Mechanical Behaviours of Intervertebral Discs: Clinical Implications of Loading, Injury and Treatment

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Abstract

Back pain is a significant public health concern with an increasing socioeconomic cost due to lost working days and direct medical expenditure. The majority of these costs can be attributed to long term pain resulting from specific physiological conditions. Acute injury and chronic degeneration of the intervertebral disc have been linked with pain and can reduce mobility, negatively impacting quality of life.

Treatments using mesenchymal stem cells have been proposed as a means of repairing damaged and degenerate discs but questions remain around the effects of the invasive medical interventions required by these treatments. Understanding and categorising the changes in mechanical behaviour of the intervertebral disc when it is healthy, injured and degenerate, and having undergone treatment will provide valuable clinical evidence of the safety and efficacy of these treatments before risking human subjects in clinical trials.

The following report contains a literature survey of the field of intervertebral disc biomechanics with specific emphasis on disc degeneration, injury and stem cell treatment, and investigates loading during activities of daily living (ADLs) using *in vivo* and *in vitro* testing methods. The work investigates thermal behavior from viscoelastic loading and the mechanical performance of hydrogel injection based clinical interventions.

Damaged and degenerate discs displayed significantly altered material behaviours than healthy discs when subjected to loading simulating ADLs. Degenerate discs further injected with a proprietary hydrogel designed for stem cell interventions recovered healthy material behaviours but did not regain full tissue functionality.

Combined, the studies presented in this work narrow the search for potential mechanisms of degeneration of the intervertebral disc and show the beneficial effects of hydrogel injections on the mechanical functionality of intervertebral discs even without the addition of mesenchymal stem cells to those injections.

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Nomenclature

Abbreviations

A list of abbreviations used throughout this thesis is presented below.

ADL	Activities of Daily Living
AF	Annulus Fibrosus
CHPL	Composite Hydrogel Precursor Liquid
DMT	Dynamic Material Testing
HSP	Heat Shock Protein
ISF	Instrumented Spinal Fusion
IVD	Intervertebral Disc
MMP3	Matrix Metalloproteinase
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal Stem Cells
NP	Nucleus Pulposus
PCPG	Polymer-Clay Precursor Gel
PIF	Posterior Interbody Fusion

PF	Posterolateral Fusion
QST	Quasi-Static Testing
VEM	Viscoelastic Material
VSH	Viscoelastic Self-Heating

Terms

A list of terms used throughout this thesis is presented below.

Acute	Rapid onset or resulting from a specific event.
Anabolic	Processes which construct molecules or biological structures.
Apoptosis	Programmed cell death.
Catabolic	Processes which break down molecules or biological structures.
Chronic	Persistent, long lasting or occurring over time.
Collagen	A family of structural proteins forming the base of most soft tissues including the intervertebral disc.
Coccygeal	Of the coccyx, the tail section of the spine in mammals.
Degeneration	Age, disease or injury related breakdown in mechanical and tissue function of a biomaterial.
Gelate	The increase in viscosity of a precursor hydrogel to a solid gel state.
Fissures	Gaps or tears in the disc structure, commonly between annulus lamellae.

Fusion	Where two or more vertebrae are fused together by encouraging bone growth between them.
Heat Shock Protein	A family of proteins which respond to shock or stress within cells. Elevated temperatures (heat shock) are one such stress.
Herniation	Prolapse of nucleus material into or through the annulus wall.
Hydrogel	Hydrophilic polymer chains within a gel matrix.
Hydrophilic	Strongly attracted to water.
Hyperelastic	An idealised non-linear elastic model of stress-strain material behaviour.
Incidence	The probability of having a specified medical condition within a given timeframe.
Instrumented Fusion	Spinal fusion which utilises non-biological implants to structurally support the fusion process.
In vitro	Testing conducted on material removed from the live organism.
In vivo	Testing conducted on or in a live organism.
Involute	Reduction in size and function. To atrophy.

Kyphosis	Curvature of the spine that bends towards the rear of the body.
Lamella	Thin plate structure.
Ligamentum Flavum	Ligaments of the spine connecting adjacent vertebrae.
Lordosis	Curvature of the spine that bends towards the front of the body.
Material Functionality	A quality of a biomaterial inherent to its structural function as a bodily tissue.
Monozygotic	Identical twins from a single zygote which splits forming two embryos.
Notochord	A developmental structure in embryos which develops into the nucleus pulposus before birth.
Matrix Metalloproteinase	A family of catabolic enzymes which break down protein.
Percutaneous	A medical intervention gaining access to the body through needle puncture.
Point Prevalence	Incidence of a condition in a 24 hour window.

Poroelastic	The elastic behaviour of viscous liquids and porous solids.
Proteoglycan	Protein-Glycosamine chains in the nucleus pulposus whose hydrophilic behaviour is key to healthy disc function.
Self-Heating	Temperature increase due to heat generated internally within a material.
Tissue Functionality	A quality of a biomaterial inherent to its biological function as a bodily tissue.
Upregulation	An increase in production of a cell component in response to an external variable.
Viscoelasticity	A combination of viscous and elastic material properties.

1 Introduction

Pain in the lower back, simply referred to as “low back pain” (LBP) and injury to the intervertebral disc (IVD) is an increasing problem, however the causes of degeneration and injury to the disc are not well understood. Treatments using mesenchymal stem cells (MSC) have been proposed as a means of repairing degenerated discs but questions remain around the effects of the invasive medical interventions required by these treatments. Accurately characterising the mechanical behaviour of the IVD is an important step in understanding the potential efficacy and safety of future percutaneous MSC interventions.

Understanding the behaviour of the IVD in response to activities of daily living (ADL) may give further evidence as to why injury and degeneration occur and will provide data from which future models of the IVD can be created and/or verified against.

As a viscoelastic material (VEM) the IVD exhibits time dependent strain behaviour when subjected to loading, it is this time dependent behaviour which is highly characteristic of the discs properties and determines how much of the energy used in compressing the disc is dissipated and how much is returned by the disc during extension. This dissipated energy may be used up in microstructural changes within the disc or is otherwise converted to heat due to viscous friction. The IVD is a complex biological body which suffers from poor metabolite transfer and limited self-healing abilities. Degenerate discs show an increased level of catabolic enzymes and proteins, the activity of

which may be linked with temperature increases. If energy dissipated by the IVD under loading is enough to cause significant temperature increases within the disc then this energy may play a role in damage or degeneration of the disc.

MSC treatment interventions show promise as a means of reversing the damage and reduced quality of life resulting from degenerate discs but like all interventions are not free from risk and would require stem cells to be injected directly into the IVD in a hydrogel scaffold matrix. Understanding how, if at all, the injection of a hydrogel would affect the behaviour of IVDs could provide vital data for the safety and efficacy of such treatments and post intervention recovery.

2 Literature Review

2.1 Back Pain – A Public Health Concern

Back pain is considered to be a major public health problem with an increasing socioeconomic cost due to lost working days, medical costs and reduced quality of life having been named as a leading cause of activity limitation by the World Health Organisation [1-4]. The social cost of back pain is high and increasing year on year, the exact economic cost is difficult to determine and dependent on the methodology used in a particular survey but according to Dagenais *et al.* "by any standards must be considered a substantial burden on society" [5].

A study which used National Health Service data over a number of years to estimate the total cost of back pain, including direct medical expenditure and lost working days at £6 billion in 1993 and rising by £500 million per year [6]. A later study by a different group used a similar methodology, using detailed NHS and Government data to estimate the cost of back pain in 1998 and concluded that total medical and economic costs were £12 billion [4].

The large increases in estimated economic cost due to back pain would appear out of line with the much longer term trend in back pain prevalence (discussed in 2.1.1). It has been suggested that the alarming rise in economic cost may reflect an increasing trend in "healthcare seeking behaviour" [6]. The majority of costs are associated with lost working days and much of the increase in cost may be due to workers taking more sick leave than in past

decades but the complex sociocultural causes of this are beyond the scope of any current research in this area.

2.1.1 Short Term Incidence

The UK Office of National Statistics conducted three surveys on back pain in 1993, 1996 and 1998. The 1996 and 1998 surveys found 15% point prevalence of LBP amongst respondents aged 16-64 years and 40% of respondents had suffered from back pain lasting longer than 24 hours in the previous 12 month period [7, 8].

A 1996 survey of men and women aged 25-64 years found similar results with 19% point prevalence and 39% 12 month prevalence respectively [6]. A cross sectional study of 18-64 year olds in Manchester by Harkness *et al.* found back pain rates had more than doubled between 1956 and 1995 [9]. In both of these studies 'back pain' was well defined, with diagrams and clear descriptions of symptoms subjects should report.

A systematic review Hoy *et al.* took averages of four studies which met their quality inclusion criteria and found 18.1% mean point prevalence [10]. High quality studies, those which met the quality inclusion standards set by Hoy et al. in their systematic review, are in close agreement that back pain is a significant and common health concern globally [10]. This close agreement is demonstrated in Table 1.

Table 1 – Mean Prevalence of Back Pain from a Sample of High Quality Studies.

Study	Mean Point Prevalence	Mean 12 Month Prevalence
Office of National Statistics [7]	15%	40%
Hillman *et al.* [6]	19%	39%
Harkness *et al.* [9]	18%	Not Measured
Hoy *et al.* [10] (Review)	18.1%	38.1%

2.1.2 Lifetime Incidence

It has been estimated that 80% of the population will suffer low back pain (LBP) at some point in life [11-13]. This statistic is prevalent not only in the academic literature but also across a range of general or specific resources aimed at the lay person [14, 15]. The logical conclusion that can be drawn from this statistic is that 20% of people never experience back pain in their lifetime, an unexpected finding given how common LBP appears to be.

The difficulty in gathering data that would provide statistically relevant information of “whole life” experience raises questions about the accuracy of this statement. The high percentage of persons reporting back pain over relatively short time frames, in studies where “back pain” has been well defined raises questions about the accuracy of the 80% figure.

High susceptibility to recall error amongst those having previously suffered back pain may explain why lifetime incidence rates are not closer to 100%. Carey *et al.* found that 21% of respondents who had visited a physician regarding lower back pain “indicated that they had not had back pain when interviewed 4-16 months later” [16]. Only 3% of respondents from the control group chosen for having no documented history of LBP reported having suffered LBP when interviewed.

These findings suggest that back pain studies may be skewed in favour of lower incidence rates as 1 in 5 respondents forget or neglect to record their back pain episodes when surveyed. Self-reporting is used in a large number of studies on back pain incidence making it possible that current figures are underestimated throughout the literature.

Whether the 80% figure is accurate or an underestimate due to errors in self-reporting the consensus in the literature is that back pain is highly prevalent and economically costly.

2.2 Back Pain Causes

The complex structural nature of the back means that there are many possible causes of LBP. LBP can be either specific or non-specific, “Specific low back pain is defined as symptoms caused by a specific patho-physiological mechanism” whereas non-specific LBP has no known origin and accounts for approximately 90% of all cases [17]. Diagnosis of non-specific back pain is difficult, key MRI signatures, such as signal intensity, suggested as measures of back pain are also common in asymptomatic patients, there are no clear links between radiographic findings and non-specific LBP, poor links exist between physical strength and LBP and psychosocial links have been suggested [18-21]. Recent studies have postulated that innervation and chronic inflammation are the key components of painful IVDs [22, 23].

Only 14% of all LBP episodes last longer than 14 days and studies have confirmed the benefits of ‘active rehabilitation’ with 80% of patients who continued their daily schedule “within the limits of pain” returning to work after one week compared to 59% of those who were advised to take bed rest [18, 21, 24]. These finding suggest that the most effective course of action for most LBP episodes is simply to carry on as normal. Why then is LBP a significant health concern?

Short episode lengths and simple treatment with cheap analgesics and active rehabilitation should render the socioeconomic effects of LBP relatively small yet back pain accounts for approximately 12.5% of all lost working days in the UK as many as all other musculoskeletal problems together and half as many as all common minor illnesses like coughs and colds [1, 25].

Part of this is undoubtedly due to high incidence rates, most LBP episodes are short but incredibly common and those episodes that do last longer than 14 days can be highly debilitating.

Watson *et al.* reported that 15% of those absent from work due to LBP were still absent after 28 days and that rates of return to work approximately followed an exponential decay, the longer back pain lasted the less likely it becomes that the sufferer will recover, leading to extended periods of absence from work [26]. Frymoyer and Cats-Baril state that as few as 5% of sufferers in the USA account for 75% of all direct medical costs [27]. It is clear then that *chronic* back pain represents a significant socioeconomic burden.

The reasons that acute LBP becomes chronic LBP are complex and not fully understood although psychosocial causes such as “distress, depressive mood and somatisation” are becoming increasingly linked with chronic disability [28]. Specific back pain resulting from trauma or injury not only causes pain but limits activity, preventing work and reducing quality of life and with approximately 90% of the total cost of back pain being due to lost work days prevention or treatment of mobility reducing injury is a key issue [29].

Physical injury to the back that causes pain and reduces mobility can be muscular or skeletal in origin with skeletal injuries being of particular concern due to the close proximity to the spinal cord. Damage to vertebrae or intervertebral discs resulting in pinched nerves has been suggested as a potential pathogenesis of pain in the lower back and legs [30]. Degenerate IVDs show high levels of nerve ingrowth with the greatest growth in discs where patients express pain, 40% of all back pain has been attributed to the

IVD through such mechanisms making it a clear and significant subject for further research [31, 32].

Pain is not the only potential issue due to damaged IVDs. Loss of IVD height due to damage or degeneration has been shown to affect the spines ability to articulate and can lead to a transfer of load away from the IVD and vertebral body to the articular joints [33].

2.3 The Vertebral Column – Anatomy and Function

2.3.1 Basic Structure

The human spine is comprised of the 24 separate vertebrae of the Cervical, Thoracic and Lumbar regions (7, 12 and 5 vertebrae respectively) and the four and five fused vertebrae of the sacrum and coccyx (Figure 1) [34]. From C2 to S1 vertebrae are separated by a composite fibrocartilage disc. Congenital variations include the fifth lumbar vertebrae being fused into the sacrum (sacralisation of L5) and the first sacral vertebrae being separate of the sacrum (lumbarisation of S1); whilst incomplete 'hemi-vertebra' may occur at any point in the spine (Figure 2) [35].

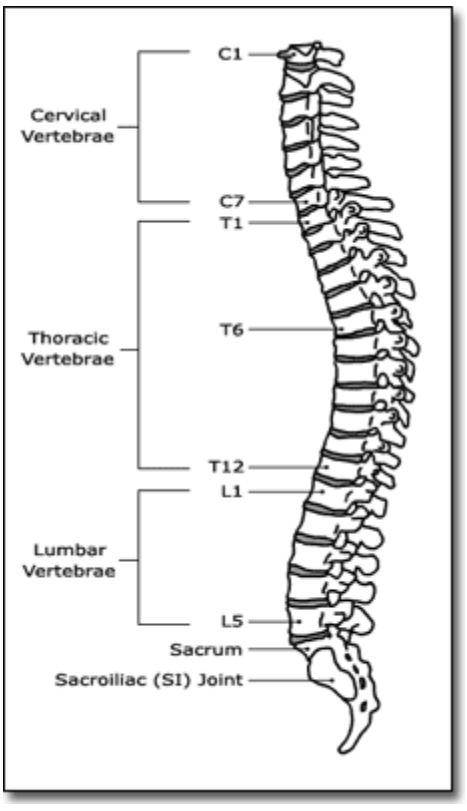

Figure 1 – The major regions of the human spine.

The vertebrae are supported by a network of ligaments and serve as the attachment point for muscles which allow upper body movements, particularly in the cervical vertebrae region due to the large range of motion possible by the head. The vertebrae form the vertebral foramen through which the spinal cord, cuada equina and meninges pass. The ratio of size of the vertebral foramen to vertebral body is naturally largest in the cervical spine where the nerve bundle is largest and the axial loading is least; this trend is reversed in the lumbar spine where large vertebral

bodies support the greatest axial loading. The thoracic vertebrae are the least flexible of the articulating vertebrae and support the rib cage.

The spine has four major curvatures, the cervical and thoracic vertebrae are concave when viewed from the posterior side (Lordosis) whilst the thoracic and sacral vertebrae are convex (Kyphosis) (Figure 1). Spinal Lordosis is affected by a range of factors including age and sex. Lordosis is most pronounced in women of childbearing age [36]; this is linked to an increase in the hormone 'relaxin' in the blood stream which relaxes spinal ligaments in the lumbar/sacral region. Lordosis naturally flattens with age although body weight and physical condition of the abdominal muscles both affect its prominence throughout life. The curvature of the spine affects load bearing with greater loads (relative to the vertebral body) carried by the apophyseal facets in vertebrae with higher curvature [35, 36]. The high loading on and rates of acute injury to the lumbar IVD makes this region of particular interest to this project.

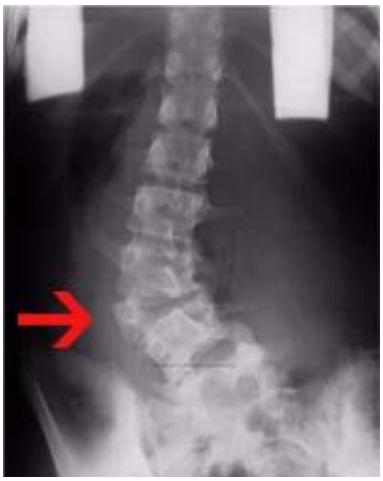

Figure 2 – Congenital Scoliosis resulting from hemi-vertebrae.

2.3.2 **Vertebrae**

Vertebrae are the irregular, articulating bones of the spinal column (Figure 3). They consist of two major components, the vertebral body and the posterior elements together these form the vertebral foramen through which the spinal cord passes.

The vertebral body is the solid, anterior part of each vertebra consisting mainly of cancellous trabecular bone which bears the majority of load, for this reason the largest vertebral bodies are found lower down in the lumbar regions whilst those in the cervical spine are smaller in dimension and smaller relative to the posterior elements. Roughly cylindrical with flat superior and inferior faces, when viewed from above the posterior surface is slightly concave in much of the lower spine. The majority of the superior and inferior faces are perforated with small holes; however a narrow ring extends from the perimeter forming the lesser perforated ring apophysis.

The posterior elements - spinous process, transverse process and superior and inferior articular facets –are attached to the vertebral body via two short, thick struts known as pedicles. Together the pedicles and posterior elements form the neural arch, a ring of bone which creates the vertebral foramen, the opening through which the spinal cord passes.

Two laminae extend posteriorly from the pedicles toward the midline and it is from the laminae that the superior and inferior articular processes protrude. Each of the four processes has an area of flattened bone, the articular facets, which form the zygapophyseal joints between vertebrae in the spine.

Extending posteriorly from the midline of the laminae is the spinous process whilst the transverse processes extend laterally from the meeting point of the pedicles and laminae. These relatively large outgrowths of bone serve as attachment points for the muscles and ligaments of the spine.

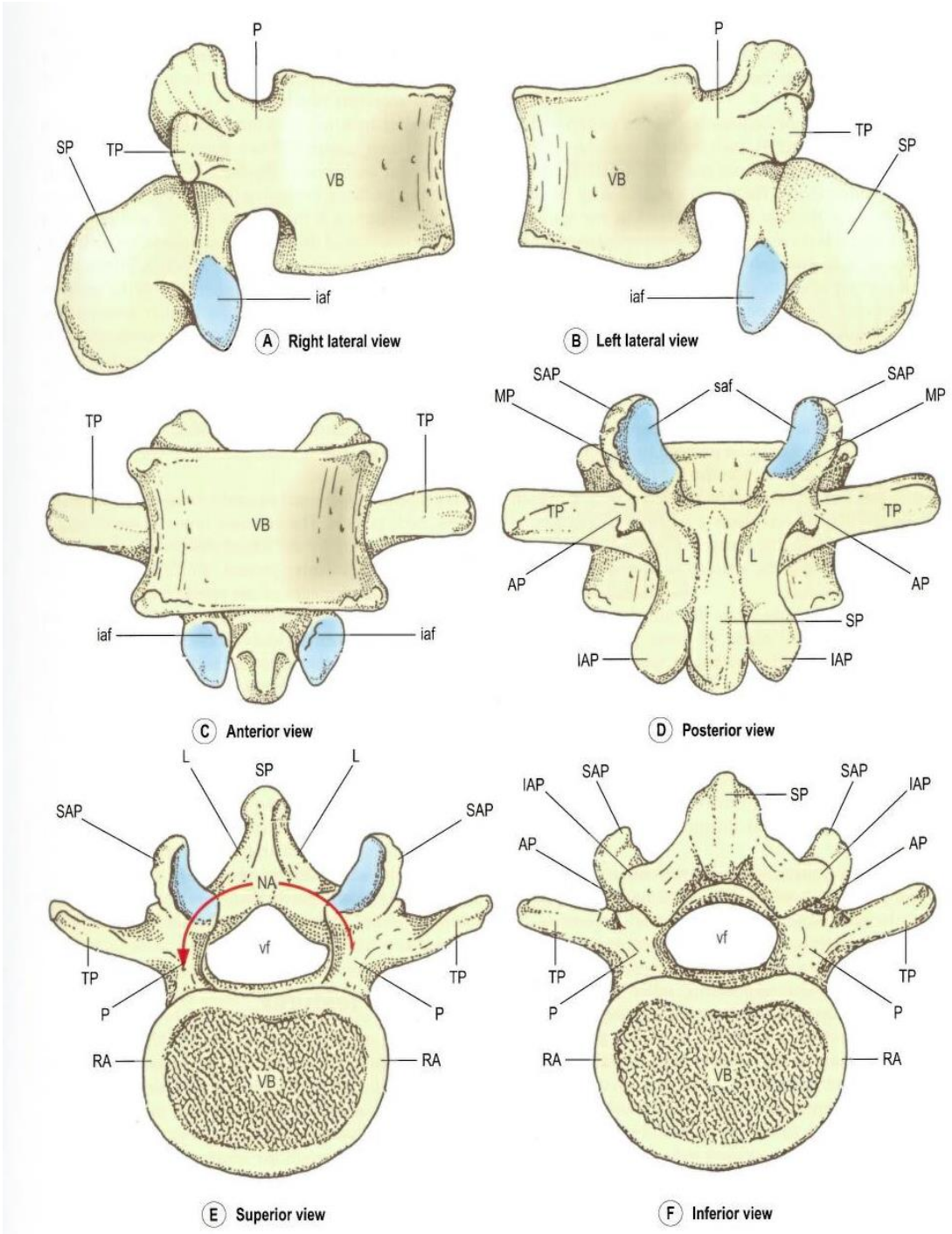

Figure 3 - Anatomy of the human lumbar vertebrae. The vertebral body (VB) is the main load bearing component of the vertebrae and connects directly to the intervertebral disc. The anterior process (AP), transverse process (TP), spinous process (SP) and articular facets (SAF, IAF) form the posterior processes, the joint which allows spinal movement. From Bogduk, page 3.

2.3.3 **Intervertebral Disc**

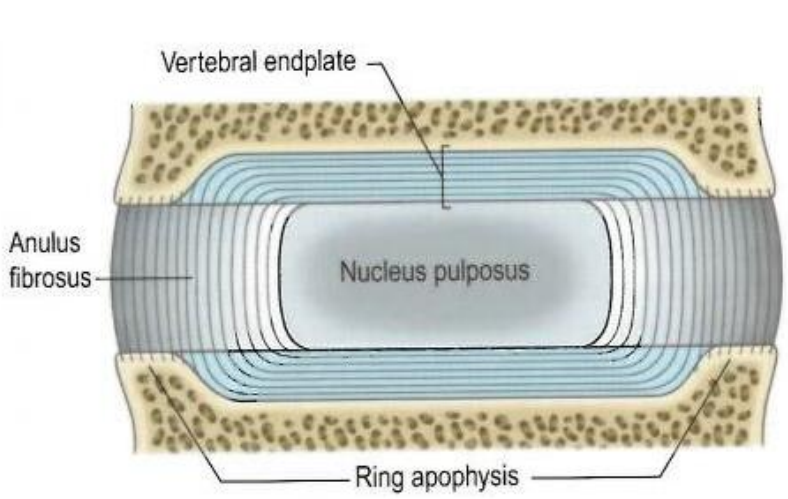

Figure 4 – Sagittal view of the IVD with adjacent vertebrae. From Bogduk, page 40.

The intervertebral disc (Figure 4) is a non-homogenous, composite structure comprising collagen fibres in a hydrous proteoglycan gel matrix and consisting of two distinct structures, the Annulus Fibrosus surrounding the Nucleus Pulposus. The IVD both allows and restrains movement between vertebrae. Outer layers of fibre-filled lamellae surround a semi-liquid nucleus which serves to equally distribute loading across vertebral bodies. The annular fibres of the disc hold vertebrae together much like ligaments. Although lamellae propagate throughout both the annulus fibrosus (AF) and cartilage endplate the two structures are considered to be distinct structures within the disc as demonstrated by the horizontal lines in Figure 4.

2.3.4 **Annulus Fibrosus**

AF forms the outer layer of the IVD and consists of concentric lamellae of highly organised collagen fibres prevented from buckling by a proteoglycan gel which binds the fibres and adjacent lamellae together (Figure 5). Fibres within a single lamella are parallel whilst those in adjacent lamella are oriented

obliquely, this layout allows for angular movement (flexion, extension) whilst stabilising against shear and torsional forces.

Fibre angle can vary from 40 to 70 degrees from the horizontal and this angle becomes more acute under compressive loading providing elasticity in the IVD [35, 36]. Fibres are aligned more closely to parallel posteriorly whilst the lamellae are thinner and more tightly packed despite less binding proteoglycan gel. In the lumbar spine the posterior lamellae are only half the thickness of the anterior and lateral lamellae predisposing it to degenerative changes.

Although the AF forms a complete disc, in any single quadrant approximately 40% of the total 10-20 lamellae are incomplete. Lamellae which pass the terminus of an incomplete lamella may either fuse or become opposed (Figure 5) [35].

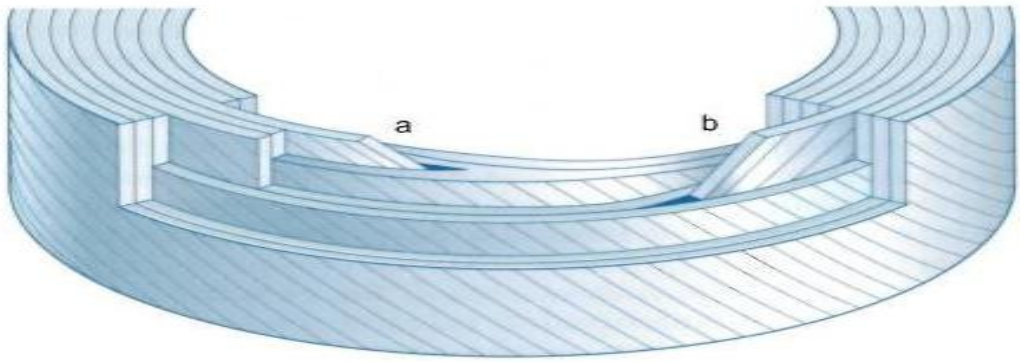

Figure 5 – Lamellae forming the annulus fibrosus, are not all complete rings. Those which pass the end point of an incomplete lamella can become (a) fused or (b) opposed. Bogduk, page 13.

2.3.5 **Nucleus Pulposus**

A semi-fluid gel which accounts for between 40% and 60% of the whole disc, the nucleus pulposus (NP) distributes hydraulic pressure evenly across the IVD. At birth the NP is greater than 85% water and is clearly distinct from the rest of the disc, with age collagen content increases whilst water content and its distinction from the AF decrease. The NP is centralised in thoracic discs whereas it is positioned slightly posteriorly in cervical and lumbar IVDs.

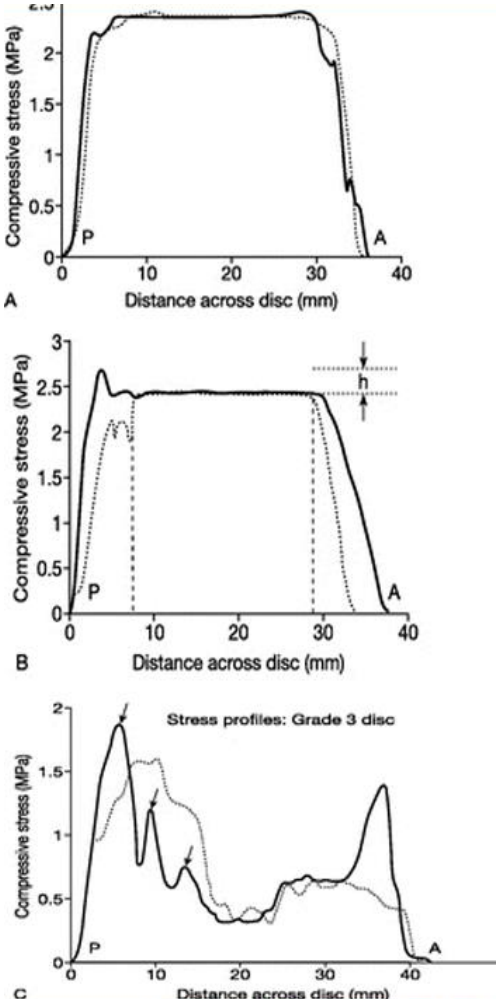

Figure 6 – Vertical (bold) and horizontal (fine) compressive stress profile across full width of three IVDs; young (a), middle aged (b) and degenerate (c).

The hydrostatic pressure of the NP equalises compressive stress across the disc (Figure 6a) with the Annulus Fibrosus (AF) acting to contain the proteoglycan fluid of the nucleus. With age the water content of the NP decreases and axial loading is taken up by the walls of the annulus leading to stress concentrations in those regions (Figure 6b) [37]. A disc which is physically disrupted supports load in a haphazard manner that is not uniform across the surface of the disc (Figure 6c).

2.3.6 **Vertebral Endplate**

Approximately 1mm thick the endplate separates the IVD from adjacent vertebrae. A harder epiphyseal ring surrounds the main hyaline cartilage plate which covers the nucleus; the inner lamellae of the annulus are firmly anchored to the end plate. Well vascularised in infancy the endplate blood vessels involute in the first 10 – 15 years leaving weakened areas, under compressive loading the endplates are the most common site of failure and are therefore considered to be the weakest part of the disc [35, 38, 39].

2.3.7 **Disc Size and Shape**

The shape of the IVD corresponds with that of the adjacent vertebral bodies. Discs which are flattened posteriorly have a greater number of fibres in that region than kidney shaped or concave discs resulting in better protection in flexion but lesser intorsion.

The height of the IVD relative to the adjacent vertebral body varies according to the region of the spine. The cervical spine has the largest IVDs relative to the vertebral body due to the high level of movement required by the upper spine whereas the IVDs in the lumbar spine are the largest in real terms due to the high loading found there. IVDs in the lordotic regions are thicker anteriorly with the largest variation in the L5-S1 disc which is 6-7mm thinner posteriorly [36].

2.4 Disc Degeneration and Treatment

Whilst IVDs are known to degrade with age, degeneration is considered to be a distinct condition from age related changes, this in spite of the fact that no clear consensus exists on the exact definition of either condition [40]. No standard system for grading the health of IVDs has been universally adopted although several have been proposed, an example is shown in Figure 7 [41]. There is broad agreement in each of these systems that degeneration involves the loss or reduction of key material and tissue functionalities but which functionalities, how to measure them and how to distinguish the results are still open to debate.

Table 1. Classification of Disc Degeneration*

Grade	Structure	Distinction of Nucleus and Anulus	Signal Intensity	Height of Intervertebral Disc
I	Homogeneous, bright white	Clear	Hyperintense, isointense to cerebrospinal fluid	Normal
II	Inhomogeneous with or without horizontal bands	Clear	Hyperintense, isointense to cerebrospinal fluid	Normal
III	Inhomogeneous, gray	Unclear	Intermediate	Normal to slightly decreased
IV	Inhomogeneous, gray to black	Lost	Intermediate to hypointense	Normal to moderately decreased
V	Inhomogeneous, black	Lost	Hypointense	Collapsed disc space

Figure 7 – Classification of disc degeneration, an example of a five tier grading system.

It is unclear whether IVD degeneration and injury is mainly a biological or mechanical issue. Battie *et al.* conducted a study on 115 male identical twin pairs, 35-70 years, and by comparing disc health with a thorough evaluation differences in physical activity between twin pairs were able to determine that genetics explained 77% of variation in disc health leading to the conclusion that inheritance is the largest single determinant in disc degeneration [42]. However a study by Lotz *et al.* found that compressive loading of vertebrae in mice resulted in dose dependent harmful biological responses including increased apoptosis within the NP [43]. Mechanical loading has been shown

to induce herniation and radial fissures in otherwise healthy discs if that loading is severe or prolonged enough [44, 45]

IVDs are the largest avascular structure in the body [46]. The networks of blood vessels in the IVD endplate involute between the approximate ages of 10-15 years and are restricted to the outer most layers of the AF [47]. The lack of blood vessels entering the disc means that nutrient absorption is limited to that which can transfer from adjacent vertebrae, resulting in poor self-healing properties [35]. Although metabolic activity in discs increases post trauma, deficiencies in metabolite transport, mean that adult discs are limited in their ability to recover from mechanical injury [40, 48]. This limited regenerative ability has been linked to a loss of stem cell potency within the nucleus with notochord cells disappearing by approximately four years of age [49, 50].

Studies have repeatedly shown that mechanical damage to the disc leads inexorably to further degeneration, Adams *et al.* comment that biological degeneration always occurs after 'scalpel induced' damage in animal studies [37]. Osti, Vernon-Roberts and Moore made cuts to the outer annulus of 21 live sheep and found significant degeneration of the inner annulus in all of the sheep at a later date with most having degenerated within 4-12 months but no longer than 18 [51]. Holm *et al.* found significant degeneration in the IVDs of 6 pigs 3 months after perforating the NP *In vivo* [52].

Whether this phenomenon extends to humans and whether damage caused by injury results in the same effect as that artificially induced with scalpels or needles is the subject of debate. Kerttula *et al.* found 8 out of 14 adolescents (mean age 15.5 years) who had suffered wedge compression fractures to

show significant signs of IVD degeneration after a minimum of 1.1 years post trauma (Figure 8) [53]. Wang *et al.* concluded that “Endplate fracture is strongly associated with disc degeneration” [54].

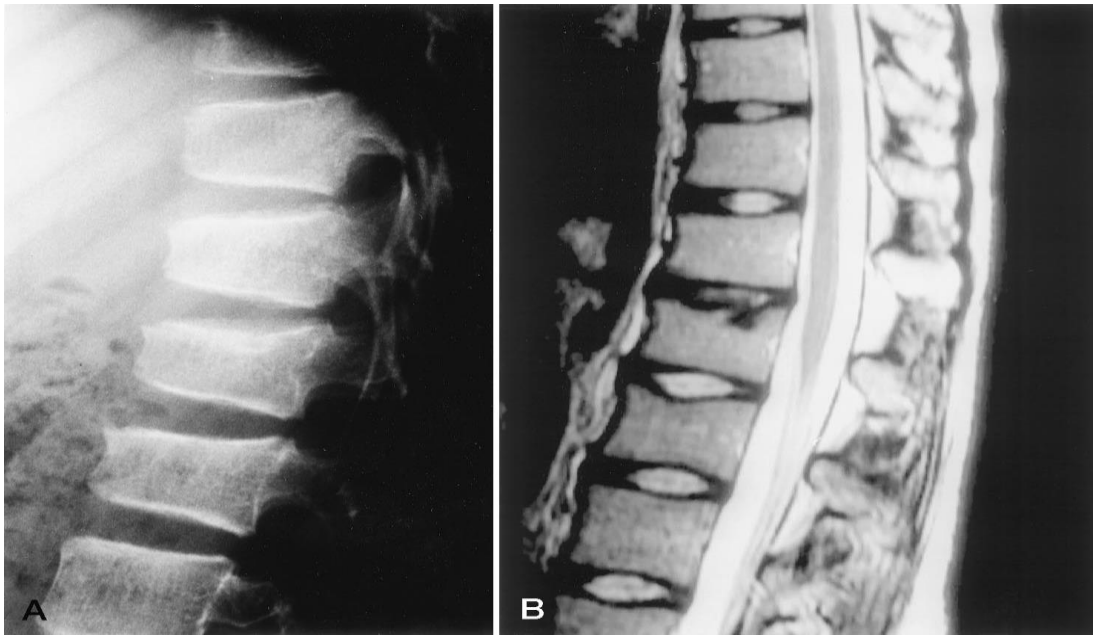

Figure 8 - Radiography showing wedge compression fractures to the T11-L2 vertebrae, note the Schmorl’s node on the upper surface of L1. B – MRI scan 3.9 years post trauma, vertebrae show signs of wedge shape and considerable degeneration is visible in the L1-L2 disc.

However Moller *et al.* found no evidence that vertebral fractures in childhood lead to increased degeneration of the IVD [55] whilst Dickson and Butt were particularly critical of the Kerttula paper citing a lack of control for familial factors and questioning the assumption that abnormalities in radiographic images were the result of injury [56]. Hancock *et al.* used 37 pairs of monozygotic twins, mean age 50.2 years (SD, 9.7) to account for genetic variation and controlled for a number of other confounding variables such as weight and smoking habits when investigating whether recalled back injuries are a predictor in lumbar disc degeneration; they found patient reported back injuries were not an important factor in future degeneration [57].

The use of a large number of monozygotic twins adds significant credibility to the Hancock study however the use of patient reporting of back injury is problematic as the reliability of patient recall has been shown to be highly inaccurate [16]. In spite of the significance of genetic factors much lumbar degeneration is still unexplained by genetics [58] and Carragee *et al.* found significant links between disc puncture with small gauge needles and accelerated disc degeneration [59]. The Hancock paper itself also found significant (P=0.007) differences in the heaviest weight lifted in a month between the twin reporting previous back injury and the non-injured twin. The mean average heaviest weigh lifted by the injured twin was 15.6 kg more than the non-injured twin, a clear link between mechanical loading and degeneration.

2.4.1 Mechanical Characterisation of Degeneration

It is clear that there is no consensus on the exact definition of disc degeneration but the investigation of mechanical behaviours of the IVD must consider and define degeneration by quantifiable changes in material behaviours and properties such as elastic stiffness, strain and pressure in the disc. Such mechanical behaviours have previously been shown to clearly differentiate healthy and damaged discs (Figure 6, page 16) [37].

The hydrostatic pressure of the IVD nucleus equalises compressive stress across the disc (Figure 6a) with the outer annulus structure acting to contain the proteoglycan fluid of the nucleus. With age the water content of the NP decreases and axial loading is taken up by the walls of the annulus leading to stress concentrations in those regions (Figure 6b). A disc which is physically

disrupted supports load in a haphazard manner that is not uniform across the surface of the disc (Figure 6c).

