Colloids and Surfaces B: Biointerfaces 152 (2017) 432–439

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Protocols

Coaxial electrospinning multicomponent functional controlled-release vascular graft: Optimization of graft properties

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ARTICLE INFO

Article history:
Received 11 August 2016
Received in revised form 20 January 2017
Accepted 24 January 2017
Available online 27 January 2017

Keywords:
Coaxial electrospinning
Vascular graft
Sustained release
Mechanical properties
Biocompatibility

ABSTRACT

Small diameter vascular grafts possessing desirable biocompatibility and suitable mechanical properties have become an urgent clinic demand. Herein, heparin loaded fibrous grafts of collagen/chitosan/poly(L-lactic acid-*co*-ε-caprolactone) (PLCL) were successfully fabricated via coaxial electrospinning. By controlling the concentration of heparin and the ratio of collagen/chitosan/PLCL, most grafts had the heparin encapsulation efficiency higher than 70%, and the heparin presented sustained release for more than 45 days. Particularly, such multicomponent grafts had relative low initial burst release, and after heparin releasing for 3 weeks, the grafts still showed good anti-platelet adhesion ability. In addition, along with the excellent cell biocompatibility, the fabricated grafts possessed suitable mechanical properties including good tensile strength, suture retention strength, burst pressure and compliance which could well match the native blood vessels. Thus, the optimized graft properties could be properly addressed for vascular tissue application via coaxial electrospinning.

1. Introduction

To fabricate a functional small diameter vascular graft that possesses desirable biocompatibility and suitable mechanical properties has become an urgent clinic demand. Synthesized polymeric materials of Dacron and expanded poly tetrafluoroethylene (ePTFE) have been successfully applied in clinics as large-diameter vascular grafts [1,2], yet they are failed to be utilized as small diameter vascular grafts due to the acute thrombogenicity and poor mechanical properties [3].

Grafts loading with heparin have been studied over the past decades in order to promote the antithrombotic performance [4–9]. After graft implantation, sustained release of heparin is desired during the entire endothelialization process of the lumen. Unfortunately, fast release of heparin and incomplete endothelialization are the main obstacle in current vascular grafts design, which could ultimately result in the graft occlusion.

Coaxial electrospinning is a facile technique which could make core-shell fibrous graft with drugs encapsulated into the inside of the fibers, inducing prolonged release time in vivo [7,10–13]. In these studies, poly(L-lactic acid-*co*-ε-caprolactone) (PLCL) grafts could load various drugs by coaxial electrospinning, and drugs could be sustained with relatively lower initial burst release compared to PLCL grafts blended with drugs. However, drug releasing study showed that most of the drugs were fully released within two weeks, indicating further research is still needed for better controlled release. It is likely that multicomponent grafts loaded with drugs constructed by coaxial electrospinning might be able to improve the release behavior through adjusting the degradation rate of polymers and diffusion rate of drugs. So it is speculated that heparin would be encapsulated into multicomponent vascular grafts fabricated by coaxial electrospinning with controlled released to achieve long-term antithrombotic performance in vivo. Apart from anti-thrombosis property, it is also very important to have desired mechanical properties for a vascular graft, which is crucial for the performance of graft after implantation. A functional vascular graft should possess enough strength to withstand arterial pressure as well as be elastic to match the compliance of native blood vessels [14–16].

In our previous study, electrospun collagen/chitosan/PLCL nanofibrous graft had been fabricated which had great physical properties and biocompatibility [17]. However, the antithrombotic property of the graft had not been considered comprehensively. In this paper, we fabricated a heparin incorporated collagen/chitosan/PLCL vascular graft with both good antithrombotic performance and good mechanical properties. Heparin with different concentration encapsulated into the vascular graft was

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http://dx.doi.org/10.1016/j.colsurfb.2017.01.045
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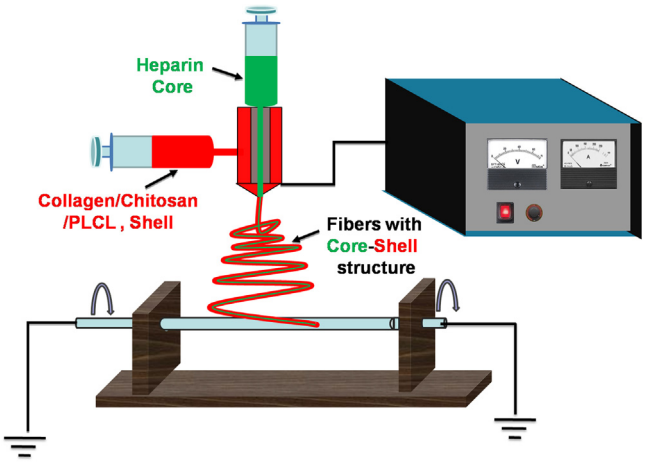

Fig. 1. Schematic diagram of the coaxial electrospinning process.

fabricated by coaxial electrospinning, and the effect of different concentration of collagen/chitosan/PLCL and heparin on the structure of fibrous grafts was further investigated in detail. Meanwhile, heparin release behavior and endothelial cells growth in vitro were examined. In addition, the mechanical properties of grafts in terms of tensile strength, suture retention strength, burst pressure, and compliance were also measured.

