Insights into the function of the knee meniscus

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March 2019

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Declaration of Authenticity and Author's rights

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Signed: Fahd Mahmood

Date: March 2019

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1. Preface

1.1 Acknowledgements

Whilst I am the author of this work, there are numerous individuals without whom it would have been impossible. Primary amongst these are my supervisors – Dr Philip Riches and Mr Jon Clarke.

I would firstly like to thank Phil for his guidance at every stage of this work – from its inception to its analysis and write up, as well as for allowing me sufficient independence to develop my own thoughts and ideas. His advice around analysis was vitally important to all aspects of this work and I could not have completed it without him. Equally, his help in procuring cadaveric samples for me to test was indispensable. Particular thanks are also due to the West of Scotland Orthopaedic Research Society, the Golden Jubilee Research Foundation and the University in funding the purchase of these samples.

Equal thanks must be given to Jon for guiding me in designing my study and allowing me a route to undertake this PhD whilst taking time out of my clinical training. Again, his help for all aspects of this work, and especially in helping with knee arthroscopy was indispensable. He has also provided a role model for me, demonstrating that it is possible to tread this somewhat challenging path as both an academic and a clinician.

Thanks also to Stephen Murray for his advice on fabricating my testing apparatus and for his time in building the jig used for testing. Again, this project would not have been possible without his help. The support of Alwayse Engineering in donating ball transfer units for my jig is much appreciated. Thanks to John Wilson for the numerous trips to abattoirs and the Golden Jubilee National Hospital to collect my myriad samples. To Katie Henderson for her advice on making up solutions and running assays and to Matt Banger for his assistance with analysing data collected using the Mako probe. To Prof Phil Rowe for allowing me to borrow critical pieces of equipment at short notice! Thank you also to Brian Cartlidge for making sure I was safely out of the tissue lab every evening.

Special thanks is also due to the staff at the Golden Jubilee National Hospital for aiding me in collecting tissue samples and identifying patients, as well as for lending me an arthroscopy stack and instruments. Thanks in particular to Prof Frederic Picard and Mr Jason Roberts for their efforts in trying to obtain cadaveric samples for my experiments.

Thanks is also due to Mr Bryn Jones, my training programme director for facilitating my time out of Orthopaedic training, for providing me financial support and for his support and advice on my return to training. Thank you also to the NHS Greater Glasgow & Clyde Endowment Fund for their support.

I would like to thank my wife Sana and my children Ibrahim and Ayesha for their patience and encouragement during my PhD as well as for pushing me to take some time out when I needed to. Finally, and most importantly, I would like to thank my parents – it is their investment in me over the course of my life which has led me to where I am today.

1.2 List of abbreviations

Abbreviation	Definition
ACL	Anterior Cruciate Ligament
ADL	Activity of Daily Living
ANOVA	Analysis of Variance
BMI	Body Mass Index
CE	Conformité Européene (European Conformity)
CMI	Collagen Meniscus Implant
CSV	Comma Separated Value
CT	Computed Tomography
DSLR	Digital Single Lens Reflex
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
GAG	Glycosaminoglycan
IKDC	International Knee Documentation Committee
IRAS	Integrated Research Application System
ISO	International Organisation for Standardisation
KOOS	Knee Injury and Osteoarthritis Outcome Score
KSS	Knee Society Score
MCL	Medial Collateral Ligament
MeSH	Medical Subject Headings

MRI	Magnetic Resonance Imaging
NA	Not Applicable
NHS	National Health Service
OA	Osteo-Arthritis
OR	Odds Ratio
PBS	Phosphate Buffered Saline
PC	Personal Computer
PCL	Posterior Cruciate Ligament
S.D.	Standard Seviation
S.E.	Standard Error
SF-36	Short Form – 36
US	United States
VAS	Visual Analogue Score
WOMET	Western Ontario Meniscal Evaluation Tool

1.3 List of tables

1.4 List of figures

1.5 Abstract

The knee menisci are understood to have a variety of roles including load transmission and stability of the knee joint. To date, there has been no exploration of the role of radial tears of the menisci in inducing kinematic changes in knee joint movement. Furthermore, the function of proteoglycans in maintaining mechanical meniscus has not been explored.

Load was applied to cadaveric knees in the intact state and following both a 50% and 100% radial tear of the medial (5 knees) or lateral (6 knees) meniscus. A coordinate system was developed to allow analysis of joint kinematics. Concurrently,

confined compression techniques were used to apply 10% strain to meniscal samples from cadavers (30 samples) and patients suffering osteoarthritis (36 samples) in solutions of varying ionic concentration. 7 samples from an Actifit meniscal scaffold were also tested in deionised water. Resultant relaxation curves were fit using finite element modelling techniques. Human tissue samples were assayed for proteoglycan content.

Radial tears of the meniscus did not induce significant changes in knee joint kinematics.

Finite element modelling demonstrated that the electrostatic effect of proteoglycans contributed to ~40% of the stiffness of the meniscus. No significant difference in proteoglycan content was observed between solutions. The Actifit meniscal scaffold is stiffer than native meniscal tissue but displays similar permeability.

Although radial tears do not alter the kinematics of the knee joint, there is evidence they result in abnormal loading of articular cartilage and it is hence important that they are repaired where possible. Proteoglycans play a critical role in maintaining stiffness of the meniscus – current repair strategies such as meniscal scaffolds do not attempt to recreate this function and hence may not prevent cartilage degradation. The stiffness of the Actifit meniscal scaffold may help protect a nascent meniscal repair but may also contribute to abnormal joint loading; its similar permeability will help mimic meniscal function.

2. Introduction

2.1 Background

The knee is the most commonly injured joint in the human body, with injuries to the meniscus the commonest form of structural knee injury [1]. These injuries often affect the working age population, resulting in significant time off work, prolonged rehabilitation and resultant societal costs.

The first reported meniscal surgery was that of a meniscal repair, performed in 1883 [2]. The same surgeon subsequently published a report a few years later advocating total removal of the meniscus, suggesting that it resulted in restoration of joint movement[3]. This view was widely adopted and consequently patients with meniscal injury underwent resection of the entire organ until the late 1940s, when unfortunately, these patients were subsequently found to develop early onset osteoarthritis[4].

Contemporary management of meniscal injuries has therefore come full circle, with treatment for such injuries undertaken through knee arthroscopy – a keyhole surgery procedure which allows inspection and management of the meniscal injury [5]. As would be expected, knee arthroscopy for meniscal injury is the most common procedure performed by orthopaedic surgeons [6]. Most such procedures are undertaken to perform limited resection of injured meniscal tissue. This approach has been adopted due to the poor healing potential of meniscal tissue and focuses on preservation of the meniscus to the largest extent possible.

This standpoint is further bolstered by evidence that removal of the meniscus in its entirety results in significant instability of the knee [7], as well as evidence that numerous partial meniscal injury states result in increased peak contact pressures coupled with decreased contact areas in the affected knee joints[8,9]. Traditionally, the meniscus is viewed as a 'secondary stabiliser' of the knee [10], coming into play when structures such as the anterior cruciate ligament are damaged, however there is some evidence that a limited number of isolated meniscal injury states [11–13] results in instability of the knee joint in particular injury states. Our understanding of this area is developing, and if present, these altered biomechanics may lead to symptomatic instability of the knee joint or contribute to accelerated wear of the chondral surfaces of the knee, resulting in osteoarthritis.

Coupled with the developing understanding of the meniscus’ macroscopic role in the biomechanics of the knee, we have also developed an understanding of how the meniscus aids load transmission through the generation of hoop stresses in the collagen fibres which comprise the majority of the solid portion of the organ [14]. Proteoglycans are also present in significant number in the meniscus. In articular cartilage, proteoglycans help maintain stiffness of the cartilage through ionic effects dependent on the negative charge associated with these proteins. It is estimated that two thirds of the stiffness of articular cartilage is attributable to electrostatic effects [15]. To date, it is unclear whether the same mechanism is present in meniscal tissue, nor do we know what proportion of the stiffness of the meniscus might be attributable to such effects.

Recognition that partial meniscal injury results in altered biomechanics of the knee joint, with the potential for accelerated wear of the articular surfaces has also led to interest in repairing meniscal injuries where possible. This option is challenging due to the limited healing potential of meniscal tissue – a reflection of its poor blood supply in adulthood [16]. Whilst meniscal replacement in the form of transplantation is an option for patients who have suffered complete meniscal injury [17], meniscal scaffolds offer the potential of allowing meniscal regeneration in regions where this would previously have been impossible [18]. Although these implants are in clinical use, there is limited data in the literature regarding their outcomes – the majority present patient reported outcome measures and to date, no study has been able to determine whether these are chondro-protective. Furthermore, these scaffolds are understood to remain in situ for up to 4 years prior to reabsorption [19]. There has been no independent investigation of the mechanical properties of such scaffolds – in particular, how they compare to those exhibited by the native meniscus.

2.2 Literature Review

This literature review seeks to summarise our current knowledge regarding the meniscus. In particular, it aims to identify the various functions of the meniscus and how these are influenced by injury. As well as this, it reviews current strategies in treatment of meniscal injury, with a focus on the use of meniscal scaffolds to augment meniscal repair. In doing so, the literature review identifies gaps in the literature which may be addressed by this work.

2.2.1 Search strategy

The literature search was conducted based on a number of themes. Search strategies on PubMed/Medline made use of the MeSH (Medical Subject Headings) operators for accuracy. Searches were restricted to humans and only results in English were examined. Initial searches were performed using the following operators:

- (("Joint Instability" [Mesh]) AND "Knee Joint" [Mesh]) AND "Meniscus" [Mesh] – 363 results
- (("Biomechanical Phenomena" [Mesh]) AND "Knee Joint" [Mesh]) AND "Meniscus" [Mesh] – 345 results

Perusal of these results allowed several more detailed search strategies to be developed using BOOLEAN operators, in particular:

- Meniscus AND properties – 150 results
- Meniscus AND permeability – 49 results
- Meniscus AND strain – 78 results
- Meniscus AND lubrication – 20 results
- Meniscus AND friction – 76 results
- Meniscus AND laxity – 127 results
- Meniscus AND proprioception – 40 results
- Meniscus AND extrusion – 163 results
- Meniscus AND anterior cruciate ligament – 1069 results
- Meniscus AND posterior cruciate ligament – 148 results
- Knee simulator – 294 results

- Collagen meniscus implant – 60 results
- Actifit – 11 results
- Polyurethane AND meniscus AND scaffold – 37 results

Databases searched included:

- MEDLINE
    - Medical Literature Analysis and Retrieval System Online – maintained by the United States National Library of Medicine, large database of life sciences and biomedical journals.
- Google Scholar
    - Hosted by Google, this search engine indexes peer reviewed journals across numerous disciplines. Scholar has functionality allowing citing articles for a journal entry to be viewed.

The abstracts of all results were reviewed to determine their suitability for inclusion in the literature review. Where studies explored the effect of ligamentous injury combined with meniscal injury, the study was only included if meniscal injury was used as the primary intervention.

Furthermore, the reference lists of full text papers were perused to identify any further relevant publications. As well as this, the ‘citing articles’ function of Google Scholar and the ‘related articles’ function of PubMed was found to be useful in identifying further publications of relevance.

Full text of relevant publications was obtained using either the University of Strathclyde/ University of Glasgow libraries or the NHS (National Health Service) Open Athens service. A few articles were not obtainable via these methods and these were requested directly from the British Library via the University of Strathclyde’s interlibrary lending service.

Publications were read by the primary author and used to construct the literature review.

2.2.2 Gross anatomy of the menisci

The knee is a synovial joint between the tibia and femur, with a further articulation between the femur and patella. Traditionally, the knee is described as a hinge joint, however, this is an oversimplification of its true function. As well as flexion/extension, the knee allows rotation as it flexes to allow the joint to function in varied conditions [20].

The menisci are two paired structures lying within the human knee joint, between the tibia and femur. The menisci are roughly crescent shaped, lying between the tibial plateau inferiorly and the femoral condyles superiorly in the medial and lateral compartments of the knee respectively (Figure 2-1 & Figure 2-2). They are triangular in cross section – thicker at the periphery and tapering to a free edge centrally (Figure 2-3 - this image is taken with a Mezoscope (Tensortek) and shows the collagen fibril network in the coronal plane). The superior border of both menisci is concave to accommodate each of the femoral condyles. The inferior borders conform to the convex tibial plateau laterally and the concave tibial plateau medially.

2.2.2.1 The medial meniscus

The medial meniscus is crescent shaped and approximately 3.5cm in diameter [21]. It is broad posteriorly and narrows anteriorly. The medial meniscus is attached to the medial collateral ligament (MCL) as it passes across the joint surface posteromedially. This attachment has been thought to limit mobility of the meniscus, potentially leaving it more prone to injury. However, anatomical studies have revealed limited structural connection between the two structures and raise questions as to the influence of the ligament on meniscal function [22]. Indeed, recent work has shown that releasing medial meniscal attachments to the MCL does not significantly affect meniscal motion in the anteroposterior or mediolateral direction [23].

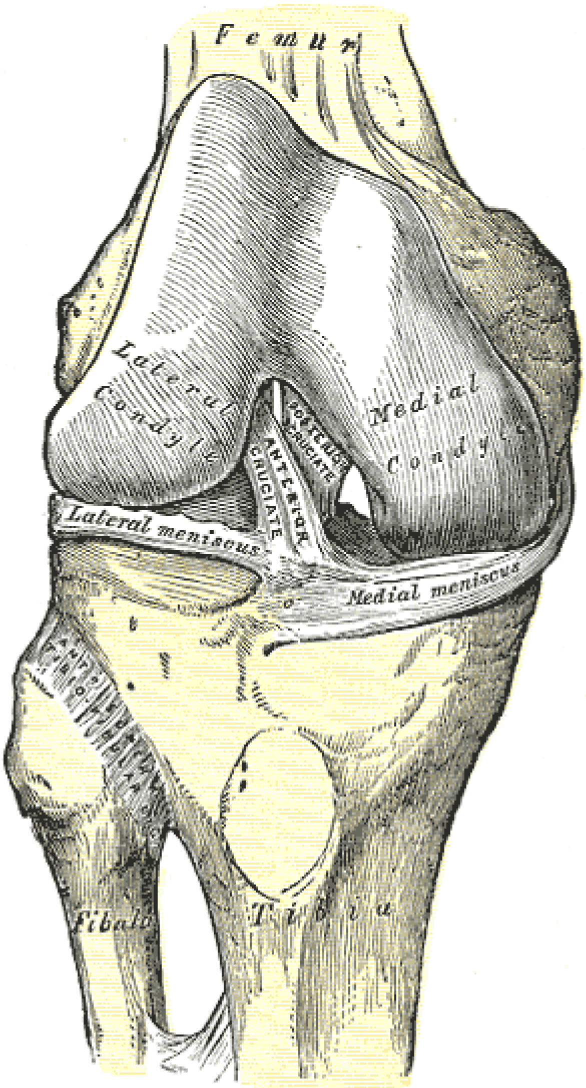


Figure 2-1 - Anatomy of the knee joint [24]

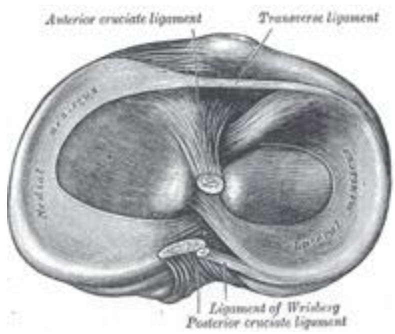


Figure 2-2 - An axial view of the tibial plateau, showing the crescent shaped menisci [24]

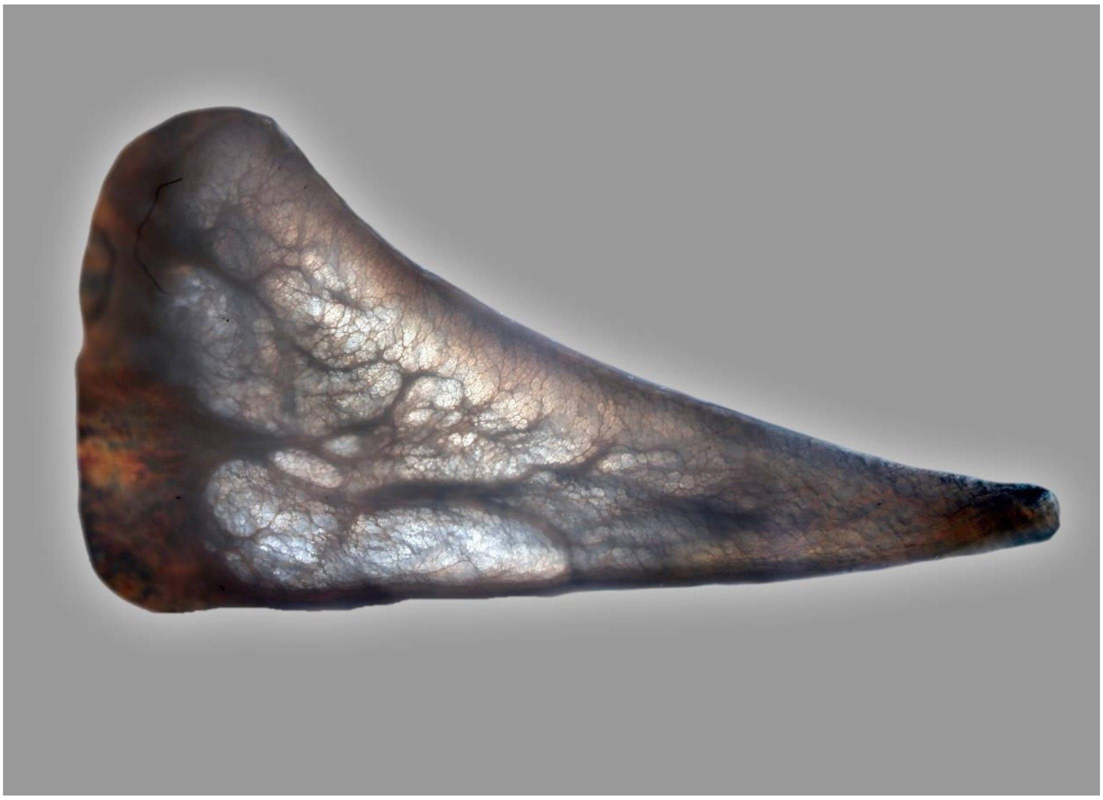


Figure 2-3 - The meniscus in cross section (image from: http://www.tensortek.com)

2.2.2.2 The lateral meniscus

The lateral meniscus adopts a roughly circular shape and covers more of the tibial plateau than the medial meniscus. There is a gap in the attachments to the capsule of the lateral meniscus' at the popliteal hiatus, where the popliteus tendon indents the meniscus. The lateral meniscus moves more during knee flexion than the medial meniscus due to less capsular attachment as well as the influence of surrounding ligamentous and muscular attachments.

2.2.2.3 Meniscal attachments

The anterior horn of the medial meniscus attaches to the tibia in front of the anterior cruciate ligament (ACL); the posterior horn attaches anterior to the posterior cruciate ligament (PCL) in the posterior intercondylar fossa. Kohn and Moreno found the anterior insertion was often marked by a distinct tubercle [25] whilst a depression marked the posterior insertion.

The anterior insertion of the lateral meniscus coincides with the ACL, whilst the posterior insertion also lies between the intercondylar tubercles of the tibia [25]. Notably, the transitional zone of the meniscal insertions has been found to be weakest in tensile testing, which would correspond to clinical findings of meniscal root tears [26].

There are two meniscofemoral ligaments arising from the lateral meniscus – both of which are prone to anatomical variation. They serve to connect the posterior horn of the lateral meniscus to the lateral aspect of the medial femoral condyle. Gupte et al.. [27] found at least one ligament present in 93% of 78 specimens. This incidence does appear to vary across the literature [28]. The anterior meniscofemoral ligament, or ligament of Humphry, passes anterior to the PCL, whilst the posterior meniscofemoral ligament, or ligament of Wrisberg passes posterior to the PCL. It should be noted that the term ligament is a misnomer here, as these structures connect meniscus to bone, not bone to bone.

The meniscofemoral ligaments are thought to play a role in controlling the movement of the posterior horn of the lateral meniscus during deep knee flexion [28]. There is also some suggestion they may play a role in knee stability, acting as a secondary stabiliser, supplementing the function of the PCL [29].

2.2.2.4 Meniscal embryology

The menisci are mesenchymal derivatives, developing between 8-10 weeks of gestation. They are initially highly vascularised and cellular through till birth. However, as a child begins weight bearing, the menisci lose their vascularity, with only a thin peripheral region remaining well vascularised. There is a concomitant decrease in cellularity, with increased collagen deposition [30].

2.2.2.5 Vascular anatomy

As noted, the adult meniscus is largely avascular, with only a peripheral vascular supply derived from the joint capsule. This blood supply is derived from branches of the medial and lateral geniculate arteries, though the lateral supply predominates. These vessels lead to a perimeniscal capillary plexus which supply the periphery of the meniscus, penetrating between 10-30% of meniscal depth through radial branches [16]. Bird and Sweet [31] examined menisci of human infants using Indian ink staining and scanning electron microscopy to demonstrate the presence of canals in the menisci, communicating both with the synovial lining and its avascular surface. They suggest these canals may serve as a means for transport of nutrients and/or synovial fluid to nourish the meniscus.

In clinical practice, the vascular anatomy of the menisci is divided into three zones – the red-red zone, which is well vascularised; the red-white zone is at the border of the vascular supply and the white-white zone is essentially avascular. Meniscal tears in the red-red zone have an excellent healing prognosis whilst those in the white-white zone have a poor prognosis [32]. The zones are illustrated in Figure 2-4.

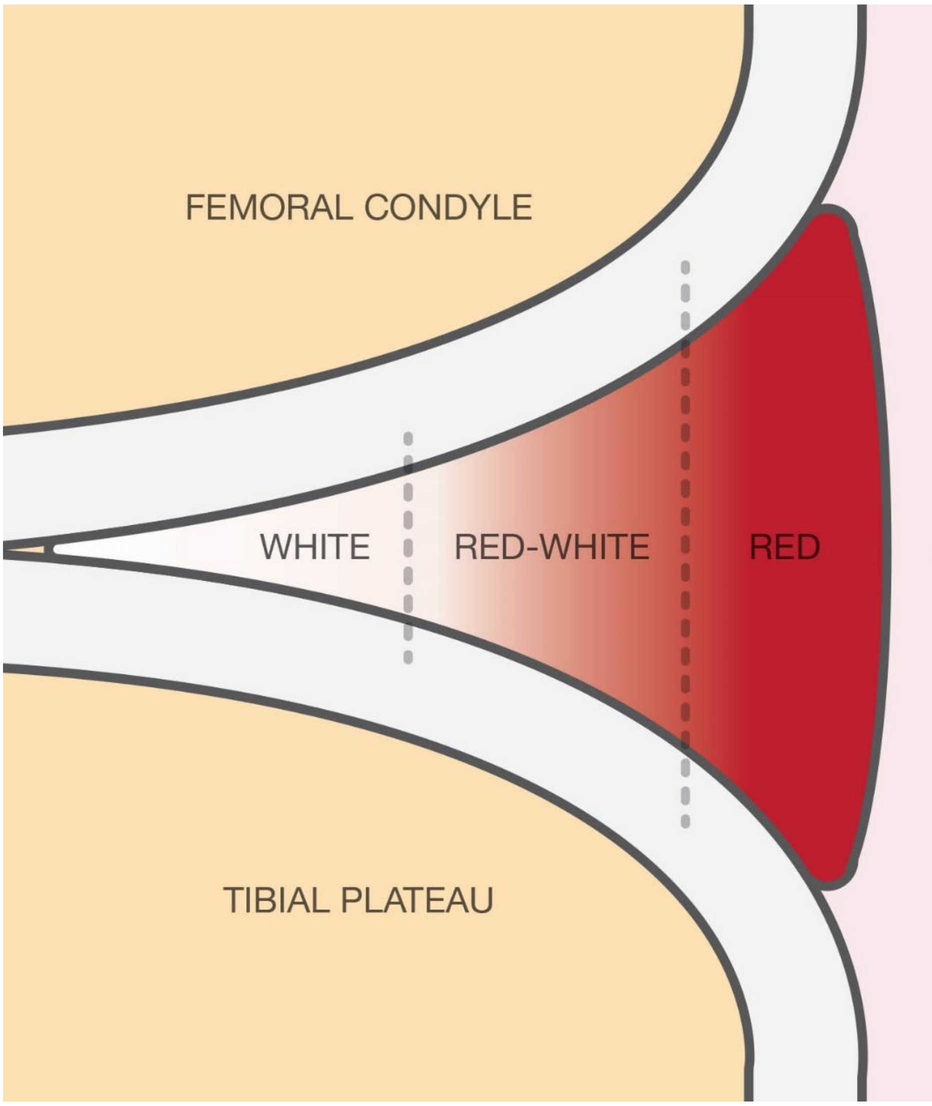


Figure 2-4 - Vascular zones of the meniscus

2.2.2.6 Innervation

Several different neurological receptors have been identified within meniscal tissue. These include free nerve endings [33] as well as mechanoreceptors such as Ruffini corpuscles, Pacinian corpuscles and Golgi organs [34]. These receptors are concentrated in the vascularised area of the meniscus, often associated with blood vessels [35]. It is suggested that this concentration allows stimulation of these receptors to occur in situations where there is significant pressure on the peripheral part of the meniscus, indicating the need for alteration of joint position to prevent injury [36]. Electrical stimulation of the medial meniscal posterior horn has been found to correlate with cortical evoked potentials during knee arthroscopy [37]. Furthermore, this stimulation was found to provoke contraction of the semimembranosus muscle in a reflex arc. The function of this arc is unclear. Multiple other roles have been suggested for these receptors, including proprioception and slow pain conduction [38]. Indeed, some have suggested that the pain relief seen after meniscectomy may be secondary to a denervation effect of removing these nerve endings [33].

2.2.2.7 Histology

The menisci are composed of largely of collagen, interspersed with fibrochondrocytes. These are of two further subtypes – fusiform and ovoid cells. These cells maintain the collagenous extracellular matrix.

Water/electrolyte content of the meniscus is estimated at 74%, the remaining dry content is 75% collagen. Type I collagen is predominant, though this is thought to decrease as osteoarthritic change occurs [39]. These collagen fibres are oriented circumferentially in deep layers of the meniscus, parallel to the meniscal border. This arrangement allows generation of circumferential hoop stresses when the meniscus is exposed to tensile stress as discussed below. More superficially fibres are oriented radially, interweaving between the circumferential fibres to provide structural support and enveloping fibres in the deeper zones [14].

Proteoglycans in the meniscus aid resistance against compressive loading of the meniscus. These large proteins are immobilised in the menisci by the aforementioned collagen fibres [40] and are thought to aid fluid distribution. No detailed study of their function in preserving meniscal stiffness was identified.

Ling and Levenston [41] used optical coherence tomography and histological techniques to identify a distinct surface layer in bovine menisci between 75-145μm thick. This layer was thicker in the centre of the menisci. Other work has suggested that the biomechanical properties of the surface layer of the meniscus differ from the deeper layers. Hence meniscal histology is variable in depth as well as position.

2.2.3 Meniscal injury

Meniscal injury, through tearing of the fibrocartilage tissue is thought to have an incidence of 8.27 per 1000 person years [42]. It is more common in men and incidence increases with age. Younger patients tend to suffer ‘traumatic’ tears – often twisting injuries landing awkwardly (i.e. loading) on a flexed knee. Sportspeople requiring pivoting/slicing movements are particularly at risk. In patients >40 years old, tears tend to be ‘degenerative.’ These are thought to be a reflection of the meniscus losing elasticity and becoming friable with age [43]. These can occur with in relatively benign situations, such as squatting.

Meniscal tears can cause symptoms such as pain, swelling, locking, and giving way of the joint [44]. Investigation is usually performed through MRI scanning; which has a sensitivity and specificity of between 70-80%. It is also useful in excluding other pathology. Definitive diagnosis is made at arthroscopy. Treatment can be conservative, or it may take the form of partial/total meniscectomy, meniscal repair or reconstruction. It should be noted that in patients with arthritis, the evidence for undertaking meniscal debridement where mechanical symptoms such as locking and giving way are not present is limited [45].

Meniscal tears vary in shape, location and size. There is some suggestion that the morphology of the knee joint can be associated with certain types of tear, with an increased size ratio of the femoral to tibial condyles medially thought to predispose to root tears in this location [46]. There are various classification systems. The O’Connor classification is often used for description [5]:

- Longitudinal/Vertical tears (I in Figure 2-5) – Usually post-traumatic and vertically oriented, the tear is parallel to the edge of the meniscus. If large, it can form a 'bucket handle' tear (see Figure 2-6). If occurring in the more vascular part of the meniscus (red-red or red-white zones – see Section 2.2.2.5), these tears are amenable to repair.

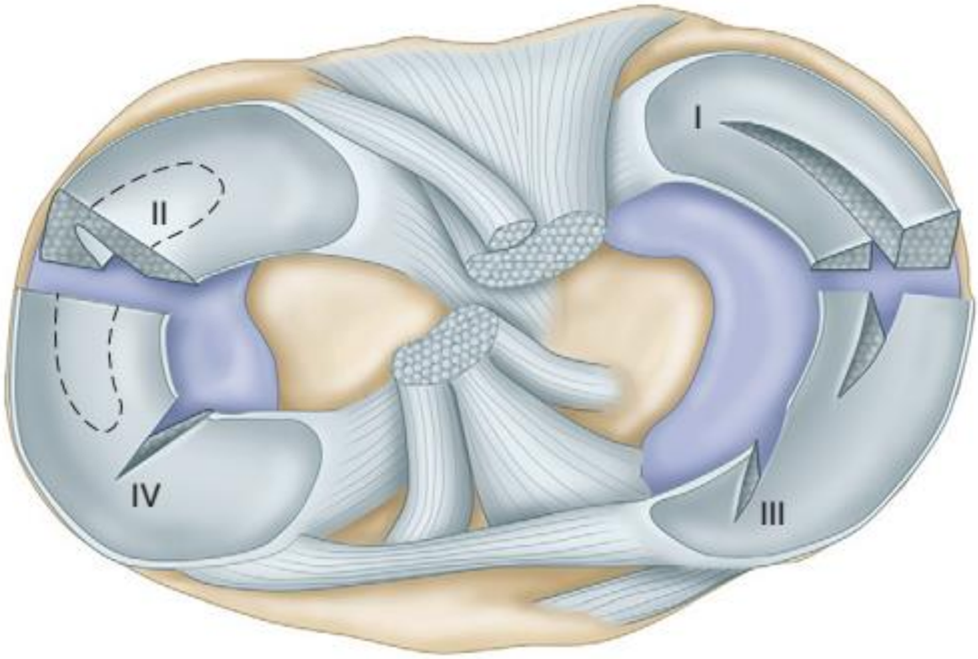


Figure 2-5 - Types of Meniscal tear[5]

- Horizontal tears (II in Figure 2-5) – These are more commonly degenerate in nature, it is thought that shear stresses lead to delamination between the superior and inferior surfaces of the meniscus. They can lead to more complex tears often requiring partial meniscectomy. If asymptomatic, these tears are often managed conservatively. Indeed, a study of the prevalence of meniscal tears found that horizontal tears were most often asymptomatic, whilst other types of tears were more likely to cause symptoms [47]. Horizontal tears have been found to widen and deform during knee flexion and recent work suggests that larger tears correlate with inability to perform activities of daily living and worse patient reported outcomes [48].
- Oblique tears (III in Figure 2-5) – These run obliquely from the inner edge into the meniscal body. A variation occurs in a flap tear, which has a

horizontal element also, resulting in a flap of tissue detached from the superior or inferior meniscal surface.

- Radial tears (IV in Figure 2-5) – These tears run from the inner edge directly to the periphery and can be complete, resulting in transection of the meniscus. Radial tears of the posterior medial meniscus have been found to be associated [49] with osteoarthritis and cartilage loss.
- Complex tears - Contain elements of the above and are often degenerate in nature.
- Root tears – Tears of the meniscal root, where the meniscus attaches to the tibial surface, are thought to result in effective defunctioning of the meniscus.

It has been demonstrated that meniscal tear patterns differ between patients with stable and unstable (ACL deficient) knees with peripheral tears occurring more readily in unstable knees [50]. Furthermore, horizontal and complex tears are associated with concomitant cartilage damage [51], though it is unclear whether either observation can be deemed a cause or effect. Other authors [52] have used logistic regression techniques to show meniscal damage being independently associated with cartilage loss. In unstable knees, most surgeons will perform stabilisation of the knee prior to or alongside a meniscal repair. This is thought to afford protection to the meniscal repair. Notably, if a stable meniscal tear is encountered during surgery, there is evidence to suggest that conservative management of such injuries does not have a deleterious effect on patient outcomes [53].

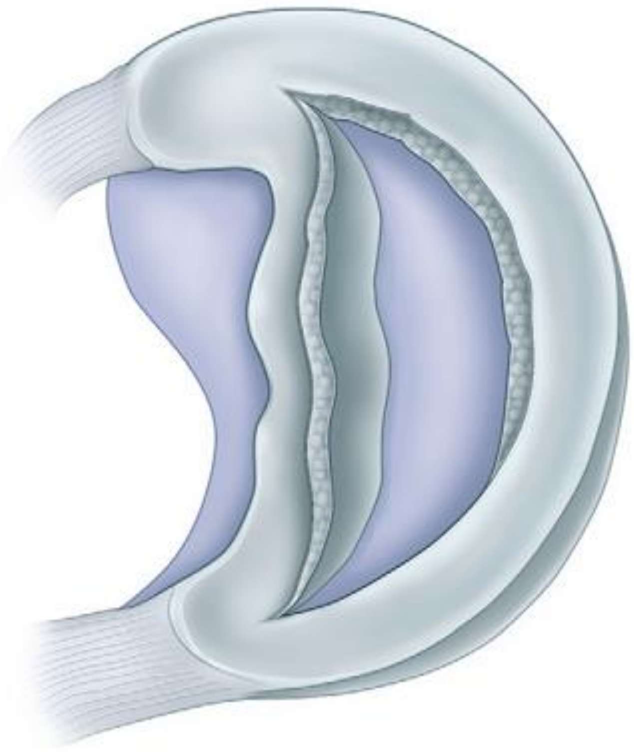


Figure 2-6 - A bucket handle tear[5]

2.2.4 Function of the menisci

2.2.4.1 Load transmission

King initially proposed a role for the menisci in load transmission through study of canine specimens [54]. Shrive [55] later conducted experiments in human and porcine knees by loading them using an Instron materials testing machine and measuring load deflection curves with both intact and divided menisci. He suggested that the menisci carried 40-60% of the load at the knee joint. In a contemporaneous study, Seedhom et al. [56,57] used a similar technique to suggest that the lateral meniscus carried 70% of the load in the lateral compartment whilst the medial meniscus carried 50% of the load in the medial compartment. Shrive et al. provided further evidence for these claims in a later study [58].

In absolute terms, the knee joint is thought to carry between 2-4 times body weight during normal gait. An average value of 3.03 times body weight has been measured in 12 subjects [59]. More recent work using a force platform has suggested a greater tibiofemoral compressive force of an average 3.9 times body weight for level walking. Downhill walking was found to increase the force transmitted to 8 times body weight [60]. Tibiofemoral joint forces during squatting have been estimated at between 4.7 and 5.6 times body weight [61].

2.2.4.1.1 The meniscus in osteoarthritis

In the early 1900s, clinical practice supported routine removal of the meniscus if it was thought to be causing symptoms [62]. Furthermore, it was suggested that a total meniscectomy was more likely to allow full regeneration than a partial resection and hence total meniscectomy was prescribed for any meniscal symptoms [63]. It was not till Fairbank's [4] seminal paper observing osteoarthritic radiological changes in post meniscectomy knees that a load bearing function for the meniscus was widely accepted. A large number of studies were subsequently undertaken to provide more evidence for this. Allen et al. [64] demonstrated worsening radiological arthritis in 231 meniscectomised knees between 10 and 22 years following surgery, finding a significant increase in both radiological and clinical arthritis compared to normal knees. 6.9% of patients reported clinical symptoms whilst 18.3% had radiological arthritis. Similar results were reported by Hulet et al. [65], who studied 74 partial meniscectomies in 57 patients over 12 years. They found radiological osteoarthritis to be present in 16% of patients where the opposite knee was radiologically normal. Factors such as age or cartilage damage at the time of initial surgery did not correlate with radiographic osteoarthritis. Importantly, radiological arthritis also did not correlate with clinical findings, with all patients achieving normal or near normal function and 95% satisfied with the outcome of their surgery.

Burks et al. [66] conducted a larger retrospective analysis of 146 patients an average 14.7 years following partial meniscectomy with the opposite knee used as a control. They used a subjective 0-IV grading system and found that knees undergoing partial meniscectomy were grade 0.23 worse than normal opposite knees. They did not report whether a significant difference was present between groups. Interestingly, this difference was more pronounced for varus knees undergoing medial meniscectomy (again suggesting a load transmission role for the meniscus). Hulet et al. [67] have published a further more recent study, with 22 year follow up of 89 patients undergoing only lateral meniscectomies. This study showed a 44% higher prevalence of radiographic osteoarthritis. Using logistic regression techniques, they suggested that factors including BMI >30, degenerative cartilage lesions present at index procedure and age >38 were predictive of osteoarthritis (though it might be argued that the latter is in fact a confounding factor). The authors do not note whether they factored in pre-existing chondral damage in their regression analysis but do note that patients with chondral damage were more likely to be symptomatic. On this occasion, significantly more knees were found to be in valgus than varus. They did not attempt to correlate degree of radiographic osteoarthritis with observed symptoms.

Observations regarding an increased risk of osteoarthritis post meniscectomy have been reported by numerous other authors [68–74].

It should be noted that none of these studies use standardised methodology for their radiological assessment. Some have suggested measuring joint space in millimetres, but do not note correction for magnification or calibration of their measurements using templating markers. Furthermore, it is broadly understood that radiographic change is not always reflective of the patients' symptoms, which ultimately determines whether any medical or surgical intervention is necessary. Nevertheless, these works suggest that significant meniscal injury is likely to accelerate the development of osteoarthritis.

2.2.4.1.2 The effects of meniscal injury on load transmission

The effects of meniscal injury on load transmission have been investigated in vitro by several groups. Pressure gradients and contact areas across the joint have been recorded in a number of meniscal injury states in an effort to determine the potential effects of such injuries on the load transmission to articular cartilage.

Ihn et al. [8] used pressure sensitive film to record contact areas and pressures in five cadaveric knees at full extension between 30-300 kg (294.2N to 2942N) of load (one knee was subsequently excluded). Two specimens underwent partial and total medial meniscectomies, the other two underwent the same procedure on the lateral side. Both then underwent bilateral total meniscectomies. In the intact knee, the authors found a mean contact area of 6.1cm^2 medially and 4.5cm^2 laterally. Focal areas of pressure loading were not seen with an intact medial meniscus even at 300kg of load, though on the lateral side stress concentration began to occur at 60kg. Progressive meniscal injury was associated with decreased contact areas and stress concentration. Of note, total meniscectomy unilaterally resulted in pressure increases on the opposite (normal side). Bilateral total meniscectomy resulted in 3x the load concentration seen with intact menisci.

Arno et al. [9] used pressure sensors to record contact area and pressure in knees undergoing arthroscopic horizontal tears in the posterior medial meniscus. Tears extended across ~55% of the width and 59% of the length of the posterior horn. A custom rig was used to apply compression, shear and allowed a range of motion from 0-135 degrees. Testing states included:

- 500N compressive force
- 500N with
    - 100N posterior shear
    - 100N anterior shear
    - 2.5Nm external rotation
    - 2.5Nm internal rotation

These were intended to recreate in vivo conditions such as walking, stair climbing, squatting and rising from a chair. Their results showed statistically significant increases in peak contact pressure for all conditions tests, except for 500N compression with 2.5Nm external rotation. These peak pressures most often occurred in the uncovered and central regions of the tibial plateau. Contact area decreases were statistically significant only for 500N compression and 500N compression with

2.5Nm internal rotation. The greatest increase in contact pressures was found to occur between 45-90 degrees of flexion. Again, the authors suggest that these changes in contact mechanics may be responsible for development of early osteoarthritis. However, they also note that the magnitude of pressure increase was lower than has been reported for other types of tear.

Bedi et al. [75] used a knee joint simulator in 6 cadaveric knees to investigate the effect of posterolateral tears of the lateral meniscus. The knee joint simulator aimed to recreate the gait cycle. Measurements were taken at 14% and 45% through the gait cycle, as maximal pressure on the tibial plateau was thought to occur at these points. Tekscan pressure scanners were used to monitor contact area and pressure across the articular surface – in common with other authors, these sensors were sutured in place beneath the menisci. Conditions modelled the following tears of the posterior lateral meniscus, created at the level of the popliteal hiatus:

- 30/60/90% meniscal width (measured with a digital caliper)
- Meniscal repair with sutures
- Partial meniscectomy in the 90% tear group.

No significant differences in contact pressure were found at 14% of the gait cycle. At 45%, peak contact pressures were statistically significantly higher at the lateral tibial plateau for 90% tears and partial meniscectomy versus all other states. Repair resulted in a decrease in peak contact pressure, though the difference between the resultant pressure and the contact pressure in the normal knee was not statistically significant. When considering contact area, statistically significant decreases were seen in all meniscectomy states at both stages of the gait cycle. Repair did not restore contact area. Notably, the authors removed all soft tissues except ligaments and capsule, which may influence kinematic measurements and result in increased instability of the knee. In addition, they performed a lateral epicondylar osteotomy to allow access to the joint to perform their meniscectomies – this too may have impacted their results. Furthermore, only normal gait was modelled.

The same group conducted an almost identical study of the medial meniscus [76], performing a medial femoral epicondylar osteotomy on this occasion. On this occasion, eight knees were used (a power calculation was performed, and this number would allow detection of a 25% increase in peak contact pressure). Tears were created as described above at the junction between the posterior horn and meniscal body. Despite tears of up to 90% of the radial width of the meniscus, statistically significant changes in contact area and pressures were seen only when a partial meniscectomy was performed. However, a 90% radial tear did cause peak contact pressure to move to a more posterocentral location. The authors highlight that despite a large tear, their study suggests the medial meniscus does still function in aiding load transmission and hence treatment should focus on meniscal preservation if possible. Zhang et al. [77] also investigated posterior horn medial meniscus tears in 6 cadaveric knees, applying 1000N load at 0/8/15/30 degrees of flexion. Again, statistically significant changes in peak pressures were demonstrated only with partial meniscectomy.

Similar work was undertaken by Lee et al. [78], who used 12 cadaveric knees and performed sequential medial meniscectomies. These were of 50% and 75% of meniscal width, followed by a segmental meniscectomy of the posterior horn and finally a total meniscectomy. Pressure sensors (Tekscan) were used to record contact area and contact pressures. The knee joint was loaded at 1800N for each state. Testing was performed at 0/30 and 60 degrees of flexion. The authors found that each meniscectomy state decreased contact area significantly. All states also increased both peak and mean contact stress significantly, though the difference between a segmental and total meniscectomy was not significant. Notably, the authors only performed a single measurement at each state. Furthermore, the jig used to mount the cadaver restricted motion in the varus/valgus plane – which may have resulted in abnormal loading of the tibial plateau.

Medial meniscal root tears were investigated by Kim et al. [79] in 10 cadaveric knees using a pressure sensor placed submeniscally. Knees were tested at 300N of axial load at 0/30/60/90 degrees of flexion. Meniscal root tear and repair was undertaken before a complete meniscectomy and subsequent re-transplantation. No statistically significant differences were seen in contact pressures at 0 degrees, however at both 30 and 60 degrees, significant increases in contact pressures were seen in all states. Notably, root repair or re-transplantation did not restore the mean contact pressures to normal. Statistically significant decreases in contact area were seen at all flexion states following total meniscectomy. These were restored by re-transplantation. This study can be criticised for using a sub-physiological axial load in their testing. Furthermore, all testing was performed on a single cadaveric specimen, hence reproducibility of the results is unclear.

Allaire et al. [80] used 9 cadaveric knees and again measured load using sub meniscal pressure sensors. Flexion was tested at 0/30/60/90 degrees. Conditions tested were intact meniscus, posterior root tear, repaired posterior root tear and total medial meniscectomy. A markedly higher axial load of 1000N was applied in all testing states. Here, the authors found statistically significant increases in peak contact pressures (up to 25%). Once more, this was not evident at 0 degrees, but could be measured in all other flexion states. They also noted no difference in peak contact pressures recorded following a root tear and those found in total meniscectomy. Repair was found to restore contact pressures to normal. Once more, this group also found that a total medial meniscectomy resulted in a significant increase in peak contact pressures in the *lateral* compartment. The authors suggest that this effect is secondary to the kinematic instability resulting from posterior medial meniscal injury as explained below (see 2.2.4.4 Stability). Contact area was only significantly decreased by total meniscectomy. The study is one of few which aims to use a minimally invasive approach to the cadaveric knee – performing (<1cm) small arthrotomies and preserving structures such as the knee capsule. However, meniscal repairs were performed using an open technique.

Padalecki et al. [81] conducted a further study, this time exploring radial tears adjacent to the medial meniscal root. 9 conditions were modelled:

- Intact
- Root avulsion
- Root repair

- Radial tear at 3mm/6mm/9mm from root attachment site
- Repair at all 3 sites

6 cadaveric knees were testing under 1000N axial load at flexion angles of 0/30/45/60/90 degrees. Pressure sensors were placed submeniscally to measure contact area and contact pressures. A medial femoral condyle osteotomy was performed to allow access to the joint. Repairs were performed with transosseus sutures tied over a button. The authors found that all meniscal injury conditions significantly decreased contact area and that this decrease was restored to normal (i.e. no significant difference) following repair. Similar findings were evident when contact pressures were measured, except that contact pressures were not restored to normal by repair of the 3mm tear at 30 degrees of flexion and the 9mm tear at 60 degrees of flexion. No differences were observed in the contact area or pressures observed in the lateral compartment.

A further study of medial meniscal root avulsions was conducted by Marzo and Gurske-DePerio [82]. 8 cadaveric knees were studied, with soft tissues as well as the patella removed. Testing was conducted in full extension with 1800N of load using Tekscan pressure sensors. Testing states were the intact knee, a posterior medial meniscus root avulsion and subsequent transosseus repair. The authors demonstrated significant increases in peak contact pressure and a significant decrease in contact area following root avulsion. Both these parameters were restored by repair. No changes were observed in the lateral compartment. Notably, the authors also observed meniscal extrusion with load in the root avulsion state.

Radial and vertical tears of the medial meniscus were also explored by Muriuki et al. [83]. A custom testing apparatus was used to study 11 cadaveric knees – 6 underwent radial tears and 5 underwent vertical tears. Subcutaneous tissues were removed, but the knee joint itself was approached through only four sub-meniscal arthrotomies. Meniscal tears were created arthroscopically with radial tears extending from the central portion of the meniscus to the peripheral third. Vertical tears were created along the peripheral third of the meniscus to involve the entire posterior horn and part of the meniscal root. Repairs were performed using arthroscopic technique.

1000N of axial load was applied. Range of movement tested included 0/30/60/90 degrees of flexion. Four conditions were tested, intact, medial meniscal tear, subsequent repair and total medial meniscectomy. Fuji pressure sensitive film was used to record contact area and pressure. Radial split tears were found not to cause significant changes in either contact pressure or area, whereas total meniscectomy causes significant changes in both. Tears were found to cause a trend towards increased pressure and decreased contact area, however, these results did not reach significance. However, total meniscectomies in this series causes neither significantly increased contact pressures nor decreased contact areas. This result differs significantly from the remainder of the literature and reasons for it are unclear. The authors suggest that the reason radial tears did not cause significant changes was due to the fact that they did not violate the outer third of the meniscus, allowing hoop stresses to be conducted. Notably, although the authors repaired these tears, they occur in the 'white-white' zone of the meniscus and have poor healing potential in the clinical setting in any case.

Meniscal root avulsions and subsequent repair were also explored by LaPrade et al. [84], who used pressure sensors in 6 cadaveric knees to record contact mechanics under 5 conditions. These were:

- Intact medial meniscus
- Posterior medial meniscal root tear
- Anatomical transtibial (i.e. transosseus) repair
- Non-anatomical repair – performed 5mm posteromedially to insertion site
- Root tear + ACL defunctioning

Soft tissues were again removed. 1000N of axial load was applied and knees were tested at 0, 30, 60 and 90 degrees. Non-anatomic medial meniscal root repair resulted in significantly lower contact area, higher peak contact pressure and higher mean contact pressure compared to an anatomic repair, with a 27% decrease on average across all flexion angles. Anatomic repair restored 85% of the normal contact area. As would be expected, meniscal root injured knees showed significantly decreased contact areas and higher mean contact pressures than intact states. Sectioning of the ACL caused increases in the absolute measured mean contact pressure and a decreased mean contact area, but these were not significant.

The same group undertook a similar study of the lateral meniscus [85], though in this case non-anatomic repair was not performed. Eight cadaveric knees were studied using pressure sensors. A lateral femoral condyle osteotomy was performed, and soft tissues were removed, as well as the patella. A custom jig was used to control knee movement – in particular, varus/valgus alignment was maintained to provide equal load in both compartments throughout the experiment. Conditions of the lateral meniscus explored included:

- Intact
- Footprint tear – created at widening between meniscal root and medial tibial eminence
- Root avulsion and repair
- Radial tear at 3mm from root and repair
- Radial tear at 6mm from root and repair

Repairs were performed using transosseus sutures in an open fashion. Flexion angles tested were 0/30/45/60/90 degrees and 1000N of axial load was applied. The authors found that mean contact area was significantly reduced by root avulsions and radial tears at 3/6mm at all angles but not by footprint tears. Repair restored contact area in the root avulsion state, but not in either of the radial tear states. Mean contact pressures were significantly increased in root avulsion and radial tears but not by footprint tears at all flexion states except 0 degrees. Repair restored contact pressures in all states at all angles. Similar results were seen with regards to peak contact pressures.

Vertical tears of the lateral meniscus were investigated by Goyal et al. [11], who measured peak contact pressures in the lateral compartment using pressure sensitive film placed submeniscally. Soft tissues around the joint were again removed. An Instron machine (Illinois Tool Works Inc, Norwood, Massachusetts, United States) was used for testing and axial loads were applied to mimic squatting. Testing was performed at 0/30 and 60 degrees of knee flexion. They tested the lateral meniscus in 5 states:

- intact
- short vertical tear
- extended vertical tear
- partial meniscectomy
- subtotal meniscectomy

Although no differences in contact pressure were noted at 0 degrees, there were statistically significant differences between partial/subtotal meniscectomy states and the intact or short/extended tear states at both 30 and 60 degrees of knee flexion.

Perez-Blanca et al. [86] explored injuries to the posterior root of the lateral meniscus in 8 cadaveric knees under 1000N at 0/30/60/90 degrees of flexion. They demonstrated that both posterior root injuries and lateral meniscectomy led to significant decreases in contact area and increases in both mean and peak contact stresses, though the magnitude of effect observed was larger following complete meniscectomy. Notably, repair resulted in normalisation of the changes observed in full extension, but the effectiveness of repair decreased with increasing knee flexion. The methodology can be criticised in that a formal arthrotomy was made, which was left open during the testing procedure. In addition, the sub meniscal placement of the sensors used may have led to alteration of meniscal function. Finally, removal of the quadriceps muscle during the testing protocol will have influenced the inherent stability of the knee – the authors note that they ‘prevented’ anteroposterior displacement of the knee – potentially leading to non-physiological loading conditions across the joint.

Horizontal cleavage tears of the posterior third of the medial meniscus were investigated by Koh et al. [87] who tested 12 cadaveric knees under 800N of load at 0 and 60 degrees flexion. Tekscan pressure sensors were used to measure contact area and pressure. Tear states investigated included a horizontal cleavage tear, repair of the tear, inferior leaf resection and resection of both inferior and superior leaves of the resection. Although the tear states and subsequent repairs did not significantly affect the outcomes measured, resection of the inferior leaf resulted in a significant decrease in contact area and increase in contact pressure. Resection of the superior leaf caused a further significant change in the outcomes measured. The study can be criticised for removing all soft tissue, along with the patella from the knees during testing. They also performed an osteotomy of the medial femoral condyle to allow meniscal injuries to be created. The authors suggest that such tears, if requiring surgical intervention, should in fact be treated through minimal resection. Notably, these injuries mostly occur in a middle-aged population and arthroscopies for partial meniscectomy are widely performed to undertake resections of these injuries, even when mechanical symptoms are not present. This practice has come under recent criticism [88].

Several criticisms can be made of all these studies. Firstly, nearly all make formal arthrotomies to access the joint and cause meniscal injury – which in itself may alter joint stability through damaging the soft tissues surrounding the knee joint. A significant proportion also make a condylar osteotomy, which is then reattached, hence potentially altering the bony or ligamentous anatomy. This has significant potential for altering joint stability through altering the tension, length or function of attached soft tissue structures. In addition, pressure sensors are placed submeniscally in all cases – necessitating some detachment of the meniscus from the tibial plateau to allow placement, which may have implications for the ability of the meniscus to transmit load. The degree of axial load varies markedly between studies and approaches physiological levels in only a few cases. The role of knee rotation is also only explored in one case. As with any in vitro model, these experiments are also unable to account for muscle forces across the joint.

Finite element modelling has also been used to investigate load distribution following meniscal injury. Atmaca et al. [89] constructed 11 finite element models using CT/MRI scans from volunteers. They modelled anterior, longitudinal and posterior meniscectomies, considering removal of 25/50/75 and 100% of tissues. Menisci were assumed to be a single phase linear elastic and isotropic, with an elastic modulus of 80mPa. Results were grouped into amount of meniscus removed across all regions. The stress/load distribution on tibial articular cartilage was then calculated, assuming a 1000N load across the mechanical axis of the lower limb and a further 500N load along the vector of the hip abductors. The authors found an increase in the stress/load distribution which was statistically significant across all types of meniscectomy. Their results are summarised in the table below (Table 2-1), all changes were statistically significant:

Percentage meniscus removed	**Percentage increase in stress/load distribution**
25%	78%
50%	177.9%
75%	473.8%
100%	752.6%

Table 2-1- Stress distribution following meniscal resection

Bae et al. [90] conducted a similar analysis from scans taken from a single patient. They explored the cartilage to cartilage contact area occurring with meniscal resection, showing increases in peak contact pressures with progressive meniscal resection. Interestingly, contact pressures in the lateral compartments also increased with medial meniscectomies.

The finite element analysis undertaken by Bao et al. [91] focussed on lateral meniscal posterior root tears, again finding increased contact pressures and decreased contact areas. Again, images were taken from a single volunteer. Menisci were modelled to be isotropic in the transverse plane, with linear elasticity. Once more, decreased contact areas and increased contact pressures in the affected compartment were predicted.

These finite element analysis studies have some limitations. Firstly, the menisci are modelled as isotropic structures, however, as explained above, this is not accurate. Furthermore, the biomechanical parameters used to model menisci differ from one study to another. As well as this, studies are based on scans taken from single or small numbers of volunteers, limiting the generalisability of the results produced.

There is also limited clinical study of the outcomes of specific tear types (though the effect of meniscectomy has been investigated as noted above). Krych et al. [92] explored outcomes in 52 patients with medial meniscal posterior root tears managed non operatively at a mean 62 month follow up. 31% of patients underwent total knee arthroplasty during the study period, whilst radiographic arthritis also significantly worsened in 87% of cases. Although this is an observational study in a small cohort, it adds further credence to the suggestion that significant meniscal injury predicts earlier osteoarthritis. This is perhaps particularly relevant in the case of root tears, which defunction the meniscus through inhibiting its ability to generate hoop stresses.

The table below summarises the literature in relation to meniscal injury and load transmission (Table 2-2). Studies are presented in chronological order. A graphical representation of the regions of the meniscus studied in relation to meniscal injury is shown in Figure 2-7 below. It appears that most of the studies in relation to this area are focussed on the posterior elements of the meniscus. Broadly speaking, it can be said that progressive meniscal injury leads to increases in contact pressure and decreases in contact areas. It is evident that there are areas which have not been explored in relation to the role of the meniscus in load transmission. Firstly, no studies have been performed with all soft tissues around the knee intact, using a fully arthroscopic technique to mimic the physiological state as closely as possible. Although loads of ~1000N (and up to nearly 3000N) have been investigated, as discussed previously, normal daily activities can cause loads of up to 5.5x body weight (~3750N for a 70kg body) to be transmitted through the knee joint. As well as this, the role of shear stresses on the joint has only been explored in a single study using a sub-physiological axial load. Figure 2-7 also highlights that large parts of the meniscus have only been studied for specific injury subtypes or not at all. For example, vertical tears have not been explored in the medial meniscus, nor are horizontal tears explored laterally. There has been no study of anterior injury (though clinically this has limited relevance), nor of bucket handle tears in either meniscus. Hence there are significant areas requiring further exploration.

Table 2-2-Summary of literature relating to meniscal load transmission

Authors	**Technique used**	**Meniscal region studied**	**Outcomes**	**Number of knees tested**	**Axial load applied**	**Flexion angle (degrees)**
Ihn et al., 1993 [8]	Pressure sensitive film	Partial and total medial or lateral meniscectomy	Progressive injury lead to decreased contact area and increased contact pressures	4	294.2-2942N	Full extension only
Lee et al., 2006 [78]	Tekscan pressure sensors	50/75% medial meniscal width resected, segmental meniscectomy posterior horn and total meniscectomy	Each state caused sig decrease in contact area and increase contact pressure.	12	1800N	0/30/60
Allaire et al., 2008 [80]	Fujifilm pressure sensors	Posterior root medial meniscus tear and repair + medial meniscectomy	Increased contact pressures at 30/60/90 with root tear restored by repair. Decreased contact	9	1000N	0/30/60/90

Authors	Technique used	Meniscal region studied	Outcomes	Number of knees tested	Axial load applied	Flexion angle (degrees)
			area with medial meniscectomy only.			
Marzo and Gurske-DePerio, 2009 [82]	Tekscan pressure sensors	Medial meniscal root avulsion	Increased peak contact pressures and decreased contact area with root avulsion, restored following repair	8	None	0 only
Bedi et al., 2010 [76]	Knee joint simulator recreating gait cycle. Tekscan pressure sensors	Radial tears posterior third medial meniscus	Only partial meniscectomy causes higher peak pressures and lower contact area.	8	ISO standard gait cycle	Results at 8 and 15 degrees of flexion – maximal axial force

Authors	Technique used	Meniscal region studied	Outcomes	Number of knees tested	Axial load applied	Flexion angle (degrees)
Bedi et al., 2012 [75]	Knee joint simulator recreating gait cycle. Tekscan pressure sensors.	Radial tears posterolateral meniscus	Increased contact pressures and reduced contact area at 45% gait cycle with 90% tear – meniscectomy caused same. Contact pressure but not contact area reduced by repair	6	ISO standard gait cycle	Results at 8 and 15 degrees of flexion – maximal axial force
Bae et al., 2012 [90]	Finite element analysis	Progressive resection of medial meniscus	Showed increased contact pressures in both compartments			
Arno et al., 2013 [9]	Tekscan pressure sensors	Medial meniscus posterior third horizontal tears	Increased peak contact stresses and decreased contact area	10	500N with ant/post shear or ext/int rotation	0-135 degrees

Authors	**Technique used**	**Meniscal region studied**	**Outcomes**	**Number of knees tested**	**Axial load applied**	**Flexion angle (degrees)**
Atmaca et al., 2013 [89]	Finite element analysis	25/50/75 and 100% resection of medial meniscus, grouped various tears into analysis	Statistically significant increase in stress/load distribution – all states			
Bao et al., 2013 [91]	Finite element analysis	Postero-lateral meniscal root tear	Decreased contact area & increased contact pressure in lateral compartment, some increase medial compartment			
Kim et al., 2013 [79]	Pressure sensor	Medial meniscal root tear	No changes in full extension, however at 30/60 degrees – significant increase contact pressure and decreased contact area	10	300N	0/30/60/90

Authors	Technique used	Meniscal region studied	Outcomes	Number of knees tested	Axial load applied	Flexion angle (degrees)
Padalecki et al., 2014 [81]	Pressure sensor	Medial meniscal root avulsion/repair and radial tear close to root attachment site	All meniscal injuries caused sig decrease in contact area and contact pressure. Repair restored contact areas but not all pressures	6	1000N	0/30/45/60/90 degrees
Goyal et al., 2014 [11]	Pressure sensitive film	Lateral meniscus – intact, short vert tear, extended vertical tear, partial meniscectomy and subtotal meniscectomy	Partial and subtotal meniscectomy states caused increased contact pressures	10	350N	0/30/60
LaPrade et al., 2014 [85]	Pressure sensors	Lateral meniscal footprint, root and radial tears 3/6mm from root	Root and radial tears decreases contact areas and increased contact pressures. Repair decreased pressures to non-	8	1000N	0/30/60/90

Authors	Technique used	Meniscal region studied	Outcomes	Number of knees tested	Axial load applied	Flexion angle (degrees)
			significant levels. Contact areas were restored by repair in root avulsions but not in radial tears.			
LaPrade et al., 2015 [81]	Pressure sensors	Medial meniscal root with anatomic/non-anatomic repair	Root tear caused decreased contact areas and increased pressures. Anatomical repairs were significantly less effective than non-anatomical repairs in restoring these parameters.	6	1000N	0/30/60/90
Zhang et al., 2015 [77]	Pressure sensors	Radial tears in posterior third medial meniscus, partial meniscectomy	Significant increase in peak contact pressures with partial meniscectomy.	6	1000N	0/8/15/30

Authors	Technique used	Meniscal region studied	Outcomes	Number of knees tested	Axial load applied	Flexion angle (degrees)
Koh et al. 2016 [87]	Pressure sensors	Horizontal cleavage tears in posterior third medial meniscus	Resection of the inferior lead of a horizontal cleavage tear resulted in sig increased contact pressure and decreased contact area.	12	800N	0/60
Perez-Blanca et al. 2016 [86]	Pressure sensors	Lateral meniscal posterior root detachment, lateral meniscectomy and repair	Root detachment and meniscectomy caused significant contact area and increased mean/maximum contact pressures across all angles tested.	8	1000N	0/30/60/90

Medial/lateral meniscal resection (partial or complete)

Ihn et al., 1993

Lee et al., 2006

Bae et al., 2012

Atmaca et al., 2013

Goyal et al., 2014 (also vert tears, see below right)

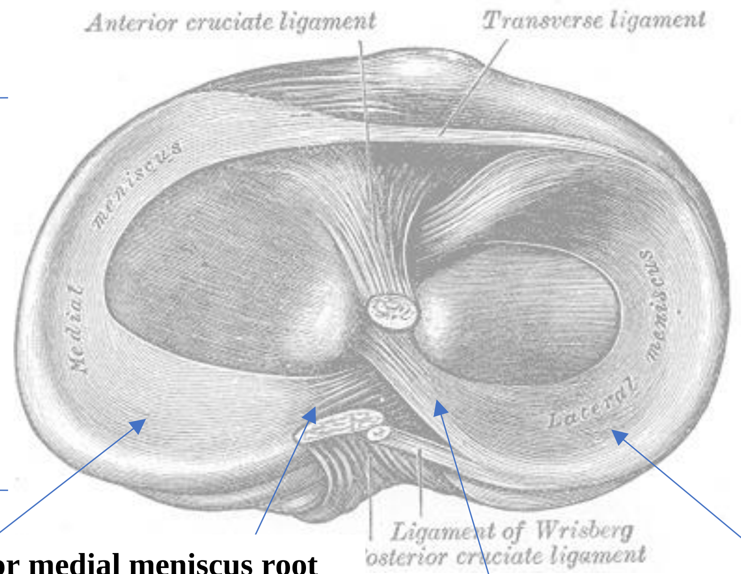


Figure 2-7 - Meniscal regions studied in relation to load transmission (Image from Gray's Anatomy[24])

Posterior medial meniscus root injuries

Allaire et al., 2008

Marzo & Gurske-DePerio, 2009

Kim et al., 2013

Padalecki et al., 2014

LaPrade et al., 2015

Posterior third medial meniscus

Bedi et al. 2010 – Radial tears

Zhang et al. (2015) – Radial tears

Arno et al. 2015 – Horizontal tears

Koh et al. 2016– Horizontal tears

Posterior lateral meniscus root injuries

Bao et al., 2013

LaPrade et al., 2014

Perez–Blanca et al., 2016

Posterior third lateral meniscus

Bedi et al., 2012 – radial tears

Goyal et al., 2014 – vertical tears

2.2.4.1.3 The meniscus in compression

Fithian et al. [14] suggest that meniscal tissue behaves in a biphasic manner similar to that described for articular cartilage by Mow et al. [93] in their ‘linear biphasic theory’ and notes the importance of creep and stress relaxation in meniscal function. He suggests that as the meniscus is loaded in compression, the creep response of the solid phase allows contact area to gradually increase with time – hence increasing contact surface area. Simultaneously in the fluid phase, fluid is extruded from the meniscus into the intra-articular space; this is subsequently reabsorbed when the load is removed. This mechanism may also aid meniscal nutrition as previously described, as well as lubricating the joint to minimise friction. In this model, stress relaxation through fluid distribution prevents high stresses in the collagen-proteoglycan solid phase for extended periods of time. Hence, the response of meniscal tissue can be summarised to occur in two phases: an elastic response of the solid phase and a stress relaxation response through the fluid phase. It is suggested that the proteoglycan component of the meniscus plays a role in regulating this fluid phase. Indeed, Subburaj et al. [94] performed MRI scans in runners, showing reduced T1 relaxation times in their medial menisci. This finding is correlated with an increase in proteoglycan content and the authors suggest that this reflects a relative increase in proteoglycan concentration due to fluid loss.

