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Electrospun Hemocompatible PU/ Gelatin-Heparin Nanofibrous Bilayer Scaffolds as Potential Artificial Blood Vessels

Heyun Wang[1], Yakai Feng[1,2], Haiyang Zhao[1], Ruofang Xiao[1], Jian Lu[1], Li Zhang[1], and Jintang Guo*[,1,2]

[1]School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, P. R. China
[2]Tianjin University-Helmholtz-Zentrum Geesthacht, Joint Laboratory for Biomaterials and Regenerative Medicine, Weijin Road 92, Tianjin 300072, P. R. China, Kantstr. 55, 14513 Teltow, Germany

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Introduction

Cardiovascular diseases are one of the most frequent causes of death all over the world. Particularly in recent years, coronary heart diseases showed a significantly increasing trend. Nowadays artificial blood vessels have become an important implant for the treatment of a serious stenosis or occlusion of blood vessels in clinic. Synthetic polymers, such as polytetrafluoroethylene and polyurethanes, have been successfully used as large diameter artificial blood vessels, but long term patency of small artificial diameter vessels (<6 mm) is not satisfactory due to thrombosis and intimal thickening. With the development of regenerative medicine, tissue engineering strategies were explored to bioengineer blood vessels.[1] An artificial scaffold shall be applied to generate for the basis to create a small diameter blood vessels. The inner layer of such vascular scaffolds is designed to form a continuous layer of endothelium to prevent thrombosis and consecutive clogging. Furthermore, it is essential that the bioengineered scaffolds should have adequate mechanical properties similar to those of native vessels.

Electrospinning technique is known as an efficient processing method to manufacture nanofibrous structures. This processing method is being explored as well for drug release systems, wound dressings, and scaffolds for bone regeneration.[2,3] A non-woven nanofibrous structure shall mimick the nano structure of fibrous extracellular matrix components.[4,5] Moreover a tubular geometry of desired diameter could be created by electrospinning for seeding smooth muscle cells and endothelial cells as basis to bioengineering a vessel.[6] Polyurethanes (PUs) have been widely applied in many fields, especially as biomedical materials owing to their excellent elasticity, mechanical properties and biocompatibility. PUs often posses a micro-phase separated structure, which is thought to contribute to hemocompatibility of these polymers.[7,8] Recently, we reported about nano- or microfibrous PU-membranes and tubes with a uniform structure and controllable fiber diameters by electrospinning. But the low hydrophilicity and cell affinity of PUs became a bottleneck when they were used as scaffolds for artificial blood vessels.

Gelatin is a natural degradable polymer derived from collagen. Gelatin has many merits, such as biodegradability, biocompatibility, and commercial availability at relatively low cost. Gelatin has been found to improve spreading and proliferation of endothelial cells.[9] Therefore, it has been widely explored for medical applications such as scaffolds and microspheres for tissue-engineering and drug delivery systems.[10] Heparin is a highly-sulfated linear glycosamine, which plays a critical role in the regulation of the blood clotting cascade and therefore has been widely used as an anticoagulant.[11] The application of antiproliferative agents to the localized adventitial surface of injured blood vessels has been previously found to be effective in reducing stenosis.

The ideal artificial blood vessel should have appropriate mechanical properties, compliance matching of nature vessels, non-thrombogenicity, high tissue compatibility. Constituents based on biopolymers and PUs could contribute to the mimicry of the characteristics of native blood vessels meanwhile the PUs nanofibrous scaffolds could improve the mechanical properties of the scaffolds. In this paper, we aimed to develop a bilayer nanofibrous scaffold, which mimicks the morphological and mechanical properties of a native blood vessel. A bilayered construct was prepared by sequential deposition of gelatin layer and PU layer by electrospinning on a rotating mandrel-type collector. Bilayered tubular scaffolds composed of elastic PU fibers as the outside-layer and hemocompatible gelatin-heparin fibers as the inner-layer. Heparin retained its biological activity after the electrospinning process. The release of heparin over an appropriate period of time *in vitro* was achieved, which makes the tubular scaffold a potential candidate as scaffold for artificial blood vessels.

