Impact of functionalized Gelatin-Methacrylate hydrogels on hematopoietic stem cell biology

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Statement of Purpose: The bone marrow niche microenvironment is known to provide a complex set of extrinsic cues that regulate hematopoietic stem cell (HSC) behavior. An artificial marrow would have significant clinical value for expansion of HSCs or study of the onset and treatment of hematologic disease. While multiple niche cell types and soluble factors are proposed as key niche regulators, the direct influence of matrix biophysical properties and cell-cell signaling (paracrine vs. autocrine) mechanisms remains poorly understood. Stem cell factor (SCF) is known to impact HSC homing and proliferation in the marrow [1]. Recent efforts have shown tethering SCF to a hydrogel can impact adhesion and spreading of an anchorage-independent hematopoietic cell line [2]. However, the utility of incorporating SCF within an engineered bone marrow biomaterial to impact the expansion and bioactivity of primary HSCs remains unknown. Here we investigate incorporation of soluble or covalently immobilized SCF within a 3D methacrylated gelatin (GelMA) hydrogel and its effect on HSC bioactivity. Niche cells within the marrow also provide a valuable source of biomolecular signals to regulate HSC fate. Therefore our secondary objective was to explore the response of HSCs to co-cultures with Lin^+ marrow and mesenchymal stem cells (MSCs) in the presence of the immobilized SCF. Using a microfluidic mixing platform we create overlapping pattern of cell and biomolecular cues to investigate the impact of spatially-graded environment on HSC quiescence vs. activation.

Methods: GelMA hydrogels were polymerized by the addition of a photoinitiator and exposure under UV light. The resulting hydrogel is stable at 37 °C and contains adhesion (RGD) and degradation sites to facilitate cell attachment and remodeling. SCF was functionalized on the GelMA backbone by first integrating the SCF with a PEG-NHS ester, and adding it to GelMA prior to photopolymerization. The resulting SCF-GelMA was characterized via confocal microscopy and scanner imaging after conjugation with a fluorescent dye. Primary HSCs ($Lin^-Sca1^+cKit^+$ marrow cells) were isolated from the marrow via FACS and cultured in GelMA hydrogels in StemSpan SFEM media (Stem Cell Technologies, Vancouver, Canada) with soluble or immobilized SCF for up to 7 days. Similarly, co-cultures of niche cells and HSCs were generated. The cultures were assessed via proliferation assays, surface antigen expression and colony-forming unit (CFU) functional assays to determine HSC response. Hydrogels containing gradients in cell and biomolecular content were created using a microfluidic mixer as previously described [3]. The design permits analysis of the impact of multiple microenvironmental signals (MSCs, immobilized SCF) in small hydrogel (60 μL) volumes.

Results: Fluorescent imaging of the SCF-GelMA indicated retention of the SCF within the GelMA hydrogel up to 7 days after polymerization. Addition of soluble SCF (100 ng/mL) to the culture media led to an increase in HSC proliferation after 2 days in culture. Notably, immobilization of SCF to the GelMA hydrogel led to a dose-depended increase in HSC proliferation able to replicate the effects of soluble supplementation (Fig. 1). Ongoing efforts are examining differences in HSC immunophenotype and functional capacity as a result of exposure to SCF.

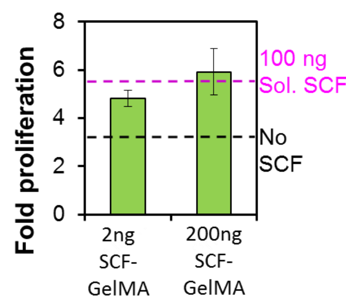

Figure 1 Proliferation of HSCs for varied SCF conditions

Co-culture of HSCs with Lin^+ bone marrow cells suggest competing effects of niche cell density and gel diffusivity on HSC proliferation and lineage specification. Early fate decisions (self-renewal) appear to be more strongly impacted by autocrine feedback loops while later stage fate decisions (lineage specification) may be more strongly impacted by niche cell mediated paracrine signals. Ongoing efforts are exploring the use of gradients of immobilized SCF and niche cells within the GelMA matrix to impact HSC fate.

Conclusions: We have demonstrated the ability to functionalize a GelMA hydrogel with a cytokine regulator of HSC fate, and maintain the activity of the factor in culture. Further, via selective alteration of matrix diffusivity we have established a biomaterial platform to explore the influence of niche cell and cytokine mediated paracrine signaling on HSC fate decisions. Ongoing experiments are examining longer culture periods to alter the balance between lineage specification and quiescence.

References:

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3. Mahadik BM. Adv Healthc Mater. 2013

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