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In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form and Deposit Licence.
The regional susceptibility of the intervertebral disc to mechanically induced disruption and herniation

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Chemical and Materials Engineering, the University of Auckland, 2017.
Abstract

Internal disc disruption and herniation are associated with low back pain and while it is widely accepted that the posterior and posterolateral regions of the disc are the primary herniation sites, much less is known about mechanisms of herniation operating in other regions of the disc. The aim of this thesis was therefore to investigate whether other regions of the disc might be implicated in mechanically induced disruption and herniation. This thesis reports the findings of three interrelated studies.

The first study investigated mechanically induced herniations in flexed and compressed ovine lumbar motion segments by employing a progressive transverse sectioning technique, enabling examination of the entire disc volume. Disruption in the lateral annulus was commonly observed, associated with circumferential tracking of nucleus within the annulus towards the posterolateral and posterior regions. The vulnerability of the lateral annulus to disruption was thought to arise from the overloading of its differentially recruited oblique/counteroblique fibre sets, resulting from induced anterior shear.

The second study investigated whether localised damage created by a needle puncture, as performed during discography, provides a preferred passage for nuclear material through the annulus, independent of any longer-term degenerative changes. Herniations were mechanically induced in discs that had been punctured with either a 25-gauge or a larger 18-gauge needle. Transverse sectioning of the whole disc volume revealed that there was no association between the 25-gauge puncture and disc disruption/herniation. In contrast, nuclear material was observed to migrate through the 18-gauge puncture. Further, disruption in the lateral annulus was commonly observed independent of the presence of a puncture thereby reinforcing the fact that this previously unreported site of initial disc disruption is of fundamental importance for the development of a more rigorous understanding of disc failure.

The final study investigated the response of the annulus to anterior shear and flexion. Micro-
structural analysis of motion segments fixed in anterior shear or flexion demonstrated that in the lateral regions anterior shear differentially recruits the oblique and counteroblique fibres, while flexion recruits these fibres more equally.
Acknowledgements

Firstly, Neil Broom and Ashvin Thambyah, thank you for providing me with the opportunity to undertake the studies for the degree of Doctor of Philosophy here in Auckland, New Zealand.

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To my parents, Harry and Ingrid, thank you for your understanding to undertake my PhD studies at the other side of the world. Irrespective of the distance between us, I have always felt your care and support. Thank you for always being there for me.

To my partner, Stijn, thank you for helping me to find a good balance between work and life, your never-ending support and the many hugs along the way. I admire your positive view on things and am very happy that we went on this adventure of living in Auckland together.

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Publications and prizes

Peer-reviewed Journal Publications


Presentations

2014


2015

van Heeswijk VM, Robertson PA, Thambyah A, Broom ND ‘Does localized disc wall disruption from a needle puncture influences susceptibility to herniation?’ 1st Workshop on Expanding International Collaborative Links in Mechanobiology, 12-13 April 2015, Melbourne, Australia.

Publications and prizes

2016

van Heeswijk VM, Robertson PA, Thambyah A, Broom ND ‘Disruption in the lateral inner annulus can lead to posterior or posterolateral herniations in flexed compressed discs’. Adelaide Centre of Spinal Research (ACSR) Spinal Research Symposium XIV, 11-13 August 2016, Adelaide, Australia.

van Heeswijk VM, Robertson PA, Thambyah A, Broom ND ‘Mechanically induced lumbar disc herniation: towards a global understanding of annular failure’. Chemical and Materials department PhD symposium, 7 September 2016, Auckland, New Zealand. 
**Awarded the Chemical and Materials Departmental PhD Presentation Prize.**

van Heeswijk VM, Robertson PA, Thambyah A, Broom ND ‘Mechanically induced posterior or posterolateral herniations in flexed ovine discs can arise from an inner annulus disruption in the lateral regions’. Australian and New Zealand Orthopaedic Research Society (ANZORS) 22nd Annual Scientific Meeting, 13-15 October 2016, Melbourne, Australia. 
**Awarded the ANZORS PhD Student Award.**

van Heeswijk VM, Robertson PA, Thambyah A, Broom ND ‘Mechanically induced posterior or posterolateral herniations in flexed ovine discs can arise from an inner annulus disruption in the lateral regions’. In 8th Annual Mechanobiology Symposium, 17 November 2016, Auckland, New Zealand.

2017


**Awarded the Rob Johnston Award.**


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<td>Neil D. Broom</td>
<td>Developed methodologies. Advised on experimentation, analysis and interpretation of results. Assisted in preparation of the manuscript.</td>
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Chapter 1

Background

1.1 - The lumbar spine

The spine is the part of the skeleton that connects the skull to the buttocks. The spinal column can be divided from top to bottom into the following five regions: cervical, thoracic, lumbar, sacrum and coccyx (see Figure 1.1). The terms ‘low back’ or ‘lower back’ relate specifically to the lumbar region of the spine. There is much interest in studying the lumbar region because low back pain is a global burden of disease and is estimated to be experienced by approximately 80% of all people at least once in their lifetime. The human lumbar spine consists of five vertebrae, four intervertebral discs, the anterior and posterior longitudinal ligaments that run over both the disc and the vertebrae, the zygapophysial joints and other ligaments that connect the vertebrae only. The lumbar vertebrae are identified from top to bottom as: L1, L2, L3, L4 and L5 (see Figure 1.1). The identifications of the vertebra are used to indicate the intervening intervertebral discs by their two adjacent vertebrae as follows: L1-2, L2-3, L3-4 and L4-5 (see Figure 1.1). The intervertebral disc below the L5 vertebra connects the lumbar region of the spine to the sacrum and is indicated by L5-S1, this disc is often taken into account in investigations of the lumbar spine. In an upright posture, the lumbar vertebral column has a lordotic curvature which develops when a toddler starts to walk. Lumbar lordosis compensates for the forward curved sacrum. The vertebral column gives axial rigidity to the trunk, permitting a range of movements in this part of the body and providing points of attachment for muscles.

Both the vertebrae and the intervening intervertebral discs resist compression caused by the upper body weight and muscle tension. Investigators who study the intervertebral disc in vitro, employ motion segments, also known as functional spinal units. A motion segment consists of an intervertebral disc, its two adjacent vertebrae, the anterior and posterior longitudinal ligaments connected to both the disc and the vertebrae, the zygapophysial joints and the ligaments that connect the vertebrae like the ligamentum flavum, the supraspinous and inter-
Figure 1.1: The spinal column. The five regions of the spinal column from top to bottom are: cervical, thoracic, lumbar, sacrum and coccyx. In the lumbar region, the identification of the vertebrae is shown in black and the intervertebral discs in blue. Reprinted from Paulsen\textsuperscript{11} with permission from Elsevier.
1.1. The lumbar spine

Figure 1.2: The motion segment. The elements comprising a motion segment are illustrated in a lateral view of a motion segments that has been dissected sagittally. Note that the zygapophysial joints are not shown as the fully functioning joints. The elements situated on the left side of the pedicles: the intervertebral disc, the vertebral bodies of the vertebrae, the anterior and posterior longitudinal ligaments comprise the anterior elements of a motion segment. All elements on the right are classified as the posterior elements. Reprinted from Paulsen with permission from Elsevier.

spinous ligaments. Figure 1.2 illustrates the different elements of a motion segment that has been dissected sagittally and viewed laterally. This illustration can be used to nicely explain that a motion segment can be classified into anterior and posterior elements. The intervertebral disc, the vertebral bodies of the vertebrae, the anterior and posterior longitudinal ligaments comprise the anterior elements of the motion segment. The other parts of the vertebrae together with the attached ligaments and the zygapophysial joints comprise the posterior elements. It should be noted that the fully functioning zygapophysial joints are not included in Figure 1.2. The posterior elements mainly control the position of the vertebral bodies relative to each other and provide connection points for muscles. Each vertebra has a pair of superior and inferior articular processes (see Figure 1.2). Next to the posterior ligaments, the inferior and superior articular processes from adjacent vertebra connect the posterior elements, and form the zygapophysial joints, also known as facet joints. These joints will be referred to as facet joints in the current thesis. The anterior and posterior elements enclose a vertebral canal in which the spinal cord is situated.
1.2 - The intervertebral disc

The intervertebral disc, also frequently referred to as ‘the disc’, is classified as fibrocartilaginous. Before discussing the different elements that comprise the disc, the three predominant biochemical constituents of those elements, namely collagen, proteoglycan and elastic fibres are now described in the following sections.

1.2.1 - Biochemical constituents

**Collagen**

The collagen fibre is the primary element used to form the structural architecture of the disc. The fundamental building block for the collagen fibril is the tropocollagen molecule, which is assembled from three polypeptide chains. The three polypeptide chains are configured into a triple helix and contains at both ends short non-helical sections, also known as procollagen. Enzymes cleave a portion of the non-helical ends releasing the residual non-helical and central helical portions (i.e. tropocollagen). Tropocollagen molecules self-assemble side-by-side and end-to-end. When they assemble, local charges present in the tropocollagen molecule cause regular displacements between adjacent molecules creating a systematic staggered overlap. Multiple layers of tropocollagen molecules form the collagen fibril and multiple fibrils form the collagen fibre. Electron microscopy of a collagen fibril reveals a characteristic 64-67 nm periodic banding which is a reflection of the gaps between (black in Figure 1.3D) and the overlap of (grey in Figure 1.3D) the tropocollagen molecules.

Variations in the repeating amino acid sequences comprising the polypeptide chains will result in different types of collagen. The main collagen types in the intervertebral disc are I and II, however collagen types III, IV, V, VI, IX, X, XI, XII, and XIV are also present. Collagen types I and II are, next to types III, V and XI, fibril forming collagens. Collagen type I is normally found in tensile load-bearing tissues like ligaments and tendons. Collagen type II is usually found in structures that are often compressed, for example articular cartilage. A previous investigation of tendon tissue has shown that compression can cause the pre-existing collagen type I to change into type II. This suggests that the distributions of collagen types I and II in the disc provide information relating to the mechanical function of its different structures.

When relaxed *in vitro*, collagen generally has a sinusoidal wave-like morphology referred to as crimp. Crimp allows stretching of the collagen with the application of relatively low tensile loads and represents the “toe” region in the tensile stress-strain curve. After the crimp morphol-
1.2. The intervertebral disc

Figure 1.3: Schematic illustrating the hierarchical structure of collagen. (A) Procollagen consists of three polypeptide chains configured into a triple helix with at both ends short non-helical sections. A portion of the non-helical ends are cleaved by enzymes to produce (B) tropocollagen. A well-organised assemblage of tropocollagen molecules forms a (C) collagen fibril. (D) Electron microscopy of a collagen fibril reveals a characteristic periodic banding pattern due to the gaps between (resulting in darker areas here indicated with black) and the overlap of (resulting in lighter areas here indicated with grey) the tropocollagen molecules. Author’s drawing based on a range of sources:20,21,24–26

...technology has disappeared, much higher loads are necessary to stretch the collagen, represented by a rapid incline of the stress-strain curve (see also illustrated in Figure 6.14).39,43 The applied load strains the bonds within the hierarchical structure of the collagen fibrils.44–46 At relatively high strains, the collagen fibres permanently deform and eventually break, this happens generally when strained 14-16 %.47 Whether crimp also exists in vivo remains unknown. The modern imaging techniques of today are unable to obtain images with sufficient resolution to investigate the existence of crimp in vivo. This currently leaves us only with a very cruel and unethical experiment of chemically fixing an animal or human alive to approximate the existence of crimp in vivo. Whether crimp exists in vivo would be an interesting research topic for the future if an ethical and non-harmful method is available.
Proteoglycans

Proteoglycans electrostatically attract and bind water which, due to the incompressibility of water, provides the intervertebral disc with good compressive resistance. Proteoglycans are composed of a core protein to which one or more glycosaminoglycans (GAGs) are bound covalently (see Figure 1.4A). The GAGs that are predominantly present in the intervertebral disc are keratan sulphate and chondroitin sulphate. The sulphate groups in these GAGs are negatively charged and charge repulsion effects cause the GAGs to expand to their maximum dimensions. Also, the fixed negative charges attract positive counter ions in order to achieve electroneutrality. This, in turn, results in a high chemical species concentration within the GAGs, leading to the osmotic in-drawing of water (an entropy effect) and resultant swelling. When the swelling is restricted, it creates a hydrostatic pressure within the tissue. The hydrostatic pressure is dependent on the concentration of sulphate groups present in the disc, thus dependent on the GAG concentration. Proteoglycans and collagen can bind, electrostatically or covalently, to each other. Proteoglycans can bind directly to collagen type I and II or indirectly via collagen type IX. The bonds between proteoglycans and collagen contribute to the strength of the tissue.

The intervertebral disc proteoglycans can be classified as whether or not they can aggregate with hyaluronic acid. Via link proteins, twenty to one hundred proteoglycans can bind to hyaluronic acid (see Figure 1.4B), resulting in large macromolecular aggregates. Those proteoglycans with the ability to aggregate with hyaluronic acid in the intervertebral disc include aggrecan and versican. Biglycan, fibromodulin, lumican, and decorin are non-aggregating proteoglycans that are present in the intervertebral disc. These proteoglycans can cluster but without a central filament like hyaluronic acid.

Elastic fibres

An elastic fibre consists of an elastin interior of covalently crosslinked tropoelastin molecules enclosed by a mantle of microfibrils comprised predominantly of fibrillins. The elastin interior imparts to the elastic fibre a near-linear elasticity and as its name indicates is elastically highly extensible, able to stretch 100-200% prior to the onset of failure. The elastin and fibrillin form long and relatively straight elastic fibres with a diameter of approximately 1 µm or less. Although elastin constitutes only 1.7-2% of the disc’s total dry weight, they form an extensive network throughout all disc regions. The elastic fibre network has been suggested to aid movement, maintain integrity and assist full recovery of the collagenous disc structures following unloading.
1.2. The intervertebral disc

Figure 1.4: Schematic of a proteoglycan and an aggregate. (A) Proteoglycans are composed of a core protein to which glycosaminoglycans (GAGs) covalently bind. The GAGs that are predominantly present in the intervertebral disc are keratan sulphate and chondroitin sulphate. (B) Some proteoglycans can bind to hyaluronic acid via link proteins to form an aggregate. Author’s drawing based on a range of sources:18,48,59,62

1.2.2 - Basic disc structure

The intervertebral disc consists of three distinct but integrated macro-anatomical regions, each with their own specialised structure: the nucleus, the annulus and the endplates. The nucleus is the central component of the disc and is surrounded by the annulus (see Figure 1.5). The superior and inferior aspects of the nucleus and annulus connect via endplates to the adjacent vertebrae (see Figure 1.5A).

Nucleus

The nucleus, also known as the nucleus pulposus, consists of 70-90 % water,18,59 resulting from the high percentage of proteoglycans present (i.e. 50-70 % of the nucleus’ dry weight).18,59,74,75 About 69-76 % of the water attracting molecules present in the nucleus are non-aggregated proteoglycans, only a minor amount being aggregated with hyaluronic acid.76,77 Aggrecan is the primary aggregated proteoglycan present in the nucleus.78 Collagen comprises 20-30 % of the dry weight of the nucleus18,74,75 of which Type II constitutes 80-85 % of the total collagen content.19,74,75 Other collagen types present in the nucleus are III, VI, IX, and XI.56,74,75 Elastic fibres and non-collagenous proteins comprise the remaining dry weight of the nucleus.74,75

Previously, the nucleus has been described as an entity distinct from its surroundings and amorphous in structure.79–81 More recently, Wade et al82 used mechanical manipulation of ovine nucleus tissue in vitro to reveal the existence of a convoluted but highly structured fibrous network. These same authors also showed integration of nuclear fibres with the adjacent annulus
Figure 1.5: The intervertebral disc. (A) The exposed surface, when a disc has been cut sagittally, shows the three distinct but integrated macro-anatomical regions of the disc. The nucleus situates in the centre of the disc and is surrounded by the annulus. The nucleus and annulus are connected to the adjacent vertebral bodies via a superior and an inferior endplate. (B) Transversely, five different circumferential regions of the disc can be identified: anterior, anterolateral, lateral, posterolateral and posterior. Note that two anterolateral, two lateral and two posterolateral regions exist in each disc.
and endplates.\textsuperscript{81–83} The fibre integration at the endplates occurs in the form of nodes and Wade et al.\textsuperscript{83} suggested these nodes provide the nucleus with a form of “tethered mobility”.

As previously described, elastic fibres are distributed throughout the disc.\textsuperscript{66,70,71} The orientation of the elastic fibres differs depending on their location within the disc. The nucleus proper contains elastic fibres longer than 150 µm and these are oriented either radially from the central nucleus to the annulus or axially spanning approximately half the discs height from nucleus to endplate.\textsuperscript{71}

**Annulus**

The nucleus is radially constrained by the annulus, this also known as the annulus fibrosus. The annulus comprises multiple concentric layers, often referred to as annular layers or lamellae.\textsuperscript{40,84} These layers consist primarily of collagen fibres connecting the inferior vertebra to the superior vertebra.\textsuperscript{84} At the disc periphery, the collagen fibres are tilted about $62^\circ$ relative to the vertical axis of the disc. Towards the inner annulus this angle decreases to $45^\circ$. The angle of the collagen fibres remains approximately the same within each circumferential annular layer.\textsuperscript{40} The inclined collagen fibres in the annulus are often referred to as oblique fibres. The orientation of the inclined fibres alternates from layer to layer, resulting in a criss-cross or oblique/counteroblique pattern.\textsuperscript{18,40}

Transversely, five different circumferential regions of the disc can be identified by the following: anterior, anterolateral, lateral, posterolateral and posterior (Figure 1.5B).\textsuperscript{6,85} Note that two anterolateral, two lateral and two posterolateral regions exist in each disc. In the previous paragraph the annular layers have been described as being concentric however, they often terminate, merge or separate (see Figure 1.6).\textsuperscript{86,87} As a consequence differences exists in the number of annular layers in, and radial thickness of, the annulus between the disc regions. For example Cassidy et al.\textsuperscript{40} has shown that in the human lumbar disc the anterior annulus consists of approximately 38 layers spanning a combined thickness of 7.5 mm whereas the thinner posterolateral annulus of about 5 mm comprises of 33 layers. In general, both the individual layers and annulus are thicker in the anterior compared to the posterior region.\textsuperscript{40,84,86,88} The lateral annulus is similar in thickness to, or even slightly thicker than, the anterior annulus as this region consists of the highest number of thickest lamellae.\textsuperscript{40,86} The highest frequency of lamellar discontinuity is found in the posterior and posterolateral regions.\textsuperscript{18,40,84,86} Failure is frequently observed in the posterior and posterolateral annulus,\textsuperscript{89–92} therefore these lamellar irregularities may well increase the vulnerability of these regions to disruption.\textsuperscript{86,87}
Chapter 1. Background

Figure 1.6: Microscope image of annular layers sectioned sagittally. Lamellae with alternating oblique/counteroblique fibre directions (here visualized with two different shades of brown) can run from vertebra to vertebra but can also terminate, merge or separate.

Radially, from the disc periphery to the nucleus, the annulus can be classified into three zones: outer, mid and inner annulus. While the annulus is mainly composed of collagen, proteoglycan and water, differences in structure and composition between the radial annular zones exist. Structurally, the outer annulus has clearly defined lamellae because of the well aligned oblique/counteroblique collagen fibre sets. The collagen fibre sets in the inner annulus are less ordered, therefore making the inner annular layers less clearly differentiated. Furthermore, the crimp morphology of the collagen fibres changes from the outer to inner annulus. The sinusoidal morphology of crimp in the outer annulus has a relatively long period in combination with a low amplitude but has a shorter period and a higher amplitude in the inner annulus. Differences in both crimp morphology and the composition within the annular zones is thought to give rise to different regional mechanical properties. The lesser degree of crimp in the outer annulus allows little deformation and thus making this region relatively stiff whereas the greater amount of crimp in the inner annular layers allow more deformation, making this region more compliant.

Compositionally, the outer annulus consists primarily of collagen fibres (i.e. 60-70 % of its dry weight) and also contains proteoglycans (i.e. 10 % of its dry weight). The remaining dry weight is made up of non-collagenous proteins and elastic fibres. From the outer annu-
lus towards the inner, the collagen content decreases to 15-32% of its dry weight. While the collagen content decreases, the proteoglycan content increases towards the inner annulus. The inner annulus therefore contains more water than the outer annulus. The collagenous structural architecture of the intervertebral disc is primarily made of collagen Types I and II. The outer annulus comprises mostly collagen type I, but the amount decreases toward the inner annulus. This decrease in collagen type I towards the inner annulus coincides with an increase in collagen Type II. This results in the inner annulus mainly comprising collagen type II but it has been reported also to contain collagen types III, V, VI and XI. Due to the differences in composition, the outer annulus is thought to be mainly responsible for the tensile strength of the disc. The higher level of hydration of the inner annulus arising from its increased proteoglycan content in combination with the nucleus, is thought to have a role more in providing both viscoelastic properties and load absorption.

Structural elements exist within the annulus that radially connect lamellae, and have been described as translamellar bridges, also known as cross-bridges, or bridging elements. The radial connections between two annular layers with the same oblique fibre orientation, thus traversing only one lamella, are referred to as bridging elements. Translamellar bridges connect and traverse two or more annular layers and range in length between 0.8 and 1.4 mm. The translamellar bridges consist of collagen and elastin fibres and their functional purpose remains somewhat unresolved. It has been suggested that they provide cohesion and probably protection against undue interlamellar shear, thereby reducing the risk of delamination. By contrast, Smith et al have proposed that the translamellar bridges are remnants of blood vessels.

Annular layers are separated by less than 30 µm of inter-lamellar matrix. The inter-lamellar matrix is made of a non-fibrillar matrix (i.e. proteoglycans, water, lipids and elastic fibres) and elastic fibres and are radially traversed by translamellar bridges. The proteoglycans of the non-fibrillar matrix keep the annulus hydrated and provide lubrication between adjacent lamellae. The elastic fibres from annular layers branch into the inter-lamellar matrix where they form a complex meshwork. Elastic fibres present within the outer annular layers lie mostly parallel to the oblique collagen type I fibres and many have been seen, in the foetus, to penetrate into the cartilaginous vertebral body rudiments. In the region where the annulus transitions into nucleus, the elastic fibres form a criss-cross pattern.