Furthermore, the proteoglycans carry a negative charge due to the dissociation of chondroitin sulphate and keratin sulphate groups when bathed in solution [15]. This fixed charge density, coupled with the relative immobility of these proteins, leads the tissue to act as a semi-permeable membrane to ionic solutions. A marked difference in the concentration of ions across the tissue boundary results due to the requirement to reach an electroneutral equilibrium. This, in turn, results in an osmotic pressure referred to as the Donnan osmotic pressure gradient [95]. The role of ionic effects in modulating the mechanical response of soft tissues has been investigated in articular cartilage [15] and intervertebral disc [96] with results suggesting that ionic effects are responsible for 98% and 70% of the equilibrium modulus respectively. To date, no such investigation of the role of proteoglycans in meniscal tissue has been undertaken.

Fithian et al. also highlight the importance of low permeability of the meniscus in load transmission. A number of studies have subsequently used the linear biphasic theory to explore the permeability of different parts of the meniscus.

The meniscus is also understood to be anisotropic in its stress response, depending on the location of the meniscal tissue sampled. Notably, the superficial layers of the meniscus do not display anisotropy, instead showing statistically similar steady state moduli (1.47-1.63 MPa) when studied using nanoindentation techniques. This holds true for samples from both femoral and tibial surfaces [97]. It is suggested that the surface layer is adapted to supporting compressive states.

However, studies using larger indentation apparatus show heterogeneity across the meniscal surface. Sweigart et al. [98] used micro level indentation with a 0.8-1mm indenter tip in a similar study. On this occasion, the anterior portion of the human meniscus was found to be stiffer as compared to central or posterior portions. However, they found no significant difference in the permeability coefficients across six different meniscal areas from both femoral and tibial surfaces.

Chia and Hull [99] conducted a further study using 2mm specimens from different parts of the medial meniscus using unconfined compression applied in axial and radial directions. They found, at an estimated physiological strain rate of 12%, that both axial and radial compressive moduli were increased compared to measurements at equilibrium. Moduli were again found to be higher in the anterior as compared to the posterior region of the meniscus.

The material properties of bovine menisci were examined by Proctor et al. [100] who took 14 circular specimens from 4 regions of the medial meniscus. They performed confined compression tests on 63 sectioned samples from these specimens, with samples taken from different meniscal regions, using the linear biphasic theory to determine a mean aggregate modulus across all samples of 0.41 +/- 0.088 MPa and a mean permeability coefficient of 0.81 (+/-0.45) x10^{-15} m^4/N-s. Statistically significant differences were found based on depth of samples (the authors do not quantify depth further than superficial/deep) and location. In particular, the authors noted that deep posterior meniscal samples had a higher modulus than deep anterior/central samples, an area which correlated with significantly higher water content than the anterior/central regions. This group also performed tensile testing, which is detailed below. As well as this, the intrinsic permeability of meniscal samples was determined by forcing Ringer's lactate at 0.17 MPa through a suspended tissue sample. A value of 0.64×10^{-15} m^4/N-s was found. The authors suggest that the proximity of the two permeability coefficients calculated suggests that creep of meniscal tissue in confined compression is due to fluid exudation.

Fischenich et al. [101] found that decreases in both instantaneous and equilibrium compressive moduli of meniscal tissue were observed in 24 macroscopically degenerate medial and lateral menisci. Notably, no change in tensile modulus was observed across any sample. The authors again suggest a role for GAGs – suggesting loss of GAGs in degenerate menisci leads to a fall in compressive moduli. Tensile moduli, maintained by collagen fibres, remain unaffected. It should be noted that the authors had only a small sample from each 'group' of degenerate menisci, with few numbers of 'normal' menisci – they were derived from osteoarthritic joints.

More recent work has demonstrated strain distributions to vary across 8 human medial menisci using an electronic speckle pattern interferometry technique [102]. Strain was measured in ten 5mm sections of meniscus. Aggregate strain was measured at 0.14% but varied from 0.03% to 0.7%. Elevated strain levels were seen in menisci derived from older donors and there was a statistically significant increase in shear and von Mises strain in the mid substance of older menisci. However, the authors sample size was small (only 4 per group in older/younger menisci). Furthermore, sectioning of the menisci would necessarily disrupt circumferential fibres and may alter the measurements taken.

Martin-Seitz et al. [103] used confined compression to measure tissue properties of medial and lateral human menisci. 150 samples were taken from the anterior horn, posterior horn and pars intermedia (boundary between anterior and posterior horns). Uniaxial confined compression was used to determine the behaviour of the tissue during stress relaxation. Strain was varied between 10-20%. The linear biphasic model was used to calculate aggregate moduli and permeability coefficients. Increasing strain was found to cause a fall in the aggregate modulus of between 3% (lateral) and 9% (medial). The lowest aggregate modulus was measured at the medial pars intermedia with the largest located at the lateral posterior horn. No difference was noted between menisci. Permeability was lowest for samples at the lateral posterior horn and highest for samples from the medial posterior horn, again with little difference between menisci. An increased strain did not influence permeability. The authors suggest that differences between their reported measures and other studies is likely secondary to differences in test conditions and mathematical models between groups.

Danso et al. [104] used a combined in vitro and finite element modelling approach to determine biomechanical properties of 13 medial and 13 lateral menisci. Indentation tests were conducted in different areas of the meniscus and the data derived used to develop finite element models. The authors predicted no difference in compressive moduli at any site in the lateral meniscus, whereas in the medial meniscus, significant differences were found between anterior, middle and posterior sampling sites. The instantaneous modulus of the anterior medial meniscus was ~200% that predicted in the lateral meniscus. Equilibrium moduli also differed between menisci at all sites. Permeability differed significantly between the middle and posterior medial meniscal sites, being lowest in the middle of the meniscus. The authors suggest that low permeability of the meniscus allows increased fluid support by preventing exudation under load and hence maintaining fluid pressure. An initial permeability across all samples of 0.08 $x10^{-15}$ m^4/Ns was determined. This is lower than described by other groups and the authors suggest this may relate factors in their model, especially because they included the role of collagen fibrils in aiding fluid pressurisation.

Studies of the properties of the meniscus in compression are markedly varied. Various authors have used markedly different techniques and studied different areas of the meniscus itself. This has resulted in a variety of results for the compressive properties of the meniscus, which are summarised in **Error! Reference source not**

found.. Studies are presented in chronological order. Regions of the meniscus studied are represented graphically in Figure 2-8. Several criticisms can be levelled at the studies presented. The large variety of techniques used makes it difficult to compare their results and likely explain the variation in the results obtained. However, results obtained are all of a similar order of magnitude, suggesting that the true value may well lie within the range described. As well as this, the theoretical model used for calculating the biomechanical properties of the meniscus was initially developed to describe articular cartilage and its utility in describing the meniscus is questionable. Also, no studies have explored whether the compressive properties of the meniscus vary in samples taken in a radial rather than anteroposterior direction. Further work may well explore these areas.

Table 2-3- Compressive loading of the meniscus

Authors	Technique used	Part of meniscus studied	Outcomes
Proctor et al., 1989 [100]	Confined compression	1mm discs from different regions of **bovine** medial meniscus	Aggregate modulus (MPa) 0.41 +/- 0.088 Permeability (m^4/N-s) 0.81(+/-0.45) x10^{-15}
Joshi et al., 1995 [105]	Confined compression	4mm cylinder from posterior medial meniscus	Aggregate modulus (MPa) ~0.21 (reported graphically) Permeability (m^4/Ns) 1.99(+/-0.79) x10^{-15}
Sweigart et al., 2004 [98]	1mm indenter, creep indentation	Anterior, central and posterior regions, femoral and tibial surfaces, medial meniscus	Aggregate (equilibrium) modulus (MPa) 0.09-0.16 Shear modulus (MPa) 0.05 – 0.08 Permeability (m^4/Ns)

Authors	Technique used	Part of meniscus studied	Outcomes
			1.32-2.74 $x10^{-15}$ across different regions (large confidence intervals)
Chia and Hull, 2008 [99]	Unconfined compression at varying degrees of strain	2mm cubic specimens from anterior, central and posterior regions medial meniscus	Equilibrium modulus (MPa) at 6% strain Axial 0.012-0.052 Radial 0.019 - 0.019
Moyer et al., 2012 [97]	Nanoindentation	**Superficial** meniscal surfaces, both menisci	Instantaneous elastic modulus 3.17-4.12 MPa Equilibrium elastic modulus 1.47-1.69 MPa
Kessler et al., 2015 [102]	Electronic speckle pattern interferometry	5mm sections of medial meniscus	Aggregate strain 0.14%

Authors	**Technique used**	**Part of meniscus studied**	**Outcomes**
Martin-Seitz et al., 2013 [103]	Confined compression	Anterior horn, posterior horn, pars intermedia of medial and lateral menisci	Equilibrium modulus (MPa) 0.06-0.07 (10% strain) Permeability (m^4/Ns) Lateral - 3.62 $x10^{-15}$ Medial - 4.24 $x10^{-15}$
Fischenich et al., 2015 [101]	1.59mm indenter, indentation/relaxation tests Tensile testing (see below)	1mm strips from anterior and posterior section of degenerate medial and lateral menisci	Instantaneous modulus (MPa) Lateral ~0.4 – 2 Medial ~0.5 – 1.8 Equilibrium modulus (MPa) Lateral ~0.04-0.27 Medial ~0.05 – 0.3
Danso et al., 2015 [104]	1.19mm indenter /finite element modelling	Anterior/ middle/ posterior samples from medial/lateral meniscus	Initial permeability (m^4/Ns) 0.08 $x10^{-15}$

Authors	Technique used	Part of meniscus studied	Outcomes
			Elastic moduli showed site specific variation in medial meniscus only (absolute values not reported)

Medial meniscus

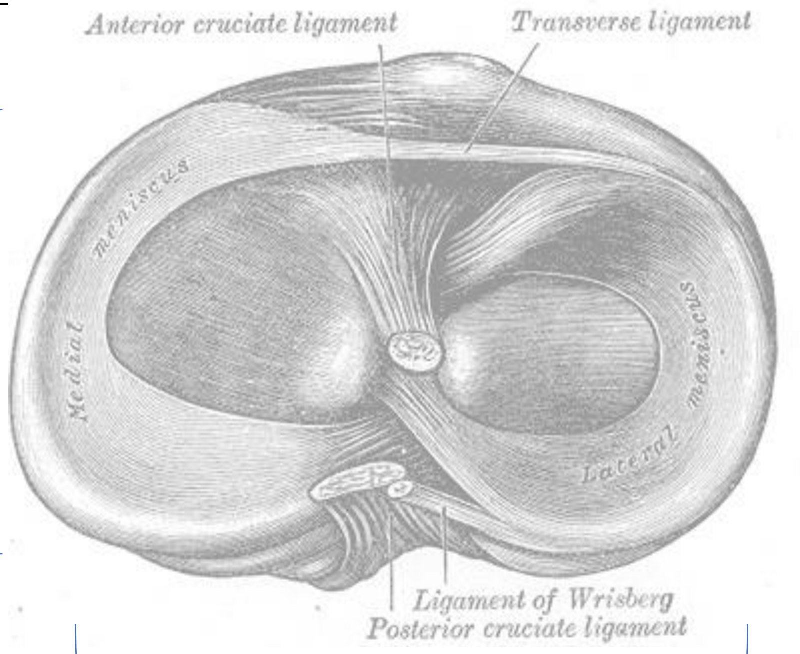
Proctor et al., 1989 – bovine

Joshi et al., 1995 – posterior

Sweigart et al., 2004 – anterior/central/posterior regions

Chia and Hull, 2008 - anterior/central/posterior regions

Kessler et al., 2015



Medial and lateral meniscus

Moyer et al., 2012 – superficial surfaces

Martin-Seitz et al., 2013

Fischenich et al., 2015

Danso et al., 2015

Figure 2-8 - Study of the meniscus in compression

2.2.4.1.4 Shear stress

As is the case elsewhere, the behaviour of the meniscus in shear stress is adapted for its function. When the meniscus is subjected to shear stress, the tissue is 25% stiffer when the plane of stress is perpendicular to the collagen fibre bundles than when it is parallel. Increasing shear strain causes a *decrease* in elastic stiffness and an increase in energy dissipation. This allows the meniscal tissue to conform to the applied stress, maximising joint congruity and allowing maximal range of motion [14].

Zhu et al. [106] studied shear stresses in bovine menisci at different levels of compressive strain. Oscillatory shear was applied. At low compressive strain (<10%), circumferentially oriented specimens were stiffer than axial and radial specimens. These differences were not present at higher strains. The authors suggested that responses to shear stresses in menisci are anisotropic and dependent on collagen fibre orientation.

There are no published studies of the behaviour of the human meniscus in response to shear stress.

2.2.4.1.5 Tensile stress

It is suggested that the macroscopic configuration and location of the menisci causes them to be extruded from the joint when it is loaded [107]. This extrusion is resisted by the firm attachments of the menisci to bone as described above, generating circumferential tensile (hoop) stresses in the parallel collagen fibres [14,108,109]. Changes in these tensile stresses have been measured using strain gauges placed in the anterior, middle and posterior sections of the medial menisci of 19 cadaveric knees by Jones et al. [110]. At three times body weight (the authors do not provide a numerical value), less strain was seen in the posterior section of the meniscus than the anterior/middle. Testing was performed in full extension and 30 degrees of flexion. A complete radial tear was found to defunction the meniscus – with no strain generated. Longitudinal tears in the anterior horn increased strain posteriorly. The authors suggest that the generation of hoop stresses is critical to meniscal function. Interestingly, study of patients with osteoarthritis notes disorganisation of the collagen matrix with concurrent subluxation/extrusion suggesting a loss of function of the circumferential fibres [111] and the ability to generate hoop stresses. Furthermore, radial displacement of menisci in knees with osteoarthritis has been observed on MRI [112,113]. Ghosh et al. [114] investigated the effect of hydrolysing proteoglycans in meniscal isolated, noting no change in stress strain relationships of the meniscus following this intervention. He suggests that proteoglycans do not play a role in resisting tensile stress.

In tensile stress, again, it is found that stiffness varies depending on the region from which the meniscal sample is taken. Fithian et al. [14] noted a greater tensile modulus in the anterior region of the meniscus as compared to central and posterior regions. However, these results were not replicated by other authors [115,116] in human and animal studies. Neither of these studies investigated the effect of samples taken from different radial locations nor the effect of different cross section sizes. These limitations were addressed by Lechner et al. [117] who applied tensile stresses to 0.5/1.5/3mm thick slices from anterior/central or posterior human meniscus. They demonstrated that although radial or circumferential location did not influence circumferential tensile modulus, samples taken from the anterior part of the meniscus showed a higher tensile modulus than central or posterior samples. The authors suggest that this may be due to decreased macroscopic width of the anterior meniscus as compared to the central/posterior regions. As the same number of collagen fibres would likely be present in each region, the higher concentration of fibres in the anterior region may translate to an increased tensile modulus. One would also expect higher tensile stresses in the peripheral areas with a correspondingly higher concentration of circumferential collagen fibres. It should be noted, however, that this group discarded the inner 2-3mm of tissue as it was felt to be too narrow to test, hence their study largely focussed on more peripheral parts of the meniscus. The same authors [117] also noted that the tensile modulus was inversely proportional to sample thickness with significant differences between their samples. Notably, they tested less than ten samples in each group.

Studying bovine medial menisci, Proctor et al. [100] conducted tensile stress testing of 400 micrometre thick samples from 6 menisci and tested with either circumferential or radial stress. Strain was determined using stereomicroscopy. The

Young’s modulus was noted to be significantly higher in circumferentially oriented samples. Furthermore, this modulus increased from surface to deep meniscus, before reducing at the base of the sample. This variation was statistically significant. Radial samples showed higher moduli in the surface samples, with smaller moduli recorded in the deep samples. As well as this, although surface samples were similar in terms of moduli across meniscal location, deep samples showed statistically significant variation dependent on location. In the deep meniscus, moduli in posterior samples were significantly greater than the anterior samples. The authors suggest that the surface of the meniscus acts homogenously in tension, but the deeper layers are specialised for functional load bearing and are anisotropic in their tensile response. They also suggest a mode for meniscal tears, suggesting that initial failure of a proportion of circumferential fibres due to increased stress or fibre degradation leads to additional load being placed on adjacent circumferential and radial fibres. This then leads to failure of radial fibres and/or circumferential fibres, eventually leading to various types of meniscal tear as described below.

LeRoux and Setton [118] conducted tensile testing and used biphasic finite element modelling of circumferential and radial samples from canine menisci to determine their tissue properties using a custom uniaxial testing system in increments of strain from 0.02 to 0.08. The finite element model was based on the biphasic mixture theory. Twenty menisci, both medial and lateral were obtained from 10 dogs. Young’s moduli were determined in plane with meniscal fibres and also transverse to them. Similarly, permeability coefficients were determined isotropic to and transverse to collagen fibres. Their results confirmed the meniscus acted anisotropically in tension. The ‘in plane’ modulus of circumferential samples was significantly higher than the ‘transverse’ modulus of radial samples. No significant difference in Poisson’s ratio was found across samples. 5 of the radially oriented samples were discarded as they showed negligible viscoelasticity and would not fit the finite element model. Permeability results were 0.089 +/- 0.077 x10^{-16} m^4/Ns in plane and 0.097+/-0.072 x10^{-16} m^4/Ns in the transverse plane. Coefficients were found to significantly decrease with increasing strain. The authors suggest that although the Young’s moduli appear to be vary anisotropically, the same is not true of the permeability coefficients. Notably, the values for the permeability coefficients measured in tension are an order of magnitude lower than those in compression. The authors query whether this difference is secondary to physical differences in sample dimensions or configurations but acknowledge that the underlying reason is unclear.

Table 2-4 below summarises the literature in relation to tensile stress of the meniscus. Table 2-5 allows comparison of the Young's moduli reported by different studies of human menisci. Studies are presented in chronological order. It would appear that the Young's modulus of circumferential samples is far higher than radial samples, supporting the theory of hoop stresses being generated in the meniscal collagen fibres. It also seems that the modulus varies with depth within the meniscus, though this has only been explored in a single study. There has only been a single (though comprehensive) study exploring radial moduli. Also, possibly due to friability of the tissue, it appears that the tensile moduli of the inner circumference of the meniscus. This area may be subject to the largest tensile stresses and further investigation is required into its role.

Table 2-4 - Tensile stress of meniscus samples

Author	Technique used	Part of meniscus studied	Outcomes
Proctor et al., 1989 [100]	Uniaxial elongation at 0.08mm/s	400 micrometre thick samples from anterior/posterior **bovine** medial meniscus – surface to deep	Young's modulus (MPa) Circumferential samples Surface – 48.3 Deep – 198.4 Base – 139 Radial samples Surface - 71.4 Deep - 2.8 Base - 4.6

Author	Technique used	Part of meniscus studied	Outcomes
Fithian et al., 1990 [14]	Uniaxial elongation at 3% per minute strain	Circumferential samples from anterior/central/posterior regions of both menisci	Young's modulus (MPa) Medial 93.18-159.58 Lateral 159.07-294.14
Tissakht and Ahmed, 1995 [115]	Uniaxial elongation at 5% strain/minute	Radial and circumferential samples from anterior/central/posterior regions divided into proximal/middle/distal layers, taken in both radial and circumferential directions from both menisci	Young's modulus (MPa) **Radial samples** *Lateral* Proximal – 12.93 Middle – 6.14 Distal – 15.83 *Medial* Proximal – 9.86

Author	Technique used	Part of meniscus studied	Outcomes
			Middle – 3.74 Distal – 16.21 **Circumferential samples** *Lateral* Proximal – 119.73 Middle – 83.83 Distal – 131.42 *Medial* Proximal – 88.61 Middle – 72.85 Distal – 87.50
Lechner et al., 2000 [117]	Uniaxial elongation at 0.006mm/s	0.5/1.5/3mm circumferential slices from anterior/central/posterior medial menisci	Young's modulus (MPa)

Author	Technique used	Part of meniscus studied	Outcomes
			0.5mm – 119.8 MPa 3mm – 49.8 MPa
LeRoux and Seton, 2002	Uniaxial elongation from 2% to 8% strain	Circumferential and radial samples from medial/lateral menisci of **canines**	Young's modulus (MPa) 67.8+/-27.9 circumferential, in plane 11.1+/-4.3 radial, transverse plane
Fischenich et al., 2015 [101]	Uniaxial elongation at 0.1mm/s	1mm circumferential strips from both medial and lateral menisci	Young's modulus (MPa) Lateral anterior – 128.8+/-62.5 Lateral posterior – 119.4+/-75.6

Author	Technique used	Part of meniscus studied	Outcomes
			Medial anterior – 112.5+/-56.6 Medial posterior 95.9+/-55.6

		Young's modulus (MPa)			
Meniscus	**Fibre orientation**	Fithian et al., 1990	Tissakht and Ahmed, 1995	Lechner et al., 2000	Fischenich et al.
	Circumferential	93.18-159.58	Anterior – 91.2 Central – 76.8 Posterior – 82.9	Anterior– 104.6 Central– 93.9 Posterior– 60.7	Anterior – 112.5 Posterior – 95.9
	Radial		Anterior – 6.6 Central – 10.5 Posterior – 12.7		
Lateral	*Circumferential*	159.07-294.14	Anterior – 108.3 Central – 103.6 Posterior – 123.1		Anterior – 128.8 Posterior – 119.4
	Radial		Anterior – 9 Central – 12.5 Posterior – 11.6		

Table 2-5 - Comparison of reported Young's moduli across human studies

2.2.4.2 Congruity

The menisci perform a ‘socket forming’ function in the knee [119]. As previously mentioned, the menisci act to improve congruity of the tibiofemoral joint surfaces. This is especially true in the lateral compartment, where both bearing surfaces are convex in shape. As the knee flexes, the anterior horns of the menisci move posteriorly to accommodate ‘rollback’ of the femoral condyles, whilst maintaining close contact with the femur anteriorly [107]. The lateral meniscus is more mobile than the medial, with the posterior portion of the medial meniscus being the least mobile (which may contribute to the frequency with which it is torn). It has been suggested that this function also has a role to play in minimising osteoarthritic change through cadaveric studies [120].

2.2.4.2.1 Meniscal extrusion

Meniscal extrusion is understood to have occurred when the meniscus protrudes beyond the tibial margin [113]. In such a situation, the ability of the meniscus to function is limited. However, MRI studies of 22 healthy volunteers [121] have demonstrated that a degree of meniscal extrusion can occur during normal flexion/extension, with extrusion occurring most commonly in the anterior horn of the medial meniscus during full extension, with corresponding posterior movement during flexion. The posterior horn of the medial meniscus was found to be least mobile, though it was found to commonly ‘lift off’ during extension. All menisci moved posteriorly during flexion. In the mediolateral plane, the medial meniscus was found to be extruded only in situations where the knee was extended, whilst the opposite was true of the lateral meniscus. Both meniscus moved laterally during flexion. It is likely therefore, that a degree of meniscal movement is normal in the asymptomatic knee and progressive extrusion occurs during normal loading of the knee [122]. However, knee pain has been independently correlated with meniscal extrusion, suggesting that extrusion may have a role to play in the arthritic process [123].

Interestingly, a 3D finite element analysis study suggests that the small cross sectional slope of the lateral meniscus (as compared to the medial meniscus), makes it less prone to extrusion [124]. As well as this, tears of the posterior horn of the medial meniscus have been associated with posteromedial extrusion of the medial meniscus [125]. Knee joint effusion has also been shown to be predictive of meniscal extrusion [126].

Notably, meniscal extrusion is also seen to occur both with and in the absence of meniscal tears [127]. Puig et al. [128] reviewed arthroscopies in knees with normal menisci and ‘non-advanced’ arthritis. In total 54 medial menisci and 83 lateral menisci in patients with an average age of 36.6 years were reviewed. MRI scans were performed in each case. The authors found extrusion in 37 medial menisci (68.5% of total), with mean extrusion 28% of total meniscal anteroposterior size. With regard to the lateral meniscus, 15 cases (18.8%) showed extrusion, mean 15% of total meniscal size. The authors question whether anatomical variation may predispose to meniscal extrusion, rather than it being a finding occurring secondary to traumatic pathology.

A further study by Rennie and Finlay [126] showed that meniscal extrusion was associated with meniscal tears, knee effusion and ACL injuries in professional athletes, but not in members of the general population. However, numbers in both groups were small – 40 knees in the athlete group and 36 knees in the control group. A study of 57 patients undergoing ACL reconstruction [129] found that extrusion of the lateral meniscus was associated with ACL reconstruction, with patients undergoing reconstruction exhibiting increased extrusion of the lateral meniscus which did not resolve following ligament reconstruction. This was not associated with any increased instability, measured using a KT-1000 dynamometer.

Injury to the PCL (in particular, its posteromedial bundle) has been found to increase radial displacement of the lateral meniscus in a cadaveric study at 0-90 degrees of knee flexion, under 200-1000N of load [130]. There was a trend towards increasing extrusion (>3mm) with increased load and flexion angles. This increased extrusion could potentially limit the lateral meniscus’ function in providing congruity. Notably, the authors admit to removing a 1.5 cm^2 x 1 cm^2 area of soft tissue at the lateral edge of the meniscus – it is unclear what this represents and what implication the removal of this tissue may have on meniscal extrusion. These results are also supplemented by Pearsall et al. [131], who demonstrated increased strain across both menisci following PCL injury using strain gauges placed in the anterior/posterior horns of both menisci, with a significant increase in strain seen at 60 and 90 degrees of flexion. This was observed to fall to non-significant levels following PCL reconstruction.

Of note, the recent ‘rediscovery’ of the anterolateral ligament [132] has prompted suggestion that it too may have a role in lateral meniscal stability. Recent work suggests that this ligament aids the ACL in controlling internal rotation, especially past flexion of 30 degrees [133].

Numerous other studies have linked meniscal extrusion to meniscal injury. A retrospective review [113] of 105 MRI scans with meniscal extrusion revealed the extent of extrusion to vary from 2-10mm. Degree of extrusion correlated with severity of degeneration. On MRI scan, longitudinal and horizontal tears were not associated with extrusion. Oblique tears predicted minor extrusion. Radial and complex tears were present with larger degrees of extrusion. Indeed radial tears have been associated with greater extrusion by other authors [134]. Finally, root tears were also associated with major extrusion, a finding which has been replicated elsewhere [135]. Furthermore, lateral meniscal root tears have been shown to correlate with lateral meniscal extrusion in a retrospective case control study of 412 patients with ACL injuries and concomitant lateral meniscal injuries [136].

More broadly, the multicentre osteoarthritis study [127] – a prospective MRI based observational study of 3026 subjects aged 50-79 years – showed correlation between medial meniscal tears (OR = 6.3), varus malalignment (OR = 1.3) and cartilage damage (OR = 1.8) and medial meniscal extrusion. In the lateral compartment, similar findings were evident also (meniscal tears OR = 10.3, valgus malalignment OR= 2.2 and cartilage damage OR=2.0). Hence, it may not be solely meniscal tears that predispose to meniscal extrusion. Interestingly, a smaller, study [137] of 687 knees in middle aged (50-60) women with a BMI >27 found that a diet and exercise programme reduced the odds ratio of meniscal extrusion progressing over the 2.5 year study period. Dietary changes were advised by dieticians whilst exercises were composed of low impact sports. Female sex has also been independently associated with increased meniscal body extrusion [138], with a further suggestion that an elevated body mass index may contribute to meniscal extrusion. Furthermore, a significant increase in medial meniscal extrusion (7.58mm vs 5.88mm) has been demonstrated to be associated with pain using ultrasound in 38 patients with knee osteoarthritis [123].

Finite element analysis based work has also demonstrates [124] correlation between mal-congruency of the articulating surfaces of the knee and meniscal extrusion, further suggesting that load transmitted through the meniscus is reduced in such a state.

In an MRI based study of patients with knee osteoarthritis, Allen et al. [139] found that meniscal tears predisposed to meniscal extrusion in a predictable manner. 161 patients were reviewed and those without knee osteoarthritis were compared with those that had radiological/symptomatic osteoarthritis. Both groups had similar rates of meniscal tears (including intrasubstance tears), though the osteoarthritis group had more meniscal maceration and more lateral meniscus tears. In the medial meniscus, medial and anterior extrusion was significantly associated with complex tears, cysts and meniscal maceration. In the lateral meniscus, a horizontal tear predicted lateral extrusion. The authors suggest that these conditions may predispose to earlier cartilage loss and subsequent osteoarthritis. There are, however, several criticisms in relation to this study. All subjects were female. The osteoarthritis and control groups had significantly different BMIs at baseline, which may have acted as a confounder. Furthermore, a few their findings are based on a single patient (for example, only a single patient had a horizontal tear which in turn caused lateral extrusion of the lateral meniscus). Finally, MRI scans were performed with the patient supine, hence the study fails to account for physiological loading of the meniscus. Indeed, meniscal extrusion has been demonstrated to be larger with the patient standing than in the supine position [140].

Nevertheless, there is evidence that suggests that meniscal extrusion does indeed predict symptomatic knee osteoarthritis. Serial MRI studies of 32 patients with knee osteoarthritis over 2 years were reviewed to identify degree of meniscal extrusion and cartilage volume change [141]. Notably 24 (75%) demonstrated some degree of meniscal injury. Although degree of meniscal extrusion did not progress or change over the study period, multilinear regression analysis demonstrated that extrusion of the middle/anterior horn of the medial meniscus was predictive of global cartilage volume loss and medial compartment cartilage loss at 2 years. Lateral meniscal middle/anterior horn extrusion also predicted cartilage loss at 2 years, though not at earlier time points. However, the study has a small number of participants and did not include a comparator group. The MRI scans were also not assessed in a blinded fashion. Similar techniques have been used to identify an association between medial meniscal extrusion and cartilage loss in the medial compartment [142,143] and the tibiofemoral compartment, with corresponding bone cyst formation and subchondral bone lesions [144].

Guess et al. [145] provide some insight into how meniscal extrusion may lead to earlier osteoarthritis by producing computational models based on gait analysis and MRI scans of two female volunteers. Their model suggested that varying horn laxity by +/-20% resulted in medical meniscal extrusion of 2.7-3.9mm and a decrease in force transmission through the meniscus from 51-8% in one subject and 36-14% in another.

It should be noted that the definition of meniscal extrusion does vary between studies. Most define it as extrusion beyond the tibial rim, grading it in millimetres. Some studies will measure extrusion as percentage of meniscus extruded. Furthermore, the MRI slices at which extrusion was measured also differ – some authors used the slice that had the largest volume of medial tibial spine visible [127], others used a ‘central slice of the medial tibia, 6mm posterior to the centre [142].’ This variation limits comparability of these works.

An in vitro study of meniscal extrusion used medial meniscal root avulsion to mimic meniscal injury in 7 cadaveric knees [146]. Linear variable differential transformers were used to measure meniscal displacement. Specimens were loaded to 1800N and measurements were taken in the intact state, following root avulsion and after meniscal repair. Medial displacement of the avulsed root medial meniscus was significantly greater than that observed in the intact or repaired state. Assuming a definition of >3mm protrusion would be termed ‘meniscal extrusion,’ this observation demonstrated that such injuries could in themselves cause meniscal extrusion. However, the study only studied cadavers in full extension. Furthermore, the linear variable differential transformers could only measure movement in one plane, hence any additional meniscal movement would not be captured.

It appears then, that meniscal extrusion is associated with both osteoarthritis and meniscal injury. It is unclear what role meniscal extrusion plays in the genesis of osteoarthritis. It could be purported that extrusion may defunction the meniscus, limiting its load transmission role and exposing the underlying cartilage to larger forces, leading to accelerated arthritis. However, there has been no correlation of meniscal extrusion with biomechanical parameters such as contact areas or pressures. Further study in this area will expand our understanding of what role extrusion plays.

2.2.4.3 Shock absorption

The role of the meniscus in shock absorption is debated. In 1976, Krause et al. [108] conducted force displacement testing of cadaveric human knees using an Instron materials testing machine. They calculated the energy absorbed in three states: intact knees, those which had undergone only medial meniscectomies and those with both medial and lateral meniscectomies, concluding that meniscectomised joints absorbed less energy that intact ones. This led them to suggest an energy absorption function for the meniscus. This study has been widely cited as evidence of a shock absorption role of the meniscus. However, Andrews et al. [147] have criticised this work for failing to account for the viscoelasticity of meniscal tissue in their data. This shortcoming was addressed by Kurosawa et al. [148], who conducted a similar experiment, but recorded both loading and unloading paths for their force displacement curves. They found that the total work done to cause deformation was higher in knees without menisci – that meniscectomised knees actually dissipated more, not less energy. Furthermore, the magnitude of force absorbed by the knee (in both intact and meniscectomised states) is thought to be sub-physiological [147]. The authors suggest the meniscus' role lies in stress distribution rather than shock absorption.

In an attempt to investigate a shock absorption role for the meniscus in vivo, Voloshin and Work [149] conducted a comparison of energy dissipation using accelerometers strapped to patients with intact, painful and meniscectomised knees. They demonstrated attenuation of vibration in the knee was decreased by 20% in meniscectomised knees. This difference was significant. The authors claimed this was evidence of a shock absorbing function for the meniscus, however, their results also showed a significant decrease in attenuation for painful knees. Hence, it is not possible to assert with any confidence that the lack of meniscus is responsible for this change.

Given the above findings, Andrews et al. [147] claim that the oft mentioned role of the meniscus as a shock absorber is, in fact, a fallacy.

2.2.4.4 Stability

The role for the meniscus in conferring stability to the knee is debated. Historically, no such role was recognised (hence the widespread use of meniscectomy). Subsequent work established the meniscus to act as a ‘secondary stabiliser’ of the joint – coming into play when other structures, in particular the anterior cruciate ligament, were injured. More recently, however, questions have been raised as to whether the meniscus may have a larger role to play.

In 1974, Wang et al. [150] explored the rotatory laxity of the knee using 27 cadaveric specimens mounted on a custom rig and subjected to load using an Instron machine. The custom rig allowed flexion from 0-75 degrees and translated load from the Instron machine to rotatory torque at 3.2 degrees/second. The authors aimed to determine primary laxity – ‘looseness in the joint before soft tissue restraint’ and secondary laxity – ‘a measure of the restraint provided by soft tissues at the extremes of rotatory motion.’ Laxity was measured in the intact state, following medial and lateral meniscectomies and following sectioning of individual ligaments. Axial load was also applied in increments of 25kg up to 100kg. The authors found marked differences between specimens and noted that double meniscectomy caused a 25% increase in rotatory laxity. Sectioning of the cruciates was found to cause a 23% increase in rotatory laxity whilst collateral injury caused a 49% increase. Furthermore, rotatory laxity was found to decrease with increasing axial load, falling to 20% of the unloaded value at 100kg.

This work was followed up by Markolf et al. [7], who conducted investigation into the structures contributing to stability of the knee joint using a custom testing apparatus on cadaveric knees. Various anatomical structures were sequentially removed or defunctioned to ascertain their function. His work suggested that the menisci contributed to both anteroposterior and varus/valgus stability if the knee was flexed and that the magnitude of this effect was small. However, the authors admitted to an error of ~20% in their testing apparatus. A further study [151] by the same group measured stability in 8 cadaveric knees in the intact state and following bilateral total meniscectomy. They measured anteroposterior and varus-valgus instability at 20 degrees of flexion, through forced tibial rotation and anteroposterior translation. Anteroposterior (AP) stability was decreased following meniscectomy with the knee in full extension, though this only occurred if axial applied load was increased to 925N. They also noted an increase in the varus/valgus laxity – though their data points showed significant scatter (and did not reach significance), thought to be due to repositioning of the cadaver in the apparatus for measuring. No changes in torsional laxity were found. However, some have reported increased incidence of varus/valgus instability following total meniscectomy compared to partial meniscectomy, suggesting that the meniscus may have more than a secondary role to play [152].

Chen et al. [153] evaluated the role of the menisci in stability of porcine knees in 4 medial meniscal states – intact, anterior horn resection, posterior horn resection and total meniscectomy. Results were recorded using a universal force/moment sensor and load applied using a robotic system. The authors measured anterior tibial translation (30/60 degrees of flexion) and internal/external rotation at 4 N.m of torque. They found that internal rotation significantly increases sequentially with increasing medial meniscal injury (except for anterior horn resection at 60 degrees). External rotation was increased significantly at 30 degrees flexion for all injury states except posterior horn resection. Differences at 60 degrees were not significant. The authors do not report the absolute magnitude of the rotational differences observed, though they do note that these were small and their effect may be subclinical. No difference in anterior tibial translation was noted. Notably, the authors did not apply any axial load to the knee joints.

Allaire et al. [80], in a study which also explored contact pressure/areas in posterior medial meniscal root tears, used a robotic system to measure the effect of such injuries on joint kinematics. Results were based on 6 cadaveric knees. Data was collected at 0/30/60/90 degrees of flexion but pooled for analysis. Their results suggested that there was a significant increase in external rotation and lateral translation of the tibia on the femur following root tears as compared with repaired or intact states. Interestingly, the authors suggest that the latter may be due to a 'buttress' effect of the intact medial meniscus against the medial portion of the medial femoral condyle. Trends towards varus alignment and anterior tibial translation were also seen with root injury. In a similar vein, the authors suggest that anterior translation coupled with loss of a stabiliser (in the medial meniscus) results in increased external rotation whilst varus alignment results from loss of the 'spacer' effect of the meniscus. The authors go on to suggest that a combination of these kinematic changes is responsible for the increase in *lateral* compartment pressures they identified following medial meniscal root injuries.

Arno et al. [12] performed arthroscopic partial medial meniscectomies in five human cadaveric knees. Sequential removal of 30%/60%/100% of the posterior third of the medial meniscus was performed. MRI scans were used to validate the percentage of meniscus removed (notably they found that less tissue was being removed than intended in the first two scenarios, with an average of 22% and 46% being removed). Fiducial markers were used in the tibia and femur to record movement and laxity testing was performed at 0/15/30/60 and 90 degrees of flexion. The knee was loaded with 500N of axial load during laxity testing, conditions tested included 50N of anterior/posterior shear, 2-N.m of internal and external rotation and 500N of anterior/posterior shear. Laxity was defined as 'the average difference in AP position for all flexion angles between anterior and posterior shear (deemed AP laxity) and between internal and external torque (deemed rotational laxity).' The authors found no significant difference in laxity or AP position of the medial femoral condyle at 22% resection. However, at 46% resection, AP position of the medial femoral condyle was significantly different compared to both no resection and 22% resection with the average AP position (for all conditions) being 3.28 +/- 1.67mm posterior than normal. Furthermore, at 46% resection rotational laxity under 50N compression was also statistically significantly different compared with the normal meniscus. All other measurements showed no difference. At 100% resection, AP translation remained significantly different. No significant differences in rotational laxity were observed at 100% resection. The authors suggest that intact collateral ligaments would have limited any rotational laxity in all states. The relevance of the result at 46% resection is unclear and may reflect the small number of knees studied. The authors also suggest that increasing load on the knee would limit joint laxity, which is reflected in their results – rotational laxity was lower when larger axial loads were applied. However, the AP translation of the femur noted may have significant clinical relevance in that it may lead to abnormal loading of the tibial plateau and early development of osteoarthritis. The 50N load applied by the authors is significantly sub physiological and these results have limited clinical relevance .

Shoemaker et al. [154] also studied the role of progressive removal of the menisci. This time testing was performed in 11 ACL deficient knees at 20 degrees of knee flexion. He suggested that anterior tibial translation increased by 10% in response to a 200N force following medial meniscectomy and that a lateral meniscectomy would increase translation a further 10%, both of which were statistically significant. In a contemporary paper, Levy et al. [155] conducted an in vitro study of cadaveric knees in the following states – intact, medial meniscectomy, ACL injury and following both medial meniscectomy and defunctioning of the ACL. They measured anteroposterior stability, with force applied through a custom testing apparatus. Nine specimens were used, and testing was performed at 0/30/60/90 degrees of flexion. Their results suggest that isolated injury to the medial meniscus did not affect AP stability of the knee, but that isolated sectioning of the ACL caused a statistically significant increase in anterior displacement of the knee joint. Combined injuries of both the ACL and medial meniscus allowed significantly larger anterior translation of the tibia compared to ACL injury alone. These findings have been further qualified by McCulloch et al. [156], who conducted a similar study in ACL deficient cadaveric knees to demonstrate that a medial meniscal root transection caused increased tibial translation compared to ACL deficiency alone – increased translation was not seen in ACL deficient knees that underwent partial or subtotal medial meniscectomy. Levy et al. undertook a further study [157], again using a custom testing apparatus in a study of 11 cadaveric knees. They reviewed tibial displacement on the femur before and after either lateral meniscectomy or sectioning of the ACL. Knees undergoing lateral meniscectomy underwent subsequent sectioning of the ACL and 2 such knees also underwent medial meniscectomy. Forces applied to the tibia were 100Nm of anteroposterior displacement, 6Nm of internal/external rotation and 20Nm of varus/valgus torque. Range of movement tested ranged from 0-90 degrees. The authors found that isolated lateral meniscectomy did not influence anterior translation. Sectioning of the ACL caused a significant increase in anterior tibial translation, which was mirrored by subsequent sectioning of the ACL in the lateral meniscectomy knees. A further small significant increase in anterior tibial translation (post ACL sectioning) was noted after medial meniscectomy. No differences in posterior translation were noted after any of the studied interventions. The authors suggest that the relative mobility of the lateral meniscus as compared to the medial prevent it from acting as a restraint to anterior tibial translation. Notably, none of this work was conducted with the knee under any load. Furthermore, there is no mention of the accuracy of the custom testing apparatus in measuring translation.

Ahn et al. [158]conducted a study of ten cadaveric knees, demonstrating that longitudinal tears of the posterior horn of the medial meniscus resulted in increased anterior tibial translation in ACL deficient knees at flexion angles up to 90 degrees (notably, increased translation was not evident at 90 degrees). Repair of the meniscal tear restored translation to the ACL deficient state. Notably, there was no difference between total meniscectomy and longitudinal tears of the medial meniscus. Hence, it is understood that the posterior horn of the medial meniscus acts to block anterior translation of the tibia on the femur in ACL deficient knees [43].

However, the medial meniscus is prone to injury in this situation [155,158], this is likely due to the increased meniscal shear stresses in this state, as it acts as a bumper against the femoral condyle. Indeed, increased force transmission through the medial meniscus has been demonstrated in ACL deficient knees using a universal force moment sensor [159]. This has been further localised by Markolf et al. [160], who demonstrated increased tensile stress in the posterior horn attachment of the medial meniscus following sectioning of the ACL during anterior tibial translation (0-90 degrees flexion, no axial load) and external rotation (0-50 degrees flexion, 500N axial load). They used a novel technique involving a load cell attached to a bone cap comprised of the posterior horn medial meniscal attachment, a technique which has not been validated elsewhere.

An interesting study by Shefelbine et al. [161], conducted MRI scans in 8 ACL deficient patients and 10 healthy controls. 125N of axial load was applied during scanning. The authors demonstrated the position of the femur to lie more posterior in ACL deficient knees during full extension. During flexion to 45 degrees, the ACL deficient knees translated 4.3mm anteriorly, no significant translation was seen in normal knees. No significant difference was noted in meniscal movement between the groups. The authors suggest this provides evidence for the menisci being more prone to injury in the ACL deficient state – as they are less mobile. Although the study is prone to interobserver variability in assessment of the MRI scans, utilises a sub-physiological load and has small numbers, it provides more evidence for the potential for meniscal injury in the context of ACL deficiency. Poor clinical [162] and laboratory [163] results have been reported in patients undergoing partial meniscectomy in the context of ACL deficiency and undertaking such a procedure without considering stabilisation of the knee is thought to be ill advised. As well as this, up to 50% increased strain has been measured in the menisci of cadaveric models following transection of the ACL – this is restored to normal by ACL grafting [164]. Interestingly, it seems the opposite may also hold true – increased strain has been measured in the ACL following medial meniscectomy; with strain reducing towards normal following allograft transplantation [165]. It may be that the role of the medial meniscus is not solely as a secondary stabiliser.

The role of the lateral meniscus has more recently been investigated by Musahl et al. [166] who conducted a mechanised pivot shift test on cadaveric knees – a test which uses combined axial load, valgus stress and flexion. The authors found that although simple anterior tibial translation of ACL deficient knees increased following medial meniscectomy, it had no effect on anterior tibial translation during the pivot shift test.

In fact, lateral meniscectomy increased the anterior tibial translation during the pivot shift manoeuvre. The authors suggest that the lateral meniscus may also act as a secondary restraint to anterior tibial translation when valgus strain is applied alongside axial loading. Similar results are reported in a subsequent study by Petrigliano et al [167].

However, these results are contradicted in a study conducted by Lorbach et al. [168] who used a universal robotic testing system to study 18 cadaveric knees. The robotic system was used to record knee kinematics in an intact state. A medial parapatellar approach was then utilised to create an ACL tear. This was followed by creation of a vertical bucket handle tear of the medial meniscus and subsequent repair of this tear. Kinematics were recorded at each stage with anterior tibial translation measured in response to a 134N load at 30/90 degrees of flexion. A pivot shift test was also mimicked by combined loads of 10Nm valgus torque and 4Nm internal rotation at 30 degrees of knee flexion. In common with several other studies, the authors found that medial meniscectomy increased anterior tibial translation significantly compared to ACL deficiency alone. Meniscal repair reduced this translation. The mechanised pivot shift test did not show increased tibial translation with ACL deficiency alone, but a significant increase was demonstrated when there was concurrent medial meniscal injury. Furthermore, partial medial meniscectomy resulted in similar results to those seen with meniscal tears in regard to both anterior tibial translation and the pivot shift test. The reason for the differing results between these studies likely relates to the manner in which the pivot shift test is performed – this is a complex clinical test which combines valgus strain, internal rotation and knee flexion. Furthermore, Lorbach’s study describes anterior tibial translation during the pivot shift occurring during full extension – this is not where one expects to see an abnormal result during the pivot shift manoeuvre. Rather, the pivot shift test seeks to observe tibial subluxation during flexion of the knee. It is quite likely that the two studies differed in their application of forces in this test and quite possible that neither accurately reproduces the test as conducted in a clinical setting.

A further study has tried to clarify this effect with regard to the lateral meniscus. Shybut et al. [10] performed a mechanised pivot shift test and measured anterior tibial translation on 8 cadaveric knees. Soft tissues were not removed from around the knee joint. Three-dimensional position of the tibia and femur were recorded using navigation software utilised in conventional total knee replacement surgery. The pivot shift test was simulated through a combination of internal rotation, valgus stress and tension on the iliotibial band. Anterior tibial translation was measured following application of 90N of anterior force. Arthroscopic injuries to the ACL and subsequently the lateral meniscal root were created. As expected, tibial translation during the pivot shift manoeuvre increased significantly following ACL injury. With additional injury to the lateral meniscal root, there was a further statistically significant increase in tibial translation compared to the ACL only injury state. However, this difference was not evident when simple anterior tibial translation was measured. The authors suggest that the lateral meniscus also plays a role as a secondary stabiliser of the knee and warrants repair in a similar fashion to the medial meniscus if encountered in the context of an ACL injury.

As part of a study investigating the effect of vertical tears of the lateral meniscus, Goyal et al. [11] investigated joint kinematics in five different injury states of the lateral meniscus – intact, short vertical tear, extended vertical tear, partial meniscectomy (all in posterior third) and subtotal meniscectomy. Skin and subcutaneous tissues including muscles around the knee joint were removed. The authors recreated muscle tone using a pulley system and applied a 350N load across the cadaveric knee joint. Fiducial markers were used to record kinematics, with a projected accuracy of 0.3mm and 0.3 degrees. The authors found no statistically significant difference in measurements including AP translation, lateral-medial translation, internal and external rotation or varus/valgus angulation at 0/30 and 60 degrees of knee flexion.

In a recent study exploring the combined role of the anterolateral ligament and injuries to the posterior root of the lateral meniscus, Lording et al. [133] applied either a 5 Nm internal rotation torque or a 90N anterior load in 16 cadaveric knees, all of whom had ACL injuries and underwent subsequent injury to either the lateral meniscal root or anterolateral ligament. No differences in anterior translation were noted in any of the injury states explored. Significant differences were noted in internal rotation when both the anterolateral ligament and posterior root of the lateral meniscus were injured in all flexion states. Comparing results between the anterolateral ligament injury and posterior root injury group, the authors suggest that the lateral meniscal root contributes to resisting internal rotation in the context of ACL injury when the knee is in full extension, whilst the anterolateral ligament plays a similar role past 30 degrees of flexion. Notably, the authors did not apply any axial load to their specimens.

Almost all these studies perform arthrotomies to create meniscal injury, which may result in altered capsular tension, in turn leading to a bias in the recorded results. Furthermore, the axial load applied varies significantly and no study has attempted to recreate a full range of physiological weight bearing conditions – particularly states such as squatting, pivoting or stair climbing. As well as this, flexion has not been tested past 100 degrees – likely due to limitations in measuring equipment at this point.

Fewer in vivo studies have explored the role of the meniscus in knee joint stability. Thorlund et al. [169] reviewed 60 patients with isolated partial medial meniscectomies and a similar number of matched healthy controls. Patients were reviewed at 3 months post meniscectomy. Laxity was measured using a dynamometer, with the knee at 20 degrees of flexion and the ankle at 90 degrees. Varus/valgus angles obtained at the point where 12Nm of resistance was recorded. There was a statistically significant difference in the varus/valgus angles measured between patients and controls as well as the midrange stiffness recorded between the two groups. However, the study can be criticised on a few counts. Firstly, no data was available from patients pre-surgery, hence it is not clear whether their knees were perhaps more ‘lax’ in the first instance. Furthermore, there were differences at baseline between the two groups, in that the patients had a higher BMI than controls. Additional soft tissue around the knee joint may have reduced accuracy of readings taken. Finally, as the subjects were conscious, muscle contraction could account for some of the differences observed, thought this probably represents the physiological situation more accurately.

Bargar et al. [170] found no significant effect on anteroposterior movement of the knee after either medial or lateral meniscectomy in an in vivo study ACL intact knees. They tested varus-valgus and AP stability using a custom clinical examination apparatus in 25 patients. Results were compared with both the patient's contralateral knee and a group of normal knees. Patients with both ACL and medial meniscal injuries showed increased AP translation. However, meniscectomies did not result in laxity in and of themselves. However, the authors' technique, although more accurate than clinical examination, was unable to accurately measure tibial rotation and hence the results should be interrupted with caution.

Harato et al. [171] explored the effect of medial meniscal injury in patients with pre-existing ACL injury (mean 10.8 months post injury). Thirty six young athletes were enrolled, outcomes measures included anterior knee laxity and gait analysis. Notably, 'meniscal injury' was defined as those patients having tears >50% meniscal width at arthroscopy (performed following the above analyses). In addition to instability caused by ACL injury, concurrent meniscal injury was found to result in a statistically significant decrease in internal rotation during the whole gait cycle. It has been suggested that this is a 'pivot shift avoidance gait' adapted by patients to prevent the knee becoming unstable. No difference was seen in terms of anterior knee laxity. A statistically significant increase in axial excursion during the gait cycle was also noted, it was suggested that this might lead to earlier osteoarthritis in the meniscal injury group due to abnormal loading patterns. This study explored a select population of young, fit adults which is not representative of the general population but provides an interesting insight into the potential effects of meniscal injury in the context of ACL damage.

Further insight into this issue is provided in a study by Zaffagnini et al. [172], who used a navigation system to test stability in ACL deficient patients, just prior to ACL reconstruction. Fifty six patients, with a mean age of 33 were included in the study. It is unclear whether the patients were anaesthetised or awake during the study (and hence, whether muscle tone was a factor). Patients were known to have no injury to the medial meniscus or one of either a bucket handle tear of the medial meniscus, a posterior horn partial meniscectomy. Stability was tested manually, including anteroposterior displacement (30 and 90 degrees of flexion), internal/external rotation (30 and 90 degrees of flexion) and varus/valgus stability (0 and 30 degrees of flexion). A pivot shift test was also performed. Significant differences were observed in anterior tibial translation in both meniscal injury states at 30 and 90 degrees as well as in the anterior displacement component of the pivot shift test. This work has a few weaknesses including small numbers, non-standardised application of manual force for testing and the inevitable difference that will exist between patient tears, it provides further evidence for the medial meniscus acting as a stabiliser in ACL deficient knees.

The effect of medial / lateral or combined meniscal injuries in ACL deficient knees was investigated by Zhang et al. [173] in 28 young patients (20-30 years) an average 3 months following injury. They investigated patients in 4 states, ACL deficient only, ACL deficient with either medial or lateral meniscal injury and ACL deficient with both medial and lateral meniscal injury. The uninjured knee acted as a control. The subjects underwent CT scans to allow construction of 3 dimensional models of their knees, followed by fluoroscopic imaging during stair ascent. The 3-dimensional models were then matched to fluoroscopic images using an automated technique, with an accuracy of 0.2mm for in plane translation and 1.57 degrees for in plane rotation. Accuracy for out of plane movement was not reported. The authors found statistically significant decreases in external rotation in the patients with isolated medial and lateral meniscal injuries at 0, 15 and 30 degrees of flexion. However, knees with both medical and lateral meniscal injuries appeared to externally rotate more. Combined injuries also showed increased anteroposterior translation compared to all other states at full extension and 5 degrees of flexion. No significant differences were observed in terms of mediolateral translation or varus/valgus angulation. This work suggests that both menisci have a role to play in knee stability, however, the small number of patients and the potential error introduced by out of plane translation make it difficult to generalise their results. Furthermore, with the uninjured knee acting as control, it is possible that the subjects may have altered their gait pattern due to their injury and that this, in fact, was the cause of the differences observed.

Table 2-7 summarises the literature to date relating to contribution of the menisci to knee stability in vitro. Studies are presented in chronological order. Figure 2-9 shows the areas of the human meniscus studied in relation to stability in graphical form.

A review of the literature with regard to the stability of the knee demonstrates that a large proportion of the studies relate to complete removal of the meniscus. Although this is of historical relevance and patients with total meniscectomies are still present in the population at large, the prevalence of this procedure has been greatly reduced and hence the clinical relevance of these studies going forward is limited, though they do aid our biomechanical understanding of meniscal function. Studies exploring different regions within the meniscus are varied. A significant proportion of them study the meniscus in the context of ACL injury. Although this state does occur commonly in clinical practice, these works shed little light of the role of meniscal injury to stability in isolation. One wonders whether this may be due to the fact that the authors failed to see any significant results with isolated meniscal injury and hence reported only those following ACL disruption (which were positive). Nevertheless, there are a few studies which study meniscal injury in isolation. Of these, one is conducted in a clinical setting using a dynamometer with no pre-injury data (Thorlund et al. [169]). In vitro works are focussed on particular meniscal regions only as illustrated in Table 2-6.

Author	**Region studied**
Arno et al., 2013 [12]	Progressive posterior **medial** meniscectomy
Allaire et al., 2008 [80]	Posterior **medial** meniscus root tear
Goyal et al., 2014 [11]	Vertical tears posterior lateral meniscus, progressing to partial meniscectomy

Table 2-6 - Studies exploring meniscal injury in-vitro in isolation

There is limited study in the literature of the role of isolated bucket handle tears of either meniscus or lateral meniscus root tears. Furthermore, although radial tears have been studied in relation to load transmission, there is no study of their role in knee instability. Vertical tears have only been studied in the posterior lateral meniscus. There has been no study of horizontal or oblique tears. Notably the former is thought to be associated with a degenerate state and the latter are likely to be difficult reproduce reliably.

More broadly, the range of movement explored in these studies is only up to 90 degrees (60 degrees in Goyal et al. [11]). This likely reflects logistical challenges in testing a knee in flexion beyond this range, though results obtained in this state would be of significant clinical interest. Furthermore, the testing states vary between all studies, with most developing a custom apparatus to test the knee. This makes comparing their results difficult. Although commercial knee simulators do exist [174], costs associated with their use can prove prohibitive. In addition, only Goyal et al., 2014 [11] subjected their cadaveric apparatus to a physiological axial load (1000N), though this does not approach the degree of force transmitted through the joint during activities such as downhill walking or squatting. As well as this, these studies disrupt the joint through performing arthrotomies, removing the medial femoral condyle and removing soft tissue from around the joint.

It would appear, therefore, that there are several areas which merit further investigation in relation to the role of the meniscus in knee stability.