Experimental

Materials. PU (Chronoflex C) with a weight average molecular weight (M_w) of 110,000 g·mol^{-1} was supplied by Cardio International Incorporated, USA. Gelatin type A (approximately 220 Bloom, viscosity 4.5 mPa·s) was purchased from Guangfu Chemical Co. (Tianjin, China). Other reagents were

*Corresponding Author. E-mail: jintang_guo@hotmail.com

of analytical grade without further purification.

Characterization of Polymer Solutions. The conductivity of the polymer solutions was determined by means of a conductivity meter (Model DDS-307A, Shengbang Scientific Instruments Co. Ltd. Tianjin, China) at 25 °C. The rotational viscosity of polymer solutions was measured using rotational viscometer (Model NDJ-79, Shanghai, China) at 25 °C.

Electrospinning. Electrospinning apparatus included a high voltage DC power supply (Model DW-P503-1ACCC, Tianjin Dongwen high voltage power supply company, China). The polymer solutions were delivered from a syringe pump (ML-749000-50, Cole-Parmer Instrument Company) fitted with 10 mL syringes having blunt ended metal needles of 21 gauges. The positive lead from the high voltage supply was attached *via* an alligator clip to the external surface of the metal syringe needle (Figure 1). The mandrel target (10 cm in length and 4 mm diameter) was mounted with a distance of 20 cm from the syringe tip, and rotated with a fixed speed. The mandrel was reciprocally moved at a speed of 45 $cm \cdot min^{-1}$ to fabricate nanofibrous vessels with uniform thickness. Gelatin-heparin solution and PU solution were sequentially electrospun onto the mandrel. The electrospinning parameters for each polymer were set as follows (listed in the order of solvent, solution concentration, voltage, air gap, volume flow rate, spinning time, and rotation speed of mandrel): (a) gelatin-heparin (inner-layer): acetic acid, gelatin 18 w/v-%, heparin 1 or 5 wt%, 18 kV, 20 cm, 0.6 $mL \cdot h^{-1}$, 60 min, and 50 $r \cdot min^{-1}$; and (b) PU (outside-layer): DMF/THF (1:1 volume ratio), 10 w/v-%, 20 kV, 20 cm, 0.9 $mL \cdot h^{-1}$, 180 min, and 250 $r \cdot min^{-1}$. Crosslinking of the gelatin-heparin fibrous scaffolds was achieved by placing the scaffolds in a glutaraldehyde ethanol solution (1/100 (v/v)) for 24 h at 20 °C.

Microscopic Analysis. The samples were coated with platinum using a sputter-coater operated at 15 kV for 3 min. The morphologies of the PU/gelatin-heparin composite tubular scaffolds were observed by SEM (S2300, Hitachi, Japan).

Tensile Tests. Mechanical properties of the wetted scaffolds with PU 98.8 wt% and gelatin 1.2 wt% (heparin 1 wt% in gelatin/heparin solution) were characterized using an Instron tensile tester (Model 3365 Issaquah, WA) at room temperature. The crosshead speed was 10 $mm \cdot min^{-1}$, gauge length was set at 30 mm and a load cell of 100 N was used. Five samples of the scaffolds were tested.

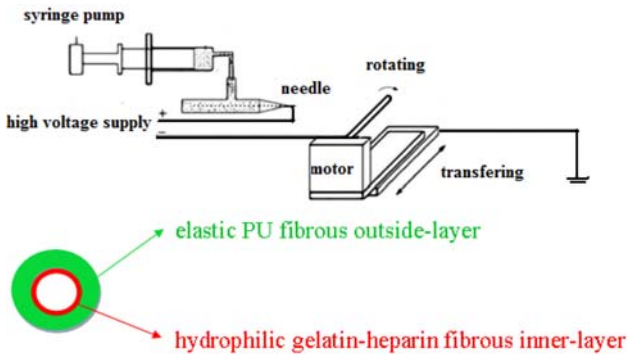

Figure 1. Schematic illustration of the electrospinning equipment and PU/gelatin-heparin tubular scaffolds.

