T1\(\rho\) Dispersion in Articular Cartilage: Relationship to Material Properties and Macromolecular Content

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Abstract
Objective. This study assessed T1\(\rho\) relaxation dispersion, measured by magnetic resonance imaging (MRI), as a tool to noninvasively evaluate cartilage material and biochemical properties. The specific objective was to answer two questions: (1) does cartilage initial elastic modulus (\(E_0\)) correlate with T1\(\rho\) dispersion effects and (2) does collagen or proteoglycan content correlate with T1\(\rho\) dispersion effects? Design. Cadaveric patellae with and without visible cartilage damage on conventional MR were included. T2 and T1\(\rho\) relaxation times at 500 and 1000 Hz spin-lock field amplitudes were measured. We estimated T1\(\rho\) dispersion effects by measuring T1\(\rho\) relaxation time at 500 and 1000 Hz and T2 relaxation time and using a new tool, the ratio T1\(\rho\)/T2. Cartilage initial elastic modulus, \(E_0\), was measured from initial response of mechanical indentation creep tests. Collagen and proteoglycan contents were measured at the indentation test sites; proteoglycan content was measured by their covalently linked sulfated glycosaminoglycans (sGAG). Pearson correlation coefficients were determined, taking into account the clustering of multiple samples within a single patella specimen. Results. Cartilage initial elastic modulus, \(E_0\), increased with decreasing values of T1\(\rho\)/T2 measurements at both 500 Hz (\(P = 0.034\)) and 1000 Hz (\(P = 0.022\)). 1/T1\(\rho\) relaxation time (500 Hz) increased with increasing sGAG content (\(P = 0.041\)). Conclusions. T1\(\rho\)/T2 ratio, a new tool, and cartilage initial elastic modulus are both measures of water–protein interactions, are dependent on the cartilage structure, and were correlated in this study.

Keywords
T1\(\rho\) dispersion, articular cartilage, initial elastic modulus, proteoglycan, collagen

Introduction
Quantification of material properties and macromolecular content of articular cartilage is important in understanding joint health and disease.\(^1\) A method for noninvasive assessment of cartilage material properties and macromolecular content would be valuable to evaluate disease modifying treatment strategies for osteoarthritis and to diagnose osteoarthritis prior to extensive cartilage damage.\(^2\) Magnetic resonance imaging (MRI) is one noninvasive method that could be used to determine cartilage material properties and thus evaluate cartilage health.

Cartilage modulus is a measure of normalized cartilage deformation for a given mechanical stress and may characterize cartilage health.\(^3\) Cartilage moduli have been correlated with cartilage macromolecular content, that is, proteoglycan and collagen.\(^3,5\) Cartilage macromolecular content has also been shown to be correlated with MRI measurements, including delayed gadolinium-enhanced MRI of cartilage (dGEMRIC),\(^6\) T1\(\rho\),\(^7\) T2,\(^8\) sodium,\(^9\) glycosaminoglycan chemical exchange-dependent saturation transfer (gagCEST),\(^10\) diffusion,\(^11\) and quantitative magnetization transfer.\(^12\) Thus, MRI measurements are likely to correlate with cartilage modulus. Previous studies in human cartilage relating MRI measurements with cartilage modulus, however, report inconsistent results. While some studies have reported a

