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Electrospinning collagen/chitosan/poly(L-lactic acid-*co*-ε-caprolactone) to form a vascular graft: Mechanical and biological characterization

Anlin Yin,[1,2] Kuihua Zhang,[3] Michael J. McClure,[2] Chen Huang,[1] Jinglei Wu,[1] Jun Fang,[1] Xiumei Mo,[1] Gary L. Bowlin,[2] Salem S. Al-Deyab,[4] Mohamed El-Newehy[4,5]

[1]State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Materials Science and Engineering, Donghua University, Shanghai 201620, China
[2]Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, Virginia 23284-3067
[3]Department of Textile Engineering, Jiaxing University, Zhejiang 314001, China
[4]Petrochemical Research Chair, Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[5]Department of Chemistry, Faculty of Science, Tanta University, Tanta 31527, Egypt

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Abstract: For blood vessel tissue engineering, an ideal vascular graft should possess excellent biocompatibility and mechanical properties. For this study, a elastic material of poly (L-lactic acid-*co*-ε-caprolactone) (P(LLA-CL)), collagen and chitosan blended scaffold at different ratios were fabricated by electrospinning. Upon fabrication, the scaffolds were evaluated to determine the tensile strength, burst pressure, and dynamic compliance. In addition, the contact angle and endothelial cell proliferation on the scaffolds were evaluated to demonstrate the structures potential to serve as a vascular prosthetic capable of *in situ* regeneration. The collagen/chitosan/P(LLA-CL) scaffold with the ratio of 20:5:75 reached the highest tensile strength with the value of 16.9 MPa, and it was elastic with strain at break values of ~112%, elastic modulus of 10.3 MPa. The burst pressure strength of the scaffold was greater than 3365 mmHg and compliance value was 0.7%/100 mmHg. Endothelial cells proliferation was significantly increased on the blended scaffolds versus the P(LLA-CL). Meanwhile, the endothelial cells were more adherent based on the increase in the degree of cell spreading on the surface of collagen/chitosan/P(LLA-CL) scaffolds. Such blended scaffold especially with the ratio of 20:5:75 thus has the potential for vascular graft applications. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 101A: 1292–1301, 2013.

Key Words: electrospinning, collagen/chitosan/P(LLA-CL) blend, mechanical properties, biocompatibility, vascular graft

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INTRODUCTION

Over the past 50+ years, Dacron™ and expanded-polytetraflouroethylene (ePTFE) vascular grafts used as large diameters replacements or bypass grafts have been applied successfully. However, their use as small diameter vascular grafts (inner diameter < 5 mm) remain a challenge clinically due to acute thrombus formation and chronic hyperplasia brought about by the poor mechanical properties and compliance mismatch.[1] However, tissue engineering offers a potential "ideal" small diameter vascular graft with one concept creating biodegradable polymeric structures that would be used to provide mechanical support and continuous blood supply while promoting vascular tissue development *in situ*.

Electrospinning is a versatile technique, which can generate fibers with diameter ranging from nanometer to microns. The electrospun fibrous structures possess a large surface area to volume ratio, high porosity, and potential for mimicking the structure and function of natural extracellular matrix (ECM).[2] It has been shown that electrospun scaffolds can promote cell attachment, spreading and proliferation which could potentially enhance tissue regeneration.[3,4] Recently, a variety of biodegradable polymers have been electrospun to fabricate tissue engineering scaffolds including collagen,[5,6] collagen/elastin,[7] silk fibroin,[8] chitosan,[9] and synthetic polymers including poly (lactic acid) (PLA),[10,11] poly(glycolic acid) (PGA),[12,13] polycaprolactone

Correspondence to: Xiumei Mo; e-mail: xmm@dhu.edu.cn and G. L. Bowlin; e-mail: glbowlin@vcu.edu
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(PCL),[14] polydioxanone,[15,16] and poly(L-lactic acid-*co*-ε-caprolactone) (P(LLA-CL)).[17,18] Collagen is one of the most abundant ECM proteins in mammals. It has been reported that collagen has been utilized as a biomaterial for a breadth of medical devices and tissue engineered scaffolds.[19] Chitosan, derived from chitin, is an abundant polysaccharide that may be used to replace glycosaminoglycan. Because of the good biocompatibility and biodegradability, both collagen and chitosan have been applied in the field of tissue engineering. Although previous studies have shown that collagen/chitosan scaffolds demonstrate good cell viability,[20–22] the insufficient mechanical properties were not conducive for use as a vascular scaffold. P(LLA-CL) is a biodegradable copolymer of L-lactic acid and ε-caprolactone possessing good mechanical properties. Hence, P(LLA-CL) is an excellent candidate for use as a vascular scaffold. However, P(LLA-CL) is a synthetic material, which means it lacks the natural integrin binding domains for proper cellular interactions.[23] Therefore, the hypothesis that electrospinning of a collagen/chitosan/P(LLA-CL) blended structure will possess cellular compatibility as well as mechanical properties for application as a vascular tissue engineering scaffold.

The utilization of a synthetic vascular graft was first reported by Dr. Voorhees and associates where they reported the successful clinical application of arterial prostheses fabricated with Vinyon-N cloth tubes.[24] Following that work, Dr. Wesolowski's research utilizing a partially resorbable vascular graft illustrated the potential for the design of a completely bioresorbable vascular graft. Overall from these studies, it was considered that a vascular graft structure should possess high porosity, as well as be a temporary vascular scaffold composed of slowly absorbable polymeric materials capable of promoting vascular regeneration.[25] Since these early demonstrations, a fair amount of effort has been placed in developing an ideal, bioresorbable vascular graft. In one such example, Stock et al. utilized poly(glycolic acid) and polyhydroxyoctanoates to fabricate vascular grafts and got successful application.[26] Regardless of composition or indication for use, building an ideal vascular graft requires not only biodegradability, it should including mechanical properties, biocompatibility, also must stimulate cell attachment, proliferation to allow for the proper vascular remodeling and regeneration.

The objective of this study was to generate a tissue engineered vascular graft (TEVG) that possess enough strength to withstand arterial pressure[27] yet be elastic to match the compliance of native blood vessels.[28] Additionally, biological properties will be evaluated in terms of endothelial cell adhesion and proliferation to examine the regenerative capacity. A successful TEVG must support endothelial cell growth and development of a neointima on the graft luminal surface to reduce or eliminate the failure upon clinical use. Thus, the focus of this study was characterizing the mechanical properties and cell compatibility of the vascular scaffolds composed of different ratios of collagen/chitosan/P(LLA-CL). Specifically, the scaffolds were characterized in terms of tensile properties, burst pressure, dynamic compliance, and endothelial cell proliferation.

MATERIALS AND METHODS

Materials

Collagen type I was purchased from Sichuan Minrang Biotechnology (molecular weight $\sim 10^5$ Da). Chitosan was purchased from Jinan Haidebei Marine Bioengineering (85% deacetylated, molecular weight $\sim 10^6$). The P(LLA-CL) (50:50), which has a composition of 50 mol % L-lactide, was supplied by Gunze Limited (Japan, molecular weight $\sim 3 \times 10^5$). The 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) from Fluorochem (United Kingdom) and 2,2,2-trifluoroacetic acid (TFA) from Sinopharm Chemical Reagent (China) were used to dissolve the collagen, chitosan, P(LLA-CL) and their blends. A crosslinking agent of aqueous glutaraldehyde (GA) solution (25%) was purchased from Sinopharm Chemical Reagent (China). Porcine iliac artery endothelial cells (PIECs) were obtained from the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, China). Except specially explained, all cell culture media and reagents were purchased from Gibco Life Technologies (China).