Such changes are clear and quantifiable measures of the effect of damage and degeneration on the IVD and present a clear theoretical case for why degeneration is such a significant health concern as the discs ability to function is reduced.

2.4.2 **Spinal Fusion**

Current methods to prevent further damage or pain due to disc degeneration centre on spinal fusion, an invasive medical intervention that results in permanent loss of motion between adjacent vertebrae [60, 61]. Spinal fusion surgery is increasingly common with over 400,000 fusion surgeries conducted in the USA each year at a cost of $34 billion, this represents a 137% increase in surgeries between 1998 and 2008 [61, 62].

Two main forms of spinal fusion surgery are used, posterolateral fusion (PF) which results in fusion of the transverse processes of adjacent vertebrae and posterior interbody fusion which fuses the vertebral bodies of adjacent vertebrae and requires a discectomy to allow fusion. Anterior interbody fusion (AIF) also exists but it is less common due to the challenges of accessing the spine through the anterior side of the body [63]. Fusion of two adjacent vertebras are known as “single level” whereas fusion joining three or more is called “multi-level” fusion. PF benefits from avoiding the spinal cord during surgery which must be retracted during PIF surgery, PIF presents a greater

surface area for fusion but whether this results in improved patient outcomes is not clear [64, 65].

Fusion is achieved by implanting bone chips, which may be harvested from the patient or a donor, into the area that fusion is desired in order to encourage bone growth between vertebrae [66, 67]. Instrumented fusion provides additional support to stabilise the joint during the fusion process. Instrumented PF utilises rods between vertebrae, attached via screws into the pedicle, whilst PIF additionally requires interbody 'cages' to maintain a vertebral gap following discectomy (Figure 9) [68].

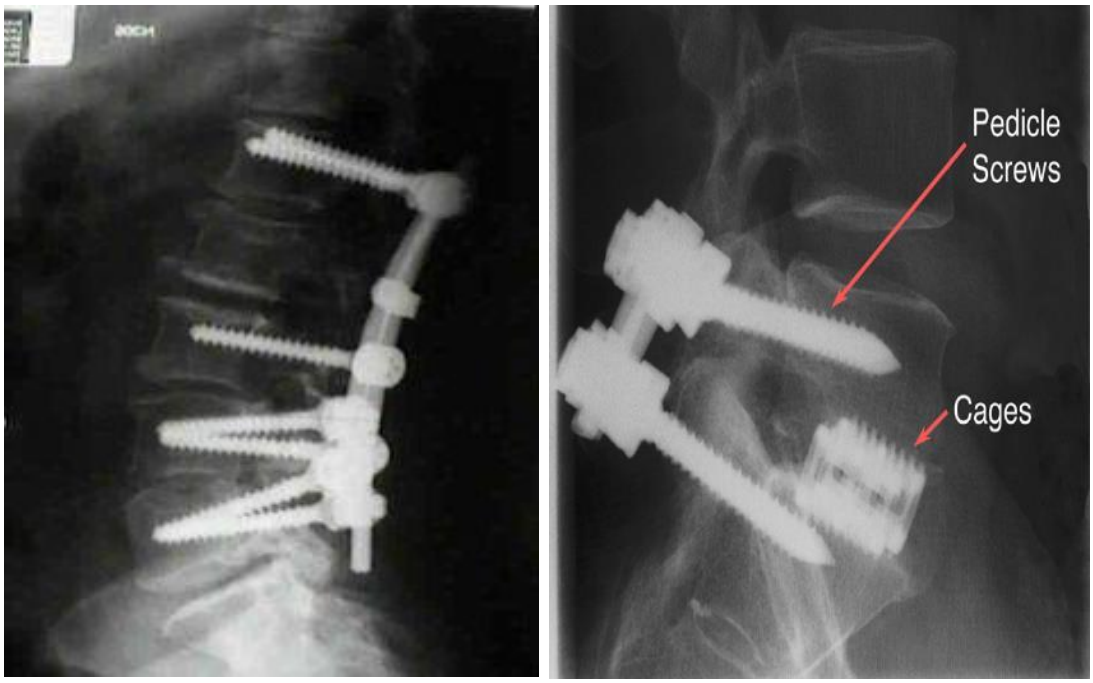

Figure 9 – Multi-level instrumented cervical fusion (left). Pedicle screws attached to a rod between the vertebrae secure the spine during the ossification process. Cages fill the IVD space following discectomy during interbody fusion (right).

Spinal fusion is highly invasive and results in a partial or total loss of mobility in the vertebral joint at the fusion site [69]. Loss of mobility in the joint is a

significant quality of life issue and patients with lower postoperative mobility demonstrate worse clinical outcomes [70].

Fusion surgery is only minimally successful in reducing back pain with only 20% of patients pain free within 5 years and 22.2% of patients suffer unsatisfactory outcomes from spinal fusion [60, 71]. Posterior lumbar interbody fusion (PLIF) in particular has a high rate of failure with 13.2% of patients requiring follow up surgery within 5 years [72].

Mortality rates for spinal fusion are 0.2-0.29% and fusion surgery mortality was significantly ($P < 0.001$) higher than other forms of spinal surgery [62, 73].

2.4.3 **Regenerative Medicine**

Regenerative medical interventions aim to reverse the degeneration process by aiding disc regrowth and preventing the need for discectomy and fusion. A regenerative approach being explored by a number of groups is the use of mesenchymal stem cells (MSC) to induce regrowth of a degenerate nucleus pulposus (NP). These treatments could recover the disc properties without the loss of mobility resulting from spinal fusion [74-78].

Such treatments would require MSCs to be introduced into the NP and one potential method is to inject the cells directly into the IVD within a scaffold matrix hydrogel, a significantly less invasive treatment intervention than the open surgery required by spinal fusion [63, 66, 69, 75-77, 79].

2.5 The Importance of the Lumbar Spine

The lumbar spine is of particular interest in relation to back pain and degeneration due to the high incidence of LBP and the increased likelihood of problems with the IVD in this region [80].

The human spine has undergone significant adaption in comparison to apes to enable bipedal movement [81]. The upright posture requires that the spinal column takes axial loading and this is naturally higher in the lumbar spine due to its lower position in the loading column. The ability to balance whilst walking requires body weight to be evenly spread over the legs; this is achieved through the spinal curvatures of which the lumbar lordosis is the most significant. This is particularly true of the wedge shaped L5 vertebrae and the adjacent wedge shaped L4-5 and L5-S1 discs.

95% of disc herniations occur in the L4-5 or L5-S1 discs [82], the combination of the wedge shape, which increases shear loading on the disc, and the relatively high axial loading is a likely factor in the high level of disc degeneration in this region. The L5 vertebrae is also the most common site for both Spondylolysis – Degeneration of the facet joint – and Spondylolisthesis – displacement of vertebrae with respect to adjacent vertebrae [83].

These facts make the lumbar spine the key area for research in reference to specific back pain, disc degeneration and treatment.

2.6 **Bioengineering – Engineering in Biology**

Although the IVD has long been the subject of medical interest, investigation of its properties as an engineering material began in earnest in the mid-20th Century when IVDs began to be subjected to basic *in vitro* mechanical tests, primarily concerned with its elastic behaviour. Virgin measured deflection due to progressive static loading and hysteresis after unloading on cadaveric discs (Figure 10) [84]. Hirsch and Nachemson monitored the load deflection behaviour of human cadaveric lumbar discs (0-90 years), finding that degenerate discs were more easily compressible than healthy discs with deflections of 2 mm and 1.5 mm respectively under 100 kg load [85]. Brown, Hansen and Yorra loaded human cadaveric lumbar discs to failure and reported on how mechanical stress resulted in the failure of disc integrity with a 150% increase in disc volume before failure [86].

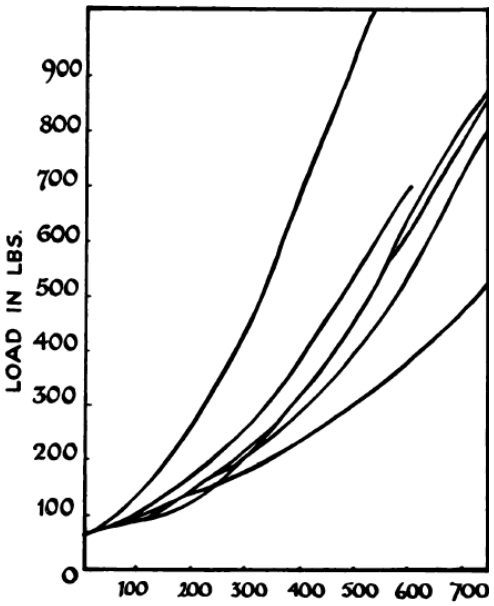

Figure 10 – Load deflection curves of six discs under static loading.

Markolf and Morris applied traditional engineering techniques to human cadaveric lumbar discs (18-58 years) which had been structurally altered; damaging the annulus wall, injecting the discs with saline and removing the nucleus completely ”to determine the contributions of the various parts of the intervertebral disc to its ability to withstand compressive forces” [87]. They found greater compliance in the first loading cycle than those subsequent cycles and noted that no significant

effects were caused by saline injection. They also observed the many expected behaviours of VEMs, increased stiffness with load and creep under constant load. Due to the limitations of data acquisition tools of the time their results were only reported graphically making comparison with modern tests difficult.

The complex viscoelastic behaviour of the IVD means that the results of static or quasi-static testing are of limited value in assessing IVD behaviour under physiologic conditions; loading conditions became more complex in order for boundary conditions to more closely represent those found *in vivo*. Adams, Hutton and Stott applied realistic loads to induce flexion in intervertebral joints and investigate strain in the interbody ligaments [88] finding the IVD to play a greater role in resisting flexion than the Ligamentum Flavum or the supraspinous/interspinous ligaments. The intervertebral joint was found to resist as much as 50% of the bending moment due to trunk flexion. Nuckley *et al.* demonstrated a clear positive relationship between displacement rate and both disc stiffness and ultimate failure load but found no relationship between displacement rate and failure strain [89]. They showed that stiffness increased from 161.4 ± 30 N/mm at 0.5 mm/s to 351.4 ± 40.9 N/mm at 5000 mm/s with failure load 468.3 ± 177.3 N and 2189.5 ± 659.5 N respectively. Having already analysed the creep response of human cadaveric lumbar segments under static loading [90], Hansson, Keller and Spengler applied cyclic compression to lumbar motion segments simulating “rigorous activity” [38]. They noted that age and disc degeneration was correlated not only with stiffness and the number of cycles required to induce fatigue failure but also of the bone mineral

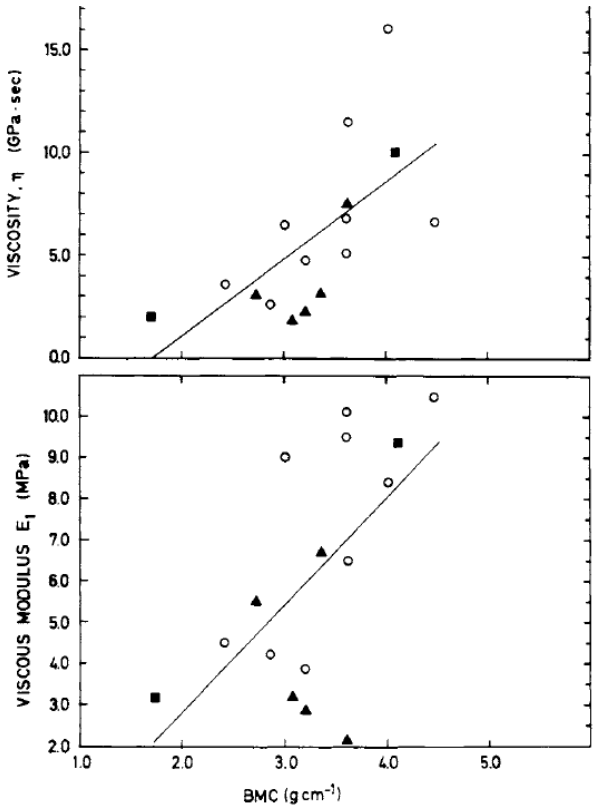

Figure 11 - Correlation between bone mineral content and material properties was shown by Hansson, Keller and Spengler. Increased BMC was linked to higher viscous modulus in the disc.

content (BMC) of the vertebrae segments to which the discs were attached (Figure 11).

As loading routines became more complex in order to simulate boundary conditions more reflective of activities of daily living the inter-disciplinary nature of IVD research became apparent as connections between mechanical and biological phenomena were investigated. Keller *et al.* had based their loading levels on vertebrae BMC [90] which has been shown to correlate with typical loads applied [91, 92]. Ishihara *et al.* found proteoglycan synthesis rates were affected by the hydrostatic pressure within the disc [93], 3atm pressure stimulated synthesis whilst 30atm inhibited synthesis suggesting a complex relationship between IVD biochemistry and mechanical loading.

Due to *in vivo* testing being limited in scope by practical and ethical concerns, much of the research on the IVD had been conducted *in vitro* before Keller *et al.* demonstrated that physiologic conditions affect the mechanical response of the IVD by showing that discs inside living subjects being more compliant than those tested *in vitro* at loads of 300N [94]. Intradiscal pressures had first

been measured *in vitro* by Nachemson in 1960 [95] and little further work was conducted in this area before Sato, Kikuchi and Yonezawa and Wilke *et al.* conducted *In vivo* pressure measurements in 1999 [96, 97]. Although estimates of vertebral loading can be made using a mechanics approach the insertion of a pressure transducer into a disc remains the only direct measure of spinal loading *in vivo* and as such the Sato and Wilke papers, along with a further 2006 study by Lisi *et al.* [98], form the bedrock of knowledge regarding spinal loading.

Sato et al. measured IVD pressure in a range of static postures including supine, seated and standing. Wilke et al. measured a wider range of dynamic activities gaining insights into the upper and lower pressure limits during those activities including many ADLs. Lisi et al. conducted a study measuring the pressure experienced by the IVD during chiropractic manipulation [98]. The baseline data they recorded of subjects in the prone and lateral decubitus positions offer an important comparison against the Sato and Wilke papers. Data where these papers overlap can be seen in Table 2, page 42 and full results from each study are included in the appendix.

The increasing ubiquity of MRI scanners offered researchers new ways of investigating the IVD, both *in vitro* and *in vivo*, although limited to a snapshot rather than a dynamic view, detailed investigation of IVD size, shape and orientation of the IVD became possible. Wisleder *et al.* and Kimura *et al.* measured the acute response of the spine under body weight loading, 100% and 50% respectively, using a spine compression unit (SCU) [99, 100]. The ability of SCUs to replicate physiologic conditions was questioned by Hioki *et*

al. which found varying lumbar alignment between scans taken in the upright position and those in the supine position using an SCU [101].

With most MRI scanners limited to prone or supine positions and only able to capture ‘snapshots’ investigators have focused on the effects of prolonged and/or diurnal loading. Botsford, Esses and Ogilvie-Harris measured disc height loss in male participants subjected to a simulated diurnal loading cycle compared to a control group [102] finding a 21.6% mean decrease in disc volume. Malko *et al.* investigated volume changes in lumbar discs of subjects also under a simulated diurnal loading [103] with discs gaining 5.4% in volume in the 3 hours after loading was removed. Kingsley *et al.* conducted lumbar scans before and after subjects underwent 30 minutes of moderate intensity running, finding a 6.9% mean decrease in disc height suggesting that disc height is affected by relatively short periods of loading.

2.7 Biomaterials Testing and Analysis

Understanding the load/deflection behaviour of the IVD and how it varies with age and degeneration for activities of daily living is vital due to the major structural role of the disc therefore measuring the strain and elastic stiffness of the disc under loading is a priority.

It is clear that the measurement or calculation of loading and deformation to derive stress-strain relationships will be necessary to answer key research questions.

The IVD is only one component in the spinal structure and the vertebrae are also highly involved in withstanding compressive loading during ADLs. It might

therefore be reasonable to include vertebral bone in testing except for the fact that Young's modulus of trabecular bone has been at 14.8 GPa and cortical at 20.7 GPa [104]. The stiffness of bone is several orders of magnitude greater than that of the IVD and therefore of negligible importance to compression under loading.

An additional factor of complexity is that the IVD is a VEM which will dissipate energy through viscous friction within the material making its behaviour time, load and velocity dependent. Measuring this energy dissipation is another key priority and will provide a measure of tissue functionality which should vary with condition of the IVD.

These material and tissue behaviours must be comparable between discs in different states of health, degeneration or having undergone hydrogel treatment intervention to ensure the work is clinically relevant. Any test methodology therefore needs to not only simulate ADLs but also be highly repeatable and consistent.

2.7.1 *In vivo* Testing

In vivo testing is experimentation conducted in or on a living organism.

In vivo experimentation has the primary advantage that it is, by default, an accurate reflection of biological and physiological conditions. Any experiments conducted *in vivo* account for the complex relationships between various bodies and systems within a living organism and are true reflections of physiological boundary conditions. In theory designing an *in vivo* experiment

which reflects ADLs is as simple as getting participants to engage in ADLs, in practice *in vivo* techniques have significant challenges and limitations.

In vivo experimentation is limited by the inherent difficulty in taking measurements from internal bodies. Imaging techniques such as X-ray computed tomography (CT) and Magnetic Resonance Imaging (MRI) allow a detailed view inside the body but have several limitations:

- Only static snapshots gained. No dynamic, real time data.
- Only physical dimensions (length, area, volume etc.) can be measured from images.
- Subject position and posture is limited by the physical space within the imaging device.
- Non-instantaneous scan time, bodies under loading are liable to creep during scans.
- X-ray and CT require the use of ionising radiation.

Real time *in vivo* techniques such as Fluoroscopy, Dynamic CT and Dynamic MRI overcome *some* of these issues allowing real time image capture but are much less common than static imaging methods and are not available at MMU [105-108]. Ultrasound imaging is a more readily available tool and offers a relatively simple dynamic image capture method, it is available at the Manchester Metropolitan University School of Healthcare Science for research but because it interacts strongly with bone it is unsuitable for investigation of IVDs due to the proximity of the adjacent vertebrae. Bone strongly reflects and distorts the acoustic waves used by ultrasound scanners greatly reducing

image quality and making it impossible to gain useful images of other bodily structures like IVDs.

Beyond the use of imaging it is possible to gain real time *in vivo* data from internal bodies, however the methods required are invasive, require clinical specialists and are potentially ethically questionable.

A number of studies have inserted needle thin pressure transducers into the IVD of live volunteers in order to measure the internal pressure during ADLs and these studies provide the foundation of *in vivo* knowledge of spinal loading [96-98, 109]. Such studies have been limited in terms of participant numbers (1-8) due to the highly invasive test protocol and the high requirement for specialist supervision during testing. In light of the large body of evidence showing that any damage to the IVD may lead to future degeneration, any invasive test protocol is highly questionable ethically and should be avoided [37, 53, 54, 110, 111].

2.7.2 ***In vitro*** **Testing**

In vitro testing is experimentation conducted on tissues or bodies removed from the organism.

In vitro experimentation removes and isolates the body under consideration; this method greatly increases test and measurement possibilities at the expense of true physiological boundary conditions.

Many of the challenging limitations of *in vivo* testing are negated by using *in vitro* methods due to the greatly reduced ethical considerations. Although

there are clear ethical practices required in obtaining tissue for *in vitro* experimentation, once tissue has been acquired participant comfort is no longer a factor making invasive test protocols acceptable and allowing the investigation of test loads and durations that might be uncomfortable or even harmful in live participants.

In vitro testing of material properties of tissues is typically conducted on a mechanical test rig such as the materials testing machine in Figure 12 although specially designed custom test rigs are also used (Figure 13) [112]. Materials testing machines are controlled either by the force exerted on the sample or by the displacement of the rig. Materials testing machines provide relatively easy access to the tissue sample allowing for separate measurement and recording devices to be used in conjunction with the rig itself.

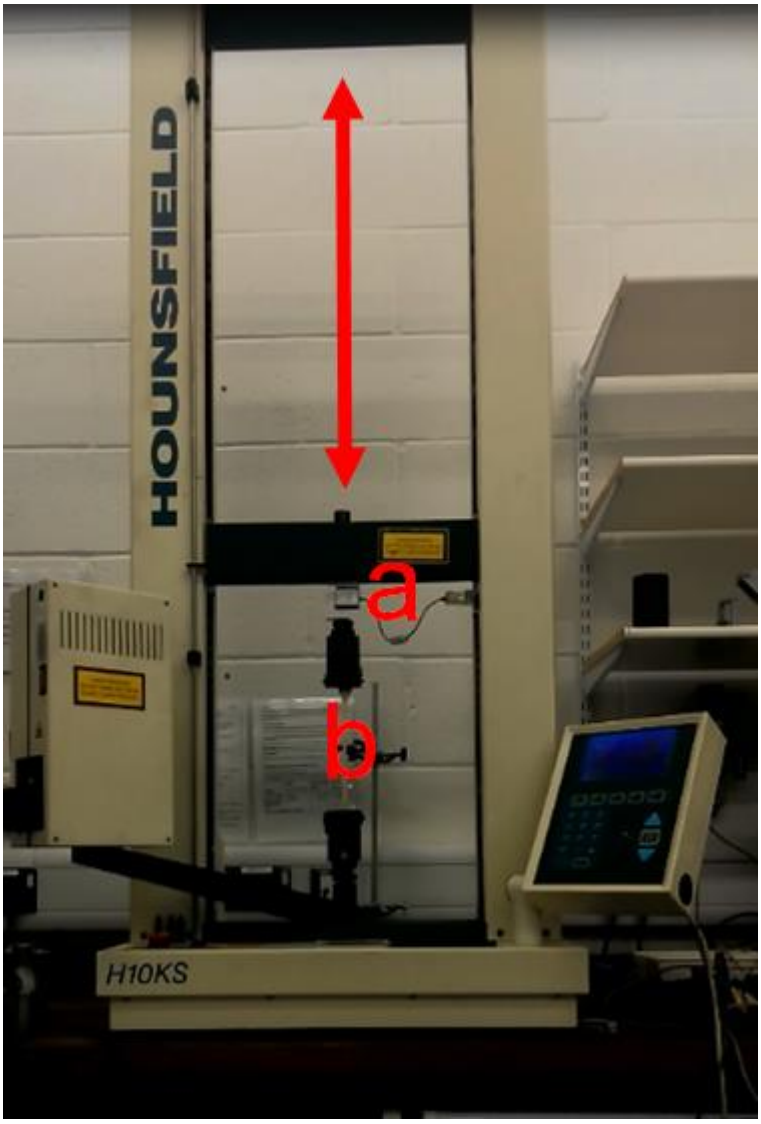

Figure 12 – A quasi-static tensometer, a type of mechanical test rig which uses a load cell (a) to measure the force on a sample (b) as a fixed rate of displacement (tension or compression) is applied.

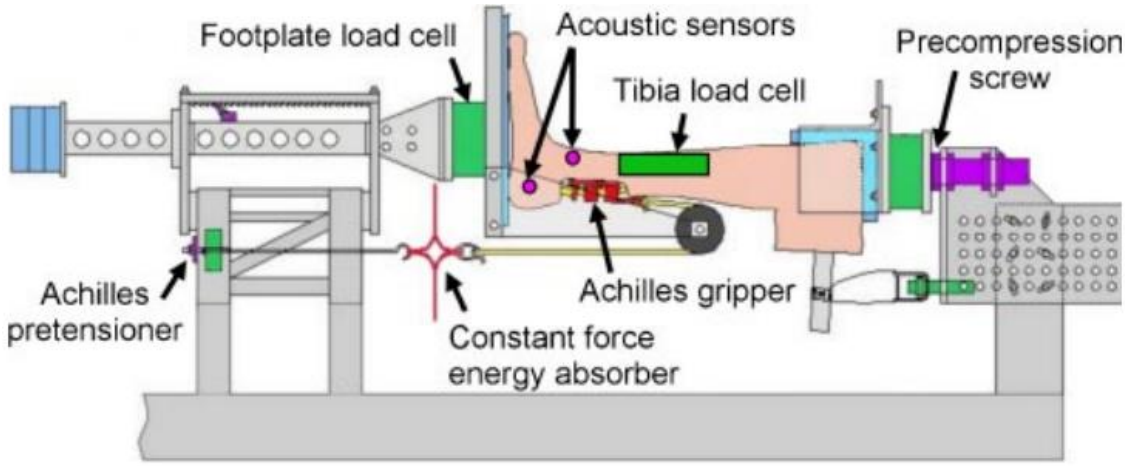

Figure 13 – A highly specialised test rig purpose built to investigate the human foot and Achilles tendon, from Funk et al. 2002.

Although this level of measurement and control possibility represents a huge benefit of *in vitro* test methodologies there are a number of key disadvantages to *in vitro* experimentation. The removal of tissues from the body changes not only the boundary conditions of an experiment but may fundamentally alter the material properties of the tissue itself. *In vitro* mechanical testing may only represent a simplified proxy of *in vivo* behaviour as the many complex, multi-directional force interactions which occur in the body cannot always be replicated *in vitro*.

2.7.3 **Modelling**

Modelling is the creation of a mathematical or conceptual representation of a tissue based on previously determined data (material properties etc.) which allows for theoretical experimentation without the need to physically conduct tests *in vivo* or *in vitro*.

Modelling of tissues can broadly be split into two categories:

2.7.3.1 Spring-Damper Models

Spring-damper models are combinations of springs and dampers combined to create “mechanical analogues” of VEMs including tissues [113]. Spring-damper models such as Kelvin-Voigt models (Figure 14) provide force-deformation relations which attempt to mirror the one dimensional behaviour of VEMs and can predict or simulate the strain behaviour of VEMs under some simple conditions [114].

A simple Maxwell model consisting of a spring and damper in series will exhibit relaxation behaviour, an exponential decrease in stress over time following an applied strain. A Kelvin-Voigt model such as that in Figure 14 can model material creep, a continuing deformation of the material under constant stress.

Increasingly accurate material behaviour can be achieved by adding Maxwell models in parallel with a single spring. This generalised Maxwell model can be customised to match material behaviour but is artificially contrived with the number, order and nature of the various spring and damper elements attempting to mimic viscoelastic behaviour rather than being based on actual properties of the material [115].

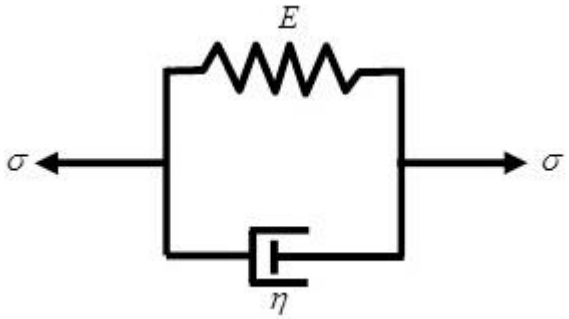

Figure 14 – A Kelvin-Voigt model represents a simplified analogue of a viscoelastic material.

2.7.3.2 Finite Element Analysis

Finite element analysis (FEA) is a theoretical modelling approach to analysing how an object reacts to inputs (loads, temperatures etc.) that splits the body into small parts or 'finite elements'. By applying the material properties to each element, constraints and inputs to the larger body the computer software can calculate the effect of those inputs throughout the material based on constitutive equations of the materials the model is built from.

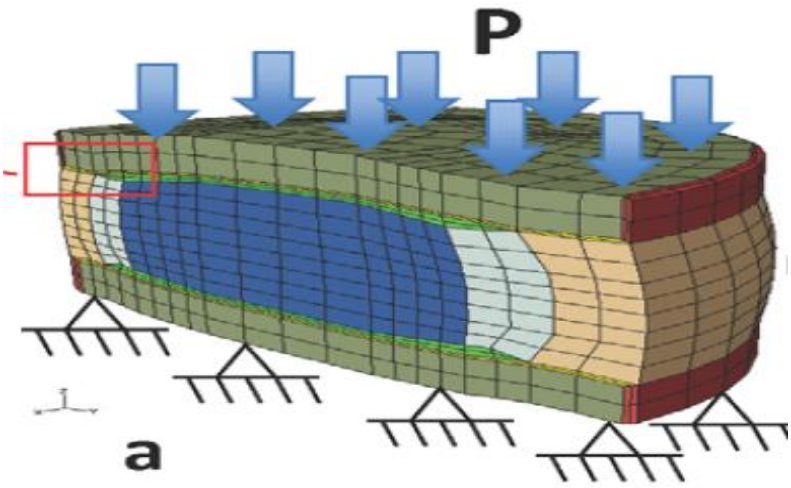

Figure 15 –A 3D FEA model of the IVD showing applied forces and constraints [116].

FEA approaches go beyond spring-damper models in allowing 3D analysis of material behaviours rather than simple, 1D force-deflection behaviour (Figure 15). FEA models of VEMs also make use of more complex material responses such as non-linear hyperelastic or poroelastic material behaviour allowing for more accurate and nuanced simulation of tissues under loading.

Modelling, whether spring-damper or FEA based offers a number of benefits in materials analysis:

- No invasive experimentation required, entirely computer based.
- Tests can be altered and re-run multiple times without consuming materials.
- Highly complex boundary conditions can be investigated negating the limitations of test rigs.
- FEA provides material behaviour throughout the material allowing for highly detailed behavioural analysis not otherwise possible.

However modelling also has limitations:

- Limited range of behaviours can be modelled with spring-damper systems
- The more detailed or complex an FEA test the more computationally expensive it becomes greatly increasing solution times.
- Material and boundary condition data must either come from experimental work in the literature or otherwise be “guessed” and then verified against experiment.
- Modelling of heterogeneous materials, such as the IVD, is highly complex and represents a field of research in its own right. Such demanding work is not feasible within the scope of this project.

The requirement for pre-existing data on which to base models is a significant limitation of modelling in regards to answering the questions asked by this thesis. Existing data on the IVDs material behaviour under ADLs is severely limited and no previous investigations have observed material behaviours of discs injected with hydrogel. The work contained within this thesis should

provide valuable, clinically relevant material property and verification data for computer modelling rather than being used to determine such data.

2.8 Material Properties of the Intervertebral Disc

2.8.1 Intradiscal Pressure

Early work on the properties of the IVD focused on the pressure within the disc during various loading situations, an influential *in vivo* study by Nachemson [95] found human cadaveric discs to have an intrinsic pressure of 70 kPa when unloaded which he attributed to the compressive effect of the Ligamentum Flavum.

More recently both Sato, Kikuchi and Yonezawa and Wilke *et al.* conducted *In vivo* studies of disc pressure; the Sato paper reports on 8 asymptomatic patients (22-29 years of age) in prone, sitting and standing postures including flexion and extension of the spine whilst the Wilke paper only included a single male participant (45 years) but tested across a range of day to day activities [96, 97]. Together these studies form the foundation of knowledge for intervertebral disc loading *in vivo*.

Despite the low number of participants the similarity in magnitude between studies gives some confidence in the accuracy of the results, a further study by Lisi *et al.* which focused on Intradiscal pressures in 2 subjects (41 and 42 years of age) undergoing chiropractic spinal manipulation also shows broad agreement on intradiscal pressures in the prone and lateral decubitus position [98] (Table 2).

Whether sitting results in higher pressure in lumbar IVDs than standing has been contentious since Nachemson reported 40% higher intradiscal pressures in the sitting position [95]. Wilke showed a small, 40 kPa reduction in pressure

in the seated position but only had a single participant, with a larger number of participants Sato is in agreement with Nachemson that sitting raises Intradiscal pressures though the average increase was less than half of that suggested by Nachemson.

Table 2 – Average Intradiscal Pressures and Standard Deviations for Various Body Positions

Position/Pressure(kPa)	Sato[96]	Wilke[97]	Lisi[98]
Prone	91 (± 27)	110	110
Lateral Decubitus	151 (± 53)	120	150
Standing Upright	539 (± 179)	500	N/A
Standing Flexion	1324 (± 222)	1100	N/A
Sitting Upright	623 (± 158)	460	N/A
Sitting Flexion	1133 (± 254)	830	N/A

The possibility that sitting places additional stresses on the lumbar spine raised the hypothesis that more sedentary lifestyles are to blame for rising levels of LBP; however there is no consensus on whether sedentary lifestyles are a risk factor. Some reviews show no association between pain and sedentary lifestyle [117] whilst others show a clear dose dependent association between “high-intensity low back pain” and sedentary lifestyles [118]. The results of recent longitudinal studies suggest that sedentary lifestyle is a result of LBP rather than the cause of [11] and Smuck *et al.* have reported that the role of physical activity in mitigating back pain is “…shown to be of greater consequence in the overweight and obese populations.” [119].

Despite the lack of consensus on these issues there is complete agreement that there is a significant increase in loading relative to the baseline due to flexion of the spine in all cases as well as due to external loading (carrying weights) and by using poor lifting technique [95-97].

2.8.2 **Loading Characteristics**

Several studies have focused on comparing the loading behaviour of ‘healthy’ discs to those which have degenerated or been damaged. Markolf and Morris tested human cadaveric lumbar discs (18-58 years) under a range of *in vitro* loading conditions comparing intact discs with those which had undergone various procedures including injection with saline, radial fissure and complete removal of the NP. They found that load/deformation characteristics were reduced in the first static loading cycle but returned to normal levels within a few cycles [87].

Adams *et al.* damaged cadaveric lumbar discs by subjecting them to compressive loading far higher than that found during typical ADLs (6.7 ± 2.5 kN). Damage was evidenced by a reduction in the load deflection curve gradient and the results of cyclic loading of those discs was compared with baseline results obtained prior to damage[37]. They note that the compressive damage affected the stress profile of the discs with high stress concentrations resulting in the disc annulus and a near complete loss of pressure in the disc nucleus [37]. These results suggest that damage to the nucleus limits the IVD’s ability to withstand loading and that compressive loading is taken up by the AF potentially risking further damage and injury. Unlike the Markolf and Morris paper these changes in stress profile were reported to become worse as loading continued.

2.8.3 **Bulk Properties**

A large number of papers have reported or used values for the bulk mechanical properties of the IVD and its constituent parts but the magnitude

of these values vary widely across the literature [116, 120-132]. Elastic modulus of the AF has been reported between 100 kPa and 8 MPa [129, 133], of the discs collagen fibres between 60 and 500 MPa [128, 130] and Poisson's ratio of the NP between 0.1 and 0.49 [121, 129].

This enormous variation in material properties of the disc highlights the difficulty in categorising material properties of viscoelastic tissues and that various different researchers have employed different test methodologies.

2.8.4 **Viscoelasticity**

As a polymer composite the IVD exhibits both elastic and viscous characteristics when subjected to loading stresses, this viscoelasticity results in time dependent strain behaviour. When subjected to a sinusoidally varying stress, the strain is out of step with the stress by a phase angle between 0 and 90. The phase angle, δ, is described by the storage modulus, E', and the loss modulus, E'', such that [134].

$$\delta = tan^{-1}\frac{E''}{E'} \quad (1)$$

The storage modulus characterises the elastic properties of a viscoelastic material and is the component of the material's stress-strain relationship which is in-phase with applied strain. The loss modulus characterises the viscous properties of the material which result in strain behaviour that is out of phase with applied strain. The double prime signifies that the loss modulus is imaginary, and combined with the storage modulus forms a complex modulus [113, 135, 136].

The viscoelastic behaviour of individual parts of the IVD, both human and animal, has been researched in several papers [124, 137, 138] and of the whole disc in others [139, 140]. Izambert *et al.* subjected human lumbar IVDs to loading sinusoidal cyclic loading from 5-30 Hz and found the stiffness and damping values ranged from 0.19 to 3.66 MN/m and 32 to 2094 Ns/m respectively when the disc was approximated as a Kelvin-Voigt model [140].

Bovine IVDs have been shown to be stress stiffening, having an elastic modulus which increases at higher stresses [141]. Race, Broom and Robertson demonstrated the increasing stiffness of bovine coccygeal discs when subjected to increasing strain rates in quasi-static compressive tests (Figure 16) . These changes have also been demonstrated in baboon [89], pigs [142] and humans [143].

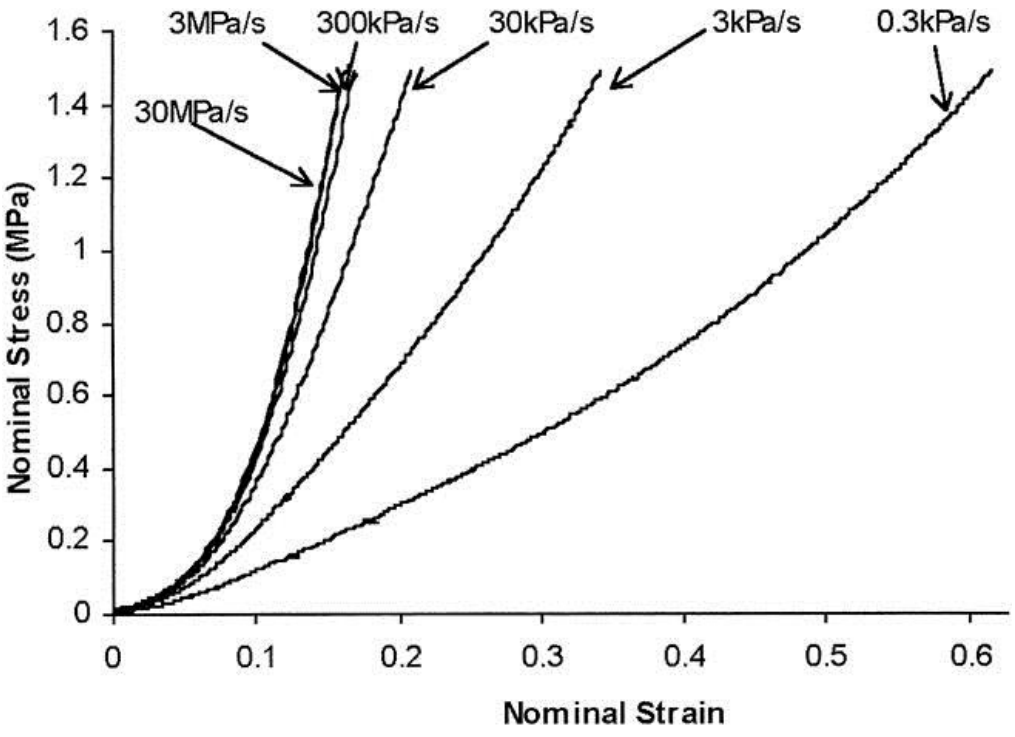

Figure 16 – Stress/strain curves for bovine IVD at increasing loading rates from Race, Broom and Robertson. Increased gradient means a higher elastic modulus; higher strain rates clearly demonstrate higher stiffness in the IVD.

