Accelerated Chondrocyte Extracellular Matrix Deposition through Dextran Sulfate Polystyrene, and Hyaluronic Acid

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Abstract:

During chondrogenic differentiation of human mesenchymal stem cells (hMSCs) for tissue engineering purposes, the accumulation of macromolecular extracellular matrix (ECM) components, specifically collagens and glycosaminoglycans (GAG), plays an important role in the generation of mechanically competent tissue. The ECM components are synthesized as individual molecules, and their assembly into a cohesive matrix depends largely on their local concentration. In tissue, these concentrations can be quite high locally, while in cell-culture experiments, the bulk of the ECM components synthesized by the cells is lost by diffusion into the large volume of cell culture medium. Others have reported that collagen incorporation by fibroblast cultures is promoted by the addition of exogenous macromolecules to the medium. Macromolecular crowding has been proposed as the underlying mechanism of this phenomenon; the exogenous macromolecules occupy space and consequently limit the volume available to other molecules (crowding) causing drastic effects on the other molecules' reaction kinetics and molecular assembly.

The goal of this project is to determine whether large (≥300 kDa) macromolecules such as polystyrene, dextran sulfate, and hyaluronic acid can enhance chondroid matrix deposition by differentiating hMSCs. We used aggregate culture as a model-system for chondrogenic differentiation and tested three different exposure times (3, 5, or 7 days after pellet formation) on two different types of aggregate cultures using multiple cell preparations. The first type was pelleted on day 0, and maintained as aggregates for the duration of the experiment. The second type was pre-differentiated in aggregate culture for 7 days, dissociated and then re-pelleted on day 7.

After harvesting, the aggregates were analyzed for DNA content (a measure of cell number). Proteoglycan deposition was localized by toluidine blue-O staining, and quantified by a colorimetric GAG assay performed on lysates of the aggregates. Collagen type II was also localized by immunohistochemistry and quantified using western blots scanned with a Bio-Rad gel documentation system. Culture supernatant was also harvested and cryopreserved to test for ECM molecules at a future date.

To further examine the effects of the crowding molecules, we are using quasi-elastic laser light scattering (QELS) to probe the diffusion coefficient of the polymer in solution. The approach is based on the fact that the intensity of light scattered from the polymer solution observed at a given angle fluctuates based on the motion of the polymer molecules, and can be used to give an estimate of the diffusion coefficients of the dissolved polymer. In theory, as the polymer molecules get closer together due to an excessive polymer concentration, the diffusion coefficient will also vary depending upon repulsive interactions between the molecules. Thus, there is likely an optimal concentration for each crowding molecule that can be determined empirically.

Overall, we found no significant changes in the DNA content (cell number) of the aggregates, consistent with a lack of toxic effects. Aggregates grown in the presence of crowding molecules were noticeably larger than control. With respect to ECM deposition, we noted increases in glycosaminoglycan accumulation, both per aggregate and normalized to cell content. The results also showed greater accumulation of type II collagen in the aggregates grown in the presence of crowding macromolecules. Quantitatively, we found that the addition of crowding macromolecules had a much greater effect on the day 7 reconstituted aggregates than on the day 0 aggregates. Although all three macromolecules showed positive results, polystyrene caused a greater increase in GAG and collagen deposition than either dextran or hyaluronic acid.

By allowing us to modulate the deposition rate of collagen and glycosaminoglycan molecules, crowding macromolecules might have useful applications in tissue-engineering. QELS experiments ongoing with Dr. Cheung at the University of Akron should help optimize the concentration of the macromolecules for effect on the ECM. Future directions will include evaluating the mechanism of action of polystyrene compared to the other macromolecules tested to determine whether these are due to intrinsic properties of the polystyrene or are a dosage effect. It will also be interesting to test other organic and synthetic macromolecules to determine if any are more efficient than polystyrene. Longer-range goals include extending these studies to larger-scale tissue engineered cartilage constructs.