Electrospinning

Collagen and P(LLA-CL) were dissolved in HFP, while chitosan was dissolved in HFP and TFA (v/v, 9:1) all at weight concentration of 8% and stirred (30RPM) at room temperature for 6 h. Before electrospinning, the three solutions were blended at different volume ratios of collagen/chitosan/P(LLA-CL). The solutions were placed into a 2.5-mL plastic syringe with a 21-gauge blunt-end needle. The syringe was located on a syringe pump (789100C, Cole-Pamer, Chicago, IL) and dispensed at a rate of 1.0 mL h^{-1}. A voltage of +14 kV using a high voltage power supply (BGG6-358, BMEICO, China) was applied to the needle opposite the grounded plate of aluminum foil collector (4 cm $\times$ 4 cm), which was placed at a distance of 12–15 cm. To fabricate the tubular scaffolds, the collector was a 3–4 mm in diameter, solid stainless mandrel. The deposited fibrous sheet or conduit was dried in a vacuum oven at room temperature for several days to removal any residual solvents.

Fiber characterization

The morphology of the fibers was observed utilizing a scanning electronic microscope (SEM) (JEOL JSM-5600, Japan) at an accelerated voltage of 10 kV. Samples were dried under vacuum and sputtered coated with gold. The mean fiber diameters were determined using image analysis software (Image-J, National Institutes of Health) and calculated by selecting 100 fibers randomly.

Pore size measurements

A CFP-1100-AI capillary flow porometer (PMI Porous Materials, USA) was used to measure the scaffold pore size (3 cm $\times$ 3 cm square samples).[29] Galwick with a defined surface tension of 21 dynes cm^{-1} (PMI Porous Materials, USA) was used as the wetting agent for porometry.

Crosslinking

The crosslinking process was carried out by placing the collagen/chitosan/P(LLA-CL) scaffolds in a sealed, dual-layered

desiccators. The round glass plate frame with some pores divides the desiccators into two layers. About 10 mL of 25% GA solution in a Petri dish was placed inside the bottom layer of the desiccators. The electrospun structures were fixed on a glass frame and were crosslinked in an atmosphere of GA vapor at room temperature for 24 h. After crosslinking, the samples were placed in the vacuum oven at room temperature for 1 week.

Contact angle measurements

Surface hydrophilicity of the electrospun fibrous structures was characterized by water contact angle measurement. The images of the droplet were visualized through the image analyzer (OCA40, Dataphysics, Germany) and the angles between the water droplet and the material surface were measured. The measurement used distilled water as the reference liquid and was automatically dropped onto the structures. To confirm the accuracy, the contact angle was measured three times from different positions and averaged.

Tensile testing

Mechanical properties were obtained by applying tensile loads to specimens prepared from the electrospun collagen/chitosan/P(LLA-CL) scaffolds (0:0:100, 20:5: 75, 40:10:50, 60:15:25,80:20: 0). For mechanical testing, five specimens ((30 mm $\times$ 10 mm, $n = 6$) were prepared according to the method described by Huang et al.[30] Mechanical properties were determined by a universal materials testing machine (H5K-S, Hounsfield, England) at ambient temperature and a relative humidity of 65% with an elongation speed of 10 mm min^{-1}. Before testing, all samples were soaked in PBS for 2 h. Ultimate tensile stress, elastic modulus, and strain at break were determined.

Burst pressure

Burst strength testing of electrospun scaffolds was completed using six different ($n = 6$) grafts and a device designed in accordance with section 8.3.3.3 of ANSI/AAMI VP20:1994.[31,32] Tubes, 4 cm in length, were hydrated in PBS for 12 h, fitted over 2.5 mm diameter nipples attached to the device, a thin latex balloon (Party Like Crazy, Target) was inserted, and the balloon/scaffold was secured with 2-0 silk suture to the nipples. Pressurized air was introduced into the system, increasing the pressure at a rate of 5 mmHg s^{-1} until the tubes ruptured. Results were recorded as the pressure (mmHg) at which the structures ruptured.

Dynamic compliance

Dynamic compliance was determined for 2.5 mm inner diameter tubular grafts taken from six different electrospun grafts ($n = 6$) at a length of 4 cm under simulated physiological conditions in accordance with section 8.10 of ANSI/AAMI VP20:1994.[32,33] The specimens were tested in an intelligent tissue engineering via mechanical stimulation (ITEMS^{TM}) Bioreactor developed by tissue growth technologies filled with PBS at 25°C. The bioreactor provided a cyclic (1 Hz, representing 60 beats per minute) pressure change to the inside of the graft at a pressure level of 120/80 mmHg systolic/diastolic. Grafts were soaked in PBS at 25°C for 12 h before testing. Briefly, specimens were secured at either end to a nipple with 3-0 silk suture and placed in the bioreactor chamber. PBS then filled the chamber and was run continuously on the outside of the graft to maintain a temperature of 25°C and a pressure of 0 mmHg. Simultaneously, PBS was pumped through the inside of the graft and an actuator in the bioreactor created the difference in pressure. Prior to compliance measurements, all grafts were allowed to stress relax for 600 cycles. Internal pressure was measured with a pressure transducer capable of measuring dynamic pressure up to 200 $\pm$ 2 mmHg, while the external diameter of the graft was recorded with a laser micrometer system with an accuracy of $\pm$0.001 mm. Compliance was calculated from recording of pressure and inner diameter as:

$$\%\text{Compliance} = \frac{R_{P_2} - R_{P_1}}{R_{P_1}} \frac{1}{P_2 - P_1} \times 10^4 \quad (1)$$

while R is the internal radius, p_1 is the lower internal pressure, and p_2 is the higher internal pressure.[32]

Cell adhesion and proliferation

PIECs were cultured in DMEM medium with 10% fetal bovine serum and 1% antibiotic-antimycotic (100 U mL^{-1} Penicillin and 100 μg mL^{-1} streptomycin) in an atmosphere of 5% CO_2 and 37°C with the medium replenished every 3 days. Electrospun scaffolds were prepared on circular glass cover slips (14 mm in diameter) and the cover slips fixed into 24-well plates with stainless rings. Before seeding the cells, scaffolds and control (cover slips) were disinfected by immersion in 75% ethanol for 2 h, washed three times with phosphate-buffered saline solution (PBS), and then washed once again with the culture medium.

Cell proliferation on electrospun scaffolds was determined by the standard MTT assay ($n = 3$). After 1, 3, and 7 days postseeding, the cells and matrices were incubated with 5 mg mL^{-1} 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) for 4 h. Thereafter, the culture media were extracted and 400 μL dimethylsulfoxide (DMSO) was added for 20 min. When the crystal was sufficiently dissolved, aliquots were pipetted into the well of a 96-well plate and tested by a microtiter plate reader (Multiskan MK3, Thermo, USA), at an absorbance of 492 nm.

