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Mechanical damage to the intervertebral disc annulus fibrosus subjected to tensile loading

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Abstract

Damage of the annulus fibrosus is implicated in common spinal pathologies. The objective of this study was to obtain a quantitative relationship between both the number of cycles and the magnitude of tensile strain resulting in damage to the annulus fibrosus. Four rectangular tensile specimens oriented in the circumferential direction were harvested from the outer annulus of 8 bovine caudal discs ($n = 32$) and subjected to one of four tensile testing protocols: (i) ultimate tensile strain (UTS) test; (ii) *baseline* cyclic test with 4 series of 400 cycles of baseline cyclic loading (peak strain=20% UTS); (iii & iv) *acute* and *fatigue* damage cyclic tests consisting of 4 × 400 cycles of baseline cyclic loading with intermittent loading to 1 and 100 cycles, respectively, with peak tensile strain of 40%, 60%, and 80% UTS. Normalized peak stress for all mechanically loaded specimens was reduced from 0.89 to 0.11 of the baseline control levels, and depended on the magnitude of damaging strain and number of cycles at that damaging strain. Baseline, acute, and fatigue protocols resulted in permanent deformation of 3.5%, 6.7% and 9.6% elongation, respectively. Damage to the laminate structure of the annulus in the absence of biochemical activity in this study was assessed using histology, transmission electron microscopy, and biochemical measurements and was most likely a result of separation of annulus layers (i.e., delamination). Permanent elongation and stress reduction in the annulus may manifest in the motion segment as sub-catastrophic damage including increased neutral zone, disc bulging, and loss of nucleus pulposus pressure. The preparation of rectangular tensile strip specimens required cutting of collagen fibers and may influence absolute values of results, however, it is not expected to affect the comparisons between loading groups or dose-response reported.

Keywords

Intervertebral disc; Fatigue; Collagen damage; Tensile properties; Permanent deformation

1. Introduction

Damage of the annulus fibrosus is implicated in intervertebral disc degeneration and may be attributed to mechanical causes, biological remodeling, loss of nutrition, and accumulation of cellular waste products. Whole body vibration causes muscular fatigue, a latency in the muscular response, and viscoelastic creep of soft tissues which may predispose the spinal

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motion segment to injury (Yoganandan et al., 1988; Adams and Dolan, 1997; Pope et al., 1998; Solomonow et al., 1999, 2000). Repetitive mechanical loading on motion segments can result in significant damage to the disc and vertebrae. Cadaveric lumbar motion segments subjected to flexion and fatigue resulted in distortions of the lamellae of the annulus fibrosus and occasional radial fissures (Adams and Hutton, 1983). Hyperflexion has long been known to result in disc prolapse (Adams and Hutton, 1982) but sub-catastrophic injuries can also occur at loads below failure (Nightingale et al., 2002). Cyclic axial compression of motion segments resulted in failure of the vertebral endplate and subchondral bone under subfailure load magnitudes (as low as 50% of failure load) with less than 1000 cycles of loading (Hansson et al., 1987, 1988). The dynamic axial stiffness of the motion segment decreased for aged and degenerated lumbar spinal segments suggesting progressive damage may be a factor in disc degeneration (Hansson et al., 1987).

Failure of the annulus fibrosus or damage to the collagenous network is a potential cause of disc herniation. Clinical failure of the annulus in the posterolateral region of the disc was attributed, in part, to a reduced tensile failure stress in that region relative to the anterior region in both single layer (Skaggs et al., 1994) and multiple layer annulus fibrosus specimens (Galante, 1967; Acaroglu et al., 1995). With disc degeneration, failure stresses were significantly reduced, however failure strains were relatively constant with values of approximately 20% in the circumferential direction (Acaroglu et al., 1995). Sub-catastrophic failure and damage accumulation and progression was also predicted using finite element models implicating peripheral tears of the annulus (Natarajan et al., 1994; Kim, 2000). In vertical slices of annulus and bone (approximately 5 mm thick $\times$ 30 mm wide) subjected to axial tension, fatigue failure was measured in less than 10,000 cycles if the load exceeded 45% of the ultimate tensile strength (Green et al., 1993). However, this study did not report material properties of the annulus in the circumferential direction, nor did they demonstrate if sub-catastrophic failure could occur with fewer cycles. At the composition level, the percent of denatured (damaged) collagen increased with intervertebral disc degeneration (Antoniou et al., 1996).

