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Orientational Dependent Sensitivities of T_2 and $T_{1\rho}$ towards trypsin degradation and Gd-DTPA^{2-} presence in bovine nasal cartilage

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Abstract

Object—To study the orientational dependencies of T_2 and $T_{1\rho}$ in native and trypsin-degraded bovine nasal cartilage, with and without the presence of 1 mM Gd-DTPA^{2-} .

Materials and Methods—Sixteen specimens were prepared in two orthogonal fibril directions (parallel and perpendicular), treated using different protocols (native, Gd treated, and trypsin-treated, combination), and imaged using μMRI at 0° and 55° (the magic angle) fibril orientations with respect to the magnetic field B_0 . Two-dimensional (2D) T_2 and $T_{1\rho}$ images were then calculated quantitatively.

Results—Without Gd, native perpendicular tissues demonstrated significant $T_{1\rho}$ dispersion (including T_2 at the zero spin-lock field) at 0° and less dispersion at 55° , while native parallel specimens exhibited smaller $T_{1\rho}$ dispersion at both 0° and 55° . Trypsin degradation caused a minimum 50 % increase in $T_{1\rho}$. With Gd, trypsin degradation caused significant reduction in $T_{1\rho}$ values up to 60%.

Conclusion—The collagen orientation in nasal cartilage can influence T_2 and $T_{1\rho}$ MRI of cartilage. Without Gd, $T_{1\rho}$ was sensitive to the proteoglycan content and its sensitivity was nearly constant regardless of fibril orientation. In comparison, the T_2 sensitivity to proteoglycan was dependant upon fibril orientation, i.e., more sensitive at 55° than 0° . When Gd ions were present, both T_2 and $T_{1\rho}$ became insensitive to the proteoglycan content.

Keywords

T_2 ; $T_{1\rho}$; nasal cartilage; collagen fibril; proteoglycan; magic angle; anisotropy; spin lock

Introduction

Articular cartilage is a type of connective tissue covering the end of bones in joints and consists mainly of water, proteoglycans (PG) and collagen fibrils [1–3]. A loss of negatively charged PG macromolecules from the extracellular matrix of cartilage is a hallmark in the early development of osteoarthritis (OA), a disorder affecting a significant portion of the

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human population. Magnetic resonance imaging (MRI) is the most sensitive medical imaging technique for noninvasive assessment of soft tissue such as cartilage. However, conventional MRI, i.e., intensity based, is insensitive to the subtle biochemical changes associated with the early stage of cartilage lesions [4–6]. Recently, several MRI-based protocols such as gadolinium-enhanced MRI (dGEMRIC) [7–11], sodium MRI [12, 13], anisotropy of T_2 relaxation [14–16], and $T_{1\rho}$ relaxation (spin-lattice relaxation in the rotating frame) [17–20], were found to be sensitive to PG content in cartilage.

Transverse relaxation T_2 measures the decay in phase coherence between individual nuclear spins and is sensitive to the dipolar interaction due to the anisotropic motion of water molecules, which is primarily modulated by the orientational structure of collagen fibrils in cartilage [14]. Due to the dipolar interaction, shortening T_2 values can cause a laminar appearance of articular cartilage in MRI when the local collagen fibrils are orientated parallel with the external magnetic field B_0 [14, 21–23]. By rotating the fibril orientation by approximately 55° with respect to B_0 , the dipolar interaction is minimized thus eliminating the laminar appearance; a result known as the “magic-angle effect” in cartilage MRI literature [15, 16, 24]. As previously stated, a number of studies demonstrate that T_2 experiments are sensitive to PG content in tissue [25, 26].

