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Manipulating the structure and mechanical properties of thermoplastic polyurethane/polycaprolactone hybrid small diameter vascular scaffolds fabricated via electrospinning using an assembled rotating collector

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ABSTRACT

The success of blood vessel transplants with vascular scaffolds (VSs) highly depends on their structure and mechanical properties. The fabrication of small diameter vascular scaffolds (SDVSs) mimicking the properties of native blood vessels has been a challenge. Herein, we propose a facile method to fabricate thermoplastic polyurethane (TPU)/polycaprolactone (PCL) hybrid SDVSs via electrospinning using a modified rotating collector. By varying the ratio between the TPU and the PCL, and changing the electrospinning volume, SDVSs with a wavy configuration and different properties could be obtained. Detailed investigation revealed that certain TPU/PCL hybrid SDVSs closely resembled the mechanical behaviors of blood vessels due to the presence of a wavy region and the combination of flexible TPU and rigid PCL, which mimicked the properties of elastin and collagen in blood vessels. The fabricated TPU/PCL SDVSs achieved lumen diameters of 1–3 mm, wall thicknesses of 100–570 μm, circumferential moduli of 1–6 MPa, ultimate strengths of 2–8 MPa, over 250% elongation-at-break values, toe regions of 5.3–9.4%, high recoverability, and compliances close to those of human veins. Moreover, these TPU/PCL SDVSs possessed sufficient suture retention strength and burst pressure to fulfill transplantation requirements and maintain normal blood flow. Human endothelial cell culture revealed good biocompatibility of the scaffolds, and cells were able to grow on the inner surface of the tubular scaffolds, indicating promising prospects for use as tissue-engineered vascular grafts.

1. Introduction

The increasing incidence of cardiovascular disease has amplified demand for new artificial grafts for bypass surgery. Although synthetic conduit materials, such as polyethylene terephthalate (Dacron) and expanded polytetrafluoroethylene (ePTFE), have been successfully used to replace large diameter blood vessels in clinical studies, they are unsuitable for small diameter blood vessels (< 6 mm) due to the risk of thrombosis, calcification, and restenosis (He et al., 2009; Hoenig et al., 2005). Thus, vascular tissue engineering scaffolds (VTESs or VSs) have been attracting attention in recent years. Numerous studies are ongoing to develop small diameter vascular scaffolds (SDVSs) for the replacement of autologous saphenous veins and mammary arteries in bypass surgery. Different from Dacron and ePTFE, SDVSs are intended to provide a conduit not only for normal blood flow but also for the regeneration of new blood vessels (Seifu et al., 2013). However, unresolved challenges still hamper the creation of optimum SDVSs due to an inability to match the biomechanical behavior of a synthetic implant to that of a human artery. Human arteries have a heterogeneous structure with unique, non-linear, anisotropic mechanical features (Singh et al., 2015). A human artery experiences low stress deformation at low pressures and shows a steep increase in stress as the pressure is increased. The initial elastic region (a.k.a. toe region) is believed to be a function of elastin, while the latter high modulus region is due to woven collagen fibrils (Roach and Burton, 1957). Hence, mimicking the special mechanical properties of blood vessels is challenging. Furthermore, residual strain has not normally been considered in the design of SDVSs in most studies. Yet blood vessels show a wavy configuration at a zero-stress state, which is caused by residual strain, and is believed to be responsible for the special mechanical properties of blood vessels (Fung

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and Liu, 1992).

To date, various methods have been developed to fabricate SDVSs, such as freeze drying (Zhu et al., 2014), particle leaching (Landau et al., 2017), electrospinning (Elsayed et al., 2016), and braiding (Wang et al., 2014). Among them, electrospinning is a versatile technique that produces fibrous SDVSs with a defined diameter and thickness using a conductive mandrel as the collector. It is the most widely used method to date (Zhao et al., 2015). However, removal of the electrospun tubes from the mandrel without interfering with their microstructure has been as problem. Winding the mandrel with copper wire is an effective way to facilitate scaffold removal, but it causes unnecessary topography on the SDVSs and the produced SDVSs cannot mimic the mechanical properties of blood vessels (Liu et al., 2016a). We have previously developed an approach to fabricate triple-layered SDVSs that consist of a fibrous TPU inner layer, braided silk intermediate layer, and porous TPU outer layer. These SDVSs were able to mimic the non-linear mechanical properties of blood vessels because of the flexible TPU and rigid woven silk filaments (Mi et al., 2015c). However, this complex method is time consuming. Hence, developing a simple method to produce SDVSs that mimic the biomechanical properties of native blood vessels is a goal that needs to be accomplished.

Because of the limited SDVS fabrication methods, considerable research is currently focused on varying the materials of SDVSs. Biodegradable synthetic and natural polymers, such as polylactic acid (PLA) (Li et al., 2017), PCL (Sun et al., 2016), PU (Mi et al., 2016), poly (lactic-*co*-glycolic acid) (PLGA) (Landau et al., 2017), chitosan (Fukunishi et al., 2016), silk fibroin (Zhou et al., 2015), and collagen (Wu et al., 2015), have been used to produce SDVSs. Different materials are often combined to produce hybrid SDVSs as well. Most common is the combination of synthetic materials that provide mechanical strength with natural materials that enhance biocompatibility (Fu et al., 2014). Previously, we found that adding small amounts of polyethylenimine (PEI) and graphene oxide into PCL and TPU enhanced the adhesion and proliferation of vascular endothelial cells (Jing et al., 2015b, 2015c). However, the effect of combining these materials with different stiffnesses on the biomechanical properties of hybrid SDVSs has not been established. We also found that simply blending soft and hard materials was an effective way to tailor the properties of hybrid materials; thus, it is expected that the combination of a flexible TPU elastomer with rigid PCL would be able to mimic the properties of elastin and collagen components in blood vessels to some extent (Jing et al., 2014; Mi et al., 2015a).