Damage progression in the circumferential direction of the disc annulus is likely to occur in vivo in response to extreme loading with associated degradation in tensile material properties, yet the threshold when this damage initiates is not available in the literature. We hypothesized that damage of the annulus will increase with the number of cycles and magnitude of strain applied to the disc. Furthermore, damage will occur at the weakest mechanical link which we hypothesized to be the interlamellar connections and therefore result in delamination. The primary objective of this study was to obtain a quantitative relationship between both the number of cycles and the magnitude of tensile strain resulting in damage to the annulus fibrosus. A secondary objective was to assess how damage affected the laminate structure of the annulus using histology, transmission electron microscopy (TEM) and biochemical measurements. Mechanical damage to the annulus was assessed through measurement of the normalized peak tensile stress and permanent deformation (% elongation).

2. Methods

Four rectangular tensile specimens oriented in the circumferential direction were harvested from the outer annulus of 8 mature bovine caudal discs ($n = 32$) that were approximately 4 years of age and subjected to one of four tensile testing protocols: (i) ultimate tensile strain (UTS) test; (ii) *baseline* cyclic test with 4 series of 400 cycles of baseline cyclic loading (peak strain=20% UTS); (iii, iv) *acute* and *fatigue* cyclic tests consisting of 400 cycles of baseline cyclic loading with intermittent loading (of 1 and 100 cycles, respectively) to peak tensile strains of 40%, 60%, and 80% UTS. The loading protocol was modified from a similar experiment that tested the inferior glenohumeral ligament of the shoulder (Pollock et al., 2000).

Bovine tails were obtained from the slaughterhouse within 2 h of the animal's death. Soft tissue was removed and the disc was isolated using a scalpel. The annulus of all specimens had discrete lamellae and were similar in appearance to healthy human tissue (grade 2, Thompson et al., 1990). Thin rectangular sections of outer annulus fibrosus tissue were isolated, soaked for 30 min in PBS with protease inhibitors (2 mM EDTA, 5 mM benzamidine HCl, 10 mM *N*-ethylmaleimide, 1 mM phenylmethylsulfonyl fluoride; Sigma Chemical, St. Louis, MO) to equilibrate water content, and frozen with liquid nitrogen. Annulus sections were microtomed using a cryostat to obtain uniformly thick sections of annulus similar to a previous study (Acaroglu et al., 1995). While frozen, 4 adjacent rectangular test specimens of identical size (Fig. 1) were harvested using a specially designed die cutter and stored at -20°C .

On the day of testing, width and thickness dimensions were measured using a dissecting microscope and calibrated reticle with mean $\pm$ SD dimensions of 3.36 ± 0.41 and 1.54 ± 0.38 mm, respectively. The annulus layer thickness was measured for ten of the specimens (randomly chosen) by taking three measurements of thickness for each layer (1 mm from each end and at the center). While minimal variation in the thickness of a single annulus layer was detected at different regions of the specimen, the total specimen thickness and thickness of adjacent layers (mean $\pm$ SD = 0.31 ± 0.028 mm) was not completely uniform, resulting in 5 annulus layers in each test specimen on average. Sandpaper grips were carefully cut and glued to the ends of the test specimens using cyanoacrylate with an additional parallel block of sandpaper glued to the ends that mated with the grips to prevent specimens from slipping (Fig. 1). The specimen was then mounted into custom tensile grips of a testing machine (ELF-3200, Enduratec, Minnetonka, MN) that were surrounded by an environmental chamber containing PBS with protease inhibitors at room temperature. Sandpaper grips were marked, photographed before and after testing, and then evaluated to insure there was no slippage in the grips.