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correlation of dGEMRIC index and T2 relaxation time with
dynamic and equilibrium elastic moduli, other studies
have reported no relationships or mixed relationships.17,18
T1ρ relaxation correlated with proteoglycan content at 500
Hz spin-lock field amplitude, was visually related to pro-
teoglycan content at 1000 Hz spin-lock field amplitude,19 and
was related to changes in collagen orientation.22,23 One study
found a relationship between T1ρ relaxation at 500 Hz with
phase angle, but not with other material properties.24 The
relationship between T1ρ relaxation and elastic modulus has
yet to be established for human cartilage.
T1ρ dispersion is an interesting property of T1ρ that may
be related to cartilage macromolecular content and thus,
cartilage elastic modulus. T1ρ dispersion is characterized
by an increase in T1ρ relaxation time with increasing spin-
lock field amplitude. Previous studies quantified T1ρ
relaxation dispersion by determining the slope of the dis-
per curve, which varied for several biochemically distinct tissues.29 Another study used a power law model to
quantify T1ρ relaxation dispersion.25 In bovine nasal carti-
lage, native and trypsin degraded (to decrease macromo-
lecular content) samples had different T1ρ relaxation
dispersion curve shapes over 0 to 6000 Hz spin-lock field
amplitudes.23 Based on this relationship between cartilage
macromolecules and T1ρ dispersion, we reasoned that car-
tilage elastic modulus may be related to T1ρ dispersion
effects.
This study assessed the potential of T1ρ dispersion as a
noninvasive method to evaluate changes in cartilage lead-
ing to degeneration. The specific objectives were to answer
two questions: (1) does initial elastic modulus correlate
with T1ρ dispersion effects and (2) does proteoglycan or
collagen content correlate with T1ρ dispersion effects?

Methods
We took the following steps: specimen acquisition and
preparation, MRI, mechanical testing, biochemistry, and
statistical analysis. In agreement with previous stud-
ies, only the upper region of cartilage, the region most
affected by the indentation test, was included in our analy-
sis. Specific determination of the region of interest is dis-
cussed in the Magnetic Resonance Imaging and Biochemical
Measurements subsections.

Specimens
We examined 17 cadaveric patellae from 10 males and 7
females ranging in age from 20 to 90 years (median age 57
years). Seven cadavers contributed 1 patella and 5 cadavers
contributed both patellae to this study. Fresh-frozen cadav-
eric knee joints (mid-femur to mid-tibia) were acquired
from the National Disease Research Interchange (Philadelpahia, PA), Anatomy Gifts Registry (Glen Burnie,

Figure 1. Photograph of a representative patella and illustration
of the 7 regions. Figure adapted from Keenan et al. (2011).19

MD), and the University of California San Francisco Willed
Body Program (San Francisco, CA). Healthy patellae and
those with varied degrees of degeneration were included in
this study (photograph of representative patellae, Fig. 1). Any
patella regions with full-thickness defects were excluded.

Specimen Preparation
The patella was dissected from the knee joint, and the ante-
rior patella bone was removed with a band saw, leaving the
intact cartilage surface attached to a layer of subchondral
bone. The subchondral bone was bonded to an acrylic plate
using ethyl-2-cyanoacrylate adhesive (Krazy Glue, New
York, NY) as described by Keenan et al.,19 and then MRI
was done. Between imaging and mechanical testing, the
specimens were stored on their plates surrounded by gauze
soaked in phosphate-buffered saline plus protease inhibitors
(0.005 M benzamidine–HCl, 0.01 M N-ethylmaleimide,
0.001 M phenylmethylsulfonylfluoride, 0.005 M disodium
ethylenediamine tetra-acetic acid; Sigma Aldrich, St. Louis,
MO)31 at −20 °C. Specimens were brought to room tem-
perature prior to imaging and mechanical testing; biochemical
measurements immediately followed the mechanical
testing.

Magnetic Resonance Imaging
MRI at 3 T was performed using a GE HDx system (GE
Healthcare, Milwaukee, WI) with a transmit/receive quadra-
ture wrist coil (Mayo Clinical Medical Devices, Rochester,
MN). The plate-mounted patella specimen was placed in a
secondary container, which was filled with phosphate-buff-
ered saline plus protease inhibitors. The patella was
oriented to the normal to the most prominent point of the subchondral bone surface perpendicular to $B_0$; the image plane was also oriented perpendicular to $B_0.$ A multislice, multiecho spiral 2-dimensional sequence was used to acquire T1ρ (spin-locking field amplitudes 500 and 1000 Hz) images with 3.0 mm slice thickness, 0 mm slice spacing, 10 cm field of view, 0.3 mm in-plane pixel size, 2-second repetition time, 6 ms echo time, and 5 spin-lock times: 0, 14, 29, 59, and 118 ms. T2 images were acquired with the same parameters as T1ρ images and echo times: 6, 20, 35, 65, and 124 ms. The total acquisition time for the independently acquired T1ρ 500 Hz, T1ρ 1000 Hz and T2 scans was approximately 21 minutes. A 3-dimensional spoiled gradient echo (SPGR) sequence with 3.0 mm slice thickness, 0 mm slice spacing, 10 cm field of view, 0.3 mm in-plane pixel size, repetition time/echo time 13.5/2.5 ms and 30° flip angle was obtained for anatomic reference and modified Noyes scoring.33

