Platelet adhesion on peptide fluorosurfactant polymers

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Statement of Purpose: Current synthetic graft materials including expanded polytetrafluoroethylene (ePTFE) exhibit unacceptably high rates of graft occlusion and thrombosis when employed in small-diameter bypass applications. To enhance ePTFE hemocompatibility, we have investigated a novel surface modification in which peptide fluorosurfactant polymers (PFSP) were stably adsorbed onto underlying ePTFE substrates. We have developed three PFSP systems, which present different short peptide sequences that possess different affinities for various integrin receptors. The objective of such a biomimetic construct is to create an ePTFE interface that promotes hemocompatibility by adhering functional vascular endothelial cells (EC) while minimizing platelet adhesion and thrombus formation. One such polymer which has demonstrated marked potential is CRRETAWAC PFSP which exhibits higher affinity for EC expressed $\alpha_5\beta_1$ compared with platelet expressed $\alpha_{IIb}\beta_{III}$ integrin receptors. To characterize an important aspect of the hemocompatibility of these constructs, we investigated the adhesion of platelets onto PFSP surfaces under both static and dynamic conditions.

Methods: PFSP synthesis was performed as previously described[1]. Three peptides were conjugated to the fluorosurfactant polymer backbone: GSSSG-**RGD**SPA (RGD), **cyclic(RGD**fE)-SSSK (cyclic(RGD)), and GSSS-**CRRETAWAC** (CRRETAWAC) and adsorbed onto perfluorodecyltrichlorosilane derivatized glass. Fibronectin (FN, 1μg/cm^2) coated glass served as a positive control. All experiments were conducted using a suspension of washed platelets isolated from venous blood drawn from adult donors and diluted to a concentration of 50,000 platelets/μl. To test the adhesion of platelets under static conditions, a washed platelet suspension was exposed to surfaces (n = 3) for 30 min. To test platelet adhesion under dynamic conditions, surfaces (n = 3) were rotated in a washed platelet suspension using a rotating disk system for 1 h. Following platelet exposure, surfaces were rinsed, fixed, and stained with FITC-labeled anti-$\alpha_{IIb}\beta_{III}$ monoclonal antibody.

Results/Discussion: Under static conditions (Fig. 1a), CRRETAWAC PFSP demonstrated significantly less ($p<0.01$) platelet surface coverage (11%) than FN coated glass (33%), RGD PFSP (46%), and cyclic(RGD) PFSP (50%). Platelet surface coverage on RGD PFSP versus cyclic(RGD) PFSP was found to be significantly greater ($p<0.05$) than FN coated glass. Under dynamic conditions (Fig. 1b), platelet surface coverage decreased as shear stress values increased for all surfaces. At 2.5 dynes/cm^2, FN (30%) and CRRETAWAC PFSP (11%) induced significantly less platelet surface coverage ($p<0.05$) compared with RGD PFSP (56%) and cyclic(RGD) PFSP (74%). In addition, other data have indicated that EC interactions with these three PFSP surfaces are promising.

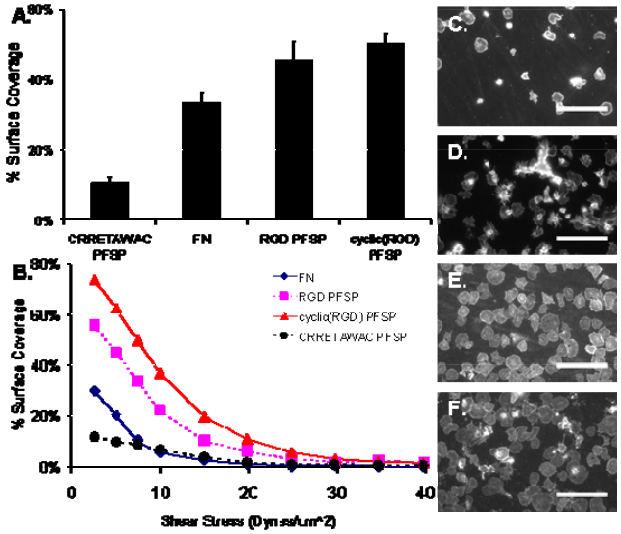

Figure 1. A) Platelet surface coverage on PFSP and FN surfaces after static exposure to platelets. Error bars indicate standard deviation. B) Platelet surface coverage on PFSP and FN surfaces after exposure to platelets under dynamic conditions. Representative images of platelet morphology of FITC-labeled platelets after static exposure [C) CRRETAWAC PFSP, D) FN, E) RGD PFSP, F) cyclic(RGD) PFSP]. Scale bars represent 30μm.

Differences in platelet adhesion are heavily dependent on ligand density and affinity. Since all PFSPs possess the same polymer backbone and identical peptide attachment chemistry, it is likely that ligand density varies little between PFSP surfaces. We also believe that a higher ligand density is present on PFSP surfaces compared with whole protein adsorbed surfaces, such as FN, since whole proteins are considerably larger than PFSPs. It is likely due to the PFSP surfaces' higher ligand density that more platelet surface coverage is observed on RGD and cyclic(RGD) PFSPs compared with FN. Finally, since CRRETAWAC ligand density is approximately equivalent to other PFSP surfaces and higher than FN surfaces, lower levels of platelet surface coverage observed on CRRETAWAC PFSP is likely due to a decreased affinity of this peptide for platelet receptors compared with RGD, cyclic(RGD), and FN.

Conclusions: CRRETAWAC PFSP surfaces exhibited the lowest degree of platelet surface coverage of all surfaces tested under static and dynamic conditions. RGD and cyclic(RGD) PFSP surfaces demonstrated higher levels of platelet surface coverage compared with CRRETAWAC PFSP and FN surfaces. These results support the conclusion that CRRETAWAC PFSP exhibits greater hemocompatibility compared with RGD PFSP, cyclic(RGD) PFSP, and FN. This study highlights the promise of CRRETAWAC PFSP as a modification to improve the haemostatic properties of ePTFE grafts.

References: 1. Larsen CC, et al. Biomaterials. 2006; 27:4846-4855.