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Co-electrospun blends of PU and PEG as potential biocompatible scaffolds for small-diameter vascular tissue engineering

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ABSTRACT

A small-diameter vascular graft (inner diameter 4 mm) was fabricated from polyurethane (PU) and poly(ethylene glycol) (PEG) solutions by blend electrospinning technology. The fiber diameter decreased from 1023 ± 185 nm to 394 ± 106 nm with the increasing content of PEG in electrospinning solutions. The hybrid PU/PEG scaffolds showed randomly nanofibrous morphology, high porosity and well-interconnected porous structure. The hydrophilicity of these scaffolds had been improved significantly with the increasing contents of PEG. The mechanical properties of electrospun hybrid PU/PEG scaffolds were obviously different from that of PU scaffold, which was caused by plasticizing or hardening effect imparted by PEG composition. Under hydrated state, the hybrid PU/PEG scaffolds demonstrated low mechanical performance due to the hydrophilic property of materials. Compared with dry PU/PEG scaffolds with the same content of PEG, the tensile strength and elastic modulus of hydrated PU/PEG scaffolds decreased significantly, while the elongation at break increased. The hybrid PU/PEG scaffolds demonstrated a lower possibility of thrombi formation than blank PU scaffold in platelet adhesion test. The hemolysis assay illustrated that all scaffolds could act as blood contacting materials. To investigate further in vitro cytocompatibility, HUVECs were seeded on the scaffolds and cultured over 14 days. The cells could attach and proliferate well on the hybrid scaffolds than blank PU scaffold, and form a cell monolayer fully covering on the PU/PEG (80/20) hybrid scaffold surface. The results demonstrated that the electrospun hybrid PU/PEG tubular scaffolds possessed the special capacity with excellent hemocompatibility while simultaneously supporting extensive endothelialization with the 20 and 30% content of PEG in hybrid scaffolds.

1. Introduction

In the last three decades, the demand for artificial blood vessels significantly was increased with a higher incidence of cardiovascular diseases [1]. The main source of the clinical small diameter (<6 mm) vascular grafts was synthetic and autologous blood vessels. The autologous blood vessels could not meet with the supplied need of the small-diameter vascular grafts because of the restrictions on the source, diameter and length [1,2]. The synthetic vascular grafts are encountered in a lot of problems such as thrombosis, intimal hyperplasia and lower long-term patency rates in clinical application [3]. With the development of tissue engineering, it provided a promising access to the source of small-diameter vascular grafts. The structure of artificial blood vessels that could mimic the supramolecular structure and biological functions of the extracellular matrix (ECM) was a key issue in the tissue engineering. Therefore, a significant guideline for the artificial blood vessels could form nano-scale building blocks such as nanofibers [3,4] and its appropriate spatial organization on the mesoscopic scale. Simultaneously, a tissue engineered blood vessel scaffold should have the necessary mechanical strength to withstand the circulatory high pressures and good blood compatibility, which were the breakthrough on the technology of artificial small-diameter vascular grafts [5,6].

From this view point, electrospinning had recently drawn increasing attention as an enabling nanotechnology process for making seamless tubular scaffolds of various diameters and lengths from an assortment of synthetic polymers and natural polymers for vascular graft applications [7,8]. Apart from its simplicity, the tubular scaffolds also can be tailored to be flexible and strong enough to withstand pressurized physiological conditions by controlling the solution composition, properties and spinning conditions, thereby exhibiting properties that resemble native blood vessels [7]. Electrospun scaffolds display a high porosity, with highly connective pores for the exchange of cell nutrients and metabolic waste [7]. Many synthetic polymers, alone or in conjunction with vascular proteins, have been experimentally explored for the construction of small diameter tubular scaffolds by electrospinning. Drilling et al. [9] had used polycaprolactone (PCL) for the fabrication of a burst pressure-competent vascular graft via electrospinning. Pektok

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et al. [10] had reported that electrospun PCL-based small diameter grafts (internal diameter 2 mm) showed better healing characteristics and faster endothelialization in vivo than an expanded polytetrafluoroethylene (e-PTFE) counterpart. Stitzel et al. [11] had created tubular scaffolds based on ternary blends of collagen, elastin and polyglycolic acid (PLGA) with compliances similar to native artery.

Polyurethane (PU), with excellent mechanical property, had been widely applied in many fields of polymeric materials, especially in the field of biomedical materials [12]. It had better biocompatibility than other synthetic polymers because of micro-phase separated structure [13]. However, the poor hydrophilicity and cell affinity of PU became the bottleneck when it was applied in manufacturing the artificial vessels. Polyethylene glycol (PEG) was well known as a biocompatible material and was often used as a hydrophilic polymer for surface modification [14,15]. PEG could be grafted onto biomaterial surfaces and provide a biocompatibility layer which could reduce the absorption of plasma albumen and red blood cell [16,17]. We hypothesized that appropriate mechanical properties and nonthrombogenic scaffolds for application as small-diameter vascular grafts could be produced by blending this two kind of synthetic polymers. Currently, many studies have attempted to investigate the vascular grafts composed of PU, however, to the best of our knowledge, no report involves the small-diameter vascular grafts made of PU and PEG.

