Heparin-eluting Vascular Suture for Preventing Thrombosis and Stenosis

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Statement of Purpose: The most significant complication for vascular surgery is thrombosis, occurring in over ten percent of patients, which is the formation of hard blood clots at the surgical site. Another significant complication is stenosis - an overgrowth of cells during the healing process, which narrows the same artery the surgeons were trying to open. These complications often lead to additional surgeries and carry increased morbidity and mortality for the patients. Numerous pharmaceutical agents have been tested to prevent these complications, but the results have been disappointing. The agents either have unacceptable systemic effects, or they cannot achieve a controlled, timed release in the circulation. Heparin is the most commonly used anti-coagulant for anastomotic thrombosis prevention, with numerous studies employing heparin-immobilized or coated biomaterials for improved thrombo-resistance. Specifically, immobilized heparin on biomedical devices is known to reduce platelet adhesion, increase plasma re-calcification time, and increase activated partial thromboplastin time (APTT) [1,2]. We hypothesized that the negatively charged heparin is immobilized or sustained by the positively charged electrospun nanofibers via electrostatic interactions, and then released out from the scaffold in a controlled fashion.

Methods: Positively charged PLGA/PEO/P-AC nanofiber yarns were fabricated using electrospinning technique. Briefly, the PLGA/PEO/P-AC (13.0:2.0:0.39 weight ratio, w/v %) polymer (PPP) solution was dissolved in dimethylacetamide (DMAC) then loaded in a 10 ml syringe and electrospun to form nanofibers under a high voltage with a consistent feed rate. After collected, nanofiber yarns were prepared by using a custom-made twisting device [3]. To evaluate heparin loading efficiency, fluorescein dye-conjugated heparin (F-Hep) was synthesized by a method modified from Luong-Van et al. [4]. For surface immobilization of heparin, PPP nanofiber yarns were rinsed in PBS and incubated in 0.5% F-Heparin/PBS solution for 4 hours at room temperature, then washed several times to remove the non-bound heparin. F-Heparin loading efficiency was calculated using a standard curve and fluorescence was measured using a microplate reader (Biotek Synergy H1 Hybrid). The morphology and diameter of the nanofiber yarns were characterized by field emission-scanning electron microscopy (FE-SEM). The mechanical properties were evaluated by tensile testing (MTS Synergie 100) equipped with a 10 N load cell.

Results: Electrospun nanofibers composed of PLGA, PEO, and P-AC (PPP) and were successfully prepared by the electrospinning method. Electrospun nanofibers composed of PLGA and PEO (PP) (13.0:2.0 weight ratio, w/v %) were prepared as a control. The diameter, Young's Modulus, and elongation at break of manufactured electrospun yarns ranged from 200 to 500 μm, from 300 to 600 MPa, and from 2 to 28 %, respectively. The morphology of electrospun nanofibers was observed by FE-SEM (Fig. 1), which showed no significant difference between nanofibers with and without P-AC. After incubation in 0.5% F-heparin solution, 348 μg and 455 μg of heparin were immobilized on the PPP and PP, respectively. The release profiles of heparin from two types of nanofiber threads are shown in Figure 2. The results showed that PP fiber has more amount of immobilized heparin than PPP fiber, because PPP fiber contained a little amount of P-AC on it, due to the difficulty in dissolving higher amount of P-AC in organic solvent.

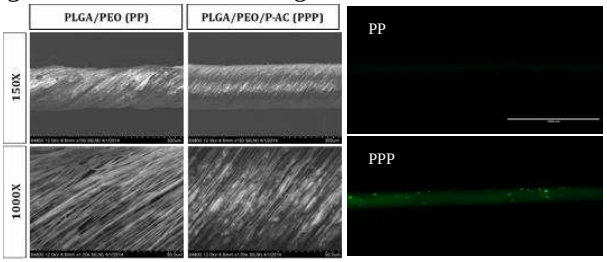

Fig. 1 (left). SEM images of electrospun nanofibers

Fig. 2 (right) Fluorescent microscopy images of fluorescent-labeled heparin immobilized on electrospun nanofibers (4x magnification)

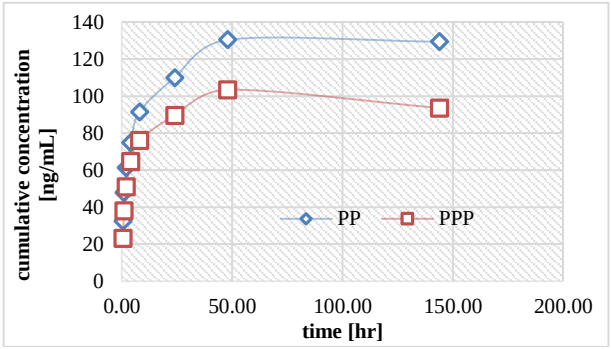

Fig. 2. In vitro cumulative release profile of heparin from electrospun nanofibers with P-AC (PPP)(squares) and without P-AC (PP) (diamonds).

Conclusions: In this study, PLGA/PEO/P-AC (PPP) nanofibers were successfully prepared using electrospinning technique and characterized using FE-SEM and tensile testing. Fluorescein conjugated heparin was immobilized on the positively charged PPP electrospun nanofibers via electrostatic interactions, and released out from the nanofibers. Currently, we are investigating the optimization for increased immobilization efficiency and sustained release of heparin, and evaluating the anti-coagulant activity of released heparin from PPP nanofibers.

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References: 1. Murugesan et al. Curr Top Med Chem 2008;**8**:80–100. 2. Jee et al. Biomacromolecules 2004;**5**:1877–81. 3. Tsai et al. Langmuir 2013;**29**(33):10596–602. 4. Pham et al. Tissue Eng 2006;**12**:1197–211.