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Dependencies of multi-component T_2 and $T_{1\rho}$ relaxation on the anisotropy of collagen fibrils in bovine nasal cartilage

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ABSTRACT

Both NMR spectroscopy and MRI were used to investigate the dependencies of multi-component T_2 and $T_{1\rho}$ relaxation on the anisotropy of bovine nasal cartilage (BNC). The non-negative least square (NNLS) method and the multi-exponential fitting method were used to analyze all experimental data. When the collagen fibrils in nasal cartilage were oriented at the magic angle (55°) to the magnetic field $\mathbf{B_0}$, both T_2 and $T_{1\rho}$ were single component, regardless of the spin-lock field strength or the echo spacing time in the pulse sequences. When the collagen fibrils in nasal cartilage were oriented at 0° to $\mathbf{B_0}$, both T_2 and $T_{1\rho}$ at a spin-lock field of 500 Hz had two components. When the spin-lock field was increased to 1000 Hz or higher, $T_{1\rho}$ relaxation in nasal cartilage became a single component, even when the specimen orientation was 0°. These results demonstrate that the specimen orientation must be considered for any multi-component analysis, even for nasal cartilage that is commonly considered homogenously structured. Since the rapidly and slowly relaxing components can be attributed to different portions of the water population in tissue, the ability to resolve different relaxation components could be used to quantitatively examine individual molecular components in connective tissues.

1. Introduction

The transverse relaxation time T_2 measures the decay in phase coherence between the individual nuclear spins and is sensitive to the structure and orientation of the collagen fibrils in connective tissues (e.g., articular cartilage) in the external magnetic field $\mathbf{B_0}$ [1,2]. This strong T_2 anisotropy in articular cartilage is depth-dependent [2,3], which causes the laminar appearance in MRI of articular cartilage; this is also known as the magic angle effect since the tissue laminae would disappear when the fibril orientation was set at 54.7° to $\mathbf{B_0}$ [3–7]. Because of its sensitivity to the fibril orientation, T_2 relaxation and its anisotropy have been used to study the degradation of articular cartilage [8–11], which is the hallmark of degenerative joint diseases, such as osteoarthritis, that affect a significant portion of the adult population.

In addition to T_2 relaxation, $T_{1\rho}$ relaxation (the spin–lattice relaxation in the rotating frame) is found to be sensitive to the proteoglycan content in osteoarthritic cartilage [12–15] because of its sensitivity to the slow motional interactions between local macromolecular environments and the confined water molecules [13,16]. Different from the anisotropy of T_2 relaxation, which can mainly be manipulated by the fibril orientation, the anisotropy of $T_{1\rho}$ relaxation can also be manipulated by the spin-lock technique. Consequently, $T_{1\rho}$ could have more uniform sensitivity toward the detection of osteoarthritis, regardless of the local fibril orientation, which is a welcome advantage over T_2 in clinical diagnose using MRI [17].

Another important aspect of T_2 relaxation is its multi-component characteristics in connective tissues including nasal cartilage, articular cartilage, tendon, and muscle [1,11,18–29]. In bulk tissues measured by NMR spectroscopy, T_2 seems consistently multi-component. For example, Fullerton et al. found the T_2 relaxation in bovine tendons to be bi-exponential (4 ms and 22 ms) [18], which was also confirmed in a later study [11]. In bovine articular cartilage, Henkelman et al. [1] showed that the distribution profiles of bulk T_2 relaxation had at least two peaks, centered around 20 ms and 55 ms at 0°, and that the 20-ms peak largely disappeared when the tissue's orientation was about 55° to $\mathbf{B_0}$. In bovine nasal cartilage (BNC), Reiter et al. [26] found three T_2 components (2.3 ms (6.2%), 25.2 ms (14.5%), 96.3 ms (79.3%)).

Unlike the multiple T_2 components in bulk specimens, the multi-component analysis of T_2 relaxation in high-resolution MRI remains highly inconsistent. One of the first studies on the issue was carried out by Keinan-Adamsky et al. [24], who noticed that the deep part of swine articular cartilage had two age-dependent T_2 components (e.g., 12 ms (39%) and 45 ms (61%) for 12-month-old tissue) by MRI. A similar imaging result in both young and mature bovine nasal cartilage was recently reported by Reiter et al. that T_2 had

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two major components in BNC [30]. However, this multi-component T_2 in nasal and articular cartilage was not observed in two microscopic MRI (μMRI) work in our lab, where T_2 in both types of tissue was found to be single components [11,29]. Reiter et al. recently attempted to discover the cause of this controversy; they considered the disruption of cartilage microstructure by the freeze-thawing storage procedure [42].

Our hypothesis concerning this controversial issue of whether T_2 in nasal cartilage by MRI was single or multiple component came from a different direction – the recent observation that nasal cartilage, which had been largely known as a homogeneous connective tissue [31,32], actually had weak but measurable anisotropy in its fibril structure. The first observation on the topic, to the best of our knowledge, was a biomechanical work that noticed the mechanical modulus of the tissue to be different if the tissue was compressed from three orthogonal directions [33]. A comprehensive set of experiments in our lab [34] that measured the same bovine nasal cartilage block using μMRI, polarized light microscopy, and mechanical indentation led to the re-discovery that the collagen fibrils in nasal cartilage were anisotropically oriented.

The goal of this project was to investigate the multi-component issue of both T_2 and $T_{1\rho}$, with the knowledge of the collagen fibril orientation in bovine nasal cartilage. The T_2 and $T_{1\rho}$ in the specimens were measured when the tissue block was oriented at 0° and 55°. Furthermore, the influences of two additional experimental issues on the measurement of T_2 and $T_{1\rho}$ relaxation were also studied: the influence of different echo spacing on T_2 relaxation in NMR spectroscopy and the influence of different spin-lock fields on multi-components of $T_{1\rho}$ in both NMR spectroscopy and MRI at microscopic resolution.

