

High-resolution/high-contrast MRI of human articular cartilage lesions

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Background Magnetic resonance microscopy (MRM) is an important experimental tool in the identification of early cartilage lesions.

Methods Normal and degenerated cartilage samples were imaged at 11.74 T using a standard spin echo sequence. Quantitative MR measurements for T_1 , T_2 , and ADC were obtained and mapping for T_2 and ADC was performed. The bi-exponential model for T_2 relaxation was also explored. Histology was carried out for comparison with MR images.

Results MR images of cartilage samples displaying early stages of degeneration were positively correlated to their histological appearance in 23- μ m high-resolution images and also with much shorter imaging times at 47- μ m resolution. T_2 maps enable delineation of the actual cartilage zones, distinguishing the superficial zone in particular. The bi-exponential model can reflect cartilage components at different stages of degeneration.

Interpretation At 11.74 T, with 23- μ m resolution or with 47- μ m resolution and shorter imaging times, MRM provides images that allow visualization of early stages of cartilage degeneration, including superficial fibrillation. This has not been shown previously. The images also allow quantitative measurements (T_1 , T_2 , and ADC) in each cartilage region, which can be indicative of different stages of cartilage degeneration.

The lack of adequate methods for imaging the early stages of the cartilage degeneration in osteoarthritis in vivo has hampered advancements in understanding the pathogenesis of this common disease, and

also the effects of therapy. Various studies have shown that MRI is capable of delineating articular cartilage and allowing detection of cartilage damage and maturation in both human and animal joints. These include, but are not limited to, T_2 and $T_{1\rho}$ mapping (Dardzinski et al. 1997, Nieminen et al. 2001, Menezes et al. 2004), gadolinium-enhanced MRI (Nissi et al. 2004, Samosky et al. 2005, Williams et al. 2005), magnetization transfer contrast (Vahlensieck et al. 1994), measurement of the diffusion coefficient (Xia et al. 1995, Kerttula et al. 2001), quantification of glycosaminoglycan content using delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) technique (Tiderius et al. 2003, Williams et al. 2005), analysis of diffusivity, porosity, and structure through diffusion tensor imaging (Filidoro et al. 2005) and q-Space imaging (Othman et al. 2006), and recently by following cartilage maturation through the bi-exponential T_2 relaxation time (Keinan-Adamsky et al. 2006).

In the present study, we report on the morphological appearance of articular cartilage at representative stages of degeneration using MRM, in vitro. Although this technology is used on small cartilage samples and is not currently useful for large samples or in the clinic, it is of value to pursue this route of high resolution MR imaging with the assumption that the associated cartilage imaging parameters will require explanation as the technology eventually advances into the clinical realm. Here, we report on MR characteristics of human articular cartilage in actual stages of degeneration, as in osteoarthritis, with a spatial resolution of 23 μ m $\times$

23 $\mu\text{m} \times 500 \mu\text{m}$ at 11.74 T (500 MHz for protons). This was correlated with histological analysis. For quantitative analysis, we report the spin-lattice relaxation time (T_1), the spin-spin relaxation time (T_2), and the apparent diffusion coefficient (ADC) measured for specific regions of interest within the three cartilage zones: superficial, middle, and deep. We also map the T_2 relaxation and apparent diffusion coefficient with in-plane resolution of 47 $\mu\text{m} \times 47 \mu\text{m}$. In addition, we investigated the feasibility of fitting the bi-exponential T_2 relaxation time to extract the fast and slow compartments for different stages of degeneration.

Material and methods

Human samples

Human tali were obtained within 24 h of death of the donor through the Gift of Hope Organ and Tissue Donor Network (with Rush University IRB approval), and frozen at -80°C until experimentation. Just prior to experimentation, 10-mm cubes containing full-thickness cartilage with subchondral bone were harvested from the talar dome using a band saw with a diamond-tip blade. To eliminate any saw blade artifacts, the samples were then trimmed with a sharp scalpel blade to cubes 3 mm in width. The cartilage/bone cubes were placed in NMR sample tubes filled with physiological saline. An initial gross morphological grading and specimen selection was carried out by author JL, who was not involved in the MR imaging or histology, to ensure variation in cartilage integrity. For comparisons between histological and MR images, there were 4 specimens displaying no signs of cartilage degeneration (grade 0), 3 specimens showing fibrillation (grade 1), and 3 specimens showing deep fissuring (grade 2). These cartilage degeneration scores were later verified through histology, as described below. After MR imaging, these specimens were then processed for conventional histology and re-graded by an observer who was blinded to the macroscopic and MR grades.

Magnetic resonance imaging system

MRI experiments were conducted at 11.74 T (500 MHz for protons) using a 56-mm vertical bore magnet (Oxford Instruments, Oxford, UK) and a

Bruker DRX Avance Spectrometer (Bruker Instruments, Billerica, MA) controlled by a Silicon Graphics SGI2 workstation (Mountain View, CA). MR images were acquired using a Bruker Micro 5 imaging probe with triple-axis gradients (maximum strength 200 G/cm) and a 5-mm diameter RF saddle coil was used to transmit/receive the nuclear magnetic resonance signals. The scanner was controlled by ParaVision imaging software version 2.1.

High-resolution MRI and measurement of MR parameters

6 samples of each grade with subchondral bone were loaded into a 5-mm diameter saddle coil. The samples were placed in a 5-mm diameter NMR tube with the cartilage imaging plane perpendicular to the main magnetic field. In all cases, the B_0 was perpendicular to the plane of the image. This orientation eliminated the dependence on the magic angle in our study, i.e., the same orientation effect was observed for all samples. High-resolution MR magnitude images were obtained in the axial plane (revealing information along the depth of the cartilage) with a standard spin echo pulse sequence (repetition time $TR = 1$ sec, echo time $TE = 60$ msec, slice thickness = 0.5 mm, number of averaged experiments $NEX = 64$). Squared field-of-view (FOV) of 0.6 cm, and a matrix size of 256×256 were used, resulting in a spatial resolution of $23 \mu\text{m} \times 23 \mu\text{m}$. The slice was positioned at the center of the specimen with the frequency encoding in the horizontal direction of the cartilage plane. After imaging, samples were fixed in 10% formalin and processed for histology.

