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MRI properties of a unique hypo-intense layer in degraded articular cartilage

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Abstract

To investigate the characteristics of a hypo-intense laminar appearance in articular cartilage under external loading, microscopic MRI (μ MRI) T_1 , T_2 and $T_1\rho$ experiments of total 15 specimens of healthy and trypsin-degraded cartilage were performed at different soaking solutions (saline and 100 mM PBS). T_2 and $T_1\rho$ images of the healthy tissue in saline showed no load-induced laminar appearance, while a hypo-intense layer was clearly visible in the deep part of the degraded tissue at the magic angle. Significant difference was found between T_2 values at 0° and 55° (from 16.5 ± 2.8 ms to 20.2 ± 2.7 ms, $p=0.0005$), and at 0° and 90° (16.5 ± 2.8 ms to 21.3 ± 2.6 ms, $p<0.0001$) in saline solution. In contrast, this hypo-intense laminar appearance largely disappeared when tissue was soaked in PBS. The visualization of this hypo-intensity appearance in different soaking mediums calls for caution in interpreting the data of relaxation times, chemical exchange, and collagen fiber deformation.

Keywords

T_2 ; $T_1\rho$; T_1 ; MRI; cartilage; loading; magic angle; anisotropy; laminar appearance

Introduction

Articular cartilage is a thin layer of connective tissue, the extracellular of which is mainly composed of water, a network of collagen fibers, and abundant amount of negatively charged glycosaminoglycans (GAG) (Venn and Maroudas, 1977; Maroudas, 1975). Cartilage has a unique zonal structure according to its collagen fiber orientations: superficial zone (SZ) at the surface with fibers parallel to the tissue surface, transitional zone (TZ) in the middle with the random fiber orientation, and radial zone (RZ) that connects the tissue and bone with fibers perpendicular to the surface (Xia, 1998; Lechner *et al.*, 1989; Xia *et al.*, 2001). The highly negative charged GAG, interacting with polar water molecules and generating a swelling pressure, plays the key role for the viscoelastic properties of articular cartilage that provides joints with sufficient resilience to everyday load-bearing activities (Mow *et al.*, 1980; Moger *et al.*, 2009; Mayerhoefer *et al.*, 2010; Xia *et al.*, 2011). In addition, the GAG concentration in articular cartilage has a well-defined linear gradient

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through the tissue depth (increasing from SZ to TZ) (Xia *et al.*, 2008). High GAG content and intact collagen architecture are essential for normal mechanical functions of cartilage and joints, while a reduced GAG can result in poor mechanical properties, which could be identified as the early degradation of articular cartilage (Rubenstein *et al.*, 1996).

The relaxation parameters in magnetic resonance imaging (MRI) have been used extensively in both research labs and clinics to evaluate cartilage degradation (Bashir *et al.*, 1996; Lesperance *et al.*, 1992; Nieminen *et al.*, 2001; Akella *et al.*, 2004; Li *et al.*, 2007; Nag *et al.*, 2004). Spin-lattice relaxation time T_1 can be used to quantify the GAG content in cartilage with the use of gadolinium based contrast agent (Wang *et al.*, 2013; Bashir *et al.*, 1996). Spin-spin relaxation time T_2 is sensitive to the collagen orientation and water content in cartilage (Nag *et al.*, 2004; Mosher *et al.*, 2005; Xia *et al.*, 2002). For example, T_2 images of articular cartilage can show a laminar appearance when the collagen fibrils in RZ are oriented parallel with the external magnetic field B_0 ; while the image of the same cartilage can appear homogeneous and brighter when the tissue is oriented at the magic angle ($\sim 55^\circ$ to the magnetic field), due to the minimization of the dipolar interaction to spin relaxation (Xia *et al.*, 1997). The immersion of articular cartilage in high concentration phosphate buffered saline (PBS) solution was found to result in a significant reduction in this laminar appearance in MRI due to the catalyzing effect of phosphate ions on the proton exchange among water molecules. Furthermore, Spin-lattice relaxation time in the rotating frame $T_1\rho$ has also been used to investigate early cartilage degradation, owing to its weaker influence from the dipolar interaction and its high sensitivity to GAG loss (Li *et al.*, 2007; Wang and Xia, 2012a; Souza *et al.*, 2012; Wang and Xia, 2013; Du *et al.*, 2010).

