Published in final edited form as:

J Magn Reson Imaging. 2015 May ; 41(5): 1281–1290. doi:10.1002/jmri.24658.

The molecular origin of a loading-induced black layer in the deep region of articular cartilage at the magic angle

Nian Wang, PhD, David Kahn, BSc, Farid Badar, BSc, and Yang Xia, PhD*

Department of Physics and Center for Biomedical Research, Oakland University, Rochester, MI 48309

Abstract

Purpose—To investigate the molecular origin of an unusual low-intensity layer in the deep region of articular cartilage as seen in MRI when the tissue is imaged under compression and oriented at the magic angle.

Materials and Methods—Microscopic MRI (μ MRI) T_2 and $T_{1\rho}$ experiments were carried out for both native and degraded (treated with trypsin) 18 specimens. The glycosaminoglycan (GAG) concentrations in the specimens were quantified by both sodium ICP-OES and μ MRI Gd(DTPA) $^{2-}$ -contrast methods. The mechanical modulus of the specimens was also measured.

Results—Native tissue shows no load-induced layer, while the trypsin-degraded tissue shows clearly the low-intensity line at the deep part of tissue. The GAG reductions are confirmed by the sodium ICP-OES (from 81.7 ± 5.4 mg/ml to 9.2 ± 3.4 mg/ml), MRI GAG quantification (from 72.4 ± 6.7 mg/ml to 11.2 ± 2.9 mg/ml). The modulus reduction is confirmed by biomechanics (from 4.3 ± 0.7 MPa to 0.3 ± 0.1 MPa).

Conclusion—Both T_2 and $T_{1\rho}$ profiles in native and degraded cartilage show strongly strain-, depth-, and angle-dependent using high resolution MRI. The GAG reduction is responsible for the visualization of a low-intensity layer in deep cartilage when it is loaded and orientated at 55° .

Keywords

T_2 ; $T_{1\rho}$; cartilage; loading; magic angle; laminar appearance; anisotropy; MRI

Introduction

Articular cartilage, composed in its extracellular matrix mainly of collagen fibers, water, and negatively charged glycosaminoglycans (GAG), is a thin layer of connective tissue that covers the articulating surfaces of bones in synovial joints (1,2). Morphologically, cartilage is known to consist of three distinctly structured zones—the superficial zone (SZ) at the surface, the transitional zone (TZ) in the middle, and the radial zone (RZ) that interfaces the tissue with the underlining bone. Each of the three zones has a distinctly different orientation of the collagen fibers (3-5), parallel to the tissue surface in SZ, random in TZ, and

*Corresponding Author and Address: Yang Xia, PhD, Department of Physics, Oakland University, Rochester, Michigan 48309, USA, Phone: (248) 370-3420, Fax: (248) 370-3408, xia@oakland.edu.

perpendicular to the surface in RZ. During everyday load-bearing activities such as standing or walking, healthy cartilage protects the underlying bone by deforming its collagen architecture and extruding water molecules from its extracellular matrix (6-9). When the structure of this collagen architecture is damaged and the concentration of GAG is reduced, the load-bearing ability of cartilage will be compromised, which thus initiates arthritic diseases such as osteoarthritis (OA) (10).

Magnetic resonance imaging (MRI) has been used in both clinics and research labs to study cartilage and its degradation (11-13). In addition to the commonly used T_1 and T_2 relaxation, $T_{1\rho}$ has become popular in recent years in MRI of cartilage, owing to its weaker influence from the dipolar interaction between the organized macromolecules (e.g., collagen fibers) and the external magnetic field (14-16). One well-known feature in MRI of articular cartilage is the so-called magic angle effect (17), where normal cartilage can have a laminar appearance when the local collagen fibrils are oriented in parallel with the external magnetic field B_0 . The same cartilage would appear homogeneous with higher intensity when it is oriented at the magic angle ($\sim 55^\circ$ to the magnetic field), due to the minimization of the dipolar interaction (17-19).

Since articular cartilage is a load-bearing tissue, imaging cartilage while loaded is a functional study of the tissue (20). Nearly ten years ago, two high-resolution MRI reports in the literature documented a *different* kind of laminar appearance in articular cartilage (21,22), where an unusual low-intensity laminar layer (or black line) could be observed in the deep region of cartilage when a compressed tissue was oriented *at the magic angle*. In other words, the loading could manipulate the homogeneous appearance of cartilage at the magic angle. The mapping of T_2 in the compressed cartilage revealed the formation of a double peak profile, which was used to interpret the deformation of the collagen fibers in cartilage when the tissue was compressed (21,22).

Since loading a piece of soft tissue, such as articular cartilage, could have a number of complicated consequences, including loss of water and some ions, increase of solid component (including both collagen and GAG), and change in fiber orientations, this project was designed to investigate the molecular origin of the load-induced laminar appearance in compressed articular cartilage. The hypothesis of this project was that the GAG content in cartilage, which was responsible for the tissue's stiffness, might play a decisive role in the appearance of the load-induced laminar structure.

Materials and Methods

Specimen Preparation

Five Humeral heads were harvested shortly after the sacrifice of mature and healthy dogs that were used for an unrelated research project, where the institutional animal care committee approved the animal handling. A total of thirty cartilage specimens were harvested; each was about $3.5 \times 2.5 \times 6$ mm where the intact cartilage was still attached to the underlying bone. Eighteen specimens were divided equally into two groups (native, degraded) for μ MRI experiments; another six specimens were divided equally into two groups (native, degraded) for biochemical analysis; the remaining six specimens (native,

degraded) were used for biomechanic measurements. For MRI experiments, the nine native specimens were freshly harvested samples, which were immersed in physiological saline (154 mM NaCl in deionized water) with 1% protease inhibitor (Sigma, Missouri). The nine degraded specimens were fresh samples first soaked in 10 µg/ml trypsin solution (Sigma, Missouri) for more than 8 hours, then soaked in saline with 1% protease inhibitor to remove excess trypsin. Identical handling procedures and storage conditions were used for both groups (native and degraded) in all three types of experiments (µMRI, biochemistry, biomechanics). No specimen was frozen at any time.