For quantitative analysis, the spin-lattice relaxation time (T_1), the spin-spin relaxation time (T_2), and the apparent diffusion coefficient (ADC) were measured for the specific regions of interest, representing the three specific zones as shown in Figure 3 using in-house MATLAB 7.0 (MathWorks Inc., Natick, MA) code. The ROI was defined based on the depth from the subchondral bone (supported by visual inspection) where the superficial and the deep zones have lower signal intensities than the middle zone in the T_2 weighted images. The T_1 was measured using a saturation recovery spin echo imaging sequence in 7 steps, with TR s of 50, 100, 200, 500, 1,000, 2,000, and 4,000 msec ($TE =$

7 msec, NEX = 1, FOV = 0.6 cm, and matrix = 128×128 , resulting in a spatial resolution of $47 \mu\text{m} \times 47 \mu\text{m}$). The T_2 was measured by applying a multi-echo spin echo imaging sequence to acquire 32 echoes with a 7-msec echo spacing (TE) from the chosen axial slice (TR = 4 sec, $TE_{\min} = 7$ msec, NEX = 1, FOV = 0.6 cm, and matrix = 128×128 , resulting in a spatial resolution of $47 \mu\text{m} \times 47 \mu\text{m}$). The ADC was measured using a spin echo diffusion weighted imaging (SEDWI) sequence. The diffusion gradient was applied along the read direction (in the plane of the articular surface) by varying the b-values in 16 steps linearly, corresponding to diffusion-weighted gradient strength ranging from 0 to 30 G/cm (TR = 1 sec, TE = 30 msec, $\delta = 3$ msec, $\Delta = 18$ msec, matrix = 128×128 , resulting in a spatial resolution of $47 \mu\text{m} \times 77 \mu\text{m}$, NEX = 1, and 16 b-values up to $1,513 \text{ sec/mm}^2$). The sequence and fitting procedure were tested on a water phantom with a measured diffusion coefficient of $2.2 \times 10^{-3} \text{ mm}^2/\text{s}$ (at $31 \pm 2^\circ\text{C}$).

The T_1 relaxation time was extracted from the experimental data using a least-squares single exponential fitting. The T_2 and ADC data were analyzed using both monoexponential and bi-exponential fitting models. The T_2 bi-exponential decay was fitted using the formula $S = A_f e^{-TE/T_{2f}} + A_s e^{-TE/T_{2s}}$, where T_{2f} and T_{2s} represent the extracted “fast” and “slow” T_2 relaxation components, while A_f and A_s are the amplitude coefficients of the fast and slow compartments, respectively. Customized MATLAB code was used to extract both relaxation times and ADC. All quantitative data were expressed as mean (SD); the SDs were calculated from different samples for each grade. Student’s t-test was used to compare between different grades and $p < 0.05$ was considered statistically significant.

Histology

The tali samples were decalcified in aqueous formic acid/sodium citrate (50:50), paraffin embedded, sectioned to $6 \mu\text{m}$ thickness, and stained with safranin O and fast green for conventional light microscopy (Rosenberg 1971), or with picosirius red for examination with polarized light microscopy. All sections were then examined under $20\times$, $40\times$, $100\times$, and $400\times$ magnification and graded by an observer who was blinded to the MR images,

according to a cartilage histopathology scale as outlined below.

Grading of the histological sections and MR images

Both MR images and histological images were graded according to the cartilage histopathology grading scale described above under “Human samples”. The MR images were examined by 2 individuals blinded to both the macroscopic and microscopic results and an overall score, agreed upon by both observers, was recorded based on the most severe lesion observed in the image. For histology grading, 6 sections from each specimen were observed by two other individuals blinded to the macroscopic and MR images, and an overall score, agreed upon by both observers, was recorded based upon the most severe lesion in the sections. The graders were not told how many samples there were at each grade of degeneration. For histology, there were 4 individual specimens from 4 separate tali at grade 0, where the thickness of the superficial zone in the 4 normal samples was measured as a percentage of the total uncalcified cartilage height and compared to that detected in the corresponding high-resolution MR images.

Results

Comparison between high-resolution MR images and histology

Representative examples of high-resolution MR magnitude images for normal (grade 0), superficially fibrillated (grade 1), and fissured (grade 2) cartilage were acquired with a voxel size of $23 \mu\text{m} \times 23 \mu\text{m} \times 500 \mu\text{m}$, as shown in Figure 1 (a, c, and e) with their corresponding histological sections (b, d, and f). Normal cartilage (Figure 1a) has a trilaminar appearance revealing three distinct zones (superficial, middle, deep), based on the signal intensity of the MR magnitude images. In the MR images, the actual depth of the superficial zone as measured in histological sections of the normal, undegenerated cartilage was $3.8\% \pm 0.4$ of the uncalcified cartilage thickness as compared to the $4\% \pm 0.6$ measured in the MR image. The superficial zone could only be measured for the grade 0 specimens, as this was the only grade in which