In several previous studies using high-resolution MRI, a unique hypo-intensity laminar appearance (a black line) was noticed in the deep region of articular cartilage when it was oriented at the magic angle with external compression. Such loading-induced laminar appearance signaled the load-induced deformation of collagen fibers in cartilage (Alhadlaq and Xia, 2005, 2004). The molecular origin of this unique laminar appearance, however, was not clear. Since a freeze-and-thaw cycle of cartilage specimen without any cryopreservation procedure could cause a GAG loss and since GAG is largely responsible for the tissue stiffness (Zheng *et al.*, 2009), the initial observation of this unique laminar appearance in cartilage likely comes from a reduced GAG in the specimens. A recent study has confirmed that the GAG content is indeed the molecular origin of this load-induced black line in the deep region of articular cartilage at the magic angle (Wang *et al.*, 2015). However, the influences of many experimental factors to this unique hypo-intensity layer are unclear.

Loading of cartilage could result in many consequences, including the reduction of water content, the enhancement of the macromolecules contents, the deformation of the collagen fibers, and the modification of the water-macromolecule and inter-macromolecular interactions. All these load-induced consequences in articular cartilage will have their own depth dependencies. To investigate the relaxation characteristics of the load-induced laminar appearance in compressed cartilage, both healthy and degraded (treated by trypsin degradation) specimens were imaged under loading, using quantitative T_1 , T_2 and $T_1\rho$ relaxation measurements throughout the entire depth of articular cartilage at microscopic

resolution. In addition, the effect of soaking solutions to the laminar appearance was also investigated.

Materials and Methods

Solutions of Saline and PBS

The solutions of normal (physiological) saline and phosphate buffered saline (PBS) for all the experiments were prepared in the laboratory. A normal saline was prepared by dissolving 9 grams of sodium chloride in one liter of deionized water (154 mM NaCl). A PBS solution was prepared as follows. First, 276 grams of sodium phosphate monobasic (monohydrate) (Sigma, Missouri) were dissolved in deionized water. After adjusting the pH value to 7.3 by NaOH (Sigma, Missouri), the volume of the phosphate buffer was finalized to one liter. Then 9 grams of NaCl was added to 50 ml of the phosphate buffer and diluted to one liter using deionized water. The final PBS solution contained a high phosphate concentration of 100 mM with the pH corresponding to ~ 7.4 (Wang and Xia, 2013).

Specimen Preparation

Humeral heads were harvested shortly after the sacrifice of mature and healthy dogs that were used for an unrelated research, where the institutional review committee approved the animal handling. A total of fifteen cartilage specimens were harvested; each was about $3.5 \times 2.5 \times 6$ mm in size where the intact cartilage was still attached to the underlying bone. Three healthy specimens were freshly harvested samples, which were soaked in physiological saline that contained 1% protease inhibitor (Sigma, Missouri). The twelve degraded specimens were fresh samples first soaked in 10 μ g/ml trypsin solution (Sigma, Missouri) for more than 8 hours to remove GAG, then soaked in saline with 1% protease inhibitor to remove excess trypsin (Wang and Xia, 2012b). All specimens were never frozen.

Microscopic MRI (μ MRI) Protocols

The specimen compression in μ MRI used a homemade unconfined loading device (Alhadlaq and Xia, 2004). T_2 and T_2^* experiments for healthy specimens were carried out without loading and with a ~ 13% strain at the magic angle. T_1 , T_2 , T_2^* experiments of degraded specimens were carried out when the specimens were compressed at a ~ 25% strain, respectively. The strain values were measured in the T_1 images by the reduction in cartilage thickness, with an error of about $\pm 4\%$. Each specimen was allowed half an hour to reach viscoelastic equilibrium upon loading.

All μ MRI experiments were performed at room temperature on a Bruker AVANCE||300 NMR spectrometer equipped with a 7 Tesla/89 mm vertical-bore superconducting magnet and microimaging accessory (Bruker Instrument, Billerica, MA). A 5 mm solenoid coil was used in the μ MRI experiments, which had a 90° hard pulse of 6.5 μ s. 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 $\times$ 0.45 cm, resulting in the 2D in-plane pixel size 17.6 μ m. The repetition time TR was 2 s for all experiments.