Endplate

At each superior and inferior side of the nucleus and the annulus lies an endplate. The
endplate connects the flexible nucleus and annulus structures to the stiffer adjacent vertebral bodies.\textsuperscript{104–106} In progressing from close to the nucleus or annulus to the vertebral body each endplate can be classified axially in two layers, namely the cartilaginous endplate and the vertebral endplate.\textsuperscript{104,105,107}

The vertebral endplate is a layer of dense, perforated cortical bone which is on average thinner than 1 mm\textsuperscript{107,108} and contains a high level of collagen type I.\textsuperscript{33} As the name suggests, this layer is usually treated as being an element of the vertebral body.\textsuperscript{7,106} The vertebral endplate in humans can be divided into two regions: a central region and a surrounding raised epiphyseal ring.\textsuperscript{106,109} Compared to the epiphyseal ring, the central region is more porous and contains channels joining bone marrow to the cartilaginous endplate.\textsuperscript{104,106,108,110–112} These channels allow easier exchange of nutrients between the bone marrow and the disc.\textsuperscript{106,111} The epiphyseal ring is often referred to as the ring apophysis or vertebral rim, and is situated at the edge of the vertebral endplate.\textsuperscript{106,109} Topographically, the raised epiphyseal ring makes the periphery of the endplate convex towards the disc while the central region is concave.\textsuperscript{106,113,114} The superior vertebral endplate is thicker than the inferior endplate.\textsuperscript{108} The thicker superior endplate and the adjacent denser trabecular bone indicates that it can sustain higher compressive loading than the inferior endplate.\textsuperscript{108,115}

On top of the porous central region of the vertebral endplate situates an approximately 0.6 mm thin\textsuperscript{112} sheet of hyaline cartilage termed the cartilaginous endplate.\textsuperscript{105,112} As with articular cartilage, this sheet comprises mainly of collagen Type II, proteoglycans and water.\textsuperscript{112} The fibres and fibrils in this layer are horizontally aligned.\textsuperscript{106,112} Radially and axially, the composition of the three main biochemical constituents in the cartilaginous endplate differs. Radially, from the edge to the centre, the composition of the endplate parallels those changes in the disc. As with the adjacent annulus, at the periphery the endplate has a high collagen but low proteoglycan and water content.\textsuperscript{112} And as with the adjacent nucleus, the centre of the endplate has a high amount of proteoglycans and water but low level of collagen.\textsuperscript{112} Axially, the cartilage close to the disc contains a higher amount of proteoglycans and water than that present close to the vertebral endplate, while the collagen content is higher close to the vertebral endplate and decreases towards the disc.\textsuperscript{112}

The region of the cartilaginous endplate close to the vertebral endplate is mineralized but the axial span of calcification varies from the nucleus towards the outer annulus.\textsuperscript{112} Calcification of the cartilaginous endplate at the nucleus is either very low or undetectable.\textsuperscript{112} The calcification increases towards the mid annulus where the cartilaginous endplate becomes fully mineralized.\textsuperscript{116,117} The fully calcified cartilage layer at the mid-to-outer annulus has a clear boundary
1.2. The intervertebral disc

Figure 1.7: Microscope image of the cartilaginous and vertebral endplate. Annular fibres penetrate into the cartilaginous endplate (CEP). The boundary between the annulus and CEP is often referred to as tidemark and the boundary between the CEP and vertebral endplate (VEP) as cement line.

with the annulus and is often referred to as tidemark (see Figure 1.7). With ageing, the cartilage adjacent to the vertebral endplate becomes increasingly calcified. The boundary between the cartilaginous and vertebral endplates is commonly referred to as the cement line (see Figure 1.7).

Fibres of the outer annular layers have been reported to penetrate into the vertebral endplate, while those of the inner annulus reach into the cartilaginous endplate. When an inner annular layer penetrates the cartilaginous endplate, the lamella branches into sub-bundles. The branching increases the contact area between the annular fibres and cartilaginous endplate matrix, thereby increasing the ability of the tissue junction to withstand tensile stresses.

1.2.3 - Cells

Cells in the intervertebral disc are responsible for the maintenance of the extracellular matrix. During foetal development, the nucleus derives from the notochord and therefore contains a syncytium (i.e. cluster of cell nuclei contained within one membrane) of notochordal cells after birth. The notochordal cells then part from the syncytium and vanish before adulthood. When the notochordal cells vanish, approximately round cells are left in the nucleus. These
nucleus cells are frequently reported as resembling the chondrocytes in articular cartilage. The cells in the nucleus and the chondrocytes in articular cartilage contain similar phenotypical characteristics, however distinct differences also exists. While the inner annulus contains chondrocyte-like cells, the remaining annulus has thin and elongated fibroblast-like or fibrocyte-like cells. The long axis of these cells align with the collagen fibre orientation. The fibroblast-like cells in the annulus respond to deformation and produce mostly collagen type I, while the chondrocyte-like cells in the nucleus respond to hydrostatic pressure and produce mainly proteoglycans and collagen type II. As with the nucleus, the cartilaginous endplates also have round chondrocyte-like cells.

Only 0.5% of the volume of the nucleus in an adult consists of cells. Of the three primary structural regions comprising the disc, the lowest concentration of cells is found in the nucleus. The annulus contains around twice the amount of cells present in the nucleus, while there is an approximate 4-fold increase in the number of cells in the endplate.

To be metabolically active, cells require oxygen, glucose, substrates and cofactors. The intervertebral disc lacks blood vessels which limits the metabolic rate of the cells. For nutrients to reach the cells within the disc, cells rely on diffusion and fluid flow.

1.2.4 - Function

The nucleus, annulus and endplates together are sufficiently strong to both reversibly deform and sustain the weight of the upper body over normal ranges of loading and movement without suffering damage or failing. The following sections provide information relating to both the weight-bearing properties of the disc and movements of the motion segment.

Weight-bearing

The different composition and organisations of the biochemical constituents in the nucleus, annulus and endplate provide them with specific mechanical functions.

The high proteoglycan content of the nucleus attracts a significant amount of water, providing this structure with hydrostatic pressure that counteracts compressive loading or weight of the upper body. Sustained loading induces water to flow out of the disc, causing a reduction of its height and outwards bulging of the annulus. When relatively relaxed, the disc has the ability to absorb water and recover to its previous volume. Daily activity reduces the disc height and volume by about 20%, which is generally restored during sleep. An internal hydrostatic pressure always exists in vivo even when laying supine.
1.2. The intervertebral disc

The collagen Type I fibres within the annulus and especially those in its outer annular layers provide the disc with its tensile strength. The crimped collagen allows some compliance but there is a swift increase in stiffness when the crimp has been eliminated. The tensile resistance of the annular fibres also restricts disc bulging. The oblique/counteroblique fibre pattern of the annulus enables it to withstand multi-directional tension.

The endplates encapsulate the disc superiorly and inferiorly and restrict axial bulging of the nucleus and annulus and have the primary role of transmitting loads uniformly between the disc and vertebrae. The porous cartilaginous endplates and the perforated vertebral endplates permit two-way flow of water.

The different mechanical properties of the nucleus, annulus and endplates combined, enable the disc to bear the weight that is exerted onto it. When a disc is compressed, the hydrostatic pressure in the nucleus increases. The increase in hydrostatic pressure causes the nucleus to expand radially, bulging the annulus outwards. This bulging is limited by the tensile resistance of the annular fibres. The restricted radial expansion causes the nucleus to exert pressure back onto the endplates, counteracting the compression induced for example by an axial load applied to the disc.

Movement

The disc allows the adjacent vertebrae to rotate, twist or translate relative to each other to a limited degree. To protect the disc, movement of the vertebrae is restricted by the facet joints.

While motion segments can rotate in multiple planes, three principle directions are normally investigated, i.e.: flexion (i.e. bending anteriorly), extension (i.e. bending posteriorly) or lateral bending. Bending occurs when vertebrae move around a centre of rotation. When the centre of rotation is situated in the middle of the disc, flexion compresses the anterior annulus and tensions the posterior annulus (see Figure 1.8A). The decrease in disc height anteriorly and the increase posteriorly probably induces a decreasing pressure gradient towards the posterior region. When a motion segment is extended, the opposite occurs. Lateral bending affects the disc structures similarly in the coronal plane.

When the centre of rotation is located slightly posteriorly and within the inferior vertebral body, the rotation of the superior vertebra during flexion also induces a slight forward displacement (i.e. anterior shear, see Figure 1.8B). Forward displacement of the vertebra also occurs in the upper levels of the lumbar spine when fully flexed in vivo. MRI scans that visualize the hydrated nucleus of the disc were obtained from human subjects sitting in an upright position or when
Figure 1.8: Schematic illustrating the influence of the location of the centre of rotation on how motion segments deform during flexion. (A) When the centre of rotation (COR) situates in the centre of the disc, flexion compresses the anterior annulus and tensions the posterior annulus (see arrows in vertebrae). (B) When the COR is located slightly posteriorly and within the inferior vertebral body, the rotation of the superior vertebra during flexion, next to compressing the anterior and extending the posterior annulus, also induces a slight forward displacement (see difference between the dashed lines in front of the vertebrae in B while not present in A).

bending forward, and showed that flexion causes the nucleus to move slightly posteriorly.\textsuperscript{130,131}

The effect of anterior shear on the annular fibres has been theoretically explained\textsuperscript{7,10} and investigated with models representing the annulus\textsuperscript{132} or the disc.\textsuperscript{133} Due to the anisotropic microstructure of the annulus, shear increasingly strains those fibres that are approximately aligned with and tilted in the displacement direction.\textsuperscript{7,10,133} In contrast, shear relaxes the counteroblique layers,\textsuperscript{133} thus effectively only half of the lamellae contribute in resisting shear in this region.\textsuperscript{10,132} The annular regions with the fibres situated approximately perpendicular to the displacement direction also resist shear, however to a lesser extent compared with the differentially recruited regions.\textsuperscript{10} It should be noted that the effects of anterior shear on the annular fibres in the motion segment have not been fully studied. Uneven straining of the oblique and counteroblique annular fibres has also been described as occurring when the disc is twisted (i.e. torsion).\textsuperscript{10,133} The facet joints protects the disc by preventing excessive torsion and shear between two vertebrae.\textsuperscript{7,128,134–136}
1.2. The intervertebral disc

1.2.5 - Age-related changes and degeneration

The following sections will provide information about age-related changes of the disc and the difference between age-related changes and degeneration.

Age-related changes

From birth to the age of 16, cell density in all disc structures decreases significantly but thereafter remains unchanged.\textsuperscript{137} Although the cell density after 16 years of age does not significantly change,\textsuperscript{137} old cells become increasingly senescent and their vitality decreases.\textsuperscript{110} This means that the cells synthesize a lower concentration of proteoglycans.\textsuperscript{138} Soon after birth the process of fragmentation of aggregated proteoglycans commences.\textsuperscript{74} These smaller proteoglycans fragments can escape the disc more easily than the large aggregates. Providing the annulus and endplates are able to retain a sufficient concentration of proteoglycans within the disc, the nucleus will remain hydrostatically functional.\textsuperscript{17} However, age-related changes in the annulus and endplates (see further explanation later in this section) reduce their ability to entrap proteoglycans, resulting in a decreasing concentration with ageing.\textsuperscript{50,139} This decreased proteoglycan concentration reduces the hydration of the disc’s extracellular matrix, especially that of the nucleus.\textsuperscript{139}

A decreased cell vitality also means a decrease in the rate of collagen turnover (i.e. its synthesis and degradation),\textsuperscript{140} allowing the collagen to become progressively more cross-linked.\textsuperscript{110} Nonenzymatic glycation, the process by which sugar molecules bind covalently to matrix proteins, gives the normally white-coloured disc a brownish appearance when aged.\textsuperscript{110} The nonenzymatic glycation is also believed to make the disc more brittle and vulnerable to injury.\textsuperscript{7} Other changes take place with ageing: the collagen type I content increases, the new type I fibres penetrate into the nucleus decreasing its size.\textsuperscript{110} The vertebral and cartilaginous endplates also become increasingly permeable and thinner.\textsuperscript{110,141,142} In older discs, the shape of the central region of the endplate has been observed to become more convex.\textsuperscript{114} This change in shape of the endplates is possibly associated with a progressive increase in lesions in the adjacent trabecular bone with ageing.\textsuperscript{143} Nonenzymatic glycation also occurs in the cartilaginous endplate.\textsuperscript{110}

With ageing, the nucleus decreases in size and increases in fibrosity, dehydration and stiffness.\textsuperscript{7} The hydrostatic pressure in the inner region declines\textsuperscript{7} and both the formation of cross-links between collagen fibres and nonenzymatic glycation make the overall annulus structure stiffer.\textsuperscript{110} These changes cause the annulus to carry more of the load than the nucleus.\textsuperscript{12} Although the annulus becomes stiffer due to increased cross-linking, the strength of the outer annulus actually decreases,\textsuperscript{144} especially in men.\textsuperscript{145} This is thought to result from accumulated annular defects
that have been induced over time.\textsuperscript{7,17}

**Degeneration**

Whereas ageing generally does not cause a decrease in the disc height,\textsuperscript{114} a narrowing of the disc space is generally considered to be a sign of degeneration.\textsuperscript{7} And unlike age-related changes, degeneration can occur at any stage in life but happens more often in older discs.\textsuperscript{7} Adams and Roughley\textsuperscript{17} have defined degeneration as “an aberrant, cell-mediated response to progressive structural failure” and a degenerated disc “one with structural failure combined with accelerated or advanced signs of aging”. Although both old and degenerate discs involve age-related changes, they can be distinguished based on the absence or presence of structural failure.\textsuperscript{7,17} When a degenerated disc is also painful, Adams and Roughley\textsuperscript{17} suggested that this defines degenerative disc disease.

Using pattern recognition,\textsuperscript{17,85} the healthy or degenerate state of a disc has traditionally been classified on a four\textsuperscript{144} or five point scale.\textsuperscript{146,147} The lowest scale represents a healthy disc, while the highest scale represents severe degeneration. These classification scales do not take into account whether a disc is painful\textsuperscript{85} and do not provide insight into what is actually happening within the disc. Amongst others, these classification scales can be useful in investigating correlations between degeneration and back pain.

Longitudinal \textit{in vivo} studies of ovine lumbar discs that had been injured by a scalpel blade, damaging only the outer-to-mid annulus, showed limited healing by scar tissue only in the outer annulus near the disc periphery.\textsuperscript{148,149} The majority of the induced injuries did not fully heal, probably due to the low cell density of the disc.\textsuperscript{7} More importantly, various \textit{in vivo} animal studies have shown that degeneration develops over a period of time after injuring the disc with a scalpel blade\textsuperscript{149,150} or a needle.\textsuperscript{151–160} These \textit{in vivo} animal studies indicate that artificial structural damage can induce degeneration over time. Structural failure can be recreated by mechanical loading of cadaver motion segments \textit{in vitro}.\textsuperscript{161} In accordance with the results of previously mentioned \textit{in vivo} and \textit{in vitro} studies, Adams and Roughley\textsuperscript{17} suggested causality of degeneration as follows: “excessive mechanical loading disrupts a disc’s structure and precipitates a cascade of cell-mediated responses, leading to further disruption.”

1.3 - Structural failure

Various different types of failure can occur in the disc. Structural failure generally refers to damage of the endplate or the annulus. Regarding failure of the annulus, three classifications are generally recognized: annular defects, internal disc disruption and herniation. This section
1.3. Structural failure

Figure 1.9: Microscope image of endplate failure. An ovine lumbar motion segment that was axially compressed *in vitro*, failed via the endplate which was accompanied by nuclear migration into the inferior vertebral body. This will provide more information on the different types of failure observed in discs.

1.3.1 - Endplate failure

Mechanical testing of a motion segment in its neutral posture *in vitro* has revealed that overloading by compression almost always causes the endplate to fail, \(14,134,162,163\) thus indicating that the endplate is weaker than the annulus in this posture. \(17,164\) Failure of the endplate can be accompanied with nuclear migration (see Figure 1.9). When the endplate fails and nuclear tissue migrates vertically into the vertebral body, the hydrostatic pressure in the disc drops. The decompression of the nucleus causes the annulus to become the main load bearing structure which leads to inwards bulging, i.e. towards the nucleus. \(164,165\) In the sagittal plane, the decrease in hydrostatic pressure of the nucleus causes high stress concentrations in the posterior annulus. \(12,165\) The loss of hydrostatic pressure in the nucleus and the stress concentrations in the annulus, alters cellular behaviour \(166,167\) and may accelerate disc degeneration. \(164,165\)

When an endplate fails and nucleus migrates into the vertebral body, a Schmorl’s node can form if calcification follows. \(17\) As previously described, the superior endplate can sustain higher compressive loading than the inferior. In accordance, Schmorl’s nodes are more frequently observed at the inferior endplate. \(168\) The occurrence of Schmorl's nodes does not correlate with
1.3.2 - Annular failure

The three different classifications of annular failure are: annular tears, internal disc disruption and herniation. Annular tears are defects that occur within the annulus. Internal disc disruption involves migration of nuclear tissue through the annulus but without it causing a change in the disc contour. When a disc contains an observable change in its periphery it classifies as a herniation.\textsuperscript{169,170}

**Annular tears**

Three types of tears within the annulus can be identified: circumferential delaminations, rim lesions and radial fissures (see Figure 1.10).\textsuperscript{7,85,171} Circumferential delamination refers to the separation of adjacent annular layers (see Figure 1.10A).\textsuperscript{171} The separation of adjacent lamellae is thought to arise from high interlamellar shear stresses.\textsuperscript{172} Rim lesions situate close to the epiphyseal ring of the vertebral endplate (see Figure 1.10B).\textsuperscript{171} This type of tear occurs more often anteriorly than posteriorly\textsuperscript{173,174} and may be accompanied by sclerosis and osteophytes.\textsuperscript{173} It has been reported, based on histology, that rim lesions could result from trauma.\textsuperscript{174} Radial fissures are tears that extend from the inner annulus outwards (see Figure 1.10C) and at times reach the disc periphery.\textsuperscript{7} Radial fissures occur most frequently in the posterior and posterolateral annulus, and have been frequently observed together with a degenerated nucleus.\textsuperscript{174} Osti et al\textsuperscript{174} has suggested that the radial tears could have influenced the structure of the nucleus. The three types of annular defects generally tend to appear after ten years of age, after which the number increases.\textsuperscript{175}

**Internal disc disruption**

As the name suggests, internal disc disruption refers to a disc that contains irregularities but only internally thus leaving the disc’s contour unaltered.\textsuperscript{170} A typical feature of internal disc disruption is the development of radial fissures originating from the nucleus and extending radially into the annulus.\textsuperscript{10} Based on the extent of radial fissuring, internal disc disruption can be classified using 4 grades. For the first three grades, the radial thickness of the annulus is divided in approximately three equally thick regions. These three regions, from the nucleus outwards, are then used to indicate how far the nucleus has radially migrated into the annulus with grade 1, 2 and 3 respectively (see schematics of grade 1, 2 and 3 in Figure 1.11).\textsuperscript{10} The Dallas Discogram Description\textsuperscript{176} comprising grades 1, 2 and 3 was later extended to include a 4th grade.\textsuperscript{177,178} This latter grade classifies a radial fissure that had penetrated into the outer third of the an-
1.3. Structural failure

Figure 1.10: Schematics illustrating the three types of annular tears. Pink shapes in the transverse view (left schematics) and sagittal view (right schematics) represent the three types of annular tears: (A) circumferential delaminations, (B) rim lesions and (C) radial fissures. Author’s drawing based on the following sources:7,85
Figure 1.11: Schematics illustrating the four internal disc disruption grades. The radial thickness of the annulus is divided in approximately three equally thick regions. These three regions, from the nucleus outwards, are then used to indicate how far the nucleus has radially migrated into the annulus with grade 1, 2 and 3 respectively. Grade 4 classifies radial fissures that had penetrated into the outer third of the annulus in which the nuclear material has then migrated circumferentially over an arc of at least 30°. Author’s own drawing based on a range of sources:10,176,177

The clinical procedure referred to as disc stimulation (for more information about disc stimulation see the provocative discography section) has demonstrated that the internal disc disruption grades correlate strongly with the provocation of pain.10,179,180. The likelihood of fissures causing pain is almost nil when dealing with a grade 1 fissure but increases when it has penetrated further into the annulus, especially when reaching the outer third.10 It has been previously reported that 75% of discs classified with the third grade are painful when clinically stimulated.179 Furthermore, of all discs with a painful sensation, 77% contain a fissure that reaches the outer third of the annulus.179 The association between pain and the extent of propagation of the radial fissure correlates anatomically with where nerve endings are situated in the disc.10 In the annulus, nerve endings are situated in the outer third181–184 which, upon stimulation by a grade 3 fissure, and sometimes a grade 2 fissure, provoke pain.10
1.3. Structural failure

Stress profiles of healthy cadaver discs *in vitro* revealed that both the nucleus and annulus exhibit an equal level of relatively high stress.⁷ Stress decreases rapidly only in the 2 to 4 mm of the annulus at the disc’s periphery.⁷ An *in vivo* study investigated the stress profiles of discs that were painful upon stimulation and revealed that they are different, in that the stress in the nucleus is reduced while increasing in the annulus.¹⁸⁵ Stress profilometry conducted on mechanically tested cadaver motion segments *in vitro*, revealed a similar sequence in the distribution of stress after a vertebral endplate had failed.¹⁶⁵,¹⁸⁶ These *in vitro* measurements indicate the possibility that fracture of a vertebral endplate causes internal disc disruption.⁷

**Herniation**

A herniation is characterized by a change in the disc's contour.¹⁶⁹,¹⁷⁰ The change in the periphery can result from migrated nuclear material through a radial fissure,¹⁸⁷,¹⁸⁸ but can also arise from bulging of the annulus only.¹⁸⁹ A herniation caused by migration of nuclear tissue can be described by the following four definitions: protrusion, subligamentous extrusion, transligamentous extrusion or sequestration (see Figure 1.12).¹⁹⁰ Protrusions involve nuclear migration that ends somewhere within the annulus (see Figure 1.12A). When nuclear migration is constrained only by the posterior longitudinal ligament, the herniation is classified as a subligamentous extrusion (see Figure 1.12B).¹⁹⁰ For both protrusion and subligamentous extrusion, the resulting change in the contour of the disc itself is generally referred to as a bulge. When nuclear tissue also penetrates the full thickness of the posterior longitudinal ligament the herniation can be described as a transligamentous extrusion or sequestration (see Figure 1.12C, D). The difference between the latter two types of herniation is that the extruded nuclear tissue is still in contact with that in the disc in a transligamentous extrusion but separated in sequestration.¹⁹⁰ The schematics in Figure 1.12 and descriptions of the different types of herniation are, of course, somewhat simplified because the extruded material may also contain annulus and/or endplate material.¹⁹¹–¹⁹⁴

Although herniations can occur in discs of all spinal regions, they are observed predominantly in the lower back,¹⁹⁵ especially in the L4-5 and L5-S1 levels.⁸⁹,¹⁸⁸,¹⁹⁵ Herniations primarily occur in the posterior and posterolateral regions of the disc,⁸⁹–⁹² via direct radial rupture of the annulus.⁹¹,⁹²

From a mechanical point of view, a herniation can happen when the annulus is incapable of withstanding the increase in hydrostatic pressure of the nucleus when loaded.⁸⁵,¹⁶¹,¹⁹⁶ Mechanical testing of cadaveric lumbar motion segments with discs of different degeneration grades *in vitro*, has shown that slightly degenerated discs (grade 2)¹⁴⁴ of 40-50 year old adults are most vulnerable to herniation.¹⁶¹,¹⁹⁶ The nucleus of a grade 2 disc is still relatively hydrated and there-
Figure 1.12: Schematics illustrating the four herniation definitions. (A) Protrusions involve nuclear migration that ends somewhere within the annulus. (B) When nuclear migration is constrained only by the posterior longitudinal ligament then the herniation is classified as a subligamentous extrusion. (C) In a transligamentous extrusion nucleus also penetrates the full thickness of the ligament but the extruded tissue is still in contact with the internal nuclear material. (D) Sequestration is a herniation in which the extruded tissue has parted from the internal migrated nuclear tissue. Author’s drawing based on a range of sources:6,7,85,190
fore hydrostatically functional but is surrounded by a slightly weakened annulus.\textsuperscript{196} Interestingly, severely degenerated discs (grade 4) do not herniate, probably due to the dehydrated nucleus which lacks hydrostatic pressure to exert onto the annulus.\textsuperscript{17,196}

1.3.3 - Pain

The origin of back pain is difficult to determine because of the anatomical position of the spine within the body, hindering close observation and palpation.\textsuperscript{161} Low back pain has been proposed to arise only from components of the lumbar spine that contain nerves, are prone to painful injuries or diseases, and the symptoms assessed using proven diagnostic tools.\textsuperscript{10} Momentary or transient pain in the back is suspected to originate from the surrounding muscles, however reliable evidence has yet to be provided.\textsuperscript{161} Severe and chronic low back pain has previously been reported to arise frequently from the following spine components: the discs, the facet joints and the joints that connect the sacrum to the pelvis.\textsuperscript{161}

The disc has been reported to be the source of chronic low back pain in approximately 40-45\% of patients.\textsuperscript{197–199} Pain originating from the intervertebral disc is often referred to as being disco-genic,\textsuperscript{7,110,178,197} which can arise upon stimulation of nerve endings situated in the outer third of the annulus (as previously described in the section on internal disc disruption).\textsuperscript{181–184} The surrounding vertebral endplate and posterior longitudinal ligament are also innervated.\textsuperscript{200,201}

Based on epidemiological evidence Adams\textsuperscript{161} has nicely summarised the relationships that exist between back pain and disruptions or abnormalities in the disc. These include endplate failure, radial fissures or herniations, as well as disc space narrowing, but not biochemical changes that occur with ageing. Disc disruptions that cause the stress profile to change and give rise to localized stress concentrations in the innervated outer annular layers have also been reported as able to provoke pain.\textsuperscript{161,185} Internal disc disruption is considered to be the main cause of disco-genic pain, but in a minor number of cases it can also arise from herniation. Focal disc bulging or extrusion of nuclear material may irritate, inflame and/or compress the adjacent nerve roots causing sciatica (i.e. a symptom of radiating pain in the leg).\textsuperscript{202,203} In fact, approximately 90\% of sciatica cases are caused by lumbar disc herniations.\textsuperscript{203} Interestingly, severely degenerated discs in humans can be asymptomatic.\textsuperscript{161} This could indicate that perception of pain may involve biochemical mechanisms but more research will be required to understand how this would work.\textsuperscript{161,204} Pain could also have been avoided by transfer of a substantial amount of loading from the disc to the neural arch when the degenerated disc is sufficiently narrowed.\textsuperscript{161}
1.4 - Provocative discography

During the late 1940’s, Lindblom introduced diagnostic disc puncture, a procedure involving injection of radiopaque medium into the nucleus revealing ruptures and protrusions. The increase in pressure from the injection also confirmed whether experienced pain was discogenic in origin. Since then the procedure has been further developed and is often referred to as discography or provocative discography.

