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Characterization of a co-electrospun scaffold of HLC/CS/PLA for vascular tissue engineering

Chenhui Zhu[a,b], Xiaoxuan Ma[a,b], Li Xian[a,b],Yang Zhou[a,b] and Daidi Fan[a,b,*]
[a]*Shaanxi Key Laboratory of Degradable Biomedical Materials, School of Chemical Engineering, Northwest University, Xi'an, Shaanxi 710069, China*
[b]*Shaanxi R & D Center of Biomaterials and Fermentation Engineering, School of Chemical Engineering, Northwest University, Xi'an, Shaanxi 710069, China*

Abstract. Novel scaffolds for vascular tissue engineering were fabricated by co-electrospinning human-like collagen/chitosan and polylactic acid at room temperature and normal pressure. By studying the effects of composition and collecting distance on the morphology of electrospun meshes, we determined that the proper collecting distance and the concentration of the solution are the keys to the success of the co-electrospinning process. The scaffolds were characterized by scanning electron microscopy (SEM) and distribution of the fibrous diametrs was analyzed. Also, Hemocompatibility of the scaffolds were evaluated. The results indicated that scaffolds fabricated by co-electrospinning: (1) had a more biomimetic structure than polylactic acid, as the fiber diameters approached the size of the extracellular matrix; (2) showed better hemocompatibility. This study demonstrates the feasibility of using two different solutions to construct a scaffold for blood vessel tissue engineering by co-electrospinning.

Keywords: Tissue engineering, blood vessel scaffold, co-electrospinning, biocompatibility

1. Introduction

Fibrous scaffolds are commonly used in tissue engineering due to their characteristic high porosities and interconnected pores. Electrospinning [1] is the only cost-effective and mass production way to prepare fibrous structure, with large surface area to volume ratio and nanometer scale fiber, which is similar to the extracellular matrix (ECM) and have ability to guide cells to attach and proliferate on the surface of the scaffold. So, electrospinning gained much popularity recently to construct different tissue engineering scaffolds, such as blood vessel [2], cartilage [3], dermis [4].

Electrospun nanofibers are mostly manufactured from natural polymers due to their similar chemical and physicological properties with ECM [5,6]. However, some biomaterials show lower mechanical integrity. In comparison, synthetic polymer are much easier to electrospin and have higher me-

*Corresponding author: Daidi Fan, Shaanxi Key Laboratory of Degradable Biomedical Materials, School of Chemical Engineering, Northwest University, Xi'an, Shaanxi 710069, China. Tel.: 13809193640; Fax: 029-88302223; E-mail: fandaidi@nwu.edu.cn.

chanical property, but absent of signal molecules and leads to poor cell adhesion. Therefore, co-electrospinning of two polymers has been investigated for the preparation of multi-functional electrospun fibers. In this study, we used human-like collagen and chitosan as biomaterials and polylactic acid (PLA) as synthetic to fabricate tubular blood vessel.

Human-like collagen (HLC) is expressed by recombinant *Escherichia coli* with a modified cDNA fragment transcribed from the mRNA coding for human collagen. Compared to animal derived collagen, HLC is of lower immunogenicity, free of pathogens, easily soluble in aqueous solutions [7]. Due to its excellent biocompatibility, HLC has been used for construction of different scaffolds, such as bone [8], blood vessel [7].

Chitosan is now widely used in tissue engineering for its analogous structure with glycosaminoglycan (GAG) in the ECM and cationic nature. In our previous work, HLC/chitosan as scaffold material using freeze-drying and electrospinning has been investigated and showed good compatibility. Though HLC/chitosan electrospun nanofibrous scaffold [9] presented poor mechanical property, it was the first time to confirm that HLC/chitosan could be electrospun into nanofibrous structures using aqueous solution.

Following the work of Lan Chen et al, in this study, we combine HLC/chitosan and PLA using a dual extrusion electrospinning setup with a rotating mandrel to fabricate tubular blood vessel scaffold, and improve the mechanical properties of vascular scaffold. In addition, the physicochemical properties and biocompatibility were investigated.

2. Materials and methods

2.1. Material

HLC (Mw=97 kDa) was expressed by recombinant *Escherichia coli* in our lab (China patent number: ZL01106757.8).

Chitosan (CS,Mw = 550 kDa, 75%-80% degree of deacetylation) was obtained from QingDao Ocean Ltd. Co. (China). Poly (ethylene oxide) (PEO) (Mw = 900 kDa) was purchased from Changchun Dadi Fine Chemical Ltd. Co. (China). Polylactic acid (PLA) was obtained from Shandong.

Glutaraldehyde (GA), as a 50% water solution, was purchased from BBI. All other solvents and reagents used were of analytical grade.