Based on these ideas, in this study, we combined TPU and PCL at various ratios to produce hybrid SDVSs with properties comparable to a human artery. We designed a new collecting setup to fabricate SDVSs with a wavy configuration to mimic the wavy configuration of the zero-stress state of blood vessels. The structural and mechanical properties of fabricated SDVSs have been investigated in detail and compared with reference blood vessels. As such, TPU/PCL hybrid SDVSs with mechanical properties closely mimicking human native blood vessels were successfully prepared.

2. Experimental methods

2.1. Materials

Medical grade TPU (Rx85A, M_n = 48,000) was purchased from Bayer Corp. PCL (CAPA 6500, M_n = 50,000) was purchased from Perstorp UK Ltd. N,n-dimethyl formamide (DMF) and chloroform were purchased from Sigma–Aldrich. All materials were used as received.

2.2. Preparation of TPU/PCL hybrid SDVS

TPU and PCL pellets with defined weight ratios were dissolved in a solvent mixture of chloroform/DMF (vol./vol. = 3/2). Table 1 lists the ratio of TPU and PCL in solution and the optimized solution concentrations for different solutes. The abbreviations T7P3, T1P1, and T3P7 represent TPU:PCL ratios of 7:3, 1:1, and 3:7, respectively.

Table 1
Optimum TPU/PCL solution concentration for electrospun bead-free fibers.

Solute	TPU	T7P3	T1P1	T3P7	PCL
Concentration	10 wt%	12 wt%	14 wt%	18 wt%	20 wt%

Note: The solvent was a mixture of chloroform and DMF at a volume ratio of 3:2 for all solutions.

Electrospinning was carried out on a custom-built electrospinning setup. Completely dissolved solution was loaded in a polypropylene syringe that connected to an 18-gauge blunt-end needle. The syringe was mounted on a digital pump to control the flow rate. The collector used was a bundle of 7 assembled copper rods (diameter of 0.8 mm each) that were connected to a rotating motor. Electrospinning was performed at a 20 cm needle-to-collector distance, a 0.5 mL/h flow rate, and an 18 kV applied voltage. Three electrospinning volumes (0.6, 1.1, and 1.8 mL) were used for each solution to fabricate SDVSs with different wall thicknesses.

2.3. Human umbilical vein endothelial cell (HUVEC) culture

Human umbilical vein endothelial cells (HUVECs) (Lonza) were maintained on T75 tissue culture-treated polystyrene flasks. Cells were fed every other day with an endothelial cell growth medium EGM-2-MV bullet kit (Lonza). Before cell seeding, the tubular scaffolds were first sterilized with 70% ethanol for 30 min, followed by a series of phosphate buffer solution (PBS) washes, and then sterilized with ultraviolet (UV) light for another 30 min. Scaffolds for the live/dead assay were fixed in 96-well plates with sterilized polyester double-sided adhesive tape (ARcare®90106, Adhesive Research Inc., USA). Scaffolds for the proliferation assay were cut open and placed in 24-well plates with their inner surfaces facing up. Cells were then seeded at a density of 5×10^4 cells/cm^2 on the scaffolds. Spent medium was aspirated and replaced with 1 mL of fresh medium daily for screening samples.

2.4. Characterization

2.4.1. Fourier transform infrared spectroscopy (FTIR)

The chemical structures of SDVSs were characterized by Fourier transform infrared (FTIR) spectroscopy (Bruker Tensor 27). The fabricated SDVSs were analyzed in transmittance mode in the range of 600–4000 cm^{-1}. The functionalities corresponding to each of the absorption bands were analyzed.

2.4.2. Scanning electron microscopy (SEM)

The structures of the prepared SDVSs were observed via SEM. The samples were quenched in liquid nitrogen for 30 min and fractured to image the cross section. The inner and outer surfaces of the samples were also imaged. The samples were sputtered with a thin film of gold for 40 s prior imaging with a JEOL Neoscope SEM (Nikon) with an accelerating voltage of 10 kV. The lumen size, tube thickness, and fiber diameter were measured using Image Pro-Plus software.

2.4.3. Circumferential tensile tests and cyclical tensile tests

Tensile tests were performed on a universal mechanical testing machine (Instron 5967) in wet conditions at ambient temperature (23 °C). The SDVSs were soaked in PBS for 1 h and then stretched in the circumferential direction using two U-shaped clamps at a crosshead speed of 1 mm/min until the sample fractured. Statistical results were the average of five samples.

Cyclical tensile tests of SDVSs were performed using the same instrument. The samples were stretched to a 25% strain at a crosshead speed of 5 mm/min and then fully released. The change of stress