In addition to T_2 , $T_{1\rho}$ has recently been used in cartilage MRI [17–20, 27–32], since it is sensitive to slow motional interactions between confined water molecules and the macromolecular environment [27, 30]. Recent studies show that the $T_{1\rho}$ relaxation rate ($R_{1\rho} = 1/T_{1\rho}$) decreases linearly with decreasing PG content and appears more sensitive than T_2 toward PG variation in both osteoarthritic human specimens and bovine patellae [29]. It is also reported that the spin-lock technique can eliminate the effect of residual dipolar interaction, which may lead to a more accurate diagnosis of degenerative changes in cartilage [33]. Although $T_{1\rho}$ shows great potential for clinical applications in detecting cartilage degradation, specimen orientation with respect to the main magnetic field B_0 has not been extensively discussed.

Another factor related to clinical application of relaxation time is the possible introduction of Gd-DTPA^{2-} ions into cartilage in medical protocols such as dGEMRIC [9]. Several studies in literature discuss T_2 dependency on Gd-DTPA^{2-} ions present in tissue [34–36], but rarely $T_{1\rho}$ dependency on Gd-DTPA^{2-} ions. This project has three aims. First, the sensitivities of both T_2 and $T_{1\rho}$ relaxation times are studied in native and trypsin-degraded specimens of bovine nasal cartilage (BNC), with and without Gd-DTPA^{2-} ions present. Second, although BNC is commonly used as a model material in the study of connective tissue due to its more homogenous structure, BNC was recently found to possess a certain heterogeneities in fibril organization which can cause measurable anisotropies in polarized light microscopy (PLM), microscopic MRI (μMRI) and mechanical experiments [37, 38]. This μMRI project is carried out with full knowledge of fibril orientation in bovine nasal cartilage specimens. Finally, the effects of specimen orientation in the magnetic field and the dependency of $T_{1\rho}$ on spin-lock field strength are also investigated in this project.

Materials and Methods

Bovine nasal cartilage specimens

Fresh bovine nasal cartilage was obtained from a local slaughter house (C. Roy, Yale, MI). The tissue was immersed in physiological saline (154 mM NaCl in deionized water) with 1 % protease inhibitor (Sigma, Missouri) and stored at -20°C before experimentation. Sixteen individual specimens, each approximately $1.5 \times 1.5 \times 8 \text{ mm}^3$ in size, were harvested from the central part of a large nasal septa in two different orientation groups (Fig 1). Eight specimens were cut such that the collagen fibrils were oriented perpendicular to the tissue block length (perpendicular specimens) and eight were cut such that the collagen fibrils were oriented parallel to the tissue block length (parallel specimens). The collagen fibrils followed a previously determined orientation such that the fibril long axes were oriented parallel to the medial axis of the nasal septum [38].

All specimens were imaged individually using the same MRI protocol prior to any chemical treatment, providing a control MRI. Prior to additional MRI experiments, four specimens from each group were immersed in 1 mM Gd-DTPA²⁻ (gadolinium diethylene triamine pentaacetic acid) solution (Magnevist, Berlex, NJ) in saline with 1 % protease inhibitor (Sigma, Missouri) for approximately 10 hours [10]. The other four specimens from each group were first soaked in 10 $\mu\text{g/ml}$ trypsin solution (Sigma, Missouri) for more than 6 hours to digest proteoglycans [39] then soaked in fresh saline with 1 % protease inhibitor to remove excess trypsin. After repeating MRI, these PG-depleted specimens were immersed in a Gd-DTPA²⁻ solution for more than 10 hours and imaged a third time using the same protocol.

Microscopic MRI (μMRI) Protocols

All μMRI experiments were performed using a Bruker AVANCE II 300 NMR spectrometer equipped with a 7-Tesla/89-mm vertical-bore superconducting magnet and microimaging accessory (Bruker Instrument, Billerica, MA). A homemade 4 mm solenoid coil was used in the μMRI experiments, which produced a 90° hard pulse of 5 μs duration. Quantitative T_2 -imaging experiments were performed using a CPMG magnetization-prepared T_2 imaging sequence [40]. The $T_{1\rho}$ imaging sequence proceeded with a 90° hard pulse followed by a spin-lock pulse. The power of the spin-lock field varied from 0.5 – 6 kHz, i.e., 500, 1000, 2000, 3500, 5000, 6000 Hz, and the spin-lock field strength was calibrated by adjusting the 90° pulse strength.

