CAN INTERNAL ELASTIC PROPERTIES OF CARTILAGE BE MEASURED USING MR ELASTOGRAPHY?

By

Anne C. Ridler

A thesis submitted in conformity with the requirements for the degree of Master of Science Graduate Department of Medical Biophysics University of Toronto

© by Anne C. Ridler 2001
The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.
Can Internal Elastic Properties of Cartilage Be Measured Using MR Elastography?

Degree of Master of Science 2001
Anne C. Ridler
Graduate Department of Medical Biophysics
University of Toronto

Abstract

Cartilage has internal variations in molecular structure, composition and possibly mechanical properties depending on position within and location of the tissue within the joint. I have examined the possibility of using MR Elastography as a way to measure the internal variations in elastic properties of cartilage. An apparatus was built to compress cartilage and coils were made to obtain the necessary SNR and resolution. Strain data was obtained in homogeneous gel samples. Changes in strain on the order that would be expected for samples differing in Young's modulus by a factor of 2 were measured. Strain was then measured in Young bovine cartilage samples and found to vary both within the sample and between samples. Cartilage proteoglycans were degraded with trypsin, softening the tissue, and the corresponding increase in strain was measured. Strain images and plots representing variations in elastic properties of cartilage were obtained using MR Elastography.
ACKNOWLEDGEMENTS

I would like to thank:

My parents and brothers for their never ending support, encouragement and faith that this was possible.

My friends from Massey, MBP and NB without whom this would have been a miserable experience.

NSERC for sponsoring me.

Nike for producing their catchy slogan "Just Do It" which became my mantra.

My supervisor R. Mark Henkelman and committee members DB Plewes and Joel Rubenstein, thanks to whom I now know I can get through anything.
# Table of Contents

**Preface**
- Abstract  
- Acknowledgment  
- Table of Contents  
- List of Figures  

**Chapter 1: Introduction**

1. Background Information on Normal Cartilage
   1.1 Cartilage Anatomy  
   1.1.2 Cartilage Composition
     - 1.1.2.1 Proteoglycan Aggregates  
     - 1.1.2.2 Collagen  
     - 1.1.2.3 Chondrocytes  
   1.1.3 Cartilage Function  
   1.1.4 Arrangement of Molecules Within Cartilage
     - 1.1.4.1 Variation in Composition  
     - 1.1.4.2 Mechanical Variation  
     - 1.1.4.3 Structural Variation

2. Changes in Cartilage with Osteoarthritis
   1.2.1 Composition and Structural changes with disease
     - 1.2.1.1 Early Osteoarthritis  
     - 1.2.1.2 Late Osteoarthritis  
     - 1.2.1.3 Repair Capabilities of Cartilage
   1.2.2 Mechanical Measurement of Cartilage Content and Disease
     - 1.2.2.1 Basics of Compression Mechanics  
     - 1.2.2.2 Mechanical Properties of Cartilage  
     - 1.2.2.3 The Impact of Collagen and Proteoglycans on Stiffness  
     - 1.2.2.4 Changes in Mechanical Properties with OA
   1.2.3 Summary  

3. Measurement of Cartilage Changes with Osteoarthritis
   1.3.1 Plain Film Radiography  
   1.3.2 Arthroscopy  
   1.3.3 Magnetic Resonance Imaging (MR)
     - 1.3.3.1 Background  
     - 1.3.3.2 MR Thickness and Volume Measurements  
     - 1.3.3.3 T2 Imaging  
     - 1.3.3.4 T1 Imaging and Gd(DTPA)$^{2-}$  
     - 1.3.3.5 MR Pressure Cell Measurement
   1.3.4 Ultrasound  
   1.3.5 Imaging Conclusion  
   1.3.6 Conclusion to the Introduction
Chapter 2: Method for Cartilage Strain Measurement

2.1 Introduction to Elastography
2.2 Elastography Measurements
2.3 Tissue Requirements for MR Elastography
2.4 Apparatus Requirements
2.5 Description of Apparatus
2.6 Design of Coils to Maximize SNR
2.7 Sample Holder for Cartilage
2.8 Data Collection Using STEAM Sequence
2.9 Apparatus Arrangement

Chapter 3: Validation of the Method

3.1 Imaging Parameters
3.2 Phase Data
3.3 Displacement Data
3.4 Gel Deformation Physics
3.5 Strain Data in a Homogenous Gel
3.6 Strain Data in a Gel with an Inclusion
   3.6.1 Relative Strain and Its Relation to Relative Young's Modulus
   3.6.2 Young's Modulus Ratio According to MR Measurements
   3.6.3 Strain Ratio According to Stress-Strain Measurements
   3.6.4 Comparison of Actual and MR Derived Y Ratios
3.7 Discussion and Conclusion Regarding the Method

Chapter 4: Application of MR Elastography to Cartilage

4.1 Normal Cartilage
   4.1.1 Contact Piece Geometry
   .2 Strain in 3 Cartilage Samples
4.2 Degraded Cartilage
   4.2.1 Degrading Cartilage
   4.2.2 T1 Results of Degradation
   4.2.3 Strain Results of Degradation
4.3 Usefulness of the Method
4.4 Conclusion as to the Application of MR Elastography to Cartilage

Chapter 5: Future Direction of Cartilage MR Elastography

5.1 Further Development of the Method
   5.1.1 Resistance to Shearing
   5.1.2 Measuring Flow During Compression
   5.1.3 Hysteresis Loop
5.2 Application In Vivo
5.3 Conclusion Regarding the Use of cMRE
LIST OF FIGURES

1.1 Anatomy of the Knee 3
1.2 Cartilage Composition 7
1.3 Mechanical Properties of Bovine Cartilage 8
1.4 Variation in Collagen Structure Around the Joint 10
1.5 Relationship between Modulus and Collagen and PG Concentration 14
1.6 Y and Thickness Changes With Degradation of Collagen or PG 15
1.7 Changes in Cartilage Stiffness with Osteoarthritis 16
1.8 Plain Film Radiography of Diseased Joint Contact Geometry 19
1.9 Arthroscopy of Cartilage Surface 20
1.10 T1 Gd(DTPA) Measurements of GAG Concentration 23

2.1 Displacement Over Time After Compression (Creep) 28
2.2 Cartilage Compression Device 30
2.3 SNR for Coils of Different Shapes and Sizes 31
2.4 Diagram of Cartilage Holder 33
2.5 Plot of STEAM Imaging Sequence 36
2.6 Diagram of Phase Accumulation Due to Gradient 37
2.7 Diagram of Equipment Set Up 39

3.1 Phase Wrapped Images of Gel Under No Motion 42
3.2 Phase Unwrapped Images of Gel Under No Motion 42
3.3 Phase Averaging 43
3.4 No Motion Gel Data Before and After Zero Correction 44
3.5 Displacement Data for Homogeneous Gel 44
3.6 Gel Deformation Physics 46
3.7 Strain Plots Through 3 Homogeneous Gels 47
3.8 Magnitude and Strain Image of Gel With Inclusion 49
3.9 Displacement and Strain Plots for Gel With Inclusion 50
3.10 Plots of Stress Versus Strain for Gel 53

4.1 Effects of Smaller Contact Area on Strain Plots 55
4.2 Edge Identification and Zero Mapping 56-57
4.3 Strain Image for Cartilage Sample A 58
4.4 Strain Image for Cartilage Sample B 59
4.5 Strain Image for Cartilage Sample C 60
4.6 T1 Cartilage Measurements During Degradation 63-64
4.7 Strain Images Before and After Degradation 65
4.8 Strain Image of Cartilage at the Growth Plate 67

5.1 Hysteresis Loop for Cartilage 70
CHAPTER 1: INTRODUCTION

Cartilage is a tissue that has long been studied in the annals of biomechanics and biochemistry and yet is not completely understood. The function of cartilage is based on an elaborate arrangement of macromolecules that provide stiffness and mechanical absorption of stresses in the joint. Macromolecules and hence stiffness change with damage or disease. There does not exist a method capable of measuring internal mechanical properties of cartilage. I have proposed, developed, and applied a way to measure these properties with MR Elastography (MRE).

1.1 Background Information on Normal Cartilage

Of the three types of cartilage, hyaline, fibrocartilage and elastic, hyaline articular cartilage is known for its stiffness and ability to absorb loads. It is found in all joints, however the knee is most susceptible to disease and injury, so has been studied in this work. Cartilage consists of four main components: water, proteoglycans (PG), collagen and chondrocytes, which change in quantity and
distribution throughout the depth of cartilage and around the joint. Their arrangement varies both within cartilage and around the joint and provides strength to the cartilage. However, the corresponding variation in mechanical properties is not known, due to lack of a method that can measure it. As cartilage ages and degenerates, proteoglycan loss and collagen degeneration creates softer pockets, but these variations in elastic properties can not currently be detected in vivo.

1.1.1 Cartilage Anatomy

Cartilage lines the patella and the end of the long bones (femur and tibia) in the knee joint (Fig 1.1). It provides a frictionless surface for gliding and dissipates pressure, sparing the subchondral bone from damage. Normal human cartilage thickness ranges from 2 to 5 mm [1], with the patella having the thickest cartilage (3.57 +/- 1.12 mm) and tibia the thinnest (2.26 +/- 0.56 mm) [2], although other works report variation of as much as 50% over a surface [3]. Only two small areas of cartilage are in contact between the Femur and Tibia. The menisci that surround the contact points absorb stress and add stability, as do the cruciate ligaments that limit bone motion. The entire joint is enclosed in a synovial lined capsule with fluid, providing hydration and nutrients for the cells within cartilage. Cartilage has no blood vessels or lymphatic system, so all nutrients must enter through diffusion [4]. Nerve fibers are found in the subchondral bone, but not in cartilage. The complicated geometry and variable thickness of cartilage make evaluating changes in its thickness and with disease hard to measure.
Fig 1.1 Anatomy of the knee [38]. The top images is of the soft tissue around the joint with the cartilage in white lining the tibia and femur. In the bottom image, the two areas of contact in the joint can be seen.
1.1.2 Cartilage Composition

Of the 15-35% solid phase of cartilage, proteoglycans (PG) account for 20-50% and collagen 50-75% dry weight [1,4,5,6], leaving only small quantities of other molecules and chondrocytes. Proteoglycans are a highly electronegatively charged macromolecule distributed throughout the cartilage. They provide resistive forces to compression and attractive ones to the fluid phase [7,8]. Collagen forms large fibers with high tensile strength that hold the matrix together [8]. Chondrocytes, the only cells in cartilage, are responsible for producing the extracellular matrix (ECM) and repairing damage to cartilage [9].