The four specimens from each disc were randomly assigned to one of four tensile testing protocols. All specimens were subjected to a uniform preloading protocol, that consisted of a 10 min creep at 1 N (~ 0.1 MPa) after which a length measurement (LM) was taken to define the initial length (mean $\pm$ SD for all specimens = 10.22 ± 2.05 mm). All specimens were pre-conditioned (Pre) with 30 cycles to 5% absolute strain at a strain rate of 0.025 s^{-1} under displacement control. Protocol one consisted of a quasi-static ramp to failure at a strain rate

of 0.025 s^{-1} to define the *ultimate tensile strain* (UTS) for the outer annulus of that disc. Protocol 2 was a *baseline* cyclic test that consisted of 4 series of 400 cycles of cyclic loading to baseline levels (i.e., peak strain = 20% UTS, denoted BL on Fig. 2). Protocol 3 was an *acute* damage cyclic test that consisted of the same 4 series of 400 cycles of baseline cyclic loading with intermittent loading of 1 cycle with peak tensile strain to damaging levels (i.e., 40%, 60%, and 80% UTS, denoted DL on Fig. 2). Protocol 4 was a *fatigue* damage cyclic test that consisted of the same 4 series of 400 cycles of baseline cyclic loading with intermittent loading of 100 cycles with peak tensile strain to damaging levels (i.e., 40%, 60%, and 80% UTS, denoted DL on Fig. 2). All cycles used a triangular waveform and a strain rate of 0.025 s^{-1} . After each of the 3 cyclic test protocols, the testing machine was switched to force control, and specimens were allowed to recover at 0 N for 60 min. After 20 and 60 min of recovery a 10 min creep test at 1 N was performed to measure specimen length, and determine if any recovery in specimen length occurred (LM, Fig. 2). A time delay was added to the damage load (DL) periods of the baseline and acute cyclic tests (where the tissue was ramped to the displacement level corresponding to a force of 0 N and held at that displacement) so that all 3 cyclic test protocols had identical time durations of approximately 220 min.

Normalized peak stress, normalized length, and percent elongation were all calculated as output measurements. The last ten cycles of each baseline loading period (i.e., test segments 1, 2, 3, & 4, Fig. 3) were averaged to obtain the steady state peak stress before and after the damage loading periods. Normalized steady-state peak stresses were calculated by normalizing the steady-state peak stress values by the value before damage loading occurred (i.e., test segment 1). Normalized length and percent elongation were calculated from the specimen length relative to the initial length measured after the first length measurement.

Normalized peak stress was analyzed using a two factor analysis of variance (ANOVA) with repeated measures on the factors test condition (baseline, acute, fatigue) and amplitude of damage loading (40%, 60%, and 80% of UTS) as the independent variables. A one factor ANOVA with repeated measures on test condition was used to analyze normalized length and percent elongation. The value $p < 0.05$ was considered significant.

The effects of loading on the integrity and composition of the specimens were determined biochemically by assessing the total collagen content, fraction of those fibers that were denatured (damaged), and proteoglycan content. In addition to testing all of the loaded specimens, three control groups were assayed to determine if specimen preparation procedures affected the amount of denatured collagen. Soaked control specimens were harvested from tissue adjacent to the mechanical test specimens and treated identically to loaded specimens without mechanical testing. For the remaining two controls, tissue was harvested from bovine tail discs within 2 h of death, and either frozen at -20° for 7 days (*frozen*) or assayed immediately (*fresh*). To determine the collagen composition, specimens were digested with 0.5 ml of 1 mg/ml alpha-chymotrypsin in 50 mM Tris, pH 7.6 (with proteinase inhibitors: 1 mM EDTA, 1 mM iodoacetamide and 10 $\mu\text{g/ml}$ pepstatin A, Sigma Chemical Co., St. Louis, MO) overnight at 37°C to solubilize denatured type II collagen. The disc residue was separated from the alpha-chymotrypsin extract by centrifugation and remaining native collagen chains were digested with 0.5 ml of 1 mg/ml proteinase-K in the

same Tris buffer with protease inhibitors overnight at 56°C. Hydroxyproline and glycosaminoglycan (GAG) contents were determined from extracts from the alpha-chymotrypsin and proteinase-K digests on an ELISA as previously described (Burleigh et al., 1974; Farndale et al., 1986). The percent denatured collagen was calculated as the ratio of hydroxyproline in the alpha-chymotrypsin digest relative to total hydroxyproline measured in both digests (Hollander et al., 1994; Antoniou et al., 1996).