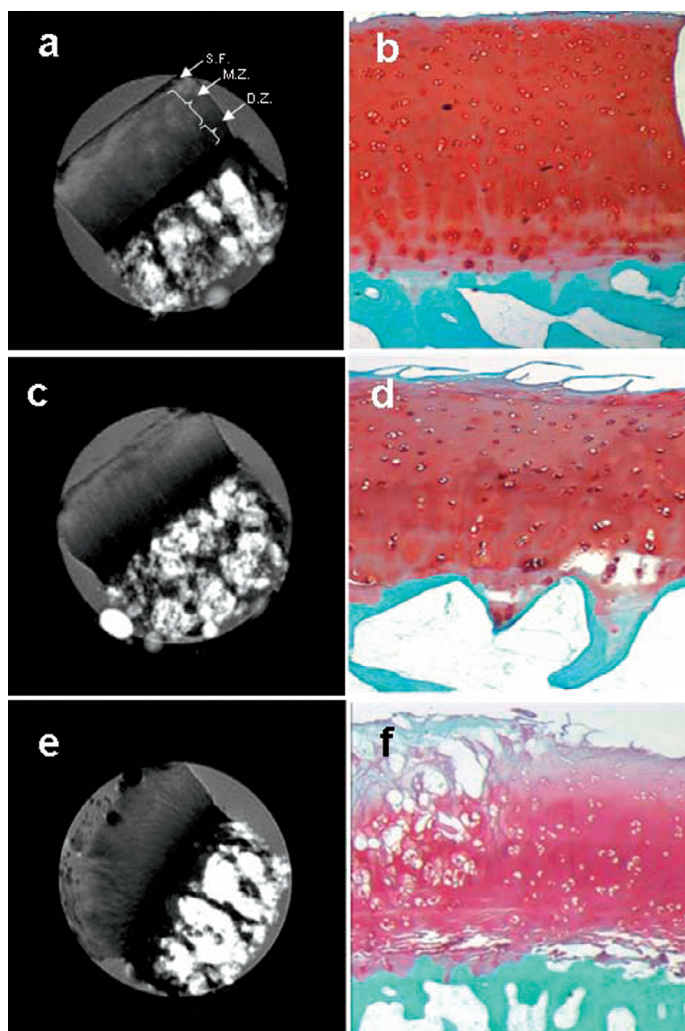


Figure 1. High-resolution MR images of different cartilage grades: (a) grade 0, (c) grade 1, (e) grade 2; and their corresponding safranin-O/fast green-stained sections ($\times 40$) for the same grades: (b), (d), and (f), respectively. The different zones are labeled superficial (S.F.), middle (M.Z.) and deep (D.Z.). Experimental parameters are: TR / TE = 1000 / 60 msec; FOV = 0.6 cm; slice thickness = 0.5 mm; in-plane resolution = $23 \mu\text{m} \times 23 \mu\text{m}$; NEX = 64. B0 was perpendicular to the plane of the image.

the superficial zone was intact. Grade 1 cartilage showed a loss of dark contrast, correlated with loss of proteoglycan (PG), in the superficial zone, while grade 2 was characterized by a loss of the characteristic signal for the superficial and middle zones. The smooth, intact surface seen in the histological sections of the grade 0 cartilage was seen as such in the MR images. The fibrillation and fissuring characteristic of the grade 1 and 2 cartilage, respectively, were accurately represented in their

MR images. This was exemplified by a one-to-one correspondence between the MR and histological images of the 4 specimens of grade 0, 3 specimens of grade 1, and 3 specimens of grade 2. This is of particular interest for the fibrillation, as it occupied less than $50 \mu\text{m}$ of the cartilage surface. For example, in the MR image of the grade 1 specimen shown in Figure 1c, the superficial zone seen in Figure 1a is nearly completely absent and the surface is slightly irregular. This corresponds to the replacement of the normal superficial zone with irregular strands of cartilage without stainable proteoglycan, seen in the histological section of the same specimen in Figure 1d. An MR image of a grade 2 cartilage specimen is shown in Figure 1e. In this case, there is a severe disruption of the surface that continues down into the middle zone. The collagen fiber architecture is also visible, probably indicating the loss of proteoglycan and thus the “opening up” of the matrix and exposure of collagen fiber bundles. This breakdown of the cartilage matrix is apparent in the histological section of the same specimen in Figure 1f. It must be kept in mind that because the partial volume filling in MR images is greater than with histology ($500 \mu\text{m}$ as compared to $6 \mu\text{m}$), identical

image registration is not possible. To ensure a credible comparison between MR and histology, the depth of the superficial zone of the cartilage was compared with the maximum attainable depth of the superficial zone in the histological sections to account for the larger partial volume effect in the MR images. In addition, the architecture of the collagen fiber bundles, observed as organized, longitudinal light and dark bands in the grade 0 and 1 specimens, was disrupted in the grade 2 speci-

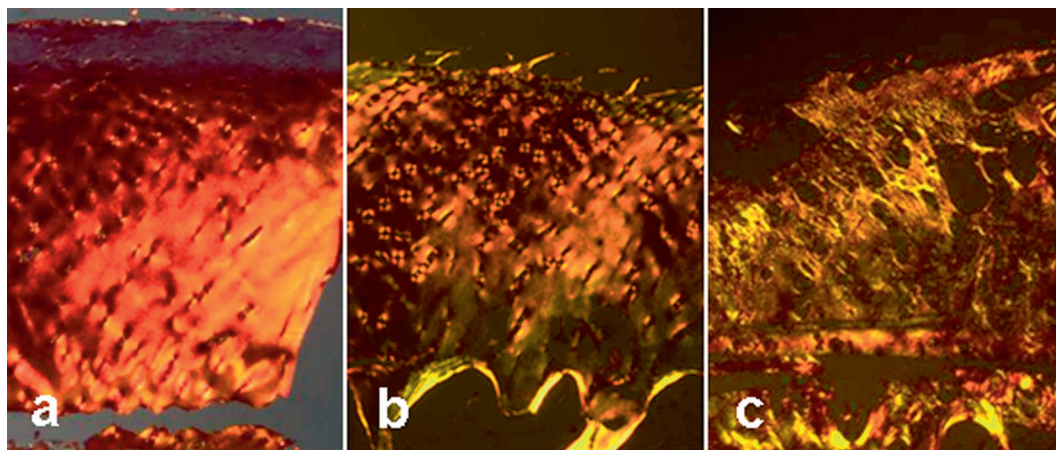


Figure 2. Polarized light microscopy of the same sections as in Figure 1: (a) grade 0, (b) grade 1, and (c) grade 2. These were stained with picosirius red and viewed with polarized light. There is loss of birefringence (panel c) in the grade 2 specimen, denoting breakdown of the normal cartilage architecture that could be visualized in the MR image of the same specimen in Figure 1e.

mens—as noted also in the polarized light microscopy images shown in Figure 2.

