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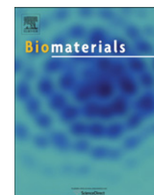

Development of a composite vascular scaffolding system that withstands physiological vascular conditions

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ABSTRACT

Numerous scaffolds that possess ideal characteristics for vascular grafts have been fabricated for clinical use. However, many of these scaffolds may not show consistent properties when they are exposed to physiologic vascular environments that include high pressure and flow, and they may eventually fail due to unexpected rapid degradation and low resistance to shear stress. There is a demand to develop a more durable scaffold that could withstand these conditions until vascular tissue matures *in vivo*. In this study, vascular scaffolds composed of poly(ε-caprolactone) (PCL) and collagen were fabricated by electrospinning. Morphological, biomechanical, and biological properties of these composite scaffolds were examined. The PCL/collagen composite scaffolds, with fiber diameters of approximately 520 nm, possessed appropriate tensile strength (4.0 ± 0.4 MPa) and adequate elasticity (2.7 ± 1.2 MPa). The burst pressure of the composite scaffolds was 4912 ± 155 mmHg, which is much greater than that of the PCL-only scaffolds (914 ± 130 mmHg) and native vessels. The composite scaffolds seeded with bovine endothelial cells (*b*ECs) and smooth muscle cells (*b*SMCs) showed the formation of a confluent layer of *b*ECs on the lumen and *b*SMCs on the outer surface of the scaffold. The PCL/collagen composite scaffolds are biocompatible, possess biomechanical properties that resist high degrees of pressurized flow over long term, and provide a favorable environment that supports the growth of vascular cells.
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1. Introduction

Large numbers of patients suffer from diseases of the vascular system, resulting in a clear clinical need for developing functional arterial replacements [1]. Currently, the use of autologous vascular graft is considered as the standard for small-diameter vessels, however, availability of suitable vessels has been a limiting factor clinically. Although allografts and xenografts may offer long-term patency, their clinical utility is limited by the potential immunogenic response and availability [2]. Synthetic grafts such as woven poly(ethylene terephthalate) (Dacron) and extended polytetrafluoroethylene (ePTFE) have been widely used for peripheral vascular reconstructions. However, these grafts tend to fail when they are applied to small-diameter (<5 mm) vessels such as the coronary artery [3–5].

Tissue engineering technology has emerged as a promising approach to address the shortcomings of current therapies [6]. Vascular tissue engineering attempts to create functional small-diameter grafts by combining cells with a natural and/or synthetic scaffold material under suitable culture conditions, resulting in a tubular construct that can be used *in vivo*. These vascular scaffolds combined with viable cells to allow the graft to remodel when implanted *in vivo* [7–9]. An ideal vascular scaffold should be biocompatible and infection-resistant, have appropriate biomechanical properties, and be readily available in a variety of sizes for grafting applications [10].

Numerous fabrication techniques have been used to produce ideal vascular scaffolds [11–13]. Recently, electrospinning technology has gained much attention because it provides a biomimetic environment with fiber diameters in the nano- to micrometer range and easy processing techniques for fabricating vascular scaffolds [14–17]. We have previously shown that the composition and mechanical properties of electrospun vascular scaffolds can be controlled [14,15]. We show that scaffolds fabricated by electrospinning naturally derived substances, such as collagen and elastin, and synthetic biodegradable polymers, including poly(lactide-*co*-glycolide) (PLGA) and polylactide (PLA), possess adequate characteristics for vessel scaffolds. These composite scaffolds show adequate biocompatibility that supports cell adhesion and growth, and display comparable compliance matching with native blood vessel tissue when tested *in vitro*. Although vascular scaffolds fabricated from PLGA and PLA polymers provide adequate

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biomechanical properties and dimensional stability *in vitro*, testing of these scaffolds under physiological blood pressure and flow conditions is necessary prior to implantation *in vivo*. This statement is based on our preliminary results that showed a total collapse of electrospun vascular scaffolds composed of PLGA and PLA when they were placed under physiologic conditions in a vessel bioreactor system due to low resistance against shear stress. Our previous study mandates that a more durable polymeric material is required to withstand high pressure and flow environments without collapse or degradation until vascular tissue develops and matures *in vivo*.

Polycaprolactone (PCL) is an aliphatic polyester that degrades slowly and possesses high tensile and elongation properties for vascular grafts. PCL has been used in various tissue engineering applications due to its biocompatibility, biodegradability and adequate cell binding properties [18,19]. In this study we fabricated a composite vascular scaffolding system by electrospinning PCL and type I collagen. These composite scaffolds are designed to provide sufficient biomechanical properties and are configured to accommodate vascular endothelial and smooth muscle cells for use in vascular tissue engineering applications. Therefore, the objectives of this study were to develop a composite scaffolding system which has sufficient biomechanical properties for vascular applications and maintains mechanical strength under physiological conditions. In addition, this study was designed to evaluate vascular cell–scaffold interactions in their initial stage.

2. Materials and methods

2.1. Scaffold preparation

Vascular scaffolds were fabricated by electrospinning using a 1:1 (weight ratio) polymer blend of poly(ε-caprolactone) (PCL, inherent viscosity = 1.77 dL/g, Lactel Absorbable Polymers, Pelham, AL, USA) and collagen type I derived from calf skin (Elastin Products Co., Owensville, MO, USA) in 1,1,1,3,3,3-hexafluoro-2-propanol (99+%) (HFP, Sigma Chemical Co., St. Louis, MO, USA). The electrospinning set-up included a syringe pump, a high voltage supply, and a rotating mandrel (Custom Design & Fabrication, Richmond, VA, USA) to collect the fibers. A positive voltage (20 kV) was applied to the polymer solution by the power supply (Spellman High Voltage, Hauppauge, NY, USA). The PCL/collagen blend solution was delivered through an 18 1/2 gauge blunt tip syringe needle at a constant flow rate of 3 mL/h using a syringe pump (Medfusion 2001, Medex, Inc., Carlsbad, CA, USA). The collecting mandrel was a 303 stainless steel rod (4.75 mm diameter). The distance between the syringe tip and the mandrel was 10 cm and the rotation rate was 1000 rpm. After the electrospinning process was complete, the PCL/collagen composite scaffolds were cross-linked in the vapor of a 2.5% glutaraldehyde solution for 6 h to increase stability and strength. Electrospun scaffolds made from PCL only were used as a control, and these were fabricated using the same procedure.

