Endothelial Cell Attachment and Shear Response on Biomimetic Polymer Coated Vascular Grafts

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Statement of Purpose: Thrombus formation and intimal hyperplasia are major mechanisms of failure for synthetic small diameter vascular grafts. Rapid *in vivo* endothelialization of the vascular graft material would help address these issues and contribute to the patency of the graft. However, clinically used graft materials, such as extended polytetrafluoroethylene (ePTFE), resist endothelial cell (EC) adhesion. Therefore, we have developed a biomimetic fluorosurfactant polymer (FSP) coating that consists of a poly(vinyl amine) backbone with fluorocarbon side chains to allow for stable adherence to the ePTFE substrate and RGD peptide ligands to attach endothelial cells. This study investigates the ability of endothelialized ePTFE grafts to retain cells when exposed to physiological shear stress, utilizing a clinically relevant vascular graft. ePTFE was coated with the extracellular matrix protein fibronectin, or with fluorosurfactant polymer. The graft was then sodded with ECs in an *in vitro* perfusion system and exposed to 8 dynes/cm^2 of shear stress. The cell retention, morphology, and gene expression of human ECs on shear exposed ePTFE grafts were explored.

Methods: RGD-FSP was synthesized as previously described.[1] The composition of the surfactant polymer was characterized by combining the results of 1H-NMR, IR spectroscopy, and XPS. RGD-FSP was dissolved in water and adsorbed to ePTFE for 24h. Fibronectin (FN) was allowed to adsorb on ePTFE for 1h. Human pulmonary artery ECs were sodded on the coated surfaces at 75,000 cells/cm^2 using a pressure sodding technique, and were incubated overnight before shear stress was applied. To create 8 dynes/cm^2 of shear stress, EC growth media thickened with 2% (w/v) 100 kDa poly (ethelyene oxide) was pumped through the graft at 2 mL/min for 4 or 24 h. The graft was then fixed with 4% (w/v) paraformaldehyde and stained with ethidium homodimer for cell nuclei, and AlexaFluor488-phalloidin for actin. Epifluorescent images were taken from the graft and the cell population was quantified. To calculate cell retention, the number of adherent cells present after shear stress was divided by the number of cells on a non-sheared graft sodded under the same conditions. Real time PCR was used to measure the gene expression of cells on the grafts.

Results / Discussion: Endothelialized grafts coated with RGD-FSP retained significantly more cells than grafts coated with fibronectin. Cell retention on RGD-FSP coated grafts was about 70% after 24h, while retention on FN coated grafts was about 30%. Additionally, ECs on RGD-FSP exhibited a more spread morphology and oriented in the direction of shear stress, as demonstrated by actin fiber staining. On FN coated grafts, cells remained rounded. This spread morphology appears to be consistent with cells that are adapting to shear stress. We also explored the expression of genes involved in endothelial cell hemostatic function before and after shear stress. ECs on grafts coated with both FN and RGD-FSP showed a reduction in vascular cell adhesion molecule 1 mRNA after shear stress, compared to sodded grafts with the same coating. The expression of genes, including tissue factor, inducible nitric oxide synthase, and tissue plasminogen activator for several other proteins did not significantly change with shear stress. This lack of change suggests the endothelial cell layer on the coated graft material does not change to a procoagulant phenotype in response to the applied shear stress.

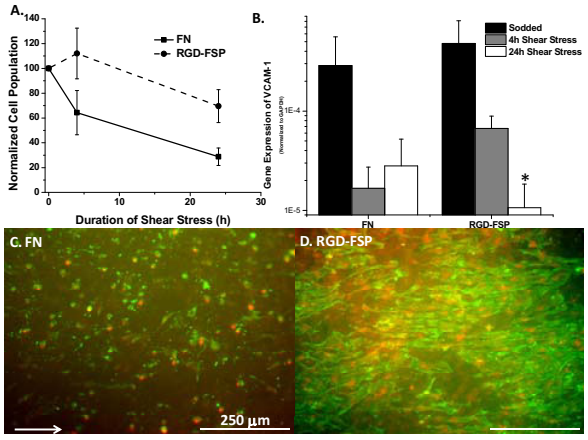

Figure 1. A. *EC retention on FN and RGD-FSP coated ePTFE grafts exposed to 8 dynes/cm^2 of shear stress* ***B.*** *VCAM-1 gene expression measured with real time PCR *p<0.05 compared to sodded* ***C.*** *and* ***D.*** *Epifluorescent images of endothelialized grafts after 24h of shear stress (arrow) with nuclei (red) and actin (green).*

Conclusions: ePTFE grafts coated with RGD fluorosurfactant polymer and fibronectin are capable of attaching ECs. However, the RGD-FSP coating allows for a cell layer that is more resistant to physiological shear stress, as shown by the increased cell retention over FN. Furthermore, gene expression profiles on RGD-FSP were similar to that on FN both at baseline and after shear stress, indicating that RGD-FSP does not alter the cells' phenotype, which is critical for preventing thrombogenesis. A shear stable EC layer is necessary for *in vivo* endothelialization of the graft material, which should increase the patency of synthetic small diameter vascular grafts.

Reference: 1. Larsen CC, et al. Biomaterials. 2006.

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