Two unloaded control and two fatigue damage specimens were imaged using histology and TEM to assess any discernible changes in the organization or structure of the collagenous structure. Sections of soaked control and fatigue loaded annulus samples were fixed overnight at 4°C in Karnovsky's fixative (2.5% glutaraldehyde, 1.0% paraformaldehyde in 0.1 M Millonig's phosphate buffer, pH 7.2). Specimens were then cut into 1 mm³ pieces, twice rinsed in Millonig's buffer at 4°C, dehydrated through graded ethanols, cleared in propylene oxide and embedded in Spurr's epoxy resin. Semi-thin sections (1 µm) were cut with glass knives on a microtome, stained with methylene blue—azure II. Histology of semi-thin sections was inspected by viewing 4 sections from 5 different regions in each of the four test specimens to define areas of interest for further TEM evaluation. Four sections were chosen from the control and fatigue damage specimens which were then prepared for TEM analysis. Ultra-thin sections (60–80 nm) were cut with a diamond knife, retrieved onto mesh copper grids (size 150), contrasted with uranyl acetate (2% in 50% ethanol) and lead citrate, and examined with a JEOL 1210 TEM (JEOL USA, Inc, Peabody, Ma) operating at 60 kV. Finally, approximately ten fields of view for each of four sections were evaluated using TEM at magnifications ranging from 2500–20,000 × .

3. Results

Quasistatic ramp tests demonstrated a nonlinear stress–strain response (characteristic of that reported for human annulus tissue) with mean±SD values for UTS of 21.3±2.1% corresponding to failure stresses of 2.94±1.05 MPa. Acute and fatigue damage loading caused significant decreases in the normalized steady-state peak stresses (Fig. 4). Acute and fatigue loading to 40% of UTS (i.e., strain of 8.52%) reduced the normalized stress to 0.81 and 0.55, respectively (corresponding to segment 2 in Fig. 3). However, the baseline loading group also resulted in a reduced normalized peak stress to 0.88 at the end of segment 2 (i.e., after 2 baseline loading periods without intermittent damaging load). Loading to larger magnitudes or with greater numbers of cycles resulted in a further decrease in steady-state peak stresses with a maximum reduction in normalized peak stress to 0.11.

Normalized specimen length was significantly greater for the fatigue damage loaded specimens compared to the baseline and acute damage loaded specimens, and significantly greater for the acute damage than that for the baseline specimens (Fig. 5). Importantly, there was no recovery in the specimen length between 20 and 60 min of relaxation for any of the load groups ($p > 0.74$).

There was no detectable hydroxyproline from the alpha-chymotrypsin digestions (i.e., no denatured type II collagen) of either the soaked controls, or the loaded specimens. Significant differences in percent denatured collagen were detected however between fresh

and frozen controls (Table 1). No significant differences in collagen content (i.e. total collagen) nor proteoglycan content were detected between any of the groups (i.e., soaked control, UTS, baseline, acute, fatigue loaded). The mean $\pm$ SD GAG for all specimens was 2.79 ± 0.46 μ g GAG/mg wet tissue weight. The fine collagen structure (e.g., total fibril diameter, and fibril diameter along the fibril length) appeared similar with fibril diameter of approximately 50–150 nm for both the control and fatigue loaded tissue (Fig. 6) when observed under light microscopy and with high power ($20,000\times$) TEM (Fig. 6). Cracks between adjacent annulus layers in the fatigue loaded specimens were more apparent in light microscopy and TEM images than for unloaded control specimens (Figs. 7 and 8).

4. Discussion

In order to evaluate mechanical damage to the intervertebral disc, annulus fibrosus specimens were tested in tension using 4 different protocols. Damage to the annulus was assessed through measurement of permanent deformation (% elongation) and normalized peak stress. A secondary objective was to assess how damage affected the laminate structure of the annulus using biochemical measurements, histology and TEM evaluation. The most significant results of this study were that tensile stress reduction and permanent deformation: occurred for baseline, acute, and fatigue loaded specimens; depended on the magnitude of damaging strain and number of cycles at that damaging strain; and were greatest for the fatigue loaded specimens.