1.1.2.1 Proteoglycan Aggregates

Proteoglycan aggregates are huge molecules up to $5 \times 10^7$-$5 \times 10^8$ in molecular weight and 8 mm long [10]. They consist of a long hyaluronic acid (HA) backbone, to which around 200 aggrecan proteoglycans are attached in cartilage [4]. Each aggrecan proteoglycan is a protein backbone to which around 100 glycosaminoglycan (GAG) chains are attached [5,11]. In cartilage the two main GAG types are keratan sulfate (KS) and chondroitin sulfate (CS) [4]. Each of these consist of 80-100 closely spaced (1-1.5nm) [1] negative charges from COO- or SO3- groups [4,5]. Some proteoglycan aggregates are held in place with one end of the HA backbone attached to the cell through receptors [12], but the rest of the molecule is evenly distributed in cartilage. Proteoglycans are compressed in cartilage by collagen to 20% of the volume they would normally occupy when in solution [4,13]. Aggrecan proteoglycan aggregates are a densely packed, highly electronegatively charged molecule, which does not form a structured arrangement.

1.1.2.2 Collagen

Collagen forms fibers with a high tensile strength that hold the ECM together in cartilage [8,14,15]. In adult cartilage 90% of the collagen is type II, but types IX, XI and VI are also present [8]. Type II collagen is made of three $\alpha$ chains tightly wound in a left hand triple helix 3000A long and 15A thick [5].
These strands are then staggered and bound through cross links to form a much larger cigar shaped fiber which is a right hand helix. The cross links [16] and opposing helical formations create a molecule that is hard to stretch. Type IX collagen is a short fiber that binds both the outer type II fiber and PGs, forming a bond between the two molecules [17,18]. Its outer surface also contains positive domains making it attractive to the surrounding highly electronegative PGs [19]. The role of type XI and type VI collagen remains unknown, although type XI is thought to play a role in determining type II thickness [4,5]. The type II thick structure provides strength to the matrix and type IX brings cohesiveness by binding type II and PGs.

1.1.2.3 Chondrocytes

Chondrocytes are the only cells in cartilage. They are often seen in pairs just above the bone. Chondrocytes are more numerous at the base of the cartilage, where they are thought to originate and are sparser at the top, becoming disk shaped as they migrate towards the surface [4]. Their role is to regulate cartilage by producing collagen and PGs, or to stimulate its breakdown depending on the state of the ECM [9]. The chondrocyte’s response to physical stimulus of static loading or underloading is associated with depression of matrix synthesis and over-loading results in damage [23,24]. However, cyclic loading around 1 Hz increases PG content [20], with up to a 50% increase at higher frequencies [21,22]. Chondrocytes are thought to respond to changes in water content, pH, fixed charge density and osmotic pressure that follow compression [22,24]. As Osteoarthritis or disease progresses and the viscoelastic properties of cartilage change, proper signaling pathways are either over or under stimulated leading to cumulative damage of the matrix [23]. Osteoarthritis (OA) is thought to originate from an imbalance in chondrocyte activity, where the damage exceeds the cell’s repair capabilities [25].
1.1.3 Cartilage Function

Collagen, PGs and chondrocytes come together to form a tissue with a near frictionless surface and a high resistance to compression. Cartilage is considered biphasic and can be broken down into solid and fluid phases [13,26]. In the solid phase, PGs, with their high number of closely packed negative charges, provide a strong repulsive or expansionary force for the cartilage [7]. Collagen with its thick fibers and high tensile strength holds down the PGs [14,15], resisting the expansion, although the detailed structure of the collagen remains unknown.

The fluid phase consists of water with high Na\(^+\) concentration in interaction with the very negative PGs. There are two interactions at play: the first is the attraction of the Na\(^+\) to the PG, that draws water from the synovial fluid into cartilage [27] and the second is osmotic pressure, due to the lower water concentration within cartilage than externally. Combined, these forces push water into cartilage, so that it expands until the balance point is reached with collagen resisting the expansion [28]. Fluid pressure also adds to the compressive strength, since the force of the fluid must be overcome in order to push down on cartilage and for water to flow out. The complex interactions between collagen, PGs, water and Na\(^+\) create a strong material resistant to compression, which can not yet be duplicated by human engineering.

1.1.4 Arrangement of Molecules Within Cartilage

1.1.4.1 Variation in Composition

The composition of cartilage is not uniform, and the density as well as arrangement of molecules varies with depth [29,30,31,32,33] (Fig 1.2). Cartilage is divided into four sections, the superficial or tangential zone at the surface, the middle or intermediate zone, the deep or radial zone and the tidemark or calcified zone which marks the transition into subchondral bone. The superficial zone is known for its tangential arrangement of collagen [31]. The intermediate zone has generally been known for its isotropic or random collagen arrangement [34],
although recent evidence discussed later suggests collagen is structured [31]. It is the area with the highest PG content and swelling pressure [35,30]. The deep has radial collagen orientation [36] and the highest concentration of chondrocytes. The tidemark is a layer of calcified cartilage, through which radial collagen strands are anchored [36], marking the transition between cartilage and subchondral bone. The variation in arrangement of molecules through the depth of cartilage suggests changes in mechanical properties, however they have not been measured in detail due to lack of a method.

Fig 1.2. Cartilage Composition. A. Sections through the depth of cartilage and their corresponding structure. B. Change in composition of collagen, proteoglycans and water through depth of cartilage (Modified from [29]).
1.1.4.2 Mechanical Variation

Internal mechanical measurements have been made of bovine cartilage by cutting 3 sections 250μm thick through the depth of the cartilage [37]. A force was applied to the sample and the displacement was measured. These measurements show a possible variation in stiffness with depth (Fig 1.3). Unfortunately, this method cannot provide a continuous assessment of properties through the depth of the cartilage and one cannot see how they vary within the sample. Measurements of Young’s Modulus based on these experiments are on the order of 0.04 MPa, significantly lower than the average of 1 MPa for equilibrium modulus previously reported in the literature [1]. The low Young’s Modulus brings into question the validity of the experiment and may be a result of the thin samples. It is also not clear what impact disruption of the collagen structure from cutting of the slices will have on the mechanical properties measured. Another drawback to these experiments is that only 2 samples were tested, making it difficult to believe a conclusion about the variation in elastic properties. However, these measurements are the only ones that exist looking at the variation in properties through the tissue and do indicate a possible variation in stiffness through the sample that would be interesting to investigate further.

![Equilibrium Stress vs. Applied Strain](image)

Fig 1.3 Mechanical properties of cartilage as a function of depth for 2 bovine samples distinguished by a hollow or filled symbol, where o is the top slice at the cartilage surface, Δ is in the middle and is at the depth or subchondral bone [37]. The samples appear to be softest at the surface and hardest in the middle.
1.1.4.3 Structural Variation

Recently work has been done to elucidate the collagen structure within cartilage. The first evidence that cartilage contained underlying structure came with polarized light and electron microscopy and was then seen through horizontal signal variations from the magic angle effect in MR [39]. When the surface of cartilage is rotated 55° from the main magnetic field in TE weighted images (distinguishes areas with different T2), its signal is homogeneous, whereas at other angles bright horizontal bands can be seen at the surface and depth. Less signal is present in the center [39]. This variation of signal decay indicates interactions of water with macromolecules in different structural arrangements. It is not known why there is a signal variation in the central region of cartilage dependant upon the sample angle with the main magnetic field. With the advent of higher strength magnets (7T), more detail could be seen within cartilage and images show vertical columns of alternating light and dark bands [40]. These images have been correlated with structure through freeze fracture experiments, however the nature of the bands remains unknown [41]. The arrangement of collagen and hence PGs also varies within cartilage depending on its location in the joint [42] (Fig 1.4). The complicated and unresolved structure of cartilage remains an area of research to which measurement of elastic properties could be applied, since structure is likely correlated with mechanical properties.
Fig 1.4. Variation in collagen structural arrangement around the joint and through depth of cartilage [42]. Collagen maintains its tangential (blue) arrangement at the cartilage surface but varies internally. At the tibia-femur contact point internal collagen fibers are oriented radially (yellow) whereas at the femur-patella internal fibers are tangential. The structure of collagen varies depending on position within the joint. The structure was deduced from MR images based on the magic angle effect of a sample from that location in the joint.
1.2. Changes in cartilage with disease

1.2.1.1 Early Osteoarthritis

The initial stages of cartilage degradation are not known, but are likely a combination of factors. Once the chondrocyte repair rate is too slow to match degeneration, damage is naturally irreversible [43] and cartilage becomes osteoarthritic. Mechanical damage to cartilage will precipitate and predispose it to deterioration. With age there are less PGs in cartilage and they lose negative charges as the number of GAG branches and charges on each decrease [23,44,45,46]. This lowers the resistance of cartilage to compression [47] as the repulsive forces and fluid force pushing in decrease [13,48,49,50]. Loss of PGs occurs in pockets, so that there are softer areas within cartilage at early stages of the disease [51,52,53]. The loss of PGs leads to a softening of the matrix because the loss of negative branches decreases the attraction of the sodium rich water from the joint cavity into cartilage, which in turn lowers the expansionary forces on the matrix. At later stages, these are palpable from the cartilage surface (grade I), and are often used during surgery to indicate tissue that is diseased. Collagen is known to lose tensile strength with age [54], as seen through sagging of the skin and disruption of its structure [23,55]. In cartilage, this results in a matrix that is less tightly held in place and thus cartilage swells early in OA when it is not under compression [23].

