Colloids and Surfaces B: Biointerfaces 82 (2011) 307–315

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Electrospun collagen–chitosan–TPU nanofibrous scaffolds for tissue engineered tubular grafts

Chen Huang[a,b], Rui Chen[a,b], Qinfei Ke[a,b], Yosry Morsi[c], Kuihua Zhang[a,d], Xiumei Mo[a,*]

[a] Key Laboratory of Textile Science & Technology, Ministry of Education, Donghua University, Shanghai 201620, China
[b] College of Textiles, Donghua University, Shanghai 201620, China
[c] Faculty of Engineering and Industry Science, Swinburne University of Technology, Melbourne, Victoria 3122, Australia
[d] College of Biological Engineering and Chemical Engineering, Jiaxing College, Zhejiang 314001, China

ARTICLE INFO

Article history:
Received 30 July 2010
Received in revised form 26 August 2010
Accepted 1 September 2010
Available online 15 September 2010

Key words:
Electrospun
Collagen–chitosan–TPU
Nanofibrous scaffolds
Cell morphology
Tissue engineering

ABSTRACT

The objective of this study is to design a novel kind of scaffolds for blood vessel and nerve repairs. Random and aligned nanofibrous scaffolds based on collagen–chitosan–thermoplastic polyurethane (TPU) blends were electrospun to mimic the componential and structural aspects of the native extracellular matrix, while an optimal proportion was found to keep the balance between biocompatibility and mechanical strength. The scaffolds were crosslinked by glutaraldehyde (GTA) vapor to prevent them from being dissolved in the culture medium. Fiber morphology was characterized using scanning electron microscopy (SEM) and atomic-force microscopy (AFM). Fourier transform infrared spectroscopy (FTIR) showed that the three-material system exhibits no significant differences before and after crosslinking, whereas pore size of crosslinked scaffolds decreased drastically. The mechanical properties of the scaffolds were found to be flexible with a high tensile strength. Cell viability studies with endothelial cells and Schwann cells demonstrated that the blended nanofibrous scaffolds formed by electrospinning process had good biocompatibility and aligned fibers could regulate cell morphology by inducing cell orientation. Vascular grafts and nerve conduits were electrospun or sutured based on the nanofibrous scaffolds and the results indicated that collagen–chitosan–TPU blended nanofibrous scaffolds might be a potential candidate for vascular repair and nerve regeneration.

1. Introduction

Autologous vein and artery segments have been claimed as the gold substitution for the repair of diseased vessels and peripheral nerve [1–3]. While vascular and nerve-related diseases can occur at any age in males and females, they become increasingly common as people get older. In those cases, the use of prosthetic vascular grafts can be offered as alternatives, as suitable autologous substitutions are probably not available. Tissue engineered grafts have been proposed as a promising solution, which involves the incorporation of isolated living cells from patients into three-dimensional scaffolds, followed by the transplantation of this scaffold back into the patient via surgery.

As a recently developed technology, tissue engineering is a multidisciplinary subject that combines genetic engineering of cells with chemical engineering to create artificial organs and tissues, such as skin, bones, blood vessels and nerve conduits [4]. The main challenge for tissue engineered scaffolds is to design and fabricate customizable biodegradable matrices that can mimic the componential and structural aspects of extracellular matrices (ECM) [5]. Native ECM include the interstitial matrix and the basement membrane. Gels of polysaccharides and fibrous proteins (collagen in particular) fill the interstitial space and act as a compression buffer against the stress placed on the ECM. Basement membranes are sheet-like depositions of ECM on which various epithelial cells rest [6]. Based on these facts, this study selected collagen as the protein part and chitosan as the polysaccharide part to fabricate ideal tissue engineered scaffolds.

As the main protein of connective tissue in animals and the most abundant protein in mammals [7], collagen is widely used as biomaterials in wound dressing and medical fields. Chitosan, a massive natural polysaccharide derived from chitin, could be used to replace glycosaminoglycan, which is the main component of natural ECM [8]. Both collagen and chitosan possess good biocompatibility, appropriate biodegradability and commercial availability. Various studies have found that collagen–chitosan complex might be an excellent candidate for tissue engineering due to its good cell viability [8–10]. However, there remains a non-ignorable gap between

* Corresponding author at: Biomaterials and Tissue Engineering, Donghua University, 2999 Renmin Rd. North, Songjiang District, Shanghai 20160, China. Tel.: +86 21 67792653; fax: +86 21 67792653.
E-mail addresses: xmm@dhu.edu.cn, med@dhu.edu.cn (X. Mo).

0927-7765/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2010.09.002

lab activities and clinical trials for the application of this type of complex, as the two materials are both too fragile to provide sufficient mechanical strength, which is indispensable for a successful tubular scaffold. Therefore, thermoplastic polyurethane (TPU) would be a good candidate for reinforcement. As a thermalplastic elastomer, TPU has been widely used as coating materials for breast implants, catheters, and prosthetic heart valve leaflets because of its supreme mechanical properties [11]. Although conventional TPUs are not intended to degrade, they are susceptible to hydrolytic, oxidative and enzymatic degradation in vivo.

In native ECM, interstitial matrix is presented as a three-dimensional structure formed by nanofibers. To architecturally mimic that structure, electrospinning technique was used because electrospun nanofiber matrices are characterized by ultrafine continuous fibers; high surface-to-volume ratio; high porosity and variable pore-size distribution, all of which are morphologically similar to the natural ECM [12]. As a simple but productive method, in recent years electrospinning technique has been widely used in biomedical fields for the production of both nonwoven and regulated matrixes [13–18].