Figure 3 shows MR images with T_2 weightings of TE = 7, 21, and 42 msec with a voxel size of $47 \mu\text{m} \times 47 \mu\text{m} \times 500 \mu\text{m}$ for the three cartilage grades (0, 1, and 2). At TE = 7 msec, the middle and deep cartilage zones cannot be distinguished from one another, although the superficial zone is delineated by a dark line. The different zones can be better visualized by increasing the T_2 weighting. Dark regions are apparent where the cartilage is compressed against the glass tube (which was also noticeable in Figure 1). The time to acquire these images was 4.5 min, while the time required to acquire the high-resolution images of Figure 1 was 4.55 h. Thus, the general morphological features attainable at high resolution are also attainable with lower resolution (specifically at TE = 21 and 42 msec), with a twofold magnitude reduction in acquisition time. The correlation coefficient (r) when comparing the MR images and their histological sections was 1, thus demonstrating a strong correlation.

Quantitative MR results

The MR relaxation times (T_1 and T_2) and the ADC were measured in the three cartilage zones and are shown in Tables 1, 2, and 3, respectively. The MR measurements were based on a single ROI in each cartilage zone, where the superficial and deep zones have lower signal intensities than the middle

zone—especially in grade 0. Figure 3a shows the typical location for ROIs selected in each zone. For grade 2, there were no distinguishable superficial or deep zones; thus, one single ROI corresponding to the middle zone (as measured from the distance from the subchondral bone) was determined for each sample. The measured MR parameters were not constant along the depth of the cartilage, moving from the superficial zone to the deep zone. No specific pattern was observed for the T_1 relaxation time in comparison with T_2 and ADC values, although T_1 relaxation times decreased from grade 0 to grade 1 in the superficial and middle zones, but this was not statistically significant.

For grade 0 cartilage, the T_2 relaxation time (monoexponential fitting; Table 2) remained almost the same (a statistically insignificant change) from the superficial zone to the middle zone and then decreased significantly ($p = 0.05$) from the middle zone to the deep zone. The early degeneration, characteristic of grade 1, was apparent in each of the zones, but particularly in the deep zone where there was a decrease from 24.1 (0.5) for the grade 0 tissue to 13.5 (2.7) for the grade 1 tissue. This was representative of what was seen in the MRI magnitude images of Figure 1—in which even at grade 1, the matrix in the upper half of the tissue appeared to be somewhat “opened up” so that there was greater visualization of the collagen fiber bundle architecture. At grade 2, the cartilage showed deep fissuring and loss of the characteristic zones; thus,

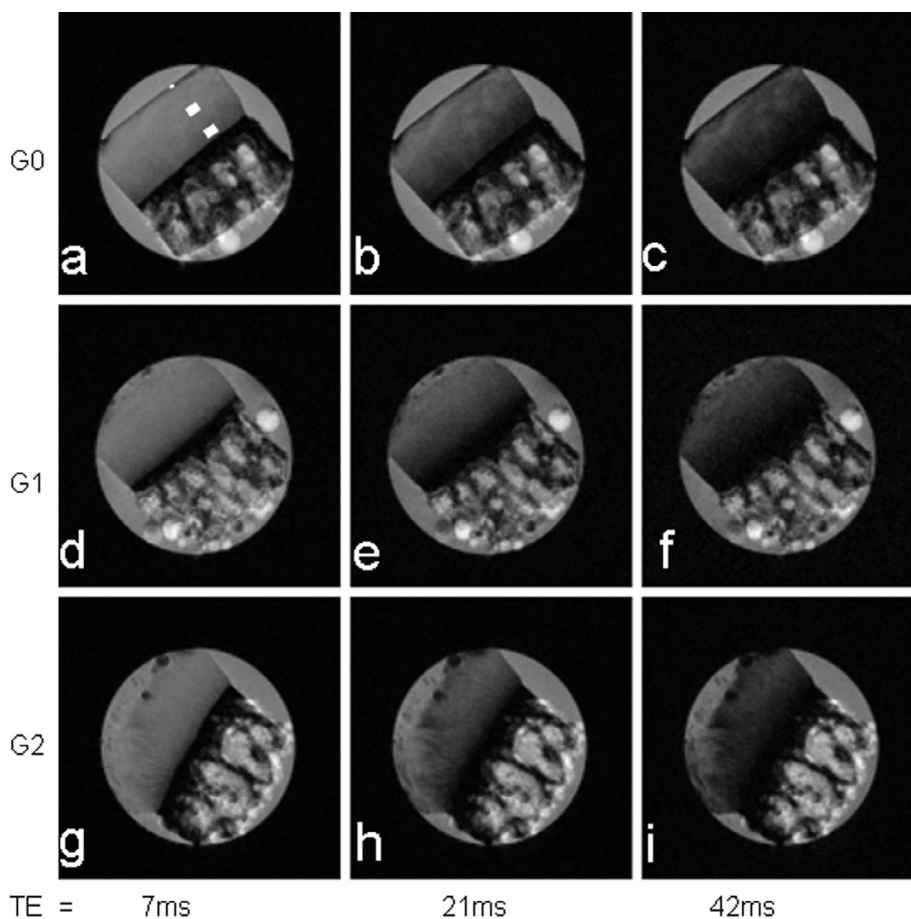


Figure 3. MR images with different TEs of 7, 21, and 42 msec for the 3 grades of degeneration. TR = 4000 msec; FOV = 0.6 cm; slice thickness = 0.5 mm; in-plane resolution = $47 \mu\text{m} \times 47 \mu\text{m}$; acquisition time = 4.5 min; NEX = 1. The white rectangles represent the ROI selected for the superficial, middle, and deep zones respectively.

Table 1. T_1 relaxation times (sec) for different grades in the three distinct cartilage zones. For grade 2 only one ROI could be determined, and this was in the middle zone. Thus, the determination of superficial and deep zones for grade 2 cartilage is not applicable (NA). 6 samples from each grade were studied ($n = 18$). Values in parentheses are SD

Zone	Grade 0	Grade 1	Grade 2
Superficial	1.32 (0.23)	1.14 (0.22)	NA
Middle	1.56 (0.22)	1.21 (0.19)	1.77 (0.35)
Deep	1.31 (0.13)	1.62 (0.54)	NA

only one ROI in the middle zone was measured. Here, the T_2 relaxation time was 27.9, which lies between the values for grades 0 and 1.

