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Inter-lamellar shear resistance confers compressive stiffness in the intervertebral disc: An image-based modelling study on the bovine caudal disc

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ABSTRACT

The intervertebral disc withstands large compressive loads (up to nine times bodyweight in humans) while providing flexibility to the spinal column. At a microstructural level, the outer sheath of the disc (the annulus fibrosus) comprises 12–20 annular layers of alternately crisscrossed collagen fibres embedded in a soft ground matrix. The centre of the disc (the nucleus pulposus) consists of a hydrated gel rich in proteoglycans. The disc is the largest avascular structure in the body and is of much interest biomechanically due to the high societal burden of disc degeneration and back pain. Although the disc has been well characterized at the whole joint scale, it is not clear how the disc tissue microstructure confers its overall mechanical properties. In particular, there have been conflicting reports regarding the level of attachment between adjacent lamellae in the annulus, and the importance of these interfaces to the overall integrity of the disc is unknown. We used a polarized light micrograph of the bovine tail disc in transverse cross-section to develop an image-based finite element model incorporating sliding and separation between layers of the annulus, and subjected the model to axial compressive loading. Validation experiments were also performed on four bovine caudal discs. Interlamellar shear resistance had a strong effect on disc compressive stiffness, with a 40% drop in stiffness when the interface shear resistance was changed from fully bonded to freely sliding. By contrast, interlamellar cohesion had no appreciable effect on overall disc mechanics. We conclude that shear resistance between lamellae confers disc mechanical resistance to compression, and degradation of the interlamellar interface structure may be a precursor to macroscopic disc degeneration.

1. Introduction

The intervertebral disc (IVD) is a unique soft tissue structure which provides support and flexibility in the axial skeleton of vertebrates. Due to the economic and social burden of disc pathologies (Hoy et al., 2014), how microstructural disease and degeneration processes in disc tissue affect the overall mechanical response of the disc is of great importance (Urban and Roberts, 2003). Previous microstructural investigations of the mammalian intervertebral disc have identified two distinct regions, the inner gel-like nucleus pulposus (NP) which is rich in proteoglycans, and the tough, fibrous, annulus fibrosus (AF) which comprises a series of concentric annular layers (lamellae), containing alternately angled collagen fibres embedded within a soft ground matrix. The annulus confines the proteoglycan-rich nucleus gel, allowing generation of high hydrostatic pressures in response to in vivo compressive loads (Nachemson, 1960). From a structure-function perspective, the alternately aligned layers of collagen fibres in adjacent lamellae serve to resist both internal NP pressures during joint loading and torsion through fibre tension. However, what is not currently clear is the role of the interface between adjacent lamellae, and the extent to which interface mechanics governs overall disc behaviour. The importance of interlamellar mechanics is further highlighted by the findings of Marchand and Ahmed (1990) in which the authors reported a high proportion (40–50%) of circumferentially discontinuous lamellae in human lumbar IVDs. A substantial proportion of the lamellae is therefore not rings, but circumferentially incomplete curved sheets, which raises the question as to how the interface allows shear stress transfer between discontinuous lamellae.

Despite several careful investigations of the microanatomy of the interlamellar interface, existing knowledge on interlamellar mechanics remains sparse and somewhat contradictory. Lamellar

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layers have been readily separated in several dissection studies (Marchand and Ahmed, 1990; Holzapfel et al., 2005), and Bruehlmann et al. (2004) show evidence of inter-lamellar sliding ('discrete deflection') in the inner AF of the bovine disc under 8° flexion, both of which suggest that interlamellar interfaces are mobile. On the other hand, the presence and extent of the inter-lamellar fibre network (Yu et al., 2005, 2007; Pezowicz et al., 2006; Schollum et al., 2009) suggests a structural role in limiting slip between lamellae, and Smith et al. (2008) demonstrated that the inter-lamellar elastin network of human lumbar discs confers substantial tensile stiffness of the interface. Two quantitative studies on the shear resistance of the interlamellar interface are those of Michalek et al. (2009) who reported that sliding did not occur between AF layers in bovine discs, and Gregory et al. (2011), who found substantial interlamellar load carrying ability in porcine cervical discs. We note also the T-peel tests of Gregory et al. (2012) who reported ~30% higher peel strength in the superficial annulus of human lumbar discs than in the inner annulus.

