The Dependence of NMR Measured Diffusion, Magnetization Transfer, and T2 Relaxation on Fractional Water Content in Bovine Articular Cartilage.

by

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Submitted to the Department of Electrical Engineering and Computer Science, in partial fulfillment of the requirements for the degree of Master of Science in Electrical Engineering

Articular cartilage is a dense, relatively acellular and avascular tissue which functions as a load bearing material and provides a smooth surface for articulation in joints. The major constituents of cartilage are water, cells, and an extracellular matrix. The functional properties of the tissue depend greatly on the integrity of the extracellular matrix. Changes in the matrix, such as those seen in the degenerative cartilage disease osteoarthritis, may compromise those properties. In addition to the matrix deterioration, there is an observed increase in fractional water content.

One of the imaging modalities used for the diagnosis of arthritis is magnetic resonance imaging (MRI). Some MRI parameters that are becoming more widely used in the clinical setting are diffusion and magnetization transfer. Diffusivity of water can be inferred directly from measurements of their brownian motion using nuclear magnetic resonance (NMR). Magnetization transfer (MT), expressed as Ms/Mo, examines the transfer of magnetization between proton pools of varying mobility. In the case of cartilage, two major pools are presumed to include protons associated with the matrix and protons in the bulk water. A more commonly used parameter is T2 relaxation, the process of the decay of the transverse magnetization.

The pathological changes that occur in osteoarthritis are complex and varied, and may affect the MRI measurements. To better understand how pathologic alterations are reflected in MRI measurements this thesis focuses on one particular change, the increase in fractional water content observed in diseased cartilage. Thus, the purpose of this thesis was to examine the specific effect of changes in fractional water content, without altering the matrix constituents, on NMR measured diffusion, magnetization transfer, and T2 relaxation in bovine articular cartilage. Fractional water content was specifically altered by compressing the cartilage samples, forcing water out of the tissue.

Using theoretical considerations and previous experimental measurements, preliminary estimates of changes in the NMR parameters were made, and these predictions were compared to the results. For comparison purposes the increase in the parameter for a change in fractional water content from 70% to 77% was determined. These values roughly represent the water content of normal and arthritic tissue. For all three parameters, an increase in fractional water content resulted in an increase in the parameter.

Diffusivity predictions based on models gave a range of increases from 8% to 28%, showing reasonable agreement with the observed increases of 21% and 23%. The expected increase based on previous data, in Ms/Mo (11%) matched well with the experimentally measured increase of 13%. The large discrepancy between the predicted increase based on previous data, in T2 (42%) and the experimentally measured increase (83%) may have been due to T2 dependence on magnetic field strength and differences in sample type and composition.

Using these results and measurements from others on cartilage that varies in both matrix constituents and fractional water content, preliminary determinations of the specificity of the NMR parameters were made. Diffusion results suggest that the

measurement reflects fractional water content and does not depend on the precise macromolecular composition. It was not possible to compare MT results to data from others due to the normalization used. T2 appears to be affected by fractional water content and matrix constituents.

In conclusion, the relationship between fractional water content and NMR measured diffusion, MT, and T2 in bovine articular cartilage has been characterized. These data provide the framework for examining the effect of variations in other cartilage matrix constituents on the NMR measurements.

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Introduction

Articular cartilage is a dense, relatively acellular and avascular tissue which functions as a load bearing material in joints. Cartilage consists mainly of water, cells called chondrocytes, and an extracellular matrix. Water comprises 60% - 80% of the wet weight of cartilage. Chondrocytes, which are responsible for the maintenance of the extracellular matrix, make up less than 10% of the total tissue volume. The extracellular matrix has two major constituents, collagen and glycosaminoglycans (GAGs).

The ability of the cartilage to distribute load in the joint and to provide a smooth surface for articulation depends upon the integrity of the extracellular matrix. Therefore, changes in the matrix may compromise the normal functioning of cartilage. Osteoarthritis (OA), a disease characterized by the progressive degeneration of cartilage, significantly changes the content and the characteristics of the extracellular matrix. (For a review see Mankin and Brandt, 1984.) In OA, there is an increase in fractional water content. There is also an increase in collagen synthesis, although collagen content appears unchanged. In comparison, GAG content decreases while GAG synthesis increases.

Clinical and laboratory evaluations, conventional radiography, arthroscopy, and magnetic resonance imaging (MRI) techniques are just some of the tools used in the diagnosis of OA (Resnic and Niwayama, 1988). Although invasive, arthroscopy is often considered the "gold standard" of the various imaging modalities (Fife 1992). MRI, a promising non-invasive technique, allows a more direct visualization of cartilage and can provide earlier detection of arthritis than conventional radiography (Kaye 1990). MRI is used clinically to detect arthritis, to determine the severity and activity of the disease, and to evaluate the progression of the disease (Kaye 1990).

Some MRI parameters that are becoming more widely used in the clinical setting are diffusion and magnetization transfer (MT). An NMR technique is able to infer the self diffusion coefficient of water from measurements of the brownian motion of the water molecules (Stejskal and Tanner 1965). Magnetization transfer examines the transfer of magnetization between proton pools of varying mobility (Forsen and Hoffman 1963, 1964). In the case of cartilage, two major pools of protons are assumed to be those associated with the large macromolecules of the solid matrix and those in the bulk water. A more commonly used parameter in the diagnosis of OA is T2 relaxation. T2 relaxation, also referred to as spin-spin relaxation, is the decay of the net transverse signal in the x-y plane.

The changes that occur in cartilage with degenerative diseases such as osteoarthritis are both varied and complex. Many of those changes, either alone or in conjunction with one another, may affect the previously mentioned NMR parameters. It is the goal of this thesis to examine the specific effect of changes in fractional water content, without altering the solid content, on NMR measured diffusion, magnetization transfer, and T2 relaxation. The results will allow the further analysis of the effect of other matrix constituents on the NMR measurements. The method used to alter fractional water content is compression. As a cartilage sample is compressed, water is forced out of the sample, and as a result the fractional water content decreases.

Previous NMR measurements of diffusion, MT, and T2 relaxation in cartilage have been reported by various authors. Diffusion of water in bovine cartilage was previously measured by Hartman (1991). The measurement was made on free swelling samples and samples compressed to approximately 40% of the initial height. The diffusivity in the compressed samples measured slightly lower than in the free swelling case. Lesperance (1993) measured MT (expressed as a ratio Ms/Mo) in trypsin digested bovine cartilage that was placed under various levels of compression. Lesperance found that as the cartilage was

compressed and the fractional water content decreased, Ms/Mo decreased slightly (the MT effect increased). T2 weighted images of bovine articular cartilage were produced by Lehner (1989). Two different zones of intensity were noted. The surface layer had a higher fractional water content and longer T2 relaxation times. The deeper layer had a lower fractional water content and shorter T2 relaxation times.

In terms of the dependence of the NMR measurements on fractional water content, the previous NMR measurements are limited in various ways. In the case of the diffusion and T2, measurements at only two different fractional water contents were made. In addition, the T2 measurements compared two different zones of cartilage that differed in fractional water content, but also may have differed in other characteristics that may have affected the T2 times. The MT measurements were made over a range of fractional water contents. However, the samples were degraded with trypsin which make it difficult to determine the effect of changes in fractional water content alone. This thesis attempts to address these limitations by measuring the NMR parameters on normal bovine articular cartilage over a range of fractional water contents. The results from this work will provide a more exact determination of the effect of changing fractional water content on the NMR measurements of diffusion, MT, and T2. In addition, since changes in other matrix constituents are often coupled with a change in fraction water content, these results will allow the further determination of the effect changes in matrix constituents, other than water, on the NMR measurements.

Background

Cartilage

Articular cartilage is a dense, relatively acellular and avascular tissue which functions as a load bearing material. Approximately 60%-80% of the wet weight of cartilage is water. The extracellular matrix is mainly composed of collagens, 12%-18% of the wet weight, and glycosaminoglycans (GAGs), 4%-6% of the wet weight (Mankin and Brandt 1984). Chondrocytes, which make up less than 10% of the total volume, are responsible for the formation, maintenance, and resorption of the extracellular matrix. Collagens form a fibrous network that provides tensile strength. GAGs provide compressive strength due partly to electrostatic repulsion of the highly negatively charged side chains (Buckwalter 1987). GAGs play a primary role in normal cartilage function. The charged GAGs repel one another and are highly hydrophilic, generating considerable swelling pressure. These attributes of the GAGs help resist water loss from the cartilage under compression. The pressure of the GAGs to expand is constrained by the elastic collagen network (Maroudas 1976). A schematic of the cartilage structure is shown in Figure 1.

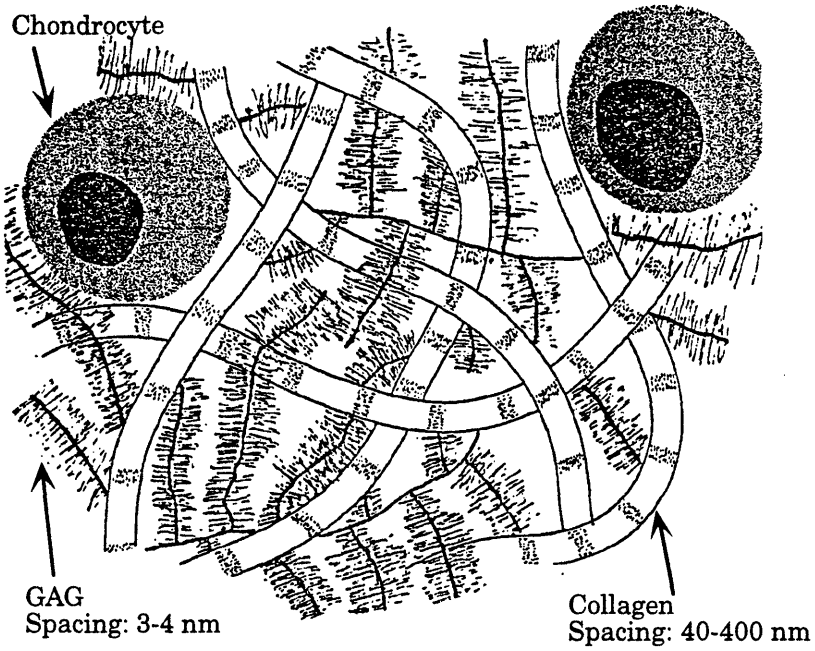

Figure 1: Schematic of cartilage structure. Spacing between GAGs is 3-4 nm (Byers 1983). Spacing between collagen fibers is 40-400 nm (Byers 1983).

In degenerative cartilage diseases such as osteoarthritis, loss of matrix constituents is observed. (For a review see Mankin and Brandt 1984.) GAG concentration is found to decrease in osteoarthritic cartilage, with the decrease proportional to severity (Mankin and Brandt 1984). In contrast, the collagen content per weight wet or dry weight in normal and osteoarthritic cartilage does not vary (Mankin and Brandt 1984).

Another characteristic differentiating normal and diseased cartilage is fractional water content. Fractional water content is defined here as the absolute water content divided by the wet weight and is expressed as a percentage. The fractional water content of osteoarthritic cartilage has been found to be significantly higher than the fractional water content of normal cartilage (Mankin and Thrasher 1975, Maroudas and Venn 1977, Venn and Maroudas 1977, Grushko 1989). The fractional water content measurements of full thickness cartilage samples are summarized in Table 1.

	Normal Cartilage Fractional water content (±SD)	OA Cartilage Fractional water content (±SD)	Source of Normal / OA cartilage
Mankin and Thrasher (1975)	66.2% ±2.2%	72.1% ±1.4%	FNF / THR
Maroudas and Venn (1977)	71% ±0.3%	76% ±2.0%	PM / THR
Venn and Maroudas (1977)	71.5%	80%	PM / THR
Grushko (1989)	70%	76-82% (depending on severity)	PM & FNF / THR

Table 1. Summary of full thickness fractional water content measurements (±SD) of normal and osteoarthritic cartilage. No standard deviations were given for Venn and Maroudas (1977) or Grushko (1989). All cartilage samples were from human femoral head. The Grushko measurements for osteoarthritic cartilage were taken from diseased cartilage of varying severity, characterized by the degree of fibrillation. Explanation of cartilage source: FNF = femoral neck fracture, THR = total hip replacement, PM = post mortem.

There is some discrepancy in the absolute fractional water content of normal and diseased tissue between Mankin and Thrasher and the others. The lower measured fractional water content of Mankin and Thrasher may be due to their procedure. Cartilage slices were resected and placed in a bath of Eagle's medium for five minutes, after which the fractional water content was measured. In the other three studies, the equilibration time was not specified. One possible cause for the lower fractional water content seen by Mankin and Thrasher may be the incomplete equilibration of the samples. However, it is clear from all the studies that there is an increase in the fractional water content of diseased cartilage.

The amount of the increase in fractional water content has also been correlated to the severity of the cartilage degradation (Venn and Maroudas 1977, Grushko 1989). Venn and Maroudas used decreasing GAG concentration as a measure of increasing disease severity. GAG concentration was measured by measuring the fixed charge density (FCD), which they defined as the amount of negatively charged fixed groups per weight of tissue. In cartilage at physiological pH, the measured FCD is due mainly to the negatively charged GAGs (Maroudas 1969, Maroudas and Thomas 1970). The mean fractional water content of normal cartilage was 71.5%. Fractional water content was found to increase with

decreasing FCD. The fractional water content of the samples with the lowest FCD measured greater than 80%. Grushko (1989) also compared normal human femoral head cartilage to osteoarthritic samples of varying severity. The osteoarthritic samples were placed into the following three groups based upon the degree of fibrillation: (1) intact surface, (2) surface fibrillation, and (3) deep fibrillation. The fractional water content of normal samples was 70%. The fractional water content of the osteoarthritic samples ranged from 76% for the samples with intact surfaces (least severe) to 82% for samples with deep fibrillation (most severe). From these results it can be seen that fractional water content varies in the presence of disease and with the severity of disease.

In addition to the variation in tissue fractional water content between full thickness samples of normal and diseased cartilage, fractional water content also varies with depth in both normal and osteoarthritic cartilage (Maroudas 1976, Venn and Maroudas 1977, Roberts 1986 A). The results of Venn and Maroudas (1977) are shown in Figure 2.

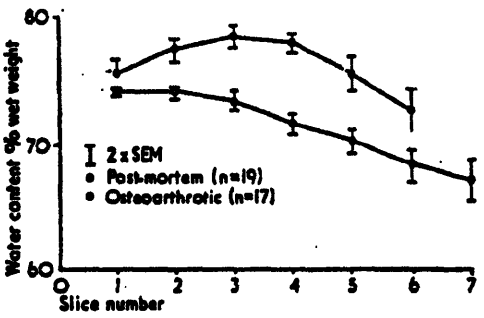

Figure 2: Venn and Maroudas (1977) Fractional Water Content (% Wet Weight) versus Slice Number for normal and osteoarthritic human femoral head cartilage. Top curve is from the osteoarthritic samples. Slice Number increases with depth from surface of the cartilage. Slice thickness was 200 μm.

In normal cartilage, fractional water content decreases with increasing depth from the surface. Osteoarthritic cartilage fractional water content increases from the surface towards the middle zone and then decreases with increasing depth. At all depths, osteoarthritic cartilage has higher fractional water content than normal cartilage. Roberts (1986 A) also made measurements of fractional water content versus depth for normal and osteoarthritic human femoral head cartilage. Roberts found a similar trend of fractional water content as a function of depth for normal and osteoarthritic cartilage. Based on all of these results, fractional water content has been shown to depend on the presence and severity of disease, and the depth within the cartilage independent of disease.

Nuclear Magnetic Resonance

One of the diagnostic tools used in the diagnosis of degenerative cartilage diseases is nuclear magnetic resonance (NMR) (Kaye 1990, Resnic and Niwayama 1988). The diagnostic usefulness of magnetic resonance imaging (MRI) has been demonstrated in many studies (Recht 1993, McCauley 1992, McAlindon 1991, Modl 1991, Kaye 1990, Verbruggen 1990, König 1987, Sabiston 1987). MRI provides greater soft tissue contrast than conventional radiography (Kaye 1990). Clinically images of cartilage are graded for the presence of osteoarthritis and the severity of disease using a visual inspection of intensity (Recht 1993, Modl 1991). The changes that occur due to arthritis in cartilage are varied, and many of those changes may affect NMR measurements which result in changes in magnetic resonance images. One of the pathological changes is the increase in fractional water content that is believed to be an indicator of both the presence of osteoarthritic disease and the severity of disease. This thesis specifically examines the effect of specifically changing fractional water content without altering solid content on NMR measurements of diffusion, magnetization transfer, and T2 relaxation. These three NMR parameters were

selected for their clinical relevance. Diffusion and MT are becoming more widely used in the clinical setting, compared to T2 which is already commonly measured.

