Numerical and Experimental Characterisation of Articular Cartilage – A Study on Biomechanics and Biotribology, Osteoarthritis and Tissue Engineering Solutions

By

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Abstract

Articular Cartilage (AC) is a soft tissue covering the articulating surface of human and animal joints. The tissue has remarkable and highly complex mechanical and wear properties allowing the joint to undergo complex kinematics and function correctly for several decades. However, trauma and degenerative joint diseases such as osteoarthritis (OA) can cause damage and excessive wear of the tissue and due to its limited regenerative capabilities, can severely compromise joint movement and impair the quality of life. OA is the most common type of degenerative joint disease and the primary cause of joint replacement surgery leading to high associated healthcare costs. Although the exact cause of this pathology remains unknown, it is thought to be mechanically induced via excessive and abnormal stresses and strains in AC which cause altered biochemical properties and a gradual decrease in the mechanical quality of the tissue.

There is currently no available cure for OA and the disease is currently being diagnosed only via imaging techniques which are based upon morphological changes of the tissue, when the pathology is already in its advanced stages and has caused irreversible changes to the AC. In this respect, one of the greatest challenges to now remains the early diagnosis of OA, potentially by assessing biochemical and mechanical changes, allowing early treatments and prevention of disability thus improving the patient’s life. Hence, there is a need to apply fundamental engineering principles to the medical world in order to shed light on the pathogenesis and progression of OA. Furthermore, the need for artificial substitutes of AC has called for a deep understanding of the mechanical behaviour of the tissue in order to design and mimic the response of the real tissue in the most accurate manner.

In this research a combination of numerical (finite element) and experimental techniques involving mechanical and tribological tests were used to fully characterise the mechanical behaviour of the tissue. Selective degradation of the AC constituents was then induced to simulate OA (OA-like AC) and the effect of different stages of degradation on the mechanical and tribological response as well as the wear properties of the tissue was investigated. The mechanical properties of osteoarthritic AC were then evaluated and compared to the OA-like AC in order to correlate similarities in the variations to the structure and the mechanical response as a result of degradation. Quantifying the mechanical response of the tissue at different stages of OA and different levels of degradation was done to ensure both a thorough understanding of the effect of the pathology’s progression on AC as
well as to provide a potential map of mechanical quality and degradation, contributing to the potential future diagnosis of OA via mechanical parameters rather than morphological alone. Having investigated structural and mechanical variation in early OA, a promising solution to treat localised early OA and AC defects was also investigated as part of this research. In particular, novel microfibrous tissue engineered scaffolds have been mechanically and tribologically assessed and compared to AC demonstrating the strong potential of matrix-assisted autologous chondrocyte implantation (MACI).

Finally, the numerical models developed to characterise the AC using numerical – experimental methods, namely advanced biphasic models incorporating fine material descriptions such as intrinsic viscoelasticity as well as transverse isotropy, were applied to a patient specific 3D menisectomised tibio-femoral joint contact model in order to demonstrate the implications that the implementation of different AC models have for the prediction of the joint response to repeated walking cycles. The results obtained from the models were then used to predict the most likely location for the origin of mechanical damage and OA.
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I would like to give my acknowledgements to Dr Daniele Dini for having given me the invaluable opportunity to embark on a life changing path; first by carrying out my third year undergraduate literature project on articular cartilage under his supervision followed by a continuation into a final year undergraduate project on articular cartilage mechanics and modelling, and finally for believing in me and allowing me to further continue my studies on the topic with a PhD. I would like to thank him for his patience, his genuine, unique and extraordinary support he has given me making my PhD a truly memorable experience. Thank you Daniele, I am deeply indebted to you.

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Disclaimer

The author hereby declares that the work contained within this thesis is original and his own, except where specifically declared or acknowledged. This thesis is a collection of research work carried out solely by the author and in some parts, due to the multi disciplinary nature of the work, in collaboration with other researchers and institutions. Some of the work described in this thesis has been previously published in peer reviewed journals and conference proceedings.

Chapter 3 presents the development of the various experimental techniques used in this study. The development of the Multiaxial Compression and Shear Testing Rig (MCSTR) was done in collaboration with Dr Richard Wayte.

Chapter 4 constitutes part of the Journal article “Accardi, M.A., Cann, P.M., Dini, D. Experimental and numerical investigation of the behaviour of articular cartilage under shear loading - Interstitial fluid pressurisation and lubrication mechanisms. Tribology International. 2011 May;44(5):565-78.”

Chapter 5 is part of a collaboration with Dr Ngee Han Lim, Dr Kazuhiro Yamamoto and Professor Hideaki Nagase from the Kennedy Institute of Rheumatology, Imperial College London and Professor Justin P. Cobb from the Department of Orthopaedic Surgery, Imperial College London, Charing Cross Hospital. The chapter constitutes part of a Journal article currently being submitted for publication.

Chapter 6 is part of a collaboration with Dr Seth McCullen, Dr Anthony Callanan, Dr Sangwon Chung and Professor Molly Stevens from the Department of Materials, Department of Bioengineering and Institute of Biomedical Engineering, Imperial College London. The Chapter constitutes part of a Journal article submitted to Tissue Engineering Part A: “Accardi, M.A., McCullen, S.D., Callanan, A., Chung, S., Cann, P.M., Stevens, M.M. and Dini, D. Effects of fibre orientation on the frictional properties and damage of regenerative articular cartilage surfaces.”

Chapter 7 is part of a collaboration with Dr Lorenza Mattei from the Department of Mechanical, Nuclear and Production Engineering, University of Pisa and Dr Eleonora Campioni from the Department of Mechanical and Civil Engineering, University of Modena and Reggio Emilia, who both joined the research group as visiting research students during my PhD studies. The chapter constitutes part of the Journal article currently under press: “Mattei, L., Campioni, E., Accardi, M.A. and Dini, D. Finite Element Analysis of the Meniscectomised Tibio-Femoral Joint: Implementation of
Advanced Articular Cartilage Models. Computer Methods in Biomechanics and Biomedical Engineering.”
“Per correr miglior acque alza le vele
omai la navicella del mio ingegno
che lascia dietro a sè mar sì crudele”

- La Divina Commedia, Purgatorio, Canto I, Versi 1-3
  Dante Alighieri
“It is not the critic who counts; not the man who points out how the strong man stumbles, or where the doer of deeds could have done them better. The credit belongs to the man who is actually in the arena, whose face is marred by dust and sweat and blood; who strives valiantly; who errs, who comes short again and again, because there is no effort without error and shortcoming; but who does actually strive to do the deeds; who knows great enthusiasms, the great devotions; who spends himself in a worthy cause; who at the best knows in the end the triumph of high achievement, and who at the worst, if he fails, at least fails while daring greatly, so that his place shall never be with those cold and timid souls who neither know victory nor defeat.”

- “Citizenship in a Republic”

Speech at the Sorbonne, Paris, April 23, 1910

Theodore Roosevelt
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<th>Description</th>
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<tbody>
<tr>
<td>AC</td>
<td>Articular Cartilage</td>
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<tr>
<td>BC</td>
<td>Boundary Condition</td>
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<tr>
<td>BLE</td>
<td>Biphasic Linear Elastic</td>
</tr>
<tr>
<td>BNLE</td>
<td>Biphasic Non Linear Elastic</td>
</tr>
<tr>
<td>BPVE</td>
<td>Biphasic Poroviscoelastic</td>
</tr>
<tr>
<td>BTI</td>
<td>Biphasic Transversely Isotropic</td>
</tr>
<tr>
<td>BTINLE</td>
<td>Biphasic Transversely Isotropic Non Linear Elastic</td>
</tr>
<tr>
<td>CAT</td>
<td>Computed Axial Tomography</td>
</tr>
<tr>
<td>ECM</td>
<td>Extra Cellular Matrix</td>
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<tr>
<td>FCD</td>
<td>Fixed Charge Density</td>
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<tr>
<td>FE</td>
<td>Finite Element</td>
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<td>FEM</td>
<td>Finite Element Model/Modelling</td>
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<td>GAG</td>
<td>Glycosaminoglycan</td>
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<td>HFRR</td>
<td>High Frequency Shear Testing Rig</td>
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<tr>
<td>M</td>
<td>Monophasic</td>
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<tr>
<td>MCSTR</td>
<td>Multiaxial Compression and Shear Testing Rig</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>OA</td>
<td>Osteoarthritis</td>
</tr>
<tr>
<td>PG</td>
<td>Proteoglycan</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>SAL</td>
<td>Surface Amorphous Layer</td>
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<tr>
<td>SF</td>
<td>Synovial Fluid</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscope/Microscopy</td>
</tr>
<tr>
<td>TFJ</td>
<td>Tibio-Femoral Joint</td>
</tr>
<tr>
<td>TI</td>
<td>Transversely Isotropic</td>
</tr>
<tr>
<td>UHMWPE</td>
<td>Ultra High Molecular Weight Polyethylene</td>
</tr>
<tr>
<td>WLI</td>
<td>White Light Interferometry</td>
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<tr>
<td>§</td>
<td>Section</td>
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</table>
## Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>$E$</td>
<td>Young's Modulus</td>
</tr>
<tr>
<td>$\nu$</td>
<td>Poisson Ratio</td>
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<tr>
<td>$E_{eq}$</td>
<td>Equilibrium Young’s Modulus</td>
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<tr>
<td>$\nu_{eq}$</td>
<td>Equilibrium Poisson Ratio</td>
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<tr>
<td>$E_{ins}$</td>
<td>Instantaneous Young’s Modulus</td>
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<tr>
<td>$\nu_{ins}$</td>
<td>Instantaneous Poisson Ratio</td>
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<tr>
<td>$k$</td>
<td>Permeability</td>
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<tr>
<td>$e$</td>
<td>Void Ratio</td>
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<tr>
<td>$M$</td>
<td>Permeability Coefficient</td>
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<tr>
<td>$G(t)$</td>
<td>Reduced Relaxation Function</td>
</tr>
<tr>
<td>$c$</td>
<td>Magnitude of Relaxation Power Spectrum</td>
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<tr>
<td>$G_i$</td>
<td>Discrete Relaxation Function</td>
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<tr>
<td>$\tilde{G}$</td>
<td>Discrete Spectrum Magnitude</td>
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<tr>
<td>$\tau_s$</td>
<td>Short Term Relaxation Time</td>
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<tr>
<td>$\tau_l$</td>
<td>Long Term Relaxation Time</td>
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<tr>
<td>$\tau_i$</td>
<td>Discrete Relaxation Time</td>
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<tr>
<td>$\mu$</td>
<td>Friction Coefficient</td>
</tr>
<tr>
<td>$\mu_{eq}$</td>
<td>Boundary Regime or Equilibrium Friction Coefficient</td>
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</tbody>
</table>
$\mu_{\text{mix}}$ Mixed Regime Friction Coefficient

$\mu_{\text{film}}$ Hydrodynamic Regime Friction Coefficient

$\mu_{\text{eff}}$ Effective Friction Coefficient

$\mu_0$ Start-up Friction Coefficient

$R_a$ Average Roughness

$R_q$ Root Mean Square (RMS) Roughness

$R_z$ Average Maximum Height

$R_t$ Maximum Surface Height

$S_{sk}$ Surface Skewness

$S_{ku}$ Surface Kurtosis
Chapter 1 – **Introduction**

1.1 - General introduction

The continuous progress in science and technology in order to achieve a higher quality of life has been the focus of human kind for the past thousands of years. Giant leaps in science have indeed been made, pushing the technology envelope further and further. Yet, the more we pursue these advancements as engineers, scientists and medical doctors, the more we realise that there is still no technology which can match the functioning of the human body, an utterly ‘perfect machine’.

This perfect machine, however, can sometimes fail for a number of reasons such as diseases or injuries, which can unfortunately severely affect it and compromise its functioning. In this respect, our knowledge of science and technology can help us understand why it failed and how to attempt to restore it (if at all possible) to normal operating conditions. This however, can only be attempted and, hopefully, ultimately achieved by integrating the fundamental engineering principles with the medical and the biological world.

This thesis focuses on studying articular cartilage (AC), the associated damage mechanisms and pathologies as well as potential artificial substitutes.

AC, also known as hyaline cartilage, is a soft tissue which covers the articulating surfaces of human and animal diarthrodial joints (Figure 1.1). It is an essential constituent of the muscoskeletal system, fundamental for humans and animals to be able to move and perform complex kinematics. The term hyaline comes from the Greek word “hyalos” meaning glass and it is due to the glassy and smooth appearance of the tissue. The thickness of AC in adult human joints varies from 1 mm in finger joints to 6 mm on the retropatellar surface [1, 2].
Chapter 1 - Introduction

The main purpose of AC in the musculoskeletal system is to provide a smooth surface to facilitate movement and avoid wear and abrasion between joint surfaces. It does this by allowing joint movement whilst at the same time reducing in-joint friction and wear to a minimum by providing excellent and incomparable lubrication properties. Additionally, it absorbs and attenuates stresses in the joint via increasing the contact area through deformation, hence also improving joint congruency [3, 4].

AC is a truly remarkable material, designed to perfection by nature; in fact, the thickness of human AC is believed to remain unchanged during adult life as long as its surface remains intact [5]. Hence, physiological joint activity is not believed to cause thinning and wear of the tissue, highlighting its exceptional wear properties. Nevertheless, many people do suffer from worn out or damaged AC and articular diseases. In this respect, a pathology known as osteoarthritis (OA) which is thought to affect 85% of people by the age of 55 years [6, 7], can severely affect the mechanical quality and the wear properties of the tissue. OA is the most frequent cause of disability affecting the elderly population and it is the primary cause of artificial joint replacements. The exact origin of this pathology is as-yet unknown, however, it is thought that abnormal or excessive stresses formed in the AC causes the tissue to change structure and composition leading to changes in tribological and mechanical properties which eventually lead to premature AC wear and eventually joint degeneration. Thus, OA is thought to be mechanically induced and has therefore sparked strong interest amongst the engineering community [8].

With these considerations in mind, this thesis firstly seeks to understand and comprehend more about the mechanical behaviour of AC; with the technological advances in medicine and engineering, there has been an increase in artificial tissue replacements and reconstructive surgery. However, artificial tissue replacements require a detailed understanding of cartilage mechanics, as well as an understanding of lubrication mechanisms occurring in the joint, in order to create a biocompatible material as similar as possible to the real AC tissue. The goal is thus to be able to...
mimic the tissue and hence restore full function to the joint which has been compromised via loss of AC either through OA, injury or other factors. Remarkable leaps forward in this field are being made in the last few years as will also be demonstrated from the research performed by the author and presented here.

Secondly, this thesis thrives to gain more understanding about the mechanical effects of OA as well as damage initiation mechanisms in AC in order to shed light on how this pathology develops and how it could potentially be diagnosed earlier and to avoid a long term disability. Clearly, a good knowledge of the properties of the native tissue is needed before a complete understanding of the damaged tissue can be made. Furthermore, being able to accurately predict the onset of damage could potentially allow us, in the near future, to determine which articular exercises and activities could be beneficial for the AC and which detrimental, so as to prevent sport related injuries for example, and hence propose physiotherapy rehabilitative protocols that consider the influence of loading conditions on the joint biomechanical response.

It should be borne in mind that for an ultimately successful investigation, the research undertaken must encompass all the necessary disciplines to allow a complete understanding of the subject, namely engineering, clinical medicine and biological sciences; this is the main challenge for this type of research, however, it is also the cardinal point for ensuring its success.

1.2 - Research objectives

In the last decades, research involving the investigation of AC mechanical properties has been carried out using a variety of techniques, whether experimental, analytical or numerical. What seems to be evident, however, is that its complex mechanical properties and, in particular, its tribological and wear properties are still far from being completely understood. Furthermore, mechanisms of damage in AC and OA initiation are still relatively new field of research, which require substantial studies from a combination of different scientific disciplines if we aspire to better understand tissue degeneration and its development.

Although fundamental research of the mechanical behaviour of AC has been the backbone of this research programme, the reader will appreciate the overall practical scope of the study, aimed at providing a future clinical contribution to early intervention in arthritis as well as designing artificial AC replacements. Moreover, the aim of this study was to utilise a research framework encompassing all the major scientific disciplines needed to fully appreciate the multifaceted nature of AC and OA. Thus, the objectives of this study were as follows:

- To investigate the mechanical and tribological behaviour of AC using a combined numerical and experimental approach.
- To correlate the mechanical, structural and biochemical changes in damaged AC in order to better understand the pathogenesis of OA.
• To assess the performance of some of the state of the art artificial AC replacements; biocompatibility, mechanical behaviour and wear resistance are dominant factors in the design and development of such materials.
• To apply numerical techniques to patient specific knee models in order to attempt to correlate the patient’s AC wear location to region of high in-joint stresses and assess the need of advanced biphasic constitutive laws to accurately describe the AC response.

1.3 - Thesis outline

Chapter 2 provides a description of the complex structure and composition of AC. A description of the mechanical behaviour and the biotribology of the tissue is then provided. The various mechanical measurement techniques used to assess the properties of the tissue are then discussed. OA and the effects on the mechanical quality of the tissue are also explained; this is followed by a brief introduction to a promising solution to treat localised AC degeneration, namely tissue engineered AC. Also in Chapter 2, an overview of the analytical and numerical methods used to study the mechanical behaviour of AC is given. In Chapter 3, the developments of the experimental and numerical techniques used in this investigation are described.

In Chapter 4, a combined numerical and experimental study was made to investigate the frictional response and the lubricating mechanisms in AC. In Chapter 5 the mechanical properties of native, enzymatically degraded (OA-like) AC and osteoarthritic AC were evaluated, compared and discussed; this work was carried out in collaboration with the Kennedy Institute of Rheumatology and the Musculoskeletal Lab in the Department of Orthopaedic Surgery at Imperial College London. Furthermore, using the fundamental findings on AC tribology of Chapter 4, the frictional response and the evolution of damage during shear of OA-like AC was investigated.

In Chapter 6, the assessment of the performance of tissue engineered AC when subjected to shear was studied and evaluated. The findings of Chapter 4 and 5 were used as a benchmarks in the comparison with the findings of Chapter 6. In Chapter 7, some of the biphasic AC models, which have been used to study the mechanical behaviour in Chapter 5, were implemented in a patient specific tibio-femoral contact. The potential location of origination of OA was studied and the importance of implementation of biphasic models in the study of OA and damage mechanisms was evaluated. Finally, in Chapter 8 conclusions to this multi-faceted and multi-disciplinary research study and suggestions for future research to be carried out in this area were made.
Chapter 2 – Articular cartilage

2.1 - Introduction

This chapter presents the reader with the fundamentals needed to fully understand the studies carried out during this research. The chapter begins by providing a through overview of the “structure – function” relationship in AC; here, the role of the individual constituents of the tissue as well as its structure, are used to explain the unique mechanical and tribological properties of the tissue. A description of the most significant mechanical measurement techniques which are nowadays used to evaluate the tissue’s mechanical properties is then given. OA and the effect of structural degradation on the mechanical properties of the tissue is then explained followed by a brief introduction to tissue engineered AC, a promising solution for early OA and the replacement of damaged tissue. Finally, an overview of analytical and numerical techniques used to investigate the mechanical properties of AC is provided, being invaluable tools used for deepening our understanding of AC, the associated diseases and the potential treatments of the latter.

2.2 - Structure and function

The excellent mechanical and frictional properties of the tissue are dictated by the tissue’s unique and incredibly complex structure and composition, which seems to be perfectly “designed” in every aspect in order to provide such an effective and extraordinary bearing surface.

AC is constituted from a relatively low number of cells called chondrocytes, embedded in an extracellular matrix (ECM). The ECM is formed from a collagen fibre network and a non-fibrous substance made up of proteoglycans (PGs) and glycoproteins. The dry weight of normal adult human AC is formed from approximately 70% collagen and 20% PGs [9, 10]. Water is the main constituent of AC, being around 75% - 80% of the wet weight of adult human patellar AC [10, 11]. AC is surrounded by synovial fluid (SF), which acts as a lubricant in the joint. SF consists of water, hyaluronan, proteins, proteoglycans and lipids [12]. The permeability of AC is however, such that it is permeable to water and ions but impermeable to all the other constituents of the SF.

Unlike most biological tissues, AC is aneural, alymphatic and avascular although few blood-vessels may be found in proximity of the bone [4, 13]. The nutrients needed by the chondrocytes are
transported by the diffusion process of the synovial fluid through the tissue, which is encouraged by pressure gradients formed as a result of loading the tissue [4].

The combination of the avascularity of the tissue, together with the fact that the chondrocytes have a very low mitogenic potency, essentially means that AC has very low regenerative capabilities [4]. The tissue is thus prone to damage and wear throughout its lifetime. This is the main reason why OA and damage in the tissue presents a real problem to the correct functioning of the joint.

The structural macromolecules of AC (collagens, proteoglycans and glycoproteins) as well as the chondrocytes are arranged in an anisotropic and an inhomogeneous manner throughout the tissue, as will be discussed in the following section. Furthermore, the composition of AC does vary with age as well as location in the joint; studies have shown a decrease in PG aggregate size with increasing age, as well as a decrease in permeability (water content) and a decrease in collagen content [14-16].

### 2.2.1 - Zonal classification of articular cartilage

The composition of AC varies the depth from the surface; if a cross section of AC is considered, then based upon the orientation and the composition of the constituents, the tissue can be subdivided into four distinct zones [17, 18]. Figure 2.1 shows the structural arrangement of the constituents of AC as well as the different zonal classifications: the superficial tangential zone, the middle zone, the deep zone and the calcified cartilage.

![Figure 2.1 – Schematic of the cross section through articular cartilage, adapted from [19].](image)

This classification is particularly convenient for descriptive purposes [13]. A brief overview of the characteristics of the different zones is given in the following section.
2.2.1.1 - Superficial (Tangential) zone
The superficial zone is the thinnest of the four zones and accounts for 10 – 20% of the tissue’s thickness [20]. The collagen fibrils are oriented parallel to the articular surface and have a banding pattern which is specifically attributed to collagen [21]. The fibrils which exist in this zone have a smaller diameter and form a finer network than the fibrils present in the deeper zones [22].

The fine network allows the zone to sustain high tensile stresses and limit the rate of fluid exudation out of the tissue which occurs during loading and deformation; permeability is lowest in this zone [20, 23, 24]. The PG content is also lowest in this zone whilst the water content is highest reaching 85% of the total wet weight of the tissue [25]. This is true even though it is normally the PG content which dictates the water content of the tissue. However, in this particular zone the correlation is somewhat different due to the particular organization of the collagen fibre network [26]. The chondrocytes are present in the lower part of the superficial zone. Their long axes are aligned parallel to the surface and they tend to have a more ovoidal or discoidal shape with respect to chondrocytes in the lower parts of the tissue [13, 27].

The surface of the superficial zone is referred to as the surface amorphous layer (SAL), lamella splendens or surface lamina. Its existence has long been debated and studies have in the past even suggested that it could be a visual artefact of imaging or processing the tissue [28]. However, the advent of new experimental techniques, such as Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM), has made possible to verify its existence [29-31]. The SAL layer has a maximum thickness of only a few micrometres and it extends directly from the superficial tangential zone. Its composition is somewhat different from the rest of the tissue; it contains no fibrils, it is acellular and it has been suggested to contain proteins and glycoproteins, PGs, chondroitin/keratin sulphates, (phospho)lipids and/or hyaluronic acid-protein complexes [32, 33].

2.2.1.2 - Middle (Transitional) zone
The middle zone is the thickest of the four zones (40-60% of the total thickness) [24, 26]. Here, the collagen fibrils have a larger diameter than in the superficial zone and their orientation is random, thus no longer aligned with the surface [34]. With respect to the superficial zone, the chondrocytes are more spherical in shape, larger in diameter and biosynthetically more active [9]. The cell density is also much lower. The PG content is highest in this zone [35].

2.2.1.3 - Deep (Radial) zone
In the deep zone, the collagen fibrils are largest in diameter and they are predominantly aligned perpendicular to the surface of the tissue. The underlying calcified zone and subchondral bone serve as an anchoring point for the fibrils. The chondrocytes are present in groups of 4 to 8 cells called isogenous groups. These are aligned in a columnar fashion, perpendicular to the articular surface [36].

The PG content and the water content are lower than in the middle zone. The cell density is also the lowest of all the three purely hyaline cartilage zones [37].
2.2.1.4 - Calcified zone

The calcified zone, or zone of calcified cartilage (ZCC) is a calcified (mineralized) zone of AC in between the hyaline cartilage of the deep zone and the subchondral bone. It is separated from the deep zone from a thin line called the tidemark [38]. Here the cells are very low in number and the crystals of calcium salts are present in the ECM [13]. The calcified zone minimizes the stiffness gradient and integrates the structure of articular cartilage with the subchondral bone [24, 34].

2.2.2 - The constituents of articular cartilage

2.2.2.1 - Collagen

Collagen is a protein responsible for providing structural and mechanical integrity to tissues in human and animal bodies [39]. More than half of the tissue’s dry weight can be accounted by the collagen present in the ECM [20]. The collagen molecule exists as a long triple-helical structure composed of repeating amino acids such as glycine and hydroxyproline [24]. The molecules assemble together to form fibrils. A bundle of collagen fibrils is then referred to as a collagen fibre [6]. From a mechanics standpoint, as with all fibres, they are very stiff in tension but offer no resistance in compression [40, 41]. In AC, collagen fibres hold a fundamental role of supporting tensile forces as a result of tensile strains due to swelling or loading of the tissue, as will be discussed in further detail in §2.2.3.

Collagen can exist in various structural forms depending on the amino acid composition and the glycosylation. The most abundant type of collagen present in AC, representing 90-95% of the total collagen content, is type II [42]. The rest is composed of different types of collagen mainly type V, VI, IX and XI [43].

The collagen fibrils are primarily constituted from type II collagen but do contain a core formed from type XI collagen, which regulates fibril size [44]. Type IX collagen, also known as a short-chain collagen, is located along the fibril’s surface and is thought to regulate the fibril diameter [39, 45]. Human adult articular cartilage contains fibrils with a diameter of around 100-200 nm [46]. Animal fibrils are generally smaller although SEM studies have shown that bovine and porcine AC contain a collagen architecture very similar to human AC [47].

As previously discussed, the collagen fibres have a given orientation depending on the “zone” or depth of the tissue. These fibres in each of the zones are not separate entities but are actually continuous with one another. Thus, the fibres in the tangential zone are continuous with the fibres in the deep zone. The fibres originate perpendicularly from the calcified zone, to which they are attached, and run vertical towards the surface of the tissue. In the transitional zone the fibres then rotate in order to align themselves to the tissue’s surface in the tangential zone. This is known as Benninghoff’s arcade model (Figure 2.2) and it was first proposed, as a theoretical model, by Benninghoff in 1925 [48]. This model was later supported experimentally by several SEM studies [47, 49-51].
Although the collagen fibres are oriented plane to the surface of the tissue, as previously discussed, their direction about the articulating surface varies depending from the joint location. The orientation of the fibres can be identified via the use of a split line technique, first introduced by Hultkrantz [52] whereby a sharp needle, usually a dissecting needle, is dipped in India ink and inserted into the joint AC [13, 53, 54]. The reader is referred to §6.4 or an example of a split line pattern.

With the use of SEM, Jeffery proposed a theory for the split line phenomenon whereby the collagen fibrils are organised into three dimensional closely packed leaf like layers termed collagen “leaves” [50]. The collagen leaves are oriented vertically in the deep zone and then arch in the transitional zone to align themselves to the surface on the superficial zone (Figure 2.3). The fibrils in between the leaves hold the structure together.

On the superficial zone, the split lines form an elongated crack along the collagen leaves along the surface. However, the split lines do also run vertically along the leaf direction in the transitional zone of the tissue. It is thought that the split lines are a consequence of friction and compression causing interlocked stresses and maximum tensile strains supported along the leaf direction, resulting in an elongated crack along the direction of the leaf [13, 56].
2.2.2.2 - Proteoglycan

PGs are complex macromolecules consisting of a hyaluronic acid protein core with covalently bonded side chains called glycosaminoglycans (GAG), which are stabilised via a link protein [57]. Several types of PG’s exist and their differences are due to the type of GAG attached [20, 58]. AC contains three different types of GAGs; dermatan sulfate, keratin sulfate and chondroitin sulfate 4- and 6-isomers. The most abundant types of PGs are the ones containing chondroitin sulfates; these type of PGs are termed Aggrecans [22].

![Figure 2.4 – The proteoglycan structure, adapted from [24].](image)

The PGs assemble together to form a mesh in between the collagen network. The PGs can have number of roles such as providing some resistance to compressive deformation as well as supporting the extension of collagen fibres during tension [59, 60]. However, their main role is attributed to the high negative charge associated to the GAG side chains, which causes repulsion of the aggrecan molecules (this causes the brush type structure of the PG (Figure 2.4). The high negative charge of the GAG is known as a fixed charge density (FCD) [61]. This gives rise to fluid imbibition in the tissue and to a swelling behaviour caused by the Donnan effect; these are fundamental mechanisms which are responsible for resisting compressive forces when the tissue is subject to loading. This is a very important aspect of AC mechanics and will be discussed in detail in §2.3.

2.2.2.3 - Chondrocytes

Chondrocytes are cells which are responsible for creating, maintaining as well as degrading the ECM [62]. They are low in number compared to other molecules, however, they are considerably active metabolically [63]. The cells are not anchored to the ECM but exist in cavities within the ECM, termed cell lacunae [51]. When analysing the structure of AC at a cellular level, it is often done so using structural units named chondrons. These are in actual fact chondrocytes which are structurally integrated into their pericellular environment meaning individual chondrons can be released from AC.
The pericellular capsule associated with the chondrocyte can be described as a complex microenvironment containing a high concentration of PGs and a fine network of collagen fibrils [21]. It is well acknowledged that the ECM’s composition and properties are directly affected by the synthetic activity of the chondrocytes. Although the exact processes still remain unknown, the synthetic activity is influenced by external conditions and the environment surrounding the cells such as stresses, strains and chemical factors. It is thought that abnormal or excessive mechanical stimulation, such as static loading of the tissue or alterations in the joint biomechanics, could lead to degradation of the ECM as demonstrated in several experimental studies [65-69].

2.2.2.4 - Non-collagenous proteins and other molecules
Additionally to PGs and collagen fibres, non-collagenous proteins and other molecules are also present in AC. Glycoproteins and other link proteins are amongst these. Although the role of these molecules is still not well understood, it is thought that they are involved in preserving the ECM as well as in the interaction between cells and other molecules [22, 24].

2.2.2.5 - Interstitial fluid
This fluid present in the tissue is commonly known as interstitial fluid and it is essentially water containing mobile ions [13]. The distribution of this fluid in the tissue is such that 30% is found in the interfibrillar space of the collagen and the rest in the molecular pore space of the chondrocytes and the PGs [70, 71]. Water largely governs the mechanical behaviour of AC as will be described in the following section.

2.3 - Mechanical properties of articular cartilage
The mechanical properties of AC are influenced by its structural components described in §2.1. Each constituent contributes to a very specific part of the mechanical behaviour of the tissue and it is the combined effect of these that makes AC such a mechanically complex material. In this section, the influence of each constituent on the mechanical behaviour of the tissue is explained.

2.3.1 - Compressive behaviour
AC is a very unique material in that it comprises time dependent and stress dependent mechanical properties when subject to compression [72]. This is mainly a consequence of the interstitial fluid which is allowed to exude in and out of the tissue through the porous and permeable ECM, thereby varying its mechanical properties [39]. At first application of the load, the load is supported by the interstitial (hydrostatic) fluid pressurisation which builds up due to the low permeability of the ECM. At this point in time, the solid constituent of the tissue is largely preserved and the stresses on the ECM are minimal. As the load is kept on the tissue, interstitial fluid exudes out of the contact area and the hydrostatic pressure drop; a load shift to the solid constituent gradually occurs. The fluid
exudation out of the contact zone causes an additional secondary deformation phase which reaches a maximum when most of the fluid has been exuded out, and the interstitial fluid pressurisation falls to zero. At this stage the load will be supported entirely by the ECM and the solid stresses will be at a maximum. Thus, the stiffness of the tissue strongly depends upon the interstitial fluid pressurisation. The quantity of water present in AC at a given time thus determines the compressive stiffness of the tissue and it is considered one of the major determinants of its mechanical properties.

The permeability and the amount of water present in AC are related to the swelling pressure and the fluid motion under compression. The swelling pressure and fluid motion is dictated by the FCD and, not surprisingly, by the PG content. Thus, the compressive behaviour of the tissue is also influenced to a large extent by the PGs [73]. Studies have demonstrated that an increase in the density of PGs causes a reduction in water content and an increase in the compressive modulus [74, 75]. Furthermore, the static compressive properties are mainly due to the electrostatic repulsion of the GAGs, which counteract compression [76].

As one would expect, the compressive modulus of the tissue increases with depth, due to the increasing PG content and the decreasing water content [77]. The permeability however decreases with the depth of the tissue and the increasing FCD (the inverse is true for the superficial zone due close packing of collagen fibrils which resist fluid flow) [78, 79]. Interestingly, the tissue also exhibits a compression non-linearity [80-82].

### 2.3.2 - Swelling behaviour

As discussed in Section 2.1, PGs influence the swelling characteristics of the tissue through two distinct phenomena; chemical expansion and osmotic swelling, also named the Donnan effect [6]. The former is due to the tightly packed arrangement of the PGs in the ECM. This causes a repulsion force between these molecules, causing the material to swell and to draw in fluid from the surrounding areas as a consequence of a pressure difference being formed [83]. The latter is caused by a difference in ionic concentration inside and outside of the tissue. Due to the FCD of the GAG, in order for AC to achieve electro-neutrality, positive counterions must be present in the interstitial fluid of the tissue. A Donnan osmotic pressure, which is the result of the excess of ion particles in the AC compared to the surrounding fluid, causes fluid to flow into the tissue and hence swelling to occur [73, 84, 85]. The swelling of the tissue comes to a halt when an ionic balance between the ions within the solid matrix and the ions in the synovial fluid surrounding the tissue is created [86].

Complete deformation recovery is only possible if AC is submerged in fluid as it would be in the synovial joint (the fluid being SF or a saline solution containing ions) [87]. This is due to the fact that the indentation properties of the tissue are also dependent upon the ionic concentration and ionic balance between the ions within the tissue and the ions in the fluid surrounding the tissue. If AC is not submerged in a bath of fluid, water cannot enter the tissue due to swelling pressure and ionic
attraction, and, hence, a complete recovery where ionic balance is achieved is not possible [88]. Thus, in this case only a partial recovery would be possible, whereby the elastic properties of the collagen structure allow the tissue to recover some of the deformation. The collagen structure also restrains the maximum swelling [89].

2.3.3 - Tensile behaviour

The collagen fibrils are responsible for the tensile behaviour of the material. The amount of collagen fibrils present, as well as the fibril orientation and the level of collagen cross linking, are major determinants in the tensile properties of the tissue [40]. Studies have demonstrated a higher tensile modulus in the split line direction than perpendicular to the split lines as well as a decreasing tensile modulus with the tissue’s distance from the surface [40, 90]. Another important aspect is the tension nonlinearity which occurs as a result of the collagen arrangement. When deformations are small, the alignment of the fibres occurs in the direction of the loading. As strain increases, however, the tensile modulus increases due to the stretching of the cross links in between the fibres. A higher stiffness is thus shown at higher strains [24, 41, 91].

2.3.4 - Viscoelasticity

AC can be classified as a viscoelastic material as it possesses three distinct mechanical features typical of viscoelastic materials: creep, stress relaxation as well as hysteresis [39]. Accordingly, there are two distinct contributions to the viscoelastic behaviour, namely flow dependent and flow independent (or intrinsic) viscoelasticity. The former is considered the main mechanism driving creep and stress relaxation in the tissue and it is caused by the diffusional drag arising from the flow of the interstitial fluid relative to the solid matrix; this is also referred to as strain dependent permeability [72]. The reason for this being that the permeability of the ECM is not constant; this can be explained from the fact that when AC is subjected to compression, the ECM compacts restricting the flow of fluid in and out of the tissue. As the tissue’s deformation increases, the permeability decreases and the flow of fluid through the solid matrix becomes more difficult. The relationship between permeability and deformation (compressive strain) is exponential [92].

Flow independent viscoelasticity is instead caused by the intrinsic viscoelastic properties of the ECM which is mainly related to the collagen fibrils; collagen content has been shown to have a positive correlation with the magnitude of the dynamic shear modulus [70, 93].

The combination of the two mechanisms has been shown to cause a mechanical response of the tissue which is a function of both rate of loading and duration [72, 94, 95].

2.3.5 - Anisotropy

The inhomogeneity and anisotropy of the tissue is given by the variation of the PG and collagen content through the depth of the tissue. The through-depth variation of the PG content causes a variation in compressive modulus whilst the change in orientation of the collagen fibrils causes a
variation in the material stiffness [40, 82]. Furthermore, compressive and tensile mechanical properties have been shown to vary according to the joint location [96]. AC also exhibits a strong compressive and tensile nonlinearity arising from the fact that the stiffness in tension is higher than the stiffness in compression [97].

2.4 - Biotribology of articular cartilage

Even after decades of complex loading and motion regimes to which joints are subjected, the damage in healthy AC remains very small [98]. This is due to the remarkable lubricating mechanisms which occur between AC surfaces, preserving the tissue from wear and tear. Due to the biphasic nature of the tissue, recalling that it is composed of a significant interstitial fluid constituent as well as a solid component, the interaction at the contact interface between AC surfaces can be fluid-fluid, solid-fluid and solid-solid, depending on the loading conditions as well as the interstitial fluid pressurisation in the tissue [99]. Hence, several theories for the lubricating mechanisms occurring in the natural joint have been proposed. Some of the theories which have been put forward are more likely to occur than others, however, the type of lubricating mechanisms arising in the joint will strongly depend upon the loading conditions. Furthermore, due to the biphasic nature of the tissue, “standard” lubricating mechanisms as well as “biphasic” lubricating mechanisms which are unique to biphasic tissues, take place. Thus, due to these various factors, any given combination of the various lubricating mechanisms described below is likely to occur in the natural joint [100].

