Peptide fluorosurfactant polymer modification of ePTFE facilitates in vitro adhesion and growth of endothelial cells

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Statement of Purpose: Currently used large-diameter vascular graft materials like expanded polytetrafluoroethylene (ePTFE) suffer from early occlusion and thrombosis when employed in small-diameter bypass applications. Our approach to improve the biocompatibility of ePTFE vascular graft material is to facilitate the formation of a monolayer of autologous endothelial cells on the lumenal surface. We have investigated the ability of a novel peptide fluorosurfactant polymer (PFSP) modification to promote endothelial cell (EC) attachment, growth, and function on ePTFE. This PFSP consists of a poly(vinyl amine) backbone with short, integrin binding RGD peptides and fluorocarbon pendant ligands for adsorption and stable adhesion to the underlying ePTFE polymer.

Methods: For PFSP synthesis, a poly(vinyl amine) (PVAm) backbone was synthesized and reacted with a cell adhesive, aldehyde-modified peptide (GSSSG**RGD**SPA) or its negative control analog (GSSSG**RGE**SPA). Next, perfluorocarbon pendant groups were added to the PVAm backbone through a reaction with N-(perfluoroundecanoyloxy) succinimide. Reaction products were characterized at various steps using proton NMR, IR, mass, and X-ray photoelectron spectroscopic methods. An aqueous PFSP solution was used to modify the surface of substrates: perfluorodecyltrichlorosilane derivitized glass (denoted FSAM)- a model, transparent fluorocarbon surface, solid PTFE, and ePTFE. Surface modification was assessed using water contact angle measurements and XPS. Following subconfluent seeding, human pulmonary artery endothelial cell (EC) adhesion and growth on PFSP was assessed by determining cell population at different time points using a colorimetric metabolic assay or a cell counting technique based on enumeration of fluorescently labeled EC nuclei. Fibronectin (FN, 1 $\mu g/cm^2$) coated glass served as positive control for all experiments while unmodified FSAM and PTFE served as negative controls. EC function on PFSP was assessed by fluorescently labeling the EC cytoskeleton or observing uptake of acetylated low density lipoprotein (AcLDL). The RGD dependence of EC adhesion to PFSP was investigated by incubating ECs in solution with soluble GRGDSP or GRGESP before attachment to PFSP.

Results / Discussion: Spectroscopic results indicated successful synthesis of PFSP with a final composition ratio of peptide:fluorocarbon on the PVAm backbone of 1.7:1. PFSP modification of ePTFE reduced the receding water contact angle measurement from 120° to 6°, indicating successful surface modification. Quantifying endothelial cell population on the experimental and control surfaces (Figure 1a) demonstrated reduced EC attachment efficiency but increased growth rate on RGD PFSP compared with FN. Five day cell population on the RGD PFSP surface approached confluence and was significantly greater than on FN surfaces. There was no appreciable cell population on unmodified FSAM and PTFE surfaces for all time points.

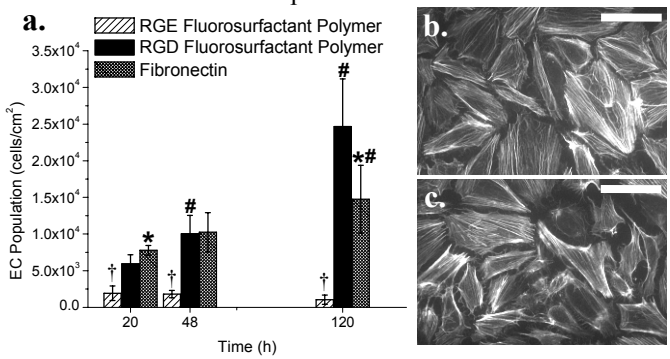

Figure 1. a.) EC adhesion and growth on RGE peptide fluorosurfactant polymer (PFSP), RGD PFSP, and fibronectin (FN) surfaces. * indicates difference ($p<0.05$) in EC population from RGD PFSP surface at same time point. # indicates difference ($p<0.05$) in EC population from identical surface at 20 h post-seeding. † indicates difference ($p<0.05$) in EC population from RGD PFSP and FN surfaces at same time point. Error bars are 95% confidence intervals from 7 FN samples, 2 RGE PFSP surfaces, and 10 RGD PFSP surfaces at each time point. b.) EC morphology on RGD PFSP after 120 h. c.) EC morphology on FN surface after 120 h. Scale bars are 100 μm.

The increased attachment efficiency on FN is likely due to higher affinity and greater diversity of attachment mechanisms compared to the RGD peptide. The increased growth rate and higher end population on RGD PFSP may be the consequence of a two-fold higher RGD density compared to a FN monolayer. The RGD-dependence of EC interaction with the RGD PFSP is demonstrated by the significant reduction in EC population on the RGE PFSP compared with FN and RGD PFSP surfaces. Additionally, incubating EC with soluble GRGDSP as opposed to GRGESP prior to seeding resulted in a 70% attachment reduction ($p<0.001$) to RGD PFSP surfaces. ECs on RGD PFSP (Figure 1b) contained organized and longitudinally aligned actin cytoskeletal components, similar to EC morphology on FN (Figure 1c). Cells on RGD PFSP stained positively for AcLDL uptake indicating physiologic endothelial cell function.

Conclusions: These data indicate successful synthesis of a novel PFSP that contains peptide ligands for EC integrin binding and fluorocarbon ligands for hydrophobic interaction with underlying ePTFE. Endothelial cells are capable of attaching to and proliferating on the PFSP with growth rates exceeding those of ECs on a native extracellular matrix protein. All of these data taken together indicate that PFSP are an attractive and effective approach to modifying ePTFE to encourage endothelial cell attachment, growth, and function. Stable endothelial cell attachment and function on PFSP modification provides tremendous promise for overcoming the early occlusion and thrombosis that have limited the use of synthetic small-diameter ePTFE vascular grafts.