An NMR technique is able to infer diffusion coefficients from the measurements of the brownian motion of molecules (Stejksal and Tanner 1965). The process of diffusion in a hydrated matrix is affected by the ratio of water to matrix volume . In cartilage, the matrix provides impediments to the motion of the solutes which reduces the average distance moved by the solutes in a given time interval. The reduction, which can be characterized by a decrease in the effective diffusivity, can be largely accounted for by the reduction in area available to the solutes and the increased tortuosity of the diffusion path for the solutes (Maroudas 1976). An increase in the ratio of water to matrix would result in a decrease in the effect of the obstacles thereby increasing the effective diffusivity of the solutes. This relationship has been seen experimentally. Maroudas and Venn (1977) measured the diffusivity of tritiated water in normal and fibrillated cartilage. A 12% increase in diffusivity was seen in the fibrillated cartilage (77% fractional water content) when compared to normal cartilage (71% fractional water content). NMR diffusion measurements of free swelling and compressed bovine cartilage have also been made (Burstein 1993). A single compression level of approximately 35% of the original thickness was used. The NMR measured diffusion coefficient in the compressed sample was lower than the free swelling samples.

Another NMR experiment that is now being used for imaging of cartilage is the magnetization transfer (MT) experiment. Using MT, improved contrast can be generated between cartilage and synovial fluid (Wolff 1991). One of the factors that affects the MT measurement (expressed as a ratio Ms/Mo) is the concentration of macromolecules in the sample, with increasing concentration resulting in a decrease in Ms/Mo. Thus, a decrease in fractional water content should result in a decrease in Ms/Mo (Sepponen 1992). The dependence of the MT measurement on fractional water content of cartilage has been

suggested experimentally (Gray 1994, Lesperance 1993). Both Gray and Lesperance made MT measurements of collagen suspensions varying in concentration from approximately 1 - 30 g/100 ml. As the concentration increased (fractional water content decreased), Ms/Mo decreased. Lesperance also made MT measurements of trypsin digested cartilage under a range of compression levels. The trypsin was used to remove the GAG content from the cartilage. As the cartilage was compressed, decreasing fractional water content, Ms/Mo measured as a function of estimated collagen content of the sample decreased slightly.

NMR measured diffusion and MT are relatively new experiments in the clinical domain. A more commonly used parameter in clinical imaging is the T2 relaxation time. Fractional water content in tissues greatly affects T2 relaxation times. Protons that are associated with macromolecules have shorter relaxation times than protons in the bulk water. The measured T2 relaxation time is a weighted average of the different groups of protons. Therefore, an increase in fractional water content would result in an increase in the T2 relaxation time measurements (Fullerton 1992).

This relationship between relaxation times and fractional water content has been demonstrated in many tissues, including cartilage. Decreases in T2 times have been correlated to decreases in fractional water content in tissues such as muscle and tendon (Scholz), intervertebral disc (Weidenbaum), uterus (McCarthy 1989) and cerebral white matter (Sappey-Marinier 1990). Lehner (1989) produced magnetic resonance images of bovine articular cartilage that displayed two different layers. The layer with the longer T2 time corresponded to the superficial layer with a fractional water content of 82%. The deeper layer with the shorter T2 time had a fractional water content of 76%. Both fractional water contents were computed by measuring wet weights and dry weights of cartilage slices from different depths. These data demonstrate a rough relationship between decreasing fractional water content and a measurable decrease in T2 times.

Purpose

Fractional water content has been shown to vary between diseased cartilage and normal cartilage, and as a function of depth in both diseased and normal cartilage. Based on the previous NMR experimental data, changes in fractional water content have been associated with changes in NMR measurements of diffusion, magnetization transfer, and T2 relaxation. However, those measurements are limited in several ways: (1) the diffusion and T2 measurements were limited to a few values of fractional water content, (2) the T2 measurement reflects tissue which may have varied in solid content in addition to fractional water content, and (3) the magnetization transfer measurements as a function of compression were made on degraded cartilage not normal cartilage, which adds the effect of degradation to the effect of varying fractional water content on the measurement. The purpose of this thesis is to determine the functional relationship between fractional water content and NMR measurements of diffusion, magnetization transfer, and T2 for constant solid composition. (For a more complete description of the NMR experiments, refer to the **NMR Theory** and **NMR Experiments** sections.) These results will allow the further examination of the effect of other matrix constituents on the NMR measurements.

The specific control of fractional water content is achieved through compression of the sample. An assumption made with compression is that the solid content does not change with compression. Any changes in weight in a compressed sample are due to loss of water, which result in a decrease in fractional water content. Absolute water content will monitored in two ways : (1) using the one pulse NMR experiment and (2) using wet weight and dry weight measurements.

Preliminary Sensitivity Comparison of NMR Measured Diffusion, MT, and T2

The purpose of this section is to provide some preliminary estimates on the magnitude of change that might be measured by the various NMR experiments for a given change in fractional water content. For computation purposes, a change in fractional water content from 70% to 77% (10% increase in fractional water content) was chosen. Those values are representative of the change in fractional water content seen pathologically in osteoarthritis. An initial solid cartilage content density of 1.4 g/cc is also assumed (Lipshitz 1976). All predicted values are summarized in Table 2 at this end of the section.

Diffusion

In order to estimate the sensitivity of diffusivity changes to changes in fractional water content several theoretical models were considered. In particular, three analytical models for diffusion are commonly compared to experimental measurements of diffusivity in cartilage - the phenomenological and stochastic Ogston models (1973) and the Mackie and Meares model (1955). All three models assume that obstacles cause distortions to the diffusion path and thereby increase diffusivity. Previous non-NMR measurements of diffusion coefficients of small solutes in chondroitin sulfate solutions (Maroudas 1988) found that for some solutes (proline, Na^+) the stochastic Ogston model fits the data well. Also, from a measurement of the tritiated water diffusion coefficient in uncompressed femoral head cartilage, Maroudas (1977) concluded that the Mackie and Meares model also described the measured diffusion coefficient well. Thus, these models are assumed to provide some indication of the sensitivity of diffusion to fractional water content. They have not, however, been rigorously tested for cartilage.

Ogston (1973) developed a theoretical explanation for an empirical equation of Laurent (1963) that described diffusion of various globular particles in hyaluronic acid (HA) solutions as a function of the solute radius and concentration of hyaluronic acid. Laurent's empirical relation was:

$$\frac{D}{D_o} = Ae^{-kr\sqrt{l}}$$

D_o = the diffusion coefficient of the solute in free solution (no polymer)

A = constant greater than 1 and usually less than 2

k = dimensionless constant for HA determined experimentally to be 1.4

l = length of HA chain/volume

r = radius of solute

Ogston began with two different theoretical premises and developed two different models - the phenomenological model and the stochastic model. The phenomenological model treats diffusion as the continuous solute movement that results from a potential gradient. The stochastic model assumes diffusive motion occurs as a series of small unit steps.

In the phenomenological model, obstacles will increase the diffusion path. This results in a change in diffusivity as:

$$\frac{D}{D_o} = \frac{1}{(1+\alpha\phi)^2}$$

D_o = the diffusion coefficient of the solute in free solution (no polymer)

α = coefficient depending on the geometry of the obstacles

ϕ = solid volume fraction

The form of this model did not match the form devised by Laurent, leading to the development of a second model based on a different theoretical premise. The

phenomenological model has been included here for comparison because of its similarity to the Mackie and Meares model.

The stochastic model makes an assumption that the unit step motion either occurs or does not occur. There is no intermediate step length. Therefore only a fraction of steps are successful. To develop this model, Ogston began with the probability distribution for the distance a solute would move (x) before the first collision with obstacles:

$$g(x) = \tfrac{1}{2}\pi l r e^{-\frac{1}{2}\pi l r x}$$

The probability of successful completion of the unit step (λ) is the probability that the distance moved before the first collision is greater than λ. To compute the probability of success (P), g(x) can be integrated with respect to x from λ to ∞, which results in:

$$P = e^{-\frac{1}{2}\pi l r \lambda}$$

Assuming a step length of $2\sqrt{\pi l}$:

$$\frac{D}{D_o} = e^{-r\sqrt{\pi l}}$$

Note that the form of this model matches well with the empirical form of Laurent. In the stochastic model k = 1.77 ($\sqrt{\pi}$), which is reasonably close to Laurent's 1.4.

The stochastic form does not take into account the actual thickness of the obstacles. To include the thickness, the r would be replaced by (r + a) where a is the radius of the obstacle. Ogston also assumed that the volume fraction of the obstacles is :

$$\phi = \pi a^2 l$$

ϕ = solid volume fraction

a = radius of obstacles

l = length of obstacle/volume

The stochastic Ogston model can now be rewritten as :

$$\frac{D}{D_o} = e^{-\frac{a+r}{a}\sqrt{\phi}}$$

Another model of diffusion was developed by Mackie and Meares (1955). They postulated that obstructions would cause an increase in path length of θ, which would modify mobility and diffusivity as $\frac{1}{\theta^2}$. Again let ϕ represent the solid volume fraction. The obstructions occupy ϕ sites, leaving $(1-\phi)$ sites available for diffusion. The diffusing solute either moves forward or encounters an obstruction and moves laterally. At that new position the probability of a site being available for diffusion is

$$\tfrac{1}{2}(1+\phi)$$

Therefore the fraction of solutes that require another extra jump is

$$\phi\left(\tfrac{1}{2}(1+\phi)\right)$$

The fraction that would require n extra jumps is

$$\phi\left(\tfrac{1}{2}(1+\phi)\right)^n$$

The increase in path length can now be expressed as :

$$\theta = 1+\phi\sum_{n=0}^{\infty}\left(\tfrac{1}{2}(1+\phi)\right)^n$$

Since $\frac{1}{2}(1+\phi) < 1$, the expression for θ converges to :

$$\theta = \frac{1+\phi}{1-\phi}$$

For a given time, a solute in a system of obstructions will move $\frac{1}{\theta}$ as far as a solute in free solution. Since the diffusion coefficient (D) is proportional to x^2 (Crank 1975), the Mackie and Meares model as a function of solid volume fraction is :

$$\frac{D}{D_o} = \frac{(1-\phi)^2}{(1+\phi)^2}$$

D_o = the diffusion coefficient of the solute in free solution (no polymer)

ϕ = solid volume fraction

These three particular models were derived for polymer systems characterized by the solid volume fraction of the polymer. To relate the solid volume fraction of cartilage to the fractional water content, a solid content density of 1.4 g/cc was assumed (Lipshitz 1976). Thus a change in fractional water content from 70% to 77% represents a change in solid volume fraction from 23% to 18%. Also, the diffusion coefficient of water at 25°C (2.3×10^{-5} cm^2/s, CRC 1980-81) is the value used for D_o. For the phenomenological Ogston model α was computed to be 2.4 using data from NMR diffusion measurements on bovine cartilage (Burstein 1993). (For a diffusing time of 25 ms, D/D_o was measured to be 0.60, and ϕ was computed to be 0.12 from a hydration of 84% and a solid density of 1.4 g/cc, implying α = 2.4.) The radius of the GAG matrix molecule (a in the stochastic Ogston model) was assumed to be 0.5 nm (Maroudas 1988), and the radius of the diffusing solute, water, was computed from the Stokes-Einstein relation (Bird 1960) to be 0.1 nm.

Using the three models, diffusion estimates can be made for the given change in fractional water content. For a decrease in solid volume fraction from 23% to 18%, the phenomenological Ogston model predicts a change in the diffusion coefficient from 0.94 x 10^{-5} cm^2/s to 1.14 x 10^{-5} cm^2/s, and the stochastic Ogston model predicts a change from 1.29 x 10^{-5} cm^2/s to 1.39 x 10^{-5} cm^2/s. For the same change in solid volume fraction as above, the Mackie and Meares model predicts an increase in the diffusion coefficient from 0.88 x 10^{-5} cm^2/s to 1.13 x 10^{-5} cm^2/s. These predicted sensitivities for NMR measured diffusion especially should be taken only as a preliminary estimates. It is not clear which diffusion model, if any, will predict actual experimental NMR measurements of diffusion.

In addition to the theoretical models, there exists some experimental NMR measurements of diffusion in bovine cartilage that allows estimation of the change in diffusivity for a given change in fractional water content. Burstein (1993) measured the diffusivity of water in free swelling calf articular cartilage and cartilage compressed by approximately 35% of the free swelling height. Using a diffusing time of 25 ms, the diffusivity of the free swelling cartilage measured 1.43 x 10^{-5} cm^2/s, and the diffusivity of the compressed cartilage measured 1.10 x 10^{-5} cm^2/s. Using a linear extrapolation, the predicted diffusivity at fractional water contents of 70% and 77% are 0.66 x 10^{-5} cm^2/s and 1.05 x 10^{-5} cm^2/s respectively.

Magnetization Transfer

There is no obvious theoretical approach to predicting the change in magnetization transfer due to a change in fractional water content. Relevant experimental data do, however, exist. Lesperance (1993) measured MT as a function of % collagen content (grams of collagen / 100 ml of tissue water) in bovine cartilage that was digested with trypsin. Trypsin was used to remove the GAG content, leaving only the collagen content of the

extracellular matrix. The % collagen content was determined by measuring the dry weight of the sample, and assuming that all of the dry weight represented collagen. The % collagen content was altered by compressing the sample. Based on Lesperance's data, it appears that for fractional water contents of 70% and 77% (corresponding approximately to % collagen contents of 43% and 30% respectively), the measured MT was approximately 0.18 and 0.20 respectively.

T2

Similar to MT, the predicted changes for T2 are based, not on analytical models, but on experimental measurements. Lehner (1989) measured the T2 relaxation time at 0.5 T and fractional water content in bovine articular cartilage. Within a single sample, two zones of different T2 times and fractional water contents were found. The superficial zone had a higher fractional water content of 82% and a T2 time of 77 ms. The deep zone had a lower fractional water content of 76% and a T2 time of 51 ms. From these data, the T2 times at 70% and 77% fractional water content were estimated by fitting a line to 1/T2, resulting in T1 times of 38 ms and 54 ms respectively. This particular method of extrapolation was chosen based on data from Weidenbaum (1992) that found a linear relationship between 1/T2 and fractional water content.

Summary

A summary of the predicted sensitivity to pathological changes in fractional water content for the NMR measured diffusion, MT, and T2 is shown below in Table 2.

NMR Experiment	Fraction Water Content - 70%	Fraction Water Content - 77%	% Increase
Phenomenological Ogston Diffusion Model	0.94 x $10^{-5}cm^2/s$	1.14 x $10^{-5}cm^2/s$	21
Stochastic Ogston Diffusion Model	1.29 x $10^{-5}cm^2/s$	1.39 x $10^{-5}cm^2/s$	8
Mackie and Meares Diffusion Model	0.88 x $10^{-5}cm^2/s$	1.13 x $10^{-5}cm^2/s$	28
Diffusion (Burstein)	0.66 x $10^{-5}cm^2/s$	1.05 x $10^{-5}cm^2/s$	59
MT (Lesperance)	0.18	0.20	11
T2 (Lehner)	38 ms	54 ms	42

Table 2: NMR Experimental Value at Fraction Water Contents of 70% and 77% and the % Increase in the measurement from 70% to 77%. The fractional water contents were chosen to approximately represent normal (70%) cartilage and osteoarthritic (77%) cartilage. Assuming a density of the solid portion of cartilage of 1.4 g/cc, 70% and 77% fractional water contents correspond to 23% and 18% solid volume fractions respectively. The first three diffusion predictions are from analytical models, not actual experimental measurements. The last diffusion prediction is based on a linear extrapolation of measurements of Burstein (1993) at two different fractional water contents. The MT values were taken from Lesperance (1993). The T2 values were taken from Lehner (1989). The Lehner values are extrapolations from measurements at only two values of fractional water content.

For the difference in fractional water content seen between normal and osteoarthritic cartilage, the MT experiment shows the smallest change, suggesting that it will probably be the least affected by changes in fractional water content. The experimental diffusion measurements (Burstein 1993), and the T2 measurements (Lehner 1989) appear to have the largest changes for the given change in fractional water content, although these three predictions were based on extrapolations from a limited number of measurements.

NMR Theory

These descriptions of basic NMR theory and the one pulse experiment are taken from Experimental Pulse NMR: A Nuts and Bolts Approach, Fukushima and Roeder (1981).