2D MRI experiments were carried out using an acquisition matrix of 32×32 ($100 \times 100 \mu\text{m}$ pixel resolution) and a slice thickness of 1 mm. The specimen was loaded into the receiver coil and ensured that the long axis of the tissue block was perpendicular to the magnetic field B_0 direction, which was used as the reference angle (Fig 1). The specimen blocks were imaged at different orientations with respect to B_0 , i.e., the 0° and 55° orientations of the perpendicular block were such that the orientations of the collagen fibrils were parallel or at 55° to B_0 , respectively. The orientations of the parallel blocks were imaged in a similar manner. Since the fibril axis was parallel with the long dimension of the tissue block, the 0° – 55° rotation did not change the fibril orientation in the magnetic field for the parallel blocks.

A repetition time (TR) of 2 s was used for specimens without Gd-DTPA²⁻ and 0.8 s for specimens treated with 1mM Gd-DTPA²⁻ since the presence of Gd-DTPA²⁻ shortened the T₁ significantly [10]. The echo spacing in the CPMG T₂-weighting segment was 1 ms and the number of echoes was 2, 30, 60, 120, 240, which yielded the echo times (TE) of 2, 30, 60, 120, and 240 ms, respectively. The spin-lock pulse durations were 2, 30, 60, 120, and 240 ms for each T_{1ρ} measurement.

2D T_{1ρ} images were calculated pixel-by-pixel using a common equation: $\text{Sig}(\text{TSL}) = \text{Sig}_0 \exp(-\text{TSL}/T_{1\rho}) + K$, where TSL was the time of spin-lock pulse, Sig was the signal intensity of the observed signal, Sig₀ was the thermal equilibrium magnetization, and K was a constant offset. 2D T₂ images were calculated pixel-by-pixel using a similar equation: $\text{Sig}(\text{TE}) = \text{Sig}_0 \exp(-\text{TE}/T_2) + K$, where TE was the echo time. Both calculations were done off-line using a custom Matlab (MathWorks, Natick, MA) code. Since there was no significant variation within the 2D image slice, an 8×8 pixel region-of-interest was chosen from the center of each image and averaged to yield one relaxation value and standard deviation. The averaged relaxation values and standard deviations calculated from each image were used to generate line plots.

Results

Perpendicular BNC specimens

Fig 2 shows the T₂ images, the 2 kHz spin-lock field T_{1ρ} images and the dispersion curves of all T_{1ρ} and T₂ imaging data from perpendicular specimens. When the spin-lock field is zero, a T_{1ρ} imaging sequence essentially becomes a T₂ imaging sequence. For this reason, the T₂ results are grouped together with the T_{1ρ} results and T₂ is labeled as T_{1ρ} at zero field in the dispersion curves. Several distinct characteristics can be identified in these complex relaxation results. First, the anisotropic effects of both T₂ and T_{1ρ} exist in nearly all cases since the relaxation values are always higher when the specimen is oriented at 55° than when at 0° with respect to B₀. The greatest relaxation difference as the function of specimen orientation occurs in samples containing no Gd ions and when the spin-lock field is zero (T₂ values in Fig 2a and 2c). For tissue subject to an increasingly larger spin-lock field or tissue treated with Gd, the orientation-dependant relaxation variation decreases. Second, T_{1ρ} relaxation is found to be a function of the spin-lock field: the larger the spin-lock field, the longer the T_{1ρ} relaxation. The largest increase in T_{1ρ} relaxation time occurs at low spin-lock fields (less than 2 kHz). Additionally, T_{1ρ} relaxation values increase only slightly when the spin-lock field is higher than 2 kHz. Third, the largest difference between native and degraded tissue occurs when the tissue contains no Gd ions (comparison between Fig 2a and 2c). With the immersion of tissue in Gd solution, both T₂ and T_{1ρ} sensitivity toward tissue degradation reduces markedly (comparison between Fig 2b and 2d).