The tensile strains used in the baseline, acute damage and fatigue damage protocols were increasing percentages of the ultimate tensile strain of adjacent tissue (i.e., 20% baseline; and 20%, 40%, 60% and 80% of UTS for acute and fatigue damage specimens). This loading protocol was used to insure that loading levels were sub-catastrophic and within physiological ranges. Damage, however, was detected in all loading groups including the baseline group subjected to 4×400 cycles at 20% of the UTS. The values for ultimate tensile strains (21%) and ultimate failure stress (2.94 MPa) measured in this study were similar to those reported for human annulus fibrosus (Galante, 1967; Acaroglu et al., 1995; Ebara et al., 1996). While fiber strains in the outer annulus of human cadaveric motion segments were measured to be 6% or less (i.e., <40% of UTS as reported in this study) under physiological loading conditions (Stokes, 1987), finite element models calculated fiber strains in the annulus to be 10% under axial loading and even greater than 20% under combined compression, bending, and twisting, or under dynamic loading (Shirazi-Adl, 1989; Kusra et al., 1992) which are similar to values applied in this study.

A loss of peak stress and some plastic deformation (permanent elongation) was detected with as little as a single cycle of loading at 40% UTS (Fig. 4) and increased with both the magnitude and number of cycles of loading suggesting that matrix damage can occur with a single cycle of loading well below the UTS of the tissue in the absence of biological repair. The plastic deformation of the annulus tissue appeared to be permanent since there was no recovery in the length measurement between 20 min and 1 h after cyclic tensile loading. Permanent deformation in the annulus *in vivo* may result in an increase in the neutral zone of a motion segment, disc bulging, or a loss of nucleus pulposus pressure. In contrast to our results, a recent study of fatigue loading on whole sheep motion segments under axial

compression found structural properties changed with loading but returned to baseline levels following a 24 h recovery and rehydration period (Johannessen et al., 2004). It is expected that the damage loading protocol in our study subjected the annulus to larger magnitudes of tensile hoop strain than for intact motion segments subjected to a 'moderate' level of axial compression. Furthermore, it is possible that a small amount of permanent deformation of the annulus may not be detectable in axial stiffness tests on healthy sheep motion segments which can likely maintain 'healthy' levels of swelling pressure under a tare force.

To better understand how collagen content or integrity may have impacted the permanent elongation and reduction in peak stress, biochemical analysis and TEM were performed. Values of denatured type II collagen measured in fresh tissue were similar to that reported for healthy human intervertebral discs (Antoniou et al., 1996). As expected, a comparison of fresh and frozen tissue showed a significant increase (from $4.3 \pm 1.7\%$ to $7.12 \pm 0.6\%$) in the percentage of denatured collagen fragments. There were, however, no detectable damaged collagen fragments from any of the soaked control, or loaded test specimens. The fact that not even the 4% denatured collagen which was found in the fresh control could be detected, suggests that these fragments were leached from the tissue during soaking. Measurement of the amount of collagen in the test solution bath in future tests may allow for detection of differences in denatured collagen between mechanically loaded specimens and biochemical controls, if differences exist. It should also be noted that the protocol used to measure damaged collagen fragments is measuring only those fragments in which the triple helix structure of the fiber has been denatured (Hollander et al., 1994), which happens during normal enzymatic degradation of the tissue and was reported to be affected by aging and degeneration (Antoniou et al., 1996). Therefore, if mechanical damage to the fibers does not occur in this manner, it is possible that it would not be detected with this assay. TEM analysis further demonstrated that the collagenous fibril structure appeared similar for unloaded control and fatigue tested specimens suggesting that the fibrils remained intact during our analysis. The highly localized sampling (due to the extremely high magnification with TEM) and freezing prior to chemical fixation, prevented us from decisively excluding fibril damage as a source of altered mechanical behavior.