The aim of this study was to investigate the extent to which interlamellar interface mechanics governs overall disc resistance to compression, the fundamental loading state to which the disc is subjected in vivo. This aim was pursued through the development of an image-based micro-scale FE model of the disc incorporating both interlamellar sliding and separation.

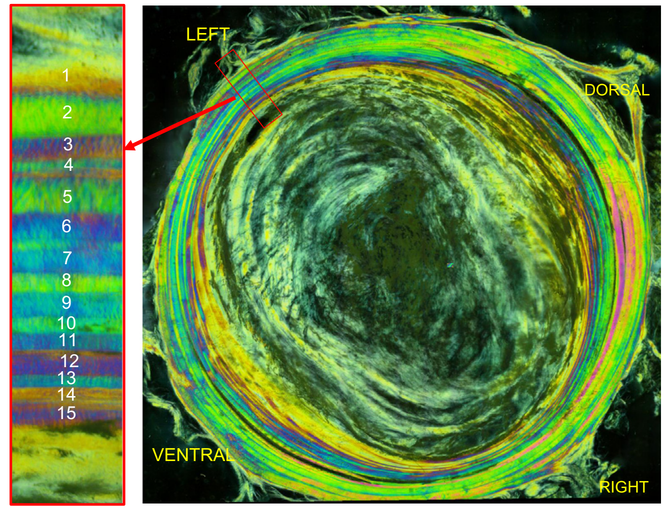

Fig. 1. Stitched polarized light image of entire transverse cross-section of the bovine intervertebral disc used for FE model generation. The inset at left shows an example of lamellar counting from outer to inner edges of the annulus fibrosus.

2. Methods

The FE model developed in this study was based upon the bovine tail disc. Bovine tails provide a consistent and readily available source of tissue with AF collagen content comparable to human discs (Showalter et al., 2012), and there is a growing body of basic science studies on the microarchitecture and mechanics of the bovine tail disc (Bruehlmann et al., 2004; Pezowicz et al., 2006; Yu et al., 2007; Michalek et al., 2009).

A single motion segment (second intervertebral joint) of an adult bovine tail was freshly obtained from a local butcher and frozen at −20 °C prior to use. Lateral and frontal radiographs were taken for subsequent endplate curvature and disc height measurement. The excised motion segment was fixed, dehydrated and embedded en bloc in Spurr resin (Electron Microscopy Sciences, Hatfield, PA, USA). A thick (~300 μm) mid-height transverse section was prepared by cutting and milling the embedded disc, before mounting to a large format glass microscope slide. By using a thick section, the need for staining to enhance collagen birefringence in polarized light could be avoided. This was desirable in order to avoid potential artefacts due to uneven staining (Kiraly et al., 1997).

The transverse disc section was imaged using a custom polarized light imaging system with white LED (6500 K colour temperature, Lightune Inc, France) illumination, and crossed circular polarizer and analyzer (Edmund Optics Ltd., Europe) positioned beneath and above the specimen. A custom mounted tube-scope was equipped with a 4× finite conjugate plan objective (Edmund Optics Ltd., Europe) and a five megapixel colour CMOS microscope camera (DCM-510, ScopeTek, China). The computer controlled imaging system was used to take a regularly spaced grid of microscope images which covered the entire disc cross-section, and these were post-stitched using the algorithm of Preibisch et al. (2009) provided as part of the Fiji distribution of the ImageJ software (NIH, USA).

Disc microstructure (lamellar count, thickness and continuity) in the stitched transverse plane image was measured as follows. Firstly, the origin of radial coordinates was defined by a vector oriented from the centroid of the disc cross-section toward the ventral edge of the disc. Angles were defined relative to this axis, so that the left and right lateral directions were at ±90° to the ventral axis respectively, and the dorsal (posterior) direction was at 180°. Using this polar coordinate system, lamellar number and thickness were counted and measured along axes from the disc centroid at angles of 0° (ventral), ±90° (left and right lateral) and 180° (dorsal). Lamellar layers were counted from the outer to the inner annulus (Fig. 1). In the transverse cross-section, a lamellar origin or termination was defined as a clearly visible 'Y' junction (Fig. 2). Lamellar discontinuities were manually marked by measuring the x, y coordinates of the tip of each discontinuity using the ImageJ point measurement tool. All measurements were performed by a single observer.