The time dependent, viscoelastic behaviour of the IVD is the probable cause of the wide variation in material property values seen in the literature and highlights the importance of test methodology. With tests conducted variously on human or animal tissue, quasi-statically or dynamically, in saline solution or exposed to air and at a multitude of frequencies, forces and durations it is little wonder that different studies find different results.

This highlights the importance of designing experiments to accurately reflect physiological behaviour and activities of daily living, poorly designed experiments may result in data which is irrelevant to human experience.

Animal tissue is typically used for experiments because of the difficulty in obtaining human tissue, the reduced ethical considerations and the fact that the majority of human cadaveric tissue is from elderly subjects who are in the majority of cases already displaying age related degeneration or damage of the IVD and surrounding bone. The wide variation in tissue condition found in cadaveric discs also requires a larger number of samples be tested despite this being harder to achieve.

Can animal tissue provide an accurate surrogate for human tissue?

When tested under the same protocol the elastic modulus of bovine discs has been observed to differ from that of human by only 30%, far less than the several orders of magnitude variation between human discs in the literature [132]. Bovine discs were found to be stiffer than their cadaveric human counterparts, cadaveric discs are mostly taken from elderly subjects and therefore likely to suffer the effects of ageing which is known to reduce disc

modulus [37, 40, 84, 144]. The elastic modulus of healthy, young human IVDs may be even closer to that of bovine discs than current experiments suggest and bovine discs present an ideal surrogate in place of human discs.

2.8.5 **Viscoelastic Heat Production**

Whereas an elastic material can be described using an elastic modulus and a viscous material using a viscosity a VEM must be described by a complex modulus which accounts for both the in phase storage modulus, E', and the out of phase loss modulus, E''. At a temperature, T, and dynamic excitation frequency, ω, VEMs have a complex modulus E* such that:

$$E^*(\omega,T) = E'(\omega,T) + iE''(\omega,T) = E(\omega)\{1 + i\eta(\omega)\} \quad (2)$$

Where:

$$E' = \left(\frac{\sigma_0}{\varepsilon_0}\right) cos\delta \quad (3)$$

E' is the storage modulus, the relationship between the in phase stress and strain and is related to the elastic potential energy stored by the material. σ_0 is the peak stress and ϵ_0 the strain at peak stress.

$$E'' = \left(\frac{\sigma_0}{\varepsilon_0}\right) sin\delta \quad (4)$$

E'' is the loss modulus, the relationship between the out of phase stress and strain and is related to the viscous dissipation of energy by the material.

The ratio of energy stored to that lost is given by the loss tangent [134]:

$$\delta = tan^{-1}\frac{E''}{E'} \quad (1)$$

For a given rate of work applied to an IVD, $\dot{W}$, the rate of work dissipated, $\dot{W}_d$, is equal to:

$$\dot{W}_d = \dot{W} \times \frac{1}{\left(\frac{1}{\tan\delta}\right) + 1} \quad (5)$$

Given that some fraction of $\dot{W}_d$ is used up in microstructural changes within the material [145] the fraction of dissipated work converted to heat, $\dot{q}_{th}$, is given by β such that.

$$\dot{q}_{th} = \dot{W}_d \times \beta \quad (6)$$

If the thermal properties of the IVD are known as well as the size and loading conditions then predictions or models of any temperature change can be made.

2.9 Disc Biochemistry and the Effects of Temperature

The IVD produces both anabolic proteins and catabolic enzymes, the production of which has been shown to be affected by both mechanical loading and temperature [39, 146-151]. Levels of catabolic enzymes are higher in degenerate discs than healthy ones [148-150].

Korecki *et al.* found significant increases in levels of both Collagen Type 1 and Matrix Metalloproteinase 3 (MMP3) in the NP of discs cycled from 0.2-1MPa at 1Hz for an hour and significant decreases in those cycled from 0.2-2.5MPa [39] raising the important question of whether the down regulation of Collagen 1 and MMP3 due to high loading is a beneficial process of remodelling or a harmful restriction of the discs regular metabolic activity.

The hypothesis that low levels of loading results in increased levels of catabolic enzymes and therefore increased degeneration is appealing, prevalence of low back pain (and musculoskeletal pain in general) has increased significantly in previous decades [9] whilst manual labour has reduced. New research has also shown that astronauts suffer increased risk of lumbar disc herniations, concluding that this was a result of low levels of loading on the discs [152].

Maclean *et al.* (2004) found a complex relationship of anabolic and catabolic production in discs cycled at a range of frequencies and loads (0.01-2 Hz, 0.2-1 MPa) [153]. Maclean *et al.* (2008) found time dependent response to loading with “inhibition of tissue breakdown, followed by synthesis of aggrecan and

matrix degrading enzymes, and eventually collagen metabolism days following loading" [39].

As well as increased innervation and increased catabolism, diseased IVDs have elevated levels of heat shock proteins (HSPs), with increased levels of HSP72 observed particularly in cellular clusters within herniated tissues [154] and during degeneration within ageing [155]. Heat shock proteins are increased in cells in response to stress including heat, load, hypoxia and catabolic enzymes [156] and IVDs heated to 43 degrees for 20 minutes show upregulation of HSP72 [157] however to date no studies have investigated whether increased heat could occur within IVD during daily loading, and whether this could be a cause of HSP induction during degeneration.

2.10 Key Questions, Aims and Objectives

It is clear from the literature that many questions remain outstanding regarding the IVD. Stated material properties of the disc vary widely and the test methodologies used previously often lack clinical relevance. Numerous degeneration mechanisms have been postulated but clear evidence for these is sorely lacking. Current treatment options for significantly degenerated discs are not only highly invasive but result in complete loss of joint motion and regularly result in poor patient outcomes. New stem cell based treatment interventions offer the promise of recovered IVD health and joint function without invasive surgery but the mechanical implications of the hydrogel injections required are unknown. Given the vital load bearing function of the IVD these implications must be better understood before clinical trials in human subjects are ethically justifiable.

Three key questions that remain are:

1. *What are the intervertebral discs key material behaviours when loaded during activities of daily living?*
2. *Does this material behaviour effect intervertebral disc health?*
3. *Is that material behaviour affected by disc injury, degeneration and treatment interventions?*

3 Methodology

3.1 Aims

After a thorough examination of the literature and determination of the state of the art three general questions have been identified which are key to further understanding of IVD behaviour and degeneration as well as potential treatments for degenerate discs.

This work aims to answer or contribute to aspects of those questions through experimental investigation. The following section analyses the three questions in more detail resulting in three highly specific thesis objectives that this work will study.

3.1.1 *In vivo* or *In vitro*?

In vivo experimentation on the IVD ensures that boundary conditions are accurate to real life experience but is greatly limited in testing and measurement possibilities, structures or tissues under investigation are often hidden inside the body and methods to overcome this are either invasive to trial participants or unable to provide the level of real time data capture that is desired. *In vitro* experimentation results in fundamentally altered boundary conditions when compared with *in vivo*, not least due to the removal of adjacent tissues and structures which may impact on the overall behaviour of a biomaterial, however *in vitro* testing opens a vast array of experimental possibilities and greatly increased measurement options, it is for this reason that the majority of work in this thesis is conducted *in vitro*.

Whilst *in vitro* experimentation offers greater measurement possibilities it is necessary to understand how those results may differ from that found *in vivo*. The first study presented directly compares *in vivo* and *in vitro* results obtained from both IVDs separated from tissue samples and compressed in a mechanical test rig and live volunteers standing in a magnetic resonance imaging (MRI) scanner. Although both such tests have previously been conducted, the work has been carried out by multiple groups with potential issues due to varying methodology making comparisons between work difficult. This work presented in this thesis takes a step further than any previous work in ensuring that experiments closely reflected conditions relevant to ADLs.

Previous *in vivo* MRI studies had been limited to the supine position and/or a maximum loading equivalent to the participant's bodyweight, the study presented in this thesis tests participants in the standing position and at loads greater than bodyweight, simulating those experienced in the lumbar spine whilst walking.

3.1.2 **The Effects of Material Properties on Disc Health**

There are many potential ways in which the behaviour of the IVD under ADLs might affect its health, resulting in acute injury or chronic degeneration and this work focuses on one potential mechanism.

An interesting by-product of material viscoelasticy is the heat generated when VEMs dissipate energy through viscous friction within the material. Cell biochemistry is highly temperature dependent and a number of potentially

degenerative changes in cell function could arise if sufficient heat was generated by the IVD. Although the body should regulate temperature, the lack of blood flow to the IVD could result in reduced temperature regulatory ability. If sufficient heat is generated within the disc under loading localised elevated temperatures could occur. This potential degeneration mechanism is investigated in this work.

The viscoelasticity of the IVD means that in order to better understand how the disc behaves during ADLs and how that behaviour may affect both the mechanical properties of the disc and its biochemistry, through changes in temperature due to viscoelastic self-heating, it is necessary to subject IVDs to dynamic loading cycles that properly reflect those found *in vivo* during ADLs.

Previous dynamic testing has been conducted at a wide range of frequencies (0.01-30 Hz) [140, 153], typically using protocols from more traditional materials testing methods. The *in vitro* work in this present study was conducted at specific loading levels and frequencies that simulated walking and running in the human lumbar spine in order to cover a range of ADLs.

Having compared load/deflection characteristics of the IVD *in vivo* and *in vitro* the second study presented aimed to determine whether the viscoelastic behaviour of the IVD can affect disc health and degeneration through viscoelastic self-heating (VSH). IVDs have a complex biochemistry that, like all biological cells, is temperature dependent, VSH presents a possible mechanism for disc degeneration if ADLs are sufficient to generate temperature changes that lead to upregulation of catabolic enzymes and/or heat shock proteins (HSP). Discs are dynamically cycled at loading relevant

to ADLs and temperature of the discs measured directly as well as energy dissipation calculated from mechanical data.

3.1.3 Disc Degeneration and Treatment

The final study presented looks at whether degeneration and treatment have significant effects on the IVDs material behaviours. MSC treatment interventions require the injection of cells directly into the IVD within a hydrogel scaffold matrix. The safety and efficacy of any medical intervention relies on carefully weighing positive and negative outcomes and determining whether the addition of hydrogel to the disc nucleus affects its material properties would significantly add to this understanding. Discs in a range of health and treatment conditions were dynamically cycled at loads simulating walking and material properties and tissue functionality compared. Discs were not cycled at rates equivalent to running in this study as this would be a highly unlikely ADL for patients immediately post intervention.

3.1.4 Specific Thesis Objectives

1. Compare the load/deflection behaviour of the intervertebral disc when tested *in vivo* and *in vitro*.
2. Determine whether activities of daily living result in viscoelastic self-heating of the intervertebral disc and whether subsequent temperature change is significant enough to effect disc health.
3. Compare the material behaviour of discs that are healthy, degenerated and treated in order to determine whether hydrogel injection has significant effects on disc behaviour during loading.

3.2 Pilot Studies to Develop Research Methodology

Several pilot studies were conducted in order to develop effective methodologies to answer the specified research questions.

3.2.1 *In vivo* Pilot Study: Magnetic Resonance Imaging

MRI offers a potential means to analyse changes in IVD height due to changes in loading. Although dynamic activity cannot be captured, data gained from MRI could be used to give upper limits to disc compression due to loading. Four asymptomatic male subjects, aged 22-32 years (Table 3), were selected for an initial trial of methodology based on previous work in the literature [101, 158, 159].

Subjects under 35 years of age were chosen to reduce the potential effects age related damage or degeneration to discs and female subjects were excluded due to the possible variation in lumbar curvature in women of childbearing age [46, 80].

Table 3 – Age, height and weight of subjects in the MRI pilot study

Subject	Age (years)	Height (m)	Mass (kg)
1	32	1.88	98.0
2	22	1.72	67.5
3	28	1.85	92.2
4	28	1.93	87.3

Subjects underwent two sagittal plane MRI scans of their lumbar spines in two loading positions in order to measure changes in IVD height in response to different levels of axial force. The first scan was taken in the supine position to

give a minimally loaded baseline, subjects were then rotated through 90 degrees to a standing position and a second scan taken.

Subjects were scanned using a positional 0.25 Tesla MRI scanner (G-Scan Esaote, Genoa). A T1-weighted spin echo was used with repetition and echo times of 440 ms and 18 ms respectively, slice thickness and inter-slice gap were both 5 mm. Scan time was approximately 5 minutes and 30 seconds, rotation from supine to standing position took 60 seconds and the second scan was taken immediately once subjects were upright.

Estimates of axial force in the spine are based on biomechanical models developed by Don Chaffin [160] using 3DSSPP software (Michigan, USA) and using anthropometric data from each subject. Distance between adjacent vertebrae was measured at the anterior and posterior edges of the vertebral body at the mid-sagittal plane, the mean average of these two measurements was used as IVD height in line with previous work in the literature [100, 101, 158, 159, 161]. This method has been shown to offer consistent repeatability [158].

Compression of the IVD due to bodyweight loading was successfully measured *in vivo* using MRI, a mean increase in load of 522.5N at the L4/L5 disc resulted in a mean disc compression of 0.87mm. All four subjects showed increased lordosis in the lumbar region in the standing position, a finding consistent with previous studies [99, 101]. Increased lordosis results in ‘pinching’ of vertebrae posteriorly and widening of the vertebral gap anteriorly (Figure 12) to allow for increased curvature. It is clear that spinal posture

makes disc compression due to loading more complex than simple axial mechanical testing available *in vitro*.

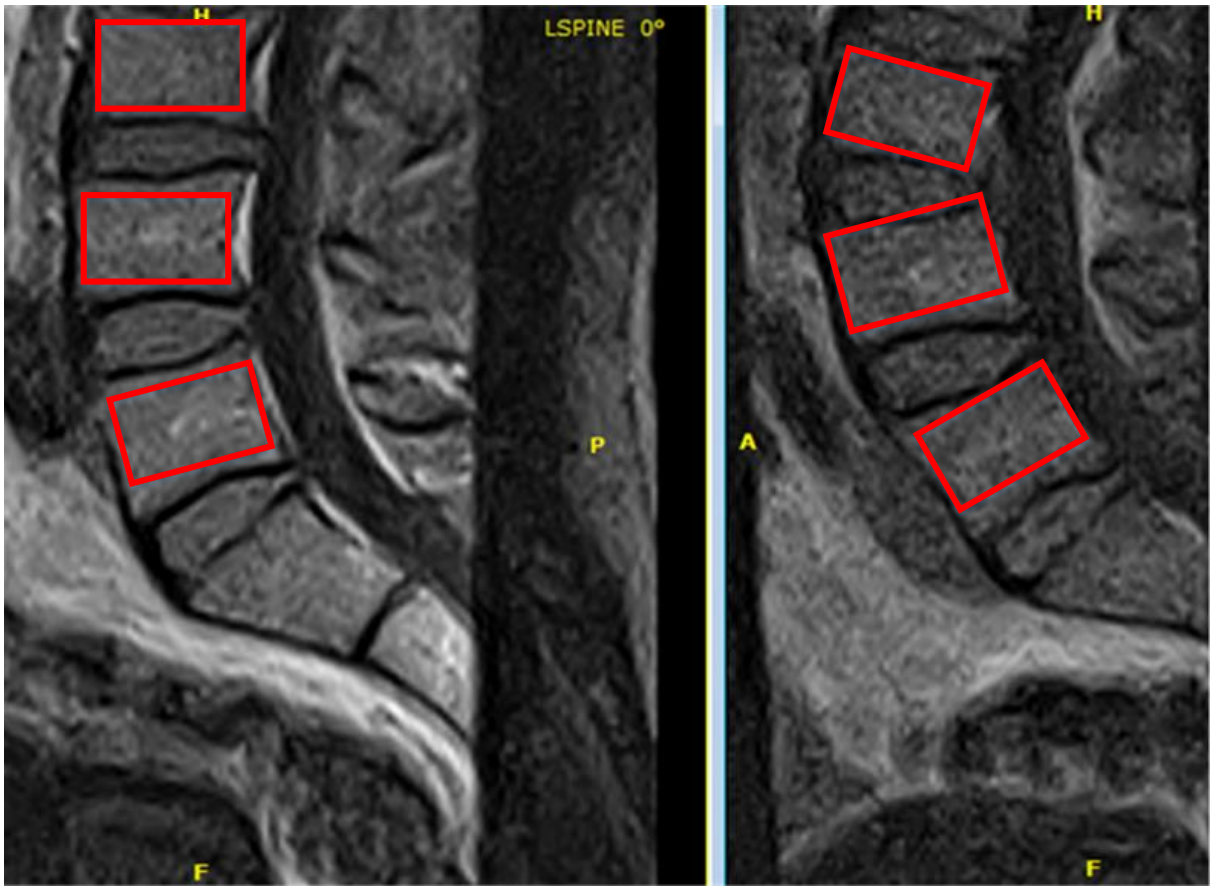

Figure 17 – The lumbar spine in the supine (L) and additional loading positions (R). Axial loading of the spine resulted in increased lordosis as well as axial compression of the IVD.

This pilot study demonstrated that *in vivo* methods can be used to provide data on load-deflection behaviour of the IVD and also provide valuable information on the complex, multi-axis behaviour of discs in the body but are limited to capturing static behaviour that is not truly representative of more dynamic ADLs.

3.2.2 *In vitro* Pilot Study: Quasi-Static Testing

Quasi-static testing (QST) is the most basic form of machine based mechanical testing available and widely used to characterise materials. QST applies displacement, whether compression or extension, at a fixed speed and measures the reaction force using a load cell [114, 134, 162]. Knowing the dimensions of the sample, stress and strain can be calculated from the load deflection data and for an elastic material stiffness and Young's modulus can also be found. Therefore QST is a potential method to determine key material properties of the IVD. A preliminary study was conducted on five bovine coccygeal intervertebral discs using similar methodology to previous work in the literature [86, 87, 140, 163].

Bovine tail sections were obtained from a local abattoir working in accordance with animal welfare regulations and MMU ethical guidelines. Tail sections were frozen at -20°C shortly post sacrifice and stored for no longer than one month before testing, sections were thawed at 5°C for 24 prior to disc harvesting. Discs were individually cut from tail sections using a scalpel and visually assessed for damage with any discs having been torn, cut or punctured being disposed of before testing.

Digital callipers were used to measure disc dimensions. Unloaded disc height was used to calculate strain under compression and disc diameter for the discs approximately circular cross sectional area and thereby stress.

Discs were compressed centrally between two flat, parallel platens on a Hounsfield H10KS quasi-static rig (Croydon, UK) running QMat software

(Tinius Olsen, Horsham, USA). The 1 kN load cell had been calibrated during a recent service to within 0.1% of magnitude with a repeatability error of <0.2%. The parallel platens applied no lateral force to the discs making physical attachment between discs and test rig unnecessary.

Discs were initially compressed at 0.5 mm s^{-1} to a pre-load of 10 N, once the pre-load had been reached compression rate increased to 2 mm s^{-1} until loading reached 100 N. Once peak loading was reached platens returned to the zero strain position and the compression cycle restarted. Each disc was compressed a total of ten times.

Test speed is limited on the rig to fixed speeds and changing from loading to unloading results in significant inertial effects on the load cell, for this reason unloading data was not recorded.

Stiffness was calculated from the stress-strain curve tangent at the maximum load in line with previous protocols from the literature [87, 140].

The IVD exhibited non-linear behaviour, typical of a viscoelastic material, when subjected to quasi-static compression at 2mm s^{-1}. Discs showed a characteristic upward curve as stiffness increased with loading (Figure 18). All discs showed a positive trend in progressive disc height loss with increasing cycles; these findings are consistent with previous studies on IVDs [39, 89, 140, 141, 164]. Most previous studies have found higher values of stiffness at peak force (Table 4) however the peak force was higher than the current study in all cases.

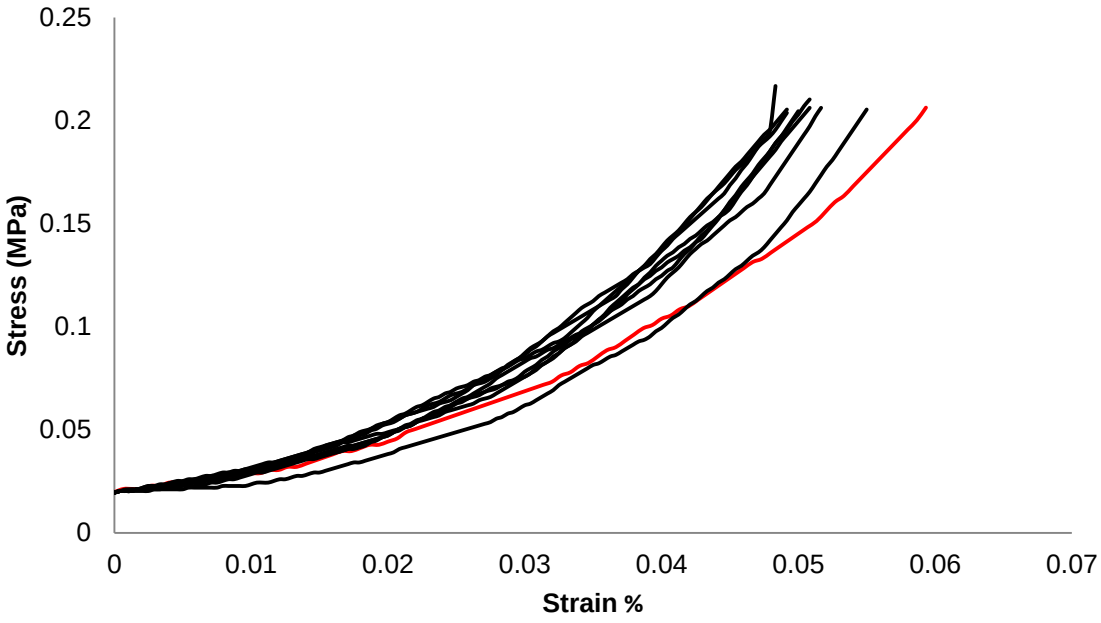

Figure 18 - Stress/strain curves for disc 5. Cycle 1 highlighted in red is more compliant than subsequent cycles. Discs demonstrate non-linear behaviour typical of a viscoelastic material.

Table 4 – Stiffness values of the IVD from different studies in the literature

Paper	Stiffness (N/mm)	Max Load (N)
Izambert et al. [140]	600-940 tangent at max load	400
Asano et al. [163]	490 (40) gradient 0-0.5mm 730 (60) gradient 0.5-1mm 1180 (90) gradient 1-1.5mm	1500
Brown et al. [86]	2100-3600 gradient major slope	450-900
Markolf [87]	1230-3320 tangent at max load	220-670
Current Study	837.9 (154.9) tangent at max load	100

The pilot study, combined with results from the literature highlights the importance of test methodology in obtaining clinically relevant data. VEM material behaviours are non-linear and highly dependent on force level and displacement rate. It is clear that bio-materials are subjected to different

displacement rates during different activities (walking, running, climbing stairs etc.) but also that the rate of displacement is continuously changing and non-linear. Any linear speed testing protocol such as QST will therefore be ineffective in characterising VEMs.

3.2.3 *In vitro* Pilot Development: Load Profiles of Activities of Daily Living

To ensure clinical relevance the material behaviours investigated in this work should reflect human experience during ADLs. QST uses a constant displacement speed which makes it unsuitable for simulating ADLs but what are the loading profiles of ADLs that testing must aim to simulate?

Although a number of papers have investigated IVD loading *in vivo* [96-98] and biomechanical models of spinal loading exist, only one study by Wilke et al. provides meaningful *in vivo* data of IVD loading during activity [97] and it is the results from this paper that provide loading profiles of the IVD during ADLs.

ADLs cover a range of activities from getting out of bed to doing housework, investigating every possible activity is not feasible so it is important to test those with a high clinical significance. Walking is a basic ADL for able bodied persons and benefits from being a simple, consistent and repeated loading cycle.

From the limited data available in the literature it is known that during walking the IVD cycles between stresses of 0.53 and 0.65MPa [97]. Average walking cadence has been measured at 120 steps per minute [165, 166], a disc load cycle frequency of 2Hz (Table 5). Running provides a higher loading situation

that can be used to investigate higher stress and loading frequency on the IVD [167]. Previous *in vitro* studies have shown that axial pressure is linked with intradiscal pressure making axial loading an appropriate method for replicating *in vivo* findings [37].

Table 5 – Stress Range and Frequency of Intervertebral Disc Loading During Activities of Daily Living

Activity	Stress Range (MPa)	Cadence (Steps min^{-1})	Frequency (Hz)
Walking	0.53-0.65	120	2
Running	0.35-0.85	180	3

What *in vitro* test methodologies can simulate these loading profiles?

3.2.4 *In vitro* Pilot Development: Dynamic Test Rig

Dynamic mechanical testing (DMT) strategies allow for variable, non-linear test speeds ensuring that a test protocol can be designed which more accurately simulates *in vivo* behaviour during ADLs than QST can achieve. Testing can be controlled by force or displacement and much like QST a range of other measurement and recording devices can easily be used in conjunction with the test rig.

Work was undertaken to refurbish and recommission a hydraulic, dynamic test rig for this work (Figure 19). Consisting of a hydraulic pump, piston and Instron Labtronic 8800 dynamic hydraulic controller (Instron, Mass, USA). A 5 kN load cell was purpose bought for the experiment and calibrated by Instron specifically for the compressive loading routine designed. Error was measured at <0.2% of load magnitude with a repeatability error <0.05% ensuring highly

accurate and repeatable results for each disc and comparison between discs. The LVDT used to measure displacement was calibrated with a <0.1% error.

Using Instron Wavematrix 1.8 control software the piston can apply any desired loading profile (within machine limits), a test protocol was developed that sinusoidally cycles discs between 0.53 and 0.65 MPa at 2 Hz, simulating the *in vivo* loading profile of walking and thereby ensuring the most clinically relevant data is obtained [97, 165, 166].

Figure 19 – A hydraulic piston (a) allows for variable displacement rates on a sample (b) with force measured by a load cell (c).

The load and displacement data collected during testing allows the determination of the IVD strain and elastic stiffness during ADLs, two of the three key measures required to achieve the stated aims of this thesis.

Elastic disc stiffness under loading is defined in this work as the difference in upper and lower force levels of the sinusoidal loading profile divided by the displacement of the IVD during this loading period.

E.g. A disc cycled between 1 and 2 Newtons which compresses a total of 1 mm during this loading has an elastic stiffness of 1 Nmm^{-1}

Strain is defined in this work as the difference in disc height between the upper and lower stress values during cyclic loading divided by the disc height at the lower stress level.

E.g. A disc cycled between 0.53 and 0.65 MPa has a height of 10 mm at 0.53 MPa loading and reduces to 9 mm at 0.65 MPa. The disc strain is 0.1 or 10%.

Each cyclic test returns large amounts of real time data and strain and stiffness values cannot be calculated for each individual loading cycle by hand. A custom MATLAB code has been used to determine these values for every cycle of every test and an average strain and stiffness calculated for each disc.

DMT on this rig can also be used to determine the third key measure, energy dissipation. Energy dissipated by the disc in each loading cycle manifests itself as a hysteresis loop on a force/displacement curve. Data from the load cell and LVDT was captured at 100 Hz providing high resolution force/displacement (and therefore stress/strain) data for each test cycle. Integrating the force/displacement curve for both the loading and unloading portion allows the energy dissipated to be calculated.

High frequency data acquisition hardware connected to the DMT rig allows the collection of sufficient data to calculate this energy dissipation using a time stepping midpoint rectangle integration method. A custom code written in MATLAB calculates the energy dissipation for each single load event in a period of cyclic testing and the average of all cycles over the entire period.

DMT made possible by this test rig represents an ideal *in vitro* testing method capable of determining all three key measures required by the research aims of this work.

3.2.5 Statistical Methods

Once these key measures have been determined for groups of discs in various damage, degeneration or treatment states one-way ANOVA with a Tukey post-hoc test has been used to look at differences between groups and whether those differences are statistically significant and therefore represent a meaningful variation between groups.

3.3 Study Design to Answer Research Questions

3.3.1 Viable methodologies

The preliminary work conducted in this chapter has highlighted the limitations of modelling and quasi-static *in vitro* testing as viable methodologies to answer the research questions of this thesis; however both *in vivo* imaging and dynamic *in vitro* testing are able to provide relevant data towards the research aims.

A DMT test rig has been refurbished which is not only able to accurately simulate the loading profiles of ADLs but also collect real time data which can be used to answer each of the three research questions.

MRI, whilst significantly limited in its ability to simulate or measure ADLs has highlighted the complex nature of IVD loading *in vivo* and provides important context against which to compare *in vitro* results.

On the basis of these findings three studies have been designed in order to answer the stated research questions.

3.3.2 **Study 1: Comparing *In vivo* and *In vitro* Loading. Chapter 4**

Axisymmetric loading *in vitro* can be used to determine the material properties of the IVD but as seen in preliminary testing, true *in vivo* loading of the IVD is not axisymmetric.

The first study will compare dynamic *in vitro* loading with an *in vivo* loading protocol allowing results from future *in vitro* loading protocols to be understood in the context of *in vivo* behaviour.

3.3.3 **Study 2: Viscoelastic Self-Heating and Disc Health. Chapter 5**

A key research question of this thesis is to understand possible ways in which disc mechanical behaviours may affect disc health and/or injury. Viscoelastic materials dissipate energy under loading and some fraction of this energy is converted to heat through viscous friction within the material. A number of disc biological functions are sensitive to temperature and if heat generated in the disc is significant it is a possible mechanism for degeneration.

The dynamic test rig is able to simulate loading equivalent to that in the IVD during ADLs and measure energy dissipated by the disc allowing this potential degeneration mechanism to be investigated.

The study has been designed which subjects discs to periods of loading simulating that of either walking or running and uses mechanical data collected to model temperature changes in the disc.

3.3.4 **Study 3: Effects of Hydrogel Intervention on Disc Functionality. Chapter 6**

Understanding whether the injection of hydrogels into degenerative discs has significant effects on the IVD's mechanical and tissue functionalities is of major clinical importance.

The dynamic test rig is able to subject discs to highly repeatable load profiles which not only allows for accurate simulation of ADLs but makes it possible to readily compare discs of differing health or treatment conditions.

A study has been designed which compares healthy, degenerate and hydrogel injected discs in order to determine if there are clinically significant differences between these groups.

4 Study 1: *In vivo* and *In vitro* Loading

Comparing and Understanding the Differences in Load/Deflection Behaviour between Static, *In vivo* and Dynamic, *In vitro* Test Methodologies

4.1 Abstract

Static, *in vivo* test methodologies such as magnetic resonance imaging have been widely used to investigate the effects of loading on the spine and intervertebral disc but it is unclear whether these methods are useful for the investigation of loading associated with dynamic activities of daily living such as walking or climbing stairs.

In vitro testing offers the ability to accurately simulate and investigate the effects of dynamic activities on the intervertebral disc but there is little or no *in vivo* data conducted at appropriate loading levels against which *in vitro* tests can be compared and verified.

To address these challenges participants were subjected to loading equivalent to that found during walking and MRI scans taken of the lumbar spine and compared against baseline scans of participants in the standing position without additional loading. Disc compression *in vivo* was then compared against that in bovine IVDs which were compressed dynamically *in vitro* between 0.53 and 0.65 MPa at rates simulating walking in the lumbar spine.

Disc compression and lumbar posture was not significantly altered between standing and axial loading simulating walking however magnitude of disc compression was substantially greater *in vivo* than *in vitro*. The study represents a comparison of greater than body weight loading *in vivo* and *in vitro* and the data presented suggests that static MRI is a poor analogue for activities of daily living due to the altered material behaviours when compressed dynamically *in vitro*.

4.2 Introduction

Acute injury or chronic degeneration of the intervertebral disc (IVD) has been linked with long-term pain [30]. The IVD works to transfer load along the spine whilst allowing movement between adjacent vertebrae and understanding the mechanical response of the disc to loading is a key factor in understanding and predicting its behaviour, understanding this behaviour should give insights into injury and degeneration [140].

In vitro testing of IVDs allows for the collection of a range of real time data that is difficult or impossible *in vivo* without highly invasive test protocols. However boundary conditions *in vitro* are inherently altered and therefore it is important that *in vivo* data exists in order to understand *in vitro* results in context.

Magnetic resonance imaging (MRI) has previously been used to monitor the effects of static loading on the lumbar spine up to and including body weight and postural changes, increased disc compression and lumbar lordosis are well documented under these conditions [99, 100, 168]. However MRI has not previously been used to observe loading which is greater than body weight.

Dynamic *in vitro* loading simulating activities of daily living (ADL) applies loading that is greater than body weight and *in vivo* studies of this behaviour have been limited to intradiscal pressure measurements [96, 97]. Understanding whether greater than body weight static loading produces different postural changes *in vivo* than body weight loading alone will provide important reference data for the analysis of greater than body weight *in vitro* loading.

Previous dynamic *in vitro* studies have been conducted at a wide range of frequencies (0.01-30 Hz) [140, 153] and loads (100 N – 3.5 MPa) [37, 153]. These often exceed those found in activities of daily living [39, 140] or did not cycle discs until after they had been intentionally damaged through excessively high compressive loading [37].

As a viscoelastic material the mechanical properties of the IVD are dependent on the rate of loading and load/deflection characteristics are liable to differ when load is applied dynamically rather than statically. Dynamic *in vivo* imaging techniques exist but are limited by cost, availability and field of vision; dynamic CT and fluoroscopy in particular require the use of ionizing radiation increasing ethical and safety considerations when designing experimental protocols. If the load/deflection characteristics obtained using static MRI are significantly different from those in dynamic *in vitro* loading then static MRI will be of limited use in investigating dynamic activities of daily living and more advanced imaging techniques such as fluoroscopy will have to be relied upon.

This study aims to investigate whether lumbar spinal posture is significantly altered by loading greater than body weight and whether load/deflection characteristics of IVDs are significantly different between static MRI of human discs and dynamic *in vitro* loading of bovine discs.

4.3 Methods

4.3.1 *In vivo* posture and stiffness measurements

Full ethical approval was granted by the MMU Faculty of Science and Engineering ethics committee in accordance with principles laid out in the

Declaration of Helsinki for trials on human participants before any work in this study was conducted. (See Appendix)

Nine asymptomatic male volunteers (22-32 years, mean 27 years) underwent three MRI scans representing unloaded, body weight and greater than body weight loading after giving informed consent for participation. Subjects were asked to conduct their usual routines prior to their scans but to avoid strenuous exercise or exertion. All participants were scanned between the hours of 10 and 11am.

The unloaded scan was taken in the supine position, the MRI bed was then rotated through 90 degrees and a body weight scan taken in the standing position. The greater than body weight scan was then taken in the standing position with an additional 12 kg load provided by subjects holding a 6 kg weight in each hand. Scans were begun immediately after participants took hold of the weights.

Hand weights were chosen as the limited space in the MRI scanner made the use of wearable weights (backpack, weight vest etc.) impossible. 6 kg per hand was an upper limit of what could be reasonably expected for a participant to comfortably support for the duration of the scan and heavier weights are typically constructed from ferrous metals unsuitable for MRI scanners.

Subjects were scanned using a positional 0.25 Tesla MRI scanner (G-Scan Esaote, Genoa). Scan settings were optimised during preliminary testing in as a compromise between multiple factors including scan time, subject comfort, image resolution and accurate capture of mid-sagittal plane. A T1-weighted

spin echo was used with repetition and echo times of 440 ms and 18 ms respectively as these produced a high contrast between vertebrae and surrounding tissues which was required to take accurate measurement of disc height. Slice thickness and inter-slice gap affect image resolution with small values resulting in higher resolution images but requiring longer scan times, 5 mm was chosen for both as an optimum compromise between the contradictory considerations.

Loading on the lumbar discs in the standing and additional weight positions was calculated based on biomechanical models developed by Don Chaffin [160] using 3DSSPP software (Michigan, USA). Using anthropometric data from each subject and known loading in the subject's hands forces transferred axially through the spine to the L4/L5 disc are calculated. This data is then used to compare disc loading against disc height in each loading position.

To determine whether spinal posture was changed between bodyweight and greater than bodyweight static loading two measures were chosen. At the base of the lumbar spine the angle of the L5 vertebrae relative to the S1 vertebrae was measured as the angle between the posterior edges of each vertebral body. At the top of the lumbar column the rotation of the L3 vertebrae was measured as the relative angle between the superior face of the vertebral body relative to the vertical axis. These two measures allow for comparison of lumbar posture, particularly lordosis, in each loading position. Ideal rotation of the L1 vertebrae would have been used but the available field of view was such that the L3 vertebrae was the highest visible in all scans.

Disc deflection was measured as the distance between adjacent vertebrae at the anterior and posterior edges of the vertebral body at the mid-sagittal plane, the mean average of these two measurements was used as IVD height in line with previous work in the literature [100, 101, 158, 159, 161].

All MRI images were analysed using OsirX (Geneva, Switzerland).

4.3.2 **Dynamic *in vitro* loading measurements using animal tissue**

Ten Coccygeal discs were harvested from 5 bovine tail sections obtained from local abattoirs operating in concordance with animal welfare regulations from cows aged approximately 18 months at time of sacrifice. Sections were stored at -20°C shortly post sacrifice and tested a maximum of one month post storage. Sections were thawed at 5°C for 24 hours prior to sample preparation.

Discs were dissected whole from tail sections and allowed to reach room temperature for 12 hours before testing. Discs were stored in sealed bags to prevent dehydration during this time and tested immediately following thawing and removal from bags.

Bovine discs were chosen due to their structure and loading properties which have been shown to be similar to healthy human discs when subjected to the same test protocol [132]. Human cadaveric discs are likely to be harvested from older persons making them more liable to degeneration. Bovine discs therefore represent a good surrogate for healthy human discs and have previously been used in the literature [39, 141]

Discs were loaded cyclically using a hydraulic, dynamic test rig incorporating a hydraulic piston controlled by Wavematrix 1.8 test software and a Labtronic 8800 hydraulic controller (Instron. Mass, USA) between 0.53 and 0.65 MPa at a frequency of 2 Hz in order to approximate loading found during human walking [97, 165, 166]. Real time load and position data was gathered at 100 Hz.

Discs were ramped to a load midway between the upper and lower values at a rate of 0.02 N s^{-1} before being subjected to 200 loading cycles. 200 cycles were chosen to simulate a short, 100 second, period of walking typical of the daily routines of all but those with significant infirmities or disabilities. A 0.02 N s^{-1} pre-load ramp rate was found to offer a controlled ramp that accurately achieved the desired load value during preliminary testing.

Each disc was tested three times to ensure initial compliance typical in recently harvested discs was averaged out over multiple tests without individual discs drying out during repeated testing. An inter-test time of approximately 2 minutes was found to be the fastest consistently achievable and was maintained across all discs.