Provocative discography requires the insertion of a needle into the intervertebral disc. Often a two-needle technique is used, because the use of one needle increases the likelihood of inducing discitis. Using one needle for intradiscal injection can introduce bacteria into the disc which can result in discitis (i.e. intradiscal infection), a condition that is intensely painful. In the two needle technique, often an 18-gauge needle is positioned near the disc to guide a smaller needle into the disc. A smaller needle; 20-gauge, 22-gauge or 25-gauge, is often inserted posterolaterally until the centre of the disc is reached. X-rays are used to visualize the position of the needle in the disc. When appropriate positioning of the needle is confirmed, a contrast agent is injected into the nucleus. During injection the patient experience of concordant pain is monitored. This part of the procedure is frequently referred to as disc stimulation. After disc stimulation discography is performed in which the morphology of the disc is investigated based on the spread of the injected medium. The combination of the two procedures, disc stimulation and discography, is best described as provocative discography but still most frequently referred to as discography.

The merits of provocative discography have been widely discussed and some clinicians still query its reliability because of mismatches between morphology, complaints and false-positive rates. Despite the controversies, provocative discography still remains the most informative procedure for diagnosing discogenic pain. Nowadays, with the available advanced imaging modalities, i.e. computed tomography (CT) and magnetic resonance imaging (MRI), provocative discography is still considered to be part of the clinician’s diagnostic toolkit and this is because information concerning the location and reproduction of pain can be obtained, something that is not possible with CT and MRI. It is generally employed when more conservative or other minimally invasive treatments are ineffective, and it also serves as a guide for surgical intervention. As Adams et al has noted MRI images can reveal circumferentially tracked nuclear material in the outer annulus as in a grade 4 fissure (see Figure 1.11), when imaged along the long axis of the circumferentially migrated nuclear tissue. The nuclear tissue in the outer annulus can be detected as a high intensity zone (i.e. small bright region in a MRI image). Although MRI imaging does not in itself provide information about pain, provocative discography...
performed on discs that reveal a high intensity zone on the MRI image does correlate with a painful disc.\textsuperscript{177,217,218}

Another point of controversy is whether provocative discography makes the disc more prone to degeneration over a longer period of time. In 2009, Carragee et al\textsuperscript{219} published results of a ten year cohort study and reported an increase in both new herniations and degeneration in lumbar discs that had undergone provocative discography compared to untreated discs. Nassr et al\textsuperscript{220} reported that patients who had received incorrect needle localization procedures (i.e. the localizing needle was placed in a cervical disc space which was not included in the surgical procedure) had a three-fold increased risk of developing disc degeneration after approximately 2 years. However, a more recent study published in 2013 by Ohtori et al\textsuperscript{221} reported no significant increase in degeneration in lumbar discs that had undergone provocative discography compared to untreated discs, five years post treatment. The contrasting findings from Carragee et al\textsuperscript{219} and Ohtori et al\textsuperscript{221} regarding degeneration in lumbar discs post provocative discography, has been proposed by the latter authors to possibly be due to the shorter time interval between the measurements and the lower number of participants included in the study of Ohtori et al.\textsuperscript{221}

That a needle puncture induces disc degeneration over time has been widely demonstrated in various \textit{in vivo} animal studies.\textsuperscript{151–160} It should be noted that for these \textit{in vivo} studies relatively large needle diameters (ranging from 31- to 16-gauge but mostly 18-gauge) were used on relatively small animal discs, and therefore would have inflicted considerably more structural damage compared to that arising from discography on the human disc. Paradoxically, the employment of an annular puncture is considered in future therapeutic interventions that include protein,\textsuperscript{222,223} gene,\textsuperscript{224–228} and cell therapies\textsuperscript{229–231} for the treatment of disc degeneration. Based on a literature review of \textit{in vivo} animal puncture studies and their \textit{in vitro} investigations, Elliot et al\textsuperscript{232} proposed that a needle diameter less than 40 % of the disc height would not significantly alter the disc properties, and thereby offered a potential guide to minimize disruptive consequences.\textsuperscript{233}

Next to increased degeneration, Carragee et al\textsuperscript{219} also reported an increase in new herniations ten years post discography. Interestingly, new herniations were most frequently located at the site of the needle puncture. It is unclear whether nuclear tissue had migrated through the localized annular damage created by the puncture performed during discography. Whether an annular puncture provides a preferred path for nuclear migration independent of longer-term degenerative consequences remains a topic of interest for further investigation.
1.5.1 - *In vivo*

More than one hundred years ago Middleton and Teacher\textsuperscript{234} reported a possible association between flexion and herniation for the first time. In their report, it was stated that a thirty eight year old man sensed a “crack” in his back while raising a heavy plate. After the incident, the man could not stand up straight due to severe pain. The physical state of the patient progressively deteriorated and he passed away sixteen days after the incident primarily from the effects of bedsores and cystitis. Post-mortem examination revealed that intervertebral disc material had extruded into the vertebral canal, and was suggested to be the origin of the unfortunate sequential events leading to his death. The association between flexion and herniation was reinforced in 1937, by the suggestion that symptomatic herniation resulted from trauma, mostly when the spine is flexed\textsuperscript{235} or more specifically by lifting a heavy weight.\textsuperscript{236} Interestingly, Love and Camp\textsuperscript{235} also noted that herniations do not necessarily occur upon one traumatic event but that accumulation of injury caused by multiple events could eventually also lead to extrusion of nuclear material.

Intradiscal pressures have been previously measured *in vivo* by the insertion of a pressure sensor into the nucleus of L4-5 discs.\textsuperscript{126,127} These intradiscal measurements showed that flexion generally increases the pressure in the nucleus. Pressure in the nucleus is always present and ranges between 0.09-0.12 MPa when laying supine, prone or on one side.\textsuperscript{126,127} During standing, the intradiscal pressure ranges between 0.50-0.54 MPa and increases to 1.10-1.32 MPa when standing with the back in a flexed posture.\textsuperscript{126,127} Flexing the spine while sitting also increased the intradiscal pressure to 0.82-1.13 MPa compared to the pressure range of 0.55-0.63 MPa when sitting upright.\textsuperscript{126,127} Lifting a weight of approximately 20 kg with bended knees and a moderately straight back generated an intradiscal pressure of 1.7 MPa.\textsuperscript{127} Again, a higher intradiscal pressure of 2.3 MPa was measured when lifting the same weight but with the back in a flexed position.\textsuperscript{127}

The *in vivo* range of motion in flexion for each lumbar disc has been investigated by analysing radiographs of healthy volunteers.\textsuperscript{129,196} Two radiographs of each volunteer were compared, one while in an erect standing posture and the second when fully flexed by reaching for their toes. Measurements from these sets of radiographs revealed that the range of motion increases from the L1-2 to the L4-5 joint from 8.3° to 14.5°, while the L5-S1 joint has a range of motion of approximately 10.1°.\textsuperscript{129,196} Pearcy et al\textsuperscript{129} also measured the amount of anterior displacement (i.e. shear) induced when flexing the spine for each lumbar joint. The induced forward displacement was highest at the L1-2 disc with 3 mm but was 2 mm for the L2-3, L3-4 and L4-5 joints. The induced forward displacement was lowest at the lumbosacral joint with 1 mm.\textsuperscript{129} The centre of rotation is located slightly posteriorly and within the inferior vertebral body for the L1-2 until
1.5. The association between flexion and herniation

the L4-5 levels but lies within the disc for the L5-S1 motion segment.\textsuperscript{237,238}

1.5.2 - \textit{In vitro}

This section will provide an overview of \textit{in vitro} cadaver and ovine studies that are most relevant for the work presented in this thesis.

\textbf{The human cadaver model}

\textit{In vitro} cadaver investigations have demonstrated the association between flexion and herniation. It has been shown that compressing cadaver motion segments in their neutral posture \textit{in vitro} ends almost always in failure of the vertebral endplate,\textsuperscript{134,162,163} while mechanical testing of motion segments in a flexed posture increases the probability of failure by herniation.\textsuperscript{196,239,240}

Flexing cadaver motion segments \textit{in vitro} and then sequentially eliminating different structures has shown that the range of flexion of the disc is limited by the supraspinous and interspinous ligaments.\textsuperscript{241} These ligaments connect adjacent spinous processes (see Figure 1.2). Flexing motion segments with the posterior elements removed \textit{in vitro} will permit the disc to flex an additional 3.5° or thereabouts beyond what can be achieved in an intact motion segment.\textsuperscript{242} This suggests that the supraspinous and interspinous ligaments provide a protective factor of safety for the intervertebral disc when flexed \textit{in vivo}.\textsuperscript{242}

In 1982, Adams and Hutton\textsuperscript{196} performed compression tests on cadaver motion segments that were bended anterolaterally (i.e. plane of flexion was rotated 15° relative to the anterior-posterior disc direction) to its normal limit of flexion (i.e. the flexion angle normally allowed by the supraspinous and interspinous ligaments). When after a first compression test no failure of the disc was detected, the bending angle was increased and a second compressive loading was performed. The compressive loading of 8 kN that Adams and Hutton\textsuperscript{196} employed in their study was thought to represent the probable loading generated in a young man of average weight when performing heavy lifting. Overall, the test was repeated with increasing angle of flexion until failure of the disc occurred. After mechanical testing, the discs were bisected to assess both the induced structural failure and the degree of degeneration. Out of 61 motion segments tested, 26 intervertebral discs had failed via posterior herniation. Although most herniations were induced in slightly degenerated (grade 2)\textsuperscript{144} discs, herniation also occurred in healthy (grade 1) and moderately degenerated (grade 3) discs. No herniations occurred in severely degenerated (grade 4) discs. Thus, this study revealed that compression of motion segments when bended anterolaterally can induce herniations in healthy discs. A different study using a similar setup also reported herniations occurring in healthy discs when flexed excessively and compressed
Chapter 1. Background

with loads in the range generated by the back muscles.\textsuperscript{239}

Although less relevant for the work presented in the current thesis, it should be noted that previous research has shown that both monotonic and cyclic loading, also known as fatigue loading, can induce herniations in discs \textit{in vitro}.\textsuperscript{196,243} Cyclic loading of motion segments allows the process of gradual herniation to be studied. Through cyclic loading of cadaver motion segments \textit{in vitro}, Adams and Hutton\textsuperscript{243} reported a gradual injury process beginning with distortion of the annular layers creating radial fissures through which the nucleus then penetrated towards the disc periphery. Their results suggest that young and healthy discs with a posteriorly positioned nucleus are more at risk to this type of gradual failure.

The ovine model

Veres et al\textsuperscript{13,93,244,245} were able to achieve herniations \textit{in vitro} in ovine lumbar motion segments using internal pressurization. They used a special hollow screw to inject radiopaque gel under pressure into the nucleus axially through one of the endplates. CT-scans of pressurized motion segments visualized the spread of the radiopaque gel in the total disc volume and revealed that disruption can occur at more than one location in the same disc when tested in its neutral posture.\textsuperscript{13} In this study all discs exhibited disruption in the posterior inner annulus, but penetration of radiopaque gel was also observed in the anterior, lateral and posterolateral regions. Gel penetrating the posterior annulus was confined between the posterior-posterolateral boundaries. Gel penetration in all other regions was associated with a distinctive circumferential gel path. Structural analysis of the discs using differential interference contrast (DIC) microscopy demonstrated that the circumferential path was “formed by gel flow within and along the length of numerous consecutive fibre bundles of an individual lamella”.\textsuperscript{13} When the gel was injected by sudden impulses in neutrally positioned discs Veres et al\textsuperscript{245} demonstrated that failure can occur via a circumferential tear that initiates in the anterior or lateral annulus and becomes externally evident posterolaterally or posteriorly.

Veres et al\textsuperscript{244,245} also investigated the effect of flexion on the failure morphology induced by internal pressurization. As with Gordon et al,\textsuperscript{246} the authors preferred the use of flexion angles experienced by the spine in daily life. Veres et al\textsuperscript{244} decided on using angles of 7° or 10°, this latter angle approximating the physiological limit of flexion for the ovine lumbar motion segment.\textsuperscript{14,247} Internal pressurization studies involving a flexed posture showed disruption in the posterior annulus in all discs, with gel rarely penetrating other regions of the disc.\textsuperscript{93,244,245}

More recently, Wade et al\textsuperscript{14,247} investigated whether herniations could be induced by compress-
1.5. The association between flexion and herniation

In ovine lumbar motion segments with a custom-built compression rig. Compared to the rig used for internal pressurization, the rig of Wade et al. allows deformations of the disc much more akin to that in vivo. Wade et al. reported that compressing ovine lumbar motion segments in their neutral posture, using the loading rates of 4 mm/min or 40 mm/min, resulted in vertebral endplate failure, as also reported for cadaveric studies. Herniations were only achieved when the motion segment was flexed 10° in combination with a higher compressive rate of 40 mm/min. Wade et al. reasoned that the loading rate of 40 mm/min is sufficiently high to minimize creep and is therefore thought to approximate traumatic loading. 

Compressing motion segments flexed 10° with a loading rate of 400 mm/min induced a higher percentage of herniations than when the lower compressive rate of 40 mm/min was used. This higher loading rate represents “surprise” loading generated for example by a sudden reactive response required to catch a significant weight as demonstrated from electromyographic studies.

Wade et al. used the herniated discs to microstructurally investigate the mechanisms of annular wall/endplate failure. It is important to emphasize that both studies focused on well-known herniation areas: the posterior and posterolateral annulus. These regions were sagittally sectioned and studied with DIC microscopy. Based on the microstructural analysis, Wade et al. addressed the importance of the topography of the endplates when considering the relative strain distribution across the posterior annulus in flexed and compressed motion segments. The microscopic structural analysis revealed that initial failure occurred in the mid annulus where the strain is highest, either at the endplate or in the annulus at mid disc height. Microstructural analysis of herniations induced with the higher compressive rate of 400 mm/min also supported the herniation mechanism initiating at the mid annulus; however failure then occurred more often at the annulus-endplate junction. Although the average loads at failure for the 40 mm/min and 400 mm/min compressive rates were not significantly different, Wade et al. showed that the different loading rates had a major influence on where within the disc-endplate system damage occurred.
Chapter 2

Objectives and overview

The production of physiologically relevant herniations by compressing flexed ovine lumbar motion segments in vitro, allowed Wade et al.\textsuperscript{14} to investigate the annular wall/endplate prolapse mechanism micro-structurally. However, the microscopic structural analysis focused only on the generally accepted herniation sites namely the posterior and posterolateral disc regions,\textsuperscript{89–92} leaving possible sites of initial failure in other disc regions and their associated herniation paths unexplored. Furthermore, to the author’s knowledge no other study has sought to investigate structurally whether a clinically relevant annular puncture as employed in discography could provide a preferred passage for nuclear material through the annulus and therefore whether it influences the herniation path. This led to the idea to explore whether a needle puncture would provide a preferred passage for nuclear material through the annulus by puncturing the disc just prior to mechanical testing followed by structural analysis.

Based on results of preliminary investigations, a puncture protocol and a macroscopic structural analysis method were developed. The method of macroscopic structural analysis enabled examination of all disc regions. A more detailed description of the preliminary investigations, as well as materials and methods used for the research presented in the current thesis are provided in Chapter 3. As a total surprise, the assessment of all disc regions with the macroscopic structural analysis of a punctured disc that had been mechanically tested revealed the possibility of the involvement of regions of the disc other than the posterior and posterolateral annulus in the herniation process. Therefore, to be able to resolve whether a needle puncture influences the herniation path it was decided to first explore the possible existence of alternative herniation paths. This led, in turn, to a major new emphasis in my doctoral research focussing on exploring the regional vulnerability of the disc to mechanically induced disruption and herniation, hence to the study presented in Chapter 4.

The objective of Chapter 4 was to verify whether there are mechanisms of herniation operating
in other regions than the posterior and posterolateral aspects of the disc that do not necessarily involve direct radial rupture. Macroscopic structural analyses of mechanically tested ovine lumbar motion segments, presented in Chapter 4, provided insight into possible internal disruption sites and their associated herniation paths in intact discs. This new gained knowledge then presented the opportunity to perform a study, presented in Chapter 5, with the aim of investigating whether localised damage created by an annular puncture provides the nuclear material with a preferred passage through the annulus and, in turn, whether it increases the risk of herniation at the puncture site. This research investigated the effect of localised damage created by an annular puncture independent of any longer-term degeneration-related effects.

The comprehensive macroscopic structural investigations of mechanically induced herniations in intact (Chapter 4) or punctured (Chapter 5) ovine lumbar discs identified sites of the disc that are vulnerable to disruption and herniation. In Chapter 4 a possible mechanism was proposed that could influence the vulnerability of annular regions to disruption which led to the objective of Chapter 6, to investigate the possible underlying mechanisms that increase the vulnerability of identified annular regions to disruption using microscopic structural analysis.

The last chapter, Chapter 7, provides an overview of the journey leading to the various aspects of the research presented in this thesis. This chapter also provides a summary of the key findings of the three studies, an overview of the limitations of these studies, and suggestions for future research.
Chapter 3

Background information for materials and methods sections

Several preliminary investigations influenced the journey that has led to the research reported in this thesis, and aided in the development of both a puncture protocol and a means of conducting a macroscopic structural analysis of the whole disc volume. Accordingly, the results of these preliminary investigations are described in the current chapter. This chapter also includes a more extended description of the materials and methods used for the research reported in Chapters 4, 5 and 6.

3.1 - The ovine lumbar spine model

The ovine model has been employed for all research presented in the current thesis. For clinical relevancy the employment of human cadaveric tissue would be most appropriate. However, the required ethical approval, but more importantly, the limited availability of healthy cadaver discs, led to the decision to use a readily available ovine model instead. Furthermore, the employment of the ovine model allowed comparison with closely related previous published work of Wade et al.\textsuperscript{14}

The most common concern with the use of the ovine spine is the validity of translating its relevance from quadrupeds to bipeds. However, the spine in a quadruped is mainly loaded in the long axis just like the human spine.\textsuperscript{249} A higher bone density of the ovine vertebrae compared to the human, even suggest that the spine in the quadruped are exposed to higher axial compressive stresses.\textsuperscript{249} Anatomically, the slightly kyphotic lumbar ovine spine contains six or seven vertebrae,\textsuperscript{250} whereas the human lordotic lumbar spine generally only has five.\textsuperscript{10,251}

The vertebra is the component of the motion segment that differs the most between human
Chapter 3. Background information for materials and methods sections

and ovine. Firstly, the human vertebral body is more squat.\textsuperscript{250,252} Secondly, the growth plate in humans is an integral part of the cartilaginous endplate, while the growth plate in ovine is separated by bone from the endplate and thus is located within the vertebral body.\textsuperscript{253} During skeletal maturation the growth plate in the ovine spine ossifies and fuses into the vertebral body.\textsuperscript{254} But similarities also exists: the human and ovine lumbar vertebral endplates are both kidney shaped\textsuperscript{10,250,255} and have a similar axial topography: close to the disc periphery the endplates have a convex profile and are centrally more concave.\textsuperscript{6,256}

Despite the difference in the morphology of the human and ovine vertebra, their intervertebral discs are similarly shaped.\textsuperscript{256} The nucleus of both the human adult grade II disc\textsuperscript{147} and the ovine disc are white (i.e. have lost their transparent appearance, see Figure 1.5) and relatively firm resulting from the disappearance of notochordal cells over time.\textsuperscript{56} When the nucleus contains notochordal cells it is more gelatinous and transparent.\textsuperscript{6,157,257} Some quadrupeds such as mice, rats, cats, minks, dogs, pigs and rabbits retain notochordal cells far into their adult life.\textsuperscript{253} The nucleus of ovine and human discs contain some notochordal cells at birth, but these cells disappear long before adulthood.\textsuperscript{6,253,258} Mature ovine discs seem to retain cell populations similar to that in the adult human.\textsuperscript{259}

The anterior annulus in both the human and the ovine lumbar disc is thicker than that in the posterior region (see Figure 1.5).\textsuperscript{6,86,144} The inclination angle of the oblique annular fibres at the disc periphery is similar in human adult\textsuperscript{40} and young ovine (9-12 months) discs.\textsuperscript{260} However, the inclination angle decreases more towards the inner annulus in the human than in the ovine disc.\textsuperscript{40,260} Although the annular layers in the human disc are less homogeneously distributed than in the ovine,\textsuperscript{121} the integration of the annulus fibres by sub-branching into the cartilaginous endplate is similar.\textsuperscript{119,121} The endplates of both human and ovine contain a calcified cartilage layer.\textsuperscript{33,117,118,261} This layer increases in thickness from the inner annulus (defined by the first visible annular lamella) until the mid annulus.\textsuperscript{112,118} In the outer two thirds of the human annulus, fibres penetrate into the vertebral bone underneath the growth plate,\textsuperscript{79,106} this has also been observed in the ovine model.\textsuperscript{6,120}

Both healthy human and ovine discs are generally considered to have comparable biochemical\textsuperscript{260,262,263} and axial mechanical\textsuperscript{262} properties. \textit{In vitro} flexibility tests have shown compatibility in range of motion of the human and ovine lumbar spine.\textsuperscript{256,264} Also, load sharing between the disc and the posterior elements is similar for the human and ovine model.\textsuperscript{256}

Despite some differences between the human and ovine vertebrae, the similarities of the intervertebral discs make the ovine lumbar spine a suitable alternative model for disc investigations.
3.2. Tissue preparation

The ovine model is widely employed for spinal investigations. However, as others of the 'Experimental Tissue Mechanics Laboratory' at the University of Auckland have also noted, caution is required when seeking to relate the results obtained with the ovine model to the behaviour of the human spine.

3.2 - Tissue preparation

Soon after sacrifice, lumbar spines of ewes aged 3-5 years were collected from the abattoir, wrapped in plastic film and stored in a freezer at -28°C. Following thawing, the extraneous tissues like muscles, fat, the anterior longitudinal ligament and the spinal cord were removed from each lumbar spine and dissected into L1-2, L3-4 or L5-6 motion segments. From each motion segment, the transverse processes and the posterior elements were removed, except for the facet joints. All posterior elements, including the facet joints, were removed from motion segments used for Chapter 6 (segments held in extreme flexion or extreme anterior shear). All motion segments were submerged in physiologic saline at 4°C for at least 20 hours to ensure a consistent level of hydration. The vertebrae were mounted in metal cups using dental plaster, while the motion segments were maintained in a neutral posture.