Quantitative T_1 , T_2 , and $T_{1\rho}$ imaging experiments without fat suppression at different orientations respect to the main magnetic field (0° , 55° , 90°) followed the previously established protocols in μ MRI of cartilage (Wang *et al.*, 2013; Wang and Xia, 2012a). T_2 -imaging experiments were performed using a CPMG magnetization-prepared T_2 imaging sequence. 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 had the same magnetization-prepared structure, preceded with a 90° hard pulse followed by a spin-lock pulse. The strength of the spin-lock was 1 kHz, which was calibrated by the strength of the 90° pulse. The T_1 experiments also had a magnetization-prepared structure, which has an inversion-recovery sequence with five inversion points (0, 0.4, 1.1, 2.2, 4.0 s). All quantitative relaxation images were calculated subsequently by a single-component fit on a pixel-by-pixel basis. A 10-pixel column was chosen from the center of each image and averaged to yield one relaxation profile and standard deviations.

Statistical Analysis

Imaging data were evaluated for significance using the commercial software KaleidaGraph (v. 4.0, Synergy Software, Reading, PA). One-way analysis of variance (ANOVA) test was performed to compare the T_1 , T_2 , and $T_{1\rho}$ values of degraded tissues after loading. The comparison was based on ROI-wise, and the ROI area was shown in Figure 1 (the small rectangle in the enlarged figures). The significance level between the data was set to $P < 0.05$.

Results

Quantitative T_1 , T_2 , and $T_{1\rho}$ images of loaded cartilage in Saline and PBS

Quantitative T_1 , T_2 and $T_{1\rho}$ images of degraded tissues soaked in both Saline and PBS were shown in Fig 1. The specimens were loaded under $\sim 25\%$ strain and oriented at three orientations (0° , 55° , and 90°) with respect to the main magnetic field. T_1 images (Fig 1a, 1d) exhibited homogenous appearance no matter soaked in what solution or set at what orientation. In contrast, T_2 and $T_{1\rho}$ images in saline (Fig 1b, 1c) lost the homogenous appearance when loaded - an unique hypo-intense layer or a black line could be seen in the deep part of the tissue at both 55° and 90° . The same hypo-intense layer was not clearly visible in the images at 0° . In comparison, when the specimens were soaked in 100 mM PBS, the black line appearance largely vanished and the T_2 and $T_{1\rho}$ images became rather uniform at any of the three orientations (0° , 55° , and 90°).

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

Fig 2 illustrates the depth-dependent profiles of T_2 and $T_{1\rho}$ (spin-lock field of 1 kHz) in healthy cartilage set at the magic angle, where the relative depth 0 is set at the articular surface and 1 at the cartilage-bone interface. T_2 profiles were homogeneous in depth without loading, since it was imaged at the magic angle. The reduction of T_2 under a modest loading (13% strain) was mainly in the upper part of the tissue. The results of the $T_{1\rho}$ profiles were qualitative similar to the results of the T_2 profiles, except that the $T_{1\rho}$ values were longer than the T_2 values regardless of loading condition.

T₁, T₂ and T_{1ρ} profiles of degraded tissues under external loading

The depth-dependent T₁, T₂, and T_{1ρ} profiles at all orientations (0°, 55°, and 90°) were plotted in Fig 3 when the specimens were soaked in saline and 100 mM PBS. Comparing with the T₂ and T_{1ρ} profiles of the healthy cartilage (Fig 2), several features of the degraded tissues were worth noting. First, T₁ profiles of the degraded and loaded cartilage (Fig 3a, 3b) showed little depth- and orientation-dependencies, no matter immersed in saline or PBS. Second, T₂ and T_{1ρ} profiles of the degraded and loaded cartilage varied extensively with both orientation and depth (the variation were from 43.4 ms to 6.9 ms in T₂ profiles at 55°, and from 79.3 ms to 24.5 ms in T_{1ρ} profiles at 55°) in saline solution. Third, T₂ and T_{1ρ} profiles of the degraded and loaded cartilage in the PBS solution (Fig 3d, 3f) showed little orientation-dependency and weak depth-dependency (the variations were from 32.3 ms to 20.9 ms in T₂ profiles at 55°, and from 34.5 ms to 20.2 ms in T_{1ρ} profiles at 55°). It's interesting to observe that two well resolved peaks in the T₂ profiles of the degraded and loaded cartilage (at both 55° and 90°): at the depths of ~ 120 μm and ~ 350 μm from the tissue surface (Fig 3b). When the specimens were oriented at 0°, T₂ profiles showed only one single-peak curve since the deepest part of the tissue could not be detected due to the low signal-to-noise ratio (SNR) when the dipolar interaction had a strong effect on T₂. Finally, the results of T_{1ρ} profiles (at the spin-lock frequency of 1 kHz) was qualitative similar to the results of T₂ profiles at all orientations (Fig 3e). The difference in the profiles existed when the tissue was oriented at 0°, where the single-peak curve in T_{1ρ} profiles was not as obvious as in T₂ profiles.