Parallel BNC specimens

Fig 3 summarizes the T₂ images, the T_{1ρ} images at 2 kHz, and the dispersion curves of all T_{1ρ} and T₂ imaging data from the parallel specimens. Comparing with the perpendicular samples, the most significant difference is the lack of anisotropic variation between 0° and 55° for both T₂ and T_{1ρ} values, which clearly indicates that the fibril orientation in the

parallel specimens are parallel with the rotation axis (Fig 1b). Consequently, a rotation of the parallel specimen does not change its fibril orientation in the magnetic field. Apart from this distinction, other features seen in the perpendicular specimens are also present in the parallel specimens.

The differences between perpendicular and parallel BNC

To quantify variations of T_2 and $T_{1\rho}$ dispersion for different orientations, different spin-lock fields and when under the influence of Gd ions, the complex data shown in Fig 2 and Fig 3 are summarized together in Fig 4 as the percentage ratios (percentage ratio = $100 \times (\text{treated}/\text{control})$), where the control is any base value of the tissue before the tissue was treated in any procedure (e.g., soaked in Gd, digested in trypsin, or combination treatment).

In comparing degraded and native tissues (Fig 4a and Fig 4b), the trypsin digestion causes $T_{1\rho}$ values to increase by at least 50 % (from the 100 % base value to 150–180 %), regardless of (a) whether it is cut in perpendicular or parallel sections, or (b) tissue orientation, as long as Gd ions are not present in the tissue. The only exception is the T_2 data of the perpendicular section at 0° , which shows no sensitivity toward tissue degradation. The weakly spin-locked $T_{1\rho}$ data (e.g., less than 1 kHz) appear to have a similar exception as T_2 , but on a less scale. Fig 4a and Fig 4b also demonstrate that the immersion of tissue in Gd solution completely eliminates the sensitivity of T_2 and $T_{1\rho}$ toward tissue degradation. In fact, the ratios of degraded/native are less than the 100% base value, indicating the T_2 and $T_{1\rho}$ relaxation values are reduced when Gd ions are present in trypsin-degraded tissue. These data suggest that both T_2 and $T_{1\rho}$ are most sensitive to PG loss in cartilage as long as the tissue is not oriented at 0° and there are no Gd ions present.

The effect of Gd ion presence in cartilage is illustrated clearly in Figs 4c and 4d. With the immersion of tissue in a Gd solution, the $T_{1\rho}$ values are reduced by about 20–25 % in native tissues and about 50–60 % in degraded tissues, regardless of specimen orientation in the magnetic field or whether parallel or perpendicularly cut. The only exceptions are the T_2 and weakly spin-locked $T_{1\rho}$ (less than 1 kHz) at the 0° specimen orientation. In these specific cases, the influence of Gd on relaxation values is minimal.

The sensitivity of T_2 and $T_{1\rho}$ toward tissue degradation

Since there is little difference after the spin-lock field is increased beyond 2 kHz, three representative relaxation parameters, T_2 , $T_{1\rho}(0.5 \text{ kHz})$, and $T_{1\rho}(2 \text{ kHz})$, were closely examined for their sensitivities toward tissue degradation in Fig 5, which revealed three distinct features. First, there is little sensitivity in T_2 to tissue treatment for all relaxation parameters when the perpendicular specimens are at 0° (the first group of columns in Fig 5a), which illustrates the strong influence of dipolar interaction on the relaxation mechanism. When the dipolar interaction is strong, the T_2 is uninfluenced by either native or degraded tissue. Second, with the presence of Gd ions in the tissue, these parameters show little sensitivity toward both native and degraded tissue (white columns in Fig 5), regardless of the specimen orientation (0° , 55°). Finally, these three parameters have good sensitivities in all other situations, with the greatest sensitivity, i.e., the biggest difference, between two

distinct situations: (1) degraded tissue with and without the presence of Gd (gray columns), and (2) native tissue versus degraded tissue (shaded columns).

