Creating a Stem Cell Niche Using Extracellular Matrix Proteins to Drive Osteogenic Differentiation

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Statement of Purpose: We have developed a protein microbead-based system to direct the differentiation of human mesenchymal stem cells (hMSC). The *ex vivo* expansion and specific induction of differentiation potential are prerequisites to robust tissue regeneration when transplanted *in vivo*. The microbead format has the advantage that cells are surrounded by a small amount of physiologically relevant matrix, and can be cultured and injected into a wound site while still buffered and controlled by this defined environment. In addition, growth factors and other biochemicals can be added directly into the bead matrix, providing highly local stimulation to the entrapped cells.

Methods: To create cell-loaded microbeads, hMSC were collected, mixed with solubilized agarose and defined amounts of collagen, fibronectin, and vitronectin. The suspension was injected into a spinning PDMS bath and emulsified. This procedure consistently produced 50-150 µm diameter beads that could be maintained in culture. Second harmonic generation (SHG) imaging was used to visualize collagen fibers. Following 21 days in culture, constructs were assayed for calcium deposition (Alizarin Red), lipid formation (Oil Red O) and proteoglycans (Alcian Blue) to determine the differentiation specificity of each culture system.

Results: The ability of hMSC to interact with their extracellular matrix/niche in a dynamic fashion is necessary for improved differentiation potential. hMSC cultured in 3D collagen gels showed increased differentiation relative to 2D controls and simultaneously upregulated a specific collagen activated receptor, discoidin domain receptor I (DDR1), which is critical in matrix remodeling (Fig. 1).

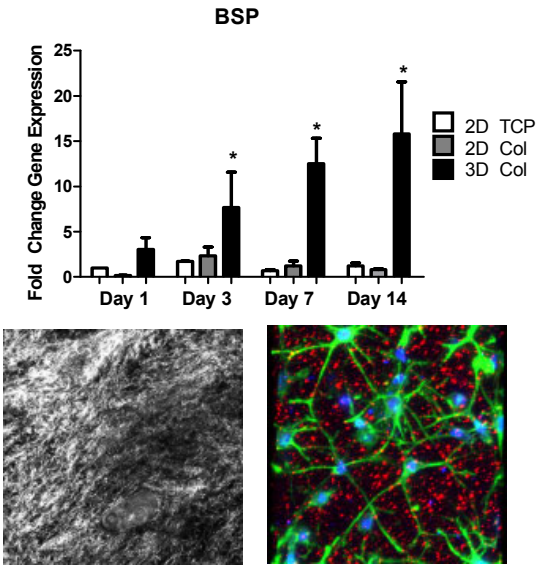

Fig. 1: 3D collagen I culture enhances the osteogenic potential of hMSC (A) and is accompanied by significant matrix remodeling shown using SHG (B) and distinct DDR1 localization in 3D space (C).

hMSC cultured in a variety of bead compositions *in vitro* for 21 days remained viable (>65 %), showed increased spreading as a function of protein incorporation and remodeled their matrix at the cellular interface, as characterized by SHG. After 21 days, hMSC showed increased osteogenic gene expression and calcium deposition (Fig. 2) when cultured in beads supplemented ECM proteins and exhibited the highest potential with 5% vitronectin, 5% fibronectin and 30% collagen I.

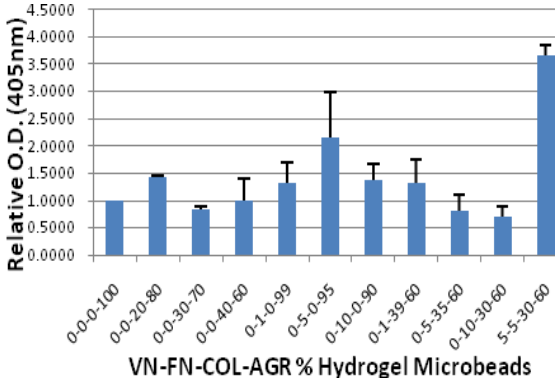

Fig. 2: Calcium deposition after 21 days as detected by Alizarin Red.

Conclusions/Discussion: Our results show that defined protein environments can be used to stimulate hMSC differentiation, though the specific cues needed for highly focused differentiation still need to be identified. These bead environments provide structural and chemical cues in a localized fashion to direct hMSC phenotype. Extracellular matrix proteins, in the absence of osteogenic supplements, can be used to direct hMSC differentiation consistent with the *in vivo* importance of the structural niche. The beads can be injected in a minimally invasive manner to fill the whole site of the defect and provide void spaces for the ingrowth of a vascular network. This bead system has broad tissue application, given the proper inductive stimuli, and may better replicate the complexity of the natural niche, thereby promoting functional tissue regeneration *in vivo*.

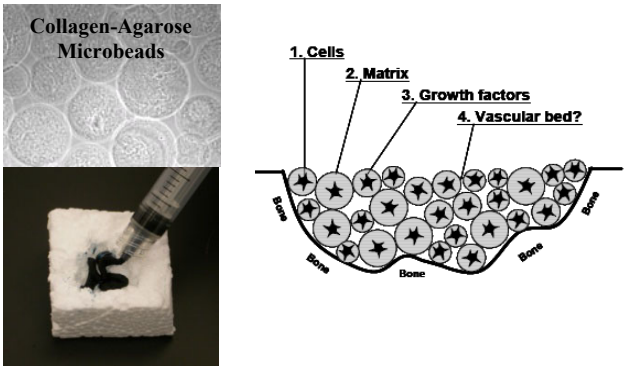

Fig. 3: Hydrogel microbeads can be concentrated into a paste and injected into the site of the defect where they will direct and induce hMSC phenotype.