Endothelial Cell Attachment and Shear Dependent Retention on Biomimetic Polymer Coated Vascular Grafts

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Statement of Purpose: Thrombosis 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. Therefore, we have investigated the ability of a biomimetic fluorosurfactant polymer to adhere and retain endothelial cells (ECs) under physiological shear stress. Extended polytetrafluoroethylene (ePTFE) was coated with extracellular matrix protein, fibronectin, or fluorosurfactant polymer. The fluorosurfactant polymer consists of a poly(vinyl amine) (PVAm) backbone with both fluorocarbon side chains to allow for stable adherence to the ePTFE substrate and an RGD peptide ligands to attach endothelial cells. The graft was then seeded with ECs in an *in vitro* perfusion system and exposed to 8 dynes/cm^2 of shear stress. The cell retention was calculated by comparing the number of cells on the graft surface after the seeding period to the number of cells remaining after exposure to shear stress.

Methods: The fluorosurfactant polymer (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. Fibronecin (FN) was allowed to adsorb on ePTFE for 1h. ECs were seeded on the coated surfaces at 75,000 cells/cm^2, using the pressure sodding technique, and were incubated overnight before shear stress was applied. To create 8 dynes/cm^2 of shear stress, endothelial cell growth media thickened with 2% (w/v) poly (ethelyene oxide) was pumped through the graft at 2 mL/min for 4 h. The graft was then fixed and stained with ethidium homodimer for cell nuclei, and Alexa Fluor488-phalloidin for the cytoskeleton. Epifluorosecnt images were taken from the graft and the cell population was determined by counting the number of nuclei in a 0.6mm^2 region of interest on each image. To calculate cell retention, the number of adherent cells present after shear stress was divided by the number of cells present on a graft seeded under the same conditions, but not exposed to shear stress.

Results / Discussion: Exposure to 8 dynes/cm^2 of shear stress reduced the cell population on FN by about 40%, but the number of ECs on RGD-FSP after shear stress remained similar to that present after seeding. Thus, grafts coated with the RGD fluorosurfactant polymer showed higher cell retention than the grafts coated with fibronectin. In addition, the cells on RGD-FSP appeared to spread more than cells on fibronectin, as demonstrated by the cytoskelatal staining. This staining also indicated that the ECs exposed to shear stress on RGD-FSP took on morphology consistent with adaptation to shear stress, while ECs on FN remained rounded after shear stress.

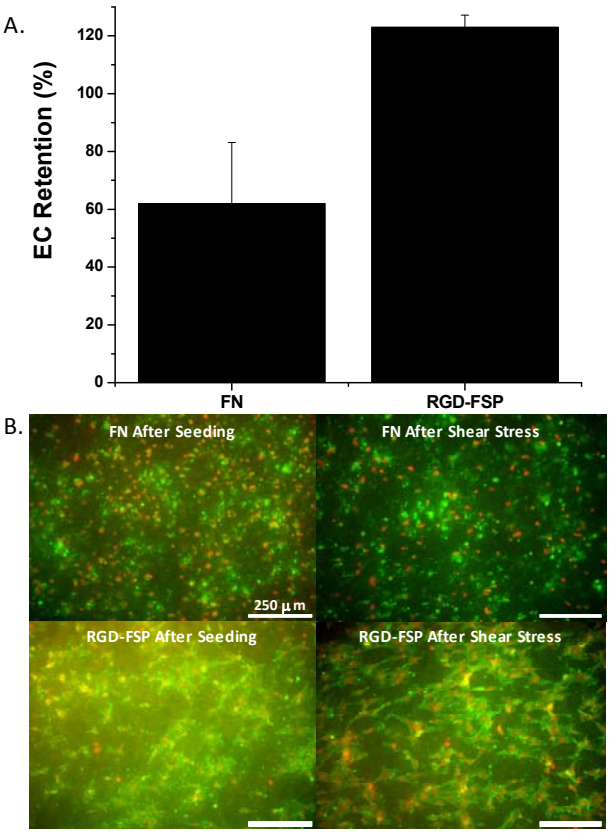

Figure 1. A. EC retention on FN and RGD-FSP coated ePTFE exposed to 8 dynes/cm^2 of shear stress for 4 h expressed as a percentage of the after seeding population. **B.** Epifloursecent images of EC seeded grafts with nuclei (red) and cytoskeleton (green).

Conclusions: RGD fluorosurfactant polymer and fibronectin coated ePTFE grafts 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. Shear stable EC adhesion 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; 27:4846-4855.

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