Development of Multigradient Hydrogels to Decode Extrinsic Regulation of Hematopoietic Stem Cell Fate

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Statement of Purpose: Hematopoietic stem cells (HSCs) are responsible for the generation of all blood and immune cells of the body. HSCs are primarily found in specific microenvironments (niches) within the bone marrow[1, 2]. The HSC niche, composed of other cell types, the ECM and soluble biomolecules, is thought to be dynamic, providing stochastically and temporally mutable extrinsic signals that influence HSC fate decisions. However, surprisingly little is known about the mechanisms that underlie niche regulation and the minimum constellation of cues needed to engineer HSC behavior. The two primary candidates for HSC niches within the bone marrow (BM), the endosteal and vascular niche[3, 4], exhibit several biophysical parameters and cell-cell interactions that can potentially influence HSC fate. Here, we are developing novel 3D biomaterial systems that mimic aspects of the complex niche microenvironment in order to systematically assess the influence of various extrinsic cues on HSC biology. We hypothesize that co-modulating the local niche cell and hydrogel densities within the chip and applying biomolecule agonists/antagonist of known HSC signaling cascades will enable us to directly quantify the role direct (cadherin-mediated) vs. indirect (paracrine signaling) interactions between niche cells and HSCs play in early HSC fate decisions.

Methods: We have created a biomaterial tool that enables a high throughput approach to address difficulties in assessing the role cell-cell interactions play in the HSC niche: 1) the multitude of potential cell types within the niche; 2) multiple mechanisms (juxtracrine, paracrine) of HSC-niche cell interaction; 3) presence/absence of additional soluble biomolecules in the BM. We create multiple opposing gradients of cells and/or hydrogel biomaterials in a novel multi-gradient microfluidic experimental chamber to encapsulate a small number of HSCs and niche cells (~180 uL volume) in a collagen hydrogel. Discrete points along the gradients within this chamber contain defined ratios of HSC:niche cells or ECM proteins, thereby providing a homologous series of microenvironments for HSCs. The linear gradient is generated from a suspension of cells in a collagen solution using a microfluidic device[5]. The intermixed solution is allowed to gel in a teflon mold (15 x 8 x 1.5 mm) with a glass bottom, enabling *in situ* imaging. The HSCs are isolated as $Lin^{-}c\text{-}kit^{+}Sca\text{-}1^{+}$ from murine bone marrow. Niche cells of interest are osteoblasts (OBs), vascular endothelial cells, $CXCL12^{+}$ stromal cells etc. Discrete regions within the mold can be isolated, post culture, to probe HSC biology using tools such as surface antigen expression, MTS assay, gene expression and functional assays[6].

Results: We have successfully created opposing gradients of fluorescent microbeads (1 μm dia. FluoSpheres, Invitrogen) and OBs vs HSCs/OBs in distinct collagen suspensions (1 – 2.5 mg/mL). We have used fluorescent image scanning (Typhoon 9400 Scanner, GE) as well as FACS and imaging of discrete regions to quantify the resultant gradients. Based on this data, we can correlate the average fluorescence intensity to a cell/bead population, which can be used for future experiments as a standard for cell densities within sections of the mold. These gradients can be created at various initial cell densities (0.2 – 2 million cells/mL). Initial studies on the OBs indicate no immediate detrimental effect of the gradient making process on cell viability (> 80%).

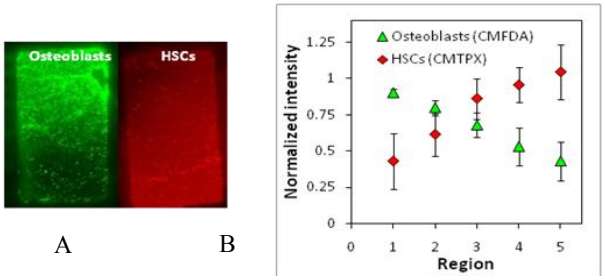

Figure 1. A) Opposing 2-color gradient of cells (within the same mold). B) Population ratios of HSCs:OBs at distinct regions within a 1 mg/ml collagen mold, based on mean fluorescence intensity.

Conclusions: We have created a novel class of collagen-based biomaterials that mimics patterned heterogeneities found in the native BM while also enabling *in situ* analysis of the HSCs. Gradient generation with different materials (cells/beads) using different collagen densities and their subsequent analysis demonstrates the versatility of this biomaterial system. This project will develop transformative new tools to systematically explore the significance of cell-based cues on HSC fate and provide significant new insight into the relationship between extrinsic cues and the internal signaling cascades regulating HSC biology. Improved understanding of HSC fate decision is critical for optimizing biomaterial systems for *ex vivo* expansion of clinically relevant hematopoietic cells and for studying the etiology, niche regulation and treatment of hematopoietic pathologies.

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