Comparison of Hydration Characteristics and Macro-mechanical Properties of Spine segments between species

By

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ABSTRACT

Disc hydration is an important factor to be considered in the mechanical aspects of the spinal column. This is linked to the time dependent mechanical behaviour of the intervertebral disc (IVD), therefore it's viscoelastic properties. The role of facet joints is also an important factor to consider as it is responsible as it restricts uncontrolled movement of the vertebral column. Although research has been conducted on the effects of hydration on mechanical properties, further research is required to see how hydration is affected based on the presence of facet joints, as well as investigating the mechanical properties based on these differing conditions.

The purpose of this study was to identify how hydration properties affect disc mechanics and how the presence or absence of the facet joints can cause further implications on these mechanics. This study was conducted using the methodology outlined which was extracted from literature, however the dehydration factor of the study was conducted based on practise and pilot testing. 3 porcine (pig) and 3 ovine (sheep) specimens were used, as this study was to further investigate what roles different species played in terms of mechanics based on their functional spinal unit (FSU).

Although further research is required to finalise the results evaluated from this study due to limitations in sample number, the overall trend seen in the results were that ovine specimen showed greater torsional stiffness compared to porcine. The most interesting finding from the results evaluated was the hysteresis loss coefficient (HLC) based on compression testing showed differing coefficients with increasing loading rates and increased HLC with the presence of facet joints. Not much could be evaluated in terms of hydration, which could be due to limitations outlined. This study can be further ventured into the future, where with appropriate set up and the limitations are kept at minimum, can produce promising results. This has the potential to address in vivo motion mechanics.

DECLARATION

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2. and the research within will not be submitted for any other future degree or diploma without the permission of Flinders University; and
3. to the best of my knowledge and belief, does not contain any material previously published or written by another person except where due reference is made in the text.

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I certify that I have read this thesis. In my opinion it is/is not (please circle) fully adequate, in scope and in quality, as a thesis for the degree of Master of Engineering (Biomedical). Furthermore, I confirm that I have provided feedback on this thesis and the student has implemented it minimally/partially/fully (please circle).

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CHAPTER 1: INTRODUCTION

The intervertebral disc (IVD) is a vital anatomical feature of the spinal column that provides flexibility without the loss of strength (Waxenbaum and Futterman, 2018). The IVD is composed of fibrocartilaginous tissue that is highly hydrated and has the functionality to absorb or distribute forces applied onto the spine (Bezci, Nandy and O'Connell, 2015). The anatomical structure of the IVD comprises of the annulus fibrosis (AF) which is the outer peripheral sector, and the central region is known as the nucleus pulposus (NP). The NP has higher water content, which allows for the distribution of hydrostatic pressure created by the compressive forces felt by the spine (Arun et al., 2009).

The importance of hydration of the IVD is highlighted through the NP, the most hydrated sector of the disc, as it distributes hydrostatic pressure. If the NP were solid, this would irradicate the distribution factor of forces thereby increasing the risk of trauma on the spine (Waxenbaum and Futterman, 2018). Therefore, the hydration factor is explored in this study to investigate the mechanics implications of a dehydrated disc vs a healthy hydrated disc.

As aforementioned, the loss of hydration in the IVD reduces the ability of the NP to distribute forces efficiently, which in turn leads to significant effects on the mechanics of the IVD, where hydration is a strong indicator of changes in the disc such as disc degeneration (Costi, Hearn and Fazzalari, 2002). IVD degeneration is the leading cause of neck and lower back pain, with a prevalence of 71% and 77% in men and women, respectively, aged 50 and younger. Additionally, those aged over 50, had a prevalence of 90% when it came to acquiring disc degeneration (Teraguchi et al., 2014).

Due to the IVD having fluid like and solid characteristics, this can be linked with viscoelastic behaviour. The disc is exposed to torsional, axial, and bending forces in vivo, and these are related to the equilibrium levels of hydration, hence the mechanical and hydration properties influence each other (Costi, Hearn and Fazzalari, 2002). The influence that these characteristics have with one another, lead to the main investigation of this study, which is the difference in mechanical properties based on disc hydration levels. There was further differentiation related to the presence of facet joints and the species.

The facet joints, also known as the zygapophysial joints, are vital elements of the spine that aid in the support of spinal motion and stability (O'Leary et al., 2018). The facet joint is a structure that can frequently be fractured, impairing the functionality of the spine, leading to pain and degeneration. Facet joint degeneration is quite common, occurring in individuals as young as 15 years of age and is prevalent in the L4/5 region of the spine (Li et al., 2011). Facet joints were incorporated into this study, for the purpose of understanding how the mechanics is affected with and without the presence of these joints, and to give a more in depth understanding of the mechanics of a functional spinal unit (FSU) in vivo. Most studies completely remove facets as they purely observe the disc and it's relevant mechanics, hence it was a contending factor to include facets in this study.

This study will examine the mechanical and hydration properties of porcine (pig) and ovine (sheep) specimens. These animal models are used to study the IVD as they are easily available and have reduced varying factors that will aid in the reduction of inconsistency of results (O'Connell, Vresilovic and Elliott, 2007). Factors that can lead to inconsistent results include extent of degeneration, sex, spinal level and age of specimen, which animal specimen do not have much variability in (Costi, Ledet and O'Connell, 2021).

Previous studies were analysed based on existing mechanical testing procedures and their results, also incorporating hydration factors of the IVD. It is important to note that not many studies exist on the differences in mechanical and hydration properties between an intact FSU, where the facet joints are present, versus an isolated FSU. This is an important factor to be considered as it delves into the actual representation of how the spine works in vivo, with facet joints intact not just investigating disc mechanics. The study focuses on two different hydration levels, fully hydrated which incorporates immersion in a saline bath, versus a dehydrated or air condition where the disc would be lightly sprayed with saline solution to avoid complete dehydration. These two hydration levels are commonly used in experimental IVD research. The main aim of this study was to investigate differences in FSU mechanics based on factors such as hydration, presence of facet joints and species. current mechanical testing methods and their associated results relating to IVD hydration factors. This critical assessment allows for the bridging of gap in existing literature, outlining the importance of anatomical features such as the presence of facet joints.

CHAPTER 2: LITERATURE REVIEW

Anatomy of Functional Spinal Unit (FSU)

The functional spinal unit (FSU) is composed of two adjacent vertebral bodies, which includes the intervertebral disc (IVD) and two posterior facet joints (Welch et al., 2012). The IVD is a vital component of the FSU, as they link vertebral bodies, where they cushion loads that are applied onto the spinal column due to muscle activity and the weight of the body.

Their other functions include facilitating flexibility by allowing movements such as bending, flexion and torsion (Raj, 2008). Facet joints are symmetrical and are the only synovial joints found in the spine. They contain fibrous capsules that connect the articular facets found on the vertebrae (Almeer et al., 2020).

Figure removed due to copyright restrictions

Figure 1a: Functional spinal unit, (Kim-Soon, 2015). Figure 1b: The Intervertebral disc, (Waxenbaum and Futterman, 2018).

The IVD consists of two main components as aforementioned, the annulus fibrosus and nucleus pulposus. The AF has an anatomical breakdown of fibrocartilaginous and fibrous connective tissues, and the NP contains proteoglycans, in particular aggrecans, which attract and retain water, which in turn allows for good disc turgidity that is a key factor in the aid of distribution of compressive loads (Yilmaz et al., 2017). Another important component part of the IVD are the cartilaginous end plates (CEP), which are bonded above and below the vertebral bodies, connecting the IVD to the vertebral body (Newell et al., 2017).

Hydration characteristics of Intervertebral disc (IVD)

The driving components of the hydration characteristic are performed by proteoglycans and water, where water constitutes to roughly 60-90% of the total volume of the IVD. Water is stored in two forms within the IVD, that is bound and free. These forms are also known as intrafibrillar, that forms a bond with protein structures and extrafibrillar that is the free form of water, which are water molecules that are integrated with proteoglycans (Żak and Pezowicz, 2016). Proteoglycans are negatively charged macromolecules, which is linked with fixed charge density (FCD) that is the concentration of charged groups on proteoglycans (Baldoni and Gu, 2019).

The NP behaves as a hydrostatic cushion when responding to compressive loads that are applied onto the spine, distributing these loads onto the AF and vertebrae. Hydration is an important factor that is responsible for this mechanism of the nucleus, as a reduced concentration of water present in the IVD in turn reduces the hydrostatic cushioning effect of the nucleus (Costi, Hearn and Fazzalari, 2002).

IVD hydration is related to its proteoglycan content, this is due to the net increase in the negative charge that in turn increases the osmolarity of the nucleus pulposus enabling the mechanical capability of cushioning and distributing compressive loads (Oichi et al., 2020).The concentration of water is highest in the NP and the inner AF regions, as the inner layers of AF have more proteoglycans integrated in comparison to the outer layers, and the presence of fewer proteoglycans also indicates less extrafibrillar water, (Żak and Pezowicz, 2016).

Mechanical Properties of the IVD

One of the main functions of the IVD is to allow the motion of adjacent vertebrae and to be able to resist bending and compressive loads, including shear and torsion-based loads (Alkalay, 2002). When comparing healthy and pathological discs, there are differences in terms of load response, where mechanical properties such as range of motion, stiffness and neutral zone are affected (Costi, Ledet and O’Connell, 2021). When the disc was subjected to mechanical dehydration, it resulted in a 20% decrease in volume and 35% decrease in height (Yang, Wendland and O’Connell, 2020).

The viscoelastic behaviour in IVD is influenced by strain rate in accordance to Holzapfel et al., 2004. The faster the pace of the IVD compression or rotation, the higher the magnitude of stiffness, however these properties are non-linear, which relates to stiffness being influenced by compressive preloads, (Newell et al., 2017).

Mechanical testing research on IVD

In a study conducted by Race, Broom and Robertson, 2000, a compressive test was performed on sample IVDs, which was under a bath condition submerged in 0.15M saline, done with the aid of a servo-hydraulic testing machine. The displacement, load and time were logged at 0.01 increments in terms of displacement. The results conclude that stiffness had increased with the load and its rate of loading. Another key finding was that hysteresis energy loss was lowest at higher loading rates, which shows an inversely proportional relationship between energy loss and loading rates.

A study conducted by Arun et al., 2009, investigated the impact of sustained mechanical loading on the diffusion of small solutes through the human IVD, on live human volunteers. This was done in 2 phases, where an (Magnetic Resonance Image) MRI was taken prior to the actual testing mechanism was applied and then phase 2 which was a month later where the individuals were subjected to axial loads with the use of an axial spinal loading device that is MR compatible. They were subjected to 50% of their body weight as the supine loading as this was the most viable option in MRI. The conclusive results were that sustained mechanical loading slows down the effect of diffusion for solutes to move through the disc.

In a study, where ovine IVDs were investigated for their hydration characteristics that is linked with their stiffness. It was seen that there was a sharp increase in fluid intake by the IVD during the initial hour, followed by a reduction in the rate of hydration until a plateau had reached. The use of a saline bath increased the hydrations levels drastically when compared to an air only condition, hence this influenced the stiffness of the IVD. This was evident in terms of loading in the torsional, flexion and left bending directions (Bezci, Nandy and O'Connell, 2015).

During the investigation of the viscoelastic behaviour of the nucleus pulposus, it was found that it relaxed to nearly zero during stress-relaxation tests. However, under dynamic conditions, the NP behaved solid or "solid-like", where values of dynamic modulus ranged from 7- 20 KPa and loss angle from 23-30 degrees over frequency loadings of 1-100 $rads$ -1 (Latridis et al., 1997).

