Mechanical and Structural Changes to the Annulus Fibrosus in Response to Repetitive Loads Representative of Military Rotary Wing Exposures

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I. INTRODUCTION

Lower back pain (LBP) is experienced by a large population of military helicopter aircrew [1], and in some cases it can affect mission readiness [2]. Spine-mediated pain is likely influenced by soft tissue damage accumulation from repeated whole-body vibration (WBV) exposures during flight over the course of a military career [3]. Specifically, damage to the annulus fibrosus (AF) has been associated with LBP [4]. We aimed to quantify how military-relevant repetitive loading can structurally damage the AF and what effect that has on its tissue mechanics. We hypothesised that structural changes would occur in the tissue in response to elevated mechanical loads and the elastic, viscoelastic, and ultimate strength response of the AF would degrade relative to the magnitude and duration of applied loads.

II. METHODS

Fresh porcine cervical spines were used in this laboratory study due to their anatomic and mechanical similarities to human lumbar spines. AF test specimens were dissected from the outermost layers of the anterior AF from C3/4 to C6/7 intervertebral discs (IVDs) and fixed to a load frame (ElectroForce 3200, TA Instruments) oriented such that tension was applied in the anatomical circumferential direction (Figure 1, left). Specimens were submerged in physiologic saline with protease inhibitors for the duration of testing and the solution was maintained at a constant temperature of 37°C.

Mechanical changes from cyclic loading were assessed with a three-step experimental protocol (Figure 1, right) that included assessment of the variation in damage strain magnitude and number of cycles (Step 2) using separate cohorts. One control group was exposed to the same loading protocol except for damage loading (Step 2). All cyclic loading steps (1B, 2, 3B) were strain rate matched at 1.12 s^{-1}. Specimens were allowed to recover between each loading step (not shown to scale in Figure 1). The change in response between loading steps one and three were compared for each test specimen and the ultimate properties of each damage group were compared to determine the influence of each prescribed damage protocol.

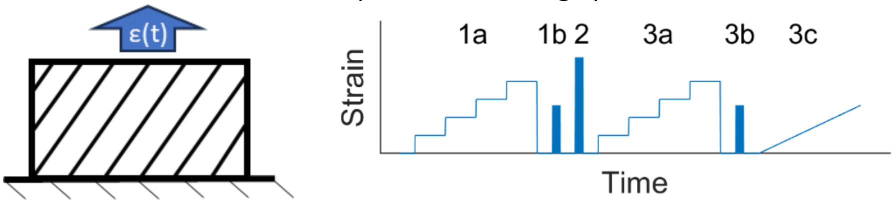

Figure 1: AF Test specimens (**left**), with collagen fiber bundle orientation represented by diagonal lines, were exposed to a three-step strain-controlled experiment protocol (**right**): (**1a**) Incremental strain step and holds (20 minutes each at 4%, 8%, 12%, 16%); and (**1b**) 100 tensile cycles to 11% strain at 5 Hz. (**2**) Damage: Specimens were assigned to 1 of 16 possible damage protocols, each with constant strain magnitude (11%, 20%, 28%, 44%) for a set number (400, 1600, 6400, 12800 cycles) of tensile loading cycles at 1.3-5 Hz. (**3**) Post-Damage: (**3a**) Incremental strain step and hold for 20 minutes at four discrete strain magnitudes (4%, 8%, 12%, 16%); (**3b**) 100 tensile cycles to 11% strain at 5Hz; (**3c**) Quasi-static distraction to failure.

A separate cohort of specimens was used to assess structural change associated with cyclic loading. Specimens were assigned to 1 of the 16 possible damage loading protocols (Figure 1, legend), then fixed, processed, sliced either parallel or perpendicular to the AF lamella, and stained with Verhoeff-van Gieson (VVG) stain.

