Peptide-grafted Hydrogels to Capture Endothelial Progenitor Cells under Shear for Endothelialization

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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, YIGSRG, CRRETAWAC, P11, PR_b and RGES, were grafted on the surface of the PEG-DA hydrogels. Interactions between ECFCs and the peptides were assessed in two ways: dynamic adhesion and maintenance of adhesion under shear. Through observation and quantification of ECFC rolling and retention on 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 0.7 µmol/mL of PEG-peptide was grafted onto the surface of the PEG hydrogel base. 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. Finally ECFC retention on the RGDS-grafted PEG hydrogels was quantified under superphysiological shear stress.

Results: ECFCs were able to form monolayer and maintain cobble stone morphology on RGDS, CRRETAWAC and PR_b-grafted hydrogels, but not on REDV, YIGSRG, and P11. 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}. All bioactive peptides supported ECFC rolling as the velocities were well below the cutoff for rolling velocity. ECFC rolling velocity was found to be significantly lower on REDV-grafted hydrogels. This suggests that $\alpha_4\beta_1$ integrins may be important in ECFC rolling. ECFC capture events were only observed on hydrogels grafted with $\alpha_5\beta_1$ binding peptides, CRRETAWAC and PRb, at 20 s^{-1}. Combination of REDV and CRRETAWAC have significantly increased the capture rate of ECFC under shear.

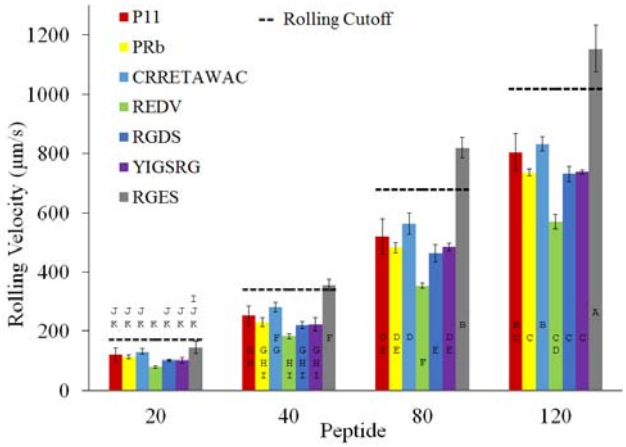

Figure 1. Comparison of ECFC rolling velocities on peptide-grafted PEG hydrogels. REDV-grafted hydrogels had significantly lower ECFC rolling velocities. Means that do not share the same letter are significantly different ($p<0.05$).

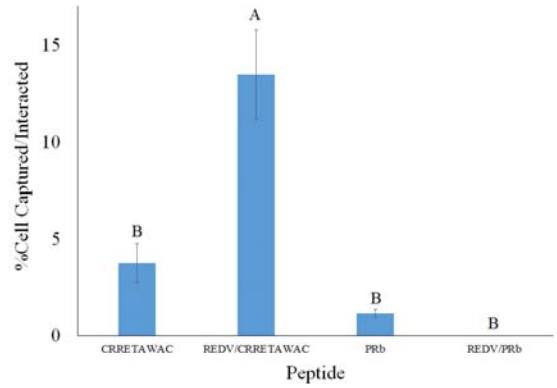

Figure 2. Comparison of ECFC captured rate on CRRETAWAC, PR_b and with REDV combinations.

Conclusions: All tested bioactive peptides supported ECFC rolling, whereas PEG-DA alone and RGES did not. 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. Capture on CRRETAWAC and PRb suggests that $\alpha_5\beta_1$, rather than $\alpha_v\beta_3$, is the major integrin that is responsible for ECFC capture under shear. CRRETAWAC was found to be superior in capturing rolling ECFCs under shear, and this effect was enhanced by the combination of REDV. 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.