A common experimental approach in understanding the disc function in axial compression is to conduct creep testing, both static and dynamic. The kelvin solid viscoelastic model is best used for the description of static creep, however in their research, Race, Broom and Robertson, 2000, found that the IVD and its material properties did not compare well with the model in terms of disc behaviour.

The intervertebral disc experiences hysteresis when put under cyclic loading conditions (Galante, 1967), where different frequencies are used to identify stiffness and energy loss. Hysteresis is at its peak during the first cycle of cyclic loading when compared to other cycles. After a certain number of cycles have been completed, and there is little to no hysteresis associated the specimen is said to have been "preconditioned" (Bezci, Nandy and O'Connell, 2015). According to Costi et al., 2008, the preconditioning technique increases the neutral zone and decreases the stiffness of the IVD, when compared to previous cycles of dynamic loading. Hysteresis is dependent on loading rate as there is strain rate dependence involved which in turn affects hysteresis and stiffness (Bezci, Nandy and O'Connell, 2015).

In a study conducted by Amin et al., 2015, where they tested degeneration effects of mechanical properties based on 6 degrees of freedom utilising a hexapod machine. In this study the FSU was investigated rather than just the IVD, where the facet joints were involved, where later the IVD was extracted for individual study, where the specimens were immersed in a 0.15M phosphate buffered saline (PBS) bath. Their study underwent dynamic loading, creep testing, stress relaxation and compression testing. The concluding results were that degeneration affected mechanical properties in the anterior shear and axial rotation sector.

Effect of Facet joints in normal discs

In the research conducted by Costi, Ledet and O'Connell, 2021, the mechanical properties of the motion segment can have drastic changes, if the facets are altered during the sample preparation period or if they are pathologic in nature, as they are also a driving factor in motion segment mechanical properties, sharing the load with the IVD. If the facet joints are pathologic, they affect stability, stiffness, and the motion coupling of the cervical section of the spine. The changes in orientation of facet joints also affects the mechanical and kinematic aspects of the spine.

While the IVD, is needed for the motion and flexibility of the spine, the facet joints aid in the limitation of the spine's range of motion, (Waxenbaum and Futterman, 2018). According to O'Leary et al., 2018, facet degeneration occurs as a results of disc degeneration, as due to the limited capability of the disc's functions, there is now an overload placed on the facet joints.

Differences in FSU and Disc anatomy between species

A study conducted by O'Connell, Vresilovic and Ellior, 2007, aimed to compare disc geometry of different species with human disc geometry, to have the ability to compare animal model results to those of human models. Animals models are more widely used due to their lack in variability in terms of mechanical properties based on factors such as degeneration or gender for instance. In their study, they utilised disc height, AP width area, lateral disc width and the lateral width of the NP as well as the centroid offset as measures to compare with human IVD. These were conducted on the following species, mouse, rat, mouse tail, baboon, bovine tail, rabbit, sheep, and rat tail, which are ranked according to their percentage of anatomy association with human IVD, from lowest to highest.

Though animal models can aid in comparative aspects of results to an extent, careful precautions must be taken as there are anatomical differences that need to be considered, as well as the position the animal's spine is oriented towards, (Alini et al., 2007).

Gap in research

The major gap found based on the literature review is the lack of mechanical and hydration-based evaluation between the FSU where the facet joints are intact and isolated. While there exists some research on the effects that facet joints have on IVD disc mechanics and their importance in terms of functionality of the spine, majority of the literature focuses solely on the properties of IVD. The information present with regards to disc mechanics and hydration characteristics is vital, but the inclusion of the presence of facet joints for mechanical evaluation and the changes associated with hydration levels would be a steppingstone in understanding how the motion segment functions in vivo. Due to the limited research in the differences of mechanical and hydration properties between the FSU and IVD, and comparing these differences between species, led to the purpose of this research study.

The main experimental aims extracted from the literature review portion of this thesis was to compare the mechanical properties on a macro level scale of the FSU, where comparison is made based on hydration factors, the presence and absence of facet joints and the differences in results based on species, which were ovine and porcine specimens.

CHAPTER 3: METHODOLOGY

The methodology for this study was adapted from the papers published by Newell et al., 2020 and Bezci, Nandy and O'Connell, 2015, with regards to specimen preparation in terms of hydration and potting. The method for dynamic compression testing was influenced by Amin et al., 2016. The information gathered from these papers then influenced the decision-making process for the current methodology based on the Instron machine, model 68SC-5 (Instron, Massachusetts, U.S.A), that was utilised and the mechanical testing that was carried out, which were axial torsion and dynamic compression.

This study was conducted on porcine and ovine FSU specimens, which were provided by the Biomechanics and Implants laboratory, situated at Flinders University, Tonsley. There were 5 ovine and 3 porcine specimens used during the experiment, out of which 2 ovine specimens were used for practise and pilot testing. 3 specimens of each species were used in formal testing, where these were the results that were used for analysing and determining the trends in mechanical properties. The specimens were categorised based on their species initially, then based on sample preparation, were classified by their hydration factor and the presence or absence of their facet joints, refer to methodology (Figure 2).

Briefly, each specimen underwent the same protocol: sample preparation and potting of the specimen using PMMA, potting rig and cups. The specimen is then placed in a PBS bath where an LVDT is attached to monitor changes in disc height as water is absorbed by the IVD. The hydration process occurs overnight after which mechanical testing follows of the hydrated specimen. The mechanical testing procedure consists of axial torsion and dynamic compression, where frequencies of 0.01, 0.1 and 1Hz are followed throughout the entirety of the testing procedure. The specimen is then dehydrated overnight, following the same period of time taken to hydrate the specimen. Mechanical testing was conducted on the dehydrated specimen, after which the facet joints were removed. Once the FSU is isolated, an isolated dehydrated mechanical testing was conducted after which the hydration process is repeated. This followed an isolated hydrated mechanical testing procedure after which the testing phase is completed for that specimen (Figure 2).

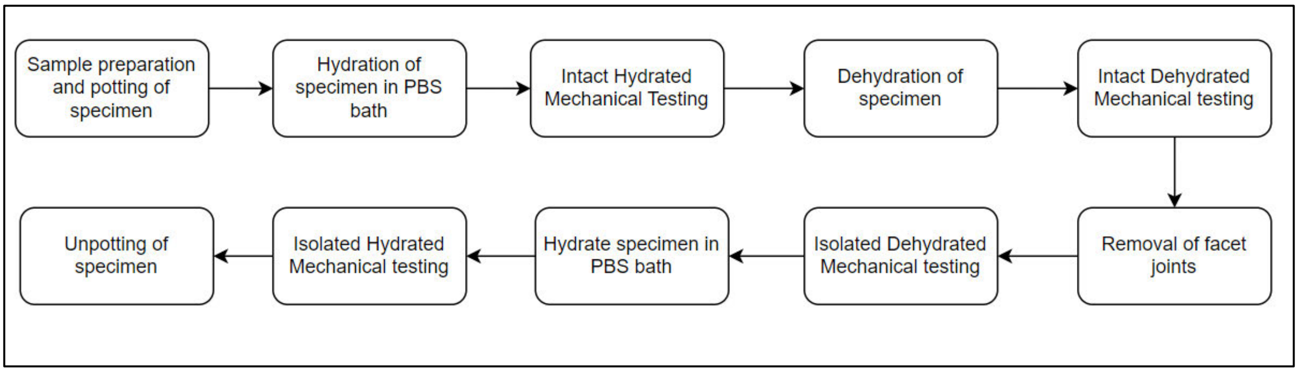


Figure 2: Flowchart of Methodology

Assumptions

The important assumptions to be considered in this study were that the given FSUs are healthy with no degenerative properties. Prior to mechanical testing, it is assumed that there will be differing results for specimens in terms of species, the presence of facet joints and the hydration factor, as the aim states that these elements will influence mechanical properties. The data obtained from specimens under each species, following the same sample preparation condition (i.e includes facet joints, ovine and hydrated) should give consistent results.

There was existing research for the hydration sample preparation methodology, however the dehydration method required practise and pilot testing to finalise the sample preparation method, which is assumed to be the correct manner to dehydrate the specimen. Bezci et al., 2018 outline facet joint mechanics in their paper, from which the methodology for intact vs isolated FSU was formulated in order to identify how mechanical properties are affected by facet joints. It is assumed that the angled wedge cut performed is the appropriate method to isolate the FSU.

Sample Preparation

3 ovine (sheep) and 3 porcine (pig) FSUs were extracted from the whole spinal column by supervisor, Mr. Michael Russo, using a bandsaw (Figure 3, a). Soft tissue removal was then conducted on the FSUs, including the removal of the spinal cord using forceps and a scalpel. A bone cutter was used for the trimming of the spinous processes, to allow the specimens to fit in the testing cups. Throughout the whole duration of sample preparation, the specimens were sprayed with saline solution to avoid complete dehydration which would permanently alter disc mechanics, this was adapted from the paper by Race, Broom and Robertson, 2000. The specimen's width and height measurements were input onto an excel template, which was used to calculate the area of the specimen. The area was calculated by using the width and height measurements multiplied by 0.84 which was extracted from the study done by Amin et al., 2016.

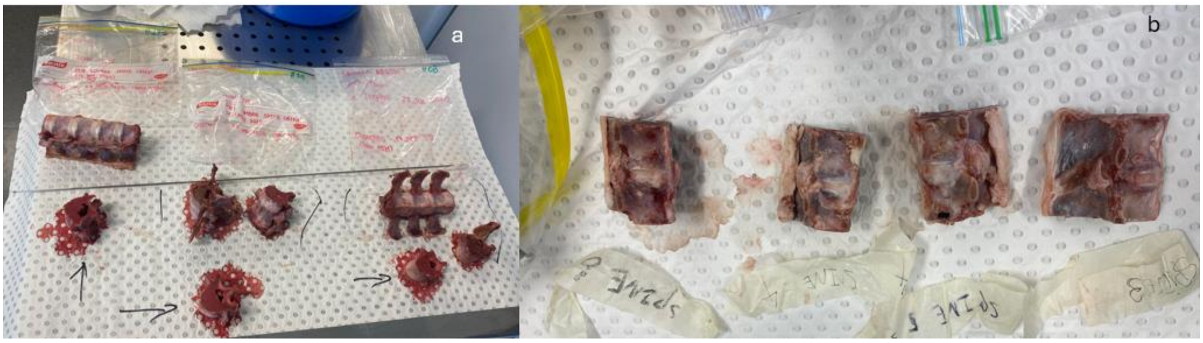


Figure 3:Porcine FSU, b: Ovine FSU

For testing, Stainless steel potting cups were used. The cup was taped (figure 4,c) and 3 greased screws (figure 4,a) were partially inserted into the potting cups. Using a potting rig (figure 4, b) for alignment reference, the specimen was placed, inferior vertebra first, central to the potting cup, which would align with the neutral axis, which is important when hydration and mechanical testing is conducted. Once the specimen was aligned the screws were adjusted such that the specimen was held in place (figure 4, d). After the screws are fixed, poly(methyl) methacrylate (PMMA) is poured until the screws are submerged and allowed to cure for 20 minutes. Once the bottom cup is cured, the same procedure is repeated for the top cup and vertebra, however the screws are aligned in accordance to the specimen's location, which is deduced when the bottom cup on the potting rig is dropped until the end of the specimen reaches the top cup. The potting process was conducted under a fume head due to toxic gases released by PMMA (figure 4,b).