2.2. Scaffold characterization

The electrospun scaffolds were observed under scanning electron microscopy (SEM; Model S-2260N, Hitachi Co. Ltd., Japan). Briefly, the electrospun scaffolds were sputter-coated with gold (Hummer™ 6.2, Anatech Ltd, Denver, NC, USA) to a thickness of 10–15 nm. Images were acquired using an environmental SEM operating at an accelerating voltage of 20 kV with a 10 cm working distance. The SEM images were analyzed with UTHSCSA ImageTool 3.0 (Freeware provided by the University of Texas Health Sciences Center at San Antonio, USA) to determine average fiber diameters. Sixty random fibers per image were used to calculate the mean and standard deviation of fiber diameters. Pore areas were also measured by a subjective approximation of surface pores in the SEM images (average and standard deviation calculated from 25 measurements per image).

The degree of cross-linking, determined by Ninhydrin assay, was defined as the percentage of free amino groups in the electrospun PCL/collagen composite scaffolds that reacted with glutaraldehyde vapor during the cross-linking process described above.

Fluid uptake was also determined to evaluate the hydrophilicity of the electrospun scaffolds. The scaffolds were weighed after drying (W_0) and then immersed in water for 30 s. Subsequently, the scaffolds were taken out of the water, wiped dry with tissue paper, and weighed again immediately (W_s). The percentage of fluid uptake was calculated using the following equation:

$$\text{Fluid uptake}\ (\%) = (W_0 - W_s)/W_0 \times 100.$$

2.3. Biomechanical evaluations

2.3.1. Tensile properties

Electrospun specimens were configured into tubular scaffolds of 15 mm length, 4.75 mm inner diameter, and 0.3 mm thickness. After immersing the specimen in phosphate buffered saline (PBS) at room temperature for 12 h, tensile properties were measured using a uniaxial load test machine (Model #5544, Instron Corporation, Issaquah, WA, USA) equipped with a maximum 2 kN load cell at a crosshead speed of 0.5 mm/s. The ultimate tensile strength, yield strength, Young's modulus, and elongation at break were obtained from the stress–strain curves. Porcine coronary artery was served as native vessel control.

2.3.2. Suture retention strength

To determine the suture retention strength of the electrospun scaffold material, the samples were cut to a rectangular shape of 5 mm width, 15 mm length, and 0.3 mm thickness. After soaking the specimen in PBS at room temperature for 12 h, one end of the specimen was fixed to the stage clamp of the uniaxial load test machine (Instron) and the opposite end was connected to another clamp by a common suture material (5-0 Prolene, Ethicon Inc., Piscataway, NJ, USA). The suture was placed 2 mm from the edge of the specimen using a tapered noncutting needle. It was also stitched to a silicone plate to prevent slippage. The distance between the clamps was 2 cm. The specimen was pulled at a crosshead speed of 8 mm/min until either the suture ripped or the scaffold fractured. Suture retention strength, which was defined as fracture strength, was obtained.

2.3.3. Burst pressure strength

The burst pressure strength for the electrospun scaffolds was measured by increasing the pressure within the tubular vascular scaffold until failure occurred. A pressure transducer catheter (thin latex tube) was inserted through a cannulating fixture at one end of the tubular scaffold. A 60 mL pressure syringe was inserted through a custom cannula at the other end of the scaffold. The pressure was gradually increased until failure occurred and the pressure change was recorded.

2.3.4. Compliance

To calculate compliance, which is expressed as the dimensional change with respect to luminal pressure change, the electrospun scaffolds were immersed in a water bath and cannulated at either end. To prevent water leakage from the pore structure of the material, a thin latex tube was inserted into the electrospun scaffolds. One cannula was connected to a column of water and the other to a drainage tube. The column of water was high enough to create a pressure within the electrospun scaffold of 120 mmHg. Water was drained through the scaffold to lower the pressure in increments of 10 mmHg. At each increment, the diameter of the scaffold was recorded using a digital camera.

Compliance is traditionally defined as

$$C = \Delta D / D_0\, \Delta P$$

where D_0 is the external diameter in diastole, ΔD is the change in external diameter, and ΔP is the pressure increment.

2.4. Maintenance of tensile properties in a perfusion bioreactor system

To evaluate the long-term mechanical integrity of the electrospun PCL/collagen composite scaffolds under high pressure and flow, the scaffolds were pre-conditioned in a perfusion bioreactor system at 37 °C for 1, 2, and 4 weeks. The scaffolds (4 cm length, 4.75 mm inner diameter, and 0.3 mm thickness) were placed in the bioreactor system and a steady culture medium flow was applied. The flow rate was set at 2 L/min and the shear stress at 31 dyne/cm^2, which is higher than the maximum shear stress occurring in normal human artery (26 dyne/cm^2) [20]. The shear stress τ_w (dyne/cm^2) was calculated using the following equation:

$$\tau_w = 4Q\mu/\pi R^3$$

where Q is the volumetric flow rate (2 L/min), μ is the viscosity of the medium (1.002×10^{-3} N s/m^2), and R is the vessel radius (2.375×10^{-3} m). Scaffolds incubated under static conditions served as controls. The scaffolds were collected and tensile testing was performed to evaluate changes in mechanical strength at various time points. The tensile strength of scaffolds conditioned in the perfusion bioreactor was compared to the tensile strength of scaffolds maintained in static conditions.