4.4 Results

4.4.1 Human Intervertebral Disc Stress from *In vivo* loading

Spinal loads calculated for L4/L5 disc in the standing and additional weight positions are shown in Table 6. With only sagittal plane images taken disc cross section area was estimated from anterior-posterior width of the L4/L5

disc and disc stress calculated for standing and additional weight positions (Table 6).

In all cases the final stress and change in stress in the L4/L5 disc exceeded 0.65 MPa and 0.12 MPa respectively, the upper limit and stress range observed in the L4/L5 disc during walking [97].

Table 6 – Loads in the L4/L5 Disc in Standing and Additional Load Positions with Stress Increase between the Two Positions

Subject	Standing Load (N)	Weighted Load (N)	Standing Stress (MPa)	Stress Increase (MPa)	Weighted Stress (MPa)
1	409	569	0.58	0.23	0.81
2	564	736	0.54	0.16	0.70
3	521	701	0.51	0.17	0.68
4	428	590	0.63	0.15	0.88
5	440	601	0.48	0.17	0.65
6	385	541	0.44	0.17	0.61
7	428	595	0.57	0.22	0.79
8	506	674	0.60	0.20	0.80
9	558	728	0.51	0.15	0.66
Mean	**471**	**637**	**0.54**	**0.18**	**0.73**

4.4.2 Disc Compression

Mean disc height across all subjects decreased between supine and standing position in all three lumbar discs (L3/L4, L4/L5 and L5/S1). Mean compression in the L3/L4, L4/L5 and L5/S1 discs were 0.3, 0.6 and 0.6mm respectively (**Error! Reference source not found.**). Mean strain across subjects in the tanding position relative to supine was 2.7%, 4.4% and 5.1% respectively (**Error! Reference source not found.**).

Mean disc height across subjects decreased between the standing and additional loading positions in the L3/L4 and L5/S1 discs by 0.1 and 0.4mm respectively. No change in mean disc height was observed for the L4/L5 disc

between the standing and additional loading positions (Figure 20). Strain in the additional weight position relative to the standing position was 1.4% and 4.0% in the L3/L4 and L5/S1 discs respectively. There was no mean strain in the L4/L5 disc (Figure 21).

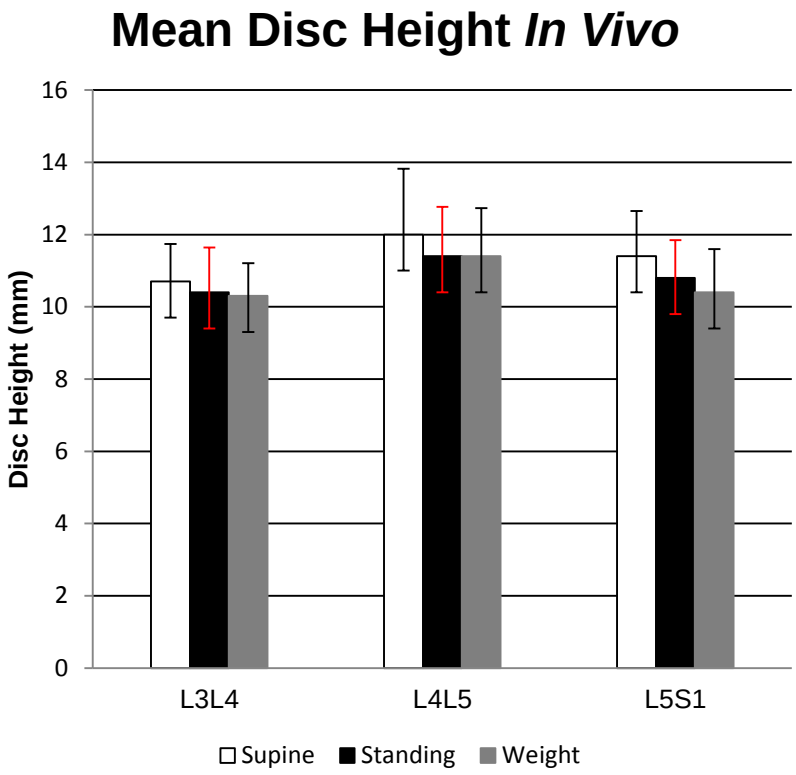

Figure 20 - Mean measured height for all postural loading conditions for IVDs between L3 and S1 vertebrae. Error bars show 95% confidence intervals.

In vitro mean compression of discs was found to be 0.024 mm (range 0.020 – 0.029 mm). Mean strain at 0.65 MPa relative to 0.5 MPa was found to be 0.46% (Figure 21). *In vivo* strain in the L3/L4 disc was more than twice that found *in vitro* and strain in L5/S1 more than eight times the *in vitro* strain almost certainly due to the effects of creep strain in the static loading case. No strain was observed in the L4/L5 disc.

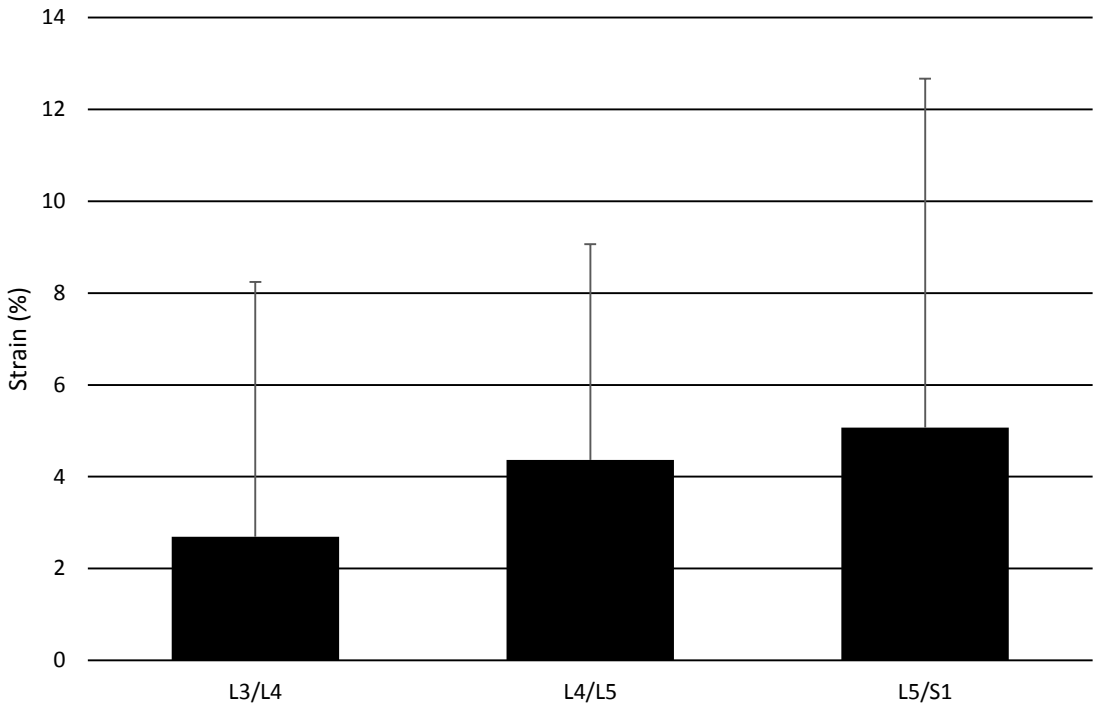

Figure 21 - Mean disc strain due to increased loading between supine and standing positions. Error bars show 95% confidence intervals.

4.4.3 **Lumbar Lordosis *In vivo***

Lumbar lordosis increased with axial load as defined by an increase in both lumbar/sacrum angle and posterior rotation of the L3 vertebrae. Mean lumbar/sacrum angles were 140, 144 and 145 degrees in the three loading positions (Figure 23) whilst the L3 vertebrae was rotated 0.7, 4.4 and 4.8 degrees posteriorly from the vertical axis in each loading position (Figure 24).

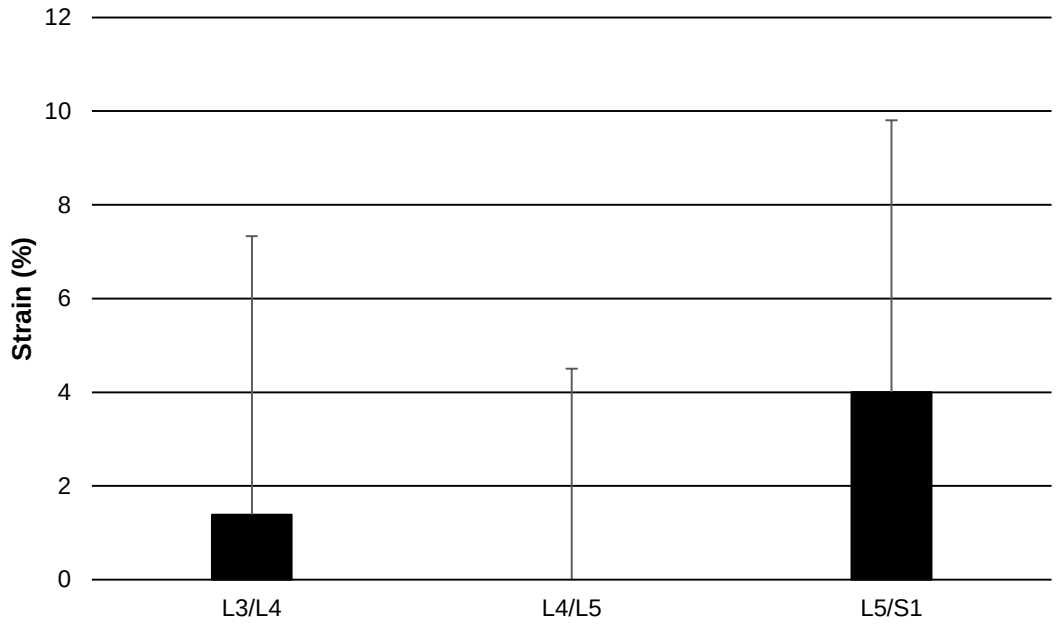

Figure 22 – Strain in discs between standing and additional loading positions. Mean strain in the L4/L5 disc across all subjects was zero but 95% confidence interval extends to over 4%.

This trend was matched by disc compression being heavily weighted towards the posterior of the disc with greater compression in this region than the anterior portion of the disc (Figure 25).

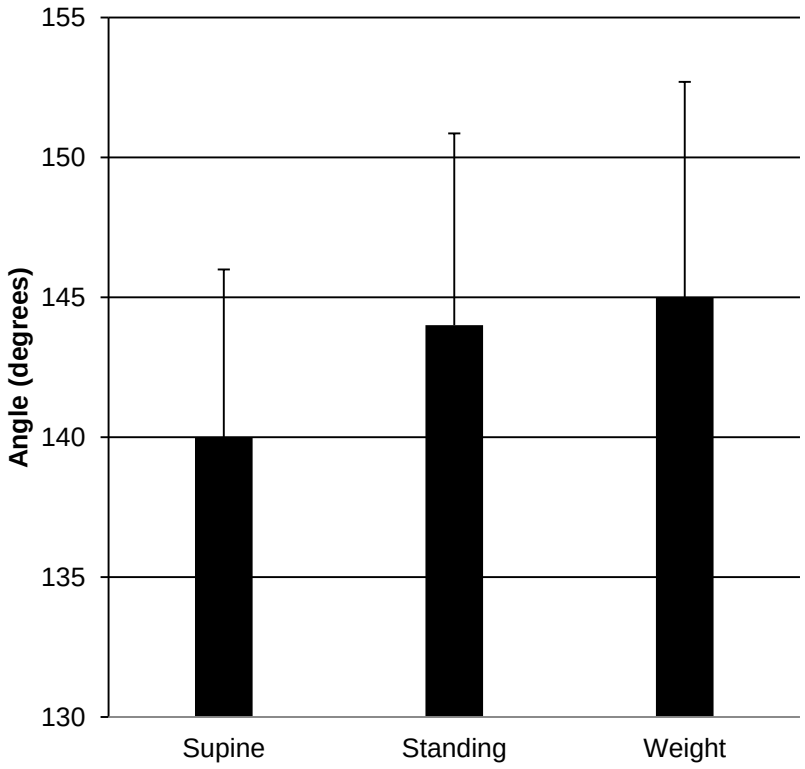

Figure 23 – Angle between the L5 and S1 vertebrae in each of the three loading positions. Error bars show 95% confidence intervals.

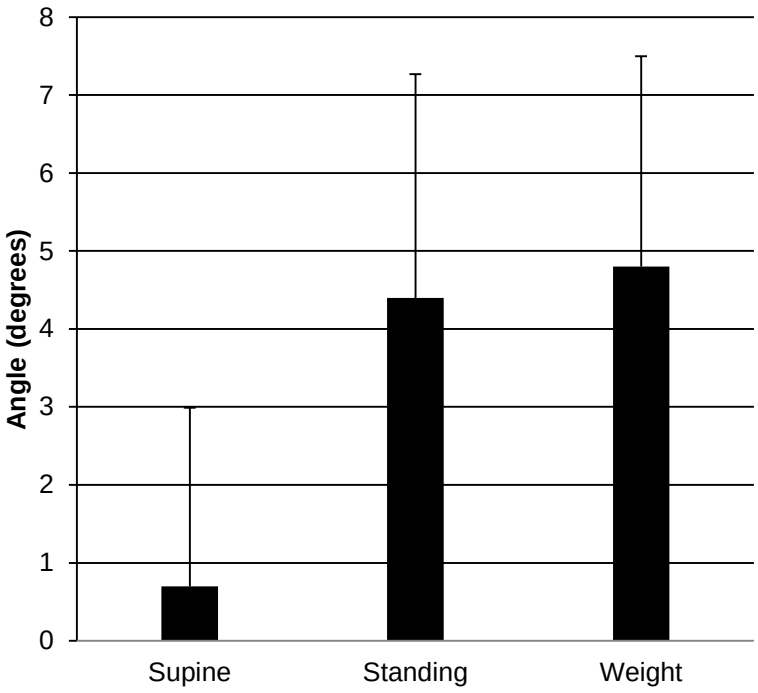

Figure 24 – Axial loading resulted in the L3 vertebrae rotating posteriorly from the vertical. Error bars show 95% confidence intervals.

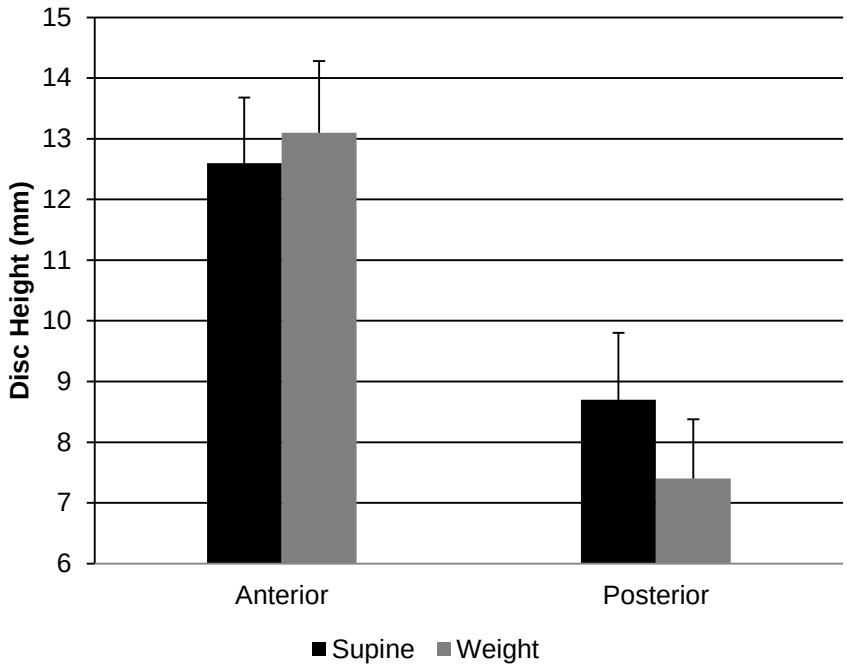

Figure 25 – The mean anterior disc height across all 9 subjects was found to increase in the L3/L4 disc even though total disc height, calculated from the mean of anterior and posterior measurements, reduced overall. Error bars show 95% confidence intervals.

4.5 Discussion

This study investigated whether loading due to ADLs resulted in significantly altered lumbar spinal posture from body weight loading alone by comparing lumbar spinal posture in the standing position with that in the standing position with additional loading applied. The present data suggests that lordosis of the lumbar spine and compression of the L3/L4 and L5/S1 discs increased with axial loading a finding in line with work previously conducted in this area [99-101, 168].

Mean disc height of the L4/L5 disc across all subjects was equal in both standing and greater than body weight loading positions but the standard deviation was large (7.2%) and each individual subject experienced change in L4/L5 disc height.

When disc height is analysed individually 5 out of 9 subjects showed *greater* L4/L5 disc height in the additional weight loading position than in the standing position (Figure 26). With the L4/L5 disc positioned at the apex of the lumbar lordotic curvature it is understandable that any effects of curvature may be greatest in the L4/L5 disc.

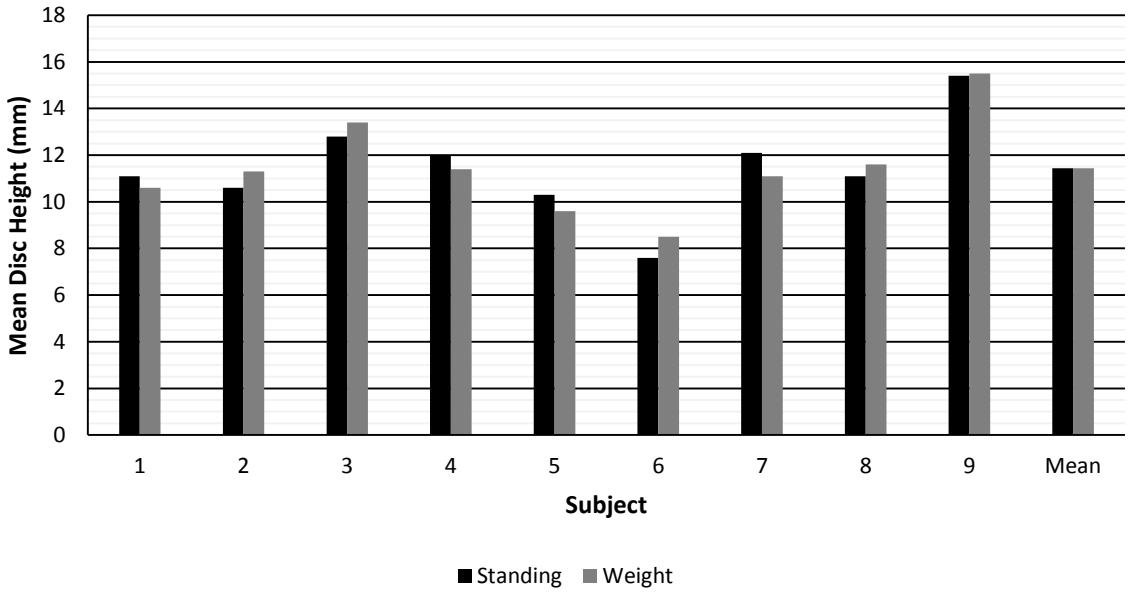

Figure 26 – L4/L5 disc heights for each subject and the mean value across all subjects. Although the mean value does not change from standing to additional weight loading, each individual did see change in disc height.

The results from this study suggest that the L4/L5 disc has a more complex biomechanical relationship than simple load/deflection with slight rotation that is observed in other discs.

Mean *in vivo* disc height change across the three lower lumbar discs was found to be 0.5 mm between supine and standing and a further 0.2 mm between standing and additional weight loading.

Table 7 Change in Disc Height Relative to the Supine Position in Studies which Measured Compression Due to Axial Loading

	Loading/Measurement Conditions	Disc Compression Measurements
Kimura *et al.* [100]	Supine Position 0.5 x body weight MRI Scan	0.5mm average lumbar disc compression
Hioki *et al.* [101]	Supine/Standing Body weight X-Ray	8.1% compressive strain
Kingsley *et al.* [158]	Supine Position Scans before and after 30 minutes of jogging MRI Scan	0.29mm creep after 30 minutes
Present Study	Supine/Standing Greater than body weight MRI	0.5mm/4% at body weight Further 0.2mm/1.8% at greater than body weight

A comparison of the present study with previous studies in the literature can be seen in Table 7.

Kimura *et al.* found a mean disc height change of 0.5 mm across all five lumbar discs when 50% bodyweight loading was applied in the supine position [100].

Hioki *et al.* found large relative deformation between supine and standing positions with an average strain of 8.1% over the lower three lumbar discs [101]. This is significantly higher than the average of 4% in the present study however subjects in the Hioki study spent 1 hour in the supine position before scans whereas subjects in the present study underwent their regular activity until shortly before their first scan. This poses the question of whether the large scale deformations seen in Hioki *et al.* are only relevant to the IVD shortly after extended periods of rest.

Kingsley *et al.* found a mean compression of 0.29 mm across lumbar discs after 30 minutes of treadmill running [158]. Subjects were tested within 30

minutes of rising from bed after a minimum of 10 hours of rest. Botsford *et al.* found mean volume reduction in the lower three lumbar discs to be 16.2% after a period of loading equivalent to a day's activity [102]. This creep effect due to periods of axial loading would suggest that studies where subjects are given large periods of rest immediately prior to imaging are not accurate representations of the response to ADL.

The present study is limited by the small number of subjects, though this is in line with previous studies which used between eight and fourteen subjects [99-102].

The present study went beyond previous work by loading subjects with an additional 12 kg, simulating maximum lumbar loading during walking. However, this only represented a 12.9-18.1% increase relative to body weight loading and some activities of daily living are known to result in loading several times body weight, future work should ideally investigate these higher loading values. The loading protocol used in this study would not be suitable for loading of that magnitude, and ethical concerns about sustaining high loading for extended periods of time would need to be addressed when considering any future protocol.

Dynamic *in vivo* methods such as dynamic CT and fluoroscopy require ionising radiation, if static MRI could replicate the load deflection characteristics of dynamic loading then it would present a more ethical and less invasive experimental protocol but load deflection characteristics of IVDs were found to be different between static *in vivo* and dynamic *in vitro* loading protocols.

Static loading of the spine *in vivo* resulted in greater compression than that due to dynamic *in vitro* loading whether expressed as absolute magnitude or strain a result of the creep during the long loading periods the discs are subjected to during static MRI scans. This suggests that current static MRI methods are a poor analogue of dynamic load deflection characteristics.

The use of bovine IVDs rather than human may be perceived as a limitation, however in the literature values for the compressive modulus of the IVD vary by as much as 3 orders of magnitude whereas studies which have compared human and bovine discs under identical protocols show close similarity between the two [121, 128, 132, 133]. Therefore given the importance of test protocol in observed disc material properties and behaviours the authors believe that bovine discs present an acceptable surrogate for human discs in this study which investigated differences in *in vivo* and *in vitro* experiments.

As has been observed, in vitro testing with an axisymmetric test rig cannot truly replicate *in vivo* loading due to the influence of rotation of adjacent vertebral bodies and the variable compression of discs which are subjected to large vertebral rotations when the spine is loaded *in vivo*.

In vitro testing on an axisymmetric rig cannot truly replicate the observed rotational behaviours of the IVD *in vivo* but still has significant advantages which make it a more suitable test methodology.

The large deformations of disc *in vivo* are undoubtedly influenced by the static loading protocol of MRI resulting in creep behaviour in the disc. Dynamic *in*

vitro testing ensures that loading rates as well as loading magnitudes are clinically relevant to ADL.

The large inter-subject variation observed in the MRI data brings the potential repeatability and accuracy of MRI studies into question due to the small numbers typically seen in such studies. A big data approach may be needed to gain better insights into general trends as current small scale studies are too easily affected by individual subjects. A systematic review of current studies may not be sufficient due to the different methodologies between studies and a large scale study by a single group may not be feasible. *In vitro* loading, whilst perhaps limited in some ways is a highly repeatable methodology and therefore ideal for detailed studies of material behaviours.

Previous dynamic *in vitro* studies have been conducted at frequencies and loading levels that far exceed activities of daily living. Discs have previously been cycled at frequencies as high as 5 - 30 Hz, well outside the typical range for human activity [140]. Others have loaded discs cyclically to 2.5 MPa, an extreme stress not typical of daily activities and not cyclical in nature [39]. The data presented in this study represents the cyclic compression of intervertebral discs at loading levels and frequencies typical of activities of daily living and therefore the clinical relevance of this data exceeds that previously determined.

4.6 Conclusions

In vivo loading at levels equivalent to that during walking resulted in increased compression and lordosis in the lumbar spines of subjects. Magnitudes of

static *in vivo* compression were approximately 4 times greater than those in discs cycled dynamically *in vitro* under a loading profile simulating walking in the lumbar spine due to the effects of creep and the different strain rates in the two methods.

As investigation of the IVD increasingly moves beyond bodyweight loading to higher forces typically experienced during dynamic activity the effects of creep and strain rate will increasingly present differences between loading in static MRI and those from dynamic tests. Although future improvements in scan times may lead to improved investigation of static loading using MRI, static MRI will only form a part of a fuller investigation into the complex behaviour of IVDs during ADLs.

Axisymmetric *in vitro* test protocols cannot fully reflect the complete complex behaviour of the IVD *in vivo* but their ability to simulate the general stress-time behaviours of discs accurately and repeatedly make them a valuable tool for clinical investigation.

5 Study 2: Viscoelastic Self-Heating and Disc Health

Does Repeated Cyclic Loading Contribute to Mechanisms of Degeneration Through Heat Generation Within The Intervertebral Disc?

5.1 Abstract

Degenerative intervertebral discs show elevated levels of catabolic enzymes and cytokines including heat shock proteins. Increased temperatures are one potential cause of elevated levels of heat shock proteins and such temperatures have been demonstrated to result in heat shock protein upregualtion in the intervertebral disc *in vitro* but whether such temperatures occur within the disc is not known. Viscoelastic self-heating of the intervertebral disc under mechanical loading during activities of daily living presents a possible mechanism for increased temperatures within the disc.

This study dynamically cycled bovine intervertebral discs at loading simulating both walking and running in order to determine if energy gained by the disc during activities of daily living is a potential mechanism for heat shock protein upregulation. A thermal model of the disc was created in order to determine potential temperature changes within the disc due to the dissipated energy. Observed energy dissipation was small, being less than 4 mJs^{-1} even in the higher loading condition simulating running and the resulting potential temperature increase of 0.025°C was found to be well within normal core

temperature variation. These results suggest that heat generated in the disc during activities of daily living is not a potential mechanism for heat shock protein upregulation.

5.2 Introduction

IVDs demonstrate a complex relationship of anabolic and catabolic enzymes after being subjected to cyclic loading [39, 153]. The production of catabolic enzymes and/or cytokines has been shown to be heightened in degenerate discs [149, 150, 169-173]. This relationship is time dependent with “inhibition of tissue breakdown, followed by synthesis of aggrecan and matrix degrading enzymes, and eventually collagen metabolism days following loading” [146].

As well as increased innervation and increased catabolism, diseased IVDs have elevated levels of heat shock proteins (HSPs), with increased levels of HSP72 observed particularly in cellular clusters within herniated tissues [154] and during degeneration within ageing [155]. Heat shock proteins are increased in cells in response to stress including heat, load, hypoxia and catabolic enzymes [156] and IVDs heated to 43 degrees for 20 minutes show upregulation of HSP72 [157] however to date no studies have investigated whether increased heat could be generated within the IVD as a result of daily loading, and whether this could be a cause of HSP induction during degeneration.

IVDs are a viscoelastic composite with a complex modulus comprised of both an elastic modulus and a viscous modulus. When work is done on the disc during compression loading, some of the energy put into the disc is lost to viscous friction and therefore not recovered when the disc does work on its surroundings to return to its uncompressed state. Part of this energy is dissipated by the disc which can cause microstructural changes within the disc whilst the rest will be converted to heat [145]. Of the total energy dissipated,

W_d, the amount which is dissipated as heat, W_{th}, is given by the thermal dissipation fraction, β, such that [174]

$$W_{th} = W_d \times \beta \quad (1)$$

Therefore, for a given rate of work on the disc the rate of heat production, $\dot{q}_{th}$ is:

$$\dot{q}_{th} = \dot{W}_d \times \beta \quad (2)$$

With degenerate discs having elevated levels of HSPs and HSPs being known to result from elevated temperatures and/or heat shock within the IVD, it is possible that high temperatures could be a precursor to degeneration. Here we test the hypothesis that compressive loading experienced during activities of daily living (ADL) could result in elevated temperatures in the IVD

This study aims to determine if there is a significant generation of heat within the IVD when subjected to cyclic loading at levels and frequencies relevant to ADL. To the authors knowledge no previous study has attempted to answer this question.

5.3 **Method**

Twenty Coccygeal discs were harvested from bovine tail sections and randomly assigned to two equal sized test groups to be tested at loads simulating those in the human lumbar spine during either walking or running. All tail sections were obtained from local abattoirs from cows aged

approximately 18 months at time of sacrifice. Tails were stored at -20°C shortly post sacrifice and tested a maximum of one month post storage. Tails were thawed at 5°C for 24 hours prior to sample preparation. Discs were carefully dissected whole from tail sections and allowed to reach room temperature for 12 hours before testing. Discs were visually inspected for signs of damage, degeneration or general size and shape that would not be practical for testing and unsuitable discs were disposed of. Discs were stored in sealed bags to prevent dehydration during this time.

Bovine discs were used because they demonstrate similar material behaviours to human IVDs when tested under the same loading protocol [132]

Discs were loaded cyclically using a hydraulic, dynamic test rig incorporating a hydraulic piston controlled by Wavematrix 1.8 test software and a Labtronic 8800 hydraulic controller (Instron. Mass, USA) for a period of one hour. Discs were compressed between two flat steel platens at loads and frequencies designed to simulate either walking or running behaviour in humans using know values of such activities of daily living from the literature [97, 165-167] (Table 8).

Table 8 – Test Cycle Stress Values and Frequencies

Test Group	Stress Range (MPa)	Approximate Force Range (N)	Frequency (Hz)	Cadence (Steps/min)
Walking	0.53 – 0.65	200-450	2	120
Running	0.35 – 0.85	120-550	3	180

Each disc was ramped to a preload of at a rate of 10 N/s, the preload was 0.59 MPa for walking discs and 0.60 MPa for running discs and once this load

had been reached cycling began immediately. Force values on each disc were based on individual disc diameter (19.8-29.2 ± 2.9 mm) in order to ensure correct stress values were achieved.

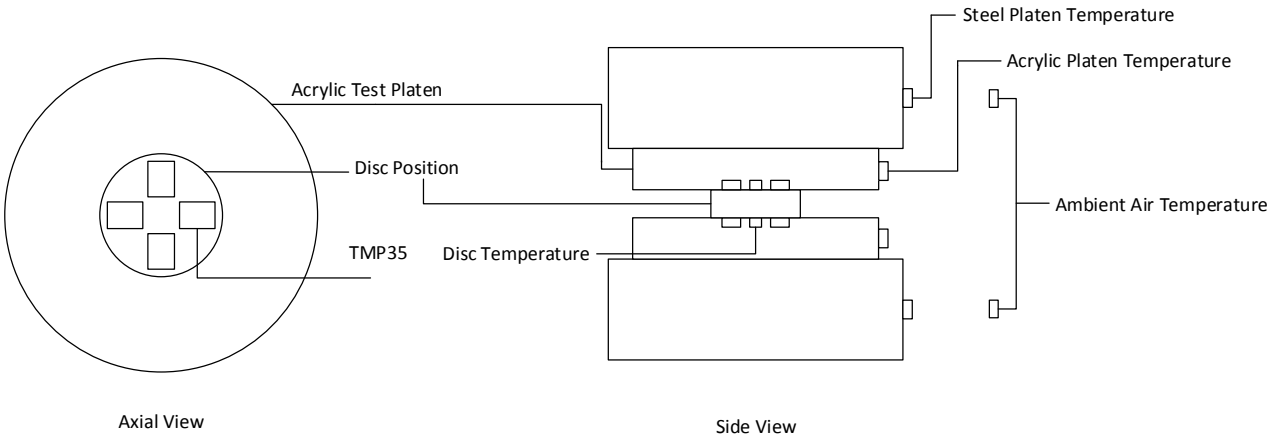

Figure 27 - Flat acrylic plates hold temperature sensors in place to measure disc temperature. Further temperature sensors monitor equipment and ambient temperatures.

Temperature sensors (TMP35, low voltage precision temperature sensors. Analog Devices, USA) connected to myRIO live data acquisition hardware (National Instruments. Texas, USA) were used to monitor temperature during test cycles at a 100 Hz acquisition rate. Four sensors, held in place by an acrylic plate, were used to measure temperature at the disc surface, whilst four measured the ambient around the disc: 1) the acrylic plate which was in direct contact with the disc 2) the steel platen of the test rig and 3) measuring ambient air temperature in close proximity to the disc and 4) measuring the temperature of the room at large (Figure 27, Figure 28). This setup was mirrored above and below the disc making a total of sixteen temperature sensors.

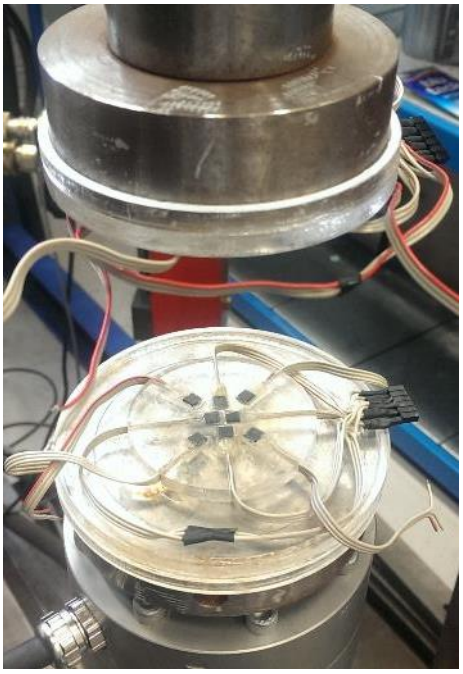

Figure 28 – Test rig with sensor array. Acrylic plates attached to the test rig with a low tolerance push fit secured sensors to the test platens. Ambient and rig temperature sensors were arranged as in Figure 27.

Mechanical energy dissipation by the disc under loading was calculated from force/deflection data collected by the Instron during testing. Data was acquired at 100 Hz allowing midpoint rectangle rule numerical integration to be performed on the data. A custom code (See appendix 11.5) developed with Dr Prabhav Nadipi-Reddy and written in MatLab (MathWorks. Mass, USA) was used to integrate both the loading and unloading portion of each individual loading cycle with the difference in these two values being equal to the energy dissipated by disc in each cycle. The mean average energy dissipated per cycle over the total loading period was then calculated. Hand calculations of several individual load cycle hysteresis values were used to verify outputs from the code.

5.3.1 **Modelling**

Using this data an idealised time stepping model was run to estimate theoretical change in temperature in the disc.

Several assumptions have been made in the model. The first is that starting temperature of the disc is 37 degrees based on typical core body temperatures from the literature [175-178] and that tissue around the disc is maintained at this temperature throughout due to regulatory blood flow. The model then calculates whether dynamic excitation of the disc can create enough energy to result in a temperature rise in the disc despite regulation from tissue surrounding the disc.

Secondly heat is assumed to be concentrated at the centre of the disc, this is a simplification to allow use of simple Fourier heat transfer equations and results in underestimated heat loss from the disc and therefore favours higher disc temperatures than a more complex 3D FEA thermal model.

Lastly it is assumed that all energy is dissipated as heat, ($\beta=1$). Some portion of dissipated energy will be used within the disc as bonds are broken and reformed within the tissue but this percentage cannot be calculated within the scope of this study or with the available equipment. Assuming $\beta=1$ provides a worst case, maximum temperature increase scenario.

Heat loss was calculated in two 2-dimensional directions, radially through the side wall of the disc and linearly through the circular faces of the disc using Fourier's law and combining this data created a simplified 3-dimensional model (Figure 29). On a second by second basis the internal energy of the

disc at time t=n, q_n, is calculated as the internal energy at time t=n-1 plus the summation of all energy gained from mechanical loading, $q_{mechanical}$, and energy lost by the disc to its surroundings, q_{linear} and q_{radial}, in the previous second. Using disc mass and thermal properties it is then possible to calculate disc temperature due to mechanical loading.

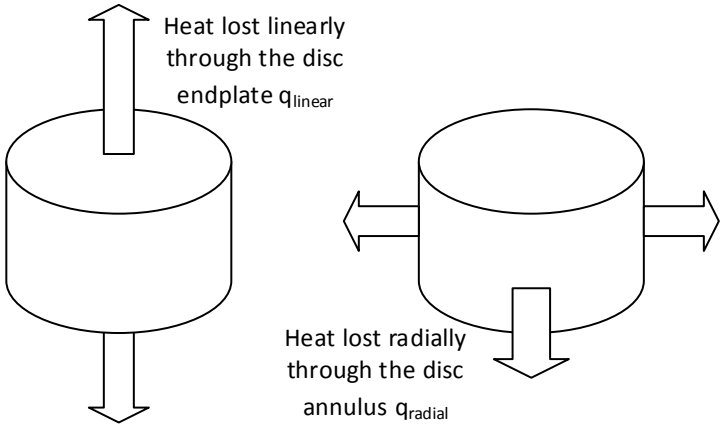

Figure 29 – IVD heat loss was calculated in two 2-dimensional directions (L) linearly though the disc end plate and (R) radially through the side walls of the disc annulus to create a simplified 3-dimensional model of heat loss. The model assumes that all dissipated energy is converted to heat within the disc (β=1) and that all heat is at the centre of the disc.

At time t=n:

From Fourier's law:

$$q_{lineral} = kA\frac{dT}{dx_{linear}} \quad (3)$$

$$q_{radial} = kA\frac{dT}{dx_{radial}} \quad (4)$$

$$q_n = q_{n-1} + q_{mechanical} - q_{radial} - q_{linear} \quad (5)$$

Where:

k is the thermal heat transfer coefficient.

A is the surface area where heat loss occurs.

dT is the temperature difference between the body and its surroundings.

dx is the linear or radial material thickness through which heat must be conducted.

Therefore

$$T_n = T_{n-1} + \frac{q_n + m}{c} \quad (6)$$

Where:

q_n is the internal heat energy of the disc at time t=n.

T_n is the temperature of the disc at time t=n.

m is the mass of the disc.

c is the specific heat capacity of the disc.

IVD specific heat capacity (3568 $J.kg^{-1}.K^{-1}$) and thermal conductivity (0.49 $W.m^{-1}.K^{-1}$) were taken from the IT'IS Foundation tissue database [179].

