Effects of Chondroitin Sulfate Incorporation on Chondrocyte Morphology and Metabolism in Mechanically Stimulated Poly(ethylene glycol) Hydrogels

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ABSTRACT:
The structure and composition of the extracellular matrix (ECM) plays an important role in translating the applied mechanical cues into biomechanical cues, which are then sensed by the chondrocytes. Biomechanical cues are known to influence chondrocyte function including the synthesis, degradation and overall maintenance of cartilage. One of the main ECM molecules in cartilage is aggrecan, which primarily consists of sulfated glycosaminoglycans (e.g., chondroitin sulfate) that contribute to the high fixed charge density found in cartilage. Under dynamic mechanical loading, streaming potentials result as mobile positive ions are forced in and out of the tissue. Streaming potentials are believed to be one mechanism by which chondrocytes sense mechanical forces [1]. However the exact mechanisms are not well understood in part due to a lack of suitable models. Therefore, the goal for this study was to develop 3D hydrogel models from which to study the effects of a highly negatively charged 3D environment on chondrocyte response under dynamic loading [2,3]. Synthetic 3D hydrogels were fabricated by co-polymerizing poly (ethylene glycol) (PEG) with multifunctional chondroitin sulfate (ChS) macromers where the ratio of PEG:ChS was varied to control the ChS concentration and fixed charged density within the hydrogel. Specifically, we examined cell morphology and ECM synthesis as a function of chondroitin sulfate concentration and mechanical loading.

METHODS:
Chondrocytes were isolated from the metacarpalphalangeal joints of six 1-2 year old steers. PEG dimethacrylate (PEGDM, 3000MW) was synthesized following standard protocols with an 88% reaction efficiency as determined by 1H NMR. Multivinyl chondroitin sulfate A (ChSA, Sigma) was synthesized by reacting ChS (25% w/v) with methacrylic anhydride (4°C and pH=8) in DI-H2O for 24 hrs and purified by precipitation in methanol (1:15). The degree of methacrylation substitution was 23% as determined by 1H NMR. PEGDM and ChSA macromers were combined in PBS to make a 10% w/v macromer solution in varying ratios of ChSA:PEGDM:0/(100, 20/80, 40/60) containing 0.05% w/w photoinitiator (Irgacure 2959, Ciba Specialty Chemical) then photopolymerized by 365 nm light at ~2 mW/cm² for 1 hr. The gels were characterized, in the absence of cells, with respect to their mechanical properties. The gels were transparent and had a gel strength of ~2 mN/cm².

CONCLUSION:
PEG:ChS gels can be used to control the level of cell deformation. In the presence of mechanical load, an increase in ChS content led to an increase in cell deformation which stimulated the metabolic activities in chondrocytes. The results from this study suggest the role ECM components such as ChS in regulating cell function and metabolism.

REFERENCES: