Hydrogel-based Platforms for Co-Culture and On-Demand Cell Retrieval of Human Mesenchymal Stem Cells

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Introduction: Diabetes mellitus can have debilitating orthopaedic consequences, possibly due to effects of sustained hyperglycemia on human mesenchymal stem cells (hMSCs)[1]. Traditional studies of hMSC behavior in pathological conditions have been conducted independently of the cell types that make up their niche, providing a limited view of hMSC behavior. Furthermore, culture systems that do include multiple cell types often prevent retrieval or separation of distinct cells populations post-culture. Thus, we have developed a unique hydrogel-based co-culture platform with on-demand recovery of hMSCs to examine phenotypic differences after exposure to normal and hyperglycemic glucose levels in the context of other cells known to populate the bone marrow niche, specifically osteoblasts and adipocytes. In combination with multivariate modeling of gene expression, we demonstrated correlation of hMSC response with culture condition and glucose levels. Thus, this platform has the unique potential to provide a tissue-level perspective of hMSCs behavior in the context of a chronic pathology.

Methods: PEG-diacrylate (PEG-DA, Mn = 8kDa), PEG-RGD and PEG-YIGSR (Mn = 3.4kDa), and PEG-LGPA-DA (Mn=3.4kDa, collagenase-sensitive) were synthesized per previous methods [2,3]. To fabricate cultures, hydrogel solutions at 15×10^6 cells/mL were formulated with10% w/w PEG-DA (human osteoblasts and adipocytes) or PEG-LGP-DA (hMSCs) and 0.05% w/w D2959 photoinitiator, with 1 mM RGD and YIGSR for hMSCs and adipocytes, respectively, and photopatterned into 1.5 × 1.5 × 1 mm laminated gel structures (Figure 1).

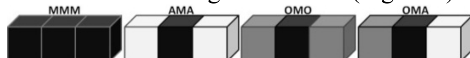

Figure 1: Blocks represent hydrogels co-culture systems with encapsulated cells. Degradable hMSC (M) blocks are black, osteoblast (O) blocks are grey, and adipocyte (A) blocks are white.

Gel structures were cultured in DMEM with 10% FBS, 5.5 mM glucose (fasting serum glucose), 2 mM L-glutamine, 70 µM L-ascorbate, 45 pM insulin (fasting serum insulin), 1% amphotericin B, and 0.1% gentamicin. After 1 day, a subset of cultures were switched to 22.3 mM glucose (hyperglycemic). For analysis, all four culture types under both normal and high glucose conditions were evaluated. On days 1 and 7, hMSCs were extracted from gels (n = 5) and gene expression was evaluated using qPCR for genes indicating osteogenesis (RUNX2, OCN, OPG), adipogenesis (PPARG2, CEBPB, LEP, ADIPOQ), and glucose responsiveness (ATF2, FOX01, JUN, NFKB1). Data was incorporated into multivariate models using principal component and partial least squares discriminant analyses. To evaluate clonogenicity, hydrogel constructs (n = 3) were subjected to 1,100 U/mL collagenase at day 7, digesting the hMSC-containing PEG-LGPA-DA block. Recovered hMSCs were re-plated and clonogenicity was evaluated by counting 2 mm colonies (stained with 3% crystal violet, 100% methanol) after 14 days. For histological analysis, gel constructs were serially cryosectioned at a 20 µm thickness. Sections were stained for lipids (10 µg Nile Red/mL in 1% acetone) and alkaline phosphatase (ALP) (ALP staining kit, Vector Labs). Sections were visualized with epifluorescence microscopy.

Results: Multivariate statistical modeling of gene expression data for adipogenic, osteogenic, and glucose responsive markers indicated that observations clustered primarily by culture condition and secondarily by glucose conditions, thus demonstrating that the response to glucose is dependent on the types of neighboring cells (Figure 2).

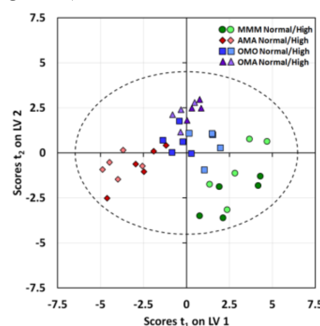

Figure 2. Multivariate statistical modeling based of covariance in gene expression data. Culture conditions are represented as different shapes and are shown to cluster. Glucose levels within each culture condition are represented in light and dark shades and cluster within each culture condition. $R^2Y = 0.842$, $Q^2 = 0.649$.

After enzymatic degradation of the MSC-containing hydrogel block after 7 days in different culture conditions, colony forming assays indicated that high glucose conditions significantly decreased colony forming ability when cultured in the absence of adipocytes (Figure 3).

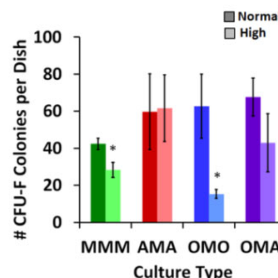

Figure 3. Number of hMSC colony forming units for each culture condition and glucose level after seven days in culture. Statistically significant differences between glucose levels within one culture type are indicated by an asterisk (*), $p<0.05$, n=3.

Lipid and ALP staining showed maintenance of cell phenotype in adipoctyes and osteoblasts, respectively, over the culture period (data not shown).

Conclusions: We have developed a dynamic culture platform on which we can both study the interactions between human MSCs, adipocytes, and osteoblasts under different physiologically relevant conditions and retrieve live cells using on-demand enzymatic degradation of specific hydrogel blocks. Through statistical modeling of gene expression and clonogenicity assays, we have shown that communication with neighboring cell types can influence hMSC response to hyperglycemic glucose levels. Thus, our data suggests that it is possible that diabetes disease pathology is not just a sum of individual cell responses but rather a manifestation of cellular communication between multiple cell types.

References: [1]Cramer et al. Stem Cells Dev. 2010; 19:1875-84. [2]Hammoudi TM. et al. Tissue Eng Pt A. 2012; 18:1686-97.[3]Yang P. et al. Tissue Eng Pt A. 2012; 18: 2365-75.

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