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Fabrication of cell penetration enhanced poly (L-lactic acid-*co*-ε-caprolactone)/silk vascular scaffolds utilizing air-impedance electrospinning

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ABSTRACT

In the vascular prosthetic field, the prevailing thought is that for clinical, long-term success, especially bioresorbable grafts, cellular migration and penetration into the prosthetic structure is required to promote neointima formation and vascular wall development. In this study, we fabricated poly (L-lactic acid-*co*-ε-caprolactone) P(LLA-CL)/silk fibroin (SF) vascular scaffolds through electrospinning using both perforated mandrel subjected to various intraluminal air pressures (0–300 kPa), and solid mandrel. The scaffolds were evaluated the cellular infiltration *in vitro* and mechanical properties. Vascular scaffolds were seeded with smooth muscle cells (SMCs) to evaluate cellular infiltration at 1, 7, and 14 days. The results revealed that air-impedance scaffolds allowed significantly more cell infiltration as compared to the scaffolds fabricated with solid mandrel. Meanwhile, results showed that both mandrel model and applied air pressure determined the interfiber distance and the alignment of fibers in the enhanced porosity regions of the structure which influenced cell infiltration. Uniaxial tensile testing indicated that the air-impedance scaffolds have sufficient ultimate strength, suture retention strength, and burst pressure as well as compliance approximating a native artery. In conclusion, the air-impedance scaffolds improved cellular infiltration without compromising overall biomechanical properties. These results support the scaffold's potential for vascular grafting and *in situ* regeneration.

1. Introduction

In 2009, there were 416,000 surgical procedures performed involving coronary artery bypass surgery in USA as an example of the clinical need for small diameter (<6 mm inner diameter (I.D.)) vascular grafts [1]. Today, the saphenous vein remains the gold standard to replace diseased vascular tissue, but it may not be suitable for some patients because of vascular disease, amputation and previous harvest. Additionally, the use of this vein requires a secondary surgical procedure to obtain the vessel [2–5].

Due to limited autologous vessels for grafting, synthetic vascular prosthetics composed of Dacron (polyethylene terephthalate (PET)) and expanded polytetrafluorethylene (ePTFE) were developed but have had limited success when used as small-diameter arterial substitutes whereas these materials as large-caliber arterial substitutes have succeeded. The reason for this limited success is lower blood flow velocities and increased flow resistance within these smaller vascular grafts leading to the failure modes of acute thrombogenicity and anastomotic/intimal hyperplasia. Thus, there remains a clear clinical need for a functional small-diameter arterial graft. Tissue engineered blood vessels (TEBV) are promising alternatives as a method of treatment for blood vessel defects. Over the past two decades, much effort has therefore been devoted to developing a viable TEBV in terms of biomechanical and biological performance, however with limited success [6].

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Electrospinning is an attractive process which can generate micro- to nano-fibrous vascular scaffolds with a high surface area to volume ratio mimicking the architecture of the natural extracellular matrix (ECM) [7]. Moreover, electrospinning affords the opportunity to incorporate drugs, growth factors and other biomolecular signals into the polymeric solutions before electrospinning. A variety of natural and synthetic biodegradable polymers have been electrospun as manufacturing materials for the fabrication of scaffolds, such as collagen [8], elastin [9], silk fibroin [10–12], chitosan [8,10,13] and synthetics including poly(lactic acid) (PLA) [14,15], poly(glycolic acid) (PGA) [16,17], polycaprolactone (PCL) [18], polydioxanone [19,20] and poly(L-lactic acid-*co*-ε-caprolactone) (P(LLA-CL)) [8,21]. Pore size, pore interconnectivity, and porosity are important characteristics for any scaffold to provide sufficient cell infiltration, exchange of nutrients and metabolic products of host cells, and three-dimensional (3-D) tissue regeneration [22,23]. However, a historic limitation of electrospinning is the inability to control the pore size and porosity of the scaffolds attributed to the random deposition of fibers. It is logical that cells should be distributed throughout the entire scaffold to allow for the development of a functional tissue engineered product. Thus, when fabricating a scaffolding to promote tissue regeneration, cell and capillary infiltration must occur with the pore size and interconnectivity determining success or failure [24].

In order to overcome the limitations of uncontrolled porosity and pore size that hinder cells from penetrating into electrospun scaffolds, several methods have been attempted to enhance cell infiltration upon seeding. Ju et al. developed a bilayered vascular scaffold where the inner layer yielded small diameter fibers and the outer layer consisted of large diameter fibers to provide different pore sizes to facilitate adequate cellular interactions [25]. The bilayered scaffolds permitted endothelial cells (EC) adhesion on the lumen and smooth muscle cells (SMC) infiltration into the outer layer. Wu et al. developed a novel nanoyarn scaffold by dynamic liquid electrospinning [26]. Through this method, aligned nanoyarns were fabricated and formed a 3-D scaffold. This scaffold possessed large pore sizes or interfiber distance and high porosity which facilitated cell infiltration into the structure. Baker et al. used water soluble polyethylene oxide (PEO) as sacrificial fibers intermingled with PCL fibers through electrospinning [27]. After immersing the PEO/PCL scaffolds into water, the PEO dissolved leaving only the PCL fibers, thus increasing the effective pore size of the scaffold, and enhancing cellular infiltration. Unfortunately, the mechanical properties of these scaffolds decreased significantly with increasing PEO composition.