Delaminations (i.e., separation of annulus layers) were more apparent with light microscopy and TEM of fatigue damage specimens than control specimens. While it is likely that multiple failure patterns were present in annulus specimens, delaminations are most likely the dominant failure mechanism based on our results and a previous analysis on damage mechanisms in the annulus (Iatridis and ap Gwynn, 2004). Interlaminar shear stresses can be large near stress free edges and are a likely mechanism for delaminations (representative of circumferential tears in the annulus) in tensile test specimens of isolated annulus tissue (Iatridis and ap Gwynn, 2004). There were on average 5 annulus layers in each test specimen, and based on theoretical results it is anticipated that the interlaminar shear stress will increase if fewer layers are present (Iatridis and ap Gwynn, 2004). The variance in normalized peak stress at 20% and 40% UTS loading was quite small (Fig. 4) suggesting that any difference in the number of annulus layers in our specimens did not impact our comparisons between groups, however, differences in the number of annulus layers in our specimens may partially explain the relatively large variance in the normalized peak stress for the damage loading groups at the 60% UTS level.

Tensile testing of isolated annulus fibrosus specimens was first used in 1967 (Galante, 1967) and is still a commonly accepted technique to determine regional variations in the material properties and strength of intervertebral disc tissue (Adams and Green, 1993; Green et al., 1993; Skaggs et al., 1994; Acaroglu et al., 1995; Ebara et al., 1996; Fujita et al., 1997; Elliott and Setton, 2001). The strength of the methodology used in the current study is that the isolation of annulus tissue provided a direct method to evaluate the effects of circumferential tensile loading on mechanical damage accumulation in the annulus material without the additional structural complexities of the full motion segment. In order to prepare tensile test specimens of uniform dimensions, however, it was necessary to microtome the specimens which required freezing and cutting.

Testing of isolated tensile specimens of annulus fibrosus is limited in that fibers are cut, stress-free edges are created, and proteoglycan loss may be increased. The influence of specimen geometry on structural properties of isolated vertebra-annulus-vertebra sections was previously described (Adams and Green, 1993) with the finding that interactions between fibers and annulus layers contributes substantially to tensile strength, consistent with our results. We anticipate that the presence of stress free edges in isolated tensile test specimens (that are not present in the intact disc) may reduce magnitudes of stress required for damage initiation as compared to an intact disc and microfailure mechanisms may also differ. A loss of proteoglycans due to equilibrating isolated specimens in PBS with protease inhibitors has been described previously (Urban and Maroudas, 1981) and can result in a loss in swelling pressure and associated collagen tension and water content which may account for some of the measured mechanical degradation seen in the baseline group. However there was no detectable difference in GAG content in any of the 4 loaded groups, therefore differences between baseline, acute, and fatigue loaded specimens are not the result of proteoglycans loss, and it is important to note that the dose-response results do suggest that alterations in normalized peak stress and permanent elongation beyond the baseline group may be explained by mechanical damage. Plastic deformation and a loss in stiffness were both implicated in the loss of normalized peak stress found with increased mechanical loading to the tissue, yet we cannot isolate these two effects for this nonlinearly elastic material in the current test protocol.

In conclusion, repetitive subfailure tensile loading of the annulus fibrosus resulted in damage to the annulus fibrosus tissue which was expressed as a loss of normalized peak stress and plastic deformation. The most significant findings of this study were that tensile stress reduction and permanent deformation were: greater in both acute and fatigue loaded specimens compared to the baseline control; depended on the magnitude of damaging strain and number of cycles at that damaging strain; and were greatest for the fatigue loaded specimens. Mechanical damage was most likely a result of separation of annulus layers which may be a mechanism for failure in the circumferential annulus fibrosus. This mechanical damage can be a cause of increased laxity that may manifest in the motion segment as an increase in neutral zone, disc bulging, loss of nucleus pulposus pressure, or sub-catastrophic damage to the disc in vivo.

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Fig. 1. Schematics and photographs of bovine caudal intervertebral disc, sample harvest region, specimen preparation, and test specimen loaded in grips. The diameter of the whole caudal intervertebral disc was roughly 30 mm.

