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Optimization of Extracellular Matrix Production and Gene Expression by Human Annulus Cells in Three-Dimensional Constructs

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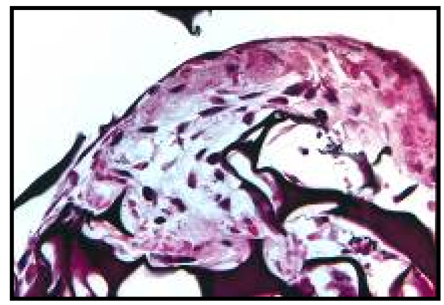
Introduction: Tissue engineering offers the potential to correct a number of conditions fundamental to disc aging and degeneration: low cell numbers in the aging disc, altered cell-cell and cell-extracellular matrix (ECM) interactions, and production of inappropriate ECM. Tissue engineering for the disc is a relatively new field, and knowledge of how disc cell microenvironments influence disc cell proliferation, ECM gene expression and ECM production is only now being obtained. This information is needed to help formulate criteria for successful cell-carrier interactions.

Objectives: To optimize gene expression, cell proliferation, and proteoglycan production by human annulus cells in three-dimensional carrier constructs.

Methods: Studies were approved by our human subjects Institutional Review Board. Annulus cells were obtained from surgical procedures performed on individuates with herniated discs or from discs procured from the Cooperative Human Tissue Network. Agarose, alginate, fibrin gel, collagen gel, and collagen sponges were used as cell carriers for annulus cells cultured from discs of 28 individuals (mean age 45.3 years, 5 Thompson grade II discs, 12 grade III discs, 12 grade IV discs). Cells were cultured in a standardized experimental protocol with 2-5 replicates for 10-14 days using previously published protocols from our lab. In situ hybridization assessed ECM gene expression of types I and II collagen, aggrecan and chondroitin-6-sulfotransferase. Assays were carried out to determine cell proliferation and proteoglycan production (S-GAG) (using the 1,9-dimethylmethylene blue (DMB) technique), and paraffin-embedded specimens were evaluated for cell and ECM features.

Results: In situ hybridization showed optimum expression of types I and II collagen, aggrecan and chondroitin-6-sulfotransferase by cells cultured in the collagen sponge microenvironment. Morphologic assessment showed production of abundant ECM between and around cells. Although collagen gels could often support good growth, these constructs did not result in either abundant ECM production or good EMC gene expression. Growth and ECM production in alginate and fibrin microenvironments were inferior.



Although agarose culture showed high S-GAG levels compared to the collagen sponge (2.94 $\pm$ 2.2 µg/ml (mean $\pm$ S.D. vs 0.94 $\pm$ 0.77), this was off-set by the significantly lower proliferation rate associated with culture of cells in agarose (agarose: 6,127 $\pm$ 2,150 cpm ^{3}[H]thymidine/µg DNA) compared to rates in the collagen sponge (12,729 $\pm$ 6,729, $p = 0.032$). As shown in the figure to the right, abundant ECM is produced by cells cultured within a collagen sponge.

Discussion and Conclusions: Human disc cells offer special challenges for in vitro studies because of the slow-growing nature of these cells. This work shows that collagen and S-GAG production can be modulated by choice of the carrier type into which human annulus cells are seeded. Cells seeded into the collagen sponge microenvironment attached well, proliferated, showed abundant gene expression for types I and II collagen, aggrecan and chondroitin-6-sulfotransferase, and produced abundant ECM evident upon histologic examination. These findings may have future application in cell-based tissue engineering strategies for biologic therapies for disc degeneration.