Table 2-7 - Meniscal contribution to knee stability

Author	Technique	Part of meniscus studied	Findings	Number of knees tested	Axial load applied	Range of movement tested (degrees)
Markolf et al., 1976 [7]	Manual testing apparatus	Whole cadaveric knees following meniscectomy (open) and sectioning of other structures	AP and varus/valgus instability in flexed knee (however 20% error)	35	Not mentioned	0/10/20/45/90/135
Bargar et al., 1981 [175]	Custom clinical examination apparatus	25 patients following total meniscectomy compared to contralateral knee and healthy controls	No sig effect on AP movement of the knee following medial or lateral meniscectomy.	25	NA	0/20/90
Levy et al., 1982 [155]	Custom testing apparatus	Cadaveric knees – medial meniscectomy,	AP stability not affected by medial meniscectomy alone. Combined ACL injury and	9	None	0/30/60/90

Author	Technique	Part of meniscus studied	Findings	Number of knees tested	Axial load applied	Range of movement tested (degrees)
		ACL injury and combined	medial meniscectomy allowed sig increase in AP stability cf. ACL injury alone			
Markolf et al., 1986 [154]	Manual testing apparatus at 20 degrees flexion	8 cadaveric knees following open bilateral total meniscectomy	Sig increase in AP laxity at 925N of axial load	8	925N	20
Shoemaker et al., 1986 [154]	Custom testing apparatus	ACL deficient knees, following medial and then lateral meniscectomy.	10% increase in anterior tibial translation following total medial meniscectomy. Further 10% increase following lateral meniscectomy.	11	320N / 925N	20

Author	Technique	Part of meniscus studied	Findings	Number of knees tested	Axial load applied	Range of movement tested (degrees)
Levy et al., 1989 [157]	Custom testing apparatus	Isolated lateral meniscectomy or ACL deficiency combined, combined lateral meniscectomy and ACL deficiency and medial/lateral meniscectomies with ACL deficiency.	Lateral meniscectomy alone did not alter anterior tibial translation. ACL sectioning significantly increased anterior tibial translation, with no difference in lateral meniscus intact/meniscectomy conditions. Medial meniscectomy allowed additional anterior translation in ACL/lateral meniscus deficient knees.	11	None	0-90

Author	Technique	Part of meniscus studied	Findings	Number of knees tested	Axial load applied	Range of movement tested (degrees)
Allaire et al., 2008 [80]	Custom testing apparatus	Medial meniscal root tear/repair and total medial meniscectomy.	Significant increases in external rotation and lateral translation of tibia on femur with root tears. Trends towards increased anterior tibial translation and varus malalignment in root tears, significant following meniscectomy.	6	1000N	0/30/60/90
Musahl et al., 2010 - [166]	Mechanised pivot shift test (combined axial load, valgus stress and flexion)	ACL deficient knees, following medial/lateral meniscectomy	In pivot shift test, anterior tibial translation affected by lateral meniscectomy but not medial meniscectomy	16	68N	NA

Author	Technique	Part of meniscus studied	Findings	Number of knees tested	Axial load applied	Range of movement tested (degrees)
Ahn et al., 2011 [158]	Custom testing system	ACL deficient cadaveric knees with longitudinal tears of posterior horn medial meniscus and meniscectomy	Increased anterior tibial translation at all angles but not at 90 degrees. Repair restored translation. Total meniscectomy caused same increase as posterior horn tear	10	200N	0/15/30/60/90
McCulloch et al., 2013 [156]	Custom activity simulator	ACL deficient cadaveric knees with medial meniscal root transection	Root transection caused increased AP translation, this was not evident in partial or subtotal medial meniscectomy.	6	Muscle contractions applied to mimic ¼ body weight.	30/60
Arno et al., 2013 [12]	Custom rig, fiducial markers	Progressive posterior medial meniscectomy	Significant increase in AP translation of the medial	5	500N	0/15/30/60/90

Author	**Technique**	**Part of meniscus studied**	**Findings**	**Number of knees tested**	**Axial load applied**	**Range of movement tested (degrees)**
			femoral condyle with >46% resection			
Chen et al., 2014 [153]	Universal force/moment sensor, robotic system to apply load at 30/60 degrees flexion	Medial menisci in **porcine** knees meniscus intact, anterior horn resected, posterior horn resected and total meniscectomy	Internal rotation significantly increases with meniscal injury. External rotation increases at 30 degrees of flexion but not at 60 degrees. No difference in anterior tibial translation.	20	None	0/30/60/90
Goyal et al., 2014 [11]	Pulley system used to apply load to cadaveric knee, fiducial markers	Intact lateral meniscus, short vertical tear, extended vertical tear, partial meniscectomy (all in posterior third)	No change in AP translation, lateral/medial translation, internal/external rotation or varus/valgus angulation	10	350N	0/30/60

Author	Technique	Part of meniscus studied	Findings	Number of knees tested	Axial load applied	Range of movement tested (degrees)
	to measure laxity	and subtotal meniscectomy)				
Thorlund et al., 2014 [169]	Dynamometer with knee at 20 degrees flexion and ankle at 90 degrees	60 patients 3 months post partial medial meniscectomy and 60 healthy controls	Sig increase in varus/valgus angles between groups. However, no preoperative data and differences in baseline characteristics.	60	NA	Tested at 20
Lorbach et al., 2015 [168]	Universal force/moment sensor	ACL deficient knees with bucket handle tears of medial meniscus/subsequent repair	Medial meniscal injury caused additional anterior tibial translation following ACL sectioning as well as increased anterior tibial translation during a pivot shift manoeuvre. These	11	None	0/30/90

Author	Technique	Part of meniscus studied	Findings	Number of knees tested	Axial load applied	Range of movement tested (degrees)
			were both restored by meniscal repair.			
Shybut et al., 2015 [10]	Mechanised pivot shift test	ACL deficient knees, following, lateral meniscal root injury	Lateral meniscal root injury causes significant increase in anterior tibial translation during the pivot shift test as compared to ACL injury alone	8	Up to 175N	NA
Lording et al., 2017 [133]	Custom rig, optical trackers	ACL deficient knees with injury to either anterolateral ligament of lateral meniscus posterior root	Significant increase in internal rotation when either structure injured, effect of posterior root injury more pronounced in extension, anterolateral ligament past 30 degrees of flexion	16	0N	0-90

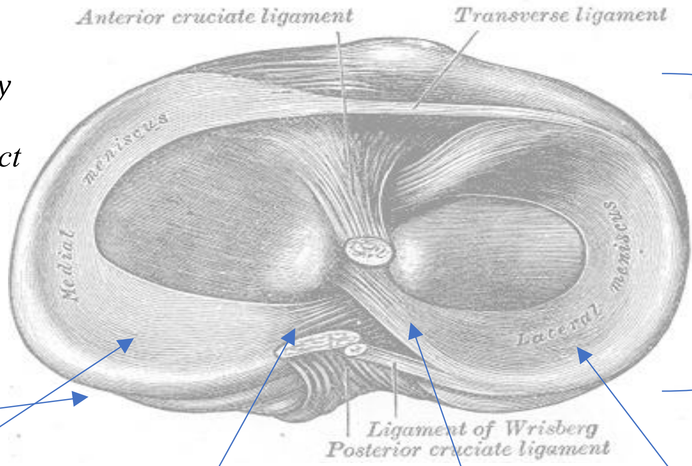
Denotes concurrent ACL injury

ǂ Denotes study where ACL intact and deficient state investigated

Medial meniscal body

Lorbach et al., 2015 – bucket handle tears*

Thorlund et al., 2014 – partial medial meniscectomy



Total meniscectomy – medial/lateral or bilateral

Markolf et al., 1976

Bargar et al., 1981

Levy et al., 1982 ǂ

Markolf et al., 1986

Shoemaker et al., 1986*

Levy et al., 1989 ǂ

Musahl et al., 2010*

meniscus

Ahn et al., 2011 – longitudinal tears*

Arno et al., 2013 – progressive posterior medial meniscectomy

Posterior medial meniscus root tear (or variations)

Allaire et al., 2008

McCulloch et al., 2013*

Posterior lateral meniscus root tear (or variations)

Shybut et al., 2015 *

Lording et al.,2017 *

Posterior third lateral meniscus

Goyal et al., 2014 – vertical tear progressing to partial meniscectomy

Figure 2-9 - Studies relating to meniscal stability in humans

2.2.4.5 Lubrication

A role for the menisci in joint lubrication is asserted based on work showing the coefficient of friction at the joint increases by 20% post meniscectomy [176]. Interestingly, the friction coefficient for bovine meniscus compared to cartilage was found to be higher during earlier stages of loading by Pickard et al. [177].

McCann et al. [178] measured friction coefficients in the medial compartment of bovine knees using a pendulum friction simulator. Testing was performed under 1000N loads, pre and post meniscectomy. Removal of the meniscus was found to cause a statistically significant increase in friction coefficient. Absolute measurements of the friction coefficient were 0.06-0.09 with the meniscus intact and 0.08-0.12 post meniscectomy.

Baro et al. [179] measured boundary friction coefficients in 6 bovine menisci with a mobile contact area using a microtribometer with the meniscal sample submerged in PBS. Friction was found to be site and rate dependent. Increasing speed (from 0.05 to 5mm/s) was found to decrease the friction coefficient by 65%. Friction was significantly higher at the lowest speed (0.05) as compared to all other sliding speeds (~0.02). A trend towards lower friction coefficients with increasing speed was seen in all measurements. No statistically significant difference was demonstrated between tibial and femoral surfaces. Following treatment with papain (to digest proteoglycans), a two-fold increase in friction coefficient was evident.

There is limited data on the frictional properties of human menisci in the literature.

2.2.4.6 Proprioception

The aforementioned mechanoreceptors in the meniscus are thought to have a role in relaying proprioception to the central nervous system and relaying pain. MRI studies of patients with meniscal tears has shown correlation of meniscal injury with poor proprioceptive accuracy – measured through detection of passive joint motion. Indeed, reducing accuracy was associated with both higher numbers of meniscal regions affected and extend of abnormality [180]. Karahan et al. [181] performed a case control study of 39 patients, 19 with posterior medial meniscal tears that had undergone partial meniscectomy an average of 2 years prior. They tested joint position sense using a modified continuous passive motion machine, assessing the participants' ability to identify the degree of knee flexion they had undergone. The authors found statistically significant differences between partial medial meniscectomy patients and controls at identifying joint position at 60 and 75 degrees of flexion. No significant difference was found at lower flexion angles (15/30/45 degrees). The authors suggest that the neurological apparatus known to exist in the menisci – such as mechanoreceptors and Golgi organs, may relay proprioceptive information from the knee. This information may also have a role in preventing knee injury through allowing reflexive knee stabilisation in injury prone situations.

A larger study by Al-Dadah [182] recruited 50 patients in each group. On this occasion, meniscal tears were present both medially and laterally. Notably, they performed MRI scans to confirm normal menisci in the control group. A custom platform for assessing dynamic postural stability (Biodex Balance SD system [Biodex Medical Systems Inc.]} was used. Both cases and controls were tested, with both legs tested in each group. Cases were tested both pre and postoperatively at 3 months. Statistically significant differences in proprioception (measured as an overall stability index) were found between the injured knee and both contralateral and control knees. This deficit was present preoperatively and postoperative results did not show any significant difference in stability, though the postoperative follow was quite short.

Interestingly, Thijs et al. [183] have demonstrated improved knee joint position sense at 70 degrees of flexion at 6 months following meniscal allograft transplantation. It is not clear whether this is through the implanted meniscus regaining neurological function of its mechanoreceptors or another mechanism.

2.2.5 Meniscectomy

As previously discussed, meniscal injury usually manifests as pain and mechanical symptoms in the affected joint. Partial meniscectomy aims to remove unstable meniscal fragments, which are causing mechanical symptoms in the knee joint[5]. The aim is to create a stable rim of meniscal tissue whilst minimising the amount of tissue removed to preserve meniscal function. This is thought to minimise the risk of developing subsequent osteoarthritis. However, MRI studies [184] have demonstrated that cartilage defects are more common in patients who have undergone partial medial meniscectomies as compared to age matched controls. It is unclear whether this reflects concomitant injury due to the tear or is an effect of the cartilage being ‘unprotected’ by the meniscus. Nevertheless, partial meniscectomy forms the bulk of current practice in the United Kingdom.

A subtotal meniscectomy is undertaken in a tear which requires excision of a portion of the peripheral rim of the meniscus. Remaining tissue (often in the anterior horn and middle third of the tissue is not resected).

A total meniscectomy involves removal of the entire meniscus. Although this procedure was historically performed quite often, it is now reserved for situations where the meniscus has been detached from its peripheral attachments with extensive intrameniscal damage. It is not justifiable if the meniscus is repairable or salvageable in any form. A concurrent allograft transplant may be undertaken, especially in younger patients to protect articular cartilage in the affected compartment.

2.2.6 Meniscal repair

Meniscal repair is indicated in specific tear subtypes, in particular longitudinal peripheral tears. Degenerate tears can be impossible to repair, if symptomatic, partial meniscectomy is the currently preferred treatment. As previously mentioned, a tear in the vascularised zone of the meniscus if much more likely to be successful than a ‘white zone’ tear. Repair is performed with sutures placed in the meniscus through arthroscopic surgery. Consideration of the detailed techniques of these repairs is not in the scope of this dissertation.

2.2.7 Meniscal reconstruction

2.2.7.1 Meniscal allograft transplantation

Meniscal allograft transplantation was first described in 1989 [185]. It involves transplant of harvested menisci (with or without bony plugs) onto the tibial plateau. Meniscal allograft transplantation is considered in young healthy patients who have undergone a previous meniscectomy and are suffering from pain in the affected compartment. Malalignment of the knee, ligamentous instability or pre-existing chondral damage are contraindications [186]. The procedure aims to prevent chondral damage which is understood to occur following meniscectomy and delay the onset of osteoarthritis. Indeed, meniscal allograft transplantation has been shown to restore contact area and contact pressures of the knee joint towards normal (though not *to* normal) [8].

Fixation techniques for the allograft vary, these include soft tissue fixation through sutures or bony plug fixation of the meniscal horns. The latter technique has been shown to be most effective in restoring contact mechanics close to normal [187].

Hergan et al. [186] conducted a systematic review of the literature related to meniscal allograft transplantation. They identified 14 studies, one of Level III evidence (a case control study) and the remainder of Level IV evidence. Only studies conducted using bone block fixation were considered. In total 352 grafts were reviewed in 323 patients, both medial and lateral, with a mean 53.8-month follow-up. Most studies included concurrent procedures, often in the form of knee stabilisation, osteochondral reconstruction or high tibial osteotomies. Patient satisfaction ranged from 62.5% to 100%. Improvements in outcomes such as the IKDC, Lysholm and Tegner scores were reported. 62 of the patients reviewed underwent second look arthroscopies and some required partial or total meniscectomies. An early (<2 years) failure rate of 10% was reported. The authors also noted that one investigator had authorship of 4 of the performed studies, suggesting that there may in fact be significant crossover of the patient populations considered and hence the total number of subjects might actually be lower than reported. This likely reflects the fact that these procedures are performed in small numbers globally and most occur in a few specialised academic centres. The authors highlight that although their work identifies good early results of the procedure, there is no consensus on whether it delays the onset of arthritis. There is need for a large scale prospective, randomised trial with long term follow up to evaluate the utility of the procedure.

A subsequent review by Verdonk et al. [188] considered 39 studies relating to meniscal allograft transplantation. 1145 patients with 1226 allografts were considered. Mean follow up was 5.5 years. Notably, only 340 isolated transplantations were identified, the remainder were performed with other procedures. These were most often ACL reconstructions or a corrective osteotomy. All studies considered showed significant improvements in patient reported outcomes, with no difference identified between isolated or combined procedures. Fixation technique did not influence subjective outcomes.

Clinical outcomes were assessed using examination findings, documenting range of movement and functional testing. Most outcomes reported improvements in factors such as range of motion, stability, effusion and functional tests.

Radiological progression of osteoarthritic change was seen in a significant proportion of patients and increased with length of follow up. Without a comparator group, it is difficult to draw any firm conclusions from these findings. At 10 years, survivorship of 70% was predicted for medial or lateral allografts.

The authors concede that it is challenging to compare studies due to differences in operative technique, large number of concurrent procedures, variation in outcomes measured and different follow up times. Again, the need for further study is highlighted.

A recent in vitro study [189] of meniscal transplants utilised seven cadaveric knees and measured tibial contact stresses using Tekscan pressure sensors in intact, meniscectomy and transplant states. A knee simulator was used to mimic human gait. ‘Transplants’ were performed with the excised meniscus using a bone plug. The authors found three areas of regional loading in the intact meniscal state. These occurred:

- At 14% of the gait cycle at the posterior aspect of the medial plateau
- At 14% and 45% of the gait cycle in the central/posterior aspect of the lateral plateau.
- At the anterior aspect of the medial plateau

After medial meniscectomy, load concentration was seen in the posterior aspect of the medial plateau, whilst load at the anterior aspect of the medial plateau fell to zero. Lateral plateau load shifted anterolaterally. Meniscal transplantation restored load on the medial plateau to the intact condition. A different loading pattern, located more posteriorly was seen in the lateral plateau. The authors suggest that this change in loading pattern despite transplantation may cause accelerated cartilage degeneration in meniscal transplant patients.

Unfortunately, there is limited data on the long-term survivorship of such grafts. Noyes et al. [17] presented a case series of 58 patients with meniscal transplants an average of 11.9 years postoperatively. Survivorship was measured based on criteria including reoperation, extrusion on MRI or radiographic osteoarthritis. Despite all patients reporting improvements in functional outcomes in the postoperative period, survivorship was 85% at 2 years, 77% at 5 years, 69% at 7 years, 45% at 10 years and 19% at 15 years (note that the number of cases falls with prolongation of follow up). Concurrent osteochondral defect repair was associated with lower survival, whilst factors including the operated knee compartment, age (<30 years or 30-49 years) and existing osteoarthritis (Grade II/III vs none) did not affect survival. Larger cohorts will provide more data on the long-term outcome of this procedure, though even at this low survivorship, the benefit of delaying arthroplasty in younger patients is undeniable.

Xenograft meniscal implants have also been explored – the use of decellularised meniscal implants was explored by Abdelgaeid et al. [190], who found the decellularisation process lowered elastic moduli and increased permeabilityof such implants. These changes were thought to be secondary to decreased GAG content. The clinical role of these implants is, as yet, unexplored.

2.2.7.2 Meniscal scaffolds

Meniscal scaffolds provide a biodegradable framework implanted in the knee with the aim of inducing biological ingrowth to restore meniscal function. Filardo et al. [191] reviewed the literature in relation to meniscal scaffolds, finding that the majority of patients were treated with one of two implants:

- The Collagen Meniscus Implant [CMI] (Ivy Sports Medicine GmbH, Grafelfing, Germany)
- The Actifit prosthesis (Orteq Ltd, Wimbledon, London, United Kingdom)

76.2% of patients treated in the literature were male, with a mean age of 36.5 years with both chronic and acute tears. Again, patients often underwent combined procedures, with concurrent procedures including ligament reconstruction, osteotomies, cartilage regeneration amongst others. These procedures are major confounding variables in all the studies reported. Complications such as pain, knee joint effusion, infection and repeat arthroscopy for implant debridement or stabilisation were noted in 12.6% of cases.

Although these scaffolds are not impregnated with any biological material, there has been some preliminary work into seeding them with mesenchymal stem cells, theoretically broadening their healing potential [192].

2.2.7.2.1 Actifit

The Actifit meniscal scaffold is composed of a polymer developed by Orteq Sports Medicine, receiving CE approval in 2008. The scaffold is a polydisperse combination of polyester and polyurethane components, the proportion of these constituents is chosen to closely mimic the mechanical properties of meniscal tissue [193]. Polyester components form the ‘soft’ segments of the implant whilst ‘stiff’ segments are composed of polyurethane [194]. Pore size of the foam is between 100-300 micrometres, with interconnecting pores of 10-30 micrometres [195].

Poluyurethanes, which form 20% of the implant, are large polymers with urethane linkages in the polymer structure. Hydrogen bonds between the urethane moieties link the hard segments, allowing the softer segments (see below) to form a network, which can then fill with blood to allow tissue regeneration. Significant variation can occur in the other components of the substance; hence the physical properties of polyurethanes can be varied. As a whole, polyurethanes have high tensile strength, toughness and resistance to degradation alongside biocompatibility [196]. Notably, concern has been raised regarding production of toxic diamines upon degradation of commercially available polyurethane. The Actifit implant is composed using 1,4-butane-diisocynate, which releases putrescine on degradation, a polyamine which occurs naturally in the body [197] and is hence thought to be non-toxic. It is excreted in the urine. The remaining 80% is composed of the poly ε-caprolactone polymer, which is more flexible. This polymer is used routinely in medical practice in sutures such as Monocryl (Ethicon Inc, Somerville, New Jersey, United States).

Development of the scaffold was based on animal studies to allow determination of factors such as pore size [198], porosity, degradation products (and their toxicity) and degradation time as well as to determination the combination allowing maximal tissue ingrowth [198]. In particular, the tear strength of the polymer has been adapted to prevent tear out of sutures, which might render the scaffold ineffective [199]. Notably, both the compression modulus and pore size within the scaffold has been found to influence the degree of fibrocartilage ingrowth, with up to 100% ingrowth shown at moduli of 150MPa [200] and macropore size between 150-300µm. The scaffold is designed to degrade over time, whilst allowing ingrowth of tissue to replace the damaged meniscus. Histological studies in rabbit and mouse models suggest that this degradation takes 3.5-4 years and is facilitated by macrophage phagocytosis[19]. Canine studies indicate no significant inflammatory response occurs following implantation. During surgery, channels are created in the joint capsule with the intention of providing a vascular supply to the scaffold region. Study in dogs [201] have demonstrated ingrowth of connective tissue derived from the synovial lining of the knee and supplied by the vasculature in the same region. Hence, the scaffold provides a thoroughfare allowing access of these factors to a region which is normally avascular. The infiltrating tissue has been found to be a fibrocartilage composed of type I and II collagen, with the relative concentration of these not dissimilar to that found in the meniscus [202] and markedly dissimilar to the fibrous scar tissue normally generated in response to soft tissue defects. However, the collagen ingrowth has been observed to mimic the mesh like appearance of the scaffold rather than the circumferential arrangement seen in the native meniscus [203]. Interestingly, once mature fibrocartilage had formed, the tissue was found to become avascular. The cause for this is unclear – it may reflect anti-angiogenic factors released by fibrochondrocytes.

Mechanical properties of the implant show a reported tensile modulus of 65-264MPa, ultimate tensile strain of 650-1200% and a compression modulus of 200kPa, which allows regeneration of fibrocartilage [193,204,205]. Notably, the compression modulus of the scaffold was calculated by means of a stress strain curve, with little detail on the methods of such measurements. Unlike meniscal tissue, which has been studied using the poroviscoelastic model (see Section 2.2.4.1.3), there is no study in the literature which seeks to model the behaviour of the scaffold using a biphasic material model, though arguably it’s construction - a foam construct bathed in a liquid solution, mimics the qualitative nature of a biphasic model.

No significant difference in the stress strain curves of the native canine meniscus and the implant have been observed prior to implantation and at 6/24 months, though

Figure 2-10 - The Actifit meniscal scaffold (Orteq Ltd)

only 13 menisci were studies [203]. Implantation of the Actifit prosthesis in an ovine knee model has been shown [206] to reduce contact stresses caused by partial meniscectomy. However, the same study showed that the coefficient of friction against cartilage for the Actifit was higher than that for stainless steel. Hence, the prosthesis could potentially cause abrasion against articular cartilage and lead to early degeneration.

Spencer et al. [207] studied 11 Actifit patients alongside patients undergoing treatment with theCollagen Meniscus Implant. A proportion of patients underwent concurrent stabilisation procedures. In the Actifit patients, at a mean 19.4 month follow up, data was available for 5 patients. This showed improved Lysholm, IKDC and KOOS scores. All results were statistically significant except for the activities of daily living and sport components of the KOOS. Patients underwent repeat arthroscopy at an average 12.8 months, 4 of the 5 were thought to have >50% infill of biological growth on macroscopic inspection. This study has small patient numbers and significant loss to follow up in the Actifit group.

Efe et al. [208] conducted a similar study in 10 patients, again, some had undergone a recent (within 12 weeks) surgical procedure on the study knee. The authors reported significant improvements in the KOOS score and KSS score at 12 months. Improvement was also seen in the VAS score, but this was not significant. All patients also underwent MRI scans at 6 and 12 months, with 4 showing gaps between the implantation site and the implant and a further 2 demonstrating extrusion. The authors also noted that subchondral oedema and bone bruising visualised on 4 scans subsequently resolved at 12 months.

Verdonk et al. [209] reviewed a series of 52 patients with both medial and lateral Actifit implantation in 9 centres. 38 patients were followed up to 24 months. Notably, nearly a third (32.7%) underwent concurrent ACL reconstruction. Significant improvements were seen in the VAS/IKDC/KOOS and Lysholm scores at 6/12 and 24 months. 9 patients required reoperation, 2 of these were thought to be due to the scaffold - requiring repair/debridement of the scaffold.

De Coninck et al. [210] conducted a study exploring whether radial displacement of meniscal scaffolds was evident on MRI post implantation, compared with pre-implantation scans. They also studied clinical outcomes. Data was collected for 23 patients followed up to 2 years, with implantation performed on both medial and lateral menisci. Again, some patients had undergone recent knee stabilisation surgery. The authors' data suggested that statistically significant radial displacement (in the order of 2mm) of meniscal implants occurred from that observed preoperatively in the medial, but not the lateral meniscus. They also demonstrated a negative correlation between medial meniscal rim thickness (i.e. that of the pre-existing meniscal tissue to which the implant is fixed) and radial displacement. In common with other authors, this group also found statistically significant improvements in clinical scores including the VAS, IKDC, Lysholm and KOOS. There was no correlation between radial meniscal displacement and clinical outcome score postoperatively, although some correlation was noted with elements of the VAS, KOOS and Lysholm score for the medial meniscus preoperatively. This may reflect symptoms secondary to meniscal instability. The authors suggest longer term follow up would be useful to ascertain whether increased radial displacement of the meniscus has any influence on development of osteoarthritis. Notably this group conducted MRI scans with the patient supine – hence the meniscus was not physiologically loaded.

The role of preoperative meniscal extrusion in the longevity of Actifit implants has been investigated in a case series of 20 patients [211]. Interestingly, preoperative coronal meniscal extrusion correlated with frontal extrusion post implantation at 1 year. Furthermore, both patient reported outcomes and MRI measured cartilage coverage were significantly worse in patients with pre-existing meniscal extrusion. The authors suggest that the presence of such extrusion indicates that meniscal function is compromised such that despite Actifit implantation, the meniscus is unable to function normally. Such extrusion would be thought to correlate with degree of meniscal tissue removed. It is possible that excessive debridement leads to inability to generate hoop stresses and maintain function. The authors suggest that the presence of such extrusion on preoperative MRI scans should serve as an exclusion criterion for Actifit implantation. The study should be interpreted with caution though, due to the small patient numbers and significant loss to follow up (only 14 of the 20 enrolled in the study underwent MRI scans). Again, a significant proportion of patients have also undergone concurrent procedures.

A further small study by Kon et al. [212] presented 18 patients undergoing both medial and lateral meniscal Actifit implantation, alongside stabilisation procedures. Only 1 patient had not had previous knee surgery. Outcomes reviewed at 2 years included IKDC and Tegner scores. Statistically significant improvement was seen in all scores.

A larger study by Bouyarmane et al. [213] conducted a similar study using the Actifit scaffold to treat patients with lateral meniscal injuries, studying 54 patients from multiple centres over 4 years. Again, this group showed significantly improved clinical outcome scores across multiple outcome measures in all patients at 2 years follow up. Outcome scores reported included a visual analogue score for pain, the IKDC score and the KOOS clinical outcome score. All showed statistically significant improved at 24 months. Three patients underwent repeat surgery – all had tears in the scaffold trimmed. The authors noted that most improvement was seen in the first year of the study. It should be noted that a significant proportion of patients in this study had undergone another procedure on the knee in question (often ligamentous reconstruction) within 12 weeks of their Actifit implantation. Once again, this is a significant source of bias. Furthermore, this study was conducted across multiple centres, hence there may be unaccounted differences in operative technique.

Baynat et al. [214] conducted a prospective study of 18 patients, all of military backgrounds. Actifit implantation was performed in all 18, though notably 12 underwent concurrent ACL reconstruction. At 2 years follow up, this group noted a mean Lysholm score of 92, indicating a good outcome. Significance was not reported. Baynat’s group also performed MRI scans in their patients at 1 year and found the prosthesis to be macroscopically well positioned in all but 1 patient, where some extrusion of the prosthesis was evident; however, this was not thought to have any clinical effect. Concurrent arthroscopic biopsy in 3 patients demonstrated infiltration of the Actifit scaffold by chondrocytes and fibrochondrocytes, indicating biological ingrowth.

Verdonk et al. [215] conducted an MRI and histological analysis of 52 patients undergoing Actifit implantation, using a contrast enhanced MRI scan at 3 months and biopsy at 12 months. 81.4% of patients showed proliferation of blood vessels within the implant region at 3 months, suggesting tissue ingrowth at this juncture. MRI at 12 months showed 76.1% of patients showed no degradation in cartilage from the preoperative state. Histological analysis demonstrated 3 layers (the first of which was only present in about a third of samples). The first, superficial layer was vascularised and hypercellular with a surrounding extracellular matrix. The second layer remained hypercellular but was avascular, consisting of type I collagen and fibroblasts. The third layer was hypocellular and avascular, with a fibrochondroblast like cell type. The authors liken these layers to the vascular zones of the meniscus, with ingrowth starting in the first layer and maturing to the third. A lack of controls limits interpretation of this work. Furthermore, it should be noted that these results were not replicated in a later study comparing the Actifit with the Collagen Meniscus Implant [216] (see below).

The longest follow up following Actifit implantation is presented by Leroy et al. [217], who studied 13 patients a mean 6 years following implantation. Five patients underwent concurrent procedures. Three patients required reoperations for worsening symptoms, all within the first 30 months. Outcomes included patient reported measures and MRI analysis. Intention to treat analysis revealed improvement in the subjective IKDC score and the visual analogue score for pain. All implants showed reduced size and intermediate signal on MRI scanning. No worsening of cartilage status was observed throughout the follow up period. No improvement was seen in the KOOS score in relation to sporting activities. The authors raised concern regarding the high failure rate, with those patients undergoing removal of the implant showing no improvement of function and worsening cartilage condition. However, no such cartilage degeneration was evident in the group whose procedure was deemed successful.

In common with many other studies on the topic, a major source of bias here are the concurrent procedures performed in two thirds of the patient population. Furthermore, it should be noted that the population studied here is likely to be fitter and more motivated than the general population given their military background.

A few studies relating to the Actifit implant review radiological outcomes. Schuttler et al. [218] conducted a study of 18 patients undergoing Actifit implantation. Outcomes were patient reported outcome scores and MRIs at 6/12 and 24 months. Notably, this is the sole study in the literature which did not perform concurrent procedures on patients undergoing Actifit implantation. Improvements were shown in the VAS, KSS and KOOS. All were statistically significant at 6, 12 and 24 months. MRI scans demonstrated extrusion in three cases, though all these patients were satisfied with their outcomes. One patient showed complete reabsorption of the prosthesis at 24 months – this patient was not satisfied with their clinical outcome. This group also showed that any signs of inflammation in the knee settled by 12 months. Interestingly, two patients showed worsening articular cartilage degeneration between 6 and 24 months. It is not clear what role the high coefficient of friction of the prosthesis might have here. It should be noted however, that 5 patients showed improvement in chondral wear over the same period. None of these differences were statistically significant.

In a subsequent study [219] following up the same patient population, it was noted that all three clinical outcome scores reported above continued to improve at 48 months also. Repeat MRI scans at 48 months showed bone bruising occurring in 2 patients with extrusions, raising the question as to whether such implants should be debrided or stabilised even if asymptomatic. Again, no significant changes in articular cartilage were seen.

Gelber et al. [220] conducted a prospective non-randomised controlled trial of 60 patients undergoing open wedge high tibial osteotomy with for early medial compartment osteoarthritis. All patients had posterior medial meniscal defects

>25mm in size. 30 patients underwent high tibial osteotomy only whilst the remaining 30 underwent concurrent Actifit implantation. The authors did not demonstrate any additional benefit across multiple scoring systems in patients undergoing Actifit implantation combined with a high tibial osteotomy.

An MRI based study was conducted by Gelber et al. [221] in 54 patients following Actifit implantation using the Genovese criteria (Table 2-8) [222]. They also identified patients within their group who had chondral injury. Again, this group showed significant improvement in patient reported outcome scores including the WOMET score, the IKDC and VAS. Interestingly, they also noted that more severe chondral injury showed worse MRI characteristics on the Genovese score. It should be noted that the Actifit is contraindicated in patients with advanced articular cartilage injuries. Furthermore, the Genovese score has been criticised [223] for having poor intra observer (0.58) and interobserver (0.35) intraclass correlation coefficients, leaving the utility of this method of assessment questionable.

Characteristics	**Type 1**	**Type 2**	**Type 3**
Morphology/size	Totally reabsorbed	Small implant, regular or irregular morphology	Identical shape and size to normal meniscus
Signal intensity	Markedly hyperintense	Slightly hyperintense	Isointense to normal meniscus

Table 2-8- The Genovese criteria

A summary of the literature relating to patient reported outcomes following Actifit implantation to date is presented in Table 2-9.

Unfortunately, there are no Level 1 studies related to the Actifit prosthesis. Indeed, none of the cited studies were randomised, most have small case numbers, with significant loss to follow up. No study cites a power calculation. There is no blinding in any of the studies (which would be challenging in any case) and a proportion of them are published by investigators with links to the manufacturer. As well as this, a small number of authors have published most of the articles in this field, raising concern that there might be significant crossover between study populations presented. Finally, as already mentioned, the performance of other procedures alongside scaffold implantation in the same treatment cycle is a significant confounder. In addition, there is only a single study of the prosthesis performed in the in vitro setting. This is performed in an animal model and the role of the implant in conferring stability to the knee, which in turn would aid earlier recovery and function is not explored. These limitations all reflect the fact that the use of this prosthesis is currently limited in clinical practice, as it is accepted more widely, one would hope further data to become available.

Table 2-9 - Summary of Actifit literature

Author	**Study type**	**Number of subjects followed up**	**Length of follow up**	**Concurrent procedure (12 weeks)**	**Primary outcome**
Spencer et al., 2012 [207]	Case series	5 (Actifit only)	19.4 months	Yes	Improved Lysholm, IKDC, KOOS ($p<0.05$)
Efe et al., 2012 [208]	Case series	10	12 months	Yes	KOOS, KSS improved ($p<0.05$). VAS improved ($p>0.05$)
Verdonk et al., 2012 [209]	Case series	38	24 months	Yes	VAS / IKDC / KOOS / Lysholm improved ($p<0.05$)
De Coninck et al., 2013 [210]	Case series	23	48 months	Yes	VAS / IKDC / Lysholm / KOOS ($p<0.05$)
Baynat et al., 2014 [214]	Case series	18	24 months	Yes	Lysholm score (Sig not reported)

Bouyarmane et al., 2014 [213]	Case series	54	24 months	Yes	IKDC / KOOS improved ($p<0.05$)
Kon et al., 2014 [212]	Case series	18	24 months	Yes	IKDC / Tegner ($p<0.05$)
Gelber et al., 2014 [221]	Case series	54	39 months	Yes	IKDC / VAS / WOMET ($p<0.05$)
Schuttler et al., 2014, 2015 [218,219]	Case series	18	48 months	No	VAS / KSS / KOOS improved ($p<0.05$)
Faivre et al., 2015 [211]	Case series	20	24 months	Yes	Preoperative meniscal extrusion correlates with worse functional (patient reported outcome) and radiological results.
Gelber et al., 2015 [221]	Case series	54	39 months	Yes	WOMET / IKDC/ Kujala improved ($p<0.05$). Worse chondral injury correlated with poorer Genovese score ($p<0.05$)

2.2.7.2.2 Collagen Meniscus Implant

In contrast to the Actifit prosthesis, the Collagen Meniscus Implant (CMI) has been in use for close to two decades. Development of the prosthesis was driven by the observation that collagen bridges allowed regeneration of nervous tissue and skin across gaps [224] as well as work showing that meniscal fibrochondrocytes infiltrated a scaffold of fibrin clot to lay down a fibrocartilaginous repair in the avascular portion of the meniscus [225,226]. The prosthesis is developed from tissue harvested from bovine Achilles tendons, with the tissue undergoing mechanical disintegration, removal of water, lipid and salt soluble material and limited proteolysis to produce purified type-1 collagen [227]. This step limits any potential immunogenic response to the implant, resulting in weak antigenicity [228]. The collagen produced is then fit into a mould, with further addition of glycosaminoglycans alongside thermal crosslinking. Modulation of this step allows control of the degradation rate of the prosthesis through collagenases [229], ensuring an optimal balance between tissue ingrowth and implant degradation. Electrospinning is used to attempt to arrange the collagen scaffold such that during subsequent ingrowth, collagen fibre orientation mirrors that displayed by the native meniscus [230]. The final matrix produced is composed of approximately 75% collagen and 25% glycosaminoglycans by dry weight. Pore size of the matrix is between 75-400μm [231]. Suture pull-out from the implant occurs at 2.5 +/- 0.5kg of load, higher than the suggested standard 1kg value for arthroscopic surgery [232]. Interestingly, a sheep model [233] seeding fibrochondrocytes into a CMI demonstrated accelerated scaffold remodelling, less cellular infiltration and more extra-cellular matrix compared to the normal implant, though this has not yet been explored in humans. These findings have been replicated in vitro, using mesenchymal stem cells in a later study [234].

Testing [235] of the implant performed in a canine model demonstrated that fibrochondrocyte ingrowth occurred in >60% of cases, with collagen bundles forming both parallel and perpendicular to the long axis of the meniscus and samples taken at 1 year following implantation resembling the native canine meniscus on histological analysis – though cells were found to be more fusiform and elongated than observed in the native meniscus [229]. Chromatography suggested that proteoglycan synthesis within this model also mirrored that of the native meniscus. The authors suggested that progressive loading of the implant lead to orientation of developing meniscal fibres along stress lines, whilst also proposing that an inability to prescribe a period of non-weightbearing lead to the significant failure rate. Further work established addition of fibronectin aided fibrochondrocytes infiltration into the scaffold. A study in a canine models [236] showed angiogenic and inflammatory infiltration at 6 weeks, with concurrent deposition of granulation tissue. This was seen to reabsorb to allow replacement with fibrochondrocytes at the 1-year mark. Scaffold degradation was ongoing at this point. Subsequent human trials [231] showed similar fibrochondrocytes proliferation and confirmed no significant inflammatory infiltrate, confirmed through lack of antibodies in biopsy samples through enzyme linked immunosorbent assay (ELISA).

Numerous clinical trials have been undertaken with the CMI implant. Initial work [237] demonstrated improved patient reported outcomes in most patients with no patients reporting deterioration of their symptoms. Arthroscopic evaluation at 6 months showed stable integration of the implant, with subsequent biopsy at 1 year (in only 2 cases) showing mature fibrochondrocytes and no inflammatory infiltrate; though the degree of infiltration varied from 40-100%. ELISA testing also demonstrated no immunological response. Progressively decreased signal intensity of the implant on serial MRI scans up to 1 year was also evident. A subsequent, more detailed study of 40 patients reported MRI outcomes up to two years following implantation [238], demonstrating falls in signal intensity and morphology similar to that of the native meniscus between 6 and 24 months which were statistically significant. However, although signal intensity did fall significantly, it was similar to native meniscus in only 26.6% of cases, suggesting that integration of the implant was still ongoing. Although 2 ‘new’ chondral lesions were found between the initial review and 24 months follow up, the authors suggested that these were in fact present throughout and detected on the latter review due to the use of magnetic resonance arthrography rather than simple MRI, leading them to suggest that there was no evidence of chondral deterioration over the study period. However, without a control group, it is difficult to identify whether the implant made a significant difference. Further evaluation of a series of 8 implants at 5-6 years showed no further degenerative change in the implanted knees and integration of the prosthesis. No remnant of the CMI was found on histological analysis of 3 samples, suggesting complete resorption.

Zaffagnini et al. [239] reviewed eight patients undergoing CMI implantation a mean 6.5 years following either a subtotal or total meniscectomy, with follow up at 6-8 years. Two had previously undergone ACL reconstruction. Five patients were observed to have chondral lesions at the time of implantation. All patients showed improvements in the IKDC score (significance not reported). Evaluation of pain was undertaken using a subjective scale but showed an overall trend towards reduction. Two of the patients showed worsening arthritis at 6 years following implantation on weight bearing X-rays. All patients reported subjective normal knee function. The study is limited by its small numbers, confounding variables and the use of non-standardised outcome measures.

Following phase I and II trials, Rodkey et al. [240] conducted a randomised controlled trial of 311 patients receiving either a partial medial meniscectomy (control group) or a CMI implant (intervention group) with follow up to 7 years. Patients were subdivided into either acute or chronic groups, the latter group had undergone previous meniscal surgery. Inclusion criteria were previous partial meniscectomy or an irreparable medial meniscal injury. Computer generated randomisation was undertaken. Neither patient nor surgeon were blinded to the intervention. ACL reconstruction was undertaken in a non-controlled manner of patients within 12 weeks of the meniscal surgery, although there were no significant differences in the numbers between groups. Postoperative rehabilitation was markedly different for both groups, with the CMI group requiring knee bracing for 6 weeks and required to remain non-weightbearing for 3 weeks and partially weight bearing for a further 3 weeks. The control group underwent standard physiotherapy only. No gross mechanical failures of the CMI were seen at arthroscopy 1-year post implantation and significant increases in the surface area of the meniscus visible as well as the percentage of meniscal defect filled with tissue were observed. However, there were no significant differences recorded in patient reported outcomes between the groups. A significant difference was reported in the degree of activity regained (based loosely on the Tegner activity scale) by patients in the chronic injury group receiving a CMI implant as compared to the chronic partial meniscectomy group. Histological evaluation, as well as showing similar findings to those described above, suggested <5% of patient suffering sub-clinical synovitis. Notably, the risk of re-operation was higher in the CMI group, though this is perhaps unsurprising given this group was undergoing implantation of a prosthetic device. Although this study has numerous strengths, including randomisation, second look arthroscopy and biopsy, one wonders whether a longer follow up may have shed more light on differing patient outcomes.

Monllau et al. [241] presented 22 patients with a minimum 10 year follow up after CMI implantation. Their patient population was somewhat heterogenous, with indications for implantation either joint line pain following medial meniscal resection or large irreparable tear at arthroscopy. 13 patients also underwent concurrent ACL reconstruction. Significant improvement in patient outcomes were reported using both the Lysholm score and VAS for pain at 1 year, these persisted to 10-year review. Radiographic evaluation showed no patients with pre-existing arthritic changes developed any radiographic signs of arthritis during the 10 years follow up (n=11). Of those showing signs of arthritis at the time of the CMI implantation, 7 showed no progression, whilst 5 showed worsening arthritis. Two of the implants required removal/salvage procedures during the follow up period, giving a failure rate of 9%. MRI showed 89% of implants were reduced in size at 10-year review, with 21% having isointense signal to the native meniscus. Although the study provides longer term outcomes for patients undergoing CMI implantation, without a control group, randomisation or matching of baseline characteristics, it is difficult to derive firm conclusions from the results.

These shortcomings were addressed in part by Zaffagnini et al. [242] who undertook a prospective cohort study of 36 patients with either acute or chronic medial meniscal injuries. Patients were given the option of either CMI implantation or partial medial meniscectomy. Of the total, 33 patients were available for final follow up. Two patients in each group underwent either ACL reconstruction or medial femoral condyle microfracture for chondral lesions. Statistically significant improvements were seen in the CMI patient outcome scores at 10 years, including in the VAS, Lysholm, SF-36, IKDC and Tegner scores, with significant difference observed between the meniscectomy and CMI groups with regard to the VAS, IKDC and Tegner scores. This study demonstrated significant joint line narrowing in the meniscectomy group as compared to the CMI implantation group. MRI showed reduced size and irregular shape of most of the implants at 5 years, which remained unchanged at 10 years. Complication rates were similar between groups. The authors suggest this study provides surrogate evidence of the chondro-protective effect of the CMI through the preservation of medial joint space on radiographs in the prosthetic implant group. However, the study is limited by its small numbers, lack of randomisation, unmatched baseline characteristics (the meniscectomy group was older than the CMI group, significance not reported) and small numbers of patients recruited.

Implantation of the CMI implant into the lateral meniscus was reviewed in a case series of 25 patients, reviewed at 2 years [243]. Similar results were reported as seen for the medial meniscus, with significant improvements in the VAS, Lysholm, Tegner and IKDC scores. MRI evaluation using the Yulish score showed no significant deterioration in cartilage in the lateral compartment. Genovese scoring of MRI scans demonstrated most implants showed a small CMI (relative to the normal meniscus) with irregular shape. One patient complained of persistent pain following the procedure and was considered a failure. The study is again limited by lack of a control group, randomisation and short follow up period.

Hirschmann et al. [244] reviewed 67 patients undergoing either medial or lateral CMI implantation following subtotal meniscectomy of the meniscus in question. Twenty-five patients had ongoing pain at the time of surgery, the remainder were asymptomatic. The majority of patients underwent concurrent procedures at the time of implantation. At a follow up time of 12 months, 60 patients were available for review. Although significance is not reported, improvements were seen in the Tegner, VAS and Lysholm scores. No differences were observed between medial and lateral implants.

Bulgheroni et al. [18] undertook a retrospective analysis comparing 2 groups of 17 patients undergoing ACL reconstruction surgery combined with either partial medial meniscectomy or implantation of a CMI device for a concurrent medial meniscal tear. At a follow up of 9.6 +/- 2.5 years, they found that both groups showed improved patient reported outcomes with no differences between the groups. The only significant difference observed was in a subgroup analysis of acute (< 6months since ACL rupture) vs chronic injuries (>6 months since ACL rupture) which demonstrated that the chronic CMI group had significantly lower pain scores and less knee laxity in terms of anteroposterior translation.

Grassi et al. [245] conducted a systematic review of clinical studies related to the CMI. They reported on 396 patients at a mean follow up of 59 months. Notably, 79% of the patients reported on were male. Close to half of the procedures (48.2%) were for acute tears, the remainder (51.8%) were for chronic tears. The vast majority of CMI implants were medial (90.2%). Only 3% of reported patients with no additional procedure. They found that the pooled mean Lysholm score value increased at 6 months and persisted in the longer term. Reduction in the visual analogue scale for pain was also reported at 6 months and maintained to 10 years. The Tegner activity score again increased at 6 months, peaking at 12 months before decreasing from 2 to 10 years (though it did not reduce to the preoperative level). The IKDC score found the number of patients reporting their knee as ‘normal’ to increase between 6 months and 2 years following implantation, which decreased over the longer term. Complications were reported in 7% of patients, the majority of which related to swelling and pain. A similar number (6.8%) required some form of revision procedure. Despite the heterogeneity of the studies reported, overall this work demonstrates a trend towards improved clinical outcomes in patients undergoing meniscal repair with the prosthesis. The high number of concurrent procedures, the preponderance of male patients and the fact that the vast majority of implants were in the medial meniscus does limit the generalisability of the reported results. Indeed, van der Wal [246] et al. reported their experience of CMI implantation in the lateral menisci of three female patients – all of whom required some form of revision procedure due to persisting symptoms. However, Zaffagnini et al. [247] subsequently published a series of 43 patients undergoing lateral CMI implantation, followed up for a period of 2 years. Improvements in the Lysholm score, Tegner activity score and VAS score for pain were noted. Furthermore, a 95% satisfaction rate was reported. Complication rates were similar to those reported above at 6%. Notably, the authors also found that an elevated BMI predicted worse knee function and increased pain. Again, a large number of patients in this study underwent concurrent procedures.

A similar review [248] of MRI outcomes reported 6 studies, with outcomes for 194 patients. Again, the majority (83%) were medial implants, concomitant surgery was reported in up to 52% of cases and once more there was a male preponderance (79%). Significant differences were noted between 6 and 12 months of implantation. Most implants were the same size as the native meniscus at 6 months, whilst the same was true of <10% of implants at 10-year follow-up. Signal intensity from the CMI implant was also seen to reduce from initial hyperintensity relative to the normal meniscus at 6 months to isointensity at 1-2 years before becoming hyperintense again at 10 years. Small numbers made comparisons between medial and lateral implants difficult. The authors suggest that implant size reduces after six months due to the careful rehabilitation up to this point, after which joint forces compress the scaffold and it begins to be reabsorbed. The isointensity at 2 years suggests remodelling to this point, with subsequent maturation of the regenerative process and degradation occurring at some point after this, leading to hyperintensity at 10 years. Again, this review includes a small number of patients, with outcomes being reported for different patients at different times, which makes any insight gleaned from progressive changes observed questionable.

Microscopic evaluation [249] of 4 CMI implants at 6 months following implantation demonstrated that the scaffold was still visible on light microscopy, with connective tissue, extracellular matrix and blood vessels all evident. Scanning electron microscopy showed lacunae within the implants surface filled with fibroblasts surrounded by new collagen matrix with uniform diameters organising into bundles. Transmission electron microscopy demonstrated a high metabolic rate, with poor nuclear condensation and pseudopodia organising collagen bundles. A further series [230] exploring biopsies of 75 implants at 1 year following implantation showed similar results, with vascular and fibroblast invasion of 30-80% of the tissue sampled with subsequent collagen fibrils produced arranged in bundles. Resorption of the implant was found to be variable, with an average of 38% of the implant remaining, though a large variation was seen between samples, with a range of 13-69%. Six samples demonstrated mixed inflammatory infiltration, with 3 of these showing focal necrosis. The implications of this inflammatory response were unclear. The authors suggest that the infiltration of the implant is a result of synoviocytes infiltrating the implant.

2.2.7.2.3 Comparing implants

Due to limited clinical experience, especially with the Actifit prosthesis, there is limited work comparing outcomes from both implants. Spencer et al. [207] published a case series of 23 patients, 12 underwent CMI implantation whilst the remainder underwent Actifit implantation. Both groups showed statistically significant improvements in clinical outcome scores, though more scores reached significance in the CMI group. Two patients reported no improvement – one of these had undergone CMI implantation and suffered a recurrent tear, she was revised to an Actifit prosthesis. The other patient had significant arthritic change at the time of implantation and suffered ongoing pain – the implant used is not reported. Arthroscopic evaluation performed in 14 patients at a mean of 12.8 months, showed >50% tissue ingrowth in 4 of the 5 evaluated patients in the Actifit group, the same was true of only 4 of 9 assessed patients in the CMI group. No patients showed progression of arthritis using MRI scans at a mean of 19 months post-surgery. The reported series is small, with no effort to match baseline characteristics. There is also a significant male preponderance overall, it is unclear how this is spread between groups.

More recently, Bulgheroni et al. [216] have demonstrated no significant difference in patient reported outcomes between the two implants (28 CMI and 22 Actifit). MRI evaluation was performed in 26 CMI and 21 Actifit patients. This group reported characteristics between groups in more detail, such as a similar size of graft and time from injury in each group. Notably, there were more significantly more chronic injuries in the Actifit group. There was a trend towards younger patients in the Actifit group. and However, they noted more vascular tissue was evident in the CMI group as compared to the Actifit group, which were completely avascular. The latter represents the state usually found in the meniscus. Both implants showed significant improvements in the Lysholm and Tegner scores between preoperative levels and one year follow up. Multiple regression suggested that no differences in Lysholm and Tegner scores existed between implants at maximal follow up of 2 years, but that female patients and those with concomitant procedures showed less improvement. Rates of complications were similar between groups. MRI at 2 years showed that all but one CMI implant were partially reabsorbed. Scaffolds isointense to the native meniscus were seen in 39% of CMI patients and 21% of Actifit patients. Arthroscopic evaluation showed no progression of arthritis in either group. Biopsy specimens showed more fibrous tissue rich in cells and blood vessels in the CMI group, whilst the Actifit group demonstrated avascular tissue with some cellular infiltration. This is the largest study to date reporting on outcomes between the implants and is limited by its small numbers, as well as the dominance of chronic patients in the Actifit group. Again, concomitant procedures were also performed in both groups. There is need for a prospective, randomised trial exploring outcomes related to both implants to establish their comparative clinical effectiveness.

2.3 Literature review summary and study rationale

Following the recognition of the various roles of the meniscus, in particular its role in load transmission, there has been renewed interest in the organ. We now understand that the meniscus plays a variety of roles and its removal has significant consequences. However, partial resection of the meniscus is still the preferred treatment for patients of middle age, with repair limited to younger patients with tears amenable to repair techniques. Although total meniscectomy is now rarely practiced, arthroscopy for partial meniscectomy secondary to meniscal tears is a common procedure. The reason for undertaking resection of an organ with proven function is the limited healing potential of the meniscus, coupled with a developing understanding of meniscal function and a lack of proven prosthetic replacements.

There is recognition that a paradigm change in treatment may be necessary – that we should aim to ‘save the meniscus [250].’ To do so, we require detailed evidence on the contribution of the meniscus to knee function. We have such evidence present in a number of sources which demonstrate that progressive meniscal injury results in altered tibiofemoral contact mechanics, increasing peak contact pressures and decreasing contact areas, which will presumably result in accelerated wear of both chondral surfaces. Coupled with this, we have some evidence that isolated meniscal injury results in instability of the knee. However, this evidence is limited to particular injury states – Arno et al. [12] explore partial meniscectomy of the posterior horn of the medial meniscus, whilst Ahn et al. [158] explored longitudinal tears of the medial meniscus posterior horn. Goyal et al. [11] explored vertical tears of the lateral meniscus, progressing to a partial meniscectomy, whilst Allaire et al. [13] explored posterior root tears in the medical meniscus. Whilst the role of radial tears in contact mechanics has been explored [83], to date, no detailed study of the role of radial tears in static tibiofemoral stability has been conducted. Hence, this thesis aims to explore the role of such tears as related to injuries in both the medial and lateral menisci.

As well as this, although we understand the role of the collagen fibres within the meniscus in generating hoop stresses, no study has been undertaken of how the proteoglycans within the meniscus function. Study of both cartilage and intervertebral disc has demonstrated that proteoglycans within these tissues contribute to the tissue stiffness through ionic effects, hence this thesis will seek to investigate whether a similar mechanism is at play within meniscal tissue. This knowledge will aid development of meniscal substitutes – whether in the form of synthetic replacements or meniscal scaffolds.

Indeed, meniscal scaffolds have emerged as potential treatment option for patients with irreparable meniscal tears. There are numerous clinical studies, albeit with small numbers, which present improved patient outcomes following implantation of these prostheses. In the longer term, we require studies exploring whether these implants are indeed chondro-protective – do they delay the onset of osteoarthritis? However, In addition, it must also be ensured that these prostheses adequately mimic meniscal function whilst they remain in situ and also safeguard the structure and function of the surrounding joint. This thesis will also aim to explore the mechanical properties of the Actifit meniscal scaffold.

The goals of this thesis are therefore to broaden our understanding of meniscal function, both at a macroscopic and microscopic level. As well as this, this work seeks to evaluate whether existing meniscal regeneration options, in the form of meniscal scaffolds, help mimic the function of the native meniscus.

2.4 Aims

To achieve the goals described above, a three-pronged approach will be adopted:

2.4.1 The role of the meniscus as a primary stabiliser of the knee

To date, there has been no investigation of whether radial tears of the meniscus result in altered kinematics of the knee. Radial tears are most common at the junction of the anterior two thirds and posterior third of the medial meniscus, whilst in the lateral meniscus they most often occur at the junction of the anterior third and posterior two thirds, hence these are the tear states that will be investigated. The null hypothesis of this element of the thesis is as follows:

Radial tears of the medial/lateral meniscus do not affect the kinematics of the cadaveric human knee.

The aims of this element of the thesis are as follows:

- To develop and test a jig which will allow a human knee to be fixed in it and will itself articulate with a materials testing machine
    - The jig will allow flexion of the knee from 0-90 degrees. Rotation in the flexion/extension axis will be fixed by the jig.
    - Load will be applied through the femur, hence the femur will move in the supero-inferior plane.
    - The tibia will be free to move in the antero-posterior and mediolateral planes. It will also be able to rotate into varus/valgus and internal/external rotation.
- To test the jig apparatus using synthetic or animal cadaver knees and develop a method for optical tracking.
- To obtain human cadaveric knees for testing.
- To introduce meniscal injury using arthroscopic instruments to allow preservation of the soft tissue envelope around the knee.
- To measure kinematics of the knee in the intact and injured states using a motion analysis system.
- To analyse this data using a local joint coordinate system and hence test the null hypothesis.

2.4.2 The role of proteoglycans in material properties of the meniscus

Whilst the histology of the meniscus is well understood, the functional role of proteoglycans in the meniscus, in particular with regard to their ionic effects, has yet to be determined. If the mechanical properties of the meniscus are influenced by ionic effects, which in turn are most likely driven by a Donnan osmotic pressure created by proteoglycans, testing these properties whilst the tissue is bathed in solutions of varying ionic concentrations should result in alteration of such properties. Hence, the null hypothesis is:

Mechanical properties of meniscal tissue do not vary in solutions of varying ionic concentration.

The aims of this element of the thesis are as follows:

- To develop an apparatus to allow testing of meniscal samples in a confined compression configuration within a materials testing machine.
- To test this apparatus using animal meniscal samples
- To conduct testing on cadaveric human menisci.
- To conduct testing on menisci taken from patients suffering osteoarthritis during total knee replacement surgery.
- To quantify the proportion of proteoglycans present in samples tested.
- To use finite element modelling to inversely engineer mechanical properties of the meniscus samples tested and thus test the null hypothesis.

2.4.3 Material properties of meniscal scaffolds

Whilst the scaffold is not a meniscal substitute, it is designed to remain in situ for up to 4 years before it is fully degraded. Weight bearing is commenced in the weeks following implantation, hence similar properties to the native meniscus will help fulfil the scaffold fulfil some of its functions. The null hypothesis here is:

There is no significant difference between the mechanical properties of the Actifit meniscal scaffold and human meniscal tissue.

- To obtain samples of the Actifit meniscal scaffold to allow testing.
- To conduct confined compression testing as described for the meniscus samples above to investigate the mechanical properties of scaffolds as compared to the meniscus.

- To use finite element modelling techniques to inversely engineer mechanical properties of the samples tested.

3. The role of the meniscus as a primary stabiliser of the knee

3.1 Methods

Prior to commencing the project, ethical approval was sought and granted from the University of Strathclyde Ethics Committee. Furthermore, all work was undertaken in concordance with The Human Tissue (Scotland) Act 2006.

3.1.1 Jig development

In order to allow load to be applied to the knee in a reproducible and measurable manner, a custom jig apparatus was developed. The requirements for this were:

1. To contain a human knee within the jig;
2. To allow the native kinematics of the knee to be recreated. Hence the tibia of an implanted cadaveric knee would be allowed to move in all 6 degrees of freedom relative to the femur; with the exception of the flexion/extension axis, which could be fixed between 0-90 degrees. This arrangement mirrors the physiological state in that the tibia can move in all 6 degrees of freedom relative to the femur, though extension is possible from -10 to 150 degrees of flexion;
3. To allow fixation of the knee despite application of loads up to 2000N;
4. To allow fixation of the jig, containing a cadaveric knee to an Instron E10000 materials testing machine;
5. To allow access to the knee joint to allow fixation of passive tracking spheres to allow tracking of movement;
6. To allow access to the knee joint such that an arthroscope could be inserted to introduce meniscal injury.

Design for the jig was based loosely around custom testing apparatus described by Allaire et al., Ahn et al. and Arno et al. [12,80,158]. To allow the effect of knee flexion to be tested, the jig was designed to allow locking of the flexion angle between 0-90 degrees. The superior and inferior elements of the jig were designed to interface with an Instron E10000 (Illinois Tool Works Inc, Norwood, Massachusetts, United States) materials testing machine to allow application of axial load. This was achieved through the use of large screws to secure the jig – the necessary dimensions were obtained from the Instron E10000 specification sheet.

To accommodate any inherent varus/valgus within knees tested, the jig was designed to allow the orientation of the femoral component to be varied in varus/valgus between -8 to +8 degrees in 2 degree increments from the vertical to account. This could be adjusted such than any load was applied with the tibia vertical.

Initial sketches of the proposed design were made by hand (Figure 3-1).

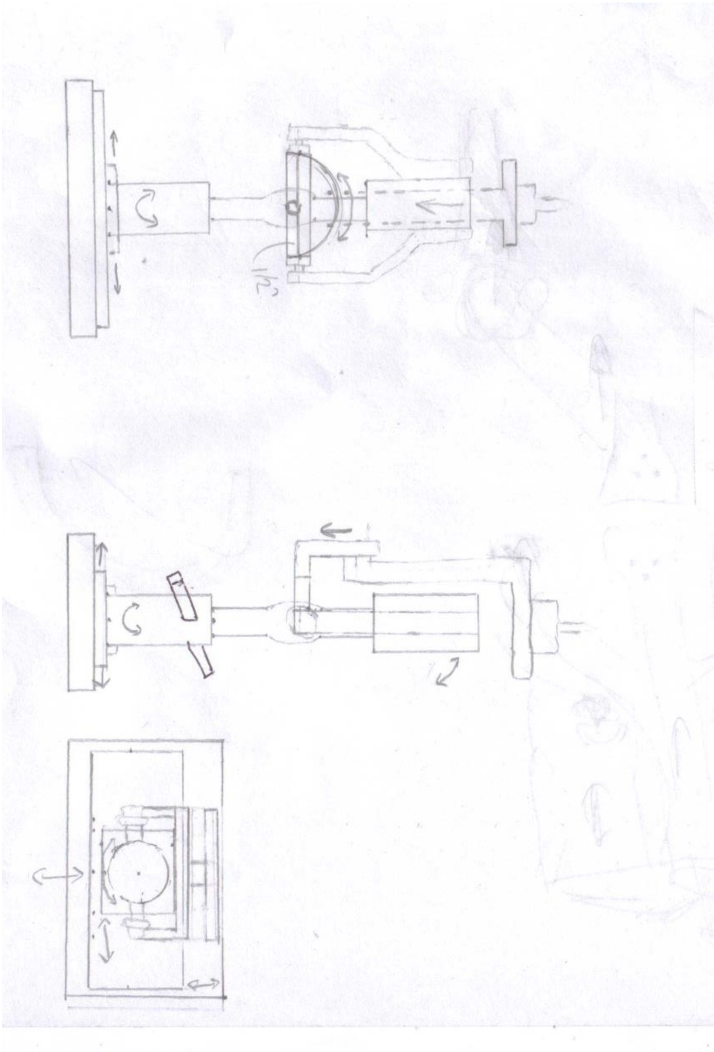


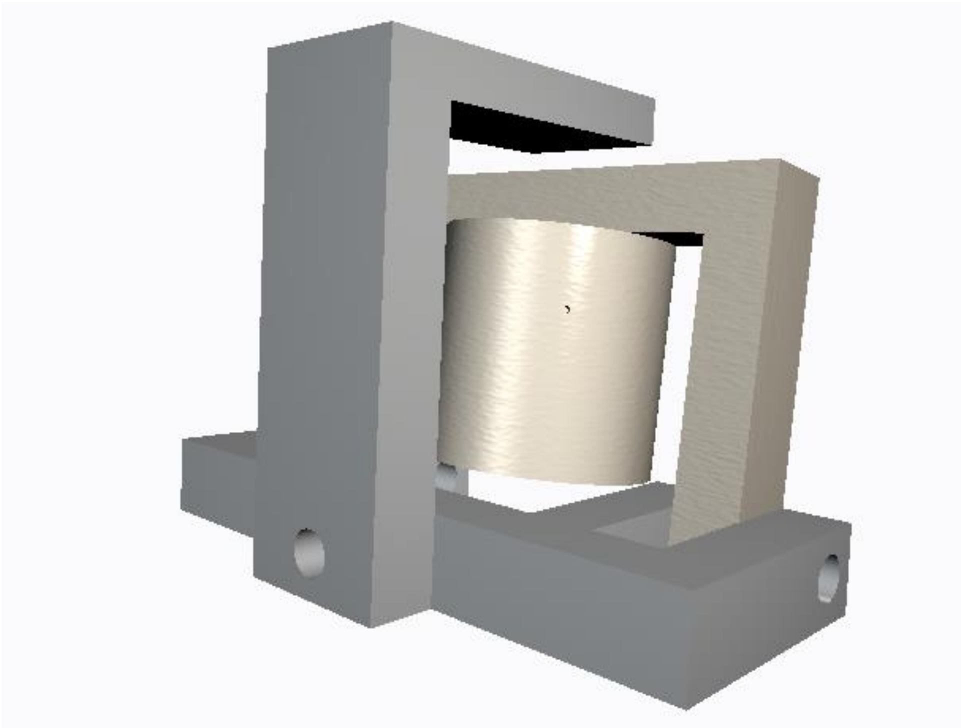
Figure 3-1 - Concept drawings of jig.

The jig had two components, with two cylinders – one to accommodate the femur and the other for the tibia. The movement of the superior (femoral) component was limited due to its articulation with the Instron E10000, however the bottom (tibial) component was free to move in 5 degrees of freedom except the flexion/extension axis which was restricted by the jig. The angle of the femur in the global coordinate system was fixed through use of two metal pins which fixed the position of the femur relative to the Instron either at 0 degrees (full extension) or at 15, 30, 45, 60 and 90 degrees of flexion.