Regarding statistics, motion segments obtained from a single lumbar spine were considered to be independent based on the following reasonable practical considerations: Firstly, for all studies presented in Chapters 4, 5 and 6 each motion segment was tested separately, no experimentation was performed on the total lumbar spine. Secondly, by ensuring as much as possible the use of healthy tissue the variability between samples was minimised. The ovine lumbar spines obtained from the abattoir were almost universally healthy. Also, the use of strict inclusion criteria meant that discs showing an abnormality like a cleft, rupture, focal disruption or discoloration were excluded. The inclusion criteria were employed during dissecting by assessing the discs that were transected to create the three motion segments. The major advantage when performing structural analysis of tested discs themselves is that the tissue can be assessed for normality with use of the inclusion criteria. The use of statistical tests that consider independence of samples similar to that reported in Chapters 4, 5 and 6 has been previously performed by other researchers in the 'Experimental Tissue Mechanics Laboratory' at the University of Auckland but also by other external researchers. The fact that there is a number of key publications all employing this same statistical analysis based on the assumption that motion segments obtained from the same healthy spine can be treated as independent provides additional support for the approach adopted in the present research.
3.3 - Annular puncture

This PhD started with the idea to explore the influence of a needle puncture on where nuclear migration or herniation would occur. Therefore several preliminary investigations relating to puncturing the disc were performed and are described in the following sections.

3.3.1 - Puncturing

A puncture protocol was designed to investigate the influence of a needle puncture on the herniation path (see Chapter 5). The following factors were considered to be of importance: the size of the needle, the direction of the puncture and the depth of the puncture. A preliminary study showed that the direction and depth of a puncture were better controllable when performed with a materials testing machine (Instron 5543) compared to insertion by hand. With the use of a custom-built needle holder, a needle was attached to the Instron materials testing machine's crosshead (see Figure 3.1). The motion segment was held in a custom-built clamp with the disc mounted underneath the needle and the posterolateral aspect facing upwards (see Figure 3.1). The needle was then driven at a constant rate of 0.5 mm/min into the posterolateral annulus, as often done in discography, to a depth of approximately half the disc's posterolateral-antelateral diameter to reach the nucleus proper. The use of a constant rate minimized any further variables for consideration during the analysis of the structural results.

3.3.2 - Tracing

To be able to distinguish between localized damage created by the needle puncture and that mechanically induced sequentially (see explained later in this chapter), it was investigated whether the annular puncture could be stained to assist locating the exact path of the puncture during subsequent structural analysis. A needle was coated with India ink and then inserted posterolaterally into a fresh disc. The staining of the puncture was assessed by bisecting the disc transversely (see Figure 3.2). This simple but effective experiment showed that coating the needle with India ink stains the puncture without extensive diffusion of ink obscuring the surrounding disc tissue. It is thought that the India ink stain reached far into the disc tissue from ink that was situated within the hollow needle because the majority of ink surrounding the needle would have been wiped off during insertion. Since successful staining of the puncture was achieved, coating of the needle with India ink prior to puncturing was incorporated in the methods of Chapter 5. It should also be noted that the transverse bisection of the disc punctured with a needle coated with India ink nicely revealed the orientation and the extend of penetration of the puncture (see Figure 3.2).
3.3. Annular puncture

Figure 3.1: Images illustrating the needle holder and clamp used for puncturing a disc. Image (A) provides a frontal view and image (B) provides a side view. A needle was attached to the Instron materials testing machine’s crosshead with a custom-built needle holder. The motion segment was held underneath the needle in a custom-built clamp. Note that normally both vertebrae were potted in metal cups, however to clearly show that the posterolateral aspect of the disc was facing upwards, the superior vertebra was not potted for these images.
Chapter 3. Background information for materials and methods sections

Figure 3.2: Image of a transversely bisected disc that was punctured posterolaterally with a needle coated with India ink. Effective staining of the puncture can be observed (see black line in the top transverse view) without extensive diffusion of ink obscuring the surrounding disc tissue.

3.4 - Mechanical testing

A custom-built rig (see Figure 3.3) that was designed and previously used by Wade et al.,\textsuperscript{14,247} was also used for mechanical testing of the ovine lumbar motion segments for the studies in Chapters 4 and 5. The rig was mounted in a materials testing machine (Instron 5567) and consists of three parts:

(i) The bottom part (see blue outlined parts underneath the potted motion segment in Figure 3.3) attaches the potted inferior vertebra to the Instron materials testing machine.

(ii) The middle part (see parts indicated in blue in Figure 3.3) consists of a plate, four screws and a trolley. The plate attaches to the top of the potted superior vertebra and contains four screws. The height of the screws can be altered to test the motion segment in a predetermined flexion angle. The trolley situated on top of the screws touches the top part of the rig during the compression test and allows freedom of motion of the superior vertebra.
Figure 3.3: Schematic of the loading rig. The bottom part (see blue outlined parts underneath the potted motion segment) attaches the potted inferior vertebra to the Instron materials testing machine. The middle portion (see parts indicated in blue) allows freedom of motion in the plane of flexion, limited only by the constraints provided by the disc and facets. The top part (see blue outlined parts above the middle part) attaches to the crossbar of the Instron materials testing machine. Author’s drawing based on the rig designed and used by Wade et al.\textsuperscript{14}

in the plane of flexion (anterior-posterior), limited only by the constraints provided by the disc and facets.

(iii) The top part (see blue outlined parts above the middle part in Figure 3.3) attaches to the crossbar of an Instron materials testing machine and consists of a plate to increase the contact area for the middle part of the rig.

Previous research reported the use of 10° flexion in combination with a compressive rate of 40 mm/min or 400 mm/min successful in inducing herniations in ovine lumbar motion segments \textit{in vitro}.\textsuperscript{14,247} Although the high rate of 400 mm/min induced a higher percentage of herniations,\textsuperscript{247} the loading rate of 40 mm/min was chosen because this compressive rate allowed visual examination of the disc during mechanical testing and the test to be stopped manually when disc failure had occurred. Each mechanical test was audio and video recorded from the lateral view.
3.5 - Sample preparation for structural analysis

The ‘Experimental Tissue Mechanics Laboratory’ at the University of Auckland employs a protocol for microscopic structural analysis, that has been used in many of its published studies.13,14,81–83,93,95,96,119,120,244,245,247,254,270–274 This protocol involves formalin fixation and decalcification of disc and vertebra tissue, followed by cryosectioning and differential interference contrast (DIC) optical microscopy.

3.5.1 - Chemical fixation

After mechanical testing, the vertebrae of each motion segment were trimmed and the remaining vertebra-disc-vertebra block stored for one week in 10 % formalin solution (i.e. 3.7-4 % formaldehyde solution) to chemically fix the tissue. The use of formalin to fix biological tissues was accidentally discovered by Ferdinand Blum in 1893.275 After studying the bactericidal properties of formalin,276 Ferdinand reported that the skin of his fingers had hardened, which had occurred due to contact with formalin.275 The hardening resulting from formalin was similar to that of alcohol which was then commonly used for histology. The use of formalin was swiftly incorporated in many research projects evident from the ability to cite over 50 papers only three years after Blum’s publication.277,278

Formalin fixation was performed because of the following two reasons: firstly, to fix the tissue in its state for subsequent structural analysis and secondly, to preserve and stabilize the tissue.20 This results from the fact that formalin averts autolysis279 (i.e. the process in which cells or tissues are broken down by enzymes originating from themselves) and it forms covalent bonds and cross-links.20,279 In vitro testing of formalin fixed motion segments has shown that the formation of cross-links increases the stiffness of the soft tissues.15 Therefore, formalin fixation was always performed after mechanical testing. An advantage of the resulting stiffer disc tissue is that it is easier to section and manually work with.95 It has been previously reported that formalin fixation does not alter the density,280 histology,281 and crystallinity of the apatitic mineral.26,282

3.5.2 - Decalcification

The fixed vertebra-disc-vertebra blocks were stored in 10 % formic acid solution for two weeks to decalcify the mineralized cartilaginous endplate and vertebral bone in order to cryosection these normally ‘hard’ tissues. Formic acid softens these calcified elements by extracting calcium crystallites like hydroxyapatite but retains the previously fixed non-mineral components of the tissue.283,284 The advantage of using formic acid compared to other available decalcifying media like EDTA, nitric acid, sulfosalicylic acid and DECAL is that it decalcifies tissues in a shorter
3.6. Microscopic structural analysis

3.6.1 - Cryosectioning

The vertebra-disc-vertebra blocks were rinsed with water and then mounted deep frozen on the stage of a Leica SM2000 R freeze-sledging microtome. Depending on how the tissue was mounted on the stage, different section planes could be obtained. The cryosectioned section planes used in Chapter 6 were sagittal (i.e. from anterior to posterior, see Figure 3.4A) and coronal (i.e. from lateral to lateral, see Figure 3.4B). Even more precise, the annular tissue was sectioned into off-centre sagittal or off-centre coronal slices (see Figure 3.4C, D). For the microstructural analysis described in Chapter 5 to explore puncture morphologies, the section plane was oriented perpendicular to the needle puncture axis. Previous research has shown that 30 µm thick sections are useful for microscopic structural analysis with DIC optical microscopy.\textsuperscript{13,14,81–83,93,95,96,119,120,244,245,247,254,272–274} This thickness is therefore used for microscopic structural analysis in the current thesis. The obtained sections were stored in well-plates filled with 0.15 M saline solution.

3.6.2 - Differential interference contrast (DIC) optical microscopy

For microscopic structural analysis, sections were carefully placed in 0.15 M saline solution on a glass microscope slide and sealed with a coverslip. All sections were examined in their fully

Figure 3.4: Schematic illustrating the different section planes used for cryosectioning. Sections were taken with the section plane either oriented (A) sagittally (i.e. from anterior to posterior) or (B) coronally (i.e. from lateral to lateral). Sections not taken in the middle of the disc are indicated with ‘off-centre’ as shown in (C) and (D).

amount of time.\textsuperscript{285} After two weeks of decalcification treatment the hardness of the tissue was assessed with a scalpel to determine whether or not the extent of demineralization was sufficient to allow cryosectioning. A vertebra-disc-vertebra block was considered sufficiently decalcified when the previously mineralized tissues could be cut easily.\textsuperscript{26}
hydrated state with DIC optical microscopes, Nikon Eclipse Ni or a Nikon Eclipse 80i. Objective lenses with 10x or 20x magnification were used to image sections.

In 1960, Georges Jerzy Nomarski, a Polish-born French physicist, patented Nomarski microscopy, also known as differential interference contrast (DIC) optical microscopy. This optical microscopy technique is widely used to enhance the contrast in unstained and transparent biological tissues either fixed or alive. Standard histological techniques require staining and embedding of the biological tissue in question. Previous published work of the ‘Experimental Tissue Mechanics Laboratory’ has shown that it is not necessary to stain or embed vertebra-disc-vertebra blocks to obtain informative sections. This significantly reduces the amount of tissue processing that would otherwise be required. DIC optical microscopy also provides advantages concerning optical sectioning capability in different focal planes and the ability to produce high resolution images.

A DIC microscope converts gradients in optical path length caused by a tissue section into regions of contrast in the section’s image. DIC images are obtained as follows (see Figure 3.5): A polarizer converts unpolarized light into plane polarized light. The polarized light enters a Wollastion prism that splits each light ray into two rays (see thin blue and dotted line in Figure 3.5) that are separated lesser than a micron. The two rays pass the tissue section which can induce an optical path difference between the two rays. A second Wollaston prism recombines the two rays and produces linear polarized light if no optical path difference had occurred. However, if a phase shift occurred the combined rays produce elliptically polarized light. The second polarizer, due to its orientation, will block the linear polarized light but will partially transmit the elliptically polarized light, constructing the final image.

DIC on sections of intact intervertebral discs provides excellent microscopic information about its structure. The procedure of cryosectioning with DIC microscopy in tandem has also been performed on intervertebral discs that were either internally pressurized or compressed to failure in vitro. Oblique or axial sections of those mechanically tested discs provided excellent insights in the mechanism of herniation, specifically locating where within the disc height failure had occurred and whether endplate tissue was involved in the herniation process.

A preliminary microstructural investigation was performed on discs that were punctured posterolaterally with a needle just before compressing the motion segment in vitro until disc failure occurred. To investigate whether a needle puncture provides a path for herniation, an attempt was made to cryosection the disc along the puncture to gather evidence of nuclear migration.
3.6. Microscopic structural analysis

Figure 3.5: Schematic illustrating all main components of a DIC microscope. Polarized light enters a Wollaston prism that splits a light ray into two rays (see thin blue and dotted line). The two light rays pass the tissue section which can induce an optical path difference between the two rays. A second Wollaston prism recombines the two rays and a second polarizer then partially transmits light rays with an optical path difference, forming the final image. Author’s drawing based on:26,292
Chapter 3. Background information for materials and methods sections

into and through the puncture damage. This investigation however revealed that cryosectioning along the puncture was difficult because only the external point of needle insertion was visible, and thus left the exact direction of the internal puncture damage to interpretation. Also, the sections did not provide clear evidence whether the puncture had reached the nucleus proper. Furthermore, microscopy of cryosections capturing the full posterolateral-antrolateral width of the disc sometimes revealed damage in the anterolateral annulus. This raised the question whether other annular regions could be involved in the herniation process. As previously addressed, transversely bisecting punctured discs provided a good indication of the direction and the depth (i.e. whether the puncture reached the nucleus proper) of the puncture (see Figure 3.2). Another advantage of transverse sectioning was that it provided a global view of all annular regions. Therefore, transverse sectioning was further explored as a method to analyse disruption of the disc structure.

3.7 - Macroscopic structural analysis

Preliminary work performed for the present thesis as presented in the current chapter, has developed into a novel structural analysis protocol for the 'Experimental Tissue Mechanics Laboratory' at the University of Auckland. This protocol involves progressive transverse sectioning of the intervertebral disc in combination with reflective light microscopy.

3.7.1 - Progressive transverse sectioning

Transversely bisecting intervertebral discs of mechanically tested motion segments in vitro has previously been performed by other researchers to investigate induced internal disruption. However, it became apparent early in the present studies that a straight forward bisection was only useful when gross disc disruption had occurred. Thus, in order to capture subtle or internal disruptions at different axial heights within the total disc volume, the use of multiple transverse cuts was necessary and therefore incorporated in the macroscopic structural analysis employed. The importance of examining multiple sections to assess the internal disc structure has also been previously reported by Vernon-Roberts et al. These authors argue that the performance of a bisection to determine the tissues degenerative grade is not suitable because of the possibility of missing degeneration at different locations that are not exposed. Intact formalin fixed discs when transversely sectioned never displayed any sign of ‘dragging’ of nuclear material. All transverse sectioning was performed on discs that had been fully chemically fixed and thus presented for sectioning as stable structures.

After fixation and decalcification, the vertebra-disc-vertebra block was rinsed with water and externally examined. Afterwards, the facet joints were carefully removed to enable transverse
sectioning. The disc was kept hydrated with 0.15 M saline solution at room temperature. A first cut was made transversely close to one of the vertebra (see Figure 3.6A) and the exposed surfaces were examined with a Nikon AZ100 microscope (i.e. reflective light microscopy). A progressive series of transverse cuts was performed until reaching the other vertebra (see Figure 3.6B). After each transverse cut both the exposed surface of the disc and the transectioned slice were systematically examined in their fully hydrated state with reflected light microscopy. The microscopy was coupled with gentle physical probing, where appropriate, to help differentiate between annulus and nuclear material where they had become intermingled (see example images of probing in Figure 3.7). Probing allowed assessment of the connectivity of migrated nuclear tissue with the surrounding annular layers and its stiffness to determine whether or not it originated from the nucleus. When the damage appeared less obvious the number of slices was increased by reducing their thickness to increase the level of structural resolution. On average approximately 8 slices were taken from each motion segment with a thickness range from 0.3-0.9 mm and an average thickness of 0.5 mm.

Transectioning the disc tissue manually was also preferred over cryosectioning it in 30 µm thick slices because the latter increases the chance of losing migrated nuclear tissue from the section, this due to the annular and migrated nuclear tissues not necessarily being well attached. Further, 30 µm thick transverse cryosectioning would generate large sections with large numbers required to canvass the entire disc volume. Each section would then have to be systematically analysed, significantly increasing the time necessary to assess the total disc volume. As explained in the previous paragraph, manual transectioning provided the ability to decrease the thickness of the slices when necessary. It provided sufficient evidence of internal disc disruption, and therefore ideally suited to the aims of the research.
Figure 3.7: Images of gentle probing. Transverse sectioning revealed that nuclear material had penetrated the lateral annulus (not shown in image) and then, in the outer annulus circumferentially tracked posterolaterally (see boxed region in A). (B) With a probe (see metal rod) it was gently assessed that it was nuclear material that circumferentially tracked towards the posterolateral disc region. A small schematic is included in images (A) and (B) of the disc to illustrate its orientation and the area captured.

There is currently no way of performing both macro-level transectioning and micro-level sagittal or oblique sectioning on the same disc. By performing the progressive transverse sectioning technique (i.e. paring away pieces of tissue) the possibility of performing a microstructural analysis is lost. The two sectioning methods are each mutually excluding of the other. The advantage of using the transverse sectioning technique is that all disc regions can be exposed for investigation, albeit macroscopically.

3.7.2 - Reflective light microscopy

The exposed surface of the disc and the transectioned slice were studied with a Nikon AZ100 stereomicroscope. This microscope can be used for transmitted or reflective light microscopy. However, due to the thickness of the sample, the exposed surfaces were studied with reflected light microscopy. The exposed disc surface and slice were illuminated with an external light source, a fiber optic bifurcated illuminator. Depending on the alignment of the light, the oblique and counteroblique annulus layers had contrasting and separable appearances on the final image (see colour difference of annular layers in Figure 3.7).

The employment of progressive transverse sectioning coupled with reflective light microscopy on a disc that had been punctured and mechanically tested revealed the possible involvement
3.7. Macroscopic structural analysis

of other disc regions in the prolapse event than the frequently reported herniation sites, namely the posterior and posterolateral aspects. A more detailed description of the findings of the structural analysis of this punctured and tested disc can be found in Chapter 7. Thus, to be able to properly investigate the influence of a needle puncture on disc disruption and herniation, it should first be established with intact discs which regions are involved in the prolapse event and their associated herniation paths. This lead to a shift in the overall aim of the current thesis and resulted in the research presented in the following chapter.
Chapter 4

An investigation of the herniation path

4.1 - Introduction

Both internal disc disruption\textsuperscript{178,295} and overt herniation cause low back pain by stimulating nerve endings\textsuperscript{183,296,297} situated in the outer third of the disc.\textsuperscript{181-184} When herniation occurs, either focal disc bulging or extrusion of nuclear material may irritate, inflame and/or compress the adjacent nerve roots causing sciatica.\textsuperscript{202}

\textit{In vitro} cadaver studies have shown that compressing discs in flexion increases the likelihood of herniation\textsuperscript{196,239,240} whereas compression of the disc in its neutral posture almost invariably leads to vertebral endplate failure.\textsuperscript{134,162,163} Previous \textit{in vitro} studies using ovine lumbar discs have shown that it is possible to induce herniations using a novel internal pressurization technique.\textsuperscript{13,93,244,245} While non-physiological these ovine studies did highlight the potential importance of both loading rate and posture on disc disruption and herniation.

More recently Wade et al\textsuperscript{14,247} investigated the influence of loading rate and posture by compressing intact healthy ovine lumbar motion segments under conditions much more akin to \textit{in vivo} function. Their studies showed that to achieve herniations a flexed posture of 10\textdegree{} and a minimum compressive rate of 40 mm/min was required. The same flexion but with a lower compressive rate of 4 mm/min, or a neutral posture irrespective of rate, resulted in vertebral endplate failure. Further, these investigators sagittally sectioned their herniated discs in order to investigate microscopically the mechanism of annular wall/endplate failure, particularly focusing on the posterior and posterolateral aspects.\textsuperscript{14}

While it is generally accepted that the posterior and posterolateral regions of the disc are the primary herniation sites\textsuperscript{89-92} via direct radial rupture of the annulus,\textsuperscript{91,92} the question remains as to whether there are also mechanisms of herniation operating in other regions that do not
Chapter 4. An investigation of the herniation path

necessarily involve direct radial rupture. It was considered that this latter question is best addressed by employing a method of structural analysis able to capture evidence of any more globally distributed damage within the whole disc, hence this new study.

4.2 - Materials and methods

Sample preparation

Mature ovine lumbar spines (age 3-5 years) were collected soon after sacrifice and stored at -28°C. Following thawing, extraneous tissues were removed from each lumbar spine and dissected into L1-2, L3-4 or L5-6 motion segments. A more detailed description of the tissue preparation can be found in Chapter 3. The facet joints were retained to restrict anterior shear to within physiological limits. If inspection of any adjacent transected disc revealed an abnormality such as a rupture, focal disruption, or discolouration, then the total spine was discarded. Each motion segment was submerged in physiologic saline at 4°C for at least 20 hours to ensure a consistent level of hydration and the vertebrae then mounted in metal cups using dental plaster.

Mechanical testing

Using a custom-built rig (see Figure 3.3), that was mounted in a materials testing machine (Instron 5567), each motion segment was flexed 10° followed by axial compression at a rate of 40 mm/min up until detection of an audible fibrous tearing sound. When initial failure of the disc was detected, the mechanical testing was manually stopped. The Instron materials testing machine recorded, together with displacement and time, the compressive load at a frequency of 10Hz. The tests were video recorded with a camera (Nikon COOLPIX AW100) at a frame rate of 30 frames per second from the lateral view to capture the downward and forward displacements of the superior vertebra permitted by the freedom of forward motion of the upper portion of the loading rig (see Figure 3.3) and to determine the load at initial failure. The load at initial failure was determined by performing the following steps: First, from the video recording, the time at which initial failure occurred was noted. Second, the raw data from the Instron was used to plot the load against time. Third, with the time at which initial failure of the disc occurred, obtained in the first step, the load at initial failure was determined with the load-time plot which was created in the second step. The aim of the mechanical testing was to induce internal disruption or herniation by a single overload, therefore the load at initial failure was not meant to be physiologically relevant. The displacement of the superior vertebra of each motion segment was measured by stacking two images obtained from the video recording. The first image captures the motion segment just after the imposition of flexion, while the second image captures the
motion segment just before failure of the disc. The two images were stacked in Adobe Illustrator CS6 by aligning the metal cup holding the inferior vertebra in the two images, which maintained a constant position during the mechanical testing. The horizontal and vertical displacement of the metal cup holding the superior vertebra was then measured by drawing a line connecting approximately the same spot, which was most frequently the corner of the cup, in each image. The diameter of each metal cup was 50 mm and used to convert the displacement values into millimetres. The forward displacement was then divided by the downward displacement to obtain a ‘displacement ratio’ for each sample.

With SPSS software (version 23; IBM Corp., Armonk, NY) a one-way ANOVA was performed to test for a significant difference in mean initial failure load between the three spinal levels used. A parametric statistical test was performed because a Shapiro-Wilk test revealed normal distributions for the initial failure loads of the L1-2 (p = 1.000), L3-4 (p = 0.969) and L5-6 (p = 0.490) disc levels and a Levene’s test confirmed homogeneity of variance (p = 0.217). Mean initial failure loads from this study and a previously published study by Wade et al.\(^{14}\) using the same ovine model and testing conditions was tested for significance with a t-test. This test was used because the initial failure loads of the current study were normally distributed (p = 0.434) as well as those previously published (p = 0.651)\(^{14}\) and exhibited homogeneity in variance (p = 0.117). To test for significant differences a p-value of 0.05 was used.

