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The influences of different spatial resolutions on the characteristics of T2 relaxation times in articular cartilage: A coarse-graining study of the microscopic MRI data

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Abstract

Microscopic MRI (μ MRI) T2 data from canine cartilage at different tibial locations were analyzed to investigate the influences of spatial resolution and pixel position on the T2 sensitivity to osteoarthritis (OA). Five experimental factors were investigated: inaccurate pixel position, different pixel resolutions, different specimen orientations in the magnetic field, topographical variations over the tibial surface, and different OA stages. A number of significant trends were identified in this analysis, which shows the subtle but substantial influences to our abilities of detecting OA due to T2 changes. In particular, any deviation in locating the cartilage pixels may result in erratic values near the cartilage surface. Significant differences were found in T2 values between nearly any two comparison-groups under all resolutions both in the meniscus-covered and uncovered areas, which were also showed interaction between the OA degradation stages. This multi-resolution project should help to improve the detection sensitivities of MRI towards cartilage degeneration.

Keywords

Cartilage; MRI; T2; spatial resolution; image analysis

Introduction

Osteoarthritis (OA) is a joint disease characterized dominantly by the degeneration of articular cartilage coating the ends of a joint, and it commonly affects the knee, hip and hands (Arden and Nevitt, 2006). Early detection of OA, when changes to the affected joint

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Conflicts of Interest

None.

Ethical Approval

This study was approved by the local institutional review board, IACUC.

components may still be reversible, is of great importance because this disease may develop to an irreversible stage, which eventually results in disability (Bay-Jensen *et al.*, 2010). The initial osteoarthritic changes in cartilage include proteoglycan loss and deterioration of the collagen network, which can increase the mobility of water and consequentially the water content (Dijkgraaf *et al.*, 1995; Roughley and Lee, 1994). At present, quantitative magnetic resonance imaging (MRI) techniques have been widely used in research and diagnosis of OA because of their abilities to detect pathophysiological changes of articular cartilage non-invasively (Dardzinski *et al.*, 2002; Laurent *et al.*, 2003; Borthakur *et al.*, 2006; Li *et al.*, 2007; Welsch *et al.*, 2008; Huang *et al.*, 2010; Zarins *et al.*, 2010; Okazaki *et al.*, 2014; Koff *et al.*, 2013; Ellermann *et al.*, 2014; Matzat *et al.*, 2014; Roemer *et al.*, 2014; Juras *et al.*, 2015). Among the quantifiable parameters in MRI, T2 relaxation time is particularly important because it is sensitive to fibril structure and orientation as described in previous articles (Rubenstein *et al.*, 1993; Xia, 1998; Gründer *et al.*, 1998; Gründer, 2006; Seidel and Grunder, 2005; Lammentausta *et al.*, 2006; Welsch *et al.*, 2008; Huang *et al.*, 2010; Joseph *et al.*, 2015).

Many image-processing techniques, in which different fitting methods were used on a pixel-by-pixel basis, have been developed in the clinics to analyze T2 data (Carballido-Gamio *et al.*, 2010; Watrin-Pinzano *et al.*, 2004; Raya *et al.*, 2010; Joseph *et al.*, 2015). Since articular cartilage is a thin layer of tissue (typically 0.5 – 3.5mm, depending on the particular joint), the spatial resolution of clinical MRI (typically 0.3 – 1mm) is insufficient to resolve the depth-dependent features and surface topography of articular cartilage. Consequently, routine MRI in the clinics may result in inaccurate T2 values of cartilage due to partial volume averaging and inexact delineation. Additionally, different spatial resolutions in MRI protocols also may lead to differences in T2 values in cartilage. Therefore, the assessment accuracy of T2 values in cartilage using quantitative MRI examinations is still open to discussion and improvement. In order to improve the diagnostic accuracy of T2 values in cartilage using clinical MRI, it is necessary to understand the characteristics of T2 changes in cartilage under higher spatial resolution. Based on the same physics principles and engineering architectures as in clinical MRI, microscopic MRI (μ MRI) may help us research these issues because it provides extremely high spatial resolution images of articular cartilage (Lee *et al.*, 2014; Wang and Xia, 2012, 2013).

Surgical transection of anterior cruciate ligament (ACL) in dogs is the most commonly used animal model for studying the early changes in OA cartilage that mimic those seen in human (Brandt, 1991; Teeple *et al.*, 2013). To test the influence of different resolution and partial volume effect to the characteristics of T2 relaxation time in OA cartilage, we re-analyzed a previously published μ MRI data (Lee *et al.*, 2014; Lee *et al.*, 2016) of canine cartilage-bone specimens from different locations over the medial tibia and at different disease stages after the ACL transection. The following five practical factors were investigated in this image analysis project: inaccurate pixel position, different pixel resolutions, different specimen orientations in the magnetic field, topographical variations over the tibial surface, and different OA stages.

Materials and Methods

This image analysis project re-analyzes the previously published μ MRI data (Lee *et al.*, 2014; Lee *et al.*, 2016) of canine cartilage-bone specimens from different locations over the medial tibia and at different disease stages after the ACL transection. The first two sub-sections here aim to provide the background description to the original methods; the second two sub-sections here describe the image analysis procedures in this project.

Specimen preparation

Seven healthy dogs were sacrificed as the normal group (three females and four males, weighing 19–32kg). Thirteen additional dogs were randomized into two groups: the 8X group (six dogs, one female and five males, weighing 22–30kg) and the 12X group (seven dogs, three females and four males, weighing 18–29kg). The animals in these two groups were sacrificed 8 and 12 weeks after a unilateral ACL transection surgery, respectively. All knee joints of the three groups were harvested immediately after euthanasia. The medial tibial plateau from each knee was divided topographically into five rectangular specimens. Each specimen was about 3.0mm $\times$ 2.5mm $\times$ 4–5mm in size and included the full thickness of articular cartilage still attached to the underlying bone. As shown in our previous publication (Lee *et al.*, 2014), in each knee joint three specimens from anterior medial tibia, exterior medial tibia and posterior medial tibia were covered by meniscus, whereas the two specimens from central medial tibia and interior medial tibia were not. To simplify the data analysis, the specimens from the five topographical locations were grouped into two areas: the meniscus-covered areas and the meniscus-uncovered areas. This study contains the data from 42 meniscus-covered and 28 meniscus-uncovered specimens in the normal group (N), 18 meniscus-covered and 12 meniscus-uncovered specimens in the 8X group, and 21 meniscus-covered and 14 meniscus-uncovered specimens in the 12X group. All specimens were immersed in physiological saline (154mM NaCl in deionized water) that contained 1% protease inhibitor cocktail (Sigma, St. Louis, MO) at 4°C until MRI examination. The specimens were never frozen. All procedures were conducted with the approval of the institutional animal care and use committee.