Discussion

Although nasal cartilage was commonly considered homogeneously structured, it was noticed only recently that BNC also possesses some structural anisotropy in its collagen fibril orientation, which could contribute to a number of anisotropies in tissue histology by polarized light microscopy, μ MRI and mechanical measurements [37, 38]. In this project, the BNC specimens were carefully prepared in two different fibril orientations, perpendicular and parallel, based on precise knowledge of fibril orientation [38]. Several critical issues related to the sensitivities of T_2 and $T_{1\rho}$ measurements toward cartilage degradation were investigated. These issues included: (1) the characteristics of $T_{1\rho}$ dispersion, i.e., the value of $T_{1\rho}$ as a function of the spin-lock field B_1 , (2) the specimen orientation in the magnetic field B_0 and (3) the PG content in the tissue. These issues were investigated with and without the presence of Gd ions.

Sensitivities of T_2 and $T_{1\rho}$ towards tissue degradation without the presence of Gd

T_2 in articular cartilage can be influenced strongly by the dipolar interaction due to the anisotropic motion of water molecules associated with collagen fibrils [14]. It is interesting to observe that $T_{1\rho}$ in BNC is also orientation dependent when the spin-lock field is less than 1 kHz (although it is to a less extent compared to T_2 anisotropy). This result implies the existence of *weak* residual dipolar interactions in BNC under *weak* spin-locked fields. When the spin-lock field is stronger than 2 kHz, a minimization of the dipolar interaction [33] eliminates the orientational difference of the cartilage specimen in the B_0 , resulting in a nearly constant $T_{1\rho}$.

Since T_2 is known to be sensitive to both tissue orientation and PG concentration [26, 39, 40], T_2 has been used extensively in cartilage MRI. However, in clinical MRI, the reliability of detecting of cartilage lesions using T_2 has been inconsistent [41, 42]. One major problem is the depth-dependent T_2 anisotropy in articular cartilage [14]. Since most human joints lack any simple geometric shape, it is not possible to orient a patient in the magnet such that the influence of dipolar interaction for the entire joint tissue is uniform. Consequently, T_2 values in human MRI are influenced not only by the health status of cartilage but also by tissue orientation in the MRI magnet. It is clear from this project that with a sufficient spin-lock field of 1 kHz or higher, the dipolar interaction in cartilage can be minimized in $T_{1\rho}$ measurements. We can therefore conclude that when the specimen orientation is controlled, T_2 and $T_{1\rho}$ both have excellent sensitivity toward cartilage degradation. Because the T_2 pulse sequence does not have a spin-lock pulse, the rf power deposition on the tissue in T_2 imaging is less than in the $T_{1\rho}$ case, which is a unique advantage in T_2 imaging. When the specimen orientation is unknown or cannot be controlled, i.e., human in vivo MRI, $T_{1\rho}$ is a more useful parameter than T_2 due to its insensitivity to fibril orientation.

In addition, even at the magic angle, in which the residual dipolar interaction is largely reduced, $T_{1\rho}$ relaxation time is longer than T_2 . A longer relaxation time is always a welcome feature in clinical MRI since imaging protocols commonly have a long echo time, i.e., less

attenuation of the available signal enables improved visualization of the specimen in any intensity based MRI.

