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. 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 an endothelial cell (EC) binding peptide 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 $\alpha_{IIb}\beta_3$ 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, washed platelet suspension (WPS), or a mixture of ECs and platelet rich plasma (PRP). 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. To measure EC adhesion and growth, phase contrast images were taken at 3h, and every 24h for four days. The cell populations on all the surfaces were 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). PRP was derived from whole blood and mixed with ECs (50,000 ECs/cm^2). The WPS or EC/PRP mixture was incubated with BSA blocked surfaces for 30 min. The surfaces were fixed and stained with FITC conjugated anti-CD41a, and DAPI. In all experiments, fibronectin (FN) coated surfaces served as the positive control. For platelet experiments, M7 FSP served as the negative control.

Results / Discussion: All of the FSP surfaces were able to bind ECs. After 96h, all of the surfaces had confluent EC layers with a typical cobblestone-like morphology, indicating that the addition of M7 in the combination surface did not alter the EC growth or morphology. 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 the CRRETAWAC FSP surface was unanticipated because this peptide is not expected to interact with the platelet integrin. Platelet adhesion to this surface is most likely nonspecific and could be due to a high degree of platelet activation in the WPS. The carbohydrate in the CRRETAWAC+M7 surface appears to minimize nonspecific interactions and help prevent platelet adhesion. In a competitive binding experiment, using a mixture of ECs and platelets, EC adhesion to the CRRETAWAC+M7 surface was not statistically significantly different to that on the FN surface and platelet adhesion was similar to the negative control, M7 FSP. Due to its endothelial cell binding and platelet resistant properties, the CRRETAWAC+M7 FSP surface was considered to be EC selective.

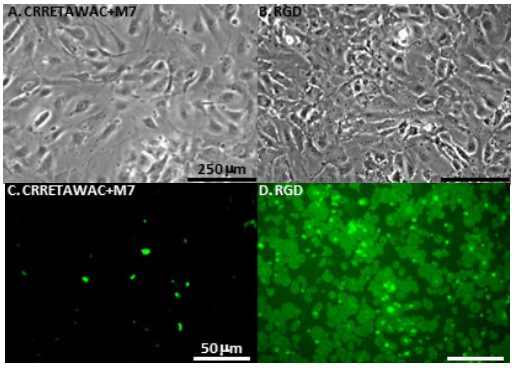

Figure 1. A. *and* ***B.*** *Phase contrast images (10x) of EC adhesion on FSPs after 3h* ***C.*** *and* ***D.*** *Epifluorescent images (40x) of platelets adhesion on FSP surfaces*

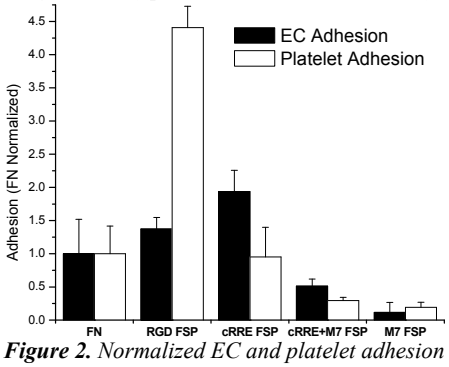

Figure 2. *Normalized EC and platelet adhesion on FSP and FN surfaces in a competitive binding experiment.*

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. 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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