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FAILURE PROPERTIES OF ANNULUS FIBROSUS: EFFECTS OF chABC AND STRAIN RATE

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INTRODUCTION

Structural failure of the intervertebral disc (IVD) is a common and painful condition associated with degenerative changes to disc composition [1]. The occurrence of tears in the annulus fibrosus (AF), including radial tears, rim lesions, and circumferential tears, increase with age and degeneration [2,3]. In particular, radial fissures can lead to prolapse of the nucleus, or disc herniation, with impingement of the spinal nerves, leading to lower back and leg pain [4].

The annulus is comprised of collagen fibrils embedded in an extrafibrillar matrix [2]. Negatively charged glycosaminoglycans (GAG) in the extrafibrillar matrix absorb water molecules, providing the tissue with its hyper-viscoelastic mechanical properties [5]. Degeneration is noted by changes in biochemical composition and structure, including a loss in GAG and water content [2]. To date, AF failure mechanics, and the interaction between failure mechanics and biochemical composition, is not well understood. A better understanding of the relationship between biochemical degeneration and failure properties of the AF may help elucidate the mechanisms of disc herniation.

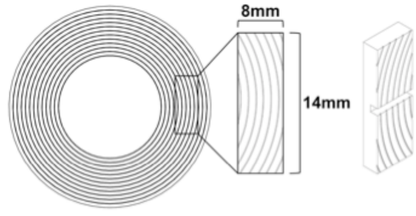

Figure 1. Schematic of test sample orientation (left) and notch geometry (right).

Previous studies [6,7] have used chondroitinase ABC (chABC) to enzymatically digest GAG from disc tissue as an *in vitro* model of disc degeneration. In this study, we examine the effect of chABC digestion on AF failure mechanics. As a decrease in GAG content diminishes the ability of biological tissues to absorb water, it is hypothesized that GAG digestion with chABC will alter both the failure properties and viscoelastic behavior of the AF. Due to the importance of water absorption to AF mechanics, our second objective was to evaluate the effect of strain rate on failure properties.

METHODS

Twelve intervertebral discs were dissected from the first three levels of four bovine caudal spines. Twenty-two circumferentially oriented annulus specimens were isolated from the discs and sliced to a thickness of 2 mm using a freezing stage microtome. To facilitate repeatability with respect to failure dynamics (i.e. avoid failure associated with gripping), samples were horizontally notched midway down their length, such that the un-cracked specimen thickness was 1mm (Figure 1). Sample notching was performed while the samples were frozen (pre-hydration) using a scalpel and depth stop. Samples were randomly divided into four test groups: healthy low strain rate (CTL-low), enzymatic digestion low strain rate (chABC-low), healthy high strain rate (CTL-high), and enzymatic digestion high strain rate (chABC-high; n = 4-8 per group). As annulus mechanical properties are known to vary with hydration state, specimens were soaked for 18 hours at 37 °C in a group specific solution. The healthy group was hydrated in phosphate-buffered saline (PBS, 0.14 M NaCl) while the degenerate group was hydrated in a PBS solution with 0.125 U/mL chABC, as per the digestion protocol described by Isaacs *et al.* [6]. Following hydration or enzymatic digestion, uniaxial tension tests were performed in PBS at room temperature using an Instron 5943 (Norwood, MA). Samples were gripped using 400 grit sandpaper super-glued directly to the specimens.

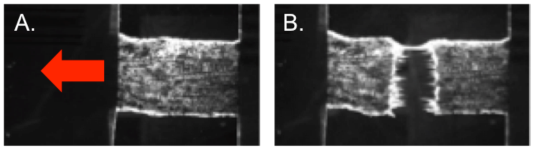

Figure 2. Representative sample (A) before and (B) after failure. Red arrow indicates loading direction.

As AF mechanical behavior in tension is highly dependent on strain rate [8], we evaluated the failure properties at two different strain rates. The high strain rate group was tested at a displacement rate of 50 mm/min, which corresponds to a strain rate of approximately 3.57 min^{-1}, while the low strain rate group was tested at a displacement rate of 0.05 mm/min, or approximately 3.57×10^{-3} min^{-1}. Strain was calculated as the change in displacement divided by the initial gauge length (i.e. grip-to-grip distance). Stress was calculated by dividing measured force by the original cross-sectional area of the notched section. The Young's modulus (E) was calculated using a bilinear fit to the stress-strain response (linear-regression optimization script, Matlab Mathworks Inc.). The stress at failure for each test was defined as the maximum stress recorded during the test, while the strain at failure was defined as the strain at which this maximum stress occurred.

