Bioactive Poly(ethylene glycol) Hydrogels for the Development of an *ex vivo* Hematopoietic Stem Cell Niche

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Statement of Purpose: Hematopoietic stem cells (HSCs) are capable of differentiating down myeloid and lymphoid lineages to become mature blood and immune cells. HSCs are used in the treatment of many blood disorders and have the potential for use in other applications. However, HSC availability is limited due to inefficient *in vitro* culture methods. We propose the development of a novel, *ex vivo* culture system that recapitulates the HSC microenvironment. This niche is comprised of various cell types and biomolecules.[1] By mimicking the *in vivo* environment, this culture system will facilitate self-renewal resulting in clinically relevant HSC populations.

Methods: Photopolymerized poly(ethylene glycol) diacrylate (PEG-DA) hydrogels were modified with two adhesive peptide sequences, RGDS and fibronectin connecting segment 1 (CS-1), according to published methods.[2] 32D or EML cells, hematopoietic progenitor cell (HPC) lines, were seeded onto the hydrogels at a density of 10,000 cells/cm^2. At predetermined timepoints, non-adherent cells were rinsed away with PBS, and adherent cells were imaged and counted using ImageJ software. Co-cultures with a murine bone marrow osteoblast line (7F2) were also conducted on PEG-RGDS hydrogels. 7F2 and EML cells were seeded at various ratios (EML:7F2: 1:1, 1:2, 2:1, 4:1, and 1:0), keeping the EML seeding density constant. Non-adherent cells were washed away after 48 hours, and adherent cells were counted as described above. Statistical analyses were performed using ANOVA and Tukey's post-hoc analysis.

Results: Cell adhesion increased on PEG-RGDS and PEG-CS-1 hydrogels when compared to PEG-DA gels (Figures 1 and 2). For the PEG-RGDS hydrogels, there was a trend of increasing adhesion as the concentration of RGDS increased. On PEG-CS-1 hydrogels, there were similar numbers of adherent 32D cells on all gels until Day 7 when we saw a sharp increase in adherent 32D cells on PEG-CS-1 hydrogels. We hypothesize that adhesion to CS-1 stimulated 32D proliferation. In the co-culture experiments, we saw more adherent EML cells on gels where 7F2 cells were present (Figure 3).

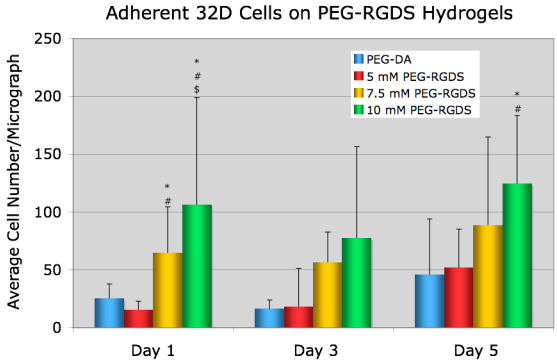

Figure 1. *: Statistically Significant (SS) from PEG-DA, #: SS from 5 mM RGDS, $: SS from 7.5 mM RGDS

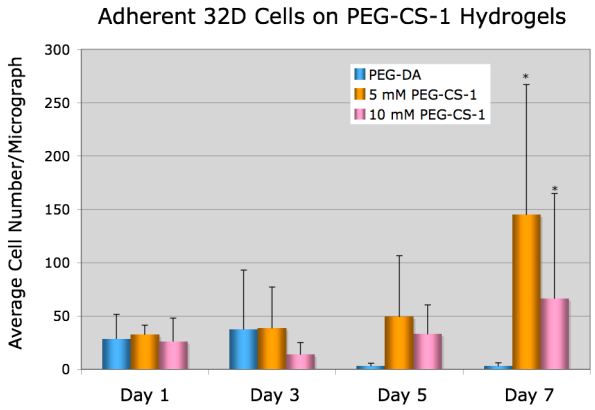

Figure 2. *: Statistically Significant (SS) from PEG-DA

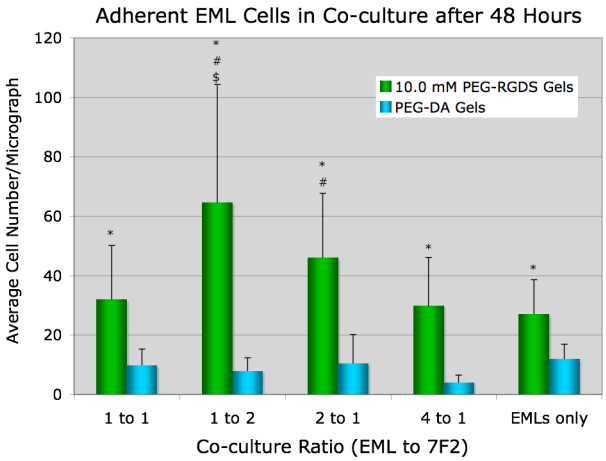

Figure 3. *: SS from PEG-DA, same ratio, #: SS from PEG-RGDS, EMLS only, $: SS from 1 to 1, &: SS from 4 to 1

Conclusions: Within the HSC niche, adhesion to extracellular matrix proteins and stromal cells via specific integrins encourages HSC self-renewal and can prevent differentiation.[3] HSCs use VLA-4 and VLA-5 integrins to anchor themselves within the niche.[1] By engaging these integrins via peptide sequences or stromal cells, we believe we can promote self-renewal *ex vivo* resulting in HSC populations that can be used therapeutically. We plan to continue our PEG-Peptide experiments to find the optimal concentration and combination of adhesive ligands that initiate self-renewal. We will also continue our work with the 7F2 cell line as well as an endothelial cell line to determine co-culture ratio(s) and cell types that support HPC adhesion and proliferation.

References:

1. Wilson, A. Nat Rev Immunol. 2006; 6: 93-106.
2. Hahn, MS. Biomaterials. 2006; 27: 2519-2524.
3. Jones, LD. Nat. Rev. Immunol. 2008; 8: 290-301.