Comparing the Transient Osmotic Swelling of Articular Cartilage and Meniscal Fibrocartilage in Confined Compression

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Disclosures:  E.G. Baylon: None. M.E. Levenston: 3B; AngioScore.

Introduction: Osmotic swelling stress resulting from electrochemical interactions between the ionic interstitial fluid and the net negative matrix fixed charge density (primarily associated with sulfated glycosaminoglycans, sGAGs) contributes to the load-bearing of articular cartilage and meniscal fibrocartilage [1], and changes in tissue sGAG content alter such contributions. Manipulation of the osmotic environment forms the basis for several techniques for indirect assessment of tissue mechanical properties in isolated samples or small animal joints, and nondestructive imaging techniques based on distribution of osmotically active CT or MR contrast agents are increasingly used to assess soft tissues in animals and humans. As cartilage and meniscus tissues have very different sGAG contents and microstructural distributions (substantially more inhomogeneous in meniscus), extension of such testing and imaging techniques to study meniscus requires a detailed understanding of osmotic behaviors of the two tissues. Effects of altered osmotic interactions on the compression and shear moduli of cartilage and meniscus in unconfined compression have been reported [2], but changes in osmotic swelling induced by altered bath conditions have not been directly explored. This study compared the effects of step changes in the osmotic environment on the swelling stress of articular cartilage and meniscus fibrocartilage tissue explants.

Methods: Full thickness cores of articular cartilage from the femoral condyles and meniscal fibrocartilage from medial and lateral menisci were removed from immature bovine stifles using an 8mm biopsy punch. The superficial and deep regions were removed and 1mm thick slices were prepared using a microtome. Samples were stored at -20C in 1X PBS with protease inhibitors until mechanical testing. On the day of testing, samples were thawed for 30 minutes in a 37C bath and 4mm diameter test samples were removed with a biopsy punch. Samples were randomly assigned to one of three compressive offset groups (5%, 10%, or 15% strain) and one of three bath concentration groups (0.1X, 1X, or 10X PBS) for a total of 9 conditions (Fig. 1), with three samples per condition for each tissue type. The hypotonic (0.1X) solution was expected to increase the equilibrium swelling stress, while the hypertonic (10X) solution was expected to decrease the swelling stress. Each sample was placed in 1X PBS within a confined compression chamber on an Instron 5848 microtester equipped with a 10N load cell, subjected to a 0.02N pre-load, and compressed at 0.001mm/s to the assigned compressive offset. After stress relaxation for 45 minutes, the bath solution was changed to the assigned concentration and continuously recirculated during an additional 90 minutes to monitor the osmotic transient and new equilibrium. After testing, samples were removed and re-equilibrated in 1X PBS for 2 hours before storing them at -20C for biochemical analysis. Samples were weighed before and after lyophilization to determine water content, and were digested with Proteinase K and assayed for sulfated glycosaminoglycan (sGAG) content via the DMMB assay.
The linear biphasic model [3] was least-squares fit to the initial stress relaxation in 1X PBS to obtain an estimate of each sample’s aggregate modulus. A decaying exponential was fit to the post-bath change equilibration to determine the time constant for osmotic equilibration and the new equilibrium stress. Due to low signal and experimental artifact, only a subset of meniscal samples (n=12) had usable data for the entire test, while all cartilage samples (n=27) had usable data. To account for an order-of-magnitude differences between tissues, final equilibrium stress values were normalized to the equilibrium stress before the bath change. Data were subjected to an optimal Box-Cox transformation to improve normality, and were analyzed with multi-factor general linear models (tissue, offset, bath). Results are presented as mean +/- SEM.

**Results:** As previously described, the compositions and moduli of cartilage and meniscus were substantially different. Meniscus samples had significantly lower water fraction (68.9±0.6% vs. 79.0±0.5%), sGAG per wet mass (0.30±0.02% vs. 5.5±0.1%) and aggregate modulus (33.6±2.3kPa vs. 405±30kPa) than did cartilage samples. Consistent with expectations, absolute changes in swelling stress were significantly greater for cartilage (+38.3±4.9kPa at 0.1X, -30.8±6.7kPa at 10X) than for meniscus (+3.48±1.00kPa at 0.1X, -2.06±0.34kPa at 10X). However, relative changes in swelling stress (Fig. 2) were similar for the two tissues. The swelling stress ratio was significantly greater at 0.1X and significantly lower at 10X (no change at 1X), with no significant difference between tissues. The swelling stress ratio at 0.1X was greatest at the 5% offset, but did not significantly vary among offsets at 1X or 10X, indicating that changes in fixed charge density due to compression had a relatively modest effect. The osmotic equilibrium time constant (Fig. 3) was significantly greater for the change from 1X to 0.1X PBS (swelling) than for the change from 1X to 10X PBS (de-swelling) for all groups except meniscus at the 5% offset (no significant difference), with an average time constant (across tissues and offsets) of 1051±271s at 0.1X vs. 280±58s at 10X. Interestingly, the osmotic equilibrium time constant was significantly greater for meniscus than for cartilage (except for 0.1X at 5% offset and 10X at 15% offset). Across offsets, the average osmotic time constant was 2204±843s at 0.1X and 534±109s at 10X for meniscus and 666±37s at 0.1X and 167±3s at 10X for cartilage.

**Discussion:** As expected, both cartilage and meniscus held at constant volume exhibited increased swelling stress in hypotonic solution and decreased swelling stress in hypertonic solution, with substantially greater changes for cartilage than for meniscus. However, the two tissues exhibited comparable relative changes in swelling stress despite substantial differences in the intratissue sGAG distributions, indicating generally consistent behaviors. The time required to reach a new osmotic equilibrium depends on multiple factors, including relative effective diffusivities of different ions, tissue permeability and water content. While the longer osmotic time constant of meniscal tissue is consistent with the lower permeability of meniscal tissue, additional experimental and theoretical studies are required to identify the specific mechanisms responsible for this effect. Ongoing studies will further explore mechanisms and examine the specific role of fixed charge distribution vs. macroscopic average content, as well as equilibration of specific solutes relevant to clinical diagnostic imaging.

**Significance:** Studying the effects of osmotic swelling interactions in cartilage and meniscus contributes to our understanding of tissue mechanics, which could be used in the development of diagnostic tools.
Figure 1: Experimental set-up

Figure 2: Swelling Stress Ratio for (A) Articular Cartilage, (B) Muscles

Figure 3: Osmotic Equilibrium Time Constant for (A) Articular Cartilage, (B) Muscles