Increasing the rate of endothelial cell migration on RGD/PEG materials without compromising cell adhesion strength

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Statement of Purpose: Endothelial cell migration is a key component to enhancing the endothelialization of cardiovascular biomaterials.[1] As demonstrated by DiMilla et al., an optimum is observed in cell speed as a function of cell adhesion strength.[2] In the presence of flowing blood, appropriate endothelial cell adhesion strengths must be balanced against the rates of endothelial cell migration. Using PEG hydrogels containing a range of concentrations of linear and cyclic RGD peptides in the presence of 20 dyne/cm^2 of fluid shear stress, we demonstrate that the delivery of a migration promoting factor promotes high cell migration speeds, even at the highest cell adhesion peptide concentrations tested.

Methods: PEG-octavinylsulfone (PEG-OVS) was produced from 8-arm PEG. Cell adhesion peptides (Ac-GCGYGRGDSPG and Ac-GCNAC*RGDGWC*G) with a single free cysteine were attached to PEG-OVS via a Michael addition prior to crosslinking. The PEG/RGD was crosslinked by reaction with fatty acid free albumin, a system previously used for the controlled delivery of the migration-promoting lipid sphingosine 1-phosphate (S1P).[3] Thin hydrogel layers were formed on mercapto-silanated glass, creating covalent linkages between the hydrogel and the glass. The hydrogels exhibit minimal swelling after crosslinking and deformation of attached hydrogels was not observed. Human aortic endothelial cells (HAEC) were seeded on the PEG/RGD hydrogels and allowed to attach for 6 h. A gravity-driven parallel plate flow chamber exposed the cells to 20 dyne/cm^2 fluid shear stress in low serum medium (0.4% FBS, 10 ng/mL VEGF, 1% ABAM in MCDB 131 medium) with 0 or 100 nM S1P. Cells were tracked by time-lapse microscopy over 12 h, manually locating the position of each cell nucleus every six minutes. Cell speeds and persistence times were determined as previously described.[4] Cell adhesion strengths were assessed by centrifugation of inverted, sealed plates containing hydrogels polymerized in the bottoms of wells. The force required to remove 50% of cells was interpolated from the data.

Results/Discussion: S1P is a signaling lipid abundantly stored in platelets and released upon platelet activation. It accounts for most of the chemokinetic activity of serum, signaling via a family of G protein-coupled receptors leading to activation of Akt and Rac.[5] Previous results showed that S1P release from the PEG/RGD/albumin hydrogels increases cell migration speeds for HUVEC on the hydrogels. This effect is due to local delivery of S1P, since with two hydrogels in the same well, only the gel loaded with S1P exhibits increased migration speeds. Developing materials for cardiovascular devices requires consideration of the effects of fluid shear stress on cell migration and cell adhesion. Under 20 dyne/cm^2, HAEC migration speeds were highest for 4 mM linear RGD. With 100 nM S1P, a decrease in cell migration speed was not seen, even when 1/10th of the PEG arms were reacted with linear RGD. With cyclic RGD, optimal peptide concentrations were 0.7 mM, while S1P tripled the mean cell migration speed without shifting the optimum.

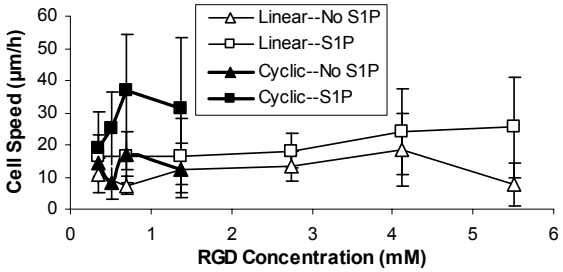

Figure 1: Migration of HAEC on PEG/RGD/albumin hydrogels with 20 dyne/cm^2 fluid shear stress. With 100 nM S1P, cell migration speeds were significantly increased at the highest RGD concentrations.

The 50% detachment force on linear RGD ranged from 850-1120 pN over an order of magnitude of RGD concentrations. On cyclic RGD, about 1300 pN were required for 50% removal of cells. No significant differences were observed in projected cell areas or cell persistence times. However, cells on cyclic RGD were less resistant to shearing forces, with detachment of 0–20% of cells after 10 hours at the highest peptide concentrations, while less than 5% of cells on linear RGD detached at similar peptide concentrations. This may reflect differences in the integrins targeted by the peptides ($\alpha_v\beta_3$ with linear RGD and $\alpha_5\beta_1$ with cyclic RGD).

Conclusions: We report findings that may be critical to the further development of engineered cardiovascular biomaterials. RGD peptides promote strong HAEC adhesion in the presence of arterial levels of fluid shear stress. The highest migration speeds were seen on cyclic RGD peptide, which also promoted stronger adhesion at lower peptide concentrations. However, cells on cyclic RGD may be less resistant to shearing forces. In future studies, combinations of linear and cyclic RGD may lead to materials that are optimized for cell migration rates and cell adhesion through the delivery of chemokinetic or chemotactic factors.

References:

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