Microscopic MRI (µMRI) Protocols

The specimen compression in µMRI used a homemade unconfined manual-loading device (21). T_1 measurements were performed for both native and degraded tissue without compression; T_2 and $T_{1\rho}$ experiments were performed at different strains. The specimens were loaded under 0% (unloaded), ~ 15%, and ~ 46% strain for native tissue, and 0%, ~ 16%, ~ 50% strain for degraded tissue respectively. The actual strain values were measured afterwards in the T_2 images by the reduction in cartilage thickness, with an error at about $\pm 4\%$. Each specimen was allowed half an hour to reach equilibrium upon loading. After the T_1 , T_2 and $T_{1\rho}$ measurements when the specimens were in saline solution, unloaded native and degraded tissues were soaked in 1 mM Gd(DTPA) $^{2-}$ solution (Magnevist, Berlex, NJ) for eight hours before the final µMRI T_{1-Gd} (soaked in the 1 mM Gd(DTPA) $^{2-}$ bath) experiments. The volume of the soaking solution was at least 100 times the volume of the specimen to ensure a nearly constant Gd concentration.

All µMRI experiments were performed at room temperature on a Bruker AVANCE II300 NMR spectrometer equipped with a 7 Tesla/89 mm vertical-bore superconducting magnet and microimaging accessory (Bruker Instrument, Billerica, MA), at both 0° and the magic angle. A 5 mm solenoid coil was used in the µMRI experiments, which had a 90° hard pulse of 6.5 µs. The imaging experiments were carried out with an acquisition matrix of 256×128 (which was reconstructed into a 256×256 matrix) and a slice thickness of 1 mm. The Field of View (FOV) was 0.45 cm×0.45 cm, resulting in the 2D in-plane pixel size 17.6 µm. (The high transverse resolution was necessary to resolve the depth-dependent features across the thin thickness of cartilage; the dimension of the slice thickness benefited from the uniform properties of the tissue along the third (surface) direction for this type of joint (23).) The repetition time TR was 2 s for the specimens without Gd(DTPA) $^{2-}$ and 0.8 s for the specimens soaked in 1mM Gd(DTPA) $^{2-}$.

Quantitative T_2 -imaging experiments were performed using a CPMG magnetization-prepared T_2 imaging sequence, where the echo time for the imaging segment was 7.2 ms (24). The echo spacing in the CPMG T_2 -weighting segment was 1 ms and the five echo times ranged from 2 ms to 140 ms, depending on the degradation, compression, and orientation of the tissue. The $T_{1\rho}$ imaging sequence proceeded with a 90° hard pulse followed by a spin-lock pulse (13,14). The strength of the spin-lock was 1 kHz, which was calibrated by the strength of the 90° pulse. The experiment time of T_2 and $T_{1\rho}$ measurement was about 2 hours at each strain. The inversion-recovery T_1 experiments (9) used five inversion points (0, 0.4, 1.1, 2.2, 4.0 s for tissue without Gd(DTPA) $^{2-}$, and 0, 0.1, 0.3, 0.5, 1

s for tissue soaked in 1 mM Gd(DTPA)²⁻). T₁ experiment without and with Gd(DTPA)²⁻ took about 6 and 2 hours respectively. All quantitative images were calculated by a single-component fit on a pixel-by-pixel basis.

Visual assessment of the cartilage appearance in the images was carried out using the following steps. First, the homogeneity of the cartilage image was inspected when the tissue was oriented at the magic angle. Second, the depth profiles of cartilage were extracted from the cartilage portion of the images through the entire tissue depth; and the mean intensity change was calculated as the difference between the high-intensity area (4*2 pixels) and low-intensity area (4*2 pixels). Finally, a laminar appearance was confirmed if the difference was larger than 20%.

GAG Quantification using Na ICP-OES

Six specimens (native and degraded) were used to measure the GAG concentration using a PerkinElmer Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) Optima 7000 DV (Waltham, MA). The bone on each specimen was carefully removed before weighing. The specimen was liquefied by concentrated 100 µl HNO₃ overnight and subsequently diluted to 10 ml by deionized water for the Na ICP-OES analysis (25). In addition to the cartilage samples, saline solution (154 mM NaCl) was used as a quality control to validate analytical results. The calculation of the GAG content in the ICP-OES experiment followed the Donnan equilibrium equation and all results were shown as the wet weight concentration with the unit of mg/ml (26,27).

Biomechanics Protocols

Six specimens (native and degraded) were used to measure the depth-dependent equilibrium modulus using an Enduratec ELF 3200 mechanical testing system (Bose Corporation, Eden Prairie, MN), which had a computer controller and a 100-µm diameter indenter. Each specimen was mounted on a homemade device that could be traversed linearly under the control of a digital micrometer. The direction of the specimen motion was along the depth of the tissue. At each test location (tissue depth), an indentation experiment was performed, where multiple indentation steps at a 15-µm displacement were applied to the tissue. At each step 20 minutes was allowed for relaxation. A description of the tissue biomechanical property was determined depth-dependently at the fixed depth increment (50 µm for native tissue, and 100 µm for degraded tissue), using the equations developed by Hayes et al. in units of MPa (28).