For cell adhesion and morphology analysis, PIECs were seeded onto scaffolds ($n = 2$) at a density of 10^4 cells $well^{-1}$ for 3 days. After 3 days, the electrospun scaffolds and the adhered cells were examined. The scaffolds were rinsed twice with PBS and fixed in 4% GA solution at 4°C for 2 h. Fixed samples were rinsed twice with PBS and then dehydrated in graded concentrations of ethanol (30, 50, 70, 80, 90, 95, and 100%). Finally, they were dried under vacuum overnight. The samples were then gold sputter coated and observed under the SEM at a voltage of 10 kV.

Confocal laser microscopy imaging was used to visualize cell distribution and morphology within the scaffolds. The cell-seeded scaffolds were fixed in 4% paraformaldehyde for 30 min and then washed and permeabilized in 0.1% Triton

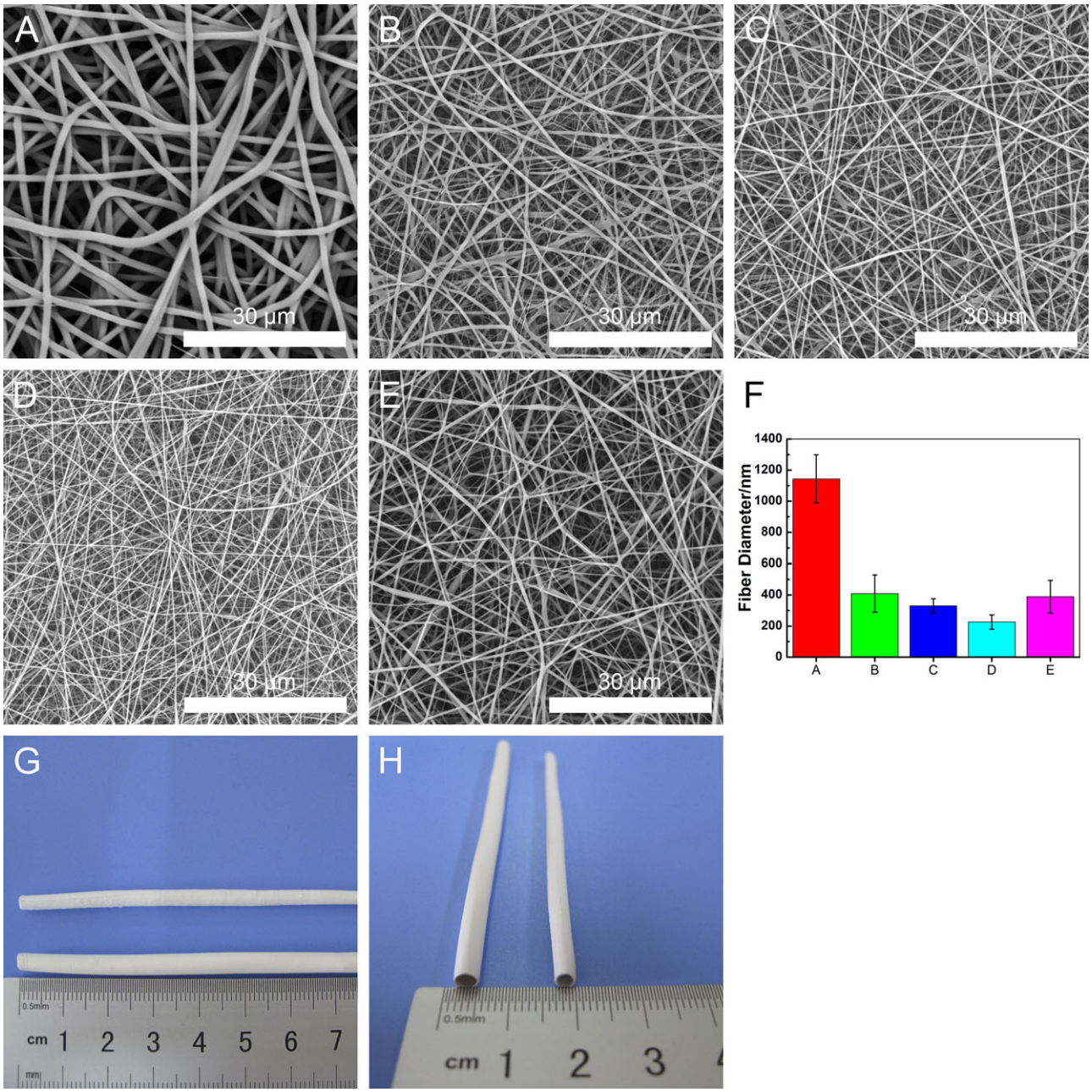

FIGURE 1. Representative morphological assessment of collagen/chitosan/P(LLA-CL) scaffold with different ratios(A) 0:0:100, (B) 20:5:75, (C) 40:10:50, (D) 60:15:25, (E) 80:20:0, (F) fiber diameter distributions of scaffolds, and (G,H) images of small diameter electrospun tubes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

X-100 (Sigma, USA) for 5 min. Rhodamine-conjugated phalloidin (Invitrogen, USA) was used to stain cell actin filaments into red. Images were captured using a confocal laser scanning microscope (Zeiss LSM 700, Germany).

Statistical analysis

All the data were obtained at least in triplicate and expressed as means ± standard deviation (SD). One-way ANOVA at a significance of $p < 0.05$ and $p < 0.01$ were performed using Origin 8.0 (OriginLab, USA).

RESULTS AND DISCUSSION

Morphology and pore diameter

The morphology of different ratios of collagen/chitosan/P(LLA-CL) fibers were observed by SEM. From Figure 1, collagen/chitosan/P(LLA-CL) scaffolds appeared macroscopically smooth and without any gross defects. From the micrographs, it is clear that the ratio of collagen/chitosan/P(LLA-CL) significantly affected the fiber diameter distributions [Fig. 1(A–E)]. The collagen/chitosan/P(LLA-CL) fiber diameter of the different ratios are shown in the Figure 1(F). Fiber average diameters gradually deceased

TABLE I. Fiber Diameters and Pore Size of Electrospun Structures

Collagen/Chitosan/ P(LLA-CL)	Mean Pore Diameter ± SD (μm)	Mean Fiber Diameter ± SD (nm)
0:0:100	2.2 ± 0.7	1144 ± 155
20:5:75	1.1 ± 0.5	409 ± 120
40:10:50	0.8 ± 0.4	330 ± 46
60:15:25	0.6 ± 0.1	226 ± 46
80:20:0	0.7 ± 0.1	389 ± 105

TABLE II. Water Contact Angle of Fibrous Structures at Different Conditions

Collagen/Chitosan/ P(LLA-CL)	Contact Angle, Degrees ($n = 6$)	
	Untreated	Crosslinked
0:0;100	136.1 ± 1.3	134.1 ± 0.5
20:5:75	110.5 ± 0.9	111.7 ± 1.5
40:10:50	105.1 ± 2.7	111.2 ± 1.2
60:15:25	99.3 ± 1.3	102.6 ± 2.3
80:20:0	79.6 ± 3.8	92.5 ± 1.9

from 1144 ± 155 nm to 227 ± 47 nm with increasing collagen and chitosan content (Fig. 1, Table I). This phenomenon could be explained by the conductivity increase of the electrospinning solution with increasing collagen and chitosan content.[34] As shown in Figure 1(G,H), we can fabricate a fibrous tube with inner diameter of 2.5 mm, length of ~9 cm, and wall thickness of ~300 μm.

