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Electrospun biphasic tubular scaffold with enhanced mechanical properties for vascular tissue engineering

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ABSTRACT

Polymer scaffolds produced through an electrospinning process are frequently explored as tissue substitutes for regenerative medicine. Despite offering desirable surface area to volume ratios and tailorable pore sizes, their poor structural mechanical properties limit their applicability in load-bearing regions. In this study, we present a simple strategy to improve the mechanical properties of a vascular graft scaffold. We achieved the formation of biphasic tubular scaffolds by electrospinning polyurethane (PU) onto an airbrushed tube made of polycaprolactone (PCL). After preparation, the scaffold was subsequently thermally-crosslinked (60 °C) to strengthen the bonding between the two materials. The tensile strength and tensile elastic (Young's) modulus of the biphasic scaffolds were significantly enhanced from 4.5 ± 1.72 and 45 ± 15 MPa (PU-only) up to 67.5 ± 2.4 and 1039 ± 81.8 MPa (PCL/PU; $p < 0.05$). Additionally, suture retention force, burst pressure, and compliance were all improved. The cytotoxicity of the fabricated samples was investigated using an MTT assay after 7 days of cell culture and found to be negligible (~100% viability). In conclusion, we have demonstrated the preparation and characterization of a stable and mechanically robust vascular graft scaffold using a novel combination of well-established fabrication techniques. This study could also be extended to the fabrication of other biphasic scaffolds to better enhance the mechanical properties of the electrospun fibers mat without deteriorating its architecture structure.

1. Introduction

Tissue engineering and regenerative medicine is a rapidly expanding field, targeting the regeneration of tissue physiology with the concurrent restoration of specific tissue functions [1]. This goal is facilitated through the development and use of mechanically supportive and biologically inductive scaffold structures. These structures form what is essentially an extracellular matrix (ECM) for the target tissue, encouraging the growth of specific tissues through relevant mechanotransduction and bioactivity [2,3]. The fabrication of scaffolds with a high porosity along with a high surface area to volume ratio is desirable in regenerative medicine, as this allows for cellular infiltration, gas and nutrient exchange, maximizes cell-surface interactions, and ideally forms a biomimetic and inductive environment [4–6].

Electrospinning is a facile and versatile process by which polymer fibers with diameters ranging from a few nanometers to several micrometers can be prepared [7]. It is highly suited to tissue engineering applications as it allows for the formation of highly porous structures with high surface areas and tailorable microenvironments. To form fibers, molten or solvated polymer solutions are extruded under pressure from a nozzle and subjected to a high electrical potential difference between the nozzle and a metal collector plate [8–10], which draws the polymer strand to a diametric range based on the process parameters and the polymer viscosity [11]. These fibers may then be deposited into a desired structural morphology through the use of a stationary collector, moving collector, or rotating mandrel.

Whilst the use of advanced manufacturing technologies for the regeneration of vasculature remains far from widespread clinical use, blood vessel substitutes and other artificial grafts have been applied clinically with varied results. It was recently reported that only 50% of artificial blood vessel grafts survive even two years after implantation [12], which indicates a great potential for growth in this field.

Within this area of research, electrospinning is a prominent approach for the formation of tailored tubular structures. Previous studies testing electrospun scaffolds for regeneration of vasculature [5,13–15] have reported that electrospun fibers provide a favorable structural template for cells, promoting cell adhesion and guiding migration despite the small pore sizes which may potentially reduce cellular

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infiltration. Whilst Papkov et al. found that smaller fiber diameters were mechanically stronger on a fiber-by-fiber scale [16], mechanical properties of porous scaffolds are overall inherently low compared to cast polymer films due to structural porosity (generally ~50–85% [14,15]) and the interactions between scaffold materials. Prominently, Lee and colleagues have attempted to mitigate this by exposing electrospun scaffolds to thermal and vapor crosslinking [5], successfully improving mechanical properties whilst avoiding ultrastructural or grossly observable changes. Overall, several groups, including ours, have reported that one of the major issues associated with the application of electrospun fiber scaffolds as blood vessel substitutes is the poor control over mechanical properties [14,17–20] (summary Table S1 in Supporting Information).

In this study, we evaluated the mechanical properties of biphasic polycaprolactone (PCL)/polyurethane (PU) electrospun fiber tubular scaffolds. PCL is an aliphatic, semi-crystalline and highly elastic polymer with reported break elongation of ~450% [1,16,21]. It is widely used in tissue regeneration research due to its availability as a medical-grade polymer, its biocompatibility, biodegradability, and FDA approval for medical use [14,22,23]. PU is a biocompatible and elastic amorphous polymer known to have desirable mechanical integrity [24,25], abrasion resistance, and fatigue life [26]. PU and PCL are miscible in solution, a phenomenon that may be utilized to create strong bonding at any interfacial regions [27–29]. Whilst Guo et al. [22] prepared vascular scaffolds from PCL/PU composites using a conventional method of solution mixing and showed acceptable cytocompatibility and high tensile strength, they, however, demonstrated a very low tensile elastic modulus. The method we propose herein is able to produce composites of PCL and PU whilst maintaining the desired mechanical properties and morphological structures of electrospun fibers.