Nuclear magnetic resonance takes advantage of the fact that nuclei with an odd number of protons or neutrons possess spin and charge, and therefore have a magnetic moment. When a sample is placed within a magnetic field B_0, the spins orient either parallel, with the direction of B_0, or antiparallel, against the direction of B_0. B_0 is assigned to be on the +z axis. The lower energy state is the parallel orientation so slightly more spins are oriented parallel. This difference in the number of spins aligned parallel and antiparallel gives rise to a net magnetization vector.

The magnetic moments are not stationary. Instead, they precess around the magnetic field. The frequency of precession is uniquely determined by the gyromagnetic ratio γ and the field B_0. This frequency, called the Larmor frequency or resonant frequency, can be expressed as:

$$\omega_0 = \gamma B_0$$

Although, the spins are all precessing at the same frequency, the phases are randomized in the x-y plane, so there is not net magnetization in the x-y plane, and the net magnetization vector is in the +z direction.

In a one pulse experiment, a magnetic field rotating at the Larmor frequency, which is in the radio frequency range, is applied in the plane (x-y plane) perpendicular to B_0 (+z axis). The field is applied for a short period of time, and this is called an "rf pulse". The rf pulse causes the moments to align with the rf pulse, similar to the parallel and antiparallel alignment due to B_0, and a phase coherence in the x-y plane is introduced. The net

magnetization vector is now a sum of the +z component and a component in the x-y plane, and still rotates at the Larmor frequency. By varying either the amplitude of the pulse or the duration of the pulse, the net magnetization vector can be rotated off the +z axis by any amount. An rf pulse that places the net magnetization vector in the x-y plane is referred to as a 90^{o} pulse. The rf pulse required to place the net magnetization vector along the -z axis is a 180^{o} pulse.

After excitation by the rf pulse, the net magnetization vector decays in the x-y plane and also simultaneously returns back toward the steady state +z axis orientation. The decay in the x-y plane, called T2 or spin-spin relaxation, is due to the dephasing of the spins in the x-y plane. The net magnetization vector simultaneously returns towards equilibrium on the +z axis as the spins exchange thermal energy with the molecular framework, or lattice, and is called T1 or spin-lattice relaxation.

The decaying net magnetization vector will generate a current in a receiver coil with its symmetry axis in the x-y plane. The signal induced (Figure 3) is called the free induction decay (FID).

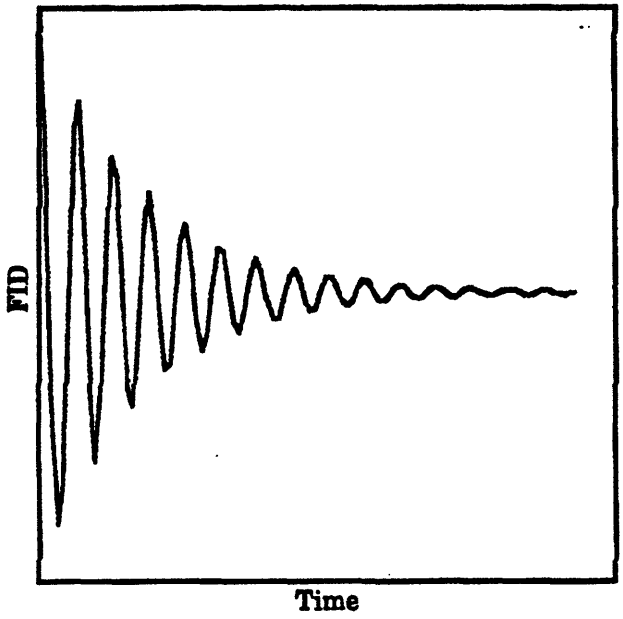

Figure 3: Example Free Induction Decay Signal versus Time

The initial value of the signal is proportional to the number of nuclei in the sample. To determine the initial value in time, the Fourier transform of the FID (Figure 4) is integrated from $-\infty$ to $+\infty$.

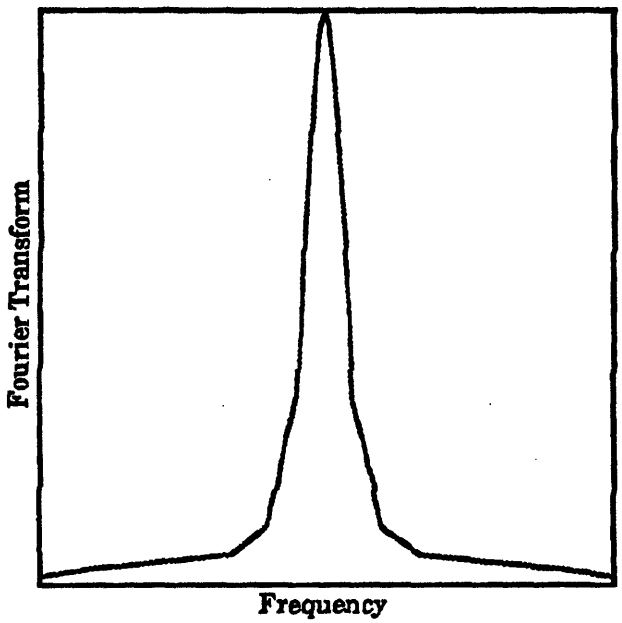

Figure 4: Fourier Transform of the FID shown in Figure 3. The area under the curve is proportional to the number of nuclei in the sample.

For quantitative measurements, the area under the curve is then calibrated to the area measured from a standard.

NMR Experiments

One Pulse

Proton content is measured using a one pulse proton experiment:

90^{o} - acquire

The 90^{o} pulse flips the net magnetization vector into the x-y plane. The net magnetization vector is proportional to the number of proton spins. To obtain quantitative information, the one pulse experiment is performed on a standard of known quantity, such as a known volume of water.

Diffusion

Proton diffusivity is measured using a stimulated echo diffusion experiment (Stejskal and Tanner 1965):

90^{o} - gradient - 90^{o} - crusher - 90^{o} - gradient - acquire

A vector diagram describing the stimulated echo diffusion experiment is shown below in Figure 5 (the vector diagram is from Callaghan 1991):

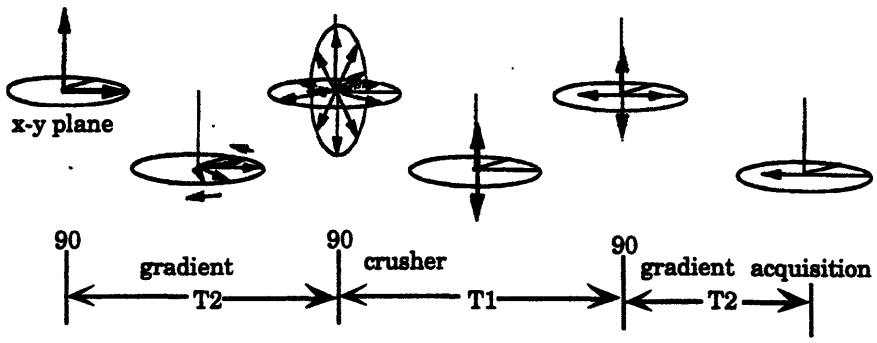

Figure 5: Vector diagram for the stimulated echo pulse sequence (from Callaghan 1991). Below the diagram is the pulse sequence and the decay rates for the various intervals.

The first 90° pulse flips the net magnetization vector into the x-y plane. Following the first 90° pulse, the first magnetic field gradient, which varies linearly with position, alters the magnetic field seen by each spin based on its spatial location. This change in magnetic field changes the frequency of each spin ($\omega = \gamma B$), again based on its location. After some time during which the magnetic field gradient is on, the spins will be out of phase with each other depending on their position. Thus the first magnetic field gradient "phase" encodes the proton spins according to location. Between the first and second 90° pulses, the net magnetization vector decays at a rate of T2.

Assuming the first 90° pulse is in the x direction, the second 90° pulse, also in the x direction, flips only the y-component of the net magnetization vector; the x-component is unaffected. The crusher gradient dephases the magnetization components that remain in the x-y plane. During the interval between the second and third 90° pulses, the magnetization in the z axis grows at a rate of T1. The remaining z axis magnetization is flipped into the x-y plane by the third 90° pulse. In this final interval between the third 90° pulse and the signal acquisition, the decay rate is again T2.

The second magnetic field gradient, which occurs between the third 90° pulse and the acquisition, also "phase" encodes by location, but the phase is opposite of the first gradient. If the protons have not moved, the acquired signal will be the same as if there were no gradients. However, if the protons have moved in the time between the two gradients, the second magnetic field gradient does not exactly cancel out the phase introduced by the first magnetic field gradient. Since each proton which has moved acquires a phase depending on how far it has moved, a phase incoherence in the sample will be introduced, and the acquired signal will decrease in comparison to the case where the protons do not move. Given that the phase encoding gradients are of duration δ and the

time between gradients is Δ, and assuming free Brownian motion, the ratio (R) of the signal acquired with gradients to that without can be written as

$$\ln (R) = -\gamma^2 g^2 \delta^2 (\Delta - \delta/3) D$$

where g is the gradient strength, γ is the gyromagnetic ratio, and D is the diffusion coefficient (Stejskal and Tanner 1965).

The advantage of the stimulated echo pulse sequence over a spin echo (90^o -180^o) pulse sequence is the T1 rate of decay between the second and third 90^o pulses in the stimulated echo pulse sequence. For most biological systems, T1 is on the order of one second, and T2 is in the tens of milliseconds. The stimulated echo diffusion experiment allows the measurement of diffusion over time intervals (Δ) that are much greater than T2.

An experimental parameter that can be varied in the NMR diffusion experiment is the time over which diffusion is measured (Δ). In a system with no obstacles, such as free solution, the measured diffusion coefficient is independent of Δ. However, if the diffusion system has obstacles to diffusion, then the effective diffusion coefficient measured by NMR varies with Δ (Chang 1975). The relationship between D and Δ developed by Chang is shown below in Figure 6.

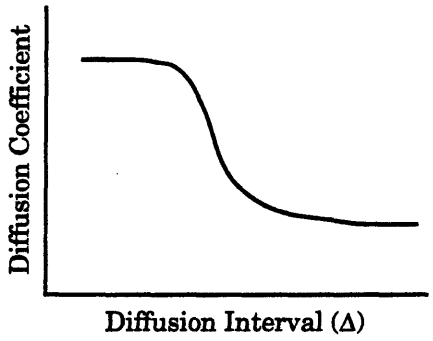

Figure 6: Chang (1975). NMR measured Diffusion Coefficient versus Diffusion Measurement Interval (Δ) in the presence of permeable obstructions to the diffusing solute. Relationship between D and Δ was developed analytically by Chang.

At very short measurement intervals, the majority of the diffusing solutes do not encounter the obstructions so that the NMR measurement of diffusivity is at a maximum. As the diffusion interval increases, more and more of the solute encounters the obstacles, decreasing the net displacement of the solutes. Movement of spins in the NMR diffusion experiment results in a decrease in the signal acquired, with greater distances moved corresponding to a larger decrease in signal and a larger NMR measured diffusion coefficient. The decrease in displacement caused by encounters with obstacles is reflected in the NMR diffusion experiment as a decrease in diffusivity. Eventually, a region at longer Δ is reached where the measured diffusion coefficient is independent of the measurement interval. At the longer Δ, the solutes have effectively encountered so many of the obstructions that the system appears homogeneous to the diffusing solute. In that interval, an increase in measurement interval (Δ) allows the diffusing solutes to move a mean distance proportional to the square root of Δ, which is equivalent to the measured diffusion coefficient being constant.

Magnetization Transfer (MT)

The magnetization transfer experiment examines the transfer of magnetization from one saturated species of protons to another unsaturated species (Forsen and Hoffman 1963, 1964). Saturation of a species is defined as a zero net magnetization vector due to equal parallel and antiparallel spins and no phase coherence. In cartilage, it is convenient to consider two species of protons. One species consists of protons associated with the large macromolecules of the solid matrix, which have restricted motion. The second species is the remaining protons in the bulk water, which are relatively unrestricted. It is possible to saturate the restricted protons without affecting the bulk water protons due to the different linewidths of the two pools. The bulk water protons have a slow T2 relaxation rate, on the order of hundreds of milliseconds, that gives a narrow (10-20 Hz) NMR linewidth, compared to the faster relaxation of the restricted protons, with a 20-40 kHz linewidth (Figure 7). The restricted protons are saturated with an rf pulse that is several kHz off the resonant frequency.

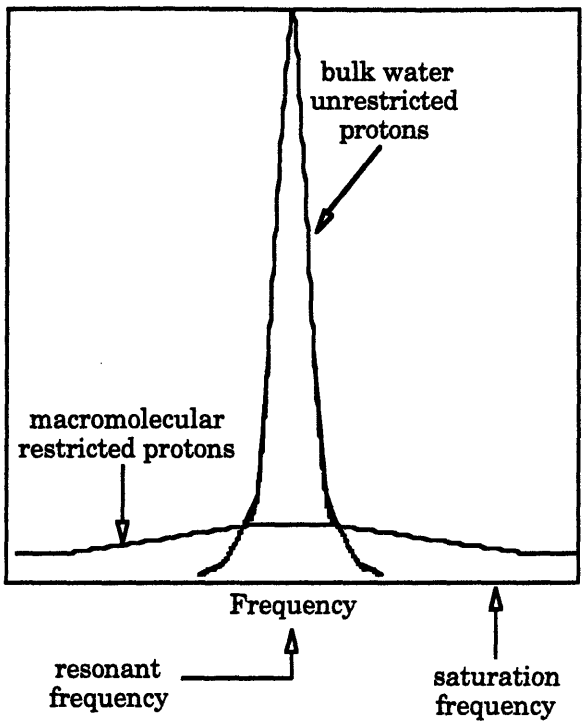

Figure 7: Frequency spectra for two proton pools in cartilage. The unrestricted bulk proton pool has a linewidth of 10-20 Hz. The restricted proton pool associated with the macromolecules has a much broader linewidth of 20-40 kHz. The frequency of saturation excitation used for the MT experiment is shown. The saturation frequency is usually offset from the resonant frequency by several kHz.

Magnetization transfer is expressed as the ratio of Ms/Mo. Ms is the bulk proton signal after saturation of the macromolecular protons. Mo is the bulk proton signal without saturation of the macromolecular protons. The MT measurement is made in two steps. First, Mo is measured on resonance using a one pulse proton experiment:

90^{o} - acquire

The 90^{o} pulse flips the net magnetization vector into the x-y plane. Mo is a measurement of the amount of water in the sample.

Ms is measured using a saturation experiment:

$$P_{sat} - 90^{o} - \text{acquire}$$

P_{sat} is defined as a square pulse, 6 kHz off resonance, with a duration of 12 seconds. The power of P_{sat} is set at the level of a 1 ms 180° pulse. The parameters of P_{sat} were characterized by Lesperance (1993). The offset frequency was chosen in a "plateau" region of the MT versus offset frequency graph where the MT effect decreased only slightly with increasing offset frequency. The saturation power was chosen to equal 12 μT to minimize tissue heating and facilitate comparison with data from other investigators. P_{sat} saturates the macromolecular protons. During P_{sat}, transfer of the magnetization occurs between the bulk protons and the macromolecular protons, resulting in a decrease in the bulk proton signal, which is measured by the 90° pulse.

T2 Relaxation

T2 relaxation, also referred to as spin-spin relaxation, is the decay of the net magnetization vector in the x-y plane. The relaxation occurs due to dephasing of the individual moments. Proton T2 relaxation is measured using a Hahn (1950) spin-echo experiment:

$$90^{o} - \tau/2 - 180^{o} - \tau/2 - \text{acquire}$$

τ is defined as the echo time. The 90° pulse flips the net magnetization vector into the x-y plane. The spins then lose phase coherence for a duration of $\tau/2$ due to magnetic field inhomogeneities and T2 relaxation mechanisms. The 180° pulse refocuses the spins after another delay of $\tau/2$ and removes any field inhomogeneity effects. Any decay in the acquired signal is now due to T2 relaxation for a duration of τ.

Methods

Cartilage Preparation

Calf articular cartilage was harvested from the femoropatellar groove according to previously established techniques to yield plane-parallel plugs 2 mm thick and 5 mm in diameter (Sah 1989). Cartilage samples were stored at -20°C until needed. The plugs were allowed to equilibrate in Hank's Balanced Salt Solution (HBSS, Gibco) at room temperature for one hour prior to the start of the experiment. The main constituents of HBSS are 138 mM NaCl, 5 mM KCl, and 4 mM $NaHCO_3$.