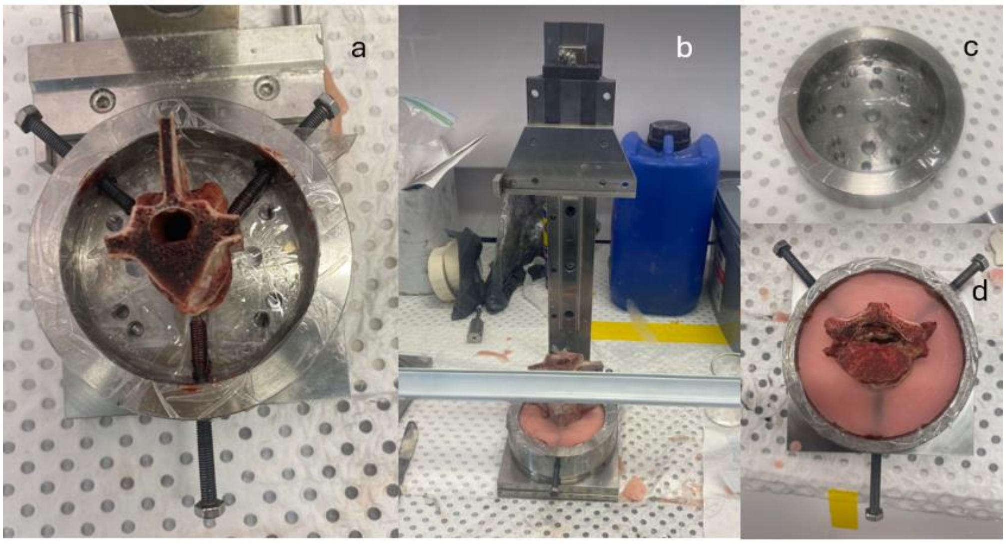


Figure 4a Bottom cup screw alignment with specimen. b: Potting process under fume head c: taped cup, d: PMMA curing of bottom cup.

Hydration procedure

For both the intake and isolated IVD the hydration protocol was kept the same. The specimen is then allowed to hydrate in a PBS bath overnight at the hydration station. The potted specimen is placed in a container and immersed in PBS solution. A custom hydration rig was used to apply a constant compressive load and allow mounting of sensors during loading. Generally, the hydration rig allows up to six loading platters, remain up right while applying an axial load (Figure 5, a). The immersed specimen and container are then placed under the hydration rig, where a specimen specific 0.1 MPa Nucleus Pressure (Wilke et al., 1999). The load was applied via a loading pattern to the estimated centre of the IVD and is the approximated same load of when a person is lying down (Recovery pre-load). A Linear Variable Differential Transformer (LVDT) was mounted to the Hydration rig such that it tracked the movement of the loading platen during hydration. LVDT signals were acquired using SignalExpress (National Instruments, Texas, U.S.A) (Figure 5,b). Refer to appendix A for graphical representation of the LVDT activity during hydration.

Figure 5a:Specimen hydration under preload, b: hydration set up

Dehydration procedure

For both intact and isolated IVD the dehydration protocol was kept the same as the procedure for hydrating the specimen, except the PBS bath was drained. While studies have not published methods for dehydration, Costi, Hearn and Fazzalari, 2002, state that specimens hydrated phased to revert back to its original weight, requiring the same time taken to hydrate.

Facet joints removal

Upon the completion of the intact testing conditions, the facet joints were removed using a hacksaw. To remove the facet joints, cuts were made on either side of the IVD using a hacksaw (Figure 6,a) , slightly below (away from the potting cup) where the facets meets the vertebra, at a slight angle towards the disc. Care was taken to make sure the cut did not go deeper, past, the posterior of the disc. Once this cut was made on both vertebra, a horizontal cut was made to connect the two (Figure 6, b,d). The facet joints were then disposed (Figure 6, c)

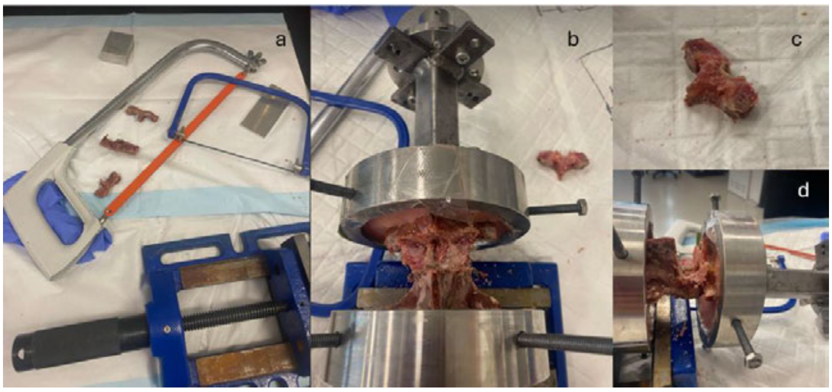


Figure 6a:Hacksaw used for cutting, b: close of isolated FSU, c: removed facet joints, d: side view of isolated FSU

Mechanical testing

Testing was performed using the Instron machine, model 68SC. The mechanical testing conducted on the specimens were dynamic axial torsion and compression, at three loading frequencies which were 0.01Hz (slow), 0.1Hz (medium) and 1Hz (fast), applied in the order presented. The load cell utilised for testing purposes was the 5KN and 25Nm cell for the Instron machine. The specimen was mounted onto the machine with the bottom plate attaching to the load cell via an adapter (figure 7, a) and the top cup attached to an adapter and chuck which is tightened (figure 7, b).

Axial Torsion

The first experiment conducted on the specimen was axial torsion, as the compression test would have the biggest impact on the specimen in terms of fluid outflow caused by activity. Prior to starting any mechanical tests, the 3 loads to be considered were the preload, follower and reference loads, which was extracted from the template based on 0.1MPa, 0.5MPa, and 0.6MPa respectively.

The testing method input onto the Instron machine was to compress the specimen until it reached the follower load (0.5 MPa), after which this load is held, and torsion is applied 2° clockwise and anticlockwise consecutively for 5 cycles, see appendix A for dual plot of cycle representation across 3 frequencies. This test is repeated for each of the outlined frequencies.

Dynamic Compression

This testing method involved compression until the follower load (0.5 MPa) was reached, after which the specimen was further compressed until it reached the sum of follower (0.5 MPa) and reference (0.6 MP) loads, referred to as the combined load (0.5 MPa + 0.6 MPa

= 1.1 MPa). The specimen was then loaded and unloaded between the follower and combined load for 5 cycles and repeated for the outlined frequencies.

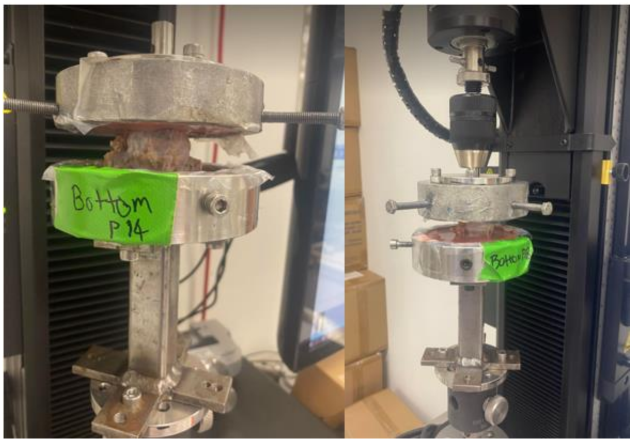


Figure 7a:specimen attached to load cell. b: chuck used to hold specimen during testing

Data Analysis

Practise and pilot testing allowed for the confirmation of whether the sequence of sample preparation and mechanical testing performed was valid based on the results obtained. The Instron machine logged and stored all data in the form of excel data sheets. These were then utilised to evaluate hysteresis loss, stiffness and peak torque in both positive and negative rotations, for the axial torsion mechanical test as well as hysteresis loss, stiffness and range of motion for the compression test. In the results section for dynamic axial torsion testing, the negative rotation results are categorised as right and positive as left. For all techniques mentioned, an excel template was developed and the final cycle of testing was isolated for analysis.

The axial torsion stiffness was calculated by plotting the torque vs rotation graph and extracting the gradient from regions -1.1° to -1.9° for negative rotation and 1.1° to 1.9° for the positive rotation region. The same procedure is performed for the compression stiffness evaluation except the region of importance was between 70% and 85% of peak compressive force extracted from the force vs displacement graph. Hysteresis for the axial torsion was evaluated by dividing the cycle between the negative (right) and positive (left) regions, as hysteresis is calculated for each direction of torsion. Then the area under the curve for the loading and unloading regions were evaluated, where hysteresis loss was calculated as the difference between the loading and unloading area. The same procedure is repeated for hysteresis loss for the compression test, excluding isolating of left and right. Peak torque was evaluated by using the maximum formula on excel for the positive region and minimum formula for the negative region. Range of motion in compression was evaluated as the distance between minimum and maximum force, hence the minimum and maximum force is extracted and the distance between these regions was found.

CHAPTER 4: RESULTS

Refer to appendix b for breakdown of results to find median result and appendix c for excel template used for results evaluation. Ovine specimens (n=3) with a median area of 645.91mm^2 and porcine specimens (n=3) with a median area of 1074.86 mm^2 were used.

Axial Torsion Test

For the axial torsion test, the median results along with IQR errors bars were plotted for both species where stiffness (Figure 8), hysteresis loss coefficient (Figure 9) and peak torque (Figure 10) were evaluated. The results are divided into left (positive) and right (negative) sectors, where for peak torque the right section was normalised as all results were negative. These were plotted for all the 3 frequencies.

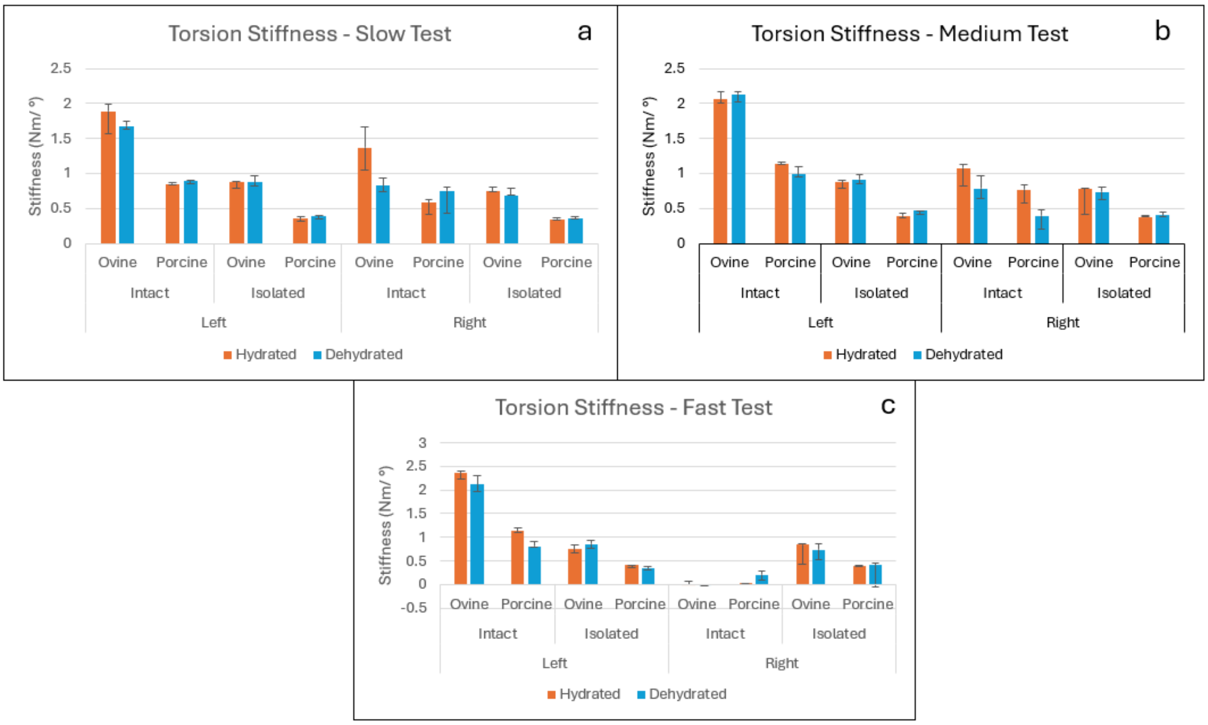


Figure 8:Median ± IQR Stiffness for Axial Torsion in left and right axial rotation for ovine and porcine specimens at, a: slow, b:medium, c: fast loading rates.