MRI protocols

Prior to μ MRI experiments, each specimen was equilibrated at 4°C in physiological saline (which contained 1 mM gadolinium contrast agent (Magnevist, Berlex, NJ)) for a minimum of 10 hours to ensure the full penetration of the contrast agent (Xia *et al.*, 2011; Lee and Xia, 2013). The specimen was then sealed in a precision glass tube with an internal diameter of 4.34mm (Wilma Glass, Buena, NJ), which was placed at room temperature for 4 hours before experimentation. (The use of the contrast agent was to reduce the total experimental time. Since all specimens in this study have been subjected to the same procedure, the comparison among them is valid.) μ MRI experiments were carried out using a Bruker AVANCE II 300 NMR spectrometer that has a vertical bore superconducting magnet (7T/89mm) and micro-imaging accessory (Bruker Instrument, Billerica, MA) and a homemade 5mm solenoid coil. The data acquisition parameters and procedures have been described in a previous publication (Lee *et al.*, 2014). Briefly, T2-weighted images were acquired at two orientations (0° and 55°) with respect to the external magnetic field B_0 , which captured the

influence of the dipolar interaction to T2 relaxation (the magic angle effect) (Xia *et al.*, 1997). In order to accurately determine the region of interest in imaging, a coronal pilot image was taken to position the slice location and a sagittal pilot image was taken to align the articular surface of cartilage to the desired orientation. A magnetization-prepared spin-echo imaging sequence was used for five T2-weighted images, which effectively minimized the influence of imaging gradients on the T2 relaxation (Xia, 1998). The echo time of the contrast segment had five increments for both at 0° and 55° (2, 8, 20, 40, 80 or 90ms). The repetition time was 0.5s, which was necessary to complete all experiments without freezing the specimens. The number of averages was 12. The field of view was 0.45cm × 0.45cm; the imaging matrix was 256×128 (then reconstructed 256 × 256); the in-plane pixel size in the direction of the cartilage depth was 17.6 μm; and the slice thickness was 0.8mm.

Image analysis

T2 images of articular cartilage were calculated on a pixel-by-pixel basis using a single exponential fitting model on MATLAB (Mathworks, Natick, MA). The quality of the T2 fitting (the correlation R between the fitted model and the data) was almost equal to 1 for most pixels (voxels) in the tissue region. Quantitative T2 characteristics of each specimen were determined in all 2D T2 images with the 17.6-μm original transverse resolution. Two particular image analysis procedures were employed in this project, based on the quantitative T2 images. (Note that comparative work was carried out between coarse-graining the T2 images and coarse-graining the T2-weighted intensity images; the difference was insignificant (data not shown).) First, in order to analyze the changes in cartilage T2 values due to different spatial resolutions, a 10-pixel-wide region of interest (176.0μm width) was determined for each T2 image, and then pixel heights of 50μm (actual size 52.8μm × 176.0μm), 100μm (actual size 105.6μm × 176.0μm), 200μm (actual size 211.2μm × 176.0μm) and 300μm (actual size 316.8μm × 176.0μm) on the cartilage depth were selected respectively from the cartilage surface to the cartilage-bone interface one-by-one manually until a part of the last pixel was located inside bone tissue. (The pixels from the cartilage surface to the cartilage-bone interface were successively denoted as Pixel-1, Pixel-2, Pixel-3, etc.) Second, in order to analyze the influences of the partial volume deviation in determining the pixel locations, the first pixel including the surface (denoted as the 'superficial pixel') was moved up (towards the articular cavity direction) by 50μm (actual 52.8μm) or 100μm (actual 105.6μm) from its correct position at each resolution, as shown in Figure 1. (It should be pointed out that the MRI signal at each pixel location comes from a 3D voxel in the specimen. Since most MRI is done on a 2D format by first selecting a slice in the third dimension, the final images become two dimensional in MRI, hence utilizing the term 'pixel'. In this project, the analyses were done on the 2D T2 images by varying the size and location of the 2D pixels, with a fixed third dimension from the slice selection. When a pixel is made larger, the size of the voxel increases accordingly.)

Statistical analysis

Values are presented as mean ± standard deviation. Differences of T2 values in the first superficial pixel (Pixel-1) between the correct position and moving-up position, and between the different spatial resolutions were analyzed by the paired t-test. One-way ANOVA with the Bonferroni correction was performed to test the differences of T2 values among the N,

8X and 12X groups from Pixel-1 to the cartilage-bone interface. Results were considered significant if p values were less than 0.05.

Results

Characteristics of T2 values on cartilage surface (Pixel-1)

Significant influences on the T2 values of cartilage's superficial pixel (Pixel-1) were found (Tables 1 and 2) from the interactions of these five factors: inaccurate pixel position (correct, moving-up 50 μ m, and moving-up 100 μ m) due to the partial volume averaging among the neighboring tissues, pixel resolution (from 50 μ m to 300 μ m) due to the depth-dependent characteristics of T2 in cartilage, specimen orientation (0° vs 55°) due to the magic angle effect, topographical location (meniscus-covered, uncovered), and OA stage (N, 8X, 12X).

Specifically, Figure 2 shows the influences of inaccurate pixel position on the T2 values of Pixel-1, when the pixel resolution was 100 μ m. A clear trend of T2 increment can be identified as the function of pixel offset, regardless of the OA stage. The same trends of T2 values as shown in Figure 2 can also be identified for the other pixel resolutions (50, 200 or 300 μ m) from the Tables.