The measurements described above were used to generate an image-based FE model to study the effects of inter-lamellar interface mechanics and lamellar discontinuity on disc compression resistance. Firstly, the external AF diameter from the transverse section was taken as the outer diameter of the model. Next, the mean thicknesses of the outer, middle and inner third of the lamellar layers in the embedded disc cross-section at mid-height were used to define the corresponding lamellar thicknesses in the FE model. Thirdly, the endplate curvatures and disc height measured from lateral and frontal radiographs of the motion segment taken prior to embedding and sectioning were used to define the endplate curvature and initial disc height of the FE model. A 3D FE model of 1/8 of the disc was then generated (one quarter of the transverse plane cross-section assuming mid-sagittal and mid-coronal plane symmetry based on the high circularity of the bovine tail disc in this plane, and assumed superior–inferior symmetry about the mid-transverse plane of the disc).

Each lamella was meshed individually, firstly with 3D continuum solid elements to represent the ground matrix, and secondly with an embedded layer of ABAQUS 'rebar' elements to represent the collagen fibre bundles within each layer. The collagen fibre alignments in successive lamellar layers were alternated between ±30° to the transverse plane (Marchand and Ahmed, 1990). The thickness and spacing of collagen fibre bundles within each lamellae were also taken from Marchand and Ahmed (1990), with a cosine correction to account for the orientation of the sectioning plane relative to the fibre orientation (fibre bundle cross-sectional area 3.212×10^{-2} mm^2, spacing 0.23 mm). The NP was meshed using 3D solid elements. Material properties for the collagen fibres and AF ground substance were based on prior literature, and the Mooney–Rivlin constants for the NP were prescribed to be an order of magnitude less stiff than the AF ground substance while maintaining near-incompressible material behaviour ($D=0.3$ corresponding to $\mu_{eff}=0.487$) (Table 1).

In order to investigate the effect of inter-lamellar cohesion and lamellar discontinuity, separate contact interfaces were defined between every pair of adjacent lamellae in the model. These interfaces could either be 'tied' (no relative slip and no separation), or allowed to slip/separate. Furthermore, in order to simulate the introduction of several circumferentially discontinuous lamellae in the model, the $\Delta x=0$ boundary condition at the mid-sagittal symmetry plane was released for three of the lamellae in the model (the 3rd, 8th and 13th lamellae from the outer annulus) thus allowing circumferential retraction under load. Six different sets of interlamellar interface conditions were simulated in all (Table 2).

The model was loaded in axial compression to 400 N using a central node which was constrained to all of the nodes on the upper surface of the model (corresponding to the vertebral endplate). The displacement at 10 N of compression was used as the reference (zero) displacement for stiffness calculation. All FE modelling was performed using ABAQUS (v6.11, Dassault Systèmes, Paris, France) using a quasi-static solution procedure with large displacement solver. Mesh sensitivity analysis was performed using an intact NP model with Interface Condition 1 from Table 2.

A small series of bovine tail disc compression experiments were then performed for comparison with the FE model predictions. Two adult bovine tails were freshly obtained from a local butcher and immediately frozen at −20 °C. Prior to dissection, specimens were defrosted overnight. Once thawed, the upper two caudal discs from each tail were carefully dissected to expose the vertebral column, leaving intervertebral discs intact. The first, second and third caudal vertebrae were sectioned transversely at mid-height, yielding two intact motion segments per tail for subsequent biomechanical testing. The cut ends of the adjacent vertebral bodies were embedded in polymethylmethacrylate. After curing, the test specimens underwent uniaxial compression in an Instron universal testing machine (Model 5566, Instron, UK). Each specimen was first subjected to three cycles of compressive preload from 10 to 50 N, followed by a single cycle of compressive load to a maximum of 400 N. The pre-cycles and the main load were all performed at a