Human Mesenchymal Stem Cell Proliferation as a Function of Scaffold Position in a Tubular Perfusion System

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Statement of Purpose: Despite recent advances, tissue engineering is still limited by the ability of oxygen and other nutrients to diffuse into the interior of 3d scaffolds. In order to create clinically relevant tissue engineering constructs, it is important to quantify human mesenchymal stem cell (hMSC) proliferation and metabolic activity as a function of scaffold position. In this study hMSC growth in static alginate beads is compared to growth in a tubular perfusion system (TPS) bioreactor. The TPS bioreactor has previously been shown to increase the differentiation and proliferation of hMSCs however the proliferation throughout the entire bead was not studied[1]. The goal of this study is to investigate cell viability as a function of scaffold position within statically cultured constructs as compared to dynamic culture in the TPS bioreactor.

Methods: Alginate constructs seeded with 100,000 cells per bead were cultured either statically or dynamically. Dynamically cultured groups were cultured in the TPS bioreactor which consists of sets of highly gas-permeable, platinum-cured silicone tubing (Figure 1). Constructs were loaded into the tubular chamber and media was perfused using a Masterflex L/S Multichannel Pump at a flow rate of 1 mL per minute. The entire tubing set was preassembled and autoclaved to ensure sterility. Cells were isolated from discrete layers by dissolving scaffolds in the calcium chelating agent EDTA. A calibration curve was constructed to determine the dissolution time that yields constructs of two-thirds, one-half, and one-third of the original diameter. Constructs were dissolved for the specified amount of time with 1 mL of 0.025 M EDTA; the supernatant, now with suspended cells, was removed and placed into centrifuge tubes.

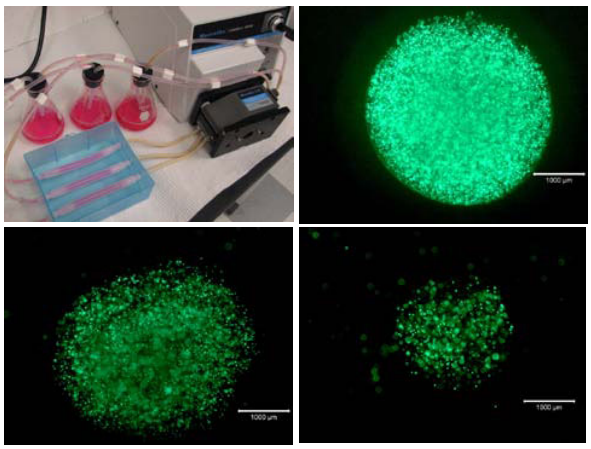

Figure 1. Photograph of TPS bioreactor (top left). Live dead staining of hMSCs in alginate beads in the entire bead (top right), middle (bottom left), and inner (bottom right) positions.

Each well was washed with PBS. Beads were transferred to a new well containing 1 mL of 0.025 M EDTA for the next dissolution period while the cells were spun at 5000 xg for 5 minutes to isolate the cell pellet. Cell pellets from discrete layers of the scaffolds were isolated and the DNA quantified using pico green. Metabolic activity was measured using an MTT-based *in vitro* toxicology kit (Sigma, St. Louis MO). Live dead staining was completed to visualize cells in scaffolds.

Results: Live dead staining indicates nearly all cells are viable throughout the alginate construct on day one (Figure 1). DNA quantification indicates cell densities throughout different annuli to be approximately equal on day one (Figure 2). On days 7 and 14 cell density was higher in the inner core of beads. The TPS bioreactor cultured constructs showed approximately two fold increases of cell density in the inner core on day 14 as compared to static culture. MTT analysis shows higher metabolic activity in bioreactor cultured beads as compared to static in all annuli, with the innermost annuli exhibiting the greatest difference in metabolic activity (Figure 2).

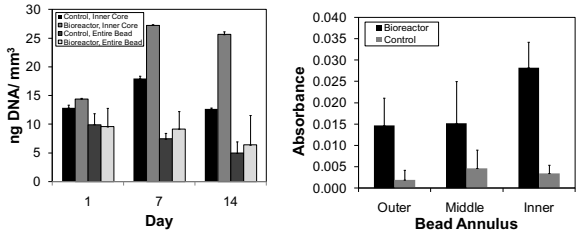

Figure 2: DNA amount normalized to volume (left). Cells density is higher in the inner core on days 7 and 14 in the bioreactor as compared to a static control. Absorbance correlating to metabolic activity after eight days of culture (right). Bioreactor groups exhibit higher levels of metabolic activity as compared to static control groups.

Conclusions: MTT results indicate that cells in the interior of statically cultured constructs have a lower metabolic activity than TPS bioreactor cultured constructs. DNA quantification indicates that cell densities are higher in the inner core of statically and dynamically cultured scaffolds, but the cell density increases almost two fold in bioreactor culture on day 14. This result can be attributed to increased mass transport through the scaffolds in the bioreactor system. The enhancement of cell proliferation in the center of alginate scaffolds in the TPS bioreactor indicates that this system can be used to enhance the *in vitro* culture of hMSCs.

References: [1]Yeatts AB, Fisher JP. Tissue Eng. 2010. In Press.