Fractional Water Content (%)

Fractional water content (FWC) was defined as:

$$\frac{\text{water content}}{\text{wet weight}} * 100\%$$

(as described below, water content was determined either by NMR or by subtracting dry weight from wet weight)

Wet weight was measured for each compression level. After the completion of the experiment, the sample was lyophilized, and the dry weight was measured. Two methods were used to measure water content: (1) the dry weight was subtracted from the wet weight, and (2) the NMR one pulse experiment. In **Results**, all NMR measurements are displayed as a function of fractional water content calculated using the first method of measuring water content.

The method chosen to control fractional water content was compression. Compression was used to specifically change fractional water content without altering the

solid content. The loss of water from the sample resulted in a decrease in fractional water content.

A custom made NMR compression chamber was designed and constructed by Steve Lin (Figure 8). The chamber was made out of Teflon so that the chamber would be NMR transparent. Early chambers were made from polysulfone, but it was determined that the polysulfone absorbed a non-negligible amount of water, giving rise to a NMR signal. The compressed tissue thickness was controlled by a pair of Teflon shims placed between the piston and the bottom surface of the upper portion of the chamber, and ranged from 200 μm to 1800 μm.

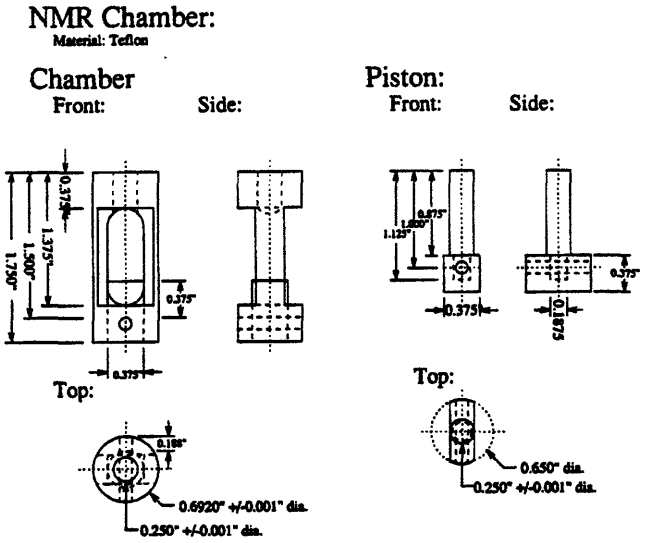

Figure 8: Schematic of the custom made Teflon NMR compression device. Teflon was chosen for its NMR transparency. Compression thickness is controlled by Teflon shims placed between the piston and the upper portion of the chamber. Compression thicknesses range from 1800 μm to 200 μm. Constructed by Stephen Lin.

It should be noted that the parameter used for analysis of the NMR parameters was fractional water content, and not the shim defined compression thickness. From visual

inspection of the plug during compression, radial expansion was obviously occurring. (For a more complete discussion of the issue of radial expansion refer to **Appendix 2**.) Also, at smaller compression thicknesses, the compression surfaces were no longer parallel to one another. Therefore, the compressed thicknesses determined by the shims may not have been exact and could not be accurately used as a measurement of sample volume or water content.

Wet Weight Measurements in Chamber

During the NMR experiments the wet weight of the cartilage sample was needed to determine the fractional water content. The sample was placed within the compression chamber, and the sample and chamber together were placed in a bath of HBSS for equilibration. Prior to performing the NMR experiments, any excess water was removed from the chamber and the exposed portions of the sample, and the sample and chamber together were weighed. The wet weight was calculated from this measurement by subtracting the weight of the chamber, which was measured alone earlier in the day.

A control study was conducted to establish the efficacy of the wet weight measurement method. Initially, the chamber alone was weighed. At each compression level, the sample was compressed to the desired height and then allowed to equilibrate in HBSS for 30 minutes. The entire chamber, including shims and cartilage sample, was then dried and weighed. The plug was then removed and weighed alone. Finally, the shims were weighed and a wet weight for the sample was computed.

NMR Measurement Repeatability

The repeatability in the NMR experiments was determined experimentally. A single cartilage plug was compressed to 1400 μm and allowed to equilibrate in HBSS (~ 1 hour). Diffusion, at both Δ's, MT, and T2 were measured. (Descriptions of the NMR experiments follow.) The plug, still at 1400 μm compression, was again placed in HBSS for 30 minutes to allow equilibration, and then the NMR measurements were repeated. A total of five repetitions were made with 30 minutes equilibrations.

NMR Experimental Protocols

All NMR experiments were performed on an 8.45 T Bruker AM Spectrometer (Bruker Instruments, Inc., Billerica MA) at a frequency of 360 MHz for proton.

NMR Measured Fractional Water Content

Each day, the one pulse experiment was calibrated to a standard of 50 μl of water. To improve signal to noise, the one pulse experiment was repeated 8 times and summed, with a delay of 12 seconds between repetitions (TR = 12 s). The TR was chosen to be greater than 5 T1 (T1 = approximately 2 s) to allow for sufficient regrowth of the net magnetization vector. The NMR measurement of water content was compared to absolute water content computed from wet weight and dry weight measurements. Also, the NMR measurement of water content was divided by wet weight to determine an NMR computed fractional water content.

Diffusion

Each day before measuring cartilage samples the diffusion experiment was performed on a standard of a small glass sphere filled with water. A source of error in measuring diffusion occurs due to the geometry of the standard. An irregular geometry, such as the meniscus formed in a 5 mm tube, causes larger inhomogeneities in the magnetic field than a sample of regular geometry. A sphere minimizes the inhomogeneities in the magnetic fields. Inhomogeneities cause additional dephasing of the protons, which is interpreted in the diffusion experiment as higher diffusivities.

The diffusion experiment consisted of two experiments. Two different times between gradients (Δ), 0.025 seconds and 0.500 seconds, were used. The duration of the gradients (δ) was 0.007 seconds for both Δ times. To determine D, the experiment was repeated for 9 values of gradient strength (g) and fit to the equation determined by Stejskal and Tanner (1965):

$$\ln (R) = -\gamma^2 g^2 \delta^2 (\Delta - \delta/3) D$$

At Δ = 0.025 seconds, the g was incremented from 0 G/cm to 13 G/cm in steps of 1.625 G/cm. At Δ = 0.500 seconds, g was incremented from 0 G/cm to 3.25 G/cm in steps of 0.4063 G/cm for the diffusion standard and from 0 G/cm to 1.64 G/cm in steps of 0.2050 G/cm for cartilage samples. To increase signal to noise at each g value, the diffusion experiment was repeated and summed 4 times with a delay between repetitions of 1 second (TR = 1 s). The delay between each value of g was 10 s.

The diffusion measurements were normalized to a temperature of 298°K by the Stokes-Einstein relationship (Bird 1960).

$$D_{298} = \frac{298^\circ K}{T} \frac{\mu_T}{\mu_{298}} D_T$$

D_{298} = diffusion coefficient normalized to 298°K

T = temperature at the time of measurement in Kelvin

μ_{298} = viscosity of water at 298°K (0.8904 centipoise (CRC 1980))

μ_T = viscosity of water at T (from CRC 1980)

D_T = diffusion coefficient measured at T

MT

To improve signal to noise Mo was measured using a one pulse experiment repeated and summed 8 times with a delay between repetitions of 12 seconds (TR = 12 s). Ms was measured using a P_{sat} 6 kHz off resonance with a duration of 12 seconds. The power of P_{sat} was set at the power level of a 1 ms 180° pulse. To improve signal to noise the Ms measurement was repeated and summed 8 times with a delay between repetitions of 12 seconds (TR = 12 s). MT was expressed as the nondimensional Ms/Mo.

The absolute Ms/Mo measurement was very sensitive to the value chosen for P_{sat} (Lesperance 1993). To remove changes due to P_{sat} the data for each day was normalized to the value measured for the unconfined sample on that day.

T2

The T2 experiment was performed at 8 values of τ. The τ values in milliseconds were 4, 8, 14, 20, 30, 40, 50, and 60. To improve signal to noise at each τ, the experiment was repeated and summed 4 times with a delay between repetitions of 12 seconds (TR = 12 s). The data were fit to the equation:

$$I(\tau) = I_o e^{-\frac{\tau}{T2}}$$

to determine the T2 relaxation time constant (Hahn 1950).

Cartilage Samples

An equilibrated cartilage plug (5 mm diameter, 2 mm thick) was placed in the NMR compression chamber (Figure 8) under no compression. This was referred to as the "unconfined" sample. Surface water was removed from the chamber and plug as thoroughly as possible and weighed before running the NMR experiments. The four NMR experiments are explained previously. After completion of the experiments, the chamber and plug were placed in HBSS and the cartilage was compressed. The sample was allowed to equilibrate for 30 minutes. The chamber and plug were again dried and weighed, and the experiments were repeated. The compression - equilibration - NMR experiments cycle was repeated at successive levels of compression with compression increased in ~200 μm steps. After all compression levels were measured, the plug was frozen to be lyophilized later to determine dry weight.

Results

Wet Weight Measurements in Chamber

During the NMR experiments the cartilage sample had to remain within the compression chamber. Wet weights of the cartilage samples were indirectly computed by measuring the combined weight of the chamber and sample and then subtracting out the weight of the chamber (previously weighed). This method was referred to as the "computed weight." To verify this approach the computed weight was compared to direct measurements (referred to as "measured weight") of the plug alone, shown below in Figure 9. The difference between computed weight and measured weight was always less than 2 milligrams, indicating that the wet weight measurements of the plug in the chamber was a reasonable substitute for direct weighing of the plug.

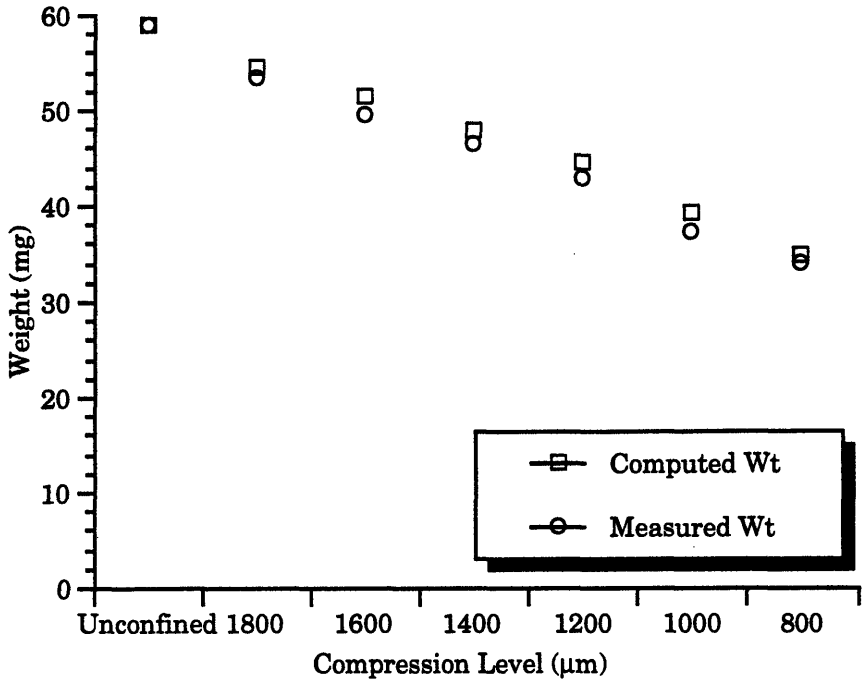

Figure 9: Verification of Wet Weight measurement technique used to measure wet weight of cartilage sample while still loaded in compression chamber (n=1). Computed weight is total weight of chamber, shims and plug minus weight of chamber and shims. Measured weight is wet weight of plug. The compression level is the approximate confined height of the cartilage sample .

NMR Measurement Repeatability

Shown below in Table 3 are the mean, standard deviation, and the coefficient of variance (COV) of the measurements of the wet weight, fractional water content, and the NMR experiments. The COV is defined as the standard deviation divided by the mean and is expressed as a percentage. All measurements were performed on a single plug and repeated 5 times. Between each repetition of the measurements, the plug was allowed to equilibrate for 30 minutes in HBSS.

	Mean	Standard Deviation	COV (%)
Wet Weight (mg)	47.5	0.4	0.9
Fractional Water Content (%)	74.9	0.2	0.3
NMR Water Content (μl)	31.7	0.9	2.8
NMR Fractional Water Content (%)	66.8	1.9	2.8
Diffusion Δ=0.025s (10^{-5} cm^2/s)	1.38	0.06	4.4
Diffusion Δ=0.500s (10^{-5} cm^2/s)	0.73	0.09	12.4
MT	0.16	0.008	4.9
T2 (ms)	19.7	0.37	1.8

Table 3: Single Sample Variation in NMR measurements. A single cartilage sample compressed to 1400 μm was measured a total of 5 times. The Wet Weight was determined by using the computed method discussed in the section Wet Weight Measurements in Chamber. COV (%) is the Standard Deviation divided by the Mean.

NMR Experiments

The results for each NMR experiment are presented as the NMR measurement versus the fractional water content (%). Also, a regression line is shown for each NMR experiment. The raw data for Figures 10 - 18 are included in **Appendix 3**.

NMR Water Content

The NMR measurement of water content (one pulse proton experiment) is compared to water content computed from wet weight and dry weight measurements (water content = wet weight - dry weight) below in Figure 10. A best fit line is shown along with a unity line. It should be noted that the calibration of the balance was not checked.

NMR Water Volume = 0.989 * Water Volume - 2.3 µl

R^2 = 0.881

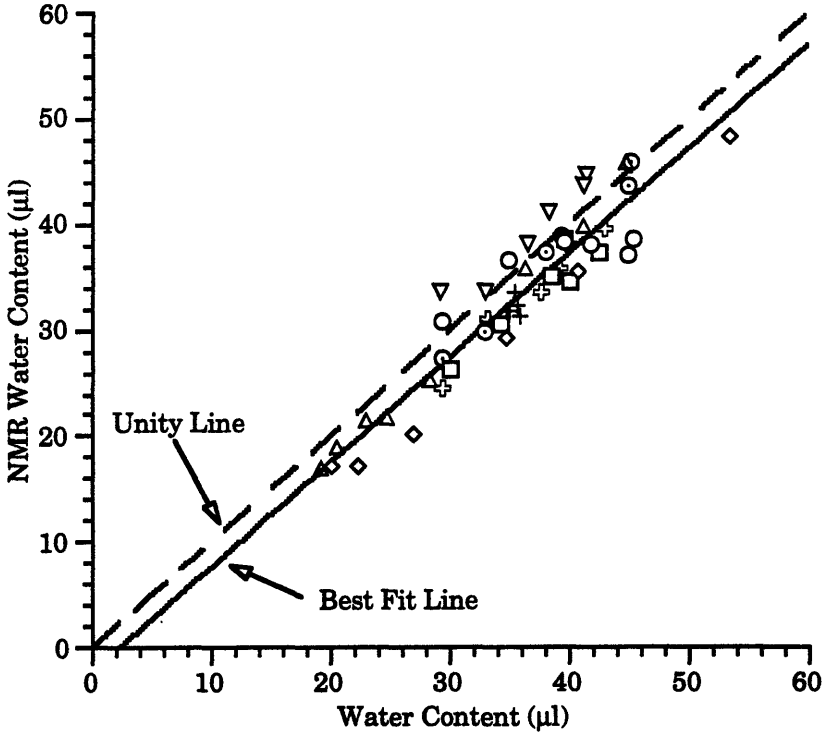

Figure 10: NMR Measured Water Content versus Water Content. Water Content is computed from wet weights and dry weights. Also shown is a unity line. Different symbols are used for each cartilage sample.

NMR Measured Fractional Water Content

The NMR measurement of water content (one pulse proton experiment) is divided by the wet weight measurement to calculate a fractional water content (Figure 11). A best fit line is also shown.