The influences of pixel resolution on the T2 values of Pixel-1 are shown in Figure 3a and 3b, when the pixel position was correct and the specimen orientation was at 0° with respect to the magnetic field. (Note that cartilage at the 0° orientation is similar to the tibial and central femur cartilage in a human knee when the person is imaged in a standard clinical MRI that has the horizontal superconducting magnet). At this orientation (0°), significant differences were found in T2 values between early any two groups under all resolutions both in meniscus-covered and uncovered areas (all $p < 0.05$). In contrast, at 55° (the magic angle (Xia, 1998)), no significant difference was found among the T2 data of Pixel-1 under different resolutions (all $p > 0.05$), as shown in Figure 3c and 3d. This beneficial insensitivity to the image resolution at the magic angle reflects the more uniform T2 profile in cartilage when the dipolar interaction is minimized (Xia, 1998).

The influences of the different OA stages on the T2 values of Pixel-1 had interesting interactions with the pixel position, also shown in Tables 1 and 2. When the pixel position is correct, significant differences could be found in T2 values of Pixel-1 among N, 8X and 12X groups (one-way ANOVA analysis inside the boxes in Tables 1 and 2, $p < 0.05$), regardless of pixel resolution, specimen orientation, or topographical location. When the pixel position has an offset that is 50 μ m, the same significances could be found if the pixel size is 100 μ m or larger. When the pixel position is inaccurate by 100 μ m, no significance can be found among different OA groups. In post hoc pairwise comparisons, T2 values in 12X meniscus-covered areas are significantly higher than that in the normal group; while T2 values in 8X uncovered area are significantly higher than that in the normal group (all $p < 0.05$). These differences reflect different stages of OA progression after the ACL surgery, as discussed previously in one of our reports (Lee *et al.*, 2014).

Characteristic of T2 values on subsurface cartilage (from Pixel-2 to the deep zone of cartilage)

Among the 135 individual specimens in this study, the thinnest cartilage was measured at 400 – 450 μ m in the meniscus-covered area and 850 – 900 μ m in the uncovered area, respectively. The meniscus-covered area, therefore, had 9 pixels under 50 μ m resolution, 5 pixels under 100 μ m resolution, 3 pixels under 200 μ m resolution and 2 pixels under 300 μ m resolution, respectively. The meniscus-uncovered area had 18 pixels under 50 μ m resolution, 9 pixels under 100 μ m resolution, 5 pixels under 200 μ m resolution, and 3 pixels under 300 μ m resolution, respectively. Figures 4–7 show graphically the changes in T2 values of all pixels. The results of subsurface pixels (from Pixel-2 to the last pixel in the deep zone of cartilage) in these figures can also be analyzed from the interactions of the same five factors mentioned earlier in this text. Some of the trends in these subsurface pixels are the continuations of the trends in surface pixel (Pixel-1) shown in Tables 1 and 2.

Figure 4 shows the changes in T2 values from the superficial pixel (Table 1A) to the subsurface pixels at 0° in the meniscus-covered area among the N, 8X and 12X groups under different resolutions. Two marked trends are clear. First, at the high resolution (e.g., Fig 4a), the trends of the T2 values have similar features as in the μ MRI T2 profiles at 13–17 μ m resolutions, where Pixel-1 represents the superficial zone, Pixel-2 and Pixel-3 represent the transitional zone, and the deep pixels represent the radial zone. When the resolution becomes low, the first loss would be the superficial zone since it is also the thinnest zone in cartilage. Second, regardless of the pixel resolution, T2 values increase from the N to 8X to 12X. The surface pixels would have the most sensitivities to the tissue degradation, while the trends in the deep pixels, although clear graphically, are not statistically significant. This difference between the surface pixels and the deep pixels is due to the strong dipolar interaction to the deep cartilage due to the well-organized fibers in the radial zone. Specially as shown in Fig 4, under 50 μ m resolution in subsurface pixels there were significant differences in the T2 values of Pixel-3 and Pixel-4 among the N, 8X and 12X groups by one-way ANOVA (all $p < 0.05$, data not shown). But in post hoc pairwise comparisons, only the T2 value of Pixel-3 in the 12X group was significantly higher than that in normal group ($p = 0.021$). Under 100 μ m resolution in subsurface pixels there was significant difference in T2 value of Pixel-2 among the N, 8X and 12X groups by one-way ANOVA ($p = 0.021$); and in post hoc pairwise comparisons the T2 value of Pixel-2 in the 12X group was significantly higher than that in the N group ($p = 0.029$). Under both 200 and 300 μ m resolutions in subsurface pixels, there was no significant difference in the T2 values of any pixel among the N, 8X and 12X groups (all $p > 0.05$).

For meniscus-uncovered cartilage (Fig 5) the observations were similar to those of meniscus-covered cartilage (Fig 4). Both areas are summarized in Tables 1 and 2. A noticeable difference in Fig 5 is that, under 50 μ m resolution, the significant differences in T2 values of subsurface pixels by one-way ANOVA extended from Pixel-3 to Pixel-12 among the N, 8X and 12X groups (all $p < 0.05$, data not shown). And post hoc pairwise comparisons showed T2 values from Pixel-3 to Pixel-12 in the 8X group were significantly higher than that in the N group (all $p < 0.05$). This extended significance along the tissue depth could also be observed at less resolution in both one-way ANOVA and post hoc

pairwise comparisons: under 100 μ m resolution from Pixel-2 to Pixel-6, and under 200 μ m resolution from Pixel-2 to Pixel-3.

Figures 6 and 7 show the similar T2 changes with the cartilage oriented at 55° for both covered and uncovered areas. One-way ANOVA showed that there were significant differences in T2 values of all pixels among the N, 8X and 12X groups under any of the four resolutions (all $p < 0.05$, data not shown). In post hoc pairwise comparisons, T2 values in the 12X meniscus-covered area were significantly higher than that of the N group (all $p < 0.05$), while T2 values in the 8X uncovered areas were significantly higher than that in the N group (all $p < 0.05$).