The initial concept was developed using computer aided design techniques with Creo Parametric (PTC Inc) software. Each part of the jig was designed separately. 3D printing was used to trial a number of different designs (Figure 3-2). 3D printing of designs allowed review of the proposed design and some modifications were made on this basis. In particular, a mortice type design was chosen for the interface between the rotating part of the top segment and the rest of the jig to ensure maximal stability and lower the risk of failure under load.

As well as this, large bolts were used to allow rotation of the femoral component such that the angle of flexion could be varied. 10mm diameter bolts were calculated to be sufficient to withstand a load of 2000N with a 2x factor of safety.

Technical drawings of initial drawings for each component of the jig are shown in Appendix 1 – Technical drawings of jig components. These were used to specify the design required and the final design was crafted from aluminium at the Department of Biomedical Engineering workshop. The jig was built in a modular fashion to allow any components to be changed or replaced as required. Notably, in the first iteration of the jig, an Igus Drylin linear bearing system was used for the bottom stage, simplifying production (Figure 3c). These systems are designed to allow linear gliding without lubrication. The system was designed to allow initial fixation of bone within the jig using 4x5mm screws placed at 90-degree intervals in each cylinder.



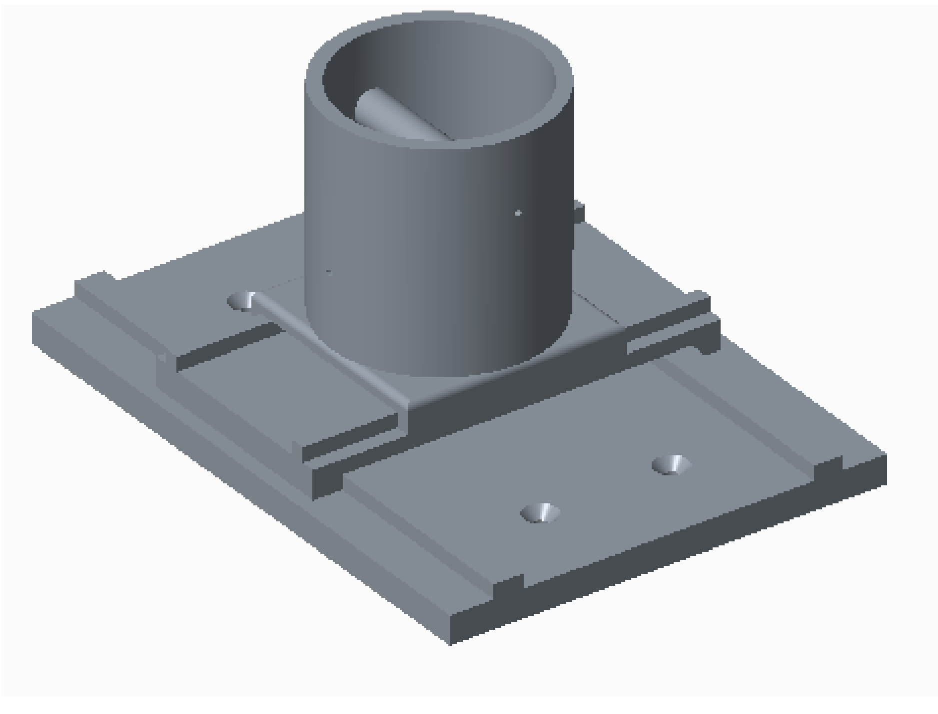


Figure 3-2 - Final drawings of jig designed using CREO software

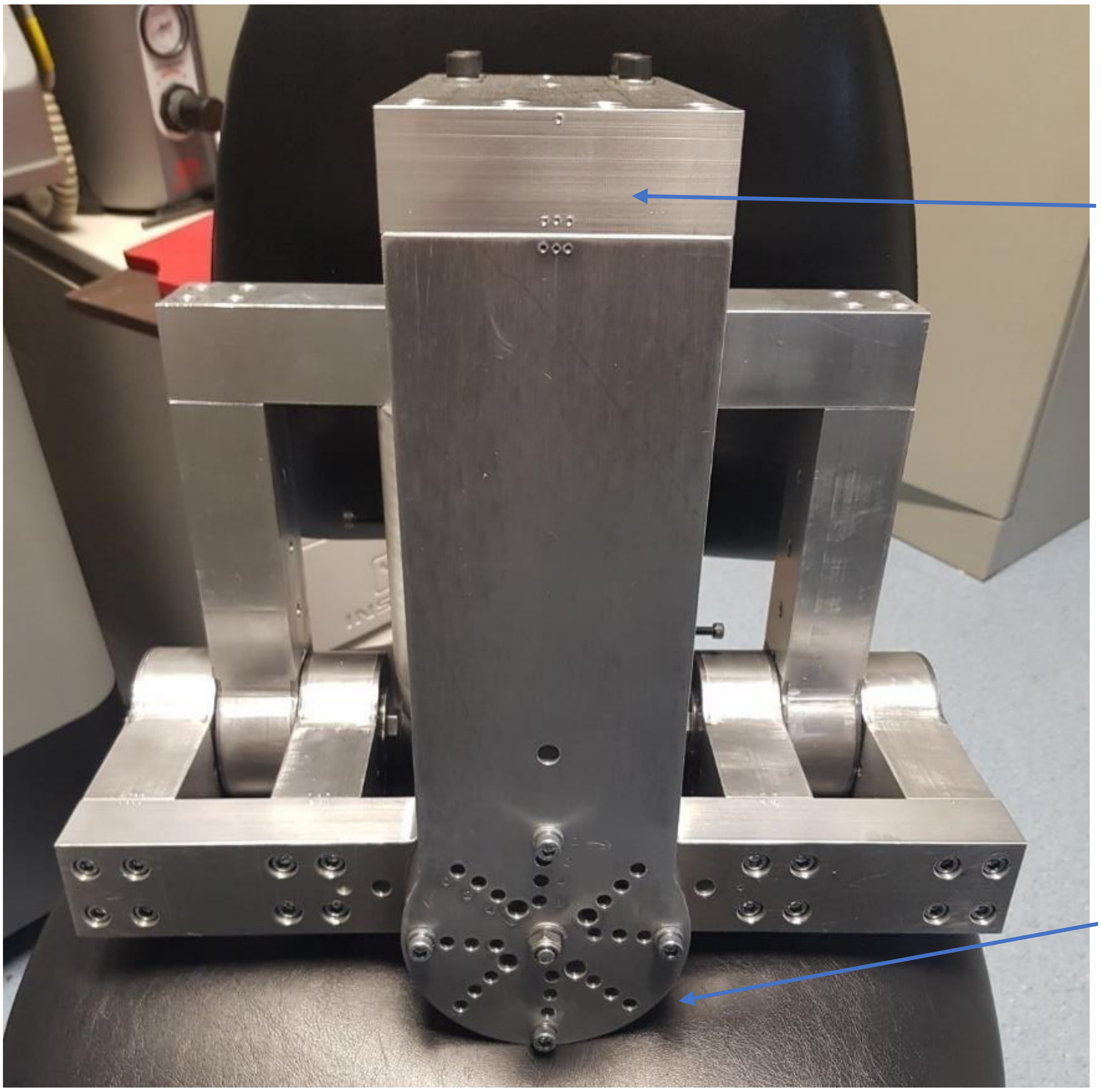


Figure 3-3 - Jig - Anterior view of the femoral component.

An anterior view of the first iteration of the jig is shown in Figure 3-3 - note the circular articulation between the superior and inferior portions of the jig to allow changes in varus/valgus angle to be locked prior to testing, Figure 3-4 shows an oblique view, note the cylinder for potting the femur. Note also the nut, which can be loosened to alter the flexion angle. Finally, Figure 3-5 shows the tibial portion of the jig, note the Igus Drylin bearing system, designed to allow linear gliding. Using this, it was possible to orient the tibial cylinder anywhere within the frame of the jig's tibial component.

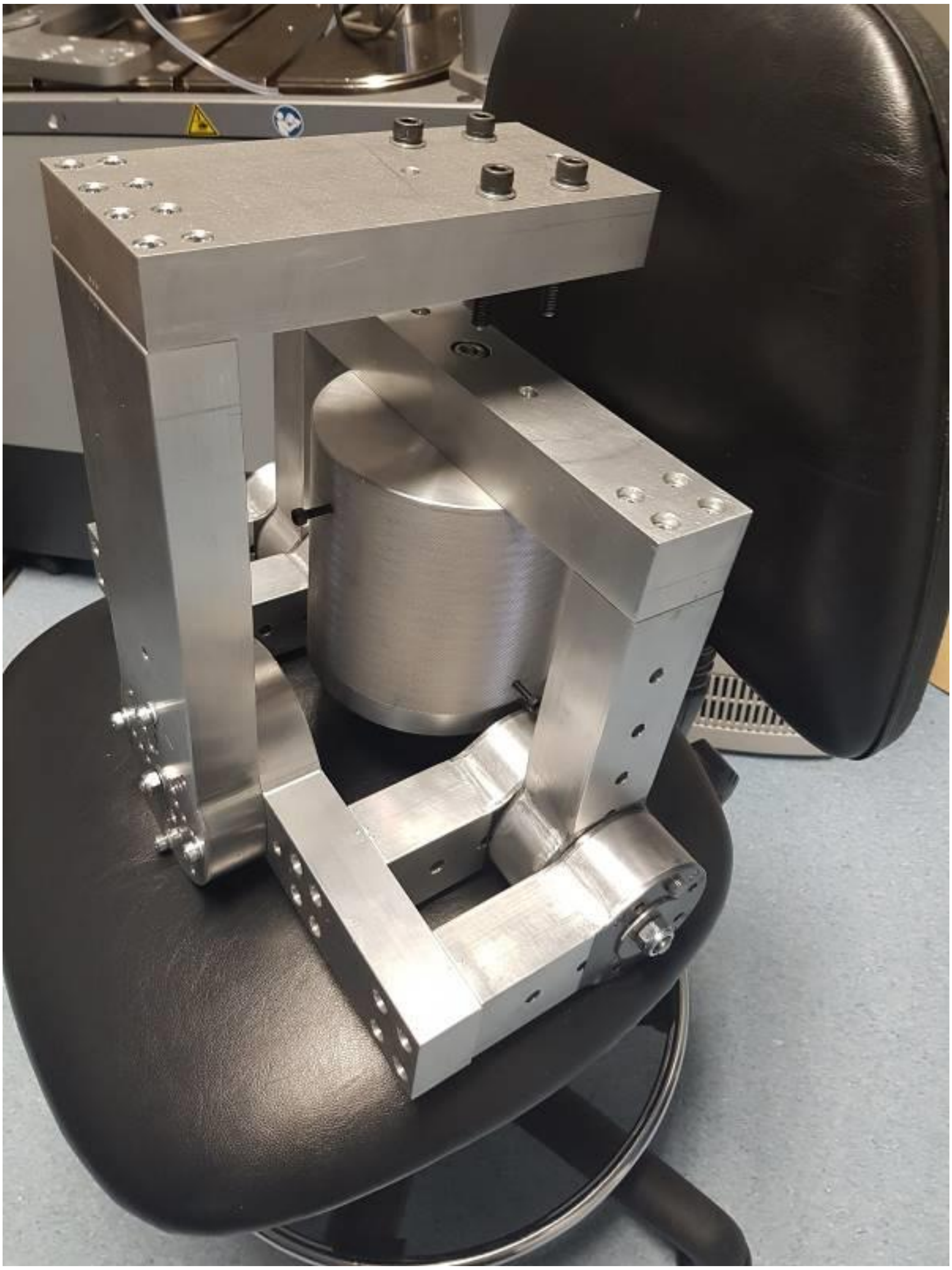


Figure 3-4 - Jig - Oblique view of the femoral component

The size of the jig was governed by two factors – that it was necessary for it to fit within the stage of the Instron materials testing machine and that it was required to contain a human knee cadaver. Required dimensions for the former constraint were obtained from the specification sheet of the Instron E10000 materials testing machine. Mean dimensions of human body thigh and calf segments, as well as leg length were obtained from a number of published sources [251,252] and used to calculate the dimensions of cylinders required. A 10mm diameter, 100mm length threaded rod was placed at the centre of the base of each cylinder, to allow the intramedullary canal of the femur/tibia to be fixed in place in the centre of the cylinder.

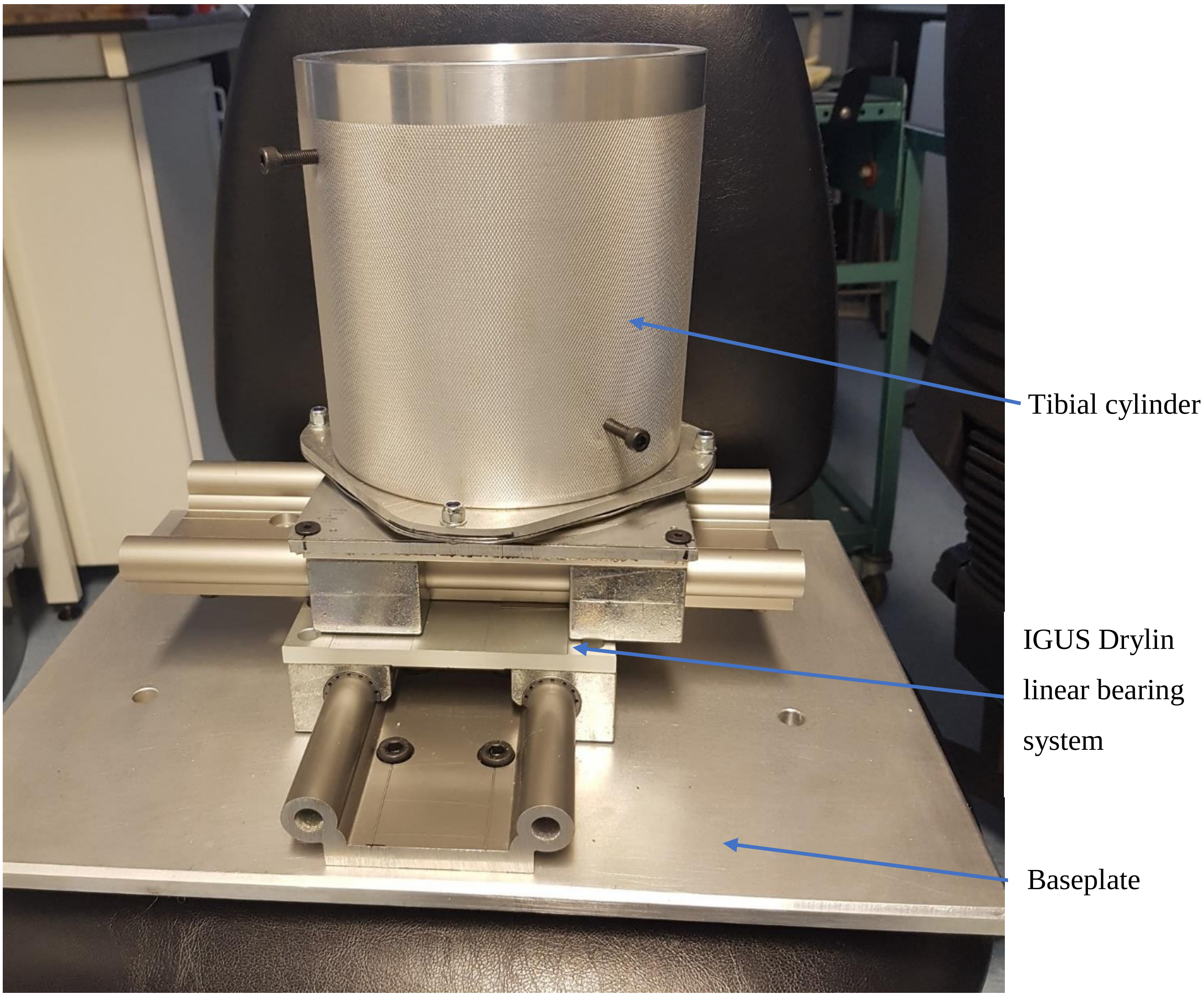


Figure 3-5 - Jig – tibial portion

The jig allowed articulation with both the stage and actuator of the Instron materials testing machine.

It was verified that the jig allowed full movement of both saw bone and human cadaveric leg from 0-90 degrees. The knee was then tested under load. It was observed that although the tibial component of the jig allowed rotation of the knee as the knee was loaded, no antero-posterior or medio-lateral movement of the tibia was observed. Manual application of shear allowed such translation with ease. It was theorised that whilst the Igus Drylin linear bearing system used in the tibial aspect of the jig allowed gliding when force was applied in the plane of movement, application of load orthogonal to the plane of movement was in fact inhibiting movement. It was therefore necessary to redesign the lower portion of the jig.

A number of options were explored. In an effort to allow maximal movement, a system which would leave the bottom cylinder free to move was preferred. As such, the use of ball transfer units, used for transport of cargo in confined spaces was explored. A set of 9 Alwayse 3-hole 32mm Steel ball transfer units were kindly donated by Alwayse for use in the project. These were arranged in a 3x3 array on a base plate designed to fit into the Instron materials testing machine (Figure 3-6). The bottom part of the tibial cylinder was redesigned with a flat plate, which would glide over the ball transfer units, allowing free movement. This design was re-tested with both saw bone and cadaveric samples, allowing both rotation and translation of the tibia under load.

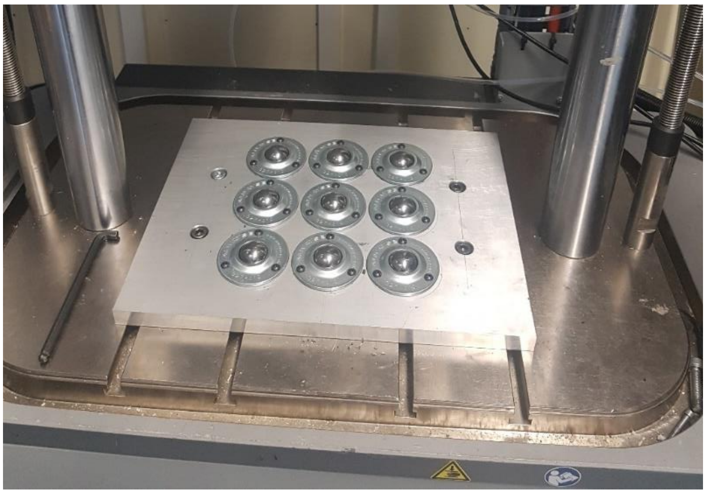


Figure 3-6 - Redesigned base plate with Alwayse ball transfer units

3.1.2 Jig testing and tracker development

Following manufacture of the jig, testing was conducted using sawbones and animal bones to ensure the experimental protocol could be performed quickly and efficiently once human cadavers were available.

3.1.2.1 Fixation

Testing using sawbones demonstrated that the knee was well contained within the cylinders, which allowed full extension and flexion of the knee. Following this, 4 bovine knees were obtained from a local abattoir. These were tested in turn. The initial sample was fixed in place using the screws placed in the jig cylinders. However, it was evident that a more robust form of fixation would be required – firstly it was possible to move the knee within the cylinder if force was applied by hand, hence it would unlikely bear physiological loads without moving. Secondly, the significant length of screw required (~100mm) risked fracture of the screws as load was applied.

Permission was sought from the University Safety Services to use bone cement to fix the legs in situ. Unfortunately, due to the lack of availability of a fume hood, coupled with the large quantities of cement required, this request was denied as there was a risk of toxic fume production with use of such cement. A decision was made, therefore, to use commercial Portland cement for fixation.

A comparison was made in using cement as compared to a concrete mix. The latter was found to provide inadequate fixation due to the aggregate material forming an inadequate seal around the tissue, hence a pure cement mixture was used. Appendix 2 - Cement mixing protocol details the cement mixing protocol used.

Due to the configuration of the leg within the jig, the femoral component was cemented first in an inverted configuration. The window cut in the femoral cylinder was obturated using a piece of foam during cementing. Through trial and error, it was found that a period of two hours allowed the cement to set sufficiently for the femoral cylinder to be turned the right way up and fixed within the jig, with no slippage of the bone within the cylinder. The tibia was then cemented in place whilst the jig was fixed within the Instron materials testing machine. The whole apparatus was left overnight to allow the cement to set fully.

Following any testing, the cement was broken up manually using a chisel and hammer to allow the next sample to be fixed. The excellent fixation achieved was evidenced by the significant amount of time required to loosen each sample.

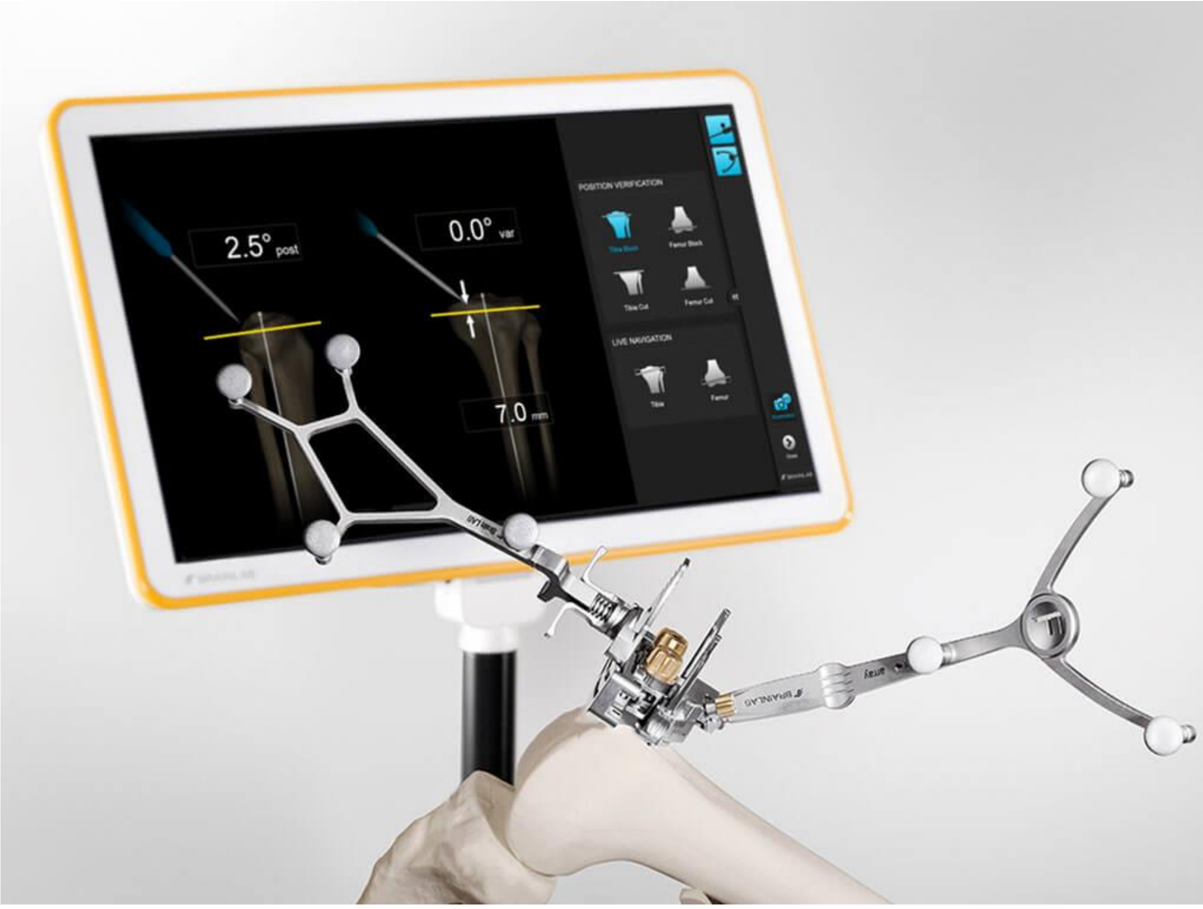


Figure 3-7 - Commercially available navigation tracker (from http://www.brainlab.com)

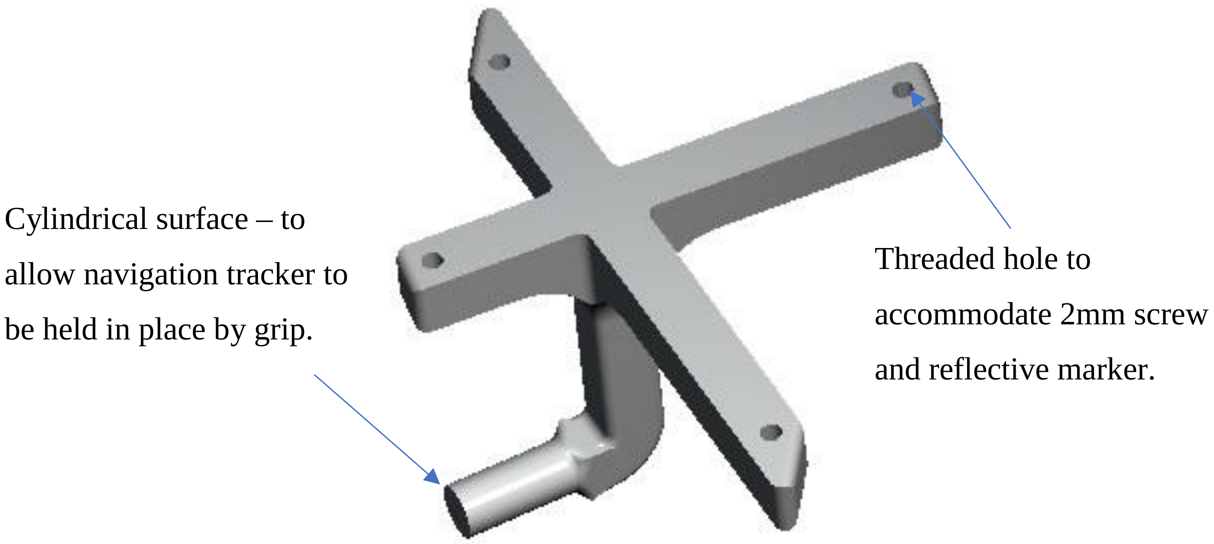
3.1.2.2 Optical tracking

To measure the degree of static instability present in each sample tested, an optical tracking system was used. This technique has previously been used for similar work in the literature [9]. To allow tracking, it was necessary to affix reflective markers to the femur/tibia respectively.

An attempt was made to source navigation trackers used in navigated total knee replacement surgery – however this was not possible, and the cost of these components made buying them prohibitive. Hence, PTC Creo software was used to design and print two unique navigation trackers which could be used for tracking purposes. The design of these was based loosely on commercially available navigation trackers, such as that shown in Figure 3-7**Error! Reference source not found.**.

The trackers were designed such that they could accommodate a 3.5 mm threaded screw, which could then be used to affix a 10mm diameter reflective circular marker to each arm. This would allow replacement of the reflective marker if contaminated or damaged.

Figure 3-8 shows a PTC Creo rendered drawing of one of the reflective markers designed. These were 3D printed at the department's workshop and manually tapped to accept the appropriate screw size.



<u>Figure 3-8 – 3D rendering of navigation tracker designed in PTC Creo</u>

The flat 10mm spherical reflective markers were threaded and four were attached to each tracker. The navigation tracker was designed with a cylinder on the opposite end to the reflective markers which could be used to grip the tracker in place. This design is similar to that used by commercial trackers, though these have custom built attachments for this purpose.

In a clinical scenario, during total knee replacement surgery, the navigation pins are freely visible from most angles around the tables, though they may be obscured by the surgeon or surgical assistant. However, in this setting there were numerous obstacles which could potentially conceal the trackers from visibility, including:

1. The jig itself, depending on configuration – at higher degrees of flexion, the jig would either obscure the trackers or, depending on their position, could cause them to overlap.
2. The Instron materials testing machine had two large pillars on both sides of the jig, which completely obscured the trackers from view.
3. The large anterior guard on the Instron materials testing machine was composed of clear Perspex – this was reflective and would cause interference with marker visibility.
4. The physical space surrounding the Instron materials testing machine was limited such that it was difficult to place cameras in a configuration such that all markers would be visible, and the cameras would not obscure each other.

Therefore, it was felt important that the navigation trackers were affixed using a method which allowed their orientation to be altered with the jig in place to ensure they were visible.

In clinical practice, these trackers are affixed to the anterior aspect of both femur and tibia as these surfaces are easily accessible, lie in the operative field and are removed from any critical neurovascular structures. However, in the current configuration, both anterior and posterior surfaces were accessible and there were no concerns regarding physiological integrity. As the knee was flexed within the jig, any tracker affixed to the anterior aspect of the femur would strike the top portion of the jig, hence it was necessary to attach the navigation trackers posteriorly. Although sufficient bone on the tibia was visible, it was necessary to cut an oblong slot through the femoral cylinder to allow access to the posterior aspect of the femur for navigation tracker fixation.

A number of techniques were trialled to allow such fixation. Firstly, a flexible arm (Figure 3-9) was purchased. This allowed the navigation tracker to be gripped at one end. Two holes were drilled in the opposite end to accommodate two 4mm bone pins. These pins were then fixed to bone to allow the navigation trackers to be attached.

Figure 3-9 - Flexible arm to allow navigation tracker fixation

Although this configuration allowed excellent control of the navigation tracker orientation throughout the range of motion of the jig, the arm was too flexible to allow reliable navigation tracking, as although it allowed secure fixation to bone and also held the tracker securely, it would oscillate following any movement, rendering it useless.

Instead, two commercially available ‘Magic arms’ designed to hold DSLR cameras were purchased (Figure 3-10). These allowed either end of each arm to be moved in 3 degrees of freedom and then secured in that position, with no movement of the arm or navigation tracker.

Figure 3-10 - DSLR 'Magic arm'

The 3d printed navigation trackers were glued to one end of the ‘magic arms,’ whilst two holes were drilled in the opposite end to accommodate two 4mm bone pins, commonly used in Orthopaedic surgery. The apparatus was secured to bone using these pins, this fixation was found to be far superior to that using the previous method, as once the screw on the arm was tightened, a rigid construct was formed. Figure 8 illustrates two magic arms in place with a bovine specimen in the jig. The arm could be loosened to allow the orientation of the navigation tracker to be altered in any dimension and then fixed in place. Figure 3-11 illustrates this system in use, with a navigation tracker attached to the femur, whilst only the

Figure 3-11 - Jig fixed in place with navigation tracker attached to femur.

‘Magic arm’ is attached to the tibia. Note that the leg is oriented such that the popliteal fossa is facing the femoral navigation tracker (i.e. the kneecap is facing away from the photographer).

Although this method was superior to that previously trialled, there were still two significant issues. Firstly, although the navigation trackers could be oriented to remain visible from a single position at any point in the flexion/extension arc, it was necessary to change their orientation past 30 degrees of flexion as either the reflective markers would become invisible or they would begin to overlap or strike one another.

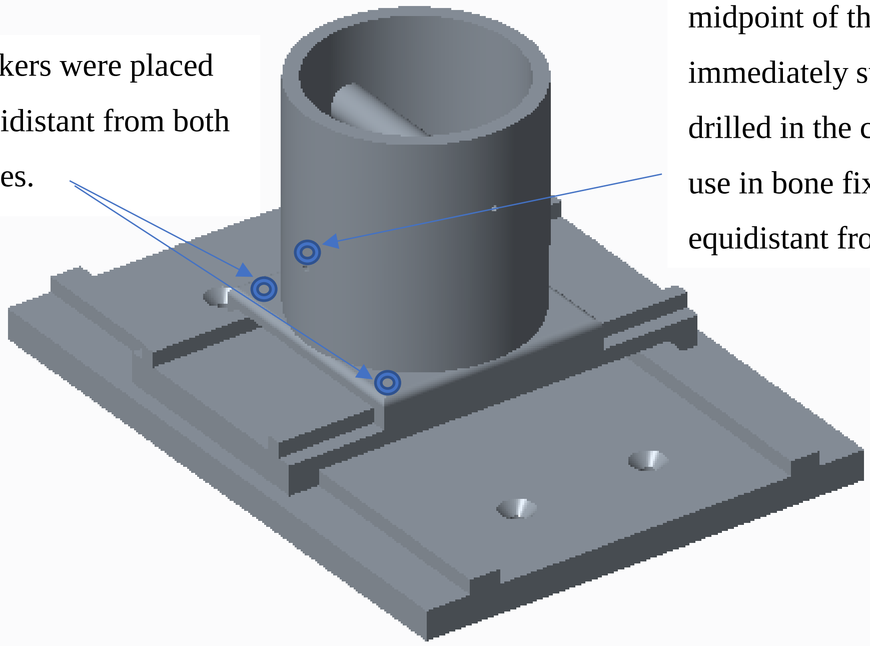
A decision was made, therefore, to attach the reflective markers directly to the surface of the jig and subsequently capture the position of relevant bony landmarks to allow calculation of their relative positions. Markers were placed as illustrated on the diagram below (Figure 3-12).

This placement was designed such that the centre marker in both halves of the jig was aligned with the long axis of the bone in question. This was achieved by placing the marker at the centre of the cylinder – the intramedullary canals of the bones themselves were aligned with the centre of the cylinder by impaling the cut ends of the bones on the threaded rods placed at the centre of each cylinder prior to cementing. Markers were changed between samples tested to ensure the reflective coating remained visible.

This configuration of markers would subsequently allow the calculation of a coordinate system to calculate relative movement of the tibial/femoral halves of the jig.

These markers were placed 20mm equidistant from both corner edges.

The third marker was placed at the midpoint of the baseplate, immediately superior to the hole drilled in the cylinder, (intended for use in bone fixation), leaving it equidistant from the other markers.



These two markers were placed at the midpoint of the two struts within the femoral portion of the jig.

The third marker was placed at the centre of the superior part of the jig, at the midpoint of the cylinder and equidistant from the other two markers.

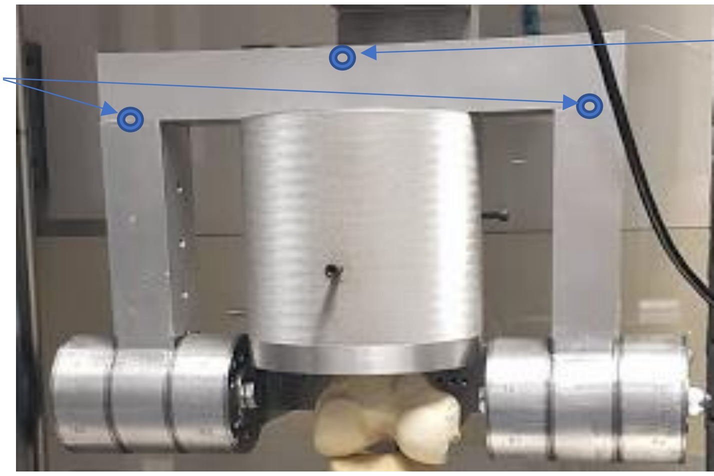


Figure 3-12 - Reflective marker placement

3.1.2.3 Camera placement

As alluded to previously, a further challenge lay in determining optimal placement of the cameras used for motion tracking. A Vicon 612 motion analysis system was used for motion capture. To allow the cameras to see both navigation trackers, it was necessary to place the cameras in front of the Instron materials testing machine, with the knee placed in the jig facing the back of the machine. A number of configurations were tested, as illustrated below (Figure 3-13).

<u>Figure 3-13 - Testing camera configurations</u>

Notably, plastic bags were required to conceal reflective components of the experimental field. Unfortunately, before any data could be collected using the Vicon system, the workstation which permitted data collection and interfaced with the system PC developed a fault. A further workstation was obtained, but this too had the same issue.

It was therefore necessary to seek a replacement motion tracking system. An Optitrack motion analysis system was sourced. The system used Optitrack Flex 3 cameras, coupled with Motive software for analysis. Notably, both the Optitrack and Vicon motion capture systems have been validated for use in capturing kinematic data during biomechanical testing [253]. The smaller size of the cameras allowed these to be wall mounted and presented a

Figure 3-14 - Optitrack camera configuration

much simpler solution for camera placement. With the knee oriented in the Instron materials testing machine such that the patella was facing forward, the Optitrack cameras were mounted in behind the Instron machine. A total of 6 cameras were used, Figure 3-14 shows the camera positions. Note the significantly smaller space required to mount the cameras. Given that the cameras were placed behind the area where the jig was removed and fixed to the Instron materials testing machine, there was no risk of dislodging or otherwise moving the cameras during testing.

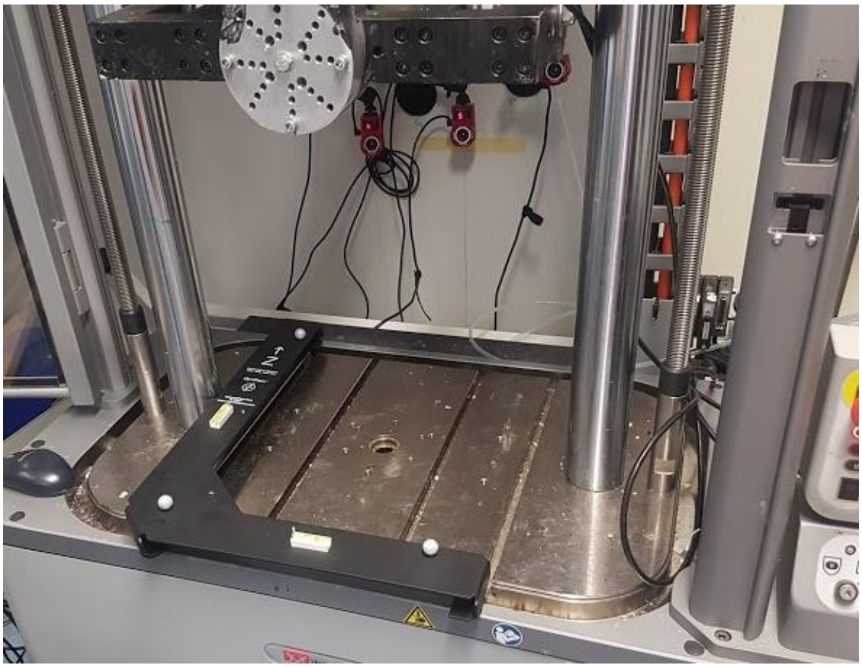


Figure 3-15 - Calibration square

Prior to each sample being tested, the Optitrack motion tracking system was calibrated as per the manufacturer's instructions. A calibration square was used to undertake static calibration, with the origin placed at the anterior corner of the Instron materials testing machine. Dynamic calibration of the system was undertaken using a proprietary wand designed for use with the Optitrack system (Figure 3-16). Calibration was undertaken using the Optitrack software, which identified areas within the capture field where more samples were required and alerted the user when sufficient samples had been taken for the system to be calibrated. No output regarding the calibration beyond the mean error was presented. Calibration was complicated by the small field within which capture was necessary, though it was possible to obtain calibration for all samples with a mean error of 1μm.

Figure 3-16 - Optitrack calibration wand

Following calibration, the top and bottom array reflective markers were registered as two ‘rigid bodies’ within the Optitrack system. A further rigid body was defined for a blunt probe with reflective markers, designed for use with the Stryker Mako surgical robot system and borrowed for the project (Figure 3-17).

3.1.2.4 Cadaveric testing

Although testing using sawbones and animal knees had allowed some validation of the experimental apparatus, it was felt important to perform some testing using a human knee prior to any experimental work. This was particularly the case as the animal samples were either too large (bovine) or too small (ovine) for the jig, such that either full flexion could not be achieved.

To allow testing of the jig and validation of the experimental set up, a cadaveric human knee was obtained from the University of Dundee anatomy department. The knee was preserved

Figure 3-17 - Mako blunt reflective probe

using the Thiel embalming technique, allowing the tissue to retain physiological movement whilst stored at room temperature for an indeterminate period.

A mid femur to mid tibia specimen from the left knee of a 72-year-old male was obtained. A soft tissue envelope of 10 cm both above and below the knee joint was left intact, with all remaining soft tissue removed to expose the femur and tibia/fibula. To accommodate the specimen within the jig, the femur was cut at 16.4 cm from the joint line, based on the distance from the centre of rotation of the jig to the roof of the femoral cylinder (Figure 3-18).

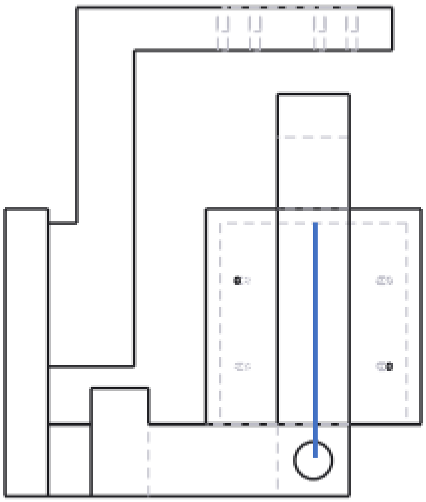


Figure 3-18 - Measurement of length of femur required to match jig centre of rotation

Following this, the exposed tibia was fixed to the fibula using a 4mm screw to prevent free movement of the bones whilst cemented (Figure 3-19). This mirrors the physiological situation where movement of these bones is prevented by their articulations at the ankle joint and the presence of an interosseous membrane. This approach is also used in a number of the studies described above [85,254].

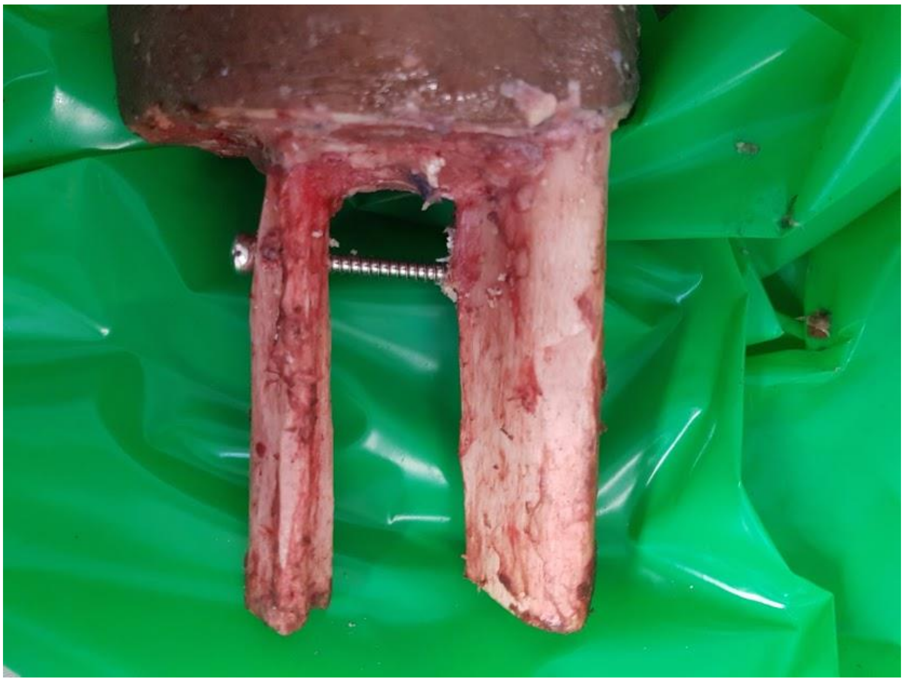


Figure 3-19 - Screw fixing tibia to fibula

As well as this, two 4mm diameter screws were placed uni-cortically in both the femoral and tibial cortices at 180-degree angles (Figure 3-20). This measure was adopted to allow rotational stability of the construct whilst cemented.

Figure 3-20 - Screws placed in femoral cortex

The intramedullary canal of the femur was then aligned with the rod in the femoral cylinder and the femoral portion of the knee placed within the femoral cylinder. Following this, cement was poured into the cylinder. The hole in the femoral cylinder (cut for placement of navigation pins) was now redundant and obscured using a large piece of foam cut to size. The cement was allowed to set for 2 hours and the femoral cylinder was then fitted into the jig, itself mounted in the materials testing machine.

The tibia was then cemented in place using a similar protocol. Notably, the length of the tibia was irrelevant as any increase/decrease required could be accommodated by moving the Instron actuator up/down. The jig was left overnight to allow the cement in both components to set.

A full range of motion was tested with loads from 0-1500N. The Thiel embalmed cadaveric knee allowed a full range of motion and the ball transfer units accommodated small movements well, allowing the tibial baseplate free movement. The reflective markers were also visible throughout the range of movement of the jig, with the Optitrack cameras arranged as noted above. Notably, reflection from the jig itself was noted to be a significant issue with the Optitrack system, hence all non-moving parts of the jig were spray painted black.

Unfortunately, the distal femur was fractured during loading of the Thiel embalmed cadaver prior to the Optitrack system becoming available. This occurred at a 90-degree angle under 1500N of load, which in retrospect was too large for the cadaveric femur to accommodate. This complication precluded any further testing with this sample – particularly the use of the sample to validate the jig by sectioning the ACL and measuring resultant instability.

Attempts were made to source a distal femoral locking plate to undertake a fracture repair, but these were unfruitful. It was also economically unviable to purchase a further cadaveric sample; hence validation was undertaken concurrently with testing.

3.1.3 Cadaveric samples

Prior studies published on the subject used 4-8 cadaveric knees per injury state tested, so we sought to test 6 knees per injury state explored. Approaches were made to all of the Scottish medical schools (with the exception of St Andrews), seeking to obtain cadaveric samples. Unfortunately, despite some interest, this proved either logistically or economically unfeasible. Quotes were therefore sought from ‘body banks’ in the US. This resulted in a smaller per sample cost but did lead to significant shipping costs. Hence, the incremental cost per knee for purchasing 12 knees was markedly less than for 6, therefore 12 cadaveric knees were purchased from the Anatomy Gifts Registry, Maryland, USA. Either right or left sided samples were accepted. Cadavers were fresh frozen, and a request was made for donors to be less than 65 years of age, to have a BMI of less than 40 (to ensure fit within the jig). Appendix 3 details the characteristics of the samples obtained. Exclusion criteria were any history of significant knee injury, knee surgery, meniscal injury or ACL/PCL rupture. Donors with history of any form of arthritis (osteoarthritis/rheumatoid arthritis/inflammatory arthritis) were also excluded. Mid-femur to mid-tibia specimens were obtained. Samples were immediately placed in the freezer on delivery and removed prior to cementing. Cementing was undertaken as described above, with the femur cemented inverted first, followed by the tibia whilst the sample was mounted in the jig. Cement was allowed to set overnight, prior to any testing.

3.1.4 Knee arthroscopy and meniscal injury

In an effort to preserve the native soft tissue envelope and minimise the risk of iatrogenic instability, we elected to introduce meniscal injury through the use of an arthroscope. Hence it was necessary to both obtain an arthroscopy stack as well as an experienced arthroscopy surgeon to undertake the surgery.

A recently decommissioned arthroscopy stack was borrowed from the Golden Jubilee National Hospital, along with some old manual arthroscopic instruments.

In a clinical setting, arthroscopic knee surgery is assisted by the use of a fluid pump which distends the joint to allow easy visualisation of anatomical structures. However, it was felt that pumping fluid into the joint space in the absence of a native circulation may lead to irreversible alteration in joint biomechanics and hence arthroscopy was undertaken 'dry,' without the use of such additional fluid.

The Thiele embalmed cadaveric knee was used to verify that arthroscopy was possible using this method and that both menisci could be visualised.

Arthroscopy was performed throughout the project by Mr Jon Clarke, Consultant Orthopaedic Surgeon at the Golden Jubilee National Hospital.

Knee stability was measured in intact/ half meniscal resection and full meniscal resection for 11 knees. Tears were made the at the junction of the anterior third and posterior two thirds of the lateral meniscus in knees 2, 8, 9, 10, 11 and 12. Tears were created at the junction of the anterior two thirds and posterior third of the medial meniscus in knees 3, 4, 5, 6 and 7.

3.1.5 Measuring knee stability

Following cementing of the knee into the jig, stability testing was undertaken using the following protocol. Due to the femoral fracture occurring during preliminary testing, it was decided to limit loads to a maximum of 1000N and avoid flexion beyond 60 degrees.

Based on these limitations, the following testing protocol was developed:

Injury state	Degree of Flexion	Load tested
Intact	0/15/30/45/60 degrees	100N/250N/500N/1000N
50% tear	0/15/30/45/60 degrees	100N/250N/500N/1000N
100% tear	0/15/30/45/60 degrees	100N/250N/500N/1000N

Table 3-1- Test states for stability testing

A review of the literature had previously demonstrated that the influence of radial meniscal tears on knee stability had not been investigated. Figure 3-21 demonstrates the typical appearance of a radial meniscal tear. Hence, we chose to investigate the effect of such tears. Radial tears commonly occur at the junction of the anterior third and posterior two thirds in the lateral meniscus, and the junction of the anterior two thirds and posterior third in the medial meniscus, hence these locations were chosen to create the injury.

Prior to any testing, two arthrotomies were made medial and lateral to the patellar tendon, akin to those normally made during arthroscopy. This step was undertaken to ensure that any incisions in the joint capsule were similar throughout testing.

Figure 3-21 - A radial meniscal tear

The knee was fixed into place within the jig and testing of the knee in the intact state at the flexion angles and loads described above was undertaken. It was not possible to simultaneously activate the Instron materials testing machine to apply load and the Optitrack Motiv system to record, hence both were commenced manually and subsequently synchronised. Prior to application of any load, the Instron E10000 was set to fix load measured at the load cell to 0N. Following the loading profile described above, a series of combined rotations of the knee in both directions were undertaken before recording was stopped. These data were not used for stability testing but instead allowed synchronisation of the recording data between various testing states.

Figure 3-22 - Arthroscopy of the cadaveric knee

Once an injury state has been tested, the knee was removed from the jig and moved to the dissection lab. Here, the meniscus in question was visualised using the arthroscopy stack (Figure 3-22). A scalpel blade was inserted using the second portal and the injury created.

Tears were initially created to 50% meniscal depth as judged by the surgeon. After this injury state was tested, the tear was extended to the full depth of the meniscus before stability testing was again conducted.

Notably, some cadavers were found to display a few degrees of constitutional varus or valgus tilt once cemented. This was accommodated for by tilting the proximal (femoral) component of the jig to allow the tibial baseplate to lie flat on the ball transfer units (Figure 3-23). Such tilt would result in concurrent tilting of the section where reflective markers were applied, hence would be recorded by the Optitrack motion tracking system.

Figure 3-23 -Sample in situ. Note the frontal plane tilt applied.

Once all three states had been tested, further incisions were made, and bony landmarks were recorded for later use in calculating a coordinate system for the knee. On the femur, the position of the medial and lateral femoral epicondyles were recorded, whilst in the tibia, the position of the edges of the medial and lateral tibial condyles were recorded using the Mako blunt probe (Figure 3-24).

Once these steps had been completed, the knee was immediately disarticulated. In the process, the state of the opposite meniscus, the ligaments and the articular surface of the joint was noted.

The menisci were then immediately removed, labelled and frozen (Figure 3-25)

<u>Figure 3-24 - Recording bony landmarks (actual recording performed with sample mounted in jig)</u>

<u>Figure 3-25 – Whole meniscus excised following stability testing</u>

Following this, cement was broken up using a hammer/chisel and the sample was placed in a clinical waste bag for disposal. Disposal of all samples took place through cremation following discussion with Her Majesty's Inspectorate for Anatomy in Scotland.

The steps undertaken during cadaveric testing are summarised below:

1. Knee cemented in jig day prior to testing and left overnight to set/defrost
2. Calibration of the Optitrack undertaken
3. Rigid bodies registered for:
    a. Femoral portion of jig
    b. Tibial portion of jig
    c. Mako blunt pointer
4. Knee fixed in jig using screws
5. One testing run under step wise increases in load at:
    a. 0 degrees
    b. 15 degrees
    c. 30 degrees
    d. 45 degrees
    e. 60 degrees
6. Knee removed, and arthroscopy performed with meniscal injury
7. Testing repeated at 50% meniscal injury
8. Knee removed, and arthroscopy performed with further meniscal injury
9. Testing repeated at 100% meniscal injury
10. Bony landmarks registered
11. Knee disarticulated, recording status of anatomical structures
12. Menisci removed and frozen
13. Cement broken up and knee disposal undertaken

A total of 11 knees were tested –testing of one sample was rendered futile due to a technical error during arthroscopy. 5 knees were tested following medial meniscal injury, 6 were tested following lateral meniscal injury.

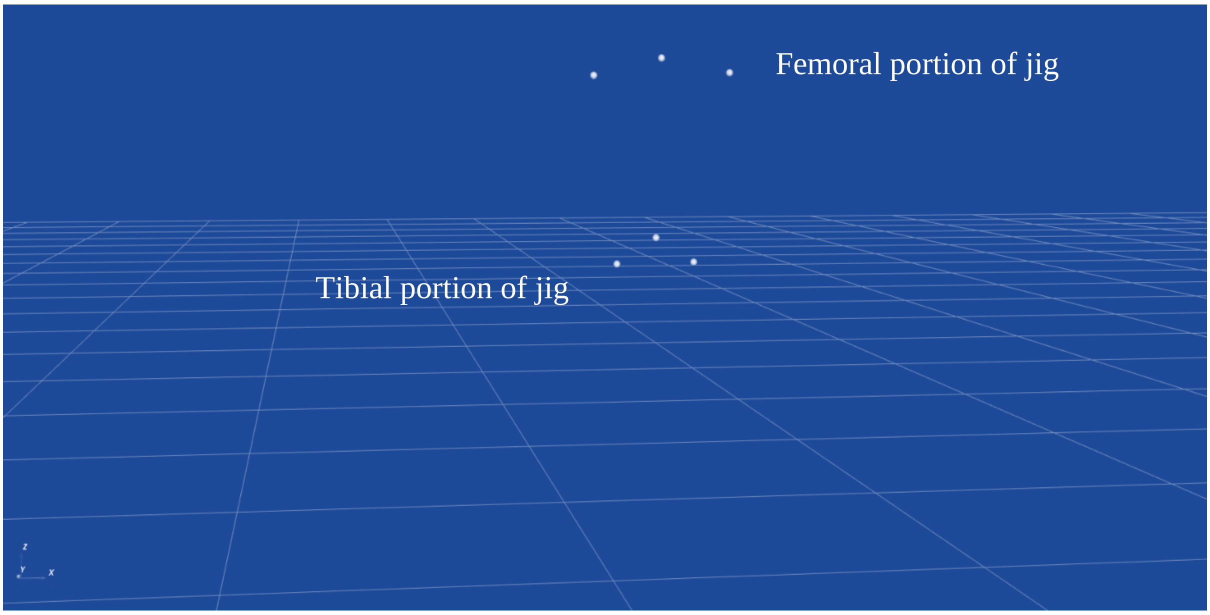
3.1.6 Data analysis

3.1.6.1 Data export and organisation

Data was saved in Optitrack and exported using the C3d file format. The C3d files were imported using the open source Mokka (Motion Kinematic and Kinetic Analyser) software [255]. This allowed inspection of the imported files in a 3d format and also allowed them to be exported as CSV (comma separated value files) with columns for x/y/z coordinate for each marker placed in columns.

As well as this, CSV files identifying the position of the bony landmarks were exported as CSV files – the Mako blunt probe was held in position for 10 seconds to determine the position of the landmark in question.

Each exported data file was manually checked to ensure marker labelling was correct. Once this step was completed, the first line of each file was inspected to ensure that labelling had been undertaken correctly. At this stage, it was identified that at time points where there was momentary signal loss, markers signals were being swapped on occasion, such that, for example the signal from the bottom left marker might start in the correct column, but then would subsequently swap to another column if there was a single frame of signal loss.



<u>Figure 3-26 - Mokka visualisation</u>

To address this issue, a MATLAB script was written to identify instances where there were sudden changes in the value of a column across all markers. This script was then run in turn for each data file. Frames where there were large magnitude changes were manually inspected and any swapped data was assigned to the correct column.

A sample visualisation from Mokka is shown in Figure 3-26. The top triad of markers represents the femoral jig, with the bottom triangle representing the tibial jig. Note that markers are placed on the posterior aspect of the jig.

The Mokka software was also used to remove extraneous markers from the data. Following CSV export, it was noted that marker labelling differed between takes, with different columns appearing in different markers in subsequent takes. Hence, markers were manually reordered in each CSV file to ensure a similar pattern existed in each file. Note that the x-axis denotes medial/lateral movement, the y-axis denotes antero-posterior movement and the z-axis denotes superior/inferior movement in the Optitrack coordinate system.

3.1.6.2 Data synchronisation

At this juncture, data files recorded identified the sample being tested, the injury state and the degree of flexion being tested. As previously noted, it was not possible to automatically synchronise recording of the Optitrack data with the loading protocol for the Instron materials testing machine. Therefore, the Instron loading protocol was developed to apply a series of

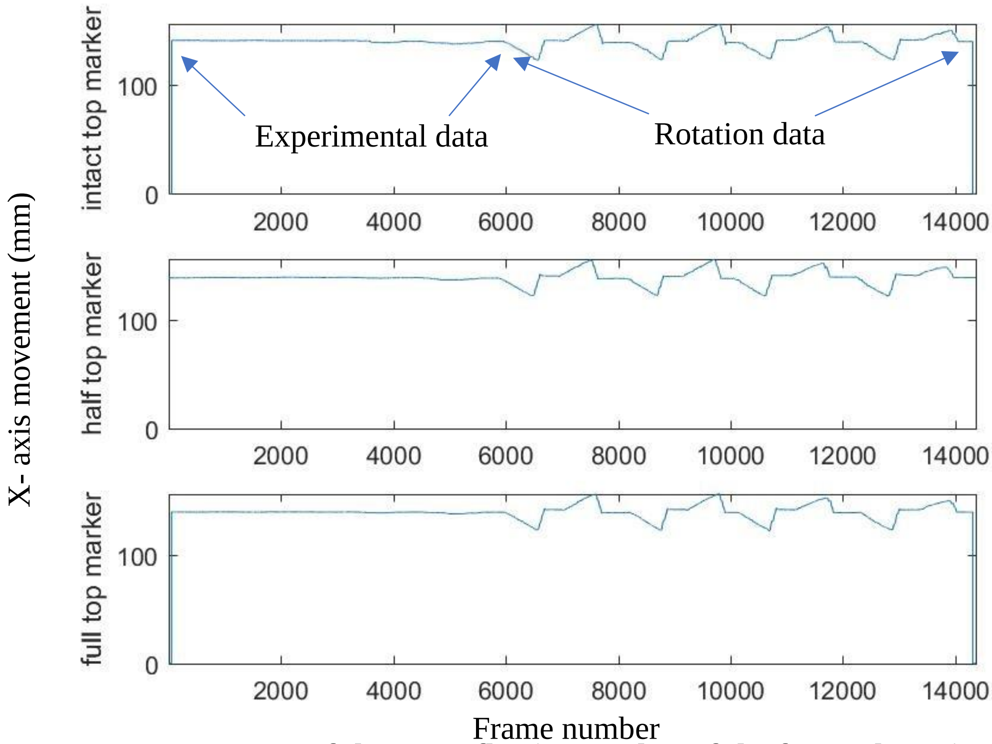


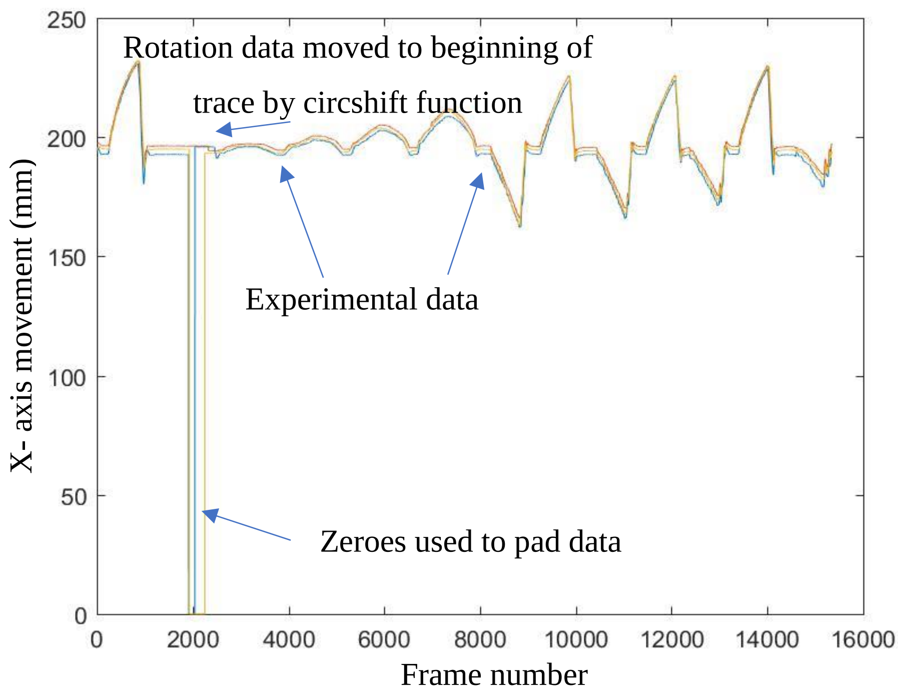
Figure 3-27 - Movement of the top reflective marker of the femoral portion of the jig in the X-axis, illustrated in each injury state.

rotations to the jig, with the intention of using this data to synchronise the files. The resultant tracking data had large magnitude oscillations in the x-axis. The marker data from the top reflective marker attached to the femoral portion of the jig was used for synchronisation. A typical trace of a complete iteration of testing is shown below in Figure 3-27. Note that the signals are out of sync. The early 'flat' part of the signal relates to the load phases, whereas the latter half of the graph contains the rotation data.

Data was imported into MATLAB 2017a (Mathworks, Natick, Massachusetts, United States) for analysis. Note that the rotation phase data was used solely for signal synchronisation and not analysed further as it was felt to represent a non-physiological movement arc, with any differences between tests potentially reflecting interaction of a number of variables such as friction between the baseplate and ball transfer units rather than stability of the knee joint.

A number of techniques were attempted to perform signal synchronisation. In particular, cross-correlation was attempted to measure the gap between signals and then time shift the signals to match. Unfortunately, this approach resulted in the signals being ‘synchronised’ out of phase from one another. Instead, the approach used was as follows:

1. The signal with the largest number of frames was identified and the other signals were ‘padded’ with zeros to ensure all three signals were of equal length.
2. All three signals were plotted as in the figure above.
3. Two points encompassing the position of the first peak in the signal within each graph were identified.
4. MATLAB was used to identify the frame number with the maximal value for each graph.
5. The ‘circshift’ function was used to time shift each graph to match the position of the first peak with the other two graphs. A typical resultant signal is shown below in Fi



<u>Figure 3-28 - Synchronised signals, note overlap of peaks/troughs in signal used to synchronise data</u>

3.1.6.3 Data segmentation

Having synchronised the data for each injury state for all samples tested, the next step was to segment the data to identify each load state. Again, this was undertaken using MATLAB software. The synchronised data shown in Figure 26 was plotted. Either the movement of the top reflective marker of the femoral rigid body in the x-axis (left/right) or z-axis (superior/inferior) was used for segmentation. Curves pertaining to the application of load at 100N/250N/500N or 1000N were identified through the baseline visible in each of the signals – this was used as the start and end point for each segment. Through segmenting the data in this manner, it was subsequently possible to calculate maximal displacements and angle changes subsequently. MATLAB was then used to save this data to separate CSV files with appropriate labelling.

