# A novel biomimetic peptide fluorosurfactant polymer engineered for endothelial cell-selective adhesion to ePTFE

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**Statement of Purpose:** There is a pressing clinical need for suitable small-diameter vascular prostheses to bypass diseased coronary arteries. A major impediment for use of artificial materials has been the lack of interface blood compatibility. The ideal blood interface is a confluent layer of healthy endothelial cells (ECs). The challenge of tissue engineering this ideal interface is that the same matrix proteins (e.g. fibronectin, FN) or FN-derived peptides that bind ECs will also bind platelets and initiate thrombosis. Here, we report a novel biomimetic construct engineered for EC-selective adhesion to vascular graft material. The construct facilitates endothelialization by utilizing a cyclic peptide ligand (CRRETAWAC) with specificity and high affinity for EC integrins[1], but low affinity for platelet integrins. The EC-selective peptide ligand is presented on a fluorosurfactant polymer (FSP) that allows for simple, aqueous self-assembly on expanded polytetrafluoroethylene (ePTFE), a clinically relevant vascular graft material that is inherently difficult to modify because of its chemical inertness.

**Methods:** EC selectivity of peptide (GRGDSP or cyclic CRRETAWAC) was assessed by investigating the affinity for platelet integrins using a solid phase $\alpha_{IIb}\beta_3$ integrin binding assay or inhibition of platelet aggregation. Peptide FSP synthesis was carried out as described[2] using a GSSS-CRRETAWAC peptide cyclized with a disulfide bond. Aqueous peptide FSP solution was used to modify fluorocarbon substrates. EC interaction with CRRETAWAC FSP was investigated by first ensuring peptide and integrin specificity. Next, EC adhesion and growth was examined. Shear stability was also investigated using a rotating disk system for 4 h of shear exposure. Finally, EC hemostatic function was examined with prostacyclin ($PGI_2$) and tissue plasminogen activator (tPA) ELISAs.

**Results / Discussion:** We found that CRRETAWAC peptide has low affinity for platelet binding. CRRETAWAC peptide inhibited fibrinogen (FG) binding to immobilized $\alpha_{IIb}\beta_3$ platelet integrin with an $IC_{50}$ = 3,054 μM, nearly 60 times higher than the $IC_{50}$ for GRGDSP peptide ($IC_{50}$=52 μM, **Fig. 1a**). Platelet aggregation was inhibited by CRRETAWAC peptide with an $IC_{50}$ = 2,700 μM, nearly 10 times higher than the $IC_{50}$ for GRGDSP peptide ($IC_{50}$=280 μM, **Fig. 1b**). These data indicate that specific CRRETAWAC-platelet interaction is very limited. EC attachment to CRRETAWAC FSP was $\alpha_5\beta_1$ integrin specific; attachment was also CRRETAWAC peptide specific. ECs attached with high efficiency to the CRRETAWAC FSP and grew as rapidly as ECs on FN (**Fig. 1c**). Cells demonstrated shear stability on CRRETAWAC FSP with no significant cell loss after 4 h of 38 dynes/cm$^2$ applied shear stress (**Fig. 1d**). Cells adherent to CRRETAWAC FSP demonstrated the EC-specific function of acetylated low density lipoprotein uptake and exhibited production of the antithrombotic mediators $PGI_2$ and tPA comparable to ECs on FN and RGD FSP.

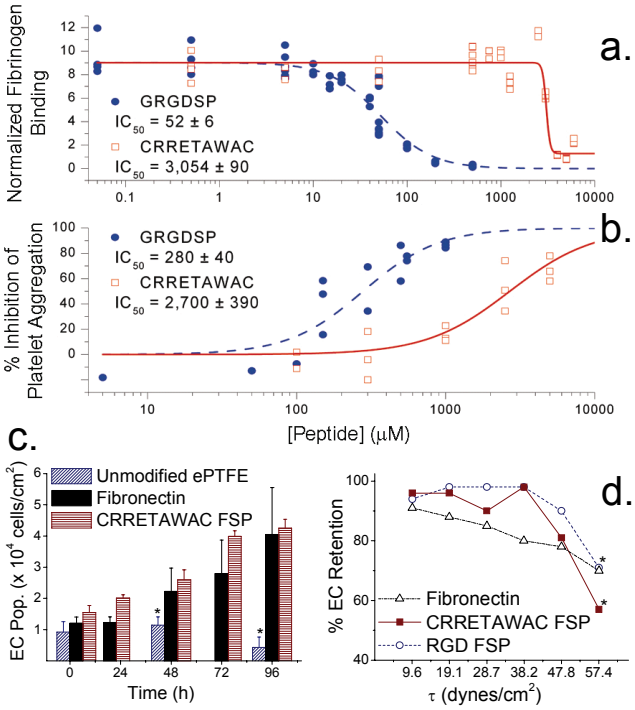


**Figure 1. a) Inhibition of fibrinogen binding to immobilized $\alpha_{IIb}\beta_3$ platelet integrin by GRGDSP (●) or CRRETAWAC (□) with nonlinear logistic regression fits. Higher $IC_{50}$ for CRRETAWAC indicates lower affinity for $\alpha_{IIb}\beta_3$ integrin. b) Inhibition of platelet aggregation by GRGDSP (●) or CRRETAWAC (□) with nonlinear logistic regression fits. Higher $IC_{50}$ for CRRETAWAC indicates lower affinity for platelet receptors involved in aggregation. c) EC attachment and growth on unmodified ePTFE, fibronectin, and CRRETAWAC FSP modified ePTFE. Cell population on CRRETAWAC FSP was comparable to fibronectin and significantly larger ($p<0.001$) than unmodified ePTFE. d) EC shear stability on fibronectin (△), CRRETAWAC FSP (■), and RGD FSP (○). ECs remained stably adherent to CRRETAWAC FSP after 4 h of 38.2 dynes/cm$^2$ applied shear stress.**

**Conclusions:** Our results demonstrate successful modification of clinically relevant ePTFE vascular graft material with an EC-selective FSP that promotes specific EC attachment, growth, shear stability, and function. This biomimetic construct has the potential to promote rapid endothelialization and healing without platelet adhesion and thrombosis of small-diameter vascular grafts.

**References:** 1. Koivunen E, et al. J Cell Biol. 1994; 124:373-380. 2. Larsen CC, et al. Biomaterials. 2006; 27:4846-4855.

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