Discussion

Previous μ MRI study has established that T2 relaxation time in articular cartilage is anisotropic and depth-dependent (Xia, 1998). These characteristics of T2 anisotropy are caused mainly by the depth-dependent structure of the collagen matrix in cartilage as well as the orientation of this collagen structure in the magnetic field when articular cartilage is being imaged (Xia *et al.*, 1997; Xia, 1998; Xia *et al.*, 2002). Since the pixel resolution in clinical MRI is insufficient to resolve the fine depth-dependent structure in articular cartilage, different spatial resolutions clearly can cause different amounts and different types of partial volume averaging among the molecular constituents in cartilage and among different tissue structures (e.g., small lesion and large background tissue) within the same pixel (Xia, 2013). In this study, we coarse-grained the high-resolution μ MRI data to reveal the influence of different resolution and partial volume effect to the characteristics of T2 relaxation time in different topographical locations of OA cartilage in early stages.

Articular cartilage is a thin and curved layer of connective tissue, located closely to many other surrounding tissues (e.g., bone, ligaments, meniscus, synovial fluid). The physiological saline that hydrated and surrounded the specimen in this experiment was a good approximation to the synovial fluid or cavity fluid found in human joint capsule, so the selection of the pixels moving up into the saline in this study (cf. Fig 2) was similar to any deviation of an imaging pixel near cartilage surface in clinical MRI. Moreover, moving up 50 or 100 μ m is a reasonable offset because it is approximately 1/8 – 1/4 of pixel size in clinical MRI and this degree of uncertainty and averaging exists commonly. From this study, we found the synovial or joint cavity fluid may greatly affect the T2 values near cartilage surface in each different resolution even though the pixels just moved up 50 μ m. This is easy to understand because the synovial or joint cavity fluid and saline all have comparably high signal intensity on T2-weighted images. Therefore, a small deviation in the localization of the imaging pixels would understandably cause an increase in the T2 values. The only in vivo situation where a deviation in pixel location becomes insignificant is when the background of the surface cartilage has a similar T2 as the tissue, which probably signal a lack of joint space separating the two opposing cartilage surfaces. In this instance, the T2 value of this cartilage pixel would be influenced by the properties of the other cartilage in the joint.

When the pixel had an accurate position on the surface, the differences in T2 values of Pixel-1 among different resolutions indicated that the T2(0°) values had a typical bell-curved shape, while the T2(55°) values were more homogeneous throughout the normal cartilage depth. These findings are consistent with previously reported depth-dependent T2 value profiles of cartilage (Xia *et al.*, 1997; Xia, 1998; Lee *et al.*, 2014). However, the bell-shaped curve of T2(0°) values may disappear in OA cartilage (both 8X and 12X groups), which might result from the structural changes in the cartilage surface during OA progression, including fibrillation of the collagen network, influx of water and swelling. The initial OA can increase the interstitial water mobility in cartilage (Buckwalter *et al.*, 2005; Mankin and Buckwalter, 1996), resulting in the T2 relaxation time to increase, as shown in our results. In clinical settings, it has been proven that the physiological or pathological increment of water content in cartilage and the concomitant change in proteoglycan and collagen would increase T2 values accordingly (Liess *et al.*, 2002; David-Vaudey *et al.*, 2004). We noted that in the meniscus-covered area the T2 values of Pixel-1 in 12X were higher than that in the N group. On the other hand, in the uncovered area, T2 values in 8X were higher than that in the N group. This phenomenon could indicate that the meniscus in the joint is capable of protecting cartilage from OA degradation due to the ACL surgery to a certain degree, and that initial swelling in cartilage tissue may reduce during OA progress (Maffulli *et al.*, 2003; Arokoski *et al.*, 2001; Pena *et al.*, 2005; Slaughterbeck *et al.*, 2009; Ichiba and Kishimoto, 2009).

At the 55° orientation, significant differences in the T2 values from every pixel (from Pixel-1 to the last pixel above the bone interface) were observed among the N, 8X and 12X groups for all resolutions; while at the 0° orientation, significant differences were only found in several pixels close to the cartilage surface. This difference could be caused by the subtle influence of nuclear dipolar interaction (Xia *et al.*, 1997). At 0° there were two tendencies found from our results. First, it was more difficult to find differences in T2 values between the N and OA groups closer to cartilage-bone interface. Second, it was more difficult to find differences between the groups at lower resolution. The reason for the first tendency could be that early changes of OA begin from the cartilage surface and the surface changes are more serious than that in the deep tissue, which was noted in one of our previous OA studies (Alhadlaq *et al.*, 2004). The second tendency arose because the same pixel in a lower resolution would include more deep cartilage when averaged. Nevertheless, there was an exception that no significant difference in the T2 values of Pixel-2 was noted under 50µm resolution. This might be due to the fact that 50µm resolution is still reasonable enough to show the bell-shaped curve of T2 profiles as seen in µMRI T2 profiles. From the data analysis we found T2 values of Pixel-2 in the N group under 50µm resolution were located in the peak of the bell-shaped curve, while in the OA group, T2 values were more homogeneous. It is worth mentioning that a resolution scaling law in MRI of cartilage was established several years ago, which suggested that the transverse resolution in MRI for cartilage should ideally be around 2% of the relative tissue depth per image pixel, which translates to about 40µm for a 2mm thick tissue (such as human knee or hip) (Xia, 2007). However presently, at present this ideal resolution is still beyond the reach of clinical MRI.