**Structural analysis**

The tested motion segments were chemically fixed in 10% formalin (one week) and decalcified in 10% formic acid (two weeks). After external examination of the disc the facet joints were carefully removed to enable transverse sectioning. Chapter 3 contains a more abundant description of the transverse sectioning method then provided here. A first cut was made transversely close to one of the vertebra (Figure 3.6A) followed by a progressive series of transverse cuts until the other vertebra was reached (Figure 3.6B). After each transverse cut both the exposed surface of the disc and the transectioned slice were systematically examined in their fully hydrated state with reflected light microscopy. The sections were always orientated for imaging such that the anterior of the disc was uppermost, a small schematic is included in images where appropriate. The microscopy was coupled with gentle physical probing, where appropriate, to help differentiate between annulus and nuclear material where they had become intermingled.

**Sample numbers**

A total of 14 motion segments from 12 ovine lumbar spines were assigned to the present study.
(see Table 4.1) with no particular preference given to a particular spinal level. All remaining segments were employed for other unrelated studies. Two other segments that were tested suffered endplate fracture but were deliberately excluded to focus on the subtle aspects of disc disruption and herniation.

4.3 - Results

Mechanical behaviour

The video recordings showed that after imposing the predetermined flexion the applied compression induced a forward displacement (see Figure 4.1A, B) of the superior vertebra that was approximately equal in magnitude to that of the axial compressive displacement (see Table 4.1). Also, just prior to disc failure, a faint definition of oblique fibres tilted towards the direction of the anterior shear was observed in the lateral disc periphery (see dashed box in Figure 4.1B) while this was not observed after the imposition of flexion only (see dashed box in Figure 4.1A). Further, while only one lateral side was imaged, it was observed in 8 of the 14 tests that even without there being any externally visible evidence of disc damage, an audible tearing sound coincided with a sudden transient disturbance or ripple in the outer wall of the lateral annulus* but without resulting in any obvious change in the lateral disc periphery following the removal of the applied compression.

The initial audible indication of disc failure in eight samples corresponded with the maximum load attained before a sudden drop in the load-displacement curve, either large or small (see curves 1, 2 in Figure 4.1C). For the remaining six samples, initial failure was manifested as a subtle discontinuity in the load-displacement curve (see curve 3). Discs failed at an average load of 8.6 kN (see Table 4.1) and there was no significant difference in mean initial failure load between the three spinal levels tested ($p = 0.116$).

Structural response

The following six categories describe the different modes of disruption observed:

(i) Two discs failed via a direct radial rupture only, either posterolaterally or posteriorly, both resulting in uncontained nuclear extrusion (Figure 4.2).

4.3. Results

Figure 4.1: Mechanical response of motion segment. Images (A) and (B) show the motion segment from a lateral view with the anterior of the disc to the left. The facet joint (see asterisks) is obscured by soft tissue. (A) Motion segment in flexed posture but still uncompressed. (B) Compressed motion segment just prior to failure. A comparison of the vertical and horizontal positions of the tips of the black reference lines in (A) and (B) indicates that the applied axial compression also induced a degree of anterior shear of the superior vertebra. Also, inspection of the lateral wall in the boxed region in (A) indicated that with flexion only, no clear fibre alignment is visible, whereas following compression a faint taut, alignment of the oblique fibres tilted in the direction of anterior shear was visible (see box in B). (C) Examples of load-displacement curves following the imposition of flexion and the corresponding points of initial failure (see arrows) which either coincided with the maximum load value before a visible drop, either large (1) or small (2), or with a subtle discontinuity (3).
### Table 4.1: Summary of mechanical and structural data

<table>
<thead>
<tr>
<th>Disruption category</th>
<th>Motion segment (Spine - Disc level)</th>
<th>Mechanical data</th>
<th>Structural data according to mode of disruption</th>
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<tr>
<td></td>
<td></td>
<td>Forward displacement of superior vertebra (mm)</td>
<td>Displacement ratio: Forward ÷ downward</td>
</tr>
<tr>
<td>i</td>
<td>S1-L56</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>S2-L56</td>
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<td>1.2</td>
</tr>
<tr>
<td>ii</td>
<td>S3-L12</td>
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<td>1.1</td>
</tr>
<tr>
<td></td>
<td>S4-L56</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>iii</td>
<td>S5-L56</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>S6-L34</td>
<td>0.9</td>
<td>1.3</td>
</tr>
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<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
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<td>0.8</td>
</tr>
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<td>1.0</td>
</tr>
<tr>
<td></td>
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<td>0.6</td>
</tr>
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<tr>
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<td>1.0</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td>N/A</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

- Modes of disruption are indicated by the transverse location and reported when externally visible by either focal bulging (+B) or nuclear extrusion (+NE).
- An externally visible herniation that initiated internally at a different location is further indicated by: begin → end locations.
- P indicates posterior; PL, posterolateral; L, lateral; 1L, one lateral side; 2L, both lateral sides; A, anterior; NE, nuclear extrusion; B, focal bulging; T, circumferential tracking; N/A, not applicable.
4.3. Results

Figure 4.2: Images of two category (i) discs that failed only via direct radial rupture. In (A) herniation occurred via a posterolateral direct radial rupture, and in (B) as a posterior herniation as viewed in a transection. Both radial ruptures (see arrows) resulted in an uncontained extrusion of nuclear material (see asterisks).

(ii) Two discs exhibited focal posterior bulging, i.e. contained herniations (Figure 4.3A). In both cases this bulging was a direct consequence of posterior radial rupture (Figure 4.3B). In one of these discs there was also clear evidence of nucleus penetrating into the mid annulus at one lateral side and then tracking circumferentially within the layers causing a large inwards-distorting bulge (Figure 4.3C). In the second disc, nucleus penetrated both lateral sides. On one lateral side, there was nuclear penetration into the mid annulus, similar to that in Figure 4.3C; on the other side the nucleus had penetrated into the inner lateral annulus and then tracked circumferentially posterolaterally some distance without further radial penetration.

(iii) One disc had a clear lateral disruption at one side which propagated as a skewed radial rupture leading to an uncontained nuclear extrusion at the lateral-posterolateral site (Figure 4.4).

(iv) In five discs, progressive transverse sectioning revealed a complete absence of any direct radial rupture. Instead, all in this group exhibited a similar pattern of annular disruption at one lateral side followed by circumferential nuclear tracking between lamellae to the posterolateral aspect. Figure 4.5 provides an example of this general pattern of failure: In Figure 4.5A nuclear material can be seen to migrate focally into the mid-to-outer lateral annulus (i.e. fully contained) and then deflect posterolaterally (see dashed arrows). Gentle probing of another transverse section from the same disc but taken at a different depth is shown in Figure 4.5B and indicates that the nuclear material had tracked deep into the posterolateral aspect as indicated by the sequence of dashed arrows.
Figure 4.3: Representative images of the two category (ii) discs containing both disruption at the lateral annulus and a direct radial rupture at the posterior. Images (A-C) are from one of these discs with its externally visible “contained” herniation at the posterior (see asterisk in A) resulting from a direct posterior radial rupture as revealed by transverse sectioning (see B). Image (C) shows how nucleus has also penetrated into the mid lateral annulus (arrow) and then tracked circumferentially within the annulus to create a large inwards-distorting bulge between layers (see plus sign).
4.3. Results

Figure 4.4: Image of a skewed lateral radial rupture in the single disc with a category (iii) disruption. The lateral annular disruption (see arrow) propagates as a skewed radial rupture resulting in an uncontained herniation at the lateral-posterolateral site (see dashed circle).

Figure 4.5: Example of category (iv) damage in a disc suffering lateral annular disruption of one side. Nuclear material penetrated the mid-to-outer lateral annulus and then deflected posterolaterally (see dashed arrows in A). Transverse sectioning of this same disc but at a different depth, combined with gentle probing, indicated that nuclear material had then tracked circumferentially deep into the posterolateral aspect (see dashed arrows in B).
One of the above five category (iv) discs exhibited a contained posterior herniation (Figure 4.6A). Progressive transverse sectioning found no evidence of any direct nuclear penetration radially into the posterior bulge (Figure 4.6B, D), the only site of penetration being the lateral inner annulus (Figure 4.6B, C). Nuclear material was also found between the outer lamellae in the posterior-posterolateral annulus (see dashed arrows in Figure 4.6D) thus indicating that the contained posterior herniation (see asterisks) has resulted from nuclear material tracking circumferentially down from the site of lateral disruption.

(v) Three discs suffered disruptions at both lateral sides and in all three cases nuclear material tracked circumferentially within the annular layers towards the posterolateral aspect. The bilateral damage in one of these discs is shown in Figure 4.7A, B. At one lateral side, nuclear material penetrated into the inner-to-mid annulus (see solid arrow in Figure 4.7B) where it then deflected posterolaterally (see dashed arrow in Figure 4.7B). The series of dashed arrows in Figure 4.7C identify the trail of nuclear material that has tracked circumferentially from the other lateral disruption shown in Figure 4.7A, eventually reaching as far as the dashed arrow in Figure 4.7A. The loose nuclear material in the posterolateral-lateral region shown in figure 4.7C has been lifted out from between the annular layers by gentle probing.

One of the three discs in this group had an uncontained extrusion on one lateral side (see asterisks in Figure 4.8A, B). Progressive transverse sectioning of this sample revealed that nuclear material had penetrated the lateral annulus at the site circled in Figure 4.8A and then tracked circumferentially between the annular layers (not shown in these images) to the lateral-posterolateral outer annulus where it breached the disc to become externally visible at the lateral-posterolateral side (see dashed line leading to asterisk in Figure 4.8B).

(vi) One disc showed disruption of the inner annulus at the anterior aspect followed by circumferential tracking of nuclear material between annular layers (marked as AL in Figure 4.9A). There were also minor disruptions found in the mid-to-outer lateral and posterolateral annulus (see dashed arrows in Figure 4.9B) and gentle probing revealed the presence of nuclear material tracking between the annular layers in the posterolateral region (Figure 4.9C). Despite systematic transverse sectioning through the entire height of the disc there was no evidence of any significant disruption of the inner annular layers in the lateral regions (see Figure 4.9B). This suggests that the nuclear material may have tracked from the anterior inner annular disruption.

Finally, in all cases where lateral disruption occurred (i.e. 11 out of the 14 discs analysed) there was no visible evidence of external bulging of the outer annulus immediately adjacent to these lateral sites. Also, at the level of structural resolution employed, there was no evidence
Figure 4.6: Disc with category (iv) damage showing a contained posterior herniation (see asterisks) imaged externally in (A) and in transectioned views in (B) and (D). Also note the disruption in the lateral annulus visible in (B) and enlarged in (C) but no evidence of any direct radial penetration of nucleus through the posterior annulus (see B and D). Dashed arrows in (D) identify nuclear material in the outer posterior-posterolateral annulus indicating that the contained posterior herniation (asterisk) has arisen from nuclear material tracking circumferentially down from the site of lateral disruption shown in (B) and (C).
Figure 4.7: Disc with category (v) damage showing annular disruptions and nuclear penetration at both lateral sides (see A and B). Note that at the lateral site shown in (B) the nuclear material that has breached the inner-to-mid annulus (see solid arrow) has then been deflected posterolaterally (see dashed arrow). Progressive transverse sectioning of the other lateral site revealed that nuclear material had tracked circumferentially (see dashed arrows in C) deep into the posterolateral aspect (see also dashed arrow in A). Note that the loose nuclear material in the posterolateral-lateral region shown in (C) has been lifted out from between the annular layers by gentle probing.
4.4 - Discussion

The use of an ovine lumbar model has the major advantage of providing an adequate number of healthy motion segments, a requirement that is difficult to meet had a human model been employed. Also, it is widely accepted that the ovine lumbar spine does provide a reasonable model for investigating aspects relevant to the human lumbar spine because of its similar anatomy, biochemistry and biomechanics.

Previously Adams and Hutton, employing in vitro testing, demonstrated a higher incidence of herniation in human grade 2 discs (slightly degenerate) compared to grade 1 discs (healthy), arguing that degeneration is an important but not crucial factor. They explained the increased vulnerability of the slightly degenerate discs as arising from the combination of a still hydrostatic
Figure 4.9: Disc with category (vi) damage showing disruption at the anterior annulus. In image (A) nuclear material has penetrated the inner anterior annulus (arrow) followed by circumferential tracking of nucleus between annular layers (AL). Although there are minor disruptions visible in the mid-to-outer lateral and posterolateral annulus (see dashed arrows in B) there was no evidence of disruption of the inner annular layers in the lateral regions. Gentle probing of the posterolateral annulus revealed the presence of nuclear material tracking between the annular layers (see C).
nucleus that can then break through the now weakened, stretched posterior annulus. While it is known that healthy ovine discs can be made to herniate,\textsuperscript{14,247} whether mild degeneration similarly increases their vulnerability would be an interesting topic for further investigation if a reliable source of mildly degenerate ovine spines were available.

The $10^\circ$ of flexion was chosen both because it lies within the range of physiological, non-damaging flexion for the ovine lumbar spine and, importantly, is also sufficient to achieve disc herniations \textit{in vitro}.\textsuperscript{14,244} Further, $10^\circ$ lies between the flexion angles measured in each level of the lumbar spine (ranging between $8^\circ$ and $14^\circ$) in an average healthy human when moving from a standing posture to full flexion.\textsuperscript{196,298}

The mean failure load of 8.6 kN obtained in the present study is not significantly different ($p = 0.795$) from the previously reported 8.9 kN by Wade et al\textsuperscript{14} for their herniated discs using the same ovine model and testing conditions. It should be noted that the latter study focused on the importance of the endplate profile and utilised sagittal sections confined to the posterior and posterolateral aspects of the disc for microstructural analysis. In contrast, progressive transverse sectioning of the whole disc was used in this study. So while the structural analysis performed in this study is confined to the macro level, the more global picture of disruption obtained has revealed a herniation path that commences in the lateral annulus with the nuclear material then tracking circumferentially to become externally evident in the more generally acknowledged posterolateral and posterior herniation regions (see Figure 4.6).

In all tests performed in this study, this lateral disruption did not cause any discernible external alteration in the lateral disc periphery following the removal of load. Clinically, discs appearing externally normal (i.e. an absence of focal bulging or nuclear extrusion) but containing an internal disruption causing low back pain are described by the pathologic entity \textit{internal disc disruption}.\textsuperscript{178,295} Interestingly, the Dallas Discogram Description (DDD)\textsuperscript{176} was extended to incorporate an additional fourth grade of annulus disruption describing an internally disrupted disc that contains a fissure extending to the outer third of the annulus followed by an additional spread of contrast medium circumferentially over an arc of at least $30^\circ$ within the annulus.\textsuperscript{177,178} Therefore the initial lateral disruption and circumferential tracking of nuclear material observed in discs of this study may well relate to a mode of internal disc disruption found during discography (DDD: grade 4\textsuperscript{177,178}) if it is assumed that the clinically imaged circumferential contrast is in fact nuclear material.

Using internal pressurization of ovine motion segments in a neutral posture \textit{in vitro}, Veres et al\textsuperscript{13,245} reported, based on computed tomography imaging, that when the radiopaque gel pen-
Chapter 4. An investigation of the herniation path

etrated the inner annulus at the anterior, lateral or posterolateral aspects it then tracked circumferentially within the annular layers. Further, two of their discs revealed that circumferential tracking of the injected gel from the anterior or lateral inner annulus was the primary path that appeared at the posterolateral or posterior disc periphery without the observation of endplate disruption. These findings, based on a more global view of damage obtained using computed tomography imaging of the whole disc, are largely consistent with the results of the present study in which the circumferential tracking of nuclear material was a common occurrence. It should be noted too that the transient disturbance (recorded by video) in the lateral wall is consistent with the internal lateral annular disruption clearly documented from the transverse sections (see e.g., Figures 4.3C, 4.5).

The high prevalence of lateral annulus disruption (11 discs out of 14 tested - see Table 4.1) obtained in the present study indicates that it is the lateral margins of the disc that are highly vulnerable under the mechanical conditions employed.

So why does lateral disruption appear so frequently in the flexed, compressed discs?

The imposition of flexion induced a forward displacement of the superior vertebra (Figure 4.1A) as is known to occur in the upper levels of the lumbar spine when flexed in vivo. This is due to the centre of rotation being located slightly posteriorly and within the inferior vertebral body. The present study has also shown that compression of the flexed motion segments up to the first indication of failure induced approximately equal amounts of forward and downward displacement (see Table 4.1). Therefore a more complete mechanism of disc disruption under the combined influence of flexion and compression will most likely need to incorporate this anterior shear component.

The combined influence of anterior shear and flexion on disc failure has been discussed by Yingling and McGill. In their in vitro study, pig cervical discs were loaded in anterior shear until failure in a neutral or 10° flexed posture. While acknowledging that their predominantly shear mode of loading may be uncommon in vivo, their neutral posture tests resulted in fractures across the pars interarticularis but with added flexion they also observed endplate avulsions in the lateral aspects. This would indicate that with the addition of flexion the annular fibres in these lateral regions are stressed to a level sufficient to create such avulsions.

Theoretical models representing the annulus or disc predict that anterior shear causes most deformation of the fibres in the lateral region. More important, anterior shear results in either an increase or decrease in the lateral fibre strains depending on the inclination of the fibre...
4.4. Discussion

Figure 4.10: Schematics illustrating how anterior shear arising from combined flexion and compression influences the local response of the annular fibres. Schematic (A) illustrates how the oblique and counteroblique fibre sets in the anterior and posterior regions are recruited approximately evenly but not so in the lateral regions. Schematic (B) compares the neutral versus the flexed and compressed posture in the lateral annulus showing that anterior shear will increase the loading on those oblique fibres tilted in the direction of forward displacement but relax those in the counteroblique orientation.

whereas only minor changes are found for the anterior and posterior fibres.\textsuperscript{133} Thus, anterior shear increases the loading on those oblique fibres tilted in the direction of forward displacement as seen in the lateral disc periphery just prior to failure (Figure 4.1B) but relax those in the counteroblique orientation. This differential recruitment means that the annular loading in these lateral regions is being carried primarily by only half of the annular layers.\textsuperscript{132}

When considering the mechanical conditions employed in this study the applied flexion, compression and induced anterior shear will inevitably increase the loading in the posterior and anterior annular walls. However, the oblique and counteroblique fibres sets in these two regions will be near-equally exposed to this loading because of their similar orientation in relation to the direction of anterior shear (see schematic in Figure 4.10A). Conversely, in the lateral regions the anterior shear will result in an uneven recruitment of the oblique and counteroblique fibres sets (see Figure 4.10A, B). With the increasing hydrostatic nuclear pressure acting on the annulus this uneven recruitment will increase the vulnerability to disruption of these lateral regions. This is consistent with the high incidence of lateral disruption reported in the present study.

Despite the observation of relatively high levels of disruption in the lateral annulus, there was
little evidence to suggest that this led to lateral nuclear extrusions via direct radial rupture. It is entirely possible that the extensive circumferential tracking of nuclear material from the lateral regions reflects a path of lesser resistance created by intra-annular weakening, this in turn arising from the uneven recruitment of the oblique/counteroblique fibre sets.

The fact that herniations, both contained and extruded, mainly appear externally at the posterior or posterolateral annulus, suggests that these two sites are more susceptible to breaching of the outer annulus. Therefore these findings highlight the importance of performing a systematic structural analysis that yields a more global view of the patterns of disc disruption and movement of nuclear material.

Interestingly, in none of the discs there was any evidence of cartilaginous endplate being attached to the nuclear material that had migrated from the lateral annulus. The present study is therefore potentially relevant to patients who have acute sciatica due to nerve root inflammation and compression but who, through treatment, have rapid resolution of the inflammatory process and a diminution of herniation size.\textsuperscript{299,300} This process would intuitively occur more rapidly where there was “pure” nucleus migration to the disc periphery as seen in this study. In contrast, herniated material that contains annulus, and in particular the “harder” cartilaginous endplate, may well be less likely to resolve clinically and require surgical removal to relieve nerve root pressure.\textsuperscript{192–194}

4.5 - Key points

- Analysis of serial transverse sections systematically throughout the full volume of the disc provides a more complete picture of both the site of initial failure and the herniation path.

- The present study revealed an additional herniation path that commences in the lateral annulus and which, via circumferential nuclear tracking, can become externally evident in the more generally recognised herniation regions, namely the posterolateral and posterior aspects.

- The vulnerability of the lateral annulus to disruption is thought to arise from the overloading of the differentially recruited oblique/counteroblique fibre sets as a consequence of the anterior shear developed in the flexed, compressed motion segment.

- The present study describes a newly identified herniation path and highlights the complex nature of the mechanisms leading to actual disc herniation.
Chapter 5

The influence of an annular puncture on the herniation path

5.1 - Introduction

A 10-year cohort study reported an increase in both new herniations and degeneration in discs that had undergone discography.\textsuperscript{219} That a needle puncture induces disc degeneration over time has also been demonstrated in various \textit{in vivo} animal studies.\textsuperscript{151–160} Paradoxically, the employment of an annular puncture is considered in future therapeutic interventions that include protein,\textsuperscript{222,223} gene,\textsuperscript{224–228} and cell therapies\textsuperscript{229–231} for the treatment of disc degeneration. Interestingly, Elliott et al\textsuperscript{232} proposed that a needle diameter less than 40\% of the disc height would not alter significantly the disc properties, thus offering a potential guide to minimizing disruptive consequences.

The herniation study presented in Chapter 4 employed an \textit{in vitro} ovine lumbar disc model and used progressive transverse sectioning of the entire disc to assess the global level of damage.\textsuperscript{267} This study showed that although actual bulging or extrusion of nuclear tissue almost invariably occurred posteriorly or posterolaterally, these herniations were commonly “fed” by nucleus that had broken through the inner-to-mid annulus in the lateral regions and then tracked circumferentially into the posterolateral regions. Thus, although lateral herniation was never observed, the lateral annulus was actually disrupted first.

Carragee et al\textsuperscript{219} found that new herniations in discs investigated previously with discography were most frequently positioned at the site of the needle puncture. This raises the question whether localised damage created by an annular puncture can redirect the path that herniation takes (i.e. whether it provides the nuclear material with a preferred passage through the annu-
Chapter 5. The influence of an annular puncture on the herniation path

lus) and thus increase the risk of herniation at the puncture site independent of any longer-term degeneration-related effects, hence the aim of this study.

5.2 - Materials and methods

Sample preparation

As previously explained in Chapter 3, mature ovine lumbar spines (3-5 years old) were collected soon after sacrifice and stored frozen at -28°C. Prior to experimentation the spines were thawed, extraneous tissues removed and dissected into L1-2, L3-4 or L5-6 motion segments. The facet joints were retained to restrict anterior shear to within physiological limits. If inspection of any intervening transected disc from a spine revealed any abnormality such as a cleft, rupture, focal disruption, or discolouration, then all motion segments from that spine were discarded. Each motion segment was hydrated in physiological saline at 4°C for 20 hours to ensure a consistent level of hydration and the vertebrae potted in metal cups using dental plaster while maintained in a neutral posture.

Each motion segment was placed in a custom-built clamp (see Figure 3.1 and description in section 3.3.1) and mounted on the base stage of an Instron materials testing machine with the left posterolateral aspect facing upwards. A custom-built needle holder (see Figure 3.1) was attached to the machine crosshead and either a 25- (diameter: 0.50 mm) or 18-gauge (diameter: 1.31 mm) needle then driven at a constant rate of 0.5 mm/min through the posterolateral annulus as in discography to a depth of approximately half the disc's posterolateral-antrolateral diameter to reach the nucleus proper. The 25-gauge needle was chosen because of its use in discography and the 18-gauge needle provided a size-related comparison. Prior to puncturing, the needle was coated with India ink to assist locating the exact path of the puncture during disc transection for subsequent structural analysis (see also section 3.3.2).

