Annulus Fibrosus Hydration and Proteoglycan Content Affect Tensile Failure Mechanics

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INTRODUCTION: Intervertebral disc degeneration is associated with tissue dehydration due to fragmentation and loss of proteoglycans from both the nucleus pulposus and annulus fibrosus (AF) [1-3]. Proteoglycans are widely believed to be partially responsible for water absorption and retention due to the osmotic gradient created by anionic subcomponents, such as glycosaminoglycans (GAGs) [2-4]. Previous studies investigated AF failure mechanics using specimens obtained from degenerated discs, but the multitude of associated structural and compositional changes that occur with degeneration makes it difficult to discern the role of individual subcomponents [5, 6]. Other studies have used enzymes to selectively digest individual molecules, isolating the effect of each constituent, such as GAGs, on tissue failure [7, 8]. However, these studies did not perform biochemical analyses to quantify the relationship between biochemical and mechanical properties. Therefore, the quantitative relationship between AF biochemical composition and failure mechanics remains unclear. The objective of this study was to determine the effect of water content, or hydration, on AF failure properties in uniaxial tension. Water content was altered through enzymatic digestion of GAGs and through osmotic loading.

METHODS: Rectangular specimens ~2 mm thick were prepared from the middle-outer AF of bovine discs from levels C1-C4. Specimens were oriented along the circumferential-radial direction (n = 37) and soaked in 0.15 M PBS (CTL, n = 12), 0.15 M PBS with 0.25 U/mL of chondroitinase ABC (chABC, n = 13), or 1.43 M PBS (OSM, n = 12) for 18 hours prior to testing. Preliminary work was performed to determine the appropriate PBS concentration to match the reduction in water content due to chABC treatment (Fig. 1A – red X). After soaking, specimens were trimmed (final width = 5.98 ± 0.71 mm) and a full-width, half-depth notch was created to facilitate midlength failure (1 mm thickness at midlength) [9]. Specimens were glued to sandpaper (gauge length = 11.96 ± 1.21 mm) and testing was performed in either 0.15 M PBS for CTL and chABC specimens or 1.43 M PBS for OSM specimens. A 0.05 N preload was applied and specimens were extended, until separation, at a rate of 0.05 mm/min. Engineering stress and strain were calculated, and the linear-region modulus was calculated using a linear-regression fit to the stress-strain response. Failure stress was defined as the maximum stress, and failure strain was defined as the strain corresponding to maximum stress. Strain energy was determined through numerical integration of the stress-strain response until failure.

After testing, two 4 mm biopsy punches were taken from the midlength of each sample. Specimen wet weight (ww) was recorded prior to lyophilization. The dry weight (dw) was then measured to calculate water content (WC = (ww – dw)/ww). Samples were digested with proteinase-K and GAG content was determined using the 1,9-dimethylmethylene blue assay [10]. A one-way ANOVA with a Bonferroni post hoc analysis was performed on mechanical and biochemical properties (significance assumed at $p \leq 0.05$).

RESULTS: All samples exhibited a nonlinear sub-failure stress-strain response. Mechanical testing data was only analyzed for samples that clearly failed at the midlength (n = 33/37, n = 11 per group). Both chABC treatment and osmotic loading had a significant effect on mechanical and biochemical properties (Fig. 1B-F). For the chABC group, linear-region modulus was 32% lower, failure stress was 33% lower, and strain energy was 46% lower versus the control group (all $p < 0.02$; Fig. 1B-D). There was no significant difference in failure strain with chABC treatment ($p > 0.1$). For the OSM group, failure stress and failure strain were not significantly different from the control ($p > 0.1$), but there was a 22% decrease in linear modulus and a 44% decrease in strain energy versus control ($p < 0.02$; Fig. 1B-C). Water content decreased by 5.9%/ww in the chABC group and by 8.3%/ww in OSM group versus control ($p < 0.001$; Fig. 1E). In contrast, there was a much greater decrease in GAG content after chABC and OSM treatment, with an 87.4%/dw decrease in the chABC group and a 49.5%/dw decrease in the OSM group versus control ($p < 0.001$; Fig. 1F).

DISCUSSION: In this study, we report quantitative measures highlighting the effect GAG and water content on AF sub-failure and failure mechanics. GAG loss caused a decrease in tissue stiffness, energy absorbance, strength, and water content. Osmotic loading was used to isolate the effect of hydration from GAG composition, resulting in similar decreases in stiffness, strain energy, and water content. Taken together, these results suggest that tissue hydration is essential for maintaining bulk tissue stiffness and capacity for energy absorption, rather than strength. These findings also suggest that GAGs may contribute to tissue strength, possibly through strengthening fiber-matrix interactions. It should be noted that the OSM group experienced a statistically significant decrease in GAG content, but had similar water content to the chABC group (Fig. 1E-F). Previous studies have observed GAGs leaching into the surrounding solution during specimen soaking, particularly when osmotic loading was used to regulate tissue swelling [11]. Lastly, we observed large changes in GAG composition (~90% decrease with chABC) that were associated with relatively small changes in water content (~10% decrease in wet weight), which may be due to alternate mechanisms for water retention, such as tissue porosity. Additional research into these mechanisms and a more comprehensive understanding of AF structure-function relationships are important for furthering our understanding of disc damage and injury, particularly as developing repair strategies aim to recapitulate the function of healthy native tissue.

SIGNIFICANCE: This study provides quantitative measures of the effects of tissue hydration on AF failure behavior, as well as the effects of enzymatic degradation and osmotic loading on tissue biochemical composition. These results are important for elucidating mechanisms of disc degeneration and injury, and help provide guidance for developing tissue-engineered repair strategies.

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IMAGES AND TABLES:

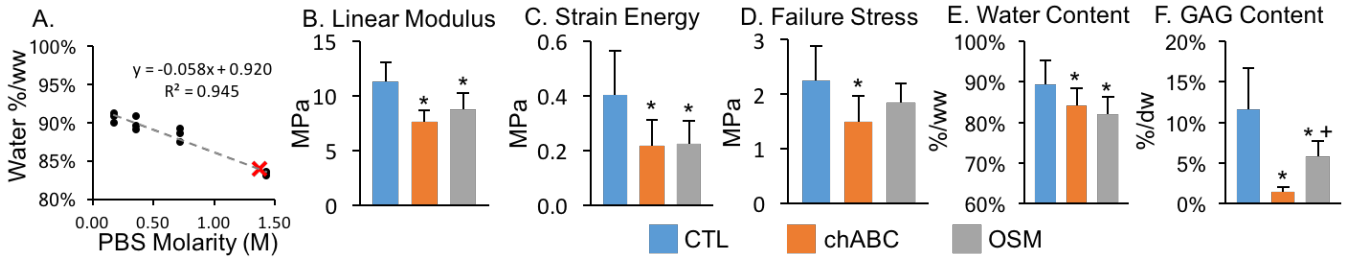

Fig. 1: A. Pearson correlation between water content and PBS molarity used to match the water content of chABC-treated samples (red X). **B-F.** Average and standard deviations for **B.** linear region modulus (MPa), **C.** strain energy (MPa), **D.** failure stress (MPa), **E.** water content (%/ww), and **F.** GAG content (%/dw). * denotes $p \leq 0.05$ *vs* control and + denotes $p \leq 0.05$ *vs* chABC.