Sensitivities of T_2 and $T_{1\rho}$ towards tissue degradation in the presence of Gd ions

The situation is entirely different with the presence of Gd ions in the tissue. First, in contrast to a significant increase of up to 80% as with tissues containing no Gd, $T_{1\rho}$ decreases in trypsin-degraded specimens, likely caused by the influx of additional Gd ions to the PG-reduced tissue. Second, T_2 becomes insensitive to tissue degradation in the presence of Gd ions, suggesting the paramagnetic Gd-DTPA²⁻ ions dominate the relaxation processes, which mask the effect of dipolar interaction. In fact, if $T_{1\rho}$ images with and without Gd are both available, the difference between these two $T_{1\rho}$ images might be a sensitive indicator of cartilage health. This is because $T_{1\rho}$ is consistently more sensitive to the presence of Gd-DTPA²⁻ in PG degraded specimens than in native specimens, i.e., the black columns in Fig 5 (which equal to " $T_{1\rho\text{-native}} - T_{1\rho\text{-native-Gd}}$ ") are always much smaller than the gray columns (which equal to " $T_{1\rho\text{-degraded}} - T_{1\rho\text{-degraded-Gd}}$ "). Please note that the use of Gd contrast agent should be limited to its necessity. If Gd ions were being administered into patients (like in the dGEMRIC protocol), however, a double $T_{1\rho}$ protocol would be more advantageous than a proper dGEMRIC protocol that requires two T_1 scans, since $T_{1\rho\text{-before}}$ has its own clinical significance but $T_{1\text{-before}}$ does not.

Conclusions

In this project, both T_2 and $T_{1\rho}$ relaxation times at both 0° and the magic angle were studied in native and trypsin-degraded BNC specimens, with and without the presence of 1 mM Gd-DTPA₂. To the best of our knowledge, this was the first μ MRI T_2 and $T_{1\rho}$ project of bovine nasal cartilage with full awareness of fibril orientation. Native perpendicular specimens demonstrated significant $T_{1\rho}$ dispersion at 0° and lesser dispersion at the magic angle while native parallel specimens exhibited less anisotropic change of $T_{1\rho}$ dispersion due to fibril axes being parallel with the rotational axis regardless of rotational angle. We have shown that $T_{1\rho}$ values are very sensitive to PG loss regardless of specimen orientation with respect to the magnet field B_0 . By comparison, the sensitivity of T_2 measurements relative to PG loss was dependant upon fibril orientation and exhibited greater sensitivity at the magic angle than 0° . Additionally, the presence of 1 mM Gd-DTPA²⁻ reduced the sensitivity of both T_2 and $T_{1\rho}$ toward cartilage degradation.

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Figure 1.

Two different ways of preparing the BNC specimens: (a) perpendicular and (b) parallel. The solid short lines inside the blocks illustrate the orientation of the collagen fibrils in BNC.

The specimens were imaged under two orientations with respect to the main magnetic field B_0 , pointing upwards. The specimen rotation was respect to the long axis of the tissue block.

Figure 2.

$T_{1\rho}$ dispersion plots of the perpendicular specimens with and without Gd-DTPA²⁻ at 0° (open circles) and the magic angle (solid squares) for (a) native specimens, (b) native specimens with Gd-DTPA²⁻, (c) trypsin-degraded specimens, (d) trypsin-degraded specimens with Gd-DTPA²⁻. All images were plotted with the same intensity limits (0 – 400 ms) in the usual gray scale.

Figure 3.

$T_{1\rho}$ dispersion plots of the parallel specimens with and without Gd-DTPA²⁻ at 0° (open circles) and the magic angle (solid squares) for (a) native specimens, (b) native specimens with Gd-DTPA²⁻, (c) trypsin-degraded specimens, (d) trypsin-degraded specimens with Gd-DTPA²⁻. All images were plotted with the same intensity limits (0 – 400 ms) in the usual gray scale.

Figure 4.

The percentage ratios of $T_{1\rho}$ dispersion plots of the perpendicular specimens (left) and parallel specimens (right), under different experimental conditions. No change would result in a ratio of 100. Increasing or decreasing with respect to the control would result in above 100 or below 100, respectively.

Figure 5. The sensitivity (i.e., difference) of (a) T_2 , (b) $T_{1\rho}$ 500 Hz, and (c) $T_{1\rho}$ 2 kHz of under different experimental conditions.