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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 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).

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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 ± 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 ± 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

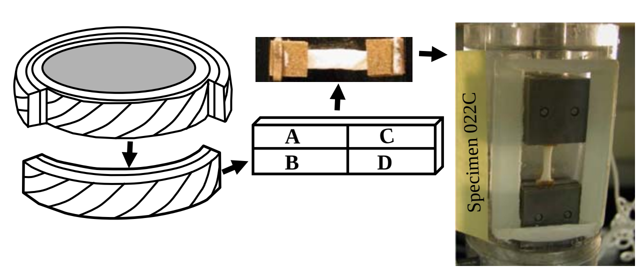

Fig. 1. Schematics and photographs of bovine caudal intervertebral disc, sample harvest region, specimen preparation, and test specimen loaded in grips. The diameter of the whole caudal intervertebral disc was roughly 30 mm.