2.4.1 - Conventional lubrication mechanisms

These are lubrication mechanisms which are largely independent of interstitial fluid pressurisation, namely: boundary, mixed, squeeze film and hydrodynamic lubrication. In conventional bearing systems, all lubrication regimes can arise depending upon the variation of velocity and fluid viscosity. The variation in conventional lubrication mechanisms as a function of velocity and viscosity can be illustrated in a typical Strubeck curve. The reader is referred to §4.3.1 for an examples of a Strubeck curve.

In a diarthrodial joint environment, the fluid film described for mixed, hydrodynamic and squeeze-film lubrication could either originate from the synovial fluid film forming between the joint surfaces (in the classical form encountered in conventional bearing systems), or from the interstitial fluid being exuded out from the tissue under load [101].

2.4.1.1 - Boundary & mixed lubrication

Boundary lubrication occurs when the asperities of the AC surfaces come into contact with each other resulting in the entire load being supported by these asperity conjunctions. In AC, the asperities are thought to be characterized by the chemical adsorption onto the surface of glycoproteins found in the SF, such as hyaluronic acid, lubricin or phospholipids (Figure 2.5) [102-105].
2.4.1.2 - Hydrodynamic lubrication

When relative tangential motion between two non-congruent articular surfaces occurs and, if the relative speed between the two is high enough, the synovial fluid will be drawn in to the wedge shaped converging gap and generate a lifting pressure which will separate the two articular surfaces (Figure 2.6) [108]. This lubricating mechanism is associated with extremely low friction since the load is entirely supported by the hydrostatic pressure of the fluid film.

For this lubricating mechanism to occur it requires high sliding speeds, low loads, and a viscous fluid [106]. As these criteria are generally not satisfied in human joints, it is unlikely for this lubricating mechanism to occur. However, studies have suggested the possibility of an elastohydrodynamic mechanism occurring which takes into account the elastic deformation of the AC due to the high fluid film pressure compared to the low stiffness of the soft tissue [109]. This mechanisms could
theoretically occur for a short period of time in a joint subjected to a low loads and during high sliding speeds. Nonetheless, several more recent studies are against this method of lubrication occurring in the natural joint. This mechanism, furthermore, fails to account for the low friction coefficient after the joint has been subjected to rest under load, which can only be explained by the boundary lubrication mechanism [100].

2.4.1.3 - Squeeze – film lubrication
This type of lubrication mechanism is thought to occur when the two articular surfaces are loaded perpendicularly. This causes a rise in the hydrostatic pressure of the fluid film which supports the applied load (Figure 2.7) [110, 111].

![Figure 2.7 – Squeeze-film lubrication, adapted from [101].](image)

Clearly, this mechanism can only last for a very limited length of time, as the fluid film is squeezed out from the gap between the two surfaces. Thus, the film is thought to be replenished cyclically via hydrodynamic lubrication, and it is the combination of tangential and vertical motion which governs the film formation and the related fluid support [100].

2.4.2 - Biphasic lubrication modes
The lubricating mechanisms described in this section are governed by the flow of interstitial fluid into or out of the tissue and exist as a consequence of the biphasic and porous nature of AC. Two main mechanisms have been proposed to occur when the AC is loaded, namely weeping and boosted lubrication. These differ mainly with respect to the direction of the fluid flow. Furthermore, a self-generating biphasic lubrication mechanism is thought to occur when a sliding component is present. These mechanisms are explained below.

2.4.2.1 - Weeping lubrication
The weeping lubrication mechanism predicts that when AC is loaded, interstitial fluid flows from inside the tissue out towards the contact zone creating a film between the contacting surfaces and reducing solid-solid interactions (Figure 2.8) [112]. A hydrostatic pressure develops as a result of interstitial fluid pressurisation which supports the initial applied load [113].
Chapter 2 – Articular cartilage

Thus, optimum lubrication would occur when the tissue is fully pressurised and interstitial fluid is able to flow into the contact zone. In weeping lubrication, AC essentially acts as a “self-pressurised” hydrostatic bearing. This mechanism, however, has not been completely validated and theoretical and experimental studies are in favour of another lubrication mechanisms termed boosted lubrication, explained below [114-116].

2.4.2.2 - Boosted lubrication

Boosted lubrication was first proposed by Walker [117] and it differs from weeping lubrication mainly from the direction in which the fluid flows normal to the articulating surface (Figure 2.9). Boosted lubrication predicts a fluid flow into the AC upon loading of the tissue [115]. More specifically, when the tissue is loaded, because AC is permeable only to water and other small solutes as a consequence of its small pores, the water found in the synovial fluid between the contact surfaces is filtrated through and into the AC. This filtration mechanism leaves a higher concentration of hyaluronate molecules in the joint space which are thought to act as a boundary lubricant on the tissue’s surface as well as increasing the load carrying capacity of the synovial fluid due to a resulting increase in viscosity [101, 106].

2.4.2.3 - Biphasic lubrication

This lubrication mechanism emphasizes the crucial role interstitial fluid pressurisation plays in reducing the coefficient of friction and minimising the wear of AC. If a tangential component of motion is applied to a normal load, a flow pattern similar to the schematic shown in Figure 2.10 is thought to occur.
Figure 2.10 – Biphasic lubrication, adapted from [101].

During sliding, the load is supported by the interstitial fluid pressurisation which, as for the weeping lubrication model, causes interstitial fluid to flow into the contact zone, thus initially preserving the solid constituent via reducing the solid-solid interactions at the articular surface. Interstitial fluid exudation seems to occur at the leading edge of the contact, thus providing a replenishing lubricating film between the articulating surfaces. At the trailing edge, on the other hand, fluid imbibition occurs, thus re-equilibrating the proportion of interstitial fluid in the tissue. Clearly, factors such as sliding speed, sliding distance and stationary loading time on the tissue would significantly impact the interstitial fluid pressurisation of the tissue and hence the friction coefficient. A migrating contact area for example, due to a continuous replenishment of interstitial fluid, would help maintain and elevated interstitial fluid pressure in a sustainable manner and thus a low coefficient of friction [114, 118]. Similarly, a high interstitial fluid support can be maintained for sliding contact velocities higher than the diffusive velocity of the interstitial fluid [119]. Some of these examples are demonstrated numerically and experimentally in Chapter 4. Thus, it can be appreciated how a lower fluid support would essentially mean more solid-solid interactions at the surface and inevitably, a higher coefficient of friction. The decrease in interstitial fluid support would eventually lead to the entire load being supported by the solid constituent and full boundary lubrication occurring at the contact interface. Thus, as long as the interstitial fluid pressurisation remains high in the tissue, the coefficient of friction remains low. The biphasic lubrication theory has been developed based upon the biphasic model first postulated by Torzilli and Mow [72, 120, 121]. After experimental and numerical studies, it is now well acknowledged that interstitial fluid pressurisation seems to be the most significant factor governing the frictional response of AC [99, 113, 115, 118, 122-128]. Understanding the tribological and mechanical behaviour of AC is crucial both for better understanding the origin and progression of damage in the tissue and for designing artificial substitutes which should ideally behave in a similar fashion as the real counterpart.
2.5 - Mechanical measurement techniques

This section provides a description of the mechanical measurement techniques which can be used to investigate the mechanical as well as the tribological behaviour of AC.

2.5.1 - Mechanical properties

The most common mechanical test used to determine the mechanical properties of AC is the compression experiment. This test, depending upon its configuration and with the use of appropriate theoretical models (numerical-experimental techniques), allows us to extract several material parameters which can be used to assess and quantify the mechanical behaviour of the tissue.

2.5.1.1 - Testing protocols

There are two types of testing protocols which can be used in a compression experiment; the creep test and the stress relaxation test [72, 129]. Both tests allow a measurement of the time dependent mechanical response of the tissue.

In the creep test (Figure – 2.11a), a prescribed load is applied to the AC specimen almost instantaneously (although a step change in load usually is reached by the means of a ramp whose slope depends on the loading mechanism) and kept constant for a given amount of time causing the AC to deform. The displacement of the indenter (or compressive strain) which is a function of time is then monitored throughout the test. The deformation of the tissue is not instantaneous due to flow dependent and flow independent viscoelasticity which governs its time dependent nature; exudation of interstitial fluid from the tissue cannot occur instantaneously due to the limited permeability of the tissue and furthermore, the viscoelastic properties of the ECM causes a time delay in the mechanical response of the tissue [34]. It is worth noting that the displacement rate is initially very high, indicating a high fluid exudation following the applied force, but then slows approaching a constant equilibrium value [55].

The stress relaxation (Figure 2.11b) is the more common of the two tests. Here, a given vertical displacement is applied to the tissue and kept constant for a given time. In ideal conditions, the displacement would be applied as a step, however, realistically, it is usually applied as a ramp, shown by the dashed line in Figure (2.11b) [130]. The reaction force is then monitored throughout the test and a force curve typical to the one showed in Figure 2.11b is produced which indicates that following the peak stresses which form in the tissue upon the sudden application of the displacement, there is a gradual reduction in stresses until an equilibrium stress level is reached [34, 72]. As for the creep test reaching the constant displacement equilibrium, the stress relaxation phenomenon is due to flow dependent and independent viscoelastic effects.
The peak reaction force, or peak stress, which forms in the tissue upon strain application, is governed by both the interstitial fluid and the collagen network [79, 132-134]. At this point, the entire load is supported by the hydrostatic pressure of the fluid which builds up as a result of the low permeability of the tissue, thus causing a minimal elastic deformation of the tissue. The stresses are highest because of the high compressive stiffness of the tissue under high strain rates: the instantaneous response of AC is similar to an incompressible material due to the incompressible properties of the interstitial fluid [135]. The collagen network however, also restrains the compressive deformation of the tissue by resisting the high tensile stresses which form perpendicular to the applied compressive strain thus contributing to an overall increase in stress levels [134, 136, 137]. As the applied compressive strain is maintained, the interstitial fluid exudes out of the tissue causing a gradual load shift to the solid constituent of AC causing elastic deformation, as well as an overall decrease in stress levels hence the term stress relaxation. The relaxation phase is dictated by the permeability of the tissue, which is mainly controlled by the PG’s, although the collagen fibrils are also thought to play a role by restricting fluid flow [79, 134]. Gradually, after a given amount of time, an equilibrium stress level is finally reached. At this stage, the tissue will have completely relaxed and the interstitial fluid pressurisation will have dropped to a negligible amount. Thus, the load at this stage is supported entirely by the ECM. The stress levels which form at equilibrium are a result of the compressive stiffness of the tissue which is in turn dictated by the PG content [76, 134, 138].

Although viscoelasticity will be discussed in greater detail in Chapter 3, briefly, the peak reaction force from a high strain rate stress relaxation test is associated with a short term viscoelastic
response of the tissue. Studies have shown that this is controlled mainly by the fluid independent viscoelasticity and hence by the viscoelasticity of the collagen fibres [139-141]. On the other hand, the long term viscoelastic response is thought to be controlled by the biphasic nature of the tissue and hence the flow dependent viscoelasticity.

Hence, it can be appreciated how the stress relaxation test is able to gives us a deep understanding of the general mechanical behaviour of the tissue as well as allowing us to grasp the role played by each constituent in the mechanical response of the tissue.

2.5.1.2 - Testing configurations

In order to subject a specimen to axial compression, three different testing configurations can be used; unconfined compression, confined compression and indentation, shown in Figures 2.12a, 2.12b and 2.12c respectively.

**Figure 2.12** – Schematics of the (a) unconfined compression, (b) confined compression and (c) indentation tests. Adapted from [4].

Unconfined compression consists of the axial compression of a cylindrical AC sample between two rigid, impermeable platens. The sample is allowed to expand radially thus a significant amount of deformation can occur. As a result of the configuration of the test, free interstitial fluid exudation occurs only from the cylindrical sides of the tissue [34]. However, due to the geometrical non-homogeneity and the surface curvature of the samples, realistically some fluid flow will also occur from the contact interfaces. Unconfined compression is usually performed with a full thickness AC sample having no subchondral bone attached. This allows the axisymmetric problem to be modelled as a one dimension problem along the radial direction, if the plates are assumed frictionless, as the radial deformation is equal and homogenous around the sample’s circumference [34, 142]. This is however not the case if the subchondral bone is left attached and agreement between experiments and theory has not been as successful in this case [34, 143, 144]. Unconfined compression together with appropriate theoretical formulations is extensively used to obtain the Young’s modulus of the tissue [145].

In confined compression, the AC sample is placed in a confining chamber preventing radial deformation as well as fluid exudation from the sides. A permeable piston is then used to axially compress the AC sample. Fluid exudation is thus only allowed through the top surface of the AC
sample via a permeable piston. This test configuration is often used to extract the permeability as well as the aggregate modulus of the tissue [34, 72].

In indentation, the axial compression is applied using either a solid or a porous rigid plane ended indenter. This testing configuration also requires an optical determination of the Poisson ratio in order to extract the Young’s modulus of the sample [34, 146]. These experimental testing configurations allow us to obtain an output typically in terms of reaction force, contact stress, displacement and time. It is, however, only by applying suitable theoretical models to the experimental results that the material parameters can be obtained. An overview of the theoretical models will be discussed in §2.9.

2.5.2 - Tribological behaviour

Assessing the tribological behaviour of materials essentially involves the investigation of wear, friction and its associated lubrication mechanisms. There is as-yet no established standard to test the tribological behaviour of soft tissues and it is thus a decision of the researcher to adopt the most appropriate experimental configuration and testing condition.

The most common and useful experimental configuration is the use of a reciprocating rig with unidirectional sliding under a constant load. The horizontal force arising from sliding and hence the friction coefficient is monitored throughout the test and a curve of friction coefficient vs. time is produced. Appropriate interpretations of the resulting friction curve are then made in order to shed light on the tribological behaviour of the material in question. In Chapter 3, the experimental configurations of various tribological tests employed throughout this investigation are discussed.

2.6 - Osteoarthritis

Osteoarthritis (OA) is a degenerative joint condition which causes irreversible damage to the structural and mechanical integrity of AC. It is thought to affect 85% of people by the age of 55 [6, 7] and it is the primary cause of joint replacement surgery as well as being the most common cause of musculoskeletal disability and pain. This pathology seems to be correlated with ageing although several other epidemiologic factors are also thought be a cause such as obesity [147], physically demanding exercises on the joints such as squatting and heavy lifting [148] as well as joint injuries leading to instability [149]. It is worth mentioning that running, in a moderate and regular fashion, does not seem to lead to OA [150-152]. On the other hand, joint immobilization has been shown to cause alterations of AC such as softening and thinning via the loss of PGs (whilst PG synthesis and AC thickness seem to increase with degree of weight bearing) [153, 154].

We can appreciate the complexity of the situation and the narrow envelope of operation for which AC is perfectly “designed” to operate in. Research in early diagnosis of OA and understanding the physiological activities which cause OA presents a fundamental opportunity to reduce or even prevent long term joint disability [150, 155, 156].
OA can be classified as primary or secondary OA. Primary OA is referred to as OA which develops in apparently normal joints with absence of any joint degeneration (e.g. healthy ligaments and meniscii) and with no predisposing factors. It tends to develop in older adults and seems to be caused by repetitive mechanical loading on the joint. Secondary OA on the other hand develops as a cause of trauma, injury, hereditary or inflammatory factors [157].

The aetiology of OA has caused a lot of debate in the recent years as to whether it is an actual AC disease or rather, purely a result of wear and tear [7]. Over the years, it has been speculated that it is not simply an AC disease but rather a pathology affecting the entire knee hence including ligaments, pararticular muscles, bones and the menisci. It is still unclear which of these is the key component responsible for the pathology although it is generally agreed that AC remains the main ‘target’ of this pathology [150]. A problem in either of these tissue components eventually leads to unstable knee biomechanics and premature degradation and wear of AC due to increase in AC stresses or even more simply due to alterations in joint kinematics [158]. It is also thought, however, that biochemical and biomechanical variations occur in the tissue due to abnormal loads, severely decreasing the mechanical quality of the tissue and hence its ability to withstand loads in the joint [156]. The response of chondrocytes to abnormal stresses and changes in the biomechanical parameters between native and osteoarthritic AC have long been reported [65, 159-161]. Many answers regarding the nature and progression of the pathology are still unknown but what is most certain is that OA is initiated by abnormal and excessive stress distributions in the joint [6].

2.6.1 - The effects of osteoarthritis on articular cartilage

The effect of OA on AC can be characterised from an initial loss of PG’s followed by collagen fibrillation and the formation of shallow fissures on the surface (Figure 2.13) [43, 156, 157]. Eventually, at later stages of the pathology, ulceration and wear causing full AC loss occurs exposing the underlying bone.

![Figure 2.13 – Human osteoarthritic tibial plateau with an evidently damaged area of AC.](image)
Three main factors are thought to be responsible for the progression of the pathology [43]: (1) mechanical wear, (2) imbalance between degradation and synthesis of the tissue and (3) tissue remodelling via proteases (cellular enzymes causing digestion of proteins).

The chondrocytes’ homeostatic activity controls the rate of degradation and synthesis of each component of the ECM [162]. Biologically, the degradation of AC is thought to be caused from matrix degrading proteases which are activated from signalling molecules, called cytokines, which are released due to environmental signals and stimuli on the chondrocytes. The degradation causes an alteration in the function and composition of the tissue [34, 163]. More specifically, studies have identified matrix metalloproteinases (MMPs) to be the main contributors towards AC degradation, being able to degrade both PGs and collagen [164, 165]. The exact mechanism in which mechanical stimuli influence the metabolic response of the cells as well as the way in which the degradation of the different AC molecules contribute towards origination and progression of the pathology still remains unknown. However, it has been shown that higher levels of a signalling protein, called β-Catenin, triggers production of high levels of MMP-13, responsible for the breakdown of collagen type-II, and other molecules which degrade AC [166, 167].

In the early stages of OA, the surface appears healthy and no observable changes can be made. The early stage of the pathology is associated with the degradation of aggrecan, which precedes the degradation collagen [168]. This is shown by an initial loss of PGs from the superficial layer of AC [169].

The loss of these PGs, more specifically decorin and biglycan, however, eventually also affects the integrity of the collagen fibrils in the superficial zone as these PGs are responsible for the stabilization of the ECM [34, 170, 171]. Furthermore, the removal of PGs seems to promote swelling of the tissue causing the collagen to become more susceptible to mechanical degradation [43, 172]. Molecular changes in the superficial zone are thought to affect the overall mechanical behaviour of the tissue as this zone plays an important role in the compression and tensile properties of the tissue [173]. The degradation and denaturation of Type-II collagen has been shown to originate in the proximity chondrocytes, confirming the role of the cells in the degradation of the tissue. The degradation of the collagen is associated with an increase in collagenase and a type of MMP, stromelysin [174]. As the pathology progresses, the degradation of the collagen is extended into the middle and the deep zone of the tissue. Although collagen degradation is thought to be an irreversible process, aggrecan degradation is believed to be reversible in OA [175].

Degradation via mechanical erosion of the articular surfaces has been shown to produce wear particles, which promote arthritis and a particular cellular activation [43]. These wear particles originate from the subchondral bone and from the lamina splendens in late stage OA and healthy joints, respectively [43, 176-178].

Figure 2.14 illustrates a hypothetical OA origination and progression sequence.
Chapter 2 – Articular cartilage

2.6.1.1 - Effect of osteoarthritis on the mechanical behaviour of articular cartilage

OA causes a significant decline in the mechanical properties of AC. The compressive modulus has been shown to decrease considerably in osteoarthritic tissue due to loss of PGs which govern the equilibrium compressive stiffness of the tissue. The tensile modulus of the tissue decreases in osteoarthritic tissue due to the disruption of the collagen network [179, 180]. The water content and the permeability have been found to be higher in osteoarthritic AC, also due to the loss of PGs [75, 181]. This is particularly important as the higher permeability would impede effective interstitial fluid pressurisation and as a consequence, affect the sophisticated load support and lubrication mechanisms in the tissue, eventually leading to abnormal damage and wear.

2.7 - Enzymatically induced damage

The link between the mechanical and the biological events is key to better understanding the origination and progression of OA. Osteoarthritic AC undergoes very complex molecular, structural and mechanical variations compared to healthy AC. It is hence very difficult to identify which constituent of the tissue is responsible for the progression of the pathology and the decline in its mechanical quality. By using proteolytic enzymes, however, we are able to selectively degrade different constituents of the ECM as well as control the amount of degradation induced in the tissue in vitro. This procedure allows us to understand how the degradation of individual constituents affects the mechanical behaviour of the tissue, shedding light on the progression of the pathology and revealing the structure – function relationship of the various constituents [182]. Hence, enzymatic digestion can essentially be used to mimic different stages of OA by respectively degrading various

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**Figure 2.14** – Hypothetical Osteoarthritis origination and progression sequence, adapted from [22].

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proportions of PGs and collagen. AC which is subjected to this treatment is referred to as OA-like degenerated AC \textit{in vitro}.

The proteolytic enzymes can either be activated using mechanical or biochemical stimuli in living AC \textit{in vitro} using cytokines such as Interleukin-1\(\alpha\) [183], or on the other hand, the proteases can be directly applied and used to degrade the AC. The chondrocytes can be necrotic in the latter configuration since the cells do not need to be stimulated to produce the enzymes.

Whilst it is easier to degrade AC directly by applying enzymes rather than stimulating living chondrocytes, due to the difficulties involved in manipulating living tissue, the main disadvantage with the direct application of enzymes is that the degradation of the tissue will take place mainly on the surface of the tissue leaving the internal part of the tissue largely unaffected from enzymatic degradation, as demonstrated from experimental studies [184, 185]. This results in a non-uniform degradation with a through thickness structure comprising both degraded and healthy tissue. Cell stimulation on the other hand causes the enzymes to be produced directly within the tissue causing a more uniform degradation.

The proteolytic enzymes which are most suitable to degrade AC \textit{in vitro} via direct application are MMP-1 and trypsin. MMP-1 is in fact part of the proteases responsible for the degradation of AC during OA, when an imbalance between synthetic and degradative processes exists; trypsin causes PG depletion without affecting the collagen in the tissue whilst MMP-1 on the other hand depletes the PGs as well as cleaving the collagen network. It is thus impossible to degrade the collagen without affecting the PGs. Osteoarthritic AC at advanced stages of the pathology can readily be obtained from patients undergoing knee and hip arthroplasty. It is however extremely difficult to obtain early OA and our understanding of the variation of the mechanical and tribological properties at an early stage is thus very limited. In this respect, OA-like AC degenerated \textit{in vitro} is particularly useful to investigate the mechanical properties of AC at an early onset of structural damage.

2.7.1 - Effect of enzymatic digestion on the mechanical behaviour of articular cartilage

Although enzymatic digestion allows us to selectively degrade the ECM, because of the complex interrelations between each constituent and the mechanical properties of the tissue, when degrading a single constituent it will consequently affect more than one mechanical property. Experimental studies have demonstrated that the depletion of PGs, as well as causing a decrease in the equilibrium compressive modulus, causes an increase in water content and an increase in permeability [34, 90, 186, 187]. However, it is also thought that collagen degradation in the superficial zone might also be responsible for an increased permeability due to the loss of tensile stiffness and thus loss of the tightly packed fibre network which would otherwise contribute in restraining fluid flow [75]. PG depletion does not seem to significantly alter the instantaneous compressive response of the tissue although it does seem to be reduced with collagen degradation [188, 189]. In terms of compressive response,
studies have concluded that PGs are mainly responsible for the equilibrium compressive modulus and the collagen for the instantaneous response [190].

2.8 - Tissue engineered articular cartilage

AC degeneration can exist in the form of lesions and defects which are sometimes caused by trauma, disease or congenital abnormalities. These lesions can severely affect the quality of life resulting in significant joint impairment. Due to the tissue’s limited repair and regeneration ability, AC defects have to be treated surgically via AC repair techniques. Such procedures include AC debridement, microfracture, osteochondral autografting and cell implantation based techniques such as autologous chondrocyte implantation (ACI) and matrix-induced autologous chondrocyte implantation (MACI) [191]. The correlation between AC defects and early OA as well as whether defects may increase the risk of OA is still at present unclear [192]. However, studies have suggested that ACI and MACI techniques could be used to treat degenerative defects as a result of OA in elderly patients, as well as young people with traumatic defects, for which these techniques are otherwise indicated [193].

The ACI technique consists of the implantation of healthy autologous cultured chondrocytes directly onto a chondral defect and using a “periosteal patch” to seal the defect keeping the new AC chondrocytes in place. A significant improvement of the ACI is the MACI technique.

In the MACI technique, autologous chondrocytes are cultured directly onto a commercially available scaffold or fibre based membrane which is then implemented directly onto the defect. This procedure enhances cellular attachment, distribution and eliminates several complications (periosteal flap harvest and implantation) while generating more predictable cell-material interactions [194-197]. The success of MACI, however, necessitates an optimal design in terms of mechanical, tribological and structural properties of the underlying scaffold.

Figure 2.15 below, shows an illustration of the MACI technique.

Figure 2.15 – Schematic of the MACI procedure [198].
From Figure 2.15, (1) autologous chondrocytes are explanted from the joint, (2) increased in number through proliferation and screened for phenotype, (3) seeded onto a scaffold with growth factors, (4) cultured to increase cell number and finally (6) implanted onto the defect.

2.9 - Analytical and finite element modelling of articular cartilage

2.9.1 - Overview
In order to investigate the mechanical behaviour of AC it is common to perform in vivo and in vitro experiments on the tissue. These however, do not give us a complete picture of the response of the tissue due to the limitations associated with such experiments. Determining the mechanical behaviour via an in vivo experiment is clearly very difficult as well as having some considerable limitations as the tests have to be done directly on the patient. An in vitro experiment unfortunately cannot provide us with a full spectrum of the stress-strain states of the tissue when subjected to loads or displacements as it would do in the intact joint [39]. This is mainly because the artificial boundary conditions are different from the boundary conditions (BCs) which occur in the natural joint [199]. Theoretical modelling allows us to approach the problem from a different perspective and can be complementary to experimental studies. Theoretical modelling of a given problem is essential in order to better understand, facilitate and explain experimental research. Advanced and sophisticated theoretical modelling, corroborated by significant experimental results, provides an extremely powerful tool which can ultimately be used to predict the outcome of a given problem or scenario. The use of advanced numerical techniques is deemed crucial in evaluating analytical models. Numerical techniques such as Finite Element Modelling (FEM) are essential in order to model AC as analytical solutions can only provide us with simple and sometimes non realistic models [200].

2.9.2 - Analytical models
AC is a very complex material to model because of its biphasic nature and its sophisticated structure and mechanical properties. When modelling the tissue one must take into account the ECM as well as the fluid component. Over the past years, several analytical models aimed at describing the mechanical response of the tissue under different loading conditions have been put forward [201, 202]. These range from the relatively simple biphasic models to more advanced models considering more or less explicitly all the main components of the tissue; among them, the biphasic poroviscoelastic model (BPVE) [203, 204], transversely isotropic model (TI) [205], the fibril reinforced models [206-208] and the triphasic models [83, 209, 210]. In order to analyse the more salient features of the tissue and attempt to model the mechanical behaviour as close as possible to the real material, more complex and detailed material description and constitutive behaviour is needed. This section provides a brief description of different AC models used throughout this investigation.
2.9.2.1 - Monophasic model

The monophasic (M) model is the simplest model which can be used to model AC. It is rarely used in AC modelling as it does not take into account the biphasic nature of the tissue. However, it is still employed for modelling the tissue in two circumstances; the first is for 3D complex geometries such as modelling AC in 3D joint contact FEMs where reducing computational expense is a requirement [158, 211-218]. The second scenario for which this model can be used is for instantaneous load applications, which for example could occur in human joints. In the monophasic model, AC is modelled as a single solid phase which is considered as linear elastic, homogenous, isotropic and almost incompressible. The model is thus characterised by only two mechanical parameters: the Young’s modulus and the Poisson’s ratio. In particular, instantaneous (i.e. dynamic) values of these two parameters are employed to represent the constitutive behaviour of the material; these are termed \( E_{\text{ins}} \) and \( v_{\text{ins}} \) respectively. The instantaneous Young’s modulus is a fictitious material parameter as it is not a true measure of the stiffness of the solid constituent of AC. It is extrapolated from indentation or compression curves obtained instantaneously after the load application on the AC and takes into account both the response of the solid and the fluid constituent. Consequently it accounts for the stiffness changes induced by the interstitial fluid pressurisation of AC and increases with the strain rate. If enough time is allowed, however, equilibrium conditions are reached and \( E_{\text{ins}} \) and \( v_{\text{ins}} \) correspond to the standard solid matrix parameters, \( E \) and \( v \), which for convenience we can also refer to as \( E_{\text{eq}} \) and \( v_{\text{eq}} \). simply to highlight the equilibrium rather than instantaneous conditions. The monophasic model does not provide a true representation of the AC behaviour as it does not in any way predict the transient response of the tissue due to load partitioning. However, this model is actually able to give a realistic response at instantaneous load applications. The reason for this being that due to the low permeability of AC, at high strain rates the biphasic response of the tissue cannot occur as interstitial fluid cannot instantaneously exude. This causes the apparent stiffness of AC or the instantaneous compressive modulus to appear higher due to the increase of hydrostatic pressure.

2.9.2.2 - Biphasic linear elastic model

The biphasic linear elastic (BLE) model is the most basic biphasic model, initially developed by Torzilli and Mow [72, 120, 121]. It is the backbone and building block of all the more advanced AC models. The tissue is assumed to be constituted by a linear elastic, homogenous, isotropic and compressible solid phase hydrated by an incompressible fluid phase.

From the biphasic theory [72], the total stress in the tissue is given by the sum of the solid stresses and the hydrostatic pressure [6]:

\[
\sigma_{\text{tot}} = \sigma_E - pI
\]  

(2.1)
Where $\sigma_{\text{tot}}$ is the total stress tensor, $\sigma_E$ the effective stress tensor and $p$ and $I$ the hydrostatic fluid pressure and the unit tensor respectively. By assuming a linear elastic isotropic material, the effective stress tensor is given by:

$$\sigma_E = \lambda e_s I + 2\mu \varepsilon$$  \hspace{1cm} (2.2)

Where $\lambda$ and $\mu$ are Lamé’s constants, $\varepsilon$ the strain tensor and $e_s$ the cubic dilation. Lamé’s constants as a function of the Young’s modulus $E$ and the Poisson ratio $\nu$ take the following form:

$$\lambda = \frac{\nu E}{(1+\nu)(1-2\nu)}$$  \hspace{1cm} (2.3)

$$\mu = \frac{E}{2(1+\nu)}$$  \hspace{1cm} (2.4)

The solid-fluid interaction in the model is described by Darcy’s law [219]. If the mass exchange through the tissue is zero, the conservation of mass takes the following form [6, 220]:

$$\nabla \cdot \vec{v}_f + \nabla \cdot \left( n_f (\vec{v}_f - \vec{v}_s) \right) = 0$$  \hspace{1cm} (2.5)

Where $\vec{v}_f$ and $\vec{v}_s$ are the velocities of the fluid and solid phases respectively and $n_f$ is the fluid interaction. The relation of the fluid flux to the hydrostatic pressure is dictated by Darcy’s law:

$$n_f (\vec{v}_f - \vec{v}_s) = -k \nabla p$$  \hspace{1cm} (2.6)

Where $k$ is the hydraulic permeability and the expression $n_f (\vec{v}_f - \vec{v}_s)$ represents the fluid flow through the surface of the tissue. By substituting Equation (2.6) in Equation (2.5), the fluid phase can be described by:

$$\nabla \cdot \vec{v}_f + \nabla \cdot (k \nabla p) = 0$$  \hspace{1cm} (2.7)

The permeability is assumed to be constant (to which the linearity of the model is due), hence independent of the void ratio, which actually varies with the elastic deformation of the solid constituent. The biphasic model uses the Young’s modulus evaluated at equilibrium, $E_{eq}$, i.e. extrapolated from the stress-strain curves characterizing the equilibrium mechanical response after total relaxation of the tissue [142].

The BLE model is satisfactory if a crude and general approximation of the tissue’s mechanical behaviour is deemed as acceptable as it captures the main characteristics of the tissue’s response due to its biphasic nature. Moreover, it is fairly simple to implement using FEM and yet not too computationally expensive. Although capable of dealing with the long term creep and stress
relaxation of AC during confined compression, this model shows non negligible deviations from the tissue’s experimental response when applied to unconfined compression tests, failing to account for the high levels of stress relaxation associated with this test, mainly due to the fact that the anisotropy of the tissue is neglected [206]. As a consequence, the BLE model tends to underestimate the stresses in the tissue which might constitute a severe limitation when investigating damage initiation [142, 221].

2.9.2.3 - Biphasic non-linear elastic model

The biphasic non-linear elastic (BNLE) model combines the BLE model with a non-linear permeability law, thus reproducing the flow dependent viscoelasticity of the tissue. The non-linearity is thus implemented within Darcy’s law and it is not a result of the non-linearity resulting from the solid constituents of the tissue. Indeed, the permeability is not constant but depends on the level of strain applied. When the tissue undergoes deformation, the porous elastic matrix deforms, thus changing the pore’s conformation and size and reducing the porous matrix void ratio; permeability thus decreases with increasing strain. Lai et al. [222] first proposed the non-linear law, which defines the permeability as a function of the void ratio and is suitable to model small strains. This law was revisited by Van der Voet and co-workers [223], assuming the following form:

\[
    k = k_0 \left( \frac{1+e}{1+e_0} \right)^M
\]

Where \( k_0 \) represents the initial permeability, \( M \) is a material parameter called the permeability coefficient and \( e \) and \( e_0 \) are the initial and current void ratio, respectively.

It is important to note that the non linearity included in this model and in all other models described in this study which are referred to using the acronym NL for “non-linear”, is purely due to the non-linear permeability law and not due to the tension-compression non-linearity which some AC models have accounted for. Such models include the Conewise Linear Elasticity (CLE) model [97] and the fibril reinforced model [206, 224] which present varying mechanical properties depending on whether the tissue is subject to tension or compression. These models have not been discussed in this thesis, however, the author has employed the fibril reinforced model in some of his earlier work [201].

2.9.2.4 - Biphasic transversely isotropic model

AC is a highly anisotropic material, although implementing full anisotropy analytically does not constitute an easy task. However, as the material response is thought to be affected by the collagen fibre orientation, modelling the tissue as TI is sought to be an acceptable assumption when modelling AC without explicitly accounting for the presence of the collagen network. Such an interpretation of the characteristic behaviour of the tissue was proposed in the biphasic transversely isotropic (BTI) model developed by Cohen and co-workers [205]. Transverse isotropy is a particular case of orthotropy and can be regarded as the invariance of material properties for rotations about a given axis.
of symmetry [225]. In AC, the plane of isotropy is thought to be oriented with the superficial collagen fibrils, which run parallel in the same direction. By assuming all the fibrils to be oriented in this way, the stresses in the tissue can be obtained by manipulating the elastic stiffness matrix to account for the planar isotropy. By invoking symmetries, it can be shown that only five independent material parameters are required in order to characterize the solid constituent of the model, as demonstrated by the solid stress matrix below, assuming fibres to be aligned in the 3rd direction [6].

\[
\begin{bmatrix}
\sigma_{11} \\
\sigma_{22} \\
\sigma_{33} \\
\sigma_{12} \\
\sigma_{13} \\
\sigma_{23}
\end{bmatrix} = \begin{bmatrix}
\frac{1}{E_1} & -\frac{v_{21}}{E_2} & -\frac{v_{31}}{E_3} & 0 & 0 & 0 \\
-\frac{v_{12}}{E_1} & \frac{1}{E_2} & -\frac{v_{32}}{E_3} & 0 & 0 & 0 \\
-\frac{v_{13}}{E_1} & -\frac{v_{23}}{E_2} & \frac{1}{E_3} & 0 & 0 & 0 \\
0 & 0 & 0 & \frac{1}{G_{12}} & 0 & 0 \\
0 & 0 & 0 & 0 & \frac{1}{G_{13}} & 0 \\
0 & 0 & 0 & 0 & 0 & \frac{1}{G_{23}}
\end{bmatrix}^{-1} \begin{bmatrix}
\epsilon_{11} \\
\epsilon_{22} \\
\epsilon_{33} \\
\gamma_{12} \\
\gamma_{13} \\
\gamma_{23}
\end{bmatrix}
\]

The 5 different material parameters are:

1) \( E_1 = E_2 \)

2) \( E_3 \)

3) \( v_{12} = v_{21} = v_{23} = v_{32} \)

4) \( v_{13} \)

5) \( G_{13} = G_{23} \)

where \( G \) is the shear modulus.

\( G_{12} \) can be calculated using; \( G_{12} = \frac{E_1}{2(1+v_{12})} \)

With regards to the fluid component, two permeability coefficients are required (in and out of plane). A total of 7 material parameters are thus required for the BTI model [144].

