Abstract

Among the problems with spinal discs, herniation is the most frequent cause of back pain (in people aged 25-45 years) and can lead to serious disability. Defects such as circumferential tears, delamination of annular layers and loss of structural integrity of the disc increase the risk of herniation. While the role of the nucleus pulposus, annular layers (lamellae) of the annulus fibrosus (AF) and endplates on initiation and propagation of herniation have been the focus of intensive studies, the inter-lamellar matrix (ILM), as a likely site for disc defects, has been poorly studied.

The ILM has an average thickness of less than 30 µm and lies between adjacent lamellae in the AF. The microstructure and composition of the ILM have been studied in various anatomic regions of the disc and it was suggested that the ILM components, mainly elastic fibres, play a role in providing integrity of the AF. However, their contribution to AF mechanical properties and structural integrity is unknown. The overall aim of this thesis was to improve the understanding of role of the ILM in structural integrity and mechanical properties of the AF and its clinical relevance in the progression of herniation for recommending a safe level of lifting load.

The first approach in this thesis was an extensive literature review that summarised the ILM composition, microstructure, and tissue level mechanical properties, which identified the potential role of the ILM in the progression of disc degeneration and herniation, and suggested areas for future investigations. This review revealed that the impact of the ILM and its structural components (mainly elastic fibres) on AF mechanical properties have been the subject of only a few studies and many questions remain. Therefore, a series of studies were undertaken to improve understanding of the structure-function relationships of the ILM.

The first study explained an optimized rapid digestion method for ultrastructural analysis of the disc elastic fibres, while preserving their structural integrity and organization. This study developed a new method for fundamental visualization of elastic fibres and their architecture in the disc. Through ultra-structural analysis, the relationship between structure and function, as well as the role of elastic fibres on the AF mechanical properties can be studied. Based on this study two important findings were revealed. First, while the rapid digestion and subsequent gradual and indirect heat treatment techniques significantly changed the overall structural organization of fibres, these changes were small and would likely have minimal impact on ultra-structural alteration of elastic fibres. Second, elastic fibres comprised of a network structure across the AF, whose size and density varied in different regions.

The second and third studies employed the technique developed from the first study to undertake ultrastructural analysis of elastic fibres in different regions of the AF, including the intra-lamellar region (lamella), ILM and the partition boundary (PB). For all three regions, it was found that the majority of fibres were oriented near 0° with respect to the circumferential direction of the lamellae, with less fibres symmetrically orientated at approximately ±45°. Visualization of the ILM under high magnification revealed a dense network of elastic fibres that has not been previously described. Within the ILM, elastic fibres formed a complex network, which were of different size and shape, and differed to those located in the intra-lamellar region. A loose network of elastic fibres was observed in the intra-lamellar region, which were comprised of almost parallel large fibres (0.5-1.5 μm diameter) and very fine interconnecting fibres of less than 0.2 μm diameter. The density of the elastic fibre network in PBs was lower, and fibre orientation was similar to both the intra-lamellar region and inter-lamellar matrix.

The fourth study aimed, for the first time, to measure the viscoelastic and failure properties of the intact ILM in both tension (radial) and shear (circumferential) directions of loading. The findings from this study identified that the stiffness of the ILM was significantly larger at faster strain rates and the viscoelastic and failure properties were not significantly different under tension and shear loading. A strain-rate dependent response of the ILM was found 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 elastic fibre orientation in the ILM.

The fifth study identified the role of elastic fibres in the mechanical properties of the ILM by removing the matrix using the digestion technique developed in the first study and repeating the testing on the same samples used in the fourth study. The results of this study confirmed the mechanical contribution of the elastic fibre network to the ILM, and likely structural integrity of the AF. A strain-rate dependent response for the elastic fibres in the ILM was found during dynamic loading, particularly for phase angle and stiffness. The elastic fibres in the ILM demonstrated a significantly higher capability for energy absorption at slow compared to medium and fast strain rates as well as in shear compared to tension loading. Also, when tested to failure, a significantly higher normalized failure force was found in tension compared to the shear direction of loading. In fact, the well-organized elastic fibres that create a highly crosslinked and orthotropic network, provide both significant viscoelastic and failure mechanical properties to the ILM.

The abovementioned studies have clarified that the ILM plays a key role in structural integrity of the AF and that the elastic fibre network contributes to the viscoelastic and failure properties of the ILM. However, the clinical relevance of the ILM is not very well known. Based on a multi-scale failure analysis of the ILM and the lamella in an ovine model, **the sixth study** explored the biomechanical properties of the ILM and the lamellae before, during (pre-herniation) and after lumbar disc herniation. Compared to the lamella, the ILM was the weaker structure during the progression of herniation in the outer posterolateral region of the disc. The finding of no differences between ILM failure stress during pre-herniation and herniation suggested that there is a loading threshold above which the ILM loses its structural integrity, which has clinical relevance for recommending safe levels of lifting load