2.5. Biological activity evaluations

2.5.1. Cytotoxicity assessment

For the biological evaluations, endothelial cells (*b*ECs) and smooth muscle cells (*b*SMCs) from bovine carotid artery were used. Both *b*ECs and *b*SMCs were seeded on scaffolds contained in cell culture plates for testing via the direct contact method [21]. Briefly, the electrospun PCL/collagen composite scaffolds were gas sterilized with ethylene oxide (Amsco®Eagle® 3017 EO Sterilizer, STERIS Co., Mentor, OH, USA) at 30 °C for 36 h. The scaffolds were incubated at 37 °C for 30 min with culture medium prior to testing. Scaffolds (5 mm × 5 mm × 0.3 mm) were placed at the center of the wells of 24-well plates that contain subconfluent monolayers of each cell type. Wells containing cells only served as negative controls. Pieces of latex cut

into the same size as the scaffold material served as positive controls. Cell–scaffold contact was maintained at 37 °C in 5% CO_2, and culture medium was changed every 3 days. MTS assay was performed on days 1, 3 and 7 to determine cell viability in each well. Briefly, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS, Promega, Madison, WI, USA) and phenazine methosulfate (PMS, Sigma) solutions were mixed at a ratio of 20:1 and the mixture was immediately added to the cell culture medium at a ratio of 1:5. The plate was incubated at 37 °C for 3 h. Absorbance was measured using a microplate reader (ELX 800, Bio-Tex Instruments Inc., Winooski, VT, USA) at 490 nm. The results were reported as the percentage of the negative controls ($n = 5$). All reagents for cell culture were purchased from Invitrogen (Gibco® Cell Culture, Carlsbad, CA, USA).

2.5.2. Cell adhesion and proliferation

For cell culture studies, PCL/collagen was electrospun onto plastic coverslips to form two-dimensional fiber structures. Briefly, plastic coverslips were sonicated in ethanol and allowed to vacuum dry. The electrospun PCL/collagen covered plastic coverslips were placed into 6-well plates, gas sterilized by ethylene oxide at 30 °C for 36 h, and incubated at 37 °C for 30 min in culture medium prior to cell seeding.

To evaluate the initial stage of *in vitro* cellular interactions with vascular scaffolds, electrospun scaffolds were seeded with *b*ECs and *b*SMCs in culture. Both cell types were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin at 37 °C in 5% CO_2, and the culture medium was changed every 3 days. Biological activity of the electrospun scaffolds was determined by testing the ability of cells to proliferate on the scaffold material. Briefly, both cell types (2×10^4 cells/cm^2) were seeded onto the scaffolds (10×10 mm^2) in the wells of a 24-well culture plate. For assay, cell-seeded electrospun coverslips were transferred to empty 24-well culture plates. Adhesion and proliferation of the cells on the scaffolds were determined by MTS assay after 1, 3, and 7 days of cell seeding. Cell proliferation was assessed by the intensity of blue color obtained, which was directly proportional to the metabolic activity of the cell population. The optical density was recorded for quantification ($n = 5$) of cells within the scaffolds.

2.5.3. Cell morphology

To evaluate cell morphology and adhesion to the scaffold material, the scaffolds (4.75 mm diameter, 0.3 mm thickness, and 1 cm length) were seeded with *b*ECs (1×10^5 cells/mL) and/or *b*SMCs (5×10^5 cells/mL). In cultures in which both cell types were used, bovine ECs were seeded in the lumen of the vascular scaffolds and bSMCs were seeded in the outer of the vascular scaffold. The cell-seeded scaffolds were incubated for 4 h to allow cells to adhere to the scaffold. Additional cell culture medium was added to the culture plate. After 48 h culture, the *b*ECs and *b*SMCs that adhered to the scaffolds were evaluated by hematoxylin and eosin (H&E) and 4′,6-diamidino-2-phenylindole (DAPI, Vector Lab., Burlingame, CA, USA) staining. Cell distribution on the scaffold was analyzed using SEM.

2.6. Statistical analysis

The differences between the electrospun PCL scaffolds and electrospun PCL/collagen composite scaffolds specific to tensile strength, elongation at break, Young's modulus, suture retention strength, burst pressure strength, and compliance were evaluated by Student's *t*-test. Data from the biological activity evaluations such as cytotoxicity, cell adhesion, and cell proliferation were analyzed by ANOVA. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Scaffold characterizations

The electrospinning parameters used for the fabrication of PCL/collagen composite scaffolds were optimized by performing a series of experiments to investigate the effects of solution concentration, flow rate, voltage levels, and distance between the syringe tip and the mandrel (data not shown). The PCL/collagen fibrous scaffolds had a length of 12 cm with a thickness of 0.3 mm. Four milliliters of polymer solution at a total solution concentration of 5% (w/v) was used in these studies. Representative gross and SEM images showing the ultrastructure of the electrospun PCL/collagen scaffold are illustrated in Fig. 1. The composite scaffolds showed randomly oriented fibers with diameters of 520 ± 14 nm and pore areas of 22.7 ± 9.6 μm^2. These findings demonstrate the feasibility of using electrospinning to fabricate vascular scaffolds, as the nanoscale structure of native vessels can be mimicked by electrospinning. In the electrospun PCL-only control scaffolds, the results and trends were similar to the PCL/collagen composite scaffolds (Table 1).

The composite scaffolds that were exposed to glutaraldehyde vapor for 6 h had cross-linking degrees of approximately 90% as determined by the Ninhydrin assay. The cross-linking degrees of composite scaffolds increased with an increase in incubation time with glutaraldehyde vapor.