Table 1 showed the mean relaxation times in healthy and degraded cartilage without loading and in saline solution. T_{1ρ} is known to be sensitive to the GAG content in the tissue. Indeed, T_{1ρ} increased on degradation regardless of the orientation, while T₂ increased more at 90° and 55°. The quantitative relaxation times (T₁, T₂ and T_{1ρ}) of degraded tissue were summarized in Table 2. T₁ values showed little variation (from 1.09 s to 1.11 s) regardless of orientation or soaking solution. T_{1ρ} values had slightly variations (47.4 ms to 49.5 ms in saline, 26.0 ms to 27.0 ms in PBS) at different orientations. There was no statistically difference for T₂ values at different orientations when cartilage was soaked in PBS, while significant difference was found between T₂ values at 0° and at 55° (p = 0.0005), and T₂ values at 0° and at 90° (p < 0.0001) when the tissue was immersed in saline.

Division of the histological zones in cartilage

According to the orientation of the collagen fibrils in articular cartilage, the total depth of articular cartilage is commonly subdivided based on the local fibril orientation into multiple structural zones, such as the superficial zone at the top surface (SZ), the transitional zone (TZ) in the middle and the radial zone (RZ) that interfaces with the underlining bone. The division of the histological zones in the healthy cartilage was well established by MRI, polarized light microscopy, Fourier-transform infrared image and confocal light microscopy methods (Xia *et al.*, 2001; Chen *et al.*, 2001). The T₂ profiles of degraded tissue under loading at 55° were plotted in Figure 4a. Based on profiles, the whole thickness of articular cartilage was divided into three zones, SZ, TZ, and RZ, in a manner that was similar to the zonal division of tissue without loading. To investigate the relaxation time changes in the radial zone, RZ was further separated to three parts: RZ1 (upper RZ), RZ2 (middle RZ), and

RZ3 (lower RZ), according to the peaks and valley (black arrows) of the profile in Fig 4a. The quantitative zonal T_2 values at different orientations (0° , 55° , and 90°) were summarized in Fig 4b. T_2 values between 55° and 90° showed similar values, there were no significant differences of T_2 values at SZ ($p=0.1025$), TZ ($p=0.0894$), and RZ2 ($p=0.1546$). Furthermore, T_2 value at 55° and 90° was always higher than that at 0° except at RZ1 (where the valley region shown in T_2 profiles at 55° and 90°). Note that there was no RZ3 (lower RZ, deep part of the RZ) when the samples were oriented at 0° due to the low T_2 values and signal-to-noise ratio (SNR) at this region.

Discussion

The glycosaminoglycans (GAG) in cartilage carry a large number of negative charges, which are responsible to the hydrophilic nature of the macromolecules. The osmotic swelling pressure generated by the GAGs provides cartilage with its efficient load-bearing properties. When cartilage is externally compressed, a sequence of complicated events would occur, including water exclusion from cartilage matrix, deformation of collagen orientations, enhancement of solid concentrations, and modification of the molecular interactions. It was shown in previous studies that the characteristics of T_2 relaxation in cartilage changed profoundly when the tissue was compressed (Alhadlaq and Xia, 2004, 2005). The purpose of this study was to investigate the load-induced properties of a hypo-intense laminar appearance in the deep region of compression cartilage using different relaxation sequences, at different orientations respect to the main magnetic field, and immersed in different soaking solutions (saline and PBS).