***In vitro* Release Experiments.** Gelatin-heparin fibrous scaffolds (3×1 cm^2) were placed in 25 mL of PBS solutions incubated and protected from light over a 14-day period in a shaking water bath (37 °C, 100 rpm). The concentration of heparin was determined using UV-2100 Visible Spectrophotometer (UNICO Instruments Co. Ltd) at 625 nm by the toluidine blue method.[12]

Blood Compatibility Test. Platelet-rich plasma (PRP) was prepared by collecting rabbit blood in plastic syringes with 3.8 w/v-% trisodium citrate solution at a ratio of 9:1, the complex of rabbit blood and trisodium citrate solution were centrifuged at 3,000 rpm for 10 min to obtain PRP. The PU scaffolds, crosslinked gelatin scaffolds and crosslinked gelatin-heparin scaffolds were rinsed with PBS (pH 7.4) and then immersed in PRP for 180 min at 37 °C to allow interaction between the PRP and the scaffolds. Subsequently, the scaffolds were washed repeatedly with PBS to remove the un-adhered platelets and then immersed in a PBS solution containing glutaraldehyde (2.5 wt%) for 1 h to fix the adhered platelets. The scaffolds were then washed again with PBS and subsequently dehydrated using a series of ethanol-water mixtures (30, 40, 50, 60, 70, 80, 90, and 100 wt% of ethanol). Finally, the scaffolds were dried in vacuum at 25 °C for 24 h and examined using SEM.

Results and Discussion

Morphology of Gelatin-Heparin Fibrous Scaffolds. In order to prepare a homogeneous solution of gelatin containing heparin for electrospinning, heparin was firstly dissolved in water and then mixed with a gelatin-acetic acid solution. The surface tension of gelatin-heparin solutions increased slightly with increasing heparin content from 0 to 1 wt%. The solution viscosity decreased significantly due to the ionic groups of heparin. Meanwhile, the conductivity of the solutions increased from 151 $\mu s \cdot cm^{-1}$ to above 1292 $\mu s \cdot cm^{-1}$ compared with gelatin solution. Heparin enhances the ionic strength and the conductivity of the electrospinning solutions, which resulted in the decrease of the average fiber diameters. Meanwhile, heparin resulted in high charge density of the gelatin jet during the electrospinning process. The increased charge density imposed higher elongation force and thinning force on the jet when it passed the electric field, resulting in fibers of smaller diameter.[13] The microstructures of electrospun gelatin-heparin fibers are shown in SEM images (Figure 2). The average diameter of the gelatin fibers was found to be 440±180 nm when gelatin-acetic acid solution without heparin was electrospun.

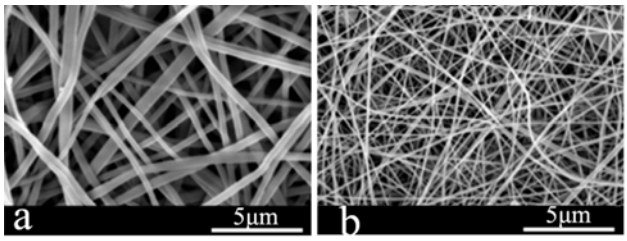

Figure 2. SEM images of gelatin fibrous scaffolds (a) and gelatin-heparin (1 wt%), (b) fibrous scaffolds.

When heparin content was 1 wt% (relative to gelatin weight), the average diameter of smooth gelatin-heparin fibers decreased significantly to 140±30 nm.

Morphology of PU/Gelatin-Heparin Tubular Scaffolds. The bilayer PU/gelatin-heparin tubular scaffolds were prepared by electrospinning to form a randomly oriented fibrous gelatin layer on the rotating mandrel and subsequently electrospinning PU solution on it. The obtained PU/gelatin-heparin bilayer tube could be easily removed from the mandrel without detachment of the layers (Figure 3). The bilayers attached well with each other, and no interlayer between them was observed, because the first layer was electrospun and dried, and subsequently PU solution was electrospun to form the second layer. The gelatin-heparin inner-layer with thickness of 5 μm showed a porous fibrous structure with interconnecting pores of 1.34 μm average pore size. The average diameter of uniform gelatin-heparin nanofibers was 140±30 nm, while the PU outside-layer with thickness of 0.4 mm consisted of fibers with diameters between 590 and 1,080 nm, which were significantly larger. The size of the interconnecting pores of PU outside-layer (around 1.60 μm) was slightly higher than that in the gelatin-heparin inner-layer.