Electrospinning technique provides a simple way to obtain nanofibers from both synthetic polymers and natural materials with the potential for tissue regeneration and repair. Collagen–chitosan electrospun complex and their biocompatibility have been reported previously [8,19], however, so far the scaffolds have not been successfully applied in blood vessel and nerve repair due to the mechanical limitation of natural materials. To overcome the problem, optimal ratio of collagen/chitosan/TPU has been selected to obtain a compromise between biocompatibility and mechanical strength in the present study. Glutaraldehyde (GTA) vapor crosslinking has been conducted to prevent collagen and chitosan from being dissolved in the water. Endothelial cells and Schwann cells were then seeded on the scaffolds to examine if the three-material based scaffold could be a suitable candidate for blood vessels and nerve repair. The orientation of electrospun nanofibers plays an important role in cell growth and related functions [20–24]. Therefore, aligned nanofibrous scaffolds were prepared to regulate cell morphology in this study. SEM and hematoxylin and eosin (H&E) staining images of cultured scaffolds demonstrated that both endothelial cells and Schwann cells have the propensity to grow along the direction of fiber alignment to some extent. Mechanical measurements of random and aligned fibrous matrices indicated that the limitation of natural materials could be solved by adding a low proportion of TPU into the mixture and such type of electrospun fibrous matrices might be a novel biomimetic tissue engineered scaffold in vessel and nerve repair.

2. Materials and methods

2.1. Materials

Collagen I (mol. wt., $0.8–1 \times 10^5$ Da) was purchased from Sichuan Ming-rang Bio-Tech Co. Ltd. (China), chitosan (85%, deacetylated, $M_\eta \approx 10^6$) was purchased from Ji-nan Haidebei Marine Bioengineering Co., Ltd. (China) and TPU polymer (Tecoflex EG-80A) was purchased from Noveon, Inc. (USA). 1,1,1,3,3,3-Hexafluoroisopropanol (HFP) from Fluorochem Ltd. (UK) and trifluoroacetic acid (TFA) from Sinopharm Chemical Reagent Co., Ltd. (China) were used to dissolve the collagen, chitosan, TPU and their blends. A crosslinking agent of aqueous glutaraldehyde (GTA) solution (25%) was purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Porcine iliac artery endothelial cells (PIECs) and Schwann cells (SCs) were obtained from the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, China). All culture media and reagents were purchased from Gibco Life Technologies Co., USA unless specified.

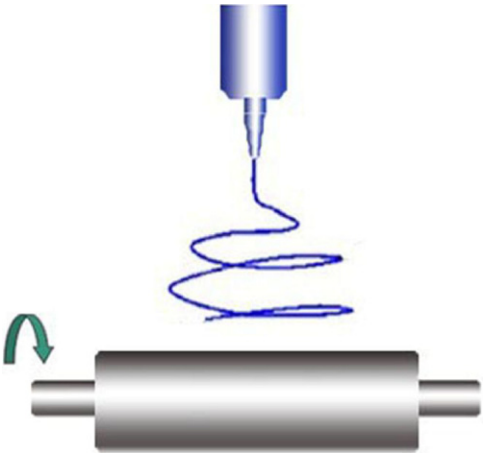

Fig. 1. Schematic diagram of electrospinning spinneret and rotating drum.

2.2. Electrospinning of collagen–chitosan–TPU scaffolds

Collagen (8 wt%) and TPU (6 wt%) were dissolved in HFP while chitosan (8 wt%) was dissolved in HFP/TFA mixture (v/v, 90/10). Before electrospinning, the three solutions were blended at a weight ratio of collagen/chitosan/TPU = 60%/15%/25% with sufficient stirring at room temperature for 1 h. The solutions were then filled into a 2.5 ml plastic syringe with a blunt-ended needle. The syringe was attached to a syringe pump (789100C, Cole-Pamer, America) and dispensed at a rate of 1.0 ml/h. A voltage of 18 kV was obtained from a high voltage power supply (BGG6-358, BMEI Co. Ltd., China) and applied across the needle and ground collector. Random nanofibers were collected on a flat collector plate wrapped with aluminum foil at a distance of 12–15 cm. Aligned nanofibers were formed on a rotating drum with a 6 cm diameter, rotation speed of 4000 r/min and 12 cm away from the tip of the syringe (Fig. 1). To compare orientation degree, pure TPU nanofibers were electrospun using the same parameters as above.

2.3. GTA vapor crosslinking

The crosslinking process was carried out by placing the collagen–chitosan–TPU nanofibrous membrane in a sealed, dual-layered desiccator containing 10 ml of 25% glutaraldehyde aqueous solution in a Petri dish. The membranes were fixed on a glass frame and were crosslinked in an atmosphere of water and glutaraldehyde vapor at room temperature for 2 days. The Petri dish was placed inside the bottom layer of the desiccator, while the nanofibrous membrane was fixed on a glass frame in the upper layer, above the semi-permeable divider. After crosslinking, samples were exposed in the vacuum oven at normal room temperature.

2.4. Characterization

Fiber morphology was observed with a scanning electronic microscope (SEM) (JSM-5600, Japan) at an accelerated voltage of 10 kV. The fibers were coated with gold sputter. Fiber diameters were estimated using image analysis software (ImageJ, National Institutes of Health, USA) and calculated by selecting 100 fibers randomly observed on the SEM images. A two-dimensional fast Fourier transform (2D FFT) approach [25] was adapted to measure fiber alignment in electrospun matrix. Surface properties of the nanofibers were examined using a nanoscope atomic-force microscope (Nanoscope IV, America), in the tapping mode and expressed as height and phase images.