Compressed healthy and degraded cartilage in saline solution

Articular cartilage shows a unique depth-dependent feature in its mechanical property due to the depth-dependent GAG concentration in cartilage. Since the surface part of articular cartilage has fewer GAGs when compared to the deep tissue, an external loading will cause more compression at the surface part (Xia *et al.*, 2011). For healthy tissue under a modest strain ($\sim 13\%$), the surface T_2 reduces more than the deep-tissue T_2 (Fig 1), which reflects a larger reduction of surface water and the deformation of the collagen structure after loading. This result also agrees with the previous T_1 results (Xia *et al.*, 2011). Trypsin treatment depletes the GAGs from cartilage (Wang and Xia, 2012a). Consequently, the collagen fibrils in a degraded tissue would have more freedom to change the orientations under loading. Indeed, T_2 and $T_{1\rho}$ in the degraded tissue change profoundly throughout the whole tissue depth after compression. The second T_2 peak in the deep part of the degraded tissue (Fig 4a) implies clearly the formation of an additional randomly-oriented fiber-zone in that part of the tissue after compressed (RZ2), which has the structure similar to the randomly oriented fibrils in the transitional zone (TZ) of healthy tissue. The ‘valley’ between these two T_2 peaks suggests the complex fibril bending after compression. Overall, the deformation of the collagen matrix in the degraded tissue differs substantially from the healthy tissue. This striking difference between healthy tissue and degraded tissue could be further explored as a biomarker for the degradation of cartilage.

In addition, the profiles of $T_{1\rho}$ relaxation time in the degraded tissue contain similar information as T_2 , which should further improve the visualization of tissue structure in clinical MRI due to its higher values than T_2 , especially T_2 values become even shorter when the tissue is under external compression. Although $T_{1\rho}$ profiles in saline at different orientation changes dramatically in μ MRI with very high resolution (17.6 μ m), this change might be omitted at lower resolution since the average $T_{1\rho}$ relaxation times (the mean value calculated from the tissue surface to the end of deep zone) at different orientations (Table 2) show smaller differences. This implies the advantages of high resolution MRI in articular cartilage structure detection, especially when the tissue thickness is reduced by the static loading.

Hypo-intensity layer appearance in different tissue-soaking solutions

This study shows for the first time that the hypo-intensity laminar appearance in compressed cartilage also depends on the solution in which the tissue specimen is immersed. This is related to the proton exchange phenomenon, which was noticed to play critical roles in relaxation as early as the 1960s (Berendsen and Migchelsen, 1965; Luz and Meiboom, 1964). Certain salts were found to be able to increase the exchange rate between water molecules: ammonium ions and phosphate ions were the most effective salts, followed by sulfate ions, but not some other salts like NaCl (Zheng and Xia, 2009; Wang and Xia, 2013). The results in this study demonstrate the important role of the tissue soaking solution in the measurement of relaxation times (T_2 and $T_{1\rho}$). The fact that the use of PBS solution can mask the hypo-intensity layer appearance provides solid evidence for the fast exchange rate between the bound water and free water in the relaxation mechanism. For the fast exchange system, the exchange rate k is commonly expressed as, $k \gg 1/T_{2A} - 1/T_{2B}$, where T_{2A} and T_{2B} are the relaxation times of two components. It is likely that the exchange rate in healthy and uncompressed articular cartilage has a strong depth-dependency, i.e., k at different tissue depth is different, depending upon the particular structure and concentrations of the local molecules involved. Comparing Fig 3c (specimens in saline) and Fig 3d (specimens in PBS) at different tissue depths, one notices that PBS soaking increased T_2 in the deep radial zone (from 200 to 400 μ m in a *compressed* cartilage) but decreased T_2 in the upper radial zone (around 100-150 μ m below the surface in a *compressed* cartilage). Assuming the deformation of collagen fibers in degraded cartilage under loading does not depend on the soaking medium, this depth-dependent change in the transverse relaxation, and hence the exchange rate, requires further investigation, to reveal the role of ions in the modulation of the relaxation process between healthy and degraded cartilage, both unloaded and loaded.

