Peptide-grafted Poly(ethylene glycol) Hydrogels Support Endothelial Progenitor Cell Rolling and Adhesion Under Shear

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Statement of Purpose: Endothelial progenitor cells (EPCs) have the potential to become a reliable source of autologous cells for endothelialization of intravascular devices and vascularization of tissue engineered constructs. In order to design biomaterials that can employ EPCs to enhance endothelialization, however, a better understanding of their dynamic adhesion to material surfaces under physiological shear is needed. In this study, late outgrowth endothelial colony forming cells (ECFCs), a type of EPCs, were investigated based on their advantages for use in endothelialization; ECFCs can be isolated from adult blood, they proliferate rapidly, and they can become mature ECs. Poly(ethylene glycol) diacrylate (PEG-DA) was chosen as the base material to test ECFC dynamic adhesion; PEG-DA is able to resist protein adsorption and therefore served as a "blank slate" for testing adhesion ligands. Peptides, including RGDS, REDV, YIGSR, and RGES, were grafted on the surface of the PEG-DA hydrogels. Through observation of ECFC rolling and retention on these peptide-grafted hydrogels under shear, dynamic and static adhesion between ECFCs and peptides was evaluated.

Methods: The cells used in this study were umbilical cord blood ECFCs. To create the peptide-grafted PEG surfaces, PEG-DA was first photopolymerized to form a hydrogel base. Peptides were conjugated to acryloyl-PEG-SVA and grafted onto the surface of the PEG-DA hydrogel base. A 6 histidine tagged RGDS peptide was synthesized, conjugated to acryloyl-PEG-SVA and then grafted on PEG-DA hydrogel base; successful grafting was then verified by immunostaining with an anti-6 histidine antibody. Shear experiments were performed to examine ECFC rolling and adhesion on the hydrogel surfaces. Using a Glycotech parallel plate flow chamber, the ECFC cell suspension was sheared over the hydrogels at shear rates of 20 s^{-1}, 40 s^{-1}, 80 s^{-1}, and 120 s^{-1}. Cell rolling events were recorded at 70 fps using a high speed camera. Cell tracking was performed using ImageJ and Matlab to determine rolling velocities. Migration of ECFCs was also performed on RGDS or RGDS/YIGSR-grafted hydrogels and migration distance was observed over a course of 3 days. Finally ECFC retention on the RGDS-grafted PEG-DA hydrogels was quantified under superphysiological shear stress.

Results: Rolling velocity of ECFCs was shown to relate to shear rates and adhesion material surface. ECFC rolling velocities increased as shear rates increased up to 120s^{-1}. ECFC rolling velocity was found to be significantly lower on REDV-grafted hydrogels compared to RGDS and YIGSR-grated hydrogels at 120s^{-1}. This suggests that $\alpha_4\beta_1$ integrins may be important in ECFC rolling. Capture of ECFCs was observed at low shear rates on RGDS-grafted PEG-DA hydrogels. No significant difference in ECFC migration was observed on the RGDS versus the YIGSR/RGDS-grafted PEG-DA hydrogels. Over 80% of adherent ECFCs were maintained on RGDS-grafted PEG hydrogels after exposure to superphysiological shear stress. These results provide a better understanding of ECFC-material interactions under physiological conditions.

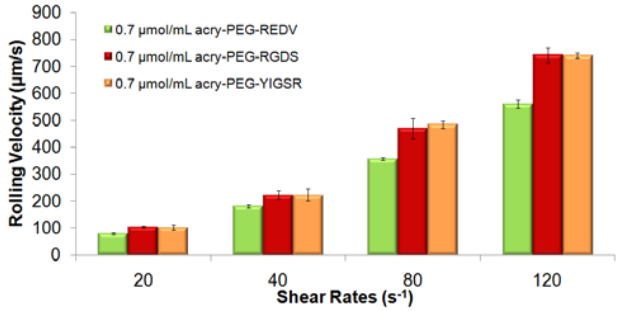

Figure 1. Comparison of ECFC rolling velocities on REDV-, RGDS-, and YIGSR-grafted hydrogels. REDV-grafted hydrogels had significantly lower ECFC reduced rolling velocities as compared to RGDS- and YIGRS-grafted hydrogels ($p < 0.01$).

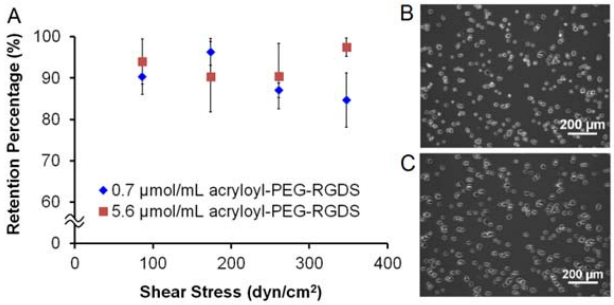

Figure 2. (**A**) Retention percentage of ECFCs did not differ between 0.7 and 5.6 µmol/mL RGDS-coupled hydrogels after 1 minute of superphysiological shear (n=3). (**B**) Image of ECFCs before shear. (**C**) Image of ECFCs on 5.6 µmol/mL of acryloyl-PEG-RGDS coupled hydrogel after shear for 1 minute at 347.4 dyn/cm^2.

Conclusions: In this study, PEG-DA was shown to be a viable "blank slate" base material for testing the ability of grafted ligands, including PEG-peptides, to interact with rolling ECFCs. Results demonstrated the ability of $\alpha_4\beta_1$ integrin-specific peptide REDV to significantly reduce ECFC rolling velocity as compared to other tested peptide sequences. Future work will include testing the ability of peptides that specifically bind other ECFC integrins to slow ECFC rolling and support ECFC adhesion under shear stress. Results of this study could be applied in the design of biomaterials for stent coating and vascular grafts to enhance endothelialization and improve EPC strength of adhesion under shear.