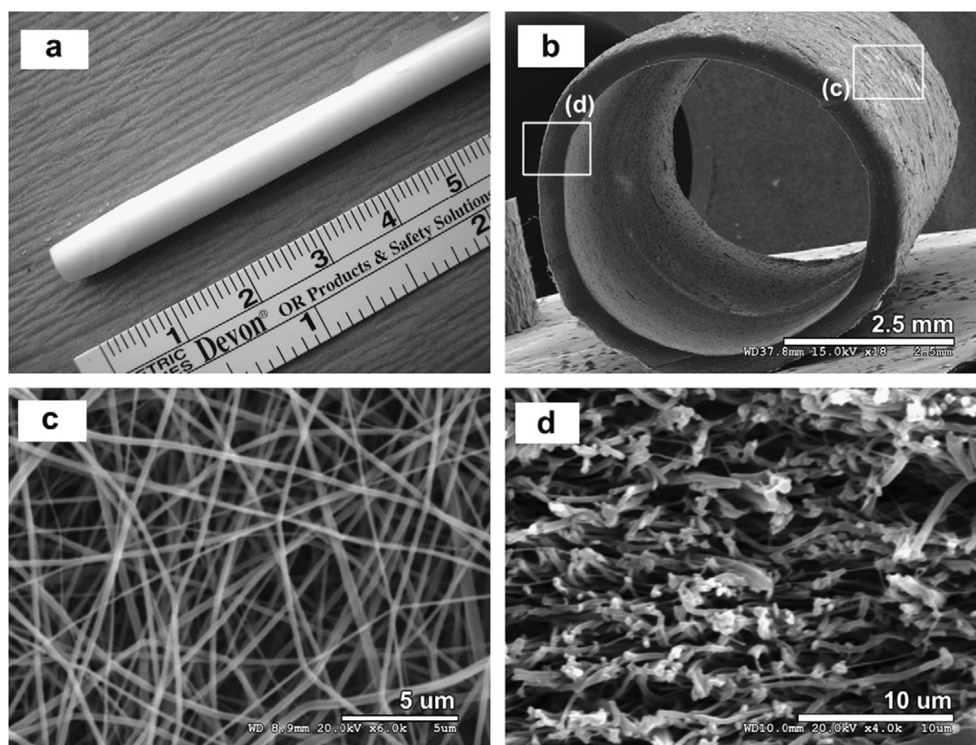

Fig. 1. (a) The gross appearance and SEM images of electrospun PCL/collagen composite scaffolds: (b) entire (×18), (c) surface (×6.0K), and (d) cross-sectional (×4.0K) morphologies.

Table 1
Physical properties of electrospun PCL/collagen composite scaffolds

Samples	Tensile testing[a] ($n=5$)				Suture retention strength (N) ($n=5$)	Burst pressure (mmHg) ($n=3$)	Compliance[b] (%) ($n=3$)	Image analysis	
	Ultimate tensile strength (MPa)	Yield strength (MPa)	Young's modulus (MPa)	Elongation at break (%)				Fiber diameter (nm)	Pore area (μm^2)
PCL[c]	5.1 ± 0.9	1.5 ± 0.7	7.5 ± 0.7	417 ± 58	4.9 ± 1.3	914 ± 130	3.5 ± 1.4	580 ± 17	26.9 ± 13.1
PCL/collagen (wet)	4.0 ± 0.4	4.0 ± 0.4	2.7 ± 1.2	140 ± 13	3.0 ± 1.1	4915 ± 155	5.6 ± 1.6	520 ± 14	22.7 ± 9.6
PCL/collagen (dry)	8.3 ± 1.2	8.3 ± 1.2	3.8 ± 5.6	62 ± 5	n/a	n/a	n/a		

n/a: not applicable.
[a] Tensile testing was carried out under dry and wet conditions.
[b] Calculated for a pressure change from 0 to120 mmHg.
[c] PCL did not show the differences between dry and wet conditions.

The fluid uptake ability of the PCL/collagen composite scaffolds (325.1 ± 7.1%) was greater than that of PCL-only scaffold (25.4 ± 8.3%) ($P < 0.01$). The fluid uptake ability of the composite scaffolds increased as the collagen to PCL ratio increased due to the hydrophilic nature of the collagen (data not shown).

3.2. Biomechanical evaluations

3.2.1. Tensile properties

The tensile property of composite scaffolds was significantly different between dry and hydrated scaffolds (Fig. 2a). In contrast, there was no difference between the tensile properties of dry and hydrated PCL scaffolds due to the hydrophobicity of PCL. Uniaxial tensile properties showed that the hydrated PCL/collagen composite scaffolds exhibited initial elastic behavior, followed by stiffening, which is similar to the tensile behavior of native tissues. Based on the stress–strain of the electrospun vascular scaffolds, tensile strength, elongation at break, and Young's modulus were calculated (Fig. 3). The ultimate tensile strength (UTS) and elongation at break of the composite scaffolds were decreased with the addition of collagen to PCL. The UTS decreased from 5.1 ± 0.9 MPa for PCL scaffolds to 4.0 ± 0.4 MPa for a 1:1 ratio of collagen to PCL under wet conditions. In addition, the elongation at break of PCL was 417 ± 58%, and this value decreased to 140 ± 13% for PCL/collagen. However, the yield tensile strength of PCL/collagen was enhanced as compared to PCL (4.0 ± 0.4 MPa vs. 1.5 ± 0.7 MPa, respectively). Moreover, the elasticity and elongation of the hydrated PCL/collagen scaffolds were more close to those of native vessels (Fig. 3). These results seem to be influenced by the amount of collagen contained within the PCL composites.

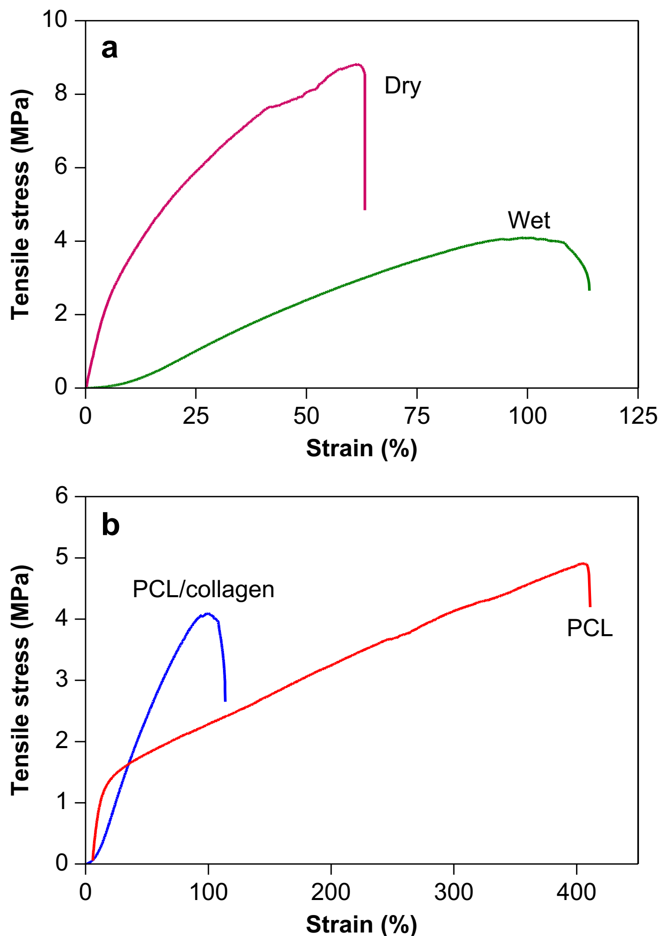

Fig. 2. Stress–strain curve from tensile testing: (a) electrospun PCL/collagen composite scaffold under dry and wet environments and (b) electrospun PCL/collagen composite scaffolds compared to PCL as control.