3.1.6.4 Calculation of joint angles/displacement

Once data had been appropriately synchronised and segmented such that data files for each injury state were directly comparable, individual data files were analysed using MATLAB to calculate a local coordinate system and work out the relative joint angles and displacements for each test iteration. Prior to this, signal noise was reduced through the use of the Butterworth filter function in MATLAB.

3.1.6.4.1 Jig coordinate system

The coordinate system for the jig was then calculated as follows. Calculations were made separately for the top and bottom segments as illustrated in Figure 3-29:

- The origin was defined as the midpoint of the inferior medial and lateral markers for both segments.
- The x axis for each segment was defined as a straight line lying between the inferior medial/lateral markers.
    - A temporary axis defined by the difference between the position of the superior marker was defined.
- The cross product of the above two axes was used to define the y-axis
- The cross product of the X and Y axes defined the z-axis

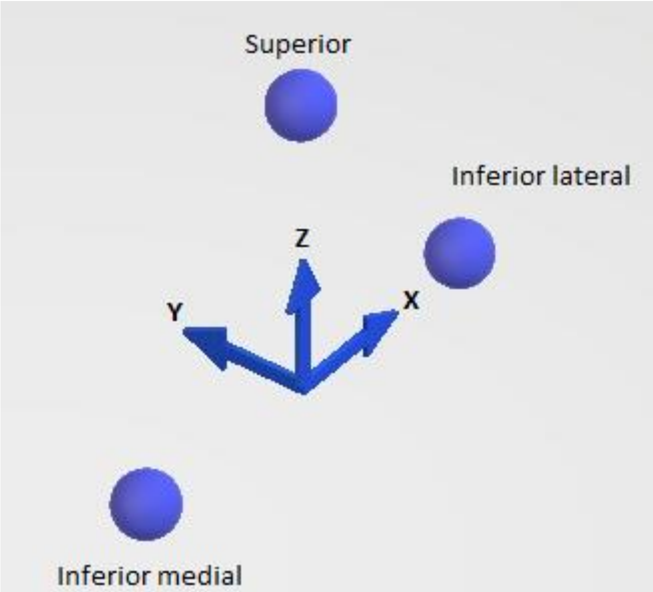


Figure 3-29 - Jig coordinate system

3.1.6.4.2 Bone coordinate system

The bone coordinate system was calculated using a combination of the bony landmarks identified and the knowledge that the centre of the intramedullary canal of the bones lay at the centre of the bone cylinders, based on the coordinate system described by Grood & Suntay [256].

The coordinates of the bony landmarks were calculated based on the known distances between the reflective markers on the Mako blunt probe.

The coordinate system for the jig was then calculated as follows. Calculations were made separately for the femoral and tibial segments, with a right-handed coordinate system:

- The origin was defined by adjusting the position of the top/bottom marker to place it in the middle of the femoral/tibial cylinder.
- The x axis for each segment was defined as a straight line lying between the two epicondyles/ edge of tibial condyles.
    - A temporary axis defined by the difference between the position of the top marker was defined.
- The cross-product of the above two axes was used to define the y-axis
- The cross-product of the X and Y axes defined the z-axis

The calculation of the x-axis was corrected based on whether the knee tested was a right or left knee.

3.1.6.4.3 Joint angle calculation

Rotation matrices were then calculated for translation from the proximal segment of the jig to the femur and the distal segment of the jig to the tibia as follows. Where B = coordinates of bone, J = coordinates of jig and R = rotation matrix.

$$[B] = [R][J]$$

$$\Rightarrow [B][J]^{-1} = [R]$$

As [J] contains only orthogonal unit vectors, $[J]^{-1} = [J]^T$

$$\Rightarrow [B][J]^T = [R]$$

The rotation matrix from the femoral segment to the tibial segment was calculated in a similar manner. Joint angles were then calculated as described by Robertson et al. [257] such that:

- Rotations about the y-axis, were given by: $\beta = \sin^{-1}(R_{3,1})$
- Rotations about the x-axis, were given by: $\alpha = \sin^{-1}(\frac{-R_{3,2}}{\cos\beta})$
- Rotations about the z-axis, were given by: $\gamma = \sin^{-1}(\frac{-R_{2,1}}{\cos\beta})$

where R denotes the rotation matrix from the femoral segment to the distal segment and the subscripted numbers denote values within the 3 x 3 rotation matrix.

The position of the bones in the first frame of each segment was used as a reference to calculate subsequent rotation.

3.1.6.4.4 Displacement calculation

Displacement between the two segments was calculated between the two bone origins as described above. The position of the bone in the first frame of the matrix was used as a reference to calculate relative displacement. Displacement values in millimetres were multiplied by the tibial rotation matrix to express displacement in the tibial coordinate system.

3.1.6.5 Calculation of differences

Following calculation of joint angles/displacements, a MATLAB script was used to find the largest maximum/minimum peak in each segmented file for all 6 degrees of freedom at each injury state. These values were subsequently compared as described below.

3.1.6.6 Statistical Analysis

Statistical analysis was undertaken using IBM SPSS Statistics v25 using repeated measures ANOVA. An assumption of repeated measures ANOVA is that of sphericity of the data, - that the variance of several repeated measures is equal and correlation between all possible combinations of trials is equal. If this assumption is violated, the risk of a type I error increases. Adopting the Greenhouse-Geisser significance value protects against this by adjusting the p-value based on the sphericity of the data. Thus, conservative Greenhouse-Geisser significance values are provided throughout. The repeated measures analysis model analysed six dependent variables: alpha, beta, gamma, x, y and z. The model utilised three main within-subjects variables (injury state, load and flexion angle), one between-subjects factor (side, i.e. medial/lateral) and all interactions, indicated by an asterisk. Bonferroni-adjusted post-hoc comparisons were made where appropriate. An error level of 0.05 was used throughout to denote significant effects. When p values were below 0.15, a potential trend was noted and discussed as, with additional samples, this may approach significance.

3.2 Results

3.2.1 Cadaveric samples

A total of 12 cadaveric knees were obtained. As described above, all patients were less than 65 years old, had a BMI below 30 and no history of knee surgery, osteoarthritis or significant knee injury.

3.2.2 Knee arthroscopy and meniscal injury

The menisci were visualised in all knees using arthroscopy. Unfortunately, during testing of the first sample (Sample ID -1), the meniscus was mistakenly injured during surgical approach to the joint due to a combination of the difficulty in using the arthroscope without fluid to distend the joint and the fact that positioning the knee was difficult. Hence, for subsequent exposures the arthroscopy portals were made largely than in routine clinical practice – 4cm in length. This step was necessary to allow the arthroscope to inspect the joint adequately – in clinical practice it is possible to reposition the leg and apply varus/valgus stress to open each joint compartment, which was not possible in the current scenario. Figure 3-30 shows the free edge of the meniscus as visualised during knee arthroscopy. All meniscal injuries in samples 2-12 were successfully created.

Free edge
of meniscus

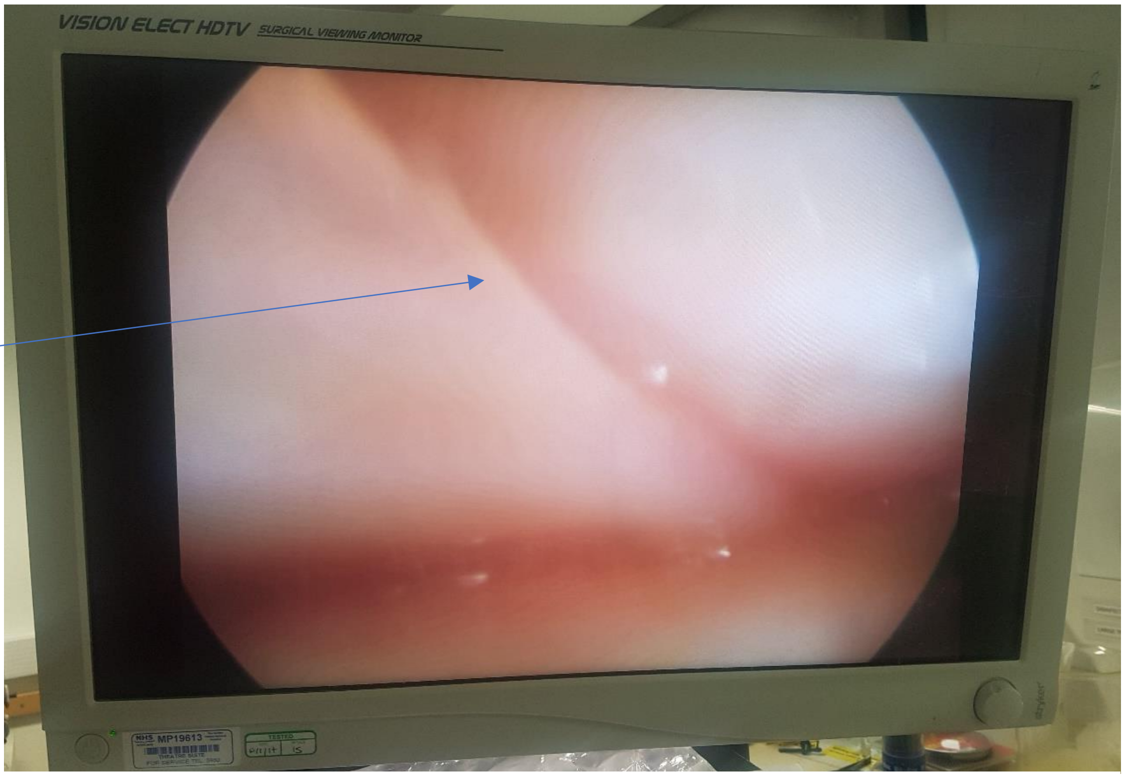


Figure 3-30 - The meniscus visualised during knee arthroscopy

3.2.3 Measuring knee stability

Following disarticulation, no evidence of significant osteoarthritis was noted in any of the samples. Furthermore, there were no tears noted in any other major joint structures, including the ACL/PCL or MCL/LCL. The remainder of the tested meniscus and the opposite meniscus were also completely intact in all cases. Furthermore, all menisci were completely transected at the location tested. It was not possible to quantify the depth of the 50% meniscal tears due to the need to leave the joint intact for further testing.

3.2.4 Data analysis

3.2.4.1 Data export and organisation

A single CSV export yielded a file with 19 columns, one for time and a further 18 for x/y/z coordinates for 6 markers. Bone landmark coordinate data was exported separately. Hence, 3 files were recorded for each injury state at each degree of flexion, resulting in 15 files of test data for each knee. A further four files containing bony landmark data were recorded for each knee, resulting in a total of 209 data files for analysis.

3.2.4.2 Data segmentation

Data were segmented into intact meniscus, 50% resection and 100% resection for 4 test loads, at each testing angle. This resulted in 60 segmented files for each knee tested, with an additional 40 files produced for knees 9 and 11, which were also tested at each joint angle/load following transection of the ACL. Hence a total of 700 data files were available for further analysis.

3.2.4.3 Calculation of joint angles/displacement

3.2.4.3.1 Jig coordinate system

Using the reflective markers attached to the jig, the coordinate system developed is illustrated at 0, 30 and 60 degrees of flexion in Figure 3-31, Figure 3-32 & Figure 3-33.

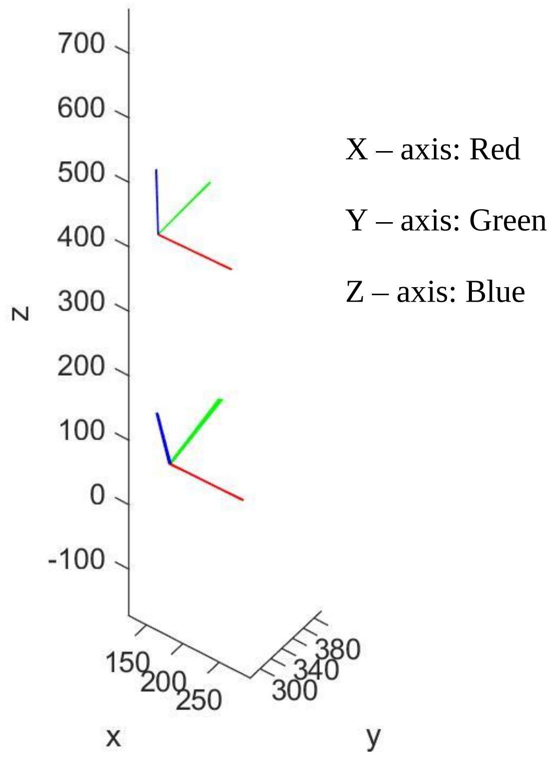


Figure 3-31 - Jig coordinate system at 0 degrees flexion

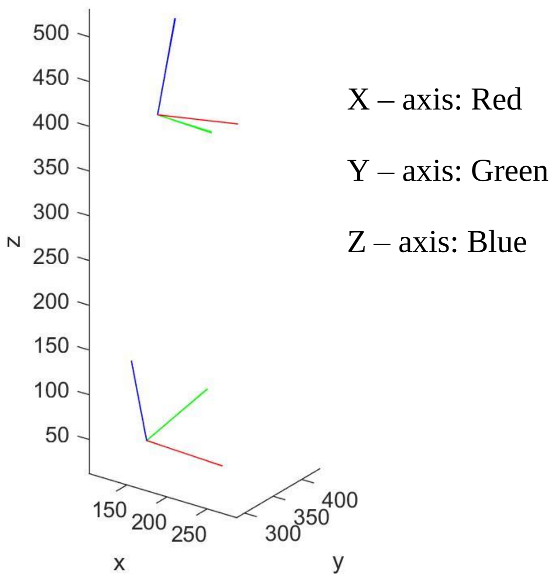


Figure 3-32 - Jig coordinate system at 30 degrees flexion

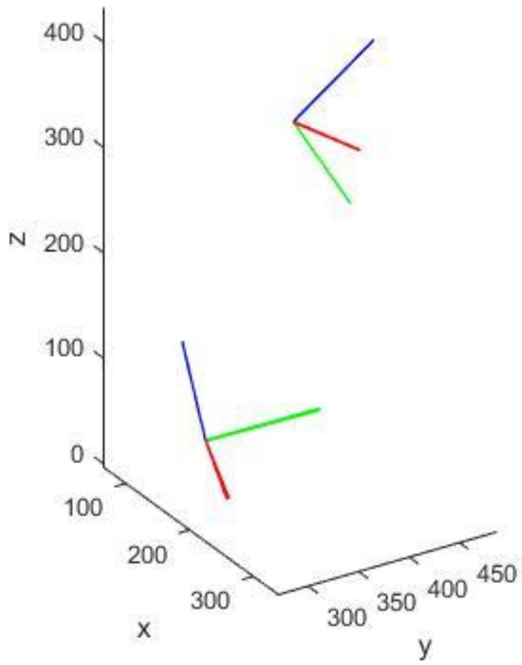


Figure 3-33 – Jig coordinate system at 60 degrees flexion

Note that whilst the top section of the jig aligns well with the global reference frame, the bottom half is tilted in the y/z-plane. This is due to the top marker on the tibial section of the jig being placed against the cylinder holding the tibia, whilst the bottom left/right markers were placed at the corners of the tibial baseplate. This is illustrated in Figure 3-34, where the

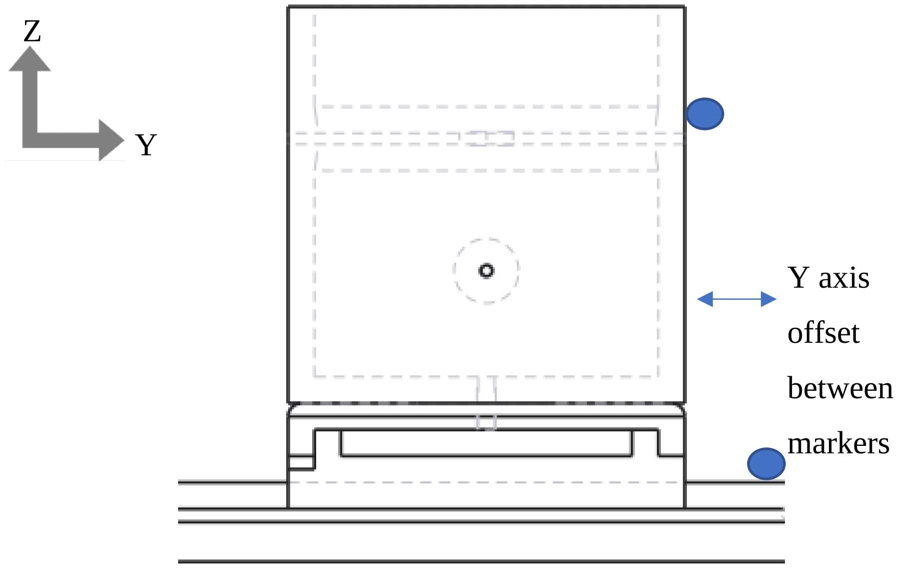


Figure 3-34 - Offset of reflective markers on tibial baseplate

base assembly is visualised from side on.

Calculation of the tibial origin (i.e. the tibial shaft) in the bone coordinate system was based on the middle position of the two bottom markers, hence this offset did not affect the bone coordinate system, as illustrated below. The rotation matrix to rotate from the jig coordinate system to the bone coordinate system accounted for this offset.

3.2.4.3.2 Bone coordinate system

The figures below illustrate the bone coordinate system, together with the jig coordinate system. To demonstrate the contrast between the two systems, a left sided knee has been chosen for illustration, the x-axis here runs in the opposite direction to that of the jig, to allow joint movements to be described accurately. Figure 3-35, Figure 3-36 & Figure 3-37 illustrate both coordinate systems at varying degrees of knee joint flexion.

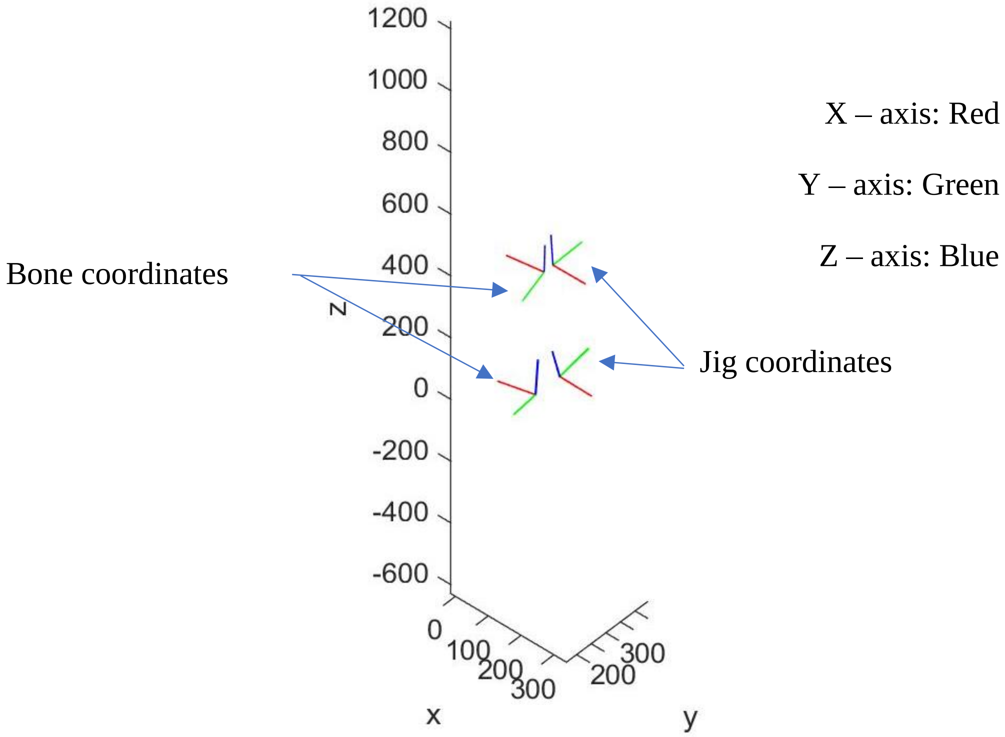


Figure 3-35 - Bone & jig coordinate system at 0 degrees of flexion

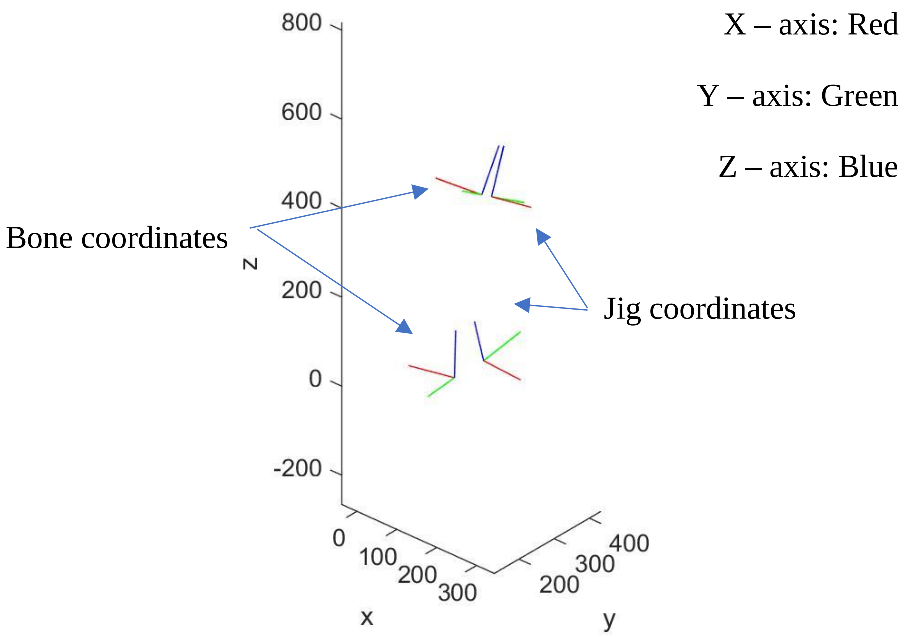


Figure 3-36 - Bone and jig coordinate systems at 30 degrees of flexion

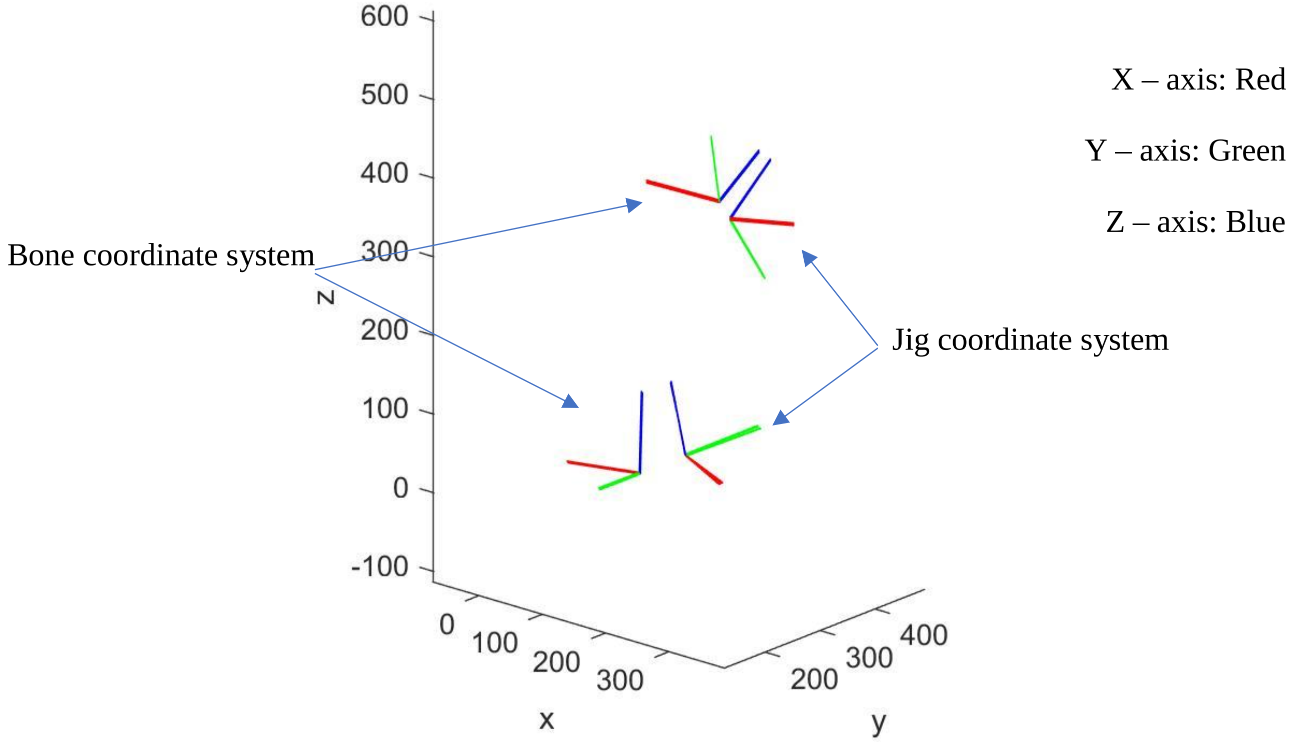
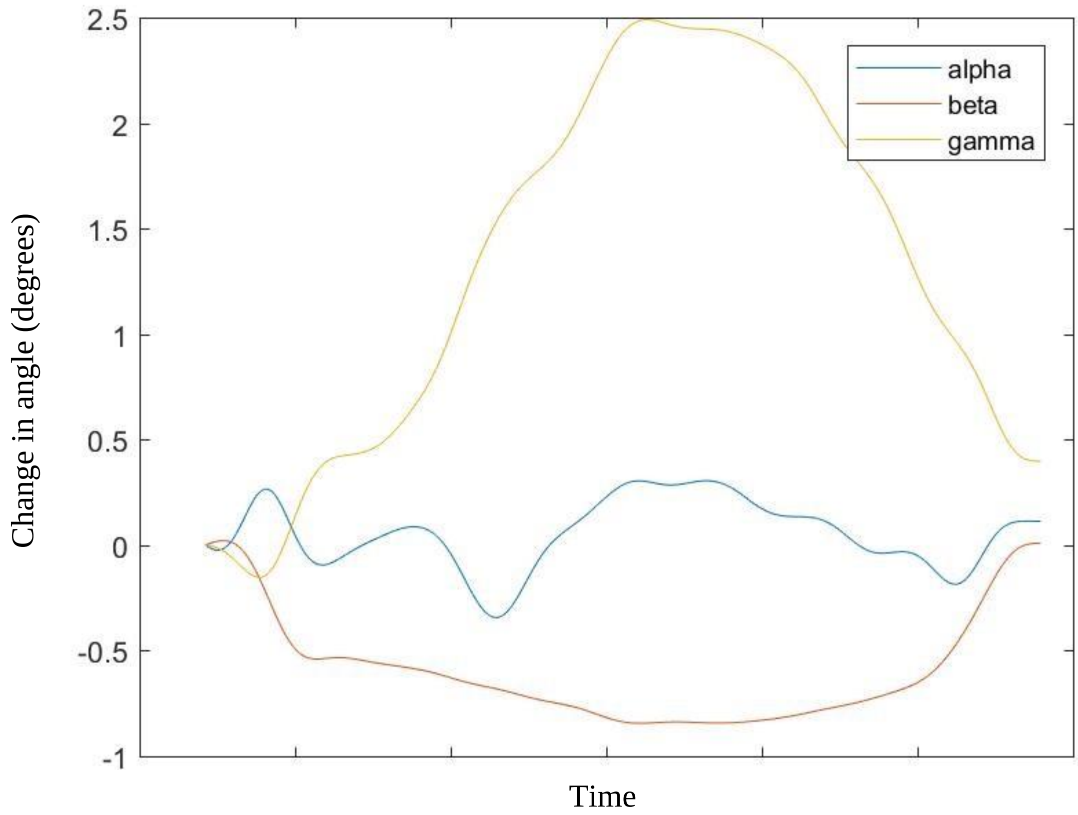


Figure 3-37 - Bone and jig coordinate systems at 60 degrees of flexion

3.2.4.3.3 Joint angle calculation

Joint angles were calculated using the above coordinate systems for all knees at each state of flexion and each load and stored to an Excel spreadsheet. A typical resultant trace is shown in Figure 3-38. Note that the x axis denotes time and is synchronised between traces as described previously.



<u>Figure 3-38 - Change in angle during application of load, alpha / beta / gamma denote rotation in flexion/extension, varus/valgus and internal/external rotation as detailed below.</u>

Note that in the coordinate system employed, joint angles are related to anatomical movement as detailed in Table 3-2.

Axis displacement	**Anatomical displacement**	**Axis rotation**	**Anatomical rotation**
Positive x	Medial	Positive alpha, around x-axis	Extension
Positive y	Posterior	Positive beta, around y axis	Varus
Positive z	Superior	Positive gamma, around z axis	Internal rotation
Negative x	Lateral	Negative alpha, around x-axis	Flexion
Negative y	Anterior	Negative beta, around y-axis	Valgus
Negative z	Inferior	Negative gamma, around z axis	External rotation

Table 3-2 - Relating the bone coordinate system to anatomical displacement/rotation

A right-handed coordinate system was employed and adapted for the side of knee tested, such that the above anatomical movements/rotations hold for both left and right knees.

Hence in the above example, minimal rotation is evident in the flexion/extension axis, < 1 degree of valgus of the tibia relative to the femur is observed and a maximum of 2.5 degrees of internal rotation is evident.

3.2.4.3.4 Displacement calculation

A similar graph showing the displacement measurement is shown in Figure 3-39.

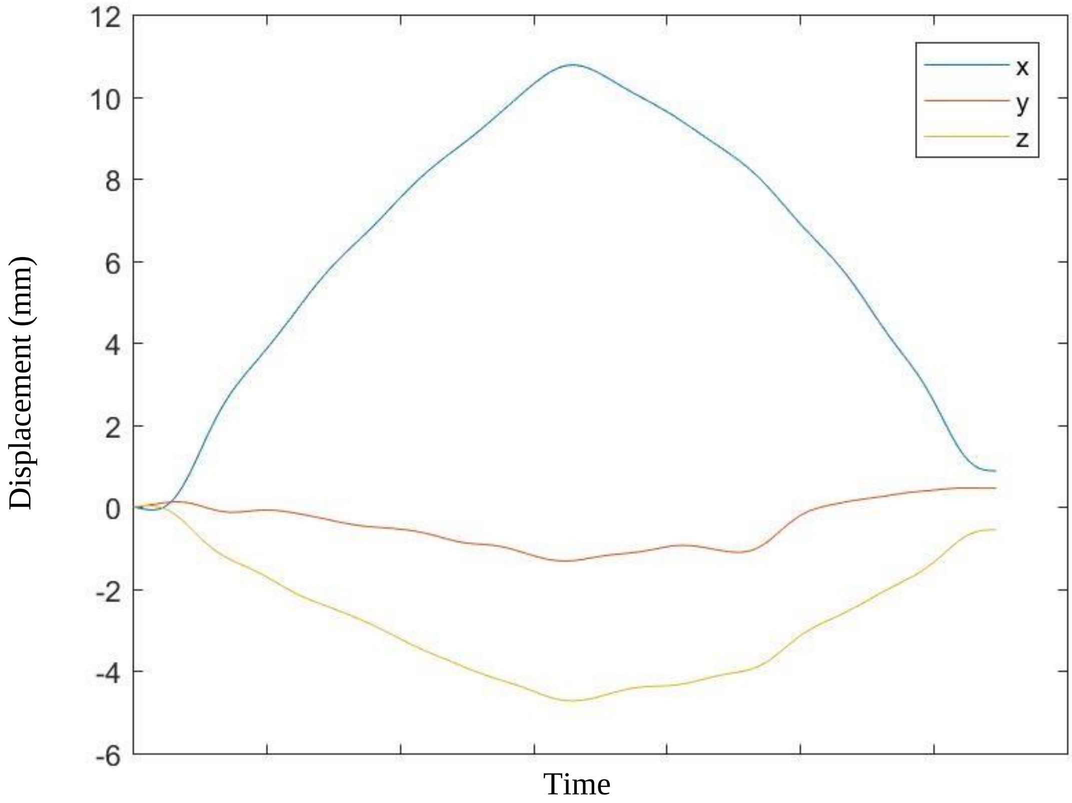


Figure 3-39 - Displacement during application of load, where x/y/z denote medilateral, anteroposterior and supero-inferior displacement respectively

Here, it can be seen that there is approximately 10mm of medial displacement of the tibia relative to the femur and approximately 5mm of inferior displacement of the knee joint as it is loaded.

3.2.4.4 Statistical analysis

The table below (Table 3-3) shows the results of the repeated measures ANOVA, highlighting the main effects and interactions between these effects.

Table 3-3 - Repeated measures ANOVA - p-values

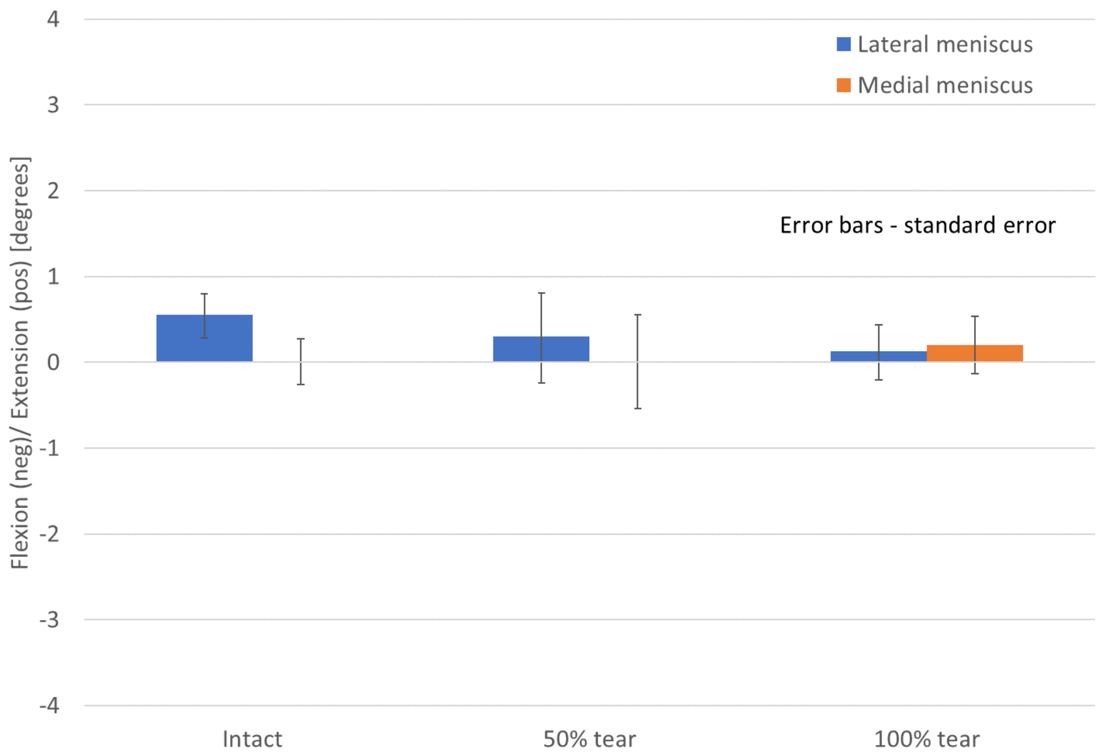
Effect	Variable of interest	Significance
Injury	Flexion/extension	0.838
	Varus/valgus	0.897
	Internal/external rotation	0.633
	Mediolateral displacement	0.310
	Anteroposterior displacement	0.400
	Superoinferior displacement	0.439
Load	Flexion/extension	0.663
	Varus/valgus	**0.002**
	Internal/external rotation	0.128
	Mediolateral displacement	**0.008**
	Anteroposterior displacement	**0.027**
	Superoinferior displacement	0.120
Flexion angle	Flexion/extension	0.471
	Varus/valgus	0.156
	Internal/external rotation	**0.001**
	Mediolateral displacement	0.145
	Anteroposterior displacement	0.204
	Superoinferior displacement	0.245
Injury*side	Flexion/extension	0.480
	Varus/valgus	0.118
	Internal/external rotation	0.660
	Mediolateral displacement	0.500
	Anteroposterior displacement	0.551
	Superoinferior displacement	0.436
Injury*load	Flexion/extension	0.722
	Varus/valgus	0.479
	Internal/external rotation	0.218
	Mediolateral displacement	0.485
	Anteroposterior displacement	0.361
	Superoinferior displacement	0.401
Injury*flexion angle	Flexion/extension	0.441
	Varus/valgus	0.739
	Internal/external rotation	0.507
	Mediolateral displacement	0.565
	Anteroposterior displacement	0.346

	Superoinferior displacement	0.374
Load*side	Flexion/extension	0.667
	Varus/valgus	0.871
	Internal/external rotation	0.945
	Mediolateral displacement	0.472
	Anteroposterior displacement	0.373
	Superoinferior displacement	0.446
Load*flexion angle	Flexion/extension	0.361
	Varus/valgus	**0.012**
	Internal/external rotation	**0.003**
	Mediolateral displacement	**0.008**
	Anteroposterior displacement	0.394
	Superoinferior displacement	0.429
Flexion angle* side	Flexion/extension	0.600
	Varus/valgus	0.684
	Internal/external rotation	0.190
	Mediolateral displacement	0.546
	Anteroposterior displacement	0.438
	Superoinferior displacement	0.337
Injury*load* flexion angle	Flexion/extension	0.476
	Varus/valgus	0.426
	Internal/external rotation	0.448
	Mediolateral displacement	0.443
	Anteroposterior displacement	0.384
	Superoinferior displacement	0.391

3.2.4.4.1 Effect of injury

Injury as an isolated variable was not found to significantly affect any of the measures of interest. There was also no interaction evident between injury and side (medial/lateral) of meniscal injury on all measures, although the varus/valgus angle approached significance with regards to this interaction. Potentially, the relationship between injury severity and varus valgus angle varied depending on which side the injury was on ($p = 0.118$) (Figure 3-41).

The effect of injury on the six degrees of freedom is shown separately for medial and lateral injuries below (Figure 3-40, Figure 3-41, Figure 3-42, Figure 3-43, Figure 3-44 & Figure 3-45).



<u>Figure 3-40 - Effect of injury states on flexion/extension, note small magnitude extension across injury states. No significant differences observed.</u>

Small magnitude variation is observed in the flexion/extension axis, with knees sustaining lateral injury extending by a mean 0.55˚ (s.e. 0.24˚) whilst intact, reducing to 0.13˚ (s.e. 0.31˚) at 100% injury. Knees sustaining medial meniscal injury extend a mean 0.01˚ (s.e. 0.27˚) whilst intact, increasing to 0.20˚ (s.e. 0.34˚) at 100% injury. No significant differences were observed.

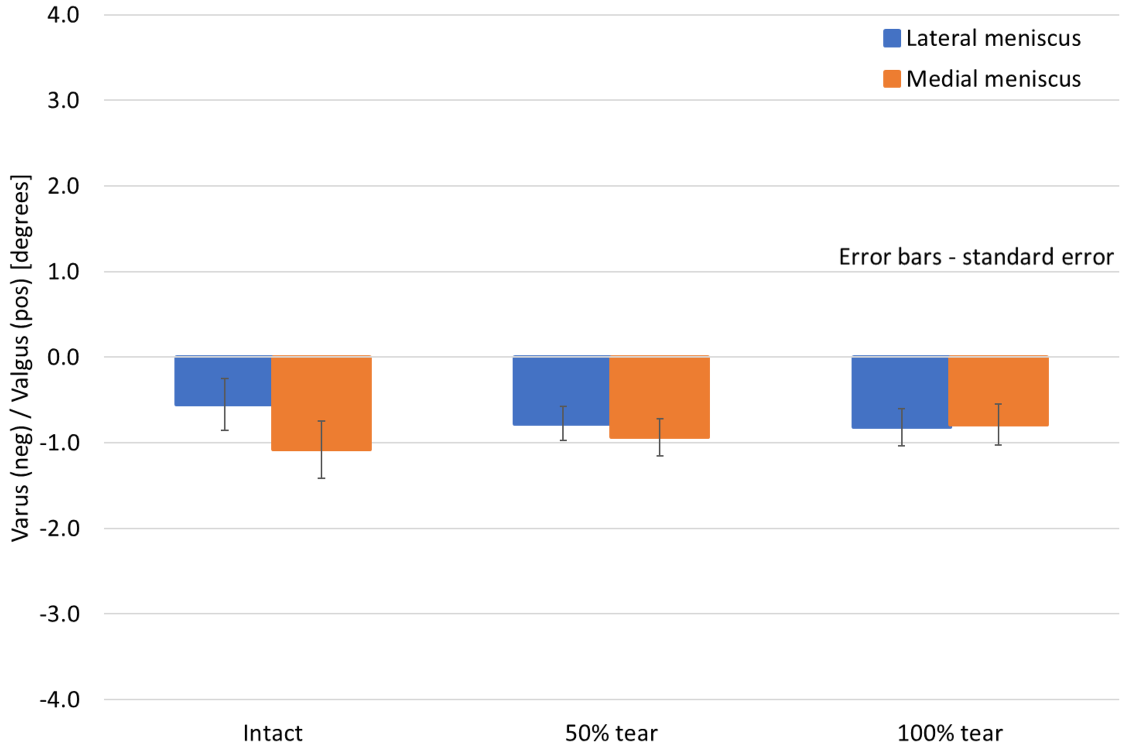


Figure 3-41 - Effect of injury states on varus/valgus, all knees tended to varus under load with no significant difference between injury states. No significant differences observed.

A similar trend in frontal plane angulation is observed, with knees sustaining both types of injury tending to varus under load. Knees sustaining lateral meniscal injury displayed 0.55˚ (s.e. 0.30˚) of varus whilst intact, increasing to 0.82˚ (s.e. 0.22˚) varus following 100% meniscal tears. Knees sustaining medial meniscal injury exhibited 1.10˚ (s.e. 0.33˚) varus whilst intact, decreasing to 0.79˚ (s.e. 0.24˚) varus following 100% meniscal tears. No significant differences were observed.

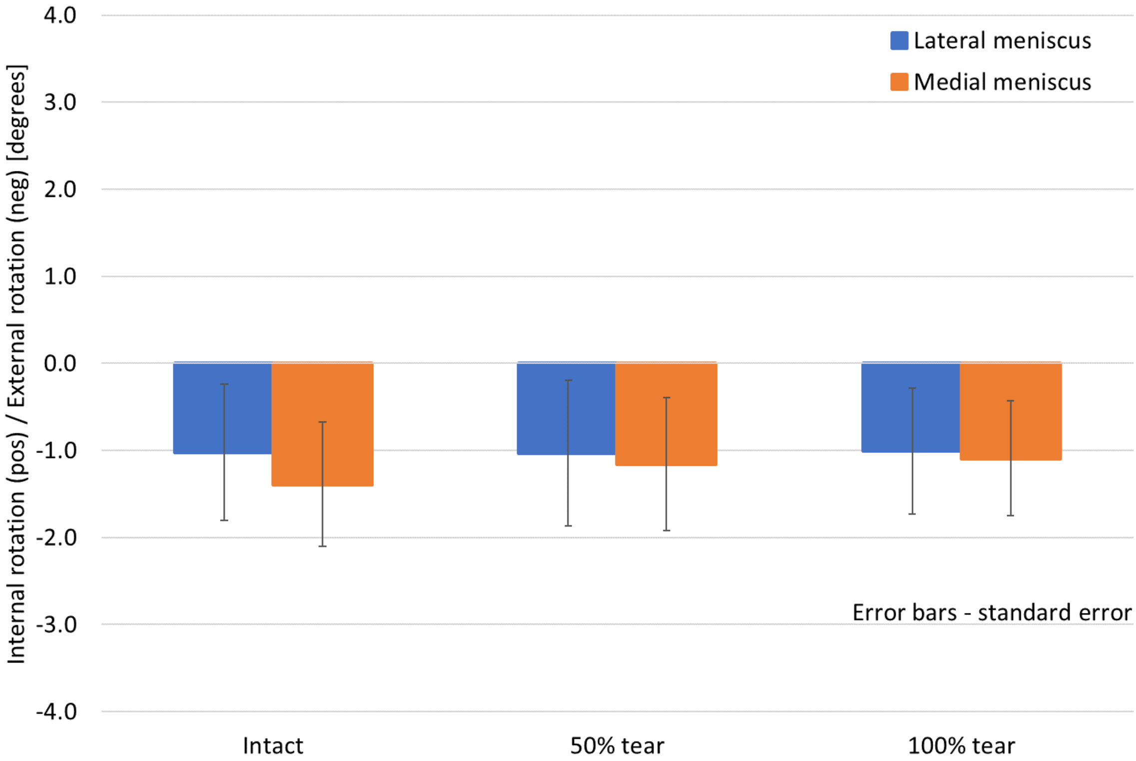


Figure 3-42 - Effect of injury states on internal/external rotation, no significant differences were observed.

Little variation in internal/external rotation is evident between groups, with the tibia exhibiting 1.02˚ (s.e. 0.71˚) internal rotation in the lateral meniscal group whilst intact, which essentially remained unchanged at 1.00˚ (s.e. 0.67˚) at the 100% meniscal tear state. Similarly, in the medial meniscal injury group, 1.4˚ (s.e. 0.78˚) internal rotation was evident in the intact state and 1.09˚ (s.e. 0.73˚) following a 100% meniscal tear. No significant differences were observed.

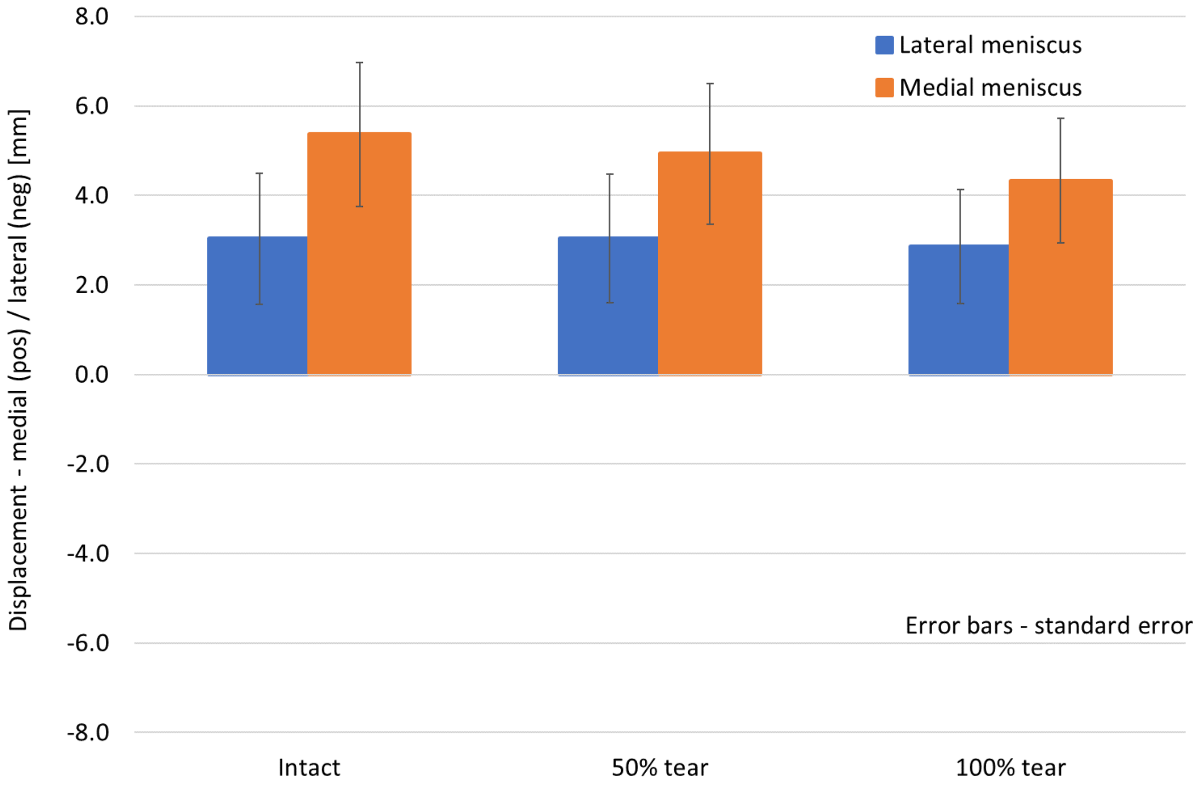


Figure 3-43 - Effect of injury states on mediolateral displacement, note the trend towards medial displacement of the tibia in all knees, more pronounced in the medial meniscus group. No significant differences observed.

All knees displayed a trend towards medial displacement of the tibia under load, though this was more pronounced in the intact state in the medial meniscus injury group (5.25 +/- 1.61 [s.e.] mm) as compared to the lateral meniscal injury group (3.03 +/- 1.47 [s.e.] mm). There was a slight trend towards lateral displacement in both groups, with medial translation reducing to 4.33 +/- 1.39 [s.e.] mm in the medial meniscus group and 2.86 +/- 1.27 [s.e.] mm in the lateral meniscal group. No significant differences were observed.

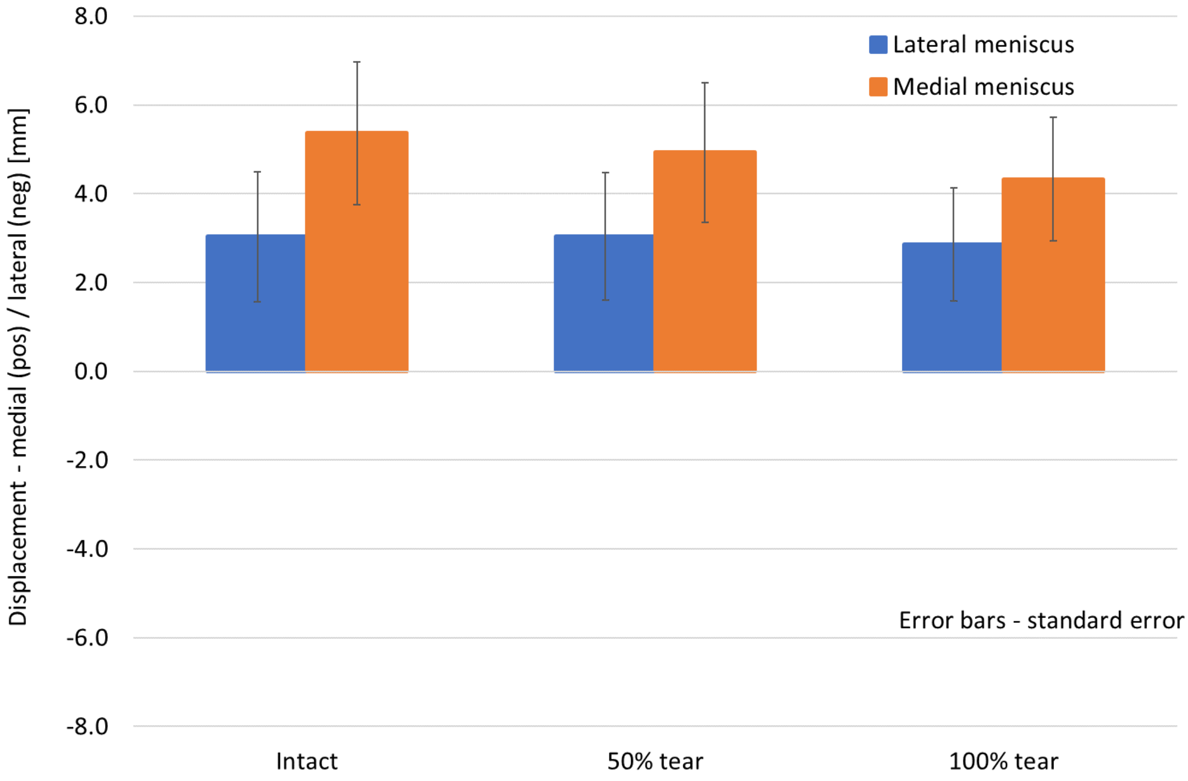


Figure 3-44 - Effect of injury states on anteroposterior displacement, all knees tended towards anterior tibial displacement, with no significant difference between groups.

Almost no effect is evident in the medial meniscal group with slight anterior displacement of the tibia relative to the femur evident in both the intact state (1.47 +/- 1.82 [s.e.] mm) and 100% tear state (1.87 +/- 0.91 [s.e.] mm) groups. More variation is evident in the lateral meniscal group, with 0.57 +/- 1.66 [s.e.] mm anterior displacement in the intact state and 2.17 +/- 0.84 [s.e.] mm in the 100% tear state. No significant differences were observed.

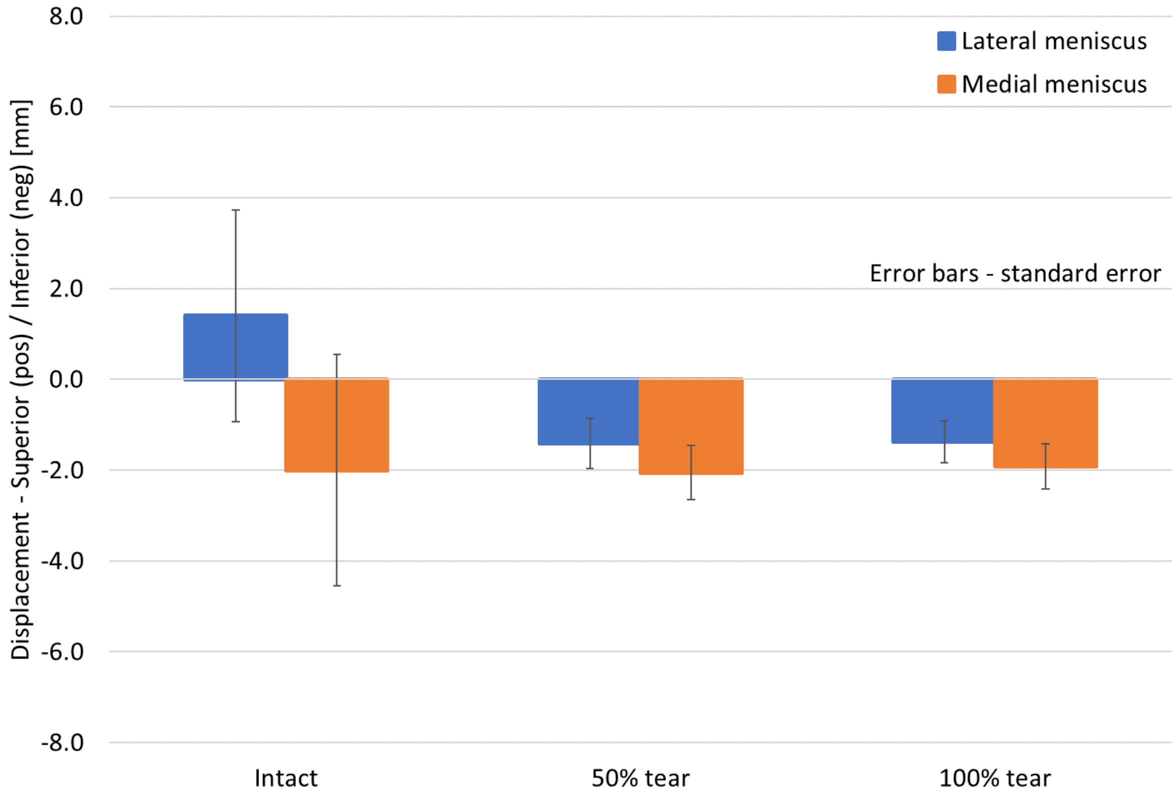


Figure 3-45 - Effect of injury states on supero-inferior displacement, inferior displacement of the tibia is observed in all states except the intact lateral meniscus group. No significant differences observed.

With the exception of the intact state, inferior displacement of the tibia relative to the femur was observed. Marked variation was seen in the intact states, the medial meniscal injury group translated 2.00 +/- 2.56 [s.e.] mm inferiorly whilst the lateral meniscal group appeared to translate 1.41 +/- 2.33 [s.e.] mm superiorly. There was almost no change in the mean inferior translation observed in the medial meniscal group (2.00 mm intact, 1.92 mm 100% tear), as compared to the lateral meniscal group (1.41mm superior translation in the intact group, 1.37 mm inferior translation in 100% tear group. No significant differences were observed.

3.2.4.4.2 Interaction of injury state and load

No significant differences were observed for all dependent variables when the interaction of injury and load tested was considered, i.e. the relationships between injury severity and the kinematic variables were the same across all loads.

3.2.4.4.3 Interaction of injury state and flexion angle

No significant differences were observed for all dependent variables when the interaction of injury and flexion angle was considered, i.e. the relationships between injury severity and the kinematic variables were the same across all flexion angles.

3.2.4.4.4 Effect of load

Load applied had a significant effect on the varus/valgus angle (p=0.03) (Figure 3-46), mediolateral displacement (p=0.01) (Figure 3-47) and anteroposterior displacement (p = 0.03) (Figure 3-48). The effect of load on superoinferior translation of the tibia approached significance (p= 0.113) (Figure 3-49).

Varus/valgus angle

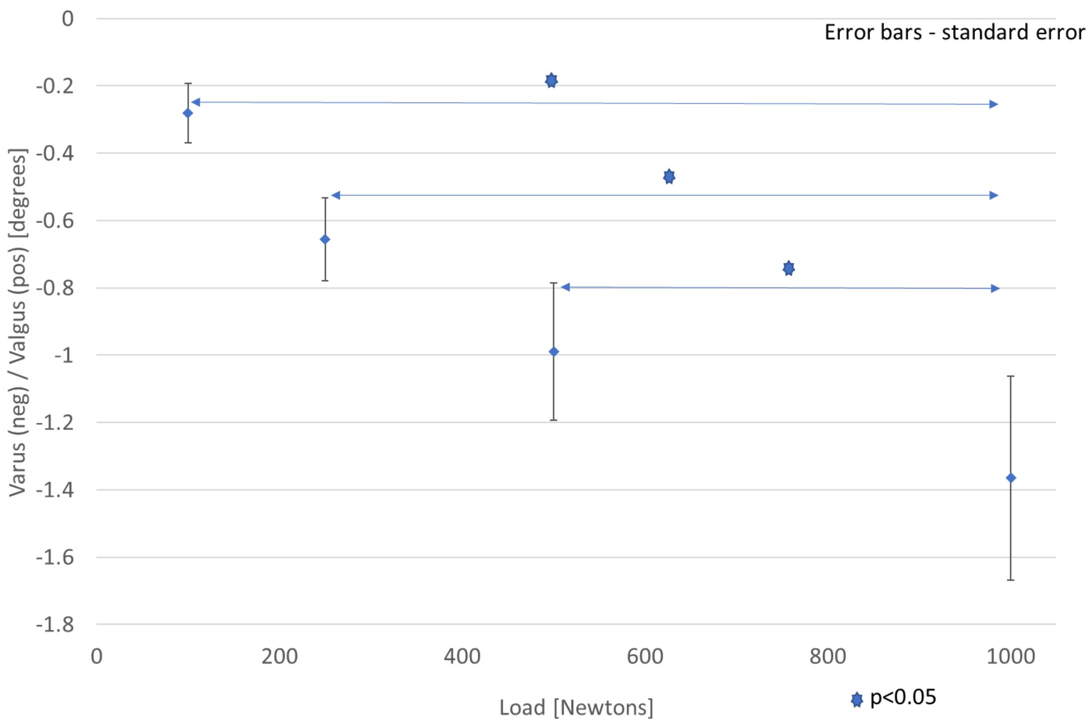


Figure 3-46 - Effect of load on varus/valgus angle, an increase in load is associated with progressive varus of the knee. Significant increases in the varus angle were observed between loads of 100/250/500 and 1000N.

Load	Varus angle (s.e.)
100N	0.28 (0.09) °*
250N	0.66 (0.12) °
500N	0.99 (0.20) °
1000N	1.37 (0.30) °
*p<0.05 compared to all other states	

Table 3-4 - Increasing varus angle with increasing load

Post hoc analyses demonstrated that there was a significant difference between the varus angle at 100N, compared with all other loading states, with increasing varus evident with increasing load (Table 3-4). The increase in varus angle with load approached but did not reach significance when comparing 250N load to 1000N load (p= 0.07) and 500N load to 1000N load (p=0.06).

Mediolateral displacement

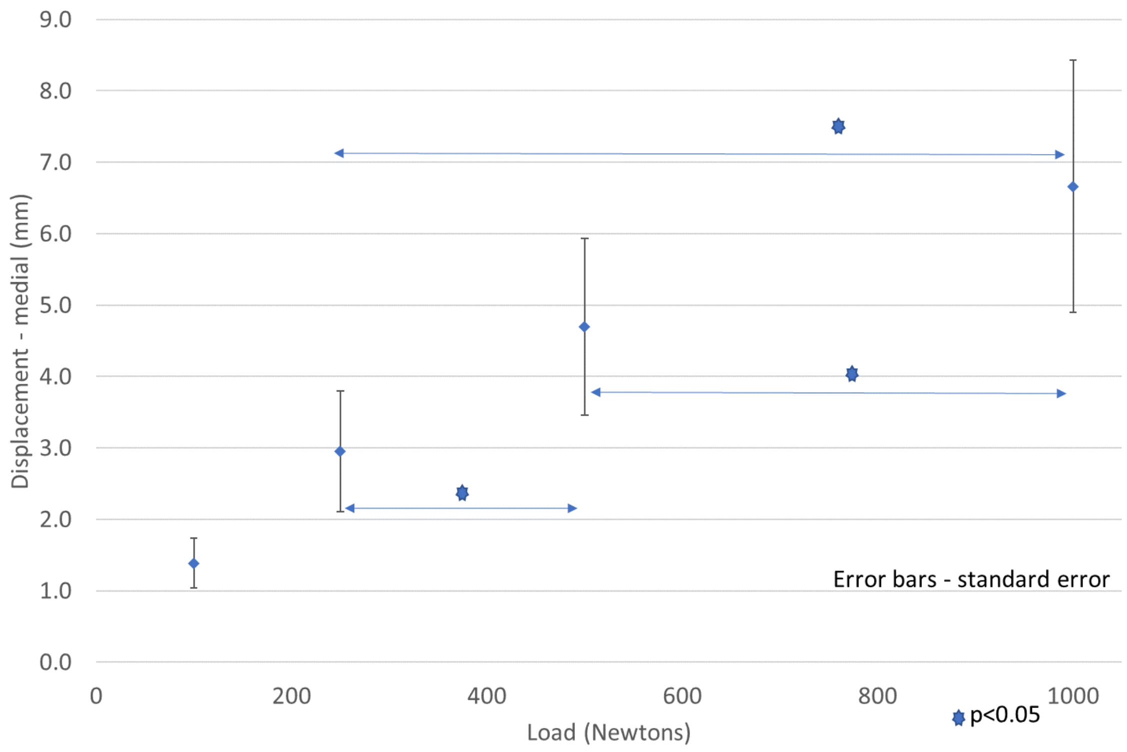


Figure 3-47 - Effect of load on medial displacement, note significant increase in medial displacement of the tibia with increasing load, between 250N and 500/1000N and also between 500N and 1000N.