Electrospun scaffolds with microscale porous structures are most favorable for tissue engineering scaffolds because they are a network of interconnected pores that provides nutrients and gas exchange and cellular infiltration, which are crucial for cell/tissue viability and tissue regeneration.[35] Pore diameters of the collagen/chitosan/P(LLA-CL) scaffolds with various blend ratios are shown in Table I and Figure 2. When blended ratios ranged from 0:0:100 to 80:20:0 mean pore diameter decreased with increasing the content of collagen/chitosan. According to a published report,[36] as expected the fiber diameter increased, the average pore size of the scaffolds increased. With increasing the content of collagen/chitosan, the fiber diameter decreased from microscale to nanoscale, therefore, the pore diameter decreased. As expected that cells infiltrated the scaffolds with lager pore diameters. The nanofibrous scaffold with small pore diameter exhibited reduced cellular infiltration, however, previous research demonstrated that the nanofibers could physical mimic the native ECM and promote cell more spreading.[36]

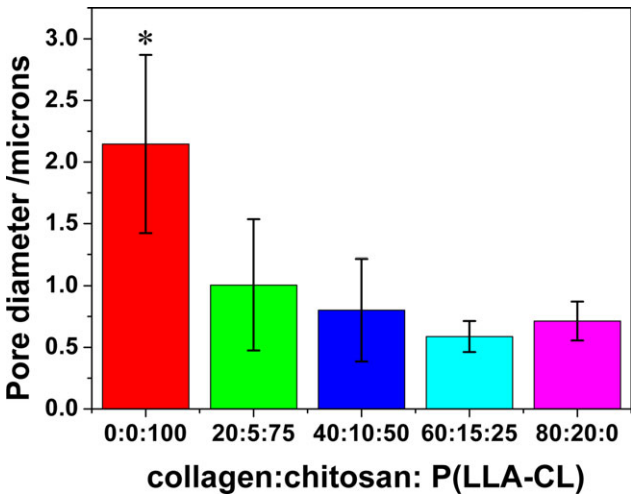

FIGURE 2. Pore diameter of the different structures. *indicates a significant difference from other scaffolds ($p < 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Contact angle

The scaffold's surface hydrophilicity will affect the attachment, proliferation, migration, and viability of many different cells.[37–39] Water contact angles were measured at two different conditions (before and after crosslinking) and shown in Table II. The P(LLA-CL) scaffolds had an average contact angle of 136.1° which indicates that P(LLA-CL) was hydrophobic. With the increasing content of collagen and chitosan, the contact angles of the blend scaffolds decreased from 110.5° to 79.6° indicating that the hydrophobic P(LLA-CL) scaffolds could be transformed to a more hydrophilic state by the introduction of the collagen and chitosan components. While compared to untreated scaffolds, the contact angle of the scaffolds after crosslinking just have a slight change that means GA solution have no significant affect on the contact angle of the scaffolds.

Tensile testing

Tensile tests were conducted on all scaffolds to determine whether the properties were conducive for use as a vascular graft. Results are showed in Figure 3 and Table III. Figure 3(A) shows stress–strain curves of scaffolds under tensile loading. All scaffolds showed onset of nonlinearity in the initial stress–strain curve. However, the scaffold of P(LLA-CL) is significantly different from 20:5:75, 40:10:50, and 60:15:25. As showed in the Figure 3(A) for P(LLA-CL), the slope of the curve decreased after the onset of nonlinearity. Compared to P(LLA-CL), scaffolds of 20:5:75, 40:10:50, and 60:15:25 have the opposite tendency, the slope of the curves increased after the onset of nonlinearity until scaffold fracture. After adding some content of P(LLA-CL), the scaffolds were significantly stronger than collagen-chitosan blended scaffold (80:20:0) [Fig. 3(B)], especially when the ratio is 20:5:75 where the peak tensile stress reached a value of 16.9 ± 2.9 MPa. As a comparison, the adventitial layer of the coronary arteries has an ultimate tensile stress of about 1.4 ± 0.6 MPa.[40] Therefore, the mechanical properties of a collagen-chitosan scaffold is too weak to match that of native artery, thus adding some content of P(LLA-CL), the elastic tensile of blend scaffold will modified.[41,42] In the meanwhile, the ultimate strain has the similar tendency that increasing the content of P(LLA-CL)