**Mechanical Testing**

Creep indentation tests were performed on a mechanical test system, as described by Keenan et al.34 Locations on the surface of each patella underwent indentation creep tests while submerged in a bath of phosphate-buffered saline plus protease inhibitors.35 The initial elastic modulus was estimated from the experimental data at an initial time, $t_0,$ of 0.15 seconds using the elastic solution for the initial modulus given by Hayes et al.36:

$$E_0 = \frac{P_0 (1 - \nu_0^2)}{2 \pi r d_0}.$$  

In Equation (1), $P_0$ and $d_0$ are the applied load (0.35 N) and resulting displacement of the creep test, respectively; $\nu_0$ is the initial Poisson’s ratio; $r$ is the indenter radius (1 mm); and $\kappa$ is a dimensionless number dependent on the indenter radius, indentation displacement, cartilage thickness and Poisson’s ratio.36 Based on the assumption of near-incompressibility,37 an appropriate value was assigned to the initial Poisson’s ratio, $\nu_0 = 0.47$ for all specimens.38 A needle-probe method was used to measure the cartilage thickness,39 and a small drop of India ink was applied at the location of the needle-probe thickness measurement.

**Biochemical Measurements**

Full-thickness cartilage cylindrical samples (3 mm diameter) were removed from locations that were both within the MR image slice and close to the indentation test sites, with care taken to avoid the site of needle-probe thickness measurement. The indentation test and needle-probe thickness measurements may breakdown the tissue and alter the cartilage properties; thus, a nearby site (within 2 mm) was selected for the biochemistry.4 A 3 mm × 3 mm grid, corresponding to the MRI slice thickness, was placed across the cartilage sample; biochemistry location was identified in the MR images based on its location on the grid. Cartilage samples were cut in half along a line drawn halfway between the articular surface and cartilage/bone interface of the cartilage sample, and only the upper region, which contributes more to the indentation test,13,15,24 was analyzed in this study. Each sample was weighed to obtain wet weight. Each sample was dried at 50 °C for 12 hours, then digested in 1 mL papain solution overnight in a water bath at 63 °C, and stored at 4 °C. Proteoglycan content was measured by their covalently linked sulfated glycosaminoglycans (sGAG). Total sGAG content was quantified using the dimethylmethylene blue assay, which measures sGAG content using chondroitin sulfate as a standard.40 Collagen content was determined from hydroxyproline content; the papain-digested samples were acid hydrolyzed, and hydroxyproline was measured using the chloramine-T/Ehrlich’s reagent assay.41 sGAG and collagen values are reported as a percentage of the wet weight of each 3 mm diameter sample.

**T1ρ Ratio**

We aim to measure T1ρ dispersion effects with a simple model, using clinically feasible measurements. Thus, we estimated T1ρ dispersion effects with a ratio, determining T1ρ dispersion as a function of the baseline T2 relaxation time: T1ρ/T2. We measured T2 relaxation and T1ρ relaxation at two spin-lock frequencies: 500 and 1000 Hz; a T2 measurement is equivalent to a T1ρ measurement at spin-lock field amplitude of 0 Hz.42

In a liquid, an unstructured environment with fast dipolar interactions, the T1ρ value will approach the T2 value, and the ratio T1ρ/T2 will approach 1.73-46 In a more structured environment, T1ρ will increase compared to T2, and the ratio T1ρ/T2 will be greater than 1. Thus, the ratio, T1ρ/T2, is a measure of the complexity of the local environment of the spins.