This model, as shown using direct comparison with experimental data in several studies [140], is capable of capturing both lateral displacements and reaction forces produced by an unconfined compression test, but it does not capture the evolution of these two variables simultaneously. This is probably caused by the absence of the viscoelasticity of the solid matrix which is included in the Biphasic Poroviscoelastic (BPVE) model. However, transverse isotropy is able to capture tension-compression non-linearity which the BPVE is not able to account for [226]. The inclusion of non linear permeability in the BTI model offers an improvement in curve fitting the
Chapter 2 – Articular cartilage

2.9.2.5 - Biphasic poroviscoelastic model

The biphasic poroviscoelastic non linear (BPVE) model takes into account both the flow dependent and the flow independent viscoelasticity, due to the viscoelastic nature of the ECM. The BPVE model was first analytically formulated by Mak [203]. It has been formulated using two different methods, namely the continuous [228] and the discrete [204] spectrum algorithms. The continuous spectrum, proposed by Fung [228], uses an integral model to represent viscoelasticity which is governed by a single relaxation function, $G(t)$. Its reduced relaxation function takes the following form [228]:

$$G(t) = \left[1 + c \left(E_1 \left(\frac{t}{\tau_s}\right) - E_1 \left(\frac{t}{\tau_l}\right)\right)\right] \left[1 + c \ln \left(\frac{\tau_l}{\tau_s}\right)\right]^{-1}$$

Where $c, \tau_s$, and $\tau_l$ are the magnitude of the relaxation power spectrum and the short and long term relaxation time respectively. $E_1$ is the exponential integral function defined by:

$$E_1(z) = \int_z^\infty \frac{e^{-t}}{t} \, dt \quad (|\text{arg } z| < \pi) \quad (2.11)$$

The continuous function provides a more descriptive differential representation of the linear viscoelasticity law than the discrete spectrum which is to be considered as an approximation [229]. However, the discrete spectrum algorithm, which is derived from a combination of Kelvin’s viscoelastic discrete models does save significant CPU time and memory compared to the continuous spectrum algorithm [204]. Furthermore, a parametric analysis of the BPVE model using the continuous function is relatively complex [204]. A slight divergence in predicting creep and stress relaxation of AC between the two algorithms exists, however, it is very small and the overall tissue behaviour is still well described. Hence the discrete spectrum is more widely used to model the viscoelastic behaviour. The discrete spectrum algorithm describes viscoelastic properties using the reduced relaxation function $G(t)$ written as a series of combinations of discrete relaxations function $G_i$ [204]:

$$G(t) = G_\infty + \sum_{i=0}^{N_d} G_i e^{-t/\tau_i}$$

Where $\tau_i$ is the discrete relaxation time constant. The mechanical response of AC following a rapid applied compressive strain is constituted from a rapid short term stress relaxation and a slower long term relaxation [230]. In order to describe such behaviour, two time constants should be adopted in defining the material characteristic. Previous studies have demonstrated that by using three relaxation time constants on a uniform decadic interval of logarithm base 10, results of discrete and continuous spectrum viscoelasticity are very similar [204]. Three discrete viscoelastic relaxation time constants...
have been assumed ($\tau_i$) which can be considered to be equally distributed with a logarithmic interval between the short term ($\tau_s$) and long term ($\tau_l$) relaxation time constants. Thus;

\[ \tau_1 = \tau_s \]  
\[ \tau_3 = \tau_l \]  
\[ \log \tau_2 = \frac{\log \tau_1 + \log \tau_3}{2} \]  

From Suh and Bai [204], $G_\infty = 1$ and it can be assumed, $G_i = \tilde{G}$ where $\tilde{G}$ is a constant named the discrete spectrum magnitude and it is equal to:

\[ \tilde{G} = \frac{G_0 - 1}{N_d + 1} \]  

Where;

\[ G_0 = G(0) \]  

And;

\[ N_d = \log \left( \frac{\tau_l}{\tau_s} \right) \]  

It is worth noting that 3 additional parameters are necessary to characterize the BPVE model with respect to the standard biphasic model, namely $\tilde{G}$, $\tau_s$ and $\tau_l$.

The BPVE model is capable of representing the tissue’s response and reproduce the experimental data obtained using confined and unconfined compression, and indentation tests. In particular, it can predict simultaneously lateral displacements and reaction forces during unconfined compression tests [139], also for different strain rates [140]. Although this model does include the fundamental characteristic of the flow-independent viscoelasticity, it does not capture anisotropy and the compression-tension non linearity of the tissue which is captured by the TI model.

### 2.9.3 - Finite element modelling

FEM is a numerical technique which allows the implementation of analytical models in complex geometries and loading configurations. Since the first use of FEM to simulate the biphasic nature of AC and practical joint contact problems [200, 231], FEM has revealed itself as being an invaluable tool for better understanding the complex behaviour of AC. With the increase of computational power, FEM has been used to simulate clinically relevant muscoskeletal problems by simulating realistic 3D human joint contact models and loading configurations [232, 233]. The decision as to which AC model to adopt for a given problem really depends upon the configuration, the geometry, the loading
conditions and the accuracy required from the simulation. Furthermore, one must take into account the computational expense of the model when solving the problem numerically, which increases with material and model complexity. Thus, the material description of the tissue is strongly dependent upon the research in question [6].

2.9.3.1 - Iterative finite element approach
The advances of constitutive models used to describe the mechanical behaviour of AC have allowed numerical – experimental methods, also called the iterative Finite Element (FE) approach, to become a valuable tool to quantify the mechanical behaviour of AC [4]. This technique involves using FEM to simulate an experiment using exactly the same geometry, BCs and testing conditions. The results obtained from the simulation are then compared to the experimental results, and, depending upon the type of model employed to model the tissue, the various mechanical parameters are varied until a satisfactory match between experiment and model is obtained. This technique requires an initial guess of mechanical parameters for the FEM as well as a geometrically and mechanically simple experiment so that it can be modelled without any convergence problems. For this reason, standard compression and tension experiments are routinely used in this procedure. The technique is illustrated in Figure 2.16.

![Figure 2.16 – Illustration of the Iterative Finite Element Approach, adapted from [4]](image)

2.10 - Conclusions
In this chapter, several aspects of AC ranging from the structural description of the tissue to the analytical modelling of its mechanical response have been illustrated. It is only by thoroughly understanding the entwinements between the biological and the engineering principles that a successful investigation into osteoarthritis and tissue engineered alternatives can really be made. Having provided the reader with the necessary foundations to comprehend such a study, the experimental and numerical techniques which have been used in this research investigation will now be described in the following chapter.
Chapter 3 – Development of experimental and numerical techniques

3.1 - Introduction

This section describes the experimental techniques and the testing protocols and procedures used throughout this research. The experimental techniques have been chosen and designed in order to assess various properties of the tissue: mechanical, tribological, and biochemical properties as well as surface topography. This allows us to obtain a complete characterisation of the tissue which is essential in order to have a complete understanding of the healthy and damaged tissue’s response under different loading scenarios.

3.2 - Extraction of animal articular cartilage

An experimental protocol to test porcine AC, as a good alternative to direct testing of human AC, was developed and will be described in this section. The reason for choosing to use animal AC for most of the experimental procedures in this investigation is due to the fact that healthy animal AC can be readily obtained unlike human AC.

Healthy adult porcine (6 – 8 months old) knee joints were obtained from a local butcher 24 hours after slaughter and, if not dissected immediately, were either refrigerated for a maximum of 24 hours or frozen at -42 °C in a PBS bath. Using a surgical scalpel, the tissue around the knee joint was carefully removed, exposing the patella, followed by the menisci, in order to expose the underlying cartilage covering the tibial plateau and the femoral condyles. Using a surgical coring device, osteochondral plugs of 10 mm diameter were removed from the various load bearing regions of the knee joint (Figures 3.1a and b) and using a surgical scalpel, full thickness AC was carefully removed from the underlying subchondral bone.
Prior to testing, the AC plugs were irrigated with Phosphate Buffered Saline (PBS) in order to prevent dehydration of the tissue. The AC plugs were either tested immediately or after they had been unfrozen from -42 °C; it should be noted that freezing is not thought to alter neither the mechanical properties nor the friction coefficient of the tissue [90, 234, 235].

**3.3 - Extraction of human articular cartilage**

Human osteochondral plugs were immediately extracted from the tibial plateau of patients which was removed following total knee replacement (TKR) surgery, as a consequence of advanced OA. The AC extraction procedure used was the same as procedure used for porcine AC described above. A typical human tibial plateau and an osteochondral plug are shown in Figure 3.2.

Clearly, the AC extracted represents osteoarthritic AC. Healthy, or visually healthy AC, could exist in the regions covered by the meniscus, as shown from the upper portion of the tibial plateau in Figure 3.2.
3.2b (denoted by an arrow). However, the AC extracted from such patients cannot be assumed to be healthy as mechanical and biochemical variations with no apparent morphologic changes could exist.

### 3.4 - Design and development of the multiaxial compression and shear testing rig

#### 3.4.1 - Overview

The Multiaxial Compression and Shear Testing Rig (MCSTR) has been designed in order to test AC or its artificial counterpart, namely tissue engineered AC, using a variety of compression and shear testing conditions. The rig, which was developed with the support of Dr Richard Wayte, consists of two fully controllable and independent loading axes in the vertical and the horizontal direction. This allows testing of the sample in both compression and shear. A schematic of the front view of the rig is shown in Figure 3.3.

![Figure 3.3 – Schematic of the Multiaxial Compression and Shear Testing Rig.](image)

Both axes include a piezoelectric load cell and a Linear Variable Differential Transformer (LVDT), allowing measurement of force and displacement respectively. The horizontal and vertical movement is driven by the action of two solenoids. The horizontal solenoid is damped by a viscous damper, silicon oil, in order to avoid instability during shear. The solenoids return to the “home” position.
upon power removal via the action of gravity in the case of the vertical solenoid and via the action of a spring pulling the horizontal slide back for the horizontal solenoid. The rig configuration is such that the upper specimen is fixed in place whilst the lower specimen is allowed to move vertically and horizontally. The rig was designed in order to test various different sample geometries, contact pairs and sizes. The shape and the surface of the upper specimen is also fully customisable allowing for different contact interfaces to be tested, e.g. a plate (for compression and wear tests), a spherical contact (for frictional tests), or another AC sample.

The AC specimen is placed in the sample holder and it is fully immersed in a solution, usually of Phosphate Buffered Saline (PBS). A standard sample size of 10 mm has been utilised throughout this investigation. This size allows a global response of the tissue to be assessed both in compression and shear, as well as providing ease of manipulation. Different samples holders with minor modification were used for compression and shear tests. The sample holder used for the shear test contains a 3 mm deep and 10 mm diameter recess where the sample is placed. Depending upon the height of the AC plug, inserts were placed between the sample and the holder in order to raise the sample from the recess thus making it viable for shear testing. The sample holder used for unconfined compression tests does not contain any recess and consists of a flat homogenous surface, in line with unconfined compression test technique BCs.

In order to control the MCSTR, an appropriate electrical circuit combined with a Data Acquisition System (DAQ) was developed. The electrical control circuits for the vertical and horizontal directions are illustrated in Figure 3.4.

**Figure 3.4** – Schematic of the (a) vertical and (b) horizontal electrical control circuit.

The rig is controlled from the computer using an *ad hoc* designed Labview control system. Signal sampling is made using the DAQ which is adopted to interface the computer and the rig.

The rig has been designed such that its optimal mode of operation is in the physiological regime corresponding to physiological contact pressures and frequencies occurring in the natural knee and hip joint.
The specifications of the rig are the following:

- Operational load frequency for vertical and horizontal directions: 0.1 – 5Hz
- Maximum vertical applied load: 100N
- Resolution of vertical and horizontal transducers over operational voltage range: ± 1mV

The design of the rig was accompanied by a structural and modal analysis using FEM. This ensured an optimal rig design and a good stability during operation with the aim of avoiding any possible disturbances which could potentially affect the accuracy of the data acquired from the experimental tests. Stresses and strains in the structure were investigated past the operating envelope of the rig, yielding a negligible elastic deformation of the structure. The fundamental natural frequency was also found to be 10.4 Hz, well above the operating frequency of the rig (Figure 3.5). The structure and the BCs were such that the vertical supporting plates were modelled as built-in between the top and bottom plates. The bottom plate was also modelled as built-in.

![Figure 3.5 – Mode Shape of the fundamental frequency of the rig’s structure.](image)

The rig and the control box are shown in Figure 3.6.

![Figure 3.6 – The rig and the respective control box.](image)
Typical tests performed by the MCSTR are unconfined compression stress, relaxation tests as well as shear tests. A description of how these tests are performed using the rig is provided below.

### 3.4.2 - Unconfined compression – stress relaxation test

The unconfined compression test was performed using the technique described in Chapter 2. Full thickness AC samples were removed from 10 mm osteochondral plugs using a scalpel. The thickness of the AC was then measured using a digital vernier caliper, by measuring the thickness at 5 different locations around the sample’s circumference and then taking an average. The AC sample was then placed in the sample holder and immersed in PBS. The sample holder was then gradually raised, until contact with the AC sample and the fixed upper specimen was made. The upper specimen for the unconfined compression test consisted of a steel plate as specified in the unconfined compression technique. Contact between the tissue and the metal plate was associated with a small peak increase in the compressive reaction force measured by the load cell, which was of the order of around 0.1N or lower. Once contact was established, the AC sample was left to equilibrate for 15 min. This was also considered to be the “zero” displacement position.

For this type of test the applied vertical strain in terms of vertical displacement, the desired ramp time for applying the chosen displacement followed by the total length of the test was accurately chosen and provided as inputs to the rig via the Labview control software. The test was then commenced and the variation of reaction force was measured and recorded at intervals of 0.05s. Once the test terminated, the sample was left to equilibrate in PBS for at least 1hour before retesting the same sample. The samples could indeed be retested in compression so long as the applied strain is not excessively high to cause any damage to the tissue and if enough time was allowed for the AC to rehydrate following the test; this was taken to be 60 minutes. The stress relaxation test was chosen as the preferred type of compression test due to the considerable amount of literature data available for comparison and validation of the results obtained using the rig, as well as the relative simplicity of the test.

### 3.4.3 - Shear testing

For shear testing, a modified sample holder was utilized having a 10 mm diameter and 3 mm deep recess which was used to press fit part of the sample in place and provide a lateral constrain during shear. If needed, the depth of the recess could be varied by inserting a thin ultra high molecular weight polyethylene (UHMWPE) disc of variable thickness to bring the surface to the required height and avoid contact between the upper specimen and the sample holder. An illustration of the cross section of the AC sample in the sample holder (without employing the UHMWPE disc) is shown in Figure 3.7.
Once the stroke length, frequency and load had been chosen, the shear test was carried out by loading the AC sample against the upper specimen and powering the horizontal solenoid which moved the tray in a reciprocating action using a sinusoidal output signal from the control software. The upper specimen used was the same as for the unconfined compression test. It consisted of a steel plate with a surface roughness $R_a = 1 \, \mu m$. The friction coefficient was averaged over a variable time period, usually taken to be equal to 0.1s, and written to a data file every second. An example of a representative AC shear test data file is shown in Table 3.1. The data represents a 10s portion approximately 40s following the start of the test.
The data above was recorded using the horizontal and vertical displacement and force transducers. The “average” and “amplitude” refer to the sinusoidal output signal. In order to extract the friction coefficient from the above data, the “Vertical Force Average” was converted into N by means of a simple (Volt/Newton) calibration which had been previously carried out for the rig. The weight of the “horizontal slide” on the load cell (see Figure 3.3) was then subtracted in order to yield the exact applied constant vertical force on the sample. This was done in order to verify that the input vertical load originally prescribed had been accurately applied to the sample. The “Horizontal Force Amplitude” resulting from running the machine with no vertical load applied was recorded prior to commencing the shear test. This was done in order to obtain a measure of friction loss associated with the MCSTR. This value was then subtracted from the “Horizontal Force Amplitude” recorded during the test. This value was then divided by 2 to yield the semi-amplitude which was then converted into N. Finally, Equation (3.1) was then applied to every data line in order to obtain friction as a function of time for every second.

\[
\mu = \frac{F_T}{F_N} \tag{3.1}
\]

Where \(F_T\) and \(F_N\) is the tangential and normal force to the surfaces respectively.
3.5 - Development of high frequency testing rig experimental protocol

Shear testing of AC was also performed using a different shear testing rig, namely a High Frequency Reciprocating Rig (HFRR) (PCS Instruments, London, U.K. – Figure 3.8). The HFRR allowed testing under different configurations and conditions with respect to the MCSTR. The HFRR allows higher testing frequencies, meaning the sample is subject to more cycles and more mechanical damage in a given amount of time. Although the HFRR's conditions are less "physiological", it does provide valuable information on the tribological performance of the sample in question.

![Figure 3.9 – Schematic of the HFRR.](image)

The HFRR consists of an electromagnetic vibrator which oscillates an upper specimen against a fixed lower specimen under controlled tribological and physiological conditions [236]. The two specimens used for the HFRR tests presented in this thesis are a 6 mm diameter steel ball (surface roughness $R_a = 0.05 \, \mu m$) and the 10 mm AC disc respectively. The AC is placed in the sample holder and is immersed in PBS (Figure 3.9).

![Figure 3.10 – AC placed in the HFRR sample holder.](image)

Similarly to the MCSTR shear test, since the customary thickness of the AC plugs is 1.0 - 3.0 mm, a UHMWPE disc of variable thickness was inserted under the AC sample to bring the surface up to the required height. A mechanical fixation method was used, whereby the AC sample was simply held in the holder via screws (Figure 3.10).
A constant normal load was applied to the specimen throughout the test using a dead weight. In order to mechanically test the AC under physiological loads, an average peak contact pressure of 1.6 MPa had to be achieved in the steel ball/AC contact, which is representative of contact pressures in the human knee joint [237]. In order to estimate the load needed to achieve the physiological contact pressures in the HFRR test, a FE BNLE AC model was used to establish the maximum contact pressure and corresponding contact width\(^1\) [238]. This will be discussed in more detail in Chapter 4. A load of 600g was found to correspond to an average contact pressure in the lower range of the knee physiological contact pressures, ~1.6 MPa. This value was also verified experimentally using Fujifilm Prescale contact film, which showed contact pressures in the range of 1.2 – 1.8 MPa; in this case, the contact pressure was obtained visually using a colour code.

The sample holder temperature was controlled and set at 37°C for the duration of the test. The stroke length and frequency was varied accordingly. The stroke lengths and the frequency range available in the HFRR are 20 \(\mu\)m – 2 mm and 10 Hz – 200 Hz respectively. Tests of different durations were performed and for the entire duration of the test, the friction was measured continuously using the centre line average (CLA) of the whole stroke. The friction coefficient is thus an average of the stroke rather than an instantaneous value and it is written to a data file every second. Direct sliding speed control is not available in the HFRR, hence, the sliding speed was controlled by altering the stroke length and keeping the frequency constant (average sliding speed = stroke \(x\) frequency). It should be noted that the sliding speed varies over the tangential loading cycle due to the reciprocating action and, therefore, the average sliding speed is considered as a measure of velocity when analysing the effect of the sliding speed on the tissue’s response.

### 3.6 - Mechanical characterisation

#### 3.6.1 - Overview

The variation of reaction force over time obtained using the unconfined compression stress relaxation technique curve fitted with numerical and analytical models, was used to obtain the relevant bulk elastic and viscoelastic parameters of the tissue reported below. The reader is referred to Chapter 2 for a detailed explanation of the parameters in question.

\[ E \quad \text{Young’s modulus} \]
\[ \tilde{G} \quad \text{Discrete Spectrum Magnitude} \]
\[ \tau_s \quad \text{Short Term Relaxation Time} \]
\[ \tau_l \quad \text{Long Term Relaxation Time} \]

\(^1\) It should be noted that Hertz contact theory cannot be used as it only rigorously applies when the half-space approximation is valid for isotropic elasticity. Here the specimens to be tested exhibit finite thickness with respect to the characteristic dimension of the contact area and are characterised by an anisotropic structure.
The permeability of the sample was also calculated indirectly from the stress relaxation response as discussed in § 3.6.3. It is however only an estimate as characterising the tissue’s permeability requires a very specific experimental set up such as the confined compression technique whereby an upper porous platen is used to compress the AC sample and a biphasic constitutive model can then be fitted to the experimental data [55]. Alternatively, more elaborate mechanical techniques can be used to measure the permeability of the tissue [239].

The Poisson’s ratio could not be directly measured in this investigation as such a characterisation requires some form of optical/mechanical measurement technique applied to the unconfined compression test in order to quantify the lateral deformation [240]. The stress relaxation curve contains a great deal of information regarding the structure-function relationship of the tissue. More specifically, viscoelastic and elastic parameters can be obtained from the different portion of the curve, as illustrated below in Figure 3.11.

![Stress Relaxation Curve](image)

**Figure 3.11** – Illustration of the stress relaxation curve.

The dynamic part of the curve is used to extract the viscoelastic parameters of the sample in question. The peak of the curve is due to the high dynamic modulus caused by the viscoelastic consolidation of the collagen fibres, which resist tensile deformation. Studies have demonstrated that the collagen fibre network is mainly responsible for the response following instantaneous deformation in unconfined compression [241]. The relaxation part of the curve is caused by both flow dependent as well as flow independent mechanisms and is used to extract the viscoelastic parameters as explained in §3.6.3. On the other hand, the equilibrium/static part of the stress relaxation curve is a measure of the stiffness of the tissue. Thus, as $t \to \infty$, the reaction force tends to a constant value yielding a measure of the “equilibrium stiffness” of the tissue and, hence, the Young’s modulus.

3.6.2 - Finite element model

The unconfined compression model of the experiment was reproduced using the commercial FEM package Abaqus v.6.10.1. The ability of Abaqus to reproduce the standard biphasic model developed by Mow and co-workers [72] applied to practical joint contact problems and geometries has been successfully demonstrated by Wu and co-workers [200]. Since then, Abaqus has been used
successfully to model AC with advanced constitutive biphasic models and ever more complex geometries and conditions (see e.g. [242-245]).

3.6.2.1 - Geometry

Modelling the unconfined compression tests entailed producing an exact equivalent of the real test using the same geometry and testing specifications. Thus, the dimensions of the sample as well as the test parameters namely, applied vertical strain, ramp time and total test time, had to be correctly reproduced by the FEM.

The FEM of the AC sample in an unconfined compression simulation is illustrated in Figure 3.12.

![FEM of the unconfined compression test on an AC sample.](image)

3.6.2.2 - Material description and interactions

Abaqus Standard was used to model the biphasic and transient response of the tissue using the built-in soil consolidation procedure. The AC was modelled using both the BNLE and the more sophisticated BPVE constitutive model. The BPVE model is well known to be able to predict the response of both native and enzymatically damaged tissue in unconfined compression and this makes it a valuable diagnostic tool for assessing degradation in AC [184]. The BNLE is the building block of the BPVE model. It is well accepted that the BNLE is not able to predict unconfined compression test but its validity is limited exclusively to the confined compression configuration. In unconfined compression, the BNLE fails to capture the stress relaxation transient response as well as severely underestimating the transition from peak to equilibrium loads [206]. It can predict a maximum ratio of 3:2 peak to equilibrium whilst experimental tests suggest a value of 10:1 or higher [142]. Nevertheless, it was decided to implement both the BNLE and the BPVE model in order to provide a comparison between simulations performed considering the inclusion and the absence of flow independent viscoelasticity.
It is worth noting that these models are in any case valid and successful in predicting the tissue’s behaviour in idealised mechanical loading conditions such as the unconfined compression test [200, 206, 231]. For a truly more accurate representation of AC, the material properties and the characteristic behaviour of a saturated mixture consisting of an ionic fluid phase, a porous solid matrix and three-dimensional fibrils embedded in the solid should be considered [206]. However, for the purpose of this study, the use of an AC model such as the BPVE model, which has been widely used to corroborate experimental findings and is relatively straightforward to implement, was deemed as satisfactory in order to analyse the experimental unconfined compression results and to gain a better understanding of the phenomena governing the tissue’s mechanical response.

The use of more advanced models involving the modelling of collagen fibres as well as changes in ionic concentrations [202] is beyond the scope of this research although these features have been considered in our previous AC modelling work [201]. Moreover, matching such advanced models with experimental data to extract the necessary parameters would also require the measurement of the lateral displacement and potentially multiaxial permeability coefficients such as in the case of the BNTI model [144].

The platen was modelled as an *analytical rigid surface* and characterised by impermeable properties. The solution to the analyses was obtained using the *surface to surface* contact interaction. The compression problem was defined as a contact between the rigid master surface of the indenter and the deformable AC slave surface [246]. The contact between the platen and the top surface of the AC was assumed frictionless. This assumption causes a uniform expansion of the sample when compressed, which guarantees an orthogonal state of deformation, in line with isotropy and incompressibility assumptions of the biphasic model [72, 247]. In reality, a certain amount of friction, although generally very small, exists between the AC and the steel platen; this could potentially lead to over prediction of stress levels by the model when the results are compared to experimental data [248]. In particular, studies have demonstrated that AC/platen interface adhesion increases peak loading and reduces relaxation time [221, 249]. In order to reduce the effect of friction, research has shown that the use of low sample aspect ratios (diameter/height) minimizes the effect of friction on the experimental results [248]. However, the use of PBS when performing the experimental test helps minimizing friction [142], as well as the reduction of the macroscopic adhesion at the interface, which has been verified in previous studies [240, 241], argue against friction being a predominant factor in the mechanical response of unconfined compression [250]. Furthermore, the use of low aspect ratios was not deemed as viable in this particular experimental set up since the MCSTR has been optimised to work in the physiological loading regime and testing very small diameter AC plugs would mean lower reaction forces and lower accuracy results. Lastly, low aspect ratios have been associated with an increase in stiffness and the occurrence of buckling [248, 251].
3.6.2.3 - Mesh & elements

The mesh of the AC layer was chosen in order to obtain a trade off between mesh density (accuracy) and computational time (efficiency). The AC layer had a total of 2480 elements and was homogenous throughout the section. The FE mesh consisted of 4 node pore pressure elements (CPE4RP). Reduced integration was applied to all elements in the model and the *Nlgeom* function in the step module was utilised in order to account for geometrical non-linearities and large deformations [252, 253]. The medial axis algorithm with the free meshing technique was employed when meshing the model.

3.6.2.4 - Boundary conditions

From Figure 3.11, the BCs for the model were assigned as follows [134]:

- At the AC surface, \( z = h \), the surface was impermeable and a vertical displacement was applied to the platen;
- At \( z = 0 \) the surface was also impermeable and no vertical displacement was allowed;
- At \( r = R \) the pore pressure was set to zero (\( P = 0 \)) in order to allow free draining of interstitial fluid;
- At \( r = 0 \) an axisymmetric BC was applied in order to simulate at best the three-dimensional effects associated with the homogenous AC plug using a two-dimensional model.

3.6.3 - Implementation of constitutive models using finite element modelling and determination of mechanical parameters from experimental findings

Stress relaxation curves of reaction force vs. time were obtained from the experimental unconfined compression test. The test was then reproduced using FEM and by using the BPVE model to represent AC. The BPVE model is essentially a standard BNLE model but with the inclusion of the non-linearity associated with the solid matrix by the means of three viscoelastic material parameters (Chapter 2). Thus, a BNLE is developed first as it represents the building block of the more advanced BPVE model.

To implement the BNLE model, three material parameters are required to define the tissue’s material properties [10]: \( E \), \( v \), and \( k \) where \( E \) is the Young’s modulus, \( v \) the Poisson ratio and \( k \) the permeability required by Abaqus, where [200]:

\[
k = yK'
\]

(3.2)

and \( K' \) is the permeability defined in the biphasic model [72]

\[
y = 9.881 \times 10^{-6} \text{Nmm}^{-3} \text{ (specific weight of water)}
\]

The variation of permeability with deformation was introduced into the material description using Equation (2.8) reported in Chapter 2. An initial void ratio and an initial permeability of the tissue was
specified accordingly. An initial “guess” of the Young’s modulus of the tissue was also assigned to the material.

Following these initial steps, the BPVE was then developed by adding the solid viscoelastic behaviour to the BNLE model. The three parameters used to assign viscoelastic properties to the model were obtained using an analytical approach directly from the unconfined compression experimental results: firstly, the axial reaction force obtained from the test was converted into stress in order to obtain the variation of contact stress as a function of time. The reduced relaxation function, $G(t)$, was then calculated by normalizing the stress data by the value of the stress extrapolated at $t = 0$ which is the point at which relaxation starts and the applied strain is kept constant (Figure 3.13).

\[
G(t) = \frac{\sigma(t)}{\sigma(t=0)}
\]  

(3.3)

This analytical method is concerned with the extraction of the viscoelastic material parameters using the relaxation portion of the curve (Figure 3.13). In theory, an analytical solution could also be applied to the compression portion of the curve in order to extract the required parameters. However, this complicates the situation even further due to the variation of applied strain with time during the compression phase. Hence, choosing the relaxation portion is analytically simpler to solve. It is worth noting that the relaxation part of the curve incorporates both the relaxation of the solid and the fluid constituents of the tissue. Yet, the viscoelastic parameters obtained from the curve are assumed to represent solely the viscoelastic behaviour of the solid constituent. This assumption can be justified by the fact that that the intrinsic viscoelastic response is considerably more pronounced compared to the viscoelastic response associated with fluid exudation, which is milder and occurs over a significantly longer time span, due to the low permeability of AC. Hence, the governing viscoelastic behaviour is due to the intrinsic component; associating the viscoelastic parameters solely to the solid component.
is deemed a suitable, although not a perfect assumption. A best-fit (smoothed) representative curve of $G(t)$ obtained for AC from experimental data using Equation (3.3) is shown in Figure 3.14.

![Figure 3.14 – Representative curve of the reduced relaxation curve of AC.](image)

Using Matlab, the experimental curve was then fitted with the continuous reduced relaxation function, Equation (2.10), in order to obtain the viscoelastic material constants $c$, $\tau_s$, and $\tau_l$, which were specified to be within a 95% confidence interval. Once these were obtained, the discrete spectrum of viscoelasticity, which is used to implement the viscoelastic behaviour into the FEM model, could be formulated.

Suh and Bai’s work [204] showed that the equivalence between discrete spectrum and the continuous spectrum necessitates $G_\infty = 1$ in Equation (2.12) (Chapter 2). This equivalent function is attained by dividing the continuous spectrum, Equation (2.10), by its solution as $t \to \infty$. Following this step, a value for $G(0)$ can be computed using this equivalent function. Combining Equations (2.16) and (2.18), the values for $N_d$ and $\bar{G}$ are then calculated. Once all the constants have been determined, the discrete spectrum formulation is represented by:

$$G(t) = 1 + \bar{G}e^{-t/\tau_1} + \bar{G}e^{-t/\tau_2} + \bar{G}e^{-t/\tau_3}$$  \hspace{1cm} (3.4)

Abaqus, however, requires a shear relaxation modulus ratio to be defined at a given relaxation time. The shear relaxation modulus ratio is defined as [254]:

$$g_R(t) = \frac{G_R(t)}{G_0}$$  \hspace{1cm} (3.5)

where $G_R(t)$ is the time dependent shear modulus and $G_0$ is the instantaneous shear modulus. Abaqus assumes that $g_R(t)$ can be defined in a Prony series expansion;

$$g_R(t) = 1 - \sum_{i=1}^{N} \bar{g}_i^R (1 - e^{-\frac{t}{\tau_i}})$$  \hspace{1cm} (3.6)

Where $\bar{g}_i^R$ is the Prony series expansion coefficient. The coefficients, at their given relaxation times, are then implemented into Abaqus to assign viscoelastic properties to the solid constituent. These
coefficients are easily calculated by curve fitting the product of Equation (3.6) and $G_0$ with Equation (3.4).

Once the viscoelastic parameters were obtained and using an initial guess of the Young’s modulus of the material, a least-squares fit was used between the experimental curve and the numerical results. In order to do so, an iterative finite element approach combined with parametric curve-fitting was utilised to obtain a prediction of the Young’s modulus of the tissue (note that the viscoelastic parameters were obtained analytically and not parametrically by curve-fitting). Only one parametric variable needed to be optimised using curve fitting, the Young’s modulus, given that the viscoelastic parameters were known and the permeability and the Poisson ratio were kept constant. In order to carry out an iterative FE approach, an initial guess of the Young’s modulus of the sample in question was made. The numerical curve was then extracted from Abaqus and fed into the software package Matlab, where it was compared to the smoothed experimental data. Due to the fact that the sampling frequency of the experimental data was higher than the sampling frequency of the numerical model’s data, an interpolation of the experimental data to the sampling frequency of the numerical data was made [255]. A coefficient of determination between the two curves, $R^2$, was used to give an indication of the quality of the fit and it was calculated using Equation (3.6) [256]:

$$R^2 = 1 - \frac{\Sigma(y-y_c)^2}{\Sigma(y-y)^2}, \quad (3.7)$$

Where $y$ and $y_c$ represent the experimental and numerical values of reaction force, respectively, and $\bar{y}$ is the mean value of $y$ [255]. The Young’s modulus of the FEM was varied by optimizing the value of $R^2$. At this stage, the Young’s modulus of the FEM was sought to be representative of the sample in question.

It is worth mentioning that the characterisation of the elastic properties of the tissue do not necessarily necessitate a biphasic model. The equilibrium response is dictated solely by the elastic properties of the solid constituent of the tissue. All biphasic and viscoelastic phenomena, flow independent and flow dependent, have faded once in the equilibrium stage (Figure 3.10). In this respect, a completely elastic model would yield the same response as a biphasic and viscoelastic model at the equilibrium stage of the test.

Material constants such as the Poisson ratio and the void ratio were assumed constant and were not extracted from the curve fitting procedure. As previously mentioned, unfortunately it was not possible to measure lateral displacement and permeability experimentally. Nonetheless, although it was not possible to obtain experimental values of permeability, once the viscoelastic and elastic parameters had been extracted using the aforementioned techniques, the permeability of the BPVE model was systematically adjusted to further optimize the agreement between experimental and numerical reaction force yielding a value and hence an indication of the tissue’s permeability. This was necessary if a good fit was going to be achieved [133].
For a more thorough and complete evaluation of the reliability of the parameters obtained via the comparison between numerical and experimental data, both axial reaction force as well as lateral displacement should be considered. However, several studies have indeed verified the suitability of the BPVE model to predict the mechanical behaviour of native and as well as enzymatically induced damaged AC [139, 140, 184, 204, 257].

3.7 - Surface characterisation of articular cartilage and tissue engineered cartilage

The surface properties and damage as a result of shear testing were assessed on both native AC and tissue engineered AC. Both White Light Interferometry (WLI) and SEM were used to assess the surface of the tissue engineered samples whilst only WLI was used for AC samples. Surface measurements were performed on the samples before and after the shear in order to quantify and visualize changes in surface topography as a result of shearing. WLI imaging of the sample’s surface was obtained using the Wyko, NT9100, Veeco with a 20X magnification objective, as utilised in other studies [115, 252, 258]. In order to use WLI, however, the surface must be completely dry to stop the light reflecting from the fluid film rather than from the AC surface. Hence, the surface of the samples were dried very gently by using soft absorbing paper, so as not to damage or disrupt the surface morphology. The imaged area obtained using the WLI was of 312 x 234 µm²; five measurements were taken in adjacent areas of the tissue’s surface and the obtained surface topography parameters were then averaged for each of the tested samples.

3.8 - Induction and quantification of OA-like damage in articular cartilage in vitro

Following extraction, the full thickness 10 mm porcine AC plugs were subjected to mechanical and tribological characterisation either as native AC or after enzymatic damage, which allows simulating OA using ex vivo specimens. Induction and quantification of OA-like damage using biochemical techniques was performed by Dr Ngee Han Lim and Dr Kazuhiro Yamamoto under the supervision of Professor Hideaki Nagase at the Kennedy Institute of Rheumatology, Imperial College London.

3.8.1 - Enzymatically induced damage

Native AC plugs were subjected to enzymatic degradation using either MMP-1 (prepared as described in [259]), trypsin (Sigma-Aldrich, Dorset, UK) or a combination of the two. Trypsin will degrade most of the components of AC, except the major structural component, collagen, whereas MMP-1 will preferentially degrade collagen but will also cause PG depletion. The reader is referred to Chapter 5 for a more in depth explanation of the role of the different enzymes on the structural degradation of AC.
Several previous studies have used a wide variety of different enzymes to simulate OA and explore the structure-function relationship on the tissue such as for example collagenase Type VII and Type IV, neutrophil elastase, chondroitinase ABC, cathepsin and stromelysin [182, 188, 260-262]. Although these enzymes do selectively degrade the ECM, they are not the most relevant enzymes to simulate OA. Collagenase for example is of bacterial origin and thus does not exist in the mammalian species. Others such as chondroitinase ABC is not a protease but a glycosidase. There is thus a strong debate regarding the relevance of these enzymes in OA. On the other hand, studies have confirmed the role of MMP-13 in degrading the collagen in osteoarthritic AC and it is thus highly relevant to the pathology [263, 264]. Hence, this justifies the use of MMP-1, which is an almost identical enzyme to MMP-13 - but rather more stable - to degrade collagen and simulate OA. No studies to our knowledge have investigated the mechanical properties of MMP-1 degraded AC. Since MMP-1 is, however, mainly responsible for collagen degradation, it is used in conjunction with trypsin in order to better control the degradation of the different components of the ECM.

The samples were incubated with the enzymes in a total buffer volume of 500 µl at 37 °C in 12-well tissue culture plates. The concentration and the length of time used to digest the samples were varied in order to create a “damage spectrum”, ranging from lightly damaged to heavily damaged samples, to simulate the different stages of OA. Lightly damaged samples were digested using low concentrations of trypsin simulating early PG loss. Heavily damaged samples were degraded using a combination of the two enzymes at higher concentrations over longer periods of time. The resulting degradation of collagen fibres causes irreversible damage to the structure of the tissue simulating the advanced stages of OA. Table 3.2 shows the details of the range of enzymatic induced damage to which AC samples were subjected to. For convenience, a colour code has been used to identify low (green), medium (blue) and high (red) structural damage.
### Table 3.2 – Enzymatic induced damage in articular cartilage samples.

At some stage in the disease, the entire spectrum of degradation reported above could occur in an osteoarthritic joint. However, the damage would not exist so as to uniformly cover the whole joint but it would be more localised, perhaps even existing or initiating at the microscopic level in some cases. It is thought that damage originates as a focal loss of aggrecan (green) before turning into a complete aggrecan loss (blue) followed by collagen degradation (red) at which point, there is a complete loss of AC structure [265]. The loss of structure would then expand from the focal point until complete loss occurs over a larger area.

#### 3.8.2 - Damage quantification

The structural damage to the ECM as a result of the various enzymatic degradations was quantified in order to identify the changes to the PG and collagen content. The percentage of degraded PG and collagen was obtained by first measuring the amount of the two main constituents of PG and collagen released into the medium following digestion, namely GAG and Hydroxyproline (hypro). GAG, being a major constituent of the PG aggrecan, is associated with the amount of aggrecan lost following enzymatic degradation. Hypro is a direct measure of the amount of collagen degradation, as hypro arises from the post translational modification of the majority of the proline residues of collagen.

