reduces the diameter of the fibers.

In terms of material properties, articular cartilage is actually a viscoelastic material. The ECM structure and composition of cartilage, which consists of water (70−80%), collagen (50−75%), and glycosaminoglycans (15−30%), are responsible for its viscoelastic properties and give it ideal compressive, tensile, and frictional features [36]. Consequently, the mechanical properties of a cartilage tissue engineering scaffold are particularly important and should match those of natural cartilage as closely as possible. In the present study, the tensile strength of the fibrous SF/PLLA scaffolds prepared using various electrospinning conditions reached as high as 1.5 MPa under the optimized electrospinning condition (i.e., collection distance 20 cm, working voltage 15 kV, and SF:PLLA mass ratio S50P50). Additionally, an enhanced tensile strength of the fibrous SF/PLLA scaffold was obtained with an increase in the collection distance, a decrease in the working voltage, and an increase in the PLLA proportion. These results are consistent with the surface morphology observations made by SEM.

Most tissue engineering scaffolds should act as a temporary ECM rather than a permanent tissue substitute. Consequently, a suitable degradation profile of the scaffold is very important, and the ideal degradation rate should match the rate at which new tissue is formed [39]. The degradation of the fibrous SF/PLLA scaffolds prepared in the present study was shown to be controllable by adjusting the SF:PLLA mass ratio, indicating that an equilibrium between the degradation of the scaffold and the formation of new tissues is achievable. It is certainly a limitation that PBS does not mimic exactly the *in vivo* cellular environment for degradation, and thus, the results observed here may not directly match the *in vivo* degradation behavior of the prepared scaffolds. However, PBS is recommended by International Standard ISO 1993-13 to simulate body fluid and is commonly used for *in vitro* evaluation of tissue engineering scaffold degradation [24].

The cytotoxicity test was performed to detect residual chemicals in the scaffolds that might be harmful to the cells. The results in the present study showed that the harmful organic solvent was almost completely volatilized during the electrospinning process, and the scaffolds showed good biosafety. The viability of chondrocytes seeded directly on the scaffolds verified more accurately the good cytocompatibility of the scaffold. TCPS was treated to provide a more hydrophilic surface to promote cell adhesion and proliferation, and thus, the cell number on this surface was greater than in the other two groups at all four time points according to the results of MTT assay. However, TCPS has a two-dimensional planar structure, and chondrocytes stopped proliferating when they reached complete

confluence due to contact inhibition. The 3D SF/PLLA scaffold had a higher surface area and better promoted proliferation of chondrocytes in comparison with PLLA scaffold, due to the hydrophilic nature of protein SF. These experiments indicated that the SF/PLLA scaffold can be used in future cartilage tissue engineering strategies.

Recently, many researchers have begun to fabricate tissue engineering scaffolds using various composite materials, generating novel composites with controllable mechanical and morphological properties [40,41]. In the present study, we prepared fibrous SF/PLLA scaffolds via electrospinning, which led to the formation of a composite with not only better hydrophobic behavior than PLLA and better mechanical properties than SF but also a tunable degradation profile achievable by varying the SF:PLLA mass ratio. More importantly, the *in vitro* cell-scaffold interactions in terms of cell adhesion and cytotoxicity indicated excellent biocompatibility of the scaffolds for chondrocyte attachment. The good spreading and ECM secretion of chondrocytes on the surface of the fibrous SF/PLLA scaffolds may be related with the promotion of protein adsorption due to the high surface area of the scaffold [42]. Because cell adhesion on a tissue engineering scaffold is considered a prerequisite for subsequent cell proliferation and differentiation, our results suggest that the fibrous SF/PLLA scaffold developed in the present study represents a promising material for potential application in cartilage tissue engineering.

In summary, electrospinning is a simple and effective technology for the preparation of fibrous scaffolds, and the morphological, mechanical, and degradation properties of the scaffolds can be controlled by varying the electrospinning conditions. Fibrous SF/PLLA scaffolds fabricated by electrospinning are biocompatible and have potential applications in cartilage tissue engineering.

Conflicts of Interest

The authors have no financial conflicts of interest.

Ethical Statement

There are no animal experiments carried out for this article.

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