**Data Analysis and Statistics**

Seven regions across the surface of each patella were examined (Fig. 1): center, lateral center, lateral inferior, lateral superior, medial center, medial inferior, and medial superior.19 To assess cartilage damage, an experienced radiologist performed modified Noyes scoring33 of all patellae at each of the seven regions using the 3-dimensional SPGR images and the first echo of T2 images. Scores were assigned based on cartilage appearance as follows: 0, normal appearance; 1, increased signal intensity on the T2 images; 2A, superficial partial thickness defect; 2B, deep partial thickness defect; and 3, full-thickness lesion. Analyses were performed on the entire data set and on subsets determined by the Noyes score.
MRI regions of interest (ROI) were determined at the locations corresponding to the biochemical measurements. In the plane of the 3.0 mm MRI slice, the ROI was approximately 3.0 mm wide and a minimum of 0.6 mm (2 pixels) tall; the height of the ROI varied with specimen thickness. The upper region was determined by drawing a line halfway between articular surface and cartilage/bone interface across the 3 mm ROI width; the upper and lower bounds of the ROI followed the cartilage morphology. To limit partial volume effects, the pixel at the apparent cartilage surface was not followed the cartilage morphology. To limit partial volume effects, the pixel at the apparent cartilage surface was not included in the ROI, as it could include the cartilage and phosphate-buffered saline plus protease inhibitors interface. Sample locations were excluded due to imaging artifacts, full-thickness defect or thin cartilage (fewer than 4 pixels through the depth of the cartilage), and potential magic angle effect. To reduce interference from the magic angle effect, all patella were viewed in the sagittal plane, the plane parallel to $B_0$. Gründer et al. and Xia et al. both showed that if there was a large curvature of the subchondral bone surface, resulting in an orientation angle greater than $10^\circ$ from perpendicular to $B_0$, the magic angle effect could alter the relaxation value; thus, the sample was excluded.

A total of 119 cartilage samples were examined; data from 79 locations were included in the analysis. Creep tests could not be performed at 13 locations because of excessive damage to the cartilage surface; thus, there was no estimate of initial elastic modulus at these locations. Twenty-one sample locations were excluded due to an image artifact (e.g., bubble), full-thickness defect or thin cartilage (3 of these sample locations were also locations where the creep indentation test could not be performed and thus already excluded from the analysis). Nine sample locations were excluded because of potential magic angle interference.

Average T1ρ and T2 relaxation times were measured for each ROI at each spin-lock field amplitude using OsiriX, and T1ρ/T2 was determined. T1ρ and T2 relaxation times were computed pixel-by-pixel and then averaged over the ROI. When determining T2 relaxation, the fifth echo was not used because the signal in the cartilage was not sufficiently different from the noise.

To assess relationships between T1ρ/T2 and initial elastic modulus, within-clusters Pearson correlation coefficients were determined; this method takes into account the clustering of multiple samples within a single patella specimen and within a donor. The data were divided into two subsets based on Noyes score. The first subset ($n = 58$) consisted of locations with a Noyes score of 0 (no visible cartilage damage based on conventional SPGR MRI). The second subset consisted of locations with visible damage on MRI, with Noyes scores of 1 ($n = 10$), 2A ($n = 10$), 2B ($n = 1$), or 3 ($n = 0$). There were insufficient data (two or fewer samples per specimen) in the subset of nonzero Noyes scores to further subdivide that subset, and we could not complete a within-clusters Pearson correlation analysis. Statistical analyses were performed using Stata Release 12 (StataCorp LP, College Station, TX). A $P$ value less than 0.05 was considered statistically significant.

### Results

Initial elastic modulus increased with decreasing values of T1ρ/T2 ratio (Table 1, top right corner). Pearson correlation coefficients were larger for the subset of samples with no visible cartilage damage on conventional MR compared with the entire data set (Table 1). In all cases, there were moderate relationships between initial elastic modulus and T1ρ/T2 (Fig. 2). sGAG and collagen contents were not related to T1ρ/T2 (Table 1).

T1ρ dispersion curves from one patella sample are shown (Fig. 3); data from a single patella that is representative of the entire data set is shown for clarity. The T1ρ/T2 ratio for this specimen increased with increasing spin-lock frequency, as expected (Table 2). In the subset of samples with no visible cartilage damage on conventional MR, T1ρ/T2 was smaller than in the entire data set. Thus, while there was T1ρ dispersion, T1ρ was not much greater than T2 (Table 3). 1/T2 and 1/T1ρ relaxation times were similar between the entire data set and the subset of samples with no visible cartilage damage on conventional MR.