NMR FWC = 1.42 * FWC - 39

R^2 = 0.655

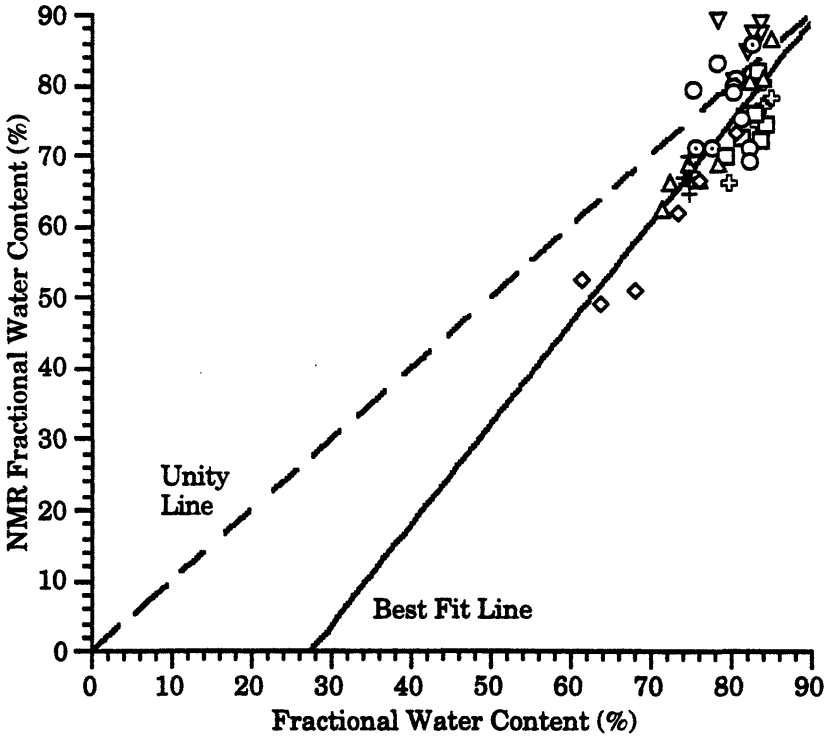

Figure 11: NMR Fractional Water Content versus Fractional Water Content. NMR Fractional Water Content computed using the NMR measured Proton content and the wet weight. Different symbols are used for each cartilage sample.

Diffusion Coefficient

The NMR diffusion measurements of the standard are shown below in Figure 12. All measurements were corrected to 298°K using the Stokes-Einstein relationship (Bird 1960).

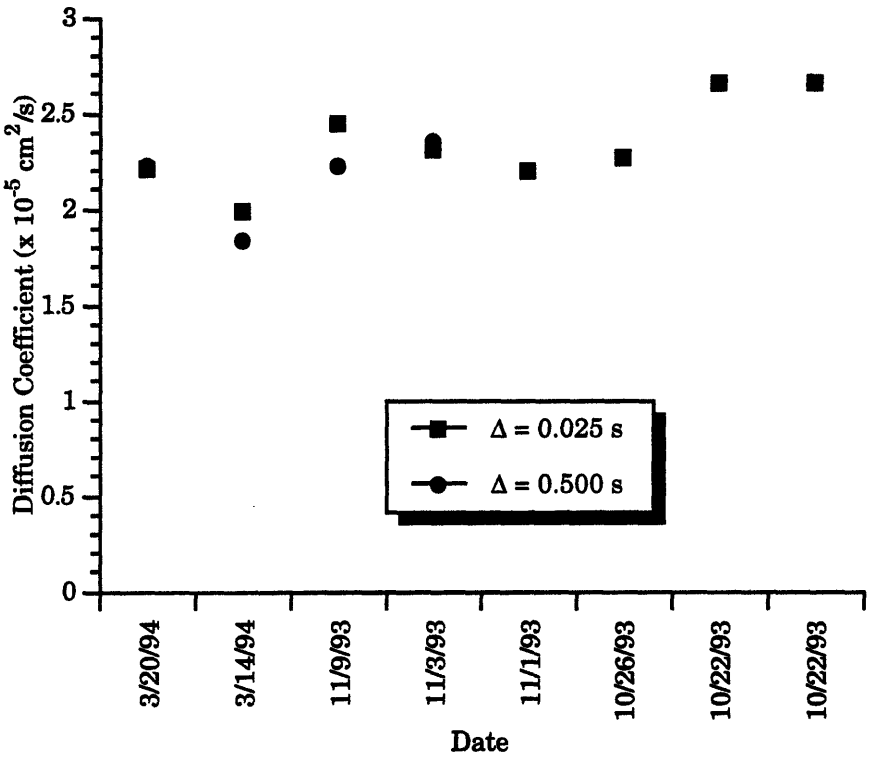

Figure 12: Diffusion Coefficient versus Fractional Water Content at Δ = 0.025 s and Δ = 0.500 s for the diffusion standard. The average value ± standard deviation (x 10^{-5} cm^2/s) for all the samples is 2.28±0.23, for Δ = 0.025 s is 2.34±0.23, and for Δ = 0.500 s is 2.16±0.22.

The diffusion experiment on cartilage was performed at two different Δ's (time between gradients) : 0.025 s (Figure 13) and 0.500 s (Figure 14). Best fit lines are also shown. A higher order fit to the data did not provide any significant decrease in residual error, so a linear fit was used.

$$D\ (\Delta = 0.025\ s) = 0.033 * FWC - 1.29\ (x\ 10^{-5}\ cm^2/s)$$

$$R^2 = 0.656$$

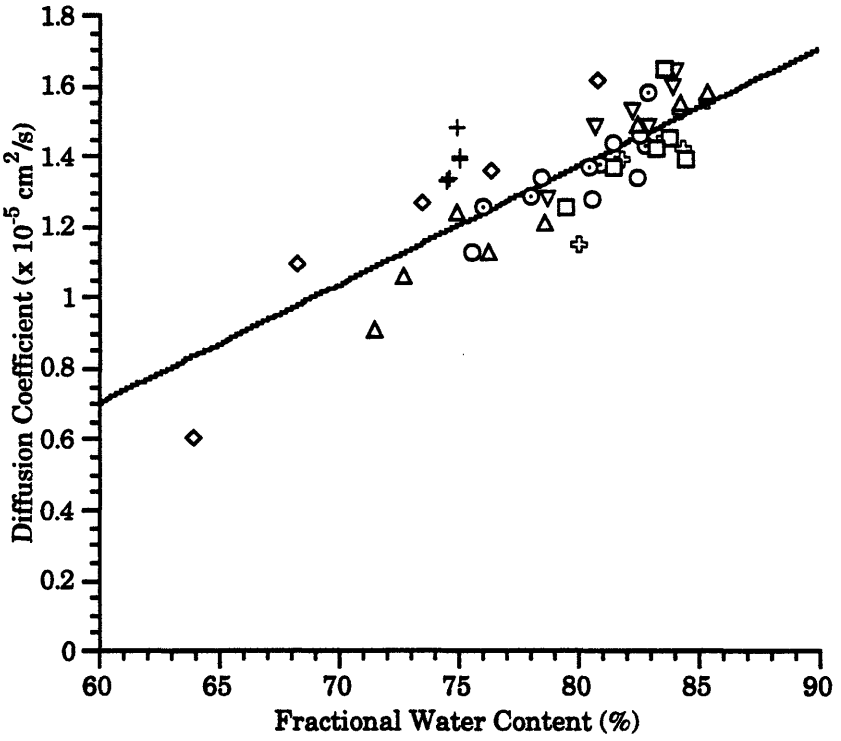

Figure 13: Diffusion Coefficient versus Fractional Water Content at Δ = 0.025 s. Best fit lines are shown. Different symbols are used for each cartilage sample.

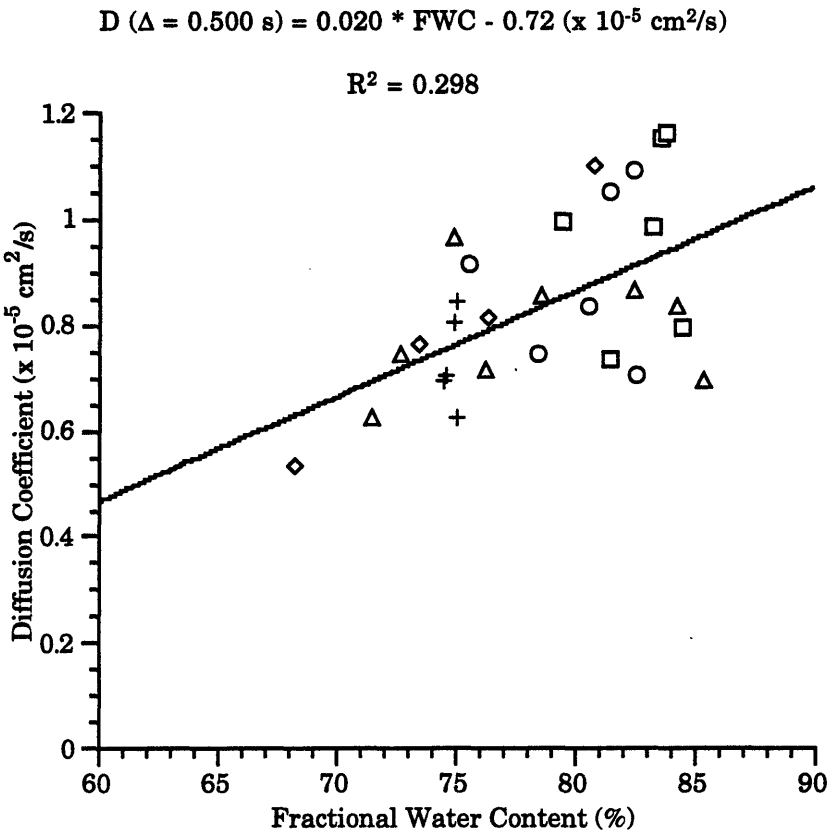

Figure 14: Diffusion Coefficient versus Fractional Water Content at Δ = 0.500 s. Best fit lines are shown. Different symbols are used for each cartilage sample.

To compare the two slopes, the diffusion results for both values of Δ are shown below in Figure 15. The equality of the two slopes was determined using the Student's t test, in a method similar to comparing two population means (Zar, 1984). The two slopes are statistically different ($p < 0.0001$).

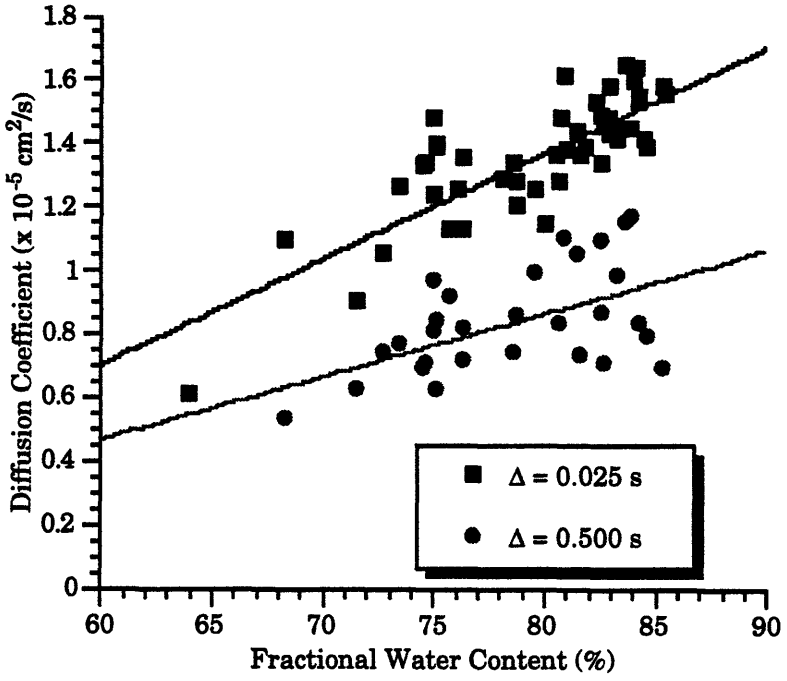

Figure 15: Diffusion Coefficient versus Fractional Water Content at Δ = 0.025 s and Δ = 0.500 s. Best fit lines are shown.

Magnetization Transfer

Magnetization transfer as a function of fractional water content is shown in Figure 16 with a best fit exponential:

$$MT\ (normalized) = 0.20 * \exp(0.019 * FWC)$$

$$R^2 = 0.639$$

MT measurements for each day are normalized to the Ms/Mo measured for unconfined cartilage on that particular day. The exponential was chosen for the best fit curve based on previous data from Lesperance (1993), who measured MT as a function of collagen content and found an exponential relationship.

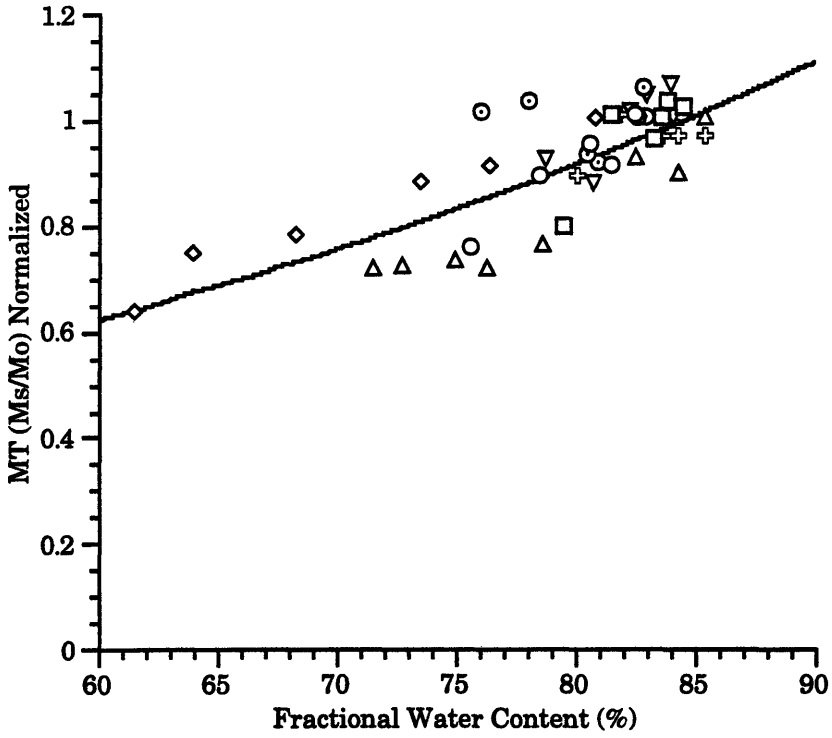

Figure 16: Magnetization Transfer versus Fractional Water Content. The MT values are normalized by the unconfined sample measurement on each day of experiments. A best fit exponential is shown. Different symbols are used for each cartilage sample.

T2

T2 relaxation time and 1/T2 (relaxation rate) as functions of fractional water content are shown in Figure 17 and Figure 18, respectively, with best fit exponentials. The 1/T2 graph is presented for qualitative comparison to Weidenbaum (1992).

$$T2 = 0.028 * \exp(0.086 * FWC) \text{ (ms)}$$

$$R^2 = 0.933$$

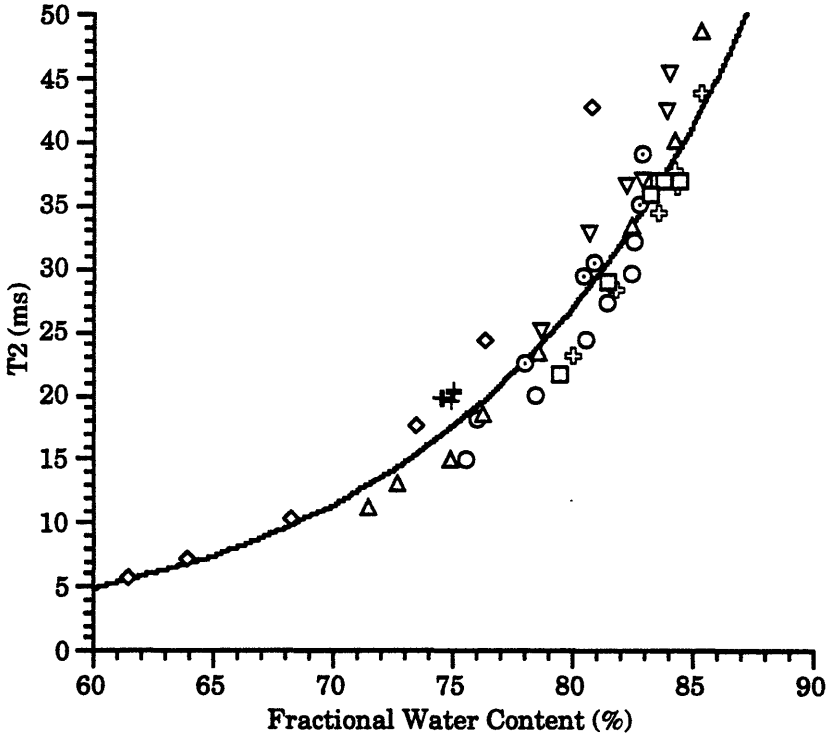

Figure 17: T2 versus Fractional Water Content. A best fit exponential is shown. Different symbols are used for each cartilage sample.

