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Diffusion tensor imaging of native and degenerated human articular cartilage

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Abstract

Diffusion tensor imaging (DTI) is potentially sensitive to collagen degeneration in cartilage. In this study, DTI was measured on human cartilage samples with interventions of trypsin and collagenase. The measured preferred diffusion direction was consistent with the zonal structure of collagen network. The glycosaminoglycan concentration decreased and apparent diffusion coefficient increased with both interventions. The fractional anisotropy (FA) was not affected by trypsin and showed a slight increase with combined trypsin and collagenase intervention. DTI in cartilage is technically challenging due to the low FA and the almost undetectable change with collagen disruption seen here.

Keywords: Diffusion tensor imaging; Cartilage; Degradation; Trypsin; Collagenase

1. Introduction

Collagen, the predominant extracellular matrix protein in cartilage, is essential to cartilage integrity and is damaged and lost in degenerative diseases of cartilage, like arthritis. Thus, with the advent of imaging technologies, considerable effort over the past decade has been devoted to finding ways of nondestructively evaluating the state of collagen within an intact joint.

Collagen fibers are composed of triple helical molecules linked by inter- and intramolecular bonds. These fibers are organized into a higher architectural structure with a well-known zonal variation. In the superficial zone, the collagen fibers are organized mainly in parallel to the cartilage surface. In the transitional zone, the fibers show random orientation. In the deep zone, the collagen fibers are mainly oriented perpendicular to the cartilage–bone interface.

The structure of collagen can be affected by many factors, including changes in the collagen per se (e.g., collagenase cleavage of the molecule) and changes in the macromolecular composition of the tissue as a whole [e.g., redistribution of water within and outside collagen and increased hydration with the loss of glycosaminoglycans (GAGs)].

Diffusion imaging offers one potential strategy that may provide information that is related to the integrity of the collagen network. An apparent diffusion coefficient (ADC) can be computed from an MR measurement of the root-mean-square (RMS) displacement of water molecule during a specified diffusion time. Macromolecules restrict the RMS displacement of water (compared with their displacement in bulk water); thus, the ADC is generally lower in cartilage than in bulk water.

While ADC studies provide an average apparent diffusivity, it is also possible to examine directional dependence of diffusivity using diffusion tensor imaging (DTI) [1]. DTI has been used to study the structure of ordered biological tissue nondestructively, such as brain [2], myocardium [3] and intervertebral disc [4]. DTI reveals the tissue structure under the assumption that the direction of the primary eigenvector of the diffusion tensor is parallel to the local fiber orientation. Diffusion anisotropy in normal human patella cartilage tissue has recently been demonstrated [5]. However, there has not yet been a report of DTI in degenerated cartilage.

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The objective of this study was to determine whether DTI was affected by interventions that were designed to mimic pathophysiologically meaningful states. Two such interventions were examined: enzymatic treatment with trypsin (to remove noncollagenous macromolecules) and with combined trypsin and collagenase (to additionally impose collagen molecular damage).

2. Methods

2.1. Sample preparation and degradation

Human femoral condyle cartilage–bone specimens were obtained from an outside vendor (Ardais, now Cytomix, Lexington, MA). Cartilage appeared grossly intact and was typically 2–3 mm thick. All samples were cut to fit the NMR tube with a diameter of 10 mm. Samples were frozen prior to use. Degradation was induced using two types of intervention: trypsin and trypsin followed by collagenase MMP-1. All samples were washed with Hank's Balanced Salt Solution (Invitrogen, Carlsbad, CA) for 1–2 h immediately after intervention.

Trypsin intervention: Six samples were submerged in 25 mg/ml trypsin solution (Invitrogen) for 24–48 h to induce loss of GAG and other noncollagenous macromolecules.

Combined trypsin and collagenase intervention: Three samples were first submerged in 25 mg/ml trypsin solution for 24 h. GAG-depleted samples were then placed in 60 nM collagenase MMP-1 (Sigma-Aldrich, St. Louis, MO) solution and incubated at 37°C for 9 days. The incubation solution was changed three times to provide fresh MMP-1. The removal of aggrecan prior to collagenase digestion is required due to its ability to prevent collagenase degradation of the matrix [6].

