Coculture of Human Adipose-derived Mesenchymal Stem Cells and Human Disc Cells Stimulates Extracellular Matrix Production

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INTRODUCTION: During disc degeneration, disc cell viability and gene expression fail to adequately maintain the extracellular matrix (ECM) resulting in proteoglycan loss, disc dehydration and annular tears, which may lead to disc herniation. During early stages of disc degeneration, stimulation of matrix synthesis may be feasible using a number of potential biologic therapies, including supplementation of the number of cells in the disc. Use of disc cells is problematic since harvest produces low yields; in addition, cells may have reduced ECM production, or be undergoing apoptosis or senescence. Stem cell therapy has potential application in the biologic treatment of disc degeneration. Stem cells are characterized by the ability to self-renew and to maintain the ability to differentiate into lineage-specific cell types. Due to ease of harvest and abundance, adipose-derived mesenchymal stem cells (AD-MSC) have shown utility in tissue engineering [1, 2]. Our hypothesis is that coculture of human intervertebral disc cells with human AD-MSC, grown in 3D culture, will stimulate ECM components important in the disc, including proteoglycans. Here we investigated the separate culture (control) vs co-culture of AD-MSC and disc cells in 3D scaffold.

METHODS: Studies were approved by our Institutional Review Board. Adipose tissue was brought directly to the laboratory. AD-MSC were extracted by collagenase digestion, filtration, centrifugation and plating. Isolated AD-MSC were confirmed to be stem cells by their ability to differentiate into lineage-specific cell types. Due to ease of harvest and abundance, adipose-derived mesenchymal stem cells (AD-MSC) have shown utility in tissue engineering [1, 2]. Our hypothesis is that coculture of human intervertebral disc cells with human AD-MSC, grown in 3D culture, will stimulate ECM components important in the disc, including proteoglycans. Here we investigated the separate culture (control) vs co-culture of AD-MSC and disc cells in 3D scaffold.

RESULTS: Prior to coculture, 100% of the annulus cells were labeled with CFSE (Fig. 1A; green = CFSE label). AD-MSC were identified in 3D cultures by anti-CD44 immunolocalization (Fig. 1B). Following the 2 week coculture of annulus and AD-MSC, both cell types were still present at harvest (Fig. 1C shows brown immunolocalization of annulus cells; arrows mark unlabeled AD-MSC). Analysis of proteoglycan production showed significantly greater proteoglycan concentrations in 3D cocultures vs separate AD-MSC or annulus cultures (p=0.04, Fig. 1D). 3D coculture of annulus and AD-MSC resulted in a 44% increase in proteoglycan production when compared to the predicted value taken as the average of the individual control stem and disc cultures (p<0.01, Fig. 2).

CONCLUSIONS: Data presented here show that coculture of human annulus cells and human AD-MSC in 3D was feasible with retention of both cell types. Coculture resulted in significantly increased proteoglycan production vs single cell culture. Data presented here point to the value of AD-MSC and disc cell coculture in future biologic therapies for disc degeneration which may utilize an autologous cell approach.

REFERENCES:

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