3.2.2. Suture retention strength

Suture retention strength is a crucial factor in the fabrication of vascular scaffolds as it directly relates to the success of the graft implantation procedure. In these experiments, the hydrated electrospun PCL/collagen composite scaffolds (3.0 ± 1.1 N) had lower suture retention strength than the control (4.9 ± 1.3 N) scaffolds, as shown by the ultimate tensile strength measurements. However, the suture retention strength of the composite scaffold was more than adequate for suturing during implantation, which is generally accepted to be greater than 2.0 N [22].

3.2.3. Burst pressure strength

To determine whether the composite scaffold material possessed adequate strength to endure physiologic forces, burst pressure testing was performed to identify the maximum pressure that the scaffolds could endure before failure (Fig. 4a). The composite scaffolds (4915 ± 155 mmHg) showed significantly increased burst pressure strength when compared to the control (914 ± 130 mmHg). This result depended on the yield strength of the scaffolds, because the composite scaffold had a significantly higher yield strength than the controls (Fig. 3b). This demonstrates that the composite scaffolds possess excellent physical strength and can be developed as substitutes for native blood vessels.

3.2.4. Compliance

The compliance showed that there were no significant differences between the compliances recorded for PCL/collagen composite scaffolds (5.6 ± 1.6%) and PCL scaffolds (3.5 ± 1.4%) within the physiologic pressure range, which is consistent with the *in vivo* mechanical behavior (Fig. 4b). However, the compliance of the composite scaffold was comparable to that of native blood vessel [23] due to enhanced elasticity (Fig. 3c).

3.2.5. Maintenance of tensile properties under a perfusion bioreactor system

The maintenance of the UTS of the PCL/collagen composite under flow and static conditions was examined (Fig. 5). After 4

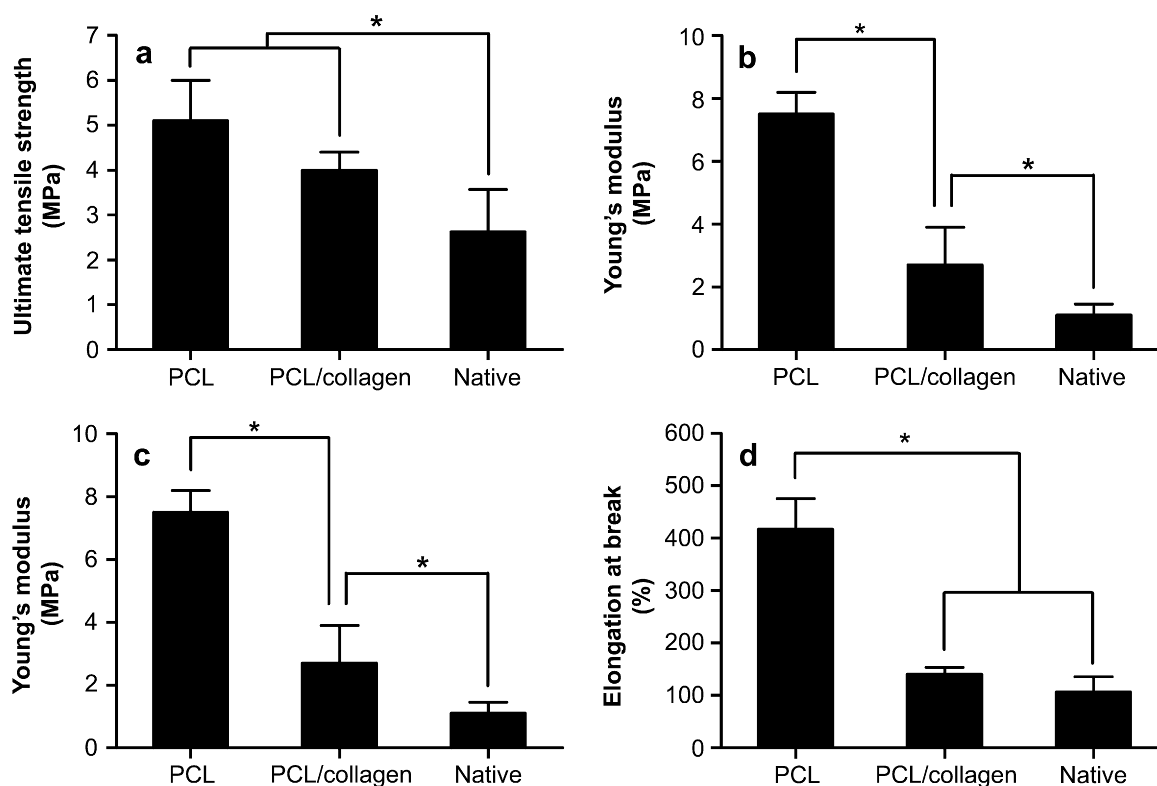

Fig. 3. Tensile properties of electrospun PCL/collagen composite scaffolds: (a) ultimate tensile strength, (b) yield tensile strength, (c) Young's modulus, and (d) elongation at break of electrospun PCL/collagen composite scaffolds compared to electrospun PCL scaffolds and porcine coronary artery as native control ($^*P < 0.05$).

weeks in flow conditions, the UTS of PCL/collagen remained at 86.3% of the original UTS. In static conditions, UTS remained at 97.3% of the control.

3.3. Biological activity evaluations

3.3.1. Cytotoxicity assessment

Cytotoxicity assessment using the MTS assay indicated that there were no significant differences in viability between the cells grown on the PCL/collagen composite scaffold and on tissue culture plates (Fig. 6). However, the latex used as a positive control was highly cytotoxic and showed that only 4.7% of latex-exposed *b*ECs and 6.5% of *b*SMCs were viable at 7 days when compared to the negative control.