### Table 1. Pearson Correlation Coefficients Between Initial Elastic Modulus (MPa), sGAG Content (% Wet Weight), Collagen Content (% Wet Weight), and 1/T2, 1/T1ρ, T1ρ/T2.

| Group (n) | 1/T2     | 1/T1ρ 500 Hz | 1/T1ρ 1000 Hz | T1ρ(500 Hz)/T2 | T1ρ(1000 Hz)/T2 |
|----------|----------|--------------|--------------|---------------|----------------|
| $E_0$ (MPa) | | | | | |
| Noyes = 0 (58) | -0.09 (0.553) | 0.16 (0.320) | 0.15 (0.349) | -0.37 (0.016) | -0.32 (0.034) |
| All (79) | -0.09 (0.490) | 0.12 (0.340) | 0.17 (0.194) | -0.27 (0.034) | -0.29 (0.021) |
| sGAG content (% wet weight) | | | | | |
| Noyes = 0 (58) | 0.31 (0.444) | 0.34 (0.024) | 0.28 (0.072) | -0.01 (0.945) | 0.08 (0.630) |
| All (79) | 0.14 (0.273) | 0.26 (0.041) | 0.22 (0.082) | -0.14 (0.272) | -0.09 (0.490) |
| Collagen content (% wet weight) | | | | | |
| Noyes = 0 (58) | 0.07 (0.678) | 0.00 (0.992) | -0.01 (0.946) | -0.12 (0.466) | -0.10 (0.525) |
| All (79) | -0.05 (0.678) | -0.18 (0.166) | -0.20 (0.119) | -0.16 (0.204) | -0.15 (0.253) |

Note: The correlation coefficient ($R$) is given with the $P$ value in parentheses. A $P$ value less than 0.05 was considered significant, and those results are given in boldface. sGAG = sulfated glycosaminoglycan.
Representative 1/T2 and 1/T1ρ relaxation time maps (Fig. 4B-D) and T1ρ/T2 maps (Fig. 4E and F) for a single axial slice from one patella specimen are shown.

There was no relationship between 1/T2, 1/T1ρ relaxation time (at either spin-lock field amplitude) and initial elastic modulus, and the relationships between 1/T2, 1/T1ρ relaxation time (at either spin-lock field amplitude) and macromolecular content were inconsistent. 1/T2 relaxation time and 1/T1ρ relaxation times at spin-lock field amplitudes 500 and 1000 Hz were related to initial elastic modulus neither in the entire data set nor in the subset of samples with no visible cartilage damage on conventional MR (Table 1). 1/T2 relaxation time increased with increasing sGAG content in the subset of samples with no visible damage on conventional MR (Table 1). 1/T1ρ relaxation time at spin-lock field amplitude 500 Hz increased with increasing sGAG content in the entire data set and in the subset of samples with no visible damage on conventional MR (Table 1). 1/T1ρ relaxation time at spin-lock field amplitude 1000 Hz had a similar relationship trend with sGAG content, though not statistically significant (Table 1). 1/T2 and 1/T1ρ relaxation times (at either spin-lock field amplitude) were not related to collagen content (Table 1).

Initial elastic modulus increased with increasing sGAG content in the entire data set (Table 4 and Fig. 5). Initial elastic modulus was neither related to collagen content in the entire data set nor to sGAG or collagen contents in the subset of samples with no visible damage on conventional MR (Table 4).

**Discussion**

In this *in vitro* study of human patellar cartilage, we found that T1ρ/T2, an estimate of T1ρ dispersion effects, may be able to noninvasively evaluate changes in cartilage initial elastic modulus. Initial elastic modulus was related to T1ρ/T2 in the entire data set and in the subset of samples with no MR-visible cartilage damage. This finding is interesting in light of prior MR work that has related T1ρ to proteoglycan in cartilage and T2 to collagen orientation and structure. T1ρ/T2 may have a complex relationship with cartilage macromolecules, explaining the relationship between T1ρ/T2 and initial elastic modulus.