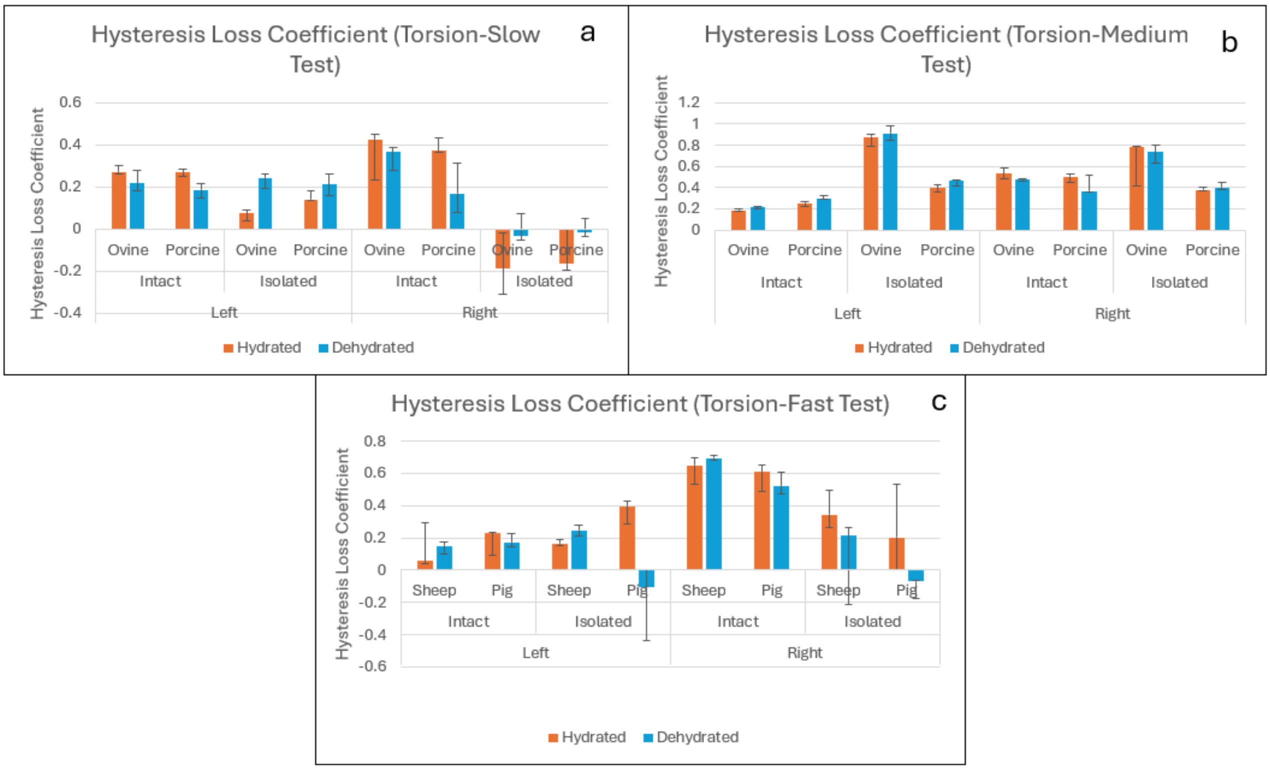


Figure 9:Median ± IQR Hysteresis Loss Coefficient for Axial Torsion in left and right axial rotation for ovine and porcine specimens at, a: slow, b:medium, c: fast loading rates.

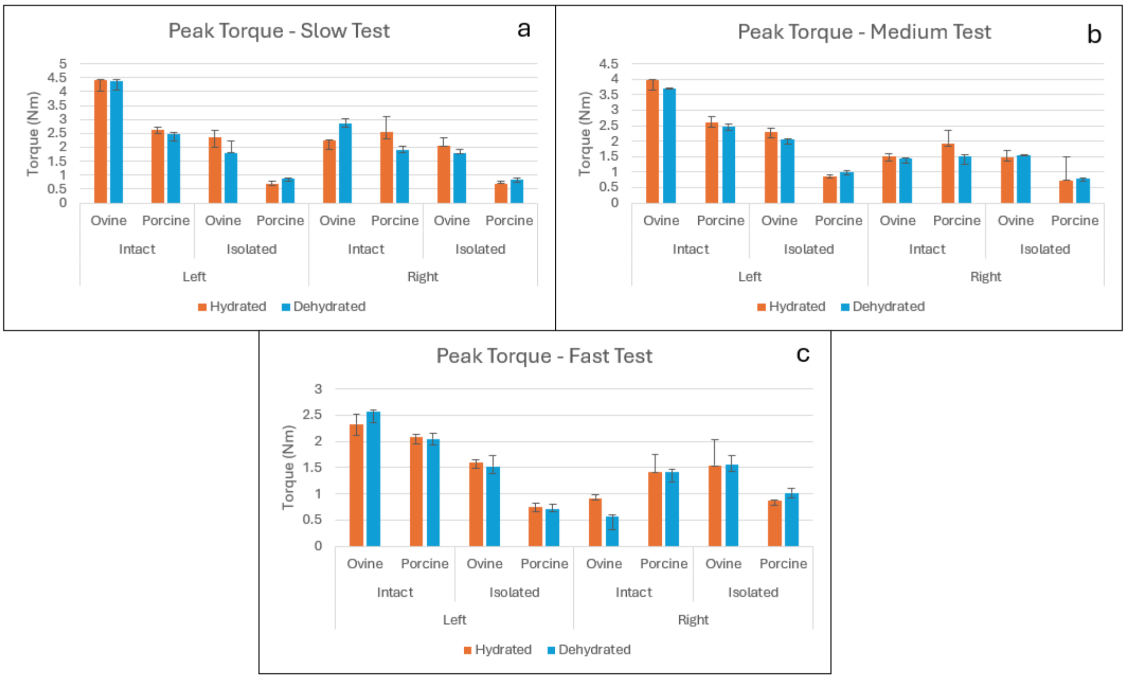


Figure 10:Median ± IQR Peak Torque for Axial Torsion in left and right axial rotation for ovine and porcine specimens at, a: slow, b:medium, c: fast loading rates.

Compression Test

For the compression test, the median results obtained alongside IQR error bars were plotted where compression stiffness (figure 11), hysteresis loss coefficient (figure 12) and range of motion (figure 13) were evaluated.

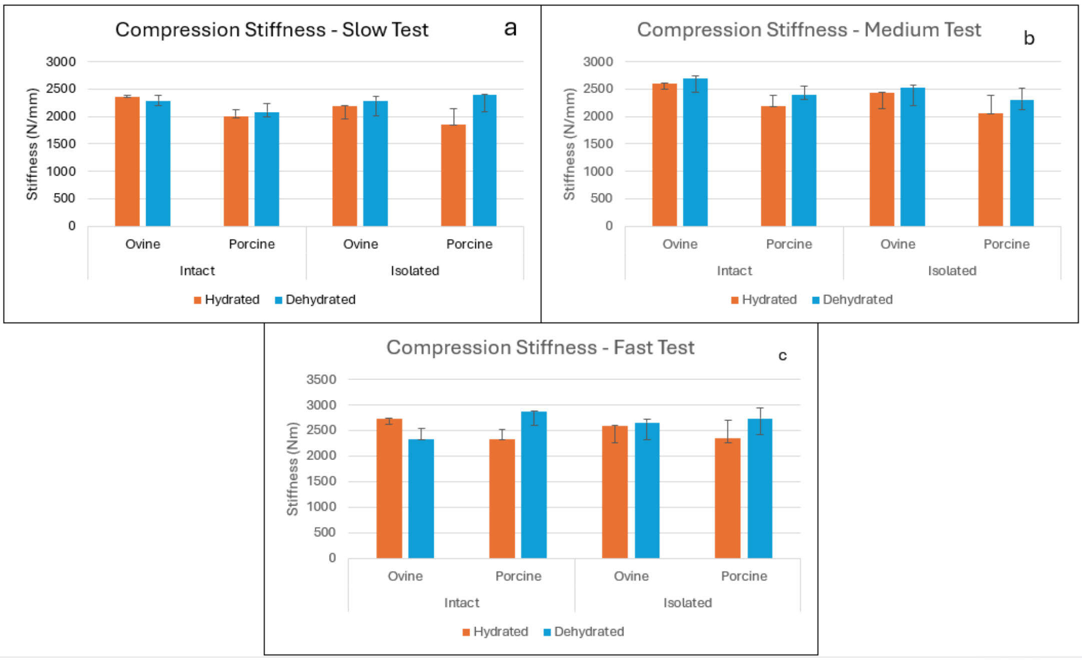


Figure 11: Median ± IQR compression stiffness for compression for ovine and porcine specimens at, a: slow, b:medium, c: fast loading rates.

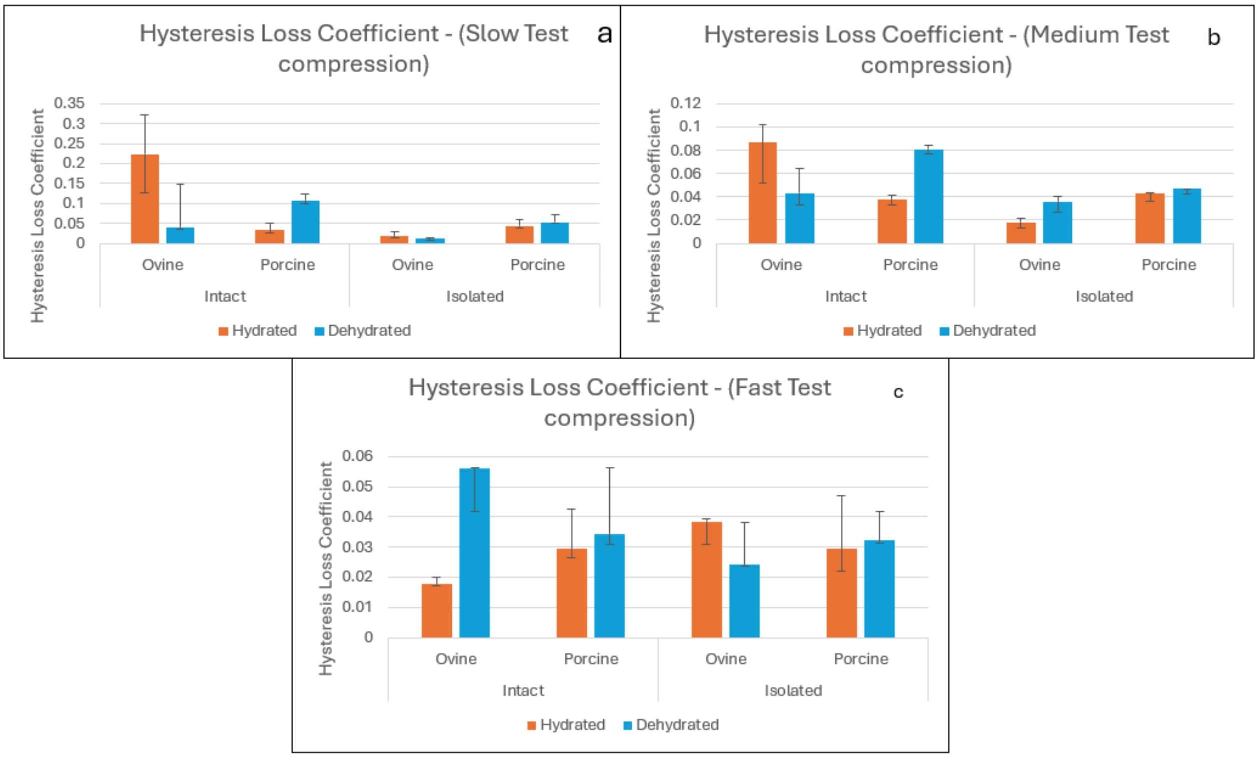


Figure 12:Median ± IQR Hysteresis loss coefficient for compression for ovine and porcine specimens at, a: slow, b:medium, c: fast loading rates.