1.2.1.2 Mid Osteoarthritis

Later stages of OA include subchondral bone stiffening. This increases the load that cartilage must dissipate, accelerating damage [56]. PGs with their loss of negative charges do not hold as much water and the cartilage becomes drier. Together with the loss of molecules and water, cartilage then becomes thinner [4]. Damage to cartilage occurs unevenly, in pockets initially, then through fibrillations forming on the surface (grade II) [57]. The fibrillations become
fissures (grade III) as the cracks extend to the subchondral bone. Blood vessels penetrate through the tidemark into cartilage [57,58] and collagen types I and III are produced [59,60]. As OA development varies throughout cartilage, differential measurement of mechanical properties would be useful in finding inclusions or pockets of softer material and hence early disease.

1.2.1.3 Repair Capabilities of Cartilage

Repair capabilities for cartilage are limited. With damage chondrocytes increase production of matrix components [46,57] and once fissures have reached the subchondral bone, cartilage grows to fill them. However, it is fibrocartilage with type I collagen, that does not have the same properties as normal hyaline cartilage and only slows the degenerative process [25,60,61]. Clinically, not much can be done until late stages, where the cartilage is already severely damaged. Treatment options include removal of loose bodies and osteophytes (bone growths in cartilage), lavage of the joint, meniscal tear and ligament repair, drilling subchondral bone, modification of contact geometry (osteotomy) and arthroplasty or joint replacement [33,60,62,63,64,64]. Recently work has been done in the area of grafting cartilage [66], or growing cartilage from chondrocytes outside the body [67]. Results are promising, but, for these methods to be viable, OA would have to be caught at early stages, making development of early detection methods important [68].

1.2.2 Mechanical Measurement of Cartilage Content and Disease

1.2.2.1 Basics of Compression Mechanics

Mechanical properties of stress (σ), strain (ε) and Young’s modulus (Y) will be explained so that they can be used to describe cartilage. Stress (Pa) is defined as the Force (N) per unit area (m²) which is applied to a material. Strain is the deformation that ensues in terms of the change in length due to the compression divided by the thickness (Δx/x) of the sample. Young’s modulus (Pa) is constant for a material and is the proportionality constant between stress
and strain so that \( \sigma = Y \varepsilon \). The instantaneous modulus (Y) for normal cartilage is 10-14 \( \text{MPa} \) [1,69]. When constant force is applied, a strain will develop in relation to the Y for the material. With 2 materials under a constant force, a softer material with a lower Y, will experience a greater strain or deformation. Strain will later be used to describe tissue properties.

1.2.2.2 Mechanical Properties of Cartilage

As mentioned earlier, cartilage consists of both a solid and a fluid phase that affects its mechanical response. When pressure is applied to cartilage there is an initial response from the solid matrix and a secondary one from the fluid. The first response is instantaneous and involves an initial compression of the matrix and fluid as a whole to accommodate the displacement. The second response referred to as creep is slower and involves the flow of fluid from cartilage. If a constant force is applied to cartilage an instantaneous displacement occurs, followed by a slow creep that takes around an hour to reach its equilibrium point where the tissue is referred to as having an equilibrium modulus or creep modulus. Creep results in a large proportion of the final displacement and is reversible when the force is removed, although this takes around an hour. With the initial solid response and secondary fluid flow, cartilage reacts quickly to applied stresses, but also accommodates long term pressure with greater compression.

1.2.2.3 The Impact of Collagen and Proteoglycans on Stiffness

Cartilage constituents PGs and collagen are known to affect mechanical properties, so their effect on Y has been measured in an experiment that will now be discussed [69]. The modulus is measured two seconds after compression, so can be considered instantaneous and hence an elastic modulus, even if it is stated as creep modulus. The modulus is seen to decrease for a decrease in PG [69] (Fig 1.5a). Collagen and proteoglycan content are measured using staining. On the other hand changes in collagen content do not appear to have an effect on Y [69] (Fig 1.5b). However, a recent experiment involving degradation of PG and collagen and the measurement of Y has shown that Y decreases with collagen.
degradation (Fig 1.6). Cartilage clearly becomes softer with decreasing PG concentrations and although it likely also softens with decreases in collagen, the relationship remains unclear.

Fig 1.5 Relationship between modulus of cartilage and proteoglycan or collagen concentration. Modified from [69].
1.2.2.4 Changes in Mechanical Properties with OA

Little work has been done in quantifying the changes in elastic properties of cartilage with disease. The decrease in cartilage modulus with osteoarthritis, indicating a softening of the matrix, has been measured. Since $Y$ represents stiffness, which determines function, $Y$ may be a better indicator of disease than superficial tears and markings. The equilibrium modulus falls from 1.0 MPa to 0.6MPa with an increase in OA to grade V, the highest stage of disease where various grades were discussed in section 2.1[70] (Fig 1.7). The method for the measurement of equilibrium modulus is not described but likely involves compression of a sample under a fixed force and measurement of the displacement after equilibrium is reached. The error bars extended below 0.5MPa, indicating that an individual modulus could be much lower. The large error range may be a result of biological variation between people so that within one person the decrease in $Y$ may involve less variability. Unfortunately, it is not currently
possible to measure the variations in $Y$ of one person over time due to lack of a non-invasive method to perform the measurements. This experiment suggests a quantifiable decrease in Young's Modulus with disease, however, the large variability and small number of samples warrants further work.

![Diagram showing changes in stiffness with Osteoarthritis](fig1.7)

**Fig 1.7** Changes in stiffness with Osteoarthritis where $n$ is the number of samples [70]. Young’s Modulus is seen to decrease with an increase in disease.

**1.2.3 Summary of Cartilage Properties**

The function of cartilage is dependent on its ability to bear loads and hence mechanical properties. Although stiffness is known to vary through cartilage and with disease, the detailed distribution of these changes remains unknown. The ability to measure elastic properties throughout cartilage would lead to a better understanding of its normal function and early changes with disease. Further refinement of these measurements could lead to a method for early detection of Osteoarthritis, allowing for human engineered cartilage to be used, other treatments to be developed and change with disease or treatment to be monitored.
1.3. Measurement of Changes with Disease

The difficulty in studying cartilage and its changes with disease is that severity of degeneration and symptoms often do not correlate [63,71]. Clinically used methods to study cartilage, X-rays, arthroscopy and MR anatomy images, focus on late stages of OA by measuring thickness changes and late stage surface focal defects. They can not detect earlier changes and do not currently have the resolution to offer further understanding of cartilage. MR and ultrasound (US) research methods are being developed to study cartilage and for the eventual application in OA early detection. These imaging methods continue to help in the understanding of cartilage and OA, however are limited and would be benefited by measures of cartilage mechanical properties and function.
1.3.1 Plain Film Radiography

Radiographs used clinically are formed through the absorption of X-Rays depending on the density of the tissue and since bone is far denser than cartilage, show bone not cartilage. Osteoarthritis is diagnosed based on decreased spacing between tibia and femur, subchondral sclerosis (hardening), osteophytes and changes in contact geometry [72]. Radiographs measure thickness of cartilage and can detect a minimum change in width of 9-15% with 95% accuracy, so detect late stage OA [73]. Radiographs do not have the sensitivity to measure changes in knee structure or anatomy over a few years [74,75]. They also lack the ability to judge internal cartilage variations as well as surface defects. Another problem is that since they are usually acquired with the patient in a supine position, spacing between cartilage “overestimates” thickness and details of contact geometry are omitted [76]. When radiographs are taken in a loaded and unloaded position, spacing varies by as much as 2-5mm, a large proportion of the 10mm spacing for normal cartilage in contact (Fig 1.8). One may think that using body weight to compress cartilage and measuring the variation in thickness with X-rays is a good way to measure overall strain in the cartilage. However, the initial thickness of cartilage is not known because of the possible spacing between cartilage in the joint, the contact geometry is not known, and the resolution is limited. Although X-Rays provide adequate details of subchondral bone deformations, they do not provide the resolution or measure a property that could be used for early OA diagnosis or judging the functional ability of cartilage in detail.
Fig 1.8 X-ray image of knee joint. The left image is taken in the standing position whereas the right one is in the supine position. A different contact geometry is seen depending on whether the patient is lying down or standing when the X-ray is taken [76].

1.3.2 Arthroscopy

Arthroscopy involves inserting an endoscope into the joint and inspecting the cartilage surface. Cartilage is judged through color and texture. Normal hyaline cartilage has a glassy white color that becomes yellow with damage and age [77]. It loses its glassy sheen and begins to appear rough as fibrillations and fissures occur. A graded scale is used to judge progression of OA based on these characteristics [33]. This method is the gold standard for judging end stages of OA that affect the cartilage surface. However, it offers no information about internal characteristics and hence is not capable of diagnosing early disease changes or being used to study long term progression of the disease due to its invasive nature since the endoscope has to be inserted into the body.
1.3.3 Magnetic Resonance Imaging

1.3.3.1 Background

Magnetic resonance imaging (MRI) acquires images through cartilage based on the different interaction of protons from water with surrounding macromolecules in each tissue and represents the structure and biochemical composition of cartilage [28]. T2 images are sensitive to the amount of surrounding organized structure. Since T2 decreases with (proton) spin-spin interaction or dephasing, structure results in more of these interactions so a faster relaxation rate and shorter T2 ensues. Cartilage, with its collagen structure, has an average T2 of 33 +/- 4 ms [78]. T1 is from individual proton spins relaxing from their excited state, so depends on specific energy transfers at a frequency of 63.9MHz and requires the tumbling of protons at that frequency to dissipate this energy. In solids, protons are more likely to tumble at the appropriate frequency, increasing the probability of an interaction occurring at 63.9MHz so shortening the time in the excited state and hence T1. Cartilage has a T1 of 674 +/- 40 ms at 1.5 Tesla [78]. T1 and T2 are used to obtain structure and composition.
information of components as well as to form images which represent cartilage's physical attributes.