The T1ρ/T2 ratio is not sensitive to a single macromolecular effect; rather it is responsive to the bulk cartilage properties, including molecular-level dynamics—the collagen/sGAG molecules themselves, the way they interact with other molecules and with water. Similarly, cartilage initial elastic modulus, while correlated with sGAG content, is also dependent on the interactions between water, proteoglycans and collagen structure.
Thus, $T_1/\rho/T_2$ and initial elastic modulus were correlated in this study. Based on the correlations between initial elastic modulus and sGAG content and $1/T_1\rho$ and sGAG content, we expected $1/T_1\rho$ relaxation time to correlate with initial elastic modulus. In this study, $1/T_2$ and $1/T_1\rho$ (500 and 1000 Hz) relaxation times were not correlated to initial elastic modulus. This might be due to the challenges of cadaveric cartilage. Previous results relating MRI measurements to cartilage moduli were varied, especially in the patella and when using cadaveric cartilage. In agreement with previous results, cartilage elastic modulus increased with increasing sGAG content. Aggregate modulus and GAG content were significantly correlated in the patella ($P < 0.001$), while collagen has ($P < 0.1$) and has not been related to cartilage creep moduli in cadaveric cartilage.

In this study, the relationship between $1/T_1\rho$ relaxation time and sGAG content varied with spin-lock field amplitude. Previous studies in human cartilage also varied: one study found $1/T_1\rho$ relaxation time at 1000 Hz is influenced by tissue hydration and molecular-level dynamics in addition to GAG content and collagen orientation, one study found a correlation with sGAG content, but not collagen content at 500 Hz, and another study found no relationship with sGAG content at 500 Hz. In previous studies, T2 relaxation was related to proteoglycan content, which could be a result of the cartilage hydration or complex molecular dynamics between sGAG, water and collagen. In this study, $1/T_2$ relaxation time was related to sGAG content only in the subset of samples with no visible cartilage damage on conventional magnetic resonance (Noyes = 0).
Figure 4. An axial spoiled gradient echo (SPGR) image from one patella specimen is shown (A); the main magnetic field, $B_0$, is perpendicular to the image plane. Representative $1/T_2$ (B) and $1/T_{1\rho}$ relaxation time maps for 500 Hz (C), and 1000 Hz (D) are shown. Representative $T_{1\rho}/T_2$ maps are shown for $T_{1\rho}$ 500 Hz (E) and $T_{1\rho}$ 1000 Hz (F).
knee joint is studied rather than focusing on the patella alone. In addition to using the entire knee joint, correlation coefficients could improve if more samples were included or if a wider range of cartilage is included; because of our mechanical test, we were limited to cartilage with an intact surface and a thickness greater than 1 mm. The majority of samples used in this study did not have visible cartilage damage on conventional MR, and there were no samples with full-thickness defects. The results of this study would therefore not necessarily apply to advanced osteoarthritis. Our results are primarily applicable to the early stages of cartilage degeneration. Between MR measurements and mechanical testing, the patella sample was frozen for no more than nine months, and this freeze/thaw cycle could have altered the cartilage properties, specifically the macromolecular content. Previous studies show that freeze/thaw cycles do and do not change the MR properties. Cartilage biochemistry may be affected by the indentation test. However, cartilage initial elastic modulus was strongly related to sGAG content in agreement with previous work; this result is a validation of our experimental methods.

In summary, the method for estimation of $T_1\rho$ dispersion is interesting and warrants further research. $T_1\rho/T_2$ has potential to noninvasively determine cartilage modulus, especially at 500 Hz. Specific absorption rate constraints prohibit in vivo imaging at 1000 Hz, but advances in $T_1\rho$ imaging techniques may be able to overcome the specific absorption rate issues and allow application of $T_1\rho(1000 Hz)/T2$ in vivo. $T_1\rho/T2$ has clinical potential to advance the understanding of osteoarthritis and other cartilage diseases by noninvasively measuring changes in cartilage initial elastic modulus in vivo prior to advanced cartilage damage.

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**Declaration of Conflicting Interests**

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Ethical Approval
This study was approved by our institutional review board.

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