3.3.2. Cell adhesion and proliferation

To evaluate the cell adhesion and proliferation on the PCL/collagen scaffold *in vitro*, *b*ECs and *b*SMCs were used. Both cell types adhered to and proliferated on the electrospun PCL/collagen composite scaffold for up to 7 days of culture, indicating that the composite scaffold is conducive to cell adhesion and proliferation. Cell adhesion on the PCL/collagen scaffold at day 1 of *b*EC seeding was significantly higher than on the PCL and culture dish ($P < 0.05$) (Fig. 7a). However, there was no significant difference in *b*EC proliferation between the PCL/collagen and the others. In contrast, cell adhesion and proliferation on the PCL/collagen scaffold after *b*SMC seeding were significantly higher than on the culture dish ($P < 0.05$) (Fig. 7b). After 7 days of *b*SMC seeding, the PCL/collagen scaffold showed higher cell proliferation than the others ($P < 0.05$).

3.3.3. Cell morphology

Histological and SEM images showed that *b*ECs and *b*SMCs are able to attach to the surface of the PCL/collagen composite scaffold after 48 h of culture (Fig. 8). The *b*SMCs formed multiple cell layers on the outer region of the tubular scaffold (Fig. 8d), while the *b*ECs formed a monolayer on the inner surface of the scaffold (Fig. 8a), which indicates that morphological reconstitution of vascular tissue is possible. SEM images also revealed a confluent layer of *b*ECs on the lumen and *b*SMCs on the outside of the scaffold after 48 h (Fig. 8c,f). These results indicate that the PCL/collagen scaffold is biocompatible and can support cell growth and proliferation *in vitro*.

4. Discussion

It is evident that scaffold materials for vascular use must be biocompatible and possess appropriate biomechanical and biochemical properties that would result in functional tissue formation. Such scaffolds should be able to maintain their structural integrity and tissue characteristics as vessels mature and remodel *in vivo*. Consequently, numerous natural and synthetic materials have been investigated in order to develop an ideal scaffold for vascular use. Naturally derived materials such as collagen and elastin have been considered due to their biocompatibility and biomimetic characteristics, however, these materials provide inadequate mechanical properties and dimensional stability. In contrast, synthetic polymers such as PLGA and PLA offer structural support and controlled degradation in a consistent manner, but lack biomimetic properties.

Previously, we have shown that a valuable vascular scaffold can be fabricated using a combination of synthetic and naturally derived substances, which possessed many of the required scaffold characteristics *in vitro*, including biocompatibility, biomechanical and biochemical properties [14,15]. However, our subsequent investigations into the durability of these scaffolds under pressurized flow conditions demonstrated that the scaffolds were inadequate,

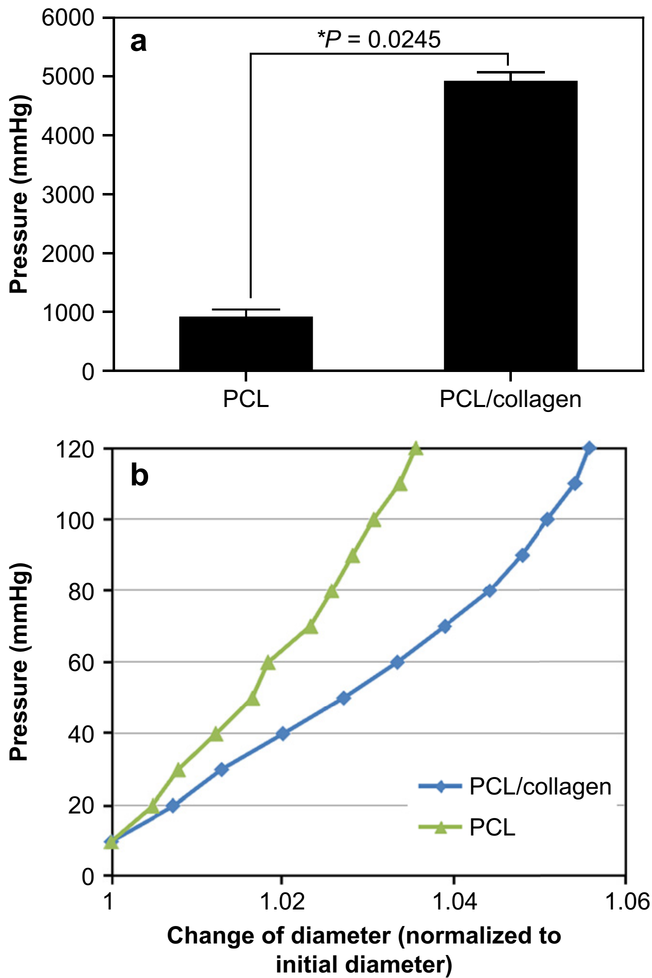

Fig. 4. (a) Burst pressure strength of electrospun PCL/collagen composite scaffolds compared to electrospun PCL scaffolds (*$P < 0.05$). (b) Compliance curves for electrospun PCL/collagen composite scaffolds compared to electrospun PCL scaffolds.

as the vascular scaffold collapsed within 1 week in a bioreactor perfusion system. This unexpected outcome was mainly due to the rapid degradation and low resistance of the vascular scaffolds to shear stress, which led to a compromise in structural integrity.

In the present study we explored the possibility of using PCL as an enduring and versatile synthetic polymer that could provide prolonged structural support under physiologic vascular conditions. PCL has good mechanical properties and degrades very slowly. It typically takes more than 1 year to degrade *in vivo*. It is thought that PCL degrades *in vivo* by hydrolysis to caproic acid and its oligomers and by enzymatic action [24]. We show that the

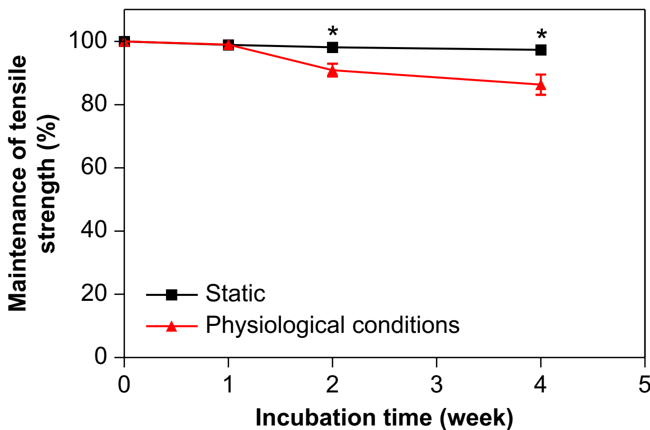

Fig. 5. Maintenance of tensile strength of electrospun PCL/collagen scaffolds under physiological vascular condition (*$P < 0.05$).