1.3.3.2. MR Thickness and Volume Measurements

The majority of currently applicable MR research involves measuring cartilage thickness, volume and focal defects [79]. Thickness and volume are generally evaluated with T1 fat suppressed images [80] or T2 contrast images that highlight cartilage [33], making it easily distinguishable from bone and synovial fluid. At a high resolution voxel size = 625x833x2000μm, volume measurements have a 95% reproducibility rate [81] meaning that volume changes greater than 10% between exams can be detected although variation in thickness readings is on the order of 8% [82]. This is too large an error range considering changes between normal cartilage and middle disease stages are minimal and that cartilage has normal fluctuations in thickness. Another problem with thickness and volume measurements is that early disease involves swelling and hence thickening of cartilage that may obscure later stage thinning in other areas [28]. Although thickness and volume measurements are useful in detecting end stage disease where severe deterioration has taken place, they can not detect subtle early disease stages or provide information as to the integrity of cartilage.

1.3.3.3 T2 imaging

Clinically, MR of cartilage currently plays the most useful role detecting focal defects. T2 is sensitive to losses in collagen [58]. T2 is sensitive to the presence of surrounding structured macromolecules that dephase the proton spins. With less collagen present and more water, there is less dephasing and signal is lost more slowly so that diseased areas appear brighter [83,84]. Research is being done to extend this technique to earlier disease detection, but so far the technique has not been sensitive enough. For large focal defects it has a sensitivity of 48-100%, specificity of 50-96% and accuracy of 52-81% [33,85,86]. The large range reflects the various stages of disease as there is a low chance of missing OA when the joint is destroyed in contrast with earlier stages of disease, that become increasingly harder to detect. Although
from the clinical perspective T2 focal defect detection of areas where large pockets of collagen are disrupted is currently the earliest OA can be diagnosed, it does not have the sensitivity to detect more subtle earlier changes in collagen. From the research perspective, T2 imaging does provide indirect information as to the functional state of cartilage, through knowledge of collagen concentration and distribution based on signal intensity and structural information from the magic angle effect discussed earlier. However, this information is not substantive enough to be used alone in studying cartilage, as it does not provide information about PG, or a complete picture of the functional state of cartilage and hence how well cartilage can absorb loads.

1.3.3.4 T1 Imaging and Gd(DTPA)2-

In cartilage T1 alone does not provide composition information as it does not correlate with collagen or PG content (Fig 1.10). Gd(DTPA)²⁻ is a contrast agent with a negative charge that is repelled by the PGs in cartilage. As cartilage becomes damaged and loses negative charges, more Gd(DTPA)²⁻ will flow into the cartilage. A Gadolidium concentration of 1mM lowers T1 of cartilage from around 1500ms to 350ms in a 8.45T magnet for a 45mg/ml concentration of GAG (Fig 1.10) [87]. T1 with Gd has a strong correlation ($r^2=0.96$, $p<10^{-15}$) with GAG concentration and hence PG. T1 increases with PG concentration from 250ms to 400ms for a GAG concentration change from 10 to 70 mg/ml. Gd(DTPA)²⁻ had been tested in vivo and was able to find focal defects, although it also highlighted regions which were not diseased and highlighted regions fluctuated between subsequent imaging sessions for unknown reasons [87]. One study has shown this method to have 93% sensitivity and 94% specificity for arthroscopically visible lesions [88]. This contrast enhancement has not been shown to be more effective than T2 weighted imaging at highlighting focal defects in vivo through direct comparison. However, this method provides an accurate way of measuring relative PG content for ex vivo cartilage samples through T1 maps.
Fig 1.10 T1 measurements of GAG concentration with (A) and without (B) Gd(DTPA)\(^{2-}\) at concentrations of 1mM [87]. For each point a sample was degraded and the T1 was then measured with and without GAG and the GAG concentration was then measured.

1.3.3.5 MR Pressure Cell Measurement

Creep defined in section 1.2.2.2, is a mechanical property of cartilage and has been measured using MR [101]. It was done with a pressure cell that compressed a bovine cartilage sample and changes in thickness were measured over time using MR and plotted. The amount of strain with fixed force was shown to change with PG degradation [89], indicating that creep may be able to differentiate osteoarthritic from normal cartilage. These measurements offer an overall assessment of mechanical properties but have not been done in vivo and are time consuming in that an experiment takes a few hours.

1.3.4 Ultrasound

The application of ultrasound to the measurement of cartilage thickness, content and changes with disease is also being studied. Ultrasound, which strongly detects the synovial fluid cartilage interface and the cartilage bone interface, can be used to measure cartilage thickness. Such thickness measurements from B-scan images have a precision of 0.1mm (2-5% depending on cartilage thickness), and have been found to correlate with Gd(DTPA)\(^{2-}\) enhanced MR images to \(r=0.86\). Conflicting results exist regarding the ability of ultrasound to characterize tissue composition. Although
slightly lower values were obtained for the speed of sound (25MHz) in OA versus normal cartilage, no correlation with water, PG or collagen content was found, whereas in other studies a significant correlation was reported for acoustic attenuation and speed of sound [90]. Ultrasound has been able to image late stage OA defects and fibrillation as a disruption to the synovial-cartilage high intensity boundary with signal that extends to the bone. Unfortunately, imaging with Ultrasound requires a direct application of the transducer perpendicular to the tissue surface, making the method invasive and excluding various cartilage areas in the joint from being imaged. The low cost of ultrasound still make it a viable option for measuring thickness and late stage defects, however its utility for studying cartilage composition, structure and functional state has yet to be proven.

1.3.5 Imaging conclusion

The need for studying cartilage stems from three areas: obtaining a better understanding of cartilage, judging its functional ability and detecting early changes with osteoarthritis. A large number of methods have been developed to study cartilage and its changes with disease, however the lack of a quantitative marker for OA has impeded the study of cartilage in this disease and assessment of the impact of therapy on cartilage function [70,91,92]. Currently clinical methods for diagnosis such as plain film radiography, arthroscopy and MR anatomical imaging highlight late stage OA by finding gross thickness changes, surface damage, large focal defects and changes to the subchondral bone. These methods do not measure characteristics that change notably early in disease such as elastic properties of the matrix or PG content and collagen structure and composition. Among the methods being developed to study cartilage and for possible clinical application, are MR T2 and T1 Gd(DTPA)\textsuperscript{2-} imaging and ultrasound. The MR methods detect mid disease stages, where focal defects have not affected the surface and correlate well with collagen and PG content respectively so far in non clinical experiments. Ultrasound shows potential for mid stage disease detection through the disruption of the synovial fluid cartilage echo band, that may be sensitive to surface disruptions before other methods. Its parameters may also be sensitive to underlying matrix composition, although results remain mixed. A need remains for a method to help describe normal cartilage function as well as early changes with disease. The measurement of elastic properties has the potential to accomplish this goal.
Cartilage has a PG and collagen matrix that elastically absorbs initial compressionnal stresses and redistributes them to the fluid that is then forced out. The distribution of components changes throughout the cartilage and around the joint resulting in variations to elastic properties that are not known. With age and disease, cartilage begins to lose its proteoglycans and the tensile strength and quantity of collagen decreases. The ability of cartilage to absorb loads is reduced and softer pockets form within the tissue. As the disease progresses cartilage thins and fibrillations occur at the surface becoming fissures as they extend to the subchondral bone, further changing the elastic properties of tissue.

Radiography, arthroscopy, and MR can detect late to mid stage changes with OA, but are not sensitive enough for earlier stages. Current methods including MR T1Gd, T2 and ultrasound are being developed to study cartilage and are able to discern certain individual aspects such as PG and collagen content and boundaries, but do not describe their combined effect on cartilage's functional ability to absorb loads.

1.3.6 Outline of the Thesis

In this thesis a method that may be capable of non-invasively measuring cartilage elastic properties with MR Elastography will be examined. The apparatus required to perform these experiments and how it was developed will be described in chapter 2. In chapter 3 the ability of measuring elastic properties in sample geometries and at resolutions that are present in cartilage will be examined. Strain images representative of elastic properties will be obtained in homogeneous gels and gels with softer inclusions representative of cartilage with Osteoarthritis in chapter 3. Strain images will be obtained in normal bovine cartilage in chapter 4.1. Elastic properties in normal bovine cartilage will then be modified through trypsin degradation of proteoglycans. These modifications will be tracked with T1 MR imaging and the corresponding variation in elastic properties will be measured with MR Elastography in section 4.2. The eventual application of knowing elastic properties in cartilage and further experiments that can be done to refine the technique will be discussed in chapter 5.
CHAPTER 2: METHOD FOR CARTILAGE STRAIN MEASUREMENT

The imaging method to study cartilage has been developed and presented in this work is called cartilage MR elastography or cMRE. It is a novel technique in that it allows elastic properties within cartilage to be measured. This method offers advantages over other techniques by measuring the elastic properties that determine the ability of cartilage to function. It also maps internal elastic properties, allowing variations within cartilage to be measured. Finally, the use of MRI makes this technique non-invasive and allows continuous measurements through the tissue without disruption of its properties.

2.1 Introduction to Elastography

Elastography is a new field that was pioneered in the early 80s using ultrasound [98]. It was first performed with MR by Muthupillai [99] and Plewes [100] on gels and with shear excitation [100]. Elastography involves obtaining elastic properties that can then be used to describe the tissue and whether it is
diseased. Elastic properties of cartilage commonly change with Osteoarthritis and are a property that doctors have long used to diagnose late stages of the disease during surgery. Elastography may extend this diagnostic method to areas that are not externally palpable and detect smaller variations in mechanical properties as well as image their distribution. MR Elastography of cartilage, not previously suggested in the literature, would allow for imaging of its internal elastic properties with several applications in both understanding function and studying early OA changes.

2.2 How elastography works

Although other methods are able to obtain gross measurements of overall properties, MRE can image internal distribution of strain. MRE necessitates that external compressions be applied and repeated cyclically as MR obtains a segment of the image per compression. The displacement must be constant while the data is collected and repeated exactly. The MR data can then be manipulated to obtain strain information through the sample, the details of which are discussed in the methods section to follow. Strain information is representative of tissue stiffness and is used to describe tissue elastic properties.

