Relationship between Quantitative T1p and T2 Relaxation Times and the Biochemical and Biomechanical Properties of Osteoarthritic Cartilage

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Introduction: Osteoarthritis (OA) is a slowly progressing disease characterized by the degeneration of articular cartilage. More specifically, the proteoglycan and collagen components within the extracellular matrix (ECM) of articular cartilage are broken down as the severity of OA increases [1]. Recent data suggests that the major contributing factors to early OA are altered mechanical loading and abnormal cartilage physiology; however, the specific contributions and interactions of these factors are not fully understood [2]. Quantitative magnetic resonance (MR) imaging techniques can be used to diagnose early onset OA in vivo [3]. T1p and T2 relaxation imaging in particular have been shown to reflect cartilage proteoglycan and collagen content, respectively [1, 4]. However, there is limited data that examines T1p and T2 imaging techniques compared to the biochemical and biomechanical properties of OA cartilage. In this study, we hypothesized that quantitative T1p and T2 relaxation times would reflect the biochemical and biomechanical properties of OA articular cartilage.

Methods: Experimental Design: In this study, medial femoral condyles (n=28) were harvested from skeletally mature female porcine knee joints obtained from a local abattoir. Condyles were harvested, visually assessed by Collins grading [5], and divided into either normal or OA groups. Sagittal T1p- and T2-weighted MR images were acquired using a 3T scanner with an 8 channel knee coil (Matrix = 256 x 256, Slice thickness = 3 mm, TR/TE = 3500/13 ms, B1 = 500 Hz; T1p: TSL = 5, 10, 20, 40, 60, 80, 100 ms; T2: TR = 3500 ms, TE = 13.8, 27.6, 41.4, 55.2, 69.0, 82.8, 96.6 ms) [6]. Three cartilage specimens (5 mm diam.) were harvested from the middle region of the normal condyles. For the OA condyles, three specimens were harvested from the degenerated area of the cartilage and three were taken from a normal appearing region on the same condyle. This resulted in three groups (Fig 1): the normal area of a healthy knee (N, n=9), the normal appearing area of an OA knee (N-OA, n=19), and the arthritic area of an OA knee (OA, n=19).

Assessment of Cartilage Explants: Histological, biochemical, and biomechanical testing were performed on the harvested cartilage explants. Explants were frozen in OCT, sectioned, and stained with Safranin O, fast green, and hematoxylin. Sections were graded by three blinded graders using a modified Mankin grading scheme [7, 8]. The explants for biochemistry were weighed, lyophilized, and then reweighed to calculate the percent water content. One half of each explant was papain digested overnight at 65°C and then sulfated glycosaminoglycan (S-GAG) content was measured using the dimethylmethylene blue assay [9]. The second half of each explant was digested overnight in α-chymotrypsin at 37°C to solubilize extractable (cleaved) collagen [10]. After chymotrypsin digestion, the supernatant was collected and the remaining tissue was digested in papain. Total collagen content was calculated as the sum of the
collagen content in the papain and chymotrypsin digested samples, using the hydroxyproline assay [11]. The percentage of extractable collagen in each explant was calculated by dividing the collagen content in the chymotrypsin fraction by the total collagen content and multiplied by 100. Aggregate modulus and hydraulic permeability of the cartilage were determined using confined compression creep experiments on an ELF 3100 (Bose) [12].

**MR Imaging Analyses:** The T1ρ and T2 relaxation times were determined by fitting an exponential relaxation curve to the image intensity versus spin lock/echo times for each pixel in the MR images. The T1ρ and T2 values for each pixel were calculated from the exponential decay to obtain the relaxation times [6].

**Statistical Analyses:** The normal group served as a reference control. Paired t-tests were performed to determine significant differences between N-OA and OA samples.

**Results:** Histological staining of cartilage explants revealed intact cartilage structure and robust Safranin O staining in the normal appearing cartilage from OA samples and substantial tissue degeneration and loss of staining in the OA regions (Fig 1). Modified Mankin grading of the tissue sections confirmed the visual delineation of groups with a significant increase in the histological grade in the OA cartilage group compared to the normal-OA group (Fig 3a). There was an approximately 7% increase in T1ρ relaxation times in the OA cartilage (Fig 2, Fig 3b), while the T2 relaxation times were significantly decreased in the OA cartilage (Fig 2, Fig 3c), as compared to the normal-OA cartilage. The S-GAG content was 44% lower in the OA cartilage compared to the normal-OA cartilage (Fig 3d). The total collagen content was not significantly different between the groups (data not shown); however the percentage of extractable collagen was significantly higher in the OA cartilage compared to the normal-OA group (Fig 3e). The percent water content was also significantly increased by approximately 5% in the OA cartilage over the normal-OA group (Fig 3f). In addition, the aggregate modulus was decreased by 65% (Fig 3g) and hydraulic permeability was increased by 35% in the OA cartilage compared to the corresponding normal-OA cartilage (Fig 3h). The normal and normal-OA groups showed similar results for all of the properties that were analyzed.

**Discussion:** Non-invasive imaging techniques are necessary to diagnose early onset OA due to the limitations of radiography. This study found a significant increase in T1ρ relaxation times that corresponded to a decrease in S-GAG content, an increase in water content, and decreased mechanical properties for OA cartilage compared to normal regions within the same joint. Interestingly, T2 mapping showed lower relaxation times in the OA cartilage, perhaps due to loss of the highly organized superficial zone in the OA cartilage [13]. Our data shows a significant decrease in the mechanical properties of OA cartilage, which is consistent with previous data that measured decreases in the compressive modulus of cartilage during OA progression [1]. The relationship between increasing T1ρ and T2 relaxation times and the properties of OA cartilage confirms that quantitative MR analysis can be used to diagnose early onset OA in vivo.

**Significance:** Quantitative T1ρ and T2 relaxation times reflect the biochemical and biomechanical properties of OA articular cartilage. MR imaging techniques can be used to diagnose early onset OA and to make a non-invasive assessment of the biochemical and biomechanical state of the articular cartilage in a site-specific manner within a joint.
Figure 1: Safranin O and fast green stained sections of cartilage from (a) a healthy knee (N), (b) the normal appearing area of an OA knee (N-OA), and (c) the OA area of an OA knee (OA). Red staining indicates proteoglycans, blue staining shows collagen, and hematoxylin stains the cell nuclei black.

Figure 2: T1p color maps for the (a) normal area of a healthy knee, (b) normal appearing area of an OA knee, and (c) OA area of an OA knee. T2 color maps for the (e) normal area of a healthy knee, (f) normal appearing area of an OA knee, and (g) OA area of an OA knee.
Figure 3: Mean (+ SD) of all three cartilage groups for the (a) Modified Mankin grades, (b) T1ρ relaxation times, (c) T2 relaxation times, (d) S-GAG content, (e) % Extractable collagen, (f) % Water content, (g) Aggregate modulus, and (h) Hydraulic permeability (*p < 0.05, **p < 0.001).

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