Epsin-Mimetic Peptide Delivery Strategies from Electrospun Meshes for Endothelialization of Vascular Grafts

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Statement of Purpose: Tissue-engineered vascular grafts (TEVGs) are a promising solution that provides patients with an alternative source for graft replacement. However, obtaining functional endothelialization and clinically viable small diameter TEVGs is a challenge. A study has suggested that a UPI peptide can target epsin – vascular endothelial growth factor receptor 2 (VEGFR2) interaction in mice, resulting in more endothelial cell proliferation and angiogenesis [1]. We hypothesize that the controlled release of epsin mimetic UPI peptides from fibrous scaffolds will also promote endothelial cell (EC) proliferation in vascular grafts. Poly(ε-caprolactone) (PCL) electrospun meshes were coated with UPI peptides via both physical adsorption and peptide-loaded fibrin coating techniques. They were tested with human umbilical vein ECs (HUVECs) *in vitro* and ECs *in vivo* to determine the impacts on cell expression and function.

Methods: Conduits were electrospun from 13% PCL, and fiber orientation and diameter were characterized with SEM. For physical adsorption, samples were emersed in UPI peptide (0, 10, 25, 50, 100, 250, and 500 µg/mL) for 24 hours, washed with saline, and dried in the desiccator overnight. For the fibrin coated technique, the conduits and meshes were emersed in fibrin and UPI solution (50 mg/mL of fibrinogen, 500 µg/mL final concentration of UPI), stored overnight, dipped into a 10:1 solution of thrombin, and then dried in the laminar hood. Optimal UPI release rate and concentration were assessed via a BCA protein assay. Primary HUVECs were used to assess the effects of UPI peptides with different concentrations on tissue culture polystyrene (TCPS) and PCL meshes in culture. Moreover, the EC-specific effects of UPI peptides were also validated using smooth muscle cells (SMCs) and normal human dermal fibroblast (NHDFs). In vivo studies were performed using UPI-loaded conduits in male rats, and the conduits were harvested one-week post-surgery. DNA assay and immunofluorescence imaging were performed to characterize cell number and phenotype on *in vivo* and *ex vivo* samples. Statistical analysis was performed via one-way ANOVA.

Results: The average fiber diameter for conduits was 0.91 ± 0.21 mm, and the fibers were randomly oriented. After fibrin coating, there was a layer of fibrin and peptide in the lumen and within the conduits' wall, as was determined with SEM and fibrin antibody staining. The UPI peptide release profile in saline was studied for seven days, showing the burst release profile. For UPI peptide release, most of the burst release occurred in the initial 12 hours (**Fig 1)**. This can be extended by including collagen within the electrospun base scaffold. There should be further sustained release that occurs after fibrin degradation [2]. In vitro tests showed that the UPI peptide had an important impact on ECs attachment. Almost no ECs attached on pure PCL meshes, but a high amount of ECs attachment and spreading occurred when UPI peptides were on the surface (**Fig 2).** Interestingly, the ECs attached much better when the peptides were physically adsorbed than when delivered in the media. Further, there was even more cell attachment and spreading in fibrin-coated meshes loaded with UPI peptide than with just physically adsorption. Our results show that this is not just due to differences in the hydrophilicity of the surface because the fibrin without peptide condition had less cell attachment and spreading than fibrin + peptide. ECs functionality was shown with the CD31 marker in all samples, including cells-cells interaction. Overall, this experiment showed that the controlled delivery of UPI peptides increased the EC attachment and function *in vitro*. Preliminary results with SMCs and NHDFs showed negligible impacts of exogenous peptides on these cell types. *In vitro* studies of peptide loaded scaffolds and controls (e.g. fibrin only) demonstrated patency after one week. We are currently analyzing the extracellular matrix remodeling response and cells phenotype for these grafts.

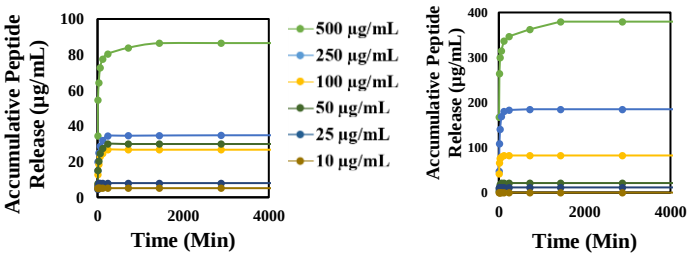

Fig 1. UPI peptide release graphs through physical adsorption (A) and fibrin layer coating (B)

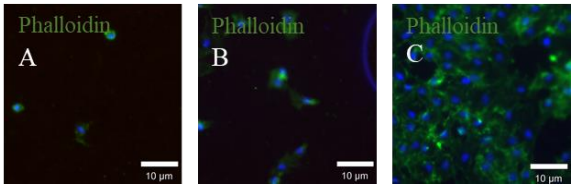

Fig 2. Representative images of phalloidin expression in pure PCL conduits (A) physically adsorbed (B) and Fibrin + Peptide (C)

Conclusion: This study showed that the UPI peptides can be successfully loaded and delivered to ECs. Further, it suggests that the peptide delivery to ECs will be more effective at TEVG endothelialization when included as part of the scaffold instead of injected into the blood. Overall, this study suggested that locally released UPI peptide can increase the viability of graft for small diameter arteries.

References:

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