The present study has several limitations. First, this in vitro study on animal models of OA using µMRI cannot simulate precisely the practical conditions in clinical MRI of human OA

(for example, the time limitation, patient movement in human MRI, physiological and biomedical specifics of a human knee in its resting environment). Second, although μ MRI and clinical MRI share the same physics principles and engineering architecture, many experimental differences do exist between μ MRI and clinical MRI of cartilage (e.g., magnetic field strength, pulse sequence, acquisition parameters, data fitting procedure, and signal-to-noise ratio) (Xia, 2013). For example, fat suppression is important in clinical MRI of human; however, it is a non-issue in μ MRI of cartilage-bone blocks since most fat-containing bony structures are trimmed off. Third, the present data analysis under different resolutions was based on the same μ MRI data instead of multiple independent experiments for each resolution. Thus the same error in the experiments may influence all data analyses. However, this current approach also excludes any other error from multiple independent experiments. Fourth, the size and aspect ratio of a voxel in the original μ MRI study is quite different from the actual size and aspect ratio of a voxel in clinical MRI of human, both anisotropic but with different ratios of transverse-resolution vs. slice thickness (hence different kinds of averaging in a voxel). At the same time, the size and topographical shape of a canine knee joint are different from these of a human knee; and the orientation of a human joint in the magnet is difficult to adjust. Consequently, the effects of the partial volume averaging among different cartilage structures and between cartilage and the surrounding tissues are different between μ MRI and clinical MRI. Further studies are desirable to investigate the practical factors in human imaging. Finally, the contrast agent was used in the original experiments to reduce the total experimental time so that all fresh specimens from one animal could be completed without being frozen. Different diffusivities of negatively charged contrast agent between healthy and OA cartilage could have a different effect on the measured T2 relaxation times depending on the degenerative status. However, such effects would not influence the assessment of practical factors investigated in this project. Despite of these limitations, the quantitative comparison and conclusions in this coarse-graining study are based on the same set of data, hence valid as an imaging analysis project.

In conclusion, to the best of our knowledge, this is the first report that coarse-grains the μ MRI data to quantify the influences of image resolution on the T2 characteristics in cartilage. We analyzed the changes in T2 values of cartilage under the influences of five practical factors: inaccurate pixel position, different pixel resolutions, different specimen orientations in the magnetic field, topographical variations over the tibial surface, and different OA stages. Many significant trends have been identified in this analysis, which shows the subtle but substantial influences on our abilities for detecting T2 changes due to OA. In particular, any deviation in locating the cartilage pixels may result in erratic measurements of T2 values near the cartilage surface, which may be damaged in early OA. This study could help us understand the features of T2 changes in cartilage under different spatial resolutions. These features are potentially useful to determine the accuracy of T2 values in cartilage using relatively low resolutions obtained from clinical instruments.

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References

- 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 the Rheumatic Diseases*. 2004; 63:709–17. [PubMed: 15140779]
- Arden N, Nevitt MC. Osteoarthritis: epidemiology. *Best practice & research. Clinical rheumatology*. 2006; 20:3–25. [PubMed: 16483904]
- Arokoski JP, Lammi MJ, Hyttinen MM, Kiviranta I, Parkkinen JJ, Jurvelin JS, Tammi MI, Helminen HJ. Etiopathogenesis of osteoarthritis. *Duodecim*. 2001; 117:1617–26. [PubMed: 12182099]
- Bay-Jensen AC, Hoegh-Madsen S, Dam E, Henriksen K, Sondergaard BC, Pastoureau P, Qvist P, Karsdal MA. Which elements are involved in reversible and irreversible cartilage degradation in osteoarthritis? *Rheumatol Int*. 2010; 30:435–42. [PubMed: 19816688]
- Borthakur A, Mellon E, Niyogi S, Witschey W, Kneeland JB, Reddy R. Sodium and T1rho MRI for molecular and diagnostic imaging of articular cartilage. *NMR Biomed*. 2006; 19:781–821. [PubMed: 17075961]
- Brandt KD. Transection of the anterior cruciate ligament in the dog: a model of osteoarthritis. *Semin Arthritis Rheum*. 1991; 21:22–32. [PubMed: 1796303]
- Buckwalter JA, Mankin HJ, Grodzinsky AJ. Articular cartilage and osteoarthritis. *Instr Course Lect*. 2005; 54:465–80. [PubMed: 15952258]
- Carballido-Gamio J, Blumenkrantz G, Lynch JA, Link TM, Majumdar S. Longitudinal analysis of MRI T(2) knee cartilage laminar organization in a subset of patients from the osteoarthritis initiative. *Magn Reson Med*. 2010; 63:465–72. [PubMed: 19918905]
- Dardzinski BJ, Laor T, Schmithorst VJ, Klosterman L, Graham TB. Mapping T2 relaxation time in the pediatric knee: feasibility with a clinical 1.5-T MR imaging system. *Radiology*. 2002; 225:233–9. [PubMed: 12355010]
- David-Vaudey E, Ghosh S, Ries M, Majumdar S. T2 relaxation time measurements in osteoarthritis. *Magn Reson Imaging*. 2004; 22:673–82. [PubMed: 15172061]
- Dijkgraaf LC, de Bont LG, Boering G, Liem RS. Normal cartilage structure, biochemistry, and metabolism: a review of the literature. *J Oral Maxillofac Surg*. 1995; 53:924–9. [PubMed: 7629621]
- Ellermann J, Ziegler C, Nissi MJ, Goebel R, Hughes J, Benson M, Holmberg P, Morgan P. Acetabular cartilage assessment in patients with femoroacetabular impingement by using T2* mapping with arthroscopic verification. *Radiology*. 2014; 271:512–23. [PubMed: 24520945]
- Gründer W. MRI assessment of cartilage ultrastructure. *NMR Biomed*. 2006; 19:855–76. [PubMed: 17075962]
- Gründer W, Wagner M, Werner A. MR-microscopic visualization of anisotropic internal cartilage structures using the magic angle technique. *Magn Reson Med*. 1998; 39:376–82. [PubMed: 9498593]
- Huang GS, Lee HS, Chou MC, Shih YY, Tsai PH, Lin MH, Lin CY, Lee CH, Chung HW. Quantitative MR T2 measurement of articular cartilage to assess the treatment effect of intra-articular hyaluronic acid injection on experimental osteoarthritis induced by ACLX. *Osteoarthritis and cartilage/OARS, Osteoarthritis Research Society*. 2010; 18:54–60.
- Ichiba A, Kishimoto I. Effects of articular cartilage and meniscus injuries at the time of surgery on osteoarthritic changes after anterior cruciate ligament reconstruction in patients under 40 years old. *Arch Orthop Trauma Surg*. 2009; 129:409–15. [PubMed: 19050905]
- Joseph GB, McCulloch CE, Nevitt MC, Heilmeier U, Nardo L, Lynch JA, Liu F, Baum T, Link TM. A reference database of cartilage 3 T MRI T2 values in knees without diagnostic evidence of cartilage degeneration: data from the osteoarthritis initiative. *Osteoarthritis and cartilage/OARS, Osteoarthritis Research Society*. 2015; 23:897–905.