The bi-exponential model for T_2 relaxation is also shown in Table 2. The values of the fast compartment relaxation time are similar to the monoexponential fitting with the highest percentage (> 95%) (for example, in the superficial zone for grade 0, $T_{2f} = 21.3$ msec as compared to T_2 monoexponential = 24.1 msec). The fast component dominated by accounting for over 95% of the total relaxation for cartilage at all stages of degeneration. The ADC increased in each of the zones at early stages of degeneration (grade 1). In grade 2, there was an increase in the diffusion coefficient as compared to all zones of grade 0 and compared to the middle and deep zones of grade 1 cartilage.

Since the ROI measurements were not sufficient to allow full characterization of the cartilage and

Table 2. The bi-exponential parameters for “fast” compartment T_{2f} , “slow” compartment T_{2s} (both in msec), and the corresponding amplitude for each compartment for different grades in the three distinct cartilage zones. For grade 2, only one ROI, the middle zone, could be determined. Three samples from each grade were studied ($n = 9$ for the entire experiment). Values within parentheses are SD

Mono- and bi-exponential fitting		Superficial		Middle		Deep	
Grade 0							
Bi-exp.	T _{2f} /T _{2s} (msec)	27.1 (1.4)	305 (0.5)	29.1 (0.2)	306 (0.8)	21.3 (0.7)	311 (9.2)
	Amplitude (%)	96.3 (0.3)	3.7 (0.3)	97.6 (0.2)	2.4 (0.2)	96.7 (0.2)	3.3 (0.2)
Mono-exp.		31 (1.9)		31.5 (0.5)		24.1 (0.5)	
Grade 1							
Bi-exp.	T _{2f} /T _{2s} (msec)	22.4 (1.2)	281 (21.3)	18.0 (1.5)	323 (41)	11.0 (2)	361 (61.7)
	Amplitude (%)	95.2 (2.1)	4.8 (2.1)	96.6 (0.6)	3.4 (0.6)	96.8 (1.2)	3.2 (1.2)
Mono-exp.		27.6 (3.9)		21.1 (2.0)		13.5 (2.7)	
Grade 2							
Bi-exp.	T _{2f} /T _{2s} (msec)	NA	NA	24.8 (7.2)	310 (7.2)	NA	NA
	Amplitude (%)	NA	NA	96.9 (0.5)	3.1 (0.5)	NA	NA
Mono-exp.		NA	NA	27.9 (7.0)		NA	NA
NA: not applicable.							

NA: not applicable.

Table 3. Apparent diffusion coefficients $10^{-3} \text{ mm}^2/\text{s}$ for different grades in the three distinct cartilage zones. For grade 2, only one ROI, the middle zone, could be determined. Six samples from each grade were studied ($n = 18$). Values in parentheses are SD

Zone	Grade 0	Grade 1	Grade 2
Superficial	0.97 (0.68)	1.83 (0.33)	NA
Middle	1.28 (0.17)	1.37 (0.28)	1.65 (0.51)
Deep	1.0 (0.19)	0.9 (0.77)	NA

NA: not applicable.

to contribute to our understanding of the changes occurring with the stages of degeneration, pixel by pixel measurements specifying the T_2 relaxation time were performed for all three stages of cartilage integrity, as shown in Figure 4. The three cartilage zones for grade 0 cartilage were better visualized in the T_2 pixel-by-pixel map than in the MR

magnitude image. For grade 1 cartilage, there was a reduction in T_2 values in all zones as compared to grade 0. While there was a general loss of structure in grade 2, the T_2 value was found to lie between the values for cartilage of grades 0 and 1, but still a trend downward remained from superficial to deep zones.

It is also interesting that at points at which the cartilage is pressed against the glass tube in which it is seated, there is a reduced T_2 relaxation time as compared to the adjacent tissue (10 msec compared to 25 msec) (Figure 5). This is due to the known changes in cartilage that occur with loading, as in this case, against the glass tube. This loading could not be avoided if the cartilage was to be well seated, so that it would not change position during imaging. In Figure 4 it can also be seen that the T_2 values are more consistent in the transverse direction in the grade 0 sample than in the other

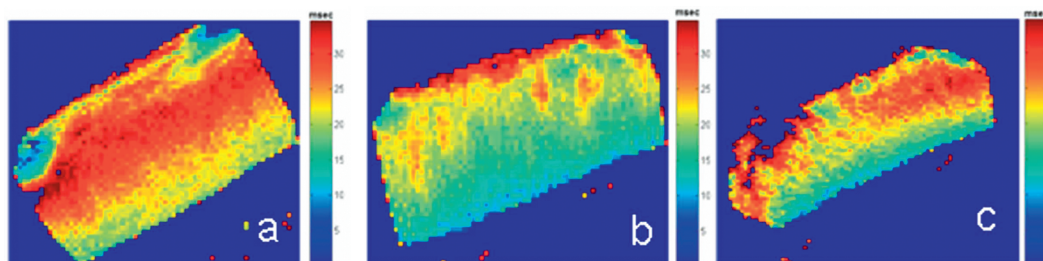


Figure 4. T_2 maps of different cartilage grades: (a) grade 0, (b) grade 1, and (c) grade 2. In (c), the grade 2 lesion seen on the left of the section can also be seen in Figure 3 g, h, and i.