In order to confirm that the MMP caused collagen degeneration, a C12C ELISA was performed on the supernatant from the digestion. The C12C assay is specific to the MMP-1 collagenase cleavage site on the alpha chain collagen fragment [7,8]. Separate studies confirmed negligible release of C12C-assay-detectable fragments from bone. The neoepitope release was normalized to cartilage tissue volume. Cartilage tissue volume was computed as a fraction of the total wet weight of the osteochondral sample. The cartilage fraction was estimated as the fraction that was cartilage in a cross-sectional image.

2.2. Imaging protocol

Prior to imaging, samples were equilibrated overnight in 1 mM Gd-DTPA^{2-} solution. The Gd-DTPA equilibration was needed in order to obtain maps of GAG distribution by MR using the delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) method [9].

All samples were placed in 10-mm NMR tubes for imaging. dGEMRIC and DTI images were made for each sample both before and after the intervention. To evaluate the accuracy of our DTI protocol, we imaged a water phantom (1 mM Gd-DTPA) using the same set of imaging parameters.

Imaging was performed on an 8.45-T scanner (Bruker Instruments, Billerica, MA) using a 10-mm birdcage coil. dGEMRIC imaging was done as previously described [9]. The DTI imaging parameters were as follows: spin-echo DTI sequence, TE/TR=16.7/1000 ms, δ/Δ=2/10 ms, field of view=1.5×1.5 cm, slice thickness=2 mm, matrix=128×128, in-plane resolution=117×117 μm, average=1. Diffusion gradients were applied in six directions: {1,0,0}, {0,1,0}, {0,0,1}, {$1/\sqrt{2}$, 0, $1/\sqrt{2}$}, {$1/\sqrt{2}$, $1/\sqrt{2}$, 0}, {0, $1/\sqrt{2}$, $1/\sqrt{2}$}; four magnitudes (25, 30, 35 and 40 G/cm) were used in each direction, and corresponding b values ranged from 100 to 300 s/mm^2. The imaging time was approximately 1.25 h/sample.

2.3. Postprocessing

DTI image processing, visualization and statistical data analysis were performed with an in-house software package developed using MATLAB (MathWorks, Natick, MA). The degree of diffusion anisotropy was quantified using fractional anisotropy (FA) [10], which is defined as

$$FA = \sqrt{\frac{2}{3}}\sqrt{\frac{(\lambda_1-\bar{\lambda})^2+(\lambda_2-\bar{\lambda})^2+(\lambda_3-\bar{\lambda})^2}{\lambda_1^2+\lambda_2^2+\lambda_3^2}},$$

where λ_1, λ_2 and λ_3 are the three eigenvalues of the diffusion tensor and $\bar{\lambda}$ denotes the mean diffusivity (mean D) and is defined as $\bar{\lambda}=\frac{\lambda_1+\lambda_2+\lambda_3}{3}$.

The FA value ranges from 0 to 1. A value of 0 indicates that the diffusion is isotropic, and a value of 1 means that the diffusion is completely restricted in one direction.

The preferred diffusion directions were visualized pixelwise using the projection of the primary eigenvector of the diffusion tensor onto the image plane and color coded with

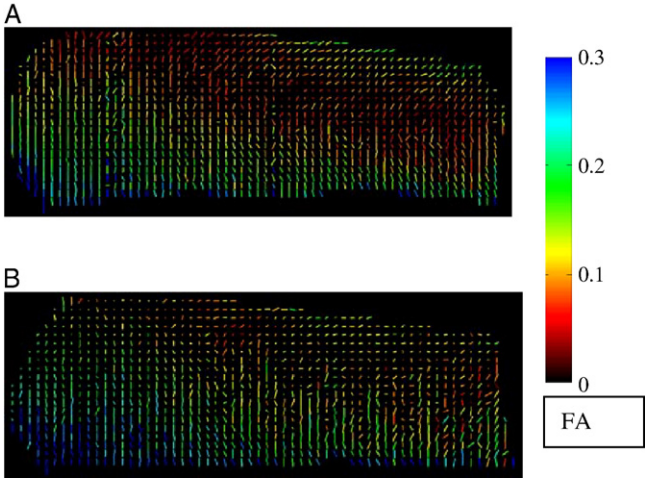

Fig. 1. Representative example of treatment effects on DTI for human articular cartilage. (A) Baseline-preferred diffusion direction map. (B) Preferred diffusion direction map for the same sample after treatment with trypsin and collagenase.