5.4 Results

5.4.1 Mechanical

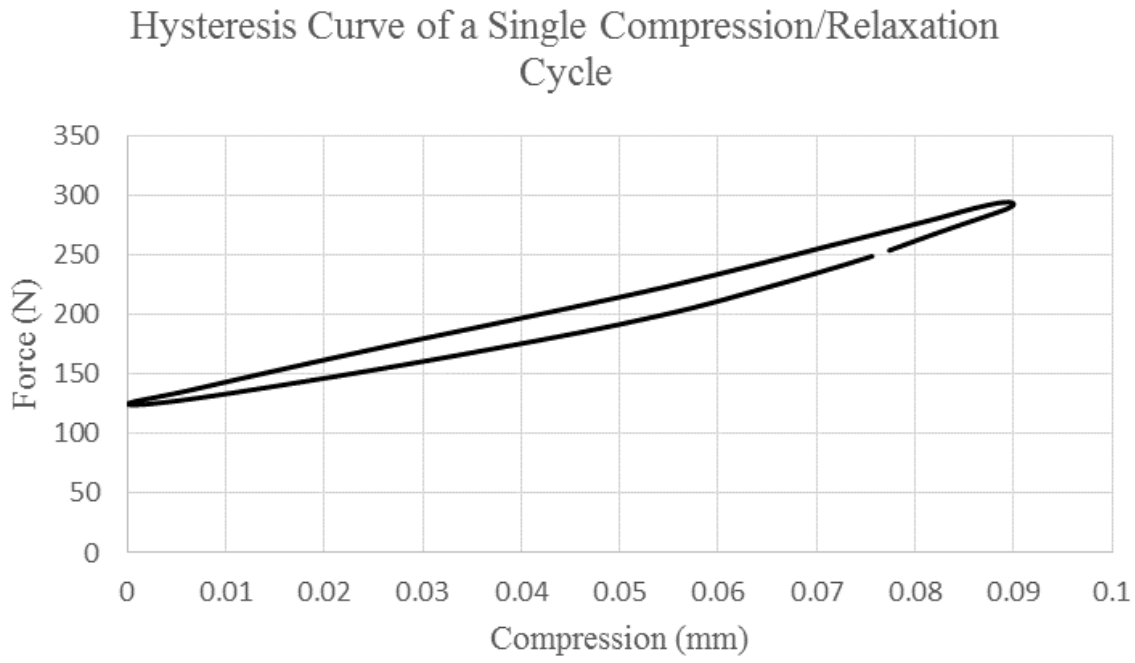

Figure 30 – Example curve from one disc (running, disc 4) showing one cycle of compression and relaxation. The area enclosed by the loop is the hysteresis and therefore mechanical energy gained by the disc. The break in the curve signifies where the software cycle registers an individual cycle beginning and ending,

Hysteresis from each compression and relaxation cycle (Figure 30) was used to calculate the total energy dissipated over the full hour along with an average dissipation per second. Under simulated walking conditions discs dissipated a mean average of 2.19 J (SD ± 0.58) at a rate of 0.608 ± 0.160 mJs^{-1}; for the simulated running condition dissipation was 12.3 ± 3.9 J over the full hour at a rate of 3.400 ± 1.090 mJs^{-1} (Table 9).

Table 9 – Total Energy Dissipated and Average Dissipation per Second for Each Disc.

	Walking		Running	
Disc	**Hour Total (J)**	**Per Second (mJs^{-1})**	**Hour Total (J)**	**Per Second (mJs^{-1})**
1	3.06	0.850	20.9	5.80
2	2.78	0.770	16.6	4.60
3	2.92	0.810	12.2	3.40
4	1.98	0.550	8.64	2.40
5	1.69	0.470	8.28	2.30
6	1.80	0.500	8.28	2.30
7	1.19	0.330	15.1	4.20
8	2.48	0.690	9.72	2.70
9	1.78	0.494	12.2	3.40
10	2.24	0.622	10.8	3.00
Mean	**2.19**	**0.608**	**12.3**	**3.40**

5.4.2 **Modelling**

Results from the model predict only minute changes in disc temperature that are well within the normal range of core body temperatures [175]. Steady state conditions were achieved within 300 seconds for running conditions suggesting no difference between short bursts of activity and prolonged exercise periods in terms of maximal temperature increase (Figure 31). Discs required longer to reach steady state under walking conditions though increase in temperature was effectively negligible.

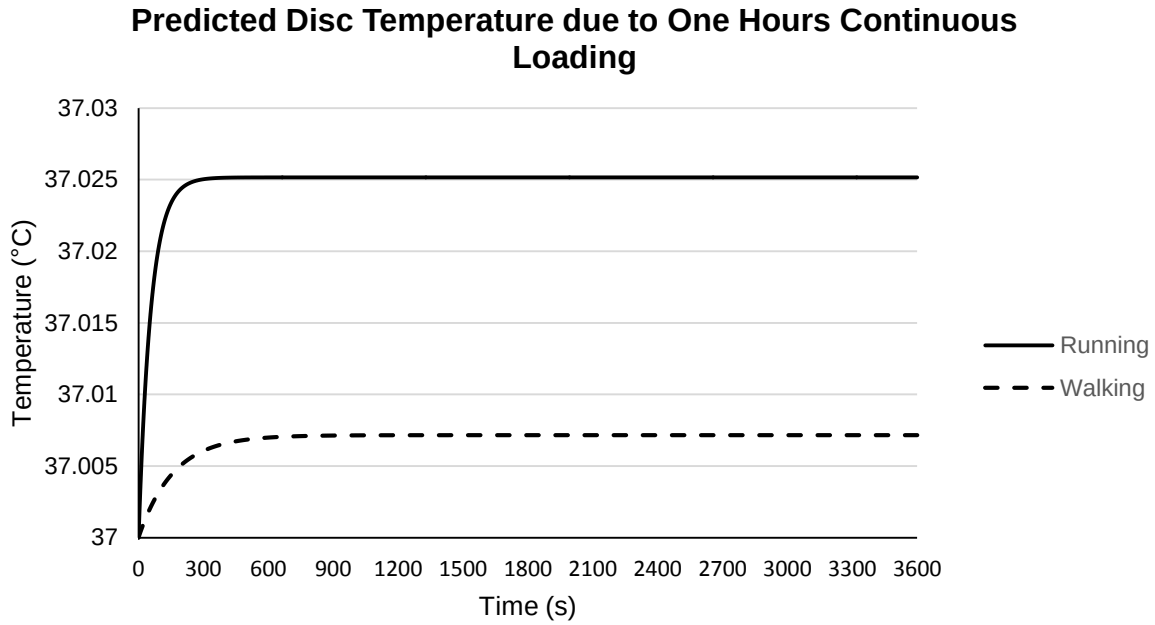

Figure 31 – Predicted temperature of an IVD subjected to sustained loading for one hour under either running or walking conditions. Temperature quickly reaches steady state and maximal increase is well within the normal range for core temperatures [175].

5.4.3 **Sensor Data**

Direct temperature measurement of the discs showed greater than 0.5 degree increases in all cases. Figure 32 shows the temperature of a single disc under walking conditions steadily increasing throughout the test.

Ambient temperatures; air, steel plate and acrylic sensor plate all showed increases throughout trials. An example of this is also shown in Figure 32

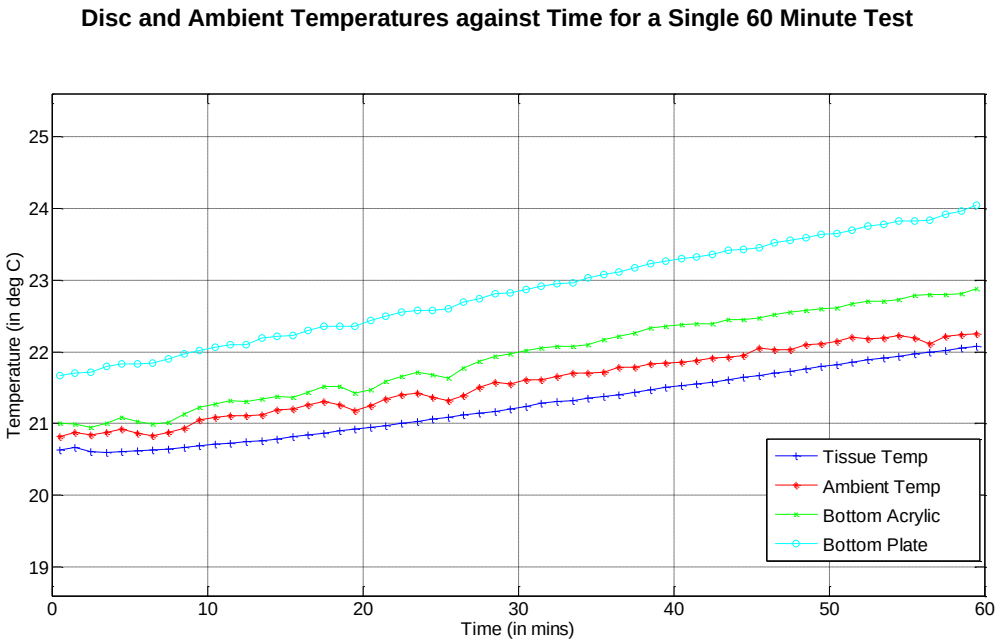

Figure 32 – Example data from one disc (Walking, disc 2) showing temperature data over a 60 minute test. The blue line represents average tissue temperature based on all 8 sensors in contact with the disc. Red is the average air temperature from sensors around the test rig. Light blue and green are from single sensors in contact with the steel rig plate and acrylic sensor plate respectively.

5.5 Discussion

Intervertebral discs subjected to mechanical loading *in vitro* at rates designed to simulate activities of daily living were shown to generate heat. The rate of heat generation (per second and per cycle) was over five and a half times greater in discs subjected to loading at levels approximating running than in those discs tested at levels approximating walking but in all cases hysteresis in each cycle and therefore heat generation was minimal. This is reflected in the model results which suggest that viscoelastic self-heating within the IVD is minimal and non-meaningful.

The mechanism of viscoelastic self-heating has been studied previously in non-biological structural and damping materials [145, 180-183] but the authors believe this study to be the first exploration of this mechanism in the IVD and possibly any biological material.

Bovine coccygeal discs were used as a surrogate for human discs, although this may be considered a limitation of this study previous work has shown that under the same test methods bovine and human intervertebral discs show markedly similar material properties when subjected to the same test protocol [132] whereas material properties of human discs tested under different test protocols vary in the literature by orders of magnitude [121, 128, 133]. It is therefore reasonable to assume that had human discs been tested under the same protocol as used in this study that results would not have been significantly different.

It has been assumed that all mechanical energy dissipated is converted to heat ($\beta = 1$). β represents the fraction of energy gained by the disc which is converted to heat through viscous friction, some of the energy gained by the disc will be used in microstructural changes such as the breaking and reforming of bonds within the polymer structures of the disc and therefore the true value of β will be less than 1.

The results of the model therefore represent a maximum possible magnitude of self-heating for the given loading and even in this worst case scenario viscoelastic self-heating due to ADLs is minimal and insignificant.

When measured directly tissue temperatures were observed to increase by amounts significantly greater than predicted by a theoretical model based on mechanical data from test cycles (Figure 32, page 103).

This increase in temperature was explained by observing ambient air, steel rig platen and acrylic sensor plate temperatures one or more of which were higher than tissue temperature at all times (Figure 32) meaning that heat was continuously moving to the disc from its surroundings. Ambient air and rig temperatures were also seen to increase throughout test cycles, the source of this increase was determined to be the hydraulic oil which achieved steady state at 37°C. The oil progressively heated the rig piston, plate and acrylic sensor plate as well as the air around the rig.

The lack of sophisticated environmental temperature control means that the relationships between mechanical loading and viscoelastic heating were difficult to determine through direct measurement and therefore direct measurement results have not been used in the analysis. More sophisticated environmental control methods would be required to accurately assess direct temperature measurements; however on the evidence of mechanical data any such temperature change would be so small as to present measurement challenges even in the most strictly controlled environment.

The current investigation made use of surface temperature data which is not a complete reflection of the temperature within a three dimensional object. Fine wire thermocouples could potentially be used to monitor internal disc temperature but the effects of piercing the disc on its mechanical behaviour and energy dissipation is not known and may alter results.

The model presented in this study assumed that temperature around the IVD remained constant at 37°C and under these conditions the calculated rate of heat generation by discs tested *in vitro* achieved steady state with heat loss from the disc after only a minimal increase in disc temperature. Although the true external temperature *in vivo* may not be 37°C or remain constant, as the key variable in determining heat loss from a disc is the difference in temperature between the disc and its surroundings the finding that the disc will not significantly increase its temperature above those of its surroundings due to mechanical loading would remain regardless of the exact temperature.

Whilst the heat generated within the disc is not enough to raise its temperature in and of itself, if external temperatures were increased above that of the disc then all heat generated by the disc would remain within the disc, shortening the time in which the disc required to achieve steady state with its surroundings. If exercise could be shown to increase body temperatures significantly then the IVD would also reach these new temperatures and potentially result in HSP upregulation.

Intramuscular temperatures have been shown to increase during exercise [177] with the quadriceps being shown to reach nearly 39°C after 30 minutes high intensity cycling [184]. However the quadriceps is a major, large and compact muscle which is highly loaded during cycling, an activity which would put less stress axially on the spine. To have a significant effect on IVD temperature exercise would need to increase core body temperature.

Tympanic temperature has been shown to increase during high intensity exercise [176] but the mean increase of 0.2°C is well within the normal range

of tympanic body temperatures [175] and far below the 43°C that resulted in HSP72 upregulation in tests by Park et al. [157].

An hours cycling has been shown to increase rectal temperature by 1.12°C above baseline measures [178] but the upper temperatures in this study still fell within the normal range of core body temperatures.

Since it is well known that core body temperature is regulated within a very narrow range and in most cases only increases minimally during exercise the present *in vitro* data does not support the hypothesis that loading designed to simulate activities of daily living would increase temperature within the IVD to a level capable of causing degeneration of the IVD through heat shock mechanisms.

5.6 Conclusion

Intervertebral discs subjected to mechanical loading *in vitro* at loading rates and frequencies approximating activities of daily living generated heat through viscoelastic damping. Those discs subjected to higher loading equivalent to running generated over five and a half times more heat than those loaded equivalent to walking. Temperature increase predicted by modelling was minimal and insignificant. The rate of heat generation measured *in vitro* in the current study would not seem to be sufficient to result in significant temperature increases if replicated *in vivo* and thus is unlikely to be a compound factor involved in initiating disc degeneration through the regulation of heat shock proteins.

6 Study 3: Damage, Degeneration and Treatment Intervention

What are the effects of damage, degeneration and hydrogel based interventions on the material behaviours and tissue functionality of intervertebral discs?

The PCHP used in this study was produced by Abbey Thorpe of Sheffield Hallam University as part of her postgraduate research under the supervision of Dr Christine LeMaitre and Professor Chris Sammon.

6.1 Abstract

Mesenchymal stem cells show promise as a treatment for degenerate intervertebral discs that could restore healthy disc function and greatly reduce the need for highly invasive surgical approaches. Mesenchymal stem cells would need to be injected into the disc within a scaffold matrix material and hydrogels have been proposed as possible materials for this purpose. However the effect of introducing hydrogel into the disc on its material behaviours is not known and the key structural function of the intervertebral disc means that without better understanding of these effects moving on to clinical trials would be unethical.

Four groups of bovine intervertebral disc representing a range of health and treatment conditions (healthy, control, degenerative and treated) were

subjected to dynamic cyclic loading at 2 Hz simulating the lumbar spine during a short period of walking and their material behaviours observed.

Bovine intervertebral discs that had been damaged or degenerated prior to testing displayed statistically significantly ($P<0.05$) reduced material and tissue functionality as defined by lower elastic stiffness, increased strain and reduced energy dissipation. Degenerate discs which had been injected with 100 µl of a proprietary hydrogel returned to pre-degeneration strain and elastic stiffness levels but made no improvement in terms of energy dissipation.

These results suggest that hydrogels designed for stem cell treatments offer an immediate benefit to the mechanical behaviours of degenerate intervertebral discs but cannot fully restore all tissue functionality to healthy levels.

6.2 Introduction

6.2.1 Pain and Degeneration

Back pain is an increasing global health concern and degeneration and/or acute injury of the intervertebral disc (IVD) has been linked with long term pain [20, 30, 144, 171, 185].

As well as being a potential pathogenesis of pain and cause of lost mobility, loss of disc height due to degeneration results in load being transferred to the posterior processes. The increased stresses are a risk factor in facet joint fracture and other serious medical issues [33].

6.2.2 Spinal Fusion

Current treatment for severe disc degeneration is spinal fusion, an invasive medical intervention which fuses one or more adjacent vertebrae together resulting in loss of motion [60, 61]. Fusion can be instrumented or non-instrumented, though both typically require an invasive discectomy [186]. Instrumented fusion inserts supporting structures, typically medical grade titanium directly into the spine to support the fusion process. Spinal fusion is increasingly common, with a 137% rise in cases in the USA between 1998 and 2008 [61, 62].

Interbody fusion methods in particular require extremely invasive protocols. Accessing the interbody cavity posteriorly requires the spinal cord to be retracted with a small hook like instrument and damage or inflammation resulting from this can lead to pain or even lowered motor control post-surgery [60, 64-67, 186]

As well as being highly invasive surgically the partial or total loss of mobility in the joint is a significant issue for quality of life and patients with lower postoperative mobility demonstrate worse clinical outcomes [70]. Only 20 % of spinal fusion patients are pain free 5 years post intervention and 22.2 % of patients have unsatisfactory outcomes from spinal fusion surgeries [60, 71]. As many as 25% of patients require re-operation following an initial spinal fusion [71]. Spinal fusion surgery mortality rate is 0.2-0.29 % and is significantly linked with increased mortality rates in spinal surgery ($P < 0.001$) [62, 73, 187]. Spinal fusion patients are increasingly over 65 years of age resulting in greater complications and comorbidities, spinal fusion surgery is more than twice as likely to result in death of those over 65 years than under and nearly 5 times more likely for those over 75 years [187].

Given the high risk of spinal fusion surgery what alternative methods exist?

6.2.3 Regenerative Medicine, Stem Cells and Hydrogel

Regenerative medical interventions aim to reverse the degeneration process by aiding disc regrowth and preventing the need for discectomy and fusion. A potential regenerative medicine approach being explored is the use of mesenchymal stem cells (MSC) to induce regeneration of the nucleus pulposus (NP) in degenerate discs [74-77]. Such treatments would require MSCs to be introduced into the NP and one potential method is to inject the cells within a scaffold matrix and hydrogel has been investigated as a potential matrix material [75-77, 79].

The IVD is significant for its role both structurally and in allowing spinal motion, the mechanical response of the IVD to loading during activities of daily living (ADL) is of key importance in quality of life. The addition of a hydrogel matrix to the NP is likely to alter the mechanical response of the IVD to a range of activities but this has not previously been investigated so the exact nature of any change is not known. Although the hydrogel's purpose is to provide a scaffold to allow MSCs to regenerate disc tissue it may have clinical benefits in and of itself. Similarly, if hydrogel results in negative mechanical effects on the disc then it would have serious implications for stem cell treatment of degenerate discs.

A proprietary pNIPAM/DMAc/Clay hydrogel has been developed by researchers at Sheffield Hallam University suitable for use as a stem cell scaffold matrix [188]. A polymer-clay precursor hydrogel (PCPH) with a viscosity (0.6-2 mPas) and density (1000-1100 kgm^3) comparable to water is injectable through small gauge needles and with a 37°C gelation temperature it can reach gel state after being injected into human or animal bodies.

Although stem cell treatments are a promising regenerative treatment for degenerate IVDs there is currently no evidence for or against the effects of hydrogel injections on the mechanical and tissue functionality of IVDs. This study investigates the differences in mechanical response and tissue functionality of IVDs that are healthy, degenerate, degenerate with PCPH injection and a control group that underwent needle puncture.

6.3 Method

Coccygeal discs were harvested from bovine tail sections obtained from local abattoirs, operating in concordance with animal welfare regulations, from cows aged approximately 18 months at time of sacrifice. Tails were stored at -20°C shortly post sacrifice and tested a maximum of one month post storage. Tails were thawed at 5°C for 24 hours prior to sample preparation. Discs were carefully dissected whole from tail sections and allowed to reach room temperature for 12 hours before testing, between 2 and 4 discs were gained from each tail section. Discs were stored in sealed bags to prevent dehydration during this time.

Discs were randomly assigned to four test groups; healthy, control, degenerate and hydrogel, containing 10 discs each.

Discs in degenerate and hydrogel groups were injected with a 2mg/ml solution of collagenase in distilled water and incubated for 2 hours at 37°C to simulate moderate degeneration, the hydrogel group discs then received an injection of the Sheffield Hallam group PCPH to simulate degenerate discs having undergone stem cell intervention, discs were left 30 minutes post injection to allow the PCPH to gelate prior to testing.

Collagenase is ubiquitous in chemistry and biology for its use in digestion of collagen and has previously been used to induce degenerative effects in IVDs [189]. Previous studies have induced degeneration through high mechanical loading [37] but collagenase digestion offers a highly repeatable protocol

which results in the internal occlusions typical of degenerate discs including degeneration which has no mechanical basis.

Injection protocol consisted of the syringe being depressed until either the internal pressure in the disc prevented any further intake of collagenase solution or a maximum of 200 µl of solution or hydrogel had been injected. Collagenase intake approximately ranged from 100-200 µl whilst hydrogel ranged from 50-100 µl. Needles were inserted through the side wall of the annulus fibrosus (AF) into the NP before injection occurred simulating *in vivo* injection protocol. Control group discs were punctured with a 21 gauge needle in the same manner but were not injected (Table 10).

Table 10 – Damage, degeneration and treatment mechanism for each test group

	Needle inserted through AF into NP	Collagenase injected and disc incubated for 2 hours at 37°C	Hydrogel Injected	Subjected to cyclic loading
Healthy				✓
Control	✓			✓
Degenerate	✓	✓		✓
Hydrogel	✓	✓	✓	✓

Discs were loaded cyclically using a dynamic test rig incorporating a hydraulic piston controlled by Wavematrix 1.8 test software and a Labtronic 8800 hydraulic controller (Instron. Mass, USA). Discs were subjected to a sinusoidal load between 0.53 and 0.65 MPa at 2 Hz (Figure 33) to simulate walking based

on known values from the literature [97, 165, 166]. Each disc was ramped to a preload at the midpoint between the upper and lower bounds at a rate of 0.01 kN/s, immediately upon reaching the preload cyclic loading began and ran for 100 seconds, a time chosen to simulate a short period of activity typical of ADLs in all but the most severely impaired persons.

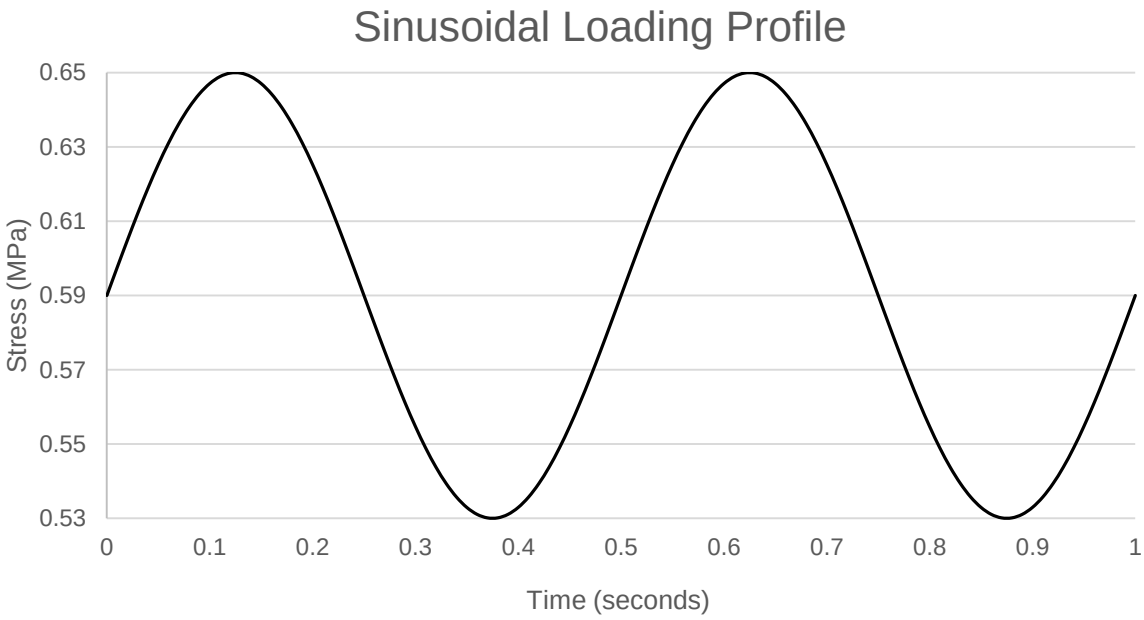

Figure 33 – Discs underwent sinusoidal loading between 0.53 and 0.65 MPa at a frequency of 2 Hz to simulate walking, these values were based on *in vivo* data from the literature.

Three measures of mechanical response were tracked by the data acquisition tools connected to Wavematrix. Engineering stiffness of each disc was measured as the change in force divided by the change in displacement from minimum (0.53 MPa) to maximum (0.65 MPa) loading. Strain was measured as the displacement between those points divided by the disc height at minimum loading. Lastly the energy dissipated by discs during each loading/unloading cycle was calculated from the hysteresis in each cycle (Figure 34), the mean value of energy dissipation per cycle was used to calculate average energy dissipation per second.

Difference between groups was analysed by one ANOVA with a Tukey post-hoc test using SPSS software (IBM, USA) with significance considered to be $P < 0.05$.

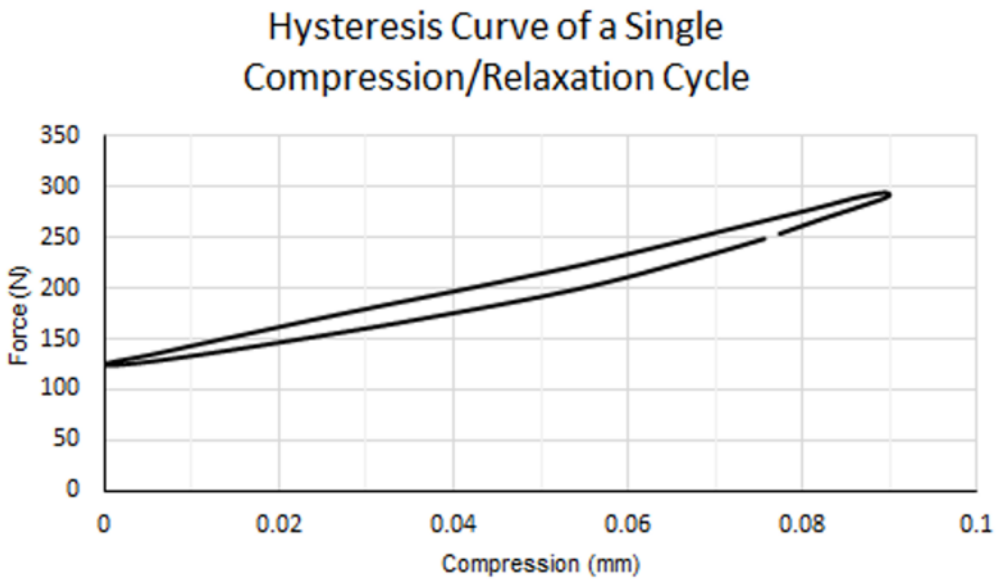

Figure 34 – An example of hysteresis typical in a single loading cycle of an IVD. The area contained within the loop represents the energy dissipated by the disc under loading. A purely elastic material would have no hysteresis whilst increasingly viscous materials display greater hysteresis as more energy is dissipated by the material.

6.4 Results

6.4.1 Raw Data

Mean strain percentage and stiffness per cycle and energy dissipated per second from raw data for all discs are shown in Table 11, mean and standard deviation of these values for each group are also reported.

Table 11 - Strain, Stiffness and Dissipated Energy for All Discs.

Healthy Discs	Strain (%)	Stiffness (N/mm)	Dissipated Energy per Second (mJ/s)
1	0.63	2097	2.6
2	0.54	2781	4
3	0.54	2565	3.5
4	0.44	2640	4.3
5	0.49	2809	5.5
6	0.67	2753	4.7
7	0.55	2326	3.6
8	0.47	2715	4.5
9	0.54	3016	6.3
10	0.55	2688	3.3
Mean	**0.54 (0.07)**	**2639 (247)**	**4.23 (1.03)**
Control Discs			
1	0.60	2597	0.39
2	0.58	1980	0.42
3	0.58	2350	0.44
4	0.61	2588	1.30
5	0.49	1978	0.44
6	0.47	2088	0.47
7	0.49	2621	0.45
8	0.47	1886	0.45
9	0.55	1662	0.72
10	0.62	2084	0.95
Mean	**0.55 (0.06)**	**2183 (336)**	**0.60 (0.30)**
Degenerate Discs			
1	1.37	762.9	0.80
2	2.32	367.6	1.16
3	7.72	224.3	1.77
4	3.64	374.2	0.75
5	3.92	459.7	0.78
6	5.99	178.2	0.76
7	0.46	2136	0.33
8	0.43	1681	0.41
9	0.52	1740	0.41
10	0.63	1652	0.66
Mean	**2.70 (2.57)**	**957.6 (755)**	**0.78 (0.42)**
Hydrogel Discs			
1	0.47	2690	0.69
2	0.52	2378	0.66
3	0.50	2406	0.70
4	0.49	2076	0.41
5	0.53	1139	0.42
6	0.48	2078	0.61
7	0.45	2266	0.51
8	0.43	2242	0.32
9	0.57	1766	0.50
10	0.52	1283	0.37
Mean	**0.50 (0.04)**	**2032 (497)**	**0.52 (0.14)**

The mean strain for each disc is shown in Figure 35 with healthy, control and hydrogel groups highlighted in Figure 36. The mean strain across the degenerate group (2.7%) was significantly larger than every other test group

(P < 0.05), there was no significant difference between the healthy group and either the control or hydrogel groups though strain in the control group was significantly larger (P = 0.022) than the hydrogel group.

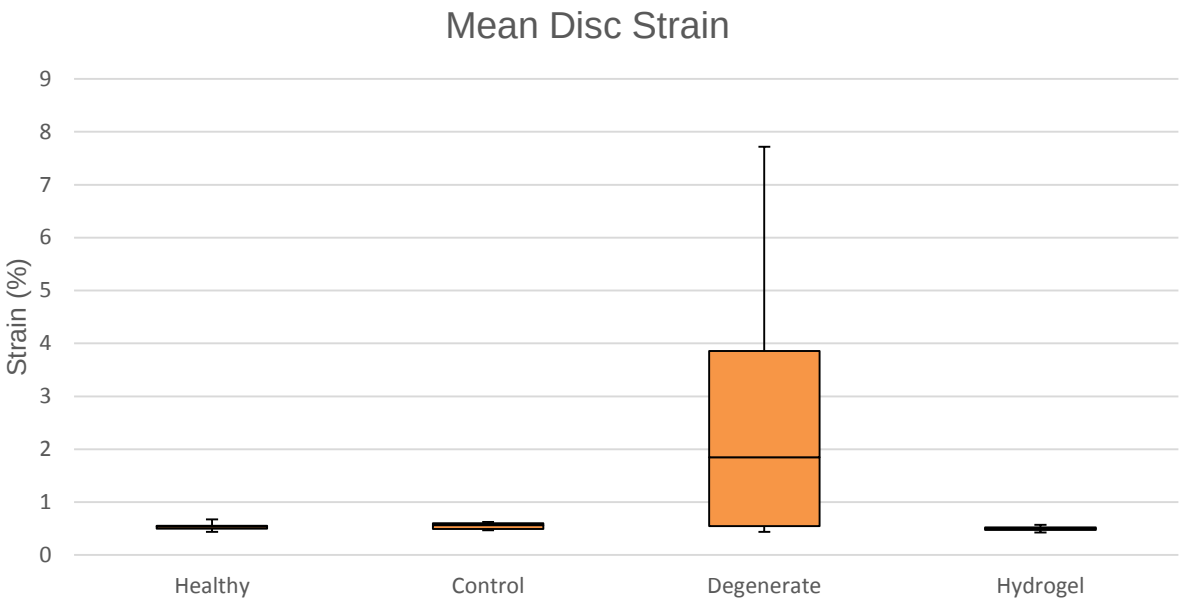

Figure 35 - Mean strain per cycle for discs in each test group. The degenerate group showed a wide spread with a mean strain across the group far larger than any of the other three groups.

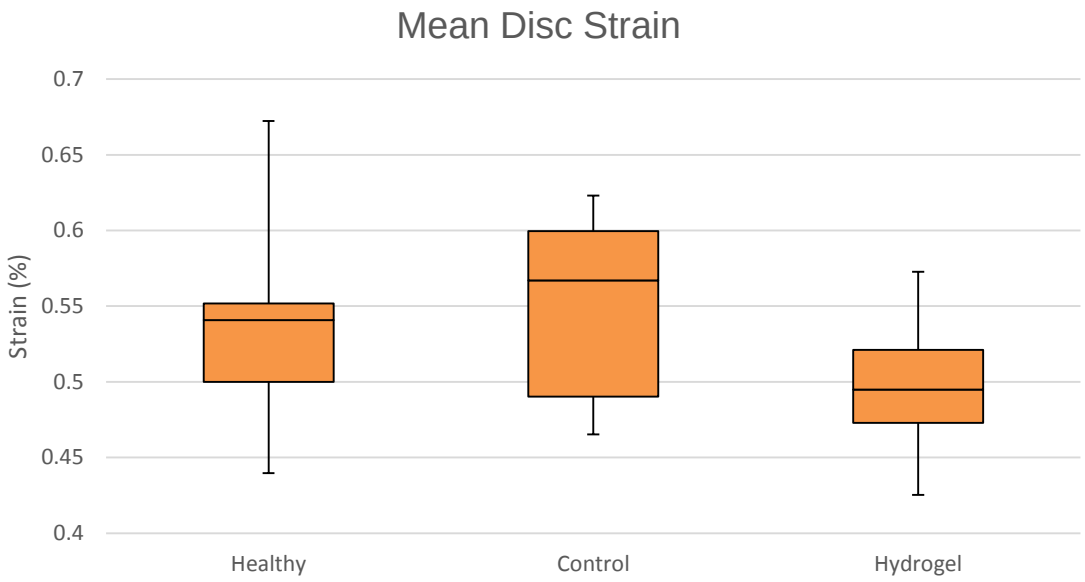

Figure 36 – Magnified results from Figure 35 excluding degenerate group discs. Healthy, Control and Hydrogel groups showed similar mean strain levels over 200 cycles.

Mean disc stiffness is shown in Figure 37. Stiffness across the degenerate group discs was significantly ($P < 0.01$) lower than all other test groups. Healthy discs were significantly ($P < 0.05$) stiffer than control and hydrogel groups but there was no significant difference between the control and hydrogel groups.

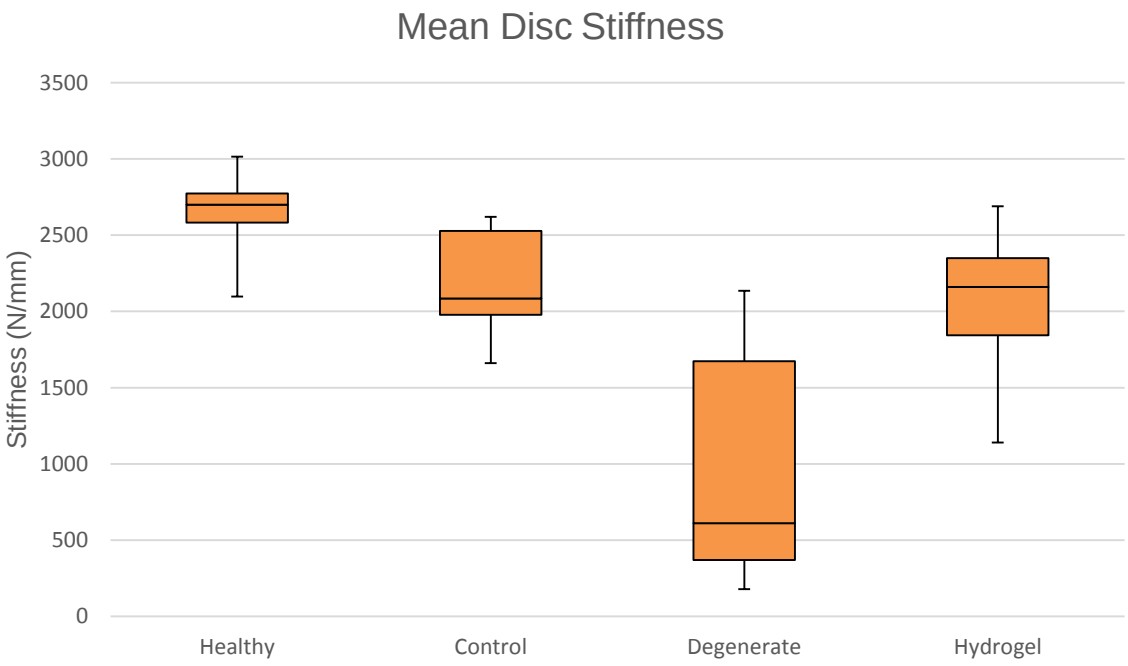

Figure 37 – Spread of disc stiffness values, degenerate discs varied significantly with mean and median stiffness far below those of the other three groups.

Mean energy dissipation per second was 4.23 mJ across the healthy test group, energy dissipation was significantly lower (P < 0.001) in control (0.60 mJ), degenerate (0.78 mJ) and hydrogel (0.52 mJ) test groups (Figure 38).