To evaluate the effect of GAG content on failure properties, a two-tailed Student's t-test was performed between healthy and degenerate groups for both strain rates. Similarly, a Student's t-test was performed to determine the effect of strain rate on failure properties. Significance was assumed at $p \leq 0.05$.

RESULTS

The full-width notch resulted in robust failure occurring at the mid-length for all samples (Figure 2). Under quasi-static loading condition (i.e. low strain rate), chABC digestion did not alter the toe- or linear region modulus (Figure 4A – shown for linear-region modulus). However, chABC did significantly decrease failure stress and strain ($p < 0.01$; Figure 4 – blue versus red bar).

An increase in strain rate increased the Young's modulus for both healthy and digested AF groups, as expected ($p \leq 0.05$; Figure 4A – low rate versus high rate). Interestingly, failure properties of chABC digested samples were not significantly different from the control when loaded at a higher strain rate ($p > 0.15$; Figure 4B, 4C). It should be noted that a higher variability in data was observed for the high strain rate cohort, and thus additional testing with a larger sample size is required to further elucidate this phenomenon.

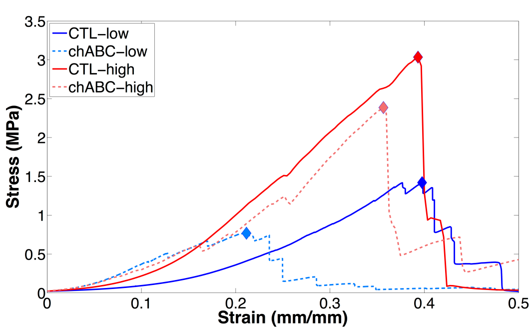

Figure 3. Representative stress-strain curve for each group. Diamond represents failure point.

DISCUSSION

GAG chains are thought to play a dominant role in the mechanical properties of biological tissues including the AF. Due to the high water contribution and the role of water composition on tissue

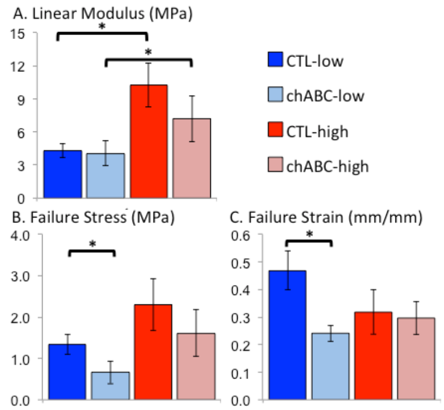

Figure 4. (A) Linear-region modulus, (B) stress and (C) strain at failure. * denotes $p \leq 0.05$.

mechanics, we evaluate AF failure mechanics at a low and high strain rates. Our study shows that for quasi-static loading, chABC digestion decreases failure stress and strain; however, these differences are not as pronounced at the higher strain rate. Our results differ from those described by Isaacs *et al.*, who reports no significant change in failure strain for similarly digested AF specimens, which is likely due to difference in strain rate (Isaac's strain rate = 0.30 min^{-1}) [6]. Taken together, these findings suggest that GAGs play an important role in AF failure mechanics that is strain rate-dependent, which is important for understanding loading conditions that may cause disc herniation.

Enzymatic digestion with chABC has been previously used to reduce GAG content in biological tissues [6,7]. Ongoing work is focused on quantifying the decrease in GAG composition in our degeneration model. Preliminary data showed significant differences in the water content of control and chABC samples, suggesting that GAG digestion was effective. Furthermore, a strong correlation was found between water content and stress at failure ($R^2 = 0.86$). Future work will focus on correlating GAG composition directly with AF failure properties in tension.

At high strain rates, there was a significant decrease in elastic modulus and a trend for a decrease in the stress at failure. The decrease in Young's modulus with chABC is likely due to fiber-matrix interactions altering tension mechanics at the higher loading rate. For healthy and chABC samples, increasing the strain rate significantly increased the stress at failure and elastic modulus; however, the strain at failure was not altered. Future work will explore whether AF failure mechanics is a strain-driven phenomenon.

GAGs comprise less than 15% of the tissues dry weight [9]. Previous sub-failure AF tensile mechanics is thought to be largely due the collagen fibers [2]; however, the findings here suggest that GAGs may play a larger role in AF failure mechanics.

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