In the initial investigation of electrospinning, various weight percents of pure HLC (0.5-20 wt%) in deionized water, pure chitosan in aqueous acetic acid solutions (10-90% v/v) (1-5 wt%) and blends of PEO (3-6 wt%) and HLC (2.5 wt%) in deionized water were prepared. Polylactic acid (PLA) was dissolved in 1, 1, 1, 3, 3, 3-Hexafluoro-2-propanol (HFIP), and the weight percent ranged from 10-18 wt%.

2.2. Preparation of the electrospinning solution

Approximately 0.2 g of HLC was dissolved in 14 mL of double-distilled water, 5% chitosan was dissolved in 80% aqueous acid, 6% PEO was dissolved in deionized water, and polylactic acid (PLA) was dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP). After the polymers were completely dissolved, the solutions were stored at 4°C. The solutions were allowed to warm to room temperature for 15 min prior to electrospinning.

Table 1

Blended solution for electrospinning

sample	HLC conc. (Wt %)	chitosan conc. (Wt %)	PEO conc. (Wt %)	(H+C+P)v/PLAv
s1	5	2.5	1	-
s2	7	2	1	-
s3	10.5	1.8	1	-
s4	5	2.5	1	4/1
s5	7	2	1	4/1
s6	10.5	1.8	1	4/1
s7	0	2.5	1.2	-
s8	0	2	1.5	-

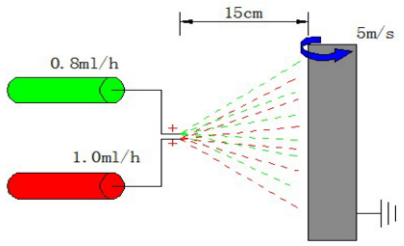

Fig. 1. Schematic diagram of the electrospinning apparatus and process.

Spinning solutions with various HLC/chitosan/PEO ratios were prepared by mixing the above stock solutions. The combined solutions required approximately 4 min of magnetic stirring to become homogeneous and were used within 24 h after blending.

Table 1 shows the different blended solutions used for electrospinning, and fibers were produced according to these formulations.

2.3. Electrospinning apparatus

The electrospinning apparatus was set up as shown in Figure 1. Electrospinning is a mature technique that is used extensively in tissue engineering. As described in previous reports, the electrospinning apparatuses consist of a syringe, a blunt needle with an outer diameter of 0.8 mm as the spinneret, a syringe pump used to provide a steady flow of solution, a high voltage supply and a collector. In our study, we electrospun two different solvents, which required us to modify and improve the traditional electrospinning apparatus. Specifically, we used two 20-mL syringes with blunt needles to separate the two solutions. One syringe discharged the solution containing HLC/chitosan/PEO, and the other discharged the PLA solution. Meanwhile, two syringe pumps (Langer, Baoding, China) were needed to provide a steady solution flow. A high voltage supply was also needed (typically in the range of 5-40kV). In addition, we had a series of collectors with diameters in the range of 3.02-5.00 mm.

The solutions were electrospun at ambient temperature, and the moisture was below 65. During the electrospinning process, a needle was connected to each syringe, which was fixed in a syringe pump. The high voltage power source was connected to the tips of the needles and supplied a steady electrostatic field between the needles and the collector. Because of the electrostatic field, the needle was set horizontal and was far away from the syringe pump and connected with long pipe insulation. The col-

lectors were cylindrical pieces of metal with diameters in the range of 3.02-5.00 mm. In addition, the tip-collector distance plays an important role in the electrospinning process, and it is crucial to have enough distance to ensure that the solvents fully evaporate.

2.4. Crosslinking of the electrospun matrix

The electrospun nanofibrous matrices on the collectors were immersed in a 95% (v/v) ethanol solution containing 0.2% (v/v) glutaraldehyde for 36 h at 37°C for crosslinking. After crosslinking, the matrices were washed in distilled water using a decolorization shaker to remove the residual glutaraldehyde. The distilled water was changed every 2 h, and the process lasted for 2 d.

2.5. Scanning Electron Microscopy (SEM)

To analyze the morphology of the scaffolds and the diameter of the nanofibers, the surfaces of all the samples were observed with a field emission scanning electron microscope (S-3400, Hitachi, Japan).