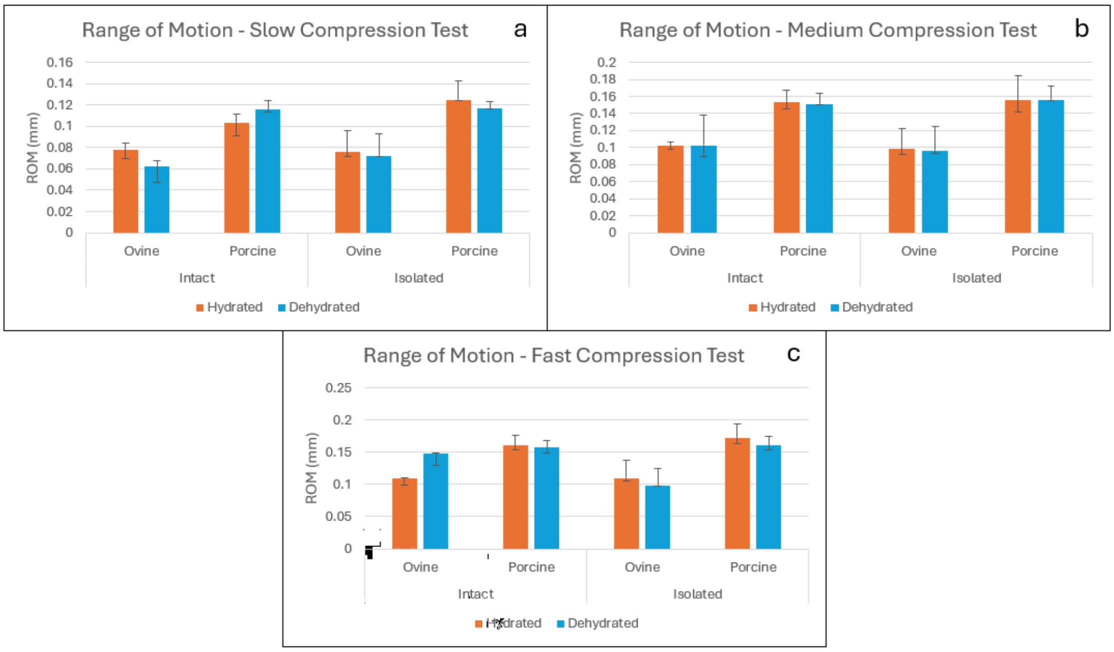


Figure 13: Median ± IQR range of motion for compression for ovine and porcine specimens at, a: slow, b:medium, c: fast loading rates.

CHAPTER 5: DISCUSSION

Axial Torsion Testing

Torsion stiffness

The general trend seen in this aspect of results was that the ovine specimen had greater stiffness compared to the porcine. Other trends that can be noticed is that stiffness is greater for specimens that are hydrated and intact. However, in accordance with Costi, Hearn and Fazzalari, 2002, stiffness was lower for specimens that were exposed to conditions of air (i.e dehydrated) hence is contradicting to literature. It can also be visualised that stiffness increased from slow to medium test, however, did not increase in the faster frequency. The results for the fast test show inconclusive stiffness, especially for the intact FSU, right region. This could be due to experimental set up, where the torsion in the negative region was not adequate due to the intact right region of testing giving rise to a very small stiffness result, which can later be linked with future work.

Hysteresis loss coefficient

Hysteresis loss coefficient (HLC) is generally larger for specimens that are hydrated. There is no trend as frequency increases, and an anomaly can be seen in both slow and fast tests for the isolated FSUs, as the HLC is negative which indicates energy being added to the specimen. These results are again consistent in the right (negative) region and can be seen in the slow test for isolated porcine and ovine specimen and fast test for the isolated porcine specimen, as well as isolated porcine FSU in the left direction. This can be related to energy being returned due to the experimental set up, however this needs to be further proved with a stiffer experimental set up in later research.

Peak Torque

Peak torque can be seen to be at its greatest for specimens that are intact and performed in the positive direction of torsion. This can be seen in specimens across all frequencies. There is no increase in torque as frequency increases, however overall peak torque is higher in the ovine specimen compared to the porcine specimen. There is very little difference seen between hydrated and dehydrated specimens, as the torque is somewhat equivalent in both conditions, varying slightly more in conditions of hydration (Inoue, Orías and Segami, 2020) .

Compression Testing

Compression stiffness

As frequency increases the change in compression stiffness is not visualised, however a slight trend can be seen with the dehydrated specimen having greater stiffness. Though it is noted that this trend is very small, therefore not much comparison can be made in terms of dehydration and hydration characteristics linked with compression stiffness, even though literature has found stiffness to be greater in dehydrated specimens. Overall, the stiffness was greater for the porcine specimen compared to ovine specimen, however the ovine specimen did have greater stiffness for intact hydrated specimens in the slow and fast tests.

Hysteresis loss coefficient

The general trend can be visually seen in this form of result evaluation, where (HLC) increases with loading rate and is consistent in all specimen conditions (i.e hydrated, intact). This can be linked with how loading rate affects mechanical properties based on (Race, Broom and Robertson, 2000). Dehydrated specimens had a greater coefficient overall, however in the slow test, the ovine specimen has a greater HLC while it was hydrated. Unlike the torsion test, there is no negative hysteresis being produced, which can indicate that the experimental set up may have played a role in any anomalies in the previous test. Another evaluation that can be made is that the coefficient is greater for specimens with facet joints compared to specimens that have been isolated. This can be used to show as an indication of the importance of facet joints, however more research needs to be done. In accordance with Galante, 1967, IVD experiences hysteresis when cyclically loaded, which can be seen with the results plotted, where varying loading rates gave rise to differing HLC values.

Range of motion

The ovine specimen varies greatly in terms of range of motion compared to porcine specimen. This is seen consistently throughout intact and isolated regions in all loading rates. There is no general trend in how range of motion is affected by the presence of facets in accordance to the results, as there is not much variability. The expected result would have been to see a difference in range of motion, where the isolated discs would have a greater range of motion.

Overall, the FSU did show viscoelastic properties based on the graphs that were plotted, where a complete cycle could be visualised. These varied with the type of test and specimen preparation, especially in torsion testing, where the hysteresis loss coefficient produced was negative, see appendix A.

Limitations

Throughout the research, limitations were faced which affected the outcome of the results. A limitation about the FSUs is that the regions they were extracted from are not consistent, where disc levels were unknown. This can affect the outcome of the results, as small differences in area can lead to differing hydration levels in turn affecting disc mechanics.

Due to time constraints, the no. of specimens used were less as only 3 per species were used, and at least 6 would have led to more consistent results. Another restriction was that once the specimen was mounted, the orientation of the testing differed for various specimens as potting affects placement of the specimen. Once mechanical testing commenced, the specimen starts to dehydrate, which cannot be controlled due to possible damage to the Instron machine if the PBS solution leaked. Another major limitation was the lack of LVDT apparatus available in the lab, as 6 specimens were hydrated in one instance, this is also linked with the lack of availability in terms of space at the hydration rig.Creep and stress relaxation tests could not be conducted to due to time restrictions, which would have been beneficial to see the trend in results for that type of testing. The experimental set up was hard to initialise, but once set up, had consistent results. However, as the results show there have been inconsistencies seen, especially in the right region of testing for torsion which in the negative direction.

CHAPTER 6: CONCLUSION AND FUTURE SCOPE

The main aim of this study was to investigate the differences between hydration factors and the presence of facet joints in an FSU and how these properties affect the mechanics of the disc. While the difference of how hydration affects the discs was not up to standard, this could have been due to the limited number of samples used. Some differences can be seen between ovine and porcine in terms of torsional stiffness, but further investigation is required. The role of facet joints can be visualised based on the results, especially based on HCL in compression testing where the intact region had a great coefficient compared to its isolated counterpart.

As there were some limitations involved in this study, future work that can be delved into would be to conduct this research with more samples of each species, where statistical analysis can be conducted to further validate results. Based on the results obtained from these results, this can then proceed to human testing where a better understanding of in vivo mechanics can be visualised.

BIBLIOGRAPHY

Alini, M., Eisenstein, S.M., Ito, K., Little, C., Kettler, A.A., Masuda, K., Melrose, J., Ralphs, J., Stokes, I. and Wilke, H.J. (2007). Are animal models useful for studying human disc disorders/degeneration? *European Spine Journal*, [online] 17(1), pp.2–19. doi:https://doi.org/10.1007/s00586-007-0414-y.

Alkalay, R. (2002). *The Material and Mechanical Properties of the Healthy and Degenerated Intervertebral Disc*.

Almeer, G., Azzopardi, C., Kho, J., Gupta, H., James, S.L. and Botchu, R. (2020). Anatomy and pathology of facet joint. *Journal of Orthopaedics*, 22, pp.109–117. doi:https://doi.org/10.1016/j.jor.2020.03.058.

Amin, D.B., Lawless, I.M., Sommerfeld, D., Stanley, R.M., Ding, B. and Costi, J.J. (2015). Effect of Potting Technique on the Measurement of Six Degree-of-Freedom Viscoelastic Properties of Human Lumbar Spine Segments. *Journal of biomechanical engineering*, 137(5). doi:https://doi.org/10.1115/1.4029698.

Arun, R., Freeman, B.J.C., Scammell, B.E., McNally, D.S., Cox, E. and Gowland, P. (2009). 2009 ISSLS Prize Winner: What Influence Does Sustained Mechanical Load *Spine*, 34(21), pp.2324–2337. doi:https://doi.org/10.1097/brs.0b013e3181b4df92.

Baldoni, M. and Gu, W. (2019). Effect of fixed charge density on water content of IVD during bed rest: a numerical analysis. *Medical engineering & physics*, [online] 70, pp.72–77. doi:https://doi.org/10.1016/j.medengphy.2019.06.011.

Bezci, S.E., Nandy, A. and O'Connell, G.D. (2015). Effect of Hydration on Healthy Intervertebral Disk Mechanical Stiffness. *Journal of Biomechanical Engineering*, 137(10). doi:https://doi.org/10.1115/1.4031416.

Costi, J.J., Hearn, T.C. and Fazzalari, N.L. (2002). The effect of hydration on the stiffness of intervertebral discs in an ovine model. *Clinical Biomechanics*, [online] 17(6), pp.446–455. doi:https://doi.org/10.1016/S0268-0033(02)00035-9.

