Novel 3D Hydrogel System Improves the Retainment of Viable MSC Populations Overtime

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Statement of Purpose: Mesenchymal stem cell-derived therapeutics are at the forefront of scientific research and discovery due to their intrinsic regenerative capabilities and their wide-ranging applications.[1] Current Mesenchymal Stem Cell (MSC) research and therapies typically utilize rigid 2D culture plastics to expand and grow MSCs. Studies have demonstrated that traditional 2D culture is not ideal for expansion of cells, particularly "stem-like" cells such as MSCs, and can result in a loss of MSC multipotency, induction of senescence, and decreased bioactivity.[2,3] Though previous data with MSC-derived therapies are promising, the utilization of standard 2D culture techniques continues to be a significant limitation to overall therapeutic efficacy. Therefore, we set out to develop a modular 3D system with tailorable properties that more closely mimics the native tissue environment and eliminates the stress of subculturing on cells. In so doing, we aimed to generate a system that improves overall MSC viability and "stem-like" phenotypic characteristics overtime in culture that results in more consistent and effective therapeutics.

Methods: Human Adipose-derived Mesenchymal Stem Cells (ASCs) were expanded in either traditional 2D plastic culture or our novel 3D hydrogel system. The customizable 3D system is a PEG-based bioprinted hydrogel that is mechanically similar to soft tissue (i.e. adipose) and contains a unique microarchitectural design that promotes cellular migration/proliferation. Additionally, the 3D hydrogel system eliminates the need for subculturing via addition of supplemental hydrogels, similar to attaching puzzle pieces (**Fig. 1A**). Characterization of ASCs was performed temporally over ten (10) passaging events in 2D, or a time-equivalence for 3D culture. Passaging events occurred every ~5 days in 2D culture. Throughout expansion, Conditioned Medium (CM) was collected and analyzed via a Proteome array, HPLC-MS/MS, or ELISA. ASCs were assessed for viability and phenotypic changes over time via senescence activity and expression of "ASC-like" phenotypic CD surface markers, respectively.

Results: Culture expansion of ASCs in a traditional 2D system resulted in increased prevalence of senescent cell populations overtime, whereas senescent ASC populations cultured in the 3D hydrogel system remained near baseline expression (**Fig. 1B**). Similarly, 2D culture resulted in a significant decrease overtime in "ASC-like" phenotypic CD markers, indicating a deterioration of "stem-like" properties. Conversely, 3D culture resulted in significantly greater expression of CD markers, relative to 2D throughout the temporal study (**Fig. 1C**).

Additionally, the secretive bioactivity of ASC populations was assessed via different proteomic analyses. The relative compositional differences between ASC Conditioned Media (ASC-CM) collected from 2D and 3D culture was initially evaluated for the relative abundance of 55 different secreted proteins. Of the 55 proteins analyzed, 8/55 (15%) were significantly increased in 3D culture relative to 2D (**Fig. 1D**). Additional proteomic analyses are currently on-going and investigating for differences in key regenerative growth factors and immunomodulatory factors of ASC-CM in 2D versus 3D via ELISAs and HPLC-MS/MS.

Conclusions: With the experimental data outlined in these studies, we introduce a novel modular 3D hydrogel system that can be customized for specific cell populations and eliminates the need for subculturing/passaging. Ultimately, this system improves the overall longevity, viability and homogeneity of cell populations expanded in this system.

References:

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Figure 1: ASCs seeded at P1 either within a 3D hydrogel system or 2D culture for ~3 weeks, or 4 passaging events in 2D. (A) Four hydrogels are attached together (*top*) and fluorescently labeled (*bottom*). (B) Quantification of fluorescently labeled senescent ASCs in 2D (***black bars***) and 3D (***teal bars***). (C) Quantification of CD markers in ASC populations at P5 in 2D (***black bars***) and 3D (***teal bars***). (D) Relative quantification of significantly increased proteins secreted in 3D relative to 2D ASC-CM at P3. *p < 0.05, ***p < 0.001, and ****p < 0.0001.