$1/T2 = 35.2 * \exp(-0.086 * FWC)\ (ms^{-1})$

$R^2 = 0.933$

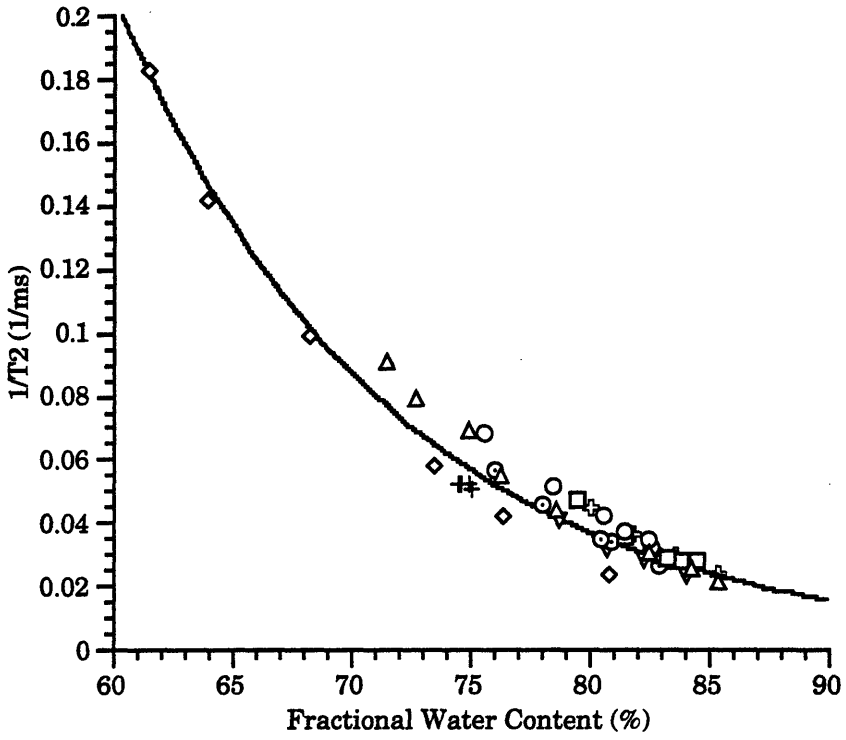

Figure 18: 1/T2 versus Fractional Water Content. A best fit exponential is shown. Different symbols are used for each cartilage sample.

Discussion

Many studies have confirmed that fractional water content increases in osteoarthritic cartilage (Mankin and Thrasher 1975, Maroudas and Venn 1977, Venn and Maroudas 1977, Grushko 1989). However, the pathological changes are not limited to fractional water content. For example, GAG content per wet weight of cartilage has also been found to decrease in diseased cartilage. The change in fractional water content, GAG, and probably many other pathological factors can affect NMR measurements on cartilage. This thesis focused on determining the effect of altering fractional water content, without changing solid content, on NMR measured diffusion, MT, and T2. A comparison of NMR and conventional measurements of water content and fractional water content was also made. The method chosen to control fractional water content was compression. The assumption was that compression forces water out of the cartilage sample without altering the solid matrix, thus decreasing fractional water content.

NMR Measured Water Content and Fractional Water Content

Both water content and fractional water content were measured using NMR and conventional scale measurements of weight, and these two different methods were compared. The NMR measured water content was measured using the NMR one pulse proton experiment. Water content was also computed as wet weight - dry weight. There was good agreement between the NMR measurement and the conventional measurement. Based on the best fit line, the NMR measurement was slightly smaller (approximately 2.3 μl) than the conventional measurement. From this data, it appears that the NMR measurement provides a reasonable measurement of water content, at least for our samples which had water contents ranging from 18 to 55 μl.

Using the NMR one pulse proton measurement for the water content and the wet weight of the cartilage sample measured conventionally using a balance, the NMR measured fractional water content was also determined. The conventional method computed a water content by subtracting the dry weight from the wet weight, and divided that water content by the wet weight to compute the fractional water content. Compared to fractional water content measured by wet and dry weight, the NMR measured fractional water content was consistently lower, with the difference increasing at smaller fractional water content.

The increasing discrepancy between NMR measured fractional water content and conventionally measured fractional water content at lower fractional water contents was consistent with the discrepancy between the NMR measured water content and conventionally measured water content. The difference in the NMR measured water content and the conventionally measured water content appeared to be constant. As the fractional water content decreased with compression, the NMR measured water content was divided by a smaller and smaller value. Thus, the constant difference in water content resulted in greater differences at lower fractional water content where the NMR water content was divided by a smaller value. When the NMR measured water content is corrected by 2.3 μl and the NMR fractional water content recalculated, there is much better agreement between the NMR measured and the conventionally measured fractional water content (Figure 19 below).

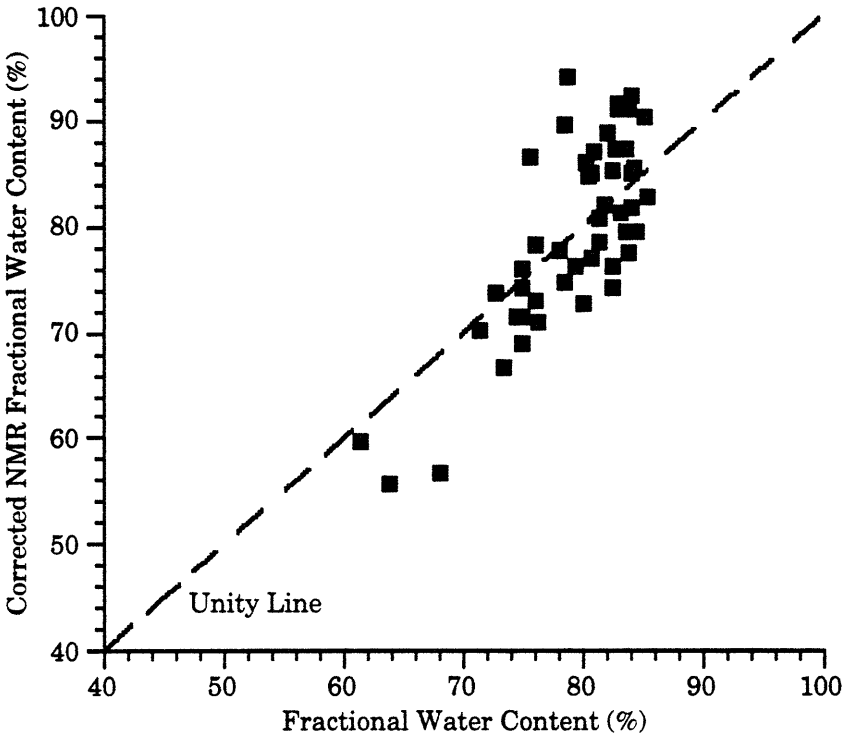

Figure 19: Corrected NMR Fractional Water Content versus Fractional Water Content. NMR Fractional Water Content computed using the NMR measured Proton content corrected by 2.3 µl and the wet weight.

It is not clear which water content measurement is in error. There may be a calibration error in the scale, or there may be an offset in the NMR measurement of water content.

NMR Measured Diffusion, MT, and T2

The relationship between changes in fractional water content and NMR measured diffusion, MT, and T2 are shown previously in the **Results** section. From those data, the effect of a change in fractional water content on the order of that seen pathologically can be determined and compared to predicted changes. Choosing fractional water contents of 70% and 77% to represent "normal" and "diseased" fractional water content respectively, and using the experimentally determined regression expressions, the NMR measurements at

fractional water contents of 70% and 77% are shown below in Table 4. Also shown in Table 4 are the experimentally determined increases and the predicted increases in the NMR measurements. Following the summary, each NMR parameter is discussed separately.

NMR Measurement	"Normal" Fractional Water Content (70%)	"Diseased" Fractional Water Content (77%)	% Change from "Normal" to "Diseased"	Predicted % Change
Diffusion Δ=0.025s ($\times 10^{-5}$ cm^2/s)	1.02	1.25	23	8,21,28,59
Diffusion Δ=0.500s ($\times 10^{-5}$ cm^2/s)	0.68	0.82	21	8,21,28,59
Ms/Mo	0.76	0.86	13	11
T2 (ms)	11.5	21.0	83	42

Table 4: Summary of Experimental NMR measurements at 70% and 77% Fractional Water Content, the % Increase and the Predicted % Increase in the NMR measurement for an increase in Fractional Water Content from 70% to 77%. The NMR values were computed from the regression lines from experimental data from each experiment.

Diffusion

Extrapolation from actual measurements (Burstein 1993) estimated a 59% increase in diffusivity for an increase in fractional water content from 70% to 77%, whereas the diffusion models of Ogston and of Mackie and Meares, predicted diffusivity increases ranging from 8% to 28% for the given increase in fractional water content . For diffusion measured at Δ=0.025 s, the measurement increased 23% for the given increase in fractional water content, and for diffusion measured at Δ=0.500 s, the measurement increased 21% for the given increase in fractional water content. The over prediction based on earlier experimental measurements is probably due to the extrapolation being based on only two measurements, or because of errors in the estimate of compression.

The actual increase in diffusivity was better matched by the phenomenological Ogston model (22% increase), and also the Mackie and Meares model (28% increase). Burstein (1993) also found that the Mackie and Meares model fit diffusion measurements at a short Δ of 0.013 s. Shown in Figure 20 are the three diffusion models and the NMR diffusion measurements at Δ = 0.025 s. The NMR measurements were converted from fractional water content to solid volume fraction for comparison to the diffusion models. For display purposes, the diffusion measurements at each value of solid volume fraction were averaged together to give a single point.

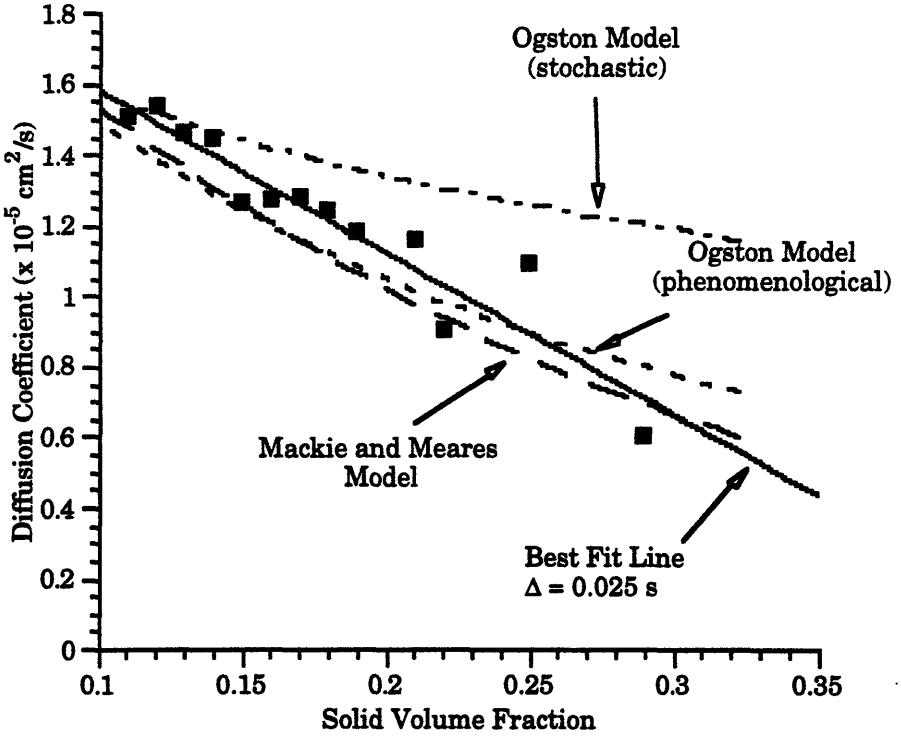

Figure 20: NMR measured Diffusion Coefficient at Δ = 0.025 s versus Solid Volume Fraction. Also displayed are a best fit line to the experimental data, and predicted diffusion coefficients by the Mackie and Meares model, the phenomenological Ogston model (α = 2.4) and the stochastic Ogston model (a = 0.5 nm; r = 0.1 nm). The parameters chosen for the Ogston models are discussed in the section **Preliminary Sensitivity Comparison of NMR Measured Fractional Water Content, Diffusion, MT, and T2 : Diffusion**.

It was interesting that the Mackie and Meares model and the phenomenological Ogston model predicted the experimental values at $\Delta = 0.025$s as closely as they did. Maroudas and Venn (1977) found that the Mackie and Meares model closely predicted experimental diffusion measurements of tritiated water in human femoral head cartilage. However, the diffusion measurements were only made on uncompressed cartilage which were at a single value of fractional water content, so it was not clear if the Mackie and Meares model would predict diffusion coefficients at different solid volume fractions.

It should be noted that the theoretical diffusion models were designed to predict steady state diffusion values, which would correspond to NMR diffusion measurements at Δ that are long enough for the tissue to appear homogeneous to the diffusing solute. Burstein (1993) made NMR measurements of diffusion coefficients at various Δ's, and found evidence of restricted diffusion, and at short Δ's, such as 0.025 s, cartilage does not appear homogeneous to diffusing protons. Therefore, the apparent correlation between the Mackie and Meares model, the phenomenological Ogston model, and the NMR measurements of diffusion appears to be due to the particular choice of the NMR diffusion parameter Δ. However, it does appear that the two models can provide some indication of the percent change in the diffusion measurement for a given change in fractional water content, since the percent change was relatively independent of the parameter Δ.

MT

From the Lesperance MT measurements on unconfined bovine cartilage from various anatomical locations and ages (calf epiphyseal, calf metacarpal, calf meniscus, calf femoropatellar, adult femoropatellar), Ms/Mo was expected to change only slightly, an 11% increase, in response to a change in fractional water content from 70% to 77%. That

insensitivity was verified experimentally, where Ms/Mo displayed the lowest % change (13% increase) of all the NMR experiments tested.

Lesperance also fit a logarithmic regression line to the MT measurements on various types of cartilage (calf epiphyseal, calf articular, and adult articular). To compare Lesperance's regression line to the regression line found experimentally, the Lesperance regression line was normalized so that at a fractional water content of 85%, the two regression lines were equal. Points computed from the two regression lines are shown below in Figure 21. There appears to be reasonable agreement with the Lesperance result.

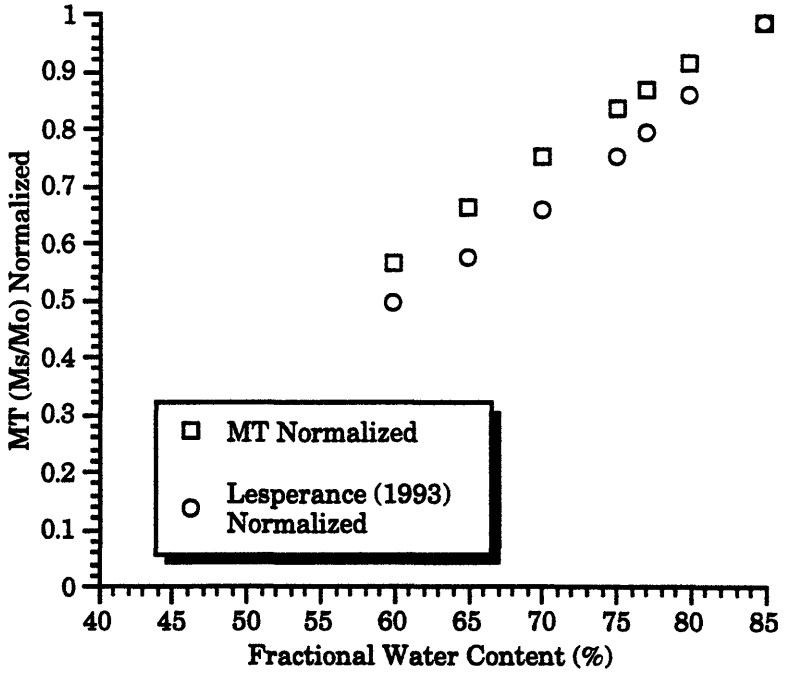

Figure 21: Normalized MT versus Fractional Water Content. The points were calculated from regression lines. For comparison purposes, the Lesperance regression line was normalized to match the MT Normalized value at a fractional water content of 85%.

T2

From the Lehner (1989) T2 measurements of intervertebral disc, T2 was expected to display a relatively large increase for the given change in fractional water content. For an increase in fractional water content from 70% to 77%, T2 was estimated to increase 42%. Experimentally, T2 was found to increase by 81% when the fractional water content was increased from 70% to 77%. Probable causes for the discrepancy between Lehner's predicted change and the experimental results are the dependence of T2 measurements on the magnetic field strength, and the differences in sample type and composition (bovine patellae of varying age versus calf femoropatellar groove).