Costi, J.J., Ledet, E.H. and O'Connell, G.D. (2021). Spine biomechanical testing methodologies: The controversy of consensus vs scientific evidence. *JOR SPINE*, 4(1). doi:https://doi.org/10.1002/jsp2.1138.

Costi, J.J., Stokes, I.A., Gardner-Morse, M.G. and Iatridis, J.C. (2008). Frequency-Dependent Behavior of the Intervertebral Disc in Response to Each of Six Degree of Freedom Dynamic Loading. *Spine*, [online] 33(16), pp.1731–1738. doi:https://doi.org/10.1097/brs.0b013e31817bb116.

Galante, J.O. (1967). Tensile Properties of the Human Lumbar Annulus Fibrosus. *Acta Orthopaedica Scandinavica*, 38(sup100), pp.1–91. doi:https://doi.org/10.3109/ort.1967.38.suppl-100.01.

Holzapfel, G.A., Schulze-Bauer, C.A.J., Feigl, G. and Regitnig, P. (2004). Single lamellar mechanics of the human lumbar anulus fibrosus. *Biomechanics and Modeling in Mechanobiology*, 3(3), pp.125–140. doi:https://doi.org/10.1007/s10237-004-0053-8.

Inoue, N., Orías, A.A.E. and Segami, K. (2020). Biomechanics of the Lumbar Facet Joint. *Spine Surgery and Related Research*, 4(1), pp.1–7. doi:https://doi.org/10.22603/ssrr.2019-0017.

Kim-Soon, O. (2015). *JaypeeDigital | Motion Preservation Surgery in the Cervical Spine*. [online] www.jaypeedigital.com. Available at: https://www.jaypeedigital.com/book/9789351525301/chapter/ch19 [Accessed 19 Oct. 2023].

Li, J., Muehleman, C., Abe, Y. and Masuda, K. (2011). Prevalence of facet joint degeneration in association with intervertebral joint degeneration in a sample of organ donors. *Journal of Orthopaedic Research*, 29(8), pp.1267–1274. doi:https://doi.org/10.1002/jor.21387.

O'Connell, G.D., Vresilovic, E.J. and Elliott, D.M. (2007). Comparison of Animals Used in Disc Research to Human Lumbar Disc Geometry. *Spine*, 32(3), pp.328–333. doi:https://doi.org/10.1097/01.brs.0000253961.40910.c1.

O’Leary, S.A., Paschos, N.K., Link, J.M., Klineberg, E.O., Hu, J.C. and Athanasiou, K.A. (2018). Facet Joints of the Spine: Structure–Function Relationships, Problems and Treatments, and the Potential for Regeneration. *Annual Review of Biomedical Engineering*, 20(1), pp.145–170. doi:https://doi.org/10.1146/annurev-bioeng-062117-120924.

Race, A., Broom, N.D. and Robertson, P. (2000). Effect of Loading Rate and Hydration on the Mechanical Properties of the Disc. *Spine*, 25(6), pp.662–669. doi:https://doi.org/10.1097/00007632-200003150-00003.

Raj, P.P. (2008). Intervertebral Disc: Anatomy-Physiology-Pathophysiology-Treatment. *Pain Practice*, [online] 8(1), pp.18–44. doi:https://doi.org/10.1111/j.1533-2500.2007.00171.x.

Waxenbaum, J.A. and Futterman, B. (2018). *Anatomy, Back, Intervertebral Discs*. [online] Nih.gov. Available at: https://www.ncbi.nlm.nih.gov/books/NBK470583/.

Welch, W., Cheng, B.C., Awad, T.E. and Macenski, M.M. (2012). *Chapter 168 - Dynamic Stabilization of the Lumbar Spine: Indications and Techniques*. [online] ScienceDirect. Available at: https://www.sciencedirect.com/science/article/abs/pii/B9781416068396101686 [Accessed 17 Oct. 2023].

Wilke, H.-J. ., Wenger, K. and Claes, L. (1999). Testing criteria for spinal implants: recommendations for the standardization of in vitro stability testing of spinal implants., 7(2), pp.148–154. doi:https://doi.org/10.1007/s005860050045.

Yang, B., Wendland, M.F. and O’Connell, G.D. (2020). Direct Quantification of Intervertebral Disc Water Content Using MRI. *Journal of magnetic resonance imaging: JMRI*, [online] 52(4), pp.1152–1162. doi:https://doi.org/10.1002/jmri.27171.

Yilmaz, A., Senturk, S., Sasani, M., Oktenoglu, T., Yaman, O., Yildirim, H., Suzer, T. and Ozer, A.F. (2017). Disc Rehydration after Dynamic Stabilization: A Report of 59 Cases. *Asian Spine Journal*, [online] 11(3), pp.348–355. doi:https://doi.org/10.4184/asj.2017.11.3.348.

Żak, M. and Pezowicz, C. (2016). Analysis of the impact of the course of hydration on the mechanical properties of the annulus fibrosus of the intervertebral disc. *European Spine Journal: Official Publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society*, [online] 25(9), pp.2681–2690. doi:https://doi.org/10.1007/s00586-016-4704-0.

APPENDICES

Appendix A

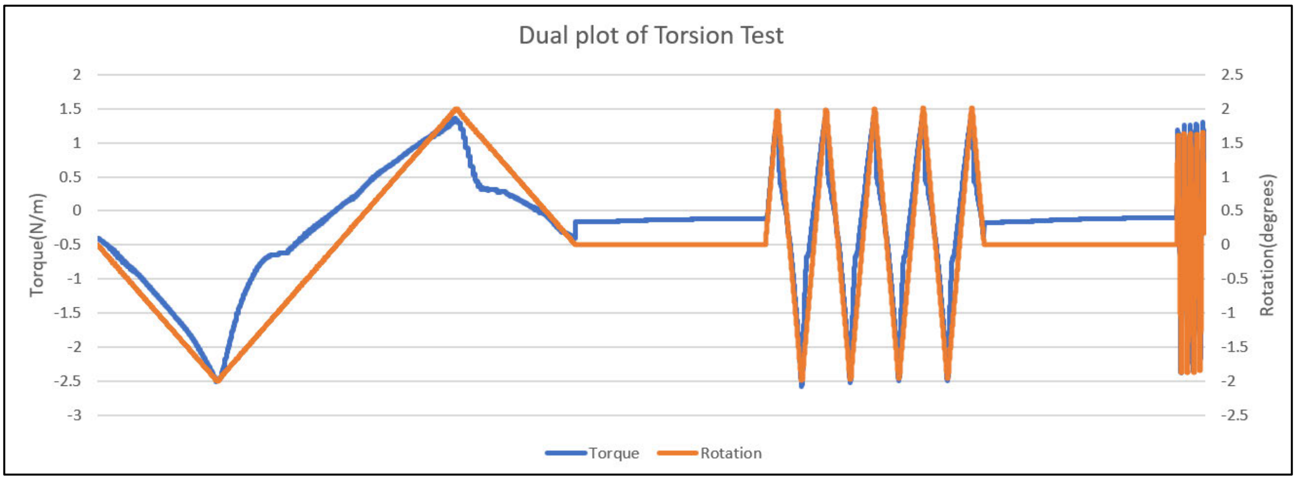


Figure 14: Dual Plot for Torsion Test

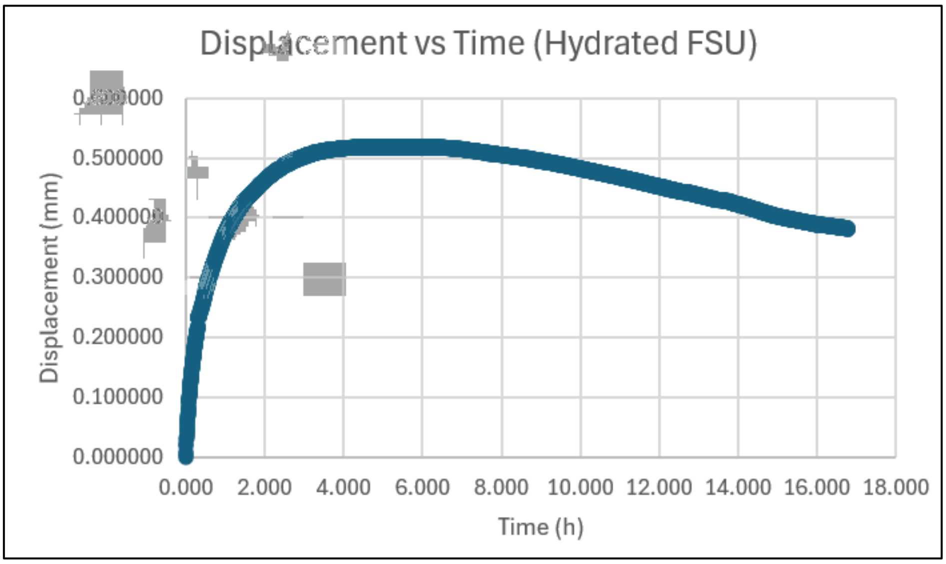
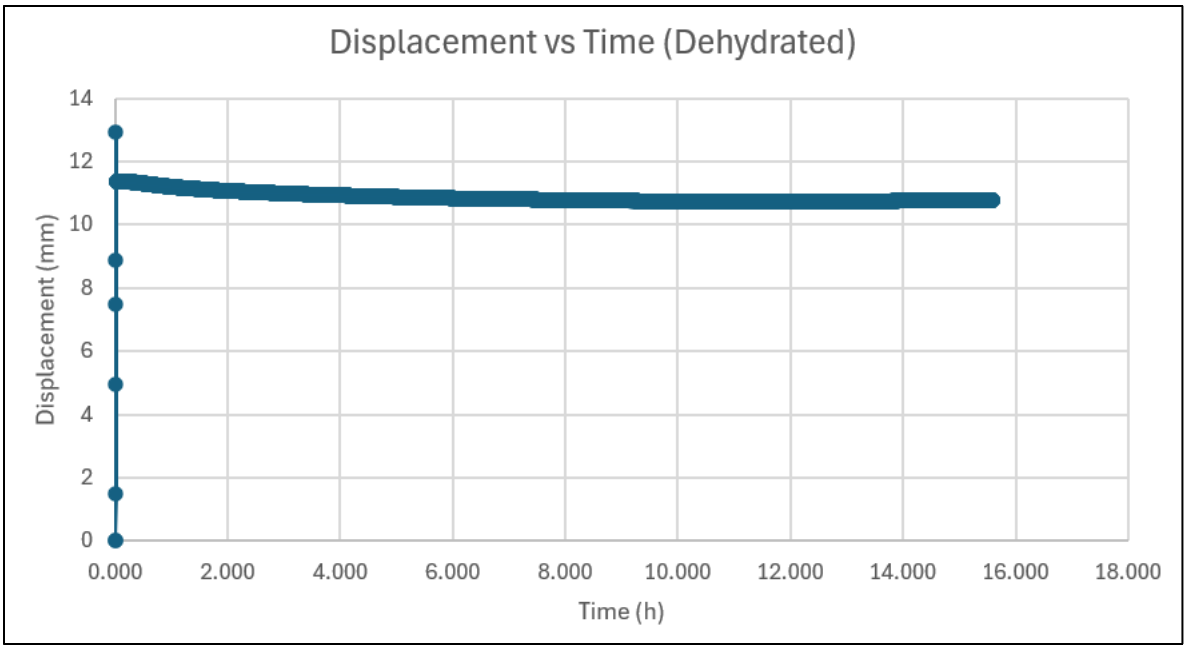
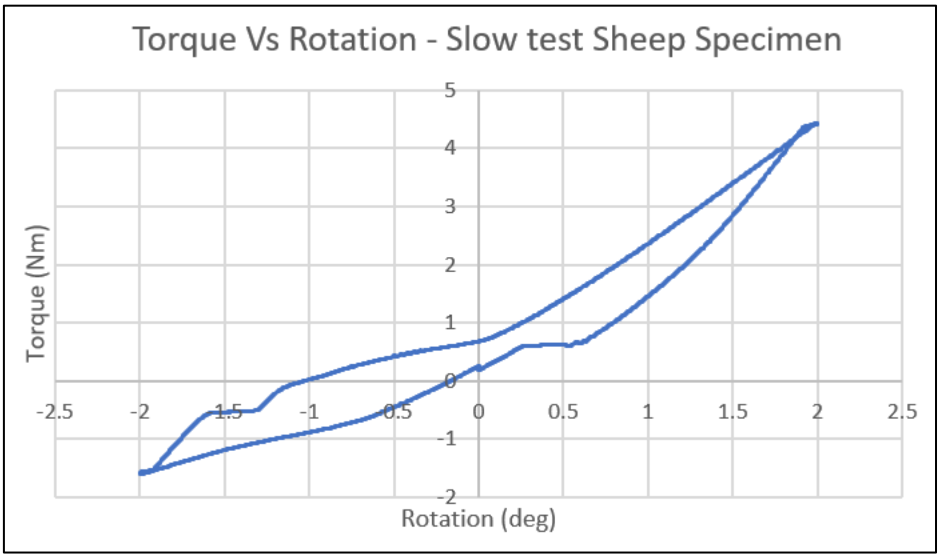