2.6. Hemocompatibility

For use of the scaffolds in vascular replacement applications, it is necessary to test their blood compatibility. For a hemolysis test, blood was drawn from healthy adult volunteers by venipuncture, collected in a potassium oxalate anticoagulant vacutainer tube and then diluted with 0.9% saline. The vascular scaffolds were washed with distilled water and incubated for 30 min at 37°C. Subsequently, 0.2 mL of diluted blood was added into a test tube with a scaffold and incubated for 60 min at 37°C. In addition, 0.2 mL of diluted blood was added to 10 mL of distilled water as a positive control, and 0.9% saline solution was used as a negative control. After 60 min of incubation, all of the samples were centrifuged at 850 rpm for 5 min. Then, the absorbance of the supernatant was measured at 540 nm using a spectrophotometer (MULTISKAN MK3, Thermo Electron Corporation). The hemolysis percentage (HP) was calculated using the following equation:

$$HP(\%) = (Dt\text{-}Dnc) / (Dpc\text{-}Dnc) \times 100\%$$

where the Dt is the absorbance of a test sample, Dnc is the absorbance of the negative control and Dpc is the absorbance of the positive control. Every sample was measured three times.

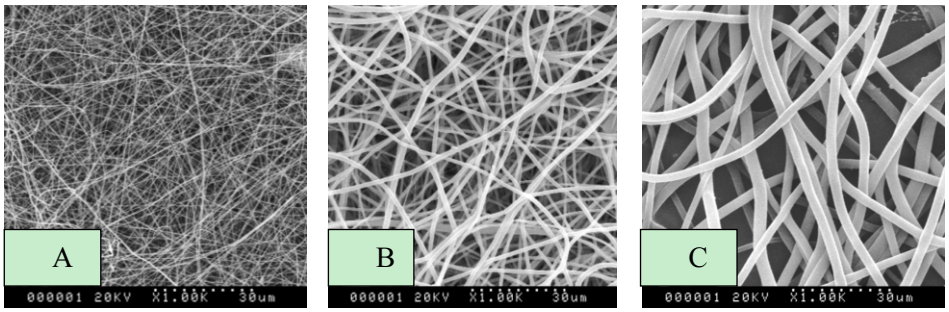

Fig. 2. SEM micrographs of electrospun vascular scaffolds from HLC/chitosan/PEO (A), HLC/chitosan/PEO/PLA (B) and PLA (C).

2.7. Statistical methods

The data are expressed as the mean plus/minus the standard deviation. The data were analyzed by a one-way ANOVA, performed using SPSS 11.0, and statistically significant values were defined as $p<0.05$.

3. Results

3.1. Scaffold morphology

SEM micrographs of electrospun meshes produced from HLC/chitosan, HLC/chitosan/PLA and PLA are shown in Figure 2.

Preliminary experiments indicated that the viscosity requirements of the electrospinning process required an HLC concentration of 8 wt%-15 wt%, a chitosan concentration of 30 wt%-70 wt% and a PEO concentration of 2 wt%-6 wt%. The PLA was dissolved in 1, 1, 1, 3, 3, 3-Hexafluoro-2-propanol (HFIP), and its optimum concentration for electrospinning was 13 wt%. Some experiments have shown that the co-electrospinning of hydrophilic and hydrophobic materials requires a more accurate collecting distance. Initial experiments showed that fibers obtained by adjusting the collecting distance had different appearances, as observed by scanning electron microscopy of the scaffolds. More homogenous fibers were obtained at a distance of approximately 15 cm.

Figures 2A and 2C show SEM micrographs of fibers electrospun individually from the hydrophilic and hydrophobic materials individual, and Figure 2b shows a SEM micrographs of fibers that consist of the hydrophilic and hydrophobic materials and were obtained by co-electrospinning. The fibers shown in Figure 2A were made of HLC, chitosan and PEO using a voltage of 20 kV. The fibers are more numerous and the diameters are smaller. The average diameter of these fibers was 148 nm, which can be observed in the SEM micrograph and was measured using Image J. The diameter size distribution in the corresponding histogram (Figure 3a) indicates that the fibers were uniform in size around the average of 148 nm. The fibers shown in Figure 3c were made of PLA at a concentration of 13wt%. As can be observed in the SEM micrograph and the diameter size distribution in the corresponding histogram (Figure 3c), the fibers were larger and exhibited an average fiber size of 2.899 μm. Obviously, the mean size of the fibers made of the hydrophobic materials is bigger than that of the fibers made of the hydrophilic materials. Figure 3b is the SEM micrograph of the fibers made from both the hydrophilic and hydrophobic materials by co-electrospinning. The histogram of diameter sizes for these fibers showed that the mean size was approximately 694 nm. In the co-electrospinning process, the evaporation of water from the hydrophilic materials leads to a local increase in the air humidity, which will decrease the evaporation rate of the organic solvent. Hence, a larger collecting distance is needed. All of the above factors result in the diameter sizes becoming thinner than when only organic solvent are used.