2. Materials and methods

2.1. Materials

Poly(L-lactic acid-*co*-ε-caprolactone) (PLCL) polymer (MW: 300,000 Da, LA to CL mole ratio at 50:50, Gunze Limited, Japan) and collagen type I (MW ~10^5 Da, Sichuan Minrang Biotechnology, China) were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, TCI America) at 80 mg/mL, respectively. Chitosan (molecular weight ~10^6 Da, 85% deacetylated, Jinan Haidebei Marine Bioengineering, China) was dissolved in HFP and 2,2,2-trifluoroacetic acid (TFA) (Sinopharm Chemical Reagent, China) (v/v, 9:1) at 80 mg/mL. Heparin (13 kDa, Runjie Medicine Chemical, China) was dissolved in distilled water at different concentration. All reagents used for cell culture were purchased from Gibco Life Technologies, USA, unless stated otherwise.

2.2. Fabrication of grafts by coaxial electrospinning

Before electrospinning, the source solutions (collagen, chitosan and PLCL) were blended at different volume ratios of collagen/chitosan/PLCL. Heparin-loaded grafts were fabricated using a special setup depicted in Fig. 1. For coaxial electrospinning, the core solution (heparin) was injected at a flow rate of 0.2 mL/h which constituted the inner layer of the fibers, while the shell solution (collagen/chitosan/PLCL) was injected at a rate of 1.0 mL/h which formed the outer layer of the fibers. The needle tip was subjected to +12 kV with an air gap distance of 12 cm between the needle and the grounded aluminum foil collector. To fabricate the tubular grafts, the collector was a solid cylindrical stainless mandrel with 4 mm in diameter. The deposited fibrous sheet or conduit was dried in a vacuum oven at room temperature for up to 7 days to remove any residual solvents.

2.3. Morphology characterization of the graft

The 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. Calibration of the Image Tool software was achieved by using the scale bar on each image. Verification of the core-shell structure of the heparin-loaded fiber was conducted by TEM (H-800, Hitachi) at 100 kV. The sample was prepared by collecting the fibers onto carbon-coated copper grids.

2.4. Mechanical properties of grafts

2.4.1. Uniaxial tensile testing

For tensile testing, specimens in a "dog bone" shape were punched from electrospun mats (sample size: 2.75 mm wide at their narrowest point with a length of 7.5 mm) and were hydrated in PBS for 6 h before testing. Uniaxial tensile testing was performed on a MTS Bionix 200 testing system with a 100 N load cell (MTS Systems Corp.) at an extension rate of 10.0 mm/min. Modulus, peak stress, and strain at break were calculated using Test Works version 4 (software).

2.4.2. Suture retention strength

Suture retention strength was measured with a rectangular test sample (10 mm in width/20 mm in length). Before testing, one end of the graft was clamped to one arm of the micro material testing machine (MMT-250N, Shimadzu Co., Japan). A loop of a 5-0 polyester suture (Shanghai Pudong Jinhuan Medical Products Co., Ltd., China) was placed 2 mm from the edge of the free end of the sample and clamped to the other arm which moved at a constant speed of 120 mm/min until failure. The suture retention strength was defined as the peak force obtained during the procedure. All samples were kept hydrated throughout the testing protocols.

2.4.3. Dynamic compliance

Dynamic compliance was determined for tubular grafts with a length of 4 cm under simulated physiological conditions in accordance with Section 8.10 of ANSI/AAMI VP20:1994.32,33 [3]. Grafts were soaked in PBS for 6 h before testing. The specimens were tested in a bioreactor developed by Tissue Growth Technologies (Minnetonka, MN) filled with PBS. The bioreactor provided a cyclic (1 Hz, representing 60 beats per minute) pressure change to the inside of the graft at a pressure level of 120/80 mmHg systolic/diastolic. Prior to compliance measurements, all grafts were allowed to stress relax for 600 cycles. Internal pressure was measured with a pressure transducer capable of measuring dynamic pressure up to 200 ± 2 mmHg, while the external diameter of the graft was recorded with a laser micrometer system with an accuracy of ±0.001 mm. Compliance was calculated through recording of pressure and inner diameter as:

$$\%\,\text{Compliance} = \frac{R_{P_2} - R_{P_1}}{R_{P_1}} \frac{1}{P_2 - P_1} \times 10^4$$

while R is the internal radius, P_1 is the lower internal pressure, and P_2 is the higher internal pressure.

2.4.4. Burst pressure

Burst strength testing of electrospun grafts was completed using a device designed in accordance with Section 8.3.3.3 of ANSI/AAMI VP20:1994.31,32 [3]. Tubes with 4 cm in length were hydrated in PBS for 6 h, fitted over 2.5 mm diameter nipples attached to the device, then a thin latex balloon (Party Like Crazy, Target) was inserted, and the balloon/graft was secured with 2-0 silk suture to the nipples. At last, pressurized air was introduced into the system with increased pressure at a rate of 5 mmHg/s until the tubes