Hypo-intensity layer appearance measured by different relaxation sequences

The hypo-intensity laminar appearance in compressed cartilage depends on the MRI relaxation parameters. Homogeneous T_1 profiles in both saline and PBS (Fig 3a, b) documents that T_1 relaxation time is more sensitive to the spin motional processes at or around the Larmor precession frequency. In connective tissue such as cartilage, the predominant contributions to relaxation are dipole-dipole interactions, chemical exchange between water and electronegative groups of proteoglycans, diffusion, spin-spin coupling, and slow rotational motions of spins on large macromolecules (Mlynarik *et al.*, 2004; Xia *et al.*, 2002; Duvvuri *et al.*, 2001). In the current experimental setting, the dipole-dipole

interaction of water protons on highly oriented collagen plays the dominant role in the formation of the hypo-intensity layer via T_2 relaxation. The strong chemical exchange plays an important role for the absence of hypo-intensity layer in 100 mM PBS. $T_{1\rho}$ results at 1 kHz are similar to T_2 results, but the effect of dipole-dipole interaction and the chemical exchange relaxation mechanism are reduced by the spin-lock technique (Akella *et al.*, 2004).

Schematic model of articular cartilage deformation

The deformation of the collagen network and the reduction of the GAG concentration in articular cartilage are shown schematically in Figure 5. When healthy tissue is loaded (Fig 5b), the obliquely oriented collagens in the upper tissue will become more parallel with the articular surface, which results in an increase of the apparent SZ thickness. The perpendicular fibrils in the upper radial zone will become more oblique after loading, which cause an increase of the apparent TZ thickness. There is no large bending in the RZ collagen due to the abundant amount of GAG molecules in the tissue, acting as a compressive buffer; although some types of localized zigzag patterns may exist in the deep cartilage (Moger *et al.*, 2009). The trypsin treatment removes a large portion of the GAG from the tissue. When a degraded cartilage is under no loading, the orientation of collagen fibrils could still keep the same shape, with a well-known 3-zone structure (Fig 5c). An external loading will deform the collagen fibers, which would have a large bending in the RZ due to the low GAG concentration in the tissue. The RZ could therefore sub-divided into three sub-RZ zones (RZ1, RZ2, and RZ3), each having a different type of fibril deformation. The middle of the RZ (RZ2) would have a similar characteristic as the original TZ, which implies the formation of a new randomly oriented fiber zone after compression.

There are a few limitations in our study. First, all the experiments were performed *ex vivo* and at high resolution. Whether a similar phenomenon can also be visible in clinical MRI of human cartilage at lower resolution needs further investigation. Second, the loading strains are different for healthy tissues (~ 13 %) and degraded tissues (~ 25 %), which makes it difficult to directly compare the relaxation times (T_1 , T_2 , $T_{1\rho}$) between healthy tissue and degraded tissue. Further loading experiments could be designed under the same strain for the direct comparison among various tissues.

In conclusion, both dipolar interaction and chemical exchange play important roles in the formation of this unique appearance. The visibility of this phenomenon depends on the tissue soaking solution, relaxation sequences, and cartilage orientation with respect to the main magnetic field. The cause of this hypo-intensity laminar appearance in the degraded tissues is the complex deformation of the collagen matrix in cartilage under external loading. The visualization of this hypo-intensity appearance in different soaking mediums calls for caution in interpreting the data of relaxation times, chemical exchange, and collagen fiber deformation.

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Figure 1.

T_1 , T_2 and $T_{1\rho}$ (spin-lock field of 1 kHz) images of degraded tissues with $\sim 25\%$ strain at three different orientations (0° , 55° , and 90°). The intensity limit of T_1 images was 0 – 2 s. T_2 and $T_{1\rho}$ images were plotted with the same intensity limits (0 – 150 ms). The arrows in (b) and (c) point to the hypo-intensity appearance in the deep part of tissue when specimens were soaked in saline. No similar hypo-intensity appearance can be seen in T_1 images (a, d). The soaking of tissue in PBS removed this appearance in T_2 and $T_{1\rho}$ images (e, f). The figures on the right showed the enlarged figures from the rectangular region of interest (ROI). A 10-pixel column (the small rectangular ROI in the enlarged figures) was chosen from the center of each image and averaged to yield one relaxation profile.

