In vivo Evaluation of an Endothelial Cell-Specific Biomimetic Peptide Fluorosurfactant Polymer Coating for Expanded Poly(tetrafluoroethylene) Vascular Grafts

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Statement of Purpose: Our goal is to improve the patency of expanded poly(tetrafluoroethylene) (ePTFE) vascular grafts by promoting endothelialization and reducing thrombosis via biomimetic peptide fluorosurfactant polymer (FSP) coating. The use of ePTFE vascular grafts is limited in small diameter applications due to the increased prevalence of thrombosis. ePTFE could be engineered to promote endothelialization and resist thrombosis by modifying the luminal surface to attach endothelial cells (EC) and limit platelet adhesion and activation. The RGD peptide has been widely used to facilitate cell attachment, but it is not endothelial cell specific. In contrast, the CRRETAWAC (cRRE) peptide has been shown to have high affinity for the $\alpha_5\beta_1$ integrin which is present in a high density on ECs and to a much lesser extent on platelets[1]. Our group has developed a cRRE FSP that adheres to ePTFE through hydrophobic interactions. We have previously demonstrated that this polymer has low affinity for platelet integrins and promotes EC attachment, growth, and shear stability, making it endothelial cell selective[2]. In this study, we evaluate the ability of the cRRE-FSP to improve ePTFE graft patency in an *in vivo* porcine model, and compare its effectiveness to uncoated, RGD-coated, and heptamaltose (M7) coated grafts.

Methods: Peptides and FSPs were synthesized as previously described[2]. Peptide-FSPs were dissolved in water and pumped through a flow system containing two ePTFE grafts (4 mm internal diameter) for 24 hr to coat the luminal surface. FSP coated grafts were ethylene oxide gas sterilized and cRRE- and RGD-FSP grafts were sodded with porcine pulmonary artery endothelial cells (PPAECs) using a pressure-sodding technique. A 4 cm graft was evaluated for cell adhesion via fluorescent labeling with DAPI and FITC-conjugated phalloidin to visualize cell nuclei and α-actin cytoskeleton, respectively. Five cm long grafts were implanted into porcine carotid arteries using a modified interposition model. At 1 month, grafts were visualized via contrast angiography then explanted and processed for histological evaluation. The fraction of the luminal area occluded by thrombus and intimal hyperplasia (IH) was quantified along 5 regions of the implant: proximal native vessel, proximal anastomosis, mid graft, distal anastomosis, and distal native vessel. In cases where a thrombus visible in histology appeared very acute, and occlusion of the sample contradicted angiography results, the thrombus was deemed to have occurred as a consequence of post-mortem processing and was removed from the quantified occlusion results.

Results: Immunofluorescent staining of RGD- and cRRE-coated grafts showed a complete layer of adherent and spread PPAECs after sodding. Angiography of the grafts after 1 month implant showed all grafts were at least partially patent. The lumen became more occluded along the graft from proximal to distal end in one uncoated and both RGD-coated grafts. Thrombus formation was present in one uncoated graft but not in coated grafts. IH developed to some extent in all grafts, and developed to the highest extent in RGD-coated grafts. Total graft occlusion was similar for uncoated and RGD-coated grafts, and lower for M7 and cRRE-coated grafts.

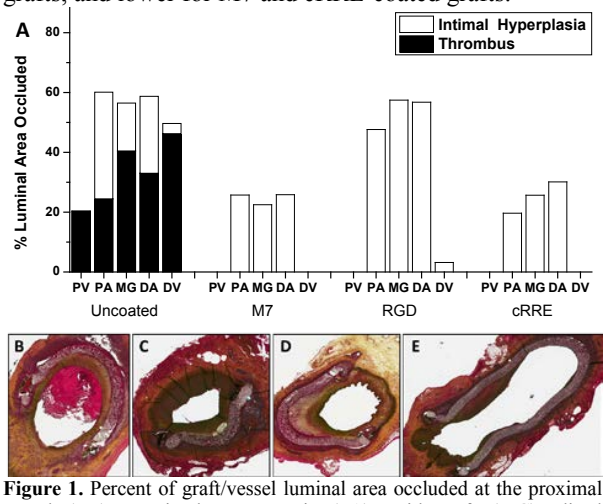

Figure 1. Percent of graft/vessel luminal area occluded at the proximal vessel (PV), proximal anastomosis (PA), mid graft (MG), distal anastomosis (DA), and distal vessel (DV) due to IH and thrombosis in uncoated, M7-coated, RGD-coated, and cRRE-coated ePTFE grafts (A, n=2). Movat pentachrome stains of the proximal anastomosis of uncoated (B), M7-coated (C), RGD-coated (D), and cRRE-coated (E) ePTFE grafts after 1 month of implantation.

Conclusions: The reduced thrombosis in M7-coated and PPAEC-sodded RGD and cRRE-coated grafts suggests that the presence of a coating reduced the thrombogenicity of these grafts compared to the uncoated grafts. Increased IH in RGD grafts suggests that RGD, a prominent cell adhesion peptide, may facilitate ingrowth of IH-causing cells compared to the other grafts. The cRRE-FSP has shown promise in improving patency of ePTFE grafts. Future work includes the analysis of endothelialization of these grafts.

References:

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