Statistical Analysis

Imaging data were evaluated for any significant difference using commercial software KaleidaGraph (v4.0, Synergy Software, Reading, PA) by two sets of statistical analysis. First, one-way analysis of variance (ANOVA) test was performed to compare the T₂ or T_{1ρ} values of native and degraded tissues at different compression strains (zero, moderate, heavy). Second, several sets of data were analyzed in pairs by student *t*-test when there was significant difference from the ANOVA result. The significance level between/among the data was set to $P < 0.05$.

Results

The 2D images of native and degraded tissues

The T_2 - and $T_{1\rho}$ -weighted proton images of both native and degraded tissues are shown in Fig 1, where the specimens are loaded under ~ 15 -16% strains and oriented at the magic angle. The native (i.e., healthy) cartilage exhibited homogenous visually when the specimen was loaded, based on the visual inspection by two of the authors. In contrast, the degraded tissues appeared to lose its homogenous appearance after loading - an unusual low-intensity layer or a black line could be seen in the deep zone of the tissue. The visibility of this loading-induced low-intensity layer depended upon the echo time of the imaging experiments. A long echo time darkened the entire cartilage image and reduced the visualization of this layer at the same display parameters (the contrast-to-noise ratio between high-intensity layer and low-intensity layer decreased from 33.7 (TE=2 ms) to 4.2 (TE=50 ms) in $T_{1\rho}$ measurements). The same unusual layer could be seen in the images at 0° (data not shown), except not as clear as at the magic angle, which is due to the fact that the deep part of articular cartilage in MRI at 0° has low intensity due to strong dipolar interaction (18).

Fig 2 summarizes the quantitative T_2 and $T_{1\rho}$ images (spin-lock field of 1 kHz) under different strains at 55° , where the differences between native and degraded tissues are clear. For native tissue, T_2 (55°) and $T_{1\rho}$ (55°) images remained largely uniform when loaded (even at a 46% strain). For degraded tissue, T_2 (55°) and $T_{1\rho}$ (55°) images no longer exhibited a homogenous appearance, even at a modest 16% strain. Instead, these T_2 and $T_{1\rho}$ images had a clear laminar appearance in the deep part of the tissue. When the strain was increased to 50%, the laminar layer could still be visible. (The laminar appearance could also be seen when the degraded specimen was oriented at 0° - data not shown.)

T_2 and $T_{1\rho}$ profiles of native and degraded tissues

The depth-dependent T_2 profiles at both 0° and 55° are plotted in Fig 3 (where the relative depth = 0 at the articular surface and 1 at the cartilage-bone interface). For native tissue, T_2 (0°) profiles (Fig 3a) showed the usual single bell-shaped curve, where the peak was shifted under loading towards the cartilage-bone interface but remained a single bell shape. The amount of the peak shift was closely related to the amount of strain. The T_2 (55°) profiles of native tissue (Fig 3c) had a homogeneous profile without loading, reduced only in the upper tissue under a modest loading (16% strain), and had an across-the-depth reduction when loaded heavily (46% strain).

For degraded tissue, the unloaded T_2 (55°) profiles (Fig 3d) were rather homogeneous, having higher values but similar shape when comparing to the native tissue profiles (Fig 3c). The modulation of the T_2 (55°) profiles under loading, however, was strikingly different between the native and degraded tissues: a well-resolved bell-shape peak appeared in the T_2 profiles at the deep part of the degraded tissue (Fig 3d). When the specimens were oriented at 0° , T_2 (0°) profiles showed a similar single bell-shaped curve when the specimen was not loaded (Fig 3b). A modest loading not only shifted the bell shape into the deep tissue but

also caused an unusual low intensity layer in the deep tissue. The heavy loading (50% strain) essentially reduced the T_2 (0°) to very low values across the entire tissue depth.

The results of the $T_{1\rho}$ profiles (at the spin-lock frequency of 1 kHz) was qualitative similar to the results of the T_2 profiles at both orientations (Fig 4). The difference in the profiles existed when the tissue was oriented at 0° , where the bell-shaped curve in the $T_{1\rho}$ profiles was not as obvious as in the T_2 profiles, due to the smaller effect of the dipolar interaction in relaxation (24).

Zonal averaged T_2 and $T_{1\rho}$ variations upon compression

Since the resolution of most MRI experiments could not reach tens of microns as in this study, the trends of T_2 and $T_{1\rho}$ as functions of both tissue depth and mechanical strain were averaged over its sub-tissue zones. The division of the histological zones in cartilage was based on the previously validated data using both μ MRI and polarized light microscopy (5). To analyze the changes of relaxation time in the radial zone (the thickest of three zones in cartilage), RZ was further divided approximately equally into an upper part (RZ1) and a lower part (RZ2). The trends of T_2 and $T_{1\rho}$ as functions of both tissue depth and mechanical strain were averaged over these sub-tissue zones on a relative depth scale, shown in Table 1 and Table 2, both from all specimens.

For both native and degraded tissues, most of the mean T_2 and $T_{1\rho}$ values, averaged over the full thickness as well as within each zone, decreased when the tissue was compressed. Two features were worth noting in Table 1. First, the mean T_2 (0°) value of the native tissue (the top left quarter of the data in Table 1) had the smallest changes after compression, which reflected the dominating influence of the dipolar interaction when the specimen was at 0° . Second, the mean T_2 (55°) value for the degraded tissue in RZ1 reduced by more than 81% when modestly loaded. In fact, the T_2 in RZ1 became lower than T_2 in RZ2. This significant reduction of T_2 in RZ1 resulted in the unusual low-intensity layer in the deep part of the degraded tissue at 55° . The mean values of $T_{1\rho}$ values in Table 2 have similar features, less clear because of the weaker dipolar interaction.