2.3 Tissue Requirements for MR Elastography

MR Elastography requires that the tissue deform reversibly for each compression. As mentioned earlier, cartilage has a biphasic nature with a solid matrix and a fluid phase. Deformations are completely reversible for the solid phase and so are considered to be elastic [93]. However, fluid exuded from the tissue takes much longer to flow back and inflate cartilage to its former thickness. Compressing cartilage at a rate where the matrix absorbs the load, but does not have a chance to redistribute it to the fluid, so that there is no flow, is essential for cMRE. Without flow occurring, cartilage loses its viscoelastic properties and hence compresses according to elastic solid deformation laws. Various experiments have reported that with cyclical compressions at 1 Hz there is no fluid flow [6,70,94]. In the experiment for the graph of displacement over time under a
certain force (Fig 2.1), after the initial compression, subsequent displacement is due to creep or fluid flow. Displacement under 5% occurs in 250ms early in the creep phase, which would be a one-time loss since fluid would not have time to flow back into the cartilage during cyclical compressions [82] (Fig 2.1). Although cartilage is a viscoelastic tissue, in square wave 2Hz compressions it behaves elastically, as only the solid matrix deforms, thereby making the application of cMRE possible.

![Graph](image)

Fig 2.1 The experiment was performed by compressing cartilage and measuring the displacement which occurred over time [82]. The points represent measurements from one sample and the line of theoretical fit. In the initial stages during the first 250ms, displacement under 5% occurs due to creep or flow of fluid from the cartilage.
2.4 Apparatus Requirements

Elastography requires repeated compression of the sample so an apparatus had to be developed for this task. Although many experiments involving oscillatory cartilage compressions have been performed, none have been done in a magnet and so are not MR compatible or non ferromagnetic. A motor was needed that was not only MR compatible, but that could also compress a sample as stiff as cartilage (10MPa instantaneous, 1MPa equilibrium) in less than 10 ms. The apparatus had to withstand these forces without dissipating any of the motion as small displacements on the order of 200μm were needed. As a result of cartilage thinness, generally 2 to 5 mm, a high imaging resolution was required. However, at the higher resolution, the signal to noise ratio (SNR) was low making obtaining sufficient signal a challenge. Cartilage had to be kept hydrated at all times throughout the experiment to maintain its signal for imaging and elastic properties. These are the design criteria that were required in order for cMRE to be possible.

2.5 Description of Apparatus

The compression apparatus (Fig 2.2) was made from plexiglass and aluminium parts. On a base sliding pieces for the motor and sample that could be bolted into the base, were used to adjust for varying sample sizes. A PZS 200 piezo electric actuator (Burleigh, NY) was used and provides adjustable displacements to a maximum of 400μm, with a force of 25N and a rise time under 1ms. The piezo electric actuator is encased in a stainless steel frame. When the actuator was placed next to a gel sample and an image was taken, the sample could not be seen. As the piezo was moved away from the sample, its SNR increased until at a 30cm separation, the SNR was the same as had the actuator not been present. To maintain this separation, a 30cm plastic rod was used between the actuator and the sample, restricting the orientation of the apparatus to along the bore of the magnet. The compressions were done horizontally from left to right according to this image, but the data is displayed with the compressed surface towards the top of the page.
For positioning of sample

Piezo Electric Actuator

Separation Rod

Stabilizing Piece

Backing against which sample was placed

Sliding piece for sample size adjustment

Base

Fig 2.2 Cartilage compression device driven by a piezo electric actuator.

Please Note: The compressions were done horizontally from left to right according to this image, but the data is displayed with the compressed surface towards the top of the page
2.6 Coils

Coils were designed for the purpose of increasing the signal to noise ratio (SNR) of the NMR measurements. Coils receive sample signal information from which phase information is calculated. The amount of signal obtained is inversely proportional to the volume of the coil. Ideally a surface or ring coil of sample diameter would have been used to obtain the highest SNR. However, the length of the pusher rod dictates that the apparatus be placed parallel to the main magnetic field (B₀). The sample is hence placed with its surface perpendicular to B₀, which would require the coil surface also be perpendicular to B₀. However, the ring coil can not function in this orientation as its surface must be perpendicular to the transverse magnetization or excited spins, in order to measure their signal. Two coils were designed to enhance signal acquisition for the apparatus.

The first one designed was a box coil, where 2 square loops are parallel to each other and B₀. For the field to be uniform over the sample, the square loop diameter needs to be equal to the separation. With a sample diameter of 15mm, the volume enclosed by the coil is 9.38cm³. Although this shape suits the gel sample cubic geometry, its volume is much larger than the .5cm²x1cm long cylindrical cartilage and bone sample. To further increase the SNR of the cartilage MR image, a half saddle coil that fit the cartilage sample geometry was built.

The SNR improvement was tested by measuring changes in SNR in Gradient Recall Echo images (GRE) of a gel with four coils. The saddle and box coils I made were compared with a 1.6cm diameter (small) surface coil (Norm Konyer) and a standard GE 3", 7.6cm diameter (large) surface coil. GRE images were taken with Echo Time (TE) 8.7ms, Repetition Time (TR) 500ms, Field of View (FOV) 4cm, Thickness 2mm, Number of Excitations (NEX) 1 and a frequency (f) x phase (p) matrix of 256x192. The SNR of the image increased with a decrease of the volume enclosed by each coil (Fig 4.3). Both the saddle coil and box coil provided a significant improvement in SNR over the 3" (large) coil. The saddle coil provided GRE images with 40% more SNR than the 1.6cm small surface coil. To maximize SNR the saddle coil was therefore used when imaging cartilage, but the gel samples were too large for the saddle coil and so the box coil was used to collect gel data.
<table>
<thead>
<tr>
<th>Coil Type</th>
<th>Dimensions</th>
<th>Signal</th>
<th>Noise</th>
<th>SNR</th>
<th>Volume cm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>r=3.8cm</td>
<td>626</td>
<td>222</td>
<td>2.8</td>
<td>230</td>
</tr>
<tr>
<td>Box</td>
<td>2.5x2.5x1.5cm³</td>
<td>1412</td>
<td>150</td>
<td>9.4</td>
<td>9.38</td>
</tr>
<tr>
<td>Surface</td>
<td>r=0.8cm</td>
<td>3987</td>
<td>296</td>
<td>13.5</td>
<td>2.14</td>
</tr>
<tr>
<td>Saddle</td>
<td>d=1.0cm l=0.7cm</td>
<td>4573</td>
<td>243</td>
<td>18.8</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Fig 2.2 B & C Comparison of SNR and coil volume for surface, box and saddle coils for data in the above table A. Plot B shows the SNR increase with smaller coils for all 4 coils, whereas C is of the 3 smallest coils. A significant SNR improvement is seen with a smaller coil volume.
2.7 Sample Holder for Cartilage

In order for accurate mechanical information to be obtained from cartilage, it must be hydrated with saline that mimics synovial fluid. SNR is also lost if the sample begins to dry. The surface of the cartilage must be covered in such a way that it can still be compressed. A plastic backing was cut, to which a cylindrical tube was glued so that it was water tight. The base of the sample was glued to the backing and the tube was filled with saline. Parafin wax was placed over the cylindrical holder and sealed with a plastic ring. The sample backing was then placed against the fixed support of the apparatus and clamped in place.

![Diagram of the holder used to maintain cartilage moisture.](image)

**Fig 2.4 GRE MR Image of Cartilage and subchondral bone in saline with a diagram of the holder used to maintain cartilage moisture.**
2.8 Data Collection (STEAM)

In order to determine elastic properties, displacement changes within the sample from the compression need to be known. These are obtained by measuring phase changes that are proportional to displacement. The relationship between phase and position of the net magnetization will now be derived. The angular frequency of the net magnetization $\omega$ is equal to the product of the gyromagnetic ratio $\gamma$ and the magnetic field $B_0$ [\( \omega = \gamma B_0 \)] (1). Gradient $G_x$ is defined as the change in magnetic field $dB_0$ over a change in position $dx$ [$G_x = dB_0 / dx$], so that $G_x x = B_0$ (2) where $x$ is a distance. The angular frequency $\omega$ describes the angle by which the net magnetization spins $d\theta$ passes in a certain time $dt$ [$\omega = d\theta / dt$ (3)]. By substituting equations 2 and 3 into equation 1, the relationship between phase accumulation and position is obtained.

\[
\frac{d\theta}{dt} = \gamma G_x x \quad (4)
\]

\[
\int_{\theta_1}^{\theta_2} d\theta = \int_{t_1}^{t_2} \gamma G_x x dt \quad (5)
\]

\[
\Delta \theta = \gamma G_x x t \quad (6)
\]

From this equation we know that the phase accumulation is dependant on the magnetic field at a position in the magnet, the gyromagnetic ratio and the time the net magnetization spends in that location.

The phase and position relationship (6) is used by the STEAM imaging sequence (Fig 2.5) to obtain displacement information. The net magnetization $M_0$ of the protons aligns with $B_0$ and is excited into the transverse plane with a $90^\circ$ RF pulse (Fig 2.5a). This net magnetization spins in the transverse plane with an angular frequency $\omega = \gamma B_0$. STEAM motion encoding is done using a bipolar gradient (Fig 2.5 d&e). As a gradient or change in magnetic field with position (dB/dx) is applied over the sample (Fig 2.5d), the precessional frequency varies according to position (Fig 2.6a). This different precessional frequency means that the magnetization at that position has spun at a different rate than $M_0$ and the
angle accumulated between the two is referred to as phase. With a bipolar gradient, the direction of the gradient is then reversed (Fig 2.5e) for an equal time t. A proton in the same position then experiences a precessional frequency opposite in direction but equal in magnitude, so that after another time t, there is no longer any phase accumulation with respect to Mo (Fig 2.6b). However, if the proton has moved it will experience a different reverse gradient and a net phase will accumulate proportional to the displacement (Fig 2.6c).

The STEAM sequence uses this technique in conjunction with an additional 90° RF pulse (Fig 2.5 b) that flips the spins along the z axis, between the lobes of the bipolar gradient (Fig 2.5d&e). This allows for slower motions to take place without being subject to T2 (20-200ms) relaxations that are much shorter than T1 (300-3000ms). It reduces the signal loss that would be present with slow motions and a SPE imaging sequence. A third 90° RF pulse (Fig 2.5 c) is then applied before the second lobe of the motion encoding gradient (Fig 2.5 e) is applied. The time between the positive and negative motion encoding gradient is referred to as mixing time T_m. In the following experiment, STEAM is used to measure displacement of proton spins that are moving with the matrix, and hence movement of the matrix.