Figure 2.

T_2 and $T_{1\rho}$ profiles (Mean $\pm$ Std) of healthy cartilage with 0% and ~13% loading strains at the magic angle as a function of the relative tissue depth (0 = articular surface, 1 = cartilage-bone interface). T_2 showed a homogeneous profile without loading, reduced mainly in the upper tissue under a modest loading (13% strain). Compared to T_2 profiles (41.7 ± 4.5 ms and 37.3 ± 3.9 ms at 0% and 13% strains), $T_{1\rho}$ values (48.9 ± 5.1 ms and 43.3 ± 4.6 ms at 0% and 13% strains) are always longer than T_2 values. The $T_{1\rho}$ profiles show similar trend as T_2 both with and without loading.

Figure 3.

T₁, T₂ and T_{1ρ} profiles of degraded cartilage (Mean ± Std) at different angles respect to the main magnetic field (0°, 55°, and 90°). T₁ profiles showed homogenous profiles no matter soaked in saline (a) or PBS (b). The arrows and arrowheads pointed to the “peak” and “valley” values in T₂ and T_{1ρ} profiles at both 55° and 90°.

Figure 4.

Division of the histological zones in degraded cartilage (a) and the corresponding zonal T_2 values (0° , 55° , 90°) (b) according to the T_2 profiles at the magic angle. This T_2 profile at the magic angle was the same dataset as shown in Fig 3 with smoothed fitting (dashed line). Three zones were divided: SZ, TZ, and RZ. RZ was further divided to 3 subzones: RZ1 (upper RZ), RZ2 (middle RZ), and RZ3 (lower RZ) according to the significant fiber orientation differences in this area. The arrows show the peaks and valley of the profiles. No significant difference of T_2 values at different orientations at SZ ($p=0.1025$), TZ ($p=0.0894$), and RZ2 ($p=0.1546$). Sample size was 6.

Figure 5.

The schematics of the collagen fibril structure and GAG distribution in both healthy (a, b) and degraded (c, d) articular cartilage when the tissue is without (left) and with loading (right). The GAG concentration of cartilage decreased dramatically after trypsin degradation (c), while the collagen fibrils still keep the same shapes when the tissue is not loaded (a, c). Some types of localized zigzag patterns may exist in RZ of the healthy tissue after loading (b), the deformation of the collagen fibers in the degraded tissue forms a large bending in the RZ after loading, the RZ2 (middle RZ) has the similar characteristics as the original TZ.

Table 1

The mean relaxation times (Mean $\pm$ Std) in healthy and trypsin degraded articular cartilage without loading in saline solution. The values in table were first obtained from the profile of each specimen and then averaged from 6 specimens (N=6).

Tissue	Healthy			Degraded		
Angle	0°	55°	90°	0°	55°	90°
T ₁ (s)	1.18±0.10	1.20±0.12	1.20±0.08	1.32±0.07	1.35±0.10	1.33±0.09
T ₂ (ms)	19.7±8.9	41.7±5.7	35.2±7.3	20.6±9.7	55.1±5.2	48.7±7.8
T _{1p} (ms)	32.5±7.4	48.9±4.0	43.7±6.2	49.2±6.0	82.6±6.4	70.8±2.9

Table 2

The mean relaxation times (Mean $\pm$ Std) in trypsin degraded articular cartilage with ~ 25% strain both in saline and PBS solution. The values in table were first obtained from the profile of each specimen and then averaged from 6 specimens (N=6).

Solution	Saline			PBS		
Angle	0°	55°	90°	0°	55°	90°
T ₁ (s)	1.10±0.05	1.09±0.06	1.10±0.04	1.11±0.04	1.09±0.05	1.11±0.05
T ₂ (ms)	16.5±2.8 ^{a,b}	20.2±2.7 ^a	21.3±2.6 ^b	25.3±0.7	25.8±0.9	25.4±0.8
T _{1ρ} (ms)	48.8±3.4	47.4±4.0	49.5±3.2	26.2±1.0	27.0±1.0	26.0±0.9

^aThere is a significant difference between T₂ value at 0° and T₂ value at 55° (p = 0.0005)

^bThere is a significant difference between T₂ value at 0° and T₂ value at 90° (p < 0.0001)