The overall aim of this study was to develop a biphasic tubular blood vessel scaffold that provides superior mechanical support than what can be achieved with a single polymer, or through traditional manufacturing techniques. We explored this aim by electrospinning PU onto an airbrushed tube of PCL [30] (Fig. 1A), and subsequently thermally bonding the two materials. We propose that using this approach, pore size and morphology of electrospun constructs may be maintained and the manufacturing process may be simplified, whilst achieving a polymeric matrix with superior mechanical properties to either polymer alone, or to what is achievable with standard blending techniques. We believe that investigations into techniques such as this will provide an avenue for advancing scaffold production technologies as well as enhancing long-term patient outcomes in clinical vascular graft applications.

2. Materials and methods

Polycaprolactone (PCL) pellets were obtained from Sigma-Aldrich Co., USA (704105) with a molecular number (Mn) of 45,000 g·mol^{-1}. Polyurethane (PU) was obtained from Lubrizol Advanced Materials, Inc. (USA). PCL and PU supplied materials were dried at 35 °C for 5 h and 80 °C for 24 h respectively, in a vacuum oven. 5 mL of PCL solution, 5 weight-% (wt%) in tetrahydrofuran (THF; Sigma Aldrich) was poured into a custom-designed airbrush reservoir. The solution was deposited layer-by-layer onto a mandrel rotating at a speed of 100 revolutions per minute (RPM) using compressed air pressure of 450 kPa and a 200 mm distance between the airbrush nozzle and the steel mandrel (Fig. 1). Further details on the airbrush system design and parameters optimization can be found in our previous reports [30,31]. The PCL film thickness was about 300 (henceforth LT) and 600 (HT) μm, and was controlled by the polymer solution spraying time [31]. Subsequently, PU electrospun fibers were directly deposited onto the PCL using the same mandrel and a speed of 100 RPM. The electrospinning fabrication process and parameters control were as previously reported [22,26]. Briefly, the polymer solution was prepared by dissolving 15 wt% of PU pellets (Lubrizol Advanced Materials, Inc., USA) in dimethylformamide (DMF)/Methyl Ethyl Ketone (MEK) solvent (DMF/MEK 50/50%, wt./wt.) (Showa Chemical Co., Ltd., Japan). The electrospinning was conducted at 15 kV with a feed rate of 1.1 mL·h^{-1} using a syringe pump, and a distance of 200 mm between the collector plate and the tip of the needle. This approach was used to form both PU-only (PU) electrospun scaffolds, as well as the two PCL-PU composites (using LT and HT; PCL-PU-LT and PCL-PU-HT). After production, all scaffolds were dried in a vacuum oven for two days to remove any residual solvents. PCL-PU tubular scaffolds were successfully produced with 3.29 mm inner diameter (ID) and a total wall thickness of 910 ± 25 μm (n = 10; outer diameter (OD) = 4.20 mm). The PCL-PU biphasic scaffolds were then heated in an oven at 60 °C for 15 min [32] to thermally fuse the PCL film with the electrospun PU layer. A PU mat was prepared using the same methods for comparison.

2.1. Characterizations

The surface properties of the PU and PCL-PU groups were examined using scanning electron microscopy (SEM; Hitachi TM-100, Japan), operating an accelerating voltage of 10 kV with a 10 cm working distance. Average fiber diameter was quantified from SEM images using ImageJ software. To determine the dimensional stability of the fabricated scaffolds after thermal treatment, the percent change in dimensions was calculated by comparing the total volume of the scaffolds before and after the treatment. The thickness of fabricated scaffolds was measured with a digital micrometer (*IP65 coolant proof*, Mitutoyo, U.S.A.) with a precision of ± 1 μm. The thermal and chemical properties of the fabricated scaffolds were characterized using dynamic scanning calorimetry (DSC; Perkin-Elmer DMA7, heating rate 10 °C·min^{-1}) and Fourier transform infrared spectrometry (FTIR; Thermo Electro AVATAR 380, USA) respectively.

2.2. Mechanical characterization

Tensile testing of the samples (10 × 25 mm^2) was conducted using a universal materials tester (H5K-S; Hounsfield, UK), equipped with a 100 N load cell and using a crosshead speed of 10 mm·min^{-1}. The ultimate tensile strength, maximum force at failure, tensile elastic (Young's) modulus, and elongation percentage at failure were determined from the test data. To investigate the interfacial integration and suture retention capabilities of the biphasic design, flat biphasic samples were prepared. Samples were fixed at one end by the PCL sheet, with the PU component connected to another grip by a monofilament surgical suture (HIDOX®-Monofilament Polydioxanone surgical suture E.P. India). The suture was held over a silicon plate to reduce slippage. Tension was induced at a crosshead speed of 10 mm·min^{-1} until either the sample fractured, or the suture tore. The force at failure was recorded as an indication of applicability to suture fixation methods (n = 3–5 per group).

Burst pressure and compliance testing were conducted on tubular scaffolds (45 mm length). For burst pressure testing, a thin latex tube was placed through a cannulated fixture at one end of the scaffold, while a 60 mL syringe was inserted through a custom cannula at the other end of the scaffold. The pressure within the scaffold was increased until failure occurred and the pressure change was recorded. For compliance testing, the tubular scaffold was immersed in a water bath and cannulated at either end. One cannula was connected to a water column and the other side was connected to a drainage tube. An initial pressure of 120 mm Hg was used, with pressure increased in increments of 10 mm Hg.

2.3. Cytotoxicity assessment

To evaluate possible cytotoxicity of the fabricated samples, a colorimetric MTT assay was used as previously described [20]. Briefly,