The bipolar STEAM acquisition is repeated twice with the direction of the gradients of the bipolar pulse reversed, so that each k-space line is an average of two acquisitions. Collecting each k-space line twice is done to eliminate any background phase not associated with motion when it is analyzed (discussed in section 3.2). Acquiring one line of k-space per compression requires the displacement and MR acquisitions be synchronized, so that data is collected each time at exactly the point in time with respect to the compression cycle. This is done through triggering of the pulse sequence and piezo at the same time.
Fig 2.5 STEAM Imaging Sequence with Physical Compression [98]. a, b and c are 90° RF pulses where a flips the net magnetization into the transverse plane, b into the longitudinal plane for time Tmixing, and c back into the transverse plane for the echo and data acquisition. Gz describes the slice select gradients and Gy and Gx are the phase and frequency encode gradients respectively. Gradients d and e are the motion encoding bipolar pulses which acquire phase proportional to displacement. The external physical compression or the sample occurs at f, between the lobes of the bipolar pulse.
Fig 2.6. (A) Phase accumulation is dependant on position within the magnet and length of time (T1) at that position. (B) When the gradients are reversed and left on for the same amount of time T1, and the spins have not moved then the same phase is accumulated but in the opposite direction. If no motion has occurred there is no net phase accumulation. (C) If there has been motion, a different amount of phase will accumulate in the reverse direction and there will be a net phase that is proportional to the displacement.
2.10 Apparatus Set-up

The piezo electric actuator creates the displacements but requires a power source. A pulse generator (Wavetek 20MHz synthesized arbitrary function generator model 95) was used to provide a square wave of 6V at 2Hz to power the piezo electric actuator and trigger the pulse sequence to start the imaging (Fig 2.7). The voltage was divided so that the acquisitions could be triggered and the sample compressed simultaneously. The 6V from the wavetek was divided between the amplifier and the magnet. This provides 1V for the amplifier (PZ-150M PZT amplifier driver, Burleigh NY) that drives the piezo (PZS-200, Burleigh NY) and can be adjusted between 0 and 150V. The remaining voltage triggered the pulse sequence to start acquiring data at each repetition, responding to a positive voltage greater than 5V. The time of compression with respect to gradients in the slice selection gradient (Fig 2.5 f) was monitored using an oscilloscope, to ensure that compression had occurred between the positive and negative lobes of the bipolar gradient pulse. This set up was used for all data acquisition.
Fig 2.7 Diagram of Apparatus Set-Up to drive piezo cartilage compressions in the magnet. A 6V 2Hz square wave is produced by the pulse generator that is then split by the voltage divider so that simultaneously 5V continues to the external auxiliary trigger to initiate the pulse sequence and 1 V passes through the amplifier and into the piezo electric actuator creating the compressions. An oscilloscope was used to monitor the timing of compression and bipolar pulse to ensure that compression occurred between the lobes of the motion encoding gradient.
CHAPTER 3: VALIDATION OF THE METHOD

3.1 Imaging Parameters

To validate the method, strain measurements were taken using cMRE on gels. Three homogeneous plastic gels (with area A 15.0x19.4mm², B 15.0x21.2mm², C 19.4x20.0mm² and thickness of 1cm) were made from plastic material (silicone polymer, M-F Manufacturing Co). STEAM images were acquired at 2Hz with a TR of 498ms. Thickness 2mm, FOV 40mm, 64x64 matrix and 20 NEX were selected to image the gels. By selecting the number of phase cycles per mm displacement (PhiD) as 5 cycles (2π)/mm, the pulse sequence determines the time the motion encoding gradients are to be played out based on a maximum G of 4.0G/cm based on the equation derived for phase (6). The piezo was adjusted to obtain 350μm motion with 150V. A 2Hz square wave of 6V was used to generate the motion and trigger the imaging sequence.

3.2 Phase Data

Understanding the Figures

In the figures, both images of the sample and plots of one pixel through the center of the image, with respect to position, are shown. The phase, displacement or strain image of the sample is on the left (Fig 3.1 T=Top, B=Bottom). It is oriented so that the surface that was compressed is facing the top of the page and the side facing the left of the page was the sample closest to the base or table. One can imagine a 90° counterclockwise shift of the sample from the horizontal position in which it was compressed. On the right are displayed plots of one line of pixels through the center of the sample, corresponding to the image on the left
from top towards bottom on the page. The data is plotted so that the left corresponds to the compressed surface of the sample and the right is the side of the sample closest to the page.

**Noise Around Data**

The noise originally surrounding the sample was set to zero. So all areas outside the sample are artificially zero.

**Unwrapping the Phase Data**

The first step involves unwrapping the images of phase that cycle from 0 to $2\pi$, so they are continuous in phase (Fig 3.1, 3.2). The unwrapping is done using Matlab where you cycle through the image from top to bottom and compares the phase value of 2 adjacent pixels (1 & 2). If the jump in phase is greater than about $1.5\pi$, it will either add or subtract $2\pi$ to the phase value of the second pixel (2) depending on whether the previous pixel (1) was positive or negative. When the nth jump is encountered, it will add $n\times2\pi$ to the subsequent phase values. When the unwrapping is finished a continuous phase variation is seen across the image from 0 to $n\pi$ (Fig 3.2).

**Isolating Phase Due to Motion**

The STEAM sequence collects 2 sets of data, but with the gradients reversed, so there are 2 images A and B of the phase data (Fig 3.1). By combining A and B, phase that is not due to motion is eliminated (Fig 3.3). Phase image B is negated, so that motion phase in A and B are now in the same direction, but phase not associated with motion is now the inverse of in A. When A and B are averaged the phase error cancels. When there is no motion the phase should average to zero, but it does not (Fig 3.4a). Since this phase offset is uniform over the sample, it is subtracted from the phase data with motion to obtain a baseline of zero (Fig 3.4b). This subtraction of no motion phase data from motion phase data is performed for all the samples measured.
Fig 3.1 Phase wrapped images (left) of gel compression with plots (right) from top to bottom of one line through the image.

Fig 3.2 Unwrapped phase images on the left with plots through the respective sample on the right.
Fig 3.3 Motion phase averaging and subtraction of phase not associated with motion. 

a) Original data, taken with opposing gradients.  
b) Data B is negated so that motion phase is in the same direction as A, but noise is opposite.  
c) A and B are added so that the motion phase averages but the non-motion phase cancels.
3.3 Displacement Data

The relationship between displacement and phase was selected using PhiD, which in this case is 10π/mm. The phase data is divided by PhiD to obtain displacement data in mm (Fig 3.5). The maximum displacement 343+/−2μm (341,343,346 μm) was then checked against a value previously obtained using a microscope 350+/−25μm and they were found to agree. The maximum displacement is obtained at the surface under the pusher and it decreases to zero at the base of the sample. However, the displacement curve is not a straight line as may have been expected and explained in the next section.
3.4 Gel Deformation Physics

To describe the displacement and strain obtained during compression it is best to start with a uniform rectangle. Imagine this 2D block broken down into smaller rectangular sections of gel and start by focusing on a piece located under the pusher plate in the center (Fig 3.6). The gel is incompressible meaning that the area of the gel segment is conserved and that if the sample is compressed in the vertical direction, it will expand horizontally. The gel experiment is not ideal in the sense that there is friction between the sample and plate. As a result of this, the piece of gel directly below the plate cannot expand horizontally and must absorb the pressure through vertical motion. This appears as a uniform displacement seen at the surface and base of the gel (Fig 3.5 & 3.6). In the center of the sample, where it is free to expand laterally, the displacement decreases linearly.

Although displacement through the tissue has been measured in section 3.3, what we are after is the elastic properties of the tissue. Strain, discussed previously in section 1.2.2.1, is related to the elastic properties or $Y$ of the tissue and can be calculated from the displacement data. Strain is defined as $\varepsilon = \left[ \frac{dw}{dz} \right]$ where $w$ is displacement in the $z$ direction and is calculated from the data as being the change in displacement between two adjacent pixels divided by the pixel size in the displacement direction ($\Delta d/p$). Since the displacement data is non linear, the strain is non uniform and there is less strain in the sample next to the compression plate and base. A plateau of uniform strain is reached in the center for a homogeneous gel, which is characteristic of the gel (Fig 3.6)
3.5 Strain Data in a Homogeneous Gel

Strain images and plots of one line of pixels through the center of the gel were obtained for three homogeneous gel samples (Fig 3.7). The gel samples were the same material but slightly different sizes. The strain plots were then normalized for height of the gel by multiplying strain by height of the sample divided by the height of the tallest sample, because a smaller sample of equivalent material will have to strain more to absorb the displacement. Strain values were measured from the plateau defined as the area in the center, starting 10 pixels away from either edge. The curves all reach a plateau at 0.0178, 0.0176 and 0.0171 +/-0.0004, although the shape of the rise depends on the gel surface. The errors are calculated from the standard deviation of the values on the plateau, however the error may be larger since the initial compression from sample positioning will vary. A sample that is already strained before the experiment, will have this strain added to the strain from the compression experiment, overestimating the strain for that sample. This initial strain may also affect the shape of the curve, as it is less likely to glide along the surface if it is compressed,
making the curve more rounded. The similar value of the maximum strain reached indicates that strain can be consistently measured.