Mechanical modulus and GAG concentration in native and degraded tissue

Fig 5a shows the depth-dependent profiles of the mechanical modulus in both native and degraded tissues. While the modulus was strongly depth-dependent in native tissue (the surface part of the tissue had the lowest modulus and hence was the softest; the deep tissue was the hardest), the enzymatic assay successfully removed most of the GAG molecules from the tissue, which reduced the tissue modulus significantly. There was no depth-dependency of GAG in the degraded tissue throughout the entire tissue depth. Fig 5b showed the actual GAG concentration in cartilage measured by the $\text{Gd}(\text{DTPA})^{2-}$ -contrast method. While GAG concentration profiles in the native tissue agreed well with several previous reports (9,29,30), the GAG concentration in the degraded tissue was reduced significantly and stayed constant through the entire tissue depth. The mean GAG concentrations (wet weight) in native and degraded tissue by μ MRI were 72.4 ± 6.7 mg/ml and 11.2 ± 2.9 mg/ml respectively, which were supported by the bulk measurements from the sodium ICP-OES experiments in this project: 81.7 ± 5.4 mg/ml for the native tissue and

9.2±3.4 mg/ml for the trypsin degraded tissue, respectively. The individual values of the measurements are shown in Table 3.

A schematic model of collagen deformation

Fig 6 contains a collagen fibril model that can explain the experimental observation of this project between the native and degraded tissues to external loading. When the tissue is not compressed (the left panel in Fig 6), its collagen matrix commonly has a well-known three-zone structure (23), from SZ where the fibers are largely parallel to the tissue surface, to TZ where the fibers orient randomly, and finally to RZ where the fibers are largely perpendicular to the surface. It is the random crossovers of the collagen fibers in TZ that minimize the dipolar interactions and result in the single bell-shaped peak of T_2 relaxation, centered at about 100 μm below the surface in this type of cartilage (0% strain profile in Fig 3a) (5). This single peak exists regardless of whether the tissue is native (i.e., healthy) or degraded (c.f., the T_2 peaks without loading in both Fig 3a and 3b).

For the native tissue under a modest strain, the surface T_2 (Table 1 55° SZ: from 37.1 ms to 15.0 ms) reduces more than the deep-tissue T_2 (Table 1: no significant difference in RZ 2), which is similar to the situation of T_1 in loaded cartilage (9) and also consistent with previous results (31). This surface reduction is due precisely to the depth-dependent modulus of healthy cartilage, where the surface tissue is softer. When the tissue is further compressed (from modest to heavy in Table 1), the additional compression mostly affects the deep tissue (no significant T_2 change between 15% and 46% strain in SZ; significant change from 38.0 ms to 11.0 ms in RZ 2). In term of the re-organization of the collagen fibers in a healthy tissue when cartilage is compressed (the mid panel in Fig 6), the obliquely-oriented collagens in the upper tissue are surely to become more parallel with the articular surface, which results in an increase of the *apparent* SZ thickness that has indeed been measured in the imaging experiment (21). Because of loading, the fibers in the upper radial zone are no longer perpendicular to the articular surface, but become more oblique (depending on the strain). This part of tissue, hence, possesses the same fibril characteristics as the TZ of uncompressed tissue, which is reflected by the shifting of the T_2 (0°) peaks towards the deep tissue (Fig 3a and Fig 3b). The large amount of the GAG molecules in the native tissue may act as a compressive buffer, which does not allow the formation of any large bending in the RZ collagen. Hence, the shape of the T_2 profiles remains largely unchanged (Fig 3). Some types of localized zigzag patterns (7,32) may exist in RZ of the native tissue when being compressed, in both modest and heavy compressions.

In contrast, the degraded tissue no longer has the compressive buffer, generated only by the ample amount of GAG in the tissue. Consequently, the collagen fibrils in RZ will have more freedom to change their orientations on a larger scale when the tissue is compressed. Indeed, for degraded tissue, even modest strain ($\sim 15 - 16\%$) causes drastic T_2 and $T_{1\rho}$ changes throughout the whole tissue depth. If the fibers in a degraded cartilage behaved the same zigzag way as in the healthy tissue (the middle panel in Fig 6), one should observe a uniform reduction of relaxation times at the magic angle, which was not the case in the degraded tissue in this project (Fig 3).

Discussion

The mechanical property of articular cartilage is principally determined by its extracellular matrix and the interaction between the matrix and water (33). The glycosaminoglycans, which carry a large number of negative charges, are immobilized in healthy tissue among the fibril structure (34). This high density of negative charges in cartilage gives the tissue its load-bearing stiffness when it is healthy (15). A reduction of GAG lowers the mechanical stiffness of the tissue and can be considered as an early sign of tissue degradation (15). The motivation of this project was to investigate the biomolecular cause of an unusual black-line in the deep-region of cartilage's intensity image *when the tissue was loaded and oriented at the magic angle* (21). The modification to the profile of T_2 relaxation was concluded earlier as the underlying cause of this MRI phenomenon (21). However, the biomolecular cause of this phenomenon has remained unclear.