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III. INITIAL FINDINGS

No changes in cyclical loading or stress relaxation properties were present between Steps 1 and 3 in the control group, indicating that elastic and viscoelastic properties were not altered by the pre- and post-damage loading protocols, or the test time (>18 hrs) in solution. Specimens exposed to damage cycles had dose dependent changes in the elastic and viscoelastic response that were each significantly dependent on strain magnitude ($p<0.001$) and number of damage cycles ($p<0.001$). During quasi-static distraction to failure, the linear modulus (16.6±6.5 MPa) ($p_{strain}=0.4$, $p_{cycles}=0.6$), ultimate stress (5.0±2.1 MPa) ($p_{strain}=0.9$, $p_{cycles}=0.6$), ultimate strain (0.58±0.2) ($p_{strain}=0.5$, $p_{cycles}=0.9$), and strain energy density (SED) to failure (1.2±0.8 MPa) ($p_{strain}=0.3$, $p_{cycles}=0.4$) were not significantly affected by damage protocol. This indicates that the mechanical response during low strain magnitudes is affected, while the high strain mechanical response remains relatively unaffected. This is further supported by the stress response in the toe and transition regions (0-30% strain) of the stress-strain curve during quasi-static distraction to failure (Figure 2). The SED of the toe and transition regions was significantly dependent on both the strain magnitude ($p<0.001$) and number of damage cycles ($p=0.003$). Specimens exposed to 44% strain for 12800 cycles were affected the most and had a SED of 0.017±0.01 MPa, 95.2% less than the baseline group. Histologic analysis of the AF tissue following cyclic loading shows cleft formation between collagen bundles as well as uncrimping of individual fibers (Figure 3).

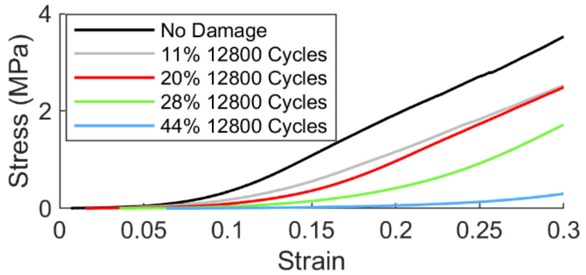

Figure 2: Average stress-strain curves (0-0.3 strain) for high cycle count groups during quasi-static distraction.

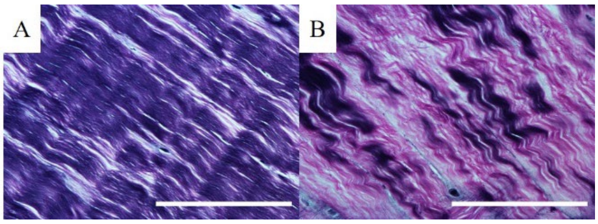

Figure 3: Control (A) and damaged (28% strain) (B) AF tissue stained with VVG. Scale bar = 100 µm.

IV. DISCUSSION

This study characterised mechanical and structural changes in AF associated with damage from sub-failure repetitive loading. It was determined that increasing the number and strain magnitude of damage cycles significantly affected the mechanical response at low strain magnitudes (0-30%). However, the linear modulus and ultimate stress and strain were unaffected, regardless of the damage protocol, suggesting the mechanical response at high strain magnitudes is maintained. Prior studies reported similar mechanical changes in functional spinal units after cyclic loading [5].

Collagen uncrimping found during histological examination of damaged AF tissue correlate with the mechanical changes reported from cyclic loading. Uncrimped collagen fibers decrease the tissue response at low strain magnitudes [6]. However, despite altered toe region mechanics, collagen fibers likely remain intact as AF ultimate properties were not affected by our damage protocol, which is consistent with prior studies [7].

Mechanical and structural changes within the AF could contribute to instability of the IVD after cyclic loading. Due to segmental load sharing between the disc and facet joints, IVD instability can cause higher stresses in the surrounding apophyseal joints and may be one contributing factor to the acute back pain many helicopter pilots experience post-flight [8]. These findings can inform the design of future technologies and exposure protocols aimed at reducing the mechanical load on the lumbar spine to prevent or limit LBP and AF degeneration.

V. REFERENCES

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