Controllable Immobilization of Heparin onto Electrospun Poly(ε-caprolactone)/Gelatin Fibers for Growth Factor Delivery

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Statement of Purpose: Electrospinning technology provides the production of continuous fibers of both natural and synthetic polymers with dimensions on the scale of nano to micrometers. Electrospun fibrous scaffolds can be combined with extracellular matrix (ECM) ligands and growth factors to modulate cellular interactions. The objectives of this study are (1) to fabricate three different fiber morphologies with variations in fiber diameter, (2) to evaluate the effects of fiber morphologies and cross-linking period on free amine groups remaining on fibers, (3) to control the amount of heparin coupled to electrospun fibers based on the free amine groups, and (4) to confirm the *in vitro* release profile and bioactivity of platelet derived growth factor BB (PDGF-BB) bound to heparin-immobilized electrospun fibers. This fundamental investigation into fiber morphology and its potential to bind heparin is crucial for predicting the delivery of various growth factors for tissue engineering applications.

Methods: Poly(ε-caprolactone) (PCL) and gelatin type A were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) at a 1:1 weight ratio. Electrospun fibers with three different morphologies were fabricated by controlling fabrication parameters. The free amine groups and corresponding heparin binding on the electrospun fibers were determined using a 2,4,6-trinitrobenzene sulfonic acid (TNBSA) and toluidine blue assay, respectively. The fiber morphologies before and after heparin treatment were evaluated using scanning electron microscopy (SEM). The amount and bioactivity of PDGF-BB released from the electrospun scaffolds were detected using ELISA and MTS assay, respectively.

Results: Electrospun PCL/gelatin fibers were fabricated into three different morphologies by controlling fiber diameters: 1.0 μm, 1.0 μm/3.0 μm (co-electrospun), and 3.0 μm (Fig. 1 Left). The morphologies of electrospun fibers were uniform at the fiber diameters of 1.0 μm and 3.0 μm. In addition, co-electrospun fibers retained both fiber diameters without any defects or size variations. The number of free amine groups in the electospun fibers (1.0 μm and co-electrospun) decreased sharply from 0 min to 30 min as the cross-linking period increased (Fig. 2A). These data suggest that the intact amine groups of gelatin decrease abruptly while participating in the cross-linking process. Higher initial amounts of free amine groups in the 1.0 μm and co-electrospun fibers were caused by the larger surface area to volume ratio in these preparations than in the 3.0 μm fibers. The fiber's corresponding heparin binding capacity exhibits the same pattern seen in Fig. 2B. *In vitro* release test showed a sustained release of PDGF-BB in a controlled manner, consistent with heparin content bound to the each different fiber morphology (Fig. 3). PDGF-BB released from electrospun fibers significantly influenced to the proliferation of smooth muscle cells (SMC), and the bioactivity of PDGF-BB immobilized onto electrospun fibers maintained in culture over time.

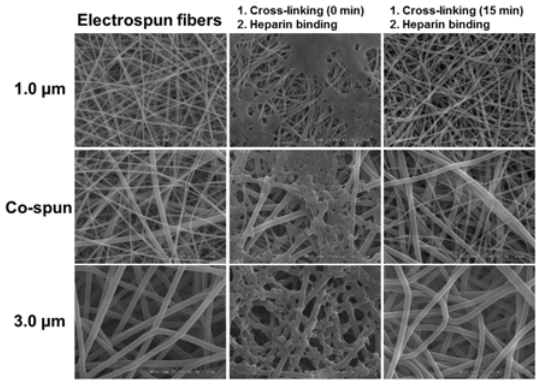

Figure 1. SEM images of before (left) and after (middle, right) heparin binding to electrospun fibers.

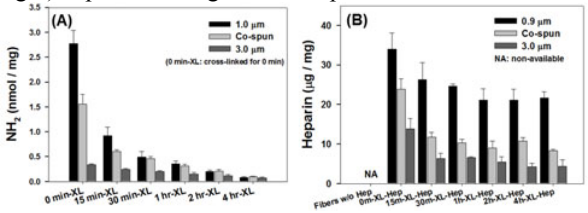

Figure 2. Determination of free amine groups with cross-linking time (A), Quantification of heparin bound to electrospun fibers (B).

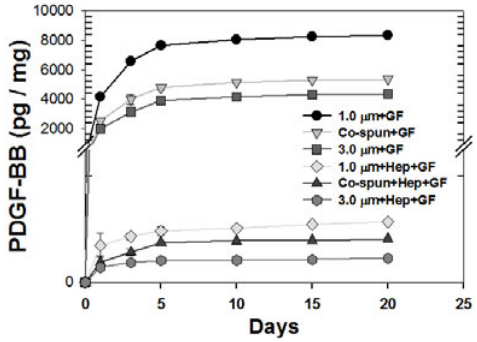

Figure 3. *In vitro* release profile of PDGF-BB incorporated into electrospun fibers.

Conclusions: In this study the content of heparin bound to the electrospun PCL/gelatin fibers was well controlled, and was dependent on the fiber morphology (diameter) and cross-linking period. The controlled heparin-immobilized electrospun fibers can be a useful tool to deliver PDGF-BB for cellular interactions while maintaining bioactivity effectively.

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