Load	Medial displacement (s.e.)
100N	1.39 (0.35) mm
250N	2.95 (0.84) mm*
500N	4.70 (1.23) mm†
1000N	6.66 (1.77) mm
*p<0.05 compared to all 500N/1000N †p<0.05 compared to 1000N	

Table 3-5 - Increase in medial tibial displacement with increasing load

Post hoc analysis revealed a significant increase in medial displacement at 500N and 1000N compared to 250N and at 1000N compared to 500N. The differences between 100N and 500N/1000N approached significance (p = 0.076 and 0.058 respectively).

Anteroposterior displacement

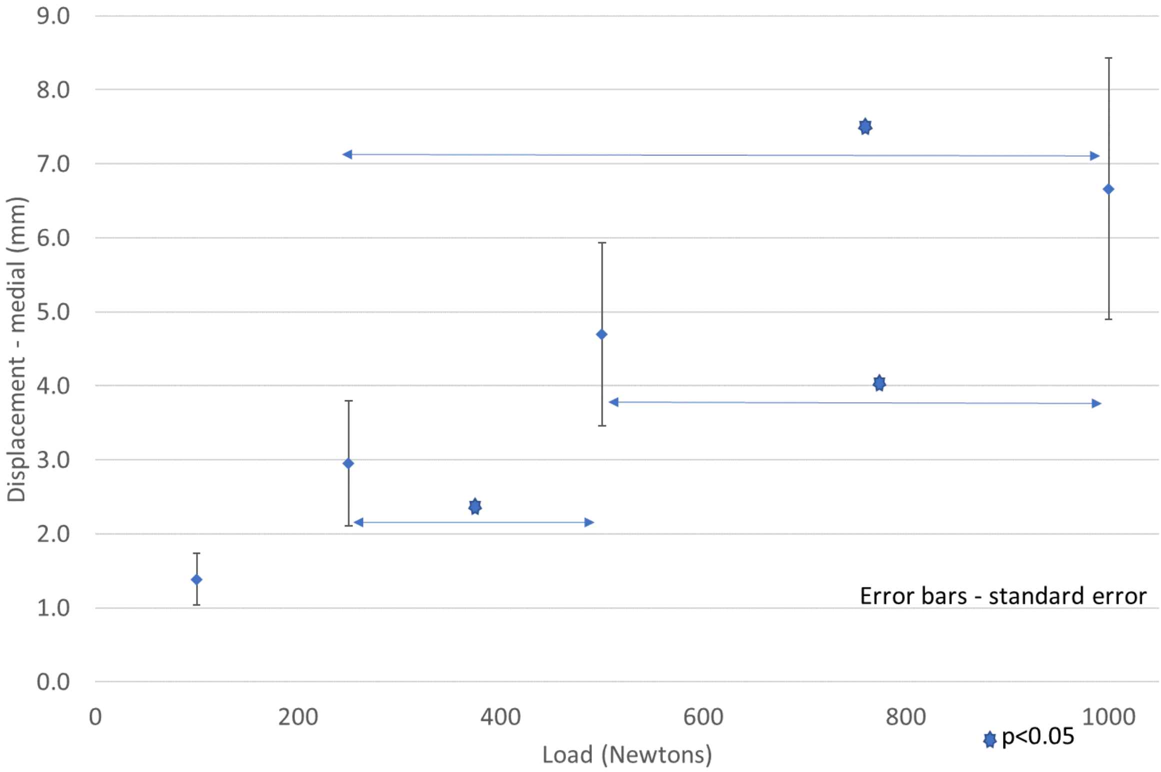


Figure 3-48 - Effect of load on anteroposterior displacement of the tibia, significantly increased anterior displacement of tibia with increasing load, between 250N and 500/1000N as well as 500N and 1000N.

Load	**Displacement (s.e.)**
100N	0.71 (1.44) mm posterior
250N	1.27 (0.43) mm anterior
500N	2.28 (0.65) mm anteriorǂ
1000N	3.97 (1.03) mm anterior*
ǂ sig increase in anterior movement compared to 250N *sig increase in anterior movement compared to 250N/500N	

Table 3-6 - Increased anterior displacement of tibia under increasing load

Once more, post hoc analyses demonstrated significant differences between the 1000N load 250N/500N load states, with progressively anterior displacement evident. The difference between 250N and 500N approached significance (p=0.062).

Superoinferior displacement

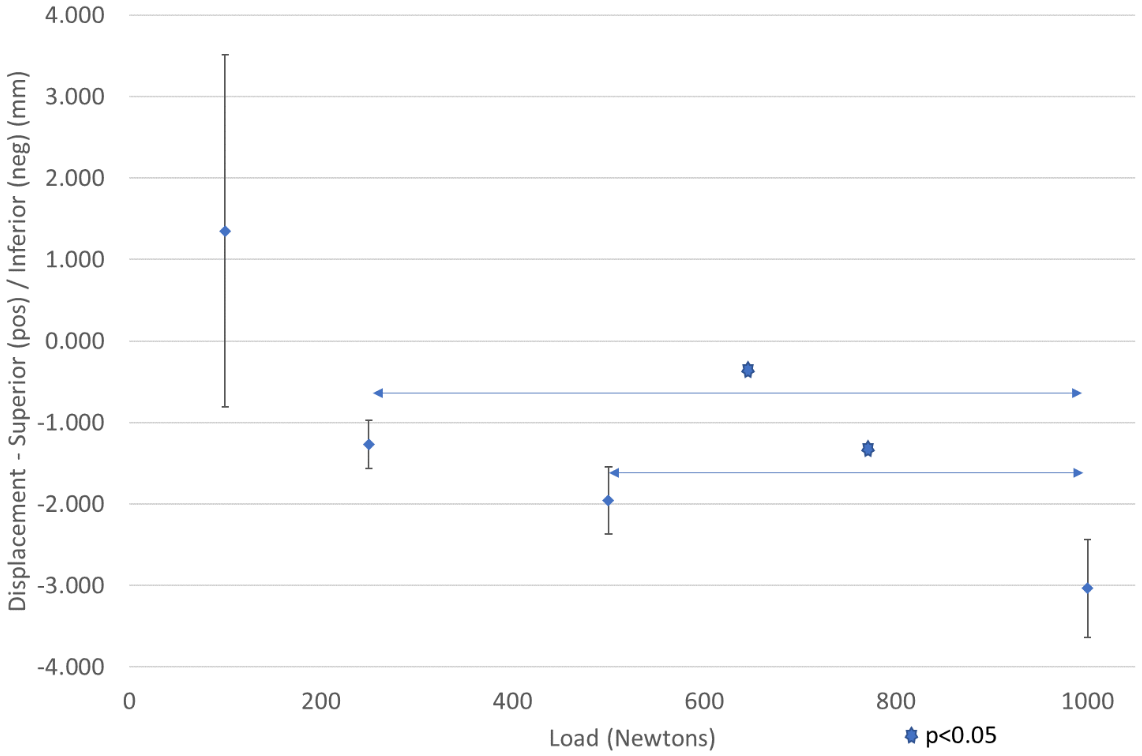


Figure 3-49 - Effect of load on supero-inferior displacement of the tibia, note progressive increase in inferior tibial displacement with load with significant differences evident between 250/500N and 1000N.

Load	Displacement (SE)
100N	1.35 (2.16) mm superior
250N	1.26 (0.30) mm inferior
500N	1.96 (0.41) mm inferior
1000N	3.03 (0.60) mm inferior*
*sig increase in inferior displacement compared to 250N/500N	

Table 3-7 – Progressive inferior movement of the tibia under load

A trend towards inferior displacement of the tibia is noted with increasing load. The univariate analysis did not show any significant differences, though post-hoc tests demonstrated a significant difference between the 1000N load state and the 250N/500N states.

No differences were noted in the effect of load stratified by side of injury.

3.2.4.4.5 Effect of flexion angle

In isolation, the angle of testing had a significant effect on the internal/external rotation of the tibiofemoral joint (Figure 3-50).

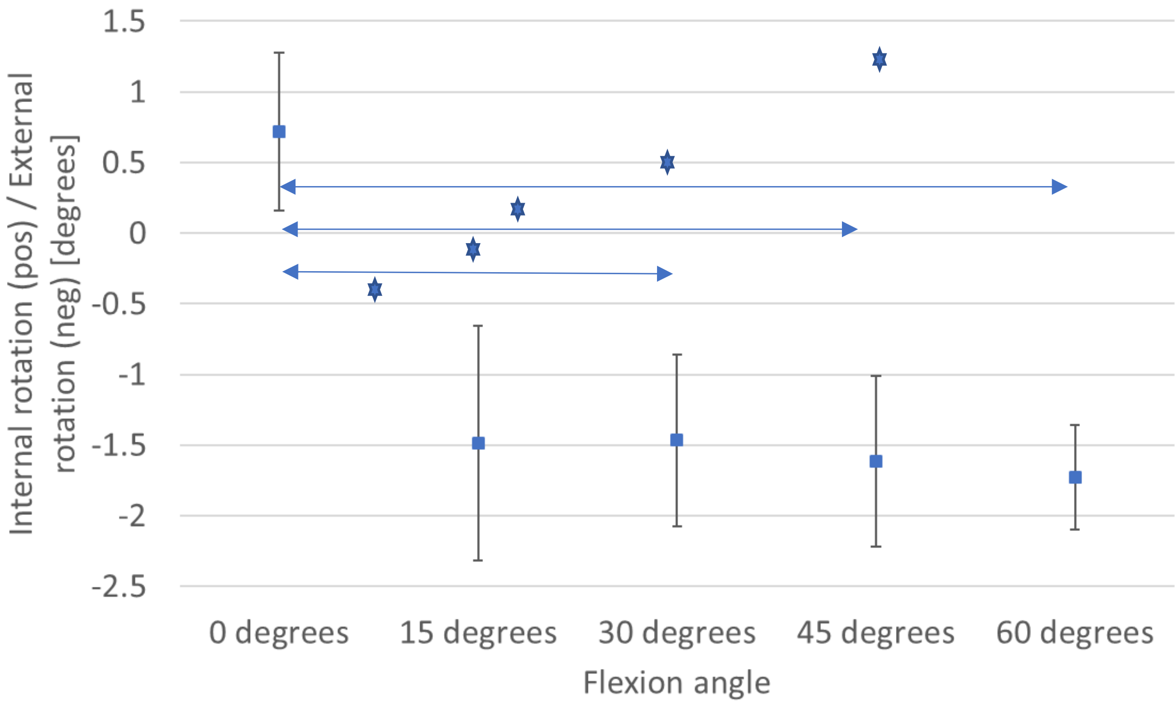


Figure 3-50 - Effect of flexion angle on internal/external rotation, note internal rotation at full extension and trend towards progressive external rotation with increasing knee flexion. Significant difference evident between rotation at 0 degrees and all other states.

Angle of testing	Rotation (s.e.)
0 degrees	0.72 (0.56) ˚ internal rotation*
15 degrees	1.49 (0.83) ˚ external rotation
30 degrees	1.47 (0.61) ˚ external rotation
45 degrees	1.62 (0.60) ˚ external rotation
60 degrees	1.73 (0.37) ˚ external rotation
*Significant difference between rotation at 0 degrees and all other flexion angles.	

Table 3-8 - Effect of flexion angle on internal/external rotation, note change from internal rotation at full extension, to external rotation with knee flexion.

At full extension, the tibia appears to internally rotate slightly under load, however, as the knee begins to flex, there is a marked shift towards external rotation of the tibia, which is maintained through the different degrees of flexion tested.

3.2.4.4.6 Interaction of load and flexion angle

When the interaction of load and angle tested was considered, a significant effect was observed on the varus/valgus angle (Figure 3-51), internal/external rotation (Figure 3-52) and mediolateral displacement (Figure 3-53).

Varus/valgus angle

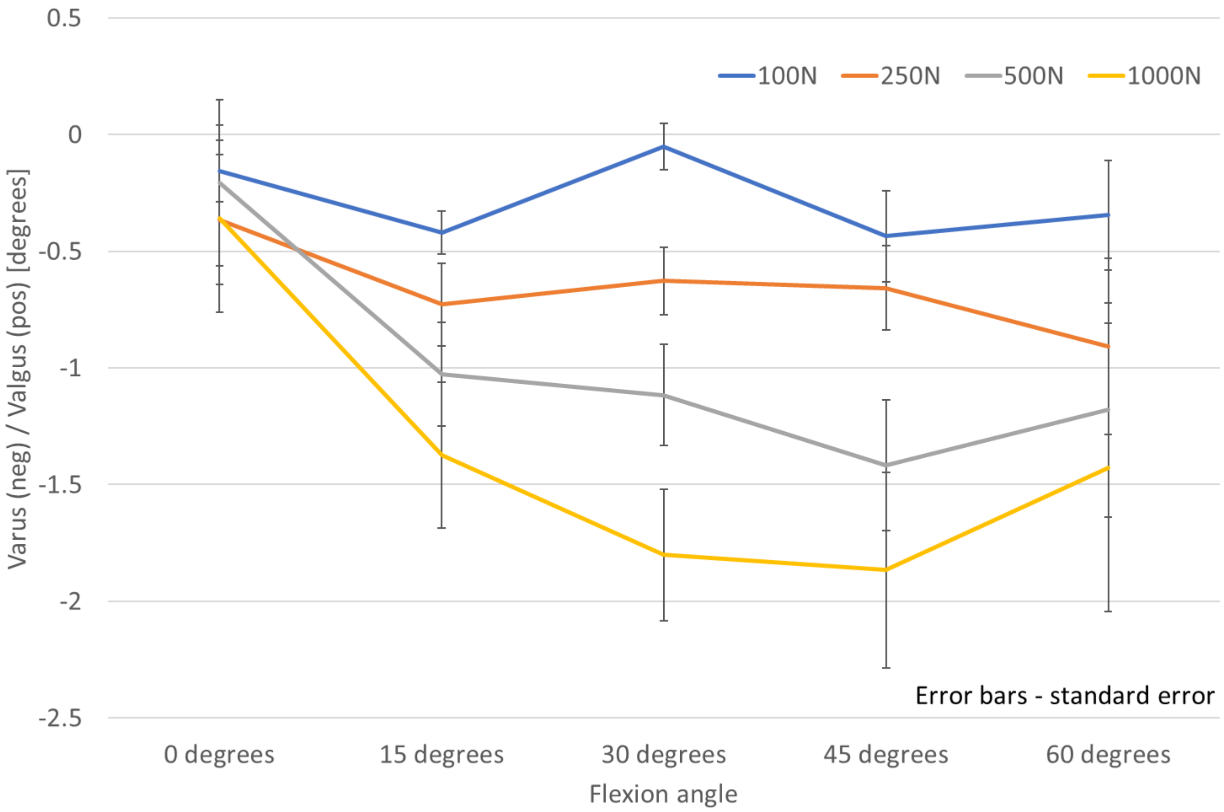


Figure 3-51 - Interaction of load and angle of testing – note progressive increase in varus angulation with increasing flexion and load.

Varus angle of tibiofemoral joint (s.e.)				
Angle of testing	**Load**			
	100N	250N	500N	1000N
0 degrees	0.15 (0.13) °	0.36 (0.28) °	0.21 (0.36) °	0.36 (0.40) °
15 degrees	0.42 (0.09) °	0.73 (0.18) °	1.03 (0.22) °	1.38 (0.31) °
30 degrees	0.05 (0.10) °	0.63 (0.15) °	1.12 (0.22) °	1.80 (0.28) °
45 degrees	0.44 (0.20) °	0.66 (0.18) °	1.42 (0.28) °	1.87 (0.42) °
60 degrees	0.35 (0.23) °	0.91 (0.38) °	1.18 (0.46) °	1.43 (0.62) °

Table 3-9 – Interaction of load and flexion, note progressive varus angulation of tibiofemoral joint with increasing load/flexion.

At full extension, there is little difference between loads on the degree of varus observed, however, as the load increases, there is a trend towards increasing varus with increasing load as the flexion angle increases (**Table 3-9**). Notably, this seems to reverse at 60 degrees, when the degree of varus observed at different load conditions appears to converge.

Internal/external rotation

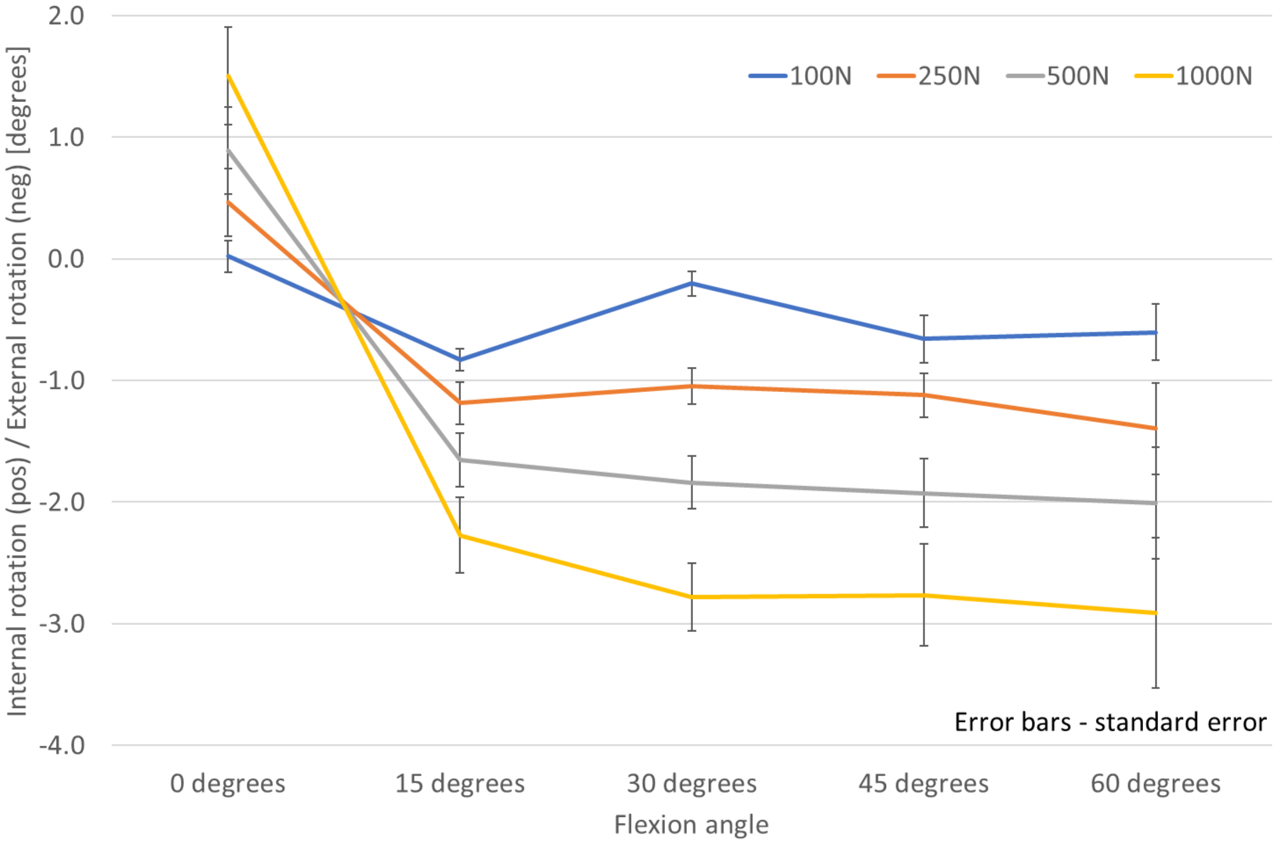


Figure 3-52 - Interaction of load and flexion angle - internal/external rotation, note internal rotation at full extension in all injury states, with progressively increased external rotation with increased load and knee joint flexion.

Internal/external rotation of tibiofemoral joint (s.e.)					
Angle of testing	**Rotation observed**	**Load**			
		100N	**250N**	**500N**	**1000N**
0 degrees	Internal	0.02 (0.27) °	0.46 (0.44) °	0.89 (0.36) °	1.50 (1.00) °
15 degrees	External	0.83 (0.36) °	1.19 (0.67) °	1.66 (0.22) °	2.27 (1.43) °
30 degrees	External	0.20 (0.19) °	1.05 (0.40) °	1.84 (0.22) °	2.78 (1.20) °
45 degrees	External	0.66 (0.26) °	1.12 (0.44) °	1.92 (0.28) °	2.76 (1.11) °
60 degrees	External	0.60 (0.22) °	1.40 (0.32) °	2.01 (0.46) °	2.91 (0.65) °

Table 3-10 – Internal/external rotation of tibiofemoral joint – interaction of load and flexion angle, note internal rotation at full extension, with progressive external rotation with increased load and flexion angle.

In full extension, increasing load appears to correlate with internal rotation of the tibia (**Table 3-10**). However, as the knee begins to flex, the tibia begins to external rotate, with the degree of external rotation correlating with the load applied.

Mediolateral displacement

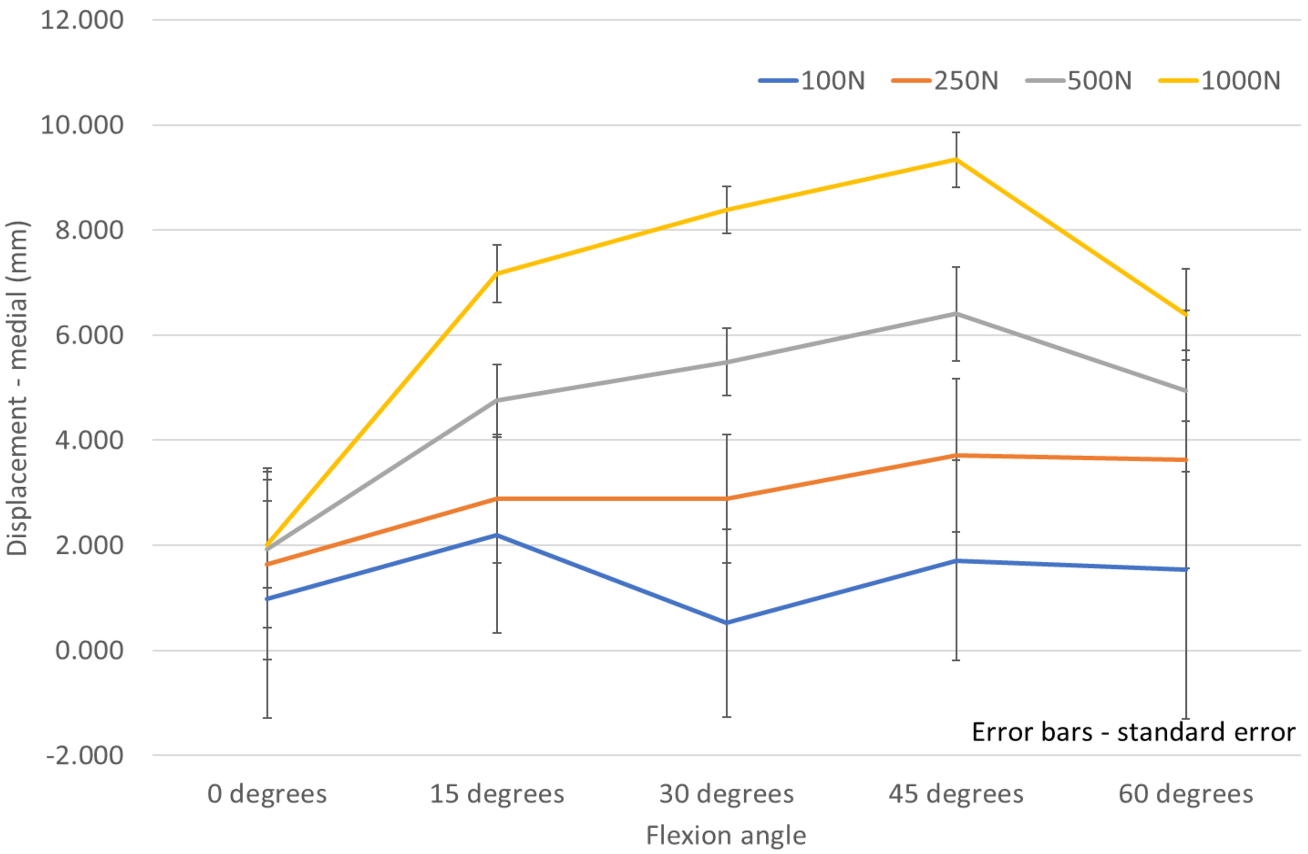


Figure 3-53 – Interaction of load and flexion angle - mediolateral displacement, progressive increases in load and flexion angle are associated with increased medial displacement of the tibia.

Medial displacement (s.e.) [mm]				
Angle of testing	Load			
	100N	250N	500N	1000N
0 degrees	0.99 (0.83)	1.64 (1.48)	1.92 (1.82)	2.01 (2.27)
15 degrees	2.20 (0.54)	2.89 (0.69)	4.75 (1.22)	7.17 (1.87)
30 degrees	0.52(0.45)	2.88 (0.64)	5.49 (1.23)	8.39 (1.78)
45 degrees	1.71 (0.52)	3.71 (0.90)	6.40 (1.46)	9.34 (1.91)
60 degrees	1.53 (0.88)	3.63 (1.54)	4.94 (2.07)	6.39 (2.83)

Table 3-11 – Medial displacement of tibiofemoral joint -, note increased medial tibial displacement with increased load and flexion angle.

Once again, there is little difference in full extension, with medial displacement fairly similar across loading conditions (Table 3-11). As the knee flexes, there is a trend towards an increase in medial displacement which appears to correlate with the load applied.

3.3 Discussion

Although the role of ligamentous structures in maintaining stability of the knee is well established, the role of the menisci in doing so has been subject to less scrutiny. Whilst there has been some study of the role of complete meniscectomy in inducing knee instability, such a situation is largely of historical interest, as a complete meniscectomy is a situation of last resort in contemporary orthopaedic practice. The role of isolated meniscal injury in stability of the knee joint has been studied by:

- Goyal et al. [11], who demonstrated no difference in stability following vertical tears of the posterior third of the lateral meniscus.
- Arno et al. [12], who demonstrated posterior translation of the medial femoral condyle following a 46% resection of the posterior horn of the medial meniscus
- Allaire et al. [13], who demonstrated external rotation and lateral translation of the tibia following a medial meniscal root tear

This work is the first study investigating the role of isolated radial tears of the menisci on stability of the tibiofemoral joint. Alongside the effect of injury, variables where significant differences (or differences approaching significance) are discussed below.

3.3.1 Effect of injury

Pooling data across all loads and flexion angles, radial tears of the menisci were not found to induce significant differences in the displacement or rotation in all 6 degrees of freedom.

In the flexion/extension axis, which was held fixed by the jig, it is reassuring to note minimal differences between states with nearly all overlapping a zero-degree difference. This suggests that the jig was successful in maintaining a fixed degree of knee flexion during application of load and the small changes seen can likely be attributed to soft tissue deformation within the knee, rather than movement at the jig-bone interface. As noted above, the base plate of the jig was redesigned to allow free movement and it is likely that the small magnitude of flexion/extension observed relates to tilting of the tibial base on the base plate during application of load, an effect that was mirrored across all knees and injury states and hence would not affect experimental validity.

The effect of injury on varus/valgus angulation approached significance. Notably, change in varus/valgus angulation was made possible as the tibial base was not fixed to the base plate – though any such changes would be limited as angulation of the base was restricted through its position over the tibial baseplate. This situation is distinct from that observed in vivo, where a change in angulation of the knee can be compensated in part by the position of the ankle/foot. Nevertheless, a trend is evident in the data whereby knees sustaining medial meniscal injury trend towards valgus angulation, whilst the opposite is true of knees sustaining lateral meniscal injury, with both groups tending to a similar mean once a full tear is sustained. The menisci are understood to aid congruity of the tibiofemoral joint, particularly in the lateral compartment – if radial tears impair the ability of the meniscus to perform this function then it is conceivable that a tear of either meniscus allows the knee to fall into an 'incongruous' alignment, dictated by ligamentous constraints, which will be the same regardless of side of meniscal injury.

There appears to be a slight trend towards external rotation in both injury states, with all knees displaying very similar characteristics. Whilst this data provides no suggestion that this variable is influenced by meniscal injury, once again, the similarity of the data across injury states and injury types adds to confidence in the reliability of the dataset.

All knees display a tendency towards medial displacement of the tibia, a trend which is also reported in the literature [258]. The knees in the medial meniscus injury group displayed a higher magnitude of medial displacement as compared to the lateral meniscal injury group – this difference most likely reflects the innate kinematics of the knees in each group – which can be dependent on a variety of factors including knee alignment and ligamentous laxity [259].

Both groups also displayed a tendency towards anterior displacement of the tibia relative to the femur, with almost no difference between groups. Again, such anterior translation is typical of that described in the literature when the knee is under load [258,259]. This work demonstrates that meniscal injury does not affect this relationship.

Supero/inferior displacement appeared to have no significant change with injury state, though there did appear to be superior translation of the tibia relative to the femur in the intact state. As described in the results relating to the effect of load, this superior translation appears to reflect loads results recorded at loads of 100N. One would expect inferior displacement of the tibia with load – though at small magnitude loads it is possible that the load applied simply causes elastic deformation of the bearing surfaces without wholesale translation of the tibia itself, hence allowing the femur to move inferiorly towards the tibia without any compensatory movement of the tibia – this manifests as superior movement of the tibia in our dataset.

3.3.2 Interaction of injury and angle/load of testing

Due to the lack of interaction effects, the effect of injury severity on knee kinematics was independent of knee angle and knee joint load. The main effects and interactions allow us to conclude that radial tears of either the medial or lateral meniscus do not manifest as kinematic instability of the knee joint.

3.3.3 Effect of load

Intuitively, one would expect the kinematics of the joint to vary with load. This was evident in many of the kinematic variables.

A progressive increase in load was associated with an increase in varus of the joint. Whilst data on the innate alignment of the knee joints tested was not available, it is well accepted that a significant proportion of the population have constitutional varus of the knee joint [260], which may manifest as an increase in varus with increasing load. Furthermore, as the knee was mounted in isolation within the jig, results cannot be extrapolated to the in vivo state, where alignment is influenced by the position of the proximal and distal joints. It is interesting to note that the standard error increases with increasing load, perhaps reflecting that the behaviour of individual knees is driven by their innate kinematics, with some knees tending to more varus than others as the load applied is increased.

A similar effect was observed in relation to medial displacement of the tibia with respect to the femur, with the standard error of the mean displacement again following a similar pattern of almost linear increase with increasing load. Gasparutto et al. [258] synthesised data from a variety of studies exploring in vivo tibiofemoral kinematics, finding that, within a range of motion of 4 degrees hyperextension to 61 degrees flexion, the motion of the tibia was mainly in medial displacement, with displacement increasing with flexion. A similar magnitude of mediolateral displacement is reported by Victor et al. [261], in an unrelated context. Notably, an increase in medial displacement of the tibia has been noted in the context of ACL injury [262] and in meniscal injury combined with ACL injury [263] – though this finding is distinct from our findings.

Interestingly, the behaviour of the knee joints under load in the anteroposterior plane showed a slight posterior displacement of the tibia at 100N load, changing to an anterior displacement of up to ~4mm at 1000N load, though it should be noted that the standard error at 100N is large and includes small magnitude anterior displacement. A variation in anteroposterior displacement has previously been reported, with up to 2mm posterior displacement and 26mm anterior displacement noted as the knee flexes [258]. Notably, in assessing solely static stability, the effect of the quadriceps muscles was not replicated. It is possible that the observed large magnitude displacement of the tibia would not have been present were stability of the tibia controlled by this group of muscles [259]. A significant difference was not observed between the 100N and 1000N load states, despite the large magnitude of difference in the load applied. This is likely due to the variability seen in anterior displacement, with some knees displaying almost no movement in the anteroposterior plane. It is possible that a 100N of load was insufficient to cause significant movement of the tibia and the results observed here reflect a small deviation from the initial position of the tibia relative to the femur as load is applied rather than movement resulting due to sliding of the articular surfaces.

Supero-inferior displacement of the tibia follows a similar pattern as that observed above, with progressive inferior displacement with increasing load – as would be expected. Once more, at 100N a large standard error and some (relative) superior movement of the tibia is seen – this likely observes compression and elastic deformation within the joint as discussed above.

3.3.4 Effect of flexion angle

At full extension, the tibia was observed to slightly externally rotate, whilst internal rotation predominated as the knee began to flex. This change in rotation is well documented in a physiological context [264,265] and has been documented by some authors as the ‘paradoxical’ screw home movement [266]. This finding adds credence and confidence as to the validity of this methodology and analysis in mimicking in vivo movements of the knee joint.

3.3.5 Interaction of angle and load

The findings observed when the combined effect of angle and load were considered separately, across all injury states to allow further insight into those described above.

As the knee is flexed, progressively more varus is observed with both an increase in flexion angle and in load. At higher loads, there is a tendency towards a reduction in the varus angle at 60 degrees. This could potentially reflect articulation of the joint bearing surfaces at these higher loads limiting the degree of varus the joint can fall into.

The effect on internal/external rotation is striking. At full extension, increasing load is associated with increased external rotation of the tibia on the femur. As the femur flexes, this trend is reversed, with both an increase in load and an increase in flexion resulting in increased internal rotation. The mechanism here is similar to that described above. Furthermore, the association between increased internal rotation of the tibia with flexion has been documented elsewhere in the literature [267,268] and is associated with roll back of the lateral condyle of the femur as the knee flexes [269]. internal rotation of the tibia with respect to the femur has been reported with increasing knee flexion and can be observed as trend in the data here – a manifestation of the well documented ‘screw home’ mechanism of the knee [270]. Although there is no documented evidence of the potential impact of this increase in external rotation of the tibia on the knee’s articular surfaces, one might propose that such altered kinematics could result in varied loading conditions, potentially resulting in abnormal loading of the articular cartilage, ultimately resulting in accelerated wear and resultant osteoarthritis. Indeed, radial tears have been demonstrated to cause such altered loading [75,76,83].

Medial displacement of the tibia appears to increase from 0-15 degrees of flexion and with increasing load, but thereafter appears largely un-influenced by degree of flexion. Indeed, Gasparutto et al. [271] noted that beyond 20 degrees of flexion, medial displacement decreased, whilst an increase was noted in the first 20 degrees. A marked decrease in medial displacement is evident at higher degrees of flexion at both 500N and 1000N, mirroring their results.

3.3.6 Summary

In summary, we have demonstrated that:

- Radial tears of the menisci do not influence kinematics of the knee. There is a trend towards valgus angulation of knees sustaining medial meniscal injury and varus angulation of knees sustaining lateral meniscal injury.
- Increasing load is associated with:

    - Varus angulation of the knee joint
    - Medial displacement of the tibia
    - Progressive anterior displacement of the tibia
    - Inferior displacement of the tibia
- Increasing flexion of the knee is associated with initial external rotation, followed by internal rotation of the tibia.
- The combined effect of increasing load and flexion angle is associated with:
    - Progressive varus, up to 45 degrees of flexion.
    - Initial external rotation, then internal rotation of the knee joint
    - Medial displacement (up to 15 degrees of flexion)

3.3.7 Strengths and weaknesses

This work has several strengths. For a cadaveric study, we have tested a large number of samples, though despite this, these are small numbers and a degree of inter-sample variation may have influenced the results obtained. All cadavers were from patients under the age of 65, with normal body mass index and no history of knee injury, minimising the risk of confounders in terms of osteoarthritis or structural abnormality of the knee. Cadavers were all derived from the same donation body, hence minimising inter-sample variation in terms of preparation. Furthermore, inspection of the knee joint following testing revealed no evidence of significant osteoarthritis or existing structural knee injury. In addition, we were able to reproduce previously described joint kinematics, such as the 'screw home' rotation of the knee joint.

Contrary to almost all other studies in the literature, we did not remove the majority of soft tissue from the knee joint during testing – with only two arthroscopic portals used to access the knee joint. This approach sought to maximise the stabilising effect of the surrounding knee joint structures. However, it must be acknowledged that we were not able to mimic the dynamic stabilisers of the knee in any manner – for example by applying a load through the quadriceps tendon. However, in doing so, we perhaps missed an opportunity to collect more data from our specimens, perhaps in terms of contact pressures/areas following meniscal injury.

The jig designed for the study was custom built to fulfil the study aims. We did not test whether the jug itself deformed under load – it was assumed that its construction of solid aluminium and resultant weight were such that such strain would be negligible. Furthermore, if such deformation were present, it would equally affect each iteration of testing.

As well as this, it is assumed that the interface between the jig and bone was perfectly rigid. Through the use of a low viscosity mix of cement coupled with allowing the cement to set overnight, it was assumed that any movement between the jig and the bone was negligible, however we did not make any direct assessment of this fixation.

As both the position of the femur and the flexion angle of the knee were fixed by the experimental apparatus, these experiments would not have identified alterations in femoral kinematics caused by meniscal tears, such as roll back of the femur during flexion.

The methodology used for the experiments was not independently validated. This could perhaps have been achieved through fixing a pre-fabricated hinged prosthesis with known kinematics within the jig and then using the testing apparatus to verify the measured kinematics were equal to those expected. This approach was considered, but not adopted due to lack of availability of such a prosthesis, coupled with the time constraints of this work. Furthermore, a number of authors have used similar techniques to ours to reliably measure knee kinematics. In addition, the observation of native knee kinematics such as medial and anterior displacement of the tibia lends credence to the validity of the methodology used here.

All knee arthroscopy was performed by a practicing Consultant Orthopaedic surgeon who performs such procedures on a regular basis, adding confidence to both accurate assessment of the joint and creation of the meniscal tears. However, we did not verify the depth of 50% resection tears and it is fair to assume that these were not all of exactly 50% meniscal depth.

The experimental design did not differentiate between left and right sided samples. There is little evidence in the literature to suggest that side specific differences in kinematics do exist, with a single study [272] in vivo suggesting any differences present relate to the swing, rather than stance phases of gait. Furthermore, previous studies of meniscal injury [9,11,80] have not accounted for differences in side of testing, assuming that knees from both sides will behave the same.

We were only able to conduct a single test in each injury state for each specimen. Ideally, testing in each state would have been repeated a number of times to improve reliability of the data. Time constraints prevented this – including the need to test samples before any tissue dehydration, the availability of the Orthopaedic surgeon conducting arthroscopy and availability of the laboratory for testing, especially in the context of a departmental refurbishment.

The study tested loads up 1000N, which is well into physiological range. However, we only tested axial load and were not able to test such load combined with an anterior draw, for example, which might reproduce more closely the forces a knee is subject to during physiological loading. Although we made an effort to preserve soft tissues surrounding the knee, we were also unable to recreate the myriad muscular forces involved in dynamic stabilisation of the knee. Furthermore, we did not make an effort to reproduce the gait cycle.

We were unable to adequately synchronise the output from the Instron materials testing machine with that from the Optitrack cameras as both systems were started manually. This precluded any combined analysis of load with displacement/angulation.

The knee joint coordinate system accurately reproduced the position of the jig in space. However, the bone coordinate system was reliant on manual collection of bony landmarks, which can result in inaccurate data [273], adversely affecting the bone coordinate system.

Finally, the kinematics of an individual knee are multifactorial in nature, reliant on factors including the geometry of the articulating bones, the type of joint loading, alignment of the joint in question as well as those above and below, body weight, soft tissue constraints and muscular action [261]. These variables likely explain some of the variability observed in our results. It is also impossible to recreate these factors in an in vitro experimental setup – ultimately clinical validation of these results is still necessary.

3.3.8 Suggestions for further work

Whilst cadaveric testing of any injury state provides insight into the potential kinematic behaviour of the knee, validation through clinical testing is required to provide a definitive insight into the effect of such injuries. As such, a clinical validation study of patients who had sustained radial meniscal tears is necessary to validate the result observed here, with the contralateral, uninjured knee acting as a control. Measurements could be conducted using a non-invasive navigation tracker, such as that described by Russell et al. [274].

Modifications could also be made to our experimental design if this study were to be repeated or if other soft tissue injuries were to be investigated. In particular, the jig design could be modified to allow tension to be applied via the quadriceps tendon, mimicking the effect of this muscle – this could be achieved using weights. As well as this, the femoral portion of the jig could be modified to allow the centre of rotation to be adjusted, limiting the need to section the femur at a particular length and potentially allowing large samples to be tested. It would also be useful to repair any tears and re-measure kinematics.

Whilst it was possible to cement the soft tissues into the bone cylinders, this was challenging due to the soft tissue obscuring much of the rim. Hence the bone cylinders could be re-designed with a larger diameter, or in an oval shape to allow more space for cementing the tissue in place.

3.3.9 Conclusions

Radial tears do not result in a clinically relevant alteration in knee joint kinematics. As noted previously, the meniscus is largely avascular and the lack of blood supply in the 'white-white' zone leads to the treatment for symptomatic tears of the medial meniscus being treated through partial meniscectomy. However, the literature suggests [9,153] that this treatment itself alters knee kinematics. Whilst there is no evidence to date of partial meniscectomy resulting in accelerated chondral wear, altered native knee kinematics could arguably result in accelerated such wear through abnormally loading the joint surfaces or causing injury to them. Furthermore, there is much recent debate over the clinical benefit of partial meniscectomy [275,276], with evidence suggesting not only that such resection is of limited clinical benefit in degenerate tears suffered by elderly patients [276], but also that it may be of little benefit to younger patients with non-obstructive traumatic tears, for whom conservative management combined with knee physiotherapy might suffice. Given this evidence, and the fact that this current study demonstrates that knee kinematics are unaltered by radial meniscal tears, it is arguable that the focus of treatment for radial tears of the menisci should shift from limited resection of damaged tissue to preservation, or where possible, repair/transplant of the tissue. This argument is further bolstered by evidence in the literature that such tears do result in abnormal loading of the articular cartilage and potential increases in peak contact pressures and concomitant decreases in mean contact areas [75,76,83]. Both restoring normal joint loading characteristics and preserving native kinematics is most important in younger patients, where the potential for chondral wear to manifest as symptomatic knee arthritis is more likely due to their likely longevity.

The challenge, however, lies in identifying a means of treatment for tears which either lie in the poorly vascularised zones of the menisci or are irreparable. It is fair to say that choosing to undertake a meniscal repair/transplant using contemporary techniques is a significant consideration, not only because of the more difficult surgical technique, but also because of the lengthy rehabilitation required and potential for causing knee stiffness and muscle wasting due to the limitations to knee movement and weight bearing which must be implemented. Hence, such procedures are usually reserved for the motivated young patient.

However, the advent of novel implants such as meniscal scaffolds or even potential meniscal substitutes may allow surgeons to aim for meniscal preservation in cases where this was not thought possible. This section of the thesis has demonstrated that radial tears of the menisci do not manifest as kinematic instability of the knee, whereas partial meniscectomy, often used as treatment for these tears, is understood to cause such instability. It is imperative, therefore, that we develop means through which the injured meniscus can be repaired or replaced. The subsequent sections of this thesis aim to collect data to further this effort.

4. The role of proteoglycans in material properties of the meniscus

4.1 Methods

Although there is extensive study of the meniscus' behaviour in confined compression, no work has been undertaken exploring the relative importance of osmotic pressures within the meniscus to withstand load. These pressures are influenced of the negative charge of proteoglycans on meniscal behaviour. To this end a number of confined compression experiments of meniscal tissue were undertaken in phosphate buffered saline with varying concentrations of sodium chloride to allow elimination of ionic effects. Notably, testing of porcine meniscal tissue in PBS and synovial fluid has demonstrated that its properties are similar – though more variability was observed during testing in PBS [277]. The authors suggested synovial fluid may allow collagen fibres to slide against one another more efficiently than PBS, leading to less variability in the relaxation modulus. However, the low number of samples tested in the study may also account for the variability observed.

4.1.1 Development of testing apparatus

In the absence of a readily available apparatus for conducting testing, a custom compression apparatus was designed to undertake the confined compression experiments using Creo (PTC Inc, Needham, Massachusetts, United States) software. An indenter, chamber and bath were designed. The dimensions of the confining chamber were based on a circle of 8mm diameter, 8mm high. Both the base of the indenter and the base of the chamber contained pores of 500 micrometre diameter. The indenter was designed with a porous surface and was hollow to contain a column of fluid. Creo drawings of the components are shown in Appendix 4.

The proximal part of the indenter was designed to allow it to be gripped by a Bose Electroforce 3100 (Bose, Framingham, Massachusetts, United States) materials testing machine (Figure 4-3). A custom sectioning device (see Appendix 4) was also designed and 3d printed to allow 5mm thick sections of meniscus to be cut. This was designed to work with commercially available razor blades. Two blades are fitted over the insert, with the entire apparatus mounted between two complementary holder components.

Figure 4-1 - Bose Electroforce 3100

Following 3d printing of the test apparatus, preliminary testing was undertaken using samples of bovine tissue. The entire apparatus was placed in a clear Perspex bath to allow the sample to be bathed in solution. However, the placement of perforating holes at the top of the indenter meant that fluid did not always filter into the indenter and there was a risk of evaporation potentially leaving one surface of the tissue dry. The indenter was therefore redesigned to place these perforating holes closer to the bottom, hence ensuring a tissue/fluid boundary at both ends of the tissue.

4.1.2 Bovine pilot tests

Bovine hindlegs were obtained from a local abattoir. Their menisci were dissected out and frozen at -20°C until use. Notably, Gelber et al. [278] conducted electron microscopy on human menisci from fresh and cryopreserved samples. They did not demonstrate a significant difference in collagen fibril diameter and degree of disarray between the two groups – leading to the conclusion that the ultrastructure of the meniscus is not altered by cryopreservative techniques and that the biomechanical properties of the tissue should remain unaltered through such preservation. However, it has been demonstrated that meniscal fibrochondrocytes undergo apoptosis when subjected to the same techniques [279].

A hollow punch (Figure 4-2) was used to obtain an 8mm diameter core section from the meniscus whilst frozen and the location of this core was recorded as anterior/ mid-anterior/ posterior or mid-posterior. The aforementioned sectioning device was used to obtain a ~5mm section from this core. Individual thickness measurements were recorded for each specimen using a micrometre. The sample was wrapped in clingfilm and allowed to thaw for 2 hours.

Figure 4-2 - Hollow punch

Experiments were conducted in 3 solutions:

1. De ionised water
2. 0.14M PBS
3. 3M PBS

Appendix 5 details the protocols for making up these solutions. The concentrations of these solutions was based on previously published work demonstrating that a 3M PBS solution negates ionic effects in both articular cartilage [95] and intervertebral disc [96] due to a hypertonicity, whilst a deionised water solution negates all mobile ion effects.

The specimen was then placed in the 3d printed chamber and irrigated with the appropriate solution, before being placed into the bath. The indenter was also submerged in this solution by placing the entire apparatus in a clear Perspex bath flooded with solution. The apparatus was then fixed in the Bose Electroforce 3100 materials testing machine (Figure 4-3).

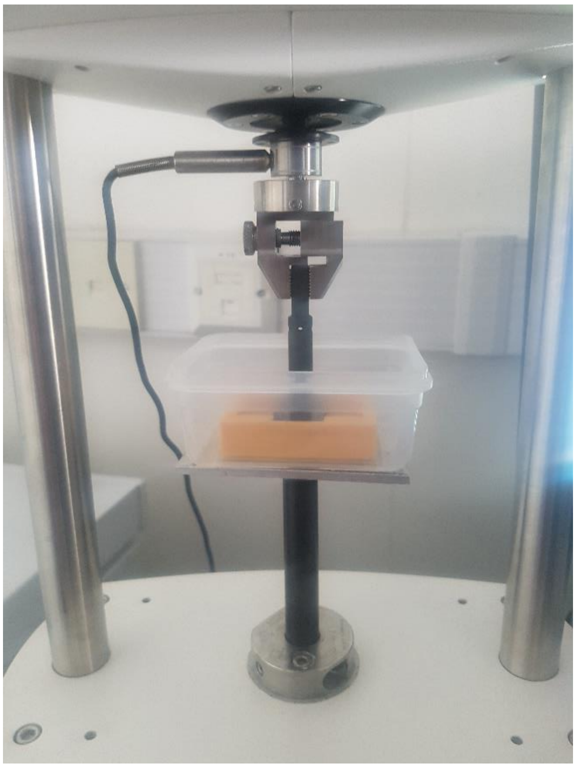


Figure 4-3 - Experimental apparatus

The Wintest 7 software was used to lower the indenter to initially apply a 0.3N preload, followed by immediate application of a 5% ramp strain to the specimen at 1% strain/second. This strain was then held for 6000 seconds whilst the load was recorded.

Preliminary results from the confined compression experiments demonstrated a typical stress relaxation curve. However, an observation of samples left in solution for a few hours (Figure 4-9) demonstrated that although samples in 0.14M/3M PBS swelled minimally, significant swelling was observed with samples in distilled water. A series of swelling experiments were undertaken to characterise the swelling behaviour of meniscal tissue in distilled water, it was demonstrated that swelling effects became significant at 5 minutes of testing (see Figure

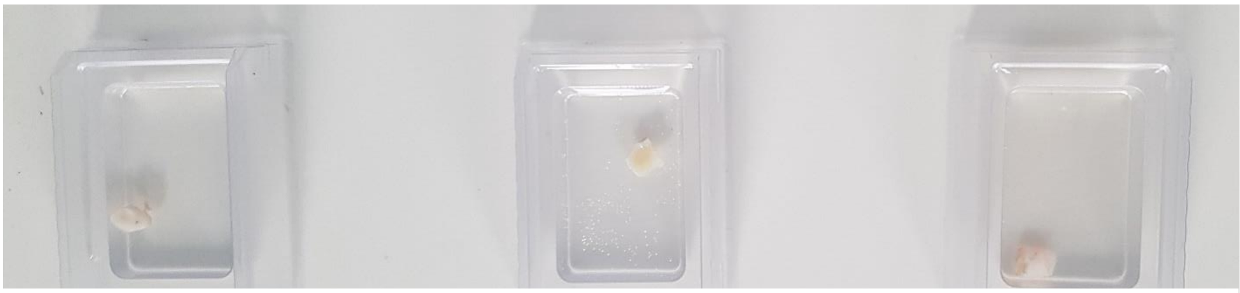


Figure 4-4 - Meniscal samples in solutions of varying ionic concentrations – deionised water, 0.14M PBS and 3M PBS

4-10) below. Hence, the protocol was modified to record relaxation behaviour for 300 seconds following a ramp strain of 5% at 1% strain per second.

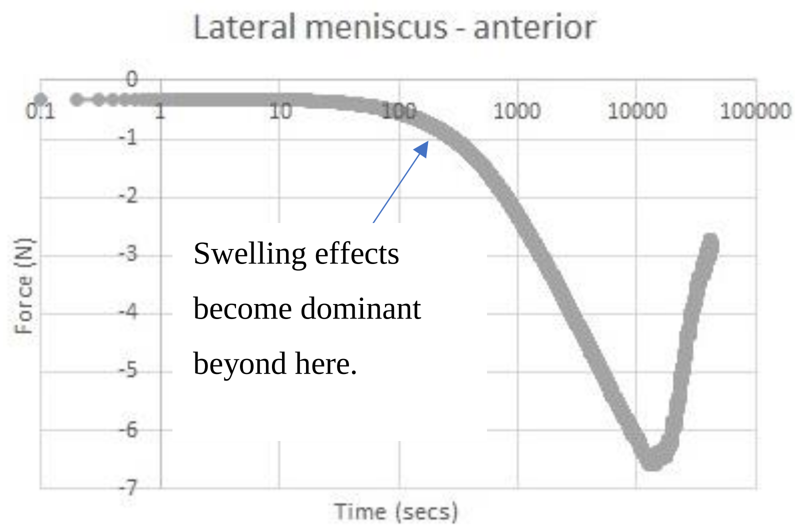


Figure 4-5 - Swelling effects

Notably, the cause for the peak in stress noted on the above curve was unclear – though it was present on all 5 samples tested.

Ten samples derived from two menisci – one medial and one lateral - were tested in each solution. Hence a total of 30 samples were tested. As alluded to previously, it is understood that the mechanical properties of the menisci vary according to region. In testing a pair of menisci in each solution, it was intended any regional variation would balance out across samples. Five samples were produced from each meniscus, one each from the anterior, mid-anterior, middle, mid-posterior and posterior regions. Mean sample thickness was 5.19mm (s.d. 0.18mm).

4.1.3 Human cadaveric tissue

Having validated the technique with bovine tissue, we sought to conduct testing with human meniscal tissue. Ethical approval was sought and granted from the University Ethics Committee.

To conduct testing with human menisci, a number of adaptations were made to the existing apparatus. Firstly, the diameter of both the confined compression chamber and indenter were reduced to 5mm to account for the smaller size of human menisci. As well as this, the permeation holes in the indenter were moved to the bottom of the indenter to ensure the indenter surface was bathed with fluid throughout experiments.

As well as this, the sectioning tool was redesigned for a number of reasons:

- A smaller thickness of tissue would be necessary
- The use of commercial razor blades was resulting in some differences between samples cut – to minimise these, the use of microtome blades was thought preferable.

The re-designed sectioning tool is shown in Figure 4-6 below.

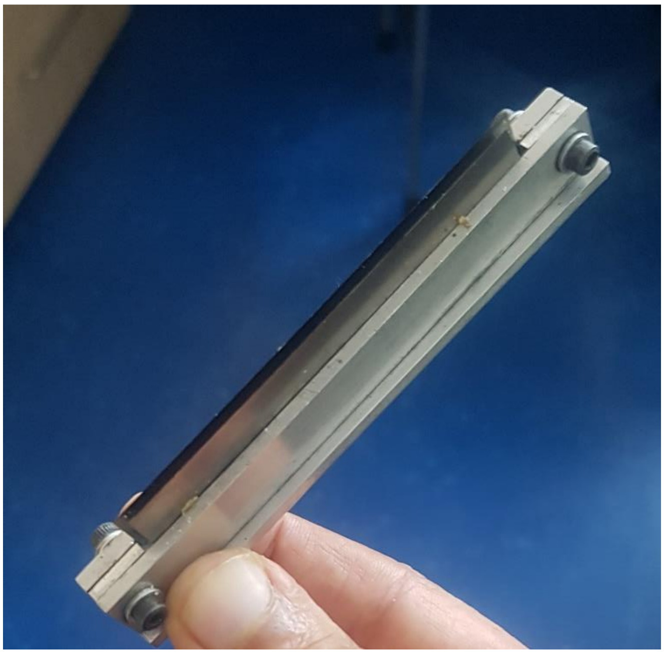


Figure 4-6 - Redesigned sectioning tool

The redesigned tool used microtome blades, held between two steel plates. A further plate placed between the blades was cut and then sanded down to ensure that tissue cut by the blades was exactly 2mm in thickness. This revised tool yielded more accurate cuts.

Human tissue samples were prepared and tested in a similar manner to that described above. On this occasion, a 5mm inner diameter punch was used to obtain tissue samples. Three samples were taken from the anterior, middle or posterior portion of each meniscus. Samples were then cut to 2mm thickness, with each sample thickness measured using a micrometre (Figure 4-7). The same concentration of solutions was used for testing as the bovine pilot tests.

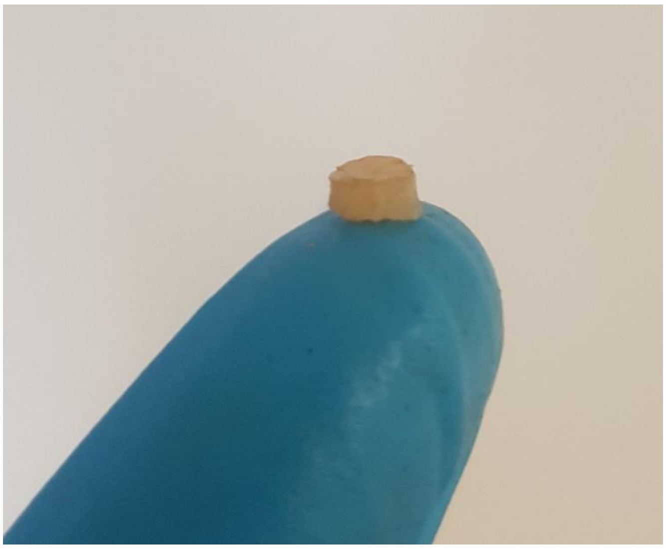


Figure 4-7 - 2mm thick sample

On this occasion, it was deemed preferable to test all samples at equilibrium to avoid any differences in test conditions. As such, a series of tests were undertaken to determine the swelling characteristics of the tissue in each of the three solutions tested. The number of tests was limited by the small amount of tissue available for testing, which had all been collected from the cadaveric knees.

Testing was conducted by placing samples in the test apparatus, lowering the indenter to 0.3N and leaving the sample overnight to observe swelling behaviour. Resulting relaxation curves were assessed to determine when equilibrium had been reached.

Based on the results of these tests, the following protocol was developed:

1. Samples were placed in the testing apparatus and bathed in the appropriate solution. The indenter was lowered to achieve a load of 0.3N and samples were then left to reach equilibrium for the following time periods:
    a. Deionised water – 13 hours
    b. 0.14M PBS – 2 hours
    c. 3M PBS – 2hours
2. A 10% ramp strain was applied at 1% strain / second.
3. Samples were allowed to relax for 2 hours whilst force data was recorded.

Following testing, samples were labelled and immediately re-frozen for proteoglycan assay.

4.1.4 Menisci from osteoarthritic knees

To ascertain whether the magnitude of proteoglycans' contribution to meniscal stiffness altered in the context of osteoarthritis, testing was also conducted on meniscus samples taken from patients undergoing total knee replacement surgery for osteoarthritis.

Ethical approval was sought and granted from West of Scotland Research Committee 4. A patient information sheet and consent form were written and used to recruit patients (Appendix 5 – Patient information sheet & Appendix 6 – Consent form). Inclusion criteria were patients undergoing total knee replacement for a diagnosis of osteoarthritis. Patients requiring total knee replacement for any other diagnosis or with a history of known tear/meniscal surgery were excluded.

Patients were identified from operating lists at the Golden Jubilee National Hospital and their medical records were scrutinised to ensure eligibility. If eligible, a participant information sheet was posted to the patient at least 1 week prior to admission. This was followed up by a telephone call a few days prior to admission to address any queries.

On admission, consent was obtained from the patient. Both menisci were retained in theatre by the operating team and immediately collected and placed in a freezer. Concurrent information on the patient's age, sex and BMI was also recorded. Patient demographics for all samples tested are shown in Table 4-1.

Table 4-1 - Patient demographics

Patient ID	Age (years)	Sex	Side of procedure
1	83	Female	Left
3	81	Male	Left
4	61	Male	Right
5	75	Female	Left
7	67	Male	Right
8	86	Male	Right
9	55	Female	Right
10	53	Male	Right
11	78	Female	Right
12	75	Male	Left

13	75	Female	Right
14	67	Female	Left
16	78	Female	Left
17	54	Female	Right
18	78	Male	Left
19	80	Male	Left
20	65	Male	Right
21	66	Male	Left
22	69	Female	Left
23	71	Female	Right
25	70	Female	Left
26	66	Male	Left
27	74	Male	Left
28	70	Male	Left
29	74	Male	Left
31	55	Female	Right
34	65	Female	Right
35	74	Male	Left
36	70	Male	Right
37	66	Female	Right

Samples were subsequently transported to the University laboratory by University transport and were placed in ice during transport. They were then frozen until experiments were undertaken.

The experimental protocol used was exactly as described for cadaveric meniscal samples. However, given the samples were taken from osteoarthritic knees, there was significant degeneration, particularly in the medial meniscus samples obtained such that less than 20% of them were useful to testing. This was due to the predominantly varus osteoarthritis affecting most of the population and resultant macroscopic damage to the meniscus. In contrast, almost all lateral meniscus samples were found to be in good macroscopic condition. As such, only samples taken from the middle portion of the lateral meniscus were tested – recruiting sufficient patients to obtain samples from the medial meniscus would have required an excessive number of patients to be recruited.

Following testing, samples were once again labelled and immediately frozen to allow proteoglycan assay testing.

4.1.5 Proteoglycan content assay

To ensure that any differences observed between samples were not due to variation in the proteoglycan content between solutions, a proteoglycan assay was undertaken to measure proteoglycan content in all meniscal samples tested. A Biocolor Blyscan Sulphated Glycosaminoglycan Assay kit was used.

Notably, results of the assay could be skewed by the presence of excessive salt. Hence, all samples were washed with deionised water 10 times to remove any salt from the samples themselves.

A papain extraction reagent was prepared by adding 800mg sodium acetate, 400mg EDTA and 40 mg cysteine hydrochloride to 100ml of a 0.2M sodium phosphate buffer. The pH of this solution was corrected to 6.4 and 250 microlitres of a papain crystallised suspension was added. This solution was stored in the fridge and used within 7 days.

Samples were defrosted by placing them in deionised water. Each sample was then cut in half using a scalpel and its wet weight was recorded – the assay required a wet weight of 20-50mg.

Individual samples were them placed in labelled microcentrifuge tubes with 1ml of the papain extraction reagent.

All samples were placed in a warm water bath (65°C) and set to shake slowly. Samples were left overnight to digest (Figure 4-8).

Figure 4-8 - Samples in water bath

The following morning, digestion of samples was confirmed visually. All tubes were then centrifuged at 10 000g for 10 minutes (Figure 4-9).

Figure 4-9 - Microcentrifuge

- A set of microcentrifuge tubes were prepared as follows:
    - 50µl of the supernatant of each test sample was added to individual tubes.
    - Tubes containing 1,2,3,4,5µg of the assay reference standard were also prepared.
    - All tubes were made up to 100µl using the papain extraction reagent previously prepared.
    - 1ml of the assay dye reagent was added to each sample.
- Samples were placed on a mechanical shaker for 30 minutes, during which time a precipitate was seen to form (Figure 4-10).