Fig 3.7 Plots of strain through three homogeneous gel samples of the same material. The plots of strain through the sample all reach a plateau at an average value of 0.0171 +/- 0.0004 although the shape of the rise differs for each of the samples.
3.6 Strain Data in a Gel With an Inclusion

3.6.1 Relative Strain and its Relation to Relative Young’s Modulus

Calculating Young’s modulus directly from strain data is not possible since the exact distribution of forces at each point in the sample needs to be known [95]. However, strain variations can be used to visualize Y variations when looking at relative values of strain [98]. Under assumptions of constant strain at the measured area and a Poisson’s ratio $\nu=0.5$, the ratio of strain $\varepsilon$ is related to relative $Y$ through [98]:

$$\frac{\varepsilon_2}{\varepsilon_1} = \frac{1}{2} \left( 1 + \frac{Y_1}{Y_2} \right) ...(8)$$

This equation is for a sphere (1) embedded in an infinitely large medium (2), where $\varepsilon_2$ is sampled far from the inclusion. Relative strain data will be obtained using MR Elastography. The strain ratio will be compared with actual strain ratios calculated by (8) from $Y$ values obtained using a traditional stress-strain analysis performed outside the magnet.

3.6.2 Strain Ratio According to MRE

To measure relative strain between two materials, a 3D rectangular gel with an area $14 \times 20 \text{mm}^2$ and thickness 8mm was made. Once the gel had hardened, a thin hollow glass cylinder, open on both ends, was forced through the gel creating an opening through which a softer gel was poured. The softer cylindrical inclusion was measured on an MR image to have a diameter of 5mm and length of 8mm (Fig 3.8a). Strain measurements were taken in the same way as for the homogeneous gel (Fig 3.8b). Looking at the strain image and plot, you can clearly see the inclusion (Fig 5.8b).
Fig 3.8 Images of a gel with a softer cylindrical inclusion through the center. A) Magnitude image  B) Strain image.
In order to calculate relative Young's Modulus, relative strain is needed. Strain for the inclusion ($\varepsilon = 0.0323 \pm 1\%$) was calculated by averaging an area in the center of the inclusion of 7x6 pixels. Error was measured by calculating the standard deviation of the strain in the measured region. Strain for the background had to satisfy 2 criteria: that it be "far" from the inclusion and that it be far from the edges to avoid the non linear region associated with sample-compression plate friction. These criteria were approximated by selecting a rectangular frame 4
pixels wide, positioned equidistant from the inclusion and edge of the sample. The Strain from this area was $\varepsilon_2 = 0.0166 \pm 1\%$. The strain ratio obtained using MR Elastography for the hard to soft region is $\varepsilon_2/\varepsilon_1 = 0.514 \pm 2\%$. Although the error is 2\% from the standard deviation of the strain values, it will actually be larger because the strain value for the background was taken from close to the inclusion and was not uniform, which were required criteria.

3.6.3 Strain Ratio According to Stress Strain Measurements

An independent stress strain relationship was crudely obtained using a balance to measure mass and calipers to apply displacement [96]. Where the force from gradual increases in displacement of .0254mm was measured and plotted versus strain. The measurements were repeated three times for the soft and hard gel to obtain error ranges. A polynomial fit was found to match the data with $\sigma_1 = 60953\varepsilon^2 + 2978.9\varepsilon - 2.1072$ for the average soft gel and $\sigma_2 = 158555\varepsilon^2 + 4957.6\varepsilon - 4.8911$ for the average of the hard gel (Fig 3.10). In order to obtain a $Y$ for the samples, the strain $\varepsilon$ of the samples in the experiment had to be approximated. This was done by calculating the overall strain for the sample by dividing the maximum displacement .324mm by the height of the sample 20mm to obtain a $\varepsilon = 0.0162$, which was then inserted into the equation to obtain $\sigma_1 = 62.15$ (Pa) and $\sigma_2 = 117.0$ (Pa). This provided $Y_1 = 3836 \pm 500$ Pa and $Y_2 = 7222 \pm 900$ Pa, for $Y_1/Y_2 = 0.531 \pm 0.133$. The error was obtained by repeating the measurements of the hard and soft gel 3 times each, fitting these curves, then calculating $\sigma$ from each one and averaging the variation from the mean. This large error is due in part to the initial contact between the plate and the sample, which was judged by eye. The sample surface was curved and smaller in the center, so that compression and strain occurred before the entire sample was in contact with the plate. The experiment was started at $t=0s F=0N$ once the entire sample was deemed to be in contact with the plate. When inserted into equation 8, a strain ratio of $\varepsilon_2/\varepsilon_1 = 0.77 \pm 0.07$ was obtained from the $Y$ ratio.
3.6.4 Comparison of Expected and MR measured Strain Values

The strain ratio obtained through MR Elastography $\varepsilon_2/\varepsilon_1=0.51+/0.01$ is much lower than the expected strain ratio $\varepsilon_2/\varepsilon_1=0.77+/0.07$ from $Y$ values obtained through stress-strain analysis and the Keller equation. The lower experimental values are expected as the cylinder length to diameter ratio needed to approximate a true 2D problem is greater than 10 but is 1.5 for this sample. As the problem is now really a 3D problem, the expected $\varepsilon_2/\varepsilon_1$ would be lower with respect to the $Y$ variation. Errors from the stress-strain $Y$ measurement and a model which was meant for an infinitely large sample and measurements of strain far from the inclusion also affect the ability to compare the strain values. Although the inclusion strain image reflects the Young's modulus and hence hardness, quantification of hardness is not straightforward and requires very complex inverse solutions which are challenging [95].

3.7 Discussion and Conclusion

Strain measurements were found to be consistent and reproducible in gel samples. Strain measurements were taken for sample sizes, resolutions of .625mm and displacements on the order that would be found in cartilage. Strain images were obtained for a gel (2) with an inclusion (1) that varied in hardness $Y_1/Y_2=0.5$, on the same order as diseased OA tissue would in cartilage. The softer inclusion was clearly visible both in the image and in plots of strain through the sample. However, quantitative interpretation of the strain ratios as it relates to Young's modulus ratios was not as straightforward. Although the MR obtained strain ratio was on the order that would be expected from the actual Young's modulus values and Keller's equation for strain ratios between a sphere and an infinitely large background medium, the two did not agree. A visual interpretation of the strain variations between inclusion and background at this stage is a more effective solution until better models and inverse solution can be found.
Fig 3.10 Stress Strain curves and polynomial fits for a hard and soft gel.
CHAPTER 4: APPLICATION OF MR ELASTOGRAPHY TO CARTILAGE

4.1 Normal Cartilage

Method

Bovine cartilage samples were obtained from the calf knee joint. The intact knee joint was obtained from a slaughter house (Regency, Toronto) and 0.5cm² samples of cartilage with the bone backing were cut. Cartilage was taken from the femoral chondyle of the patella-femoral joint as these samples are thicker, on the order of 5mm. Samples were kept in 0.5mmol Gd(DTPA)²⁻ saline solution, refrigerated and used within 2 days of being cut. Gd(DTPA)²⁻ was used initially in the solution so that it could equilibrate throughout the cartilage as this takes 24hrs. This way there was no need for a delay time between initial strain measurements and T1 degradation that requires Gd(DTPA)²⁻ in the tissue.

4.1.1 Contact Piece Geometry

Due to the stiffness of cartilage the size of the pusher plate was decreased to 2mmx3mm, which is smaller than the cartilage sample. The effect of the contact point being smaller than the sample was first tested on a homogeneous gel sample to ensure that it had no effect on the strain results. However, plots of strain show a variation both through the thickness of the gel and parallel to the surface, depending on the plot position with respect to the plate (Fig 4.1). Directly below the pusher, there is an increase in strain, which decreases and levels towards the
base of the sample. The height of this peak is maximal close to the pusher and decreases as one moves away. As a result of variations in strain due to contact geometry and not changes in stiffness, a contact plate larger than the sample was used for cartilage experiments even if it necessitated the use of smaller displacements to obtain strain due to the limited strength of the piezo.

Fig 4.1 Compression of a gel sample with contact area smaller than the sample. A) Strain Image with contact in the center. B) Strain is plotted through the depth of the sample at each pixel. The strain is highest in the center of the sample (pixel 15) just under the pusher plate and drops off at the base of the sample. The strain also decreases moving away from the center and is lowest through pixels 12 and 18.
4.1.2 Strain

Strain measurements were obtained in the same way as for the gel. A mask of the cartilage-bone sample was taken from a GRE image (4.2a) and overlaid onto the phase image (4.2b) so that strain from only the sample is measured (Fig 4.2c). By observing the strain image, the distinction between the bone and cartilage is clear based on stiffness. The experiments were repeated 3 times on each of 3 samples (S1, S2, S3) and the samples were removed from the holder and repositioned for each repetition. For each sample and each repetition, a strain line is plotted through the center 3 pixels of the image, averaging the 3 values to produce one line per repetition. As there are 3 repetitions of the strain imaging for each sample, 3 strain lines are obtained per sample. These 3 lines are plotted against each other for each sample and an example of a strain image is presented for each sample (Fig 4.3, 4.4 & 4.5). The shape of the strain plots is consistent for a sample, but varies strongly between samples. However, even within a sample there is variation in elastic properties between each repetition. This is likely due to variations in repositioning the sample and slightly different areas within the cartilage being imaged. Cartilage MR Elastography obtained strain plots that reflect elastic properties, with measurements that are repeatable and have a low enough variation that they show potential for being able to distinguish regions of differing elastic properties.
Fig 4.2 A GRE image of the sample is used as reference to identify the edge of the cartilage and the cartilage bone interface. A) GRE of Cartilage sample B) Strain image with noise C) Strain image with mask to remove the noise.
Fig 4.3 Strain image of cartilage sample for one of the repetitions (SI). Each line corresponds to one of the repeated strain measurements for the same sample. Plots are taken through the sample strain image around the center pixel line, with 3 pixel lines averaged. Cartilage at the subchondral bone appears softer than in the rest of the sample.
Fig 4.4 Strain image of cartilage sample for one of the repetitions (S2). Each line corresponds to one of the repeated strain measurements for the same sample. Plots are taken through the sample strain image around the center pixel line, with 3 pixel lines averaged. Cartilage at the surface appears softer than in the rest of the sample since it has a higher strain.
Fig 4.5 A) Strain image of cartilage sample for one of the repetitions (S3). Each line corresponds to one of the repeated strain measurements for the same sample. Plots are taken through the sample strain image around the center pixel line, with 3 pixel lines averaged.
4.2 Degraded Cartilage

Once strain data was obtained in cartilage, the ability to measure changes in strain in cartilage was put to the test. A cartilage sample (S4) previously measured with cMRE was degraded to remove PG and hence change its elastic properties. A decrease in $T_1$ Gd is known to correlate with a decrease in GAG concentration (Fig 1.10) and hence PG concentration, that in turn correlates with a decrease in Young's modulus of cartilage (Fig 1.5,1.6). $T_1$ Gd measurements were used to monitor the removal of proteoglycans in cartilage. Strain plots were then retaken and compared with the original.