The general consequences of compressing a soft connective tissue such as articular cartilage include the reduction of its water content, the enhancement of its solid molecular concentrations, and the re-organization of its fibril structure. The water reduction and the solid concentration enhancement should both reduce T_2 and $T_{1\rho}$ relaxations, which were indeed observed in both native and degraded tissues. The re-organization of the fibril structure in cartilage, however, could influence T_2 and $T_{1\rho}$ relaxations in a complex manner, depending upon the particular structure of the re-organized fibers at a particular strain. If the re-organized fibers at a particular strain and a particular depth had a more random nature, the relaxation time could become relatively high (as in TZ of a unloaded cartilage). If the re-organized fibers had a more ordered nature, the relaxation time could reduce significantly (as in RZ of a unloaded cartilage). The measured relaxation times and the visualization of the load-induced black line in cartilage by MRI reflect consequently a complex balance among these strain-dependent effects.

A unique feature of the mechanical property of articular cartilage is its depth-dependency (35), soft at the surface and hard in the deep tissue, *due to the depth-dependent GAG concentration in cartilage* (29). Hence an external loading will compress different parts of the *healthy* cartilage differently – more compression at the surface tissue and less compression at the deep tissue. This non-linear compression is also strain dependent, since the upper part of the tissue (SZ and TZ) will become harder after a modest compression and, hence, changes less in the subsequent compression (9). The experiments in this project clearly show that the deformation of the collagen matrix in the degraded tissue differs substantially from the native tissue.

The observation of the second relaxation peak and the additional black line in the deep cartilage when the tissue is under compression at the magic angle is explained by the schematic model, which implies a different bending pattern of the fibers. Due to the same reason that the randomly oriented fibers in TZ results in a T_2 peak in the native tissue, the visualization of the second T_2 peak in the deep part of the degraded tissue may imply the formation of an additional randomly-oriented fiber zone in that part of the tissue when compressed. Between the original TZ near the surface and this additional loading-induced random zone near the cartilage-bone interface, a portion of the tissue would have its fibers to

remain relatively perpendicular to the surface, which causes the ‘valley’ seen between the two T_2 peaks. A future experiment at ultrahigh resolution (e.g., by transmission electron microscopy) could be considered to directly visualize the different patterns of fibril bending.

The striking difference in the T_2 profiles between the native and degraded tissues *at the modest strain*, which is comparable to the daily strain in human activities, could be further explored as a functional biomarker for the degeneration of cartilage in human clinically. Furthermore, the data of $T_{1\rho}$ contain the same features between the native and degraded tissues as in T_2 . Since $T_{1\rho}$ relaxation time is always longer than T_2 in cartilage, it could further improve the visualization of cartilage in clinical MRI.

Having explained the differences in the matrix deformations between the native and trypsin-degraded tissue, one may consider a different kind of cartilage degradation, which does not involve any enzymatic treatment. Recently, a significant loss of GAG (as high as 56.5%) was found in cartilage that had been frozen at -20°C and -80°C and thawed without any cryopreservation procedure (36). This unintentional loss of cartilage GAG during the storage periods, which has been attributed to the formation of the ice crystals in tissue (37), could introduce inaccurate measurement to biomedical experiments, which likely resulted in the initial observation of the black line in the deep-region cartilage in MRI (21,22). Caution should always be exercised in the planning and implementation of any biomedical experiment at each step. Future studies could be designed to investigate the correlation between the patterns of the dark line and the amount of the GAG loss in tissue degradation.

There are two limitations in our study. First, we used trypsin digestion model to mimic the degraded tissue, which is a commonly used enzymatic method in tissue study (38). However, the molecular properties and structures after the trypsin degradation are likely different from the tissue situations in the idiopathic human cartilage diseases. Second, different specimens were used for the ICP-OES, MRI, and biomechanical experiments, in order to avoid the time delay in a sequential experiment of the same specimens. Since the specimens all came from the same animals, the variations among the specimens are likely small.

In conclusion, we have demonstrated that both T_2 and $T_{1\rho}$ profiles in both native and degraded articular cartilage are strongly strain-dependent, depth-dependent, and angle-dependent using high resolution MRI experiments. Although a microscopic resolution is not currently possible in clinical MRI, the fact that both μMRI and clinical MRI share the same physics principle and engineering architecture ensures the clinical relevance of this μMRI project. The reduction of the negatively charged glycosaminoglycans, which contributes to the tissue stiffness, is found to play a key role in the formation of an unusual low-intensity laminar line at the deep-region of articular cartilage when it is loaded and orientated at 55° . Native tissue that contains a high concentration of GAG shows no load-induced laminar appearance in T_2 and $T_{1\rho}$ images, while the trypsin-degraded tissue shows clearly the *additional* low-intensity line in the deep tissue. The GAG loss after the trypsin treatment is confirmed by the sodium ICP-OES measurements, MRI GAG quantification, and biomechanical experiments. The differences in the matrix deformations between the native and degraded tissue are used to formulate a schematic model for the structural changes of

collagens in the native and degraded cartilage. This study demonstrates that MRI of loaded cartilage could, in principle, be developed into a clinical procedure to detect the early degeneration of joint diseases – where the tissue is still intact but has a reduced GAG concentration. With sufficient resolution, T_2 and $T_{1\rho}$ relaxations in MRI could become sensitive markers to detect small and localized lesions in articular cartilage at different tissue depths.

Acknowledgments

Yang Xia is grateful to the National Institutes of Health for the R01 grant (AR 052353). The authors thank Drs. Cliff Les and Hani Sabbah (Henry Ford Hospital, Detroit) for providing the canine specimens, Dr. Edith Chopin (Dept of Chemistry, Oakland University) for helping the ICP-OES experiments, Ms. Janelle Spann (Michigan Resonance Imaging, Rochester Hills, MI) for providing the contrast agent, and Ms. Carol Searight (Department of Physics, Oakland University) for editorial comments on the manuscript.