Mechanical testing

As performed for the research presented in Chapter 4, using a custom-built rig (see Figure 3.3) each motion segment was flexed 10° followed by axial compression at 40 mm/min until the first audible sign of disc failure. The tests were video recorded from a lateral view to capture any visible nuclear extrusion from the puncture and to determine the downward and forward displacements of the superior vertebra induced after the imposition of flexion and just prior to disc failure. These two displacements were then used to calculate a ‘displacement ratio’ by dividing the forward displacement by that induced downward. The audio and video data were synchro-
nized with the corresponding load-time curve to determine the load at initial failure (i.e. the load at which the first sound of fibrous tearing was heard).

SPSS software (version 22; IBM Corp., Armonk, NY) was used to compare the mean initial failure loads of the two puncture groups with a Mann-Whitney U test. A nonparametric test was used because the initial failure loads of the 18-gauge punctured discs were not normally distributed (Shapiro-Wilk test: \( p = 0.022 \)). For the same reason, mean initial failure loads from the 25- and 18-gauge puncture groups of the present study and two previously published studies\(^ {14,267} \) (one of the two studies is presented in Chapter 4) of unpunctured discs using the same ovine model and testing conditions were also tested for significance with a nonparametric Kruskal-Wallis test. A one-way ANOVA was performed to test for a significant difference in mean displacement ratio between the two puncture groups from the present study and the intact ovine motion segments from Chapter 4. The Shapiro-Wilk test confirmed normal distributions for the displacement ratios of the 25-gauge group (\( p = 0.220 \)), 18-gauge group (\( p = 0.520 \)) and that of Chapter 4 (\( p = 0.534 \)). A Levene’s test revealed homogeneity of variance (\( p = 0.612 \)). Significance was found when \( p < 0.05 \).

**Structural analysis**

Following testing, the motion segments were chemically fixed in 10 % formalin for one week followed by decalcification in 10 % formic acid for two weeks. After examining whether changes in the disc periphery had occurred, the facet joints were removed to enable transverse sectioning (see Chapter 3 for a more extensive explanation of the systematic analysis method). Each disc was then transected as close as possible to one of the vertebrae followed by a progression of transverse cuts until the other vertebra was reached (see Figure 5.1). Both the exposed surface of the disc and the transectioned slices were systematically examined using a Nikon AZ100 microscope. A small schematic is included in images that do not incorporate the full transverse cross-section of the disc to illustrate its orientation and the area captured. Where appropriate, the disc tissue was gently probed to help differentiate between annulus and nuclear material where they had become intermingled.

Annular damage inflicted by the two needle sizes used in the present study was inspected independently by chemically fixing four punctured discs for one week in 10 % formalin with or without either the 25-gauge or 18-gauge needle retained *in situ*. After two weeks of decalcification in 10 % formic acid, 30 µm slices were cryosectioned perpendicular to the puncture and then microscopically examined with differential interference contrast (DIC) microscopy.
Sample numbers

The motion segments for the mechanical testing and transverse structural analysis were randomly assigned to either the 25- or 18-gauge groups. Sixteen motion segments from 10 lumbar spines were tested, 8 for each group. The remaining motion segments were reserved for unrelated spine experiments.

5.3 - Results

Mechanical behaviour

Externally visible nuclear extrusions at the posterolateral puncture site did not occur in any of the 25-gauge samples but were seen in four of the eight 18-gauge samples.

Initial failure in four of the 25-gauge punctured samples and three of the 18-gauge punctured samples was indicated by audible tearing that coincided with a sudden drop in load (similar to curves 1 and 2 in Figure 4.1C). For the remaining samples of both groups this audible tearing sound coincided with a slight irregularity in the loading curve but without any actual drop in load (similar to curve 3 in Figure 4.1C), similar to that previously reported.\textsuperscript{14,267} The mean initial failure loads for the 25- and 18-gauge groups were 9.7 kN and 8.6 kN, respectively (see Table 5.1). This difference was not significant (p = 0.293). The compression of the flexed motion segments up to the first indication of failure induced approximately equal amounts of forward and downward displacement for both puncture groups (see displacement ratios in Table 5.1).
### Table 5.1: Summary of mechanical and structural data

<table>
<thead>
<tr>
<th>Group</th>
<th>Motion segment (Spine - Disc level)</th>
<th>Mechanical data</th>
<th>Structural data according to mode of disruption and puncture involvement</th>
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<td></td>
<td></td>
<td>Forward displacement ratio: (mm)</td>
<td>Load at initial failure (kN)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forward downward</td>
<td></td>
</tr>
<tr>
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<td>0.9</td>
<td>9.9</td>
</tr>
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<td>S2-L12</td>
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<td>0.4</td>
<td>11.5</td>
</tr>
<tr>
<td>S3-L12</td>
<td>1.3</td>
<td>0.9</td>
<td>8.0</td>
</tr>
<tr>
<td>S4-L34</td>
<td>0.8</td>
<td>0.9</td>
<td>11.5</td>
</tr>
<tr>
<td>S5-L12</td>
<td>1.5</td>
<td>1.1</td>
<td>8.9</td>
</tr>
<tr>
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<td>0.5</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Average</td>
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<td>0.9</td>
</tr>
</tbody>
</table>

#### 25-gauge needle puncture

- Modes of disruption are indicated by the transverse location and reported when externally visible by either focal bulging (+B) or nuclear extrusion (+NE).
- An externally visible herniation that initiated internally at a different location is further indicated by: begin → end locations.
- P indicates posterior; PL, posterolateral; il, lateral site ipsilateral to the puncture; cl, lateral site contralateral to the puncture; biL, bilateral; NE, nuclear extrusion; B, focal bulging; T, circumferential tracking; N/A, not applicable.

### 18-gauge needle puncture

- Modes of disruption are indicated by the transverse location and reported when externally visible by either focal bulging (+B) or nuclear extrusion (+NE).
- An externally visible herniation that initiated internally at a different location is further indicated by: begin → end locations.
- P indicates posterior; PL, posterolateral; il, lateral site ipsilateral to the puncture; cl, lateral site contralateral to the puncture; biL, bilateral; NE, nuclear extrusion; B, focal bulging; T, circumferential tracking; N/A, not applicable.
Chapter 5. The influence of an annular puncture on the herniation path

Structural response

Influence of the 25-gauge needle puncture

In all eight motion segments the needle puncture had no influence on the path of herniation. The discs failed with varying degrees of complexity (see Table 5.1) as follows:

Two discs failed via direct radial extrusion through the posterior annulus (see Figure 5.2). Figure 5.2A shows an external posterior view of one such extrusion prior to transverse sectioning. A transectioned view of this disc is shown in Figure 5.2B and although above the extrusion plane, it does locate the actual needle puncture (see its faint outline in boxed region) which unfortunately is not clearly marked by India ink. The transverse section in Figure 5.2C intersects directly with the actual extrusion path thus confirming that the needle puncture had no association with the herniation.

In two discs lateral annular disruption occurred ipsilateral to the puncture and arose from direct penetration of nucleus into the mid-to-outer annulus (Figure 5.3). Multiple transverse sectioning revealed that the nuclear material then tracked circumferentially both anterolaterally and posterolaterally (see partial circumferential extension of nuclear material indicated with dashed arrows in Figure 5.3) with the latter extending to the needle puncture (not shown in image). That the puncture had no role in this movement of nuclear material is evident from both the undisturbed annular layers surrounding the puncture (see Figure 5.3) and also from inspecting adjacent transverse sections. On external inspection one of these two discs also showed a posterior bulge. Transectioning indicated that this bulge was a direct result of a posterior radial rupture (similar to Figure 5.2C).

Three distinct sites of annular penetration by nuclear material were found in two other discs and all unrelated to the needle puncture. Transections from these two discs revealed nuclear penetration bilaterally. In one of these two discs the nuclear material tracked circumferentially at both lateral sides posterolaterally and anterolaterally (similar to Figure 5.3). Ipsilateral to the puncture in the other disc there was direct penetration of nucleus laterally through the annulus resulting in an uncontained extrusion (Figure 5.4A), while the nuclear material that penetrated the contralateral annulus tracked circumferentially to the posterolateral aspect (Figure 5.4B). Although not externally evident, in both discs progressive transverse sectioning also revealed a contained posterior radial rupture (Figure 5.4C).

Two bilaterally disrupted discs contained externally visible herniations. In one disc both the lat-
Figure 5.2: An uncontained posterior extrusion in a disc punctured with a 25-gauge needle. (A) External posterior view of the extrusion. (B) Complete view of the transectioned disc locating the actual site of puncture which is faintly outlined in boxed region but not marked by India ink. (C) Location of actual radial extrusion path in the posterior (see box). Asterisks mark extruded nucleus.
Chapter 5. The influence of an annular puncture on the herniation path

Figure 5.3: Lateral annular disruption ipsilateral to a 25-gauge puncture. A large bulk of nuclear tissue (see asterisk) penetrated radially into the mid-to-outer lateral annulus then tracking circumferentially both anterolaterally and posterolaterally (see dashed arrows). The slightly “off transverse” sectioning plane captures only part of the full needle puncture (see arrow) but the image still clearly shows that the surrounding annular layers are left undisturbed.

Figure 5.4: A 25-gauge punctured disc failing at three different sites. (A) shows a lateral radial rupture that has resulted in an uncontained nuclear extrusion (asterisk) ipsilateral to the puncture (dashed lines indicate the approximate site of the puncture which is not visible in this image). (B) A second lateral annulus disruption was found contralateral to the puncture from which nuclear tissue tracked circumferentially in a posterolateral direction as revealed by gentle probing. In (C) the posterior region shows a direct radial rupture (see boxed region) which was not externally visible.
5.3. Results

Figure 5.5: External posterolateral view of a disc punctured with a 25-gauge needle. Major nuclear extrusion (see asterisks) occurred at site adjacent to but separate from the needle puncture (latter site marked by circle).

Several disruptions led to posterolateral nuclear extrusions, but again there was no evidence that the extruded nuclear material had exited via the puncture; rather, this extrusion occurred at a site adjacent to it (Figure 5.5). The second bilaterally disrupted disc contained a posterior bulge (asterisk in Figure 5.6A). While transectioning revealed clearly this bilateral disruption, there was no evidence of damage in the posterior annulus (Figure 5.6B). Most significant was the absence of any nuclear material within the puncture (see boxed region in Figure 5.6B). This indicated that the nucleus had tracked circumferentially within the outer annulus (see black arrow in Figure 5.6C) from the site of ipsilateral disruption, bypassing the needle puncture (see box in Figure 5.6C) to create this posterior bulge from which the nuclear material was able to be displaced by gentle probing (see white arrows in Figure 5.6C). Contralaterally, the inner annulus was disrupted more towards the anterolateral aspect (Figure 5.6D).

Influence of the 18-gauge needle puncture

Four of the eight discs punctured with the 18-gauge needle contained externally visible nuclear extrusions at the puncture site. However, transverse sectioning revealed that direct passage of nucleus through the puncture had actually occurred in six of the eight punctured discs. Two of these six discs had direct extrusion of nucleus only through their punctures with no obvious disruption elsewhere (Figure 5.7A, B). The other four discs, as well as showing the direct extrusion of nucleus through the puncture (Figure 5.7C) also contained a lateral annular disruption ipsilateral to the puncture in two samples and contralateral to the puncture in the other two. One of these four laterally disrupted discs also contained an externally visible posterior bulge: gentle probing of this transectioned disc revealed just how much nucleus had found its way to this
Figure 5.6: Images from disc punctured with a 25-gauge needle that exhibited an external posterior bulge. (A) External view showing a posterior bulge (asterisk). (B) Transverse sectioning revealed bilateral annular disruptions (dashed circles) and the needle puncture (see box). No disruptions were seen in the posterior annulus adjacent to the bulge (asterisk). (C) A track of nuclear material (see all arrows) was found at the posterolateral outer annulus bypassing the needle puncture (boxed region) creating the bulge at the posterior. A small part of the nuclear material is absent due to gentle probing performed to verify it as such (white arrows). (D) The inner annular disruption at the contralateral site shows clear evidence of penetration and circumferential tracking of nuclear material between lamellae.
5.3. Results

Figure 5.7: Images from 18-gauge punctured discs. (A) An externally visible extrusion of nuclear material (asterisk) through the puncture. (B) Transverse section of the same sample as in (A) showing direct passage of nucleus through the puncture (boxed region) as an uncontained extrusion (asterisk). (C) Another example of direct passage of nuclear material through the needle puncture (box). (D) Gently probing of a transectioned bulge showed that a substantial amount of nucleus tissue had accumulated (boxed region) with no disturbance of the posterior annulus. (E) The same disc as in (D) showing its contralateral disruption. Note that in image (E) the arrow marks the penetration of nucleus into the mid contralateral annulus followed by circumferential nuclear tracking (faintly visible in ellipsed site). Both images in (D) and (E) indicated that the posterior bulge (boxed region in D) has been fed by nucleus tracking from the site of major contralateral disruption in (E).
posterior site (see Figure 5.7D). There was no evidence of direct radial rupture and penetration of the adjacent annulus that could have created this posterior bulge. Rather, the transections showed that nucleus had penetrated into the contralateral mid annulus (see dashed arrow in Figure 5.7E) and then tracked circumferentially and posterolaterally within the mid annulus (see a portion of this tracked nuclear material within the dashed ellipse in Figure 5.7E). This indicated that the posterior bulge had been fed by nucleus tracking from the site of major contralateral disruption.

The two remaining discs of the eight punctured with the 18-gauge needle both contained bilateral annular disruptions (Figure 5.8A, B). In one of these discs the puncture did contain some nucleus (Figure 5.8C) but it was inconclusive as to whether this nuclear material had moved directly (i.e. radially) into the puncture or had tracked circumferentially from the ipsilateral annular disruption (Figure 5.8A) before entering the puncture site. The second disc showed no direct radial passage of nucleus into the puncture. Instead nuclear tissue penetrated into the mid-to-outer lateral annulus from the ipsilateral disruption and then tracked circumferentially to reach the puncture where it either fully extruded via the needle puncture (see circled region in Figure 5.8D) or continued to track circumferentially some distance passed the puncture (see small arrow in Figure 5.8D). In these two discs, nuclear material had also penetrated the lateral annulus contralateral to the puncture and gentle probing showed that this material had tracked circumferentially at least as far as the posterolateral outer annulus (see circled site in Figure 5.8B).

Microscopically, sections from the disc formalin fixed with the 25-gauge needle retained in situ showed an approximately circular hole resembling the needle’s cross-section that had separated and broken relatively few annular fibres (see Figure 5.9A). The sections of the punctured disc that was formalin fixed without the 25-gauge needle retained in situ had lost the circular hole because the undamaged annular fibres had recovered their original alignment (see Figure 5.9C). Furthermore, these sections showed clearly that relatively few annular fibres had been broken by the puncture. Interestingly, the inflicted damage as shown in Figure 5.9C was only observed in annular layers with the same oblique fibre orientation. For annular layers with the fibres oriented counterobliquely, the needle puncture only induced damage in the form of a slit (see dashed ellipse in Figure 5.9E). Compared to the annular damage inflicted by the 25-gauge needle, the sections of the two punctured discs formalin fixed with (see Figure 5.9B) or without the 18-gauge needle (see Figure 5.9D) retained in situ, both showed that the needle had broken more fibres and had induced a greater span of disruption.
Figure 5.8: Images of the two bilaterally disrupted 18-gauge punctured discs. (A) Lateral annular disruption (asterisk) ipsilateral to the puncture (see box) and (B) the contralateral disruption (asterisk) with nuclear material visible posterolaterally (see circle). (C) Same disc as in (A) and (B) showing evidence of nuclear material in the puncture (see box). (D) The second disc showed nuclear material (black arrow) tracking posterolaterally, then both extruding through the puncture (circle) and tracking further posteriorly (see white arrow).
Figure 5.9: DIC microscope images of punctured discs fixed either with or without the needle retained in situ. Fixation with the needle retained in situ showed an approximately circular hole resembling the needles cross-section for both the (A) 25-gauge and (B) 18-gauge needle. (A) The 25-gauge needle had separated and broken relatively few annular fibres whereas (B) the 18-gauge puncture had broken more fibres and induced a much greater span of disruption. The difference in amount of broken fibres is also evident from the images of the discs punctured with the (C) 25-gauge or (D) 18-gauge needle but fixed without the needle retained in situ. The inflicted damage by the 25-gauge needle as in (C) only occurred in the annular layers with the same oblique fibre orientation. For the annular layers with the fibres oriented counterobliquely the needle had only induced damage in the form of a slit (see dashed ellipse in E).
5.4 - Discussion

The longer-term influence of an annular puncture on degeneration, as well as risk of herniation occurring ipsilateral to the puncture, is well known. However, whether there is an increased vulnerability to herniation at the damaged site immediately following the discography puncture has not been previously investigated.

By transecting the entire disc volume this new study has enabled us to investigate whether localised damage created by an annular puncture of specific size can redirect the herniation path by allowing the passage of nucleus through the annulus and thus increasing the risk of acute herniation at the site of damage independent of any longer-term effects. The word “acute” is used to address the fact that the herniations were induced by a monotonic overload.

Our analysis shows that whereas the damage inflicted by the 25-gauge needle had no influence on the path herniation takes (Figures 5.2B & 5.6B) the larger 18-gauge needle did provide a direct passage of nucleus through the annulus (Figure 5.7B, C). Yang et al. internally pressurized punctured porcine motion segments with alginate gel in vitro and obtained gel leakage through 18- to 24-gauge punctures but not through a 26-gauge puncture. However, there is likely to be a large difference in extrudability between intact nuclear tissue that is also well connected with its surrounding annulus and endplates as in the current study and that of an injected alginate gel. Veres et al. internally pressurized unpunctured ovine discs to failure by injecting a radiopaque gel axially through one of the endplates and demonstrated the possibility of multiple disruption sites. It is therefore unclear whether the gel in the Yang et al. study extruded directly through the puncture or had disrupted the annulus at another site and then tracked circumferentially between the layers to finally exit at the puncture - a pattern of behaviour seen with the 18-gauge puncture in the present study (Figure 5.8D).

Adams and Hutton investigated in vitro the evolution of herniations in human discs under cyclic loading. They showed that a posteriorly positioned nucleus and the distortion of the posterolateral annular layers then lead to radial fissures through which the nucleus can penetrate. Although the present study used monotonic loading, the results suggest that any radial fissure (in this case a needle puncture) must be of a sufficient size to enable nuclear material to extrude directly. This accords with Elliott et al. who argued that needle size relative to disc height was an important factor in influencing disc behaviour.

As might be expected, the microscopic comparison of puncture damage revealed relatively few broken fibres around the 25-gauge puncture (see Figure 5.9A, C, E) compared to the much
greater span of disruption induced by the 18-gauge puncture (see Figure 5.9B, D). Bulging of annular fibres around the needle when retained in situ seen in this study (see Figure 5.9A, B), was also observed by Vergari et al.\textsuperscript{302} in punctured bovine caudal annulus blocks using second harmonic generation microscopy. Interestingly, the 25-gauge puncture inflicted either a hole (Figure 5.9C) or a slit (Figure 5.9E) in the annular layers dependent on the oblique fibre orientation. It is hypothesized that this results from the orientation of the needle tip in relation to that of the oblique and counteroblique fibres. When the tip is approximately aligned with the oblique fibres, the needle will most likely separate fibre sets (see Figure 5.10A) and slide through this annular layer while the counteroblique fibres form a barrier and therefore are severed (see Figure 5.10B). Using confocal microscopy on punctured and stained bovine caudal annulus blocks Michalek et al.\textsuperscript{303} also reported the occurrence of two damage morphologies of either a circular hole or a larger jagged tear. However, in that study the authors did not mention a possible relationship between the annular fibre orientation and damage morphology.

That the smaller needle puncture had little influence on where herniation occurred, even when placed posterolaterally - this being a commonly accepted herniation site\textsuperscript{89–92} - suggests that the annulus is structured so as to provide a “factor of safety” sufficient for it to cope with moderate overloading.

Although the present study did not investigate the longer-term degenerative consequences of a needle puncture, in vivo animal studies have suggested that passage of nucleus through the puncture, as seen in the 18-gauge punctured discs in the current study, could potentially accel-

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Figure 5.10: Schematic illustrating the hypothesis for the two different puncture morphologies observed for the 25-gauge needle. (A) When the tip is approximately aligned with the oblique fibres, the needle will most likely separate fibre sets and slide through this annular layer. (B) In contrast, the counteroblique fibres form a barrier and therefore are severed.
erate disc degeneration. In vivo animal studies over time have indicated a positive association between needle diameter and severity of the induced degeneration.\textsuperscript{151,155,232} Two studies, one rat and the other rabbit, reported the development of degeneration in discs that contained nuclear material in the puncture.\textsuperscript{152,156} Keorochana et al\textsuperscript{155} also found significant degenerative changes over time in rat caudal discs punctured with a 22-, 20- or 18-gauge needle with the greatest changes occurring with the 18-gauge. They postulated that because the 18-gauge puncture would permit a larger amount of nuclear material to pass through the annulus, causing more biochemical and biomechanical deterioration within the disc, this would then accelerate the degenerative process. However, the substantial mismatch between needle diameter and disc size for all of the above mentioned in vivo animal studies would mean that considerably more structural damage would have been inflicted compared to that arising from discography on the human disc.

Nassr et al\textsuperscript{220} reported that patients undergoing cervical discectomy and fusion and who had incorrect needle localization (i.e. the localizing needle was placed in a cervical disc space which was not included in the fusion) had a three-fold increased risk of developing disc degeneration after approximately 2 years. In a 10-year cohort study Carragee et al\textsuperscript{219} reported an increased progression of degeneration in human lumbar discs that had undergone discography compared to those not exposed to puncture and injection. However, these investigators did note that not all discs subjected to discography showed an increased progression of degeneration. Interestingly, the above cohort study concerned discography performed predominantly with a 25-gauge needle as used in the current study. Thus, although the 25-gauge needle did not influence the herniation path induced by acute monotonic loading in the present study, the longer-term study of Carragee et al\textsuperscript{219} indicates that the use of such a needle size in combination with an injection should be carefully considered because of the potential risk of accelerated degeneration over time.

Interestingly, there was no significant difference in the mean failure loads between the two punctured groups of the present study nor with respect to previously published data\textsuperscript{14,267} (see also Chapter 4) obtained from unpunctured discs using the same ovine model and test conditions (p = 0.543). Thus the large difference in herniation response between the two needle sizes, even when there was little difference in mean failure loads, indicates that the latter parameter is relatively insensitive to what actually happens internally to the disc structure at failure. This agrees generally with the earlier findings of Michalek and Iatridis\textsuperscript{304} who concluded that mechanical tests on whole motion segments are relatively insensitive to the presence of small defects caused by needle punctures.
Chapter 5. The influence of an annular puncture on the herniation path

Table 5.2: Indication of damage induced by needle diameter to disc height (%)

<table>
<thead>
<tr>
<th>Needle Size (Ga)</th>
<th>Needle Diameter (mm)</th>
<th>Disc height (mm)*</th>
<th>Ovine</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.5</td>
<td>3.93</td>
<td>12.7%</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.6%</td>
<td></td>
</tr>
</tbody>
</table>

- Asterisk indicates that the disc heights are taken from Elliott et al.232

The minimal difference in mean failure load between the two needle sizes used in the present study, combined with the high prevalence of lateral annular disruption, indicates that even with the structural damage created by the needle puncture it is the lateral margins of the disc that are highly vulnerable under the mechanical conditions employed. This is reinforced by the study presented in Chapter 4 of unpunctured discs tested under identical conditions.267 This study demonstrated a similar sequence of lateral annulus disruption followed by circumferential tracking of nucleus between the annular layers. In that chapter it was argued that this lateral annular disruption arises from differential recruitment of the oblique/counteroblique fibre sets resulting from anterior shear induced in the flexed and compressed motion segment.

There was no significant difference between the mean displacement ratio (i.e. forward displacement ÷ downward displacement) of the punctured discs in the present study and that of the unpunctured discs reported in Chapter 4 (p = 0.517). Therefore the same reasoning for the increased vulnerability of the lateral annulus to disruption of effectively underloading one set of fibres at the expense of overloading the other set due to anterior shear, induced during the mechanical testing, can apply.

