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Fabrication of triple-layered vascular scaffolds by combining electrospinning, braiding, and thermally induced phase separation

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ABSTRACT

Triple-layered small diameter vascular scaffolds, which consisted of thermoplastic polyurethane (TPU) and silk, were fabricated in this study for the first time by combining electrospinning, braiding, and thermally induced phase separation methods. These novel vascular scaffolds, which possess three layers of different structures (nanofibrous inner layer, woven silk filament middle layer, and porous outer layer), can mimic the structure of native blood vessels. The vascular scaffolds have a desirable toe region in tensile tests and sufficient suture retention and burst pressure for vascular graft applications. Endothelial cell culture tests showed that a cell layer could form on the inner surface of the scaffold with high cell viability and favorable morphology.

1. Introduction

In recent years, the demand for vascular scaffolds has been growing due to their increased use in bypass surgeries for various cardiovascular diseases. The demand is especially high for small diameter vascular scaffolds. Vascular tissue engineering provides an approach to fabricating artificial scaffolds that can resemble native blood vessels. A native blood vessel consists of three different layers—(i) intima, (ii) media, and (iii) adventitia—and each layer is comprised of different cells [1, 2]. Therefore, the fabrication of vascular scaffolds with a triple-layered structure has attracted significant attention from researchers. To date, most multilayered vascular scaffolds have been based on electrospinning different materials on top of each other [3,4]. However, these scaffolds lack a variety in microstructure and the ability to allow cell penetration. Thermally induced phase separation (TIPS) methods can produce scaffolds with highly porous structures, although the mechanical properties of the scaffolds are relatively low. Therefore, superior vascular scaffolds can be prepared by combining the electrospinning and TIPS processes.

Recently, silk has been promoted as a potential vascular graft due to its biocompatibility with vascular cells [5]. Braided silk tubes possess textile structures and good mechanical properties, thereby making them good candidates for vascular grafts [6]. Among all of the synthetic polymers, polyurethane (PU) is the most widely used material for vascular grafts due to its high flexibility and tear resistance [7]. Vascular scaffolds comprised of PU and silk may possess even better properties [8].

This study aimed to fabricate PU/silk triple-layered vascular scaffolds with various structures using a novel approach that combined the electrospinning, braiding, and TIPS methods. The mechanical properties and endothelial cell viability were tested and are discussed below.

2. Materials and methods

2.1. Materials

Medical grade TPU (Texin® Rx85A) was supplied by Bayer Material Science, Inc. N,N-dimethylformamide (DMF) and 1,4-dioxane were purchased from Sigma-Aldrich. Silk yarn was purchased from Danee Silk International Co., Ltd., Taiwan. All chemicals were used as received without further purification.

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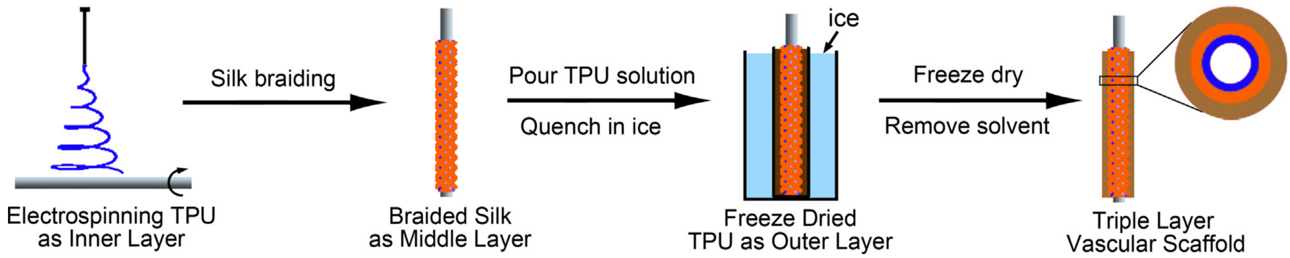

Fig. 1. Schematic illustration of the procedure to fabricate elec–TPU+silk+TIPS–TPU vascular scaffolds.

2.2. Preparation of triple-layered vascular scaffolds

The triple-layered vascular scaffold fabrication approach is shown in Fig. 1. The inner layer was produced by electrospinning the TPU solution (10 wt% in DMF) on a rotating aluminum rod with an 18 kV voltage and a 0.5 mL/h flow rate for 1 h. The silk yarn braiding was performed using a rod with the inner layer as the mandrel via a 36-carrier braiding machine (Kokubun, Japan). The outer layer of the vascular scaffold was prepared by the TIPS method using 8.5 wt% TPU in a dioxane/water (8.5/1.5 vol.) solution. Briefly, a mandrel with inner and middle layers was mounted onto a cylindrical mold and the TPU solution was poured into the gap followed by an ice quench for 20 min. Then the mold was placed in a −80 °C refrigerator (Thermo Scientific) for 6 h and the solvent was extracted by lyophilization using a freeze dryer (LABCONCO) for 5 days. The scaffold prepared was named as elec–TPU+silk+TIPS–TPU. Single-layered electrospun TPU tubes (elec–TPU), TIPS TPU tubes (TIPS–TPU), and double-layered electrospun+TIPS TPU tubes (elec–TPU+TIPS–TPU) were prepared for comparison.

