Development of Bioactive Poly(ethylene glycol) (PEG) Hydrogels to Promote Expansion of Hematopoietic Stem Cells and Maintain their Potency

Maude L. Rowland[1], Omar Banda[1], Suleyman Coskun[2], Karen K. Hirschi[2], Jennifer L. West[1].
1. Rice University, Houston ,TX 2. Baylor College of Medicine, Houston, TX

Statement of Purpose: Hematopoietic stem cells (HSC) differentiate down multiple pathways to become mature blood and immune cells. HSC are used in the treatment of blood disorders and have applications potential in other areas including regenerative medicine. However, HSC availability is limited due to inefficient *in vitro* culture. By mimicking the *in vivo* HSC microenvironment with bioactive poly(ethylene glycol) (PEG) hydrogels, we can facilitate self-renewal while maintaining differentiation potential resulting in clinically relevant HSC populations.

Methods: Photopolymerized 6 kDa PEG diacrylate (PEG-DA) hydrogel wells were fabricated by replica molding the polymer against microfabricated pillars. A fibronectin-derived adhesive peptide sequence, RGDS, was covalently immobilized on well surfaces using 0.29 mM PEG-RGDS solutions as previously described.[1] Stromal Derived Factor 1α (SDF1α - a chemokine that regulates homing of HSC to the niche), Stem Cell Factor (SCF), and Interferon gamma (IFNγ) (cytokines that promote HSC proliferation) were covalently tethered, with RGDS, to the PEG-DA hydrogels using the same technique (0.33 μM solution). Primary murine bone marrow-derived c-kit+ cells (containing HSC and progenitors) were seeded into hydrogel wells at densities of 80,000 cells/cm^2. Adherent cells were counted (ImageJ) as a function of culture time and biomolecule type. After 10-14 days in culture, a colony-forming unit (CFU) assay was performed on cultured cells. c-kit+ cells were also encapsulated within 1 mM PEG-RGDS hydrogels, molded between two glass plates, and grown in culture for two weeks. After 3, 7, 10, and 14 days, viability was assessed using a live/dead assay.

Results: After 24 hours, c-kit+ cells adhered significantly to hydrogels modified with PEG-RGDS when compared to PEG-DA. With the incorporation of SCF, SDF1α, and IFNγ onto the hydrogel surface, we observed an increase in adherent cells compared to wells with PEG-RGDS alone (Fig. 1). These differences were maintained throughout the 14 days in culture. In the CFU assay, we observed colonies in all study groups, but the most multi-lineage CFU-GEMM colonies were present in the PEG-SCF, SDF-1α, and IFNγ groups, indicating the cells' multipotency (Fig. 2). We were also able to encapsulate c-kit+ cells within PEG-RGDS hydrogels and maintain their viability for two weeks in culture (Fig. 3). We attribute cell death to the harvesting, sorting, and encapsulation procedures as opposed to the culturing conditions because the percent viability remained constant throughout the entire time in culture.

Conclusions: Within the HSC niche, adhesion to extracellular matrix proteins and stromal cells promotes HSC self-renewal and can prevent differentiation.[2] We have demonstrated the ability to mimic these interactions by the biofunctionalization of our hydrogels with RGDS, SCF, SDF-1α, and IFNγ and have observed increased adhesion on bioactive PEG hydrogels while maintaining the cells' multi-lineage potential. We also have the ability to encapsulate primary HSC within the gels and preserve their viability, providing us with a means to culture the cells three dimensionally. By recapitulation of the HSC microenvironment using the PEG hydrogel system, we can promote self-renewal *ex vivo* and generate HSC populations for therapeutic use.

References:
1. Hahn, MS. Biomaterials, 2006; 27: 2519-2524.
2. Jones, LD. Nat. Rev. Immunol. 2008; 8: 290-301.

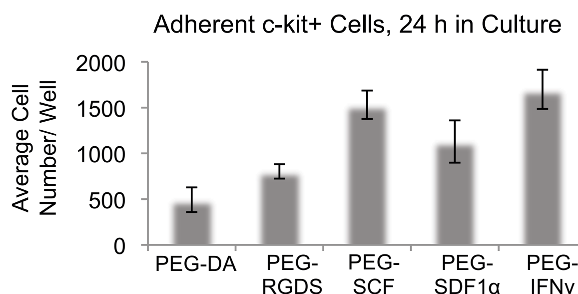

Figure 1: More marrow-derived c-kit+ cells adhere to hydrogels with surface-immobilized SCF, SDF1α, and IFNγ when compared to hydrogels with RGDS alone. Mean ± SEM

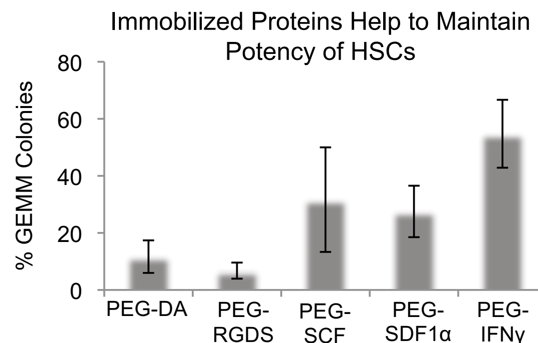

Figure 2: c-kit+ cells grown on PEG hydrogels in the presence of surface-immobilized SCF, SDF-1α, or IFNγ produce more multi-lineage CFU-GEMM colonies than those grown in the absence of these factors. Mean ± SEM.

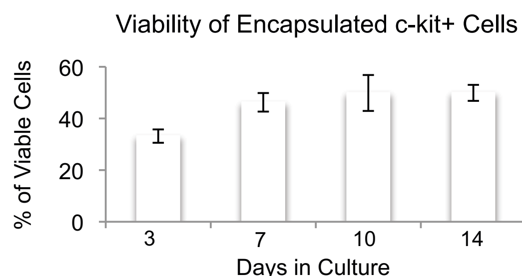

Figure 3: Marrow-derived c-kit+ cells can be encapsulated in PEG-RGDS hydrogels and remain viable for fourteen days in culture. Mean ± SEM.

©2011 Society For Biomaterials