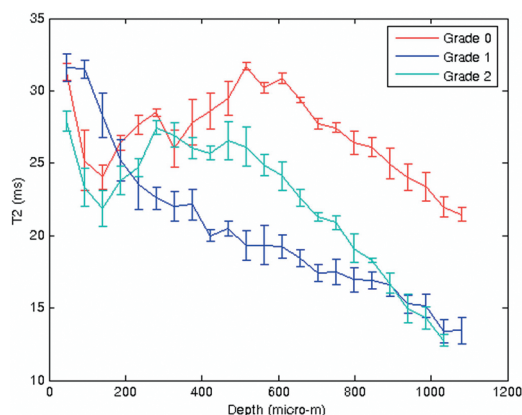


Figure 5. T₂ line profiles along the depth of the cartilage for different grades of degeneration, moving from the deep zone to the superficial zone. The lines were the average of 6 lines along the cartilage depth of the samples shown in Figure 4.

samples. This can be explained by the homogeneity across each individual zone in normal cartilage that is lost in the degenerated samples, in which there is a gradation of pathological changes across the tissue. A T₂ line profile extracted from the data of Figure 4, along the depth of the cartilage from the superficial zone to the deep zone and for the three stages of cartilage degeneration, is shown in Figure 5. For grade 1, the T₂ values were lower than for grade 0. The line profiles were obtained by averaging 10 vertical lines from the articular surface to the subchondral bone. For the purpose of determining reproducibility, this procedure was carried out for 3 samples at each grade of cartilage degeneration, although data for 1 sample per grade

are shown. The depth of the cartilage was normalized within each grade. A 2% variation in the superficial zone, 8% variation in the middle zone, and < 2% variation in the deep zone was found within each grade.

The bi-exponential T₂ relaxation times showing the fast and slow compartments and their percentages are shown in Figure 6. The values of fast compartment relaxation time are similar to the mono-exponential with the highest percentage (> 95%). This agrees with the results for the individual ROIs. It is obvious from Figure 6 panels c and g that the slow relaxation time changes in the middle zone at early stages of degeneration. ADC maps for all three stages of cartilage degeneration are shown in Figure 7. The ADC increases at the early stage of cartilage degeneration. A bi-exponential model for calculation of the fast and slow ADC compartments was not possible due to the low “b” values used (Cohen and Assaf 2002).

Discussion

Our study shows, ex vivo, that at 11.74 T, MR imaging allows visualization of the morphological state of cartilage degeneration at the microscopic level. Stages of cartilage intactness ranging from normal, undegenerated through early fibrillation, and fissuring were readily identifiable. The images produced are of high quality and with a degree of visualization of degeneration—particularly in the superficial zone—not previously reported for natu-

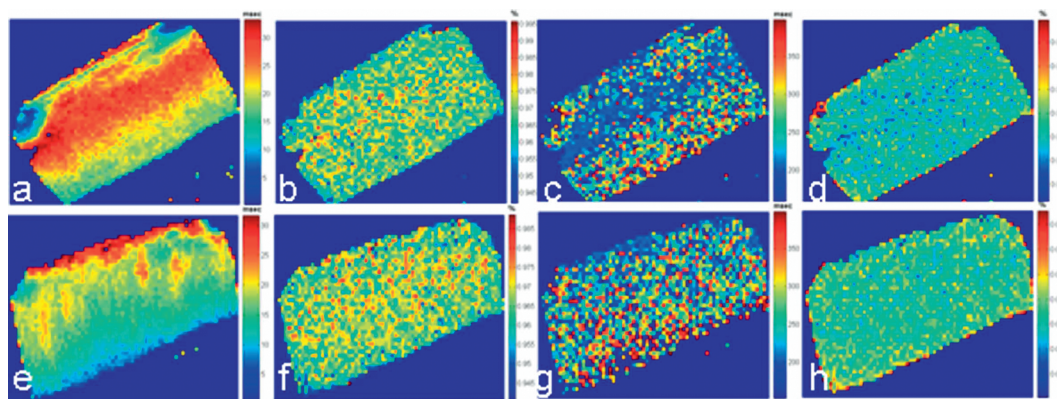


Figure 6. T₂ maps for the bi-exponential fitting for grades 0 and 1. Fast T₂ relaxation (a, e) and the corresponding percentages (b, f) for grades 0 and 1, respectively. Slow T₂ relaxation (c, g) and the corresponding percentages (d, h) for grades 0 and 1, respectively.

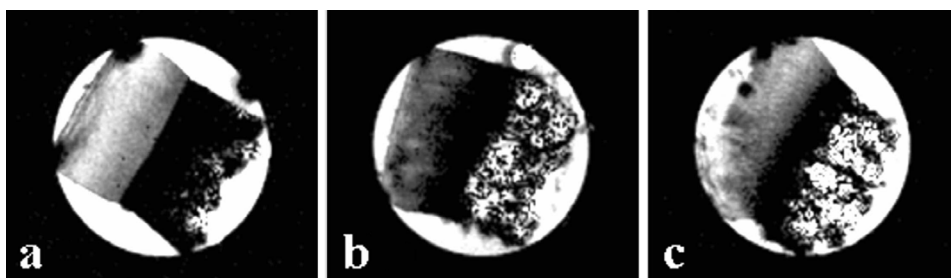


Figure 7. ADC maps of different cartilage grades: (a) grade 0, (b) grade 1, and (c) grade 2.

rally occurring cartilage degeneration. Rather than the multilaminar appearance in which the zones are not equal in depth to the actual individual cartilage zones as measured histologically (Recht et al. 1993, Rubenstein et al. 1993, Erickson et al. 1996, Frank et al. 1997, Nissi et al. 2004), the actual superficial zone (as assessed by histological comparison) was identifiable in the present study. At high contrast and high SNR, we were able to distinguish the superficial cartilage zone as an entity in itself, and could therefore determine even the most superficial fibrillation. This was discernable in the 23- μ m high-resolution images, as well as with much shorter imaging times at 47- μ m resolution. This contrasts with previous studies (Modl et al. 1991, Recht et al. 1993) in which a trilaminar appearance was detected but in which the zones were not equal in depth to the actual individual cartilage zones as measured histologically. Even the earliest stages of fibrillation, occupying less than 50 μ m in depth from the articular surface, were readily identifiable in both low- and high-resolution MR images. Thus, these data may be used as baseline information for future in vivo cartilage studies with emerging high-field MR systems (Duyn et al. 2005). These have a higher signal-to-noise ratio (SNR) that can be used to reduce acquisition times and improve the spatial resolution, combined with stronger gradients, and fast imaging techniques such as RARE or EPI.

