A Method for Repeatable Tensile Total-Life Fatigue Testing of Annulus Fibrosus

Benjamin Werbner[1], Grace O'Connell Ph.D[1]
[1]University of California, Berkeley. Berkeley, CA

Disclosures: Benjamin Werbner: none, Grace O'Connell: none

Introduction: Fiber-reinforced tissues of the musculoskeletal system, including tendons, ligaments, and the annulus fibrosus of the intervertebral disc, are regularly subjected to repetitive tensile stress. Though it is widely accepted that damage to connective tissues may occur from high frequency and large stresses [1], it is not well understood how damage accumulates over time [2]. The lack of a highly repeatable method for tensile total-life fatigue testing of fiber-reinforced soft tissues likely contributes to this dearth of knowledge, particularly in the case of annulus fibrosus.

Ensuring repeatable failure dynamics remains a major challenge in understanding the mechanical behavior of fiber-reinforced tissues [3]. For example, a previous investigator reported that only around 40% of their uniaxial monotonic tensile specimens failed within the gauge region (i.e. away from the grips) [4]. Ensuring repeatable failure dynamics can be even more challenging during cyclic loading, where stresses may remain significantly lower than the ultimate strength [3]. A reliable method of fatigue testing would not only serve to further quantify the complex behavior of native tissue, but would also provide a framework for evaluating tissue repair strategies. Therefore, the objectives of this study were two-fold: first, to develop a robust testing protocol to evaluate tissue-level tensile fatigue mechanics of the annulus fibrosus (AF), and second, to determine an S-N curve to validate our method and serve as a basis for further study of AF failure mechanics.

Methods: Intervertebral discs were dissected from bovine caudal spines. Radial-circumferential specimens were isolated from the anterior and posterior AF (n = 8) and sliced to a thickness of 2 mm using a freezing stage microtome. A razor blade was used to create parallel surfaces along the width (width = 5.3 ± 0.14 mm; length = 9.6 ± 1.3 mm). Specimens were hydrated for 18 hours in phosphate-buffered saline (0.15 M PBS). Following hydration, a full-width notch was made at the mid-length with a scalpel, such that the remaining specimen thickness was 1 mm (Figure 1). Samples were gripped by super-gluing sandpaper onto each end. Then, each sample was cycled between 0.2 N and 2.0 N for 20 cycles at a rate of 50 mm/min. Strain was calculated as the change in displacement divided by the initial gauge length. Stress was calculated by dividing the measured force by the original cross-sectional area remaining at the notch location. The Young's modulus was calculated as the slope of the linear region from the stress-strain response of the 20^{th} cycle.

A dataset from previous work was used to help estimate the ultimate tensile stress (UTS) for each specimen in this study [5]. A linear correlation was established between the Young's modulus and the UTS for each sample in the previous dataset (n=22, Pearson correlation, R^2 = 0.68), and this correlation was used to estimate the UTS of the fatigue samples based on the Young's modulus calculated during preconditioning [6]. This estimate was then averaged with the mean UTS from the previous dataset to provide a sample-specific UTS for each specimen tested. For each cyclic test, the upper stress limit (σ_{max}) was selected between 50%-100% of the predicted UTS. Specimens were then cyclically loaded between $0.1\sigma_{max}$% and σ_{max}%. The linear region modulus was calculated for each cycle, and the number of cycles to failure was defined as the cycle number when catastrophic failure occurred (i.e. complete rupture of the tissue). Finally, σ_{max}% was plotted against the number of cycles to failure, and a logarithmic best-fit line was used to approximate an S-N curve, which demonstrates a relationship between σ_{max} and the number of cycles to failure.

Results: Slipping or tearing at the grips was not observed for any sample. The full-width notch resulted in robust failure occurring at the mid-length within 20,000 cycles. All samples tested exhibited a clearly defined primary region, in which the rate of strain increased rapidly with an increase in modulus, a secondary region in which the modulus remained nearly constant, and a tertiary region where a dramatic softening precipitated catastrophic failure (Figure 2). Catastrophic failure entailed separation of the tissue at the notch site, which typically originated in the outer annulus and propagated toward the inner annulus. Complete failure occurred within one or two cycles at the end of the tertiary phase. There was an inverse relationship between the number of cycles to failure and the maximum stress applied (as % UTS; Figure 3; correlation, R^2=0.88). The linear region modulus increased by 10-15% during the first 200 cycles and remained relatively constant until it began to decrease in the last 100 cycles before failure.

Discussion: In this study we used a full-width notch method to ensure failure at the mid-substance for a fiber-reinforced soft tissue (i.e. annulus fibrosus). The classical creep and fatigue behavior observed in Figure 2, as well as the strength of the logarithmic fit in the S-N curve, suggest that our sample geometry and testing protocol produced a repeatable total-life fatigue method for a complex fiber-reinforced tissue that is both consistent with previous work [7] and supported by theory from the mechanics of materials.

The high water content in the AF contributes to its visco-poro-elastic properties, resulting in a significant creep response. The strain response over time demonstrated a slight creep response (Figure 2 – secondary phase), suggesting that creep rupture may be a dominant mechanism for failure. However, progressive separation of the tissue at the notch site was observed during the tertiary phase, which also suggests that a combination of creep deformation and accumulated fatigue damage in the form of tissue separation contributed to failure at the notch site [8].

Very few previous studies have investigated the tensile total-life fatigue properties of the AF. Previous investigators have presented a comparable S-N curve for axially-loaded bone-AF-bone segments, but found that failure initiated between the hyaline cartilage and bone [7]. While this observation has its own merits, the method and data presented would not allow for the validation of tissue-level mechanical properties of a novel engineered biomaterial. The method presented here, however, has its own limitation in the stress concentration present at the notch site, which is being investigated through ongoing finite element analyses.

With minor case-specific modifications, the method presented here could be used to perform repeatable total-life tensile fatigue tests on a variety of fiber-reinforced soft tissues. Since this method is applied purely on the tissue level, other applications may include rough life-estimates of engineered biomaterials and tissue repair strategies, as well as validation of their mechanical properties. Future work will investigate the effect of degeneration and tissue composition on total-life fatigue behavior.

Significance: Many common *in vivo* loading scenarios involve repetitive tensile loading. The repeatable method for tensile total-life fatigue testing presented in this study will be of great value when testing native tissue and validating the mechanical properties of engineered fiber-reinforced biomaterials.

References: [1] Galante, J., *Acta Ortho Scand*, 1967; [2] Iatridis, J. et al., *J Biomech*, 2004; [3] Taylor, D. et al., JMBBM, 2012; [4] Skaggs, D. et al., *Spine*, 1994; [5] Bonnheim, N. et al., SB3C, 2016; [6] LaCroix, A. et al., *J Appl Phsiol*, 2013; [7] Green, T. et al., *Eur Spine J*, 1993; [8] Bowman, S. et al., *J Biomech Eng*, 1998

Figure 1: Sample Geometry