The Effect of Sulfated Glycosaminoglycan Concentration on the Transient Osmotic Swelling of Articular Cartilage and Meniscus Fibrocartilage in Confined Compression

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Introduction: The osmotic swelling stress that results from the interaction between the negatively charged sulfated glycosaminoglycans (sGAGs) and the ionic interstitial fluid is important to the load bearing capabilities of articular cartilage and meniscal fibrocartilage [1]. Changes in the sGAG content, such as those that occur as a result of degenerative joint disease, alter the osmotic swelling stress and thus, the ability of both tissues to sustain compressive loading. Previous studies have examined effects of osmotic interactions on the compression and shear moduli of cartilage and meniscus in unconfined [2] and confined compression [3], but the influence of concentrations of sGAG in the tissues have not been explored. This study compared the effect of altered osmotic environments on the swelling stress of normal and degenerated articular cartilage as well as adult and immature meniscus fibrocartilage, tissue groups selected to span overlapping ranges of sGAG concentration.

Materials and Methods: Meniscal fibrocartilage from juvenile and adult bovine stifles and articular cartilage from the femoral condyles of immature bovine stifles were isolated using a 6mm biopsy punch. 1mm-thick slices were obtained using a microtome, removing the superficial and deep regions. In order to produce cartilage samples with a reduced sGAG concentration in the range of adult meniscal tissue, some articular cartilage explants were treated with 1U/ml of Chondroitinase ABC for 42hrs at 37˚C following by a washing step to remove the excess enzyme. Meniscus and cartilage explants were stored at -20˚C in 1X PBS with protease inhibitors until mechanical testing. Samples were thawed for 30 minutes in a 37˚C bath on the day of testing and a 4mm biopsy punch was used to reach the final sample diameter. Testing: Articular cartilage and meniscus explants were assigned to one of 9 conditions, controlling both the compressive offset (either 5%, 10%, or 15% strain) and the bath concentration (0.1X, 1X, or 10X PBS). The equilibrium swelling stress was expected to increase with the hypertonic bath solution (10X PBS) and decrease with the hypotonic bath solution (0.1X PBS). Explants were tested within a confined compression rig on an Instron 5848 microtester using a 1.1N load cell. Each sample was pre-loaded to 0.02N in 1X PBS with Protease Inhibitors and compressed at 0.001mm/s to the compressive offset that was previously assigned. After a 45 minute relaxation step, the bath solution was changed to the assigned PBS concentration group and a new relaxation step (at a constant compressive offset) of 90 minutes followed. Following mechanical testing, samples were removed from the rig and allowed to reach a new equilibrium (unconfined) in the last solution used during the test for 1hr. After recording the weight, samples were re-equilibrated in 1X PBS for an additional hour and weighed one last time before being stored at -20C until biochemical analysis. Explants were lyophilized, dry weighed, digested with Proteinase K and assayed for sulfated glycosaminoglycan (sGAG) content using the dimethyl/methylene blue (DMMB) assay. Analysis: A least-squares approach was used to fit the linear biphasic solution [4] to the data from the initial relaxation step while samples were equilibrated in 1X PBS to obtain the aggregate modulus. A decaying exponential was used to model the post-bath change data to find the time constant that best described the new osmotic equilibrium. As a result of experimental artifact and low signal in some of the mechanical tests, a subset of meniscus (n=25) and cartilage (n=17) samples were used. Data were subjected to an optimal Box-Cox transformation to improve normality, and were analyzed with multi-factor general linear models (tissue group, offset, bath). Results are presented as mean +/- SEM.

Results. sGAG per water content in adult meniscal fibrocartilage explants was 0.0217 +/- 0.0014 mg/mg (compared to 0.0054 +/- 0.0005 mg/mg in immature meniscus) while in the cartilage explants treated with Chondroitinase ABC it was 0.0185 +/- 0.0005 (compared to 0.0859 +/- 0.0034 mg/mg in immature normal cartilage), all inter-group differences were significant. The equilibrium modulus in 1X PBS increased with sGAG concentration across all samples, but the intact articular cartilage group exhibited a dependence distinct from those of the other three groups (Fig. 1). For the immature cartilage and adult meniscus groups, the swelling stress ratio (Fig. 2) was significantly greater at 0.1X PBS than 1X PBS and significantly lower at 10X PBS. For the degraded cartilage and immature meniscus groups the swelling stress ratio was significantly greater at 0.1X PBS than 10X PBS, with no significant changes observed at the 1X PBS level, regardless of the compressive offset. The swelling stress ratio was significantly greater at 10X PBS in the degraded cartilage explants than the immature (non-degraded) cartilage explants. In general, the osmotic equilibrium time constant (Fig. 3) was significantly greater for meniscus than cartilage across conditions (degraded cartilage, adult meniscus, normal immature cartilage, immature meniscus), PBS, and compressive offset groups. Across offsets, tissue type, and condition, the osmotic equilibrium time constant was greater for the 0.1X PBS groups than the 10X PBS groups.

Discussion. This study explored mechanical behaviors of tissue samples across a range of sGAG concentrations. Interestingly, the low sGAG tissues (juvenile and adult meniscus, degraded cartilage) exhibited a consistent dependence of equilibrium modulus on sGAG content, while intact cartilage (with a higher sGAG content) exhibited a distinct dependence. Overall trends in the osmotic swelling transient were similar across tissue groups; although the magnitude of swelling stress depended on sGAG content, the relative change in swelling stress was comparable for all tissue groups and varied strongly (and consistently) with bath osmolarity. Expansion of these experiments to include more graded variations will allow a detailed study of the interactions between sGAG-associated osmotic swelling and other aspects of tissue composition and structure in determining tissue mechanical behaviors.

Significance. Understanding the role of the sGAG content in the osmotic swelling behavior of meniscus and cartilage is of critical importance not only to enhance our understanding of basic tissue mechanics, but also to better understand the distribution of osmotically active CT or MR contrast agents to assess soft tissues in animals and humans.

References