Evaluation Of The Compositional And Functional Properties Of Articular Cartilage Using T1rho And T2 Relaxation Imaging

Amber T. Collins, PhD, Sophia Y. Kim, BSE, Courtney E. Cox, MS, Sophia Ziemian, Charles E. Spritzer, MD, Farshid Guilak, PhD, Amy L. McNulty, PhD, Louis E. DeFrate, ScD.
Duke University Medical Center, Durham, NC, USA.


Introduction: The extracellular matrix of articular cartilage is composed primarily of proteoglycans, their glycosaminoglycan (GAG) side chains, and collagen fibers, all of which provide structural integrity and influence the mechanical properties of cartilage [1]. One of the earliest changes associated with osteoarthritis (OA) is the loss of proteoglycans within the cartilage. Early detection of these changes may provide important information on OA progression or potential therapeutic interventions. Unfortunately standard anatomic imaging techniques do not provide information on changes occurring with early stage OA, such as proteoglycan and collagen loss. However, novel MR imaging techniques, such as T1rho and T2 relaxation mapping allow for the measurement of relative proteoglycan and collagen content, respectively, within articular cartilage [2-5]. The goal of this work is to compare T1rho and T2 relaxation times to the biochemical and biomechanical properties of articular cartilage. Specifically, we will utilize a repeated measures study design in order to examine the effect of targeted cartilage degradation to elicit isolated changes in cartilage composition, mechanical properties, and T1rho and T2 relaxation times.

Methods: Eight lateral femoral condyles were harvested from skeletally mature female porcine knee joints obtained from a local abattoir. Condyles were then MR imaged using a 3T scanner (Trio Tim, Siemens) with an 8 channel knee coil. Sagittal 3D FISP T1rho- and T2- weighted images were acquired (matrix=256x256, slice thickness=3 mm, TR/TE=3500/13 ms, B1=500 Hz; For T1 rho: spin lock pulse duration (TSL)=5, 10, 20, 40, 60, 80, 100 ms; For T2 mapping: TR=3500 ms, echo time (TE)=13.8, 27.6, 41.4, 55.2, 69.0, 82.8, 96.6 ms) [6]. Following MR imaging, three 5mm biopsies were taken from each condyle for biochemical, biomechanical, and histological analysis (untreated group). The condyles were digested with 0.15 units/mL chondroitinase ABC (cABC) (Sigma) at 37°C. After 48 hours, a second set of MR images was taken followed by the collection of 3 additional biopsies (cABC group). The condyles were then digested in 10 units/mL collagenase (Worthington) for 48 hours at 37°C. Following digestion with collagenase, condyles were MR imaged and 3 more biopsies were collected (cABC + collagenase group). To compute T1rho and T2 relaxation times, femoral cartilage was first manually segmented from T1rho- and T2- weighted images (Figure 1). Global cartilage T1rho and T2 values were then computed pixel-by-pixel by fitting image intensity to the equations S(TSL)=S0*exp(-TSL/T1rho) and S(TE)=S0*exp(-TE/T2), respectively. The explants for biochemical analysis were cut in half, wet weights were determined, samples were lyophilized, and then dry weights were measured to calculate the percent water content. 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 [7]. The other half of each sample was digested in α-chymotrypsin at 37°C overnight to release the extractable (cleaved) collagen in each sample [8]. After α-chymotrypsin digestion, the supernatant was collected.
and the remaining tissue was digested in papain. Collagen content was measured in the papain and chymotrypsin digested samples with the hydroxyproline assay [9]. Total collagen content was calculated as the sum of the collagen content in both the papain and chymotrypsin fractions for each sample. To quantify the percent of extractable collagen in each explant, the collagen content in the α-chymotrypsin fraction was divided by the total collagen content and multiplied by 100. The aggregate modulus was determined using confined compression creep experiments on a materials testing machine (ELF 3100, Bose) [10]. Explants were then cryosectioned and stained with safranin-O, fast green, and hematoxylin to visually assess proteoglycan and collagen content in the cartilage matrix. Repeated measures analysis of variance was used to determine statistically significant differences in all outcome measures between the untreated, cABC, and cABC + collagenase treated groups. Post-hoc testing was completed using the Fisher’s least square differences method with p<0.05.

**Results:** With enzymatic depletion of s-GAG using cABC, T1rho color maps (Figure 1) illustrate a statistically significant increase in T1rho relaxation times (p<0.05, Figure 2A). This corresponded with a statistically significant reduction of 25% in s-GAG content between the untreated and cABC treated groups (p<0.05, Figure 2B). T2 relaxation times also significantly increased by 21% between the untreated and collagen depleted groups (p<0.001, Figure 2D), which is demonstrated in the T2 color maps (Figure 1). There was no change in total collagen content among the treatment groups (data not shown), however the percent of extractable collagen significantly increased upon collagenase digestion (p<0.001, Figure 2E). Water content was not significantly different between treatment groups (Figure 2C). Additionally, mechanical testing showed a significant decrease in aggregate modulus between the untreated and cABC groups and between the untreated and cABC + collagenase treated groups (p<0.05, Figure 2F). Histological staining demonstrated a reduction in s-GAG content as shown through reduced safranin-O staining in the cABC treated group and little change in the fast green staining, indicative of collagen, due to enzymatic digestion (Figure 3).

**Discussion:** Targeted enzymatic depletion of s-GAG and collagen content resulted in elevated T1rho and T2 relaxation times and was confirmed through biochemical analysis. Aggregate modulus also decreased with enzymatic depletion of s-GAG and further with depletion of collagen, which may be a result of a reduction in the matrix components that contribute to fixed charge density and matrix stability. Collagenase digestion did not change the overall collagen content of the tissue but did cause the breakdown of collagen fibers within the collagen fibril network. This degradation of collagen fibers was demonstrated as increased extractable collagen in the samples and confirmed by an increase in T2 relaxation times, allowing for the detection of early collagen structural breakdown similar to that found in osteoarthritis [8]. The results of this study demonstrate the validity of T1rho and T2 relaxation mapping as powerful, non-invasive tools for quantifying and assessing changes to the biochemical and biomechanical environments of articular cartilage.

**Significance:** Early signs of osteoarthritis are often undetectable using conventional radiographic techniques. The results from this study demonstrate that MR relaxation mapping of cartilage using T1rho and T2 sequences is a powerful tool for the non-invasive assessment of the biomechanical and biochemical state of articular cartilage.
Figure 1. Representative T1rho (A) and T2 (B) color maps of femoral articular cartilage from untreated and enzymatically depleted samples.

Figure 2. Mean (±SD) of all three treatment groups for A) T1rho relaxation times; B) sGAG content; C) water content; D) T2 relaxation times; E) % extractable collagen; F) aggregate modulus. N=8 for A-D, F; N=6 for E; *p<0.05; **p<0.001.
Figure 3. Histologically stained cartilage biopsy samples following enzymatic depletion of proteoglycans and collagen. Safranin-O (red), fast Green (blue), and hematoxylin (black) staining indicate proteoglycans, collagen, and cell nuclei, respectively. Scale bar is 100 μm.