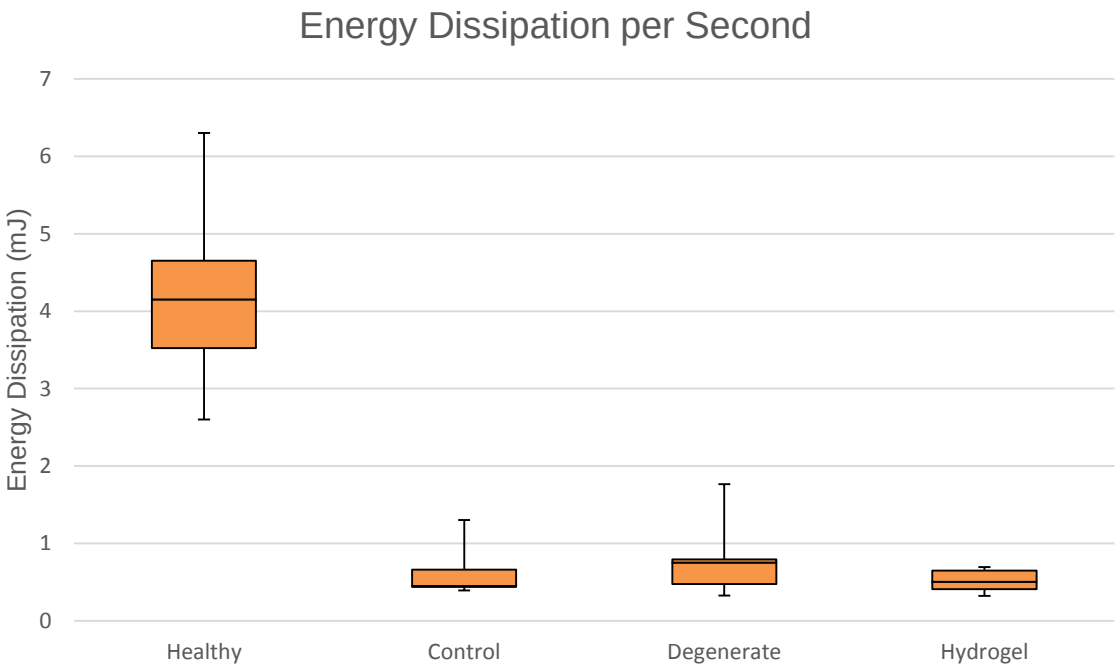

Figure 38 – Energy dissipation per second of discs in all four test groups

6.4.2 Data Normalised for Disc Size

Significant differences in disc size (P<0.001) were observed between healthy group discs and discs in all three other groups and some trends were observed between disc size and mechanical and tissue functionalities. Energy dissipation was found to significantly correlate with disc size in all four groups and stiffness and disc size were strongly correlated in the healthy and hydrogel groups. Data has been normalised by adjusting values along disc size trend lines.

Mean strain percentage, disc stiffness and energy dissipated per second for all discs after normalising data to account for differences in disc size is shown

in Table 12, mean and standard deviation of these values for each group are also reported.

The mean strain across the degenerate group (2.7%) was significantly larger than every other test group ($P < 0.05$), there was no significant difference between the healthy group and either the control or hydrogel groups though strain in the control group was significantly larger ($P = 0.022$) than the hydrogel group.

Stiffness across the degenerate group was significantly ($P < 0.001$) lower than all other test groups. Control group discs were significantly less stiff than healthy discs ($M = 455.5$ Nmm^{-1}, $P = 0.033$) and hydrogel injected discs ($M = 489$ Nmm^{-1}, $P = 0.003$). After normalising against disc size no significant difference in stiffness was observed between healthy and hydrogel injected discs ($M = 33.7$ Nmm^{-1} $P = 0.833$) (Figure 39).

Table 12 - Strain, Stiffness and Dissipated Energy for All Discs when Normalised against Disc Diameter

Healthy Discs	Strain (%)	Stiffness (N/mm)	Dissipated Energy per Second (mJ/s)
1	0.63	2097	2.6
2	0.54	2781	4
3	0.54	2565	3.5
4	0.44	2640	4.3
5	0.49	2809	5.5
6	0.67	2753	4.7
7	0.55	2326	3.6
8	0.47	2715	4.5
9	0.54	3016	6.3
10	0.55	2688	3.3
Mean	**0.54 (0.07)**	**2639 (247)**	**4.23 (1.03)**
Control Discs			
1	0.60	2597	1.38
2	0.58	1980	1.16
3	0.58	2350	1.44
4	0.61	2588	1.91
5	0.49	1978	1.37
6	0.47	2088	1.45
7	0.49	2621	1.59
8	0.47	1886	1.41
9	0.55	1662	1.64
10	0.62	2084	2.02
Mean	**0.55 (0.06)**	**2183 (336)**	**1.54 (0.26)**
Degenerate Discs			
1	1.37	762.9	1.70
2	2.32	367.6	1.72
3	7.72	224.3	2.11
4	3.64	374.2	1.67
5	3.92	459.7	1.82
6	5.99	178.2	1.38
7	0.46	2136	1.17
8	0.43	1681	0.90
9	0.52	1740	1.30
10	0.63	1652	1.31
Mean	**2.70 (2.57)**	**957.6 (755)**	**1.51 (0.36)**
Hydrogel Discs			
1	0.47	3123	0.97
2	0.52	2909	0.89
3	0.50	2943	0.90
4	0.49	2505	0.72
5	0.53	2213	0.61
6	0.48	2988	0.92
7	0.45	2696	0.80
8	0.43	2427	0.69
9	0.57	2674	0.79
10	0.52	2247	0.62
Mean	**0.50 (0.04)**	**2672 (318)**	**0.79 (0.13)**

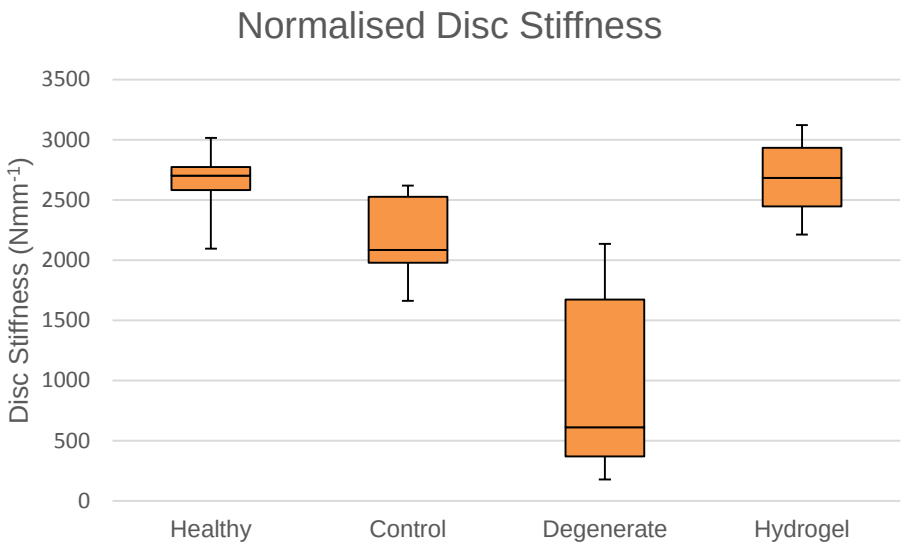

Figure 39 – When disc size was accounted for there was no significant difference in stiffness between healthy and hydrogel injected groups (M = 33.7 Nmm^{-1}, P<0.001).

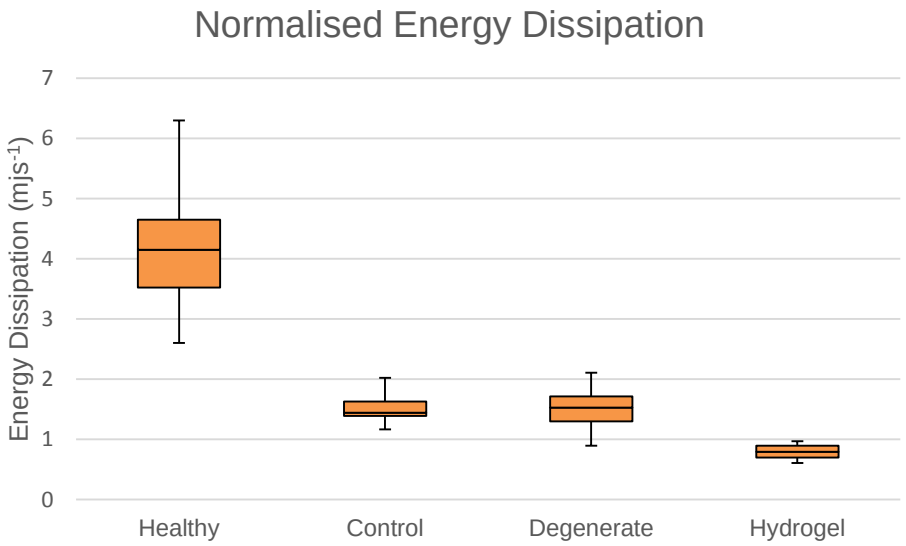

Figure 40 – Even when disc size was accounted for, energy dissipation in all three non-healthy groups is significantly lower (P<0.001) than healthy discs though the magnitude of the difference is reduced compared to raw data. Energy dissipation is lowest of all in the hydrogel injected group (0.79 mjs^{-1}).

Normalising against disc size reduced the difference in energy dissipation between healthy discs and all other groups but remained significantly different in all cases. Energy dissipation was lowest of all in the hydrogel group with a

large significant reduction compared to healthy discs (M = 3.4 mJs^{-1}, P <0.001) and significantly (P < 0.001) lower than control and degenerate groups.

6.5 Discussion

6.5.1 Recovery of Hydrogel Injected Discs

Intervertebral discs subjected to loading simulating a short period of walking demonstrated a different mechanical response and tissue functionality depending on the health condition of the disc. When differences in disc size were accounted for healthy discs had 80% lower strain (0.54% to 2.7%), 2.75 times higher stiffness (2639 to 957.6 Nmm^{-1}) and 2.8 times greater energy dissipation (4.23 to 1.51 mJs^{-1}) than 'degenerate' discs that had undergone collagenase digestion. Discs subjected to the same collagenase digestion process that were then injected with a proprietary hydrogel designed as a scaffold matrix for stem cell treatment demonstrated a significant recovery of structural properties, complete regaining back pre-degeneration levels of healthy disc stiffness (2672 Nmm^{-1}) and reverting to pre-degeneration strain levels (0.5%). No recovery of tissue functionality, defined as the discs ability to dissipate energy, was observed with hydrogel injected discs dissipating less energy than any other test group (0.79 mJs^{-1}).

This data provides a strong suggestion that the hydrogel used in this study offers a significant and near immediate structural benefit to degenerate IVDs but cannot return full disc tissue functionality in the short term. To the authors knowledge this is the first study that has investigated the mechanical response

of IVDs injected with scaffold matrix hydrogels and the results have significant clinical implications for the treatment and recovery of degenerate IVDs.

The full recovery of disc mechanical response following hydrogel injection is a highly promising result suggesting an immediate benefit in cases of degenerate discs. As well as being a possible pathogenesis for pain, degenerate IVDs affect spinal loading, shifting stress away from the vertebral body to the posterior processes and risking significant further negative health outcomes [33]. The positive mechanical effects of hydrogel injection could lessen or reverse these risks and reduce the likelihood of discectomy or fusion being required. Hydrogel injection did not recover energy dissipation to pre-degeneration levels but over time stem cells could be infused into the hydrogel which may recover this behaviour by repairing the disc tissue.

6.5.2 **Normalising Data against Disc Size**

Data was adjusted to account for disc size after significant differences in disc diameter were observed between the healthy group and control ($M = 6.85$ mm, $P < 0.001$), degenerate ($M = 5.8$ mm, $P = 0.001$) and hydrogel ($M = 6$, $P = 0.001$) group discs. Plotting the disc stiffness against disc diameter for healthy and hydrogel groups demonstrates this trend ($r = 0.88$) (Figure 41).

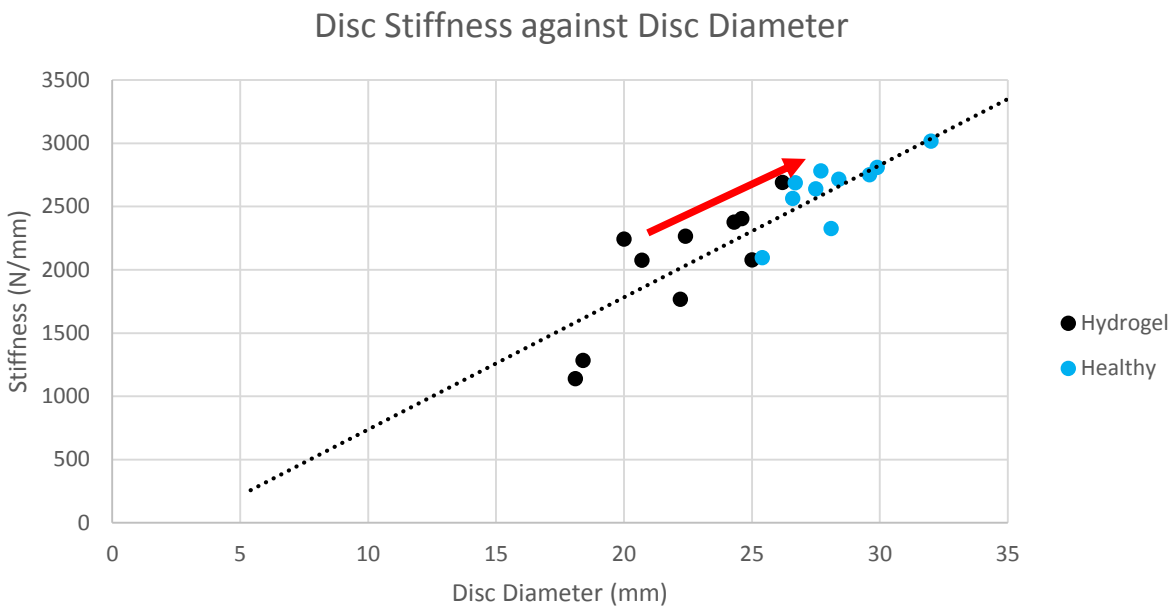

Figure 41 – Healthy and hydrogel group discs show a strong correlation (r^2 = 0.77) between disc diameter and disc stiffness and Healthy group discs had a mean diameter 6mm greater than hydrogel group discs. Hydrogel data was adjusted in line with this trend (red arrow) to account for the difference in size between groups.

When data was adjusted, by transposing each discs stiffness value along the diameter-stiffness trend-line seen in Figure 41 to account for the 6 mm difference in disc size between groups there is no longer a significant difference in stiffness between healthy and hydrogel groups (Figure 39). In the original data there was a significant difference between the groups (M = 606 N/mm P = 0.02) but after normalising against disc size this difference is small and no longer significant (33.7 N/mm P = 0.833).

Not all parameters significantly correlated with disc size. Plotting disc strains against diameter for healthy and hydrogel discs shows only a small and non-significant correlation between disc diameter and strain (r = 0.249, P = 0.289) (Figure 42). Adjusting hydrogel group disc strain in accordance with this observed relationship to account for the 6mm difference in average diameter has only a notional effect on strain. Difference in mean strain in the original

data is small and non-significant (0.047% P = 0.111) and remains so in the adjusted data (0.010% P = 0.623) and due to the non-significant trend, this adjustment was not included in final data values.

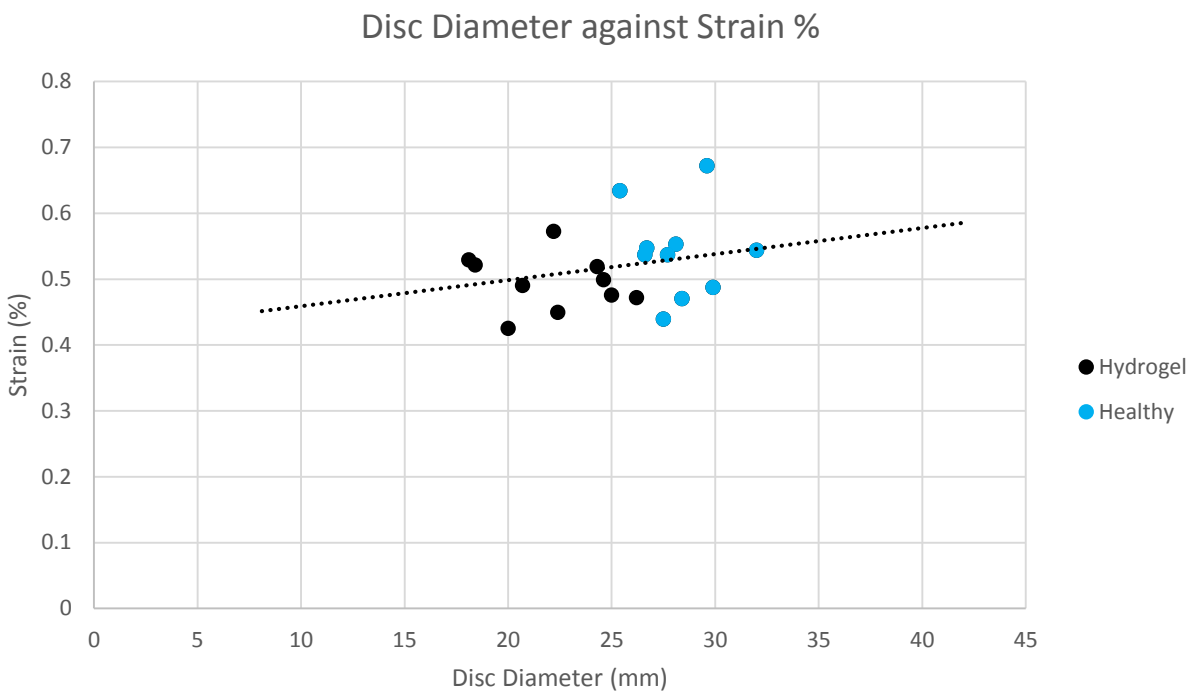

Figure 42 – Healthy and hydrogel disc specimens show no correlation between disc diameter and disc strain ($r^2 = 0.06$).

The observed difference in disc diameter is the result of natural variation in the size of cow tails used for each group. Although such variation is found in humans it could be argued that discs could have been matched for size prior to testing. This was not done for two reasons.

The first is practical, each damage or treatment method is an involved process requiring significant time and effort. Attempting to mix and match protocols during one laboratory visit based on disc size is highly impractical compared to running test groups as complete batches on different days.

The second is that this would reduce the randomisation of the overall study. Each tail section was stored, dissected and tested in the order it was received from the abattoir, any sorting protocol would have resulted in a non-random test protocol.

6.5.3 **Degenerate Outliers?**

Statistically, degenerate discs appear to have a significant correlation between disc diameter and strain (r = 0.703, P = 0.023) but this trend is questionable when observing the potted data (Figure 43).

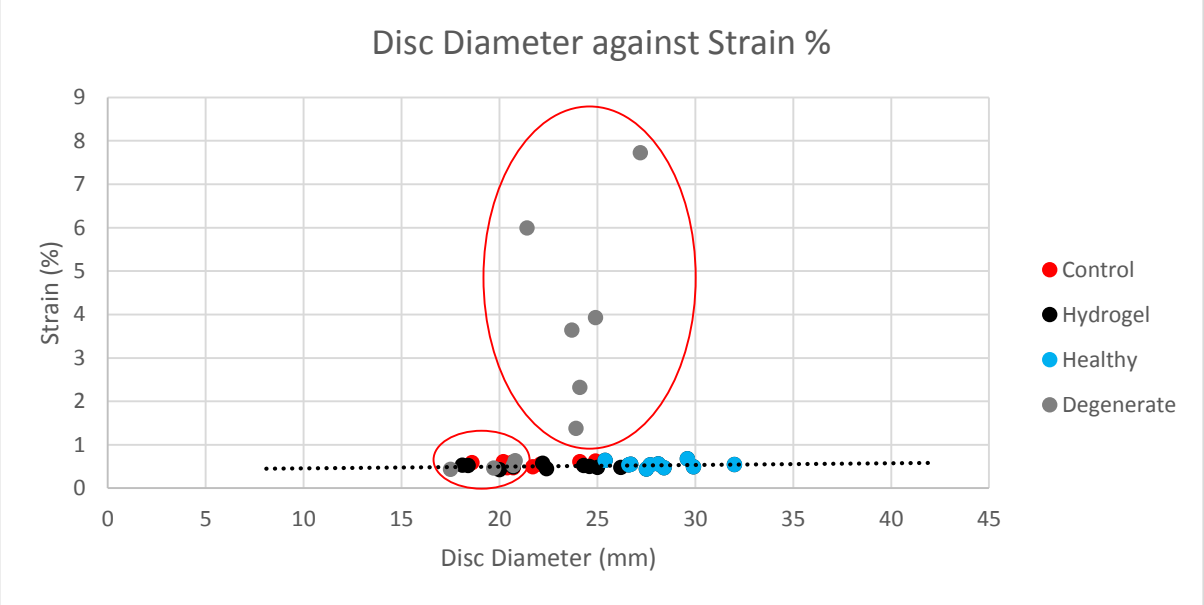

Figure 43 – Mean strain in all discs against disc diameter. Note the wide spread of degenerate discs which appear to form two distinct groups.

Six of the degenerate group showed a large variation in strain that bares no relation to disc diameter whilst four are in line with discs from the other three groups (Figure 43) a pattern repeated for disc stiffness (Figure 44). Comparing the degenerate group with the healthy group shows that degenerate discs varied widely from those which displayed strain and stiffness close to or in line

with the healthy group to those which suffered a near complete collapse of mechanical integrity compared with healthy discs.

Can these four degenerate group discs be dismissed as outliers?

If the discs were healthy they should display the same tissue functionality as healthy group discs. Whilst strain and stiffness are in line with healthy discs, energy dissipation of those four degenerate group discs was significantly lower than that in healthy discs and clearly in line with the other discs in the degenerate group (Figure 45).

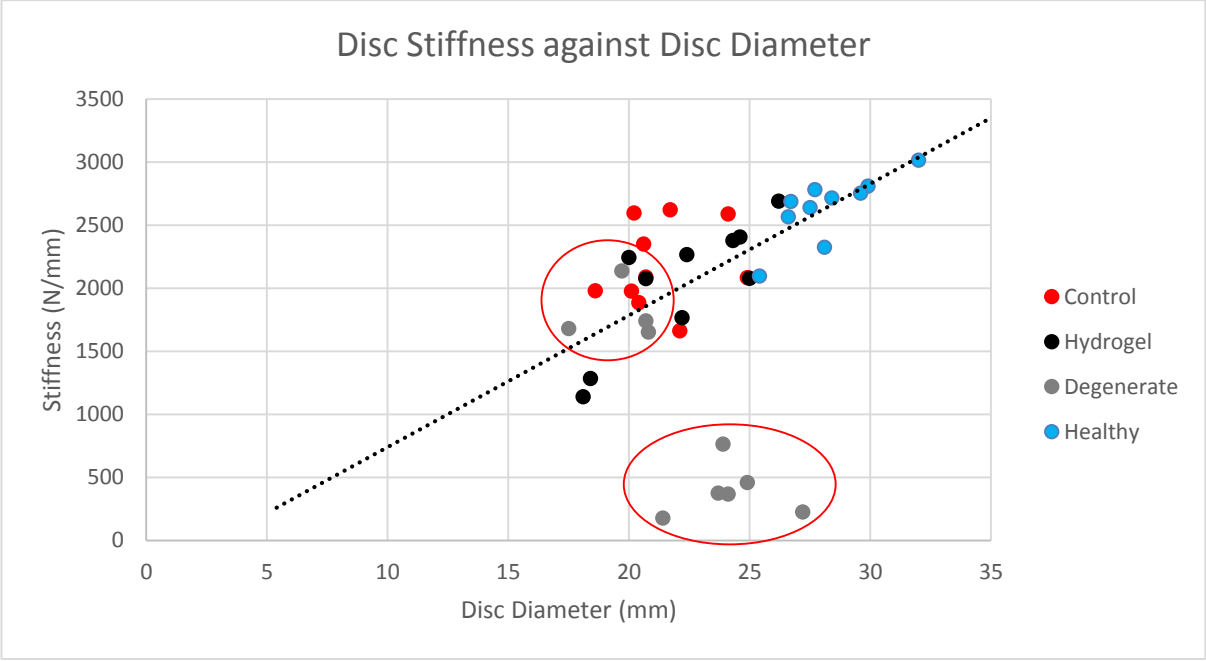

Figure 44 - Mean stiffness in all discs against disc diameter. Note the wide spread of degenerate discs which appear to form two distinct groups.

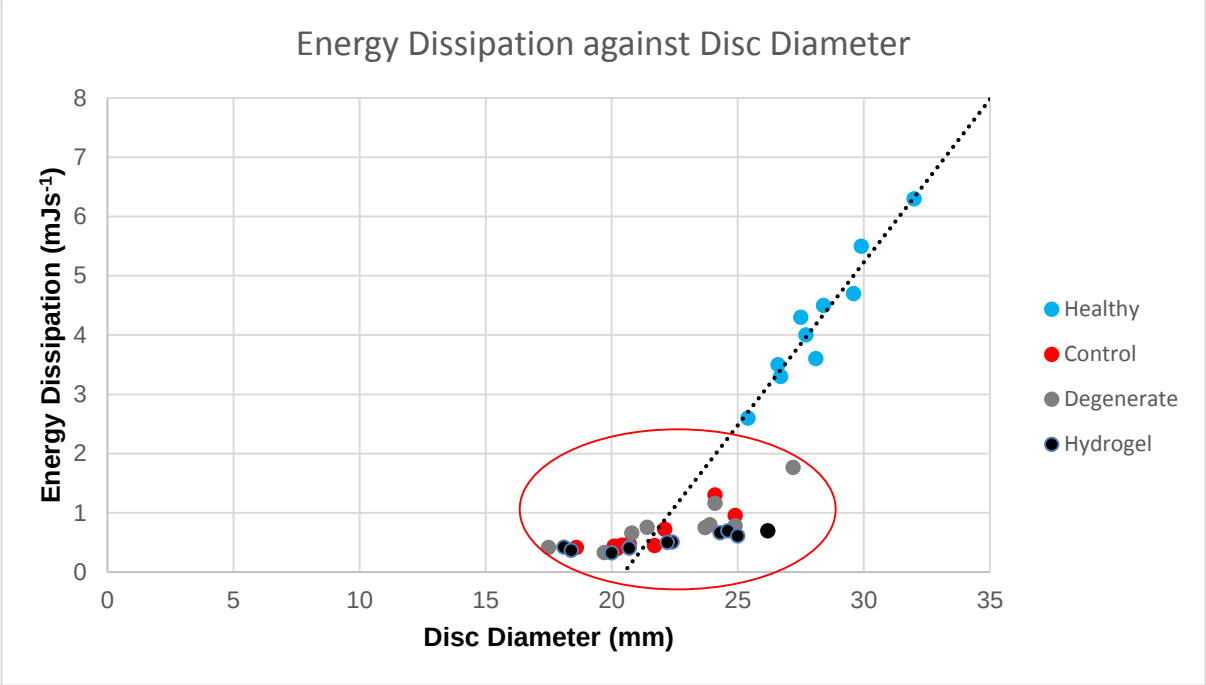

Figure 45 – Degenerate group discs had significantly lower energy dissipation than the healthy group.

6.5.4 Puncture Damage and Loss of Tissue Functionality

With all three groups showing significantly lower energy dissipation than healthy discs the data seems to suggest that needle puncture may be the key initiator of discs losing tissue functionality, needles were inserted through the side wall of the AF into the NP thereby negating the pressure vessel effect of the IVD. In the control group this puncture effectively mimics acute injury of the IVD without chronic degeneration. In the degenerate group the needle puncture could represent an acute injury or damage to the disc's structural integrity due to chronic degeneration. The wide variation in strain and stiffness in the degenerate group is likely a result of variable levels of degeneration in the NP with the loss of tissue functionality an entirely separate factor.

6.5.5 **Clinical Implications**

The data presented in this study has numerous clinical implications in respect to IVD health, injury and treatment.

There is a large body of evidence which has linked physical damage of the IVD to further degenerative breakdown with biological degeneration of the IVD always occurring following artificially induced damage *in vivo* in animal studies [37]. Several longitudinal studies on animals have observed continued degeneration of the disc in the months following damage or puncture to the IVD [110, 111]. Whether this trend holds true in humans is the subject of much debate [53-56, 190] however it is known that chronic back pain episodes which last longer than 14 days become increasingly unlikely to end following an approximate exponential decay suggesting that degeneration leads to further degeneration [26].

Discs in the control group, damaged by needle puncture, demonstrate a clear loss of tissue functionality that is separate to degeneration seen in the degenerate group discs. The exact mechanism for how damage or puncture may result in degeneration is unknown and outside the scope of this study but two possible mechanisms were observed.

Energy dissipated by the discs which had been punctured was 64% of that dissipated in healthy discs, IVDs under cyclic loading demonstrate a complex relationship of anabolic protein and catabolic enzymes [146, 153] and such a significant change in disc energy could affect cell regulation.

Needle puncture through the annulus compromises the disc as a pressure vessel, reducing internal pressure of the disc under loading [37] . Pressure has been studied as a potential factor in protein and enzyme function and damage to the disc changing the homeostatic pressure may upset the balance of catabolic and anabolic processes within the disc [191-196].

The loss of both mechanical and tissue functionality of the IVD seen in the degenerate disc group is in line with expected behaviour of degenerate discs as previously observed [37, 40-43, 48, 53, 54, 110, 111, 190]. The data presented in this study provides several interesting clinical findings. The first is the wide variance in mechanical behaviour of discs which underwent the same collagenase digestion protocol highlighting the large variation in individual discs and the complexity in categorising degeneration.

The second is that loss of tissue functionality is not related to degeneration. Energy dissipation of degenerate group discs was closely grouped despite the wide range of strain and stiffness within the group. This is further evidence that physical injury to the disc is the major factor in loss of energy dissipation by the IVD.

The most significant clinical finding in this study is the complete recovery of disc mechanical functionality in discs injected with polymer-clay precursor hydrogel when disc size is accounted for. This represents a significant positive benefit for hydrogel based medical interventions even before considering the regenerative effects of stem cells contained in injected hydrogels.

Hydrogel group discs displaying the lowest energy dissipation rates of all discs is further evidence for the damage hypothesis as these discs underwent a second injection phase. This injection related damage is potentially worrying for injection based treatments and methods to limit or repair this are key questions for future research.

The inability of PCPH to restore tissue functionality of the disc in terms of energy dissipation rates is not unexpected if loss of structural pressure integrity is the cause. Determining whether MSCs injected within a hydrogel scaffold matrix can restore tissue functionality over time is a clear direction for future work.

6.6 Conclusions

Mechanical response and tissue functionality of intervertebral discs subjected to loading simulating walking was found to vary according to disc health and treatment condition.

Mechanical response to loading was significantly altered in discs that had been degenerated through collagenase injections and these changes were not mimicked in a control group ruling out needle puncture as the cause. Discs which had undergone the same degeneration process but were then further injected with a proprietary hydrogel showed no significant difference (P=0.998) in mechanical response to healthy discs when disc size was accounted for.

Disc energy dissipation under loading was significantly reduced in all three groups that had suffered needle puncture and was lowest of all in the hydrogel injected group which had received two separate injections suggesting that

damage to the IVD which compromises the discs pressure holding integrity is a major factor in loss of tissue functionality.

Injections of hydrogel were found to recover disc mechanical response to loading but not tissue functionality.

7 Discussion

7.1 *In vivo*/*In vitro* Test Methods

The large majority of work contained in this thesis has been conducted *in vitro* due to the greater test and measurement possibilities available with this methodology compared to what can be achieved *in vivo* without highly invasive protocols. Nevertheless it is important to fully understand the results obtained by this work in the greater context of true *in vivo* behaviour.

The first study in this thesis compared discs cycled dynamically at loads simulating activities of daily living by the use of magnetic resonance images of the lumbar spines of participants loaded to similar levels statically.

The dynamic test rig developed for the work in the three reported studies is able to accurately simulate the stress-time behaviours of discs under loading due to activities of daily living. However, the one dimensional, axisymmetric loading applied by the rig and measured by the load cell is a simplification of true disc behaviour under loading *in vivo*.

The lordotic curvature of the lumbar spine means that IVD compression *in vivo* is weighted posteriorly with the rear edge of adjacent vertebral bodies pinching together whilst the anterior portion of the disc remained relatively uncompressed or even extended (Figure 46).

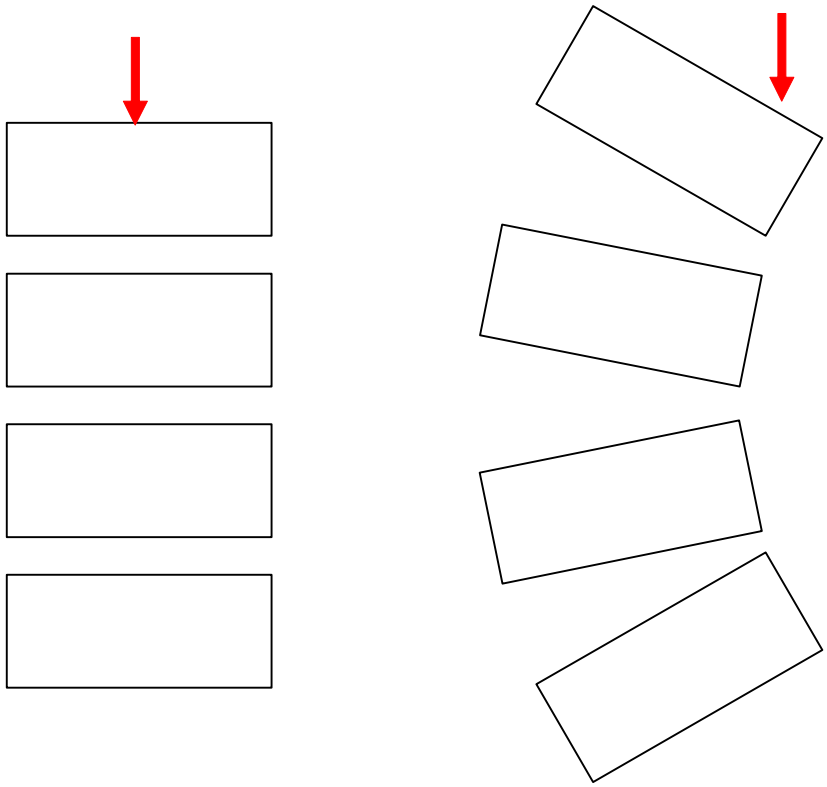

Figure 46 – *In vitro* testing in this work approximated spinal loading as axisymmetric (left). True *in vivo* behaviour is more complex with asymmetric loading weighted posteriorly (right).

An extreme case of this behaviour was observed in the L4/L5 disc where mean compression across the disc was negligible with changes in anterior and posterior cancelling out.

7.1.1 **Limitations of Present Work**

The axisymmetric loading method used for *in vitro* work in this thesis is ideal for determining bulk material properties of the IVD at stress levels equivalent to ADLs but future work may need to better simulate asymmetric compression in order to focus on smaller regions of the disc rather than the whole IVD.

Work in this thesis has focused on the compressive behaviour of the IVD and has not examined torsion. Typical human activity subjects the lumbar spine to significant torsional effects and is a significant area of interest in regards to

acute injury of the disc. Collagen fibres within the AF resist torsional forces on the disc through tensile loading and the location and orientation of these fibres is different in those discs which are subjected to the greatest torsion *in vivo* [35].

The highly specific AF structure of lumbar discs and its effect on injury and degeneration means that the circular cross section bovine coccygeal IVDs available during this work would not have been suitable for replicating the behaviour of oval or kidney shaped human lumbar discs. This fact combined with the lack of equipment suitable for applying torsional loads to a material like IVDs meant that this potential injury mechanism was beyond the experimental means of this work.

7.1.2 **Findings and Advances from Present Work**

Previous work in the literature has identified the axially asymmetric nature of IVD compression under body weight loading *in vivo* but work presented here went beyond this, examining the effects of loading greater than body weight at forces typical of those in the lumbar spine during walking, these results are detailed in Chapter 4 (Table 7) [99, 100, 159].

The findings not only go beyond what has been done previously using MRI but also highlight the difficulties in using MRI as a method for investigating activities of daily living. Strain levels in discs cycled dynamically were significantly lower than those loaded statically *in vivo* due to creep in the IVD under continuous loading and the large inter-subject variation means that findings from MRI scans are highly dependent on the individual subject (Figure 26).

The increasing ubiquity of MRI and other imaging technology will likely mean that such methods continue to receive wide use in investigation of the IVD and spinal loading. However, as investigation of the mechanical behaviour of the IVD increasingly looks at more clinically relevant, complex, dynamic scenarios at loading greater than bodyweight the effectiveness of static MRI will be greatly reduced. MRIs future role is likely to be a combined approach using MRI for static loading and *in vitro* for dynamic, continued improvements to MRI technology allowing for reduced scan times will also greatly benefit this approach.

7.2 Heat shock proteins and IVD degeneration

7.2.1 Heat Shock Proteins: Degeneration Markers

Although there is evidence that genetics are a significant determinant in disc degeneration, much lumbar degeneration is still unexplained by genetic effects and various lifestyle factors are implicated in degeneration and injury [42, 57, 59].

IVD biochemistry is a complex mix of proteins and enzymes which are regulated in response to various stimuli on the disc including mechanical loading [39, 146, 148-151, 153, 169-171]. Degenerate IVDs have significantly higher levels of heat shock proteins than healthy discs, implicating HSPs as a potential pathogenesis for degeneration within the disc or as a by-product of another mechanism which is itself responsible for the pain. The exact mechanism for upregulation of HSPs in the IVD is not yet known but it is has been shown that IVDs subjected to temperatures of 43 degrees have increased levels of HSPs [157].

The second study of this thesis was designed specifically designed in order to determine if such temperatures could be initiated in the IVD by the loading of ADLs and therefore implicate physical activity as a mechanism for HSP related degeneration of the disc. This self-heating behaviour has previously been observed in non-biological VEMs but has to date never previously been investigated in a bio-material [145, 180-183].

The study hypothesised that the viscoelastic nature of the IVD would cause temperature rises when cyclically loaded at levels simulating ADLs. However, the results showed that this was not the case and that loading levels simulating running activity would only produce a temperature rise of 0.025°C, even if loading continued for a full hour.

The results from this study rule out isolated heat generation within the IVD as a mechanism for temperature increase in the disc. Previous work in the literature also rules out changes in core body temperature due to activity

meaning that there are no known mechanisms by which temperature rises out of normal core range and it is therefore unlikely that it could result in upregulation of HSPs beyond any already resulting from core temperature fluctuations [175-178, 184].

7.2.2 Limitations of Present Work

Attempts to measure tissue temperatures directly unsuccessful due to the interference of ambient temperatures around the experimental set up. The current test rig did not allow for sufficient control of atmospheric temperatures which negated all tissue measurements.

The lack of sophisticated environmental temperature control means that the relationships between mechanical loading and viscoelastic heating were difficult to determine through direct measurement. Although maximum theoretical temperature changes were shown to be negligible making direct measurement of these changes challenging but future work must better shield against external influences.

The model used in the study was greatly simplified and relied on a number of assumptions which are detailed in Chapter 5. A more sophisticated 3D FEA thermal model would provide more detailed analysis of heat movement in the disc, however due to the “worst case scenario” heat generation modelled in the study a more accurate thermal model would not change the overall finding that insufficient heat is generated to result in meaningful temperature change.

7.2.3 **Findings and Advances from Present Work**

Heat generated within the IVD under loading simulating ADLs was not found to be sufficient to result in temperature increases within the disc (Figure 47).