3.2. Hemocompatibility

Hemocompatibility is an important property for a blood vessel substitute. Different from inflammation and migration, this property determines whether the vascular graft can exist for a long period. As shown in Figure 4, the S5 and S6 samples had low hemocompatibility, approximately 0.3%. The S7 and S8 samples had higher hemocompatibility, possibly due to interactions between the chitosan and

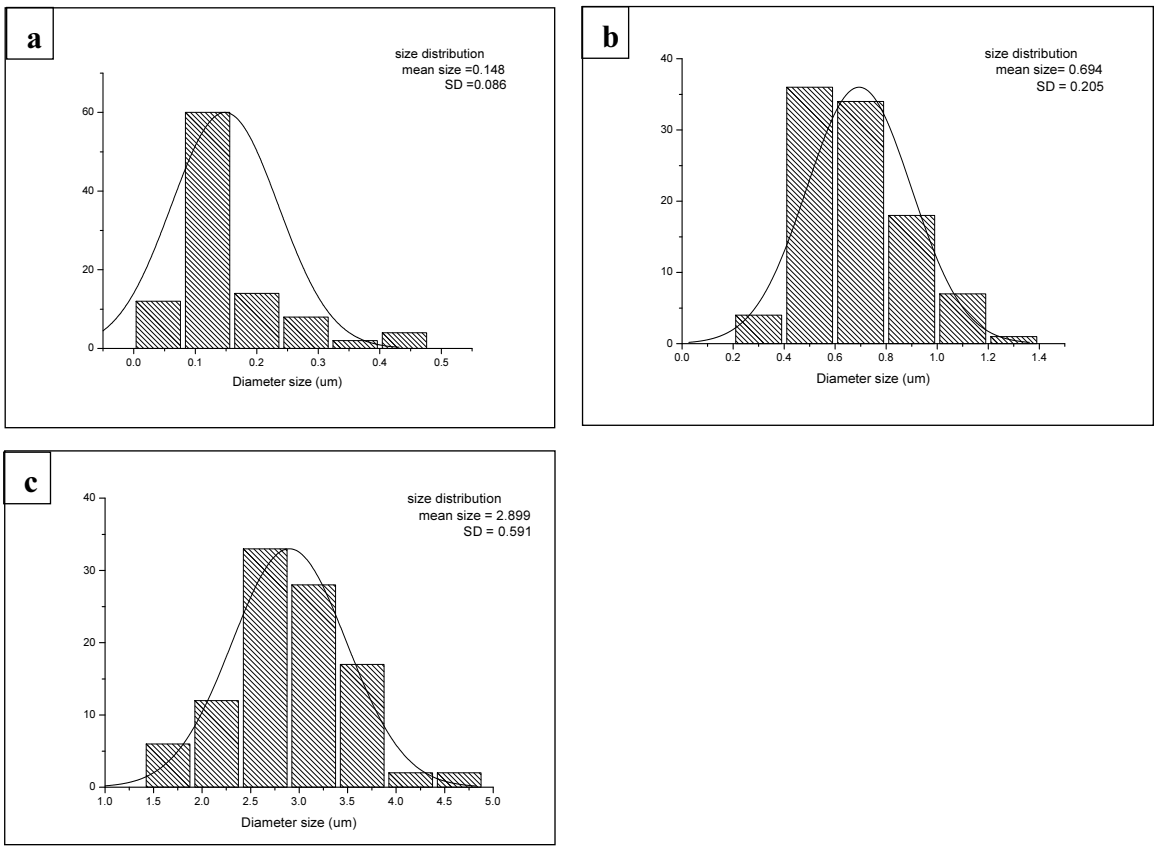

Fig. 3. Histograms showing the fiber size distributions for the images shown in Figure 2 (a to A, b to B, c to C).

blood cells. NH_3- may prevent lysis of the blood cells because HLC can interact with the NH_3- groups, thereby reducing the hemocompatibility.

4. Conclusion

This work demonstrated the feasibility for co-electrospinning hydrophilic and hydrophobic materials to fabricate blood vessel grafts. The natural materials HLC and chitosan were co-electrospun with PLA, which improved the mechanical properties of the grafts. Meanwhile, the biocompatibility was improved compared to the PLA polymer alone. This study also demonstrated that materials dissolved in two different solvents could be electrospun to make scaffolds for tissue engineering.

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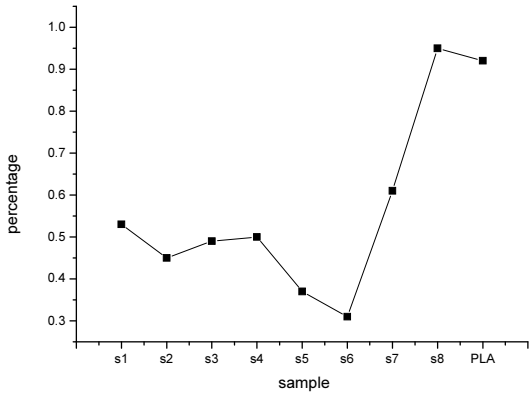

Fig. 4. Hemocompatibility test.

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