Figure 15: Displacement vs Time graph showing disc displacement for hydrated FSU (Sheep Specimen)


Displacement vs Time (Dehydrated)
Displacement (mm)
0
2
4
6
8
10
12
14
0.000
2.000
4.000
6.000
8.000
10.000
12.000
14.000
16.000
18.000
Time (h)


Torque Vs Rotation - Slow test Sheep Specimen
Torque (Nm)
5
4
3
2
1
0
-1
-2
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
Rotation (deg)

Appendix B

Intact stiffness torque

Slow test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	1.235	1.8077	1.9463	0.6678	P08	0.881	0.8208	0.2468	0.8626
S4	1.8911	1.6787	1.3723	1.0529	P14	0.8531	0.9199	0.6801	0.752
S5	2.1002	1.6035	0.7333	0.8231	P17	0.8061	0.8891	0.5772	0.1068
median	1.8911	1.6787	1.3723	0.8231	**median**	0.8531	0.8891	0.5772	0.752

Medium test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	2.0607	2.1312	1.0805	0.786	P08	1.1372	0.8961	0.379	0.3937
S4	2.1674	2.0549	1.1762	1.1445	P14	1.1758	0.9895	0.7701	0.5791
S5	2.3831	1.8378	0.5611	0.5053	P17	1.1273	1.1961	0.9176	0.005
median	2.1674	2.0549	1.0805	0.786	**median**	1.1372	0.9895	0.7701	0.3937

Fast test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	2.1197	2.1165	0.1376	0.007	P08	1.1313	0.8119	-0.0213	0.3636
S4	2.4567	2.5173	-0.0184	-0.0063	P14	1.2648	0.7767	0.0035	0.2109
S5	2.3614	1.8346	-0.0058	-0.0051	P17	1.0647	1.0162	0.0146	-0.0059
median	2.3614	2.1165	-0.0058	-0.0051	**median**	1.1313	0.8119	0.0035	0.2109

Intact HLC torque

Slow test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	0.27211	0.216355	0.425489	0.364717	P08	0.235772	0.106283	0.492256	-0.00821
S4	0.334141	0.344593	0.044455	0.412828	P14	0.300638	0.25204	0.370239	0.168619
S5	0.247895	0.144782	0.471363	0.192384	P17	0.268074	0.184743	0.360885	0.457494
median	0.27211	0.216355	0.425489	0.364717	**median**	0.268074	0.184743	0.370239	0.168619

Medium test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	0.183036	0.219783	0.428841	0.473979	P08	0.178086	0.293893	0.542286	0.367092
S4	0.218399	0.226831	0.533193	0.490876	P14	0.254083	0.355315	0.503022	0.34874
S5	0.16523	0.176552	0.629229	0.443937	P17	0.269079	0.274127	0.400434	0.661547
median	0.183036	0.219783	0.533193	0.473979	**median**	0.254083	0.293893	0.503022	0.367092

Fast test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	0.055489	0.058489	0.650721	0.663185	P08	-0.04146	0.276997	0.374589	0.423594
S4	0.025067	0.204131	0.414987	0.694594	P14	0.230983	0.172444	0.695135	0.522634
S5	0.537359	0.148862	0.745927	0.721227	P17	0.230954	0.114684	0.607335	0.684512
median	0.055489	0.148862	0.650721	0.694594	**median**	0.230954	0.172444	0.607335	0.522634

Intact peak torque

Slow test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	3.6216	4.4794	-2.2575	-2.497	P08	2.3769	1.9754	-1.4372	-2.0805
S4	4.4066	4.3591	-2.9478	-3.103	P14	2.7916	2.6039	-3.0336	-1.9016
S5	4.4415	3.7623	-2.2108	-2.8461	P17	2.6209	2.4605	-2.5592	-1.6347
median	4.4066	4.3591	-2.2575	-2.8461	**median**	2.6209	2.4605	-2.5592	-1.9016

Medium test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	3.3261	3.7221	-1.5185	-1.4349	P08	2.2873	2.2347	-1.0522	-1.9613
S4	3.9795	3.6479	-1.8657	-1.362	P14	2.985	2.4721	-2.1004	-1.493
S5	3.9941	3.7085	-1.3539	-1.7624	P17	2.6031	2.6549	-1.9213	-1.3546
median	3.9795	3.7085	-1.5185	-1.4349	**median**	2.6031	2.4721	-1.9213	-1.493

Fast test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	1.8796	2.6444	-0.7561	-0.5639	P08	1.8161	1.7983	-0.7152	-1.7876
S4	2.3301	2.1253	-0.9121	-0.4927	P14	2.2021	2.0499	-1.4091	-1.2916
S5	2.6972	2.5726	-0.9762	-1.0539	P17	2.0842	2.2441	-1.4206	-1.4147
median	2.3301	2.5726	-0.9121	-0.5639	**median**	2.0842	2.0499	-1.4091	-1.4147

Isolated stiffness torque

Slow test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	0.9095	0.7584	0.7208	0.6802	P08	0.2821	0.3238	0.3758	0.3398
S4	0.8705	1.0561	0.8633	0.8712	P14	0.3538	0.3903	0.3202	0.4259
S5	0.6955	0.8733	0.7462	0.6898	P17	0.3974	0.4087	0.3493	0.3542
median	0.8705	0.8733	0.7462	0.6898	**median**	0.3538	0.3903	0.3493	0.3542

Medium test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	0.9222	0.7791	0.0463	0.5221	P08	0.3239	0.37	0.4182	0.4021
S4	0.8774	1.0437	0.782	0.858	P14	0.3927	0.4641	0.3697	0.492
S5	0.6976	0.9135	0.7955	0.735	P17	0.4536	0.4702	0.379	0.3583
median	0.8774	0.9135	0.782	0.735	**median**	0.3927	0.4641	0.379	0.4021

Fast test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	0.9132	0.6735	0.0255	0.3274	P08	0.2867	0.2924	0.4251	0.4093
S4	0.7434	0.8552	0.8819	0.9785	P14	0.4272	0.4171	0.388	-0.5091
S5	0.5935	1.0061	0.8397	0.7305	P17	0.4042	0.3382	0.3852	0.5024
median	0.7434	0.8552	0.8397	0.7305	**median**	0.4042	0.3382	0.388	0.4093

Medium test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	0.183036	0.219783	0.428841	0.473979	P08	0.178086	0.293893	0.542286	0.367092
S4	0.218399	0.226831	0.533193	0.490876	P14	0.254083	0.355315	0.503022	0.34874
S5	0.16523	0.176552	0.629229	0.443937	P17	0.269079	0.274127	0.400434	0.661547
median	0.183036	0.219783	0.533193	0.473979	**median**	0.254083	0.293893	0.503022	0.367092

Fast test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	0.055489	0.058489	0.650721	0.663185	P08	-0.04146	0.276997	0.374589	0.423594
S4	0.025067	0.204131	0.414987	0.694594	P14	0.230983	0.172444	0.695135	0.522634
S5	0.537359	0.148862	0.745927	0.721227	P17	0.230954	0.114684	0.607335	0.684512
median	0.055489	0.148862	0.650721	0.694594	**median**	0.230954	0.172444	0.607335	0.522634

Intact peak torque

Slow test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	3.6216	4.4794	-2.2575	-2.497	P08	2.3769	1.9754	-1.4372	-2.0805
S4	4.4066	4.3591	-2.9478	-3.103	P14	2.7916	2.6039	-3.0336	-1.9016
S5	4.4415	3.7623	-2.2108	-2.8461	P17	2.6209	2.4605	-2.5592	-1.6347
median	4.4066	4.3591	-2.2575	-2.8461	**median**	2.6209	2.4605	-2.5592	-1.9016

Medium test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	3.3261	3.7221	-1.5185	-1.4349	P08	2.2873	2.2347	-1.0522	-1.9613
S4	3.9795	3.6479	-1.8657	-1.362	P14	2.985	2.4721	-2.1004	-1.493
S5	3.9941	3.7085	-1.3539	-1.7624	P17	2.6031	2.6549	-1.9213	-1.3546
median	3.9795	3.7085	-1.5185	-1.4349	**median**	2.6031	2.4721	-1.9213	-1.493

Fast test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	1.8796	2.6444	-0.7561	-0.5639	P08	1.8161	1.7983	-0.7152	-1.7876
S4	2.3301	2.1253	-0.9121	-0.4927	P14	2.2021	2.0499	-1.4091	-1.2916
S5	2.6972	2.5726	-0.9762	-1.0539	P17	2.0842	2.2441	-1.4206	-1.4147
median	2.3301	2.5726	-0.9121	-0.5639	**median**	2.0842	2.0499	-1.4091	-1.4147

Isolated stiffness torque

Slow test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	0.9095	0.7584	0.7208	0.6802	P08	0.2821	0.3238	0.3758	0.3398
S4	0.8705	1.0561	0.8633	0.8712	P14	0.3538	0.3903	0.3202	0.4259
S5	0.6955	0.8733	0.7462	0.6898	P17	0.3974	0.4087	0.3493	0.3542
median	0.8705	0.8733	0.7462	0.6898	**median**	0.3538	0.3903	0.3493	0.3542

Medium test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	0.9222	0.7791	0.0463	0.5221	P08	0.3239	0.37	0.4182	0.4021
S4	0.8774	1.0437	0.782	0.858	P14	0.3927	0.4641	0.3697	0.492
S5	0.6976	0.9135	0.7955	0.735	P17	0.4536	0.4702	0.379	0.3583
median	0.8774	0.9135	0.782	0.735	**median**	0.3927	0.4641	0.379	0.4021

Fast test

Ovine					Porcine				
	left		Right			left		Right	
Sample	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**	**hydrated**	**dehydrated**
S3	0.9132	0.6735	0.0255	0.3274	P08	0.2867	0.2924	0.4251	0.4093
S4	0.7434	0.8552	0.8819	0.9785	P14	0.4272	0.4171	0.388	-0.5091
S5	0.5935	1.0061	0.8397	0.7305	P17	0.4042	0.3382	0.3852	0.5024
median	0.7434	0.8552	0.8397	0.7305	**median**	0.4042	0.3382	0.388	0.4093