- Juras V, Bohndorf K, Heule R, Kronnerwetter C, Szomolanyi P, Hager B, Bieri O, Zbyn S, Trattnig S. A comparison of multi-echo spin-echo and triple-echo steady-state T2 mapping for in vivo evaluation of articular cartilage. *European radiology*. 2015
- Koff MF, Shah P, Pownder S, Romero B, Williams R, Gilbert S, Maher S, Fortier LA, Rodeo SA, Potter HG. Correlation of meniscal T2* with multiphoton microscopy, and change of articular cartilage T2 in an ovine model of meniscal repair. *Osteoarthritis and cartilage/OARS, Osteoarthritis Research Society*. 2013; 21:1083–91.
- Lammentausta E, Kiviranta P, Nissi MJ, Laasanen MS, Kiviranta I, Nieminen MT, Jurvelin JS. T2 relaxation time and delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) of human patellar cartilage at 1.5 T and 9.4 T: Relationships with tissue mechanical properties. *J Orthop Res*. 2006; 24:366–74. [PubMed: 16479569]
- Laurent D, Wasvary J, O'Byrne E, Rudin M. In vivo qualitative assessments of articular cartilage in the rabbit knee with high-resolution MRI at 3 T. *Magn Reson Med*. 2003; 50:541–9. [PubMed: 12939762]
- Lee JH, Badar F, Kahn D, Matyas J, Qu X, Chen CT, Xia Y. Topographical variations of the strain-dependent zonal properties of tibial articular cartilage by microscopic MRI. *Connect Tissue Res*. 2014; 55:205–16. [PubMed: 24559385]
- Lee JH, Badar F, Matyas J, Qu X, Xia Y. Topographical variations in zonal properties of canine tibial articular cartilage due to early osteoarthritis: a study using 7-T magnetic resonance imaging at microscopic resolution. *Magma (New York, NY)*. 2016
- Lee JH, Xia Y. Quantitative zonal differentiation of articular cartilage by microscopic magnetic resonance imaging, polarized light microscopy, and Fourier-transform infrared imaging. *Microscopy research and technique*. 2013; 76:625–32. [PubMed: 23533143]
- Li X, Benjamin Ma C, Link TM, Castillo DD, Blumenkrantz G, Lozano J, Carballido-Gamio J, Ries M, Majumdar S. In vivo T(1rho) and T(2) mapping of articular cartilage in osteoarthritis of the knee using 3 T MRI. *Osteoarthritis and cartilage/OARS, Osteoarthritis Research Society*. 2007; 15:789–97.
- Liess C, Lusse S, Karger N, Heller M, Gluer CC. Detection of changes in cartilage water content using MRI T2-mapping in vivo. *Osteoarthritis and Cartilage*. 2002; 10:907–13. [PubMed: 12464550]
- Maffulli N, Binfield PM, King JB. Articular cartilage lesions in the symptomatic anterior cruciate ligament-deficient knee. *Arthroscopy*. 2003; 19:685–90. [PubMed: 12966374]
- Mankin HJ, Buckwalter JA. Restoration of the osteoarthrotic joint. *J Bone Joint Surg Am*. 1996; 78:1–2. [PubMed: 8550665]
- Matzat SJ, Kogan F, Fong GW, Gold GE. Imaging strategies for assessing cartilage composition in osteoarthritis. *Current rheumatology reports*. 2014; 16:462. [PubMed: 25218737]
- Okazaki K, Takayama Y, Osaki K, Matsuo Y, Mizu-Uchi H, Hamai S, Honda H, Iwamoto Y. Subclinical cartilage degeneration in young athletes with posterior cruciate ligament injuries detected with T1rho magnetic resonance imaging mapping. *Knee surgery, sports traumatology, arthroscopy: official journal of the ESSKA*. 2014; 3:2325967115585092.
- Pena E, Calvo B, Martinez MA, Palanca D, Doblare M. Finite element analysis of the effect of meniscal tears and meniscectomies on human knee biomechanics. *Clin Biomech (Bristol, Avon)*. 2005; 20:498–507.
- Raya JG, Dietrich O, Horng A, Weber J, Reiser MF, Glaser C. T2 measurement in articular cartilage: impact of the fitting method on accuracy and precision at low SNR. *Magn Reson Med*. 2010; 63:181–93. [PubMed: 19859960]
- Roemer FW, Eckstein F, Hayashi D, Guermazi A. The role of imaging in osteoarthritis. *Best practice & research. Clinical rheumatology*. 2014; 28:31–60. [PubMed: 24792944]
- Roughley PJ, Lee ER. Cartilage proteoglycans: structure and potential functions. *Microscopy research and technique*. 1994; 28:385–97. [PubMed: 7919526]
- Rubenstein JD, Kim JK, Morava-Protzner I, Stanchev PL, Henkelman RM. Effects of collagen orientation on MR imaging characteristics of bovine articular cartilage. *Radiology*. 1993; 188:219–26. [PubMed: 8511302]
- Seidel P, Grunder W. Collagene order of articular cartilage by clinical magnetic resonance images and its age dependency. *Z Med Phys*. 2005; 15:45–51. [PubMed: 15830784]