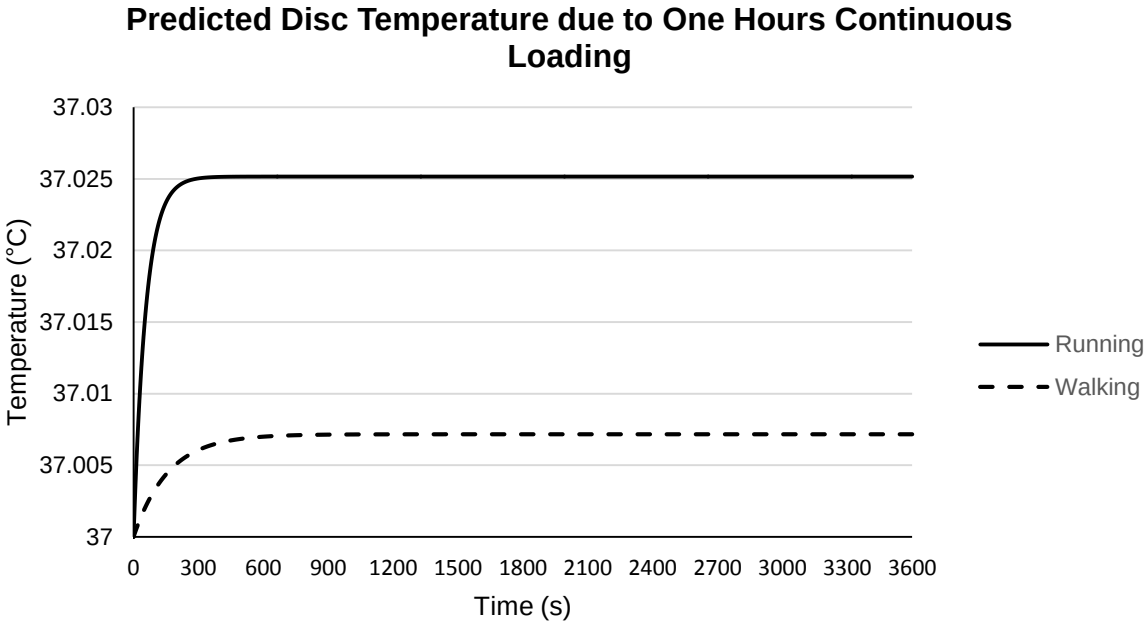

Figure 47 - Predicted temperature of an IVD subjected to sustained loading for one hour under either running or walking conditions. Temperature quickly reaches steady state and maximal increase is well within the normal range for core temperatures [175].

These findings represent a significant clinical finding in respect to IVD health and degeneration. The presence of increased levels of heat shock proteins in degenerate discs and their known regulatory response to increased temperatures represented a clear potential mechanism for degeneration of the IVD. With no previous work having investigated whether loading on the disc could generate the required heat for this mechanism this study is a first in the literature. On the basis of this finding, investigation into degeneration of the

IVD can be focused on other potential mechanisms which upregulate HSP proteins.

7.3 Injury, Degeneration and Clinical Interventions

7.3.1.1 Current Treatment Interventions

Specific back pain resulting from injury or degeneration of the IVD causes pain and limits activity, preventing work and reducing quality of life. Approximately 90% of the total cost of back pain is due to lost work days and therefore prevention or treatment of IVD injury and degeneration is a key issue [29].

Current methods to prevent further damage or pain due to disc degeneration centre on spinal fusion, an invasive medical intervention resulting in permanent loss of motion between two or more vertebrae [60, 61]. Spinal fusion surgery is increasingly common with over 400,000 fusion surgeries conducted in the USA each year at a cost of $34 billion, this represents a 137% increase in surgeries between 1998 and 2008 [61, 62].

Spinal fusion is highly invasive and results in a partial or total loss of mobility in the vertebral joint at the fusion site [69]. Loss of mobility in the joint is a significant quality of life issue and patients with lower postoperative mobility demonstrate worse clinical outcomes [70].

Fusion surgery is only minimally successful in reducing back pain, only 20% of patients are pain free within 5 years and 22.2% of patients suffer unsatisfactory outcomes from spinal fusion [60, 71]. Posterior lumbar

interbody fusion (PLIF) in particular has a high rate of failure with 13.2% of patients requiring follow up surgery within 5 years [72].

Mortality rates for spinal fusion are 0.2-0.29% and fusion surgery mortality was significantly ($P < 0.001$) higher than other forms of spinal surgery [62, 73].

7.3.1.2 Potential Regenerative Interventions

Mesenchymal stem cells represent a possible regenerative treatment intervention for degenerative IVDs that is far less invasive than current spinal fusion and without permanently immobilising the articular joint [74-78].

The most promising method for such treatment interventions would require the injection of MSCs directly into the IVD within a scaffold matrix hydrogel. These treatments are still in the development stage and have not gone through clinical trials and the biomechanical implications on their safety and efficacy are not yet understood. The study contained within the thesis represents the first time that the material and tissue functionality of hydrogel injected degenerate discs has been investigated and compared with healthy, damaged and degenerate discs.

7.3.1.3 Key Study Findings

The study made a number of key findings which to date had not previously been determined.

- Healthy IVDs damaged through needle puncture suffered a loss of tissue functionality compared to non-damaged discs that was separate and distinct from disc degeneration.

- Mechanical function of damaged discs was significantly reduced compared to healthy discs but remained significantly higher than that of degenerate discs.
- Degenerate discs suffered from greatly reduced mechanical function compared to healthy discs. Under the same loading, mean stiffness in degenerate discs was 2.75 times lower and mean strain 5 times greater than that of healthy discs.
- Degenerate discs that were injected with hydrogel showed no significant differences in mechanical function (stiffness) from healthy discs.
- Hydrogel injections did not result in a recovery of tissue functionality (energy dissipation) in degenerate discs and in fact hydrogel injected discs had the lowest levels of energy dissipation of any group.

7.3.1.4 Limitations of Present Work

A needle puncture control group was included in the study in order to rule out the damage caused by the collagenase injections as the cause of differences between healthy and degenerate discs. The results from this group highlighted key difference between healthy, damaged and degenerate discs but needle puncture represents a specific type of injury which violates the discs integrity as a pressure holding vessel. Many disc injuries, such as annular fissures and some partial herniations do not penetrate the disc annulus and may present different changes in material behaviour compared with healthy discs.

Hydrogel group discs were subjected to the same collagenase digestion process as degenerate group discs but were not tested before hydrogel

injection. Repeatedly testing the same disc can alter the material responses measured due to creep behaviour of viscoelastic materials and therefore testing the hydrogel group discs before and after hydrogel injection was not considered. As such the results are based on the assumption that discs in the hydrogel group would have displayed the same material behaviours as those in the degenerate group before being injected with hydrogel.

7.3.1.5 Findings and Advances from Present Work

The work conducted in this study represents the first investigation of the biomechanical effects of hydrogel injection on damaged or degenerate intervertebral discs and represents a significant clinical finding. The immediate recovery of IVD mechanical functionality post injection is a positive endorsement of the benefits of treatment intervention using polymer-clay precursor hydrogels as studied in this work.

The finding that tissue functionality is lost in discs punctured through the annulus even without any degeneration of disc material is of benefit in distinguishing between injury and degenerative mechanisms which previously were less clear. That discs subjected to damage from a second needle puncture had the lowest energy dissipation rates reinforces this idea.

The differences in energy dissipation between healthy discs and discs which were damaged and/or degenerate may point to potential exponential degenerative mechanisms in damaged discs and is a key future research direction.

8 Further Work

The work conducted in this thesis has various clinical implications regarding the intervertebral disc and hydrogel based stem cell treatments but has only scratched the surface of this significant issue. Follow up work may be required to determine the full implications of the studies in this thesis and the work has naturally raised further questions which may guide the direction of future work in this area.

Future and follow up work will take two major directions, experimental and computer modelling.

8.1 Experimental

8.1.1 Limitations of Current Experimental Work

Key material and tissue functionalities were characterised for discs subjected to loading simulating walking and running. The functionalities during walking were then compared with discs which had been subjected to damage, degeneration and hydrogel injection.

Although walking is a basic human activity of daily living which allowed for consistent and highly repeatable testing it is only one of a number of ADLs. Understanding disc behaviour during the full range of ADLs would provide valuable clinical data on how degenerate discs differ from healthy and how patients may respond to hydrogel based treatments.

Future test load profiles might simulate dynamic, non-cyclic activity, such as standing up from a chair, or it might simulate a typical activity period consisting of multiple different activities in sequence (Table 13).

Table 13 – An Example Multi-Activity Loading Period Simulating a Short Period of Activity

Activity	Time (s)	Total Time (s)	Load (MPa)
Sitting (pre-load phase)	60	60	0.46
Standing from chair	2	62	1.10
Standing	5	67	0.50
Walking	20	87	0.53-0.65 at 2 Hz
Standing bent forwards	60	147	1.10
Walking	20	167	0.53-0.65 at 2 Hz
Sitting in chair	2	169	1.10
Sitting (relaxation phase)	60	249	0.46

8.1.2 Human Cadaveric Discs

Although methodologically bovine IVDs represent an ideal surrogate for human discs testing on human cadaveric lumbar discs is a desirable future direction for experimental work. The nature of cadaveric disc availability means that samples would likely suffer from moderate to severe age related degeneration or damage, combined with the small number of cadaveric discs available replicating the current test protocol would not be possible.

A probable test protocol would subject discs to a loading profile in their current state before injecting the discs with hydrogel and repeating the loading. Although this would not determine the characteristic behaviour of healthy discs and whether hydrogel injection returned discs to this state, it could nonetheless demonstrate differences between degenerate and hydrogel injected human discs.

8.1.3 Live cultures and Stem Cells

Degenerate discs injected with hydrogel were found to recover their mechanical functionality but did not recover tissue functionality, having lower rates of energy dissipation than other discs. Stem cells injected within hydrogel are intended to regenerate discs by initiating regrowth of disc material but whether this successfully restores tissue functionality is not yet known and was beyond the means and scope of the current work.

Another next step is to investigate the mechanical and tissue behaviours of discs kept alive in culture, degenerated through collagenase digestion and then injected with hydrogel containing stem cells before undergoing a recovery period. If the addition of MSCs to degenerate discs was shown to beneficially alter the tissue functionality then this would be a major clinical finding.

8.1.4 Non-Axisymmetric Loading

DMT conducted in this thesis was done on an axisymmetric test rig which only applied and measured force in one direction. As determined in the first study and from data in the literature, loading on the intervertebral disc can also include rotation and torsion. Future work should aim to more accurately simulate true *in vivo* loading through the use of multi-axis test equipment or specialist test fixtures which apply more complex loading.

8.2 Finite Element Analysis and Modelling

The work in this thesis provides useful data for the creation and verification of computer models of the IVD. Two directions of possible future work are structural and thermal modelling of the disc.

8.2.1 **Thermal Modelling**

Temperature analysis of the disc was conducted using a highly simplified 2-dimensional model of heat transfer and isotropic disc thermal properties resulting in a single temperature for the entire disc. In reality the NP and AF have different thermal properties due to their different water content.

Using FEA a 3-dimensional model of the disc could provide more accurate data on how heat moves within the disc and how temperature varies throughout the disc volume.

8.2.2 **Structural Modelling**

3-dimensional FEA models of the IVD have previously been developed but the wide range of material property values used by the various research groups is a strong indication of the limited current understanding of IVD material behaviour.

This experimental work in this thesis provides verification data which can be used to verify future FEA models of the IVD.

9 Conclusions

9.1 Research Aims and Thesis Questions

This thesis aimed to address three general questions about the intervertebral disc based on the state of art of the field:

1. What are the intervertebral discs key material behaviours when loaded during activities of daily living?
2. Does this material behaviour effect intervertebral disc health?
3. Is that material behaviour affected by disc injury, degeneration and stem cell interventions?

Three specific questions were then developed that could be investigated by this thesis:

1. Compare the load/deflection behaviour of the intervertebral disc when tested *in vivo* and *in vitro*.
2. Determine whether activities of daily living result in viscoelastic self-heating of the intervertebral disc and whether subsequent temperature change is significant enough to effect disc health.
3. Compare the material behaviour of discs that are healthy, degenerated and treated in order to determine whether hydrogel injection has significant effects on disc behaviour during loading.

The three studies conducted in this thesis have provided clinically relevant results in answer to these questions.

9.2 Research Findings

1. Material behaviour of healthy disc are highly dependent on loading rate, level and frequency and changes during extended periods of loading. Bovine discs subjected to loading simulating walking had a mean stiffness of 2639 N mm^{-1} at a mean strain of 0.54 % and dissipated 4.23 mJs^{-1}.
2. The heat generated by viscous friction within the disc during loading was not sufficient to cause any meaningful temperature increase and therefore viscoelastic self-heating is not a possible mechanism for degeneration through regulation of heat shock proteins.
3. The material behaviours of the intervertebral disc are significantly affected by health and treatment conditions. Damage to the disc significantly affects tissue functionality, greatly lowering energy dissipated by the disc under loading. Degeneration of the disc results in a significant reduction in disc mechanical functionality with greatly reduced stiffness under loading. Hydrogel injections used as a scaffold matrix for mesenchymal stem cell interventions restored disc mechanical functionality (stiffness), recovering disc stiffness and strain under loading to healthy levels. Hydrogel injections were not able to recover tissue functionality (energy dissipation), energy dissipation in hydrogel injected discs remained significantly lower than healthy discs.

9.3 Clinical Implications

Tentative recommendations can be made regarding the use of hydrogel as a treatment intervention for the following reasons:

- Hydrogel injections are beneficial.

Despite not completely restoring healthy tissue function hydrogel injections nevertheless demonstrated an immediate, positive effect on the mechanical behaviours of degenerate IVDs.

- Hydrogel injections would do minimal further harm.

Whilst needle injection was observed to have a negative effect of mechanical behaviours of the disc, damaged and degenerate discs which may be targeted by hydrogel interventions would already be suffering from greatly reduced mechanical and tissue functionality. The catastrophic change observed between healthy and needle punctured discs in Study 3 is much less likely in a clinical setting.

- Current alternatives are more severe.

The current alternative to hydrogel injections, spinal fusion, requires drastic and invasive surgery to the spinal column and successful surgery results in a permanent loss of joint motion.

- Hydrogel injections do not prevent future treatment methods.

Should hydrogel injections be deemed unsuccessful there is no reason that spinal fusion could not then be attempted.

The implications of reduced energy dissipation in damaged IVDs are not yet fully understood and further work is clearly needed in this area. However, due to the fact that discs targeted by hydrogel treatments would already be heavily damaged or degenerate and possible negative impact of treatment in this respect is limited and outweighed by the potential positives.

On the basis of the work presented in this thesis and the reasons given above there is reason to believe that hydrogel injections present a net positive benefit in damaged and degenerate discs. Though questions remain and research on cultured discs injected with stem cells and investigation into minimally damaging injection methods are required hydrogels have been shown to offer significant potential mechanically in the treatment of intervertebral discs.

10 References

[1] S. Bevan, "The Impact of Back Pain on Sickness Absence in Europe " University of Lancaster2012.

[2] J. D. Cassidy, L. J. Carroll, and P. Cote, "The Saskatchewan Health and Back Pain Survey: The Prevalence of Low Back Pain and Related Disability in Saskatchewan Adults," *Spine,* vol. 23, pp. 1860-6, 1998.

[3] L. Lidgren, "The bone and joint decade 2000-2010," in *Bull World Health Organ*. vol. 81, ed Switzerland, 2003, p. 629.

[4] N. Maniadakis and A. Gray, "The economic burden of back pain in the UK.," *Pain,* vol. 84, pp. 95-103, 2000.

[5] S. Dagenais, J. Caro, and S. Haldeman, "A systematic review of low back pain cost of illness studies in the United States and internationally," *Spine J,* vol. 8, pp. 8-20, Jan-Feb 2008.

[6] M. Hillman, A. Wright, G. Rajaratnam, A. Tennant, and M. A. Chamberlain, "Prevalence of low back pain in the community: implications for service provision in Bradford, UK," *Journal of Epidemiology and Community Health,* vol. 50, pp. 347-52, 1996.

[7] T. Dodd, "The prevalence of back pain in Great Britain in 1996," *London: ONS Social Survey Division,* 1997.

[8] A. Boucher, "The Prevalence of Back Pain in Great Britain in 1998," *Department of Health. Available at http://www. doh. gov. uk/public/backpain. htm,* 1999.

[9] E. F. Harkness, G. J. Macfarlane, A. J. Silman, and J. McBeth, "Is musculoskeletal pain more common now than 40 years ago?: two population-based cross-sectional studies," *Rheumatology,* vol. 44, pp. 890-95, 2005.

[10] D. Hoy, P. Brooks, F. Blyth, and R. Buchbinder, "The Epidemiology of Low Back Pain," *Best Practice and Research Clinical Rheumatology,* vol. 24, pp. 769-81, 2010.

[11] G. T. Jones and G. J. Macfarlane, "Epidemiology of low back pain in children and adolescents.," *Archives of Disease in Childhood,* vol. 90, pp. 312-316, 2005.

[12] L. Manchikanti, "Epidemiology of Low Back Pain," *Pain Physician,* vol. 3, pp. 167-192, 2000.

[13] P. Savigny, P. Watson, and M. Underwood, "Early management of persistent non-specific low back pain: summary of NICE guidance," *BMJ,* vol. 338, 2009.

[14] L. b. pain. (2013, 4/2/2013). *Low Back Pain*. Available: http://en.wikipedia.org/wiki/Low_back_pain

[15] B. P. Overview. (2013, 4/2/2013). *Back Pain Overview*. Available: http://www.webmd.com/back-pain/default.htm

[16] T. S. Carey, J. Garrett, A. Jackman, L. Sanders, and W. Kalsbeek, "Reporting of Acute Low Back Pain in a Telephone Interview: Identification of Potential Biases," *Spine,* vol. 20, pp. 787-90, 1995.

[17] M. van Tulder, B. Koes, and C. Bombardier, "Low back pain," *Best Pract Res Clin Rheumatol,* vol. 16, pp. 761-75, Dec 2002.

[18] G. Waddell, "1987 Volvo award in clinical sciences. A new clinical model for the treatment of low-back pain," *Spine (Phila Pa 1976),* vol. 12, pp. 632-44, Sep 1987.

[19] M. C. Jensen, M. N. Brant-Zawadzki, N. Obuchowski, M. T. Modic, D. Malkasian, and J. S. Ross, "Magnetic resonance imaging of the lumbar spine in people without back pain," *N Engl J Med,* vol. 331, pp. 69-73, Jul 14 1994.

[20] K. Luoma, H. Riihimaki, R. Luukkonen, R. Raininko, E. Viikari-Juntura, and A. Lamminen, "Low back pain in relation to lumbar disc degeneration," *Spine (Phila Pa 1976),* vol. 25, pp. 487-92, Feb 15 2000.

[21] R. A. Deyo, J. Rainville, and D. L. Kent, "What can the history and physical examination tell us about low back pain?," *Jama,* vol. 268, pp. 760-5, Aug 12 1992.
[22] Y. Peng and F. J. Lv, "Symptomatic versus Asymptomatic Intervertebral Disc Degeneration: Is Inflammation the Key?," in *Crit Rev Eukaryot Gene Expr*. vol. 25, ed United States, 2015, pp. 13-21.
[23] B. A. Walter, D. Purmessur, M. Likhitpanichkul, A. Weinberg, S. K. Cho, S. A. Qureshi*, et al.*, "Inflammatory Kinetics and Efficacy of Anti-inflammatory Treatments on Human Nucleus Pulposus Cells," *Spine (Phila Pa 1976),* Apr 14 2015.
[24] A. Malmivaara, U. Hakkinen, T. Aro, M. L. Heinrichs, L. Koskenniemi, E. Kuosma*, et al.*, "The treatment of acute low back pain--bed rest, exercises, or ordinary activity?," *N Engl J Med,* vol. 332, pp. 351-5, Feb 9 1995.
[25] J. J., "131 million working days lost to sickness in 2011 - but it's falling ", ed: Office for National Statistics, 2011.
[26] P. J. Watson, C. J. Main, G. Waddell, T. F. Gales, and G. Purcell-Jones, "Medically certified work loss, recurrence and costs of wage compensation for back pain: a follow-up study of the working population of Jersey," *Br J Rheumatol,* vol. 37, pp. 82-6, Jan 1998.
[27] J. W. Frymoyer and W. L. Cats-Baril, "An overview of the incidences and costs of low back pain," *Orthop Clin North Am,* vol. 22, pp. 263-71, Apr 1991.
[28] T. Pincus, A. K. Burton, S. Vogel, and A. P. Field, "A systematic review of psychological factors as predictors of chronicity/disability in prospective cohorts of low back pain," *Spine (Phila Pa 1976),* vol. 27, pp. E109-20, Mar 1 2002.
[29] J. K. Moffett, G. RIchardson, T. Sheldon, and A. Maynard, "Back pain: its management and costs to society," 1995.
[30] K. Omarker and R. R. Myers, "Pathogenesis of sciatic pain: role of herniated nucleus pulposus and deformation of spinal nerve root and dorsal root ganglion," *Pain,* vol. 78, pp. 99-105, 11/1/ 1998.
[31] A. Freemont, T. Peacock, P. Goupille, J. Hoyland, J. O'Brien, and M. Jayson, "Nerve ingrowth into diseased intervertebral disc in chronic back pain," *The Lancet,* vol. 350, pp. 178-181, 1997.
[32] M. A. Jackson and K. H. Simpson, "Chronic back pain," *Continuing Education in Anaesthesia, Critical Care & Pain,* vol. 6, pp. 152-155, 2006.
[33] P. Pollintine, P. Dolan, J. H. Tobias, and M. A. Adams, "Intervertebral disc degeneration can lead to "stress-shielding" of the anterior vertebral body: a cause of osteoporotic vertebral fracture?," *Spine (Phila Pa 1976),* vol. 29, pp. 774-82, Apr 1 2004.
[34] S. A. o. America. (6th July). *Spine, Hip & Shoulder Diagrams*. Available: http://www.spondylitis.org/patient_resources/spine_diagram.aspx#
[35] N. Bogduk, *Clinical and Radiological Anatomy of the Lumbar Spine*, Fifth ed. London: Elsevier Churchill Livingstone., 2012.
[36] G. Schmorl and H. Junghanns, *The Human Spine in Health and Disease*, Second American ed. New York: Grune and Stratton, 1971.
[37] M. A. Adams, B. J. C. Freeman, H. P. Morrison, I. W. Nelson, and P. Dolan, "Mechanical Initiation of Intervertebral Disc Degeneration," *Spine,* vol. 25, pp. 1625-1636, 2000.
[38] T. H. Hansson, T. S. Keller, and D. M. Spengler, "Mechanical behavior of the human lumbar spine. II. Fatigue strength during dynamic compressive loading," *J Orthop Res,* vol. 5, pp. 479-87, 1987.
[39] C. L. Korecki, J. J. MacLean, and J. C. Iatridis, "Dynamic compression effects on intervertebral disc mechanics and biology," *Spine (Phila Pa 1976),* vol. 33, pp. 1403-9, Jun 1 2008.

[40] M. A. Adams and P. J. Roughley, "What is Intervertebral Disc Degeneration, and What Causes It?," *Spine,* vol. 31, pp. 2151-2161, 2006.
[41] C. W. A. Pfirrmann, A. Metzdorf, M. Zanetti, J. Hodler, and N. Boos, "Magnetic Resonance Classification of Lumbar Intervertebral Disc Degeneration," *Spine,* vol. 26, pp. 1873-1878, 2001.
[42] M. C. Battié, T. Videman, L. E. Gibbons, L. D. Fisher, H. Manninen, and K. Gill, "Determinants of lumbar disc degeneration. A study relating lifetime exposures and magnetic resonance imaging findings in identical twins.," *Spine,* vol. 20, pp. 2601-2612, 1995.
[43] J. C. Lotz, O. K. Colliou, and J. R. Chin, "Compression-induced degeneration of the intervertebral disc: An in vivo mouse model and finite-element study.," *Spine,* vol. 23, pp. 2493-2506, 1998.
[44] M. A. Adams and W. C. S. Hutton, "Prolapsed intervertebral disc: A hyperflexion injury.," *Spine,* vol. 7, pp. 184-191, 1982.
[45] S. J. Gordon, K. H. Yang, P. J. Mayer, A. H. Mace, V. L. Kish, and E. L. Radin, "Mechanism of disc rupture: A preliminary report.," *Spine,* vol. 16, pp. 450-456, 1991.
[46] A. Middleditch and J. Oliver, *Functional Anatomy of the Spine*, Second ed. London: Elsevier Butterworth Heinemann, 2005.
[47] L. T. Twomey and J. R. Taylor, "Age Changes in the Lumbar Articular Triad," *Australian Journal of Physiotherapy,* vol. 31, pp. 106-112, 1985.
[48] S. Rajasekaran, J. Naresh Babu, and R. Arun, "A study of diffusion in human lumbar discs.," *Spine,* vol. 29, pp. 2654-2667, 2004.
[49] J. P. G. Urban, S. Roberts, and J. R. Ralphs, "The Nucleus of the Intervertebral Disc from Development to Degeneration," *American Zoologist,* vol. 40, pp. 53-061, 2000.
[50] B. E. Bachmeier, A. Nerlich, N. Mittermaier, C. Weiler, C. Lumenta, K. Wuertz*, et al.*, "Matrix metalloproteinase expression levels suggest distinct enzyme roles during lumbar disc herniation and degeneration," *Eur Spine J,* vol. 18, pp. 1573-86, Nov 2009.
[51] O. L. Osti, B. Vernon-Roberts, and R. Moore, "Annular tears and disc degeneration in the lumbar spine. ," *Journal of Bone and Joint Surgery,* vol. 74, pp. 678-682, 1992.
[52] S. Holm, A. K. Holm, and L. Ekstrom, "Experimental disc degeneration due to endplate injury. ," *Journal of Spinal Disorders and Technique,* vol. 17, pp. 64-71, 2004.
[53] L. I. Kerttula, W. S. Serlo, O. A. Tervonen, E. L. Pa¨a¨kko¨, and H. V. Vanharanta, "Post-Traumatic Findings of the Spine After Earlier Vertebral Fracture in Young Patients Clinical and MRI Study," *Spine,* vol. 25, pp. 1104-1108, 2000.
[54] J. Wang, Y. Zhou, Z. F. Zhang, C. Q. Li, W. J. Zheng, and J. Liu, "Radiological study on disc degeneration of thoracolumbar burst fractures treated by percutaneous pedicle screw fixation," *Eur Spine J,* vol. 22, pp. 489-94, Mar 2013.
[55] A. Möller, P. Maly, J. Besjakov, R. Hasserius, A. Ohlin, and M. K. Karlsson, "A vertebral fracture in childhood is not a risk factor for disc degeneration but for Schmorl's nodes: a mean 40-year observational study," *Spine,* vol. 32, pp. 2487-2492, 2007.
[56] R. A. Dickson and W. P. Butt, "Post-traumatic findings of the spine after earlier vertebral fractures in young patients," *Spine (Phila Pa 1976),* vol. 28, pp. 1749-50; author reply 1750, Aug 1 2003.
[57] M. J. Hancock, M. C. Battie, T. Videman, and L. Gibbons, "The Role of Back Injury or Trauma in Lumbar Disc Degeneration: An Exposure-Discordant Twin Study," *Spine,* vol. 35, pp. 1925-1929 10.1097/BRS.0b013e3181d60598, 2010.
[58] T. Videman, M. C. Battie, E. Parent, L. E. Gibbons, P. Vainio, and J. Kaprio, "Progression and determinants of quantitative magnetic resonance imaging

measures of lumbar disc degeneration: a five-year follow-up of adult male monozygotic twins," *Spine (Phila Pa 1976),* vol. 33, pp. 1484-90, Jun 1 2008.

[59] E. J. Carragee, A. S. Don, E. L. Hurwitz, J. M. Cuellar, J. A. Carrino, and R. Herzog, "2009 ISSLS Prize Winner: Does discography cause accelerated progression of degeneration changes in the lumbar disc: a ten-year matched cohort study," *Spine (Phila Pa 1976),* vol. 34, pp. 2338-45, Oct 1 2009.

[60] A. Dower, R. Chatterji, A. Swart, and M. J. Winder, "Surgical management of recurrent lumbar disc herniation and the role of fusion," *J Clin Neurosci,* Aug 14 2015.

[61] S. S. Rajaee, H. W. Bae, L. E. Kanim, and R. B. Delamarter, "Spinal fusion in the United States: analysis of trends from 1998 to 2008," *Spine (Phila Pa 1976),* vol. 37, pp. 67-76, Jan 1 2012.

[62] M. Pumberger, Y. L. Chiu, Y. Ma, F. P. Girardi, V. Vougioukas, and S. G. Memtsoudis, "Perioperative mortality after lumbar spinal fusion surgery: an analysis of epidemiology and risk factors," *Eur Spine J,* vol. 21, pp. 1633-9, Aug 2012.

[63] J. Bartleson and H. G. Deen, *Spine disorders: medical and surgical management*: Cambridge University Press, 2009.

[64] S. Madan and N. R. Boeree, "Outcome of posterior lumbar interbody fusion versus posterolateral fusion for spondylolytic spondylolisthesis," *Spine (Phila Pa 1976),* vol. 27, pp. 1536-42, Jul 15 2002.

[65] P. Ekman, H. Moller, T. Tullberg, P. Neumann, and R. Hedlund, "Posterior lumbar interbody fusion versus posterolateral fusion in adult isthmic spondylolisthesis," *Spine (Phila Pa 1976),* vol. 32, pp. 2178-83, Sep 15 2007.

[66] J. M. Janes and R. Stifter, "Spinal Fusion," *The American journal of nursing,* pp. 1062-1065, 1955.

[67] J. J. Reid, J. S. Johnson, and J. C. Wang, "Challenges to bone formation in spinal fusion," *Journal of biomechanics,* vol. 44, pp. 213-220, 2011.

[68] A. G. Filler, *Do you really need back surgery?: a surgeon's guide to neck and back pain and how to choose your treatment*: Oxford University Press, 2012.

[69] V. V. Patel, E. Burger, and C. W. Brown, *Spine trauma: surgical techniques*: Springer Science & Business Media, 2010.

[70] D. B. Pateder and E. Benzel, "Noninstrumented facet fusion in patients undergoing lumbar laminectomy for degenerative spondylolisthesis," *J Surg Orthop Adv,* vol. 19, pp. 153-8, Fall 2010.

[71] T. Andersen, F. B. Christensen, E. S. Hansen, and C. Bunger, "Pain 5 years after instrumented and non-instrumented posterolateral lumbar spinal fusion," *Eur Spine J,* vol. 12, pp. 393-9, Aug 2003.

[72] R. Greiner-Perth, H. Boehm, Y. Allam, H. Elsaghir, and J. Franke, "Reoperation rate after instrumented posterior lumbar interbody fusion: a report on 1680 cases," *Spine (Phila Pa 1976),* vol. 29, pp. 2516-20, Nov 15 2004.

[73] J. S. Smith, D. Saulle, C. J. Chen, L. G. Lenke, D. W. Polly, Jr., M. K. Kasliwal*, et al.*, "Rates and causes of mortality associated with spine surgery based on 108,419 procedures: a review of the Scoliosis Research Society Morbidity and Mortality Database," *Spine (Phila Pa 1976),* vol. 37, pp. 1975-82, Nov 1 2012.

[74] S. M. Richardson and J. A. Hoyland, "Stem cell regeneration of degenerated intervertebral discs: current status," *Curr Pain Headache Rep,* vol. 12, pp. 83-8, Apr 2008.

[75] S. M. Richardson, A. Mobasheri, A. J. Freemont, and J. A. Hoyland, "Intervertebral disc biology, degeneration and novel tissue engineering and regenerative medicine therapies," *Histol Histopathol,* vol. 22, pp. 1033-41, Sep 2007.

[76] C. L. Le Maitre, P. Baird, A. J. Freemont, and J. A. Hoyland, "An in vitro study investigating the survival and phenotype of mesenchymal stem cells following injection into nucleus pulposus tissue," *Arthritis Res Ther,* vol. 11, p. R20, 2009.

[77] L. E. Clarke, S. M. Richardson, and J. A. Hoyland, "Harnessing the Potential of Mesenchymal Stem Cells for IVD Regeneration," *Curr Stem Cell Res Ther,* vol. 10, pp. 296-306, 2015.

[78] M. V. Risbud, T. J. Albert, A. Guttapalli, E. J. Vresilovic, A. S. Hillibrand, A. R. Vaccaro*, et al.*, "Differentiation of mesenchymal stem cells towards a nucleus pulposus-like phenotype in vitro: implications for cell-based transplantation therapy," *Spine (Phila Pa 1976),* vol. 29, pp. 2627-32, Dec 1 2004.

[79] S. M. Richardson, N. Hughes, J. A. Hunt, A. J. Freemont, and J. A. Hoyland, "Human mesenchymal stem cell differentiation to NP-like cells in chitosan–glycerophosphate hydrogels," *Biomaterials,* vol. 29, pp. 85-93, 1// 2008.

[80] K. L. Moore, A. M. R. Agur, and A. F. Dalley, II, *Essential clinical anatomy*, 4th ed. ed. Philadelphia, Pa. ; London: Lippincott Williams & Wilkins, 2011.

[81] J. Ackerman. (2006, 5 October). *The Downside of Upright. All those aching backs may be trying to tell us something: It's part of the price we pay for walking on two legs.* Available: http://ngm.nationalgeographic.com/print/2006/07/bipedal-body/ackerman-text

[82] K. L. Moore and A. M. R. Agur, *Essential Clinical Anatomy*, 3rd ed. Baltimore: Williams and Wilkins, 2007.

[83] N. Eizenberg, C. Briggs, C. Adams, and G. Ahern, *General anatomy: Principles and Applications.* Sydney: McGraw Hill Education, 2008.

[84] W. J. Virgin, "Experimental investigations into the physical properties of the intervertebral disc," *J Bone Joint Surg Br,* vol. 33-b, pp. 607-11, Nov 1951.

[85] C. Hirsch and A. Nachemson, "New observations on the mechanical behavior of lumbar discs," *Acta Orthop Scand,* vol. 23, pp. 254-83, 1954.

[86] T. Brown, R. J. Hansen, and A. J. Yorra, "Some mechanical tests on the lumbosacral spine with particular reference to the intervertebral discs; a preliminary report," *J Bone Joint Surg Am,* vol. 39-a, pp. 1135-64, Oct 1957.

[87] K. L. Markolf and J. M. Morris, "The Structural Components of the Intervertebral Disc a Study of their Contributions to the Ability of the Disc to Withstand Compressive Forces," *Journal of Bone and Joint Surgery,* vol. 56, pp. 675-687, 1974.

[88] M. A. Adams, W. C. Hutton, and J. R. Stott, "The resistance to flexion of the lumbar intervertebral joint," *Spine (Phila Pa 1976),* vol. 5, pp. 245-53, May-Jun 1980.

[89] D. J. Nuckley, S. M. Hertsted, M. P. Eck, and R. P. Ching, "Effect of displacement rate on the tensile mechanics of pediatric cervical functional spinal units," *Journal of Biomechanics,* vol. 38, pp. 2266-75, 2005.

[90] T. S. Keller, D. M. Spengler, and T. H. Hansson, "Mechanical behavior of the human lumbar spine. I. Creep analysis during static compressive loading," *J Orthop Res,* vol. 5, pp. 467-78, 1987.

[91] H. Granhed, R. Jonson, and T. Hansson, "The loads on the lumbar spine during extreme weight lifting," *Spine (Phila Pa 1976),* vol. 12, pp. 146-9, Mar 1987.

[92] A. Ireland, T. Maden-Wilkinson, J. McPhee, K. Cooke, M. Narici, H. Degens*, et al.*, "Upper limb muscle-bone asymmetries and bone adaptation in elite youth tennis players," *Med Sci Sports Exerc,* vol. 45, pp. 1749-1758, 2013.

[93] H. Ishihara, D. S. McNally, J. P. Urban, and A. C. Hall, "Effects of hydrostatic pressure on matrix synthesis in different regions of the intervertebral disk," *J Appl Physiol (1985),* vol. 80, pp. 839-46, Mar 1996.

[94] T. S. Keller, S. H. Holm, T. H. Hansson, and D. M. Spengler, "1990 Volvo Award in experimental studies. The dependence of intervertebral disc mechanical properties on physiologic conditions," *Spine (Phila Pa 1976),* vol. 15, pp. 751-61, Aug 1990.

[95] A. Nachemson, "Lumbar intradiscal pressure. Experimental studies on post-mortem material," *acta orthopaedica scandinavica, supplementum,* vol. 43, pp. 1-104, 1960.

[96] K. Sato, S. Kikuchi, and T. Yonezawa, "In Vivo Intradiscal Pressure Measurement in Healthy Individuals and in Patients with on going Back Problems," *Spine,* vol. 24, pp. 2468-2474, 1999.

[97] H. J. Wilke, P. Neef, M. Caimi, T. Hoogland, and L. E. Claes, "New In Vivo Measurements of Pressures in the Intervertebral Disc in Daily Life.," *Spine,* vol. 24, pp. 755-762, 1999.

[98] A. J. Lisi, C. W. O'Neill, D. P. Lindsey, R. Cooperstein, E. Cooperstein, and J. F. Zucherman, "Measurement of In Vivo Lumbar Intervertebral Disc Pressure During Spinal Manipulation: A Feasibility Study," *Journal of Applied Biomechanics,* vol. 22, pp. 234-239, 2006.

[99] D. Wisleder, M. B. Smith, T. J. Mosher, and V. Zatsiorsky, "Lumbar spine mechanical response to axial compression load in vivo," *Spine (Phila Pa 1976),* vol. 26, pp. E403-9, Sep 15 2001.

[100] S. Kimura, G. C. Steinbach, D. E. Watenpaugh, and A. R. Hargens, "Lumbar spine disc height and curvature responses to an axial load generated by a compression device compatible with magnetic resonance imaging," *Spine (Phila Pa 1976),* vol. 26, pp. 2596-600, Dec 1 2001.

[101] A. Hioki, K. Miyamoto, H. Sakai, and K. Shimizu, "Lumbar axial loading device alters lumbar sagittal alignment differently from upright standing position: a computed tomography study," *Spine (Phila Pa 1976),* vol. 35, pp. 995-1001, Apr 20 2010.

[102] D. J. Botsford, S. I. Esses, and D. J. Ogilvie-Harris, "In vivo diurnal variation in intervertebral disc volume and morphology," *Spine (Phila Pa 1976),* vol. 19, pp. 935-40, Apr 15 1994.