4.2.1 Degrading Cartilage

Samples were kept in 0.5mmol Gd(DTPA)$^2-$ saline solution and refrigerated for 48hrs before the degradation to allow Gd to equilibrate through the tissue. A 2.5% trypsin concentration in 0.5 mmol Gd(DTPA)2- saline solution was used to degrade cartilage. In a test tube, each sample was degraded using 2.5% trypsin covering 1/3 of the sample from the base of the bone, so as to approximately cover the radial zone of the cartilage (Fig 4.6A). The proteoglycans in the radial zone were depleted, softening that part of the sample and the container was sealed. $T_1$ images were obtained using a spin-echo imaging sequence with spiral readouts [97]. $T_1$ images were taken before ($t=0s$), during ($t=5hrs$) and after ($12hrs$) the degradation.

4.2.2 $T_1$ Results of Degradation

The $T_1$ in normal cartilage is seen to increase towards the base of the sample agreeing with an expected increase in PG concentration. The sample was left for 5hrs and 12hrs and the $T_1$ decreased (Fig 4.6D). The lines plotted (Fig 4.6D) represent an average of 15 pixels through the center of the sample, to see the average $T_1$ from the surface of the sample to the base. From the start, the $T_1$ at the sides and top of the sample are much lower ($T_1$ 350-450ms) than in the center of the sample ($T_1$ 500-800ms), indicating a higher Gd concentration and hence lower PG concentration. This indicates possible damage to the matrix from
cutting, loss of natural structure and drying during sample transfers. The lower central region of the cartilage, above the tidemark undergoes a more significant degradation as it decreases from T1 675ms to around 475ms.

Strain data was obtained after degradation had occurred (t=9hrs), between the second and third T1 measurement and compared with the original (Fig 4.7). Strain data was normalized by the height of the sample as the sample decreased in height by 2 pixels or 0.625mm. Interestingly, the elastic properties of cartilage appear uniform initially (Fig 4.7a) even though the T1 Gd measurements vary strongly through the depth of the sample. This indicates that T1 Gd measurements, which increase with increases in GAG concentrations, are not directly related to elastic properties and that other component may be varying within the sample to balance the strain.

However, variation in GAG concentration from the initial stages would still change elastic properties if the other factors remain the same. Increase in strain is clearly seen at the base of the cartilage, indicating the softer region where more degradation occurred. A decrease in strain is seen when moving up from the tidemark away from the bone, due to the diffusion of the trypsin. Small vessels were present in the cartilage, which may have facilitated distribution of trypsin to the internal regions. A greater softening of the cartilage above the tidemark was confirmed through observation. When degradation was left to occur until 24hrs and the sample removed from the holder, a light compression of cartilage resulted in a collapse of the cartilage matrix above the tidemark, whereas cartilage from the superficial and middle zone retained their structure. The increase in strain in the central radial and middle zone with respect to the superficial zone is clear and corresponds with PG degradation.
n. Gd(DTPA)²⁺ Solution

Cartilage

Trypsin. Gd(DTPA)²⁺ & Saline Solution

Tidemark

Bone

1 pixel = 0.156 mm
Fig 4.6 T1 measurements of cartilage degrading in trypsin. A) Initial image at T=0hrs shows a high T1 at the base of the cartilage. B) and C) Show progressively lower T1 values. D) Plot through each of the samples with — initial measurement T=0hrs, — intermediate T=5hrs, — final T=12hrs. The dip at the arrow is the boundary between cartilage and bone.
Fig 4.7 Strain of degraded cartilage sample. A) Strain image before cartilage is degraded B) Strain image after degradation (Both A and B are not normalized) C) Plots averaging 3 pixels through the strain images normalized for height. (1 pixel = 0.625mm).

- Before (A).  - After (B)
4.3 Usefulness of the method

The advantage of the method is its ability to distinguish tissue based on stiffness, which might otherwise not be noticeable. A sample (F) of cartilage with an uneven cartilage bone interface at the epiphyseal line or metaphysis was taken. When observing the Spin Echo Image (SPE TR 800ms FOV2cm Th3mm 256x192 1NEX), the cartilage and bone can clearly be distinguished (Fig 4.8). However, in the strain image an additional region of softer tissue lining the bone and in the metaphysis is seen. This emphasizes the usefulness of the method in distinguishing regions of tissue with different properties that might not otherwise be noticeable.

4.4 Conclusion as to the Application of MR Elastography to Cartilage

Strain images and plots were obtained in normal juvenile bovine cartilage. They were shown to be repeatable by highlighting the same regional variations in strain in repeated experiments on one sample. Strain also varied between different areas for each sample. With the degradation experiments, differential strain was measured within cartilage, highlighting regions of relatively harder and softer tissue. When strain measurements were applied to a sample, the epiphyseal line was a factor of 3 greater in strain (softer) than the cartilage, a variation that was not apparent in a SPE image. Internal variations in elastic properties of cartilage can be measured using MR Elastography, but the detail at which this can be done needs to be explored further.
Fig 4.8 A) GRE image of cartilage with bone B) Strain image of the same sample showing a much softer region coinciding with the location of the metaphysis that was not distinguishable from cartilage in the GRE image. C) Plot of strain through cartilage showing a region in the metaphysis with 4x more strain and hence 4x softer than the surrounding cartilage.
CHAPTER 5: FUTURE DIRECTION OF cMRE

5.1 Further Development of the method

Strain measurements are not the only way that mechanical information can be extracted using cartilage MR elastography. The measurement of other matrix properties such as resistance to shearing, rate of flow of fluid from cartilage under compression and energy dissipated during compression and expansion will be discussed in this section. They would provide additional information and aid in completing the picture of cartilage mechanical function. These measurements have been made on cartilage samples using traditional measures of mechanical properties, however their internal distribution and changes with disease are not known. Further experiments regarding both in vitro and in vivo cartilage experiments will be detailed.
5.1.1 Resistance to Shearing

Cartilage is thought to have an arch like arrangement of collagen that resists shearing stresses. With age related collagen deterioration, this structure may be weakened. In order to measure these mechanical changes, the apparatus would have to be redesigned so that shear forces were exerted on the tissue. For shearing the measurement would also be modified by collecting phase motion data from both the horizontal and vertical direction. Relationships between changes in strain and relative shear modulus could be examined for a standard to compare samples.

5.1.2 Measuring Flow During Compression

Flow from the sample under compression as well as re-absorption could be studied through a simple adaptation of the method. Surface flow has been measured but not its internal distribution. A STEAM imaging sequence will collect phase data from flowing protons in the same manner as for ones moving with the matrix. The adjustment would have to be made to the apparatus so that when the images are taken, flow is occurring. Since the reaction of the sample to compression is first motion of the solid matrix then flow, the imaging would have to occur after the initial compression. The amount of fluid displacement over a fixed time period and hence rate of flow could be obtained. This flow rate might change with disease and variation in PG content. In terms of imaging, all the data would have to be collected at once since there would not be cyclical compressions. The only disadvantage to this method is the length of time it would take for the sample to reach equilibrium, which is on the order of an hour. Flow of fluid is an essential property of the matrix and would be useful to understand.

5.1.3 Hysteresis Loop

A hysteresis loop (Fig 5.1) is a plot of stress versus strain, where the area enclosed would represent the energy dissipated by the tissue and hence its state. By adjusting the exerted force between experiments, strain would vary and could be measured using cMRE. This graph is a different way of presenting the compression information from an area within cartilage. The experiments I
performed applied an overall amount of strain for a certain stress, which would appear as a point on this plot. By having a variety of stress strain data, a more subtle change may be detectable. Although one looses the ability to see strain variation within the tissue, so would ideally be used in conjunction with strain compression images.

![Hysteresis loop for cartilage under compressions](image)

**Fig 5.1** Hysteresis loop for cartilage under compressions [29]

### 5.2 Application In Vivo

An area of great interest with imaging is whether the method can be used in vivo. The work presented is far from currently being applicable clinically and some of the major challenges to be overcome will be addressed. Before looking to apply this method in vivo, further work needs to be done in determining cMRE's ability to discern OA. Currently there is not a good correlation between changes in mechanical properties and grades of disease in OA. Although a vague
correlation was mentioned earlier in this work, a more detailed and exact one is needed to be able to judge OA using stiffness. One needs to know the variations in mechanical properties at each stage of the disease.

The greatest challenge in pursuing this work in vivo lies in being able to apply the force. Some sort of apparatus would be needed to push for example, the lower leg bone (tibia) onto the upper one (femur). Although force would be lost in the process, it would not matter as long as the displacements were consistent as the actual displacement can be measured using cMRE. For a joint such as the patella-femoral one, the force would be much easier to apply due to the location of the patella. Another option is to try to apply the force directly to the femoral contact cartilage that is exposed when the knee is bent. Compression would have to be applied through the skin. Besides this large physical constraint to be overcome and the usual questions of SNR and resolution, there are no reasons why this method could not be used in vivo, although it may be found to be more useful as a research tool.

5.3 Conclusion Regarding Use of cMRE

Cartilage MR elastography was found able to measure strain in cartilage samples. The strain plots for various samples were different in shape indicating a variation in elastic properties between cartilage samples as well as within them. cMRE was able to distinguish changes in elastic properties due to degradation of PG and the pattern was detailed enough to distinguish the decrease in degradation from the tidemark to the cartilage surface. The advantage of cMRE is its ability to distinguish variations in stiffness throughout the sample that can highlight regions not otherwise noticeable in the image. This may one day be applied to understand changes in elastic properties through cartilage and around the joint, as well as to measure currently undetectable early changes from disease or osteoarthritis.