Figure 4-10 - Precipitate formation following addition of dye reagent

- All samples were spun in a microcentrifuge at 12000 rpm for 10 minutes.
- The supernatant was manually removed from each tube, taking care not to disturb the precipitate which had collected at the bottom of each tube.

- 0.5ml of the assay dye dissociation reagent was added to each tube and a vortex mixer was used to allow the bound dye to dissolve into solution.
- 200µl of each sample was transferred to a 96 microwell plate (Figure 4-11).
- Absorbance was measured at 656nm.

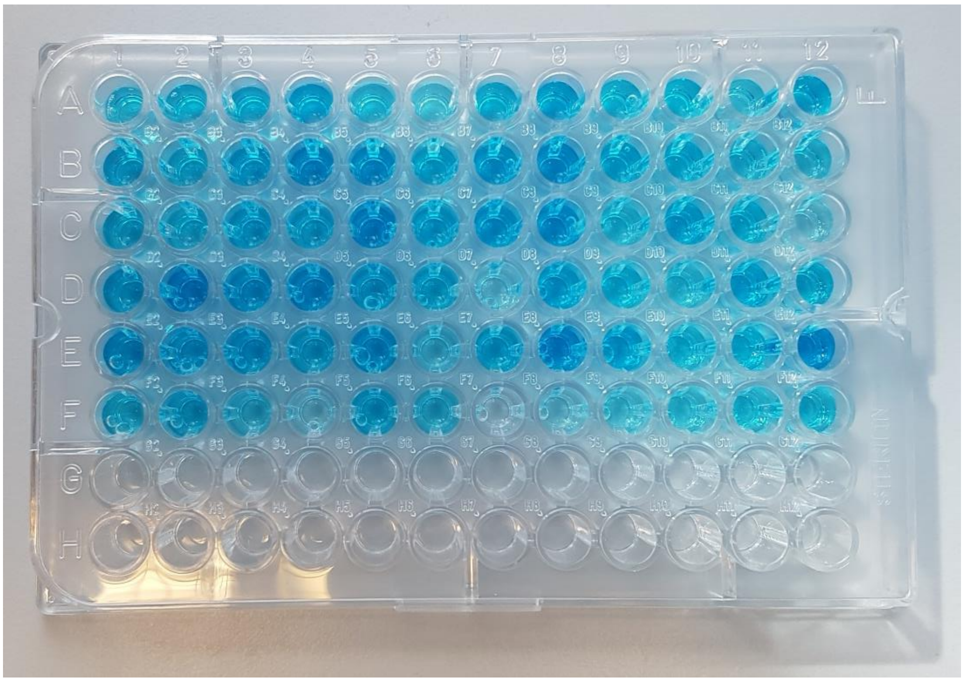


Figure 4-11 - Microwell plate with all samples

The luminescence values for the assay reference standard solutions were used to create a standard curve. A best fit line was applied to this curve using Microsoft Excel (Microsoft, Redmond, Washington, United States) – the equation for this line was then used to calculate proteoglycan content for individual samples.

The standard curve based on the assay reference standard samples is shown in Figure 4-12.

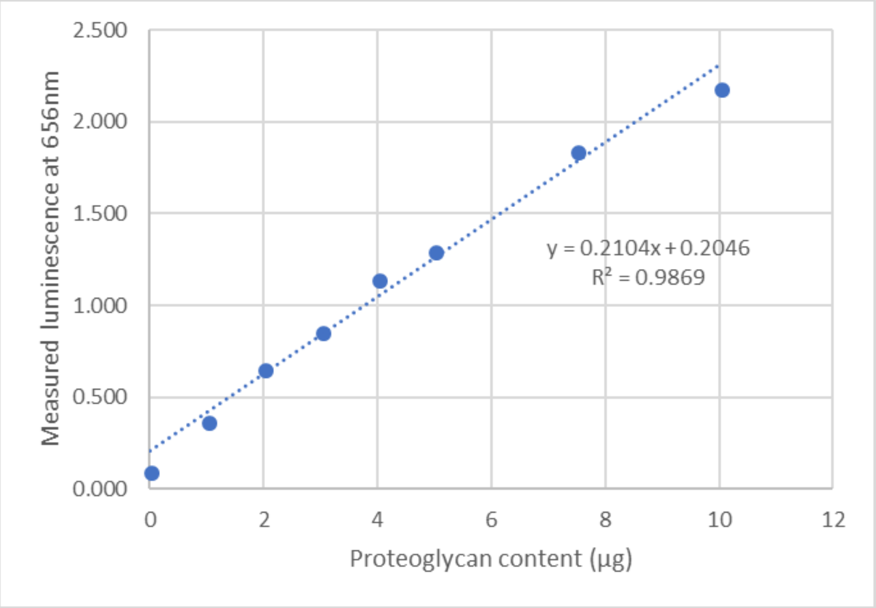


Figure 4-12 - Proteoglycan content reference curve

The equation for the line is shown below:

$$Luminescence = 0.2104 \times proteoglycan\ content + 0.2046$$

Hence, the proteoglycan content was equal to:

$$Proteoglycan\ content = \frac{Luminscence - 0.2046}{0.2104}$$

Finally, the wet weight of the samples was used to calculate the proteoglycan content (μg) per gram of tissue.

4.1.5.1 Data analysis

The resultant proteoglycan content in each solution was compared using a univariate ANOVA model in SPSS (v 25.0). Proteoglycan content was considered as the sole dependent variable, with the solution tested, the meniscus tested, the region of the sample and the source of the sample considered as fixed factors. Significance was set at $p \leq 0.05$.

4.1.6 Finite element modelling

FEBio software [280] was used to analyse the stress relaxation curves recorded. A non-linear poroviscoelastic finite element model with strain dependent permeability was used for analysis [281]. This material has previously been used to represent articular cartilage [282] and intervertebral disc [283]. A model consisting of 404 nodes and 100 elements was designed. In keeping with the confined compression experimental design, boundary conditions were set to allow only vertical displacement and the Poisson's ratio was set to zero. The coupled hyper elastic strain energy for this material is given by:

$$W(I_1 I_2 J) = \frac{1}{2} c(e^Q - 1)$$

And

$$Q = \frac{\beta}{\lambda + 2\mu} [(2\mu - \lambda)(I_1 - 3) + \lambda(I_2 - 3) - (\lambda + 2\mu) ln\, J^2$$

where I_1 and I_2 are the first and second invariants of the right Cauchy-Green tensor, J is the Jacobian of the deformation gradient and β is the exponential stiffening coefficient. λ and μ are the Lamé parameters, related to the Young's modulus (E) and Poisson's ratio (ν) as follows:

$$\lambda = \frac{E}{(1 + \nu)(1 - 2\nu)}$$

$$\mu = \frac{E}{2(1 + \nu)}$$

The relaxation function (G) was assumed to be given by:

$$G(t) = 1 + \gamma \exp(-\frac{t}{\tau})$$

where γ is the viscoelastic coefficient and τ is the relaxation time in seconds. Strain dependent permeability was described by the following function:

$$k(J) = k_0 e^{\frac{1}{2}M(J^2 - 1)}$$

where k_0 is the isotropic hydraulic permeability in the reference state and M is the exponential strain dependent coefficient.

A MATLAB function was written to iteratively reverse engineer model parameters for each relaxation curve using a Nelder-Mead function. The power law exponent was held at zero. For the bovine samples, both the exponential strain dependent coefficient and exponential stiffening coefficient were restricted from becoming negative. Based on these results obtained from the bovine tissue, these values were also held at zero in subsequent human tissue analysis.

4.1.6.1 Data analysis

4.1.6.1.1 Bovine tissue

Goodness of fit for each sample was assessed using a coefficient of determination as described by Soltz and Ateshian [284]:

$$R^2 = 1 - \frac{\sum(\sigma - \sigma_{est})^2}{\sum(\sigma - \bar{\sigma})^2}$$

where σ is the observed stress, σ_{est} is the estimated variable from the model and $\bar{\sigma}$ is the mean value of σ, summed over all samples time steps.

Comparison of resultant mechanical parameters was undertaken using one-way ANOVA in SPSS. The mechanical parameters, as well as the R^2 value were considered dependent variables, whilst the solution tested was considered as the sole fixed factor.

4.1.6.1.2 Human tissue

Goodness of fit for each sample was assessed using a coefficient of determination as described above. Comparison of samples was conducted using multivariate ANOVA in SPSS. All samples were analysed in a single model. The mechanical parameters, as well as the R^2 value were considered dependent variables, whilst the meniscus, region, solution tested and the source of the samples (cadaveric/donated) where considered as fixed factors. To minimise the risk of a type 1 error, Bonferroni adjustment for multiple comparisons was made in the univariate tests.

4.2 Results

4.2.1 Bovine pilot tests

Mean values for each of the parameters following finite element modelling are shown in Table 4-2.

Mean values (S.D.)						
Solution	**E (Young's Modulus) (MPa)**	**K_0 (zero strain dependent permeability) (m^4/Ns)**	**M (exponential strain dependent coefficient)**	***β* (exponential stiffening coefficient)**	**γ (viscoelastic coefficient)**	**τ (relaxation time) (secs)**
0.14M PBS	0.42 (0.32)	0.53 (0.39)	0.01 (0.02)	0.27 (0.85)	0.55 (0.26)	68.85 (24.39)
Deionised water	0.38 (0.23)	0.89 (0.59)	0.00 (0.00)	0.00 (0.00)	0.59 (0.18)	52.30 (19.78)
3M PBS	0.06 (0.11) †	0.01 (0.02) †	0.00 (0.00)	0.11 (0.19)	0.00 (0.00) †	-*

*Viscoelastic efficient close to zero, making value of relaxation time irrelevant

† - p=<0.05 in 3M PBS compared to 0.14M PBS/deionised water

Table 4-2 - Mechanical parameters derived for bovine meniscus

The R^2 values in PBS, deionised water and 3M PBS were 0.97, 0.98 and 0.75 respectively. One-way ANOVA suggested a significant difference ($p<0.05$) existed in the Young's modulus (E), zero-strain permeability (k) and viscoelastic coefficient (γ) between the 3M

PBS solution and the Deionised water/0.14M PBS solutions. The p-value of the exponential strain coefficient (M) trended towards significance ($p = 0.092$), whilst no significant difference was evident in the values of the exponential stiffening coefficient (β) or relaxation time (τ).

Figure 4-13, Figure 4-14 & Figure 4-15 show the mean values derived for the significant variables. Based on these results, 79% of the Young's modulus of bovine meniscal tissue is attributable to ionic effects.

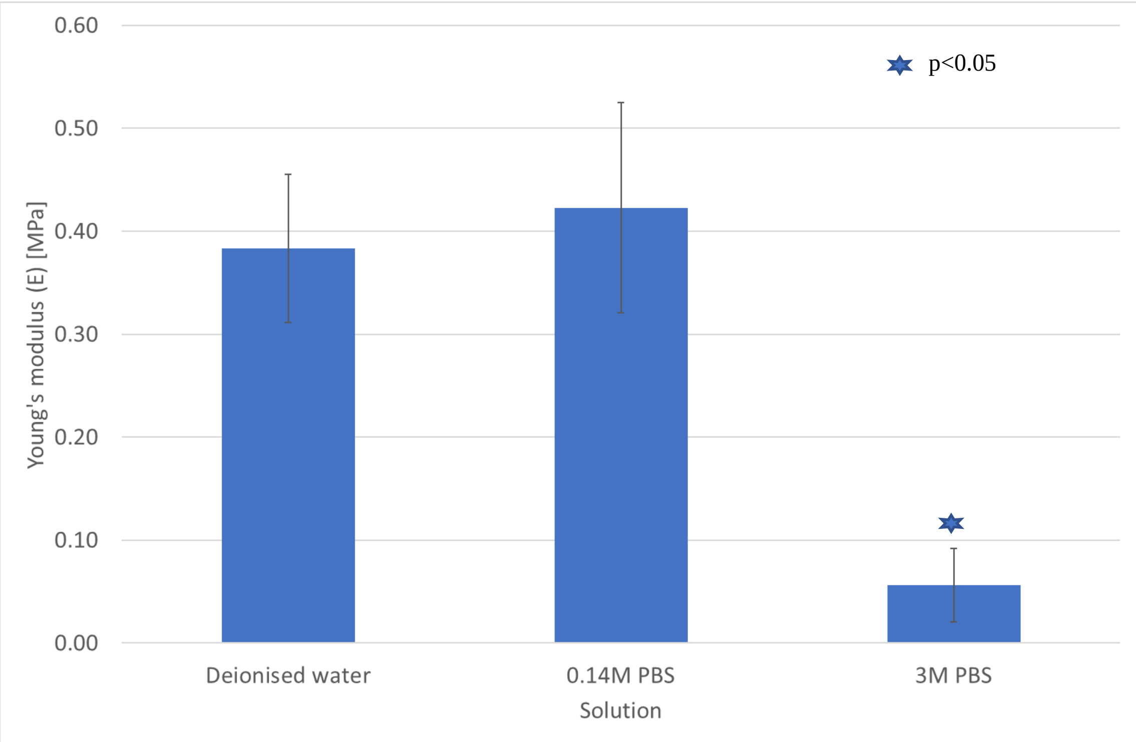


Figure 4-13 - Mean Young's modulus between solutions, note significant decrease in 3M PBS compared to other samples (error bars denote standard error).

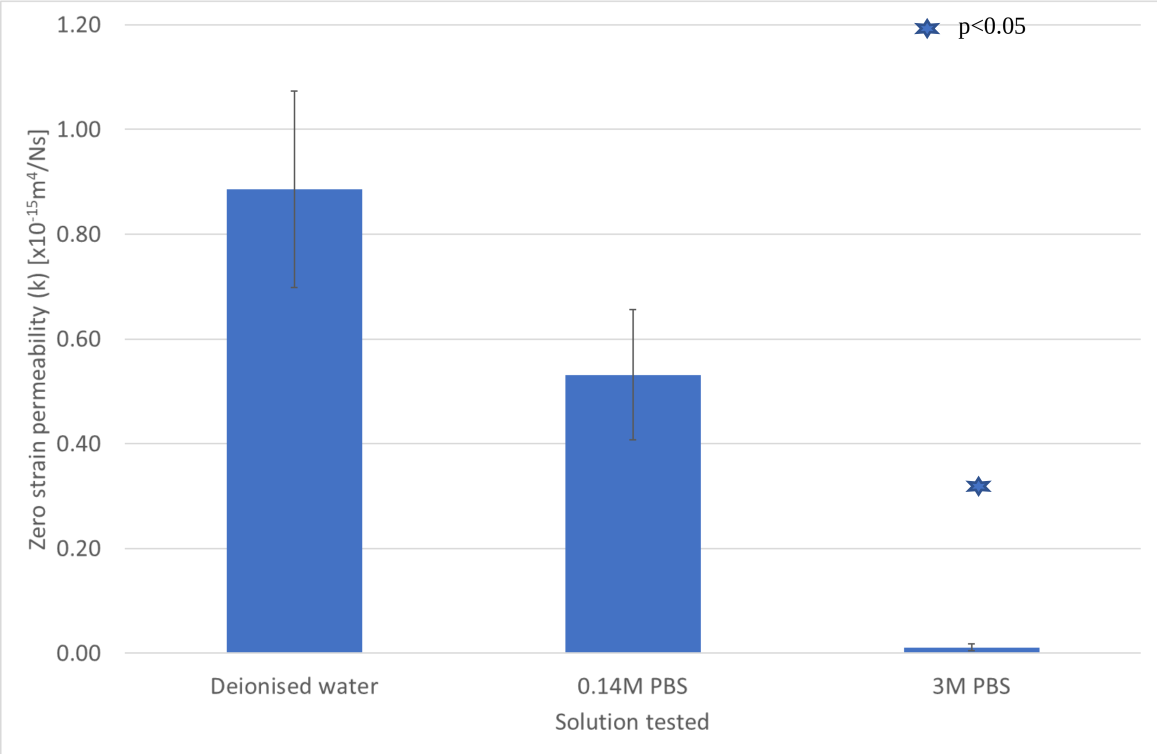


Figure 4-14 - Mean zero strain permeability between solutions, permeability significantly decreased in 3M PBS compared to deionised water/0.14M PBS (error bars denote standard error)

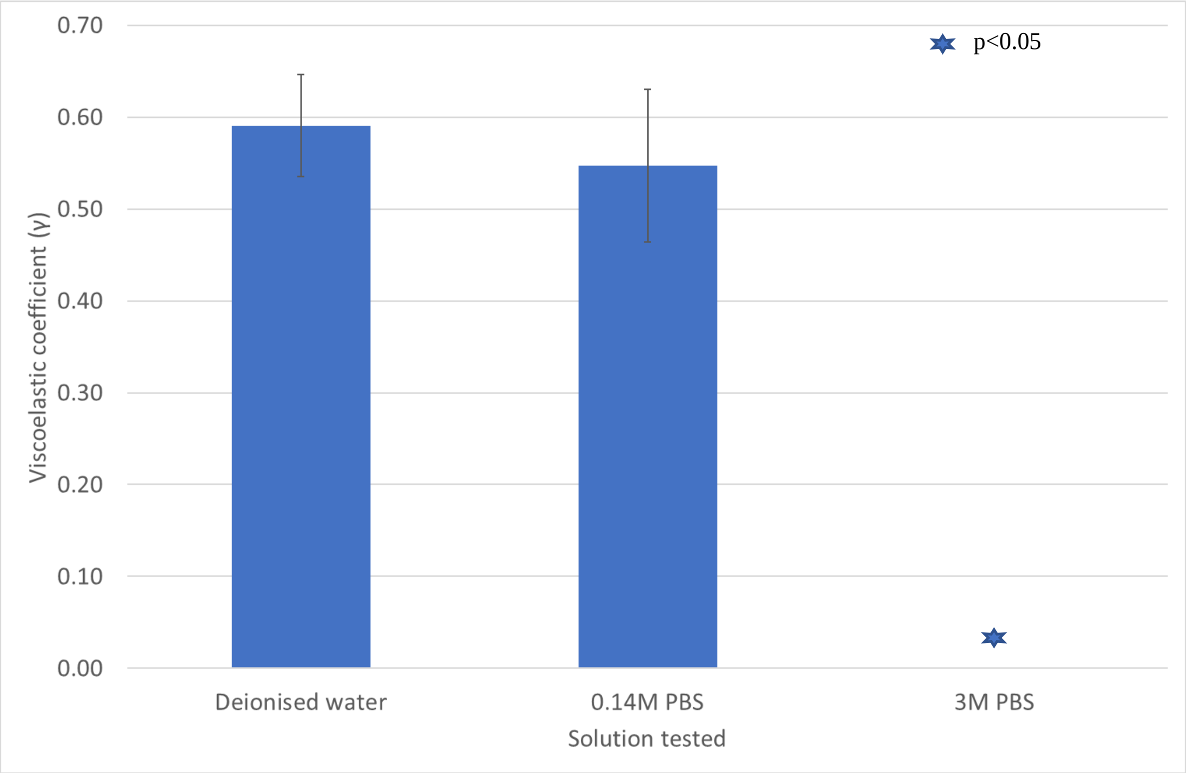


Figure 4-15 - Mean viscoelastic coefficient between solutions (error bars denote standard error)

4.2.2 Human cadaveric tissue

A total of 36 samples from cadaveric menisci were tested. Samples were derived from 6 cadavers and testing was conducted such that 12 samples from each meniscus and each region respectively were tested in each solution, as shown in Table 4-3. This step was undertaken to ensure that there would be no regional or meniscus variability between solutions tested. The mean sample thickness was 1.99 (+/- 0.04) mm. Note that the cadaver ID here is the same as that used in Chapter 3.

Table 4-3 - Cadaveric samples tested

Cadaver ID	Meniscus	Region	Solution
1	Medial	Anterior	Deionised water
1	Medial	Middle	0.14M PBS
1	Medial	Posterior	3M PBS

1	Lateral	Anterior	Deionised water
1	Lateral	Middle	0.14M PBS
1	Lateral	Posterior	3M PBS
2	Medial	Anterior	0.14M PBS
2	Medial	Middle	3M PBS
2	Medial	Posterior	Deionised water
2	Lateral	Anterior	0.14M PBS
2	Lateral	Middle	3M PBS
2	Lateral	Posterior	Deionised water
3	Medial	Anterior	3M PBS
3	Medial	Middle	Deionised water
3	Medial	Posterior	0.14M PBS
3	Lateral	Anterior	3M PBS
3	Lateral	Middle	Deionised water
3	Lateral	Posterior	0.14M PBS
4	Medial	Anterior	Deionised water
4	Medial	Middle	0.14M PBS
4	Medial	Posterior	3M PBS
4	Lateral	Anterior	Deionised water
4	Lateral	Middle	0.14M PBS
4	Lateral	Posterior	3M PBS
5	Medial	Anterior	0.14M PBS
5	Medial	Middle	3M PBS
5	Medial	Posterior	Deionised water
5	Lateral	Anterior	0.14M PBS
5	Lateral	Middle	3M PBS
5	Lateral	Posterior	Deionised water
6	Medial	Anterior	3M PBS
6	Medial	Middle	Deionised water
6	Medial	Posterior	0.14M PBS
6	Lateral	Anterior	3M PBS

6	Lateral	Middle	Deionised water
6	Lateral	Posterior	0.14M PBS

Due to the negligible effect of the viscoelastic coefficient and relaxation time observed in the bovine tissue samples, finite element modelling was conducted twice – once with the viscoelastic coefficient and relaxation time held at zero and again, with these parameters allowed to vary. No difference was seen in the derived variables between these two states – hence it was assumed that viscoelastic behaviour was not occurring in the collagen network at the level of strain tested. Therefore, the viscoelastic coefficient and relaxation time are not presented below and can be assumed to be held at zero.

To improve statistical power, results of both the cadaveric and osteoarthritic patient menisci were analysed together, hence they are presented together below. Table 4-4 summarises the characteristics of the samples tested.

		N
Meniscus	Lateral	48
	Medial	18
Region	Anterior	12
	Middle	42
	Posterior	12
Solution	Deionised water	22
	0.14M PBS	22
	3M PBS	22
Source	Cadaveric	36
	OA patients	30

Table 4-4 - Characteristics of human meniscal samples

The Pillai’s trace was chosen as the measure of choice in the multivariate model due to its conservative nature and the fact that is not influenced by assumptions about the normality of the data distribution. The multivariate model suggested that there were no significant differences in values derived between different menisci, meniscal region or the source of the sample (cadaveric vs. osteoarthritic patient), however a p-value of 0.00 was observed for the effect of solution in the multivariate model.

Further investigation with univariate testing revealed significant differences were present in the values of the Young’s modulus and zero strain dependent permeability between samples.

The Young’s modulus in 3M PBS was significantly different to that in deionised water (p=0.00) and approached significance in comparison to 0.14M PBS (p=0.067). The zero-strain permeability was significantly lower in deionised water as compared to both the 0.14M PBS and 3M PBS solutions (p=0.021 and 0.037 respectively). Mean values for parameters in each solution are shown in below.

Mean values (95% confidence interval)				
Solution	**E** **(Young's Modulus) (MPa)**	**K_0** **(zero strain dependent permeability) (m^4/Ns)**	**M** **(exponential strain dependent coefficient)**	***β*** **(exponential stiffening coefficient)**
0.14M PBS	0.623 (0.439-0.807)	0.024 (0.011-0.037)	0.011 (0.011-0.012)	0.211 (0.174-0.248)
Deionised water	1.261 (1.077-1.446)	0.004 (0.0-0.017)	0.011 (0.011-0.012)	0.232 (0.195-0.269)
3M PBS	0.370 (0.186-0.555)	0.026 (0.012-0.039)	0.012 (0.011-0.012)	0.213 (0.176-0.250)

Table 4-5 - Derived parameters for human menisci

Pairwise comparison suggested that a significant difference existed between the deionised water and 0.14M/3M PBS solutions for Young’s modulus (p=0.00). The difference between the values of Young’s modulus for 0.14M PBS and 3M PBS approached but did not reach significant (p=0.067).

Figure **4-16** shows the trend in Young’s modulus across the three solutions tested.

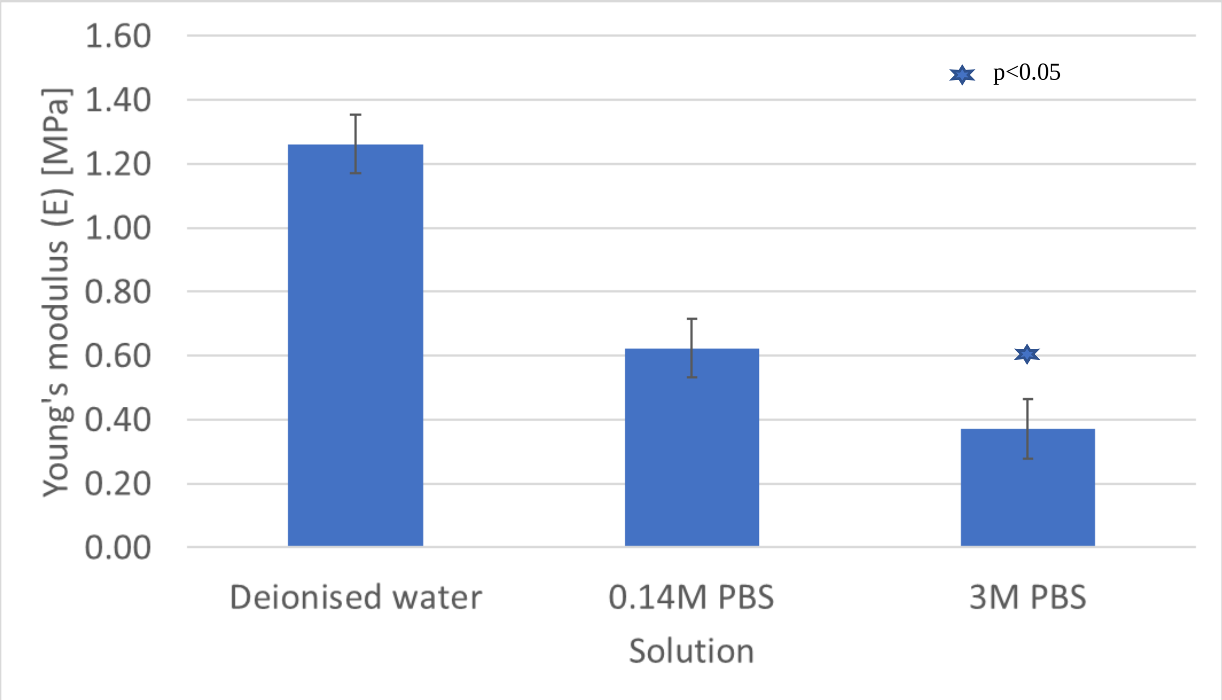


Figure 4-16 - Mean values of Young's modulus across solutions, note fall in Young's modulus with increasing salinity (error bars denote standard error). Significant difference evident between 3M PBS and deionised water.

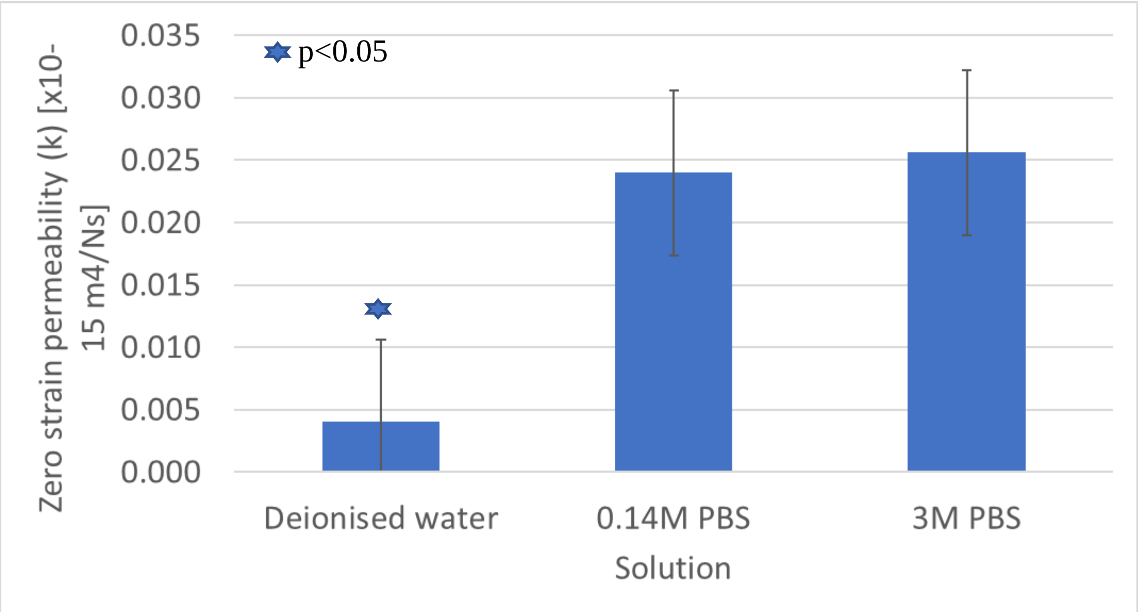


Figure 4-17 - Mean values of the zero-strain dependent permeability across solutions (error bars denote standard error). Significant difference evident between permeability in deionised water compared to other solutions.

A significant difference was also found in the zero strain dependent permeability values when comparing the deionised water solution with the 0.14M PBS/3M PBS solutions (Figure 4-17).

The mean R^2 value was 0.82 in deionised water, 0.778 in 0.14M PBS and 0.740 in 3M PBS.

4.2.3 Menisci from osteoarthritic knees

A total of 37 subjects were recruited to the study. The mean age of patients recruited to the study was 70.0 years (s.d. 8.6 years). There were 16 male and 14 female patients, and 16 left knees/14 right knees.

A number of subjects were screened but subsequently excluded from the study – this was due to a number of reasons, including surgical cancellation (6), history of meniscal surgery not previously noted (2), failure to attend (2) and refusal to take part in the study (1). 30 samples were tested under confined compression – 7 samples could not be tested due to poor macroscopic condition of the sample. This had occurred either due to osteoarthritis or due to meniscal damage sustained during surgery (the menisci are normally discarded at surgery and hence no efforts are routinely made to preserve them in their entirety). All samples tested were derived from the middle portion of the lateral meniscus. Mean sample thickness was 1.99 (+/-0.02) mm.

4.2.4 Proteoglycan content

A total of 66 samples were tested. Overnight incubation resulted in complete digestion of all samples.

Using the aforementioned formula, the proteoglycan content per gram of wet weight was calculated for each sample. Table 4-6 shows resulting proteoglycan content in each sample.

Table 4-6 - Proteoglycan content of meniscal samples

Source	**Sample ID**	**Wet weight of sample (g)**	**Proteoglycan content (µg)-entire sample**	**Proteoglycan content (µ/g of tissue)**
OA patient	1	0.044	6.11	139.91
OA patient	3	0.047	7.51	160.40
OA patient	4	0.047	8.00	170.86
OA patient	5	0.049	10.96	222.71
OA patient	7	0.044	5.48	124.31
OA patient	8	0.032	3.19	99.88
OA patient	9	0.038	8.12	212.99
OA patient	10	0.049	11.73	241.80
OA patient	11	0.046	7.33	160.31
OA patient	12	0.033	8.30	252.15
OA patient	13	0.036	4.18	115.14
OA patient	14	0.036	10.52	289.14
OA patient	16	0.046	10.40	226.01
OA patient	17	0.037	6.15	167.18
OA patient	18	0.040	9.06	224.72
OA patient	19	0.045	12.40	273.78
OA patient	20	0.041	12.17	297.54
OA patient	21	0.034	7.49	217.66
OA patient	22	0.047	11.47	246.58
OA patient	23	0.039	12.57	324.05
OA patient	25	0.036	8.94	250.34
OA patient	26	0.048	7.63	158.20
OA patient	27	0.043	5.05	118.81
OA patient	28	0.039	4.11	105.89
OA patient	29	0.047	7.78	166.90
OA patient	31	0.040	4.95	123.86
OA patient	34	0.047	7.31	155.47
OA patient	35	0.050	5.49	110.49

OA patient	36	0.045	12.56	279.71
OA patient	37	0.049	7.77	157.25
Cadaver	1	0.049	10.89	220.36
Cadaver	1	0.039	12.34	318.87
Cadaver	1	0.045	3.56	79.33
Cadaver	1	0.048	10.14	213.47
Cadaver	1	0.039	6.98	177.58
Cadaver	1	0.029	1.08	36.85
Cadaver	2	0.048	9.50	197.98
Cadaver	2	0.047	12.66	267.16
Cadaver	2	0.033	9.85	301.36
Cadaver	2	0.046	11.62	254.32
Cadaver	2	0.039	7.21	185.75
Cadaver	2	0.041	5.78	142.05
Cadaver	3	0.034	0.69	20.37
Cadaver	3	0.035	9.21	263.98
Cadaver	3	0.045	5.30	117.55
Cadaver	3	0.035	3.06	86.87
Cadaver	3	0.045	10.33	231.50
Cadaver	3	0.040	6.71	167.29
Cadaver	4	0.042	11.63	278.28
Cadaver	4	0.045	9.10	200.96
Cadaver	4	0.036	9.40	262.52
Cadaver	4	0.043	5.93	138.63
Cadaver	4	0.040	10.93	276.10
Cadaver	4	0.042	1.79	43.22
Cadaver	5	0.042	7.52	180.36
Cadaver	5	0.043	12.62	294.19
Cadaver	5	0.047	10.84	229.14
Cadaver	5	0.028	4.56	165.99
Cadaver	5	0.036	6.39	179.62

Cadaver	5	0.041	12.80	314.41
Cadaver	6	0.046	6.28	137.43
Cadaver	6	0.034	8.49	248.12
Cadaver	6	0.043	3.42	78.78
Cadaver	6	0.039	0.52	13.20
Cadaver	6	0.047	6.67	142.42
Cadaver	6	0.032	3.53	109.58

Summary characteristics for these samples are shown in Table 4-7 below.

		N
Solution	Deionised water	22
	0.14M PBS	22
	3M PBS	22
Meniscus	Lateral	48
	Medial	18
Region	Anterior	12
	Middle	42
	Posterior	12
Source	Cadaveric	36
	Osteoarthritis patient	30

Table 4-7 - Summary characteristics of meniscal samples assayed for proteoglycan content

Statistical analysis of results suggested that there was a significant regional variation in proteoglycan content ($p<0.05$)(

Figure **4-18**) and a trend towards a difference in proteoglycan content between menisci ($p=0.06$) (Figure 4-19). No significant difference in proteoglycan content between solutions was observed ($p= 0.121$) (Figure 4-20).

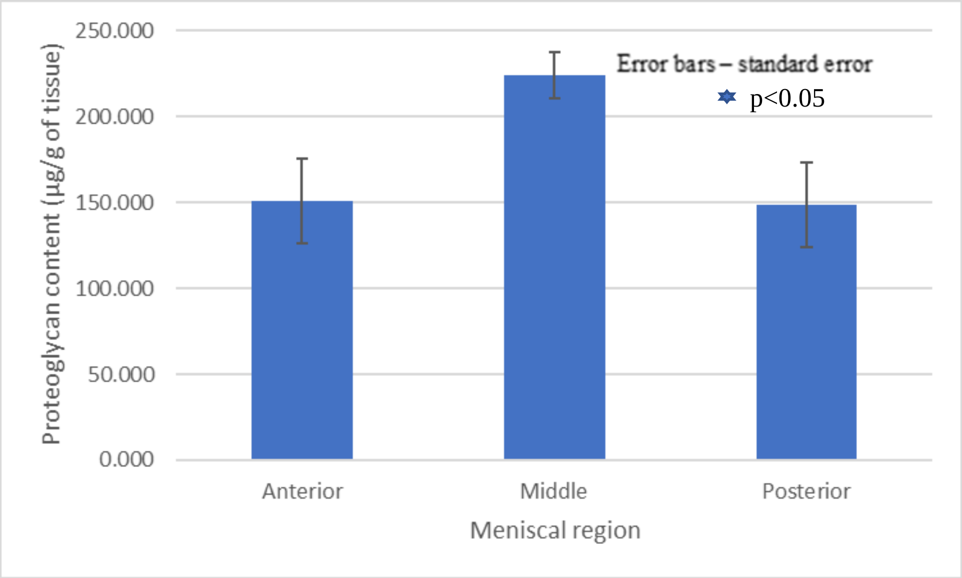


Figure 4-18 - Proteoglycan content - found to be significantly higher in middle meniscal region

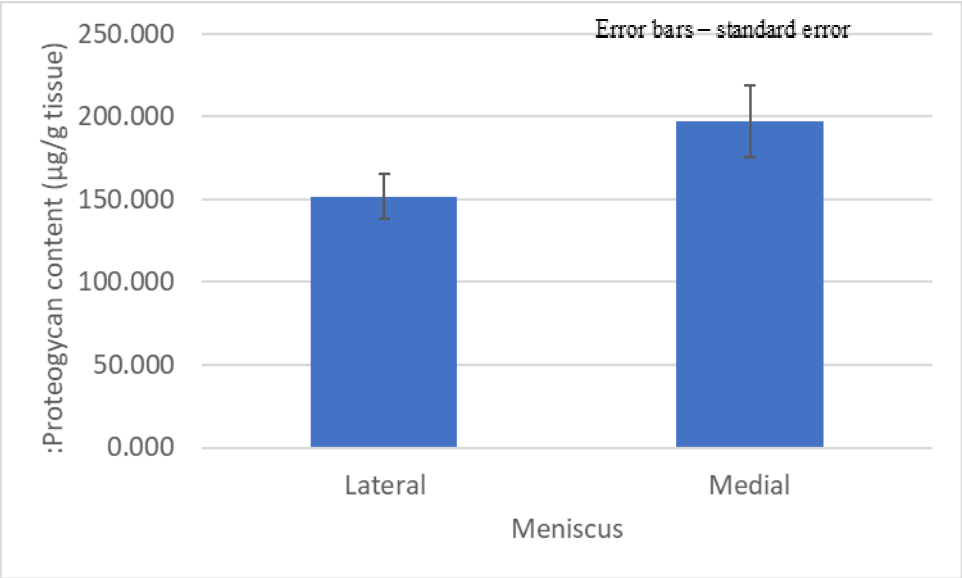


Figure 4-19 - Proteoglycan content – no significant difference observed between meniscus tested

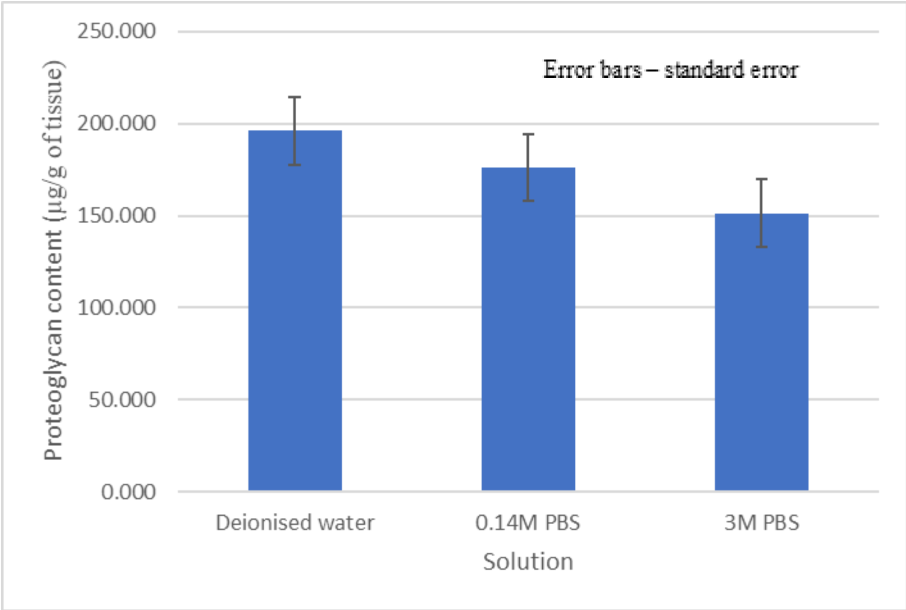


Figure 4-20 - Mean proteoglycan content in each solution, no significant differences observed

4.3 Discussion

4.3.1 Development of testing apparatus

Due to the unavailability of a functional indenter, it was necessary to 3d print an apparatus to conduct the confined compression experiments. The pore size of the indenter was restricted by the physical capability of the 3d printer to print a fine mesh – a number of iterations were printed to determine this limit. Any pore size below 500 µm would result in the mesh being 'clogged' by the polymer used to print the indenter, rendering it useless. As such, a pore size of 500µm was chosen. Data from cartilage suggests pore sizes in the order of 60 Å [285], whilst pore size in the meniscus is thought to be around 80Å [105]. Whilst the use of porous polyethylene as a mesh has been suggested [286], there is a risk of detachment from the indenter, especially at higher stress and the potential to interfere with the boundary between the indenter and compression chamber. The purpose of such an indenter is to allow application of a pre-determined strain without deforming the tissue surface, as well as free fluid flow. No deformation of the tissue surface was observed following application of strain, in particular there was no sign of interdigitation, which can cause an increase in measured tissue resistance during compression [287]. Hence the apparatus was deemed appropriate for testing.

The 3d printed sectioning device used for cutting the bovine meniscus was designed to work with commercially available razor blades (as opposed to microtome blades). The device was designed to account for the thickness of the razor blades. Although it functioned acceptably, it resulted in a marked variation in sample thickness, presumably as the blades themselves could bend slightly within the device. This variation is evident in the thickness of the bovine tissue samples, where the standard deviation of sample thickness approached 3.5%. Although the thickness was considered when strain was applied to each sample, this led to un-necessary variation between samples, hence the apparatus was re-designed to a metal device accommodating microtome blades. This resulted in less variability of the samples, with the standard deviation of sample thickness reduced to 2%.

Sample thickness was measured using a micrometre. Ideally, a laser micrometre would have been used for this purpose, as it was possible to deform the sample itself in the process of measuring it. However, such apparatus was not available. To minimise the risk of measurement error, measurements were taken at the point where both ends of the microtome contacted the tissue sample, with this approach replicated across all samples tested.

The strain applied to each sample was also dictated by the capacity of the load cell used to take measurements, which was rated at 9.9N. Testing at strains >5% for bovine tissue and 10% for human tissue resulted in the load cell being overloaded for some samples and the test being rendered invalid. Hence, sub-physiological strains were tested, which may account for the lack of viscoelastic behaviour evident in the tissue response, as discussed below. On a related note, the rate of strain was also dictated by these limits – with a high strain rate resulting a sudden increase of force, potentially overloading the load cell.

4.3.2 Meniscal testing

4.3.2.1 Bovine tissue

Testing in bovine tissue indicated that the mechanical response of the samples differed significantly between solutions. In particular the Young's modulus, zero strain permeability and the viscoelastic coefficient were markedly lower in deionised water and compared to 0.14M PBS/3M PBS. These results indicate that ionic effects play a significant role in maintaining the mechanical stiffness of bovine meniscal tissue, with 79% of the Young's modulus attributable to ionic effects. Given the composition of the meniscus is restricted to collagen, proteoglycans and meniscal fibrochondrocytes [288], it would appear that these effects are mediated through proteoglycans, which carry negative charged moieties.

Notably, the values inversely engineered for Young's modulus and permeability (equivalent to the aggregate modulus, given the Poisson's ratio in our experimental configuration is zero) are of a similar order of magnitude to those reported for meniscal tissue elsewhere in the literature [100,105].

As previously noted (Section 2.2.4.1.3), the negative fixed charges associated with lead to the development of a Donnan osmotic pressure across the tissue boundary. The effect on the Young's modulus observed here likely reflects modulation of this Donnan osmotic pressure by bathing solutions. In 0.14M PBS, a situation akin to that observed physiologically is created – the Donnan osmotic pressure exists as normal and indeed, the tissue is observed to be stiffer than in other solutions, perhaps suggesting it is optimised to work in these conditions. In deionised water, the effect of mobile ions in the solution in augmenting the Donnan pressure is eliminated – resulting in a small decrease in the mean Young's modulus; though this difference is not significant. Finally, in 3M PBS, the large number of ions flooding the solution has been predicted to negate all ionic effects [95]. The resulting stiffness is solely that of the collagen fibril network, without the contribution of electrostatic effects. This is significantly lower than that observed in the ‘physiological’ situation.

A similar effect was observed in relation to the zero-strain permeability of the tissue, with the permeability observed in 3M PBS significantly lower than that in 0.14M/3M PBS. Kaufman et al. [289] studied the permeability of articular cartilage samples which had undergone degradation of proteoglycans, finding that permeability increased with proteoglycan digestion, suggesting that these proteins play a role in modulating permeability of tissue. However, the effect of altering the ionic concentration of the bathing solution on the permeability of tissue in bovine meniscus differed from that observed in human meniscus, as discussed below. The reasons for this are unclear and are likely related to the complex association between poro- and visco-elastic effects within the tissue. Hosseini et al. [290] suggest that the results of mechanical tests obtained from tissue may depend on the pre-conditioning protocol in articular cartilage, in particular noting that poro- and visco- elastic effects may act in opposite directions. It is likely the opposing effects on permeability noted in the two types of test described reflect this effect.

The viscoelastic function was also found to differ significantly in samples tested in 3M PBS as compared to deionised water/0.14M PBS. Indeed, samples tested in 3M PBS did not display viscoelastic behaviour, whilst those tested in other solutions showed small viscoelastic coefficients, reflecting the small magnitude of strain tested. Due to the value of the viscoelastic coefficient being so close to zero in 3M PBS, the value of the relaxation time became irrelevant and was excluded from analysis. It is understood that proteoglycans aid in ‘inflating’ the collagen fibril network, pre-tensioning it to augment its elastic energy storage capability [291]. The observed result may be a reflection of the high ionic concentration inhibiting the proteoglycans’ ability to perform this function, preventing the tissue acting in a viscoelastic manner in solutions of high ionic concentration.

The values of the exponential strain and stiffening coefficients were close to zero in all solutions, with no significant differences evident. Due to the small magnitude of strain tested, tissue stiffness was likely constant throughout testing in all solutions, hence the values of these parameters would not be expected to differ.

There has been limited study of the role of proteoglycans within the meniscus to date. Danso et al. [292] conducted an assessment of proteoglycan content in the human meniscus using digital densitometry, demonstrating that proteoglycan content increased as a function of tissue depth. They also noted site specific variations in proteoglycan content, such as increased proteoglycan content anteriorly in the medial meniscus compared to the lateral. Due to the small strains applied in this current study, it is conceivable that the results observed reflect the surface properties of the tissue sampled. However, both femoral and tibial surfaces were removed from all samples, resulting in a mid-substance sample. Indeed, if the proteoglycan content in the superficial layers of tissue is lower, then it is conceivable that the magnitude of electrostatic effects observed here may in fact be smaller in magnitude.

As noted above, Danso et al. also report marked regional variation of proteoglycan content in different areas of the meniscus. This highlights a potential weakness of this work, as samples were derived from all meniscal regions. In an effort to minimise the effect of this variation, equal numbers of samples from each region and each meniscus were tested in each solution, such that any variation would be applied equally to each experimental group. This variation may explain the large standard deviations observed in some of the mechanical parameters inversely engineered through this work.

As well as this, testing of bovine meniscal tissue was undertaken without allowing the tissue to reach equilibrium. As described above, samples in deionised water were taking a prolonged period to reach equilibrium, hence we chose to conduct testing over a short time span to limit the influence of swelling effects. Nevertheless, these effects may have contributed to some of the stress measured, particularly in the deionised water solution, though any such effect is assumed to be negligible.

Finally, the coefficient of determination showed an excellent fit for data generated in deionised water and 0.14M PBS, whilst data from the 3M PBS solutions fit less well. If ionic effects do indeed play a significant role in modulating the mechanical response of meniscal tissue, then this response will not be accounted for by biphasic theory, which envisages solid and liquid phases of tissue. As such, application of triphasic or quadriphasic theory may be more appropriate for assessing meniscal tissue, accounting for ionic charge within the tissue alongside solid and liquid phases. Such an approach will pose its own challenges, in particular, the large number of variables present increase the probability of multiple best fit solutions being present.

4.3.2.2 Human tissue

Testing of human tissue followed a similar pattern to that of bovine meniscus. The smaller size of meniscus required a re-design of both the confining chamber/indenter and sectioning device as described above. This refinement in the experimental technique helped minimise the risk of sample variation, whether in terms of preparation or experimental setup.

Once again, a significant difference in the Young's modulus was observed. Whilst the tissue was once again least stiff in the 3M solution, where electrostatic effects had been eliminated, maximal stiffness was observed in the deionised water solutions. Whilst samples tested in 0.14M PBS/3M PBS reached equilibrium in 2 hours, samples in deionised water required 13 hours to reach equilibrium. It is unclear whether this prolonged period required to reach equilibrium may itself have influenced the mechanical properties of the tissue. Andrews et al. [293] investigated the swelling behaviour of meniscal tissue in deionised water, 0.14M PBS and 0.28M PBS. They found that the amount of swelling in solution following an hour of immersion was not significantly different between solutions. They also noted that the material properties of swollen and 're-compressed' samples – those that were compressed back to their original thickness following swelling, were markedly different, though there was a strong correlation between moduli measured in both states. This study sought to minimise this risk by placing the samples in confined compression as soon as they were sectioned, rather than allowing them to defrost in solution prior to testing.

In testing human meniscus, the permeability values derived were an order of magnitude lower than those calculated in bovine meniscus and are quite close to those observed by Danso et al. [104] using indentation techniques. As noted previously, the diverse preconditioning techniques applied to the two types of samples may have a role to play in the different results observed [290]. Furthermore, Gu et al. [294], in a theoretical exploration of Lai's triphasic theory, predict that a marked increase in fixed charge density results in a concurrent decrease in apparent permeability. We theorise that it is this scenario which we have observed here, where a solution bereft of balancing ions in free solution results in an increase in fixed charge density and a concurrent decreased initial permeability. The exact mechanism behind this phenomenon is unclear – meniscal tissue permeability is likely a complex interaction between the intrinsic permeability of the collagen fibril network, modulated by electrostatic effects.

Once again, the values of the exponential strain and stiffening coefficients were close to zero, with no differences between samples. Although a larger magnitude of strain (10%) was applied to these samples, the load generated was in the order of 1N, which is grossly sub-physiological, and it is hence likely that tissue stiffness remained constant in all solutions throughout testing.

In light of the results obtained from the bovine meniscus samples, when conducting finite element analysis of the human meniscal tissue, fitting was undertaken twice for each sample – once with the viscoelastic coefficient and relaxation time held at zero and again with these variables allowed to vary. The former approach did not improve the fit in any sample and hence these values were assumed to be zero. The tissue was hence assumed not to behave in a viscoelastic manner, reflecting the small magnitude of strain tested.

The coefficient of determination in the human menisci did not differ significantly between samples, though the mean R^2 value was lower than that achieved in bovine tissue across all solutions.

4.3.2.2.1 Cadaveric tissue testing

In an effort to minimise the effect of regional variation in both the proteoglycan and collagen content [292], meniscal samples were tested in each solution in an ordered fashion to ensure equal numbers of samples from each region and each donor were tested in each solution. Andrews et al. [293] also point out a marked inhomogeneity in individual tissue samples, which they propose may influence the ability to proteoglycans to absorb fluid and expand the tissue matrix. As has previously been alluded to, the meniscus is an inhomogeneous tissue in all planes. We undertook a number of steps to minimise the risk of this inhomogeneity, including taking a large number of samples, testing samples from each region in a controlled fashion and minimising variation through development of the experimental technique. Our statistical analysis did not demonstrate any significant difference in parameters between regions, however, there was a marked preponderance of samples derived from the middle region of the lateral meniscus (taken from osteoarthritic patients) and small numbers of from other regions which may have made such variation difficult to demonstrate. Nevertheless, it is possible that the compositional variation of the meniscal tissue itself may have influenced some of the results observed, in particular the variation seen in the derived parameters. It should be noted however, that the variation in mechanical parameters observed for the human tissue samples is lower than that for bovine meniscus, suggesting that these measures may have reduced the inhomogeneity between testing conditions.

It should be noted that a potential confounder in testing the cadaveric samples was the thaw-refreeze cycle the tissue was exposed to. Repetitive freezing of meniscal tissue has been shown to reduce the elastic modulus [295]. However, statistical analysis did not demonstrate any significant difference between the parameters derived from cadavers as opposed to osteoarthritic patients, hence it was assumed that the tissue was not adversely affected. Notably, as the tissue was fresh frozen, and the joint capsule was not significantly disturbed, all menisci were found to be well hydrated at time of excision.

4.3.2.2.2 Menisci from osteoarthritic knees

The innate nature of these tissue samples dictated that they would likely show some macroscopic damage. Tissue sampling was also made more challenging by the requirement to collect the samples intraoperatively without altering the course of the patient's routine care. Some surgeons would routinely section the menisci during removal and this precluded further testing. There is evidence that varus malalignment of the knee increases the risk of tibiofemoral osteoarthritis [296] and in knees with varus osteoarthritis, gross degeneration of the medial meniscal samples was evident. Although the prevalence of valgus osteoarthritis was lower, some lateral menisci exhibited degeneration also. Procuring all samples from the middle part of the lateral meniscus provided the highest probability of obtaining an adequate sample and ensured appropriate use was made of donated tissue, minimising wastage. Danso et al.'s [292] work suggests that the proteoglycan content of the middle region of the lateral meniscus is higher than is present anteriorly or posteriorly, hence this region was most suited to investigating this effect.

A potential confounder with regard to the tissue from osteoarthritic knees is the presence of calcific deposits in the tissue. Calcific deposition is well recognised in osteoarthritis, with MacMullan and McCarthy demonstrating that fibrochondrocytes in the meniscus can themselves generate calcium crystals. Large volume calcium deposits within the tissue could potentially alter the stiffness of the meniscal plugs. Care was taken to avoid any regions with gross calcium deposition and no plugs were found to have large deposits.

4.3.2.2.3 Proteoglycan content

A particular concern in undertaking these experiments was that the differences observed between solutions may in fact reflect proteoglycans leaching out from solution at different rates. A proteoglycan assay was therefore undertaken to measure concentration of proteoglycans across all human meniscal samples tested.

In keeping with the findings of Danso et al. [297], regional variation in proteoglycan content was observed, with samples derived from the middle region of menisci more likely to have a higher proteoglycan content than those derived from other regions. As previously noted, all samples taken from osteoarthritic patients were from the middle region of the lateral meniscus and it is possible that these samples maintained a higher proteoglycan content than those from cadavers. There was also a trend towards a higher proteoglycan content in the medial meniscus as compared to the lateral, although this did not reach significance. Again, these findings are mirrored in Danso's work.

Most importantly, however, there was no evidence of a significant difference in the proteoglycan content between samples – suggesting that a comparison of mechanical properties based on proteoglycan content between solutions was valid. Of note, the manufacturers of the assay highlighted that the presence of a high concentration of salt could interfere with the interaction of proteoglycans with the dye complex. As such, all samples were washed in deionised water for an equal period of time in an effort to remove excess salt from the tissue. Nevertheless, it is possible that some salt may have remained, particularly in the 3M PBS sample and may account for the small magnitude trend in decreasing proteoglycan concentration seen in each solution. It is also possible that the washing may have itself caused proteoglycan leaching – any potential such effect was minimised by exposing all samples to the wash for equal periods of time.

4.3.3 Strengths and weaknesses

In addition to the issues discussed above, there are some more general consideration which bear mentioning. A strength of this work, in relation to testing of human tissue in particular, is the large number of samples tested. As discussed, the meniscus is a profoundly inhomogeneous material and testing of multiple samples, although potentially increasing variability in the results derived, demonstrates that the effect observed holds across the breadth of the tissue. Furthermore, recent study of the meniscus has suggested that whilst the effect of proteoglycans on tissue swelling is localised, their effect on compressive tissue mechanics is a global effect [298]– validating our approach of measuring these effects on a cross-meniscal scale. Another strength of this work is the experimental technique described for the Actifit prosthesis mirrors that used for the human tissue exactly - it is well documented that a difference in experimental technique results in variation of mechanical parameters [299]. Hence, we are able to directly compare mechanical parameters between our various test samples.

However, there are also some methodological limitations to our work. As noted above, the results derived here may differ from those obtained using indentation or unconfined compression techniques. We have assumed a Poisson's ratio of zero in calculating our aggregate modulus – this may not strictly be the case. As well as this, in using confined compression, it has been previously shown that indenter geometry and boundary conditions can affect the measured reaction force, with perfect interdigitation of the indenter resulting in larger peak reaction force [300]. A related issue is that of frictional effects occurring between the sample and confining chamber, assuming a perfect fit, which were considered negligible in our model. However, frictional effects are more important to consider in a dynamic situation and are likely to be of less relevance for the equilibrium parameters we have considered [299]. Use of the same indenter and experimental technique across all samples ensures that the comparisons we have made are valid.

4.3.4 Suggestions for further work

As alluded to above, the relaxation data presented here could be fitted to a triphasic model. Indeed, the parameters derived here could be used to simplify model fitting and explore whether the fit is indeed improved through the use of a triphasic tissue model. If this was indeed the case, it would develop further the argument that multiphasic modelling is more appropriate to model behaviour of meniscal tissue.

It would also then be important to characterise meniscal tissue properties according to such models and explore whether the regional variation shown in meniscal tissue through use of the poroviscoelastic model is replicated through a triphasic approach.

As well as this, although we have explored the electrostatic effect of proteoglycans, it would also be of interest to determine whether these proteins have a similar non-electrostatic role to play in maintaining tissue stiffness as demonstrated in articular cartilage. This could be explored through testing samples before and after proteoglycan digestion, as well as in differing ionic solutions, though one would need to be wary of re-testing samples and potential confounders introduced by swelling of the tissue or differing pre-conditioning states between tests.

4.3.5 Conclusions

To our knowledge, this is the first study demonstrating that the actions of proteoglycans in the meniscus are mediated through electrostatic effects. Furthermore, assuming this electrostatic effect of proteoglycans is eliminated in 3M PBS our results suggest that approximately 40% of the stiffness of meniscal tissue in the physiological condition is mediated through these electrostatic effects. Although the meniscus has not been studied with regard to the electrostatic effects of proteoglycans, there has been study of articular cartilage in this regard. A decrease in the compressive modulus bovine humeral articular cartilage with increasing osmolarity was shown by Korhonen and Jurvelin [301]. As well as this, Canal Guterl et al. combined testing of articular cartilage in solutions of varying ionic concentration with further testing of articular cartilage following proteoglycan digestion. Doing so, they concluded that the combined contribution of proteoglycans to the compressive modulus of articular cartilage was >98%, with ionic effects representing 62% of this total.

Electrostatic effects of proteoglycans have also been noted in the intervertebral disc of the spine, within a structure termed the nucleus pulposus [96], with ionic effects thought to account for ~70% of the stress response. As is the case with the meniscus, the structure of proteoglycans in this tissue has been found to differ from articular cartilage [302].

Our results suggest that the contribution of proteoglycans to mechanical stiffness of the meniscus is lower than measured in other tissues. However, given that the proteoglycan concentration varies across the meniscus [292], with the highest concentrations found on the innermost surfaces [303]. It is possible that, had we sampled tissue from the innermost edges of the meniscus, we may have found the electrostatic contribution of proteoglycans to have a larger effect – however, obtaining such samples is physically challenging due to the thin free edge and friability of the meniscus in this region. However, the concentration of glycosaminoglycans has been found to be lower compared to articular cartilage, which may also account for the difference observed [304].

Although the poroviscoelastic model has been widely used for modelling tissues such as articular cartilage and meniscus, our work raises the question as to whether a triphasic model might be more appropriate to account for the electrostatic effect of proteoglycans. Whilst this is computationally challenging, such a model would account for the role of proteoglycans as demonstrated by our work. In the context of our study, accounting for such effects is particularly important where fluid flow or localised tissue strain is modelled.

Furthermore, from a clinical perspective, current therapies for meniscal repair or regeneration do not seek to replicate or augment the function of proteoglycans. If these proteins are indeed critical to meniscal function, then there may well be a role for implants such as meniscal scaffolds to be seeded with proteoglycans prior to implantations As well as this, the viability of proteoglycans within transplanted meniscal tissue may potentially influence their ability to replicate meniscal function and hence efforts should be made to both preserve and potentially augment the availability of proteoglycans within such tissues. Further research is necessary to determine whether such strategies are viable.

5. Material properties of the Actifit meniscal scaffold

5.1 Methods

5.1.1 Samples

The cost of buying such implants for testing was prohibitive and we are grateful to Orteq Ltd for donating Actifit samples for testing.

5.1.2 Confined compression testing

Confined compression testing of Actifit scaffold samples was undertaken in a similar manner to that of cadaveric menisci. It was assumed that the scaffold was homogenous in its composition, hence samples were taken from all regions, allowing 6-8 samples per Actifit scaffold (Figure 5-1).

Figure 5-1 - Actifit meniscal scaffold following sample extraction

Sample preparation and testing was undertaken as described for cadaveric meniscus samples in Chapter 4, with samples left to equilibrate for 2 hours at 0.3N preload before being subjected to a 10% ramp strain at 1% strain/second. All samples were tested in deionised water.

5.1.3 Mechanical parameters

The finite element modelling for Actifit samples was undertaken using the same non-linear poroviscoelastic finite element model with strain dependent permeability as used for meniscal tissue, as described in Chapter 4.

5.1.4 Data analysis

Goodness of fit was again calculated as described for meniscal tissue. Statistical assessment was undertaken using one-way ANOVA in SPSS. All mechanical parameters and the R^2

value were considered as dependent variables, whilst the solution tested was considered as the sole fixed factor.

5.2 Results

5.2.1 Confined compression testing

A total of 6 samples from one Actifit meniscal substitute were tested – it was assumed there was no inter regional variation in the composition of the scaffold. Samples were all tested in deionised water.

5.2.2 Mechanical parameters

Table 5-1 shows the parameters generated.

Mechanical parameter	**Mean value (standard deviation)**
Young's modulus (E)/ Aggregate modulus	1.28 (0.07) MPa
Zero strain permeability (k_0)	0.08 (0.03) mm^4/Ns
Exponential strain coefficient (M)	2.95 $x10^{-5}$ (2.04 $x10^{-5}$)
Exponential stiffening coefficient (β)	5.59 $x10^{-4}$ (3.78 $x10^{-4}$)
Viscoelastic coefficient (γ)	0.21 (0.04)
Relaxation time (τ)	1215.53 (690.70) seconds

Table 5-1 - Mechanical parameters of the Actifit meniscal scaffold

The mean R^2 value was 0.964, indicating an excellent fit (Figure 5-2). Note that as Poisson's ratio is equal to zero in the confined compression configuration, the value of the Young's modulus equates to that of the Aggregate modulus.