Mechanical Properties of PU/Gelatin-Heparin Tubular Scaffolds. Appropriate mechanical properties of scaffolds under physiological conditions are one of the most important evaluation criteria for scaffolds as artificial grafts. As expected, under wet conditions the stress at break and the elastic modulus decreased significantly, but the elongation at break increased, which resulted from the hydration effect of the gelatin-heparin scaffolds when immersed in water. When the PU/gelatin-heparin bilayer scaffolds were immersed in water for 1 h, the stress at break decreased slightly to 2.0 MPa, but the elongation at break increased up to 150%. Elastic PU layer could improve the flexibility and decrease the rigid property of the gelatin layer. The bilayer tubular scaffolds had both appropriate stress and high elongation at break to maintain the elasticity under a periodically loaded stress field. They had desirable tensile properties for vascular grafts, which were generally accepted to be 1.0 MPa (stress at break) and 40.0% (elongation at break).[14]

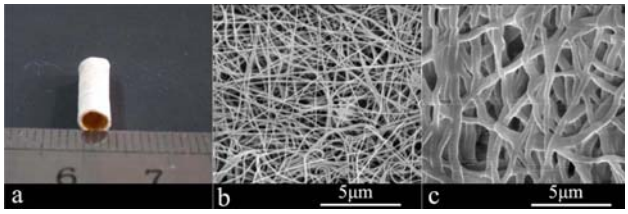

Figure 3. Photo and SEM images of the PU/gelatin-heparin tubular scaffolds. Photo of the tubular scaffold with a ruler (a), SEM image of inner-layer of scaffold (gelatin-heparin, heparin 1 wt%) (b), and SEM image of outside-layer of scaffold (PU) (c).

The Heparin Release and Hemocompatibility of the Gelatin-Heparin Fibrous Scaffolds. The immediate release of heparin from the fibrous scaffolds may be beneficial in preventing the myoproliferative response as heparin treatment already begun at the time of injury. This is more effective than delayed administration. Within the first day, 18.5% of heparin has been released from gelatin-heparin scaffolds in PBS at 37 °C when heparin was 1 wt% in the gelatin-heparin scaffolds. However, when heparin was 5 wt%, the 1st day release amount was slight low (17.2%). The loaded heparin on the scaffold surface dissolved and diffused rapidly in the 1st day. After the significant burst, the heparin was released with an approximately uniform rate until the 9th day. From Figure 4, the release rate of heparin was determined as 1.72%/d and 1.61%/d during 2nd to 9th day for 1 and 5 wt% heparin, respectively. The gelatin-heparin scaffolds with higher heparin content did not show a higher release rate. Subsequently, the cumulative release amount of heparin increased slightly to an overall value 33.0% after 14 days. This unusual result might be due to the heparin is bound by glutaraldehyde during crosslinking reaction. Owing to the gradual release of heparin, the bilayer tubular scaffolds might have high hemocompatibility for potential application as artificial blood vessels. After 14 days immersion in

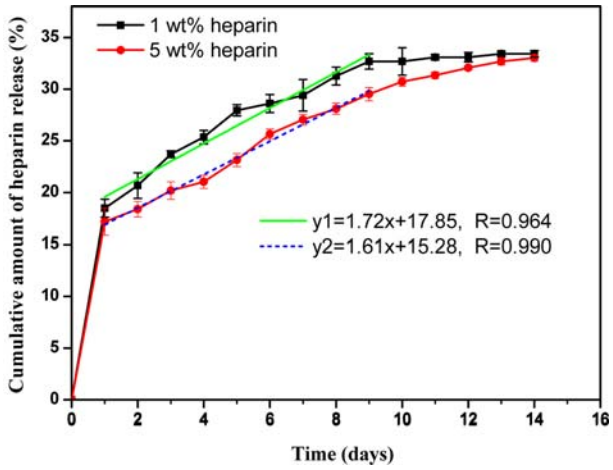

Figure 4. Heparin release curves of crosslinked gelatin-heparin fibrous scaffolds in PBS at 37 °C. The data fitting results of heaprin release from 1st to 9th day are shown in green solid line and blue dash line for 1 and 5 wt% heparin, respectively.