MR imaging of articular cartilage reflects the colligative properties of the water in each tissue layer, through local variation in the T_1 , T_2 relaxation times and the apparent diffusion coefficient (ADC). In articular cartilage, tissue zones that contain higher concentrations of proteoglycan contain less water, which implies that there should be an inverse correlation between MR signal intensity and proteoglycan content. In our study, we elimi-

nated the dependence of our comparison between different grades of articular cartilage on orientation (with respect to the static field) by placing all cartilages in the same orientation, with the static field perpendicular to the imaging plane.

We obtained in-plane resolution of 23 μ m $\times$ 23 μ m with a slice thickness of 500 μ m. Because cartilage is heterogeneous in terms of zonal variation, particularly once degeneration begins, the slice thickness of 500 μ m that we used allowed higher resolution characteristics to be discerned. For instance, proteoglycans are not necessarily depleted from the cartilage in a homogeneous manner throughout the tissue. Within a given sample, even at the μ m level, it is often the case that the degenerative changes will not be uniform from one region to another. This is why we recommend performing the same study with higher isotropic voxel resolution where the slice thickness is of the order of the in-plane resolution.

Our quantitative data show that in general, the T_2 relaxation time varies with depth—reflecting three different anatomical zones that are lost with degeneration. More specifically, we found that although the cartilage showing early fibrillation showed a smooth T_2 decline with cartilage depth, the normal (grade 0) and fissured (grade 2) cartilage displayed T_2 peaks approximately 45% and 17%, respectively, into the depth of the cartilage. These T_2 peaks also appeared at 9.4 Tesla in the work of Nissi et al. (2004) and Kurkijarvi et al. (2006), and at 7 Tesla in the work of Xia et al. (2001) on canine cartilage. However, they appeared closer to the articular surface as compared to our work, but not at the articular surface.

It is obvious from the literature that the role of T_2 relaxation time in relation to the integrity of cartilage has not yet been established, and is dependent

upon many factors such as the spatial resolution, strength of the magnetic field, and the orientation of the specimen relative to the main magnetic field (see, for example, Loeuille et al. (1997), Mlynarik et al. (1999), Borthakur et al. (2000), Wayne et al. (2003)). Indeed, it has recently been suggested that the many factors involved in cartilage degeneration may offset each other, thus resulting in the inconsistent changes in T_2 at various stages of osteoarthritis (Burstein and Gray 2006).

Because of the high contrast and high spatial resolution in the present study, we were able to retrieve T_2 relaxation times from the true superficial zone. For this reason, we are most likely getting a decreased T_2 time here because of the highly organized collagen in a direction parallel to the cartilage surface, without contamination from the much less directed collagen fiber orientation of the middle zone.

We also explored the bi-exponential model for T_2 relaxation. The fast component (inside the cartilage) dominated by accounting for over 95% of the total relaxation for cartilage at all stages of degeneration, with values approaching the mono-exponential fitting. With early degeneration, the slow T_2 component increased in the middle and the deep zones while it decreased in the superficial zone. The decrease in T_2 was accompanied by a higher percentage, which reached an amplitude of approximately 5% in the superficial zone of grade 1 cartilage. This is supported by the fact that the deep zone has the highest proteoglycan content. However, in a study on the maturation of pig articular cartilage (Keinan-Adamsky et al. 2006), it was found that the slow component was 30 msec and the fast component was 4 msec. The maximum echo time was 128 msec, which explains why these workers did not capture the slow component of the present study. Furthermore, our echo spacing was 7 msec, which explains why their fast component was not observed in the present study. These results suggest that a 3-compartment model may better fit the cartilage results representing exchange between the bulk water, collagen, and PG (Lattanzio et al. 2000).

Regarding the ADC taken in the x-direction (Table 3), our results show an increase in the ADC value in early degradations, confirming that water content is increased in cartilage of grade 1. The

exception to this is in the deep zone, where the ADC value was found to remain almost the same. Bi-exponential analysis of the ADC might reveal additional information and enable a better understanding of cartilage degeneration. To better extract the fast and slow diffusion compartments, high “b” values should be used (Cohen and Assaf 2002).

In summary, our study has demonstrated that in vitro 11.74 T MR imaging of human articular cartilage provides images that allow visualization of the state of cartilage degeneration, even at the earliest stage, i.e. fibrillation in the actual superficial zone. To study such a heterogeneous structure, T_2 maps provided better contrast, enabling delineation of the actual cartilage zones—distinguishing the superficial zone in particular. Bi-exponential maps for T_2 may indeed prove to be another tool for studying OA. In the present study, we assumed that the slow T_2 component, and its percentage, is an indicator of proteoglycan content (and also of minor components), as it provides different values for different grades. Even so, there should be further investigation involving a 3-compartment model. We measured the monoexponential diffusion coefficient, which gave an indication of changes in water concentration in different cartilage zones and between different disease states. Our results suggest that in early degeneration there is an increase in water content, possibly due to edema/swelling of the cartilage. We speculate that diffusion-weighted imaging (DWI) with its variants, such as DTI, q-space, and tractography can trace fiber orientation and water content—and hence the physical restrictions that water molecules actually encounter while diffusing within the cartilage. Extending DWI to trace fiber orientation using tractography, DTI, and q-space is our next goal.

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Contributions of authors

SH: performed the MR experiments and co-wrote the manuscript. JL: was responsible for specimen preparation for MR imaging and for histological preparation. OA: was responsible for bi-exponential analyses. JJM: was responsible for analyses of MRI relaxation time. RLM: supervised MRI

experiments. CM: was responsible for overseeing tissue harvesting and for analysis of histological and MR image data, and co-wrote the manuscript.