[103] J. A. Malko, W. C. Hutton, and W. A. Fajman, "An in vivo magnetic resonance imaging study of changes in the volume (and fluid content) of the lumbar intervertebral discs during a simulated diurnal load cycle," *Spine (Phila Pa 1976),* vol. 24, pp. 1015-22, May 15 1999.

[104] J. Y. Rho, R. B. Ashman, and C. H. Turner, "Young's modulus of trabecular and cortical bone material: ultrasonic and microtensile measurements," *J Biomech,* vol. 26, pp. 111-9, Feb 1993.

[105] S. Balter, *Interventional fluoroscopy: physics, technology, safety*: Wiley-Liss, 2001.

[106] J. D. Houston and M. Davis, *Fundamentals of fluoroscopy*: Gulf Professional Publishing, 2001.

[107] C. Claussen and B. Lochner, *Dynamic computed tomography: basic principles and clinical applications*: Springer Science & Business Media, 2012.

[108] A. Jackson, D. L. Buckley, G. J. Parker, and M. Ah-See, *Dynamic contrast-enhanced magnetic resonance imaging in oncology*: Springer, 2005.

[109] A. Rohlmann, S. Neller, L. Claes, G. Bergmann, and H.-J. Wilke, "Influence of a follower load on intradiscal pressure and intersegmental rotation of the lumbar spine," *Spine,* vol. 26, pp. E557-E561, 2001.

[110] O. L. Osti, B. Vernon-Roberts, and R. Moore, "Annular tears and disc degeneration in the lumbar spine.," *Journal of Bone and Joint Surgery,* vol. 74, pp. 678-682, 1992.

[111] S. Holm, A. K. Holm, and L. Ekstrom, "Experimental disc degeneration due to endplate injury.," *Journal of Spinal Disorders and Technique,* vol. 17, pp. 64-71, 2004.

[112] J. R. Funk, J. R. Crandall, L. J. Tourret, C. B. MacMahon, C. R. Bass, J. T. Patrie*, et al.*, "The axial injury tolerance of the human foot/ankle complex and the effect of Achilles tension," *Journal of biomechanical engineering,* vol. 124, pp. 750-757, 2002.

[113] J. Aklonis and W. MacKnight, "Introduction to Polymer Viscoelasticity. 1983," *A Wiley-Interscience Publication: New York*.

[114] A. S. Wineman and K. R. Rajagopal, *Mechanical response of polymers*. Cambridge: Cambridge University Press, 2000.

[115] G. A. Ateshian, "Tissue Mechanics: Key Note Lecture," in *Congress of the International Society of Biomechanics*, Glasgow, 2015.

[116] F. Galbusera, H. Schmidt, J. Noailly, A. Malandrino, D. Lacroix, H.-J. Wilke*, et al.*, "Comparison of four methods to simulate swelling in poroelastic finite element models of intervertebral discs," *Journal of the mechanical behavior of biomedical materials,* vol. 4, pp. 1234-1241, 2011.

[117] S.-M. Chen, M.-F. Liu, J. Cook, S. Bass, and S. K. Lo, "Sedentary lifestyle as a risk factor for low back pain: a systematic review," *International Archives of Occupational and Environmental Health,* vol. 82, pp. 797-806, 2009.

[118] A. J. Teichtahl, D. M. Urquhart, Y. Wang, A. E. Wluka, R. O'Sullivan, G. Jones*, et al.*, "Physical inactivity is associated with narrower lumbar intervertebral discs, high fat content of paraspinal muscles and low back pain and disability," in *Arthritis Res Ther*. vol. 17, ed England, 2015, p. 114.

[119] M. Smuck, M. C. Kao, N. Brar, A. Martinez-Ith, J. Choi, and C. C. Tomkins-Lane, "Does physical activity influence the relationship between low back pain and obesity?," *Spine J,* vol. 14, pp. 209-16, Feb 1 2014.

[120] A. Shirazi-Adl, *Effect of annulus composite representation on the response of a human disc-a poroelastic analysis.*, 1990.

[121] M. Argoubi and A. Shirazi-Adl, "POROELASTIC CREEP RESPONSE ANALYSIS OF A LUMBAR MOTION SEGMENT IN COMPRESSION," *Journal of Biomechanics,* vol. 29, pp. 1331-39, 1996.

[122] B. A. Best, F. Guilak, L. A. Setton, W. Zhu, F. Saed-Nejad, A. Ratcliffe*, et al.*, "Compressive mechanical properties of the human anulus fibrosus and their relationship to biochemical composition.," *Spine,* vol. 15, pp. 212-21, 1994.

[123] V. C. Mow, S. C. Kuei, W. M. Lai, and C. G. Armstrong, "Biphasic creep and stress relaxation of articular cartilage in compression? Theory and experiments.," *Journal of Biomechanical Engineering,* vol. 102, pp. 73-84, 1980.

[124] J. C. Iatridis, L. A. Setton, M. Weidenbaum, and V. C. Mow, "The viscoelastic behaviour of the non-degenerate human lumbar nucleus pulposus in shear.," *J. Biomech.,* vol. 30, pp. 1005-13, 1997.

[125] J. C. Iatridis, L. A. Setton, R. J. Foster, B. A. Rawlins, M. Weidenbaum, and V. C. Mow, "Degeneration affects the anisotropic and nonlinear behaviors of human anulus fibrosus in compression," *J Biomech,* vol. 31, pp. 535-44, Jun 1998.

[126] A. J. Beaudoin, W. M. Mihalko, and W. R. Krause, "Finite element modelling of polymethylmethacrylate flow through cancellous bone.," *Journal of Biomechanics,* vol. 24, pp. 127-36, 1991.

[127] R. Eberlein, G. A. Holzapfel, and C. A. J. Schulze-Bauer, "An anisotropic model for annulus tissue and enhanced finite element analyses of intact lumbar disc bodies," *Computer methods in biomechanics and biomedical engineering,* vol. 4, pp. 209-229, 2001.

[128] S. J. Ferguson, K. Ito, and L. P. Nolte, "Fluid flow and convective transport of solutes within the intervertebral disc," *J Biomech,* vol. 37, pp. 213-21, Feb 2004.

[129] V. Esat and M. Acar, "Viscoelastic finite element analysis of the cervical intervertebral discs in conjunction with a multi-body dynamic model of the human head and neck," *Proc Inst Mech Eng H,* vol. 223, pp. 249-62, Feb 2009.

[130] M. Fagan, S. Julian, D. Siddall, and A. Mohsen, "Patient-specific spine models. Part 1: Finite element analysis of the lumbar intervertebral disc—a material sensitivity study," *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine,* vol. 216, pp. 299-314, 2002.

[131] A. Malandrino, J. Noailly, and D. Lacroix, "The effect of sustained compression on oxygen metabolic transport in the intervertebral disc decreases with degenerative changes," *PLoS Comput Biol,* vol. 7, p. e1002112, 2011.

[132] D. Perie, D. Korda, and J. C. Iatridis, "Confined compression experiments on bovine nucleus pulposus and annulus fibrosus: sensitivity of the experiment in the determination of compressive modulus and hydraulic permeability," *J Biomech,* vol. 38, pp. 2164-71, Nov 2005.

[133] S. Umehara, S. Tadano, K. Abumi, K. Katagiri, K. Kaneda, and T. Ukai, "Effects of degeneration on the elastic modulus distribution in the lumbar intervertebral disc," *Spine (Phila Pa 1976),* vol. 21, pp. 811-9; discussion 820, Apr 1 1996.

[134] K. P. Menard, *Dynamic Mechanical Analysis: A Practical Introduction*, 2nd ed. Boca Raton: CRC Press, 2008.

[135] A. S. Wineman and K. R. Rajagopal, *Mechanical response of polymers: an introduction*: Cambridge University Press, 2000.

[136] R. S. Lakes, *Viscoelastic Solids*. Boca Raton: CRC Press, 1998.

[137] J. C. Leahy and D. W. L. Hukins, "Viscoelastic properties of the nucleus pulposus of the inter-vertebral disk in compression.," *J.Mater.Sci.,Mater.Med.,* vol. 12, pp. 689-92, 2001.

[138] G. R. Fulcher, D. W. L. Hukins, and D. E. T. Shepherd, "Viscoelastic Properties of Bovine Articular Cartilage Attached to Subchondral Bone at High Frequencies.," *BMC Musculoskel. Disord.,* vol. 10, 2009.

[139] J. J. Costi, I. A. Stokes, M. G. Gardner-Morse, and J. C. Iatridis, "Frequency-dependent behavior of the intervertebral disc in response to each of six degree of freedom dynamic loading–solid phase and fluid phase contributions," *Spine,* vol. 33, pp. 1731-38, 2008.

[140] O. Izambert, D. Mitton, M. Thourot, and F. Lavaste, "Dynamic stiffness and damping of human inter-vertebral disc using axial oscillatory displacement under af ree mass system.," *Eur.SpineJ.,* vol. 12, pp. 562-66, 2003.

[141] A. Race, N. D. Broom, and P. Robertson, "Effect of Loading Rate and Hydration on the Mechanical Properties of the Disc," *Spine,* vol. 25, pp. 662-69, 2000.

[142] V. R. Yingling, J. P. Callaghan, and S. M. McGill, "Dynamic loading affects the mechanical properties and failure site of porcine spines.," *Clinical Biomechanics,* vol. 12, pp. 301-5, 1997.

[143] A. R. Kemper, C. McNally, and S. M. Duma, "The influence of strain rate on the compressive stiffness properties of human lumbar intervertebral discs.," *Biomedical Sciences Instrumentation,* vol. 43, pp. 176-81, 2007.

[144] J. P. G. Urban and S. Roberts, "Degeneration of the intervertebral disc," *Arthritis Research & Therapy,* vol. 5, pp. 120-130, 2003.

[145] J. D. Cazenove, D. A. Rade, A. M. G. de Lima, and E. Pagnacco, "A Study of Self-Heating Effects in Viscoelastic Damping Devices," vol. Proceedings of the 11th Pan-American Congress of Applied Mechanics, ed, 2010.

[146] J. J. MacLean, P. J. Roughley, R. D. Monsey, M. Alini, and J. C. Iatridis, "In vivo intervertebral disc remodeling: kinetics of mRNA expression in response to a single loading event," *J Orthop Res,* vol. 26, pp. 579-88, May 2008.

[147] L. L. Johnson, R. Dyer, and D. J. Hupe, "Matrix Metalloproteinases," *Current Opinion in Chemical Biology,* vol. 2, p. 6, 1998.
[148] C. L. Le Maitre, A. J. Freemont, and J. A. Hoyland, "The role of interleukin-1 in the pathogenesis of human intervertebral disc degeneration," *Arthritis Res Ther,* vol. 7, pp. R732-R745, 2005.
[149] C. L. Le Maitre, J. A. Hoyland, and A. J. Freemont, "Catabolic cytokine expression in degenerate and herniated human intervertebral discs: IL-1beta and TNFalpha expression profile," *Arthritis Res Ther,* vol. 9, p. R77, 2007.
[150] A. N. Tiaden, M. Klawitter, V. Lux, A. Mirsaidi, G. Bahrenberg, S. Glanz*, et al.*, "Detrimental role for human high temperature requirement serine protease A1 (HTRA1) in the pathogenesis of intervertebral disc (IVD) degeneration," *J Biol Chem,* vol. 287, pp. 21335-45, Jun 15 2012.
[151] S. Velasco, M. Tarlow, K. Olsen, J. W. Shay, G. H. McCracken, Jr., and P. D. Nisen, "Temperature-dependent modulation of lipopolysaccharide-induced interleukin-1 beta and tumor necrosis factor alpha expression in cultured human astroglial cells by dexamethasone and indomethacin," *J Clin Invest,* vol. 87, pp. 1674-80, May 1991.
[152] D. L. Belavy, M. Adams, H. Brisby, B. Cagnie, L. Danneels, J. Fairbank*, et al.*, "Disc herniations in astronauts: What causes them, and what does it tell us about herniation on earth?," *Eur Spine J,* Apr 18 2015.
[153] J. J. Maclean, C. R. Lee, M. Alini, and J. C. Iatridis, "Anabolic and catabolic mRNA levels of the intervertebral disc vary with the magnitude and frequency of in vivo dynamic compression," *J Orthop Res,* vol. 22, pp. 1193-200, Nov 2004.
[154] C. A. Sharp, S. Roberts, H. Evans, and S. J. Brown, "Disc cell clusters in pathological human intervertebral discs are associated with increased stress protein immunostaining," *Eur Spine J,* vol. 18, pp. 1587-94, Nov 2009.
[155] T. Takao and T. Iwaki, "A comparative study of localization of heat shock protein 27 and heat shock protein 72 in the developmental and degenerative intervertebral discs," *Spine (Phila Pa 1976),* vol. 27, pp. 361-8, Feb 15 2002.
[156] S. S. Gogate, N. Fujita, R. Skubutyte, I. M. Shapiro, and M. V. Risbud, "Tonicity enhancer binding protein (TonEBP) and hypoxia-inducible factor (HIF) coordinate heat shock protein 70 (Hsp70) expression in hypoxic nucleus pulposus cells: role of Hsp70 in HIF-1alpha degradation," *J Bone Miner Res,* vol. 27, pp. 1106-17, May 2012.
[157] H. S. Park, S. G. Cho, C. K. Kim, H. S. Hwang, K. T. Noh, M. S. Kim*, et al.*, "Heat shock protein hsp72 is a negative regulator of apoptosis signal-regulating kinase 1," *Mol Cell Biol,* vol. 22, pp. 7721-30, Nov 2002.
[158] M. I. Kingsley, L. A. D'Silva, C. Jennings, B. Humphries, V. J. Dalbo, and A. T. Scanlan, "Moderate-intensity running causes intervertebral disc compression in young adults," *Med Sci Sports Exerc,* vol. 44, pp. 2199-204, Nov 2012.
[159] B. Danielson and J. Willen, "Axially loaded CT and MRI of lumbar spine—a method to achieve an accurate radiological diagnosis in patients with low back pain," in *Advances in Spinal Fusion*, ed New York: Marcel Dekker, Inc, 2003.
[160] D. B. Chaffin, "Development of computerized human static strength simulation model for job design," 1997.
[161] G. D. O'Connell, W. Johannessen, E. J. Vresilovic, and D. M. Elliott, "Human internal disc strains in axial compression measured noninvasively using magnetic resonance imaging," *Spine (Phila Pa 1976),* vol. 32, pp. 2860-8, Dec 1 2007.
[162] Y. M. Haddad, *Static and quasi-static loading*: Kluwer Academic Publishers, 2000.
[163] S. Asano, K. Kaneda, S. Umehara, and S. Tadano, "The mechanical properties of the human L4-5 functional spinal unit during cyclic loading. The structural effects of the posterior elements," *Spine (Phila Pa 1976),* vol. 17, pp. 1343-52, Nov 1992.

[164] J. L. Wang, T. K. Wu, T. C. Lin, C. H. Cheng, and S. C. Huang, "Rest cannot always recover the dynamic properties of fatigue-loaded intervertebral disc," *Spine (Phila Pa 1976),* vol. 33, pp. 1863-9, Aug 1 2008.

[165] A. Pachi and T. Ji, "Frequency and velocity of people walking," *Structural Engineer,* vol. 83, 2005.

[166] J. E. Bertram and A. Ruina, "Multiple walking speed-frequency relations are predicted by constrained optimization," *J Theor Biol,* vol. 209, pp. 445-53, Apr 21 2001.

[167] V. L. Billat, L. Mille-Hamard, B. Petit, and J. P. Koralsztein, "The role of cadence on the VO2 slow component in cycling and running in triathletes," *Int J Sports Med,* vol. 20, pp. 429-37, Oct 1999.

[168] B. Danielson and J. Willen, "Axially loaded CT and MRI of lumbar spine—a method to achieve an accurate radiological diagnosis in patients with low back pain," *Advances in Spinal Fusion*.

[169] C. L. Le Maitre, A. J. Freemont, and J. A. Hoyland, "Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc," *J Pathol,* vol. 204, pp. 47-54, Sep 2004.

[170] C. L. Le Maitre, A. J. Freemont, and J. A. Hoyland, "Human disc degeneration is associated with increased MMP 7 expression," *Biotech Histochem,* vol. 81, pp. 125-31, Jul-Dec 2006.

[171] C. L. Le Maitre, A. Pockert, D. J. Buttle, A. J. Freemont, and J. A. Hoyland, "Matrix synthesis and degradation in human intervertebral disc degeneration," *Biochem Soc Trans,* vol. 35, pp. 652-5, Aug 2007.

[172] T. Kadow, G. Sowa, N. Vo, and J. D. Kang, "Molecular basis of intervertebral disc degeneration and herniations: what are the important translational questions?," *Clin Orthop Relat Res,* vol. 473, pp. 1903-12, Jun 2015.

[173] D. S. Mern, A. Beierfubeta, J. Fontana, C. Thome, and A. A. Hegewald, "Imbalanced protein expression patterns of anabolic, catabolic, anti-catabolic and inflammatory cytokines in degenerative cervical disc cells: new indications for gene therapeutic treatments of cervical disc diseases," *PLoS One,* vol. 9, p. e96870, 2014.

[174] D. Rittel, "An investigation of the heat generated during cyclic loading of two glassy polymers. Part I: Experimental," *Mechanics of Materials,* vol. 32, pp. 131-147, 2000.

[175] M. Sund-Levander, C. Forsberg, and L. K. Wahren, "Normal oral, rectal, tympanic and axillary body temperature in adult men and women: a systematic literature review," *Scand J Caring Sci,* vol. 16, pp. 122-8, Jun 2002.

[176] F. D. Legrand, P. M. Joly, and W. M. Bertucci, "Do Changes in Tympanic Temperature Predict Changes in Affective Valence During High-Intensity Exercise?," *Res Q Exerc Sport,* pp. 1-8, Jun 26 2015.

[177] A. D. Flouris, P. Webb, and G. P. Kenny, "Noninvasive assessment of muscle temperature during rest, exercise, and postexercise recovery in different environments," *J Appl Physiol (1985),* vol. 118, pp. 1310-20, May 15 2015.

[178] G. B. Coombs, M. N. Cramer, N. M. Ravanelli, N. B. Morris, and O. Jay, "Acute acetaminophen ingestion does not alter core temperature or sweating during exercise in hot-humid conditions," *Scand J Med Sci Sports,* vol. 25 Suppl 1, pp. 96-103, Jun 2015.

[179] P. Hasgall, F. Di Gennaro, C. Baumgartner, E. Neufeld, M. Gosselin, D. Payne*, et al.* (2015, 13/01/15). *IT'IS Database for Thermal and Electromagnetic Parameters of Biological Tissues (2.6 ed.)*. Available: www.itis.ethz.ch/database

[180] T. C. Henry, C. E. Bakis, and E. C. Smith, "Viscoelastic characterization and self-heating behavior of laminated fiber composite driveshafts," vol. 66, Part A, pp. 346-355, 2015.

[181] O. Chervinko, I. Senchenkov, and N. Yakimenko, "Vibrations and self-heating of a viscoelastic prism with a cylindrical inclusion," *International Applied Mechanics,* vol. 43, pp. 647-653, 2007.
[182] F. Dinzart, A. Molinari, and R. Herbach, "Thermomechanical response of a viscoelastic beam under cyclic bending; self-heating and thermal failure," *Archives of Mechanics,* vol. 60, pp. 59-85, 2008.
[183] J. D. Cazenove, D. A. Rade, A. M. G. de Lima, and C. A. Araújo, "A numerical and experimental investigation on self-heating effects in viscoelastic dampers," vol. 27, pp. 433-445, 2012.
[184] B. C. Long, M. L. Cordova, J. B. Brucker, T. J. Demchak, and M. B. Stone, "Exercise and quadriceps muscle cooling time," *J Athl Train,* vol. 40, pp. 260-3, Oct-Dec 2005.
[185] G. Moneta, T. Videman, K. Kaivanto, C. Aprill, M. Spivey, H. Vanharanta*, et al.*, "Reported Pain During Lumbar Discography As a Function of Anular Ruptures and Disc Degeneration: A Re-analysis of 833 Discograms," *Spine,* vol. 19, pp. 1968-1974, 1994.
[186] Y. P. Ye, D. Chen, and H. Xu, "The comparison of instrumented and non-instrumented fusion in the treatment of lumbar spondylolisthesis: a meta-analysis," *Eur Spine J,* vol. 23, pp. 1918-26, Sep 2014.
[187] Y. Shen, J. C. Silverstein, and S. Roth, "In-hospital complications and mortality after elective spinal fusion surgery in the united states: a study of the nationwide inpatient sample from 2001 to 2005," *J Neurosurg Anesthesiol,* vol. 21, pp. 21-30, Jan 2009.
[188] V. L. Boyes, C. Sammon, C. Lemaitre, and C. Breen, "Composite Hydrogel-Clay Particles," ed: Google Patents, 2012.
[189] A. Barbir, A. J. Michalek, R. D. Abbott, and J. C. Iatridis, "Effects of enzymatic digestion on compressive properties of rat intervertebral discs," *J Biomech,* vol. 43, pp. 1067-73, Apr 19 2010.
[190] L. Kerttula, K. Luoma, T. Vehmas, M. Gronblad, and E. Kaapa, "Modic type I change may predict rapid progressive, deforming disc degeneration: a prospective 1-year follow-up study," *Eur Spine J,* vol. 21, pp. 1135-42, Jun 2012.
[191] M. Gross and R. Jaenicke, "Proteins under pressure," *European Journal of Biochemistry,* vol. 221, pp. 617-630, 1994.
[192] O. Drews, W. Weiss, G. Reil, H. Parlar, R. Wait, and A. Görg, "High pressure effects step-wise altered protein expression in Lactobacillus sanfranciscensis," *Proteomics,* vol. 2, pp. 765-774, 2002.
[193] M. F. Patterson, M. Quinn, R. Simpson, and A. Gilmour, "Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods," *Journal of Food Protection®,* vol. 58, pp. 524-529, 1995.
[194] R. E. Marquis and D. M. Keller, "Enzymatic adaptation by bacteria under pressure," *Journal of bacteriology,* vol. 122, pp. 575-584, 1975.
[195] R. A. Tschirret-Guth, L. S. Koo, G. Hui Bon Hoa, and P. R. Ortiz de Montellano, "Reversible pressure deformation of a thermophilic cytochrome P450 enzyme (CYP119) and its active-site mutants," *Journal of the American Chemical Society,* vol. 123, pp. 3412-3417, 2001.
[196] K. Nomura, H. Iwahashi, A. Iguchi, and T. Shigematsu, "Barosensitivity in Saccharomyces cerevisiae is Closely Associated with a Deletion of the COX1 Gene," *Journal of food science,* vol. 80, pp. M1051-M1059, 2015.

11 Appendix

11.1 Study Ethics

The work contained in this thesis was given full ethical consideration before any practical experimentation took place and where appropriate full ethical approval was gained from the relevant regulatory bodies. The following ethical concerns were considered:

11.1.1 Human Trial Participants

The first study in this thesis includes human trial participants who underwent MRI scans in order to observe their lumbar spine response to axial loading.

The use of human trail participants required the approval of the Academic Ethics Committee to ensure that the participants were not subjected to unnecessary risk of harm or discomfort and that the work was required for valid research.

Ethical approval for the study was granted on January 6th 2014 by the Academic Ethics Committee.

MMU Ethics Committee Reference Number: SE131405

11.1.2 Animal Tissue

Unlike human cadaveric tissue or live volunteers, experimentation on animal tissue sourced from the food chain does not require the express written

approval of the Academic Ethics Committee however the following steps were taken to ensure work was conducted in a safe and ethical manner:

- Head of the Ethics Committee, Bill Gilmore, was informed of the work in writing.
- Animal tissue was sourced from abattoirs regulated by the food standards agency.
- Maximum storage times of all animal tissue samples were agreed in writing with relevant technical staff. Samples were stored at -20°C for a maximum of 3 months and samples moved to the 5°C fridge were stored for a maximum of 24 hours.
- Although animal tissue used was safe for disposal in normal waste streams, best practice protocols for tissue disposal were followed and all tissue was incinerated after use by a third party contractor hired by the university.

11.2 Application for Ethical Approval

APPENDIX 2

Application Number_____
Date Received_____

APPLICATION FOR ETHICAL APPROVAL

Manchester Metropolitan University

Introduction
All university activity must be reviewed for ethical approval. In particular, all undergraduate, postgraduate and staff research work, projects and taught programmes must obtain approval from the Academic Ethics committee.

Application Procedure
The form should be completed legibly (preferably typed) and, so far as possible, in a way which would enable a layperson to understand the aims and methods of the research. Every relevant section should be completed. Applicants should also include a copy of any proposed advert, information sheet, consent form and, if relevant, any questionnaire being used. The Principal Investigator should sign the application form. Supporting documents, together with one copy of the full protocol should be sent to the Faculty/Campus Research Group Officer.

Your application will require external ethical approval by an NHS Research Ethics Committee if your research involves staff, patients or premises of the NHS (see guidance notes)

Work with children and vulnerable adults
You will be required to have an Enhanced CRB Disclosure, if your work involves children or vulnerable adults.

The Academic Ethics Committee will respond as soon as possible, and where appropriate, will operate a process of expedited review.

Applications that require approval by an NHS Research Ethics Committee or a Criminal Disclosure will take longer.

1. Details of Applicants	
1.1. Name of applicant (Principal Investigator): Gary Dougill	
Telephone Number: 07772334797	
Email address: garydougill@gmail.com	
Status:	Postgraduate Student (Research)
Department/School/Other Unit: Mechanical Engineering	
Programme of study (if applicable): MPhil/PhD	
Name of supervisor/Line manager: Dr Glen Cooper and Dr Neil Reeves	
1.2. Co-Workers and their role in the project: (e.g. students, external collaborators, etc.)	
Name:	Name:

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Application Number_____
Date Received_____

Telephone Number:	Telephone Number:
Role:	Role:
Email Address:	Email Address:

2. Details of the Project

2.1. Title: Effect of Body Position and Loading on Intervertebral Disc Height

2.2. Description of the Project:

Understanding the behaviour of the human intervertebral disc during everyday activities provides important insight in to the causes of back pain and injury as well as providing valuable information relating to the demands on the intervertebral disc (IVD) during rehabilitation from injury or treatment.

The effect of load on the compression of intervertebral discs has been routinely measured by in vitro methods both inside MRI equipment (O'Connell et al. 2007; O'Connell et al. 2011; Chan and Neu, 2013) and more traditional compressive testing machines (Adams et al. 2000; Gadd and Shepherd, 2011). Data gathered by these experiments can be used to create models of the IVD; however due to the variation in boundary conditions between in vivo and in vitro testing in vitro models may not accurately reflect the behaviour of the IVD in real world scenarios.

MRI offers a non-invasive means of collecting in vivo data from IVDs. A number of studies have measured changes in disc height; volume and bulge due to periods of loading (Wisleder et al. 2001; Hioki et al. 2010; Kingsley et al. 2012) but these studies have either measured changes after extended periods of activity or relied on artificial means of loading the spine in a supine position. Through the use of positional MRI this study aims to measure changes in disc height and volume due to natural bodyweight loading and additional loading at near immediate timescales. This data is to provide information on the behaviour of the human IVD under loading and as a comparison tool for in vitro experimentation on IVDs.

2.3. Describe what type of study this is

This is a quantitative study. IVD height and volume data will be obtained from human participants in the IRM laboratory at Manchester Metropolitan University.

2.4. Are you going to use a questionnaire?

NO

2.5. Start Date / Duration of project:

To start 1st quarter 2014, this is not a longitudinal study and the duration is dependent on availability of the MRI. For 20 participants at 2 participants a week it is estimated the study will take 10 weeks to complete.

2.6. Location of where the project and data collection will take place:

Data collection for all participants will take place within the IRM laboratory at Manchester Metropolitan University.

2.7. Nature/Source of funding:

This is a post-graduate student project and funding is through a research studentship.

2.8. Are there any regulatory requirements?

NO

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Application Number_____
Date Received_____

3. Details of Participants

3.1. How many?

The study population will consist of 20 asymptomatic male participants with no history of chronic lower back pain or injury.

3.2. Age: 18-40 years

3.3. Sex: Male

3.4. How will they be recruited? (Attach a copy of any proposed advertisement)

Participants will be recruited from the university and the student's work place via word of mouth. Each participant will be given an information sheet before consenting to take part.

3.5. Status of participants: (e.g. students, public, colleagues, children, hospital patients, prisoners, including young offenders, participants with mental illness or learning difficulties.)

Participants will be members of staff or students at the university.

3.6. Inclusion and exclusion from the project: (indicate the criteria to be applied).

Inclusion Criteria:

- Consenting male participants aged 18-40 years.

Exclusion Criteria:

- Physical disabilities of the spine.
- Any history of surgery of the spine or lower back.
- Any history of pain or reduced range of motion of the lower back which lasted for longer than 7 days or required medical treatment (GP, A&E etc.).
- Any history of acute injury of the spine or intervertebral disc (disc prolapse etc.).
- Any metallic implants (pins, plates, cochlear devices etc.).

3.7. Payment to volunteers: (indicate any sums to be paid to volunteers).

No payments will be made.

3.8. Study information:

Have you provided a study information sheet for the participants?

YES (Please attach a copy)

3.9. Consent:

(A written consent form for the study participants MUST be provided in all cases, unless the research is a questionnaire.)

Have you produced a written consent form for the participants to sign for your records?

YES. The university standard participant consent form.doc will be used.

4. Risks and Hazards

4.1. Are there any risks to the researcher and/or participants?

(Give details of the procedures and processes to be undertaken, e.g., if the researcher is a lone-worker.)

- MRI (Magnet) – The MRI contains a large fixed magnet.
- MRI (Moving parts) – The MRI has several moving parts which pose pinch points and or potential to strike nearby persons. The participant remains inside the MRI whilst it rotates.
- Participants are required to hold two weighted objects during the final scan.

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Application Number_____
Date Received_____

4.2. State precautions to minimise the risks and possible adverse events:

- All metallic items are to be removed before entering the MRI cage; persons with implanted metallic devices (pins, plates etc.) are not to enter the cage.
- Participants are positioned in the MRI before any rotation is to occur; all other persons are to be clear of the cage before rotating the MRI. Due care and attention must be taken when adjusting the bed.
- MRI screening questionnaire is used to prevent scanning any participant with contraindications to MRI scanning.
- Weighted objects to be within safe manual handling limits.

4.3. What discomfort (physical or psychological) danger or interference with normal activities might be suffered by the researcher and/or participant(s)? State precautions which will be taken to minimise them:

N/A

5. Ethical Issues

5.1. Please describe any ethical issues raised and how you intend to address these:

The study does not pose any considerable ethical issues. Data protection / confidentiality is addressed below.

6. Safeguards/Procedural Compliance

6.1. Confidentiality:

6.1.1. Indicate what steps will be taken to safeguard the confidentiality of participant records. If the data is to be computerised, it will be necessary to ensure compliance with the requirements of the Data Protection Act 1998.

Data collected will be stored using a code and no participants will be identified via their data recordings. All computers used for data storage will be password protected.

6.1.2. If you are intending to make any kind of audio or visual recordings of the participants, please answer the following questions:

6.1.2.1. How long will the recordings be retained and how will they be stored?
MRI scans may be stored indefinitely as images in papers, presentations or as part of a thesis. Participants will not be identifiable from these images.

6.1.2.2. How will they be destroyed at the end of the project?
N/A

6.1.2.3. What further use, if any, do you intend to make of the recordings?
See Above

6.2. The Human Tissue Act

The Human Tissue Act came into force in November 2004, and requires appropriate consent for, and regulates the removal, storage and use of all human tissue.

6.2.1. Does your project involve taking tissue samples, e.g., blood, urine, hair etc., from human subjects?

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NO

6.2.2. Will this be discarded when the project is terminated?
N/A

If NO – Explain how the samples will be placed into a tissue bank under the Human Tissue Act regulations:

6.3. Insurance

The University holds insurance policies in place to cover claims for negligence arising from the conduct of the University's normal business, which includes research carried out by staff and by undergraduate and postgraduate students as part of their course. This does not extend to clinical negligence.

In addition, the University has provision to award indemnity and/or compensation in the event of claims for non-negligent harm. This is on the condition that the project is accepted by the insurers prior to the commencement of the research project and approval has been granted for the project from a suitable ethics committee.

Research which is applicable to non-negligent harm cover involves humans and physical intervention which could give rise to a physical injury or illness which is outside the participant's day to day activities. This includes strenuous exercise, ingestion of substances, injection of substances, topical application of any substances, insertion of instruments, blood/tissue sampling of participants and scanning of participants.

The following types of research are not covered automatically for non-negligent harm if they are classed as the activities above and they involve:

1) Anything that assists with and /or alters the process of contraception, or investigating or participating in methods of contraception
2) Anything involving genetic engineering other than research in which the medical purpose is treating or diagnosing disease
3) Where the substance under investigation has been designed and /or manufactured by MMU
4) Pregnant women
5) Drug trials
6) Research involving children under sixteen years of age
7) Professional sports persons and or elite athletes.
8) Overseas research

Will the proposed project result in you undertaking any research that includes any of the 8 points above or would not be considered as normal University business? If so, please detail below:
N/A

6.4. Notification of Adverse Events (e.g., negative reaction, counsellor, etc.):
(Indicate precautions taken to avoid adverse reactions.)

Please state the processes/procedures in place to respond to possible adverse

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reactions. N/A In the case of clinical research, you will need to abide by specific guidance. This may include notification to GP and ethics committee. Please seek guidance for up to date advice, e.g., see the NRES website at http://www.nres.npsa.nhs.uk/

SIGNATURE OF PRINCIPAL INVESTIGATOR: Gary Dougill	Date 31/10/2013
SIGNATURE OF FACULTY'S HEAD OF ETHICS:	Date:

Checklist of attachments needed:

1. Participant consent form
2. Participant information sheet
3. Full protocol
4. Advertising details
5. Insurance notification forms
6. NHS Approval Letter (where appropriate)
7. Other evidence of ethical approval (e.g., another University Ethics Committee approval)

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11.3 **Ethical Approval (SE131405)**

FACULTY OF SCIENCE AND ENGINEERING

Manchester Metropolitan University

M E M O R A N D U M

TO Gary Dougill

FROM Elanor Henry

DATE 22nd January 2014

SUBJECT Application for Ethical Approval **(SE131405)**

On the 22nd January 2014 the Head of Ethics for Science & Engineering considered your application for Ethical Approval (SE131405) entitled "Effect of Body Position and Loading on Intervertebral Disc Height". The application has been granted Favourable Opinion and you may now commence the project.

MMU requires that you report any Adverse Event during this study immediately to the Head of Ethics (Prof Bill Gilmore) and the Administrator (Elanor Henry). Adverse Events are adverse reactions to any modality, drug or dietary supplement administered to subjects or any trauma resulting from procedures in the protocol of a study.

An Adverse Event may also be accidental loss of data or loss of sample, particularly human tissue. Loss of human tissue or cells must also be reported to the designated individual for the Human Tissue Authority licence (currently Prof Bill Gilmore).

If you make any changes to the approved protocol these must be approved by the Faculty Head of Ethics. If amendments are required you should complete the attached form and submit it to the Administrator.

Regards

Elanor Henry
Assistant Research Administrator
All Saints North

11.4 MRI Study Participant Information Sheet

PARTICIPANT INFORMATION SHEET

Manchester Metropolitan University

Title of Project: Effect of Body Position and Loading on Intervertebral Disc Height.

MMU Ethics Committee Reference number:

Chief Investigators: **Gary Dougill**

About the Study

This study focusses on the Intervertebral Disc, intervertebral discs (IVDs) are discs of cartilage that form joints between the vertebrae (bones) that make up your spine. IVDs help to transfer loading along the spinal column but can often become damaged, especially with age, which can affect the individuals quality of life. Studying how the IVD is affected by loading during everyday activities will help to improve understanding of how and why the IVD degenerates. This study aims to use non-invasive imaging techniques to understand how the size of the IVD changes due to the amount of loading upon it. With this information it is possible to make theoretical models of the IVD which will help to prevent and treat disc injuries and degeneration.

Who can take part?

We would like to recruit male participants between the age of 18-35 years. We would like people who have not had surgery, serious injury or chronic (recurring) pain in their lower back and who are physically able to hold two 6kg weights for 10 minutes.

What will I have to do if I take part?

We will ask you to attend the laboratory (IRM laboratory, John Dalton Building, Manchester Metropolitan University) for some measuerements, you will only need to attend once.

Laboratory visit 1:

After taking your height and weight measurments some images of your spine will taken using a magnetic resonance imaging scanner (MRI). Before you enter the scanner you will be asked a number of questions to determine if it is appropriate for you to enter the scanner. This scanner encompasses a permanent magnet and works on the principle of magnetizing atoms in your body, but it does not emit any radiation. You will need to lie down in the MRI scanner for around 15 minutes, this is to allow your spine to relax with no loading. After this time a scan will be taken of your spine, this will take approximately 5 minutes. Once the first scan is complete the MRI machine will be rotated 90 degrees so that you are in a standing position, a second scan of your spine will be taken immediately and will also take approximately 5 minutes. A final scan will be taken in the standing position but this time you will be asked to hold two weights, one in each hand, for the duration of the scan.

This session will take approximately 45 minutes.

Do I have to take part?

Your participantion in this study is completely voluntary. It is entirely your decision whether or not to take part. If you decide to take part you may withdraw from the study at any time without having to give a reason. If you have any questions after reading this information sheet please ask the investigator.

11.5 Matlab Energy Dissipation Code

```
% read in comma delineated rig data from csv file
ADD FINAL SQUARE BRACKET IN NEXT LINE!!!!!!!!!!!!!!!
sdata=['I:\Hydrogel Injected\Disc10','\Test12.stop.csv';
% create matrix ignoring excel column titles
a=csvread(sdata,1,0);
% read in column vectors for cycle number, deflection, force and
time
aIdx=floor(a(:,7));
pos=a(:,10);
load=a(:,11);
t=a(:,1);

% set initial values and create blank array for eLoss variable
flg=aIdx(1);
cnt=0;
temp=0;
eLoss=[;
eLossT=[;

for k=2:length(aIdx),
    % if load increasing add each rectangle to total
    if aIdx(k)==flg,
        temp=temp+(pos(k)-pos(k-1))*load(k-1);
        cnt=cnt+1;
    % if load decreasing subtract rectangle from total
    else
        eLoss=[eLoss; temp-1/2*abs(pos(t_idx)-
pos(k))*(load(k)+load(t_idx));
        eLossT=[eLossT; t(k);
        temp=0;
        cnt=0;
        flg=aIdx(k);
    end
    % Make final value equal starting value to account for
hysteresis gap
    if cnt==1,
        t_idx=k-1;
    end
end
% calculate individual cycle and mean energy dissipation
eLoss=eLoss(2:length(eLoss));
eLossT=eLossT(2:length(eLossT));
```