Grant Support: NIH R01 grant (AR 52353)

References

1. Maroudas A. Biophysical chemistry of cartilaginous tissues with special reference to solute and fluid transport. *Biorheology*. 1975; 12:233–248. [PubMed: 1106795]
2. Venn M, Maroudas A. Chemical composition and swelling of normal and osteoarthrotic femoral head cartilage. I. Chemical composition. *Annals of the Rheumatic Diseases*. 1977; 36:121–129. [PubMed: 856064]
3. Lehner KB, Rechl HP, Gmeinwieser JK, Heuck AF, Lukas HP, Kohl HP. Structure, function, and degeneration of bovine hyaline cartilage: assessment with MR imaging in vitro. *Radiology*. 1989; 170:495–499. [PubMed: 2911674]
4. Xia Y. Relaxation anisotropy in cartilage by NMR microscopy (μ MRI) at 14- μ m resolution. *Magn Reson Med*. 1998; 39:941–949. [PubMed: 9621918]
5. Xia Y, Moody JB, Burton-Wurster N, Lust G. Quantitative in situ correlation between microscopic MRI and polarized light microscopy studies of articular cartilage. *Osteoarthritis Cartilage*. 2001; 9:393–406. [PubMed: 11467887]
6. Mow VC, Kuei SC, Lai WM, Armstrong CG. Biphasic creep and stress relaxation of articular cartilage in compression? Theory and experiments. *J Biomechan Eng*. 1980; 102:73–84.
7. Moger CJ, Arkill KP, Barrett R, Bleuet P, Ellis RE, Green EM, Winlove CP. Cartilage collagen matrix reorientation and displacement in response to surface loading. *J Biomechan Eng*. 2009; 131:031008.
8. Mayerhoefer ME, Welsch GH, Mamisch TC, Kainberger F, Weber M, Nemec S, Friedrich KM, Dirisamer A, Trattnig S. The in vivo effects of unloading and compression on T1-Gd (dGEMRIC) relaxation times in healthy articular knee cartilage at 3.0 Tesla. *European Radiology*. 2010; 20:443–449. [PubMed: 19727756]
9. Xia Y, Wang N, Lee J, Badar F. Strain-dependent T1 relaxation profiles in articular cartilage by MRI at microscopic resolutions. *Magn Reson Med*. 2011; 65:1733–1737. [PubMed: 21452280]
10. Buckwalter JA, Mankin HJ. Articular cartilage. Part II: Degeneration and osteoarthritis, repair, regeneration, and transplantation. *J Bone Joint Surgery (Am)*. 1997; 79:612–632.
11. Alhadlaq H, Xia Y, Moody JB, Matyas J. Detecting Structural Changes in Early Experimental Osteoarthritis of Tibial Cartilage by Microscopic MRI and Polarized Light Microscopy. *Annals of Rheumatic Diseases*. 2004; 63:709–717.
12. Gold GE, Han E, Stainsby J, Wright G, Brittain J, Beaulieu C. Musculoskeletal MRI at 3.0 T: relaxation times and image contrast. *Am J Roentgenol*. 2004; 183:343–351. [PubMed: 15269023]
13. Wang N, Xia Y. Orientational dependent sensitivities of T2 and T1rho towards trypsin degradation and Gd-DTPA2- presence in bovine nasal cartilage. *MAGMA*. 2012; 25:297–304. [PubMed: 22071581]