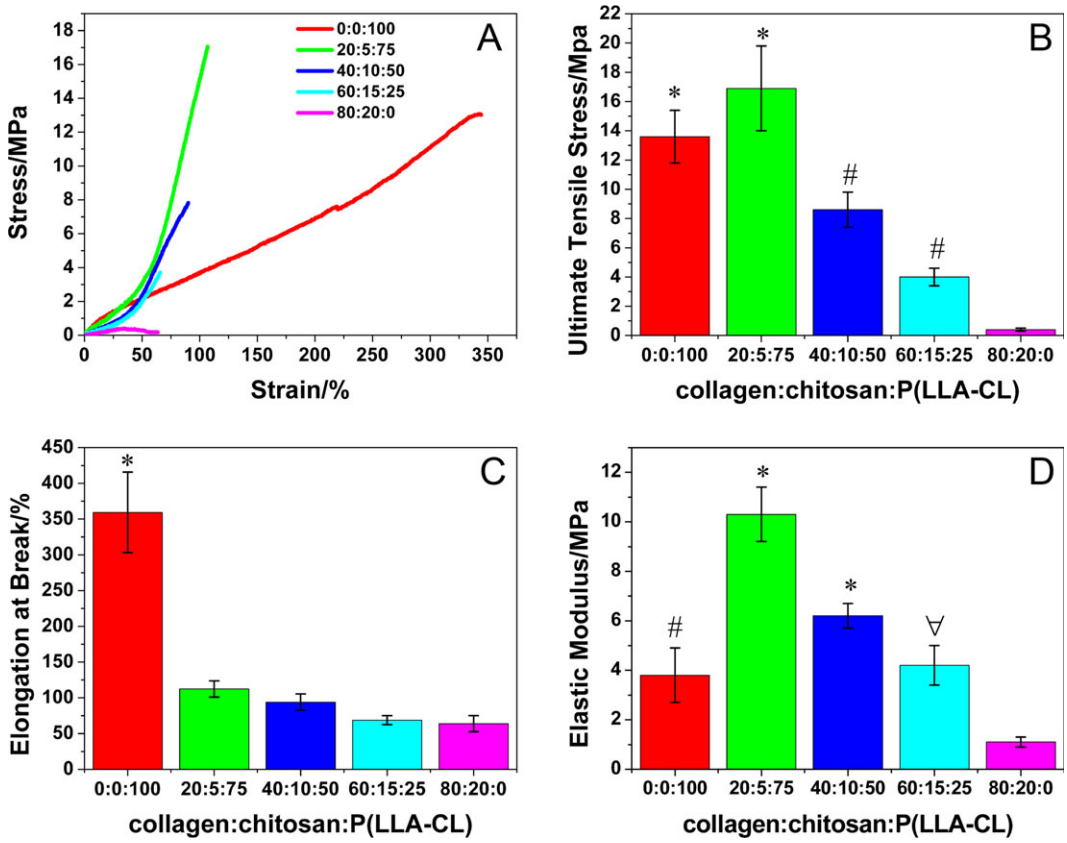

FIGURE 3. Comparison of mechanical properties of scaffolds with different ratios of collagen:chitosan:P(LLA-CL). (A) The stress–strain curves of scaffolds. (B) Tensile stress of scaffolds. *indicates a significant difference from 40:10:50, 60:15:25, and 80:20:0 ($p < 0.01$), #indicates a significant different from others ($p < 0.01$) (C) Elongation at break. *indicates a significant difference from other scaffolds ($p < 0.01$). (D) Modulus. *indicates a significant difference from other scaffolds ($p < 0.01$), #indicates a significant different from other scaffold except 60:15:25 ($p < 0.01$), ∀ indicates a significant different from other scaffold except 0:0:100 ($p < 0.01$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the strain of scaffold increased [Fig. 3(C)]. When the ratio of collagen/chitosan/P(LLA-CL) is 20:5:75, the ultimate strain of the scaffold was 112%. With increasing collagen and chitosan content, the scaffold of 40:10:50 could stretch to 94%, 60:15:25 and 80:20:0 have strain of 69 and 64%, respectively. Over all, the ultimate strain of these scaffolds was comparable to the human coronary artery (45–99%).[41,43] In terms of elastic modulus, all the scaffolds except 80:20:0 were higher than radial arteries (2.68 ± 1.81 MPa)[42] as showed in Table III and Figure 3(D), Scaffold of 0:0:100, 20:5:75, 40:10:50, 60:15:25, and 80:20:0 have modulus values of 3.8, 10.3 6.2, 4.2, and 1.1 MPa, respectively. Of particular note is that collagen/chitosan/P(LLA-CL) blended scaffolds with high stress and high strain also have high elastic modulus.

TABLE III. Mechanical Properties of Scaffolds

Specimen	Ultimate Stress (MPa)	Elongation at Break (%)	Elastic Modulus (MPa)
0:0:100	13.6 ± 1.8	359 ± 56	3.8 ± 1.1
20:5:75	16.9 ± 2.9	112 ± 11	10.3 ± 1.1
40:10:50	8.6 ± 1.2	94 ± 11	6.2 ± 0.5
60:15:25	4.0 ± 0.6	69 ± 6	4.2 ± 0.8
80:20:0	0.4 ± 0.1	64 ± 11	1.1 ± 0.2

Burst pressure

The burst pressure of a vascular scaffold is one of the most important parameters which determine the suitability of its use as a vascular graft for implantation.[44] For this study, all the samples were soaked in PBS for 12 h before testing, and the thickness of specimens were measured (ranged from 0.24 to 0.33 mm). According to the burst pressure results shown in Figure 4 and Table IV, the average values for the collagen/chitosan/P(LLA-CL) blended scaffolds was significantly different ($p < 0.01$) from P(LLA-CL). Pure P(LLA-CL) can resist an average of 1403 ± 210 mmHg of burst pressure. The P(LLA-CL) blended with collagen-chitosan scaffolds can resist burst pressure greater than 3320 ± 72 mmHg (40:10:50). It should be noted that the 20:5:75 grafts did not even burst when the pressure reached the systems maximum of 3365 mmHg. However, with higher concentrations of collagen and chitosan, the burst pressure decreased significantly to 432 ± 24 mmHg and even lower as the content increased to a point (80:20:0) where the grafts were ruptured after soaking in PBS for 12 h. It has

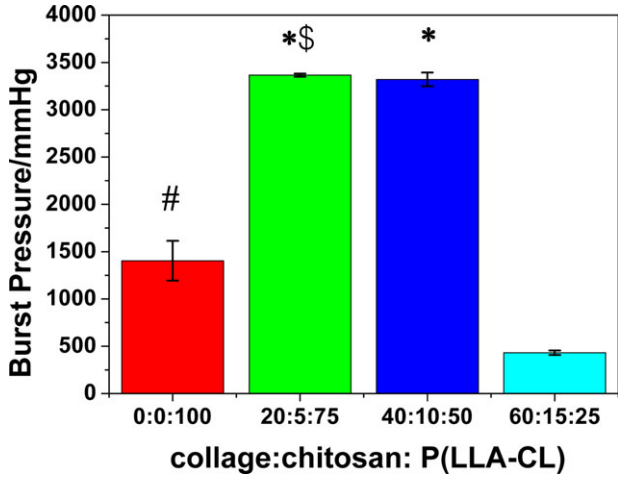

FIGURE 4. Burst pressure of scaffolds with different collagen:chitosan:P(LLA-CL) ratios. *indicates a significant difference from 0:0:100 and 60:15:25 ($p < 0.01$), #indicates a significant different from 60:15:25 ($p < 0.01$), *indicates the scaffolds have not burst under the maximum pressure achieved by the system. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

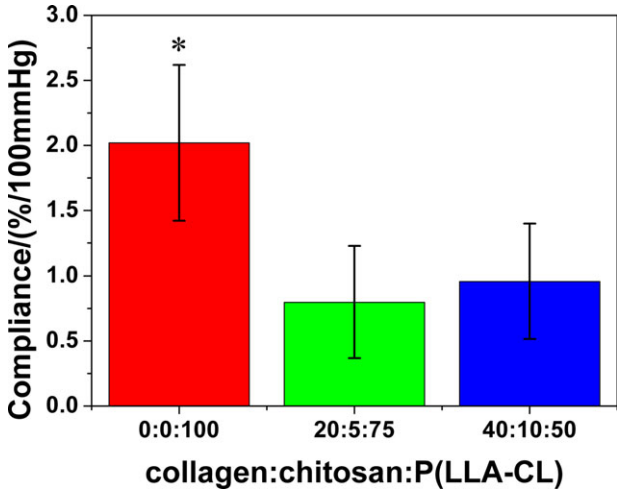

FIGURE 5. Compliance results for the different collagen:chitosan:P(LLA-CL) scaffolds. *indicates a significant difference from 20:5:75 and 40:10:50 ($p < 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

been reported that saphenous vein and mammary artery have burst pressure values of 1680–2273 and 2031–4225 mmHg, respectively.[44,45] Therefore, the scaffold of 20:5:75 can resist burst pressure over 3365 mmHg, illustrating potential use as an artery bypass graft.

