Custom bioreactor testing and effects of IVD mechanical loading on MSCs for use in tissue-engineered NP scaffold

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Degenerative disc disease (DDD) is a leading cause of backpain and occurs when the intervertebral discs (IVD) are damaged or start to degrade[1]. The IVD is made up of two parts: a stiff cartilage-like outer layer known as the annulus fibrosus (AF) and a more gel-like inner section called the nucleus pulposus (NP). When disc degeneration occurs, the NP can protrude through the AF and press on surrounding nerves which causes more pain. Current treatments include surgery to remove the herniated disc and pain management that do not address the underlying causes. A potential future treatment for DDD is the use of a tissue engineered NP scaffold that can be seeded with stem cells and inserted into the IVD to repair damage from degeneration[4]. To date, however, it is unknown which source of stem cells are best suited to use in combination with the scaffold as a new treatment for disc degeneration. The cells of normal IVDs experience between 0.1MPa and 3MPa of hydrostatic pressure during daily activity[2] while diseased IVDs experience decreased hydrostatic pressure of around 0.03MPa because of the compromised AF structural integrity[3]. The diseased discs also have a more acidic pH compared to healthy IVDs. Thus, the goal of the present study is to determine how different stem cells respond to the altered mechanical and chemical conditions of diseased IVD.

There are two objectives for the present study. The first is to test a new custom bioreactor that is capable of applying strain and hydrostatic pressure together or independently to cell cultures. Specifically, percent strains experienced by silicone membrane cell substrate under known pressures were determined by applying negative pressure to the chamber and measuring the deformation using ImageJ software. The maximum strain that can be achieved with this system was found to be approximately 14% at 0.09MPa. The second objective of the project is to expose adipose-derived mesenchymal stem cells to conditions found within the NP using the new bioreactor to examine cell viability and differentiation. The conditions used will be similar to those found in healthy and diseased IVDs. The six groups to simulate healthy and diseased conditions are: atmospheric pressure with no additional hydrostatic pressure and physiological, normal (7.4) pH (control), no HP and low (6.8) pH, 0.03MPa and normal pH, 0.03MPa and low pH (diseased), 0.3MPa and normal pH (healthy), and 0.3MPa and low pH. Each group will be exposed to the elevated pressure condition for 1 hour/day and the pH level will be maintained for the trial period. Following exposure to one of the treatment conditions for up to 10 days, one set of samples will be used for qRT-PCR to look at genes associated with cell differentiation and ECM production, specifically SOX9 and COL2A. The cell count will also be used to assess viability and cell proliferation of the MSCs under diseased and healthy IVD conditions.

References:

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