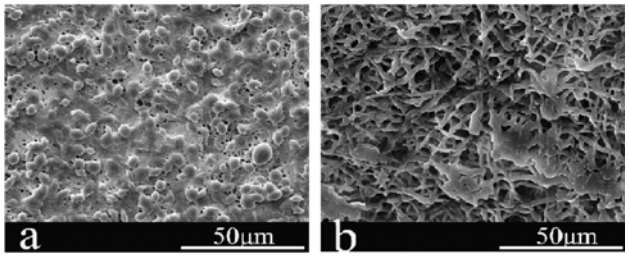

Figure 5. SEM images of platelet adhesion on fibrous scaffolds. PU scaffold (a) and crosslinked gelatin-heparin scaffold (heparin, 5 wt%) (b).

PBS, the bilayer scaffold showed high structural stability and high retention of mechanical properties.

The platelet adhesion test showed apparently that many platelets with a few filopodia adhered on the PU fibrous scaffold surface, while the gelatin-heparin (5 wt%) fibrous scaffold exhibited a significant suppression of platelet adhesion and rare platelets were observed (Figure 5). The hemocompatibility of PU/gelatin-heparin scaffolds was improved efficiently with the gradual release of heparin. The results indicate clearly that PU/gelatin-heparin scaffolds were less likely to initiate thrombosis when used as substitute of the small-diameter artificial blood vessels.

In summary, tubular scaffolds composed of a polyurethane fibrous outside-layer and a gelatin-heparin fibrous inner-layer were fabricated by bilayering electrospinning technology. The scaffolds had the desirable breaking strength and elongation at break, mimicking the elastic properties of natural blood vessels. Heparin release from the gelatin-heparin scaffolds was uniform from 2nd to 9th day, resulting in rare platelet adhesion in *in vitro* tests. Owing to the gradual release of heparin, microstructure and mechanical properties of the tubular scaffolds, the high hemocompatible fibrous scaffolds have a high potential as artificial blood vessels.

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References

(1) F. Jung, C. Wischke, and A. Lendlein, *MRS Bull.,* **35**, 607 (2010).
(2) Y. K. Feng, F. R. Meng, R. F. Xiao, H. Y. Zhao, and J. T. Guo, *Front. Chem. Sci. Eng.,* **5**, 11 (2011).
(3) S. Kidoaki, I. K. Kwon, and T. Matsuda, *Biomaterials*, **26**, 37 (2005).
(4) V. Thomas, D. Dean, M. V. Jose, B. Mathew, S. Chowdhury, and Y. K.Vohra, *Biomacromolecules*, **8**, 631 (2007).
(5) V. Thomas, X. Zhang, and Y. K.Vohra, *Biotechnol. Bioeng.*, **104**, 1025 (2009).
(6) V. Thomas, X. Zhang, S. A. Catledge, and Y. K. Vohra, *Biomed. Mater.*, **2**, 224 (2007).
(7) S. F. Zhang, Y. K. Feng, L. Zhang, J. T. Guo, and Y. S. Xu, *J. Appl. Polym. Sci.*, **116**, 861 (2010).
(8) Y. K. Feng, Y. Xue, J. T. Guo, L. Cheng, L. C. Jiao, Y. Zhang, and J. L. Yue, *J. Appl. Polym. Sci.*, **112**, 473 (2009).
(9) Z. Ma, W. He, T. Yong, and S. Ramakrishna, *Tissue Eng.*, **11**, 101 (2005).
(10) H. S. Koh, T. Yong, C. K. Chan, and S. Ramakrishna, *Biomaterials*, **29**, 3574 (2008).
(11) K. Y. Lee, L. Jeong, Y. O. Kang, S. J. Lee, and W. H. Park, *Adv. Drug Deliv. Rev.*, **61**, 1020 (2009).
(12) P. K. Smith, A. K. Malla, and G. T. Hermanson, *Anal. Biochem.*, **109**, 466 (1980).
(13) J. Zeng, X. S. Chen, X. Y. Xu, Q. Z. Liang, X. C. Bian, L. X. Yang, and X. B. Jing, *J. Appl. Polym. Sci.*, **89**,1085 (2003).
(14) Z. M. Huang, Y. Z. Zhang, S. Ramakrishna, and C. T. Lim, *Polymer*, **45**, 5361 (2004).