- Slauterbeck JR, Kousa P, Clifton BC, Naud S, Tourville TW, Johnson RJ, Beynnon BD. Geographic mapping of meniscus and cartilage lesions associated with anterior cruciate ligament injuries. *J Bone Joint Surg Am.* 2009; 91:2094–103. [PubMed: 19723985]
- Teeple E, Jay GD, Elsaid KA, Fleming BC. Animal models of osteoarthritis: challenges of model selection and analysis. *AAPS J.* 2013; 15:438–46. [PubMed: 23329424]
- Wang N, Xia Y. Depth and orientational dependencies of MRI T(2) and T(1rho) sensitivities towards trypsin degradation and Gd-DTPA(2-) presence in articular cartilage at microscopic resolution. *Magn Reson Imaging.* 2012; 30:361–70. [PubMed: 22244543]
- Wang N, Xia Y. Experimental issues in the measurement of multi-component relaxation times in articular cartilage by microscopic MRI. *J Magn Reson.* 2013; 235:15–25. [PubMed: 23916991]
- Watrinn-Pinzano A, Ruauud JP, Cheli Y, Gonord P, Grossin L, Gillet P, Blum A, Payan E, Olivier P, Guillot G, Netter P, Loeuille D. T2 mapping: an efficient MR quantitative technique to evaluate spontaneous cartilage repair in rat patella. *Osteoarthritis and cartilage/OARS, Osteoarthritis Research Society.* 2004; 12:191–200.
- Welsch GH, Mamisch TC, Hughes T, Zilkens C, Quirbach S, Scheffler K, Kraff O, Schweitzer ME, Szomolanyi P, Trattnig S. In vivo biochemical 7.0 Tesla magnetic resonance: preliminary results of dGEMRIC, zonal T2, and T2* mapping of articular cartilage. *Invest Radiol.* 2008; 43:619–26. [PubMed: 18708855]
- Xia Y. Relaxation anisotropy in cartilage by NMR microscopy (muMRI) at 14-microm resolution. *Magn Reson Med.* 1998; 39:941–9. [PubMed: 9621918]
- Xia Y. Resolution ‘scaling law’ in MRI of articular cartilage. *Osteoarthritis and cartilage/OARS, Osteoarthritis Research Society.* 2007; 15:363–5.
- Xia Y. MRI of articular cartilage at microscopic resolution. *Bone Joint Res.* 2013; 2:9–17. [PubMed: 23610697]
- Xia Y, Farquhar T, Burton-Wurster N, Lust G. Origin of cartilage laminae in MRI. *J Magn Reson Imaging.* 1997; 7:887–94. [PubMed: 9307916]
- Xia Y, Moody JB, Alhadlaq H. Orientational dependence of T2 relaxation in articular cartilage: A microscopic MRI (microMRI) study. *Magn Reson Med.* 2002; 48:460–9. [PubMed: 12210910]
- 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; 65:1733–7. [PubMed: 21452280]
- Zarins ZA, Bolbos RI, Pialat JB, Link TM, Li X, Souza RB, Majumdar S. Cartilage and meniscus assessment using T1rho and T2 measurements in healthy subjects and patients with osteoarthritis. *Osteoarthritis and cartilage/OARS, Osteoarthritis Research Society.* 2010; 18:1408–16.

Figure 1.

(a) A quantitative T2 image of a tibia cartilage specimen at the 0° orientation in the magnetic field, which points vertically up in the figure. The dashed box in (a) is the region of interest in data analysis, which is magnified in (b). The yellow, red, green and blue colored boxes in (b) show physically the size of one pixel (Pixel-1), which are 50, 100, 200 and 300 μm resolutions, respectively. The pixels are located in three different locations (the correct position and position moving up 50 and 100 μm).

Figure 2.

The influences of the pixel location (correct, moving up 50 μm or 100 μm) on the averaged T2 values in Pixel-1 when the pixel resolution is 100μm. (*P < 0.05, **P < 0.01, and ***P < 0.001)

Figure 3.

The influences of the pixel resolution (50, 100, 200 and 300 μm) on the averaged T2 values in Pixel-1(at the correct pixel location). Differences in T2 values among different OA groups (N, 8X and 12X group) were shown. (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$)

Figure 4.

The depth-dependent T2 values at the 0° orientation in the meniscus-covered area, among different pixel resolutions and among different OA groups. Pixel 1 is at the cartilage surface. (*P < 0.05, **P < 0.01, and ***P < 0.001)

Figure 5.

The depth-dependent T2 values at the 0° orientation in the meniscus-uncovered area, among different pixel resolutions and among different OA groups. Pixel 1 is at the cartilage surface.

(*P < 0.05, **P < 0.01, and ***P < 0.001)

Figure 6. The depth-dependent T2 values at the 55° orientation in the meniscus-covered area, among different pixel resolutions and among different OA groups. Pixel 1 is at the cartilage surface. (*P < 0.05, **P < 0.01, and ***P < 0.001)

Figure 7.

The depth-dependent T2 values at the 55° orientation in the meniscus-uncovered area, among different pixel resolutions and among different OA groups. Pixel 1 is at the cartilage surface. (*P < 0.05 and **P < 0.01)

Table 1

T2 values of Pixel-1 on different resolutions and positions, and their comparisons among normal, 8X and 12X group in meniscus-covered (A) and meniscus-uncovered (B) areas at 0°

A)								
Resolution	Position	N (ms)	8X (ms)	12X (ms)	P values			
					ANOVA	Bonferroni post hoc test		
						N vs. 8X	N vs. 12X	8X vs. 12X
50 μm	Correct	49.9±10.8	57.2±10.4	65.0±13.4	<0.001	0.080	<0.001	0.110
100 μm	50 μm up	82.7±13.3	87.8±14.1	89.8±12.6	0.113	0.540	0.155	1.000
	100 μm up	130.9±14.5	132.4±15.6	128.6±15.9	0.724	1.000	1.000	1.000
	Correct	53.5±11.2	57.6±12.3	64.6±11.7	0.002	0.620	0.002	0.191
	50 μm up	66.3±11.8	72.5±13.3	77.4±12.2	0.003	0.224	0.003	0.636
200 μm	100 μm up	106.8±13.6	110.1±15.2	109.2±15.0	0.662	1.000	1.000	1.000
	Correct	49.1±9.8	53.9±12.2	58.4±10.2	0.005	0.327	0.004	0.565
	50 μm up	59.6±11.7	64.1±12.5	69.0±11.8	0.015	0.550	0.013	0.619
	100 μm up	80.2±13.6	83.9±13.8	86.9±13.4	0.169	1.000	0.197	1.000
300 μm	Correct	42.6±10.8	47.2±11.3	50.2±12.3	0.040	0.470	0.044	1.000
	50 μm up	51.9±11.2	56.6±11.5	60.0±13.8	0.037	0.501	0.039	1.000
	100 μm up	68.4±12.1	72.7±12.5	75.3±12.3	0.103	0.679	0.122	1.000
B)								
Resolution	Position	N (ms)	8X (ms)	12X (ms)	P values			
					ANOVA	Bonferroni post hoc test		
						N vs. 8X	N vs. 12X	8X vs. 12X
50 μm	Correct	53.6±11.6	69.2±11.8	62.2±12.4	0.001	0.001	0.092	0.420
50 μm up								
100 μm up								