The original amount of GAG/HYPRO before enzymatic degradation had to be quantified in order to calculate the % of GAG/HYPRO degradation in the tissue. Once the sample was degraded with trypsin and/or MMP-1, this was done by subjecting the sample to a further digestion using papain (Roche, Manheim, Germany) which induced complete loss of any remaining PG or HYPRO in the tissue.
Hence, the percent of degraded ECM was quantified by calculating the proportion of degraded ECM with respect to the total quantity of ECM present in the tissue. By taking GAG as an example, we can write:

\[
\text{\% of degraded GAG} = \frac{\text{GAG released following digestion}}{\text{Total GAG in sample}}
\] (3.8)

Where:

\[
\text{Total GAG in sample} = \text{GAG reaming in the tissue} + \text{GAG released following digestion}
\]

For the papain digestion, each weighed AC sample was incubated in 500 ul of papain digest solution (0.05 mM phosphate buffer pH 6.5, 2 mM N-acetyl cysteine, 2 mM disodium EDTA and 10 ug/ml papain) at 65 °C until tissue was dissolved. The amount of GAG and hypro released into the medium of the cultured sample following enzymatic digestion was quantified using the dimethylmethylene blue (DMMB) assay and the hydroxyproline assay respectively, prepared using the method described in [266, 267].

### 3.8.2.1 - PG Damage quantification

The DMMB assay used to quantify aggrecan loss involved a modified technique [167, 266] of the original assay described by Farndale et al. [268]. The DMMB assay measures the amount of GAG released which constitutes a major portion, but not all, of the PG. The DMMB assay reagent was formed from 16 mg/ml DMMB dye, 41 mM sodium chloride, 40 mM glycine and 9.5 mM HCl. Two hundred and fifty microlitres of assay reagent was added to 5 µl of the conditioned medium containing the released GAG, the absorbance of the samples was read at 540 nm. Shark/whale chondroitin sulphate (0 - 2.5 µg) was then used to standardize the results [167]. Where samples were outside the specified linear range, they were suitably diluted with Dubelcco's Modified Eagle Medium (DMEM) (BioWhittaker, Verviers, Belgium). To more accurately track the cleavage of aggrecan, western blot analysis using the 2-B-6 antibody which recognizes the chondroitinase-cleaved chondroitin-4-sulfate stubs left on the aggrecan core protein, was carried out. This demonstrated the cleavage of aggrecan into smaller fragments, and supports the results from the DMMB assay.

### 3.8.2.2 - Collagen damage quantification

The hypro assay utilised [167] was a modification of the original assay described by Bergman and Loxley [267]. Using a microcentrifuge tube, 6 N HCl (50 µl) was added to conditioned media (50 µl) for 20 h at 95 °C to acid hydrolyse the solution. The acid was evaporated off in a fume cupboard over night at 60 °C. 100 µl of H₂O was then added to fully re-suspend the dried residue. 25 µl chloramine T reagent (1.4 % (w/v) chloramine T, 30.8 % (v/v) isopropanol, 335 mM Sodium Acetate), 100 mM tri- sodium citrate, 23 mM citric acid) was reacted with 40 µl of medium containing released hypro at room temperature and for the duration of 4 minutes. Subsequently, 150 µl of
dimethylaminobenzaldehyde (DMBA) reagent (16.7 % (w/v), 75 % (v/v) isopropanol, 52.5 % (v/v) perchloric acid) was added to the solution and incubated for 40 minutes at 70 °C. Once cooled, the absorbance at 560 nm was read. A standard curve (0 – 1.2 µg hypro) was used to calculate the amount of hypro in the solution [167].

A further quantification of damage as a result of shear testing was also carried out on both native and degraded samples. The PBS used in the shear testing bath of the HFRR was collected and DMMB and hypro assays were carried out [269]. A comparison between the amount of GAG and hypro release from native and enzymatically degraded samples as a result of shearing, could then be made. This will be discussed in Chapter 5.
Chapter 4  – Experimental and numerical investigation of the behaviour of articular cartilage under shear loading – interstitial fluid pressurisation and lubrication mechanisms

4.1 - Introduction

In this chapter, the mechanical and frictional responses of native porcine AC when subjected to alternating shearing forces under a constant load were investigated. Shear testing was performed at physiological contact pressures using the HFRR to ascertain the influence of interstitial fluid support on the evolution of frictional forces during cyclic loading. Following the tests, the change in AC surface topography was assessed using WLI. Numerical studies, in order to complement the experimental studies and to shed light on the lubrication mechanisms governing the tribological response of the tissue, were also performed using the FE software Abaqus. The scope of this investigation was thus to investigate AC lubrication mechanisms and to understand the effect of interstitial fluid pressurisation on the frictional response of the tissue. It is a combined experimental and numerical study.

4.2 - Methods

4.2.1 - Experimental investigation

Full thickness AC plugs were extracted and shear tested using the HFRR as described in Chapter 3. Two series of experimental tests were carried out to study the effect of the following test conditions on AC lubrication mechanisms:
1. Effect of sliding speed on friction evolution and lubrication regimes.
2. The effect of static loading time (before the test started) on the frictional response in order to investigate the interstitial fluid pressurisation of the tissue at start-up.
Surface analysis of the AC sample before and after shear testing was also performed.
4.2.2 - Numerical investigation

In order to investigate the effect of the interstitial fluid support and the lubrication mechanisms during shear testing, a model was created using the FEM software package Abaqus v.6.9.1. For the purpose of this study, capturing the salient features and the biphasic behaviour of AC is deemed as sufficient in order to validate the experimental findings and to gain a better qualitative understanding of the phenomena which govern articular joint responses. Hence the numerical model adopted in this study is the BNLE model discussed in Chapter 3.

The material properties used for the AC are the following [72, 200, 270]:

\[
E = 0.85 \text{ MPa} \quad k_0 = 2.787 \times 10^{-7} \text{ mms}^{-1} \\
\nu = 0.0 \quad e_0 = 4.2 \quad M = 5
\]

The UHMWPE disc, forming the support of the AC sample, was assigned the following mechanical properties [271];

\[E_{\text{UHMWPE}} = 1.258 \text{ GPa} \quad \nu = 0.2\]

Although the experimental test involved a sphere on flat configuration, a 2D simplified analysis was used to capture the qualitative behaviour of the tissue. The shear test was thus modelled in 2D plane strain configuration. The upper specimen or indenter was modelled as an analytical surface, the AC layer as porous elastic and the UHMWPE disc as elastic. The AC and UHMWPE disc were modelled as whole block of 3 mm depth and 10 mm diameter and then partitioned so as to assign the different material properties. Both the AC layer and UHMWPE disc were assigned a thickness of 1.5 mm. The BCs applied to the model were assigned in order to replicate the experiments. The two sides of the AC layer were assumed to be sealed rather than free draining unlike in our previous work [272]. This is believed to be a more accurate representation of the AC BCs since the AC sample’s circumference is fully constrained by the sample holder thus limiting the fluid flow across the sides. A user defined *FLOW subroutine was used to define the BC at the surface. This enabled contact dependent flow conditions to be simulated in the model [273-275]. Fluid is hence allowed to exude and enter the tissue only through the top surface in the regions outside the contact zone.

The sides of the AC and the UHMWPE were assigned displacement and rotation BCs so that only displacement in the vertical direction was allowed.

The seepage coefficient \((k_s)\) used for the *FLOW condition was such that [114]:

\[k_s = 1 \text{ mm}^3/\text{Ns (fluid flow)}\]

\[k_s = 0 \text{ mm}^3/\text{Ns (no fluid flow)}\]
The solution to the analyses is obtained using the *surface to surface contact interaction*. The indentation problem is defined as a contact between the rigid master surface of the indenter and the deformable AC slave surface [246].

A load of 0.5 N/mm was applied to the reference point of the indenter. This gives rise to an average contact pressure of about 0.5 MPa, which, although lower than the range of pressure applied in the experimental shear test (~1.6 MPa), allows avoiding convergence issues which occurred using models with higher loads. These problems were caused by the very large deformations that take place in the AC model when using the higher loading range, especially during the gradual load shift from interstitial fluid to the solid matrix when the simulations were run for large number of cycles. However, the flow effects and the observed overall mechanical behaviour of the tissue appeared to be qualitatively the same as that obtained for simulations performed for a more limited number of cycles at higher loads (up to 6 N/mm) and they are considered to adequately represent the behaviour captured by our experimental analyses.

Once the load was applied and contact between the AC and the indenter took place, the AC was allowed to equilibrate for 1 second prior to shear taking place. The shear test was modelled by applying an oscillating displacement to the reference point of the analytical surface in the tangential direction, whilst the prescribed normal load was held constant. The frequency of oscillation was varied by varying the displacement amplitude.

The mesh density was chosen in order to obtain a trade off between mesh density (accuracy) and computational time (efficiency). The AC layer had a total of 700 elements whilst the UHMWPE disc had 560 elements. The FE mesh used for the models consisted of 4 node pore pressure elements (CPE4RP) for the AC layer and 4 node bilinear quadrilateral 4 node elements (CPE4R) for the UHMWPE disc. The mesh in the AC layer was refined in the contact region until convergence was achieved in terms of interfacial contact pressures. Reduced integration was applied to all the elements in the model and the *Nlgeom* function in the step module was utilised in order to account for geometrical non linearity and large deformations. The *medial axis* algorithm was employed when meshing the model.

The contact was modelled as frictionless. This means that any tangential resistance encountered by the indenter is due to the work done to deform the AC layer and to “squeeze” the fluid out of the contact area in the lateral direction.
4.3 - Experimental investigation

4.3.1 - Results

The broad range of stroke lengths and frequencies available in the HFRR allowed investigation of different lubrication regimes using several different sliding velocities. The schematic of a typical Strubeck curve relating the frictional joint response to different sliding velocities is shown in Figure 4.1. The numbers on the Strubeck curve relate to the different average sliding speeds achieved by varying the stroke length, as shown in Table 4.1.

![Figure 4.1 – Schematic of a classical Strubeck curve](image)

<table>
<thead>
<tr>
<th>No.</th>
<th>Stroke (mm)</th>
<th>Frequency (Hz)</th>
<th>Velocity (mm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.25</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>10</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 4.1 – Range of parameters used for sliding tests.

Although physiological lubrication mechanisms have been reported to occur mainly in boundary and mixed lubrication with sliding speeds of 1 – 4 mm/s in the joint [276], we have explored a wide range of conditions, covering different lubrication mechanisms starting from boundary up to the initial stages of hydrodynamic lubrication. The area highlighted in Figure 4.2 is the focus of the work presented in this article.
Examples of AC frictional response for different average sliding speeds are shown in Figure 4.2.

![Figure 4.2](image)

**Figure 4.2** – Evolution of the friction coefficient for boundary, mixed and hydrodynamic lubrication regimes tests.

Three curves denoting the different response in terms of the evolution of the friction coefficient are plotted. The lubrication mechanism during the shear test is time dependent [123]. This means that a variation in the lubrication mechanisms occurs during sliding. Hence up to three distinct friction responses can be identified depending on the imposed sliding speed. In all cases the friction coefficient stabilises to a constant value after a given number of cycles. The stabilised friction coefficients decrease with increasing stroke length (increasing average sliding speed). Sliding in each test was commenced within 1 minute of static normal loading applied to the AC sample and different AC samples were used for each curve.

The stabilised frictional coefficients are termed $\mu_{eq}$, $\mu_{mix}$ and $\mu_{film}$ respectively for the boundary, mixed and hydrodynamic lubrication regime. $\mu_{eq}$ is the effective friction coefficient used to describe the coefficient of friction prior to reaching full boundary lubrication or equilibrium ($\mu_{eq}$), and exists under conditions of interstitial fluid pressurisation [99]. We term $\mu_0$ the coefficient of friction at start-up (at $t = 0$). Each test will be identified according to the lubrication mechanism which dominates once the test has stabilised. By direct observation of the coefficient of friction when a stabilised value has been reached, which we assume to be constant [113], boundary lubrication is achieved at sliding velocities of 5 mm/s mixed lubrication at 10 mm/s and hydrodynamic lubrication at speeds of $\sim$20 mm/s and above. An experimentally obtained Stribeck curve is shown in Figure 4.3. The friction coefficient measured at different average sliding speeds is representative of the stabilised condition.
In a second series of tests the effect of static loading time on the frictional response was studied. These tests are used to assess the influence of interstitial fluid pressurisation on the frictional response of the tissue at start-up. A frequency of 10 Hz and a stroke length of 2 mm were used. Although these conditions lead to a stabilised hydrodynamic lubrication regime, here only the start-up coefficient of friction is measured. At the beginning of the test not enough time has been given for a thick lubricant film to form at the interface between the spherical surface and the AC sample and therefore the contact is in boundary or mixed lubrication. The value of $\mu_0$ is a product of the boundary/mixed lubrication mechanisms developing at the contact interface as soon as the indenter starts reciprocating and this depends on the amount of time that static load is applied to the AC prior to sliding. The static loading time is referred to as the amount of cumulative time the AC is left loaded normally without lifting the ball from the AC sample. The longer the AC is loaded prior to commencing the shear test, the higher $\mu_0$, as the interstitial fluid pressurisation in the contact zone drops due to the fluid exuding out of the contact area.

Figure 4.4 shows a representative example of the initial few seconds shear testing after different static loading times using the same AC sample. The effect of the static loading time on $\mu_0$ can be clearly observed and this trend was noticed in several tests involving other AC samples. It is worth mentioning that the curves start at a cycle time of 1s because data is automatically extracted every second.
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Figure 4.4 – Effect of static loading on the start-up friction coefficient.

In Figure 4.5, the start up coefficient for each static loading time has been plotted. Three tests using three different AC samples were performed. A best-fit curve is reproduced on the plot, showing the repeatability of the trend.

Figure 4.5 – Start-up friction coefficient variation due to static loading time.

Figure 4.6 shows three boundary lubrication tests where an AC sample was subjected to different static loading times. For long static loading times, the start-up coefficient of friction seems to be higher than the coefficient of friction occurring for tests leading to a stabilised boundary lubrication regime ($\mu_{eq} \approx 0.25$). This could be attributed to adhesion and solid matrix viscoelasticity. The additional contribution of these factors towards the higher start-up friction coefficient has been termed $\mu_i$.
Figure 4.6 – Influence of static loading on the start up friction coefficient of AC in the boundary lubrication regime.

The evolution of the coefficient of friction in boundary and hydrodynamic conditions is shown in Figures 4.7a and 4.7b respectively, for two tests obtained by subjecting the same AC sample to sliding after 1min from the application of the normal load ($\mu_1$) and by subsequently repeating the test following the application of the constant normal load for 15 minutes after the interruption of the first test ($\mu_2$).
This test procedure allows us to verify the effect of the interstitial fluid support on the coefficient of friction. For the boundary case, $\mu_{eq}$ is reached after approximately 8000 cycles and for the hydrodynamic case, $\mu_{film}$ is reached after approximately 5000 cycles. The data in Figures 4.7a and 4.7b can be manipulated to obtain a measure for the interstitial fluid support (Figures 4.8a and b).
The primary mechanism regulating the friction coefficient is believed to be the interstitial fluid pressurisation [126] and not wear occurring at the surface of the AC. This can be demonstrated by observing the surface of the AC before and after the shear test. Shown in Figures 4.9 and 4.10 are the surface topographies of an AC sample before (a) and after (b) it has been subjected to a hydrodynamic and a boundary shear test respectively. The duration of the shear tests was of 2 hours at a frequency of 10 Hz (72000 cycles). The images have been obtained using WLI.

Figure 4.9 – Surface topography of AC (a) prior to hydrodynamic shear testing and (b) after hydrodynamic shear testing.
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Figure 4.10 – Surface topography of AC (a) prior to boundary shear testing and (b) after boundary shear testing.

The imaged area is 312 x 234 µm² which is much smaller than the initial contact area (1.6 mm²). The initial contact area was obtained using FEM by prescribing the same load used in the shear test and by allowing 1s of equilibration. Roughness and surface parameters before and after the shear test were calculated automatically by the inbuilt analysis software. Table 4.2 shows roughness and surface parameters after subjecting AC samples to 2 hour shear tests with a 250 µm and a 2 mm stroke length at a frequency of 10 Hz. Differences in roughness and surface topography between 250 µm and 2 mm stroke lengths can be observed. Boundary regime shear tests seem to have a slightly higher roughness than hydrodynamic tests following the shear test. Also, deep depressions on the surface (as shown from Figure 4.9b) are thought to occur only after hydrodynamic tests rather than boundary regime tests.

<table>
<thead>
<tr>
<th>Surface Parameter</th>
<th>Native AC</th>
<th>AC after Boundary Regime Shear Testing</th>
<th>AC after Hydrodynamic Regime Shear Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_a$</td>
<td>0.56 ± 0.16</td>
<td>1.02 ± 0.24</td>
<td>0.64 ± 0.12</td>
</tr>
<tr>
<td>$R_q$</td>
<td>0.71 ± 0.20</td>
<td>1.28 ± 0.28</td>
<td>0.79 ± 0.16</td>
</tr>
<tr>
<td>$R_z$</td>
<td>8.60 ± 3.32</td>
<td>10.40 ± 3.73</td>
<td>8.90 ± 5.05</td>
</tr>
<tr>
<td>$R_t$</td>
<td>9.90 ± 4.51</td>
<td>12.01 ± 6.11</td>
<td>11.50 ± 5.51</td>
</tr>
<tr>
<td>$S_{sk}$</td>
<td>0.15 ± 0.57</td>
<td>0.18 ± 0.30</td>
<td>0.11 ± 0.34</td>
</tr>
<tr>
<td>$S_{ku}$</td>
<td>3.60 ± 0.90</td>
<td>3.24 ± 0.20</td>
<td>4.20 ± 1.31</td>
</tr>
</tbody>
</table>

Table 4.2 – Roughness and surface parameters before and after subjecting AC to a 2 hour sliding test at a frequency of 10 Hz with a 250 µm and 2 mm stroke length.
Where:

\[ R_a = \text{average roughness} \]
\[ R_q = \text{root mean square (rms) roughness} \]
\[ R_z = \text{average maximum height} \]
\[ R_t = \text{maximum surface height} \]
\[ S_{sk} = \text{surface skewness} \]
\[ S_{ku} = \text{surface kurtosis} \]

It is worth mentioning that although the values of the roughness parameters are theoretically only valid to describe 2D profiles, the inbuilt software automatically computes the average of the roughness parameter for the entire surface. The roughness parameters are hence very similar to surface parameters.

The parameters have been obtained from AC samples originating from four different porcine joints. Sixteen different AC samples were used to obtain the surface parameters for the native samples and the samples subjected to sliding. In turn, 6 measurements of surface parameters were made on each of the samples. The average between the various measurements and samples was then calculated. It is worth noting that for AC following shear loading, the measurements were made on the damaged area of the sample which had been subjected to shear; this area was clearly visible to the naked eye. The downside of using this method is that it is impossible to image the exact same location prior to and after sliding. Hence, when imaging the native samples, particular attention was given to imaging the centre of the tissue where the contact during sliding was thought to occur. When imaging the samples after being subjected to sliding, different areas along the wear patch, which was clearly visible after the test, were analysed. Variation in topography and roughness within the wear patch existed.

The surface topography of the native AC (Figures 4.9a and 4.10a) is constituted by several “humps” and “pits” or bowl shaped depressions [13]. These are more clearly seen in a 2D image of AC (Figure 4.11) after a latex glove finger is gently rubbed over the surface of the AC sample. This procedure is reported to remove the surface amorphous layer (SAL) [277] and it allows to see the surface depressions more clearly. The SAL is a superficial layer extending from the superficial tangential zone, 800 nm – 2 \( \mu \text{m} \) thick in porcine AC [33]. It contains no fibrils or chondrocytes [30] and it has been suggested to contain proteins and glycoproteins, PG’s, chondroitin/keratin sulphates, (phospho)lipids and/or hyaluronic acid-protein complexes [278]. The SAL tends to cover and “hide” the characteristic pits and humps (Figure 4.10a) as also demonstrated in other studies [32].
The reason for these depressions on the surface is still not completely known however, it has been postulated that they are attributable to underlying cell lacunae [279]. Furthermore, the presence of these “pits” and “humps” might be a cause of specimen preparation causing shrinkage of the AC and the underlying chondrocytes [280] and might not actually be present in vivo [281].

An image of an AC sample obtained using an optical microscope at the boundary between the worn area and the unworn area is shown in Figure 4.12. A change in surface topography between the two areas can be clearly observed.

**Figure 4.12** – Photograph of an AC sample at the boundary where sliding occurred after 360,000 cycles at a stroke length of 2 mm and a frequency of 10 Hz (Image area 1300 x 970 µm²).

### 4.3.2 - Discussion

The frictional response of the tissue at different sliding regimes is discussed below. Each sliding regime is explained with a unique friction coefficient aside from \( \mu_0 \) which occurs at the start of all sliding tests regardless of the mode of lubrication at stabilised conditions (see Figure 4.2).

#### 4.3.2.1 - Boundary lubrication

The lowest-speed boundary lubrication results show a very distinct evolution of the friction coefficient during the shear test as compared to the mixed and hydrodynamic regimes. Two different coefficients of friction are needed to describe the curve due to the continuous rise in the coefficient of friction until an equilibrium value, namely \( \mu_{eq} \) is reached. The coefficient of friction at any time after
start-up and prior to $\mu_{eq}$ can be described by $\mu_{eff}$ [282]. This is of fundamental importance as it is unique to biphasic materials such as AC. When the AC is loaded and the shear test is commenced, a high interstitial fluid pressurisation under the contact area exists whereby the upper specimen is supported mainly by the action of the fluid. Gradually, as the shear test continues, a larger quantity of interstitial fluid is exuded out from the contact area causing a fall in fluid pressurisation which will eventually lead to full boundary lubrication. In this regime interstitial fluid pressurisation is thought to approach a decrease until it becomes negligible. Interstitial fluid exudation is not immediate due to the very low permeability of the tissue. AC is thus capable of maintaining some level of interstitial fluid pressurisation for several minutes. This is crucial in reducing wear of the solid matrix to a minimum during the natural functioning of the joint.

The theory whereby interstitial fluid pressurisation causes a decrease in the coefficient of friction also correlates very well with the idea that a biphasic lubrication mechanism is actually occurring during the shear test. Until some interstitial fluid pressurisation exists in the contact zone, then fluid within the tissue will exude out of the leading edge thus providing a replenished lubricating film. The sliding speed would however be too low to allow any stable film to form; hence it is assumed that the contact undergoes different levels of mixed lubrication ($\mu_{eff}$) evolving towards the steady-state boundary lubrication regime ($\mu_{eq}$). Therefore, we can state that $\mu_{eff}$ is representative of evolving mixed lubricating regimes while $\mu_{eq}$ is the boundary friction coefficient.

### 4.3.2.2 - Mixed lubrication

The friction coefficient evolution curve for 10 mm/s shown in Figure 4.2 is thought to correspond to mixed lubrication achieved at the steady-state. This is somewhat surprising as the stabilised coefficient of friction resulting from 10 mm/s sliding corresponds to a value lower than $\mu_{eq}$ but relatively high compared to the friction coefficient expected to develop in the case of full hydrodynamic film formation. Such behaviour, not easily attainable as it requires a balance between fluid exudation and film formation, has been achieved by increasing the stroke length from 250 $\mu$m to 500 $\mu$m. The stroke length was increased rather than decreasing the frequency because 10 Hz is the lowest frequency the HFRR will operate at and it is thus due to the machine’s limitation. With 250 $\mu$m stroke length, $\sim$84% of the initial contact zone remains completely loaded during the shear test (the initial contact area corresponding to 1.6 mm$^2$ was used to obtain the percentage of loaded tissue area). Hence, if only $\sim$16% of the contact zone is rehydrated during sliding, it is difficult for fluid replenishment in the contact zone to occur. With a stroke length of 500 $\mu$m, $\sim$37% of the contact area remains loaded, meaning that now $\sim$63% of contact area is replenished with fluid and hence more fluid is able to flow back into the tissue. This appears to be in line with the findings of recent studies [114] in which the authors, by means of numerical analyses, have argued that if the stroke length is
reduced to a value whereby at least 89% of the initial contact zone remains loaded during sliding, then fluid replenishment in the contact zone cannot occur. Studies have also shown that a migrating contact area and hence longer stroke lengths lead to a very high fluid pressurisation and hence a very low coefficient of friction [119, 283]. The fact that the value of the coefficient of friction is still relatively high and that the presence of fluid film generated might be very thin and intermittent suggests that the stabilised coefficient of friction is in the mixed lubrication regime and its value is therefore termed $\mu_{\text{mix}}$.

### 4.3.2.3 - Hydrodynamic lubrication

The test running under hydrodynamic conditions can be described by $\mu_{\text{film}}$ once sliding has commenced. $\mu_{\text{film}}$ is the coefficient of friction once the shear test has stabilised and film has formed between the contact interface. $\mu_{\text{film}}$ is considerably lower than $\mu_{\text{eq}}$ since the former is a result of hydrodynamic and not boundary lubrication conditions. The curves for the mixed and the hydrodynamic lubrication case seem to be very similar in shape but the AC frictional response is very different. According to the curve, the fluid film seems to be fully developed throughout the stroke even though the sliding speed is continuously varying. The reason for this is probably due to the high frequency employed (10 Hz) which is sufficient to allow a stable film to form. Although the contact area migrates from its initial position allowing for tissue rehydration, with a 2mm stroke length this does not affect the frictional response since a fluid film has developed between the contacting surfaces due to the high sliding speed. This fluid film masks the interstitial fluid support in the contact zone. One point worth mentioning is that due to the reciprocating action there is a translational sliding speed variation throughout the stroke during sliding. Hence the lubrication regime will be different between mid stroke and start/end stroke. This however cannot be accounted for and as previously mentioned, the friction coefficient is computed automatically via the CLA of the full stroke.

### 4.3.2.4 - The effect of static loading on the coefficient of friction

As shown in Figure 4.4, as the static loading time increases, the start-up friction coefficient increases. The start-up friction coefficient at various loading times is plotted in Figure 4.5. The shape of the curve resembles the shape of the boundary lubrication test. This indicates that $\mu_0$ indeed occurs as a result of the evolution of the lubrication regime from mixed to boundary as a function of the time available for the interstitial fluid to exude out of the contact zone. The longer the static load time, the higher the coefficient of friction until a plateau is reached. The increase in the start-up friction coefficient after a static loading time is however also due to the stress relaxation occurring and not simply due to the change in lubrication mechanism. As the AC remains loaded, fluid exudes out from the contact zone causing the solid constituent to elastically deform and load shift to the solid part to
occur. This causes the ball to “sink” into the material. Hence, when sliding is initiated, there is a higher tangential force needed to “push” the material away, hence a form of “ploughing” occurs causing the rise in start-up friction coefficient. This “ploughing” effect is directly linked to stress relaxation and can be attributed to the fall in interstitial fluid support.

The slight difference in coefficient of friction between the samples could be due to the slight variation in thickness of the AC plugs. During our investigation, although this is not discussed in detail in this study, it was noticed that thicker AC samples are able to maintain a longer interstitial fluid pressurisation. It is hypothesised that in thicker samples some fluid flows into the contact area from the deeper zones of the material thus providing more fluid to maintain pressurisation than for thin samples. Further research will need to be carried out to shed some light on these findings.

The value of $\mu_0$ appears to be slightly higher than $\mu_{eq}$ for long static loading times (Figure 4.6). Theoretically, however long the static loading time, $\mu_0$ should not exceed $\mu_{eq}$ as $\mu_{eq}$ represents the highest friction coefficient achievable in an AC shear test, recalling that $\mu_{eq}$ is assumed to be a constant depending on the contact pair. However, it was noticed that after a given amount of loading time $\mu_0$ appeared to be higher than $\mu_{eq}$, conflicting with the theory that full boundary lubrication occurs at $\mu_{eq}$. In order to investigate this, further tests were performed. Figure 4.6 shows three static load tests where an AC disc was left under static load for 1 minute, 15 min and 1 hour respectively prior to commencing a boundary lubrication test at 5 mm/s. At 1 min static loading time, $\mu_0$ appears to be well below $\mu_{eq}$, as a result of interstitial fluid pressurisation in the contact zone. After 15 min most of the interstitial fluid has exuded out of the contact area. Hence, sliding occurs in the boundary regime throughout the test. At the beginning of the 15 min static loading test however, a small peak in $\mu_0$ is observed. However, we can assume that $\mu_0 \approx \mu_{eq}$. The peak in $\mu_0$ is seen more clearly as the static loading time is increased to 60 min. Here, $\mu_0$ is indeed much higher than $\mu_{eq}$. The complete absence of interstitial fluid support can only justify the conditions whereby $\mu_0 \approx \mu_{eq}$. In order to achieve values of static friction $\mu_0 > \mu_{eq}$, there must be other contributing factors. We suggest two mechanisms which occur after long periods of static loading time which might promote the increase of $\mu_0$. The first mechanism is due to viscoelastic consolidation of the AC ECM. As explained earlier, as interstitial fluid pressurisation decreases, the load elastically deforms the solid matrix around the contact causing the ball to “sink” into the material. The ball however takes time to “sink” even if there is no fluid pressurisation because of the viscoelastic nature of the solid matrix. Hence the time dependent viscoelastic behaviour of the solid constituent plays a role in this phenomenon. The second mechanism which could occur is adhesion between the steel ball and the AC. We indeed have noticed some resistance when removing the load after completion of the test. This however, is yet to be proven experimentally.
Further investigations will be performed to shed light on the relative contribution of the two mechanisms hypothesised and described above.

A further remark which can be made by critically observing the results obtained is that $\mu_0$ is generally higher for tests characterised by hydrodynamic rather than boundary lubrication regimes (see Figures 4.7a and 4.7b). This is due to the different stroke lengths used for the tests leading to different lubrication regimes. Hydrodynamic test were carried out using a 2 mm rather than a 250 $\mu$m stroke length. This means that there is a greater resistance in the tangential direction and more work is done for the hydrodynamic case at start-up since the material surrounding the upper specimen has to be moved over a longer distance. Recalling that the friction is recorded as an average over the stroke and not instantaneously, this leads to higher values for $\mu_0$.

4.3.2.5 - Evolution of the interstitial fluid support during sliding at boundary and hydrodynamic regimes

In both the boundary and the hydrodynamic conditions, the time-dependent fluid support mechanism effect on the start-up coefficient of friction can be clearly seen (Figure 4.7a and 4.7b). The static load causes fluid exudation from the contact zone and hence a higher start up friction coefficient for the repeat test as compared to the initial test.

A further aspect to be considered is that a measure of the interstitial fluid support variation during sliding can be given by a dimensionless ratio $(\mu_1-\mu_2)/\mu_{eq}$ and $(\mu_1-\mu_2)/\mu_{film}$ for both boundary and hydrodynamic lubrication regimes respectively. This is shown in Figures 4.8(a) and 4.8(b). The normalised ratio shows, for both regimes, a continuous decrease in interstitial fluid pressurisation between the repeat and the initial test. In the boundary regime the dimensionless ratio decreases with time until the effect of fluid support vanishes and a plateau is reached. In the hydrodynamic case, there is a continuous decrease in fluid pressurisation until a fluid film is developed at the contact interface. Whether for the boundary or the hydrodynamic case, the dimensionless parameter shows the same behaviour in time confirming the decrease in interstitial fluid support in both regimes.

Figure 4.13a shows an almost linear relationship between $\mu_1$ and the measure of interstitial fluid support, represented by $(\mu_1-\mu_2)/\mu_{eq}$, for the boundary lubrication case. Figure 4.13a confirms how the lower friction coefficient is associated with a high interstitial fluid pressurisation. Such a quasi-linear dependency between fluid load support and the coefficient of friction has also been identified by Krishnan and co-workers [284] (Figure 4.13b). From Krishnan et al.’s work in Figure 4.13b, the effective friction coefficient ($\mu_{eff}$), which is equal to $\mu_1$ in this study, varies linearly and with a negative slope with the interstitial fluid load support which the authors have obtained experimentally, represented by $W_p/W_r$. 

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Hence, the similar relationship which has been obtained in this study suggests the level of interstitial fluid support could also be measured by using repeat loading tests and $\mu_i$ rather than measuring the hydrostatic pressure directly [284]. This relation is particularly useful as simply by looking at the coefficient of friction $\mu_i$ one can have an idea of the interstitial fluid support present in the tissue.

A linear relation between $\mu_i$ and the interstitial fluid support cannot be seen for hydrodynamic conditions as a fluid film develops within the contact “masking” the effect of the decreasing fluid support: in this case the coefficient of friction does not increase monotonically and therefore the linear relationship is lost.

The boundary regime test shows much smaller values for the dimensionless ratio. The reason for this is the much higher start-up friction coefficient given by the hydrodynamic case (due to the longer stroke length).

Figure 4.13 – Friction coefficient as a function of the interstitial fluid support for (a) representative sample and (b) compared to Krishnan et al.’s work.
4.3.2.6 - Surface characterisation of articular cartilage

By direct observation of the surface of AC after undergoing the shear test, we can clearly see a change in the topography before and after shear testing for both the boundary and the hydrodynamic regimes (Figures 4.9a and 4.9b, Figures 4.10a and 4.10b and Figure 4.12). The direction of the tangential sliding can be identified in Figure 4.12. Several deep “pits” occur after sliding in hydrodynamic conditions as shown in Figure 4.9b. We hypothesise these pits to originate from the shallow pits which can also be seen on the surface of native AC. However, due to the action of normal and tangential loading, the chondrocytes or chondrons are forced away from the contact and the surrounding fluid is exuded out, causing the overlaying material to be expelled out of the contact zone thus forming these deeper “pits”. Hence the depressions which occur in native AC are not removed by loading but are actually made more visible. Other studies have also demonstrated that these surface depressions are not removed by load [285]. These pits, however, are not visible after a boundary regime test (Figure 4.10b). We hypothesise that the difference between the tissue’s surface topography after boundary and hydrodynamic tests is to do with the surface material removal when adopting longer stroke lengths. With the very short stroke length present in the boundary regime’s case, the contact area hardly migrates and hence, the material under the contact cannot be expelled out but instead remains trapped in between the contact. Thus the damage, although expected to be higher than for the hydrodynamic case, remains confined and localised. For the hydrodynamic case, the particles are easily expelled from the surface of the tissue although the level of mechanically induced damage should be, comparatively higher in the sample subjected to the boundary regime test. Although a change in topography occurs after sliding, the average surface roughness is still very similar to a fresh sample (Table 4.2). This has also been demonstrated by other similar studies [269]. There is only a very small increase in the roughness and surface parameters chosen to describe the topography of the specimen before and after loading. This difference is believed to be too small to account for any rise in the friction coefficient both when performing the boundary regime test and when performing repeat static loading tests. Furthermore, although the SAL has been mechanically removed during sliding, studies have demonstrated that it does not actually reduce the friction coefficient [286].

It should also be noted that the surface and roughness parameters obtained in this study for native AC fall within the range of values obtained by other studies where WLI has been used [32, 258]. Furthermore, differences in surface topographies of native AC exist between samples and also depend on the area of the joint from which the sample was harvested. From Figure 4.9(a) and 4.10(a), differences in surface topographies between the native samples can be seen. The difference in surface topography between native AC depending on the knee joint harvesting location is beyond the scope of this study and may constitute part of future research.
4.4 - Numerical investigation

4.4.1 - Results

The numerical investigation was made in order to corroborate, where possible, the experimental findings. The shear test has been simulated using lower loads as mentioned earlier, and with lower frequencies (1 Hz rather than 10 Hz). High frequencies, as well as high loads, cause severe convergence issues. At high frequencies fluid has less time to rehydrate the tissue and hence solid stresses increase at a higher rate causing high deformations and further convergence issues. The choice of loads and frequencies does not, however, prevent the use of the FE analyses for the qualitative understanding of AC behaviour under alternating shear loading. Figure 4.14 shows the direction of the fluid exudation predicted by the FEM after 1s of static loading (a) and after 10 cycles of sliding (b). The test was simulated using a frequency of 1Hz and a stroke length of 2 mm.

Figure 4.14 – Fluid exudation predicted by the FEM after (a) 1s of static loading and (b) after 10 cycles of reciprocating sliding.
Figure 4.14 shows the load shift from the interstitial fluid to the solid constituent of the tissue for a 2 mm and a 250 µm stroke length. The contribution of the solid and the fluid support has been normalized by the contact load. These two terms have been calculated by creating a path in the middle of the AC layer (0.75 cm from the surface) and by integrating the fluid pressure and the solid axial stress at each node along the path. The contact load was obtained by integrating the values of the contact pressure established at the AC/indenter interface. It was decided to analyse fluid support in the middle of the AC layer rather than at the interface between the contact pair to minimise contribution of flow effects.

![Graph showing load shift from fluid to solid constituent](image)

**Figure 4.15** – Load support of solid and fluid constituent of AC during initial 100 cycles at 2 mm and 250 µm stroke length.

Finally, Figure 4.16 shows how the static loading time affects the start-up lateral force and thus the coefficient of friction through the stress relaxation mechanism. Again, the stroke used was 2 mm.
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4.4.2 - Discussion

Figure 4.14a shows that, as soon as the AC is loaded, fluid exudation occurs away from the contact zone. If the static normal load is kept for a longer time, eventually all fluid will be exuded out of the contact zone and the load will be completely supported by the solid matrix. This correlates very well with the increase in the coefficient of friction seen as the static loading time increases.

A boosted lubrication [112] mechanism was predicted by the FEM, as shown in Figure 4.14a, where fluid is pushed by the contacting surfaces into the tissue. Furthermore, a self-generating lubrication mechanism was observed during sliding (Figure 4.14b) as fluid exudes out of both the leading and the trailing edge of the contact, thus providing the fluid essential to replenish the lubricating film. This mechanism could help to explain the low friction coefficient of AC [112].