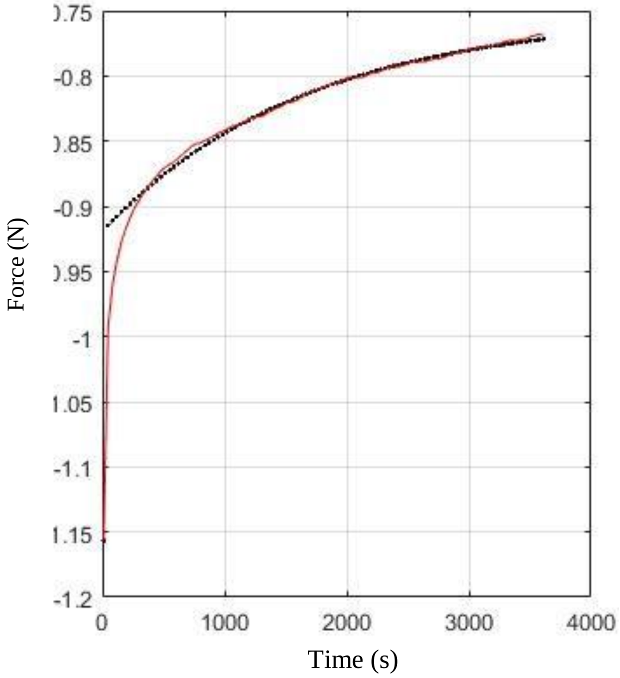


Figure 5-2 - Finite element model fitting for the Actifit prosthesis

5.3 Discussion

To date, the Actifit meniscal scaffold has not been subject to independent characterisation of its material properties. Whilst it remains a promising treatment for young patients with selected subtypes of meniscal injury, there is a need for both clinical and basic science evaluation of its function.

The aggregate modulus derived in our Actifit sample exceeds that reported elsewhere in the literature for the meniscus [98,100,103] and indeed, that shown by our own experiments in 0.14M PBS. In fact, it even exceed that reported for cartilage [305,306]. When the Actifit is implanted, a period of non-weightbearing, followed by protected weight bearing occurs, with knee flexion gradually increased over a period of weeks. During this period, a cellular infiltrate lays down nascent fibrocartilage and blood vessel proliferation to allow meniscal repair[215]. It may be that a construct stiffer than native tissue is required to protect these structures during weight bearing, prior to its eventual reabsorption. However, a stiffer implant than the native joint structures may also lead to increased contact stresses on the articular cartilage surfaces. Indeed, radiological study of articular cartilage following Actifit meniscal repair has shown conflicting results, with both improvement and degradation in cartilage status noted in the same cohort [218].Furthermore, the coefficient of friction of the implant against cartilage has been determined to be higher than that of stainless steel [206]. This finding warrants further investigation as discussed below.

The zero-strain permeability of the Actifit scaffold is similar to that reported for the meniscus. As has been discussed prior, permeability of the tissue is intrinsic to meniscal function, allowing generation of hydrostatic pressures under load [307]. The Actifit implant remains in situ for a period of years prior to full reabsorption. A similar permeability to meniscal tissue may aid the development of appropriate pore sizes in the repaired meniscal tissue, ultimately replicating the meniscus' intended function.

Both the compression modulus and pore size within the scaffold has been found to influence the degree of fibrocartilage ingrowth, with up to 100% ingrowth shown at moduli of 150MPa and macropore size between 150-300μm [200]. Hence these parameters are optimised to allow maximal ingrowth of reparative tissue.

Again, testing of the exponential strain and stiffening coefficients revealed small magnitude values for these parameters, reflecting the small magnitude of strain applied to the sample. The same is true of the viscoelastic coefficient – a value close to zero suggests little evidence of viscoelastic behaviour in the tissue at the strain tested, rendering the value of the relaxation time relatively meaningless (and also explaining the large standard deviation in this result).

Of note, the standard deviation of the other variables, particularly the Aggregate modulus and permeability were quite small. This likely reflects homogeneity of the scaffold throughout its structure. Although this differs substantially from the inhomogeneous nature of the meniscus, such homogeneity is likely a requirement for allowing uniform tissue ingrowth and adequate meniscal repair.

The mean coefficient of determination in the Actifit samples was remarkably high, suggesting a close to ideal fit. This is likely because the experimental setup mirrors the tissue organisation envisaged in the poroviscoelastic model – that of a porous solid phase permeated with fluid.

5.3.1 Strengths and weaknesses

Most of the points raised in the section concerning testing of meniscal tissue apply here. Only a single scaffold was tested in this work. It is possible that the mechanical properties of individual scaffolds may differ somewhat, depending on factors such as the manufacturing process, time since manufacture and environmental factors. Furthermore, recently published work has suggested [308] that the use of an ion containing Hartmann's solution as opposed to an electrolyte free solution during surgery resulted in a significantly higher load to failure of sutures placed in the Actifit scaffold. The reasons for this are unclear and the authors of the work suggest that surgeons irrigate joints with an ionic solution during surgery.

5.3.2 Suggestions for further work

As noted above, recently published work has suggested that the use of an ionic solution resulted in a higher load to failure of sutures placed in the Actifit scaffold as compared to a non-ionic solution. In designing our experiments, it was assumed that the response of the scaffold to ionic solutions would be negligible, however, this study raises the question as to whether this is the case. Given these findings, it would be interesting to explore whether mechanical properties of the Actifit scaffold differ in ionic solutions, particularly in 0.14M PBS.

A recently published study investigated the use of a meniscal substitute in the form of a polycarbonate urethane prosthesis within a sliding model mimicking the gait cycle [309]. The meniscal substitute and cartilage plugs to determine the coefficient of friction throughout the gait cycle. The authors found a high coefficient of friction during the swing phase of gait in their model. Applying such methodology with the Actifit implant would shed further light on whether its increased stiffness might result in adverse frictional properties.

Finally, the techniques described in the joint stability testing section could also be used to explore whether Actifit implantation results in altered peak stresses or contact areas due to its increased stiffness as compared to the native meniscus. Such a study would incorporate the use of pressure sensors, using the apparatus developed here.

5.3.3 Conclusions

The search for an effective and predictable treatment for meniscal tears has led to the development of multiple prosthetic devices to augment meniscal repair or potentially to replace the meniscus itself. Meniscal scaffolds are a promising treatment in this regard, but there is much work to be done to determine whether they do indeed aid meniscal healing and ultimately, protect articular cartilage. Notably, coverage for the Collagen Meniscus Implant was revoked by the US Medicare/Medicaid service on the grounds that it did not improve healthcare outcomes in the Medicare population [310]. More recently, Monllau et al. [311] published 5 year outcomes of the Actifit implant in a series of 32 patients, finding that although patient reported outcomes were improved, the scaffolds did not result on a normal MRI signal volume in the meniscal region, with the volume of 'repaired' tissue smaller than expected. A significant revision rate was also reported. Given the significant risk of a performance bias in the patient reported outcomes due to concomitant procedures, the authors concluded there was little evidence that scaffold implantation led to definite benefit. This study was the subject of a somewhat scathing editorial [312], with the editor noting that there was no evidence to date that the scaffold was able to recruit the patient's biology to allow a viable meniscal substitute. Although no direct evidence of cartilage degradation was noted in Monllau's work, our study does raise concerns about the potential for the Actifit implant to place abnormal stresses on the articular joint surfaces.

The results presented here highlight a number of potential challenges in the development of tissue engineered meniscal substitutes. Firstly, in terms of the implant's mechanical stiffness, there is a balance to be struck between sufficient stiffness to both protect developing reparative tissue and encourage fibrocartilage ingrowth whilst also ensuring that excessive stiffness does not itself result in chondral damage. Although there is no evidence to date that the Actifit implant causes chondral wear, implanting a prosthesis which can potentially abrade articular cartilage is a potential concern which requires further investigation – particularly due to the lengthy time it takes for the implant to be reabsorbed. It should be noted that the literature review did not identify any studies of the Actifit's aggregate modulus, with a single work [204] in the development of the prosthesis suggesting a compression modulus of 200kPa, significantly lower than we observed (it is unclear whether this value reflects the properties of the final implant or a prototype). Our results suggest that future iterations should undergo testing of their mechanical stiffness to ensure that these are similar to those of the native tissue as far as possible, ultimately reducing the risk of damaging native tissue through the use of such prostheses. However, as noted, these properties must also be optimised to encourage tissue ingrowth.

The fact that the zero strain permeability of the Actifit is similar to that of the meniscus will likely aid such ingrowth, by presenting ingrowing tissue with a similar mechanical environment to that available in native tissue. However, the previous chapter of this thesis has demonstrated the critical importance of proteoglycans to meniscal function. Notably, neither of the commercially available meniscal scaffolds are seeded with biological tissue, which may accelerate the regenerative response, through pre-loading critical components of meniscal architecture, such as proteoglycans or patient derived stem cells. Indeed, Patel et al. [313] have recently published data on a collagen /hyaluronic acid seeded scaffold used for total meniscal replacement in an ovine model, demonstrating deposition of circumferentially aligned collagen fibres and proteoglycan in regions subject to compression. In light of our work highlighting the importance of proteoglycans within the meniscus, addition of these proteins may well allow an adequate repair. Such approaches may yield the ability to deliver patient specific scaffolds, which allow regenerative potential by means of autologously derived cell lines – a technique which would also limit the immunogenic potential of such implants.

Notably, our use of the poroviscoelastic model in testing the Actifit implant has also highlighted the suitability of this model for testing meniscal scaffolds, evidenced by the excellent fit seen in our experiments. Our work indicates that future developments in meniscal substitutes would be well suited to testing through such techniques, in an effort to both determine their mechanical properties and compare them to native tissue.

6. Overall discussion

6.1 Hypotheses

Returning to consider the null hypotheses of this thesis, we are now able to conclude the following:

- *Radial tears of the medial/lateral meniscus do not affect the kinematics of the cadaveric human knee.*

The evidence collected suggests that this is indeed the case, hence we accept the null hypothesis. However, this work also highlights that the predominant treatment for such tears, in the form of partial meniscectomy, can itself induce altered kinematics. Furthermore, such tears have been found to alter contact mechanics within the tibiofemoral joint. Hence, this work suggests that the focus of management for such tears should be to reconstruct the damaged meniscal tissue, rather than remove it.

- *Mechanical properties of meniscal tissue do not vary in solutions of varying ionic concentration.*

The evidence collected suggests that the mechanical properties of meniscal tissue vary in solutions of varying ionic concentration and that this is a result of the electrostatic effects of proteoglycans within the tissue. Hence this null hypothesis is rejected. Of note, this is the first study to identify that proteoglycans within the meniscus function through electrostatic effects. This finding is of significant relevance for the development of meniscal substitutes, which currently do not aim to replenish or replace the supply of proteoglycans within regenerated meniscal tissue. Accounting for the function of proteoglycans in meniscal regeneration is of critical importance.

- *There is no significant difference between the mechanical properties of the Actifit meniscal scaffold and human meniscal tissue.*

The evidence collected suggests that there is a significant difference between the mechanical properties of the Actifit meniscal scaffold and the native meniscus, hence this null hypothesis is rejected. Of particular note is the fact that the scaffold is stiffer than both meniscus and cartilage, potentially allowing it to damage the indwelling articular cartilage. Although the zero strain permeability of the implant is similar to the meniscus, theoretically both mimicking meniscal function and providing a suitable environment for meniscal regeneration, as noted above, the lack of proteoglycans in meniscal tissue regenerated using scaffolds potentially limits their efficacy in acting as a meniscal substitute. Future efforts to develop meniscal substitutes should ensure the function of these proteins is replicated. Our work also highlights the suitability of the poroviscoelastic model in describing such tissue substitutes, suggesting that this model can be used in the development and testing of these prostheses.

6.2 Conclusions

Although the meniscus suffered a prolonged period of neglect and lack of appreciation of its innate role, there is now widespread appreciation that this organ has a critical purpose in normal function of the knee. Despite this, there are still numerous gaps in our understanding of the meniscus' function, which is important if we are to aim to treat meniscal injuries adequately to preserve knee function.

This thesis has added to the body of literature on the meniscus, expanding facets of knowledge concerning the function of organ in both macroscopic and microscopic roles. Macroscopically, we have demonstrated that radial tears of the meniscus do not adversely affect the kinematics of the knee, though previous work has shown that such tears do alter load bearing characteristics within the joint. As noted above, there is an ongoing paradigm shift in the orthopaedic literature regarding the use of partial meniscectomy as a first line treatment for meniscal tears. A combination of studies suggesting that the use of meniscectomy both alters tibiofemoral loading characteristics and knee joint kinematics, coupled with clinical studies identifying no benefit in surgery compared with physiotherapy has lead to a growing recognition that partial meniscectomy has limited utility. This work adds to this argument, highlighting the potential for inducing altered kinematics in patients with radial meniscal tears through partial meniscectomy when the primary injury does not cause such effects.

From a microscopic perspective, we have demonstrated that proteoglycans within the meniscus aid in stiffness of the human meniscus through electrostatic effects - these account for up to 40% of the stiffness of meniscal tissue. This is the first study to determine that proteoglycans within the meniscus operate through electrostatic effects. This work also indicates that proteoglycans are in fact of critical importance in maintaining meniscal stiffness, which itself plays a central role in allowing the meniscus to fulfil its function in transmitting load across the knee joint. Hence, any strategies which aim to develop meniscal substitutes or scaffolds to augment repair must account for the function of these proteins. Furthermore, tissue modelling of meniscal tissue, especially where fluid flow or localised strain is being considered, should account for these electrostatic effects.

We have also explored the mechanical properties of the Actifit meniscal scaffold, demonstrating that these match those of the meniscus in some respects and differ in others. In particular, the scaffold has been demonstrated to be stiffer than the body’s tissues, potentially risking damage to native tissues. This is also the first study to demonstrate the use of finite element modelling techniques in characterising the properties of such scaffolds and it appears the poroviscoelastic model is well suited to such a role. Hence, further efforts to develop such substitutes should consider these factors.

Ultimately, the knowledge presented in these thesis will both aid the clinical management of meniscal tears and aid the development of novel therapies for treating meniscal injuries.

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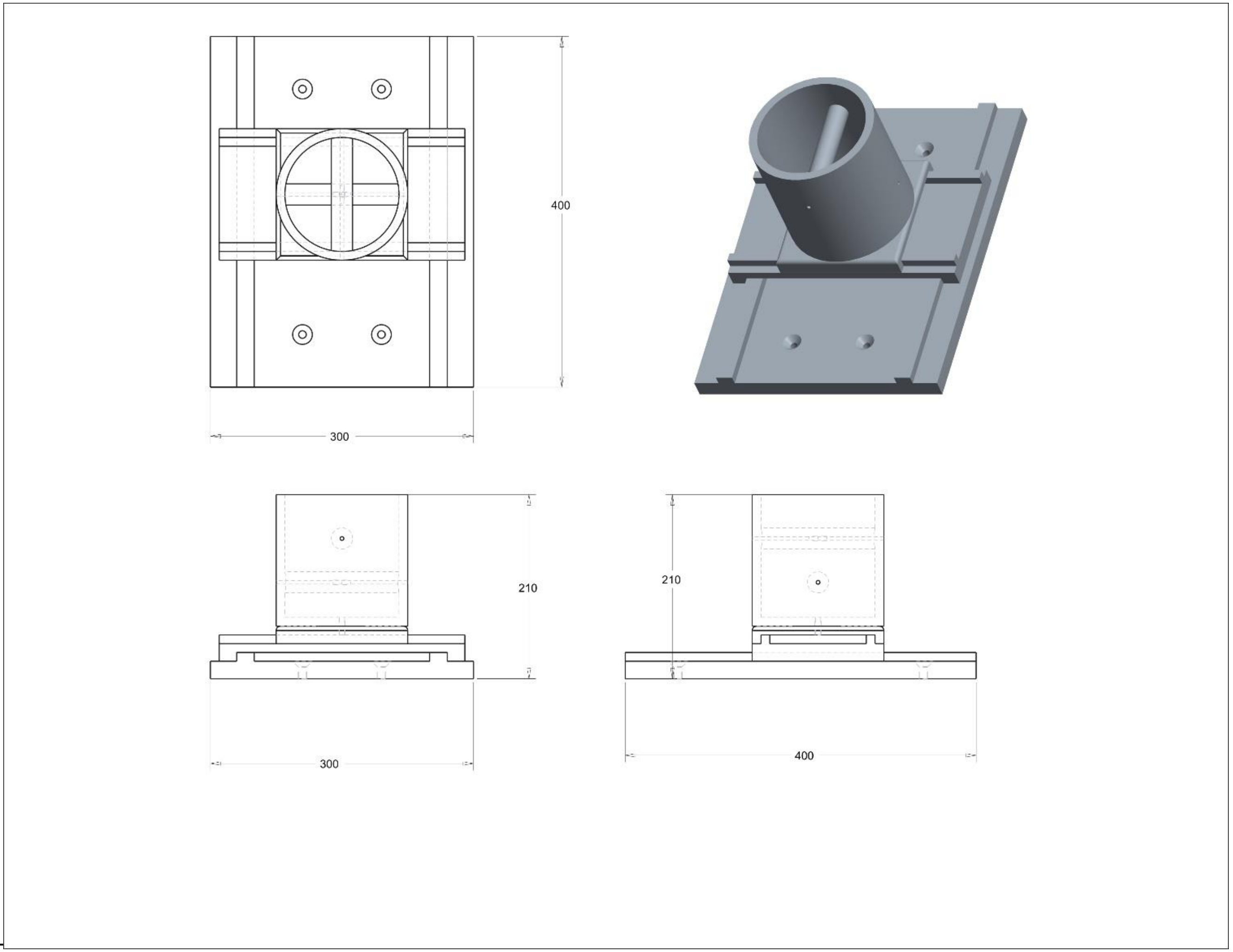
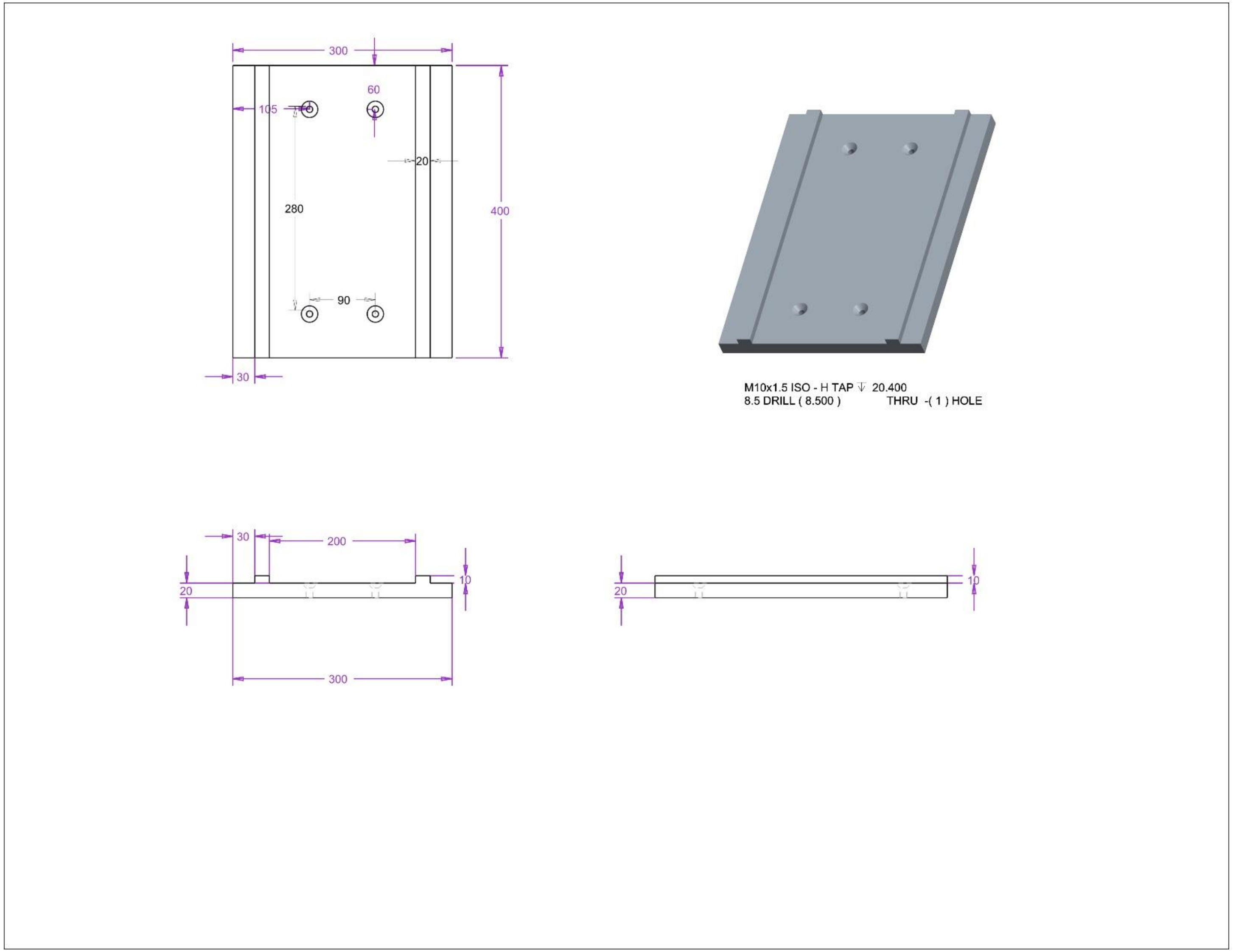
8. Appendices

8.1 Appendix 1 – Technical drawings of jig components

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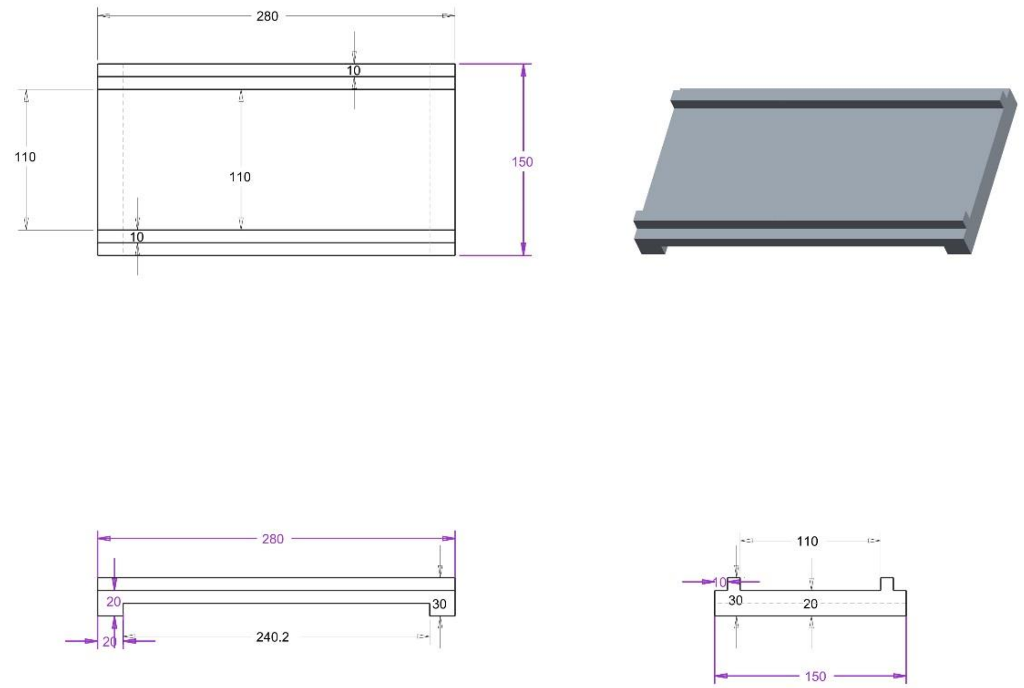
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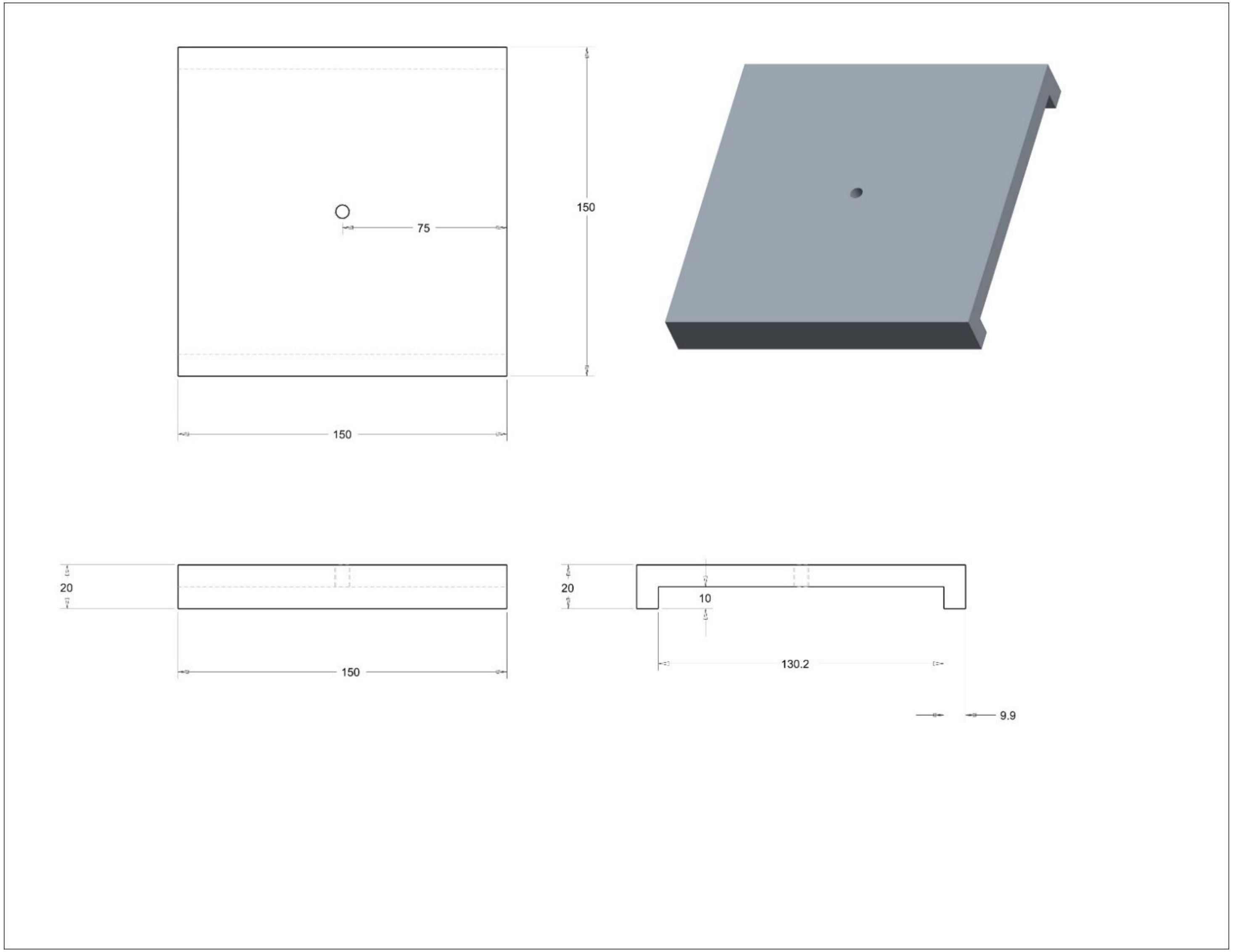
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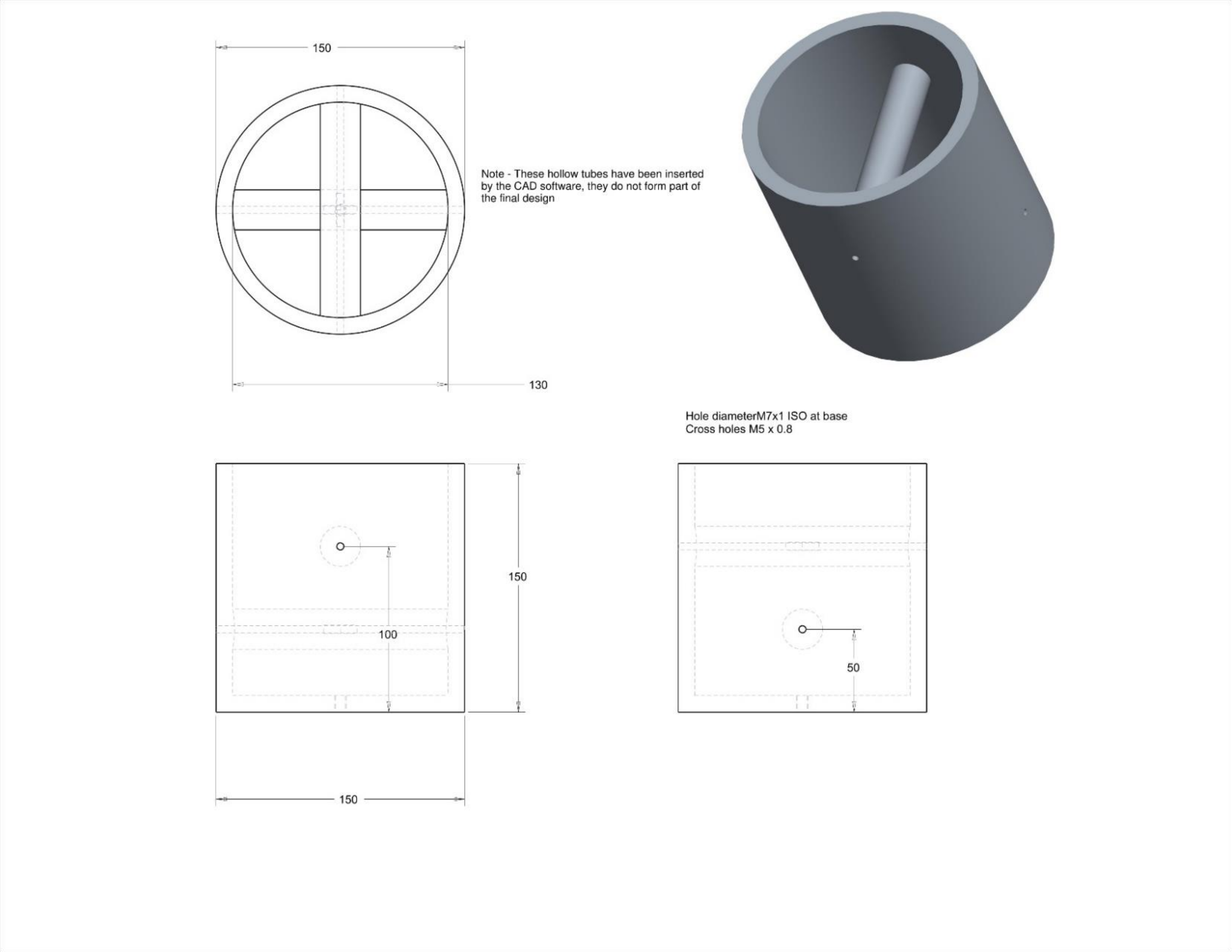
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Note - These hollow tubes have been inserted by the CAD software, they do not form part of the final design
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Hole diameterM7x1 ISO at base
Cross holes M5 x 0.8
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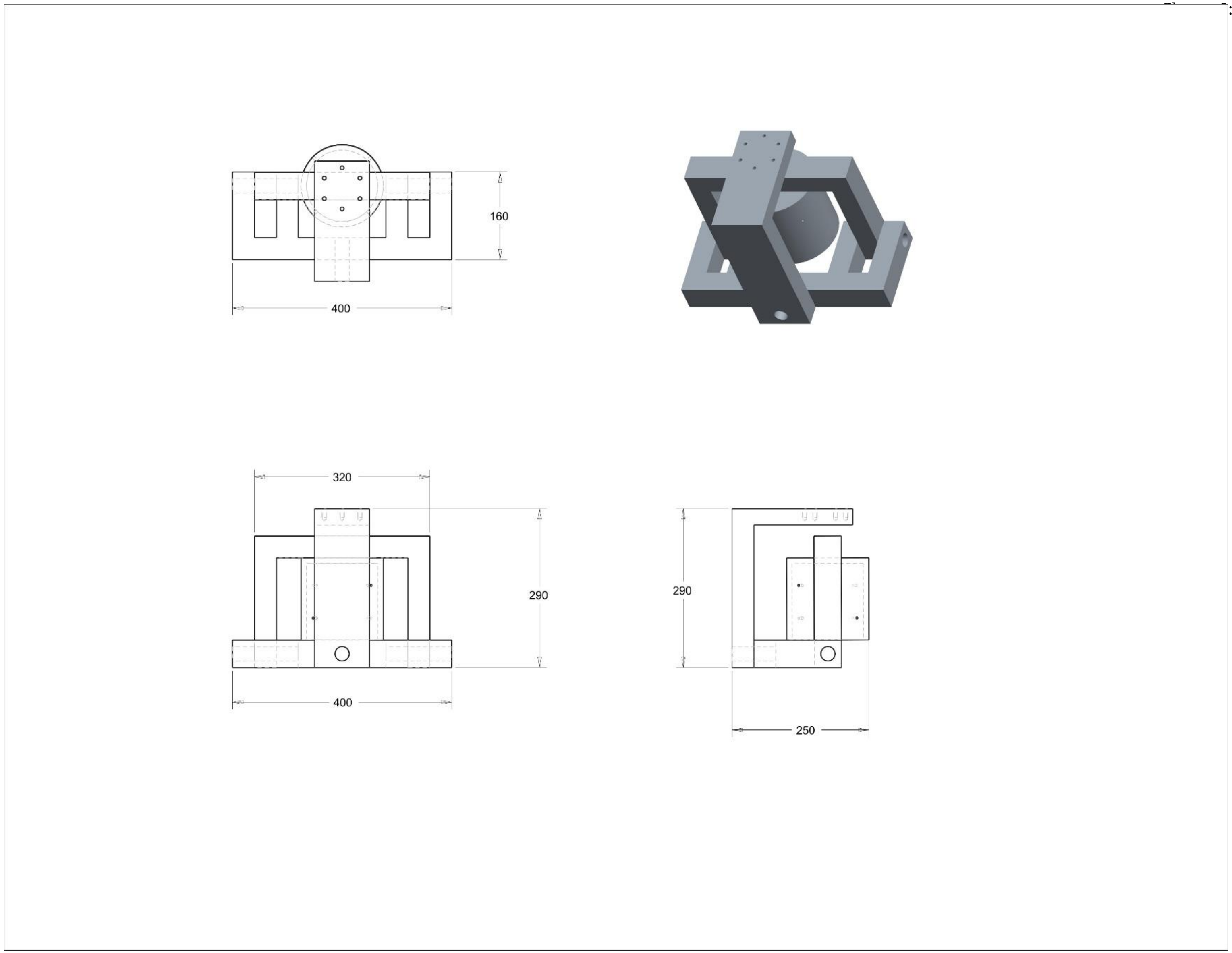
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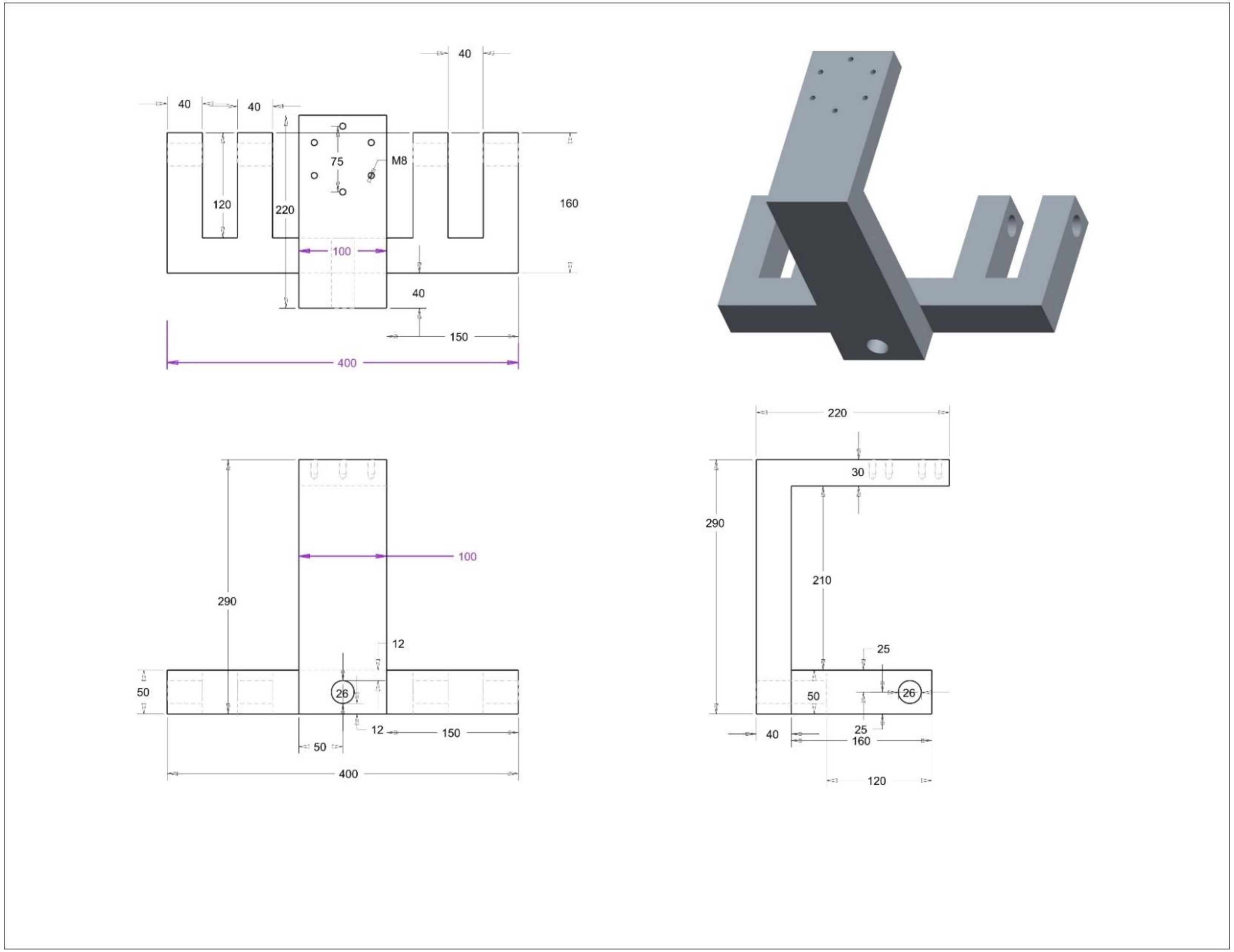

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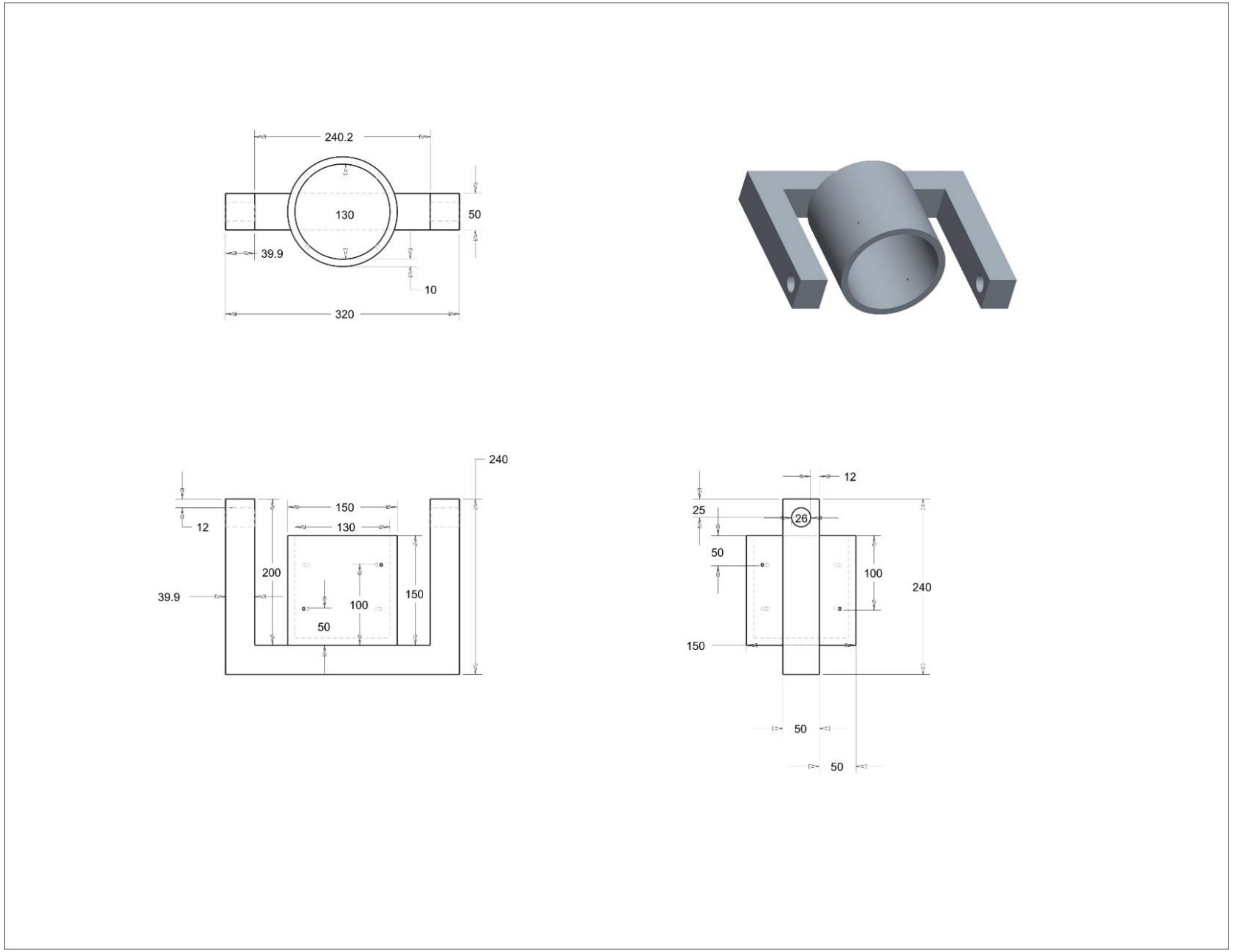
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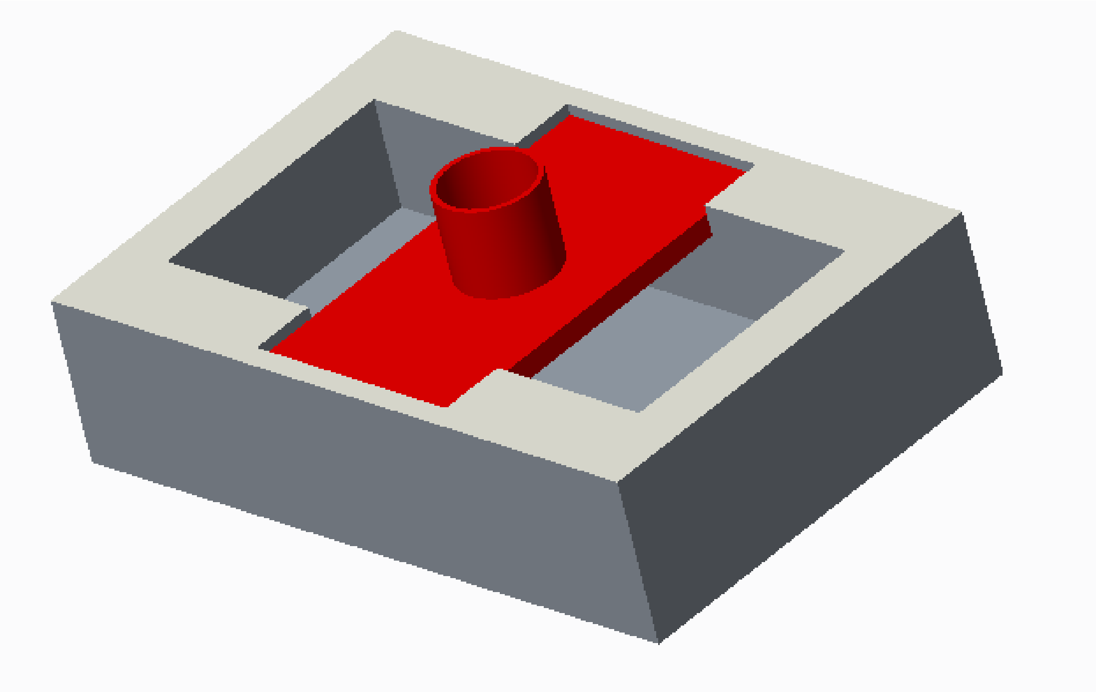
8.2 Appendix 2 - Cement mixing protocol

- For ease of use, cement was mixed by measure of volume rather than weight.
- 200ml of tap water was added to a volume of 250ml cement powder whilst constantly mixing.
- Cement was mixed until thoroughly dissolved, then immediately poured into the bone cylinders.

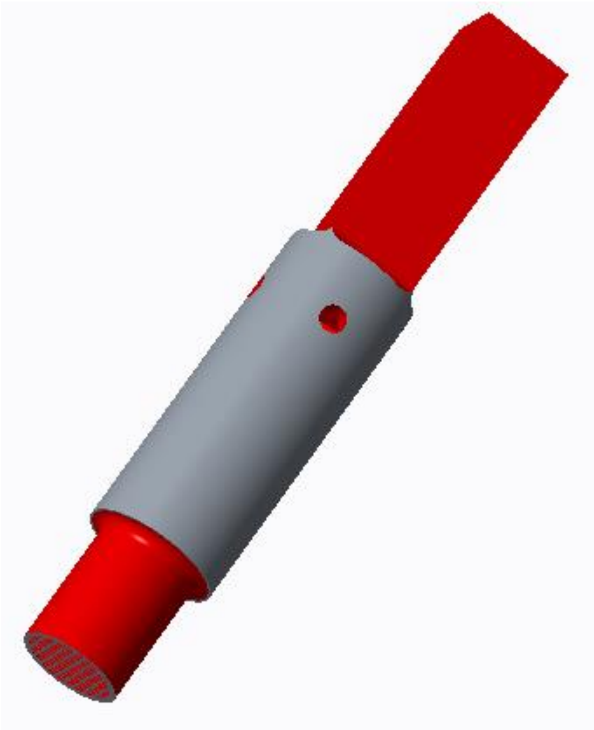
8.3 Appendix 3 - Characteristics of cadaveric donors

ID	Side	Age	Sex	Meniscus tested
1	Left	58	Male	Medial meniscus
2	Right	65	Female	Lateral meniscus
3	Left	65	Female	Medial meniscus
4	Left	61	Male	Medial meniscus
5	Left	57	Male	Medial meniscus
6	Left	32	Female	Medial meniscus
7	Right	60	Female	Medial meniscus
8	Right	32	Female	Lateral meniscus
9	Left	60	Female	Lateral meniscus
10	Right	61	Male	Lateral meniscus
11	Right	58	Male	Lateral meniscus
12	Right	57	Male	Lateral meniscus

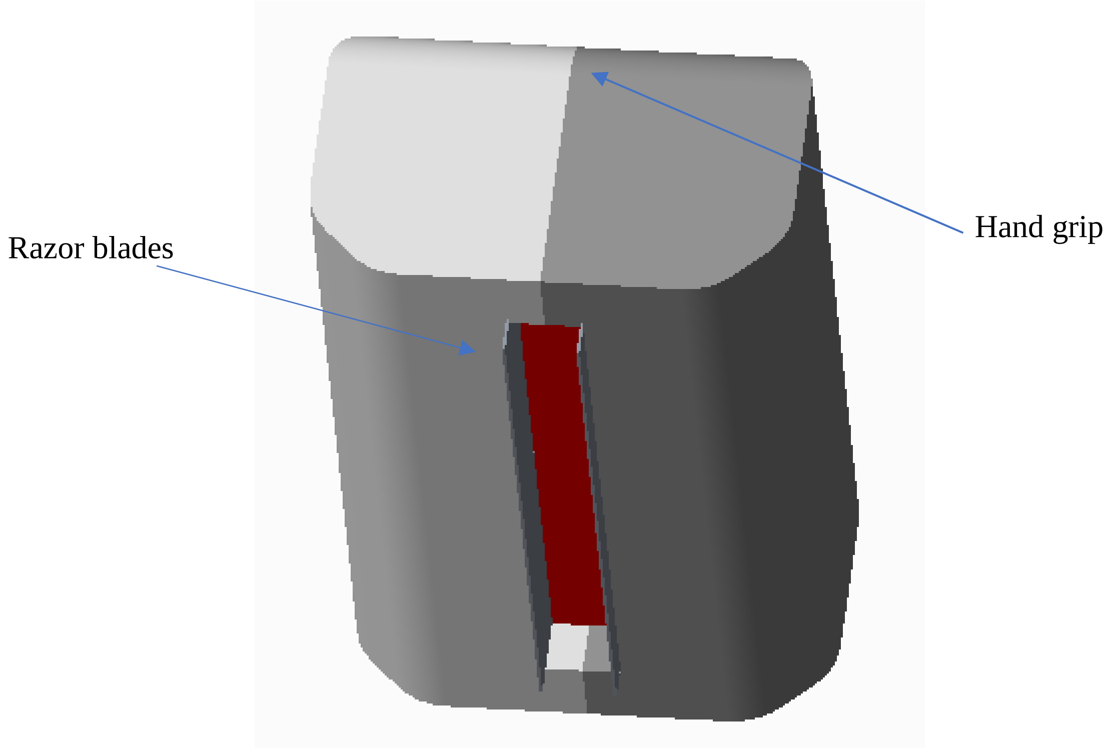
8.4 Appendix 4 – Creo drawings

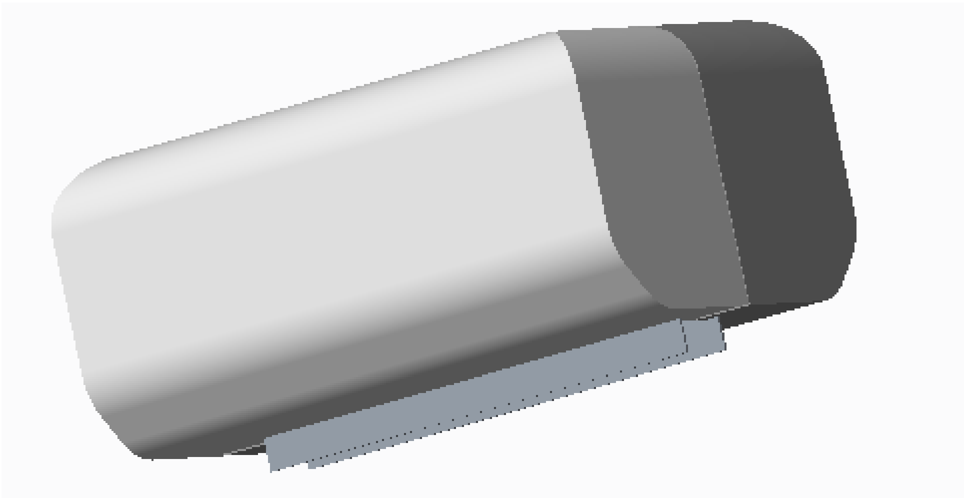


Creo drawing of confining chamber and bath

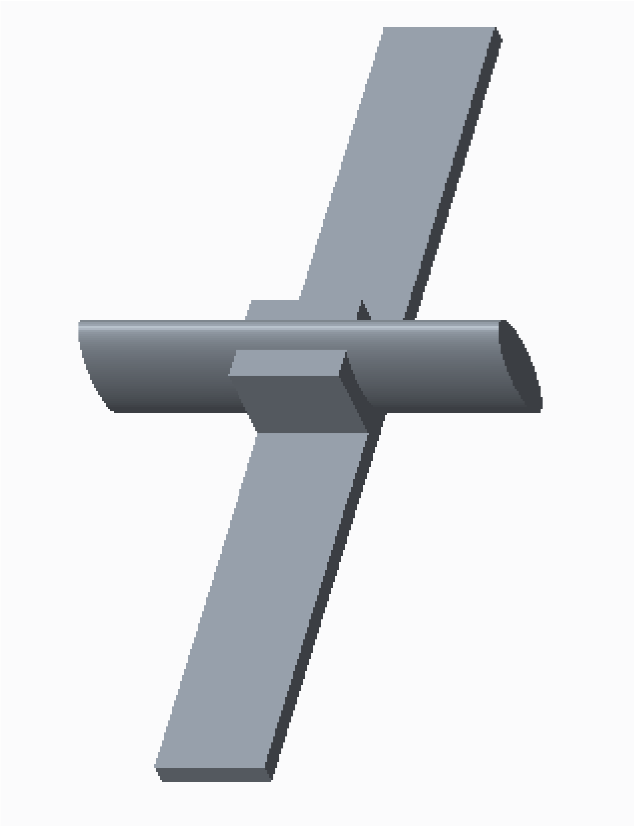


Creo drawing of indenter

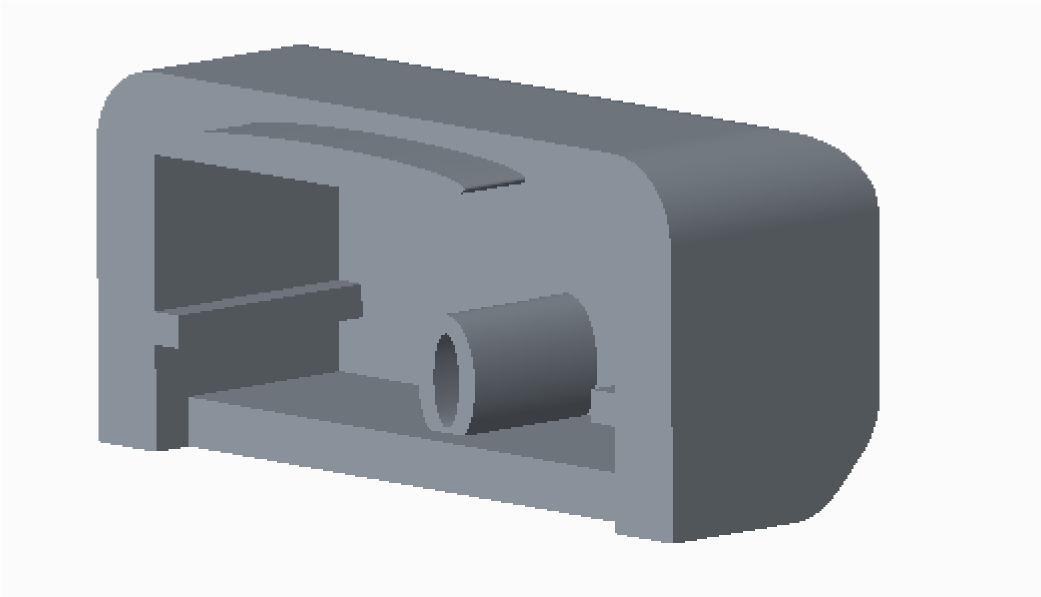




<u>Creo drawings of sectioning tool</u>



Creo drawing - Sectioning device insert component



Creo drawing - Sectioning device holder component

8.5 Appendix 5 – Preparation of ionic solutions

- Deionised water was obtained from a supply available in the laboratory.
- 0.14M PBS was prepared by dissolving 1 tablet Sigma Aldrich PBS in 200 ml water
- 3M PBS was prepared by dissolving 1 tablet Sigma Aldrich PBS in 200 ml water, then adding 33.5g NaCl
    - This mixture was agitated until all salt had been dissolved.

Solutions were prepared in batches and stored in a laboratory fridge until use. Prior to use, if any precipitate was evident, the solution was mixed until this had dissolved.

8.6 Appendix 5 – Patient information sheet

Participant information sheet

Title of project: Stress relaxation behaviour of human meniscal tissue

Invitation and brief summary

You are being invited to take part in a research study. You are under no obligation to do so and your decision will have no impact on the quality of care you are provided. The study participants are all patients undergoing total knee replacement surgery due to severe osteoarthritis. Before you decide whether to take part, it is important that you understand why the research is being done and what it will involve. Please take the time to read the following information carefully and ask us if there is anything not clear to you or you would like more information. Take your time in deciding whether or not to take part.

The study involves conducting laboratory experiments on a tissue called the meniscus which lies within the knee. This tissue is routinely entirely removed during total knee replacement surgery and discarded. If you consent to take part, your meniscus will instead be immediately frozen, transported to a laboratory at the University of Strathclyde and used to conduct experiments. After testing all tissue will be destroyed. You will not be identifiable to the researchers after you have donated your tissue and any results generated from your tissue will not be linked to you in any way.

What's involved?

This research project is a laboratory-based project which seeks to identify the role of proteins within the meniscus. Currently, we do not understand how these proteins help in transmitting the weight of the body on the meniscus (for example, when walking). We believe that this effect might be influenced by electrical charge. Therefore, we are looking to obtain samples of meniscus and subject them to tests where we squeeze the tissue and then take very accurate measurements of how it relaxes. By conducting these tests whilst bathing the meniscus in liquids with varying salt concentrations, we will be able to identify whether electrical charge influences the behaviour of meniscus tissue. This knowledge will be useful in developing artificial replacements for the meniscus in the future and also the design of computer models of the meniscus.

We are seeking to recruit 30 participants in the study. This will allow us to obtain samples from the same region of the meniscus in each participant. As described above, the meniscus is routinely removed and discarded during knee replacement surgery and your decision or refusal to take part would not alter your operation in any way.

What would taking part involve?

If you were to agree to take part, your operation would proceed as scheduled. You would not be subjected to any additional or different procedure, nor would there be any difference in your pain after the operation. Your recovery time and rehabilitation after the operation would also be the same. The function of your knee replacement would also be the same. Your decision to take part would not influence the type of implant the surgeon uses during the operation.

If you consent to take part, samples taken during the operation will be immediately frozen and then transported to a secure laboratory licensed to work with human tissue at the University of Strathclyde where they would undergo testing. Following testing, samples would be destroyed as directed by the Human Tissue Authority, who regulate research with human tissue in the United Kingdom.

As well as this, at the time of your donation, your patient notes would be used to record some data regarding you and your condition, including your age, weight, body mass index, diagnosis and any history of knee instability. This data will be recorded in anonymised form and you would only be identified by a number (e.g. Patient 12) so that your sample can be matched to your data.

What are the possible benefits of taking part?

There are no direct benefits to you of taking part in the study. However, any knowledge generated from this work may benefit patients with meniscus injuries in the future.

What are the possible disadvantages/risks of taking part?

There is no increased risk of harm to you from the surgery as the procedure you undergo will be no different whether or not you choose to take part. The routine risks of total knee replacement surgery and the associated anaesthetic will have been explained to you by your surgeon/anaesthetists.

Will my taking part in the study be kept confidential?

All information collected during the study will be immediately anonymised before it leaves the hospital site. There are strict laws governing your privacy and every effort will be made to ensure your participation remains anonymous.

There is a very limited risk that you might be identified following your donation. To minimise this risk, the samples and data collected will be identified using a number (e.g. Patient 12) and no information identifying you will leave the hospital site.

Further supporting information

- What if something goes wrong?

As the study involves collection of tissue which would be otherwise removed and discarded, it is highly unlikely that any problems will arise during collection of the samples. Your clinical care is insured both by the NHS and the indemnity provider of the doctors caring for you and the NHS complaints process remains open to you. As well as this, the research study is insured by the University of Strathclyde, who are the main sponsor. If you have any concerns regarding the study, you can contact Dr Fahd Mahmood (see below), who is the primary investigator of the study. The Chief Investigator for the study, Dr Philip Riches, can be contacted at the University of Strathclyde on 0141 548 5703. Finally, if you wish to discuss this study with an Orthopaedic Surgeon, you can speak with Mr Jon Clarke, Consultant Orthopaedic Surgeon at the Golden Jubilee National Hospital, on 0141 951 5000.

- Do I have to take part?

No, you do not have to take part. Your participation is voluntary. If you do not wish to take part in the study at any point, you are free to withdraw at any point. This will not affect the quality of care you are provided. If you have not yet undergone surgery, your procedure will proceed as normal. If you have donated meniscus samples already, these will be destroyed without any further testing. If any tests have been conducted before you choose not to carry on, the results of these tests will be used in our analysis.

- What will happen to the results of the study?

The results of the study will be communicated to other healthcare professionals via presentations at conferences and publication in scientific journals. Any data presented will not identify you in any way.

- Who is organising this study?

This research study forms part of a PhD thesis at the University of Strathclyde, who are the sponsor for the study.

- Who has reviewed this study?

The study has been reviewed by an NHS Research Ethics Committee to ensure it is appropriate, complies with regulation and ensures patient safety.

- Further information and contact details

If you require any further information, please contact Dr Fahd Mahmood on 07917 122025 or at fahd.mahmood@strath.ac.uk

- What to expect during the consent process

During the consent process, you will be asked to read the information sheet, ask any questions and then sign a form which signifies your agreement to taking part in the study. You will be provided with a copy of the information sheet and consent form to keep and the consent form will be filed in both your patient notes and research study site file.

- What if relevant new information becomes available?

It is not anticipated that these experiments will generate any new information which has clinical relevance as they are not seeking to measure any factors which would have clinical relevance.

- What will happen to the samples I give?

Samples you provide will be immediately frozen and transported to a secure laboratory at the University of Strathclyde using a University car designated for transport of tissue samples.

The laboratory has controlled access, both with a card reader and pin code. The samples themselves will be stored in a locked freezer until testing occurs. They will be identified only using a unique identifier assigned to you for the study which contains none of your personal information (e.g. Patient 12) Once samples have been tested, they will be destroyed via a disposal process approved of by the Health and Tissue Authority, who regulate experimental work with human tissue in the United Kingdom.

- Commercial

This research will not generate any intellectual property of commercial benefit to the sponsor or researchers. Any knowledge generated will be published in scientific journals, so it is available to the wider healthcare community.

Thank you for your time.

8.7 Appendix 6 – Consent form

IRAS ID: 224784

Golden Jubilee National Hospital

Study Number: ______________________

Participant Identification Number: ____________

CONSENT FORM

Title of Project: **Stress relaxation behaviour of human meniscal tissue**

Name of Researcher: Dr Fahd Mahmood

Please initial box

1. I confirm that I have read the information sheet dated 21st April 2017 (version 2.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I understand that the information collected about me will be used to support other research in the future and may be shared anonymously with other researchers. ☐

5. I agree to take part in the above study.

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature