Combinatorial 3D Hydrogels for Stem Cell Niche Optimization towards Bone Differentiation

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Statement of Purpose: A long-standing bottleneck in stem cell biology and tissue engineering field is the lack of understanding of how the complex niche signals regulate stem cell fate in 3D. While stem cell niche is a multi-factorial environment, most previous studies focus on studying the effects of individual type of microenvironmental cue on stem cell fate regulation. Furthermore, most high-throughput studies on cell-material interactions to date have been performed on 2D environments, while the architecture of the stem cell niche itself is 3D. As such, the goal of this study is to develop novel 3D combinatorial hydrogel microarray with various biochemical and mechanical properties to facilitate rapid analysis of interactive niche signaling on stem cell fate in 3D.

Methods: Synthetic photopolymerizable hydrogels with varying molecular weight and concentration were fabricated to produce a hydrogel microarray with 36 groups with a broad range of mechanical properties. Various doses and combinations of natural extracellular matrix proteins (Col I, Fibronectin, and Laminin) were incorporated into the hydrogel microarray to introduce the biochemical diversity. Passage 6 human adipose-derived stem cells (hADSC) were encapsulated in 3D combinatorial hydrogels with various biochemical and mechanical properties. All samples were cultured in osteogenic medium for up to 21 days to induce bone differentiation. Outcomes were analyzed by live-dead staining (day 1), alkaline phosphatase activity (day 7), quantitative gene expression of bone markers and calcium assays (day 21).

Results: Liver-dead staining confirmed most cells remain viable after encapsulation in 3D, and significant mineralization was deposited after 3 weeks of culture. Increased alkaline phosphatase (ALP) activity was observed in groups with higher mechanical properties (15% w/v compared to 10% w/v). Optimal group (MW5000, 15% w/v, FN10) led to over 150-fold increase in expression of mature bone marker osteocalcin. Similar to the trend observed in ALP, increasing mechanical property of the 3D network (15% w/v) led to significantly increased OCN. In the presence of low FN (10 µg/ml), increasing LM led to increased OCN. In contrast, in the presence of intermediate FN (25 µg/ml), increasing LM led to decreased OCN expression. Further increase of mechanical property of hydrogels (20% w/v) led to decreased osteocalcin expression in the presence of ECM proteins.

Conclusions: Our data suggests that various biochemical and mechanical cues interact to regulate stem cell differentiation in a non-linear manner. Our results also shows that an optimal range of mechanical cues in 3D may synergize with biochemical cues to promote hADSC differentiation towards osteogenesis. The outcomes of the proposed studies can aid in elucidating how complex niche signals regulate stem cells fate in 3D, and guide synthesis of microenvironments to promote desired stem cell differentiation.

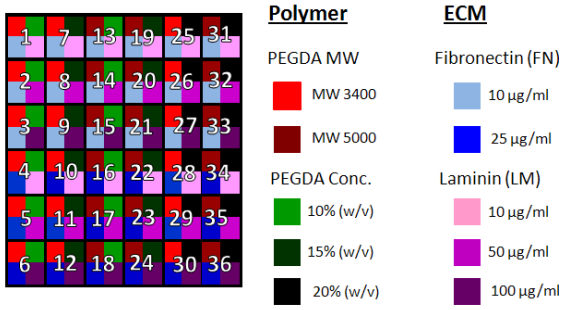

Figure 1. Schematic illustration of hydrogel compositions. A total of 36 compositions with varying biochemical compositions and mechanical stiffness were investigated.

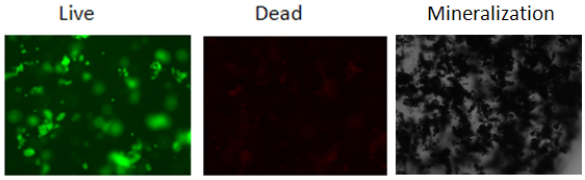

Figure 2. Most cells remain viable after being encapsulated in 3D. And significant amount of mineralization was observed in the lead group (15% w/v, MW5000, FN10, LM 50).

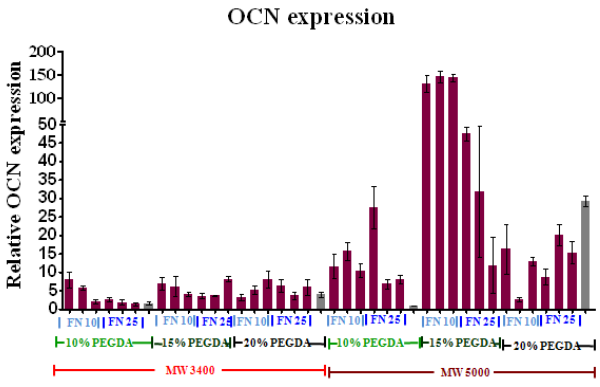

Figure 3. Quantitative gene expression of mature bone marker, osteocalcin (OCN) by hADSCs after 3 weeks of culture in osteogenic medium.

References: Underhill GH and Bhatia SN. Curr Opin Chem Biol, 2007. 11(4): p. 357-66.