Dynamic complicance

Results from Figure 5 and Table IV showed compliance values with a range from 0.7 to 2.0%/100 mmHg, where 0:0:100 had an average value of 2.0; 20:5:75 had an average of 0.7 and 40:10:50 had an average of 0.8%/100 mmHg. While 60:15:25 had also been measured, unfortunately the grafts were ruptured before reaching 600 cycles. The 0:0:100 grafts were significantly higher ($p < 0.05$) than other scaffolds. Because P(LLA-CL) is an elastic polymer, the compliance values of the P(LLA-CL) grafts approached that of native tissue (2.6%/100 mmHg).[46] According to a prior study, 20:5:75 graft produced similar compliance values as saphenous vein (0.7–1.5%/100 mmHg).[45,47,48] In addition, the grafts possess higher compliance values than standard ePTFE grafts (0.1%/100 mmHg).[49]

Cellular analysis

Scaffolding for tissue engineering was typically designed to promote cell growth, physiological functions, and maintain normal states of cell differentiation.[50] To evaluate cell proliferation and adhesion on collagen/chitosan/P(LLA-CL) blended scaffolds, PIECs were seeded on the scaffolds. The proliferation of PIECs on days 1, 3, and 7 after seeding on the various scaffolds is shown in Figure 6. All the scaffolds were conducive to cell proliferation in comparison with cover slips (control), the cell proliferation values of 20:5:75, 40:10:50, and 60:15:25 grafts were significant difference ($p < 0.05$) from control at day 1. On day 3, cell proliferation on 40:10:50 and 60:15:25 scaffolds exhibited a significant increase ($p < 0.05$) compared to cover slip. On day 7, cell proliferation on 60:15:25 scaffolds was significantly greater ($p < 0.05$) than cover slip. Meanwhile, cell proliferation on 40:10:50 and 60:15:25 scaffolds were significant different ($p < 0.05$) when comparing to P(LLA-CL). The results showed that blended scaffolds could promote increased cell growth and

TABLE IV. Burst Pressure and Compliance of the Tubular Scaffolds

Specimen	Burst Press (mmHg)	Compliance (%/100 mmHg)	Wall Thickness (mm)
0:0:100	1403 ± 210	2.0 ± 0.6	0.24 ± 0.08
20:5:75	>3365 ± 6	0.7 ± 0.4	0.33 ± 0.09
40:10:50	3320 ± 72	0.8 ± 0.4	0.31 ± 0.07
60:15:25	431 ± 23	–	0.33 ± 0.06

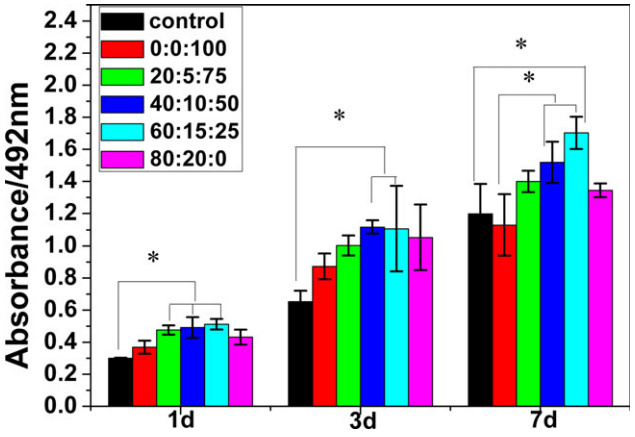

FIGURE 6. Proliferation of PIECs cultured on collagen:chitosan:P(L-LACL) scaffolds and cover slips for 1, 3, 7 days. Statistical difference between groups is indicated (*$p < 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

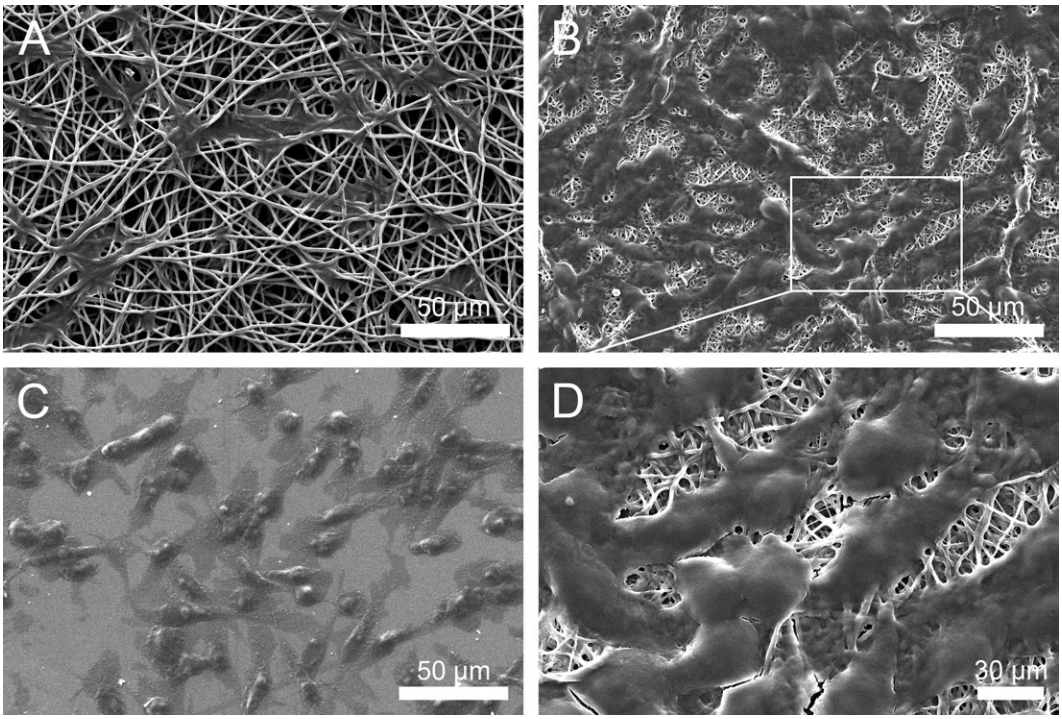

FIGURE 7. SEM micrographs of PIECs grown on scaffolds for 3 days. (A) P(LLA-CL); (B) Collagen:chitosan:P(LLA-CL) (60:15:25); (C) Cover slips; (D) is the high-resolution of an area squared in B.

proliferation in comparison with P(LLA-CL). From Figure 6, the result showed the scaffold of 40:10:50 and 60:15:25 provided more suitable cell growth condition for PIECs compare to control and P(LLA-CL). This is probably caused by the introduction of biological functional groups via collagen and chitosan in collagen/chitosan/P(LLA-CL) that enhanced the proliferation rate of PIECs. Also, the addition of collagen and chitosan improved the fibers surface wettability. Meanwhile, previous research demonstrated that the nanofibers could physical mimic the native ECM and promote cell more spreading.[36]

Cell morphology and the interaction between cells and scaffolds were studied *in vitro* for 3 days. SEM micrographs and Confocal laser microsgraphs were shown in Figures 7 and 8. After 3 days, PEICs more easily spread to develop an endothelial cell layer on the surface of 60:15:25 scaffolds compared to the control and PLLA-CL scaffolds. That is because nanofibers structures have been shown to enhance cell spreading as nanofibers mimic the native ECM.[36]

CONCLUSION

In this study, we developed fibrous small diameter vascular scaffolds with collagen, chitosan and P(LLA-CL) blended in different ratios. Grafts were assessed via mechanical properties and cell compatibility to evaluate effectiveness as

