Towards Engineering a Multi-Niche Functional Human Bone Marrow: Advancing Basic Studies and Aiding Clinical Translation

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Statement of Purpose: The bone marrow (BM) environment is a complex arrangement of physical, chemical, and cellular constituents that form distinct microenvironments within the bone organ. There are three distinct niches within BM, the endosteal niche, the central marrow, and the perivascular niche, that have different properties and play host to separate cellular processes and behavior (Wang LD. Nat Rev Mol Cell Biol. 2011;12:643-55.). A functional *in vitro/ex vivo* model, that recapitulates the major physiological and anatomical properties of human BM without being overly complex, could overcome the limitations of animal-based studies, and allow for quantitative understanding of the biophysical, chemical, ECM, and cellular characteristics of human BM, and potentially enable live-imaging of dynamic BM processes. Several groups have attempted to reproduce specific niches within the BM, but have not yet reported a multi-niche human BM environment generated *in vitro* (Torisawa Y. Nat Methods. 2014;11:663-9.). We hypothesize that the various niches present in human BM can be simulated *in vitro* using specific physical, chemical, and cellular components; and that the appropriate combination of these engineered microenvironments would allow for *in vitro* generation and subsequent culture of a functional, *in vivo*-like, surrogate BM organ. Here we present our efforts to screen physical environments for suitability in a multicomponent BM culture system**.**

Methods: Well-characterized human mesenchymal stem cells (hMSCs) (RoosterBio Inc., Frederick, MD) (>90% CD105+, CD73+, CD90+; <5% CD45+, CD34+, CD11b+, CD19+) were used to generate stromal cell populations in endosteal, central marrow, and perivascular niche constructs. Endosteal niche was reconstructed by seeding hMSCs in 3D scaffolds in basal and osteogenic media conditions. Two scaffold formats and two materials were screened for the endosteal niche; electrospun scaffolds (Roy group) and precision extrusion deposition (PED) scaffolds (3D Biotek, Hillsborough, NJ) composed of polystyrene (PS) and polycaprolactone (PCL). Cells were seeded onto scaffolds directly or after the scaffolds had been coated with collagen I. The central marrow and perivascular niche were recreated by encapsulating hMSCs in hyaluronic acid (HA)/polyethylene glycol (PEG) hydrogels (Hystem™, ESI BIO, Alameda, CA) at a density of $10x10^6$ cells/mL. The mechanical properties of the hydrogels were controlled by increasing/decreasing the percentage of PEG diacrylate (PEGDA) crosslinker in the hydrogel, while maintaining HA weight percent. Hydrogels with elastic moduli of 1-2 kPa were used for the central marrow. Hydrogels with elastic moduli of 10-30 kPa were used to fabricate the perivascular niche. The secretion/surface display of cytokines (ELISA and IHC), expression of extracellular matrix (ECM) (histology and IHC), and cellular gene expression (RT-PCR) were measured to evaluate hMSC behavior in the niche constructs.

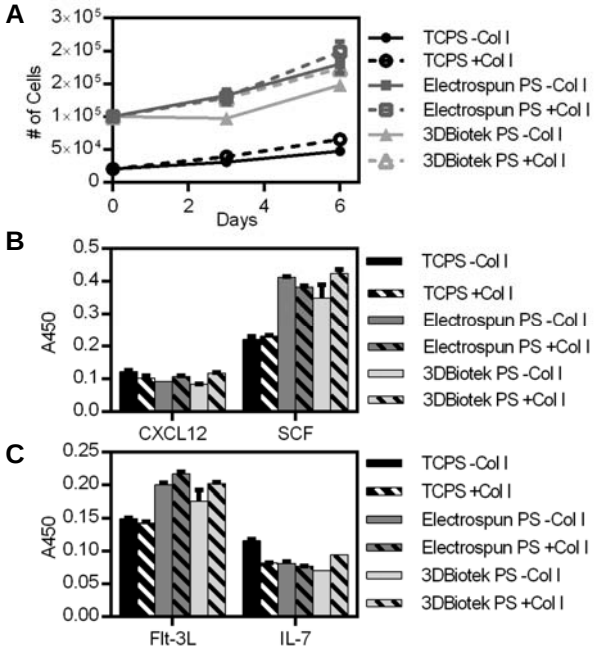

Figure 1. *The (A) expansion of hMSCs in different 3D scaffolds and the secretion of (B) CXCL12, SCF, (C) Flt-3L, and IL-7 measured by ELISA.*

Results: 3D PS scaffolds supported hMSC culture and expansion (Figure 1A). The hMSCs cultured on 3D scaffolds secreted cytokines necessary for hematopoietic cell homing (CXCL12), early hematopoiesis (SCF) (Figure 1B), as well as cytokines necessary for early B cell development (Flt-3L and IL-7) (Figure 1C). Coating the culture surface with collagen I resulted in increased hMSC expansion (Figure 1A) and altered cytokine expression of cytokines in some culture contexts (Figure 1C). The effect of mechanical properties on hMSCs encapsulated within HA/PEG hydrogels will be explored to screen for physical conditions that replicate stromal properties of the central marrow and perivascular niche. Endosteal, central marrow, and perivascular constructs will then be layered in a multi-niche culture to construct a complete BM surrogate.

Conclusions: The expression of key cytokines for the support of hematopoiesis has been found to be dependent on the physical and biochemical properties of the culture. We believe these properties can be used to create distinct niches within a single culture to create the unique niches found in BM.

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