Relevance of study

Although this present study has utilised the ovine lumbar model it is commonly used for investigating spine-related issues in humans because of its similar biochemistry,260 anatomy,121,250,260 and biomechanics.264 By employing a needle size (25-gauge) regularly used in discography for puncturing the smaller ovine disc, in fact, a greater scale of structural disruption was inflicted than would be expected from using the same needle in the larger human disc during discography (see Table 5.2). This enabled investigation of disc behaviour under more demanding disruptive conditions thus increasing the study’s potential clinical relevance.
5.5 - Key points

• Analysis of serial transverse sections exposing all regions of the disc has demonstrated the degree to which an annular puncture influences the path that herniation takes.

• Whereas the 25-gauge needle puncture had no influence on the herniation path, the 18-gauge puncture provided a passage for nuclear material to extrude.

• The fact that the 25-gauge needle had no influence on the herniation path suggests that the annulus is structured so as to provide a “factor of safety” sufficient for it to cope with moderate overloading.

• Even with the posterolateral annular damage created by either the 25- or 18-gauge needle puncture the inner lateral annulus was the region most vulnerable to disruption resulting from combined flexion and compression.
Chapter 6

The response of the annulus to anterior shear or flexion

6.1 - Introduction

The annulus consists of multiple concentric layers, with each layer containing collagen fibres that are aligned oblique to the disc’s vertical axis. The oblique orientation of collagen fibres alternates from layer to layer, resulting in an oblique/counteroblique fibre pattern. Within lamellae, collagen fibres are crimped (i.e. a sinusoidal-like wave morphology aligned with the annular fibres). Previous research has shown that crimp can be visualized with polarized light microscopy, for example through differential interference contrast (DIC) microscopy.

Tensile testing of sections taken from the annulus of caudal ox discs in vitro, showed that stretching along the collagen orientation straightens the fibres (i.e. the crimp disappears). The crimped morphology of collagen in loose rat tendons also disappeared when 4 % strain was applied in vitro and reappeared when the strain was released. Thus crimp can be used as an indicator of the state of stretch in collagenous tissues.

Macroscopic structural investigations of mechanically induced herniations in ovine lumbar discs in vitro, described in Chapters 4 and 5 of the current thesis, revealed frequent occurrence of a circumferential herniation path that was initiated in the lateral inner annulus. Based on predictions of models representing the annulus or the disc, it was suggested in Chapter 4 that the vulnerability to disruption of the lateral annulus was increased by anterior shear induced during compression of a flexed motion segment. For the lateral annulus, the forward displacement of the superior vertebra was suggested to stretch the oblique fibres inclined towards the direction
of shear, whereas the counteroblique fibres were more relaxed. The oblique and counteroblique fibres in the posterior and anterior annulus were assumed to be recruited more equally. Other in vitro herniation studies, employing a more complex posture during mechanical testing,\textsuperscript{268,308} proposed that differential recruitment of annular fibres resulting from induced shear could be the precursor to the extensive circumferential tracking of nuclear tissue observed in their overloaded ovine lumbar discs.

This new study aims to verify microstructurally the effects of anterior shear or flexion on the oblique and counteroblique fibres in the lateral and posterior regions of the disc, by examining the absence or presence of crimp with polarized light microscopy. Although the anterior annulus would also have been of interest for investigation, the limitation of time meant that this region had to be excluded. It was decided that the microstructural investigation should focus on the lateral and posterior regions, because herniations in monotonic overloaded ovine lumbar discs in vitro were initiated primarily in the lateral annulus and to a lesser extend in the posterior inner annulus,\textsuperscript{233,267} the latter being a well-known herniation site.\textsuperscript{89–92}

6.2 - Materials and methods

Sample preparation

As previously described in Chapter 3, lumbar spines of 3-5 years old ewes were collected soon after sacrifice and stored frozen at \(-28^\circ\text{C}\). After thawing the spines, the extraneous tissues were removed and the remaining tissue was then dissected into L1-2, L3-4 or L5-6 motion segments. Each motion segment was randomly assigned to be chemically fixed in one of three postures: neutral (functioning as control), anterior shear, or flexion. All posterior elements were removed from motion segments for shear and flexion postures to eliminate restrictions provided by the facet joints. With regard to the posterior elements of those motion segments functioning as controls, by chemically fixing them in their neutral posture, only the facet joints were retained. During dissection, the transected discs were inspected for abnormalities such as a cleft, rupture, focal disruption, or discoulouration and when present all motion segments from that spine were excluded from the study. Each motion segment was stored in physiological saline at \(4^\circ\text{C}\) for 20 hours to ensure a consistent level of hydration.\textsuperscript{16,265,266}

Sample fixation

\textit{Neutral posture}

Those motion segments that were used as controls, were immersed in 10\% formalin for a
6.2. Materials and methods

Figure 6.1: Images and schematic illustrating the rig used to fix a motion segment in anterior shear. (A) The anterior view and (B) lateral view show the motion segment held in anterior shear by the addition of a spacer rod (see arrow in A and B) between the metal cup of the superior vertebra and the custom-built clamp. (C) The schematic illustrates the sagittal cross-section of the rig with the spacer rod indicated in blue.

period of seven days in their neutral posture.

Anterior shear posture

For fixation of the motion segments in anterior shear, the vertebrae were mounted in metal cups using dental plaster and then placed in a custom-built clamp (see clamp in Figure 6.1). Anterior shear was applied by compressing both metal cups against the clamp, with the addition of a spacer rod (diameter: 2 mm, see arrows in Figure 6.1A, B) between the clamp and the metal cup holding the superior vertebra. The diameter of 2 mm for the spacer rod was chosen because it has been shown previously that bending forward maximally from an erect standing position in vivo induces on average 2 mm anterior shear per disc level in healthy human lumbar spines. The entire rig was then immersed in 10% formalin for a period of seven days.

Flexion posture

Because of the larger size of the flexion rig compared to the clamp used for shear, it was decided to use a plastic bag to chemically fix motion segments in flexion. Each motion segment
Figure 6.2: Images illustrating the rig used to fix a motion segment in flexion. (A) The posterior view and (B) lateral view show the motion segment mounted in the rig and held in flexion. Image (C) is a close-up view of the motion segment in (A). The inferior vertebra, while in a plastic bag, was mounted in dental plaster and after positioning the motion segment in flexion, the bag was filled with 10% formalin for tissue fixation (not shown in the images).

was placed in a plastic bag, which was tightly bound around the inferior vertebra. Both vertebrae were mounted in metal cups with dental plaster with only the inferior vertebra in the plastic bag. Inevitably, the plastic bag allowed a slight amount of movement of the inferior vertebra. In an attempt to correct for the movement allowed by the plastic bag, the degree of flexion assumed to be provided by the bag was measured for each motion segment. The motion segment was then placed in a rig previously used for internal pressurization studies (see Figure 6.2). After positioning the centre of rotation approximately within the inferior vertebral body, the motion segment was flexed and then held in this posture (see Figure 6.2). The applied flexion angle for each motion segment was the sum of 10° and the previously measured freedom of movement assumed to be allowed by the plastic bag, which was on average 2.4°. The plastic bag was filled with 10% formalin, ensuring immersion of the disc, then sealed and the motion segment was then left in 10% formalin for a period of seven days.

Structural analysis

The motion segments that were chemically fixed in flexion or anterior shear were removed from the rig or clamp, respectively. After formalin-fixation, each motion segment was decalcified in 10% formic acid for two weeks. The lateral regions were separated with two off-centre sagittal
6.2. Materials and methods

Figure 6.3: Schematic illustrating the different section planes used for the lateral and posterior disc regions. The lateral region was cryosectioned with the section plane sagittal and the posterior coronal (see blue plane in each of the two disc regions).

scalpel cuts (see Figure 6.3) and the exposed surfaces examined macroscopically using a Nikon AZ100 microscope. For the microscopic analysis, the use of one lateral site was considered sufficient to study the effects of anterior shear and flexion in this region due to the symmetry of the disc. One of the two lateral regions was cryosectioned sagittally and the posterior region was cryosectioned coronally (see Figure 6.3), into 30 µm thick slices. As explained previously in Chapter 3 slices were taken off-centre and resulted in a set of serial slices comprising the full annulus thickness. Every sixth slice of the serial set of slices was examined in its fully hydrated state with differential interference contrast (DIC) microscopy.

For each sample, on average 11 slices of the lateral and 10 slices of the posterior annulus were analysed. For consistency between the microstructural analyses of the three posture groups, it was decided for the lateral region to use “oblique” to indicate fibres inclined upward toward the anterior while “counteroblique” refers to those tilted upward toward the posterior. For the posterior region, “oblique” refers to fibres inclined upwards towards the left and “counteroblique” to those tilted upward towards the right. Because of the relative thinness of the slices not all contained both oblique and counteroblique layers. A table was therefore created for each sample to first document for each slice included in the analysis whether the oblique and counteroblique fibres were present. An example of such a table is created based on the images of Figure 6.4 (see Table 6.1) which for the purposes of describing the analysis are assumed to be images of different slices from the same sample. It was documented separately for the oblique and counteroblique fibres whether they were present. If present, a “1” was denoted in the table and a “0” when absent (see Table 6.1). For the three images of Figure 6.4 this resulted in the denotation of “1” for each of the oblique and counteroblique fibres to indicate that both were present. The fibre
Figure 6.4: DIC microscope images of oblique and counteroblique fibres in neutral posture samples. Image (A) shows oblique and counteroblique fibres not exhibiting crimp. Image (B) shows that only the oblique fibres exhibit crimp, while in image (C) both the oblique and counteroblique fibres exhibit crimp.
sets were then inspected for crimp and when crimp was observed a “1” again was denoted in the table, otherwise a “0” was denoted. For example, when the oblique and counteroblique fibre sets had similar morphologies as shown in Figure 6.4A it was documented that both fibre directions did not contain crimp by denoting “0” for both fibre directions. Figure 6.4B shows oblique and counteroblique fibres for which it was documented that only the oblique fibres contained crimp with denoting only a “1” for this fibre direction. By documenting “1” for each of the oblique and counteroblique fibres shown in Figure 6.4C it was documented that both fibre directions contained crimp. After each sixth slice of the set of slices from a sample was assessed, the sum of each column was calculated (see Table 6.1). For each of the two fibre directions, the number of times crimp was observed was then divided by the total number of slices containing the corresponding fibre direction, resulting in a crimp ratio (see Table 6.1).

Statistical tests were performed for the lateral and posterior regions separately. For both the lateral and posterior regions, Kruskal-Wallis tests were performed with SPSS software (version 22; IBM Corp., Armonk, NY) to test for significance differences between the crimp ratios of the oblique fibres of the neutral, anterior shear and flexion groups and between the crimp ratios of the counteroblique fibres of the three different posture groups. When significance was found, a Dunn-Bonferroni post hoc test was performed. Nonparametric test were used because the crimp ratios of the counteroblique fibres of the anterior shear group, each being the same value of 1 (see Table 6.2), are not normally distributed and violates the assumption of homogeneity of variance. From each disc both the oblique and counteroblique fibres were assessed to obtain two crimp ratios which were therefore considered to be independent. For that reason, for each posture the oblique and counteroblique crimp ratios were statistically tested with Wilcoxon signed-rank tests. $P < 0.05$ was considered significant.

Due to the curved morphology of the annulus, many slices did not contain full axial views of
The response of the annulus to anterior shear or flexion

lamellae. Therefore an accumulative assessment was performed into where most crimp occurred with respect to the disc height, i.e. top, midspan or bottom. For each sample the number of slices containing crimp were documented and those slices were used to indicate where crimp occurred with respect of the disc height. After examination of the slices containing crimp, the number of times crimp was observed was calculated separately for the top, midspan or bottom regions and each number was then divided by the total number of slices that were included in this analysis. For each region 5 numbers were obtained because of the inclusion of 5 samples per group in the present study and the average of these numbers was then used as an indication of where crimp occurred with respect to the disc height. No statistical tests were performed on this set of data.

Sample numbers

A total of 15 motion segments from 11 ovine lumbar spines was assigned to the present study. The remaining segments were reserved for other unrelated studies. Each of the three posture groups contained five samples.

6.3 - Results

The motion segments were permanently fixed in the desired anterior shear or flexion postures (see Figure 6.5). The anterior shear displacement was on average 1.3 mm after formalin-fixation of the motion segments. The flexion angles of the flexed motion segments after formalin-fixation ranged from 7° to 11° with an average of 8.5°.

Macroscopic structural response

Neutral posture

The exposed surfaces from the off-centre sagittal cuts revealed a moderately consistent pattern of lamellae for the anterior and posterior annulus for all five neutral posture motion segments. In the anterior annulus, the pattern of lamellae from inner region to the disc periphery, consisted of outward bulging inner annular layers, which changed into slight inward bulging and ended in moderately straight layers at the disc periphery (see Figure 6.6A). The posterior annulus had a pattern of lamellae of outward bulging annular layers (see Figure 6.6A). Two discs deviated from the pattern of lamellae in different regions of the posterior annulus. One disc showed moderately straight layers from the mid-to-outer annulus (see dashed box in Figure 6.7A) and the other disc had the inner-to-mid annular layers bulging inward, toward the nucleus (see dashed box in Figure 6.7B).
Figure 6.5: Example images of two motion segments fixed in either anterior shear or flexion. (A) Motion segment fixed in anterior shear. The forward displacement of the superior vertebra is visible by the difference in distance between the metal cup and the ruler (see also the difference in space between the tips of the white arrows at the superior and inferior metal cups). (B) Permanent fixation of a motion segment in flexion (see dashed lines) with the centre of rotation slightly posteriorly and within the inferior vertebra (see small circle on dashed lines).
Chapter 6. The response of the annulus to anterior shear or flexion

Figure 6.6: Example images of the patterns of lamellae in the neutral, anterior shear and flexion samples exposed by off-centre sagittal cuts. (A) The anterior annulus of neutral posture discs showed, beginning from the nucleus, outward bulging of the lamellae changing into slight inward bulging to a moderately straight alignment at the disc periphery. The posterior annular layers bulged outward. (B) The pattern of lamellae of the anterior shear samples was similar to the neutral posture discs, however shear had bended all annular layers anteriorly. (C) Flexion caused the anterior inner annular layers to bulge inward which changed into slight outward bulging, ending in a bulged disc periphery. The posterior inner annular layers bulged outward while the outer layers had become relatively straight.
Figure 6.7: Images of two neutral posture discs showing a different pattern in the posterior annulus. (A) Although the inner posterior annular layers bulged outward in the first disc, the mid-to-outer layers were aligned moderately straight. (B) The second disc had the inner-to-mid annular layers bulging inward which changed to outward bulging in the mid-to-outer annulus.
Chapter 6. The response of the annulus to anterior shear or flexion

Anterior shear posture

The pattern of lamellae seen in discs fixed in anterior shear was similar to that generally observed in the neutral posture samples. However, for all five discs fixed in anterior shear, the forward displacement of the superior vertebra created a skewed forward bending in all annular layers (see Figure 6.6B). This was most evident in the anterior mid-annulus, where the lamellae had a tilted forward alignment in the shear samples (see Figure 6.6B) compared to the relatively vertical aligned layers in the neutral posture discs (see Figure 6.6A). In the posterior annulus, the anterior shear had resulted in a straighter alignment of the lower half of the annular layers and distorted the upper half anteriorly.

Flexion posture

The exposed surfaces of all five flexed motion segments revealed a distinct pattern of lamellae in the anterior annulus compared to that in the neutral discs. From the inner region to the disc periphery, flexion caused the anterior inner annular layers to bulge inward which gradually changed into slightly outward bulging, ending in a bulged disc periphery (see Figure 6.6C). The posterior inner annular layers bulged outward while the outer lamellae became relatively straight.

Microscopic structural response

Lateral annulus

The study firstly considered the lateral region of the neutral posture samples. In the slices of fixed neutral posture samples, crimp was observed in approximately half of the oblique and counteroblique lateral annular layers (see Table 6.2). The crimp ratios for the oblique and counteroblique fibres were not significantly different (p = 0.066, see Figure 6.8). Figure 6.4 provides some example images of the stretched and crimp morphology of the annulus observed with DIC microscopy. Although crimp was observed throughout the disc height, it was most often seen in the middle (see Table 6.3).

For the discs fixed in anterior shear, the oblique fibres contained on average less crimp than those in the neutral posture discs (see Figure 6.8 and Table 6.2). This means that these annular fibres were stretched more often (see Figure 6.9A). Additionally, three discs showed more vertical irregularities in the stretched annular layers in two to four slices (see arrows in Figure 6.10). In contrast, significantly more crimp was observed in all annular layers with the counteroblique fibre orientation (p = 0.041, see Figure 6.8 and 6.9B). With respect to disc height, crimp was consistently observed in the top, very frequently in the middle of the annular layers and less so in
### Table 6.2: Summary of crimp data for the lateral annulus

<table>
<thead>
<tr>
<th>Posture</th>
<th>Motion segment (Spine Disc level)</th>
<th>Number of slices</th>
<th>Crimp ratio for the oblique fibres</th>
<th>Crimp ratio for the counteroblique fibres</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neutral</strong></td>
<td>S1-L56</td>
<td>15</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>S2-L56</td>
<td>11</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>S3-L34</td>
<td>8</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
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<td>S4-L34</td>
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<td>0.7</td>
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<td>S5-L12</td>
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<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Shear</strong></td>
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<td>1.0</td>
</tr>
<tr>
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<td>S7-L56</td>
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<td>0.2</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>S8-L34</td>
<td>11</td>
<td>0.4</td>
<td>1.0</td>
</tr>
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<td></td>
<td>S4-L12</td>
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<td>S5-L56</td>
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<td>1.0</td>
</tr>
<tr>
<td><strong>Average</strong></td>
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<td>12</td>
<td>0.2</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Flexion</strong></td>
<td>S6-L12</td>
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<td>0.2</td>
</tr>
<tr>
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<td>S9-L34</td>
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<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>S4-L56</td>
<td>8</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>S10-L56</td>
<td>10</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>S11-L34</td>
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<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>N/A</td>
<td>9</td>
<td>0.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

- “Oblique” represents the fibres inclined upward toward the anterior while “counteroblique” refers to those tilted upward toward the posterior.
- N/A: not applicable.
Chapter 6. The response of the annulus to anterior shear or flexion

Lateral annulus

Figure 6.8: Bar charts of the crimp ratios. The top left and top right bar charts represent the average crimp ratios for the lateral annulus organized by posture or fibre direction, respectively. The crimp ratios for the posterior annulus are represented by the bottom left and bottom right bar charts. The bar charts for the posterior annulus are organized similarly to those for the lateral annulus. The error bars indicate the maximum and minimum crimp ratios. For the lateral annulus, “oblique” represents the fibres inclined upward toward the anterior while “counteroblique” refers to those tilted upward toward the posterior. For the posterior region, “oblique” refers to fibres inclined upwards towards the left and “counteroblique” to those tilted upward towards the right. Asterisks indicate significant differences.
6.3. Results

Figure 6.9: DIC microscope images of the lateral annulus from a disc fixed in anterior shear. The images are oriented such that the anterior is at the left side and the posterior is at the right side of the image. Image (A) shows stretched oblique fibres (i.e. fibres tilted towards the direction of shear). (B) The counteroblique annular fibres have a crimped morphology. (C) Image showing both the stretched and relaxed (i.e. crimped) fibre morphologies of the oblique and counteroblique lateral annular layers when fixed in anterior shear.
Chapter 6. The response of the annulus to anterior shear or flexion

Figure 6.10: DIC microscope image of more vertical irregularities in a stretched oblique layer in the lateral annulus of a disc fixed in anterior shear. The arrows indicate more vertical irregularities in a stretched oblique annular layer.

the bottom region (see Figure 6.11A and Table 6.3). Interestingly, in the crimped layers, anterior shear had also induced a substantial amount of annular bundle separation mostly around the mid-disc height (see Figure 6.11A). Viewed at higher magnification these clefts between fibre bundles within the layers also showed a cross-over of smaller sets of fibres or even individual fibres (see Figure 6.11B, C), similar to that reported by Pezowicz et al.270,271

For the lateral annulus of the discs fixed in flexion, the crimp ratios revealed that both oblique and counteroblique layers contained crimp in approximately half of the slices (see Table 6.2). The two crimp ratios for the flexed discs are not significantly different (p = 1.000, see Figure 6.8). Interestingly, the counteroblique fibres in the flexed discs contained significantly less crimp than

<table>
<thead>
<tr>
<th>Region</th>
<th>Neutral</th>
<th>Shear</th>
<th>Flexion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top</td>
<td>0.4</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Midspan</td>
<td>0.9</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Bottom</td>
<td>0.5</td>
<td>0.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Figure 6.11: DIC microscope images of annular bundle separations in slices of the lateral annulus from two discs fixed in anterior shear. Image (A) from the first disc showed that with respect to disc height, crimp can be observed in the top and middle of the annular layer but its presence decreases toward the inferior vertebra. At mid-disc height some collagen bundle separations are visible. Bundle separation captured in the other disc (see B and a higher magnification of the area indicated with dashed box in C) revealed that smaller sets of fibres or even individual fibres cross-over the clefts.
those in the anterior shear samples (Kruskal-Wallis test: \( p = 0.005 \) and Dunn-Bonferroni post hoc test: \( p = 0.004 \), see Figure 6.8). With respect to the disc height, crimp was mainly observed in the top and middle of the annulus (see Table 6.3).

**Posterior annulus**

With respect to the posterior annulus, for each of the three postures and two oblique fibre directions, crimp was observed in approximately one third of the posterior slices, except for flexion in which one of the two fibre directions contained crimp in half of the slices (see Table 6.4). There was no significant difference between the posterior oblique and counteroblique crimp ratios of each posture (neutral: \( p = 1.000 \), anterior shear: \( p = 1.000 \), and flexion: \( p = 0.102 \), see Figure 6.8), nor between the crimp ratios for the oblique or counteroblique fibre directions of the three postures (oblique: \( p = 0.245 \), counteroblique: \( p = 0.982 \)). With respect to disc height, all three postures had most crimp at the middle, followed by the top and the least at the bottom (see Table 6.5). The neutral samples had almost no crimp at the bottom (see Table 6.5).

Interestingly, quite apart from the ready imaging of the oblique and counteroblique fibres, a set of vertical fibres were also observed (see Figure 6.12) and these were shown not to be part of the posterior ligament. In all 143 posterior slices that were studied, those vertical fibres were only observed in 1 or 2 slices of 2 neutral discs, 1 anterior shear sample and 1 flexed sample. Although vertical fibres were rarely observed, it was thought to be an observation worthy of mention in this thesis. Unfortunately, the limitation of time did not allow examination of slices adjacent to the ones in which vertical fibres were observed. The vertical fibres in the posterior annulus would be an interesting topic for future investigation.

Another important finding to address was the observation of endplate failure in two shear samples and four flexion samples. In the slices of the posterior aspects of those samples, ruptures were observed at the tidemark, the cement line or a combination of both (see Figure 6.13A). Three slices even showed ruptures beyond the cement line, evident from small portions of vertebral bone attached to the annulus (see Figure 6.13B). When rupture occurred, both fibre directions were affected (see Figure 6.13B, C).