Figure 4.14b also shows the fluid flow path on the surface during sliding: when the contact area migrates, fluid re-enters the tissue in the previously loaded area. On the other hand, on the lead edge fluid is being exuded out of the tissue. If the sliding velocity is low enough and the stroke length long enough, equilibrium between fluid exudation (flow out of the tissue) and fluid imbibitions (flow into the tissue) can be reached. Equilibrium in terms of hydrostatic fluid pressurisation and fluid support can therefore be attained.

Similar studies have also predicted boosted lubrication rather than weeping lubrication [114]. Although boosted lubrication seems to be supported by most numerical predictions [126], there is still a strong debate whether weeping or boosted mechanism occurs in the natural synovial joint. Further investigations on this topic involving different indenter geometries and loading regimes are required to achieve a better understanding of the dominating lubricating mechanism in different configurations.

In Figure 4.15, the shift in load support from the interstitial fluid to the solid constituent is shown. The curves seem to reach equilibrium after 50 cycles indicating equilibrium between fluid imbibition and

Figure 4.16 – Evolution of the ratio between the lateral reaction force and normal applied load on the indenter due to different static loading times.
fluid exudation rates. This is however not true if the stroke length is reduced to 250 µm. By reducing the stroke length to 250 µm, very little of the contact area is rehydrated. Hence, it becomes very difficult for the fluid to re-enter the tissue in the contact zone and provide some form of pressurisation. This causes a continuous decrease in the interstitial fluid pressurisation until most of the load is actually supported by the solid stresses. The qualitative agreement between the numerical model and the experiments demonstrates that the model is able to capture the behaviour of the tissue although using smaller loads and lower frequencies. However, in order to further confirm these findings, the simulation should be improved by running longer simulations at higher frequencies and loads.

The model is also able to predict the behaviour of the tissue when subjected to static loading for different periods of time prior to the application of the tangential load. Figure 4.16 shows the start-up lateral force (first 10 cycles) on the indenter after different static loading times. The longer the static loading time, the more resistance is encountered by the indenter as it moves tangentially due to the stress relaxation mechanism of AC. After 600s of static loading time, the start-up lateral force on the indenter does not seem to increase further, hence indicating that full stress relaxation occurs at around 600s of static loading time. This time, however, is strongly dependent on the static load applied and thus cannot be corroborated to experimental findings where higher loads have been used.

4.5 - Conclusions

This investigation reports a combined experimental and numerical study of lubrication mechanisms in AC. The effect of different lubrication regimes on the tissue was investigated. Whilst the mixed and the hydrodynamic regime approached a stabilised friction coefficient within very few sliding cycles, the boundary regime test exhibited a gradual and continuous rise in the friction coefficient until a plateau was reached. Damage at the surface of the tissue has been excluded to cause such a dramatic increase in friction, following examination using WLI which did not reveal the presence of significant surface damage. It has thus been postulated that this increase in friction is due to both a transition from mixed to boundary lubrication and to stress relaxation causing an increase in lateral resistance on the indenter. These factors also dictate the increase in the start-up friction coefficient which was observed with increasing static loading times.

It is difficult to quantify which factor dominates and contributes more to the rise in the friction coefficient and further investigation is required to explore this issue.

Using repeat static loading tests, a measure of the interstitial fluid support, \((\mu_1 - \mu_2)/\mu_{eq}\), for the boundary regime test was obtained. A linear relationship between the monotonically increasing coefficient of friction and the new parameter identified to characterise the fluid support was shown. This is in line with the results reported by other researchers [284].
Using FEM, boosted lubrication was predicted after statically loading the tissue and a lubrication mechanism promoted by the biphasic nature of the tissue was predicted during sliding. The gradual load shift from the fluid to the solid constituent of the tissue was also explored for different stroke lengths. Varying the stroke length from 2 mm to 250 µm caused a more rapid loss of interstitial fluid pressure. Furthermore, the effect of stress relaxation on start-up lateral indenter reaction force was investigated. An increase in static loading time was found to increase the lateral reaction force until full stress relaxation occurred. This behaviour is in line with the experimental findings reported in this study.
Chapter 5 – Mechanical and tribological characterisation of native, OA-like and osteoarthritic articular cartilage

5.1 - Introduction

Early diagnosis of OA represents an important objective for the prevention or the reduction of joint disability which is known to affect osteoarthritic patients in the long term. By identifying early signs of the joint disorder, it may be possible for the patient to be treated in a more effective manner and much before the pathology reaches advanced and irreversible stages where invasive surgical procedures such as arthroplasty are, currently, the only viable treatment.

Osteoarthritis is at present impossible to clinically diagnose at a very early stage. At more advanced stages of the pathology, the use of Magnetic Resonance Imaging (MRI) and advanced post-processing methods [156, 287, 288] allows identification of morphological changes which are associated with the disease, such as for example loss of AC thickness in the joint space or abnormal increase in joint bone density. Unfortunately however, these morphological changes are not detectable at early stages of OA where early clinical intervention could be vital in delaying the progression of the pathology.

Morphological changes of AC are due to variations in mechanical properties which are in turn due to changes in the structural and biochemical properties of the tissue. In this respect, changes in biochemical properties, and thus mechanical properties, represents one of the earliest indications of OA [149]. Quantifying changes in biochemical and mechanical properties in the tissue in vivo is extremely challenging although research in this area is ongoing [289, 290]. In order to successfully assess whether AC is indeed affected by OA, however, a detailed understanding of how the mechanical properties vary as a function of biochemical properties is fundamental.

Joints are also not static, and it is important to understand how the tissue behaves under shear in its native (or undamaged) state and once its mechanical properties have been compromised. This would allow us to better understand the onset and progression of damage of the tissue at different stages of
OA. Shear is, in fact, thought to be a major factor in causing mechanical damage to the tissue [291]. The scope of this study is thus to:

1. Correlate different forms of structural damage to the mechanical and frictional response of the tissue in an attempt to better understand the impact of ECM degradation on the mechanical response and wear resistance of the tissue.
2. Compare the mechanical properties of OA-like damage from ex vivo models to real osteoarthritic AC in order to shed light on possible similarities between enzymatically induced damage and OA.
3. Establish a complete structure-function map relating biochemical and structural variations to changes in mechanical properties, tribological performance and damage in the tissue.
4. Investigate the ability of the BPVE model to predict the mechanical response of PG and collagen depleted AC.
5. Investigate the onset and progression of damage in AC as a result of shear loading.

5.2 - Methods

5.2.1 - Experimental methods

In order to study the effect of selective structural damage as well as real OA on the mechanical response of the tissue, both human and porcine AC was tested. Porcine AC was used to obtain properties of healthy native AC and OA-like AC by subjecting it to enzymatic degradation. Human AC, extracted from the tibial plateau of patients undergoing knee arthroplasty as a result of advanced OA, was used to investigate the mechanical response of real osteoarthritic tissue.

Although some differences in mechanical properties between human and porcine AC have been reported to exist [292-294], for part of this study human and porcine AC was assumed to have equivalent mechanical and biochemical properties. This was done in order to allow qualitative but also somewhat quantitative comparisons between OA-like and osteoarthritic tissue and to demonstrate the potential use of a biochemical-mechanical map involving osteoarthritis and OA-like AC.

Full thickness porcine AC plugs were obtained as described in Chapter 3 and either left as native, or subjected to enzymatically induced damage. These were either characterised mechanically using the numerical-experimental method reported in Chapter 3, or tribologically using the HFRR technique also described in Chapter 3. Human osteoarthritic AC was extracted and characterised to obtain relevant mechanical properties. Human AC was extracted from different regions of the tibial plateau, some of which were severely damaged, with visible signs of OA such as fibrillation and abrasion [295], and some of which appeared visually and morphologically healthy.

A total of nine porcine AC samples per level of enzymatic degradation described in Table 3.1 were used. Three of these were used to obtain an indication of the percentage of degradation of ECM components via the method summarised in §3.8.2.
Following enzymatic degradation, the remaining six samples were subjected to mechanical testing. Three of which were used to evaluate mechanical parameters of the tissue using unconfined compression, as described in Chapter 3, whilst the remaining three were characterised tribologically using the HFRR. The surface condition of the samples was characterised by WLI before and after being subjected to shear loading. Moreover, in some of the samples, damage was assessed by measuring the amount of ECM degradation following sliding which was quantified using the DMMB and the hypro assays as described in §3.8.

Thus, the percentage of ECM degradation in the six samples used for mechanical and tribological testing was not directly quantified but assumed to be equal to the average of the three samples used solely for the full damage characterisation. The reason for choosing this approach lies in the fact that quantifying the original amount of GAG and hypro in the individual samples having undergone mechanical testing would have resulted in inaccurate and unreliable values; during mechanical and tribological characterisation, an unavoidable further loss of molecules into the medium occurs. Furthermore, from the time the native AC was extracted to the moment it was subject to enzymatic degradation and mechanical testing, the samples underwent up to two freeze–thaw cycles which could have caused a further release of molecules from the tissue. Uttermost care was taken to ensure a standard size between all AC samples in order to allow for the best possible and reliable comparison.

GAG content is thought to vary according to location in the joint. For this reason, all samples were extracted from the medial and lateral femoral condyle summits of the given knee joints. These sites are thought to have equal or very similar GAG content and are associated with the thickest AC layer and the highest GAG concentration [296, 297].

A total of four human AC samples were assessed for mechanical properties, due to limited availability of these. The samples were extracted from the two tibial plateaus of two different patients. Both tibial plateaus had an Outerbridge OA classification of IV [298, 299] due to the presence of exposed subchondral bone, according to the independent views of two orthopaedic surgeons. Osteochondral plugs were extracted from both visibly damaged areas as well as lightly damaged areas, where the AC was, at least morphologically, almost completely intact. Severely damaged and lightly damaged AC areas were associated with the central aspect and the anterior/posterior aspect of the tibial plateau respectively [300] (Figure 3.2). Using the International Cartilage Repair Society (ICRS) OA classification, the AC plugs were individually classified as ICRS Grade I and III, where Grade 0 and Grade IV represent healthy and osteoarthritic tissue (with severely exposed subchondral bone) respectively.
5.2.2 - Mechanical and tribological characterisation

The mechanical properties of the samples were evaluated using the unconfined compression test and numerical-experimental methods as described in Chapter 3. The testing conditions used for the unconfined compression test were kept constant for all tests. These were:

- Applied vertical strain of 10% of the tissue’s thickness
- Ramp time of 0.5s

A higher strain rate was used as compared to previous studies as this is considered more clinically relevant and comparable to physiological conditions and activities such as walking, where high compressive forces are rapidly applied to the tissue. Furthermore, differences in mechanical response under dynamic conditions between different degraded samples are more evident when using high strain rates, where the viscoelasticity of the collagen fibres dominates the material’s behaviour. Adopting lower strain rates would mean smaller variations in dynamic response and hence, increased difficulty in detecting differences between different levels of degradation of AC samples. Lastly, no studies to our knowledge have used the classic BPVE model in predicting the mechanical behaviour of PG and collagen degraded samples under such high strain rates.

In order to implement the numerical-experimental technique described in Chapter 3, AC was modelled using the BPVE. The permeability of AC was systematically varied to optimize the agreement between experiment and model. This was deemed necessary since the permeability has been found to increase at least six fold in trypsin degraded AC [184]. The Poisson ratio was assigned two different values depending on whether the tissue was native or degraded [184].

The material parameters which were kept constant for the native and degraded tissue are included in Table 5.1.

<table>
<thead>
<tr>
<th>Material Parameter</th>
<th>Native AC</th>
<th>Degraded AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poisson ratio ($\nu$)</td>
<td>0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Initial void ratio ($e_0$)</td>
<td>4.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Permeability coefficient ($M$)</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 5.1 – Material parameters associated to the native and degraded AC models.

These parameters were assumed constant for all native and damage AC compression test models. The reference initial permeability parameter used was $2.82 \times 10^{-14} \text{ m}^4\text{N}^{-1}\text{s}^{-1}$ [92, 200].

As mentioned in Chapter 2, the depletion of PG and collagen does not occur in a uniform fashion. It ranges from completely degraded AC on the surface of the sample, to potentially completely native AC in the core of the specimen, depending upon the duration of time the sample has been exposed and
the respective concentration of the enzymes. Thus, in reality a composite structure of native/degraded AC would actually arise following digestion. In the FEM however, the degradation of the tissue was assumed to be uniform and homogenous throughout the sample. Studies have demonstrated that in trypsin-treated AC plugs, no statistical difference in mechanical response and material parameters existed between inhomogeneous and homogenous digested AC models [184].

5.2.3 - Mechanically induced damage in articular cartilage – preliminary study

Finally, in order to foster some discussion and to open new avenues for future research in this topic, a preliminary study on the effect of ex vivo mechanically induced damage on the mechanical properties of AC was made. The impact of mechanically induced damage on the frictional response and the mechanical properties of AC is an interesting topic which has not yet been thoroughly investigated. To achieve this, long duration shear tests was performed on two native porcine AC samples. Following the test, the tissue was characterised mechanically using unconfined compression. The HFRR standard upper specimen consists of a sphere (see Chapter 3) which creates only local damage on the sample’s surface. In order to evaluate the mechanical response following the test using unconfined compression, the upper specimen had to be modified so as to create a uniform damage across the entire sample’s surface. Thus, a copper flat disc was used as an upper specimen, $R_a = 1 \mu m$, uniformly covering the surface of the specimen during sliding (Figure 5.1). Two AC samples were studied for this investigation.

![Figure 5.1](image)

**Figure 5.1** – Modified HFRR upper specimen to induce homogenous damage on the sample’s surface.
5.3 - Results

5.3.1 - Biochemical characterisation of ex vivo enzymatically degraded AC

The % of degradation of ECM components according to the different levels of enzymatic degradation is included in Table 5.2 below. It was calculated from an average of three porcine samples using the method described in §3.9.2.

<table>
<thead>
<tr>
<th>Enzymatic Degradation</th>
<th>% of degraded ECM components</th>
<th>% of degraded PG</th>
<th>% of degraded Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Low (1)</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Low (2)</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Medium (1)</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Medium (2)</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Medium (3)</td>
<td>60</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>High (1)</td>
<td>91</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>High (2)</td>
<td>100</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>High (3)</td>
<td>100</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>High (4)</td>
<td>100</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 5.2 – Estimate of ECM component degradation in OA-like AC.

A representative image of a degraded sample’s cross section obtained using an optical microscope is shown in Figure 5.2.

![Figure 5.2](image)

5.3.2 - Mechanical and tribological properties

5.3.2.1 - Mechanical properties of native and OA-like articular cartilage

The mechanical parameters obtained using the numerical-experimental technique applied to the stress relaxation tests are summarised in Table 5.3. These parameters were obtained from an average of three AC plugs per level of enzymatic degradation. The tested samples had a wide range of
Chapter 5 – Mechanical and tribological characterisation of native, OA-like and osteoarthritic articular cartilage

thicknesses, spanning from 1.6 mm up to 3.1 mm in some circumstances. However, the average samples thickness was of approximately 2.3 mm. Samples corresponding to High (1) and High (3) were only tested in shear and no mechanical properties have been evaluated. The stress relaxation response was found to be insensitive to variations in permeability of the Medium (3) and High OA-like samples. The permeability was hence taken to be the same for the Medium (3) and the High samples.

<table>
<thead>
<tr>
<th>Enzymatic Degradation</th>
<th>Young’s Modulus (MPa)</th>
<th>Continuous Spectrum Viscoelastic Parameters</th>
<th>Permeability ($10^{-14}$ m$^4$/Ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>0.50 ± 0.10</td>
<td>1.49 ± 0.49</td>
<td>2.88 ± 0.06</td>
</tr>
<tr>
<td>Low (1)</td>
<td>0.18 ± 0.04</td>
<td>2.16 ± 0.68</td>
<td>2.05 ± 0.39</td>
</tr>
<tr>
<td>Low (2)</td>
<td>0.09 ± 0.04</td>
<td>1.02 ± 0.38</td>
<td>2.25 ± 0.11</td>
</tr>
<tr>
<td>Medium (1)</td>
<td>0.04 ± 0.01</td>
<td>525.9 ± 161</td>
<td>1.25 ± 0.09</td>
</tr>
<tr>
<td>Medium (2)</td>
<td>0.03 ± 0.01</td>
<td>1672 ± 1369</td>
<td>0.73 ± 0.08</td>
</tr>
<tr>
<td>Medium (3)</td>
<td>0.04 ± 0.01</td>
<td>5806 ± 2141</td>
<td>0.99 ± 0.31</td>
</tr>
<tr>
<td>High (2)</td>
<td>0.06 ± 0.02</td>
<td>4234 ± 1988</td>
<td>0.70 ± 0.22</td>
</tr>
<tr>
<td>High (4)</td>
<td>0.03 ± 0.02</td>
<td>6768 ± 1628</td>
<td>0.40 ± 0.17</td>
</tr>
</tbody>
</table>

**Table 5.3** – Mechanical parameters of native and OA-like AC.

For completeness, the discrete spectrum viscoelastic constants are also included in Table 5.4:

<table>
<thead>
<tr>
<th>Enzymatic Degradation</th>
<th>Discrete Spectrum Viscoelastic Parameters</th>
<th>Permeability ($10^{-14}$ m$^4$/Ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$G$</td>
<td>$G_0$</td>
</tr>
<tr>
<td>Native</td>
<td>2.45 ± 0.79</td>
<td>10.15 ± 2.93</td>
</tr>
<tr>
<td>Low (1)</td>
<td>3.58 ± 1.16</td>
<td>13.81 ± 4.37</td>
</tr>
<tr>
<td>Low (2)</td>
<td>3.25 ± 0.27</td>
<td>12.29 ± 2.50</td>
</tr>
<tr>
<td>Medium (1)</td>
<td>23.32 ± 12.37</td>
<td>1.31 ± 0.22</td>
</tr>
<tr>
<td>Medium (2)</td>
<td>43.61 ± 21.43</td>
<td>45.11 ± 21.51</td>
</tr>
<tr>
<td>Medium (3)</td>
<td>80.80 ± 38.70</td>
<td>82.70 ± 43.34</td>
</tr>
<tr>
<td>High (2)</td>
<td>93.87 ± 30.80</td>
<td>95.87 ± 31.02</td>
</tr>
<tr>
<td>High (4)</td>
<td>602.38 ± 319.28</td>
<td>631.20 ± 123.41</td>
</tr>
</tbody>
</table>

**Table 5.4** – Discrete viscoelastic parameters of native and OA-like AC.

A control group consisting of three AC samples incubated for a period of 10 days with no proteases, was evaluated both biochemically and mechanically. No statistically relevant change in biochemical content and mechanical response was observed. Representative tests showing the raw experimental stress relaxation curves of the unconfined compression tests on native AC and degraded AC are
shown in Figures 5.3a-c for Native, Low (2) and Medium (1) AC samples. The response of the best-fitted BPVE and BNLE numerical models are also shown for comparison.

**Figure 5.3** – Experimental and numerical model stress relaxation tests of (a) native and OA-like porcine AC sample with a level of degradation corresponding to (b) Low (2) and (c) Medium (1).
5.3.2.2 - Mechanical properties of osteoarthritic articular cartilage

The mechanical properties of human osteoarthritic AC are included in the table below. Two samples (due to limited availability of OA human joints which could be used to extract samples) per ICRS grading were evaluated.

<table>
<thead>
<tr>
<th>ICRS OA Grade</th>
<th>Young’s Modulus (MPa)</th>
<th>Continuous Spectrum Viscoelastic Parameters</th>
<th>Permeability ((10^{-14} \text{m}^4/\text{Ns}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(C)</td>
<td>(\tau_s (s))</td>
</tr>
<tr>
<td>I</td>
<td>0.57 ± 0.03</td>
<td>0.42 ± 0.09</td>
<td>0.10 ± 0.08</td>
</tr>
<tr>
<td>III</td>
<td>0.15 ± 0.08</td>
<td>123.4 ± 52.1</td>
<td>0.72 ± 0.11</td>
</tr>
</tbody>
</table>

Table 5.5 – Mechanical parameters of the tested osteoarthritic human AC specimens.

The discrete viscoelastic parameters are also included in Table 5.6.

<table>
<thead>
<tr>
<th>ICRS OA Grade</th>
<th>Discrete Spectrum Viscoelastic Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\bar{G})</td>
</tr>
<tr>
<td>I</td>
<td>0.70 ± 0.71</td>
</tr>
<tr>
<td>III</td>
<td>1.63 ± 0.71</td>
</tr>
</tbody>
</table>

Table 5.6 – Discrete Spectrum viscoelastic parameters of the tested human osteoarthritic AC samples.

The range of the coefficients of determination for the reaction force of each sample category is included below in Table 5.7.

<table>
<thead>
<tr>
<th>Enzymatic Degradation</th>
<th>Reaction Force Coefficient of Determination ((R^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Best)</td>
</tr>
<tr>
<td>Native</td>
<td>0.92</td>
</tr>
<tr>
<td>Low</td>
<td>0.91</td>
</tr>
<tr>
<td>Medium</td>
<td>0.74</td>
</tr>
<tr>
<td>High</td>
<td>0.52</td>
</tr>
<tr>
<td>Human ICRS Grade I</td>
<td>0.90</td>
</tr>
<tr>
<td>Human ICRS Grade III</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Table 5.7 – Reaction force coefficients of determination of native, OA-like and osteoarthritic AC.
5.3.3 - Tribological properties of native and OA-like articular cartilage

The frictional response of native and OA-like AC is shown in Figure 5.4. The curves are representative tests whilst the error in $\mu_{eq}$ was computed from three samples per level of enzymatic degradation. As discussed in Chapter 3, the test was carried out at a constant load of 6N, 250 µm stroke length and 10Hz frequency and for the duration of 1 hr. The static loading time prior to commencing the test was 5s for all samples.

![Figure 5.4](image_url)  
**Figure 5.4** – Representative frictional response curves of native and OA-like porcine AC.

Figure 5.5 shows representative WLI images of native and OA-like AC before and after subjecting the samples to shear loading.
Table 5.8 summarises the average surface parameters obtained using WLI. The parameters were obtained from 3 samples per level of enzymatic degradation. As performed for the shear loading study described in Chapter 4, 6 surface measurements per sample were made and an average was taken. For the samples which had undergone shear loading, the WLI measurements were taken on the area of the sample which had been subjected to shear.

<table>
<thead>
<tr>
<th>Enzymatic Degradation</th>
<th>Surface Parameters (Before Shear Testing)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_a$ (µm)</td>
</tr>
<tr>
<td>Native</td>
<td>0.50 ± 0.11</td>
</tr>
<tr>
<td>Low (1)</td>
<td>0.53 ± 0.18</td>
</tr>
<tr>
<td>Low (2)</td>
<td>0.79 ± 0.21</td>
</tr>
<tr>
<td>Medium (1)</td>
<td>1.05 ± 0.22</td>
</tr>
<tr>
<td>Medium (2)</td>
<td>1.66 ± 0.26</td>
</tr>
<tr>
<td>Medium (3)</td>
<td>1.54 ± 0.31</td>
</tr>
<tr>
<td>High (1)</td>
<td>2.11 ± 0.42</td>
</tr>
<tr>
<td>High (2)</td>
<td>1.61 ± 0.34</td>
</tr>
<tr>
<td>High (3)</td>
<td>1.57 ± 0.37</td>
</tr>
<tr>
<td>High (4)</td>
<td>2.44 ± 0.40</td>
</tr>
</tbody>
</table>
Table 5.8 – Surface parameters of native and OA-like AC (a) before and (b) after shear testing.

For three of the samples, damage was also assessed by characterising the release of molecules in the PBS bath following shear testing and comparing it to the original quantity. The original quantity was assumed to be equal to the quantity of ECM calculated to evaluate the % of degradation of ECM components in Table 5.2. The % of ECM damage following shear is reported in Table 5.9.

Table 5.9 – ECM damage after a 1 hr shear test at standard conditions.

5.3.4 - Mechanically induced damage in articular cartilage – preliminary results of mechanical and tribological properties

Figure 5.6 shows a representative frictional response of an AC sample subjected to a 4 hour shear test, at a stroke of 1mm and a load of 10N using the HFRR, corresponding to a contact pressure of 0.13MPa. Although not physiological, this contact pressure was the maximum which could be achieved using the HFRR and this contact configuration, as the rig is limited to a maximum of 1Kg of applied vertical mass.
Chapter 5 – Mechanical and tribological characterisation of native, OA-like and osteoarthritic articular cartilage

Figure 5.6 – Representative frictional response test of native AC after 144000 sliding cycles.

The surface parameters and WLI images are included in Table 5.10 and Figure 5.7 respectively.

<table>
<thead>
<tr>
<th>Surface Parameters (After Shear Testing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Ra$ (µm)</td>
</tr>
<tr>
<td>0.69 ± 0.18</td>
</tr>
</tbody>
</table>

Table 5.10 – Surface parameters following shear testing.

Figure 5.7 – WLI image following shear testing.

The mechanical parameters of the sample evaluated following the shear test are included in Table 5.11.

<table>
<thead>
<tr>
<th>Young’s Modulus (MPa)</th>
<th>Continuous Spectrum Viscoelastic Parameters</th>
<th>Permeability $(10^{-14} m^4/Ns)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 ± 0.1*</td>
<td>1.86 ± 0.84</td>
<td>0.33 ± 0.09</td>
</tr>
</tbody>
</table>

*Not statistically significant as only two tests have been performed.

Table 5.11 – Mechanical parameters of AC subjected to 144000 sliding cycles.
The discrete viscoelastic parameters are also reported below in Table 5.12.

<table>
<thead>
<tr>
<th>$\tilde{G}$</th>
<th>$G_0$</th>
<th>$N_d$</th>
<th>$\tau_2 (s)$</th>
<th>$g_1$</th>
<th>$g_2$</th>
<th>$g_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.02 ± 0.84</td>
<td>11.27 ± 3.26</td>
<td>2.41 ± 1.12</td>
<td>5.2 ± 3.46</td>
<td>0.378 ± 0.08</td>
<td>0.265 ± 0.13</td>
<td>0.268 ± 0.02</td>
</tr>
</tbody>
</table>

**Table 5.12** – Discrete viscoelastic parameters of AC subjected to 144000 sliding cycles.

### 5.4 - Discussion

In this study, selective degradation of the ECM was used in order to reveal the effect of PG and collagen damage on the mechanical and frictional response as well as on the progression of damage due to shear loading in AC. A comparison with real human osteoarthritic AC was made to estimate its structural damage according to the porcine AC structure-function map. The main aim of this work was to lay the foundations for a protocol relating mechanical properties to damage and finally OA grading as well as to better understand the effect of degradation on mechanical and frictional properties of the tissue. A preliminary comparison between the mechanical response of AC subjected to enzymatically and mechanically induced damage has also been carried out; however, a more exhaustive test programme is required to shed light on similarities and dissimilarities between the two processes and potential implications for the assessment of OA. This will constitute the object of future investigations.

#### 5.4.1 - Mechanical properties

**5.4.1.1 - Mechanical properties of native and OA-like articular cartilage**

The Young’s modulus of native porcine AC (mean ± S.D.), 0.50 ± 0.10 MPa, as well as the viscoelastic parameters (Table 5.3) were found to be almost identical or in line with previous studies [184, 292, 293, 301]. The permeability of native AC was also found to be very similar to the values previously estimated by other researchers [92]. Figure 5.3(a) shows a representative stress relaxation curve for native AC. The curve clearly demonstrates the peak caused by the viscoelastic effects corresponding to the high applied strain rate, followed by relaxation until equilibrium conditions are reached. Equilibrium conditions are associated with no interstitial fluid pressurisation and a stabilised reaction force dictated by the elastic properties of the tissue. Although full relaxation (corresponding to tissue response fully in the elastic regime) occurs after approximately 1000s according to the reaction force predicted by the FEM of native AC, the curve fitting procedure was performed until 1000s following compression. Further variations in reaction force were beyond the resolution of the experimental set up and were hence considered negligible.
With PG depletion, the Young’s modulus was found to be strongly affected; only a 10% loss of PGs from the tissue yielded a 64% drop in stiffness (Table 5.3), as shown by the Low (1) sample. PGs are thought to be the ECM constituent primarily responsible for the compressive stiffness of the tissue. As demonstrated in several studies, the primary effect of PG depletion is a decrease in the equilibrium stiffness of the tissue [182, 184, 261]. This is probably due to the loss of the high repulsive forces, which exist when PGs are tightly packed in the ECM. The loss of PGs is believed to mainly affect the equilibrium and static part of the mechanical response. However, although indirectly, PGs also affect the dynamic response of the tissue. In fact, the dynamic response of AC is thought to be governed by the collagen fibres, especially in the superficial zone, as well as interstitial fluid pressurisation, which supports the initial load application. If permeability increases due to loss of PGs then a subsequent decrease in interstitial fluid pressurization and hence in the instantaneous stiffness of AC occurs. The permeability in the PG depleted sample showed 10-fold rise compared to the native AC. This value seems to be higher than previously recorded; however, it follows the general trend of permeability increasing with PG depletion [133, 184, 302, 303].

Figure 5.3b shows a representative stress relaxation test of the sample Low (2). By comparison with the native AC response in Figure 5.3a, sample Low (2) shows a drop in the peak reaction force followed by an increased relaxation rate and, finally, lower equilibrium stiffness. This behaviour is typical of PG depleted AC [133, 182, 184, 261]. However, it is worth noting that the reaction force does not provide a meaningful means of comparison due to the difference in thickness between samples.

The Low OA-like samples were subjected to a very low concentration of trypsin. Yet, the effect on the equilibrium properties was significant. Although this could seem surprising prima facie, several studies have demonstrated that PGs in the superficial zone of AC are the primary determinants of the equilibrium properties of the tissue [133, 171, 182, 188, 304, 305]. In the current study it is thought that the proteases in the Low (1) sample have mainly degraded the superficial zone since not enough time was allowed for the enzymes to penetrate into the deeper regions of the tissue. This would explain the significant decrease in the elastic modulus of sample Low (1). In Figure 5.2, a cross section of the upper part of the sample Medium (1) is shown. From the image, degraded AC seems be present only in the first 250 µm of the tissue’s depth. A depth of 250 µm from the surface corresponds only or mostly to the superficial zone of the tissue, depending on the sample’s thickness.

With regards to the viscoelastic properties of sample Low (1), PG depletion confirms an expected increase in permeability and relaxation rate as well as a decrease in dynamic stiffness (Table 5.3). This is associated with an increase in the magnitude of the relaxation power spectrum, $\tilde{c}$, and the related discrete spectrum parameters, $\tilde{G}$ and $G_0$ and a decrease in the short term and long term relaxation times, $\tau_s$ and $\tau_l$ respectively (Table 5.4). These findings provide yet further evidence that
PGs, in the superficial zone in particular, are also in part responsible for the mechanical viscoelastic behaviour of the tissue [182, 184, 261].

Further PG depletion, but no collagen degradation, as present in samples Low (2) to Medium (2), results in a further decrease in equilibrium stiffness yielding a 94% decrease in Young’s modulus when ~100% PG has been depleted (Tables 5.2 and 5.3). This demonstrates that the collagen fibre network has a minimal role in supporting compressive loads compared to PGs. Consistent with expectations, additional PG depletion also causes an increased relaxation rate and a decrease in dynamic stiffness, as shown from Figure 5.3(c). Over a 50% depletion in PG content, as seen from sample Medium (1), does not seem to cause any significant additional drop in equilibrium stiffness reaching a plateau of ~0.04 MPa in Young’s modulus (Table 5.2). This could suggest that, due to the way in which the proteases degrade the tissue, from the surface towards the centre, indeed the main part of the tissue governing the equilibrium response of the tissue is the superficial zone. The permeability is seen to severely increase as more PG is depleted although the largest step increase in permeability occurs from the initial native to Low (1).

Understanding the effect of PG depletion on the mechanical behaviour of the tissue is particularly important, especially if the depletion occurs mainly in the superficial layer. OA initiation is in fact thought to be associated with loss of PGs from the superficial layer of AC when the collagen content has not yet been altered [306].

As the collagen starts to degrade together with the PGs, as in sample Medium (3), the dynamic response of the tissue seems to be strongly affected. This is demonstrated by a significant drop in the dynamic stiffness followed by an increased relaxation rate whilst the equilibrium properties, however, remain largely unvaried (Table 5.3). The equilibrium properties remain constant due to the PGs which, having already been significantly depleted, have caused the Young’s modulus to reach a plateau of ~0.04 MPa. It is speculated that the 5% degradation in collagen content of sample Medium (3) mainly represents degradation of superficial collagen, which as previously mentioned, is a major contributor of the dynamic response of the tissue. The tightly arranged collagen network in the superficial zone restricts fluid exudation from the tissue as well as restraining the swelling pressure of the PGs [34]. If this network is disrupted following degradation [307] and the fibres lose tensile strength [188], the permeability increases explaining the increased relaxation rate [173]. Further collagen degradation, as in samples High (2) and High (4), results in a decrease in the dynamic stiffness and an increased in relaxation rate. In this investigation as well as in previous studies, collagen degradation, especially following PG depletion, has been found to create a step change in permeability as compared to PG depletion alone, as in sample Medium (3) [133, 303]. It has been hypothesized that this is due to the reduction of the electrostatic forces that arise between the collagen network and the PGs as well the induction of secondary PG loss. Another reason for the increase in permeability could be due to the decrease in resistance to fluid-flow as a result of the widening of the gaps between the collagen fibres, which is especially true for the superficial zone of
the tissue [79]. The permeability in the High-type samples was assumed to be equal as it could not be accurately calculated since the intrinsic viscoelastic values of these samples, such as the stress relaxation rate, were extremely high, causing the dynamic response to be largely insensitive to variations in permeability.

The final small decrease in Young’s modulus in sample High (4) (Table 5.3) might be due to the very high disruption of the collagen network in the deeper zones of the tissue, which, due to the high level of degradation, is no longer able to withstand compressive loads.

In summary, the results have confirmed the role of the PGs in governing the equilibrium compressive stiffness, the collagen the instantaneous dynamic compressive response and the fluid the stress relaxation of the tissue. The severely degraded samples showed altered viscoelastic effects due to a possible strong non-linearity of the tissue and were associated with a very high relaxation rate, masking the effect of the fluid response.

### 5.4.1.2 - Mechanical properties of osteoarthritic articular cartilage

Two different stages of osteoarthritic AC were evaluated in terms of mechanical properties; ICRS Grade I and III. The Young’s modulus of native human AC is thought to lie between 0.6 – 0.8 MPa. Assuming a healthy human AC Young’s modulus of ~0.60 MPa, obtained from an average of literature values [135, 292, 294], then according to the mechanical parameters obtained, Grade I AC, with a Young’s modulus of 0.57 MPa (Table 5.5), lies on the border between native AC and very early stage osteoarthritic tissue. This value is in the range of previous studies which have classified ICRS grading and mechanical properties [308]. However, it is difficult to distinguish between healthy and early ICRS Grade I AC via the comparison of the Young’s modulus alone. Thus, a range of mechanical parameters such as viscoelastic time constants should be used to further assess the level of OA. This, however, necessitates further studies involving the characterisation of healthy human AC. It is worth noting that at extremely early stages of the pathology, a noticeable and reliable difference in mechanical parameters between healthy and early osteoarthritic tissue could be very difficult to grasp. Nevertheless, identifying OA via a variation of mechanical parameters would still offer the potential of diagnosing OA much earlier than using morphological changes alone, at which stage the joint function could already be compromised.

The Young’s modulus and the long term relaxation time of native, OA-like AC and human osteoarthritic OA is visualised in Figure 5.8 for ease of comparison. The comparison is purely used to show the similar trends in mechanical properties as a result of damage in both OA-like and osteoarthritic tissue. This “stiffness plateau” discussed in the previous section can be easily seen in Figure 5.4a, which shows a practically constant value in Young’s modulus from Medium (1) to higher levels of degradation. The dotted line separates the results between animal and human AC.
Chapter 5 – Mechanical and tribological characterisation of native, OA-like and osteoarthritic articular cartilage

From Figure 5.8a and Table 5.5, it is evident that a drastic reduction in the quality of mechanical properties has occurred in the sample with ICRS Grade III. The Young’s modulus has decreased by 74% followed by a significant increase in stress relaxation rate, seen from the decrease in the long term relaxation time of approximately 99% as compared to the ICRS Grade I sample (Figure 5.8b). The elastic and viscoelastic parameters give a strong indication that the AC specimen is definitely damaged, it has undergone severe biochemical and biomechanical changes and it is certainly osteoarthritic. The sample has clearly undergone some form of PG depletion due to the decrease in equilibrium stiffness. The severe decrease in the long term relaxation time also suggests that the collagen has undergone severe disruption too. As previously discussed in §2.7.1, collagen dictates the intrinsic viscoelastic behaviour of the tissue and hence part of the relaxation behaviour. Thus, it is...
vital to use more than one mechanical parameter to assess the level of structural damage in AC. The Young’s modulus is only capable of grasping the elastic behaviour of the tissue but the dynamic response, represented by the viscoelastic parameters, also holds fundamental information on the mechanical quality of the tissue. This is why for a complete characterisation, several mechanical parameters which consider both the elastic and the viscoelastic response should be used to accurately assess the overall damage in the tissue. It is envisaged that a quantitative assessment of elastic, viscoelastic and permeability parameters could be used to establish the level of structural and damage and thus the osteoarthritic stage of the tissue.

The Young’s modulus value of 0.15 MPa is in the bottom range of what has previously been reported for ICRS Grade III [308]. The very high permeability obtained for the Grade III sample, similarly to the High OA-like samples, was due to the intrinsic viscoelastic effects which masked the fluid response upon relaxation, making the model largely insensitive to permeability variations. The results obtained from the osteoarthritic tissue confirm a decrease in mechanical stiffness and mechanical quality with increasing ICRS grading and provides yet more evidence that osteoarthritic changes of AC involve mechanical changes of the tissue as a result enzymatic degradation of the ECM [309].