This paper focused on fabricating hybrid PU/PEG fibrous small-diameter vascular grafts by the electrospinning technology. The composite of hybrid PU/PEG vascular grafts had testified by attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) and differential scanning calorimetry (DSC). The morphology and hydrophilicity of hybrid PU/PEG scaffolds were studied by scanning electron microscopy (SEM) and contact angle tests. The mechanical properties of hybrid PU/PEG electrospun scaffolds with various weight ratios of PU/PEG were investigated in dry and hydrated condition. The in vitro hemocompatibility of the scaffolds were systematically evaluated by platelet adhesion test and hemolysis assay. Human umbilical vein endothelial cell (HUVECs) morphology and proliferation were measured by SEM and thiazolyl blue assay (MTT) by a culturing period.

2. Materials and method

2.1. Materials

PU (Chronoflex AL 80A) with a number average molecular weight of 110,000 was purchased from Cardio International Incorporated, USA. PEG (Mn = 2000) was purchased from Aldrich. Dissolving solvent of N,N dimethylformamide (DMF) and tetrahydrofuran (THF) were products from sigma. All chemical solvents were of analytical grade without further purification.

2.2. Scaffold preparation

PU was dissolved in 1:1 ratio (v:v) of DMF and THF at 10% (w/v) concentration, stirring at room temperature for 12 h to make transparent solutions. PEG was added to PU solution to prepare blend PU/PEG solutions with the different weight ratios (PU/PEG: 90/10, 80/20, 70/30, 60/40, 50/50). All these different weight ratio solutions were electrospun at room temperature. Electrospinning apparatus included a high voltage DC power supply and a multi terminal distribution box (Model, 0–50 kV, Tianjin Dongwen High Voltage Inc., China). The polymer solutions were delivered from a syringe pump (KDS 220, KD Scientific Inc., USA) fitted with 10 mL syringes having blunt ended metal needles of 21 gauges. The applied voltage was fixed at 20 kV, a distance of 20 cm was maintained between the rotating mandrel-type collector (outer diameter 4 mm, length 15 cm) and spinneret. All the sample solutions were electrospun at a rate of 0.6 mL/h to a grounded rotating mandrel-type collector, and the rotating speed of mandrel was 60 rpm. The spinning time was set 8 h to gain 0.25 mm thickness of scaffolds. Meanwhile, the mandrel was moved traversely at a speed of 45 cm/min to fabricate nanofibrous vessels with uniform thickness. The hybrid PU/PEG scaffolds were placed into a vacuum oven at room temperature to evaporate residue solvents.

2.3. Characterization

The surface tension of solutions was measured with a surface tension meter (Kruss EasyDrop goniometer, Germany) at 25 °C, equipped with a digital photoanalyzer, and 10 μL of electrospun solutions was also used. For conductivity measurements, 10 mL of each solution was taken in a plastic container, and measured with conductivity meter (DDS-307A, Rex Shanghai) at 25 °C. The rotating viscosity of the electrospun solutions was measured using rotating viscometer (Model NDJ-401, Shanghai). All the electrospun solutions were kept at 25 °C to obtain uniform temperature and from which 25 mL of each well-mixed solutions was taken in the built-in stainless steel container of the viscometer. Measurements were done at 100 rpm.

The morphologies and diameters of the hybrid PU/PEG nanofibers were determined with the use of SEM (S2300, Hitachi, Japan). Based on the SEM images, fiber diameter and standard deviations were analyzed using an image analysis program (Digimizer 3.1).

The rectangular samples were cut from the hybrid PU/PEG tubular scaffolds and their length, width and thickness were accurately measured to calculate the volume (V). The dry weight of the samples (mi) was measured with an analytical balance accurated to 10^{-4} g. The density (ρ_i) of each sample was calculated from following Eq. (1) according to volume (V_i) and weight (m_i).

$$\rho = \frac{m_i}{V_i} \tag{1}$$

The porosity (ε) was calculated by using following Eq. (2).

$$\varepsilon(\%) = \left(1 - \frac{\rho}{\rho_0}\right) \times 100\% \tag{2}$$

Where ρ is the density of electrospun scaffold, ρ_0 is the density of the bulk polymer.

Tensile properties of the scaffolds were characterized using an Instron tensile tester (M350-20KN, Testmetric, United Kingdom) under both dry and hydrated conditions. Hydration was accomplished by placing the electrospun scaffolds in the distilled water for 1 h separately at room temperature. The strips ($60 \times 8 \times 0.25$ mm^3) were cut from the tubes, which were measured in tension with crosshead speed of 10 mm/min by the Instron tensile tester. Gauge length was set at 30 mm and a load cell of 100 N was used. The ambient conditions were controlled at 25 °C. All samples were stored under ambient conditions before testing. Three samples of each weight ratio were tested for tensile behavior.

The chemical structures of the hybrid PU/PEG tubular scaffolds were characterized by ATR-FTIR spectroscopy (Bio-Rad FTS-6000, USA). The transmittance of each sample was recorded with between 4000 cm^{-1} and 500 cm^{-1}, with a resolution of 2 cm^{-1}.

The melting and crystallization characteristics of the hybrid PU/PEG scaffolds were investigated by means of DSC (Perkin–Elmer system, USA). DSC measurements were carried out using a Perkin–Elmer system with a heating or cooling rate of 10 °C/min, under temperature ranging from −50 °C to 150 °C and under constant N_2 stream (30 mL/min). About 5–8 mg of sample was used for each measurement, and an empty aluminum pan was used as a reference.

To evaluate the hydrophilic/hydrophobic properties of the hybrid PU/PEG scaffolds, the water contact angle was examined. The samples were cut into a rectangular shape (15 mm × 8 mm). The contact angle