In a previous study by Dr. Bowlin and associates, electrospun PCL grafts fabricated with porous mandrel were evaluated. During the air-impedance process, traditional solid mandrel was replaced with a perforated mandrel, and added pressurized air exiting the pores to impede fiber deposition. Through this procedure, air-impedance electrospun grafts possessed larger pore diameter (interfiber distance) and the fibers formed less compacted, this kind of graft structure allowed partially increased cellular infiltration in defined regions than on traditional electrospun fiber grafts [24].

In this study, an elastic material of P(LLA-CL) and natural protein of SF were used to fabricate P(LLA-CL)/SF vascular scaffolds, utilizing air-impedance electrospinning method. The air-impedance processing method uses air flow to impede fiber deposition onto the mandrel in a controlled, patterned fashion to increase the scaffold porosity. The fabricated scaffolds were characterized to determine their potential application as a vascular scaffold/prosthetic. This study explored cell penetration into the scaffold, and calculated the depth of cellular infiltration. Furthermore, scaffold mechanical properties were evaluated in terms of tensile testing, suture retention, burst pressure and compliance.

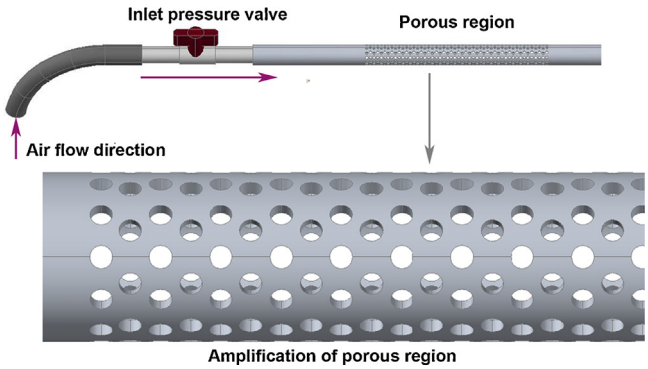

Fig. 1. Diagram of the prototype air-impedance mandrel.

2. Materials and methods

2.1. Silk fibroin extraction

SF was extracted from the cocoons of *Bombyx mori* silkworms (The Yarn Tree, NY, USA) by following a published protocol [28]. Briefly, cocoons were cut into pieces and boiled in a 0.02 M Na_2CO_3 (Sigma Aldrich) solution for 30 min to remove the sericin gum, followed by thorough rinsing in de-ionized water (DI), the boiling and rinsing processes were repeated three times, and then air-dried overnight in a fume hood. The SF was then dissolved in a LiBr (Fisher Scientific) solution at 60 °C for 1 h, (1 g silk raw fiber dissolved in 4 mL (9.3 mole/L) LiBr solution). This solution was then dialyzed against deionized water for 3 days using 12,000–14,000 MWCO dialysis tubing (Fisher Scientific). The SF solution was then frozen and lyophilized to provide pure SF for electrospinning.

2.2. Electrospinning

A polymer of P (LLA-CL) (MW: 300,000, LA to CL mole ratio is 50:50, Gunze Limited, Japan) and SF were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, TCI America) at 70 mg/mL, respectively. P(LLA-CL) and SF solution were blended at volume ratio of 70:30. To create vascular graft scaffolds, the blended solution was loaded into a 5 mL plastic Becton Dickinson syringe with a 16 ga blunt tip needle and dispensed at a rate of 5 mL/h. The needle tip was subjected to +30 kV with an air gap distance of 20 cm between the needle and the grounded mandrel. Electrospinning was performed on both solid and perforated stainless steel mandrels [24]. Fig. 1 is the sketch of perforated mandrel. The perforated mandrel was subjected to a luminal air pressure of 0, 50, 100, 200 and 300 kPa to determine the effect of a range of air-flow rates on fiber deposition and vascular graft structural properties.

2.3. Scaffold morphology characterization

Scaffold morphology characterization was performed using scanning electron microscopy (SEM, JEOL JSM-5610LV, Japan). SEM micrographs were analyzed with a software Image-J (National Institutes of Health). The average fiber diameter was determined by measuring 50 randomly selected fibers in the SEM image. Interfiber distance was measured by determining distance based on both the most superficial fibers and by measuring from one fiber to the next closest fiber (SEM image). Calibration of the Image Tool software was achieved by using the scale bar on each image. Moreover, we evaluated the alignment of fibers which typically deposited on solid and perforated mandrels (within pore regions) according to a previous procedure [29], a two-dimensional fast Fourier transform (2D FFT) approach. In brief, a quadrate region was captured from