Adhesion to Bioactive Poly(ethylene glycol) (PEG) Hydrogels Promotes Expansion of Hematopoietic Progenitor Cells

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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 diseases and disorders and have potential for other applications. However, HSC availability is limited due to inefficient *in vitro* culture. 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 6 kDa poly(ethylene glycol) diacrylate (PEG-DA) hydrogel wells were fabricated by replica molding the polymer against microfabricated pillars. Fibronectin-derived adhesive peptide sequences, RGDS and LDV, were covalently immobilized on well surfaces using 2.92, 1.46, 0.29, and 0.15 mM PEG-RGDS or 2.75 mM PEG-LDV solutions as previously described.[2] Stromal Derived Factor 1α (SDF-1α), chemokine that regulates homing of HSCs to the niche, was covalently tethered to the PEG-DA hydrogels using the same techniques (0.33 μM solution). 7F2 cells (murine bone marrow osteoblasts) were encapsulated (1 million cells/ml) within PEG-DA or PEG-RGDS (5mM) replica molded hydrogel wells. 32D cells, a murine hematopoietic progenitor cell line, or primary murine Sca1+ cells, were seeded into the hydrogel wells at a density of 20,000 cells/cm^2. Adherent cells were counted (ImageJ) as a function of culture time, biomolecule concentration, and biomolecule type.

Results: Adherent cell number increased on hydrogels modified with PEG-RGDS when compared to PEG-DA hydrogels and a fibronectin (FN) coated well plate. We also observed a dose-dependent trend of increasing cell number with increasing concentrations of PEG-RGDS (Figure 1). In samples containing higher concentrations of RGDS, cell number also increased over time indicating cell proliferation. We were also able to maintain these results when seeding primary hematopoietic cells on the gels. After 72 hours, more adherent cells were observed on gels modified with RGDS or LDV (Figure 2). A potential synergistic effect was observed on hydrogels modified with both PEG-RGDS and PEG-SDF-1α. On these scaffolds, adherent 32D cell number was increased when compared to samples modified with only one of the biomolecules (Figure 3). In coculture experiments, we observed increased 32D cell adhesion and proliferation when 7F2s were present within the hydrogel (Figure 4).

Conclusions: Within the HSC niche, adhesion to extracellular matrix proteins and stromal cells encourages HSC self-renewal and can prevent differentiation[3]. We have shown the ability to mimic fibronectin and stromal cell signaling by the biofunctionalization of our hydrogels with RGDS and SDF-1α and coculture with bone marrow osteoblasts. By recapitulation of the HSC microenvironment, we believe we can promote self-renewal *ex vivo* and generate HSC populations that can be used therapeutically.

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.

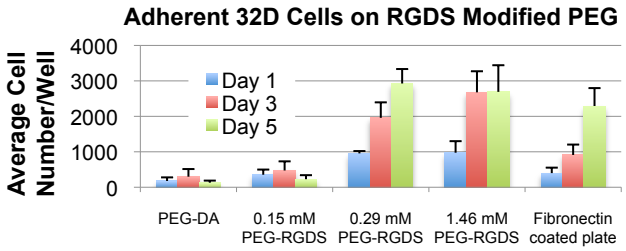

Figure 1: 32D cell adhesion and proliferation was increased on gels modified with RGDS compared to PEG-DA and fibroectin well plates

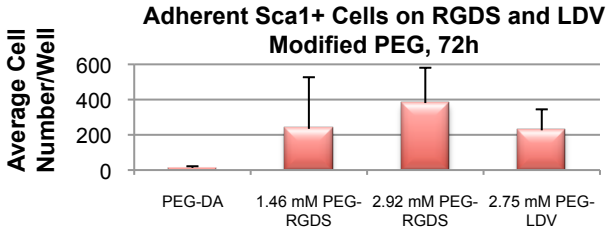

Figure 2: Sca1+ cell adhesion was increased on gels modified with RGDS and LDV compared to PEG-DA

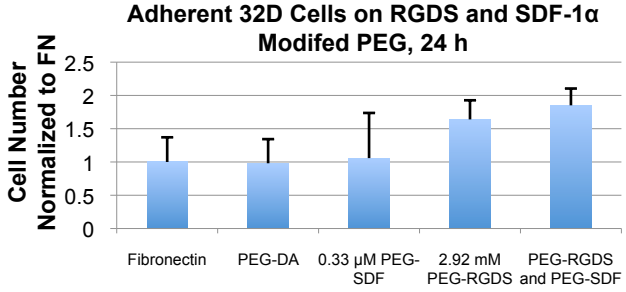

Figure 3: 32D Cell adhesion was increased on PEG gels modified with both SDF-1α and RGDS when compared to either biomolecule alone

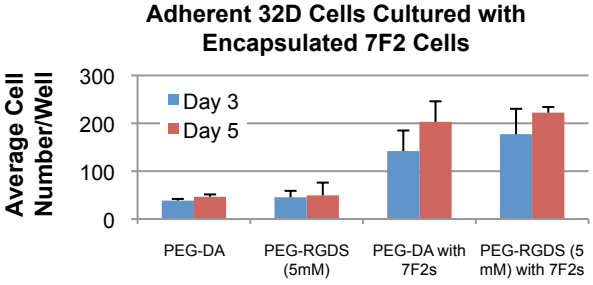

Figure 4: 32D Cell adhesion and proliferation was increased on PEG hydrogels containing 7F2 osteoblasts