In Search of an Endothelial Cell Selective Surface for Modifying ePTFE Grafts

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Statement of Purpose: Thrombus formation and intimal hyperplasia are major mechanisms of failure for synthetic small diameter vascular grafts. Endothelial cells form the natural lining of blood vessels and have anticoagulant, antiplatelet, and profibrinolytic properties. Thus, rapid *in vivo* endothelialization of the vascular graft material would help prevent thrombosis and contribute to the patency of the graft. In order to achieve endothelialization, we have developed biomimetic fluorosurfactant polymers (FSPs) designed to selectively adhere endothelial cells over platelets. The FSPs consist of a poly(vinyl amine) (PVAm) backbone with various EC binding peptides and fluorocarbon side chains to allow for stable adherence to the ePTFE substrate. The EC binding peptides we studied were RGDSPA, which is expected to interact with EC integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$ and platelet integrin $\alpha_{IIb}\beta_3$, REDV, which has been shown to bind EC integrin $\alpha_4\beta_1$, and CRRETAWAC, a cyclic peptide that binds $\alpha_5\beta_1$. We also explored a FSP that has a glycocalyx mimicking carbohydrate side chain, maltoheptaose (M7), in combination with the CRRETAWAC peptide. To test EC selectivity, FSP coated surfaces were exposed to either human pulmonary artery endothelial cells (HPAECs) or washed platelet suspension (WPS) prepared from human donors. A FSP coating that attached endothelial cells, but adhered few platelets was considered EC selective.

Methods: FSPs were synthesized as previously described.[1] Compositions of the surfactant polymers were characterized by 1H-NMR, IR spectroscopy, and XPS. HPAECs were seeded on the surfaces at 15,000 cells/cm^2 in serum free media for 2h. The surfaces were then rinsed and phase contrast images were taken. To measure EC growth, phase contrast images were also taken at 24h and 48h after seeding. The number of cells present on each surface was determined by manually counting ECs in several images on each surface. To prepare WPS, human blood was centrifuged to obtain the platelet pellet, which was resuspended in solution with EDTA and bovine serum albumin (BSA). The WPS was incubated with BSA blocked surfaces for 30 min. The surfaces were fixed and stained with FITC conjugated anti-CD41a. In both experiments, fibronectin (FN) coated surfaces served as the positive control. For the platelet experiments, maltose (M2) FSP served as the negative control.

Results / Discussion: All of the FSP surfaces were able to bind ECs. However, the REDV FSP and the CRRETAWAC+M7 combination FSP surfaces did not support cell growth to the same extent as the FN and other FSP surfaces. When exposed to WPS, only the CRRETAWAC+M7 FSP and negative control surface demonstrated platelet resistance, with very few adherent platelets. In contrast, the other surfaces had spread platelets. Platelet adhesion to FN and RGD FSP was expected due to RGD's interaction with integrin $\alpha_{IIb}\beta_3$. However, platelet adhesion to REDV FSP and CRRETAWAC FSP was unanticipated because these peptides are not expected to interact with the platelet integrin. Platelet adhesion to these surfaces is most likely nonspecific and could be due to a high degree of platelet activation in the WPS. The carbohydrates in the CRRETAWAC+M7 and M2 FSP surfaces appear to minimize nonspecific interactions and help prevent platelet adhesion. Due to its endothelial cell binding and platelet resistant properties, the CRRETAWAC+M7 FSP surface was considered to be EC selective.

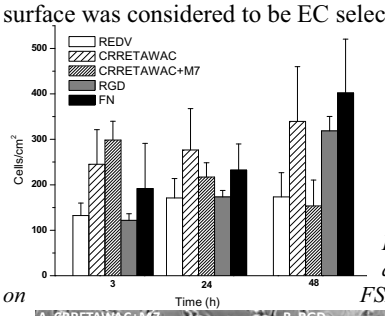

Figure 1. *EC adhesion and growth on FSPs and FN*

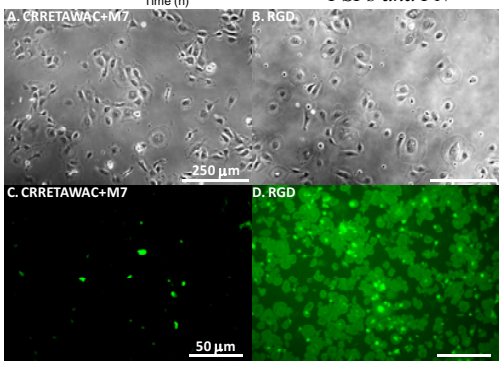

Figure 2. A. *and* ***B.*** *Phase contrast images (10x) of EC adhesion on FSPs after 3h* ***C.*** *and* ***D.*** *Epifluorescent images (40x) of platelets adhesion on FSP surfaces*

Conclusions: In this study, one EC selective fluorosurfactant polymer coating was identified, CRRETAWAC+M7. This biomimetic polymer coating allowed for EC attachment and showed very little platelet adhesion. Reducing the length of the maltose chain in future combination FSPs may allow us to further tailor the polymer properties and increase EC growth on coated surfaces. An EC selective surfactant polymer would facilitate endothelialization of ePTFE vascular grafts and may contribute to increased small diameter graft patency.

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

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