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Regulating dynamic signaling between hematopoietic stem cells and niche cells via a hydrogel matrix

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ABSTRACT

Hematopoietic stem cells (HSC) reside in unique bone marrow niches and are influenced by signals from surrounding cells, the extracellular matrix (ECM), ECM-bound or diffusible biomolecules. Here we describe the use of a three-dimensional hydrogel to alter the balance of HSC-generated autocrine feedback and paracrine signals generated by co-cultured niche-associated cells. We report shifts in HSC proliferation rate and fate specification in the presence of lineage positive (Lin^+) niche cells. Hydrogels promoting autocrine feedback enhanced expansion of early hematopoietic progenitors while paracrine signals from Lin^+ cells increased myeloid differentiation. We report thresholds where autocrine vs. paracrine cues alter HSC fate transitions, and were able to selectively abrogate the effects of matrix diffusivity and niche cell co-culture via the use of inhibitory cocktails of autocrine or paracrine signals. Together, these results suggest diffusive biotransport in three-dimensional biomaterials are a critical design element for the development of a synthetic stem cell niche.

1. Introduction

Hematopoietic stem cells (HSCs) are responsible for mediating the process of hematopoiesis, the continuous generation of the body's full complement of blood and immune cells. These events take place in unique regions of the bone marrow termed niches [1–6]. While the genetic information required to direct HSC fate specification events such as quiescence, self-renewal, or differentiation is contained within its DNA, signals from the niche – surrounding cells, the extracellular matrix (ECM), ECM-bound or diffusible biomolecules – trigger these events [7–9]. An artificial bone marrow would have significant clinical value, both for therapeutic expansion of HSCs to improve hematopoietic stem cell transplants and to facilitate studies of the etiology and treatment of hematologic diseases.

Recent efforts have focused on the development of biomaterials that regulate the presentation of matrix-based signals (*e.g.*, stiffness, composition) or exogenously-provided (soluble or matrix bound) growth factors [10–13] to direct HSC fate. However, exploring the role played by interactions between HSCs and supportive niche cells within the matrix provides a new avenue for investigation. Direct cell-cell interactions (juxtacrine signals) rely on the activation of membrane-bound cell adhesion molecules; these signals have been exploited in a biomaterial context to alter HSC survival and differentiation [14,15]. Indirect cell-cell interactions between HSCs and niche cells (paracrine signals) or autocatalytic effects from the HSC itself (autocrine signals) are mediated by diffusible biomolecules [16–20]. Recently, experimental and modeling efforts have begun to examine niche-inspired paracrine and autocrine signals, primarily using liquid culture systems [19,21]. While demonstrating the potential to alter HSC lineage specification profiles, translation of such systems into fully three-dimensional biomaterial platforms offers an opportunity for innovation but remains poorly explored.

Within the niche, HSCs and supportive niche cells secrete and subsequently interact with a large number of cytokines and growth regulators [22]. Mesenchymal stem cells (MSCs) are believed to stimulate proliferation of HSCs through paracrine-mediated interactions via several molecules such as CXCL12, IL-6 and TPO [20].

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Csaszar et al. demonstrated that paracrine-mediated IL-6 signaling between CD34$^+$ human progenitor cells can be regulated by activation of the delta-1 ligand and is responsible for HSC expansion [19]. Similarly, the transforming growth factor β (TGF-β) pathway has been shown to be activated via both niche generated paracrine signals and HSC-generated autocrine feedback to inhibit HSC self-renewal and proliferation [16,23]. *In vitro* autocrine feedback activated via vascular endothelial growth factor (VEGF) has also been shown to maintain HSC survival [17] while platelet derived growth factor (PDGF) mediated autocrine feedback can enhance proliferation [23,24]. From an engineering context, Zandstra et al. demonstrated selective inhibition of paracrine and autocrine feedback signal transduction pathways in liquid culture to alter HSC fate [21,23,25]. Recently, Müller et al. described the use of arrays of microcavities to culture single or small groups of hematopoietic stem and progenitor cells (HSPCs), and reported that while autocrine feedback within the microcavity culture may play a role in HSC quiescence, paracrine signaling provided both stimulatory and inhibitory effects [26]. While these studies highlight the importance of autocrine and paracrine signaling mechanisms for HSC biomanufacturing, significant opportunity exists to develop a framework to examine the balance of these signals within a fully-3D biomaterial platform where matrix diffusive transport plays a critical role.

