Heparin-immobilized Electrospun Nanofibers for Vascular Sutures

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Statement of Purpose: Free tissue transfer is a widely used technique for soft tissue reconstruction in which surgical sutures are used to connect the graft and implant site vasculature. One of the most common complications that can lead to graft failure is anastomotic thrombosis. Heparin is the most commonly used anti-coagulant to prevent anastomotic thrombosis. Various studies have shown that heparinized surfaces provide improved thrombo-resistance. Specifically, immobilized heparin on the surface of biomedical devices binds plasma anti-thrombin III (AT-III), concentrating and increasing its activity to reduce platelet adhesion, increase plasma re-calcification time, and (increase/reduce) activated partial thromboplastin time [1, 2]. Electrospinning technique is a simple and versatile method for developing polymeric fibers with diameters ranging from submicrons to several nanometers [3]. In addition, electrospun nanofibers provide higher surface area available for modifications with different functional groups than conventional melt-spun fibers. The objective of this study was to develop heparin-immobilized electrospun nanofibers composed of PLGA, PEO, and a proprietary positively charged amphiphilic copolymer (P-AC) as a microvascular suture. We hypothesize the positively charged electrospun fiber may interact with negatively charged heparin via electrostatic interactions and provide sustained and controlled release of heparin from the suture.

Methods: To fabricate the positively charged electrospun fibers, different ratios of PLGA/PEO/P-AC polymer solutions were prepared and placed in a syringe. A syringe pump was used to control the flow rate, and a custom-made rotating mandrel with four alumina bars was used as a fiber collector [4]. A high voltage power supply was connected to the syringe through a stainless-steel needle that was placed 15 cm from the collector. After the nanofibers were collected on the mandrel, a custom-made collecting and twisting device was used to form yarns. The resultant nanofibers were heparinized by incubation in 1% heparin solution in PBS, washed several times with distilled water to remove unbound heparin, and then lyophilized. After freeze-drying, the morphology and diameter of the collected fibers were characterized by field emission-scanning electron microscopy (FE-SEM) and attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy. The mechanical properties were evaluated by tensile testing (MTS Synergie 100) equipped with 10 N load cell. The amount of immobilized heparin on the P-AC/PLGA nanofibers was measured using the toluidine blue colorimetric method.

Results: P-AC/PLGA nanofiber sutures, made of a different composition of PLGA, PEO, and P-AC were successfully fabricated by the electrospinning technique. Table I shows the mechanical properties and diameters of electrospun nanofibers. The diameter, Young's Modulus, and elongation at break of electrospun nanofiber yarns ranged from 200 to 500 μm, from 300 to 600 MPa, and from 2 to 28 %, respectively. SEM images of the electrospun fibers showed a twisted fibrous yarn (Fig. 1), without significant difference between nanofibers with or without P-AC (data not shown). After incubation in 1% heparin solution, toluidine blue assay demonstrated that 2.13 ± 0.43 μg of heparin was present per 10 mm length of electrospun nanofibers.

Table 1. Characterization of nanofibers

Polymer (Weight Ratio, w/v%)*	Average Fiber Diameter [μm]	Youngs Modulus [MPa] **	Elongation at Break (%) ***
PLGA:PEO (13.0:2.0)	291.0 ± 10.46	502.2	2.1
P-AC:PLLA (0.67:19.3)	402.2 ± 127.87	366.5	28.2
P-AC:PLGA:PEO (0.5:12.5:2.0)	463.3 ± 8.98	404.1	7
P-AC:PLGA:PEO (0.5:11.5:3.0)	474.4 ± 41.52	555.7	2.6

* Polymer was dissolved in dimethylaceamide (DMAC)
** Youngs Modulus was found using random points selected in the linear portion of stress strain plots.
*** Elongation at break values was found from software (strain at break value × 100).

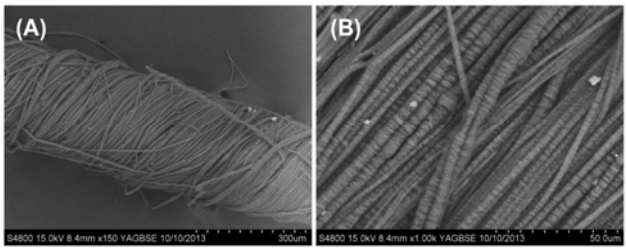

Fig. 1. SEM photography of electrospun nanofiber yarns. Magnification: 150X (A), 1,000X (B)

Conclusions: In this study, P-AC/PLGA/PEO nanofibers were successfully fabricated using electrospinning technique and characterized using FE-SEM and tensile testing. Heparin was immobilized on the positively charged P-AC/PLGA/PEO electrospun nanofibers via ionic interaction. Currently, we are preparing electrospun nanofibers with various compositions to improve the heparin loading efficiency, and evaluating the feasibility of heparinized eletrospun fibers as surgical sutures for vascular anastomosis.

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