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New findings confirm the viscoelastic behaviour of the inter-lamellar matrix of the disc annulus fibrosus in radial and circumferential directions of loading

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ABSTRACT

While few studies have improved our understanding of composition and organization of elastic fibres in the inter-lamellar matrix (ILM), its clinical relevance is not fully understood. Moreover, no studies have measured the direct tensile and shear failure and viscoelastic properties of the ILM. Therefore, the aim of this study was, for the first time, to measure the viscoelastic and failure properties of the ILM in both the tension and shear directions of loading. Using an ovine model, isolated ILM samples were stretched to 40% of their initial length at three strain rates of 0.1%s^{-1} (slow), 1%s^{-1} (medium) and 10%s^{-1} (fast) and a ramp test to failure was performed at a strain rate of 10%s^{-1}. The findings from this study identified that the stiffness of the ILM was significantly larger at faster strain rates, and energy absorption significantly smaller, compared to slower strain rates, and the viscoelastic and failure properties were not significantly different under tension and shear loading. We found a strain rate dependent response of the ILM during dynamic loading, particularly at the fastest rate. The ILM demonstrated a significantly higher capability for energy absorption at slow strain rates compared to medium and fast strain rates. A significant increase in modulus was found in both loading directions and all strain rates, having a trend of larger modulus in tension and at faster strain rates. The finding of no significant difference in failure properties in both loading directions, was consistent with our previous ultra-structural studies that revealed a well-organized (±45°) elastic fibre orientation in the ILM. The results from this study can be used to develop and validate finite element models of the AF at the tissue scale, as well as providing new strategies for fabricating tissue engineered scaffolds.

Statement of significance

While few studies have improved our understanding of composition and organization of elastic fibres in the inter-lamellar matrix (ILM) of the annulus in the disc no studies have measured the direct mechanical failure and viscoelastic properties of the ILM. The findings from this study identified that the stiffness of the ILM was significantly larger at faster strain rates, and energy absorption significantly smaller, compared to slower strain rates. The failure properties of the ILM were not significantly different under tension and shear.

1. Introduction

With a unique hierarchical structure, the annulus fibrosus (AF) of the intervertebral disc is comprised of annular lamellae with alternating orientation of collagen fibres embedded in a proteoglycan-rich ground matrix [1]. The inter-lamellar matrix (ILM), with a thickness of approximately 20 μm, lies between lamellae and consists of collagen type IV, cells, several glycoproteins and matrix; however, elastic fibres were recognized as its main component [2–6]. Microstructural studies of the ILM revealed higher elastic fibre density relative to the intra-lamellar region [7–10], while further insight into ILM elastic fibre ultrastructure identified a dense and complex network of thick (diameter of 1–2 μm) and thin (0.1 μm diameter) fibres that were not randomly distributed [11].

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The clinical relevance of the ILM is not fully understood. However, loss of structural boundaries between lamellae in scoliotic discs, which are more vulnerable to degeneration, along with evidence interpreting an increase of irregularity and interdigitating of lamellae structure with age, may show a correlation between the ILM disorganization and disc degeneration [12,13]. Investigation of AF radial cohesion identified a complex hierarchy of interconnecting fibres in the ILM-lamella boundary that demonstrates the role of the ILM in lamellae connectivity [14]. Furthermore, based on studies of AF delamination, tears, patterns of herniation of the whole disc and lamellae peel strength, it is likely that the ILM plays a role in providing AF structural integrity [15–18]. Therefore, failure of the ILM may contribute to the initial stages of herniation and disc degeneration [19–21]. Knowledge of the structure-function relationship in the ILM is crucial in identifying the loading conditions that contribute to AF delamination or increased risk of disc herniation. Development of clinical strategies including tissue engineering scaffold fabrication and herniation treatment requires a greater understanding of the ILM mechanical and structural properties.

In spite of recent progress identifying the ultrastructure of the ILM, there is limited knowledge on its mechanical role. The ILM shear strength has been found to increase with lamella thickness [22], and deform by up to 10 mm, having a mean (SD) peak shear strength of 0.03 (0.05) MPa during delamination [23]. Significantly different shear stress and strain distributions, with a trend of being greater in the lamella than across the ILM, were found in disc mechanical modelling when inter-lamellar connectivity was considered [24,25]. Also higher strain was reported in the ILM compared to the intra-lamellar region in bovine discs [26].