### 6.4 - Discussion

Disruption in the lateral annulus was frequently observed in flexed monotonically overloaded ovine lumbar motion segments *in vitro*, as described in Chapter 4. Disruption in the lateral annulus even occurred when the posterolateral annulus, one of the well-known primary herniation sites, was punctured with a needle (see Chapter 5). It remained unclear as to why
6.4. Discussion

Table 6.4: Summary of crimp data for the posterior annulus

<table>
<thead>
<tr>
<th>Posture</th>
<th>Motion segment (Spine Disc level)</th>
<th>Number of slices</th>
<th>Crimp ratio for the oblique fibres</th>
<th>Crimp ratio for the counteroblique fibres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S1-L56</td>
<td>11</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>S2-L56</td>
<td>8</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Neutral</td>
<td>S3-L34</td>
<td>8</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>S4-L34</td>
<td>13</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>S5-L12</td>
<td>12</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Average</td>
<td>N/A</td>
<td>10</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>S6-L34</td>
<td>9</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>S7-L56</td>
<td>9*</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Shear</td>
<td>S8-L34</td>
<td>8</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>S4-L12</td>
<td>9</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>S5-L56</td>
<td>8*</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Average</td>
<td>N/A</td>
<td>9</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>S6-L12</td>
<td>8*</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>S9-L34</td>
<td>9*</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Flexion</td>
<td>S4-L56</td>
<td>10*</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>S10-L56</td>
<td>13</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>S11-L34</td>
<td>8*</td>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Average</td>
<td>N/A</td>
<td>10</td>
<td>0.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

- “Oblique” refers to fibres inclined upwards towards the right and “counteroblique” to those tilted upward towards the left.
- Asterisk indicates that annular-endplate rupture occurred in the set of slices of that sample.
- N/A: not applicable.
Figure 6.12: DIC microscope images of vertical fibres observed in slices of the posterior annulus of four discs. (A) The image of the first disc shows a few vertical fibres (see arrow) next to the oblique and counteroblique fibres. (B) The second disc showed a sheet of vertical fibres (see indicated with an arrow). (C) The third disc also showed a few vertical fibres (see arrow), similar to that in (C). (D) The last disc showed vertical fibres almost spanning the full disc height (see indicated with arrows).
Figure 6.13: DIC microscope images of endplate failure in slices of the posterior annulus of flexed motion segments. Failure occurred at the tidemark (TM), the cement line (CL) or a combination of both as shown in (A). (B) Failure beyond the cement line has also been observed and was evident from the small portions of vertebral bone (see black arrows) attached to the cartilaginous endplate and annulus. Images (B) and (C) show that both oblique and counteroblique fibres were affected.
Chapter 6. The response of the annulus to anterior shear or flexion

Table 6.5: Occurrence of crimp with respect to the disc height for the posterior annulus

<table>
<thead>
<tr>
<th>Region</th>
<th>Neutral</th>
<th>Shear</th>
<th>Flexion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top</td>
<td>0.7</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Midspan</td>
<td>0.9</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Bottom</td>
<td>0.1</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Lateral disruption appeared so frequently in the flexed, compressed discs. In Chapter 4 it was theorised that the vulnerability of the lateral annulus was increased as a result of differential recruitment of the lamellae induced by anterior shear, whereas the oblique and counteroblique fibres in the posterior annulus were assumed to be recruited more equally. This new study sought to verify microscopically the effects of anterior shear and flexion on the lateral and posterior annulus and determine whether the proposed mechanism in Chapter 4 could be an explanation for the increased vulnerability of the lateral annulus when a motion segment is flexed and compressed to failure in vitro.

Fixing motion segments while flexed and compressed, with an element of anterior shear, as previously described in Chapters 4 and 5, but without inducing failure, would intuitively be the first experiment to perform. However, instead of fixing motion segments while flexed and compressed, the separate postures of neutral, anterior shear and flexion were studied. Justification for the decision to study the disc fixed in the three different postures is as follows: Firstly, it was postulated that compression would bulge the annulus and thus would stretch the annular fibres to a degree, decreasing the visibility of the effects of flexion and anterior shear on the oblique and counteroblique fibres. Secondly, the different postures should provide insight into which posture has a more dominant effect on the inclined fibres. Thirdly, the effect of single postures on the annular fibres could be used to construct an accumulative response. It should be acknowledged that anterior shear only, does not normally occur in vivo.

Using confocal microscopy, Michalek et al studied the effect of shear on parts of the annulus of bovine caudal discs in vitro. Their study reported the effect of circumferential shear on annular lamellae with the fibres inclined towards the direction of shear and proposed that shear results from uncrimping and stretching, together with rotation of collagen fibre bundles. In this study, the authors made no comment on the effect of shear on the counteroblique fibres. The advantage of studying sections, or parts, of the annulus in vitro, is that the tissue can be manipulated while being studied with microscopy. In this view, chemically fixing ovine lumbar motion segments in a posture, as performed in the current study can be considered as a limitation. However, fixation allows the effects of a posture to be studied on the internal
disc structure without compromising the intact motion segment. Although the effects of anterior shear and flexion on human lumbar discs would be of considerable interest, the present study employed the ovine model because of its use in the studies presented in previous chapters of the current thesis. The ovine model is frequently used as a substitute model for the human disc because of its similar anatomy, biochemistry, and biomechanics.

As with previous in vitro herniation investigations and the other studies reported in the present thesis, a decision was made to employ a flexion angle of 10°. However, it was decided to use a larger amount of anterior shear than that induced during the compression of flexed ovine lumbar discs, after the imposition of flexion (i.e. on average 1 mm see Tables 4.1 and 5.1). The amount of 2 mm for the anterior shear fixation was chosen based on the following three reasons: Firstly, due to the centre of rotation being situated slightly posterior and into the inferior vertebral body, flexion had already induced some forward displacement of the superior vertebra that should be taken into account over and above the 1 mm induced by combined flexion and compression. Secondly, 10° flexion is considered to be a physiologically extreme posture for the ovine lumbar spine and to be able to compare the effects of anterior shear and flexion the use of an extreme shear posture was preferred. Thirdly, application of the largest amount of anterior shear of 3 mm observed in the human lumbar spine when maximally flexed on the ovine lumbar motion segment, fractured one of the vertebra (this data is not included in this chapter). The average 2 mm shear measured in the flexed human lumbar spine did not result in gross failure of the ovine vertebra.

The macroscopic structural analysis revealed two neutral posture discs deviating from the generally observed pattern of lamellae in the posterior annulus (see dashed boxes in Figure 6.7). The microscopic analysis of the lateral and posterior regions of those two discs did not indicate any abnormalities, hence the inclusion of these samples in this study. It may be that the observed deviations indicate that the posterior annulus can have a broader range of morphologies. However, it is possible that the inward bulging posterior inner annulus is a result of failure of the disc, which with the current information available in this study cannot be excluded.

The macroscopic structural analysis of the present study also revealed that flexion causes the anterior inner annulus to bulge inward. Inward bulging of the anterior annulus has also been predicted to occur during flexion by Adams and Hutton based on an analysis of X-rays of healthy adults when their spine was maximally flexed or when standing upright. Other in vitro studies placed metal beads or a grid of metal wires into healthy human discs which were radiographically imaged to study the effect of flexion on the internal deformation of the disc. Since radiographs do not show the actual disc structure but only the metal markers, it has to
be estimated which marker represents the inner anterior annulus. Based on the displacement of approximately evenly spaced metal beads in the sagittal plane, Seroussi et al.\textsuperscript{313} reported outward bulging of the anterior inner annulus, but noted that for their analysis they did not take into account the fact that the anterior annulus is thicker than the posterior annulus.\textsuperscript{40,84,86,88} When reconsidering the displacement directions of the metal beads with a thicker anterior annulus, the inner anterior annulus seems to be situated closer to markers indicating a posterior displacement,\textsuperscript{269,312,313} as seen in the present study. Using a grid of metal wires, Tsantrizos et al.\textsuperscript{314} mapped strain measurements in the transverse plane of a disc when flexed. Their results indicated that both the anterior inner annulus and the adjacent transition area to the nucleus experienced tensile strain when the disc was flexed. This indicates that inward bulging of the inner anterior annulus could have occurred. When reconsidering the results of the \textit{in vitro} studies using metal beads\textsuperscript{312,313} or wires\textsuperscript{269,314}, they seem to hint that flexion induces inward bulging of the inner anterior annulus.

With regard to the use of collagen crimp in the oblique and counteroblique fibres as a qualitative indicator of differential loading it is important to point out that the relationship between crimp morphology and load is highly non-linear and can be represented by a J-type stress/strain response\textsuperscript{39,43} as illustrated schematically in Figure 6.14. It can be seen that the acute crimp present at zero strain is largely eliminated over a very small incremental stress range $\Delta \sigma_1$. Once the crimp is removed there is then very little change in morphology over a very large incremental stress range $\Delta \sigma_2$. Thus, it can be argued that any slight amount of residual crimp is still indicative of relatively low levels of loading. By contrast, fibres exhibiting a straight morphology are not necessarily indicative of high levels of load.

In the present study the microstructural analysis of the lateral annulus has shown that the oblique and counteroblique annular layers are recruited significantly differently by anterior shear but not by flexion (see Figure 6.8). This coincides with predictions of a theoretical disc model by Broberg et al.\textsuperscript{133} predicting that 2 mm anterior shear increases the strain in the oblique layer but decreases strain in the counteroblique layer. Broberg et al.\textsuperscript{133} also predicted that flexion would recruit the lateral annular layers more equally, as also seen in this study. Models of the annulus\textsuperscript{132} or the disc\textsuperscript{133} have also reported that anterior shear would induce the largest deformation in the lateral annulus which, although not statistically analysed, is also consistent with the crimp ratios obtained in the present study. When motion segments are fixed in anterior shear, the oblique fibres still contain an element of crimp. This could indicate that the annulus is built to be able to cope with more complex postures as occurring during daily activities.

In the present study, endplate failure in the posterior annulus was frequently observed in the samples fixed in flexion, while 10° flexion has previously been reported to be non-damaging for
Figure 6.14: Schematic illustrating the highly non-linear relationship between crimp morphology and loading. Acute crimp present at zero strain is largely eliminated over a very small incremental stress range $\Delta \sigma_1$. Once the crimp is eliminated there is very little change in morphology over a very large incremental stress range $\Delta \sigma_2$. Author's own drawing based on:39,43

It is interesting that failure in the posterior annulus involved the separation of the cartilaginous endplate or even parts of the underlying bone. This is noteworthy because involvement of the endplate in the herniation process has been previously suggested by Tanaka et al315 who noted that cartilaginous endplate separating from the vertebral bone can then migrate with extruding annular tissue. That the endplate can be involved in the herniation event is further supported by the observation of this harder tissue in the extruded material.188,194,316 For future research, it would be of interest to investigate the cause of endplate ruptures in the posterior annulus to gain more insight into factors involved in increasing the vulnerability of the endplate to rupture.

A lower crimp ratio would have been expected for the posterior annular layers in the flexed ovine lumbar motion segments in vitro.14,244 The exact cause of endplate failure is unknown. Endplate failure in the posterior region could be due to the fact that an average of $2.4^\circ$ was added to the $10^\circ$, with the aim to correct for the additional movement resulting from the plastic bag that was situated between the inferior vertebra and the plaster in order to maintain the exposure of the motion segment to the formalin solution. It should also be noted that in this study motion segments were held in flexion significantly longer than in previous studies.14,244 It could be that the prolonged flexion position in combination with the formalin fixation with the inevitable embrittlement resulted in failure of the endplate. The lower flexion angle of $8.5^\circ$ measured after fixation could be due to the combination of failure at the endplate in the posterior annulus, flexion angle setting accuracy and some relaxation effects. It should be noted that the superior vertebra was only partly submerged in 10% formalin and was thus only partly chemically fixed.
Chapter 6. The response of the annulus to anterior shear or flexion

discs compared to the neutral\textsuperscript{317} and anterior shear postures.\textsuperscript{133,269} However, no significant difference was found between the oblique and counteroblique fibre directions of the three postures ($p>0.05$, see Figure 6.8). Whether this is due to failure at the endplate is uncertain and will need to be investigated further. However, the observation that both the oblique and the counteroblique layers were affected by endplate failure hints towards an approximately equally recruitment of the posterior lamellae in flexion. For all three postures, equal recruitment in the posterior lamellae is supported by the non-significant difference between the oblique and counteroblique crimp ratios of each posture. It should be noted that the ruptures at the endplate observed in the posterior region were mostly situated close to the disc periphery and did not extend into the nucleus and is thought to have little influence on the results of the lateral annulus.

With regard to the three posture groups, overall the crimp ratios for the lateral and posterior annulus, with the qualification that the ratios for the latter region are somewhat complicated by samples containing ruptures at the endplate, support the theory proposed in Chapter 4 that flexion in combination with anterior shear could induce differential recruitment of lamellae in the lateral annulus, whereas the oblique and counteroblique fibres in the posterior annulus are recruited more equally.\textsuperscript{233,267} Based on the findings of this study, it is plausible that anterior shear plays a key role in inducing differential recruitment of the annular layers in the lateral region. This would, in turn, increase the vulnerability of the lateral annulus to disruption because the loading is then carried by only half of the annular layers.\textsuperscript{132}

6.5 - Key Points

- Formalin-fixation of motions segments in anterior shear or flexion has provided insights into the effects of those postures on the oblique and counteroblique annular fibres in the lateral and posterior disc regions.

- While the inner anterior annulus in discs in their neutral posture bulges outwards, flexion causes the inner anterior annulus to bulge inward.

- In the lateral annulus, anterior shear differentially recruits the oblique and counteroblique fibres while flexion recruits those fibres more equally.
Chapter 7

Final remarks

7.1 - Summary

This chapter will first provide an overview of the journey leading to the various aspects of my doctoral research presented in this thesis. This is followed by a summary of the key findings of the studies in Chapters 4, 5 and 6. The last section of this chapter will provide an overview of the limitations of these studies and suggestions for future research.

The original aim of this research was to investigate whether an annular puncture would provide a preferred passage for nuclear material through the annulus. Various preliminary studies were performed to gain experience in mechanical testing of ovine lumbar motion segments and to master the techniques of cryosectioning and microscopy required for structural analysis. Two observations from my preliminary studies require mentioning. Firstly, an initial microscopic examination of cryosections capturing the full posterolateral-antrolateral width of the disc, revealed damage in the anterolateral annulus in a subset of the investigated samples. Secondly, video recordings of compression tests conducted on flexed ovine lumbar motion segments detected an audible tearing sound which coincided with a sudden transient disturbance or ripple in the outer wall of the lateral annulus, this occurring without there being any externally visible evidence of disc damage. These two observations hinted at a more global involvement of the disc in the herniation event, which had yet to be discovered.

Microstructural analysis of punctured ovine lumbar discs revealed that it was difficult to determine whether the needle puncture had reached the nucleus proper, as in provocative discography. To investigate how to best orientate the needle so as to reach the nucleus proper when puncturing and to determine whether India ink would stain the puncture effectively, discs were punctured with a needle covered in India ink and then transversely bisected. The exposed surfaces clearly revealed the direction and extend of the puncture reaching the nucleus (see Figure 3.2). The
Chapter 7. Final remarks

India ink had effectively stained the puncture without extensive diffusion of ink obscuring the surrounding disc tissue (see Figure 3.2). This experiment suggested the idea of using a transverse slicing technique to analyse the disc tissue of mechanically tested motion segments. Further experimentation led to the progressive transverse sectioning technique as described in Chapter 3 and was then used to considerable advantage in the studies described in Chapters 4 and 5.

The first disc investigated by progressive transverse sectioning, was punctured with an 18-gauge needle and was compressed to failure in a flexed posture of 10°. Mechanical testing of this disc resulted in an externally visible posterior bulge. When analysing the disc’s tissue using progressive transverse sectioning to cover the full height of the disc, it was revealed that nuclear material had migrated through the puncture. However, there was no connection between the nuclear tissue that had migrated through the puncture and that in the bulge. There was also no evidence to support any suggestion that direct radial rupture and penetration of nuclear tissue in the adjacent annulus could have created this posterior bulge. Studying all disc regions, revealed that nuclear material had penetrated the lateral annulus contralateral to the puncture (see dashed arrow in Figure 5.7E) which then tracked circumferentially within the annulus towards the posterolateral region of the disc (see a portion of this tracked nuclear material within the dashed ellipse in Figure 5.7E). Thus, the serial transverse sectioning had revealed that the posterior bulge had been fed by nucleus tracking from the site of major contralateral disruption. Transverse sectioning of this disc provided insight into the possibility that a puncture can provide passage of nuclear material through the annulus. More importantly, this disc provided evidence that other regions of the disc, with a circumferential herniation path, can be involved in the prolapse event. Thus, before being in a position to investigate rigorously the effect of a needle puncture on the herniation path, it was decided that I should first investigate what herniation paths can operate in intact ovine lumbar motion segments, hence the research presented in Chapter 4.

The study presented in Chapter 4 aimed to investigate whether there are mechanisms of herniation operating in regions of the disc other than in the well-known primary herniation sites, the posterior and posterolateral aspects, and that do not necessarily involve direct radial rupture. Herniations were mechanically induced by compressing intact ovine lumbar motion segments while flexed in vitro. The analysis of serial transverse sections systematically throughout the full volume of the disc, provided a more complete picture of both the site of initial failure and the herniation path. Macroscopic transverse analyses of the tested motion segments revealed an additional herniation path that commenced in the lateral annulus and which, via circumferential nuclear tracking, could reveal itself externally in the more generally recognised posterolateral and posterior sites of herniation.
The frequent observation of disruption in the lateral annulus raised the question as to why this occurs so often in the flexed, compressed discs. Video recordings of the mechanical tests showed that compression of the flexed motion segments induced approximately equal amounts of compressive displacement and anterior shear. The latter resulted from the anteriorly directed freedom of motion allowed by the rig used for mechanical testing (see Figure 3.3). It was therefore proposed that a more complete mechanism of disc disruption, under the combined influence of flexion and compression, will most likely need to incorporate this anterior shear component. In this study it was theorised that the vulnerability of the lateral annulus to disruption arises from the overloading of the differentially recruited oblique/counteroblique fibre sets as a consequence of the anterior shear developed in the flexed, compressed motion segment. The oblique and counteroblique fibres in the posterior and anterior annulus were thought to be recruited more equally.

As detailed in Chapter 5, the analysis of serial transverse sections of mechanically tested discs, punctured with either a 25- or 18-gauge needle, demonstrated the degree to which an annular puncture influences the path that herniation takes. This study showed that a 25-gauge needle puncture had no influence on the herniation path, whereas an 18-gauge puncture did provide a passage for nuclear material to extrude. The fact that the 25-gauge needle had no influence on the herniation path suggests that the annulus is structured so as to provide a “factor of safety” sufficient for it to cope with moderate overloading. More importantly, even with the posterolateral annular damage created by either the 25- or 18-gauge needle puncture, the inner lateral annulus was the region most vulnerable to disruption resulting from combined flexion and compression.

As for the mechanically tested intact motion segments, the video recordings of the mechanical tests of the motion segments with a punctured disc revealed that compression of the flexed motion segment induced approximately equal amounts of compressive displacement and anterior shear. Therefore, it was similarly argued that the increased vulnerability of the lateral annulus to disruption was a consequence of the underloading of one set of fibres at the expense of the other set due to anterior shear.

The study in Chapter 6 aimed to verify microscopically the effects of anterior shear and flexion on differential fibre recruitment in the lateral and posterior annulus. Microstructural analysis of motion segments formalin-fixed in neutral posture, anterior shear and flexion, provided insights into the effects of those postures on the oblique and counteroblique annular fibres in the lateral and posterior disc regions. The microstructural analysis of the lateral annulus revealed that anterior shear differentially recruits the oblique and counteroblique fibres, while flexion recruits
those fibres more equally. Although the results of the posterior annulus suggest an approximate equal recruitment of the oblique and counteroblique fibres for all three postures, these results are somewhat complicated by the presence of failure at the endplate, particularly for flexion, and additional experimentation will be necessary to obtain a proper conclusion. The findings of this study support the theory proposed in Chapter 4 that flexion in combination with anterior shear, could induce differential recruitment of fibres in alternate lamellae in the lateral annulus.

In summary, the studies of the current thesis have provided evidence that other regions of the disc besides the posterior and posterolateral aspects, can be involved in the prolapse event and highlight the complex nature of the mechanisms leading to disc herniation.

7.2 - Limitations and future work

The limitations of the studies presented in this thesis are discussed in this section together with possible topics for future investigations.

The use of the ovine model introduces a limitation to the studies presented in the current thesis. As described previously in Chapter 3, despite some differences between the human and ovine vertebrae, the similarities of the intervertebral discs make the ovine lumbar spine a suitable alternative model for disc investigations. The ovine model is widely employed for spinal investigations. However, as others of the ‘Experimental Tissue Mechanics Laboratory’ at the University of Auckland have also noted, caution is required when seeking to relate the results obtained with the ovine model to the behaviour of the human spine.

Another major limitation of the studies reported in this thesis concerns the fact that there is currently no way of performing both macroscopic transverse sectioning and microscopic sagittal or oblique sectioning of the same disc; they are mutually exclusive. As argued in the present thesis, the main advantage of using the transverse sectioning technique is that all disc regions are subjected to scrutiny, albeit macroscopically. For future research, this macroscopic structural analysis can be used to identify regions of interest for further microscopic investigation. For example, the macroscopic studies presented in Chapters 4 and 5 have identified the lateral annulus to be of interest for further microscopic investigation. Microstructural analysis of the lateral annulus of ovine lumbar discs overloaded when flexed in vitro, would then provide more detailed information about the annular wall/endplate prolapse mechanism in this region.

Previously, Wade et al successfully induced herniations by monotonically overloading ovine lumbar motion segments when flexed $10^\circ$ using a compressive rate of 40 mm/min or 400 mm/min. Wade et al used the herniated discs to microstructurally investigate the mech-
7.2. Limitations and future work

Anisms of annular wall/endplate failure and focused only on the well-known herniation areas: the posterior and posterolateral annulus. As presented in the current thesis, the macroscopic transverse analysis of monotonically overloaded ovine lumbar motion segments when flexed 10° and compressed using a loading rate of 40 mm/min, revealed involvement of other disc regions in the herniation event. For future research it would be of interest to investigate whether other regions of the disc, besides the posterior and posterolateral aspects, would be involved in herniated discs induced using a compressive rate of 400 mm/min. Loads at initial failure instead of stresses were used in Chapters 4 and 5 because this allows direct comparison with previous work by Wade et al. Furthermore, to be able to correctly convert the loads at initial failure to stresses the contribution of the facet joints in load bearing should be investigated.

Although monotonic loading at the compressive rate of 40 mm/min could be viewed as a limitation of the studies presented in the current thesis, this moderately high rate would have been sufficient to minimize creep and thus approximate a traumatic loading event. An important extension of the monotonic work presented in Chapter 5 would be to explore the effects of cyclic loading at subfailure levels on similarly punctured discs and conduct a comparably global structural analysis of the herniation path. In this context, it should be noted that Adams and Hutton showed that cyclic loading produces different patterns of herniation to those resulting from acute monotonic loading.

Further, only healthy discs were used in the study presented in Chapter 5, whereas discography is commonly performed on both normal and degenerated discs. Adams and Hutton monotonically overloaded cadaver discs and reported a higher incidence of herniations in slightly degenerate (grade 2) discs compared to healthy (grade 1) discs. The studies of Chapters 4 and 5 and others certainly confirm that herniation can occur in the healthy ovine disc when compressed to failure while flexed 10° in vitro. It would therefore be of clinical interest to conduct a similar ovine-based study using discs with varying degrees of degeneration, having now established in the study presented in Chapter 5 a baseline for the influence of a puncture on the herniation path in healthy ovine discs. A major challenge would be to obtain ovine spines in sufficient numbers with varying degrees of naturally occurring degeneration.

Finally, two somewhat unexpected observations were reported in Chapter 6. Firstly, endplate rupture was observed primarily in the posterior annulus of motion segments undergoing chemical fixation in the flexed posture. It would be of interest to investigate the cause of such rupture and whether or not it arose from either or both the degree and duration of flexion while exposed to the cross-linking and stiffening effect of the fixative. Secondly, the infrequent observation of intralamellar vertical fibres in the posterior annulus has, to the author’s knowledge, not been
previously reported and could well be a species-specific phenomenon. It would be interesting to investigate in greater depth the presence of these fibres in the ovine model as well as in other species.
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