The large increase in T2 with increases in fractional water content is consistent with magnetic resonance images of diseased cartilage. A longer T2 would result in a brighter image because the signal decay due to T2 would occur slower with a longer T2. A bright region in a MR image has been correlated to disease (Recht 1993, McCauley 1992, McAlindon 1991). From these T2 measurements, it appears that the brightness of diseased cartilage may largely be due to increases in fractional water content.

The T2 measurements were also presented as 1/T2 versus fractional water content (Figure 18). Based on the conclusions of Weidenbaum (1992), 1/T2 versus fractional water content was expected to follow a linear relationship. Over the entire measured range of fractional water contents, 1/T2 was clearly not linearly related to fractional water content. However, Weidenbaum measured uncompressed samples, and if these results are examined over a range of fractional water content near free swelling, 1/T2 versus fractional water content does appear to be more linear (Figure 22).

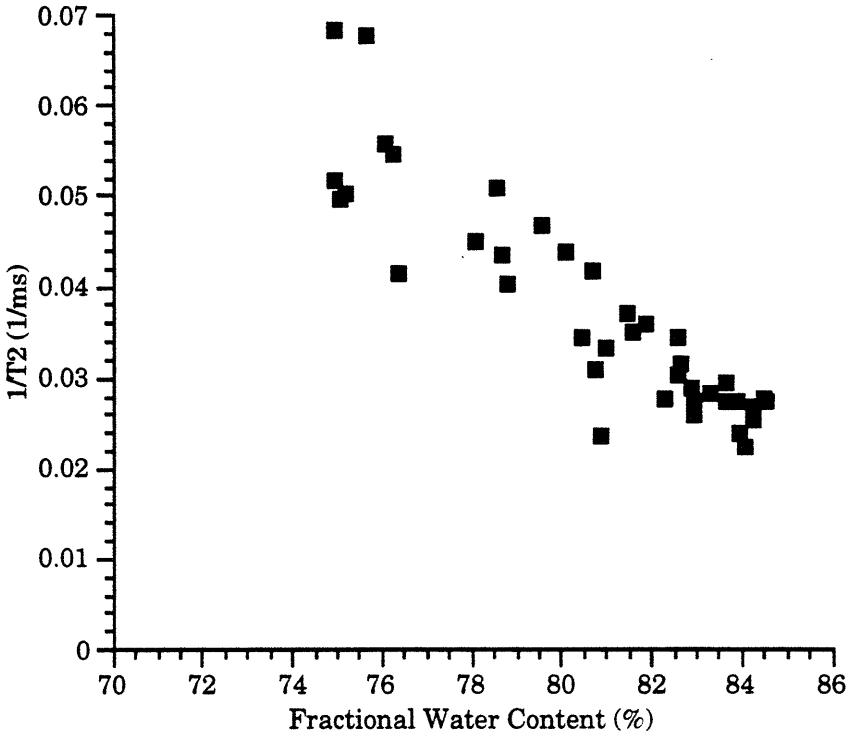

Figure 22: 1/T2 vs Fractional Water Content. The data are constrained to FWCs between 75% and 85%.

There is one final note about the T2 measurement. It is assumed that inhomogeneities in the magnetic field due to the sample are negligible. Later NMR experiments found that the assumption may not have been valid, and thus some error may have been introduced into the T2 measurement. The actual calculation of the error is difficult, due to the difficulty in characterizing the inhomogeneities. However, an analysis of the error demonstrated that the error in T2 should only increase the change seen for a given change in fractional water content, so that the positive correlation between NMR measured T2 and fractional water content is still valid. A more complete discussion of the error analysis can be found in **Appendix 1**.

Specificity

These NMR measurements alone do not address the issue of the specificity of the NMR measurements to changes in cartilage matrix constituents other than water. The effect of other matrix changes can begin to be examined by combining these results with other NMR measurements on different cartilage samples. Diffusion has been measured by Burstein (1993) on calf articular cartilage treated with trypsin to remove the GAG portion of the matrix. Raman (1995) also used NMR to measure diffusivity in normal cartilage, trypsinized cartilage, GAG solutions, and collagen suspensions. The magnetization transfer results were excluded from the comparison with other measurements, due to the normalization used. T2 measurements have been made on free swelling calf epiphyseal cartilage with and without IL-1ß treatment (Bashir and Liu, 1994). The IL-1ß treatment results in the degradation of the matrix. These other measurements were made on samples whose matrix composition differed from the samples measured in this thesis. Comparison of those two types of samples can give some preliminary indication of the effect of matrix composition on the NMR measurements, and can begin to evaluate how strong a determinate of the NMR measurements fractional water content is.

Diffusion

The diffusion measurements made by Burstein (1993) and Raman (1995) at Δ = 0.025 s, along with the previous results, are presented below in Figure 23.

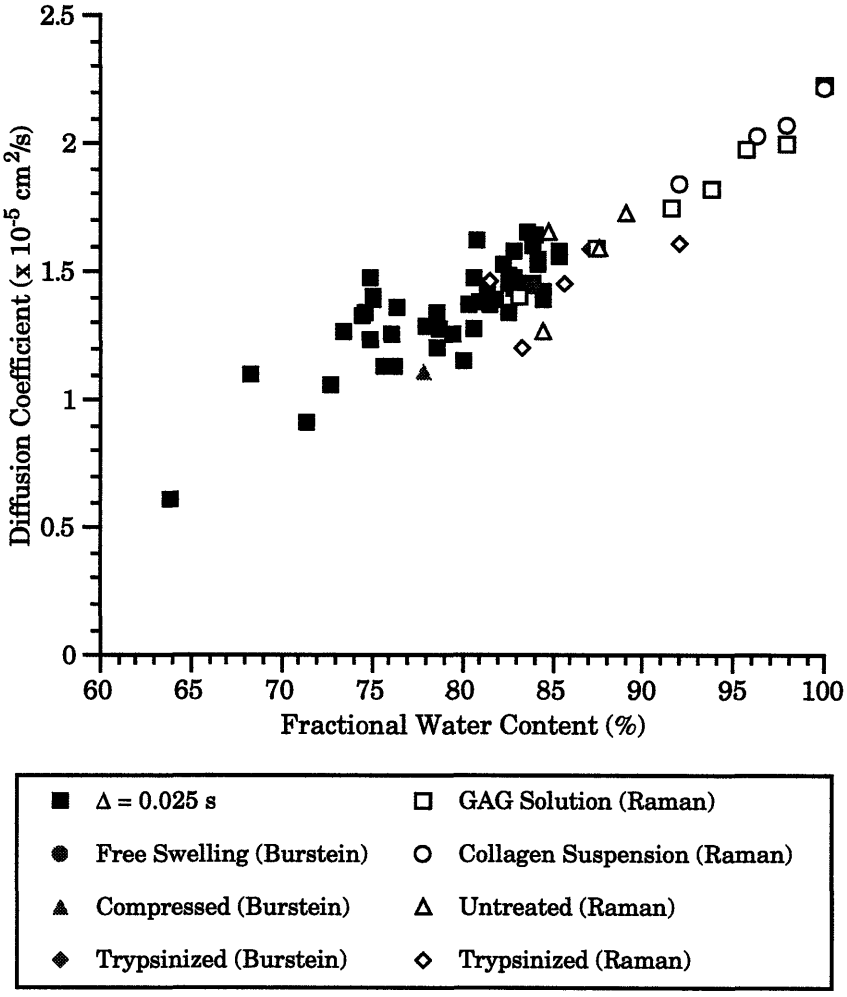

Figure 23: Diffusion Coefficient versus Fractional Water Content at Δ = 0.025 s. Measurements from Burstein (1993) are shown as gray symbols. Measurements from Raman (1995) are shown as open symbols.

Burstein and Raman made these measurements on the same type of cartilage that was used here. In addition, Raman measured the diffusivity of GAG solutions and collagen suspensions. Although these measurements varied in their specific solid matrix

composition, they appear to be in good agreement with the measurements from this thesis. It appears that NMR measured diffusion reflects the fractional water content, and not the precise macromolecular composition, of the sample.

T2

Shown below in Figure 24 are some additional T2 measurements that begin to address the specificity of T2 to various matrix changes.

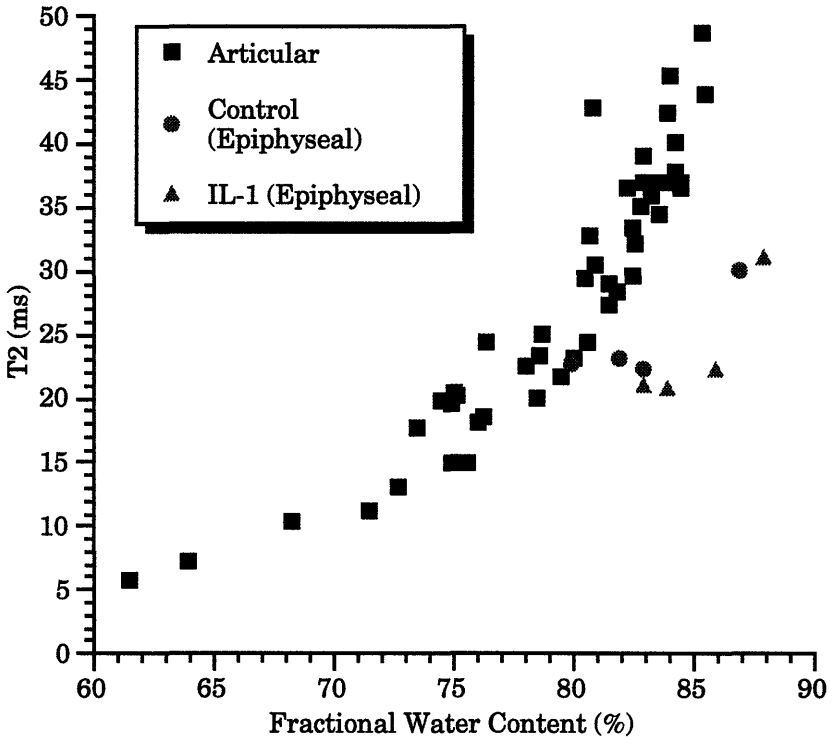

Figure 24: T2 versus Fractional Water Content. Measurements were made on bovine articular cartilage under varying compression levels (squares), free swelling bovine epiphyseal cartilage before (circles) and after (triangles) treatment with IL-1ß.

The squares are T2 measurements on bovine articular cartilage under varying compression levels that have been discussed already. The circles and triangles are T2 measurements of free swelling calf epiphyseal cartilage before (circles) and after (triangles) treatment with IL-1ß. The IL-1ß treatment results in the degradation of the matrix. In addition, the articular samples and epiphyseal samples vary in their matrix constituents. Thus, if fractional water content is the only matrix constituent that affects the T2

measurement, the epiphyseal data and the articular data should have a similar correlation to fractional water content. Based on these limited number of measurements, it appears that fractional water content is not the only matrix constituent that determines T2.

In comparison, Lüsse (1995) recently measured T2 times of pig articular cartilage and bovine nasal cartilage placed under varying levels of osmotic pressure. Fractional water contents ranged from approximately 80% (free-swelling) down to 35% for pig articular cartilage and 50% for bovine nasal cartilage. They concluded that for moderate compression levels, down to a fractional water content of approximately 45%, T2 times were only a function of fractional water content and independent of cartilage type.

It is possible that Lüsse's T2 result differs from the one determined here due to differences, or lack of differences, in the solid matrix of the various cartilage types used. However, another possibility for the discrepancy has to do with the way in which the data is presented. Lüsse's T2 results were presented as R2 (1/T2) versus Solid Weight / Water Weight. The results in Figure 24 were recomputed to match the axes of Lüsse and are shown below in Figure 25. The difference between articular and epiphyseal cartilage seen in Figure 24 has been de-emphasized due to the different data presentation.

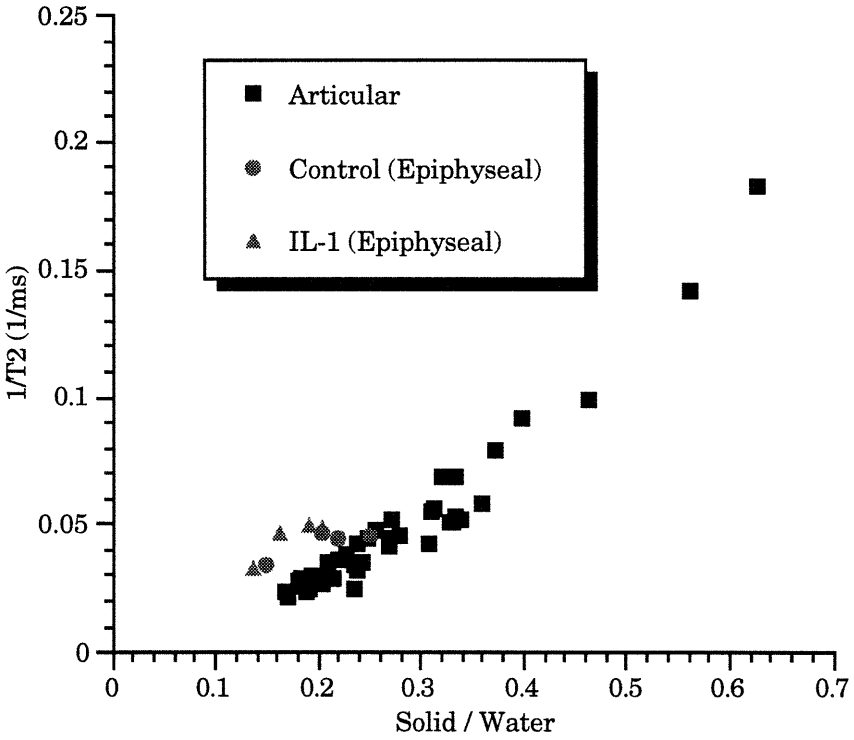

Figure 25: 1/T2 versus Solid / Water. The results of Figure 24 were recomputed so that the axes would match those of Lüsse (1995). Measurements were made on bovine articular cartilage under varying compression levels (squares), free swelling bovine epiphyseal cartilage before (circles) and after (triangles) treatment with IL-1ß.

Conclusions

In cartilage diseases, such as osteoarthritis, there are many pathological changes, one of which is a change in fractional water content. Previous NMR measurements of diffusion, MT, and T2 in cartilage were either limited in the range of fractional water content examined, or there was variation in solid content in addition to the variation in fractional water content. It was difficult to determine the specific effect of any one tissue component of the NMR measurements. The goal of this thesis was to address those limitations by examining the relationship between specific changes in fractional water content and NMR measurements of diffusion, MT, and T2.

In addition to the experimental characterization of the NMR parameters versus fractional water content, predicted changes in the parameters for a given change in fractional water content were computed using both theoretical models and previous experimental results. For comparison purposes, the % increase in the NMR measurement for an increase in fractional water content from 70% to 77% was computed.

The phenomenological Ogston model and the Mackie and Meares model closely predicted the experimentally measured increase in diffusivity. In addition, the two models well matched the absolute diffusion measurements at $\Delta = 0.025$ s. This was unexpected since the models were expected to predict diffusivities over longer time scales, and therefore the models should have best matched the NMR measured diffusivity at the longer Δ (0.500 s). It does, however, appear that the models can provide a reasonable estimate of the % change in NMR measured diffusivity over the range of fractional water contents measured. The previous diffusion measurements (Burstein 1993) were a much poorer predictor than the theoretical models, although that discrepancy was likely due to the limited number of

previous measurements, or some error in the estimate of compression in those previous measurements.

Unlike diffusion, the predictions for MT and T2 were all made based upon previous measurements. The predicted increase in Ms/Mo (11%) matched well with the experimentally measured increase of 13%. In addition, the Ms/Mo agreed well with the normalized Lesperance results. The large discrepancy between the predicted increase in T2 (42%) and the experimentally measured increase (83%) was likely due to T2 dependence on magnetic field strength and differences in sample type and composition.

It should be noted that these results do not directly address the issue of the specificity of the NMR parameters to water content, although they do provide a reference from which to begin. The comparison of NMR measurements of different types of cartilage (**Discussion - Specificity**) suggest that the NMR measured diffusion may be specifically sensitive to fractional water content, whereas the T2 measurement is affected by changes in fractional water content and other matrix constituents . These are, however, only preliminary speculations, which are based on a limited number of measurements.