A deep understanding of the relationship between mechanical and biochemical properties of AC at different stages of OA could allow us to predict the biochemical composition of the tissue and hence the OA stage, simply by measuring mechanical properties. With this in mind, assuming the native elastic and viscoelastic parameters of human AC to be similar to native porcine AC, we could have a general understanding of the level of structural damage present in the human osteoarthritic tissue. It is worth noting, however, that this assumption is necessary to demonstrate a method, described below, which one could use when using mechanical properties to obtain the level of damage in the tissue. Clearly, as previously mentioned, differences between animal and human do exist and for an accurate comparison the same species should be considered.

Thus, as equivalence in mechanical properties is assumed between human and animal AC, we can estimate the amount of structural damage in human osteoarthritic AC. According to the mechanical parameters of the osteoarthritic tissue and depending upon which reference material parameter to use for OA-like AC, the damage can be estimated and quantified. As an example, by taking the Young’s modulus of ICRS Grade III which is 0.18 MPa, it falls within the range of Low (1) to Low (2) OA-like AC indicating between 10% and 22% of PG depletion and no collagen degradation. This is clearly just an estimate and in reality, disruption to the collagen network is present as it appears in the form of fibrillation on the tissue’s surface. Unfortunately however, the ICRS Grade III falls within the trypsin treated samples of this experimental study which have no collagen degradation. Future studies could involve smaller concentrations and smaller amounts of exposure time to MMP-1 to yield less PG depletion and some collagen degradation for a more realistic comparison. In fact, due to the mechanisms of action of MMP-1, it tends to degrade collagen type II at a much higher rate than it
would otherwise deplete PG. However, the overall aim of this study was to induce significant damage on the sample and to isolate in the best possible manner PG depletion and collagen degradation in order to investigate the distinct effects of both types of degeneration on the mechanical response. When using MMP-1, collagen cannot degrade without also depleting some PG thus making it more difficult to understand the structure-function relationship of the tissue. However, the use of this enzyme was necessary if a protease truly responsible for OA was to be used to simulate OA.

The relationship between % of PG depletion (Table 5.2) and the Young’s modulus (Table 5.3) is reported below in Figure 5.5. Only samples degraded using trypsin, and hence which have no collagen degradation, have been included to yield the following curve.

![Figure 5.9](image.png)

**Figure 5.9** – Relation between Young’s modulus and PG depletion.

Hence, by choosing the Young’s modulus, $E$, as the reference material parameter for comparison, the degradation of the ICRS Grade III AC is estimated to be 13.4% of PG depletion from Figure 5.5. Although some of the viscoelastic parameters of ICRS Grade III AC, such as the long term relaxation time, seem to be in the region of OA-like AC samples Medium (2) - High (2), where collagen degradation has started to occur, it is not possible to estimate the degradation accurately due to the lack of native human AC viscoelastic data. However, with a greater understanding of the key proteases active in AC degeneration, this protocol could, with further research on human AC, be extremely useful. It could guide the development of improved correlations between structure and function in a way which has as of now not yet been explored before; using either mechanical or biochemical parameters of osteoarthritic AC, potentially obtained in a clinical setting *in vivo*, and comparing these to an OA-like biochemical-mechanical parameter map in order to estimate and quantify the level of structural damage of the osteoarthritic tissue.
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Using selective ECM degradation to investigate its effect on the mechanical properties is a valuable method for investigating the structure-function relationship of the tissue. However, one of the limitations of this present study is associated with the small number of tests performed on human osteoarthritic AC, which consisted of only four AC samples from two donors. Hence, a more rigorous study on osteoarthritic tissue should be envisaged in order to more accurately correlate the OA-like mechanical properties to the osteoarthritic mechanical properties. Furthermore, the amount of PGs and collagen should be quantified in the human osteoarthritic tissue to truly validate a trypsin / MMP-1 OA simulation.

5.4.1.3 - Numerical model suitability in predicting the mechanical response of native and degraded articular cartilage and experimental limitations

In this investigation, significant statistical differences of BPVE material parameters between native, OA-like AC and osteoarthritic AC were found indicating that numerical models have indeed the potential to be used as a tool for investigating AC degradation. The suitability of the BPVE model in predicting mechanical behaviour of native AC was confirmed also for high strain rates, where viscoelasticity plays an important role in the mechanical response. This is confirmed by the relatively high $R^2$ values of native AC. From Figure 5.3a, the BNLE model is, however, clearly unable to predict the dynamic response of the tissue but only the static response as it lacks the intrinsic viscoelastic components needed to predict the peak reaction force of the unconfined compression test.

However, looking at the values reported in Table 5.7, some variability in $R^2$ was found also using the BPVE model for native AC, indicating the presence of some inaccuracy in the prediction of reaction force by the numerical model. This is most likely due to minor differences between the model and the experimental configuration rather than due to the constitutive model itself, as the BPVE model has already been deemed suitable for the prediction of mechanical response of native as well as PG depleted AC using unconfined compression [140, 184, 301, 310].

The samples used for this investigation were considerably larger compared to those used in the work presented by other researchers in the literature. Obtaining a uniform contact between the upper cartilage surface and the platen was difficult due to the sample’s curvature and geometrical inhomogeneity. Thus, fluid could have permeated out of the upper surface as well as from the sides, which the model assumes to be impermeable, yielding an overestimation in permeability and stress relaxation rate. The sample geometry could also be one of the reasons for the higher than expected permeability value. Although the sample’s dimensions in terms of thickness were measured as accurately as possible, an average thickness was taken; this could have also yielded potential discrepancies between the model and the experiment. Furthermore, the samples tested had a relatively
high range of thickness which could have potentially caused some further discrepancy when comparing the mechanical response.

The BPVE model proved to be successful in predicting the drop in dynamic and equilibrium stiffness as well as the increased relaxation rate as a result of PG depletion. An acceptable curve fit was found in parts of the Low level of enzymatic degradation yielding $R^2$ values of up to 0.91.

However, as already observed in previous studies [184], significant variability existed in the $R^2$ values of degraded AC. A possible explanation for this is the non linearity present in some of the samples due to the alteration of the stress/strain curve caused by enzymatic degradation [184]. The variability of $R^2$ severely increased as more PG was depleted and especially as collagen started to degrade such as in the Medium (3), High OA-like samples and for the ICRS Grade III samples.

As the degradation increased, a consistent overestimation of the peak response in reaction force and in the stress relaxation rate was observed. This is already visible from sample Medium (1) in Figure 5.3c, which shows a slight ‘overshoot’ of the peak response by the BPVE model.

An even more severe overestimation of the peak response was observed as collagen was degraded highlighting the need to consider the compression-tension non linearity of the sample if a more advanced constitutive model was to be implemented to characterise damaged AC [133, 144, 206, 255]. The incorporation of compression-tension non linearities together with viscoelasticity has been found to better predict the dynamic modulus of AC, and this is thought to play an important role in the load support. The lack of this material description could explain the large deviation between experiments and BPVE fitted models for highly degraded AC. Previous studies have demonstrated that models such as the fibril reinforced poroelastic model which incorporates compression-tension non linearity, implemented as part of our previous work [201], is able to predict the stress-relaxation behaviour of PG depleted or collagen degraded AC [133].

Hence, in this investigation it was found that the BPVE model was able to predict the reaction force of degraded AC up until approximately 50% PG depletion (sample Medium, (1)). Further degradation seems to be beyond the capabilities of the BPVE model and the parameters describing the viscoelastic nature of the tissue should not be considered entirely trustworthy.

In this respect, the permeability of the degraded samples was also found to be significantly higher than previously reported in the literature. This could be due to the long incubation times employed in this investigation, which potentially caused the degradation of a much larger volume of tissue, thus yielding to higher permeability. However, the permeability values for the highly degraded samples, from Medium (1) onwards, should also not be considered trustworthy as the non-linear nature of the problem might have contributed to masking the effect of the fluid response. Permeability values should accurately be measured using a porous platen and confined compression experimental configurations.

Finally, some limitations of the experimental set up exist. The MCSTR was initially designed to test AC under physiological compressive loads, mimicking contact pressures present in the natural
joint. Testing AC in unconfined compression involved much lower applied loads, meaning that the rig was not operating under optimal conditions. This implied a low signal-to-noise ratio rendering curve fitting more difficult at very low loads. This was especially true for highly degraded AC which showed very low compressive stiffness. Testing these type of samples sometimes resulted in instability, requiring adjustments to the gain/damping functions of the control box.

A further limitation of the experimental setup was that it did not allow measurement of lateral displacements. This meant that a complete BPVE model validation with experimental data could not be carried out.

5.4.2 - Tribological properties of OA-like articular cartilage

The tribological properties have been found to be severely affected by PG depletion and collagen degradation. From Figure 5.4, a considerable difference in frictional response can be seen between native and the various OA-like AC samples.

As previously discussed in Chapter 4, the frictional response of native AC is associated with a continuous and gradual rise of the friction coefficient, represented by \( \mu_{\text{eff}} \), denoting the loss of fluid support as the load shift mechanism occurs. The coefficient eventually stabilizes and reaches equilibrium as it tends to full boundary lubrication which results in complete loss of fluid support. This portion of the curve is represented by the equilibrium friction coefficient, \( \mu_{\text{eq}} \). A third friction coefficient termed start-up friction coefficient, \( \mu_0 \), can be used to describe the friction coefficient immediately after starting the test.

As PG is depleted, the change in mechanical response of AC has been associated with a loss of dynamic and equilibrium compressive stiffness due to the loss of interstitial fluid pressurisation and the loss of the tightly packed PG structure which is responsible for supporting compressive loads.

In terms of frictional response, a low PG loss in AC is associated with a rise in \( \mu_{\text{eq}} \) as shown from samples Low (1) and Low (2) in Figure 5.4; this is confirmed in other similar studies [33, 311, 312]. At equilibrium, the lubrication mechanism consists of full boundary lubrication as the interstitial fluid pressurisation has dropped to a negligible amount and the compressive load is supported by the solid constituent of the tissue [99]. The increase in \( \mu_{\text{eq}} \) following PG depletion in samples Low (1) and Low (2) cannot therefore be a consequence of the effect of the fluid component but it is more likely due to variations of the ECM’s composition in the superficial layer. It is thought that the digestion of GAGs on the AC surface causes a decline in the lubricating action of the surface, thus influencing the frictional response [33, 311]. The increase in \( \mu_{\text{eff}} \) associated with these samples, could be due to the increased ploughing friction effect in PG depleted AC. This could be caused by the loss of compressive stiffness of the tissue, which in turn causes an increased penetration of the sphere into the sample and hence a greater resistance to motion. Similar studies have, however, shown
an increase also in a flat plate sliding configuration which is most probably attributable to loss of fluid support [311].

A comparison of interstitial fluid pressurisation between samples can be made by analysing the time taken for the friction coefficient to reach equilibrium conditions. By comparing the frictional response of Low (1) and Low (2) tests to the native tissue, no significant differences in $\mu_{eff}$ are observed although the mechanical parameters have indicated a significant increase in permeability and a drop of interstitial fluid support. A possible reason for the lack of variation of $\mu_{eff}$ between native and Low samples could be a potential “masking” effect of the frictional response caused by the decreased compressive stiffness of the tissue and the increased ploughing friction effect, although this would have to be verified with further studies.

As further PG depletion occurs following sample Low (1), the equilibrium coefficient gradually decreases although always remaining above the native tissue $\mu_{eq}$, so long as the collagen is not degraded, at which point $\mu_{eq}$ drops below the native’s tissue equilibrium response (sample Medium (3)).

OA-like AC Medium (1) represents a step change in the frictional response compared to the Low enzymatic degradation. In sample Medium (1), an early loss of interstitial fluid support is clearly visible from the shortening of the time taken to reach equilibrium conditions. Medium (2) and especially Medium (3) grade OA-like AC show a dramatic loss of interstitial fluid pressurisation as equilibrium conditions are reached after around 600s compared to native AC where the equilibrium plateau starts at approximately 1200s after commencing the shear test.

Gradually, as collagen is degraded, $\mu_{eq}$ drops below the native tissue’s equilibrium coefficient and decreases with increasing level of collagen degradation. It is hypothesized that this further decrease in the equilibrium coefficient as a result of collagen degradation is due to the formation of digested collagen in the form of gelatin on the surface of the sample. The formation of gelatin as a result of MMP-1 degradation has not been reported in in vivo studies; if it were to form as a result of enzymatic activity, it would probably be swept away instantaneously.

In the High samples, $\mu_{eff}$ is almost nonexistent as the tissue is no longer capable of sustaining interstitial fluid pressurisation due to both the lack of PGs as well as the disruption of the tightly packed collagen network which increases the tissue’s permeability. At this stage, the compressive load during shear is supported by the now structurally and mechanically altered ECM. At this level of degradation, an abnormal increase in damage and wear occurs in the tissue, as described in the following section.

5.4.3 - Damage assessment of OA-like articular cartilage

Damage as a result of enzymatic digestion was assessed using WLI. Figure 5.5 shows representative WLI surface images of native and OA-like AC after and before being subjected to shear testing.
Enzymatic degradation alone has a noticeable effect on the tissue’s surface causing a significant change in surface topography and a rise in surface roughness, as shown from Table 5.8a. The value of $R_a$ has shown a 4-fold increase between the native and the High (4) sample. Other surface parameters such as $R_q$, $R_z$ and $R_t$ have also been shown to increase as a result of enzymatic degradation. Surface parameters also increase as a result of shearing, as shown from Table 5.8b, indicating an increase in damage and mechanical disruption of the sample’s surface as compared to the samples before undergoing shear testing. Clearly, the degraded samples appear to have a more disrupted surface following shear than the native samples do. This indicates a reduced resistance to shear and damage once the structural and mechanical integrity of AC is compromised.

Nevertheless, especially for collagen degraded AC, the equilibrium friction coefficient decreases the more the tissue is enzymatically degraded irrespective of the increase in surface roughness. This suggests that the gelatine layer which has formed on the AC surface has some positive effect on the lubricating property, probably due to the reduction in “ploughing” forces required to slide the counter surface over the degraded sample, lowering the equilibrium coefficient during sliding. However, the lower friction coefficient does not necessarily imply reduced wear. Following shear testing, the surface parameters and the extent of damage on the surface is noticeably higher as compared to before the shear test.

Biochemical analyses of the PBS contained in the shear test bath following shear testing shows a 56 fold increase in hypro release from the High (1) samples as compared to subjecting the native tissue to an equivalent shear test. In a perfectly healthy tissue, damage to the collagen is minimal as the collagen fibres, which are oriented parallel to the articular surface, offer excellent resistance to shear stresses. As the collagen is degraded, however, the resistance to shear of the fibres is compromised resulting in damage initiation. The reason for the low PG release following shear testing in the High samples is due to the fact that almost all of the PGs have already been previously depleted by the enzymatic activity.

The PG damage in the native sample as a result of shear supports the idea that the onset of osteoarthritis, a mechanically induced pathology, is associated with damage of PGs first. In fact, PG damage is thought to be reversible as PG has been demonstrated to have a half life of 3.4 years whereas collagen disruption is thought to be irreversible, having a half life of 177 years [313, 314]. Damage to the collagen fibres results in a drastically changed frictional response making the tissue more prone to wear, highlighting the need to diagnose OA at a very early stage of the pathology.

5.4.4 - Insight into the effect of mechanically induced damage – a preliminary investigation

A long duration shear test was performed on native porcine AC in order to investigate any variations of frictional response and mechanical properties as a result of mechanically induced damage. The
frictional response, after stabilising to an equilibrium value, was seen to gradually decrease (Figure 5.6). This variation must be due to changes to the surface composition of the tissue since fluid support has been completely lost at the equilibrium stage of the shear test. Surface analysis using WLI indicated disruption to the tissue’s surface and a change in surface topography (Figure 5.7). Shear has almost certainly caused the removal of the SAL on the sample’s surface, as well as damaging the actual superficial zone of the tissue. The decreasing equilibrium coefficient supports the idea that the SAL has been removed as studies have demonstrated that the removal of the SAL causes a significant counter-intuitive drop in $\mu_{eq}$ [286].

Following shear testing, the mechanical properties of the tissue were evaluated using unconfined compression. The Young’s modulus decreased by 40% compared to native porcine AC and an increase in relaxation rate and permeability was also found (Table 5.10). It can be hypothesised that damage has occurred mainly to the collagen network and the PG present in the superficial zone of the tissue as this has been found to strongly affect the mechanical properties of the tissue [305]. According to other studies, collagen damage is thought to originate from excessive shear along the fibre direction causing failure of the entwinements between fibrils or failure of the fibril itself as a result of extreme strain [315]. Histological studies of the tissue following the shear tests would, however, have to be performed to confirm this hypothesis.

These initial findings are important as they suggest a variation of mechanical properties as a result of mechanically induced damage alone. As the tests concern ex vivo tissue, the change in mechanical properties is due to the removal material from the AC surface and the severe mechanical disruption of the ECM, it does not involve any biochemical and mechanical variations as a result of enzymatic activity. Although a more in depth investigation is necessary, it does suggest that a decrease in the mechanical quality of AC could also arise simply due to wear of the tissue alone rather than solely due to the biological response of the chondrocytes.

AC is a strongly inhomogeneous and anisotropic material and its global mechanical response is determined by the interplay between the different constituents and the various through-thickness zones of the tissue. A homogenous material would not undergo any sort of variation in mechanical properties following shearing, even if the surface layers of the material would be removed. In AC, however, due to the through thickness variation in structure and mechanical properties, damage on the upper layers of the tissue is expected to cause a variation in the mechanical response. Hence, inducing wear or mechanically compromising a given part of the tissue, could impact the mechanical quality of the tissue. The decline of mechanical properties of osteoarthritic AC could actually be a result of both chondrocyte response to excessive loading and thus enzymatic degradation as well as mechanical wear. OA could in fact commence with mechanical degradation due to proteases being released into the tissue. As the mechanical properties of the tissue decline, AC could become more susceptible to wear, as demonstrated from the tribological study on OA-like AC. At this point, when the tissue is
more susceptible to mechanical and shear damage, mechanically induced wear could occur further reducing the mechanical quality of the tissue eventually leading to full thickness loss of AC.

5.5 - Conclusion

In conclusion, variations of ECM composition significantly alter the mechanical and frictional response of AC. This study has shown that osteoarthritic AC resembles OA-like AC suggesting that enzymatic activity, possibly due to chondrocytes responding to abnormal stresses and strains, plays an important role in the pathogenesis of the disease. Early PG depletion, which is thought to be a reversible process, has been associated with a distinct variation in mechanical parameters. More severe depletion of PGs followed by collagen degradation has been found to have a drastic effect on the mechanical and frictional response of the tissue, including significantly decreased wear properties. Finally, inducing mechanical damage alone in the tissue, has been found to alter the mechanical properties suggesting that alterations of the ECM’s structure as a result of mechanical damage could also have detrimental effects on the overall mechanical response of the tissue. This work sets out a protocol for future work involving comparison between OA-like and osteoarthritic AC. Furthermore, from this research, it can be gathered how valuating the mechanical properties of AC could be a valuable technique for the assessment of OA. In fact, one of the limitations of the ICRS grading technique, and in general OA assessment techniques which base the progression of the pathology on morphological changes, is that biochemical and hence mechanical changes in the tissue could already exist in a morphologically healthy (Grade 0) tissue. This highlights the strong need for a technique and a device to evaluate mechanical properties of AC in a clinical setting. By assessing mechanical properties of osteoarthritic AC ideally through arthroscopy, it could allow an estimate of the structural damage of the tissue and a clearer understanding of the progression and the development of the pathology. However, such a technique would have to be thoroughly investigated and validated across several different stages of osteoarthritic AC in order to establish consistency in the measurements.
Chapter 6 – Effect of fibre orientation on the frictional and mechanical properties of tissue engineered articular cartilage

6.1 - Introduction

Regenerative medicine strategies offer a promising solution for the treatment of AC defects and potentially localised early OA. Such strategies rely on the development of a tissue-engineered cartilage replacement. In this study, microfibrous poly(ε-caprolactone) (PCL) scaffolds of varying fibre orientations (random and aligned) were cultured with bovine chondrocytes for four weeks in vitro, and the mechanical and frictional properties were evaluated. Mechanical properties were quantified using unconfined compression and tensile testing techniques. Frictional properties were investigated using a reciprocating shear testing machine at physiological compressive strains usually occurring in native AC. Scaffolds were sheared along the fibre direction, perpendicular to the fibre direction and in random orientation. The evolution of damage as a result of shear was evaluated via WLI and scanning electron microscopy (SEM). As expected from a composite material, the fibre orientation strongly affected the tensile properties as well as the compressive modulus of the scaffolds. Fibre orientation did not affect the equilibrium frictional coefficient but it was however a key factor in dictating the evolution of surface damage on the surface. Scaffolds shear tested perpendicular to the fibre orientation displayed the highest surface damage. The results suggest that the fibre orientation of the scaffold implanted in the joint should be carefully considered when using microfibrous scaffolds. This study was done in collaboration with Dr Seth McCullen and Dr Anthony Callanan under the supervision of Professor Molly Stevens from the Department of Materials, Department of Bioengineering and Institute of Biomedical Engineering at Imperial College London.

6.2 - Outline

MACI has significantly improved the traditional ACI technique by utilizing a range of commercially available fibre-based membranes, which are able to enhance cellular attachment, distribution, while
eliminating several complications (periosteal flap harvest and implantation) and generating more predictable cell-material interactions [194-197]. Current AC tissue grafts not only undergo extensive loading regimens as neo-cartilage is formed but also must provide a near frictionless surface. Thus, a key parameter of tissue scaffolds for AC is their bio-tribological performance. In this regard, reconstitution of the frictional properties of AC requires scaffolding with optimal design with regards to micro-structure, extracellular matrix (ECM) deposition/organisation, as well as binding of appropriate molecules [316-318].

Recent advances in scaffold materials have examined how the microstructure as well as the chemical composition can influence chondrocyte growth, distribution, matrix production, and the resulting biomechanical properties [319-321]. However, few studies have investigated the frictional behaviour of tissue engineered AC [316, 322-325]. Thus, this research has focused on the functionality of such tissue grafts with particular interest in their frictional response. The microstructure of these scaffolds can vary from fibre webs to dense fleeces. Fibrous scaffolds offer advantages in controlling fibre size, spacing, and orientation [317, 318]. Control of fibre orientation is known to dictate differences in bulk tensile and compressive properties and offers morphological similarities to AC [326]. For example, the superficial zone of naturally functioning AC consists of primarily flattened ellipsoidal-like chondrocytes and a very polarized dense organisation of nanoscale collagen type II fibrils oriented parallel to the plane of the articular surface [327]. The superficial zone consists of the highest concentration of collagen and the lowest concentration of proteoglycans (PGs). Based on the alignment of chondrocytes and collagen type II fibrils, the thin superficial zone has the greatest tensile strength found in articular cartilage, which is crucial for resisting shear and tensile forces from the articulating surfaces. However, the role of fibre orientation in such constructs has yet to be investigated as natural AC exhibits organisation in both depth-dependent and surface directions. Electrospun scaffolds have been investigated for fibrous and hyaline cartilage replacement [328]. Fibre alignment in the bulk scaffolds contributes significantly to the tensile properties, yet little is known regarding their frictional and wear performance [329]. Assessing the tribological performance and the evolution of damage in the scaffold is crucial as it will have to sustain complex loading in the joint prior to the formation of significant amounts of ECM. In this study we assess the effect of fibre orientation in electrospun scaffolds on the friction and wear properties of tissue engineered cartilage.
6.2 - Materials & Methods

6.2.1 - Scaffold fabrication and characterisation

The scaffolds were prepared in the Department of Biomedical Engineering by Dr Seth McCullen and Dr Anthony Callanan. PCL (Mₙ = 80 kDa) and the solvent 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) were procured from Sigma-Aldrich (Dorset, UK). Fibrous scaffolds were fabricated on a custom-made electrospinning device that included a programmable syringe pump (Kd Scientific Model KDS 100 CE, Sandbach, UK), a Glassman high voltage power supply series WR (Glassman, Bramley, UK), and a voltage-driven rotating mandrel. Scaffolds of varying fibre orientation were fabricated by dissolving 2.4 g PCL in 20 mls HFIP (12 wt%) with stirring overnight. The PCL solution was electrospun through a 19 gauge blunt tip needle at a flow rate of 2 ml/hr, at 15 kV, with a spinneret-to-collector distance of 10 cm, and collected onto a rotating mandrel (width = 6 cm; diameter = 20 cm) at a linear velocity of either 1 or 15 m/s to form either randomly oriented or aligned fibre scaffolds, respectively. All scaffolds were approximately 1 mm in thickness. Prior to chondrocyte seeding, 10 mm diameter scaffolds were punched from electrospun sheets. Scaffolds were imaged using a JEOL 5610 (Herts, UK) environmental scanning electron microscope (SEM). Specimens were coated with 100Å Au using an Emitech K550 sputter coater and observed at an accelerating voltage of 15 kV and a working distance of 10 cm.

6.2.2 - Cell culture

6.2.2.1 - Bovine chondrocyte isolation

Bovine cartilage was harvested from the lower leg joint of young calves as previously described [330]. Chondrocytes were isolated by digesting in Dulbecco’s Modified Eagle’s Medium (DMEM) + Glutamax (4.5 g/l glucose) with 0.2% w/v pronase, 10 mM HEPES, 50 μg/ml gentamycin, and 5% v/v foetal bovine serum (FBS) (all reagents from Invitrogen, Paisley, UK) for 1 hr at 37°C with agitation. This digesting solution was removed and replaced with DMEM + Glutamax (4.5g/l glucose) supplemented with 10 mM HEPES, 50 μg/ml gentamycin, 5% v/v FBS, and 0.04% w/v collagenase (Sigma) overnight at 37°C with agitation. Chondrocytes were filtered through a 70 μm pore size filter, centrifuged at 250 g for 3 minutes, and plated in DMEM (4.5g/l glucose) with 10% v/v FBS, 50 μg/ml ascorbic acid (Sigma), and 50 μg/ml gentamycin (expansion medium).

Scaffolds were sterilised in 70% ethanol for 30 minutes followed by three washes with sterile phosphate buffered saline (PBS) and then soaked in 0.01% v/v bovine serum albumin (BSA) (Sigma) in PBS overnight to assist with chondrocyte adhesion (20). Twenty μl of expansion medium containing 0.5M bovine chondrocytes (passage 1) was placed on one side of each scaffold and cells were allowed to adhere for 2 hours before seeding the other side with an additional 0.5M chondrocytes. Following chondrocyte adhesion, fibrous scaffolds were transferred to non-adherent 24
well plates and cultured in 1 ml of chondrogenic differentiation medium consisting of DMEM (4.5 g/L L-glucose), supplemented with 50 μg/ml L-proline (Sigma), 50 μg/ml ascorbic acid (Sigma), 0.1 mM sodium pyruvate (Sigma), 10 ng/ml TGF-β3 (Lonza, Slough, UK), and 1% v/v ITS Premix (BD Biosciences, Oxford, UK) at 37°C and 5% CO₂. Medium was changed twice weekly.

**6.2.2.2 - Chondrocyte-scaffold interaction**

Chondrocyte morphology on electrospun scaffolds was assessed by examining cytoskeletal organisation by immunostaining. Scaffolds were fixed in 4% w/v paraformaldehyde for 15 minutes, washed twice with PBS, and permeabilised with 0.25% v/v Triton X-100 in PBS for 15 minutes. Actin cytoskeleton was stained with Alexa Fluor® 568 phalloidin (Invitrogen; 1:160) for 20 minutes and nuclei were stained with DAPI (Sigma; 1:1000) for 2 minutes. Type I and type II collagen were detected using Collagen I antibody (rabbit polyclonal, Ab34710) and Collagen II antibody (dilution ratio 1:100, rabbit polyclonal, Ab34712, Abcam, Cambridge, UK). Both antibodies were detected using Goat polyclonal secondary antibody to rabbit IgG with a FITC conjugation (Ab97050) (dilution ratio 1:1000) and counterstained with DAPI (Sigma; 1:1000) for 2 mins. Sections were stained separately for collagens. Chondrocytes were imaged on an Olympus IX51 epifluorescence microscope equipped with an Olympus DP70 camera.

After 0 and 4 weeks of culture, chondrocyte-seeded scaffolds were digested in papain solution (2.5 units papain/ml, 5 mM cysteine HCl, 5 mM EDTA, in PBS (all reagents from Sigma) at 60°C overnight. Digested samples were assayed for total DNA content using the Quant-iT™ PicoGreen® kit (Invitrogen) according to the manufacturer’s instructions. Sulfated glycosaminoglycan (GAG) contents were determined using the Blyscan Kit (Biocolor, UK) as per the manufacturer’s instructions.

**6.2.3 - Mechanical characterization**

**6.2.3.1 - Compressive properties**

After 0 and 4 weeks in culture, chondrocyte-seeded scaffolds were assessed for compressive properties by performing unconfined uniaxial compression testing using an Instron Model 5540 testing machine equipped with a 50 N load cell. Three mm diameter samples were cored from 10 mm diameter samples, pre-loaded to 0.05 N, allowed to equilibrate for 5 minutes, and then compressed to 10% strain at a crosshead speed of 0.5% strain/min. Tangent modulus was calculated from the linear portion of the stress-strain curve.

Stress relaxation tests on the samples were also performed in order to investigate any form of interstitial fluid support. The samples used for the test were 3 mm in diameter. The test consisted of a pre-loaded to 0.05 N, and then compressed to a low strain of 2% strain to examine the contribution of ECM deposition on the scaffolds and a high strain 18±2.5% equivalent to the compressive load of 3N used in the shear tests. The crosshead speed was at 0.5% strain/min and samples were then allowed to relax for a period of 5 minutes under the high or low strain condition. Percentage relaxation was
calculated at 1 and 5 min during the relaxation period. The compressive properties and the tensile properties, reported below, were determined by Dr Seth McCullen and Dr Anthony Callanan in the Department of Biomedical Engineering.

6.2.3.2 - Tensile properties

The response of the scaffolds under tensile loading was mechanically tested using the same machine used for compression testing, operated at a crosshead speed of 10 mm/min. Specimens had a gauge length of 30 mm and width of 10 mm; thickness was measured by digital calipers. Tensile modulus was calculated from the linear portion of the stress-strain curve after all specimens were tested to failure.

6.2.3.3 - Shear testing

Shear tests were performed using an in-house built Multi-axial Compression and Shear Testing Rig (MCSTR) specifically designed to test AC using a variety of physiological pressures, testing conditions, and configurations. The MCSTR was configured such that shear tests were carried out at constant load using a steel plate with $R_a \sim 1 \mu m$ as the counter interface. For the rig the counter interface was fixed, whilst the lower specimen was able to move both vertically (compression) and horizontally (sliding). In order for the scaffolds to be viable for shear testing, they were affixed onto high density polyethylene (PE) discs using a very thin layer of cyanoacrylate glue. Cellular and acellular (control) scaffolds were press fit into the sample holder so that the PE disc was laterally constrained, raising the scaffold above the sample holder’s height. Unless specified, in this study throughout the text and the relevant figures and tables, acellular scaffolds refer to scaffolds with no cell-deposited ECM and no chondrocytes. Cellular scaffolds refer to chondrocyte seeded scaffolds after the full 4 weeks of culture time. Specimens were immersed in PBS at room temperature and equilibrated for 30 min prior to shear testing. Scaffolds were tested in shear using a 500 µm stroke length and a frequency of 2 Hz corresponding to a sliding speed of 2 mm/s. This sliding speed ensures boundary lubrication and is similar to previous studies on tissue engineered AC tribology [323, 325]. To verify that the scaffolds were undergoing shear, force displacement loops were produced confirming that shear was indeed taking place. Prior to testing, both deterministic and homogenised models developed by Scaraggi and co-workers [331-333] for the study of lubricated soft rough contacts were utilised to simulate the scaffold – steel platen interactions during sliding and to verify that the lubrication regime of the experimental test occurring during sliding was indeed boundary lubrication. The shear test parameters were input into the model as well as the mechanical properties of the scaffold (assumed to be elastic) and the measured topography of a representative sample’s surface. Testing the scaffolds in boundary regime was deemed as the most appropriate shear test conditions to use when evaluating frictional response and surface damage hence avoiding and potential masking effects which could arise in fluid film lubrication [115].
Scaffold specimens were tested at loads of 3N and 6N corresponding to contact pressures of ~ 0.04 MPa and 0.08 MPa respectively. Testing was carried out on random and aligned scaffolds (sliding parallel and perpendicular to the fibre direction). AC samples were also tested; osteochondral plugs (10 mm) were extracted using a surgical coring device from healthy adult bovine knee joints, as previously reported [115]. The native AC was used as a reference for assessing the frictional response of tissue engineered AC.

6.2.3.4 - Evaluation of surface properties and damage
Surface morphology was measured using White Light Interferometry (WLI) and SEM. Measurements were performed on the scaffolds before and after testing to quantify and visualize changes in surface topography as a result of shearing. WLI used a Wyko, NT9100 interferometric microscope, with a 20X magnification objective [115, 252, 258]. Following shear testing, the samples were blotted to remove excess liquid and then imaged. WLI was used to obtain surface parameters (e.g. Ra, mean surface roughness) as well as images of 312 x 234 µm². SEM was used to visualise micro and macro surface features.

6.2.3.5 - Statistical analysis
Scaffold groups (aligned and random fibre orientation) were analyzed for significant differences for biochemical analysis and mechanical response to compression (n=4/group/time point). Tensile and shear testing were performed using different fibre orientations: both aligned fibres (parallel and perpendicular to loading direction) and random fibres samples (n=5/group/time point) were analysed. Numerical and graphical results are displayed as mean ± standard deviation. Significance was determined by using ANOVA and accepted at a p-value < 0.05.

6.3 - Results

6.3.1 - Chondrocyte scaffold interaction
Fibre scaffolds of varying fibre alignment were produced (Figure 6.1). Chondrocytes seeded on electrospun scaffolds adopted varying morphologies based on scaffold type. The microstructure orientation of the underlying matrix directed chondrocytes into an elongated morphology for cells grown on aligned scaffolds and rounder, polygonal morphology for cells grown on randomly oriented scaffolds, as shown in Figure 6.1. After 4 weeks of culture, immunohistochemical staining revealed an intense type II collagen matrix with minimal staining for type I collagen (Figure 6.1).
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Figure 6.1 – Scaffold and chondrocyte morphology on aligned (a-d) and random (e-h) fibre scaffolds. Chondrocyte morphology was dramatically affected by fibre orientation (b,f). Immunohistochemical staining revealed intense staining for type II collagen matrix (c,g) with minimal type I collagen staining (d,h). Actin cytoskeleton = red; type I/II collagen = green, cell nuclei = blue; scale bar = 20 μm.

Chondrocyte-seeded aligned and randomly oriented scaffolds exhibited significant increases in sGAG content with no significant changes in DNA amount after 4 weeks in culture (p-value < 0.05) (Figure 6.2). Sulfated GAG amount was not statistically different between the scaffold groups (p-value > 0.05).

Figure 6.2 – Chondrocyte-seeded scaffolds did not exhibit any significant differences in DNA content following 4 weeks culture, while significant increases were noted for sulfated glycosaminoglycans (sGAG) for both random and aligned scaffolds (p-value < 0.05). sGAG content was not significantly different between the scaffold groups after 4 weeks in vitro culture.
6.3.2 - Mechanical properties

Tensile and compressive properties of the scaffolds were determined after 4 weeks \textit{in vitro}. The results are reported in Table 6.1.

<table>
<thead>
<tr>
<th>Mechanical Parameter</th>
<th>Acellular</th>
<th>Cellular</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Aligned Parallel</td>
<td>Aligned Perpendicular</td>
</tr>
<tr>
<td>Tensile Young’s Modulus (MPa)</td>
<td>28.07 ± 1.59</td>
<td>1.84 ± 0.08</td>
</tr>
<tr>
<td>Compressive Young’s Modulus (kPa)</td>
<td>179.00 ± 7.00</td>
<td>162 ± 33.30</td>
</tr>
<tr>
<td>Equilibrium Friction Coefficient at 0.04 MPa ($\mu_{eq}$)</td>
<td>0.78 ± 0.12</td>
<td>0.72 ± 0.12</td>
</tr>
<tr>
<td>Equilibrium Friction Coefficient at 0.08 MPa ($\mu_{eq}$)</td>
<td>0.48 ± 0.06</td>
<td>0.41 ± 0.05</td>
</tr>
</tbody>
</table>

Table 6.1 – Tensile and compressive modulus of cellular and acellular scaffolds. * denotes statistically significant difference in compressive modulus between acellular and cellular samples and equilibrium friction coefficient between varying fibre orientations ($p$-value < 0.05).

As expected, tensile properties were dependent on fibre orientation with the aligned fibre scaffolds having the highest tensile modulus. Tensile tests were performed for the duration of the culture period and showed no significant changes between acellular and cellular groups, for either the aligned or random scaffold groups, as shown in Table 6.1. Compressive testing demonstrated a significant reduction in the compressive modulus of cellular scaffolds for both aligned and random fibre orientation after 4 weeks in vitro culture (Table 6.1). Due to the differences in mechanical properties, scaffolds underwent different compressive strains, and these equivalent strains were calculated for the compressive shear testing loads of 0.04 and 0.08 MPa. These loads ranged from 16-31\% strain which are in the physiological range of AC. Table 6.2 shows the resultant axial strain following a 3N and a 6N load.
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Table 6.2 – Axial strain on scaffolds and equilibrium friction coefficient at 3N and 6N (0.04 and 0.08 MPa, respectively) compressive load.

Stress relaxation tests of 2% and 18% axial compressive strain were performed on cellular and acellular scaffolds in order to verify the presence of interstitial fluid support represented by the stress relaxation response. The results of these tests are reported in Table 6.3.

Table 6.3 – Stress relaxation of cellular and acellular scaffolds at 1 min and 5 min following 2% and 18% applied axial strain. * denotes statistically significance difference between stress relaxation rate of acellular and cellular samples at 2% applied axial compressive strain.