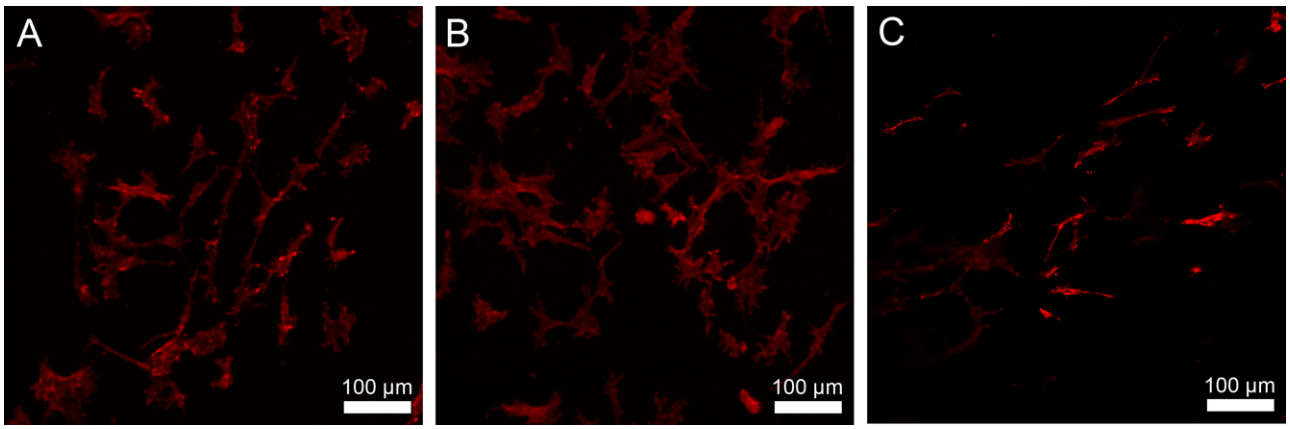

FIGURE 8. Confocal laser micrographs of PIECs grown on scaffolds for 3 days. (A) P(LLA-CL); (B) Collagen:chitosan:P(LLA-CL) (60:15:25); (C) Cover slips. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

prospective vascular grafts. The mechanical properties of scaffolds in terms of their tensile strength, burst pressure, and compliance were studied. Results showed that the graft with the ratio of 20:5:75 exhibited similar tensile strength, burst pressure and compliance properties as the saphenous vein. Meanwhile, we evaluated the cell compatibility of grafts by assessing the surface wettability and cell proliferation. The hydrophobicity of the P(LLA-CL) scaffolds could be transformed to hydrophilic by introducing collagen and chitosan. Thus, cell proliferation assays showed that cell proliferate was enhanced on the collagen/chitosan/P(LLA-CL) blended scaffolds as compared to P(LLA-CL) scaffolds. In the meantime, PEICs spread more easily to develop an endothelial cell layer on the surface of collagen/chitosan/P(LLA-CL) blended scaffolds. Overall, to balance the mechanical properties and biocompatibility, the results indicate that the collagen/chitosan/P(LLACL) scaffold with the ratio of 20:5:75 has potential application in vascular tissue engineering.

REFERENCES

1. Isenberg BC, Williams C, Tranquillo RT. Small-diameter artificial arteries engineered in vitro. Circ Res 2006;98:25–35.
2. Langer R, Vacanti JP. Tissue engineering. Science 1993;260: 920–926.
3. Sahoo S, Ang LT, Goh JC, Toh SL. Growth factor delivery through electrospun nanofibers in scaffolds for tissue engineering applications. J Biomed Mater Res 2010;93A:1539–1550.
4. Zhang YZ, Venugopal JR, El-Turki A, Ramakrishna S, Su B, Lim CT. Electrospun biomimetic nanocomposite nanofibers of hydroxyapatite/chitosan for bone tissue engineering. Biomaterials 2008;29:4314–4322.
5. Bessa PC, Casal M, Reis RL. Bone morphogenetic proteins in tissue engineering: The road from laboratory to clinic, part II (BMP delivery). J Tissue Eng Regen Med 2008;2:81–96.
6. Matthews JA, Simpson DG, Wnek GE, Bowlin GL. Electrospinning of collagen nanofibers. Biomacromolecules 2002;3:232–238.
7. Boland ED, Matthews JA, Pawlowski KJ, Simpson DG, Wnek GE, Bowlin GL. Electrospinning Collagen and Elastin: Preliminary vascular tissue engineering. Front Biosci 2004;9:1422–1432.
8. Ayutsede J, Gandhi M, Sukigara S, Ye H, Hsu CM, Gogotsi Y, Ko F. Carbon nanotube reinforced bombyx mori silk nanofibers by the electrospinning process. Biomacromolecules 2006;7:208–214.
9. Bhattarai N, Edmondson D, Veiseh O, Matsen FA, Zhang MQ. Electrospun chitosan-based nanofibers and their cellular compatibility. Biomaterials 2005;6:6176–6184.
10. Zeng J, Xu XY, Chen XS, Liang QZ, Bian XC, Yang LX, Jing XB. Biodegradable electrospun fibers for drug delivery. J Controlled Release 2003;92:227–231.
11. Yokota T, Ichikawa H, Matsumiya G, Kuratani T, Sakaguchi T, Iwai S, Shirakawa Y, Torikai K, Saito A, Uchimura E, Kawaguchi N, Matsuura N, Sawa Y. In situ tissue regeneration using a novel tissue-engineered, small-caliber vascular graft without cell seeding. J Thorac Cardiovasc Surg 2008;136:900–907.
12. Pullens RA, Stekelenburg M, Baaijens FP, Post MJ. The influence of endothelial cells on the ECM composition of 3D engineered cardiovascular constructs. J Tissue Eng Regen Med 2009;3:11–18.
13. Lim SH, Cho SW, Park JC, Jeon O, Lim JM, Kim SS, Kim BS. Tissue-engineered blood vessels with endothelial nitric oxide synthase activity. J Biomed Mater Res B Appl Biomater 2008;85B: 537–546.
14. Tillman BW, Yazdani SK, Lee SJ, Geary RL, Atala A, Yoo J. The in vivo stability of electrospun polycaprolactone-collagen scaffolds in vascular reconstruction. Biomaterials 2009;30:583–588.
15. Boland ED, Coleman BD, Barnes CP, Simpson DG, Wnek GE, Bowlin GL. Electrospinning polydioxanone for biomedical applications. Acta Biomater 2005;1:115–123.
16. Smith MJ, McClure MJ, Sell SA, Barnes CP, Walpoth BH, Simpson DG, Bowlin GL. Suture-reinforced electrospun polydioxanone–elastin small-diameter tubes for use in vascular tissue engineering: A feasibility study. Acta Biomater 2008;4:58–66.
17. Chen F, Li XQ, Mo XM, He CL, Wang HS, Ikada Y. Electrospun chitosan-P(LLA-CL) nanofibers for biomimetic extracellular matrix. J Biomater Sci Polym Edn 2008;9:677–691.
18. Mo XM, Xu CY, Kotaki M, Ramakrishna S. Electrospun P(LLA-CL) nanofiber: A biomimetic extracellular matrix for smooth muscle cell and endothelial cell proliferation. Biomaterials 2004;25: 1883–1890.
19. Di Lullo GA, Sweeney SM, Korkko J, Ala-Kokko L, San Antonio JD. Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type I collagen. J Biol Chem 2002;277:4223–4231.
20. Chen ZG, Wang PW, Wei B, Mo XM, Cui FZ. Electrospun collagen–chitosan nanofiber: A biomimetic extracellular matrix for endothelial cell and smooth muscle cell. Acta Biomater 2010;6: 372–382.
21. Chen R, Huang C, Ke QF, He CL, Wang HS, Mo XM. Preparation and characterization of coaxial electrospun thermoplastic polyurethane/collagen compound nanofibers for tissue engineering applications. Colloids Surf B Biointerfaces 2010;79: 315–325.
22. Sionkowska A, Wisniewski M, Skopinska J, Kennedy CJ, Wess TJ. Molecular interactions in collagen and chitosan blends. Biomaterials 2004;25:795–801.
23. Kim BS, Mooney DJ. Development of biocompatible synthetic extracellular matrices for tissue engineering. Trends Biotechnol 1998;16:224–230.
24. Fox D, Vorp DA, Greisler HP. Tissue Engineering of Vascular Prosthetic Grafts. Cape Town, South Africa: Landes Company; 1999. 489 p.
25. Wesolowski SA, Fries CC, Domingo RT, Liebig WJ, Sawyer PN. The compound prosthetic vascular graft: A pathologic survey. Surgery 1963;53:19–44.
26. Stock UA, Nagashima M, Khalil PN, Nollert GD, Herden T, Sperling JS, Moran A, Lien J, Martin DP, Schoen FJ, Vacanti JP, Mayer JE. Tissue-engineered valved conduits in the pulmonary circulation. J Thorac Cardiovasc Surg 2000;119:732–740.
27. Girton TS, Oegema TR, Grassl ED, Isenberg BC, Tranquillo RT. Mechanisms of stiffening and strengthening in media-equivalents fabricated using glycation. J Biomech Eng 2000;122:216–223.
28. Haruguchi H, Teraoka S. Intimal hyperplasia and hemodynamic factors in arterial bypass and arteriovenous grafts: A review. J Artif Organs 2003;6:227–235.
29. Huang C, Chen R, Ke QF, Morsi Y, Zhang KH, Mo XM. Electrospun collagen–chitosan–TPU nanofibrous scaffolds for tissue engineered tubular grafts. Colloids Surf B Biointerfaces 2011;82: 307–315.
30. Huang ZM, Zhang YZ, Ramakrishna S, Lim CT. Electrospinning and mechanical characterization of gelatin nanofibers. Polymer 2004;45:5361–5368.
31. Baker B, Gee A, Metter R, Nathan A, Marklein R, Burdick J, Mauck R. The potential to improve cell infiltration in composite fiber-aligned electrospun scaffolds by the selective removal of sacrificial fibers. Biomaterials 2008;29:2348–2358.
32. McClure MJ, Wolfe PS, Simpson DG, Sell SA, Bowlin GL. The use of air-flow impedance to control fiber deposition patterns during electrospinning. Biomaterials 2012;33:771–779.
33. Association for the Advancement of Medical Instrumentation. Cardiovascular implants- vascular graft prostheses. ANSI/AAMI VP20-94. 1994. ISBN: 1570200254.
34. Zhang KH, Wang HS, Huang C, Su Y, Mo XM, Ikada Y. Fabrication of silk fibroin blended P(LLA-CL) nanofibrous scaffolds for tissue engineering. J Biomed Mater Res 2009;93A:984–993.
35. Murugan R, Ramakrishna S. Nano-featured scaffolds for tissue engineering: A review of spinning methodologies. Tissue Eng 2006; 12:435–447.
36. Pham QP, Sharma U, Mikos AG. Electrospun poly(ε-caprolactone) microfiber and multilayer nanofiber/microfiber scaffolds: Characterization of scaffolds and measurement of cellular infiltration. Biomacromolecules 2006;7:2796–2805.