Future Work

Using these results as a baseline, it would now be possible to examine the effect of controlled changes in matrix constituents other than water on the NMR parameters. These preliminary correlations between NMR measurements and fractional water content are needed because, in practice, it is difficult to alter matrix constituents without a concomitant change in fractional water content that may obscure the change in the measurement due to changes in matrix constituents.

For example, an extension to the results discussed here would be to determine the effect of changing GAG content on the various NMR parameters. Both increases in fractional water content and decreases in GAG content are associated with cartilage degradation. GAG content can be altered by trypsin digestion, which removes the GAG portion of the matrix and also changes the fractional water content. The data from trypsinized samples can be compared to the data from normal samples. Any difference between the normal cartilage and the trypsinized cartilage would likely be attributable to the difference in GAG content. These additional results would provide further understanding regarding the effect changes associated with arthritis have on NMR measured diffusion, MT, and T2 relaxation.

Another possible application of these results might be to use NMR to provide a reflection of compression of cartilage, or other tissue, *in vivo*. Higher compression forces would result in water being forced out of the tissue, while the solid matrix composition would assumedly be unchanged. That change is fractional water content should be reflected in the NMR parameters previously discussed.

Appendix

1. Analysis of Error in the Measurement of T2

T2 was measured assuming that the inhomogeneities in the magnetic field due to the sample are negligible. For Hahn spin echo T2 measurements, the combination of diffusion of the spins and inhomogeneities in the magnetic field can cause incomplete refocusing of the spins. The additional dephasing that occurs is measured as T2 dephasing, giving rise to a Hahn T2 time that is shorter than the actual T2 time.

After the experiments had been performed, another experiment revealed that the assumption of negligible sample inhomogeneities may not have been valid. The T2 time for a single cartilage sample was measured using two different pulse sequences: Hahn spin echo (see **Methods**) and the Carr-Purcell-Meiboom-Gill (CPMG). The CPMG sequence is:

$$\mathbf{90^o - (\tau/2 - 180^o)_n - \tau/2 - acquire}$$

The CPMG sequence was used to minimize the effects of diffusion on the T2 measurement. The T2 times measured by the Hahn spin echo and the CPMG were 37 ms and 50 ms respectively, indicating that inhomogeneities in the magnetic field due to the presence of the sample could not be ignored.

In an attempt to make some estimations about the error in the T2 measurement, an "equivalent" linear error gradient was computed such that the linear gradient would produce the same difference seen experimentally in the Hahn and CPMG T2 measurement. Assuming that the CPMG value represented a "true" T2 time, the background gradient was calculated by fitting the experimental Hahn spin echo data, which measured a T2 time of 37 ms, to the equation (Fukushima and Roeder 1981):

$$I(TE) = I(0)e^{-\left(\frac{TE}{T2}+\gamma^2 Dg^2\frac{TE^3}{12}\right)}$$

T2 = CPMG measured T2 time (50 ms)

γ = gyromagnetic ratio of proton

D = diffusion coefficient

The diffusion coefficient of the particular sample was not measured, which required a diffusion coefficient to be assumed. The sensitivity of this computation to the choice of D was made by computing the gradient for three values of D: 1.3, 1.4 and 1.5 x 10^{-5} cm^2/s (Table 5).

Assumed D (x 10^{-5} cm^2/s)	Computed g (G/cm)
1.3	1.36
1.4	1.41
1.5	1.46

Table 5: Computed Gradient (g) for 3 choices of Diffusion Coefficient (D) .

It appeared that the computation is not very sensitive to the choice of diffusivity, and therefore the "equivalent" error gradient (g) used for error computations was 1.4 G/cm. The measurements of diffusion and T2 from three different fractional water contents were recomputed to include the presence of the "equivalent" error gradient.

The use of the linear error gradient should only be taken as an approximation. The inhomogeneities in the magnetic field due to the inclusion of the sample results from the geometry of the sample and the difference in magnetic permeabilities. The magnetic permeability relates the **B** field (magnetic flux density) to the **H** field (magnetic field intensity) by the following constitutive law: $\mathbf{B} = \mu\mathbf{H}$. Since cartilage is largely made up of water, the magnetic permeability (μ) of cartilage can be approximated as that of water, which is 0.99999 μ_o, where μ_o is the magnetic permeability of air. If the sample had

spherical symmetry, the magnetic field lines inside would be relatively uniform. However, in the case of a cylindrical plug, the interior field lines would be complex and spatially inhomogeneous.

To compute the error, the raw T2 data, consisting of signal intensities as a function of the echo time (TE), were fit to the combined T2/diffusion equation above, and the results are shown below in Table 6.

Hahn T2 (ms)	D (x 10^{-5} cm^2/s)	Calculated T2 (w/ Diffusion) (ms)	Absolute Error (ms)	% Error
14.7	1.29	15	0.3	2.0
32.3	1.56	37	4.7	14.6
45.8	1.62	58.8	13	28.4

Table 6: Error in T2 measurement due to background gradients.

The T2 error was dependent on the T2 value. Diffusion in the presence of magnetic field inhomogeneities produce additional dephasing that is interpreted by the Hahn T2 measurement as T2 dephasing. Therefore, the Hahn T2 measurement results in a T2 time that is shorter than the actual T2. This effect is lessened when T2 times are short because the T2 effect of dephasing is already large and the diffusive dephasing only contributes a small amount.

This error would result in an increased change in the T2 measurement for a given change in fractional water content. The actual increase in T2 for an increase in fractional water content is likely to be greater than 83%. However, the exact determination of this error would require the knowledge of the gradients present when the measurements were made. In the future, it is suggested that T2 be measured in cartilage using the CPMG pulse sequence rather that the Hahn echo.

2. Radial Expansion During Compression

From visual inspection of the plug during compression, radial expansion of the plug was obviously occurring, at least at large compressions. Direct measurement of diameter would require the use of a molding compound that could possibly become stuck in the compression chamber. Instead, to maintain the integrity of the chamber, an estimation of the plug diameter as a function of compression level was computed.

The volume was computed using the wet weight of the plug at each compression thickness and the dry weight with a density of 1.4 g/cm^3 (Lipshitz, 1976). Two different values of the compressed thicknesses were used to assess the sensitivity of the calculation to the assumed height: the shim spacing + 5%, and the shim spacing - 5%. The diameter was then computed from the volume and the assumed compression thickness. Since the thickness of the unconfined sample was not measured, it was assumed to be 2 mm. The results are shown below in Figure 26. It appears that the computed diameter is not very sensitive to the ±5% variation in assumed compression thickness.

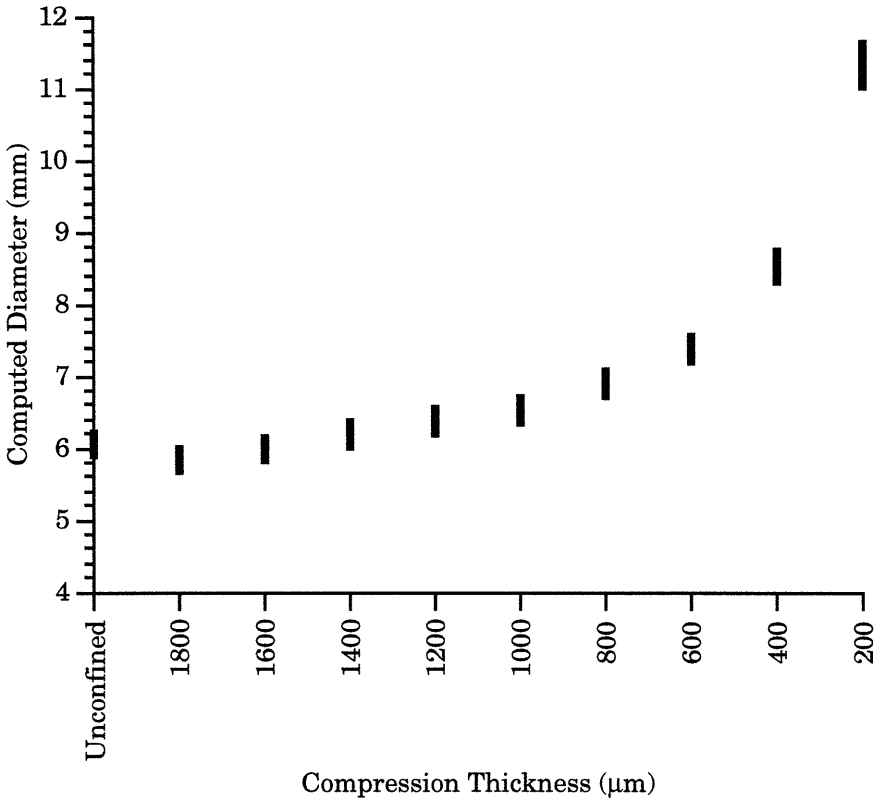

Figure 26: Estimated Diameter versus Compression Thickness (n=1). Diameter was computed from wet and dry weight measurements. The lines represent the range of computed diameters when varying the height by ±5%. Unconfined thickness of plug was assumed to be 2 mm.

There appear to be two regions of compression. From the unconfined thickness to 1000 μm the computed diameter remains approximately constant so changes in volume are mainly due to changes in height. At compression thicknesses below 1000 μm, the computed diameter increases with decreasing compression thickness. The computed diameters at the smaller compression thicknesses were clearly too large. The width of the upper compression surface is approximately 9 mm, and the compressed plug was never larger than the compression surface. The absolute values of the computed diameter may be incorrect due to the assumptions made about the density and the actual dimensions of the plug.

Errors at smaller compression thicknesses were partly due to misalignment of the upper compression surface relative to the lower surface, which resulted in a visibly non-plane parallel compression thickness. That inaccuracy in compression thickness combined with radial expansion of the sample precluded the use of the shim defined compression thickness as a measurement of volume. Therefore, the variable of interest used was fractional water content, and not the compression thickness defined by the shims.

3. Raw Data for Figures 10 - 18

Figure 10: NMR Measured Water Content versus Water Content

Water Content (µl)	NMR Water Content (µl)
36	30.92
35.2	31.53
35.7	33.23
35	31.43
35.9	32.03
53.5	48.2
40.8	35.33
34.9	29.14
27.1	20.06
22.4	17.07
20.1	17.07
44.9	45.48
41.4	39.66
36.5	35.54
28.5	24.9
24.8	21.59
23.1	21.18
20.6	18.67
19.3	16.77
45.6	38.29
45.1	36.91
41.9	37.89
39.8	38.19
35	36.22
29.6	30.41
39.7	38.39
42.7	37.1
40.3	34.23
38.7	34.72
34.4	30.16
30.2	26.09
45.3	45.69
45	43.24
39.6	38.53
38.3	37.06
33.2	29.41
29.6	26.96
41.6	44.24
41.3	43.23

38.5	40.71
36.6	37.88
33.2	33.23
29.3	33.33
40	37.85
43.2	39.24
39.4	35.66
37.8	33.27
33.3	30.78
29.6	24.3

Figure 11: NMR Fractional Water Content versus Fractional Water Content

Fractional Water Content (%)	NMR Fractional Water Content (%)
84.5	80.2
85.5	77.9
84.3	76.5
83.7	73.9
81.9	75.9
80.1	65.9
84.1	88.5
84	87
83	86.9
82.3	84.3
80.8	80
78.8	88.7
83	85.3
82.9	81.2
81	80.4
80.5	79.4
78.1	70.6
76.1	70.7
83.7	81.6
84.6	74.2
83.9	71.9
83.3	75.4
81.6	72.2
79.6	69.4
82.7	70.6
82.6	68.7
81.5	74.9
80.7	78.7

78.6	82.7
75.7	79
85.4	86.1
84.3	80.4
82.6	80.1
78.7	68.5
76.3	66.2
75	68.5
72.8	65.7
71.5	61.9
80.9	73.1
76.4	66.3
73.5	61.5
68.3	50.6
64	48.9
61.5	52.3
75.2	64.3
74.7	66.7
75	69.5
74.6	66.7
75.1	66.7

Figure 13-15: Diffusion Coefficient versus Fractional Water Content at Δ = 0.025 s and Δ = 0.500 s.

Fractional Water Content (%)	Diffusion Coefficient (x 10^{-5} cm^2/s) for Δ = 0.025 s	Diffusion Coefficient (x 10^{-5} cm^2/s) for Δ = 0.500 s
84.5	1.41	
85.5	1.55	
84.3	1.52	
83.7	1.44	
81.9	1.38	
80.1	1.14	
84.1	1.63	
84	1.59	
83	1.47	
82.3	1.52	
80.8	1.47	
78.8	1.27	
83	1.57	
82.9	1.42	
81	1.37	
80.5	1.36	

78.1	1.28	
76.1	1.25	
83.7	1.64	1.15
84.6	1.38	0.79
83.9	1.44	1.16
83.3	1.41	0.98
81.6	1.36	0.73
79.6	1.25	0.99
82.7	1.45	0.7
82.6	1.33	1.09
81.5	1.43	1.05
80.7	1.27	0.83
78.6	1.33	0.74
75.7	1.12	0.91
85.4	1.57	0.69
84.3	1.54	0.83
82.6	1.48	0.86
78.7	1.2	0.85
76.3	1.12	0.71
75	1.23	0.96
72.8	1.05	0.74
71.5	0.9	0.62
80.9	1.61	1.1
76.4	1.35	0.81
73.5	1.26	0.76
68.3	1.09	0.53
64	0.6	
61.5		
75.2	1.39	0.62
74.7	1.33	0.7
75	1.47	0.8
74.6	1.32	0.69
75.1	1.38	0.84

Figure 16: Magnetization Transfer versus Fractional Water Content

Fractional Water Content (%)	MT (Ms/Mo) Normalized
84.5	1
85.5	0.966
84.3	0.966
83.7	0.966
81.9	1.008
80.1	0.89

84.1	1
84	1.063
83	1.04
82.3	1.012
80.8	0.877
78.8	0.921
83	1
82.9	1.057
81	0.917
80.5	0.93
78.1	1.03
76.1	1.013
83.7	1
84.6	1.022
83.9	1.031
83.3	0.964
81.6	1.009
79.6	0.796
82.7	1
82.6	1.009
81.5	0.91
80.7	0.953
78.6	0.891
75.7	0.754
85.4	1
84.3	0.896
82.6	0.925
78.7	0.761
76.3	0.716
75	0.731
72.8	0.721
71.5	0.716
80.9	1
76.4	0.9101
73.5	0.884
68.3	0.783
64	0.745
61.5	0.637
75.2	
74.7	
75	
74.6	
75.1	

Figure 17: T2 versus Fractional Water Content

Fractional Water Content (%)	T2 (ms)
84.5	36.4
85.5	43.6
84.3	37.6
83.7	34.3
81.9	28.1
80.1	22.9
84.1	45.2
84	42.2
83	36.8
82.3	36.3
80.8	32.5
78.8	24.9
83	38.8
82.9	34.8
81	30.3
80.5	29.3
78.1	22.4
76.1	18
83.7	36.7
84.6	36.8
83.9	36.8
83.3	35.6
81.6	28.7
79.6	21.5
82.7	31.9
82.6	29.4
81.5	27.1
80.7	24.1
78.6	19.8
75.7	14.8
85.4	48.4
84.3	39.8
82.6	33.2
78.7	23.2
76.3	18.4
75	14.7
72.8	12.8
71.5	11.1
80.9	42.7
76.4	24.2
73.5	17.5
68.3	10.2

64	7.1
61.5	5.5
75.2	20
74.7	19.5
75	19.4
74.6	19.5
75.1	20.2

Figure 18: 1/T2 versus Fractional Water Content

Fractional Water Content (%)	1/T2 (1/ms)
84.5	0.0275
85.5	0.0229
84.3	0.0266
83.7	0.0292
81.9	0.0356
80.1	0.0437
84.1	0.0221
84	0.0237
83	0.0272
82.3	0.0275
80.8	0.0308
78.8	0.0402
83	0.0258
82.9	0.0287
81	0.0330
80.5	0.0341
78.1	0.0446
76.1	0.0556
83.7	0.0272
84.6	0.0272
83.9	0.0272
83.3	0.0281
81.6	0.0348
79.6	0.0465
82.7	0.0313
82.6	0.0340
81.5	0.0369
80.7	0.0415
78.6	0.0505
75.7	0.0676
85.4	0.0207
84.3	0.0251

82.6	0.0301
78.7	0.0431
76.3	0.0543
75	0.0680
72.8	0.0781
71.5	0.0901
80.9	0.0234
76.4	0.0413
73.5	0.0571
68.3	0.0980
64	0.1408
61.5	0.1818
75.2	0.0500
74.7	0.0513
75	0.0515
74.6	0.0513
75.1	0.0495

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