Figure 6.3 shows two representative stress relaxation tests for an acellular and a cellular aligned sample demonstrate the time-dependent behaviour of the scaffolds.
6.3.3 - Shear testing

Shear testing was performed on cellular and acellular scaffolds (as well as on native AC) to assess frictional properties at two different loads corresponding to two different axial strains (Table 6.2). The equilibrium friction coefficient ($\mu_{eq}$) was recorded at the end of the test (3600s) in order to compare between cellular, acellular and varying fibre orientation (Table 6.1). Representative shear curves for each scaffold type, fibre orientation and loading condition are shown for acellular and cellular scaffolds in Figure 6.4.
Figure 6.4 – Representative frictional response for acellular (acell) and cellular (cell) scaffolds at varying fibre orientation and contact pressure. Figure 6.4a and 6.4b show the effect of load variation according to different contact pressures between (a) acellular and (b) cellular scaffolds. In Figure 6.4c and 6.4d the effect of varying fibre orientation of both acellular and cellular scaffolds is visualised by showing the frictional response at a constant load of (c) 0.04 MPa and (d) 0.08 MPa.

The frictional response of native AC when subjected to the same sliding test conditions at 0.04 MPa and 0.08 MPa is also shown in Figures 6.4c and 6.4d. In addition, a further test using a normal load of 30N (contact pressure 0.4 MPa) was performed in order to confirm the trend between equilibrium friction coefficient and load. The effect of the increased contact pressure on the frictional coefficient of AC was also verified as shown in Figure 6.5.
Figure 6.5 – (a) Representative frictional response of AC at different contact pressures and (b) WLI images of AC before and after shear loading.

6.3.4 - Surface properties and damage assessment

Surface images of native (before undergoing shear testing) acellular and cellular scaffolds of random and aligned fibre configuration were recorded prior to shear testing using SEM and WLI (Figure 6.6).
Figure 6.6 – SEM and WLI images of native acellular and cellular scaffolds in aligned (a,e,c,g) and random (b,f,d,h) configurations. After 4 weeks in vitro (e,f,g,h), the fibre networks became distorted due to excessive extracellular matrix deposition.

Prior to shear testing and cell culture, random acellular scaffolds were found to have a significantly lower mean surface roughness compared to the acellular aligned scaffolds ($p$-value < 0.05) (Table 6.4).

<table>
<thead>
<tr>
<th>Material</th>
<th>Surface Parameters</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Native $Ra$ ($\mu$m)</td>
<td>0.04 MPa $Ra$ ($\mu$m)</td>
<td>0.08 MPa $Ra$ ($\mu$m)</td>
</tr>
<tr>
<td>AC</td>
<td>0.64 ± 0.11</td>
<td>0.79 ± 0.17</td>
<td>1.15 ± 0.38</td>
</tr>
<tr>
<td>Acell-Parallel</td>
<td>5.09 ± 0.83</td>
<td>2.82 ± 0.51</td>
<td>2.67 ± 0.95</td>
</tr>
<tr>
<td>Acell-Perpendicular</td>
<td>2.76 ± 0.95</td>
<td>1.75 ± 0.81</td>
<td></td>
</tr>
<tr>
<td>Acell-Random</td>
<td>3.29 ± 0.49*</td>
<td>3.41 ± 0.56</td>
<td>1.81 ± 0.27</td>
</tr>
<tr>
<td>Cell-Parallel</td>
<td>3.86 ± 1.05</td>
<td>3.33 ± 0.84</td>
<td>1.94 ± 0.40</td>
</tr>
<tr>
<td>Cell-Perpendicular</td>
<td>3.71 ± 0.98</td>
<td>3.57 ± 1.18*</td>
<td></td>
</tr>
<tr>
<td>Cell-Random</td>
<td>3.26 ± 0.67</td>
<td>2.87 ± 0.49</td>
<td>2.08 ± 0.80</td>
</tr>
</tbody>
</table>

Table 6.4 – Surface parameters of articular cartilage and scaffolds under native conditions and after 1 hr of shear testing at contact pressures of 0.04 MPa and 0.08 MPa. * denotes statistically significant difference in mean surface roughness between acellular and cellular samples or varying fibre orientations ($p$-value < 0.05). Acell and cell refers to acellular and cellular scaffolds respectively.

With the cell-based deposition of the ECM, the fibre network became distorted and was less visible, homogenizing the surface and yielding a very similar surface roughness between cellular random and
cellular aligned scaffolds (Table 6.4). Surface images were then obtained following shear testing at various configurations. Representative images are shown in Figure 6.7.

Figure 6.7 – Surface topography images using WLI and SEM following a 1 hr shear test with a normal load of (a) 0.04 MPa and (b) 0.08 MPa.
For the cellular scaffolds following a 1 hr shear test (corresponding to 7200 sliding cycles) WLI revealed surface disruption of the ECM and the fibre architecture underlying the deposited ECM (Figure 6.7a, 6.7b). Significant surface disruption to the acellular scaffolds follow shear is clearly visible at both loading regimes (Figure 6a, 6b). A decrease in the mean surface roughness value, $R_a$, occurred following shear testing (Table 6.4) for all fibre orientations and at both loads. A statistically higher surface roughness of the cellular perpendicular tested scaffolds at 0.08 MPa was found compared to the other orientations indicating a higher surface damage ($p$-value < 0.05). The surface topography of AC was also assessed using WLI to provide a comparison for the tissue engineered scaffolds (Figure 6.5). Contrary to the scaffolds, the surface roughness following shear testing was seen to increase as previously reported in [115]. SEM of the scaffold surface after shear testing confirmed the removal and formation of “rolls” of the ECM (Figure 6.7a, 6.7b).

### 6.4 - Discussion

The fabricated fibrous scaffolds of varying fibre orientation of either aligned or randomly oriented matrices were prepared to evaluate the effect of scaffold fibre orientation and its effect on the frictional response of tissue engineered cartilage. Tensile properties of the engineered tissues varied significantly depending upon the fibre orientation but were maintained after short term (4 week) culture duration (Table 6.1). With the addition of cell-deposited ECM to the fibrous scaffolds, the compressive properties changed significantly, with the compressive modulus decreasing by 44% (Table 6.1). Thus the axial strain on the scaffolds at loads of either 0.04 or 0.08 MPa varied between 16 and 31% (Table 6.2). This range of values represents the physiological strain which AC is subjected to in the natural joint [334, 335].

Under low compressive strains, relaxation in the acellular scaffold diminished, indicating little fluid load support due to the high permeability of the scaffold construct. Stress relaxation was significantly higher for the cellular scaffolds, contributing to higher interstitial fluid pressurisation and a stress relaxation response more similar to AC [72] (Table 6.3 and Figure 6.3). At higher compressive strains such as 18%, the mechanical responses were dictated by the properties of the substrate, in this case the scaffold itself, which offers no or very limited interstitial fluid pressurisation and hence fluid support, explaining the similarity in stress relaxation between cellular and acellular samples in Table 6.3.

The characteristic time-dependent frictional response of AC under shear testing is shown in Figure 6.5 for three different loads. The frictional changes due to shear reflect the different lubrication mechanisms associated with AC. Initially the friction coefficient is low (typically < 0.15) and is due to the fluid pressurization mechanism [99] which generates a fluid film separating the surfaces. As shearing progresses the fluid is expelled from the contact zone and the increased friction is due to the increased solid–solid interaction.
The frictional response of acellular and cellular scaffolds is very different to AC and is indicative that the underlying lubrication mechanisms are different. Low friction coefficients at the start of the test are not observed for the tissue engineered AC, which suggests the fluid pressurization mechanism present in the scaffold and in the thin ECM layer is absent. The frictional responses at both 0.04 MPa and 0.08 MPa show very similar trends regardless of cellular presence and fibre orientations (Figure 6.4). The presence of ECM on the surface as well as the orientation of the fibres appears to govern the equilibrium friction coefficient whilst not affecting the overall trend. The response of the scaffolds at 0.04 MPa can generally be described by an initial peak in friction coefficient followed by a transitional stage until an equilibrium friction coefficient is reached. The peak in friction at the beginning of the test is due to the high surface roughness of the native scaffolds. The friction coefficient, following the initial peak, quickly decreases to a minimum value as some form of ‘running in’ effect occurs. As the lubricating regime was predicted to be boundary, according to the analytical model, the frictional response is particularly susceptible to changes in surface roughness and topography. Once the minimum is reached, the friction coefficient increases and stabilizes to the equilibrium value, \( \mu_{eq} \), which is however lower than the start-up friction coefficient. The reason for the increase in friction coefficient following the minimum can be explained by the formation of debris and surface damage on the scaffold as it occurs both for acellular and cellular specimens. At this load (3 N), it is likely that after the initial ‘running in’, the formation of surface features and debris plays a role in promoting a subsequent increase of the surface roughness as the surface pressure is probably not sufficient to flatten surface features. This is witnessed by the fact that the final surface roughness of the specimens tested at this load is generally higher than the roughness measured for the specimens after testing at 6 N.

The WLI images show significant disruption to the fibre network on the surface of the scaffolds following shear testing (Figure 6.7a and 6.7b). Application of the load creates a more homogenous surface and a reduced surface roughness (\( R_a \)) as compared to the native scaffolds (Table 6.4). At 0.08 MPa load, following the peak in friction coefficient at the beginning of the test, a gradual decrease in friction occurs until an equilibrium value is reached. The “transitional” stage is absent at the 0.08 MPa load. This could be explained by the WLI results (Table 6.4) which show a significantly lower surface roughness after shear testing as compared to the native scaffolds and, as previously suggested, to the scaffolds subjected to the 0.04 MPa load shear test. The final smoother surface, due to the higher compressive load, causes the equilibrium coefficient to stabilize at a lower value with respect to the 0.04 MPa load case.

SEM images (Figure 6.7), show extensive surface damage on the scaffolds following shear testing. As suggested in previous studies, scaffold debris released during sliding could potentially be acting as a boundary lubricant lowering \( \mu_{eq} \) with increasing load [323]. Overall the surface roughness is higher for the cellular case based on 1) the ECM curling and rolling over itself across the surface in
the direction parallel to sliding restricting the motion increasing $\mu_{eq}$ and 2) the adhesion mechanisms which occur in AC [336]. Adhesion is associated with an increase in friction [337], especially after long compression times caused by the increased effect of adhesive asperity micro-contacts [336].

The tissue engineered scaffolds did not demonstrate the time dependent frictional response typical of AC (Figure 6.5). The cell-deposited ECM layer is limited in thickness (~50 µm), as determined by histological staining, and the scaffolds are under significant axial strain thus the frictional response is dominated by the scaffold (Table 6.2). Although the scaffold itself is biphasic, as it is constituted by a solid phase of tightly woven fibres hydrated by a fluid phase consisting of water, it is thought that its permeability is too high to allow any interstitial fluid pressure to form as the fluid is easily exuded out of the material, as shown by the stress relaxation values in Table 6.2. At high strains, 16 – 31% (Table 6.2), equivalent to conditions experienced during sliding, the stress relaxation was low due to the minor contribution of the cell-deposited ECM and the low fluid support of the scaffold. This agrees with the hypothesis that the sliding tests showed no sign of interstitial fluid pressurisation. Other studies [316, 322, 325] have reported interstitial pressurisation in tissue engineered scaffolds. These, however, were very different material constructs and/or were subjected to extremely small normal loads and applied strains. Under these conditions the frictional response was solely or mostly given by the response of the ECM rather than the scaffold [316, 322, 325]. Previous studies have shown a higher equilibrium friction coefficient for tissue engineered AC [316, 322] while others have shown a lower $\mu_{eq}$ compared to native AC [323, 325]. These variances can be based on testing conditions/configurations, type of scaffold used, and cell culture duration/conditions including the type of cell [324].

Fibre orientation of the scaffolds seems to only slightly affect $\mu_{eq}$ although not the overall trend of frictional response (Figure 6.4c and 6.4d). At 0.04 MPa, for the cellular scaffolds, there is no statistically significant difference in $\mu_{eq}$ between random, parallel and perpendicular fibre orientation, while the acellular scaffolds display differences based on fibre orientation (Figure 6.4c). The random orientation acellular scaffolds exhibit the highest friction with $\mu_{eq} = 0.96 \pm 0.11$ whilst the perpendicular alignment had the lowest friction with $\mu_{eq} = 0.72 \pm 0.12$ (0.04 MPa) (Table 6.1). This agrees with the surface roughness of the acellular scaffolds following sliding; the random orientation has, overall, a rougher surface compared to the perpendicular case (Table 6.1). In this study the surface roughness and damage appear to be the main governing factors of $\mu_{eq}$. The onset of damage on the scaffold’s surface is thought to be dictated by the orientation of the fibre network. We hypothesize that for the cellular case, the cell-deposited ECM layer masks the fibre homogenisation effect occurring at the surface and hence the frictional response of the cellular scaffolds compared to acellular at 0.04 MPa. At 0.08 MPa, acellular scaffolds display almost identical $\mu_{eq}$ (Figure 6.4d); possibly due to the higher normal load producing a similar surface morphology for all scaffold orientations. This is confirmed by the WLI images in Figure 6.6, where the fibre network appears
completely homogenised after shear. Only the parallel orientation seems to have some fibres still intact on the surface, probably due to superior mechanical properties when subject to tension, along the fibre direction. The parallel fibre sample following shear has a higher $R_s$ compared to the two other orientations, and this is probably due to the fibres which are still present on the surface. It should be highlighted that the frictional response of the engineered tissues is the result of the interplay between substrate morphology, surface evolution, fluid/solid interactions and applied load.

The frictional response for the cellular scaffolds is very similar at both loads (Figure 6.4c and 6.4d) with the perpendicular construct displaying a statistically significant higher friction coefficient compared to the other orientations at 0.08 MPa (Table 6.1). The high $\mu_{eq}$ for the perpendicular scaffold could be due to the excessive damage occurring as a result of sliding and is supported by the WLI measurements (Table 6.4) which indicate a significantly higher surface roughness for the perpendicular samples following sliding compared to the other orientations at 0.08 MPa. This supports the notion that surface roughness plays a dominating role in the frictional response. SEM images of the perpendicular scaffold show more disrupted fibres on the surface as compared to the other two orientations.

In summary, no significant differences in the $\mu_{eq}$ between fibre orientations were found. Minor variations between fibre orientations were attributed to differences in the evolution of surface damage as a result of shearing. This suggests that when inserting the scaffolds in the joint, the choice of fibre orientation is not particularly important in terms of frictional response but it is however fundamental with regards to damage resistance and resistance to tension arising from shearing. Collagen damage is in fact thought to originate from excessive shear and strain along the fibre direction [315]. Hence, when inserted into the joint, either the aligned scaffolds should be oriented along the direction of shear and motion or the random scaffold orientation should be implanted. Although the random fibre orientation provides significantly lower tensile properties, it yields a better topographical surface for friction and wear based on the isotropic orientation of the matrix. With this in mind, it is thought that the collagen fibres in the superficial zone are oriented in the direction of maximum tensile strain [56] and hence, the scaffold, could potentially be placed along the direction of the split line pattern of AC, as suggested in Figure 6.8.
Chapter 6 – Effect of fibre orientation on the frictional and mechanical properties of tissue engineered articular cartilage

6.4 - Results

Figure 6.8 – Aligned tissue engineered scaffold placed in the distal end of a porcine femoral joint. The black line on the scaffold indicates the direction of the fibres which has been tentatively placed in the orientation of the split line pattern, made using a dissecting needle and India ink. The red lines have been placed to help visualize the direction of the split lines.

Furthermore, this study suggests that in order for tissue engineered AC to mimic the mechanical and frictional response of AC at physiological strains, the cell culture time should be long enough to allow full thickness ECM and hence AC to form. This would allow the scaffold-AC construct to better resist in-joint physiological stresses and strains prolonging the life of the scaffold in the joint which is essential in providing the foundation for the proliferation of chondrocytes and hence the successful formation of native AC.

6.5 - Conclusions

This study assessed the mechanical and frictional properties of microfibrous scaffolds to demonstrate the potential of these materials to be used as a replacement for small AC defects. Determination of mechanical properties highlighted significant differences in tensile properties according to the varying fibre orientation of the scaffolds. The aligned fibre scaffold tested parallel to the tensile load offered the best resistance to tension. The frictional response of the tissue engineered scaffolds in the boundary regime showed a significantly different trend from AC. This was attributed to the lack of interstitial fluid support in the tissue engineered scaffolds, mainly due to very thin layer of cell-deposited ECM, as well as the high compressive strains applied during sliding. However, the equilibrium coefficient of friction for the tissue engineered AC at 0.08 MPa (Figure 6d) was found to be very similar to AC, possibly due to the similar boundary lubrication mechanism occurring at the contact interface. The equilibrium coefficient of friction was also found to decrease with increasing load, for both acellular and cellular scaffolds, probably due to a smoothing of the surface. No significant differences in the equilibrium friction coefficient between differing fibre orientation
scaffolds were found. However, surface damage was higher when the shear was perpendicular to the scaffolds and for the randomly orientated scaffolds. The frictional response of the scaffolds was strongly dominated by the surface roughness and the presence of ECM on the scaffold’s surface increased the equilibrium friction coefficient probably due to ECM damage and adhesion effects. In conclusion, the results obtained from this study suggest that aligned microfibrous scaffolds provide improved surfaces for superficial zone tissue engineering of articular cartilage, as compared to random fibre networks and offer a potentially promising solution for the treatment of articular cartilage defects and localised early OA. However, studies involving longer culture times to allow full thickness ECM growth on the scaffold surface should be made to allow a more direct comparison to AC and thus to better evaluate the true clinical potential of such a technique. Future studies should also test different scaffold materials and varying fibre sizes in order to establish the effect of such variations on the frictional response.
Chapter 7 – Finite element analysis of the menisectomised tibio-femoral joint: implementation of advanced articular cartilage models

7.1 - Introduction

This study presents advanced computer simulations aimed at the accurate modelling of the human tibio-femoral joint (TFJ) in terms of anatomy, physiological loading, and constitutive behaviour of the tissues. A realistic FE model of the TFJ contact following total meniscectomy was reconstructed from MRI and CT scans of a human knee joint. The AC in the TFJ was modelled using some of the advanced constitutive models discussed in Chapter 2. These include: the (i) BLE, the (ii) BNLE, the (iii) transversely isotropic biphasic non linear model (BTINL) and the (iv) BPVE. The main objective of this study was to demonstrate the implications that the implementation of the different AC models have on the prediction of the joint response.

Firstly, the effect of the implementation of different AC models in a simplified three-dimensional configuration is explored in §7.4, where the importance of adequately capturing the contribution of the interstitial fluid support is shown.

In §7.5, a patient specific FE model of the TFJ with AC modelled as BPVE is developed in order to compare the effect of monophasic and biphasic models of AC on the joint mechanical response and to investigate the effect of physiological loading-unloading cycles when reproducing simplified walking conditions. The time evolution of stresses, pore pressure, contact areas and joint displacements are captured and compared with existing menisectomised knee models. The results are then used to predict the most likely locations for the origin of mechanical damage which could correspond to the most likely areas for OA origination. Finally, the limitations of the current model are discussed and future research directions suggested. This piece of research was done in collaboration with two visiting researchers, Dr Lorenza Mattei and Dr Eleonora Campioni from the Department of
Chapter 7 – Finite element analysis of the menisectomised tibio-femoral joint: implementation of advanced articular cartilage models

Mechanical, Nuclear and Production Engineering, University of Pisa and the Department of Mechanical and Civil Engineering, University of Modena and Reggio Emilia, respectively.

7.2 - Outline

In the last decades a plethora of advanced constitutive models for AC have been proposed and numerically implemented using FEM in order to describe the unique mechanical behaviour of AC dictated by its biphasic nature [338], flow-dependent and independent viscoelasticity [140, 339], and its strong structural and mechanical anisotropy mainly due to fibril reinforcement [208, 340-342]. Although a linear elastic model is a long way away from an accurate representation of AC, it has been largely adopted in the study of knee biomechanics. This is because the high non linearity of both realistic knee geometries and AC mechanical behaviour result in a numerical problem which is very complex and computationally expensive. Consequently, the linear elastic model has been adopted to model AC in several patient specific FE models of the knee used for investigating stresses and strains of native [212, 214, 218, 343-346] and menisectomised [158, 213, 217, 347, 348] joints. Only very recently, sophisticated biphasic constitutive models of AC have been implemented in human knee FE models. The fibril-reinforced model proposed by Li et al. [342] was applied, however neglecting viscoelasticity effects, in knee FE models [349, 350] in order to investigate the effect of pore pressure and fibre orientation in contact mechanics of both healthy and menisectomised knee joints [350]. The effect of the fibril pattern has also been investigated by Mononen and co-workers both for healthy and osteoarthritic AC in the human knee [351]. In this case the fibril-reinforced poroviscoelastic model described in [208, 340, 352] was adopted for AC. The models mentioned above simulated compression loads with the femur in full-extension, i.e. a fast loading ramp followed by a constant load. The maximum load was relatively low in [349] and [350], being 36 N and 300 N (in 1 s) respectively, whilst a physiological load of 1000 N (in 1 s) was applied in [351]. In addition, the majority of these models rely on the implementation of constitutive laws for the description of the tissue’s behaviour by the means of user defined material subroutines, which are often not readily available and simple to code and are usually linked to high computational costs.

Meniscectomy is well recognized as risk factor for secondary OA in the knee [353] causing a severe change in the knee contact mechanics. The loss of the medial meniscus leads to a decrease of up to 75% and an increase of up to 300% in contact area and contact stress, respectively [354-357]. Many clinical studies have highlighted the significant incidence of OA following meniscectomy, which can be observed both in the short and in the long term follow-up [358-360], and affects both young [361] and older patients [362]. In addition to this, it has been demonstrated that total meniscectomy causes higher incidence of degenerative change compared to partial meniscectomy [363, 364]. Several in vivo studies on animals have confirmed this evidence: meniscectomy-induced OA models revealed biological, bio-chemical, and biomechanical degradation of knee AC centrally,
and osteophyte formation peripherally [365-369]. Secondary OA, being mechanically induced, has recently become the subject of many biomechanical and biomedical engineering studies. To this aim FEM is certainly an invaluable tool for studying stresses and strains distribution within healthy or injured knee joints, and investigating the onset of AC damage. However, as discussed in Chapter 5, this can only be successful if suitable and realistic constitutive models of AC are employed.

### 7.3 - Articular cartilage models employed

The anatomical structures to be considered and modelled when analysing human joints consist of menisci, ligament, muscles, cortical bone, trabecular bone and the AC. In this study, the presence of the ligaments and the muscles was reproduced implicitly using *ad-hoc* BCs, while the presence of the menisci were ignored as the focus has been placed on the menisectomised knee joints. As far as the mechanical properties of the materials modelled are concerned, the femur and the tibia extremities were assumed to be composed of only epiphyseal surface bone, which has mechanical properties in the range between the cortical and trabecular bone properties. These were therefore modelled as homogeneous, isotropic and linear elastic, with a Young’s modulus of 5 GPa and a Poisson’s ratio of 0.3 [218].

The real focus of this study is, however, the AC behaviour. From Chapter 2, several material models of AC can be found [202], ranging from the relatively simple biphasic models to models considering more or less explicitly all the main components of the tissue, such as the fibril reinforced models [206-208, 342] and triphasic models [83, 209, 210]. In the present study only some of the existing biphasic AC models are considered and implemented in the FEM. These models, while not explicitly modelling the microstructural features of AC, are chosen as they allow a realistic representation of the tissue’s mechanical response, they are relatively simple to implement as they do not require the implementation of user defined material descriptions, and enable to run simulations of 3D non linear models at a relatively low computational cost.

Five AC models have been considered in this study. These are the monophasic (M), BLE, BNLE, BPVE and BTINL models. Comparison between the different models allows the exploration of the suitability of different constitutive material behaviours for the study of realistic TFJ geometries. It should be noted that the non linearity arising from the non linear permeability law is included in the BNLE, BPVE and BTINL models (recalling from §2.9.2.3 Equation (2.8)). The reader is referred to §2.9.2 for a detailed explanation of the analytical models employed in this study. Three BPVE models, characterized by different relaxation functions (BPVEa, BPVEb, and BPVEc), were implemented in order to investigate the role of the viscoelasticity of the solid phase on the AC mechanical response. Collagen fibrils oriented parallel to the AC surface, i.e. running in the x and z directions (Figure 7.1), were considered in the BTINL model.
7.3.1 - Material parameters

The mechanical properties characterising each model were extracted from experimental studies reported in the literature and used to calibrate the parameters to be adopted for numerical implementation. The mechanical parameters of the various models are shown below in Tables 7.1 – 7.3.

<table>
<thead>
<tr>
<th>BLE</th>
<th>BNL E</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{eq}$ (MPa)</td>
<td>0.421</td>
</tr>
<tr>
<td>$v_{eq}$</td>
<td>0.28</td>
</tr>
<tr>
<td>$k_0$ (m$^4$/N s)</td>
<td>$6.9 \times 10^{-15}$</td>
</tr>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>1</td>
</tr>
<tr>
<td>$e^*$</td>
<td>4</td>
</tr>
<tr>
<td>$M^*$</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 7.1 – Mechanical parameters for the BLE and the BNLE models of AC [3].

<table>
<thead>
<tr>
<th>BPVEa</th>
<th>BPVEb</th>
<th>BPVEc</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{eq}$ (MPa)</td>
<td>0.55</td>
<td>0.63</td>
</tr>
<tr>
<td>$v_{eq}$</td>
<td>0.24</td>
<td>0.1</td>
</tr>
<tr>
<td>$k_0$ (m$^4$/N s)</td>
<td>$0.9 \times 10^{-15}$</td>
<td>$1.72 \times 10^{-15}$</td>
</tr>
<tr>
<td>$\tilde{G}$</td>
<td>15.180</td>
<td>17.692</td>
</tr>
<tr>
<td>$\tau_s$ (s)</td>
<td>0.45</td>
<td>0.62</td>
</tr>
<tr>
<td>$\tau_L$ (s)</td>
<td>95.9</td>
<td>85.1</td>
</tr>
</tbody>
</table>

Table 7.2 – Mechanical parameters for the BPVE models of AC (BPVEa [3], BPVEb [65], BPVEc [59]), in addition to the marked parameters (*) of Table 7.1.

<table>
<thead>
<tr>
<th>BTINL</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{eq}$ (MPa)</td>
</tr>
<tr>
<td>$E_x$</td>
</tr>
<tr>
<td>$v_{eq}$</td>
</tr>
<tr>
<td>$v_{xz} = v_{yz}$</td>
</tr>
<tr>
<td>$G_{eq}$ (GPa)</td>
</tr>
<tr>
<td>$k_0$ (m$^4$/N s)</td>
</tr>
</tbody>
</table>

Table 7.3 – Mechanical parameters for the BTINL model of AC [3], in addition to the marked parameters of (*) of Table 7.1.

For the monophasic model, $E_{ins} = 5$ MPa and $v_{ins} = 0.46$ (recalling the terminology from §2.9.2.1) were chosen from the ranges of values reported in literature [158, 211-215, 217, 218, 345], 4 - 20 MPa and 0.45 - 0.48, respectively. The parameters of the biphasic models BLE, BNLE, BPVEa, BTINL, were taken from [339]. This allowed the use of a consistent set of parameters, derived from
the same AC samples set, for different AC models and consequently allow a direct and rigorous comparison between the implemented models. It should be noted that the parameters $M$ and $e_0$ were not reported in [339] and consequently they were estimated from other literature sources [204, 370-372]. The properties of the BPVEb and BPVEc models were taken from [310] and [204], respectively, assuming the same values of $M$ and $e_0$ used for the other models.

All the AC constitutive models were implemented in the simplified TFJ FE model, whilst only the monophasic and the BPVEb models were adopted in the patient specific TFJ model. The FE models were developed using the FE package Abaqus 6.9, using predefined material models.

**7.4 - Three dimensional simplified finite element model of the tibio-femoral joint**

**7.4.1 - Model description**

As a first step towards the implementation of an advanced AC material model in realistic 3D knee geometries, a 3D simplified FE model of the TFJ was developed, reproducing the realistic local anatomy of the joint. Simulations were therefore performed with the aim of identifying the optimal mesh and the most reliable constitutive law for AC to then be implemented in the patient specific FE model presented in §7.5. The simplified three-dimensional model of the TFJ depicted in Figure 7.1a is geometrically representative of a single knee compartment and is constituted by two interacting spherical layers of AC bonded to two prismatic substrates representing the equivalent bone structures. An average thickness of the AC layers of 2.5 mm [373, 374] and curvature radius of 200 mm were selected as they correspond to the equivalent radius of curvature and cartilage thickness observed in the most heavily loaded TFJ contact regions. The bone is represented by a prismatic part characterised by a square base (16 mm x 16 mm) and height 25 mm. Only a quarter of the model was simulated since symmetric kinematic BCs were invoked (§7.4.3).

**7.4.2 - Mesh**

Hexahedral elements were employed to mesh both the AC and the bone. Different element types were used for each tissues/models: linear 3D Stress (C3D8R) for bone, quadratic 3D Stress (C3D20R) for the monophasic AC, and quadratic Pore Fluid/Stress (C3D20RP) for biphasic AC. It is worth noting that hexahedral elements are a requirement for the soils consolidation analyses performed by Abaqus; in addition, the quadratic order used for the AC elements was chosen in order to achieve better accuracy and avoid convergence issues. This is in accordance with the mesh sensitivity study performed in this study; a thorough investigation examining convergence and accuracy issues was also carried out to select the element size required to correctly capture the AC behaviour. On the basis of such analysis, which showed that the results are relatively insensitive to the choice of prismatic element base size, two mesh densities were selected: their topologies are depicted in Figure 7.1b, with
the finer one employed in the simplified 3D model to maximize accuracy and the coarser one used in the full 3D knee joint model to achieve an optimal compromise between accuracy and computational cost.

**Figure 7.1** – Simplified model of the TFJ: geometry of a quarter of a single compartment \((a)\) and two examples of mesh \((b)\).

### 7.4.3 - Boundary conditions

**Surface interactions:** Two different surface interactions occur in the TFJ: the bone-AC and the AC-AC interactions (Figure 7.2a). The bone-AC interaction was modelled as a tie constraint (i.e. no relative motion). This was thought to be a reasonable assumption since the AC surface is strongly bonded to the correspondent bone surface where the AC tidemark lies. The AC-AC interaction was modelled as a frictionless (considering only normal stresses at the contact surface) and hard contact (the contact is “total” when it is detected), taking into account the possibility of finite sliding between the surfaces.

**Kinematic and loading conditions:** The lower transversal section of the tibial bone was built-in while the femur kinematics was driven by the kinematics of a reference point (RP) linked to the top rigid surface, as depicted in Figure 7.2a. The vertical translation of the RP was left as unconstrained since this degree of freedom (DoF) was indirectly controlled by the vertical load; all the other DoF were fixed. The loading cycle consisted of a vertical load of 10 N applied to the rigid surface using a ramp of 15 s, then kept constant for 450 s (Figure 7.2b), which results in a maximum load of 80 N applied to the entire knee. Although such conditions do not reproduce the physiological conditions later adopted for the patient specific TFJ model, they were chosen for a number of reasons. First of all, the loading protocol simulated conditions similar to those of the mechanical tests carried out to extrapolate AC material properties used in this study (Table 7.3): e.g. DiSilvestro and co-workers [339] prescribed a strain rate of 0.001 s\(^{-1}\) whilst here (see §7.6.1) the loading conditions resulted in a strain rate in the range 0.002–0.007 s\(^{-1}\) at the end of the load ramp. Secondly, the simulation of low loads allowed the comparison of different AC constitutive models and provided insights into the
transient mechanical response of AC avoiding numerical difficulties and high computational costs. A similar approach has also been adopted in other studies presented in the recent literature [349].

**BCs for the pore pressure of the biphasic AC:** When the AC is modelled as a biphasic material, pore pressure BCs are implemented to regulate the fluid flow in and out of the tissue. As indicated in (Figure 7.2c), a sealed condition (i.e. no fluid flow) at the bone-AC interfaces, and a free draining condition (i.e. zero pore pressure) on the external AC surfaces were employed.

![Figure 7.2 – Scheme of the BCs applied to simplified TFJ model. The global coordinate system is shown on the bottom-left.](image)

**7.5 - Patient specific finite model of the tibio-femoral joint**

**7.5.1 - Model description**

In this study, a bicompartamental total menisectomised knee joint has been modelled with the aim to investigate the development of secondary OA. Consequently the anatomical structures of interest are the femur/tibia bone, the AC, the ligaments and the muscles. As the stabilising and the load transfer functions of ligament and muscles were reproduced by *ad-hoc* BCs, the patient specific model consists of the femur/tibia bone extremities and AC, which is modelled as a surface layer perfectly bonded to the bone.

The model geometry was obtained from high resolution medical images of a 75 years old female patient, with a body weight of 65 Kg, which had previously undergone a bicompartamental total meniscectomy surgery. The hard and soft tissue reconstructions were based on CT and MRI scans, respectively (Figure 7.3a). The 2D scans were later combined for the surface rendering by means of the AVIZO package (Visualization Sciences Group). This part of the study was done by Dr Eleonora Campioni and Dr Lorenza Mattei whilst visiting the research group at Imperial College London.

The final model consists of six deformable parts, as shown in Figure 7.3b: the two articulating bone extremities of the femur and the tibia, the lateral femoral AC (LFAC), the medial femoral AC (MFAC), the lateral tibial AC (LTAC), and the medial tibial AC (MTAC). A rigid body,
Chapter 7 – Finite element analysis of the menisectomised tibio-femoral joint: implementation of advanced articular cartilage models

corresponding to the upper transversal slice of the femur (coloured in grey in Figure 7.3c), was also included to enable the application of uniform loads on the upper bone cross-section.

Figure 7.3 – FEM process starting with the (a) reconstruction of 3D geometry of a patient specific TFJ from CT (hard tissue) and MRI (soft tissue) scans (with permission from M. Tuncer, Imperial College London), (b) the creation of the geometric parts of the model and finally (c) the meshing of the model.

7.5.2 - Mesh

The reconstructed geometry was imported in Abaqus where all remaining phases of pre-processing, solving and post-processing of FE analysis were executed. The geometric parts were individually meshed as portrayed in Figure 7.3c. The mesh characteristics are described in Table 7.4.

<table>
<thead>
<tr>
<th>Part</th>
<th>Elements</th>
<th>Geometry</th>
<th>Dimension (mm)</th>
<th>Type</th>
<th>Geometric order</th>
<th>Number</th>
<th>Nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur</td>
<td>Tetrahedral</td>
<td>1.5 – 4 (edge)</td>
<td>3D stress</td>
<td>Linear</td>
<td>5768</td>
<td>24636</td>
<td></td>
</tr>
<tr>
<td>Tibia</td>
<td></td>
<td>1.5 – 4 (edge)</td>
<td>3D stress</td>
<td>Linear</td>
<td>5508</td>
<td>23590</td>
<td></td>
</tr>
<tr>
<td>LFAC</td>
<td>Hexahedral</td>
<td>1.5 – 2.5 (edge)</td>
<td>3D Stress/ Pore Fluid Stress</td>
<td>Quadratic</td>
<td>592</td>
<td>3143</td>
<td></td>
</tr>
<tr>
<td>MFAC</td>
<td></td>
<td>+ 4 layers</td>
<td>3D Stress/ Pore Fluid Stress</td>
<td></td>
<td>924</td>
<td>4776</td>
<td></td>
</tr>
<tr>
<td>LTAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>680</td>
<td>3547</td>
<td></td>
</tr>
<tr>
<td>MTAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>684</td>
<td>3580</td>
<td></td>
</tr>
</tbody>
</table>

Table 7.4 – Main characteristics of the mesh of the patient specific TFJ model.

The bone and the AC were meshed with tetrahedral and hexahedral elements respectively (see §7.4.2). The dimension of the bone elements varied along the Y-axis, with the elements progressively reducing in size in the regions adjacent the contact region. The mesh of the AC sections was chosen according to the mesh sensitivity analysis performed using the simplified model described in §7.4; thus, 4 layers of prismatic elements of varying thickness and with a square base characterised by edge dimensions of about 1.5 – 2.5 mm were adopted for the discretisation of the AC tissue.
7.5.3 - Boundary conditions

Surface interactions: The surface interactions were modelled as described in §7.4.3. The resulting model was composed of 4 pairs of tied surfaces at the AC-bone interfaces, and two contact pairs, in the medial (MFAC-MTAC) and in the lateral (LFAC-LTAC) compartments (Figure 7.4(a)).

Kinematic and loading conditions: The kinematic and loading BCs were chosen in order to simulate the adaptation mechanism of the articulating surfaces approaching contact, therefore allowing the surfaces to reach a relative position that ensures maximum contact area and minimum contact pressure. The lower transversal section of the tibia was built-in, while the femur kinematics was driven by the kinematics of the femoral rigid part, whose reference point (RP) was positioned between the two femoral condyles (Figure 7.4a). As for the simplified TFJ model, the vertical translation of the RP was controlled via the vertical load. The abduction/adduction, i.e. the rotation of the rigid part of the femur around the Z-axis, was considered free whilst all the other DoFs were fixed. The full-extension of the TFJ was chosen as the initial condition since this is considered one of the most critical loading conditions. All simulations were executed in load control. A physiological vertical load was applied to the femoral rigid part and transmitted, as a homogenously distributed pressure, to the upper transversal section of the femoral deformable part. The loading cycle, which was repeatedly applied to the joint for 5 times during the simulations, is illustrated in Figure 7.4b. This corresponds to a simplified representation of the vertical load evolution observed during a gait cycle, which is characterised by a double peak during the stance phase and a constant low value during the swing phase. This is in agreement with both the predictions of other musculoskeletal models [375, 376] and the measurements carried out on instrumented knee prosthesis [377]. However, the peak load, which can reach up to 2.5 BW during the gait, was here set to 1.5 BW, which corresponds to the maximum force during the gait with the knee in full extension [378, 379] and is also in line with the loading protocol used in the experimental tests [380] and in other FE models [213, 214, 217, 344, 351]. It is worth mentioning that the load control can be implemented only when the joint surfaces are in contact. This condition was guaranteed by an initial phase of displacement control which consisted of a very small linear vertical displacement, applied in 0.1 s.