Here we study the coordinated effects of paracrine signals produced by co-encapsulated Lin$^+$ niche cells and HSC-generated autocrine feedback on HSC lineage specification. We hypothesize that the mode of cell communication in a diffusion-restricted environment is a function of niche cell density, responsible for establishing the concentration of paracrine signals, and the diffusivity of the hydrogel encapsulating the cells. We further hypothesize that altering the delivery of paracrine signals from niche cells or the balance of diffusive loss vs. retention of HSC-generated autocrine signals alters HSC fate specification. We explored this concept using the well-described murine hematopoietic stem cell system, choosing primary bone marrow derived Lin$^-$Sca1$^+$cKit$^+$ (LSK) sub-fraction as HSCs and Lin$^+$ marrow cells as supportive niche cells known to secrete biomolecular cues that stimulate HSC proliferation and lineage specification [23]. HSCs were encapsulated with Lin$^+$ niche cells in collagen hydrogels, varying both hydrogel density (1, 3 mg/mL) and HSC:Lin$^+$ ratio (1:0, 1:10, 1:100), with liquid culture used as a control (Fig. 1). Cultures were limited to 2 days in order to explicitly consider early HSC responses to mixed cultures and to limit the effects of dynamic feedback responses. We traced HSC response via established metrics of HSC apoptosis, proliferation, surface antigen expression, and colony forming unit capacity, and used selective inhibitors of paracrine and autocrine signaling pathways to validate our findings. We report HSC fate specification as a function of diffusion-restricted (autocrine feedback dominated) vs. diffusion unlimited (paracrine dominated) biomaterial environments, and in so doing define a biomaterial-based approach to regulate the balance of autocrine vs. paracrine signals to tune HSC proliferation vs. differentiation.

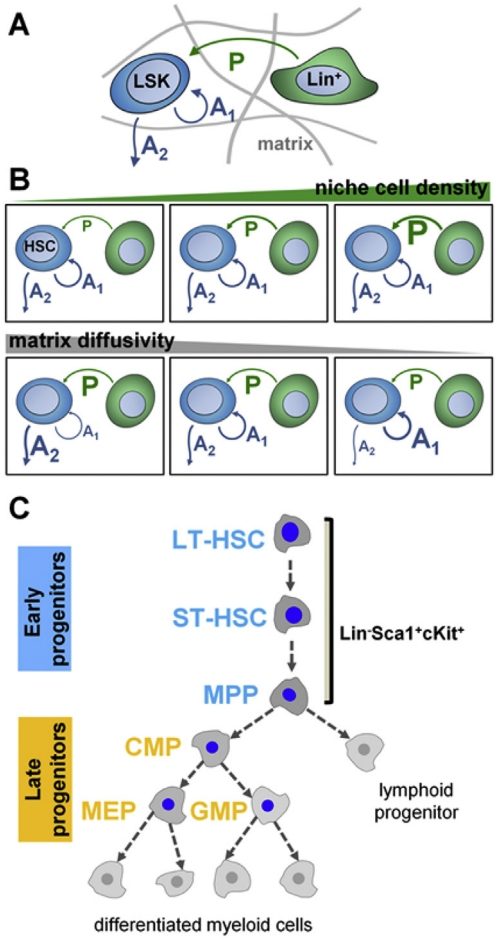

Fig. 1. Hematopoietic stem cell – Lin$^+$ niche cell interactions within a hydrogel niche. **(A)** A schematic depicting biomolecular interactions between HSCs and niche cells encapsulated within a hydrogel matrix. P: paracrine signals generated by the niche cell population. A1: Fraction of HSC-generated autocrine signals that contribute to feedback loop. A2: Fraction of HSC-generated autocrine signals that diffuse away. **(B)** Schematic of expected effects of changes in niche cell density versus matrix diffusivity on the magnitude of Paracrine vs. Autocrine feedback reaching the HSC population. Magnitude of signal depicted by the arrow width and font size. **(C)** Representative image of the HSC differentiation hierarchy depicting the starting Lin$^-$Sca1$^+$cKit$^+$ (LSK) fraction along with the early vs. late hematopoietic progenitor cell populations. LT-HSC: Long-term repopulating HSC (LSK·CD34$^-$·Flk2$^-$). ST-HSC: Short-term repopulating HSC. MPP: Multipotent progenitor (LSK·CD34$^-$·Flk2$^-$). CMP: common myeloid progenitor. MEP: megakaryocyte-erythrocyte progenitor. GMP: granulocyte-macrophage progenitor.

2. Methods and materials

2.1. HSPC and Lin$^+$ cell isolation

All work involving primary cells was conducted under approached animal welfare protocols (Institutional Animal Care and Use Committee, University of Illinois at Urbana-Champaign). Primary HSPCs were isolated from the bone marrow of the femur and tibia of female C57BL/6 mice (Jackson Labs; Ages 1–3 months) as described previously [27]. HSPCs were identified as the Lin$^-$ Sca-1$^+$c-kit$^+$ (LSK) fraction by incubating the remaining bone marrow cells with a cocktail of antibodies (eBioscience San Diego, CA): PE-conjugated Sca-1 (1:100 dilution), APC-Cy7 conjugated c-kit (1:100 dilution), and a 1:100 dilution of a FITC-conjugated Lineage (Lin) cocktail (CD5, B220, Mac-1, CD8a, Gr-1, Ter-119). Both the LSK and Lin$^+$ fraction was sorted using a BD FACS Aria II flow cytometer (BD FACS Diva software) and collected in PBS/5% FBS on ice for immediate use.

2.2. Collagen hydrogel preparation

Rat tail type I collagen (BD Biosciences, Bedford MA) was used to make the cell-laden collagen hydrogels. Collagen solutions of defined densities (1, 3 mg/mL) were prepared from the stock collagen solution of approximately 9 mg/mL (lot specific). The final concentrations were as follows: 1× M199 buffer (supplemented with 0.75% NaHCO2 and 0.01 M HEPES) in a ratio of 1:4 with collagen stock; 1 mM Dithiothreitol (DTT, Sigma); 5 mM CaCl2;