37. Altankov G, Grinnell F, Groth T. Studies on the biocompatibility of materials: Fibroblast reorganization of substratum-bound fibronectin on surfaces varying in wettability. J Biomed Mater Res 1996;30:385–391.
38. De bartolo L, Morelli S, Bader A, Drioli E. The influence of polymeric membrane surface free energy on cell metabolic functions. J Mater Sci Mater Med 2001;2:959–963.
39. Lampin M, Warocquier C, Legris C, Degrange M, Sigot-Luizard M F. Correlation between substratum roughness and wettability, cell adhesion, and cell migration. J Biomed Mater Res 1997;36: 99–108.
40. Holzapfel GA, Sommer G, Gasser CT, Regitnig P. Determination of layer-specific mechanical properties of human coronary arteries with nonatherosclerotic intimal thickening and related constitutive modeling. Am J Physiol Heart Circ Physiol 2005;289: H2048–H2058.
41. Valenta J. Clinical aspects of biomedicine. In: Jaroslav V, editor. Biomechanics. New York: Elsevier; 1993. p 142–179.
42. Laurent S, Girerd X, Mourad JJ, Lacolley P, Beck L, Boutouyrie P, Mignot JP, Safar M. Elastic modulus of the radial artery wall material is not increased in patients with essential hypertension. Arterioscler Thromb 1994;14:1223–1231.
43. Xu CY, Inai RJ, Kotaki MY, Ramakrishna S. Electrospun nanofiber fabrication as synthetic extracellular matrix and its potential for vascular tissue engineering. Tissue Eng 2004;10:1160–1168.
44. Konig G, McAllister TN, Dusserre N, Garrido SA, Iyican C, Marini A, Fiorillo A, Avila H, Wystrychowski W, Zagalski K, Maruszewski M, Linthurst Jones A, Cierpka L, de la Fuente LM, L'Heureux N. Mechanical properties of completely autologous human tissue engineered blood vessels compared to human saphenous vein and mammary artery. Biomaterials 2009;30:1542–1550.
45. L'Heureux N, Dusserre N, Konig G, Victor B, Keire P, Wight TN, Chronos NAF, Kyles AE, Gregory CR, Hoyt G, Robbins RC, McAllister TN. Human tissue engineered blood vessel for adult arterial revascularization. Nat Med 2006;12:361–365.
46. Tai NR, Salacinski HJ, Edwards A, Hamilton G, Seifalian AM. Compliance properties of conduits used in vascular reconstruction. Br J Surg 2000;87:1516–1524.
47. Dobrin PB. Mechanical behavior of vascular smooth muscle in cylindrical segments of arteries in vitro. Ann Biomed Eng 1984;2: 497–510.
48. Cambria RP, Megerman J, Brewster DC, Warnock DF, Hasson J, Abbott WM. The evolution of morphologic and biomechanical changes in reversed and in situ vein grafts. Ann Surg 1987;205: 167–174.
49. McClure MJ, Sell SA, Simpson DG, Walpoth BH, Bowlin GL. A three-layered electrospun matrix to mimic native arterial architecture using polycaprolactone, elastin, and collagen: A preliminary study. Acta Biomater 2010;6:2422–2433.
50. Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol 2005;23:47–55.