In the radial direction, the ILM structural connectivity was discussed, yet the mechanical properties of the ILM in tension were not quantified [14,19,27]. Compared to the lamella, the stiffness of the outer lamellae interface (i.e. ILM) was higher than the inner AF, (ranging between 43% and 75%).[28]. This result was consistent with a study indicating that AF peel strength was approximately 30% higher in outer lamellae compared to the inner layers [17].

While microstructural-based studies revealed that the density of elastic fibres were higher in the ILM compared to the lamellae [7–9,12,29,30], ultrastructural studies provided new insights to the organization of elastic fibres in different regions of the disc [11,31,32]. A dense network including thick (diameter of 1–2 μm) and thin (0.1 μm diameter) elastic fibres was seen in the ILM. This highly organised complex network was comprised of the majority of fibres oriented at ±45° to the circumferential lamellae, and having different size and shape fibres, compared to those in the intra-lamellar region [11].

Studies on the ILM were categorized into qualitative structural-based research where no mechanical measurement were undertaken [2,11,14,19,21,29,32], or quantitative research where a number of lamellae were used with limited mechanical properties reported [17,18,23,28,33]. While these studies have improved our knowledge of the possible mechanical role of the ILM, no studies have measured the viscoelastic and mechanical behaviour of the ILM under direct tensile and direct shear loading has not been studied, however peel tests, which would represent a more-tensile loading regime, have been performed.

Studies of multiple and single lamellae have demonstrated that these tissues exhibit increased modulus and failure stress with increasing strain rate, and a higher modulus in shear compared to tension [34] however no such studies have been conducted on the ILM. Therefore, the aim of this study was to measure the viscoelastic and failure properties of the ILM in both the tension and shear directions of loading. The following hypotheses were proposed:

1. The stiffness of the ILM will be significantly larger at faster strain rates, and energy absorption significantly smaller, compared to slower strain rates.
2. The viscoelastic and failure properties under tension and shear loading will not be significantly different.

The first hypothesis was proposed due to the combination of elastic fibres and extracellular matrix in the ILM, which most likely impart viscoelastic behaviour to the ILM. The second hypothesis is based on the dominant orientation of elastic fibres at ±45°, which suggests a similar mechanical role in both tension and circumferential directions of loading.

2. Materials and methods:

2.1. Sample preparation

Sixteen Ovine spines (18–24 months old) were obtained from a local abattoir, and discs from lumbar FSUs (L4/L5) were dissected from vertebral bodies, sprayed with saline and stored at −20 °C in cling wrap until used for sample preparation. While frozen, a 10 mm width of the anterior AF, with the depth to the nucleus pulposus region (~7mm disc height) was separated from each disc. Each AF tissue was moulded with optimal cutting temperature (OCT, Tissue-Tek®, Sakura, Japan) compound to identify the transverse cutting plane. Samples from adjacent sections (approximate thickness 1 mm and width 10 mm) were sliced using a hand microtome (Fig. 1a-c). Damaged samples or those having less than ten lamellae were excluded from the study. All adjacent samples were labelled and then divided into two groups of 10 for mechanical testing in the radial (tension) and circumferential (shear) directions.

2.2. Mechanical testing

To accomplish the ILM mechanical tests, a functional lamellae unit, which consisted of two adjacent lamellae and the ILM between them, was identified from prepared adjacent sections using a stereomicroscope (Motic, SMZ-168, China) (Fig. 1d-e). The functional lamellae units were isolated from the middle of the AF (approximately 5–6 lamellae inwards) and was consistent for all samples. Previous ultrastructural studies identified an approximate 30 μm width for the ILM. During specimen preparation the total width of the lamella-ILM-lamella complex was approximately 200 μm and careful attention was used to ensure that the ILM was located in the middle of the complex. (Fig. 1f - g). This strategy for sample preparation was consistent among all samples. Sand paper (250 grit) was bonded above and below the sample, and on each edge using cyanoacrylate adhesive. The mechanical properties of samples were measured in tension (radial) and shear (circumferential) loading directions.

Pilot failure mechanical tests for the ILM were performed at different strain magnitudes to identify the maximum strain (40%) used in this study, which was low enough (compared to the yield stress) to be non-destructive, yet high enough to include the linear region. The strain rate was estimated based on disc deformation data during compression from Costi et al., 2007 for the outer anterior radial AF displacement [35]. For every 1 mm of applied compressive displacement, the radial strain was 11%, as calculated between the two outermost anterior radial displacement vectors. In order to estimate the compressive displacement of the disc during physiological loading, an in-vivo nucleus pulposus pressure of 1.1 MPa that was measured during standing was used [36]. The equivalent axial compressive force [37]required to create the 1.1

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