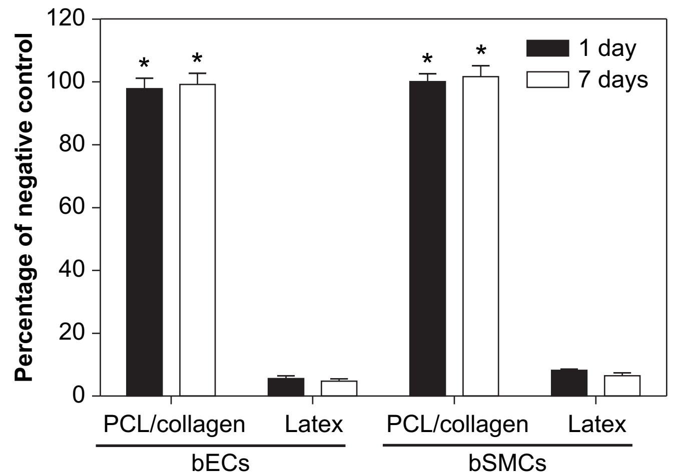

Fig. 6. Biocompatibility. The mitochondrial metabolic activity of bovine endothelial cells (ECs) and smooth muscle cells (SMCs) cultured in direct contact with electrospun PCL/collagen composite scaffolds at 1 and 7 days, as determined by MTS assay. Latex rubber is used for all positive controls (*$P < 0.01$).

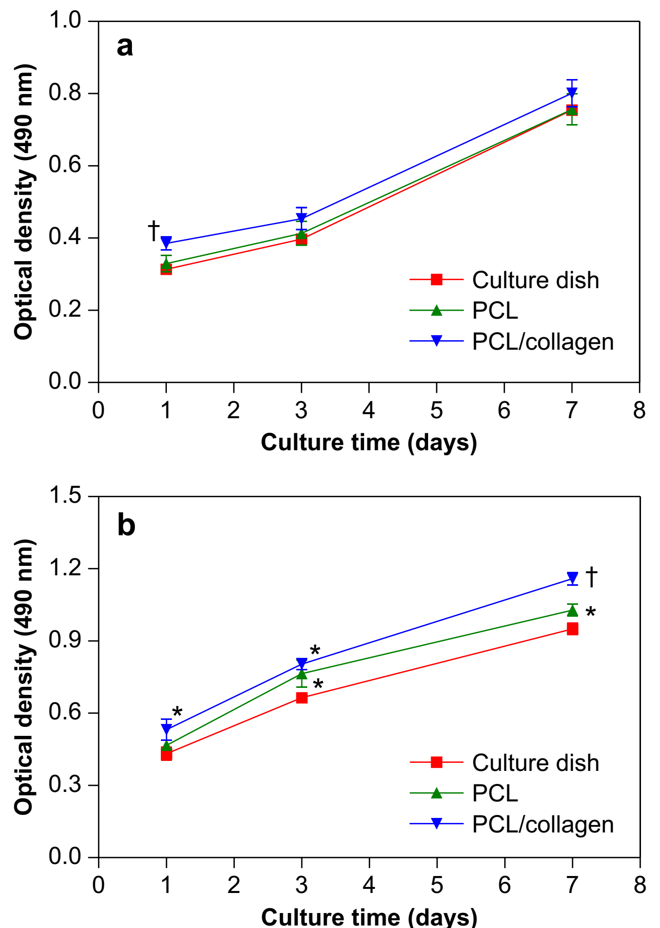

Fig. 7. Cell adhesion and proliferation. (a) Bovine ECs and (b) bovine SMCs seeded on the electrospun PCL/collagen composite scaffolds compared to PCL and tissue culture dish as measured by MTS assay (†$P < 0.05$ compared to PCL and tissue culture dish and *$P < 0.05$ compared to tissue culture dish).