14. Akella SV, Regatte RR, Wheaton AJ, Borthakur A, Reddy R. Reduction of residual dipolar interaction in cartilage by spin-lock technique. *Magn Reson Med*. 2004; 52:1103–1109. [PubMed: 15508163]
15. Li X, Benjamin Ma C, Link TM, Castillo DD, Blumenkrantz G, Lozano J, Carballido-Gamio J, Ries M, Majumdar S. In vivo T1rho and T2 mapping of articular cartilage in osteoarthritis of the knee using 3 T MRI. *Osteoarthritis Cartilage*. 2007; 15:789–797. [PubMed: 17307365]
16. Wang N, Xia Y. Dependencies of multi-component T2 and T1rho relaxation on the anisotropy of collagen fibrils in bovine nasal cartilage. *J Magn Reson*. 2011; 212:124–132. [PubMed: 21788148]
17. Xia Y. Magic-angle effect in magnetic resonance imaging of articular cartilage: a review. *Investigative Radiology*. 2000; 35:602–621. [PubMed: 11041155]
18. Xia Y, Farquhar T, Burton-Wurster N, Lust G. Origin of cartilage laminae in MRI. *J Magn Reson Imaging*. 1997; 7:887–894. [PubMed: 9307916]
19. Grunder W, Kanowski M, Wagner M, Werner A. Visualization of pressure distribution within loaded joint cartilage by application of angle-sensitive NMR microscopy. *Magn Reson Med*. 2000; 43:884–891. [PubMed: 10861884]
20. Rubenstein JD, Kim JK, Henkelman RM. Effects of compression and recovery on bovine articular cartilage: appearance on MR images. *Radiology*. 1996; 201:843–850. [PubMed: 8939241]
21. Alhadlaq HA, Xia Y. The structural adaptations in compressed articular cartilage by microscopic MRI (μ MRI) T2 anisotropy. *Osteoarthritis Cartilage*. 2004; 12:887–894. [PubMed: 15501404]
22. Alhadlaq HA, Xia Y. Modifications of orientational dependence of microscopic magnetic resonance imaging T2 anisotropy in compressed articular cartilage. *J Magn Reson Imaging*. 2005; 22:665–673. [PubMed: 16220547]
23. Xia Y. Resolution ‘scaling law’ in MRI of articular cartilage. *Osteoarthritis Cartilage*. 2007; 15:363–365. [PubMed: 17218119]
24. Wang N, Xia Y. Depth and orientational dependencies of MRI T2 and T1rho sensitivities towards trypsin degradation and Gd-DTPA(2-) presence in articular cartilage at microscopic resolution. *Magn Reson Imaging*. 2012; 30:361–370. [PubMed: 22244543]
25. Lesperance LM, Gray ML, Burstein D. Determination of fixed charge density in cartilage using nuclear magnetic resonance. *J Orthopaedic Research*. 1992; 10:1–13.
26. Bashir A, Gray ML, Hartke J, Burstein D. Nondestructive imaging of human cartilage glycosaminoglycan concentration by MRI. *Magn Reson Med*. 1999; 41:857–865. [PubMed: 10332865]
27. Zheng S, Xia Y. The impact of the relaxivity definition on the quantitative measurement of glycosaminoglycans in cartilage by the MRI dGEMRIC method. *Magn Reson Med*. 2010; 63:25–32. [PubMed: 19918900]
28. Hayes WC, Keer LM, Herrmann G, Mockros LF. A mathematical analysis for indentation tests of articular cartilage. *J Biomechanics*. 1972; 5:541–551.
29. Xia Y, Zheng S, Bidthanapally A. Depth-dependent profiles of glycosaminoglycans in articular cartilage by microMRI and histochemistry. *J Magn Reson Imaging*. 2008; 28:151–157. [PubMed: 18581328]
30. Wang N, Chopin E, Xia Y. The effects of mechanical loading and gadolinium concentration on the change of T1 and quantification of glycosaminoglycans in articular cartilage by microscopic MRI. *Phys Medicine Bio*. 2013; 58:4535–4547.
31. Mosher TJ, Smith HE, Collins C, Liu Y, Hancy J, Dardzinski BJ, Smith MB. Change in knee cartilage T2 at MR imaging after running: a feasibility study. *Radiology*. 2005; 234:245–249. [PubMed: 15550376]
32. Broom ND, Silyn-Roberts H. The three-dimensional ‘knit’ of collagen fibrils in articular cartilage. *Connective Tissue Research*. 1989; 23:75–88. [PubMed: 2632144]
33. Nag D, Liney GP, Gillespie P, Sherman KP. Quantification of T2 relaxation changes in articular cartilage with in situ mechanical loading of the knee. *J Magn Reson Imaging*. 2004; 19:317–322. [PubMed: 14994300]
34. Bashir A, Gray ML, Burstein D. Gd-DTPA2- as a measure of cartilage degradation. *Magn Reson Med*. 1996; 36:665–673. [PubMed: 8916016]

35. Chen SS, Falcovitz YH, Schneiderman R, Maroudas A, Sah RL. Depth-dependent compressive properties of normal aged human femoral head articular cartilage: relationship to fixed charge density. *Osteoarthritis Cartilage*. 2001; 9:561–569. [PubMed: 11520170]
36. Zheng S, Xia Y, Bidthanapally A, Badar F, Ilsar I, Duvoisin N. Damages to the extracellular matrix in articular cartilage due to cryopreservation by microscopic magnetic resonance imaging and biochemistry. *Magn Reson Imaging*. 2009; 27:648–655. [PubMed: 19106023]
37. Reiter DA, Peacock A, Spencer RG. Effects of frozen storage and sample temperature on water compartmentation and multiexponential transverse relaxation in cartilage. *Magn Reson Imaging*. 2011; 29:561–567. [PubMed: 21277724]
38. Xia Y, Farquhar T, Burton-Wurster N, Vernier-Singer M, Lust G, Jelinski LW. Self-Diffusion Monitors Degraded Cartilage. *Arch Biochem Biophys*. 1995; 323:323–328. [PubMed: 7487094]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.

T_2 and $T_{1\rho}$ weighted intensity images at two echo times ($TE = 2$ ms and 50 ms) and oriented 55° to the magnetic field (vertically up) of (a) native and (b) degraded tissue, with approximately the same strain. All intensity images were displayed using the same maximum (32767) and minimum (0) values on the usual gray scale.

Figure 2.

T₂ and T₁ρ (spin-lock field of 1 kHz) images of (a) native and (b) degraded tissues at different strains and the magic angle (55°). All images were plotted with the same intensity limits (0 – 150 ms). The arrows in (b) pointed to the 'valley' between the two peaks when the tissue was compressed. The rectangular region of interest (ROI) showed the profile locations in Fig 3 and Fig 4.

Figure 3.

T₂ profiles of (a, c) native and (b, d) degraded cartilage at different strains and at 0° and 55°. The boundaries of cartilage zones, shown as the vertical lines on the plot at different strains, were determined from the T₂ profile at 0° from the same specimen. Different zones (SZ, TZ, and RZ) of uncompressed tissue are marked. The long arrows in (a) and (b) pointed to the location shift of T₂ peak due to loading. The short arrows marked the second T₂ peak induced by compression.

Figure 4.

$T_{1\rho}$ profiles of (a, c) native and (b, d) degraded cartilage at different strains and at 0° and 55°. The arrows mark the second T_2 and $T_{1\rho}$ peak induced by the compression for degraded tissue.

Figure 5.

The depth-dependent mechanical modulus (a) and GAG concentration (b) profiles of native and degraded tissue as a function of the relative tissue depth (0 = articular surface, 1 = cartilage-bone interface).

Figure 6.

The schematics of the collagen fibril structure in articular cartilage when the tissue is not loaded (left), the native tissue when it is loaded (middle) and the degraded tissue when it is loaded (right). The deformation of the collagen fibers in the degraded tissue when it is loaded will form a large bending in the RZ, which has the same characteristics as the original TZ (arrow).