B)

Resolution	Position	N (ms)	8X (ms)	12X (ms)	P values			
					ANOVA	Bonferroni post hoc test		
						N vs. 8X	N vs. 12X	8X vs. 12X
100 µm	Correct	56.3±10.1	68.6±13.3	63.0±12.4	0.009 0.025	0.009 0.035	0.242	0.656
	50 µm up	67.2±11.5	78.2±13.5	74.5±12.6			0.221	1.000
200 µm	100 µm up	106.0±13.4	105.8±14.7	106.7±13.0	0.984	1.000	1.000	1.000
	Correct	51.6±11.4	65.1±12.3	59.8±12.4	0.004 0.024	0.005 0.031	0.116	0.789
	50 µm up	60.8±12.1	72.3±13.1	68.0±12.8			0.260	1.000
	100 µm up	81.2±13.9	87.2±14.2	84.8±13.1	0.416	0.631	1.000	1.000
300 µm	Correct	45.2±10.6	59.5±11.7	52.9±11.3	0.001 0.007	0.001 0.009	0.114	0.408
	50 µm up	53.9±10.7	66.7±12.4	61.5±13.5			0.161	0.819
	100 µm up	69.9±11.7	78.7±12.0	75.4±11.4	0.082	0.107	0.490	1.000

N = Normal

Dashed boxes indicated P < 0.05

Table 2

T2 values of Pixel-1 on different resolutions and positions, and their comparisons among normal, 8X and 12X group in meniscus-covered (A) and meniscus-uncovered (B) areas at 55°

A)								
Resolution	Position	N (ms)	8X (ms)	12X (ms)	P values			
					ANOVA	Bonferroni post hoc test		
						N vs. 8X	N vs. 12X	8X vs. 12X
50 μm	Correct	53.7±12.2	58.9±10.7	67.0±10.9	<0.001	0.338	<0.001	0.094
100 μm	50 μm up	72.4±13.9	74.8±12.5	80.1±14.7	0.124	1.000	0.125	0.708
	100 μm up	140.9±15.4	135.2±15.5	133.7±15.1	0.162	0.568	0.252	1.000
	Correct	55.1±12.9	60.0±10.5	66.9±10.7	0.002	0.452	0.001	0.220
	50 μm up	63.0±13.3	66.8±11.8	73.6±11.8	0.009	0.856	0.007	0.289
200 μm	100 μm up	106.7±13.1	105.0±13.6	106.9±13.1	0.875	1.000	1.000	1.000
	Correct	54.9±12.8	60.7±10.2	65.3±11.7	0.005	0.268	0.005	0.698
	50 μm up	59.5±13.0	64.2±11.5	69.5±11.4	0.011	0.534	0.009	0.539
	100 μm up	80.9±13.3	82.5±12.3	86.9±12.6	0.227	1.000	0.261	0.871
300 μm	Correct	53.6±12.3	59.6±11.4	64.5±13.1	0.005	0.265	0.004	0.661
	50 μm up	57.2±12.5	62.6±11.8	67.6±11.1	0.006	0.335	0.005	0.596
	100 μm up	72.2±11.6	75.4±12.2	79.1±11.4	0.096	1.000	0.097	1.000
B)								
Resolution	Position	N (ms)	8X (ms)	12X (ms)	P values			
					ANOVA	Bonferroni post hoc test		
						N vs. 8X	N vs. 12X	8X vs. 12X
50 μm	Correct	56.1±11.5	70.4±13.7	64.3±12.1	0.004	0.004	0.137	0.625
100 μm	50 μm up	76.5±14.1	83.8±13.9	84.1±13.4	0.158	0.409	0.311	1.000
	100 μm up	131.7±13.5	122.6±14.8	125.9±16.1	0.164	0.227	0.693	1.000

Resolution	Position	N (ms)	8X (ms)	12X (ms)	P values			
					ANOVA	Bonferroni post hoc test		
						N vs. 8X	N vs. 12X	8X vs. 12X
100 µm	Correct	58.7±11.0	72.7±14.2	66.2±12.4	0.005 0.018	0.005 0.031	0.196	0.537
	50 µm up	66.3±11.5	77.1±12.4	74.2±11.8			0.137	1.000
200 µm	100 µm up	104.1±12.9	103.2±14.2	105.0±13.6	0.947	1.000	1.000	1.000
	Correct	59.2±11.3	73.7±13.1	67.9±12.2	0.002 0.007	0.003 0.011	0.088	0.673
	50 µm up	63.9±11.1	76.5±13.7	72.1±11.7			0.120	1.000
300 µm	100 µm up	81.4±11.7	87.9±13.9	85.6±14.2	0.302	0.451	0.967	1.000
	Correct	58.1±12.5	71.6±13.6	66.4±11.5	0.007 0.007	0.008 0.009	0.143	0.870
	50 µm up	61.7±12.0	74.6±13.5	69.9±10.5			0.124	0.986
100 µm up		74.3±11.7	83.4±14.1	80.1±11.7	0.085	0.113	0.479	1.000

N = Normal

Dashed boxes indicated P < 0.05