2.3. Cell culture

Human umbilical vein endothelial cells (HUVECs) (Lonza) were used to test the cellular response on the triple-layered scaffolds. Before cell seeding, the 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. The scaffolds were placed in 24-well TCPs and fixed with sterilized polyester double-sided adhesive tape (Adhesive Research Inc.) to prevent scaffold flotation. Cells were then seeded at a density of 5×10^4 cells/cm^2. Spent medium was aspirated and replaced with 1 mL of fresh medium daily.

2.4. Characterizations

2.4.1. Scanning electron microscopy (SEM)

The whole view of the scaffold cross section was taken by a JEOL Neoscope SEM (Nikon) at 10 kV. The high magnification images were taken by a fully digital LEO GEMINI 1530 SEM (ZEISS) at 3 kV. The scaffolds were quenched in liquid nitrogen for 30 min and fractured in cross section, then sputtered with a thin film of gold before imaging.

2.4.2. Mechanical properties

Tensile tests were performed on a mechanical testing machine (Instron 5967) in wet conditions at ambient temperature. Tubular samples were soaked in PBS for 1 h and then stretched with a crosshead speed of 5 mm/min until the samples fractured.

The suture retention strength of the tubular samples was measured according to the standard ISO 7198. Samples were cut into 2 cm lengths and immersed in PBS for 1 h prior to testing. The crosshead speed used to stretch the tube was 5 mm/min until the tube fractured. The maximum load was recorded as the suture retention strength.

The burst pressure strength of the electrospun tubes was estimated using the circumferential tensile strength according to previously published methods [4]. A section of tube with a length (L) of 5 mm was cut and immersed in PBS for 1 h before being stretched in the circumferential direction by two L-shape clamps at a constant rate of 5 mm/min.

2.4.3. Cell viability

The HUVECs viability was assessed 2 weeks after seeding via a Live/Dead Kit (Invitrogen) as the percentage of living cells. This kit allows simultaneous visualization of both live and dead cells. The stained cells were imaged with a Nikon Ti-E confocal microscope.

3. Results and discussion

3.1. Structural analysis

The structure of the prepared vascular scaffolds is shown in Fig. 2. The triple- layered scaffolds had three different layers: a fibrous inner layer consisting of TPU electrospun fibers, braided silk fibrils as a middle layer, and an interconnected porous outer layer. This structure mimics the intima, media, and adventitia structure of native blood vessels. The lumen diameter of the scaffolds was 3.18 mm, and the average thickness was 1.05 mm

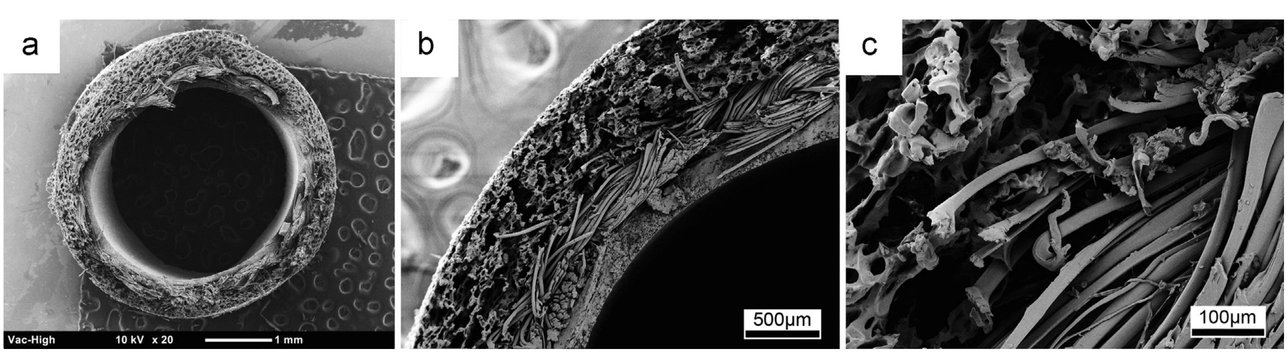

Fig. 2. Morphology of the cross section of the prepared scaffolds: (a) overview of the scaffold; (b) and (c) are images at a higher magnification.