BCs for the pore pressure of the biphasic AC: As already described in §7.4.3, sealed and free-draining conditions were imposed at the bone-AC interfaces and on the external AC surfaces, respectively. In this way we assumed that the interstitial fluid could exude out the AC across the free surfaces, as illustrated by the fluid velocity vectors computed during the simulation and shown in Figure 7.4c.
Chapter 7 – Finite element analysis of the menisectomised tibio-femoral joint: implementation of advanced articular cartilage models

7.6 - Results and discussion

7.6.1 - Comparison of AC material models

This section presents the comparison of the constitutive models of the AC implemented in the simplified TFJ model. Both global (i.e. vertical displacement of the RP and contact area) and local parameters\(^2\) (e.g. stresses, strains, pore pressure, flow velocity) were monitored for the AC and the implications that their variations had on the tissue’s response were investigated.

Initially the difference between the monophasic and the biphasic AC models was considered. The results reported in Figure 7.5 demonstrate that the monophasic material, whose properties have been calibrated as described in §7.3.1, seems to be stiffer than the biphasic model, being characterized by a lower vertical displacement of the RP (i.e. indication of soft tissue compression), a lower contact area and a higher contact pressure. The maximum mismatch between the models in terms of contact area and contact pressure are 85% and 450% respectively, as shown in Figures 7.5b and 7.5c.

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\(^2\) A local parameter of a geometric part corresponds to the value of the variable of interest extrapolated at the node/element at which the maximum absolute value was recorded during the loading cycle. If the maximum value was shared by more nodes/elements, the variable was evaluated by averaging over these nodes/elements.
Figure 7.5 – Comparison of the AC models in terms of (a) vertical displacement of the RP, (b) contact area, (c) contact pressure and (d) pore fluid pressure.

Direct evidence of the fact that biphasic AC models are required to predict the realistic transient behaviour of the soft tissue is provided by the constant values obtained for both local and global characteristics when looking at all the outputs of the monophasic model displayed in Figure 7.5 after the load reaches the plateau (see Figure 7.2b). This is obviously not the case for the biphasic model, whereby the load support is generated by both the solid matrix and the fluid phase [381] and their continuous interaction results in a time dependent material response (Figure 7.5). In particular, the ratio of the fluid/solid support varies both in time and in space within the AC thickness. This is clearly captured in Figure 7.6, which portrays the normalised solid and fluid support components along two node paths extracted under the surface of the bottom AC. The simulations, obtained assuming the BPVEb model for the AC, show that, when the load is initially ramped to its maximum value, the load is almost entirely supported by the increase in interstitial fluid pressure in the tissue, which develops due to the very low permeability of AC; gradually, as the fluid exudes out of the contact zone, a load shift is observed from the fluid to the solid phase. It is worth noting that the solid and fluid supports were calculated as the integral of the vertical axial stress and the pore pressure along the path of nodes, respectively, and were normalised with respect to the total load.
Figure 7.6 – An example of the temporal evolution of the fluid/solid load support in the biphasic AC. The fluid/solid supports for two node paths at different depths in the AC thickness are compared.

We turn now to the differences between the various biphasic AC models considered in this study. Among the implemented BPVE models, only the BPVEa is considered for the purpose of comparison. As shown by the vertical displacement of the RP in Figure 7.5a, during the monotonic application of the load the compression of the tissue increases, more or less linearly, depending on the adopted biphasic model; after the load is held constant at its maximum value, the vertical displacement keeps increasing for all models due to the fluid exudation and, in the case of the BPVEa, also due to the viscoelastic relaxation of the ECM. The comparison between the responses obtained using different biphasic AC models revealed that the compression of the tissue is largest for the BLE model and smallest for the BPVEa model, with the BNL and the BTINL producing intermediate results. This is due to the fact that the BNLE model is stiffer than the BLE model because of a lower fluid exudation due to the reduction of permeability as a function of the strains experienced by the solid matrix; furthermore, the stiffness of the BTINL material in the transverse direction is higher than in the BNL model. Moreover, with respect to all the other models, the matrix of the BPVEa material is characterised by additional stiffening due to the viscoelastic behaviour of the solid phase; this effect depends on the strain rate and is partly responsible for the behaviour displayed in Figure 7.5a. The compression of the AC is directly related to the contact area and the contact pressure, depicted in Figures 7.5b and 7.5c respectively: the higher the strain, the better the adaptation of the articulating surfaces and, thus, the larger the contact area and the lower the contact pressure. Consequently, the lower contact area and the highest contact pressure were observed for the BPVEa model, whilst the highest contact area and the lowest contact pressure for the BLE model. It is worth noting the significant dispersion of the local/global parameters predicted by the various biphasic models. For instance, a relative mismatch of 45.2% was found at the end of the loading cycle between the largest (BLE model) and the smallest (BPVEa model) contact area and a relative variation of about 54.5% was computed between the largest (BPVEa model) and the smallest (BLE model) peak contact pressure. The same data dispersion was observed for the pore (i.e. fluid) pressures of the biphasic models, compared in Figure 7.5d. All models clearly show pore fluid pressurisation as the load is applied; in particular the highest pore pressure was observed in the BPVEa model, whilst the lowest in
the BLE model. When the load is kept constant at its maximum value, the increase in fluid exuded from the contact zone corresponds to a drastic decrease of the fluid pressure and an increase of the strain in the solid matrix.

The results pointed out that the flow dependent viscoelasticity of biphasic AC enables more conformal contacts and hence allows the load to be distributed over a larger area, leading to lower contact pressure and stresses in the soft tissue. Also the flow independent viscoelasticity of BPVE models is a fundamental aspect of AC behaviour: for high strain rates due to rapidly varying dynamic loading, it enables the stiffening of the soft tissue thus inhibiting excessive deformations, in agreement with [382]. The influential role of the flow independent viscoelasticity is also highlighted by means of the comparison between the mechanical responses of several BPVE models. Indeed BPVEa-c models predicted quite different values of peak contact pressure (Figure 7.5c) and peak pore pressure (Figure 7.5d), and also different rates of stress relaxation, all in agreement with their viscoelastic parameters reported in Table 7.3. For instance the BPVEb model predicted higher contact pressures when compared to BPVEa and BPVEc, with a discrepancy of up to 17.8% and 56%, respectively.

This study further supports the need to implement biphasic models for the AC in order to predict its realistic transient mechanical behaviour, the load shift mechanism and the adaptation of the contacting surfaces together with the load redistribution. Although several TFJ contact studies have made use of the monophasic elastic model, relying on the fact that at high strain rates a biphasic material would behave as a monophasic model with a higher instantaneous elastic modulus, the implementation of biphasic constitutive models, even in their simplest form, is certainly a first step for achieving more accurate predictions of the stress state and the onset of damage of AC as this is bound to be linked to the time-varying response of the tissue under impact loading. Furthermore, it has been suggested that the instantaneous load response of AC in the knee might not be adequately predicted using the elastic model [383, 384]. The BPVE model was selected among the previously discussed biphasic AC models to be implemented in the patient specific TFJ model as it is the only one that accounts for the flow independent viscoelasticity and the predicted mechanical response shows very good agreement with several experimental findings obtained from different experimental configurations (e.g. confined/unconfined tests) [140, 310, 339].

7.6.2 - AC mechanical response during simplified walking cycles

This section presents the main results obtained by simulating the patient specific TFJ contact under simplified walking cycles. The comparison between the outputs obtained using the monophasic and one of the implemented biphasic models (i.e. BPVEb) was considered to shed light on the AC behaviour. The main findings are reported in Figures 7.7 - 7.10, which show the results obtained by the two models in terms of both global and local responses of the AC (Figure 7.7), also making use of relevant 3D contour plots (Figures 7.8 - 7.10) to aid the discussion.
Figure 7.7 – Comparison of the global and the local parameters of the M and the BPVEb (simply labelled as “B”) AC models, during 5 loading cycles: (a) vertical displacement of the RP, (b) contact area, (c) contact pressure and (d) pore fluid pressure.

Figure 7.8 – Contour plots of the contact pressure of biphasic AC layers, at several instants during the walk. The contact pressure is higher for higher loads and decreases during time due to the load redistribution on wider contact areas.
Figure 7.9 – Contour plots of von Mises stress of biphasic AC layers, at several instants during the walk. The von Mises stress is higher for higher loads and decreases during time due to the stress relaxation.

Figure 7.10 – Comparison of von Mises stresses predicted in the medial compartment implementing the (a) BPVEb and the (b) M models for the AC, at the first load peak of the first walking cycle.

7.6.2.1 - Monophasic vs BPVE articular cartilage behaviour during walking: implications for the onset of damage and OA

The comparison between monophasic and biphasic AC response during walking confirms the necessity to implement advanced AC models in order to predict a realistic transient tissue response. During each walking cycle, the mechanical response of both the monophasic and the biphasic AC reflects the transient behaviour of the load: all the curves in Figure 7.7 are characterised by two peaks which correspond to the two loading peaks. Before each loading peak, the compressive strains (Figure 7.7a), the contact area (Figure 7.7b) and the contact pressure (Figure 7.7c) rapidly increase. In the
model characterised by biphasic AC, an increase in the interstitial fluid pressure (Figure 7.7d) and of the magnitude of flow velocity is recorded, whilst both the void ratio and the permeability decrease. The opposite occurs as the load decreases after each loading peak. In particular, the fluid pressure remains always positive; this suggests a continuous fluid exudation (fluid flowing out of the tissue), in accordance with the free draining BC applied to the AC surface.

The differences between the two models appeared to be remarkable: the biphasic AC model predicted a temporal evolution of the mechanical response of the tissue, both within a single and several walking cycles, highlighting the unique capacity of the biphasic material to adapt to the load and to redistribute stresses and strains. Figures 7.7-7.9 show a significant variation of the biphasic AC response during the first three cycles, and less variation in the last two cycles. Indeed, the AC behaviour approached a pseudo-steady state after about five walking cycles, when most of the fluid has been exuded and the elastic response of the solid matrix dominates the tissue’s behaviour. The value of the pore pressure in the MTAC decreased by 22.9% between the first peak load of the first and the third walking cycle, and by 8.6% between the first peak load of the third and the fifth walking cycle. The corresponding values of the von Mises stress decreased by 21.1% and 6.8% between the first peak load of the first and the third walking cycle, and between the first peak load of the third and the fifth walking cycle, respectively. In brief, the compression of the soft tissue continuously increased (Figure 7.7a) and allowed a better adaptation of the contacting surfaces. This corresponds to increasing contact areas (Figure 7.7b) and decreasing contact pressures (Figure 7.7c). Between the first peak load of the first and the last walking cycle, the variations of the contact pressure and the contact area in the medial side were -25.8% and 34.3% respectively. The load, at first mainly supported by the fluid pressurisation, was gradually transferred to the solid matrix as the fluid exuded the pore pressure decreased (Figure 7.7d); furthermore, the von Mises stresses decreased as a consequence of the poroviscoelastic stress relaxation (Figure 7.9). The stress and strains redistribution that characterizes the behaviour of the biphasic AC is clearly described by the contour plots in Figures 7.8-7.10: during the cyclic application of the load both the von Mises stress and the contact pressure decrease, while the contact area increases.

It appears that the longer one walks, the less stressed the AC in the TF contact. A mechanism of load redistribution in the tissue occurs which is fundamental to prevent the damage of the solid constituents. During the initial loading phase of walking, the tissue is subject to the highest stresses. After just a few walking cycles, the AC adapts to the loading cycle and shows a quasi-steady behaviour characterised by a higher contact area and lower stresses. The limits of the monophasic AC model are thus highlighted: it fails to account for the load redistribution, hence overestimating the contact area, as well as underestimating the high instantaneous stresses which arise upon load application in the biphasic material.

The von Mises stress (overall stress level) does indeed decrease during the gait cycle as the compliant nature of the tissue allows it to adapt to the geometry and distribute the load over a larger
area. In this case, the increase in contact area has been found to offset the loss of interstitial fluid support, allowing for a decrease in overall solid stress levels. However, we must not disregard that although the overall stress level between gait cycles is decreasing, the solid stress in the tissue will have significantly increased following the first and initial load application, due to loss of interstitial fluid support, which is detrimental for the tissue as its solid constituent, prone to damage, will now be supporting most of the axial load. The increase in contact area and the contact adaptation mechanism would definitely help in reducing the stresses, as demonstrated from the simulation, but, however, only for the first few cycles. Once interstitial fluid pressurisation is lost and the viscoelastic material has completely relaxed, the AC would ultimately be exposed to the most damage.

It should be borne in mind, however, that the stress decrease observed in this study might not necessarily occur in every TFJ contact. This is a complex phenomenon dictated by the rising stresses due to loss of interstitial fluid support and the decreasing stresses due to the increasing contact area. The balance between the two, which ultimately dictates the overall resulting stress levels, depends upon the contact geometry and loading conditions which would vary from joint to joint. The model reported in this work to study the mechanical response of a full knee joint suffering from a total bicompartamental meniscectomy is motivated by the overall objective of investigating the initiation of AC damage and OA associated with such a procedure [385, 386]. Indeed, because of the absence of the menisci, the biomechanics are completely modified and high stresses may be experienced by the AC, therefore promoting tissue damage [357]. Some studies have addressed the damage initiation in AC using FEM [23, 291, 387, 388]; however, researchers have not yet investigated the damage initiation in a patient specific and anatomically realistic TFJ contact both using advanced AC models and under physiological loading conditions. Investigating damage initiation goes beyond the aim of the present study; however, some preliminary considerations on this issue will be reported here to discuss the initial efforts of what will constitute the basis for future investigations.

Most FE models of the knee joint, which attempt to predict AC damage initiation, typically investigate maximum shear stress [291, 388], Tresca stress distributions, maximum principal strains [23] in the AC and identify regions with the highest stresses as being the locations more prone to OA initiation and damage. Furthermore, a new criterion was recently introduced by Pena et al. [158] based on the hypothesis that the AC failure is mainly due to the local variation of shear stresses rather than their absolute magnitude; this has shown some promise and the predictions were somewhat in agreement with existing experimental studies. The exploitation of classical von Mises and Tresca failure criteria has some limitations considering that they were first established for ordinary engineering materials. These clearly have a different mechanical behaviour compared to the biphasic AC, and cannot account for the mechanisms that cause AC damage at the microstructural level. Nevertheless, from a macroscopic point of view, it is reasonable to expect that high local stresses will correspond to the most damaged areas [315]. Since the aim of the investigation is to compare AC
damage predicted using monophasic and biphasic constitutive behaviours, in the present study the locations of OA initiation suggested by the maximum of von Mises and Tresca stresses were analysed. As such locations were predicted to be almost identical according to the two criteria, only the von Mises results are here presented and discussed. The intention, however, is not to propose which criterion is most suitable to predict the onset of damage in the tissue but simply to use the von Mises or Tresca stresses to localise the most stressed region of the joint and therefore associate these with the most likely area for damage initiation. As expected, the biphasic and the monophasic constitutive laws predicted a different stress field in the soft tissue, both in terms of magnitude and location (Figure 7.10). Firstly the two models predicted the highest von Mises stresses in different TFJ compartments, i.e. in the MTAC and in the LFAC for the BPVE and the monophasic model respectively, although both the models predicted a more conformal contact and a wider contact area in the medial compartment. Secondly, a different distribution of von Mises stresses was also observed in the AC thickness. Figure 7.10a shows the map of von Mises stresses simulated by the BPVE models at the first load peak in a section of the medial AC compartment belonging to the frontal plane and crossing the point of the maximum superficial von Mises stress. The von Mises stresses for the AC is maximum at the AC surface during the entire duration of the simulations, almost at the same locations as the maximum contact pressure, while decrease through the AC depth. However, it should be noted that the maximum von Mises stress varies in magnitude and location with time (see also Figure 7.9). On the other hand, the monophasic model predicted much lower von Mises stresses in the same AC section and at the same instant (Figure 7.10b) and, in contrast to what is seen for the BPVE model, the highest von Mises stresses are approximately at the centre of the AC thickness.

In conclusion, according to the biphasic constitutive law and assuming a conventional macroscopic failure criterion, i.e. maximum von Mises stresses, the most likely location for the OA initiation in the bicompartamental menisectomised knee was predicted to be on the AC surface of the medial compartment. In particular, by analysing the superficial von Mises stresses distribution (Figure 7.9), OA could initiate in the region near the centre for the medial compartment and in proximity of the AC surface edge for the lateral compartment, almost in correspondence of the maximum contact pressures. This is in line with previous studies and clinical data which have showed that OA affects the medial compartment significantly more than the lateral compartment [389-391]. Furthermore, the central aspect of the compartment seems to be more prone to damage compared to the anterior and posterior aspects [300].
7.6.2.2 - Model validation using experimental evidence and comparison with existing numerical simulations

In the past decades several experimental studies on cadaveric/amputated human knees have been carried out to investigate the effects of meniscectomy on knee contact mechanics in terms of contact area and contact pressure, generally measured using flexible pressure-sensitive films (e.g. Prescale by Fuji Film Co. [380]). A comparison with these results can be difficult since experimental tests can differ in BCs or be affected by inter-subject variability. The closest match to the scenario modelled in this paper is provided by the experimental results obtained by Fukubayashi and Kurosawa [380]. Under a compression load of 1000 N, with the knee in full extension, they measured, for fully menisectomised knees, a contact area of about 300 ± 80 mm² in the medial compartment and 230±80 mm² in the lateral one, and a peak contact pressure around 6 MPa. These results are in good agreement with the simulations reported in this study obtained using a biphasic description of the tissue: if a load of 1000 N is applied to the BPVE model, the predicted contact areas are in the range of 203–282 mm² and 146–182 mm², for the medial and the lateral compartment respectively, while the peak contact pressure is in the range of 4.5 – 6.3 MPa. In addition, our results showed larger contact areas in the medial compartment, as also observed in several ex-vivo studies of total menisectomised knees [355, 380].

A comparison with existing numerical studies can be extremely difficult considering the wide variety of geometries, BCs and AC material laws/properties reported in literature. However, an attempt is made here to compare our results with some of the outputs obtained by other researchers using models of fully-extended knee under compression axial loads. First, FE simulations performed assuming the AC as an elastic isotropic material are compared to our results obtained using a monophasic AC model. It is worth noting that such models simulated total medial menisectomised knee and, thus, only results relative to the medial compartment are considered here. Bendjaballah et al. [213] predicted, under 1000 N load, compressive stresses of about 2.8 MPa and 5 MPa in MTAC and MFAC respectively; for a higher load of 1150 N, Pena et al. [217] computed compressive stresses of 5.3 MPa and 6.4 MPa in MTAC and MFAC respectively. Again for a compressive load of 1150 N, Pena et al. [158], when considering the Tresca stresses in order to predict the onset to OA, obtained maximum values for this parameter of 6 MPa and 3.3 MPa in MTAC and MFAC respectively. If a direct comparison with our model is sought, the results obtained for the monophasic AC description in our simulations at 1000 N show maximum compressive stresses of 3.9 MPa and 4.1 MPa in MTAC and MFAC respectively and maximum Tresca stress of about 2.8 MPa. According to Bae et al.’s work [392], simulations carried out using a load of 570 N show a peak contact pressure of 3.3 MPa and 3 MPa in MTAC and MFAC respectively, whilst the values obtained using the model presented here yield 3.5 MPa and 2.9 MPa respectively. To conclude, by assuming a monophasic description of the
soft tissue, the model developed in this investigation is in good agreement with previously published studies.

Few studies which implement the biphasic description of AC in a bicompartamental total menisectomised knee joint have been carried out. Wilson et al. [372] proposed a simplified axysymmetric knee model with biphasic AC layers: under a load of 588 N, applied in 1 s, a maximum contact pressure of 1.5 MPa for BNLE model and 1.9 MPa for a BTINLE model were obtained. Under the same load, but applied at higher loading rates, the model developed in our study predicts, as expected, a higher maximum contact pressure of about 4.7 MPa. More recently, a 3D knee model with fibril reinforced biphasic AC was developed by Kazemi et al. [350]; a load ramp of 300 N in 1 s caused a maximum principal stress of 0.52 MPa and a maximum fluid pressure of 0.6 MPa, both in the deep layer of the MFAC that is approximately at a 7/8th depth from the AC surface. The results obtained using the proposed biphasic description for a load of 300 N applied in 0.11 s were 0.7 MPa and 3 MPa respectively. As in our simulations the loading rate was significantly higher than in Kazemi et al.’s work [350] (e.g. about 3000 N/s vs 300 N/s), the higher interstitial fluid pressurisation level can explain the larger values of pressures and stresses predicted in our simulations.

7.7 - Conclusion

The present study highlights the importance of the implementation of advanced biphasic AC models to capture the fluid load support and the transient behaviour of the tissue’s response when modelling human joints. The prediction of damage initiation location in such joints can only be achieved by simulating the mechanisms governing the behaviour of the tissue and by correctly reproducing the realistic stress and strain distributions within the AC.

In particular, the comparison of different biphasic constitutive laws performed in a simplified three-dimensional model of one of the TFJ compartments is used to show that both flow dependent and flow independent viscoelasticity are important and necessary to model the realistic mechanical response of the AC. The biphasic poroviscoelastic non-linear description of the tissue was then implemented in a patient specific joint model. The model is relatively simple to implement and captures the most important mechanisms governing the AC response under loading. In particular, it has allowed the highlighting of the load shift mechanism between the fluid/solid constituents of the tissue, to study the adaptation of AC to the applied load with time, and to identify the most stressed areas in the AC, which in turn are most likely to develop OA (i.e. the medial side). For the first time, a patient specific knee model has been developed to include and compare the effect that the monophasic and biphasic models of the AC have on the joint. The results obtained using the simulations performed and their comparison with existing simulations of menisectomised joints have emphasised the need to explicitly model the biphasic nature of the AC. It is worth noting that the patient specific TFJ model has also been corroborated using experimental results obtained by studies carried out on cadaveric knees and available in literature.
However, it should be noted that once the contact adaptation mechanism has reached equilibrium, thus resulting in constant stresses and strains thereafter during the gait cycle, by assigning a Young’s modulus equal to an elastic model which would yield the same response as the biphasic model at equilibrium, an elastic solution could indeed be used to model the AC at stabilised conditions. Nevertheless, the biphasic model still remains essential to characterise the transient response at the beginning of the gait cycle and therefore the equilibrium conditions.

Future studies should be devoted to the implementation of a flow continuity across the AC tissues in three-dimensional joints, to optimize the BCs in order to simulate more realistic walking conditions both in term of load (e.g. torque) and kinematics (e.g. flexion, anterior-posterior translation), and include one or both menisci in the model in order to reproduce partial meniscectomy and healthy knees. The effect of collagen fibres orientation and the development of a micromechanical damage model to be implemented in the FE framework should also be amongst future research topics.
Chapter 8 – Conclusions and future work

8.1 - Introduction

The reader will have acknowledged that for a successful and in depth understanding of AC and the pathogenesis and progression of OA as well as for the design of potential artificial substitutes, inevitably necessitates bridging the gap between the medical, biological and engineering worlds. The main aim of this thesis was to approach the research using different methodologies and techniques with the ultimate goal of contributing to better understanding OA, to the early diagnosis of the pathology by studying mechanical and biochemical variations in the tissue and to suggest improved designs of tissue engineered AC which is a potential treatment for early OA.

The research commenced with a comprehensive study of the mechanical and frictional response of native AC which provided the essential backbone for the rest of this investigation. The following step was to study the effect of selective ECM degradation on the mechanical, frictional and wear properties of the tissue. Significant differences in mechanical response were found with only minor structural damage, simulating early OA. Further damage, simulating middle and late stage OA, was found to have devastating effects, severely decreasing the quality of mechanical, frictional and wear properties of the tissue. Using mechanical parameters, OA-like AC was then compared to osteoarthritic tissue to confirm the role of proteolytic enzymes in the pathology. Finally, mechanically induced damage was found to affect the mechanical and frictional response of the tissue suggesting a possible role in the progression of OA.

The mechanical performance of state of the art tissue engineered AC to be used in MACI procedures was then evaluated. The orientation of the micro fibres was found to be critical in dictating the evolution of surface damage in the scaffold. It was confirmed that MACI seems to be a promising solution to treat AC defects and localized early OA. However, care must be taken to understand the role played by the tissue engineered AC in the load support so as to ensure that the tissue develops as premature mechanical damage on the scaffold could inhibit full tissue recovery and hinder the success of the treatment.

To conclude the research investigation, a 3D FEM study of a patient specific menisectomised tibio-femoral contact was conducted. Some of the biphasic models previously used to evaluate the
mechanical parameters of AC were implemented for the first time in a 3D knee joint contact model. This study was made to demonstrate the importance of using biphasic models and other salient material descriptions to accurately predict stresses and strains in the AC. The study was also an attempt to show the strong potential of patient specific FEM in diagnosing early OA by predicting probable locations of OA origination and mechanically induced damage.

The main results and findings of this research investigation are summarised below.

8.2 - The unique mechanical and frictional properties of native articular cartilage

AC has a unique structure with highly complex material properties. Crucial to its role, it has remarkable friction and wear properties allowing joints to function for several decades with no degradation or thinning of the tissue unless affected by trauma or degenerative joint diseases [5]. The main feature of the tissue responsible for such an important quality is the biphasic nature and the associated interstitial fluid pressurisation which occurs in the tissue. In Chapter 4, AC was subjected to alternating shearing forces under a constant load and physiological contact pressures. Shear testing under different lubricating regimes was performed to ascertain the influence of interstitial fluid support on the evolution of frictional forces during cyclic loading. Numerical studies were also performed using the finite element software Abaqus. The tissue was modelled as a biphasic material with strain dependent permeability. The lubrication mechanisms occurring when cartilage was subject to compression and shear were studied in order to corroborate the experimental findings [115].

As already observed by other recent investigations, interstitial fluid pressurisation was found to govern the frictional response of AC. Even after several thousand cycles, very little damage was detected on the surface. This experimental findings and the FEM which showed how fluid pressurisation helps support normal loads as well as promoting a self-generating biphasic lubrication mechanism, indicated the crucial role played by fluid pressurisation in minimizing the surface wear [113]. Using two consecutive static loading tests, a direct measure of the interstitial fluid support was derived for the first time and a linear relationship between the coefficient of friction and the interstitial fluid support was confirmed [393].

The role of interstitial fluid support in sustaining compressive loads was also verified by means of unconfined compression. A combined numerical/experimental approach was used to extract mechanical parameters from AC (Chapter 5). Incorporating the intrinsic viscoelasticity in the biphasic model (BPVE model) was deemed crucial to predict an accurate the response of the tissue in unconfined compression [204].
8.3 - The effect of enzymatically induced damage, osteoarthritis and mechanically induced damage on the mechanical and tribological properties of articular cartilage

It is thought that in OA, proteolytic enzymes such as MMP-13 are involved in the gradual degradation and decrease in mechanical quality of the tissue \[166, 167\]. This leads to softening and thinning of the tissue which eventually leads to abnormal wear and full thickness loss of AC. Because the variation of biochemical and mechanical properties, as a result of OA, occur much before any visible morphological changes \[149\], there is strong potential in the future to diagnose the pathology by means of biochemical and mechanical parameters rather than using the imaging techniques currently used at present. This will allow early diagnosis of the pathology and prevention of disability \[156, 290\]. Furthermore, although studies have investigated the structure-function relationship of the tissue \[22, 132, 182, 188, 260, 262, 311\], until now, there have been no complete studies assessing the mechanical, frictional and wear performance of AC at different levels of structural degradation, from reversible PG depletion through to irreversible collagen degradation. Understanding this is crucial as it could strongly affect the type of treatments to be used for different levels of OA. In Chapter 5, a combination of proteolytic enzymes, namely trypsin and MMP-1, were utilized to selectively degrade the ECM of AC samples. This was done to simulate early to late OA by inducing PG depletion and collagen degradation respectively. Low PG depletion, which is thought to be a reversible process, was found to significantly decrease the stiffness of the tissue and to increase the stress relaxation rate of the tissue whilst also increasing its permeability. With regards to the frictional response, low PG depletion was found to cause a rise in the equilibrium friction coefficient indicating some variation to the boundary lubricating properties of the tissue. Further PG depletion (≈50% depletion) caused a greater reduction of the stiffness and considerable loss of interstitial fluid support in the early stage of the shear test. With collagen degradation as well as PG depletion, the stress relaxation rate severely increased, the dynamic stiffness sharply decreased and the presence of interstitial fluid support during sliding seemed to be somewhat nonexistent with almost no $\mu_{eff}$ present in the frictional response. The equilibrium friction coefficient was found to decrease dramatically as collagen was degraded. Counter-intuitively, however, although the coefficient of friction was lower in the collagen degraded samples, the wear resistance of the tissue drastically decreased, as proved from biochemical studies and WLI following shear, revealing a 56 fold increase in collagen damage as compared to the native tissue and extensive surface damage, respectively. At this level of degradation, damage in the tissue’s structure seems to be an irreversible process highlighting the need to diagnose OA before this stage, when the tissue’s wear properties are strongly compromised. The mechanical parameters were extracted using the BPVE applied to the numerical-experimental method. Although the model successfully predicted the response for native and low PG depleted AC \[184\], the BPVE model failed
to accurately predict the response of the more severely damaged AC and especially collagen degraded AC. This was probably due to the altered viscoelastic properties of the degraded tissue and the strong non-linear effects, calling for a more sophisticated AC model to be implemented with the inclusion of tension-compression non-linearity, such as the fibril reinforced poroelastic model [133]. Once OA-like AC was investigated and the mechanical properties quantified, real osteoarthritic AC was tested to obtain the relevant mechanical parameters. A decrease in mechanical quality was confirmed with increasing ICRS grading [308]. The osteoarthritic tissue was then compared to OA-like AC in terms of mechanical properties and similarities in the way the mechanical response has been altered following enzymatically induced damage or OA were observed. Similarities between the two included a decrease in dynamic and static stiffness, increase in permeability and increase in stress relaxation rate.

Following this, a tentative quantification of structural damage in osteoarthritic tissue was made by comparing the mechanical parameters of osteoarthritic and OA-like AC. Although several fundamental assumptions were made, such as the equivalence in native mechanical properties between porcine and human AC and assuming an equivalent type of structural degradation in osteoarthritic tissue, a method suggesting the possibility of estimating structural damage using mechanical properties was put forward. To our knowledge, no studies have proposed to directly correlate biochemical and mechanical parameters of osteoarthritic tissue to OA-like AC. This could be the starting point for more elaborate and realistic correlations between structural degradation and mechanical parameters.

Finally, AC was subject to mechanically induced damage due to shear in an attempt to investigate any variation in mechanical properties and frictional response as a result. The goal of this last section was merely to provide an insight into future work. Nevertheless, a decline in mechanical quality of the tissue as a result of mechanically induced damage was found. Stiffness was shown to decrease and permeability and relaxation rate to increase as compared to the native sample. This can be explained by the fact that AC is a strongly inhomogeneous material and damage to the surface’s structure, which is in part responsible for the global mechanical response of the tissue, can cause a significant variation in mechanical properties. Especially because PGs and collagen fibres in the superficial zone are thought to strongly influence the mechanical response of the tissue. This suggests that mechanically induced damage, as well as degradation due to enzymes, could also play a role in the degradation of AC in vivo. However, a more in depth study is needed to support this theory. The frictional response was also found to vary as result of damage. The equilibrium frictional coefficient was found to decrease as mechanical damage was induced during shear. This is explained by the SAL, whose removal from the surface has been demonstrated to decrease the equilibrium friction coefficient in AC [286].
8.4 - The impact of scaffold fibre orientation on damage and wear in tissue engineered articular cartilage

MACI seems to be a promising solution for the treatment of AC defects and potentially also localised early OA. One of the key parameters in this treatment is the ability of the scaffold to provide an ideal surface for cell attachment whilst resisting shear and compressive forces due to joint movements. Despite the strong potential of this treatment, very few studies have assessed the mechanical performance of the scaffolds and have evaluated the onset and progression of damage in the tissue engineered AC as a result of shear [316, 323-325]. In Chapter 5, mechanical and frictional properties of micro fibrous PCL scaffolds at varying fibre orientations (random and aligned fibre orientation) and cultured with bovine chondrocytes for a culture time of up to 28 days were evaluated for the first time. Mechanical properties were quantified via unconfined compression and tensile testing techniques. Frictional properties of the scaffolds were investigated using a reciprocating friction testing machine at physiological compressive strains usually occurring in native AC and at boundary lubrication regime. Scaffolds were sheared along the fibre direction, perpendicular to the fibre direction and in random orientation. The evolution of damage as a result of shear was evaluated by means of WLI and SEM. As intuitively expected, the fibre orientation strongly affected the tensile properties of the scaffolds but was not found to influence the compressive modulus. With regards to the frictional response, the fibre orientation did not strongly affect the equilibrium frictional coefficient but it was however a key factor in dictating the evolution of surface damage on the surface. Scaffolds shear tested perpendicular to the fibre orientation displayed the highest surface damage. The results suggest that when using the MACI, fibre orientation should be considered when implanted the scaffold into the joint.

8.5 - Implementation of biphasic articular cartilage models in a patient specific tibio-femoral contact and prediction of the onset of damage

Although one of the cardinal points of OA research and treatment will be the possibility of diagnosing OA via biochemical and mechanical properties in vivo, by merging imaging techniques such as MRI and CAT with state of the art patient specific FEM, a non invasive approach could actually be utilized to diagnose the pathology. The key to success here would be to reproduce identical joint contact geometries and loads in the FEM in order to accurately predict possible locations of OA origination or mechanically induced damage. For this to be possible however, AC would have to be modelled using the most advanced and complete material description possible, in order to accurately predict potential damage locations. In Chapter 7, for the first time according to the author’s knowledge, a BPVE model has been used to model AC in a patient specific 3D meniscectomised tibio-femoral joint and whose biphasic and viscoelastic properties have
demonstrated that, as a consequence of the load shift mechanism and the stress relaxation effect, a contact adaptation of AC occurs with time during walking, increasing the contact area and reducing the overall stresses in the tissue. This seems to be a fundamental factor of the mechanical behaviour of the tissue when subjected to physiological loads and motion. However, although the contact adaptation mechanism does help reduce the overall stresses in the tissue during walking, this is also the stage at which the AC is more exposed to wear as it lacks the protective mechanisms of interstitial fluid pressurisation and interstitial fluid load support. Furthermore, the dramatic reduction of fluid load support might also cause a detrimental effect on the lubrication mechanisms of AC such as the biphasic lubrication mechanisms previously discussed in §2.4.2, which might be compromised without the restoration of fluid support. This leads us to consider exploring the effect of different gait cycles and velocities on the replenishment of fluid load support in AC, as suggested in §8.3.

This study has further emphasized that implementing the biphasic model is essential in order to achieve a more accurate representation of the stresses formed and the mechanical behaviour of the tissue. Following this study, it is clear that the monophasic model is not adequate when investigating stresses and strains in the tissue and that all the necessary effort must be taken to implement advanced AC models in realistic geometries if the transient response has to be captured. Finally, using the FEM of the tibio-femoral joint and by evaluating the highest stresses in the tissue, it has been predicted that damage initiation and potentially OA could occur in the medial side of the tibio-femoral joint, in line with clinical studies [389-391].

8.6 - Future work

Although this investigation addressed and shed light on some of the most important and salient topics of AC and OA research, the complexity of the problems under investigation requires further studies and the research which has been presented here prompts the need for future developments. Included below are suggestions for future work which will ultimately help to better understand OA, to improve its diagnosis and the discovery of potential treatments.

(i) The findings of these studies as well as the protocol developed by the author and used to assess the mechanical response of damaged AC, could be used for the development of algorithms, methodologies and devices, possibly in arthroscopy, for the in vivo testing and assessment of the mechanical, structural and biochemical integrity of AC.

(ii) A more advanced AC model incorporating tension-compression non linearity is needed for the mechanical characterization of OA-like and osteoarthritic AC. Future work could involve the development and the use of the fibril reinforced poroelastic or poroviscoelastic model in the numerical-experimental methods used to characterize osteoarthritic and OA-like AC. Moreover, smaller samples and a more sensitive machine should be used to more accurately measure the response of the tissue. Lastly, different
strain rates representative of walking and running for example, should be used explore differences in mechanical response of degraded AC.

(iii) An interesting further study would be testing the frictional properties of different stages of osteoarthritic AC in order to investigate how the pathology affects the frictional response of the tissue and how differences/similarities correlate to the frictional response of OA-like AC.

(iv) The tissue engineered AC developed and tested in Chapter 6 should be subjected to longer culture times in order to favour the growth of full thickness ECM. This is needed to really compare the mechanical and frictional properties of the artificial counterpart at its mature stage to native AC.

(v) Although challenging and computationally expensive, the meniscus, with its appropriate properties, should also be modelled in the tibio-femoral contact simulation in order to create a more realistic model allowing a more accurate prediction of potential damage locations. Furthermore, more advanced AC models such as the fibril reinforced model could be used to model AC. It would also be extremely interesting to accurately model the gait cycle in a tibio-femoral contact incorporating biphasic AC models. This could allow the investigation of the interstitial fluid support in the contact at different walking and running speeds in order to establish which speed could be beneficial for AC and which detrimental. Simply by speculation, an “optimal” and beneficial walking speed would allow fluid exudation and imbibition in the AC and hence the transport of nutrients to the tissue. A detrimental exercise could be associated to high speed running, and/or high frequency and high loading scenarios, where fluid would be exuded out of the tissue due to repeated compressive forces, but not enough time would be allowed for fluid imbibition to occur. Gradually, interstitial fluid support would drop to zero, and the compressive and shear forces as a consequence of running would be entirely supported by the solid constituent of the tissue leading to abnormal wear, high stress and possibly, mechanically induced damage and OA.

(vi) Finally, one of the most recent discoveries in the pathogenesis of OA, has been the discovery of the role of the β-catenin signalling protein in the development of OA [166]. The next crucial step would be to correlate the release of this signaling protein with mechanical stress and mechanically induced damage. Future work could involve placing the MCSTR in an incubator to mechanically stress AC in vitro. Different levels of stress or damage could then be correlated to the release of the signalling protein to understand at which levels of stress OA could potentially originate.
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