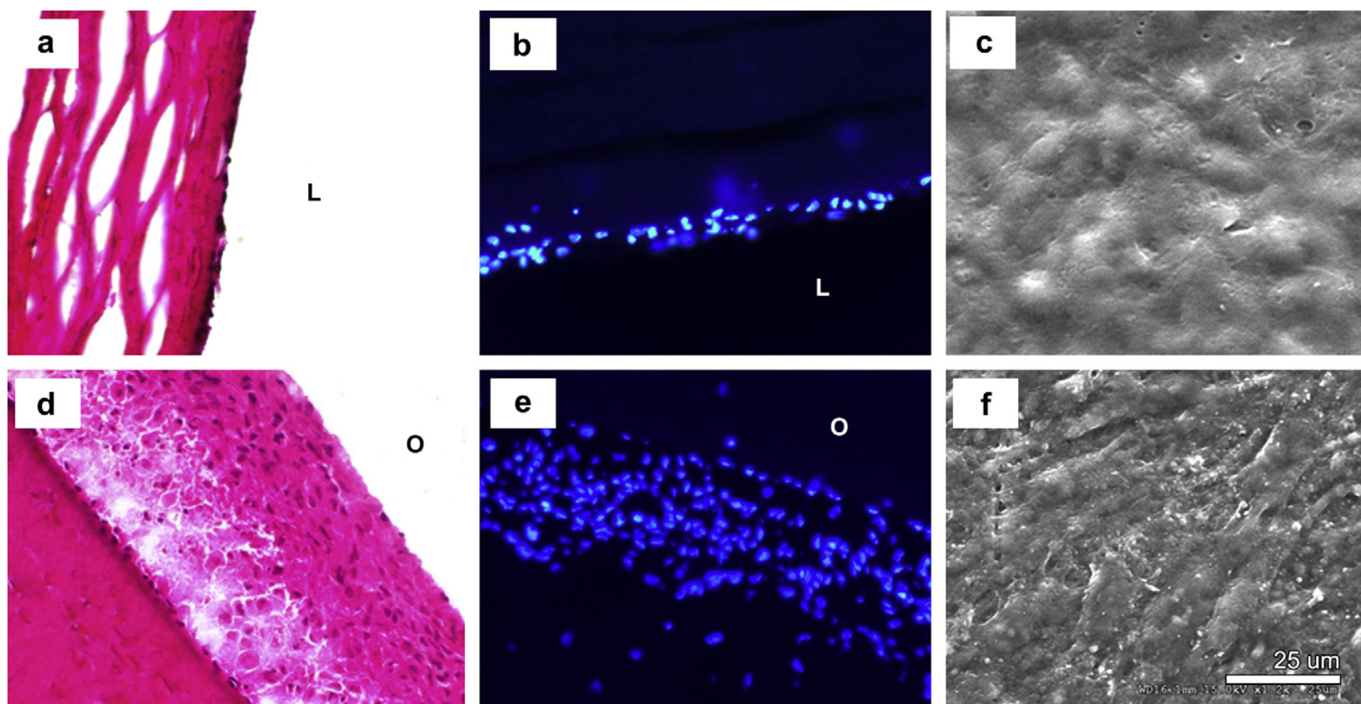

Fig. 8. (a–c) The electrospun PCL/collagen composite scaffolds were seeded with bovine ECs and (d–f) bovine SMCs at 48 h after seeding; (a, d) H&E staining (×200), (b, e) DAPI staining (×200), and (c, f) SEM images (×1.2K). (L) lumen side and (O) outer side.

composite vascular scaffolding system fabricated by hybridizing a high molecular weight PCL (>200,000 g/mol measured by GPC) and type I collagen using electrospinning techniques possesses adequate biomechanical properties that resist high degrees of pressurized flow over the long term. The long-term stability of the PCL/collagen composite scaffolds was demonstrated in a continuous perfusion bioreactor system for up to 4 weeks. In contrast to our assumption that certain mechanical properties of the vascular scaffolds would decrease when collagen is combined with a PCL scaffold, our results demonstrated increases in several properties, including yield tensile strength and elasticity, when compared to PCL-only scaffolds. The electrospun PCL-only scaffold showed a high elongation property with adequate tensile strength, but this property induced the vascular scaffold to inflate when pressure increased. The PCL/collagen composite scaffold used in this study was able to endure high physiological pressure, most likely due to the introduction of collagen to PCL, which stiffened the scaffold. Interestingly, the hydrated PCL/collagen scaffolds showed tensile properties that are similar to that of native blood vessel (Fig. 2). One of the potential problems of our composite scaffolding system is the use of glutaraldehyde as a cross-linking agent, as this might cause calcification *in vivo*. To overcome this problem, we are currently working to develop alternative cross-linking agents, including carbodiimides and naturally derived cross-linking agents.

Biomechanical properties of the fabricated vascular scaffolds cannot be overemphasized as the long-term success depends on the characteristics of engineered vessel *in vivo*. The implanted vessels should mature and remodel into tissue similar to native vessels. As such, the engineered vessels should be compliant, be able to resist kinking and compression, possess sufficient tensile and shear strength to resist fraying at cut edges and tearing out of sutures, and maintain circumferential strength sufficient to withstand arterial pressures [25]. In this study we show that the PCL based composite vascular scaffolds possess the required characteristics, including the tensile properties, suture retention strength, burst pressure strength, and compliance of the scaffolds. We show that the composite vessel scaffolds have tensile strengths of approximately 4 MPa, which far exceeds the tensile strength of a porcine artery (1 MPa) [26]. The suture retention strength of the composite scaffold was greater than the force required to suture native blood vessel, which generally is accepted to require more than 2.0 N.

The burst pressure strength is associated with the pressure that a vascular graft can be subjected to before an acute leak develops and the graft fails. For instance, the burst pressure of a typical carotid artery is approximately 5000 mmHg and 2250 mmHg for saphenous vein [27]. Our data show that the burst pressure of PCL/collagen composite scaffolds ranged from 4760 mmHg to 5070 mmHg, which is far greater than physiologic blood pressure seen in any patients.

Compliance mismatch has an important role in graft failure. It has been demonstrated that the compliance of biological grafts is more compliant than those of current noncompliant vascular grafts. Most of porous polymeric vascular scaffolds showed compliance mismatch in the initial stage after transplantation due to their rigidity. As compliance mismatch increases, graft patency decreases [27]. This may be important in determining the long-term success of vascular graft materials. For example, the compliances of human artery and saphenous vein are approximately 6.0% and 4.6%, respectively [23]. As shown in our study (Fig. 6b), the PCL/collagen composite scaffolds were adequately compliant for vascular applications.

To successfully engineer blood vessels that can be applied clinically, the vascular scaffolds must possess excellent biocompatibility to ensure that seeded vascular cells remain viable and encourage host cell infiltration into the graft. These processes are required for the development of a viable, patent blood vessel from the scaffold. In this study, the basic cellular interactions between the electrospun PCL/collagen composite scaffolds and bovine ECs and SMCs, measured by MTS assay, showed that the scaffold material did not affect cell viability in all cases. In addition, the tubular vascular scaffolds are able to support adhesion and proliferation of

ECs and SMCs in the initial stage *in vitro* as demonstrated by SEM and histological analyses. These findings indicate that PCL/collagen based composite scaffolds can be used in conjunction with vascular cells to create an engineered vessel that could withstand physiological vascular conditions. Further studies are currently being conducted in an *in situ* animal model to determine the applicability of the composite scaffolding system.

5. Conclusions

We have developed a composite scaffolding system that can withstand physiologic vascular conditions consisting of high pressure and flow. The composite scaffolds, fabricated by hybridizing a high molecular weight PCL and type I collagen using electrospinning techniques, possess excellent biomechanical properties and demonstrate a long-term stability under a continuous perfusion bioreactor system for up to 4 weeks. In addition, the composite scaffolds provide a favorable environment that supports the growth of vascular cells.

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