Isolated HLC torque

Slow test

Ovine					Porcine				
	left		Right			left		Right	
Sample	hydrated	dehydrated	hydrated	dehydrated	Sample	hydrated	dehydrated	hydrated	dehydrated
S3	0.100698	0.242567	0.148088	-0.03125	P08	0.13992	0.107633	-0.16621	-0.06216
S4	0.002853	0.275359	-0.1854	-0.07625	P14	0.22685	0.211911	0.15831	0.109304
S5	0.07348	0.146884	-0.4298	0.18148	P17	0.133697	0.308231	-0.22549	-0.01388
median	0.07348	0.242567	-0.1854	-0.03125	**median**	0.13992	0.211911	-0.16621	-0.01388

Medium test

Ovine					Porcine				
	left		Right			left		Right	
Sample	hydrated	dehydrated	hydrated	dehydrated	Sample	hydrated	dehydrated	hydrated	dehydrated
S3	0.127941	0.178076	0.247667	0.093811	P08	0.13825	0.028328	-0.14727	0.06052
S4	0.133748	0.198564	0.024204	0.12295	P14	-0.04708	0.106551	-0.06522	0.113484
S5	0.080371	0.012114	-0.24574	0.13703	P17	0.04708	0.171514	0.13433	0.08391
median	0.127941	0.178076	0.024204	0.12295	**median**	0.04708	0.106551	-0.06522	0.08391

Fast test

Ovine					Porcine				
	left		Right			left		Right	
Sample	hydrated	dehydrated	hydrated	dehydrated	Sample	hydrated	dehydrated	hydrated	dehydrated
S3	0.147552	0.306059	0.647799	0.321065	P08	0.17831	0.11727	-0.19881	-0.05244
S4	0.210282	0.248903	0.177961	0.21342	P14	0.459636	-0.77178	0.85792	-0.27778
S5	0.163723	0.178817	0.34324	-0.641	P17	0.3982	-0.10746	0.20209	-0.0704
median	0.163723	0.248903	0.34324	0.21342	**median**	0.3982	-0.10746	0.20209	-0.0704

Isolated peak torque

Slow test

Ovine					Porcine				
	left		Right			left		Right	
Sample	hydrated	dehydrated	hydrated	dehydrated	Sample	hydrated	dehydrated	hydrated	dehydrated
S3	2.8929	1.7946	-2.0608	-1.7835	P08	0.5624	0.6803	-0.7014	-0.7177
S4	2.3451	2.6914	-2.046	-1.7717	P14	0.6807	0.8829	-0.73	-0.9682
S5	1.6575	1.7987	-1.4671	-1.4531	P17	0.9191	0.9081	-0.5606	-0.8282
median	2.3451	1.7987	-2.046	-1.7717	**median**	0.6807	0.8829	-0.7014	-0.8282

Medium test

Ovine					Porcine				
	left		Right			left		Right	
Sample	hydrated	dehydrated	hydrated	dehydrated	Sample	hydrated	dehydrated	hydrated	dehydrated
S3	2.5265	1.7211	-1.0188	-1.4579	P08	0.7376	0.813	0.7921	-0.7675
S4	2.2923	2.0754	-1.7107	-1.5827	P14	0.8473	0.9835	-0.7357	-0.9481
S5	1.9183	2.0629	-1.4708	-1.5339	P17	0.9577	1.0908	-0.7563	-0.7932
median	2.2923	2.0629	-1.4708	-1.5339	**median**	0.8473	0.9835	-0.7357	-0.7932

Fast test

Ovine					Porcine				
	left		Right			left		Right	
Sample	hydrated	dehydrated	hydrated	dehydrated	Sample	hydrated	dehydrated	hydrated	dehydrated
S3	1.5965	1.2536	-0.5407	-1.2082	P08	0.5421	0.6241	-0.8656	-0.8441
S4	1.6824	1.5213	-1.5393	-1.551	P14	0.8652	0.8951	-1.0401	-1.2085
S5	1.3819	1.9354	-1.5572	-1.7864	P17	0.755	0.7045	-0.8256	-1.0126
median	1.5965	1.5213	-1.5393	-1.551	**median**	0.755	0.7045	-0.8656	-1.0126

Facet stiffness comp

Slow test

Ovine			Porcine		
Sample	hydrated	dehydrated	Sample	hydrated	dehydrated
S3	2339.6	2289.5	P08	1954.9	1900.5
S4	2348.1	2484.1	P14	2252.2	2376.6
S5	2411.6	2092.7	P17	2004.3	2083.1
median	2348.1	2289.5	**median**	2004.3	2083.1

Medium test

Ovine			Porcine		
Sample	hydrated	dehydrated	Sample	hydrated	dehydrated
S3	2402.6	2189.6	P08	2173	2212.4
S4	2603.7	2786.5	P14	2573.1	2719.6
S5	2604.6	2693.1	P17	2188.9	2394.5
median	2603.7	2693.1	**median**	2188.9	2394.5

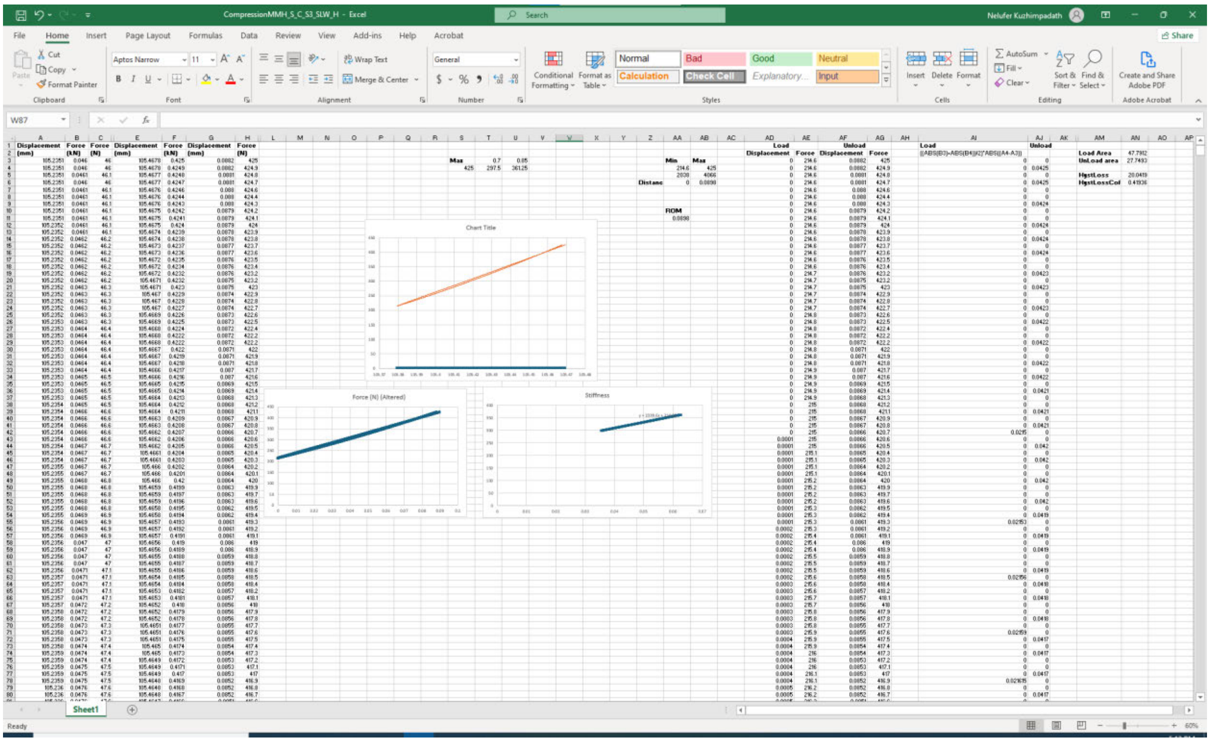
Fast test

Ovine			Porcine		
Sample	**hydrated**	**dehydrated**	**Sample**	**hydrated**	**dehydrated**
S3	2533.4	2320.8	P08	2320.8	2339.1
S4	2759.1	2320.8	P14	2727.7	2874.7
S5	2723.4	2760.7	P17	2327.2	2880.2
median	2723.4	2320.8	**median**	2327.2	2874.7

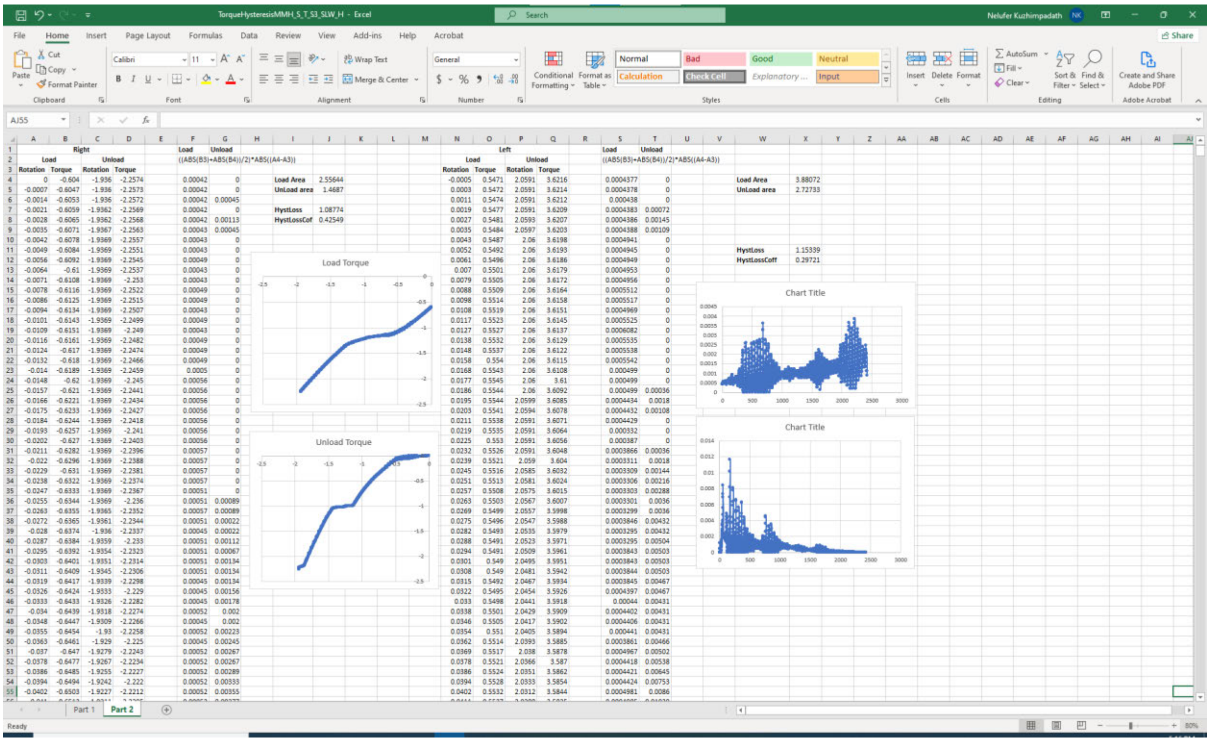
Appendix C

Excel Templates

Compression stiffness, hysteresis coefficient loss and range of motion excel template.



Torsion Hysteresis Coefficient and Peak Torque Evaluation



Torsion stiffness evaluation