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- Borthakur A, Shapiro E M, Beers J, Kudchodkar S, Kneeland J B, Reddy R. Sensitivity of MRI to proteoglycan depletion in cartilage: comparison of sodium and proton MRI. *Osteoarthritis Cartilage* 2000; 8: 288-93.
- Burstein D, Gray M L. Is MRI fulfilling its promise for molecular imaging of cartilage in arthritis? *Osteoarthritis Cartilage* 2006; 14: 1087-90.
- Cohen Y, Assaf Y. High b-value q-space analyzed diffusion-weighted MRS and MRI in neuronal tissues - a technical review. *NMR in Biomedicine* 2002; 15: 516-221.
- Dardzinski B J, Mosher T J, Li S, Van Slyke M A, Smith M B. Spatial variation of T2 in human articular cartilage. *Radiology* 1997; 205: 546-50.
- Duyn J H, van Gelderen P, Talagala L, Koretsky A, de Zwart J A. Technological advances in MRI measurement of brain perfusion. *J Magn Reson Imaging* 2005; 22 (6): 751-3.
- Erickson S J, Waldschmidt J G, Czervionke L F, Prost R W. Hyaline cartilage: truncation artifact as a cause of trilaminar appearance with fat-suppressed three-dimensional spoiled gradient-recalled sequences. *Radiology* 1996; 201: 260-4.
- Filidoro L, Dietrich O, Weber J, Rauch E, Oerther T, Wick M, Reiser M, Glaserl C. High-resolution diffusion tensor imaging of human patellar cartilage: Feasibility and preliminary findings. *Magn Reson Med* 2005; 53: 993-8.
- Frank L R, Brossmann R B, Buxton R B, Resnick D. MR imaging truncation artifacts can create a false laminar appearance in cartilage. *Am J Roentgenol* 1997; 168: 547-54.
- Keinan-Adamsky K, Shinar H, Navon G. Multinuclear NMR and MRI studies of the maturation of pig articular cartilage. *Magn Reson Med* 2006; 55 (3): 532-40.
- Kerttula L, Kurunlahti M, Jauhiainen J, Koivula A, Oikarinen J, Tervonen O. Apparent diffusion coefficients and T2 relaxation time measurements to evaluate disc degeneration. *Acta Radiol* 2001; 42 (6): 585-91.
- Kurkijarvi J E, Rieppo J, Nissi M J, Töyräs J, Kiviranta I, Nieminen M T, et al. T2 relaxation of human articular cartilage in the presence of GD-DTPA: a comparative MRI and polarized light microscopy study. *Proc Orthop Res Soc* 2006, #0741.
- Lattanzio P J, Marshall K W, Damyanovich A Z, Peemoeller H. Macromolecule and water magnetization exchange modeling in articular cartilage. *Magn Reson Med* 2000; 44: 840-51.
- Loeuille D, Gonord P, Guingamp C, Gillet P, Blum A, Sauzade M, et al. In vitro magnetic resonance microimaging of experimental osteoarthritis in the rat knee joint. *J Rheumatol* 1997; 2: 133-9.
- Menezes N M, Gray M L, Hartke J R, Burstein D. T2 and T1ρ MRI in articular cartilage systems. *Mag Res Med* 2004; 51: 503-9.
- Mlynarik V, Trattnig S, Huber M, Zembsch A, Imhof H. The role of relaxation times in monitoring proteoglycan depletion in articular cartilage. *J Magn Reson Imaging* 1999; 10: 497-502.
- Modl J M, Sether L A, Haughton V M, Kneeland B J. Articular cartilage: Correlation of histologic zones with signal intensity and MR imaging. *Radiology* 1991; 181: 853-5.
- Nieminen M T, Rieppo J, Toyraas J, Hakumaki J M, Silvennoinen J, Hyttinen M M, Helminen H J, Jurvelin J S. T2 Relaxation time reveals spatial collagen architecture in articular cartilage: a comparative quantitative MRI and polarized light microscopic study. *Magn Reson Med* 2001; 46: 487-93.
- Nissi M J, Töyräs J, Laasanen M S, Rieppo J, Saarakkala S, Lappalainen R, et al. Proteoglycan and collagen sensitive MRI evaluation of normal and degenerated articular cartilage. *J Orthop Res* 2004; 22: 557-64.
- Othman S F, Abdullah O, Magin R L, Zhou X J. Microscopic MRI Assessment of Human Articular Cartilage Degeneration Using q-Space Analysis. *Int Soc Mag Res Med 14th Scientific Meeting*, Seattle, May 2006.
- Recht M P, Kramer J, Marcelis S. Abnormalities of articular cartilage in the knee: analysis of available MR techniques. *Radiology* 1993; 187: 473-8.
- Rosenberg L. Chemical basis for the histological use of safranin O in the study of articular cartilage. *J Bone Joint Surg (Am)* 1971; 53: 69-82.
- Rubenstein J D, Kim J K, Morova-Protzner I, Stanchev P L, Henkelman R M. Effects of collagen orientation on MR imaging characteristics of bovine articular cartilage. *Radiology* 1993; 188: 219-26.
- Samosky J T, Burstein D, Grimson W E, Howe R, Martin S, Gray M L. Spatially-localized correlation of dGEMRIC-measured GAG distribution and mechanical stiffness in the human tibial plateau. *J Orthop Res* 2005; 23: 93-101.
- Tiderius C J, Olsson L E, Leander P, Ekberg O, Dahlberg L. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) in early knee osteoarthritis. *Magn Reson Med* 2003; 49 (3): 488-92.
- Vahlensieck M, Dombrowski F, Leutner C, Wagner U, Reiser M. Magnetization transfer contrast (MTC) and MTC-subtraction: enhancement of cartilage lesions and intracartilaginous degeneration in vitro. *Skeletal Radiol* 1994; 23: 535-9.
- Wayne J S, Kraft K A, Shields K J, Yin C, Owen J R, Disler D G. MR imaging of normal and matrix-depleted cartilage: correlation with biomechanical function and biochemical composition. *Radiology* 2003; 228: 493-9.
- Williams A, Sharma L, McKenzie CA, Prasad P V, Burstein D. Delayed gadolinium magnetic resonance imaging of cartilage in knee osteoarthritis. *Arthritis Rheum* 2005; 52: 328-35.
- Xia Y, Farquhar T, Burton-Wurster N, Vernier-Singer M, Lust G, Jelinski L W. Self-diffusion monitors degraded cartilage. *Arch Biochem Biophys* 1995; 323 (2): 323-8.
- Xia Y, Moody J B, 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.