Fig. 2.

Schematic of cyclic test protocols for a fatigue damage cyclic test including initial tare loading, length measurements, preconditioning, and post-test length measurements. Length measurements (LM) were taken after 10 min of creep under force control at 1 N (dashed lines): at the end of the tare loading, and at 20 min and 60 min after the cyclic testing. Under displacement control, 4 series of baseline loading (**BL**=400 cycles @ 20% **UTS**) were performed to assess the baseline mechanical response of the annulus fibrosus specimens before and after damage loading. Damage loading (**DL**) involved cyclic loading to strain magnitudes of 40%, 60% and 80% of UTS. For the *baseline* test protocol, no damage loading (**DL**) cycles were performed. For the *acute* damage cyclic test, the **DL** protocol consisted of 1 cycle of loading at each of the amplitudes. For the *fatigue* damage cyclic test, the **DL** consisted of 100 cycles of loading at each of the amplitudes. All cycles were performed with a strain rate of 0.025 s^{-1} . A time delay (where the tissue was ramped to the displacement level corresponding to a force of 0 N and held at that displacement) was added during the **DL** periods for the baseline and acute damage protocols to insure that the testing duration for all protocols was constant.

Fig. 3.

Peak stress results for the displacement control sections of a fatigue damage cyclic test including preconditioning. The maximum peak stress can be seen for baseline and damage loading periods. The steady-state peak stress under baseline loading was measured by taking the average values over 10 cycles at the end of each of the baseline loading periods (i.e., segments 1, 2, 3 & 4). The steady state peak stress at the end of the first baseline loading period (i.e., segment 1) was used to calculate normalized steady state peak stress.

Fig. 4.

Normalized steady-state peak stress (mean $\pm$ SEM) with magnitude of damage loading for baseline, acute damage, and fatigue damage loaded tissues. The steady-state peak stress at 20%, 40%, 60% and 80% of ultimate tensile strain correspond to data taken at test segments 1, 2, 3 & 4 normalized to the value at test segment 1 (Fig. 3). The loss of normalized peak stress increased with the magnitude of loading and number of cycles.

Fig. 5.

Mean $\pm$ SEM normalized tissue length (i.e., normalized by initial tissue length) for baseline, acute damage, and fatigue damage specimens. Normalized tissue length was significantly increased at 20 and 60 min after cyclic loading compared to the initial length for all 3 loading groups ($p < 0.0001$). There was no recovery between the 20 and 60 min of recovery for any specimens ($p > 0.74$). The symbol * = significant difference between groups with $p < 0.05$.

Unloaded Control Samples

Fatigue Damage Loaded Samples

Fig. 6. High magnification ($20,000\times$) TEM images of unloaded (top) and fatigue damage (bottom) annulus fibrosus specimens. TEM images are transverse to the fibril direction (left, i.e., note the fibril cross-sections) and parallel to the fibril direction (right). The fibril diameters and structure appeared similar for both control and loaded specimens under high power. The vertical scale bars (upper left of each image) are 200 nm. Collagen fibril diameters range from approximately 50–150 nm.

Fig. 7.
TEM image ($6000\times$) of fatigue damage specimens with orientation transverse to fibril direction demonstrating separation of annulus layers. The vertical scale bar is $1\text{ }\mu\text{m}$.

Fig. 8. Histological image ($20\times$ objective) of frozen control and loaded annulus fibrosus specimens. The dashed line in the background demarcates the interface between adjacent annulus layers. Loaded specimens demonstrated more evidence of cracks between adjacent annulus layers than control specimens. The scale bars are $100\text{ }\mu\text{m}$.

Table 1

Biochemical results for control and loaded specimens

	µg Collagen/mg wet tissue				Percent denatured collagen			
	Loaded	Soaked ctrl	Fresh ctrl	Frozen ctrl	Loaded	Soaked ctrl	Fresh ctrl	Frozen ctrl
Average	32.47	28.09	30.60	30.89	nd	nd	4.13	7.12
Stdev	8.81	5.29	1.77	4.76	—	—	1.72	0.69

nd = not detectable. ctrl = control.