Table 1

The zonal averaged T_2 values (RMS $\pm$ SD in ms) as the function of strain. The three strains for the native tissue were 0%, 15% and 46%; the three strains for the degraded tissue were 0%, 16% and 50%.

		0° Orientation			55° Orientation		
		Unloaded	Modest strain	Heavy strain	Unloaded	Modest strain	Heavy strain
Native	SZ	15.9 $\pm$ 3.4	7.9 $\pm$ 4.4 ^b	10.3 $\pm$ 6.5 ^b	37.1 $\pm$ 3.4	15.0 $\pm$ 7.5 ^b	15.9 $\pm$ 7.6 ^b
	TZ	32.7 $\pm$ 9.1 ^b	29.9 $\pm$ 7.4 ^b	26.8 $\pm$ 3.2	44.4 $\pm$ 3.0	33.8 $\pm$ 4.6	25.9 $\pm$ 7.8
	RZ 1	14.1 $\pm$ 7.1 ^a	17.2 $\pm$ 6.1 ^a	14.8 $\pm$ 3.9 ^a	47.7 $\pm$ 1.4	43.7 $\pm$ 1.5	20.8 $\pm$ 3.7
	RZ 2	6.2 $\pm$ 1.0 ^a	7.3 $\pm$ 1.5 ^a	6.1 $\pm$ 1.5 ^a	39.0 $\pm$ 5.4 ^b	38.0 $\pm$ 5.2 ^b	11.0 $\pm$ 1.7
	All tissue	18.5 $\pm$ 11.7 ^a	18.4 $\pm$ 10.4 ^a	17.9 $\pm$ 9.7 ^a	43.0 $\pm$ 5.7	35.9 $\pm$ 1.3	20.1 $\pm$ 7.2
Degraded	SZ	18.5 $\pm$ 3.9	11.4 $\pm$ 6.8	4.8 $\pm$ 0.6	44.7 $\pm$ 5.9	36.2 $\pm$ 17.4	10.7 $\pm$ 4.5
	TZ	47.4 $\pm$ 11.5	40.4 $\pm$ 6.5	4.9 $\pm$ 0.1	60.3 $\pm$ 3.3	42.5 $\pm$ 16.0	24.9 $\pm$ 2.4
	RZ 1	23.9 $\pm$ 12.5	18.2 $\pm$ 3.8	7.6 $\pm$ 1.0	69.1 $\pm$ 1.6	12.8 $\pm$ 5.1	24.8 $\pm$ 6.2
	RZ 2	5.6 $\pm$ 0.6	9.8 $\pm$ 4.0 ^b	9.6 $\pm$ 4.2 ^b	54.7 $\pm$ 8.1	21.5 $\pm$ 6.2	11.5 $\pm$ 1.8
	All tissue	20.1 $\pm$ 13.6 ^b	19.6 $\pm$ 11.2 ^b	8.0 $\pm$ 3.1	60.5 $\pm$ 10.0	31.5 $\pm$ 16.5	18.9 $\pm$ 7.9

^aThese three sets of the T_2 data were statistically identical.

^bThese two sets of the T_2 data were statistically identical.

Table 2

The zonal averaged $T_{1\rho}$ values ($\text{RMS} \pm \text{SD}$ in ms) as the function of strain. The three strains for the native tissue were 0%, 15% and 46%; the three strains for the degraded tissue were 0%, 16% and 50%.

		0° Orientation			55° Orientation		
		Unloaded	Modest strain	Heavy strain	Unloaded	Modest strain	Heavy strain
Native	SZ	48.4±2.7	12.8±6.9 ^b	14.8±4.1 ^b	50.9±0.9	25.9±8.7	19.0±6.2
	TZ	52.7±1.2	37.8±7.1	32.9±2.2	52.2±1.7	45.4±3.9	29.4±1.9
	RZ 1	34.7±8.4	29.4±6.4	24.2±4.5	55.4±1.9	52.1±1.3	25.9±2.5
	RZ 2	17.1±4.6 ^b	14.9±4.8 ^b	12.1±4.3	45.0±5.7 ^b	42.9±5.5 ^b	17.0±3.4
	ALL	34.7±14.5	27.0±12.0 ^b	24.4±10.8 ^b	50.7±6.0	44.1±10.5	24.0±6.4
Degraded	SZ	68.6±3.5	36.7±13.5	17.2±0.5	76.5±0.6	60.7±12.8	20.0±7.8
	TZ	80.5±1.5	61.1±1.2	22.3±4.4	80.0±2.0	67.3±10.3	37.0±3.9
	RZ 1	47.8±11.3	54.1±5.9	29.3±1.7	86.8±2.2	37.9±6.3	31.6±4.5
	RZ 2	33.6±5.6	27.3±8.2	23.5±7.3	73.4±10.0	41.0±12.2	24.4±3.7
	ALL	55.1±19.3	45.7±13.3	26.0±6.0	80.4±8.6	54.1±16.8	31.0±7.9

^b These two sets of the $T_{1\rho}$ data were statistically identical.

Table 3
The GAG concentrations in native and degraded cartilage (individual measurements and mean values)

	GAG in Native Tissue (mg/ml)		GAG in Degraded Tissue (mg/ml)	
	ICP	MRI	ICP	MRI
Sample 1	88.9±6.4	78.4±7.2	11.3±4.1	13.1±3.2
Sample 2	80.6±5.1	71.6±6.8	7.1±2.9	9.2±2.6
Sample 3	75.5±4.7	67.2±6.1	9.3±3.2	11.4±3.0
Mean±Std	81.7±5.4	72.4±6.7	9.2±3.4	11.2±2.9