2. Materials and methods

2.1. Bovine nasal cartilage

Bovine tissue was obtained fresh from a local slaughterhouse. The central part of a large piece of nasal cartilage was harvested, immersed in physiological saline (154 mM NaCl in deionized water) with 1% protease inhibitor (Sigma, Missouri), and stored at −20 °C before the experiments. Before the specimen preparation, the BNC block was thawed to the room temperature and cut into 10 specimens, each approximately 3 mm × 3 mm × 8 mm. The orientations of the individual specimens with respect to the large BNC block and the animal were noted; the fibril direction in the block was perpendicular to the long dimension of the block [34].

2.2. NMR spectroscopy and microscopic MRI

NMR spectroscopic and μMRI experiments were performed at room temperature on a Bruker AVANCEII300 NMR spectrometer equipped with a 7-Tesla/89-mm vertical-bore superconducting magnet and micro-imaging accessory (Bruker Instrument, Billerica, MA). A homemade 5 mm solenoid coil was used in the NMR spectroscopy and μMRI experiments. The BNC specimens were surface-blotted dry to remove excess surface water and subsequently immersed in Fluorinert FC-77 liquid (3 M, St. Paul, MN), which has similar susceptibility to tissue and low water solubility [29]. This immersion fluid produced no NMR and/or MRI signal while minimizing the influence of the magnetic susceptibility difference between the tissue and air. The first-order automatic shimming was performed before each imaging and spectroscopy experiment.

Each block was imaged when the fibril direction of the block was 0° and 55° with respect to $\mathbf{B_0}$ respectively. T_2 imaging experiments were performed using a Carr-Purcell-Meiboom-Gill (CPMG) magnetization-prepared T_2 imaging sequence [11,29]. The echo spacing in the CPMG T_2-weighting segment was 1 ms to avoid the spin-locking effect [35]. The number of echo times was 46, resulting in 46 delays from 2 to 600 ms. The $T_{1\rho}$ imaging sequence consisted of a $T_{1\rho}$-weighting segment, which had a 90° pulse followed by a spin-lock pulse. The power of the spin-lock pulse varied from 0.5–2 kHz (500, 1000, 2000 Hz). The strength of the spin-lock field was calibrated by the strength of the 90° rf pulse. The lengths of the spin-lock pulses were equaled to the 46 echo times in the T_2 imaging experiments. The 2D imaging parameters were consistent for all experiments: the echo time/pulse repetition = 3 ms/2 s; the number of scans = 12; the field of view (FOV) = 4.5 mm × 4.5 mm. The imaging matrix size was 32 × 32, which yielded the transverse pixel resolution of 140 μm. The slice thickness was 1 mm. A minimum SNR of 1000 was achieved for all experiments. The typical length of a 90° rf pulse was 6.5 μs.

The bulk T_2 relaxation by NMR spectroscopy was measured by the standard CPMG sequence, which was similar to that of the MRI experiments except without the 2D imaging segment. Seventy-five data points were acquired for each of the three echo spacings (0.6 ms, 1 ms, 3 ms). The repetition time was 8 s; the number of dummy scans was 8; the number of scans was 8; and a minimum SNR of about 3000 was achieved for all experiments. The parameters of $T_{1\rho}$ experiments were similar to T_2 experiments, while the length of spin-lock pulse was equaled to the 75 echo times in the CPMG experiments.

2.3. T_2 and $T_{1\rho}$ relaxation analysis

To calculate both T_2 and $T_1\rho$ relaxation times, the non-negative least-squares (NNLS) method [36,37], implemented with Matlab codes (MathWorks, Natick, MA), and the multi-variable exponential fitting in KaleidaGraph (Synergy Software, Reading, PA) were used [29]. In MRI data analysis, the central region of 8 pixels by 8 pixels was averaged to improve the signal-to-noise ratio. In the NNLS analysis of the T_2 and $T_1\rho$ spectrum, any T_2 or $T_1\rho$ component with a value below or above two constant thresholds (1.5 ms, 250 ms) was ignored to eliminate the dependence of the fit on the experimental noise [19,23,38]. The use of the NNLS meant that the results in this project were calculated without *a priori* assumptions about the number of T_2 and $T_1\rho$ components and any initial guesses of the solution.

3. Results

3.1. Proton intensity images

Forty-six intensity images were acquired for each specimen during each multi-component imaging experiment, each image at a different T_2 or $T_{1\rho}$ weighting. Fig. 1 shows the representative sets of BNC images at the specimen orientation of 0° (Fig. 1a–d) and 55° (Fig. 1e–h) with respect to $\mathbf{B_0}$. Two features could be identified from these intensity images. First, the tissue showed stronger anisotropy when the specimen orientation changed in the T_2 and the $T_{1\rho}$ at low spin-lock field (500 Hz) experiments (comparing Fig. 1a and b and e and f) – likely due to the minimization of the dipolar interaction at the magic angle. Second, $T_{1\rho}$ images at high spin-lock fields (1000 Hz and higher) appeared considerably more uniform, especially at the magic angle.

3.2. T_2 and $T_{1\rho}$ by NMR spectroscopy and MRI

Fig. 2 shows the T_2 relaxation as the functions of the echo spacing (τ = 0.6 ms, 3 ms), both at 0° (left) and 55° (right) in NMR spectroscopy. At the 0° orientation, the signal decay at any echo spacing