T1ρ magnetic resonance imaging quantification of early articular cartilage degeneration in a rabbit model

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Abstract

Background: Osteoarthritis (OA) is a serious problem in the recent aging society, and early diagnosis and intervention of articular cartilage degeneration are very important for the onset of OA. Therefore, development of newer MRI techniques is necessary and expected for detection of early articular cartilage degeneration.

Methods: 24 rabbits were randomly divided into four equal experimental groups (Group A, B, C, D) to establish articular cartilage models in different grades of early degeneration by injecting papain into the left knee joint cavity. Another 8 rabbits were considered as blank control (Group E), and then randomized into four subgroups (E1, E2, E3, E4). T1ρ and T2-weighted images of the bilateral knee joints were obtained for rabbits by using 3.0 T MRI. Group A, B, C, and D were imaged respectively at 1, 2, 3, and 4 weeks post-operation, and E1, E2, E3, E4 underwent the same period imaging. Rabbits were sacrificed after scanning and the femoral condyle cartilage (FCC) was histologically examined. T1ρ values of the femoral condyle cartilage were measured and statistically analyzed, and contrasted with the histologic results.

Results: T1ρ values of the left side in experimental groups were significantly higher than the right side (P < 0.05), and which increased gradually with the passage of post-operation time (P < 0.05). Histological examination demonstrated the proteoglycan content of the left side decreased, and indicated the occurrence of early degeneration.

Conclusions: T1ρ MRI can sensitively and quantitatively reflect the change in proteoglycans prior to the morphologic alterations of articular cartilage, and T1ρ value is gradually increased with a decrease in proteoglycan content, therefore that T1ρ could potentially act as a reliable tool to identify early cartilage degeneration.

Keyword: T1ρ, Magnetic resonance imaging, Early cartilage degeneration, Proteoglycan, Histology

Background

Osteoarthritis (OA) is the most common joint disorder worldwide, which is the major cause of mobility impaired and disabled in humans [1, 2]. It affects approximately 75% of the population over 70 years of age, while a quarter of people aged over 55 have an episode of persistent knee pain, and there is a rapid increase in the number of the cases due to the aging of the population and the obesity epidemic [2–5]. This suggests that OA will continue to be a large and growing public health problem in the future [5, 6].

The most recent accomplishments have indicated that articular cartilage degeneration is highly likely to play a key role in the pathogenesis of OA, and early diagnosis and intervention of cartilage degeneration before the onset of irreversible changes, are very important for the prevention and treatment of OA [2, 7, 8]. For this purpose, considerable efforts have recently been in search of non-invasive techniques to sensitively and accurately assess the prophase of cartilage degeneration [9]. Articular cartilage starts early to degenerate from extracellular matrix (ECM) metabolism imbalance, which mainly involves a gradual loss of proteoglycan (PG), collagen damage and increased water content, and these macromolecular (biochemical) changes are not accompanied by significant structural disturbances in
the tissue [10–13]. Among them, decreased PG content is an initiating factor and an important marker of early cartilage degeneration. Presently, it is believed that cartilage destruction results from large PG aggrecan degradation, which precedes the damage to collagen fibrillar network and is responsible for the whole changes [12–15]. In short, early cartilage changes are characterized by PGs depletion, and detection of PG content can identify early cartilage degeneration. Although conventional magnetic resonance imaging (MRI) could provide excellent detection of morphological changes in the thickness and structure of articular cartilage, it has some difficulties in discriminating minimal changes associated with the early stage of degeneration before morphological or clinical alterations [2, 12, 13]. Given as such, newer MRI techniques have emerged for investigation of the physiology and biochemistry of cartilage.

T\(_{1p}\) MRI is one of the most promising techniques focused on PG quantification [2, 12, 16]. The longitudinal relaxation time that occurs during the application of T\(_{1p}\) imaging is referred to as spin–lattice relaxation in the rotating frame, or T\(_{1p}\) relaxation time. T\(_{1p}\) relaxation time (T\(_{1p}\) value) can be measured to describe the slow-motion interactions between the macromolecule protons and bulk water protons at high static fields in vivo, and therefore indirectly reflect PG content [17–20]. As a consequence, T\(_{1p}\) imaging is mainly used for the evaluation of tissues composed partly of slow-frequency macromolecules, such as brain, articular cartilage, and intervertebral disc [21–23]. In articular cartilage, several studies have reported that a decrease in PG content is the major factor in change of T\(_{1p}\) value, and which has been shown to detect cartilage changes [13, 23, 24]. However, T\(_{1p}\) is still available on a research basic, and further studies are required to evaluate its value in early degeneration, and optimize the scanning sequence to create a reliable quantitative scale [2, 25–27]. Therefore, we designed the present study to confirm the sensibility and reliability of T\(_{1p}\) in quantification of early cartilage degeneration with histology.
Methods

Ethics statement
The experimental protocol was approved by the Animal Care and Use Committee of Jinan University, Guangzhou, China. All procedures performed in this study involving animals were in accordance with the revised Animals (Scientific Procedures) Act 1986 in the UK and Directive 2010/63/EU in Europe.

Preliminary experiment
In order to investigate the feasibility of this study, including training operations and sample size estimation, two healthy New Zealand white rabbits were performed the preliminary experiment first. Sample size was determined by the calculation of the pre-test results and the relevant literatures [12, 28].

Animal model
Thirty-two healthy New Zealand white rabbits (16 male and 16 female, with a mean age of 8 months and weighting from 2.0 to 3.0 kg) were used for the formal experiment. Rabbits were randomly numbered 1–32, and divided into 5 groups. Numbers 1 to 6 were included in Group A, 7 to 12 in Group B, 13 to 18 in Group C, 19 to 24 in Group D, and numbers 25 to 32 were included in Group E (among the 8 rabbits in Group E, 2 rabbits were randomly selected for each subgroup E_A, E_B, E_C, and E_D, which were considered as the blank controls of Group A, B, C, and D, respectively). The left knees of experimental groups (Group A, B, C, D) were served as the experimental side to establish articular cartilage models at different stages of early degeneration with papain, which is a known and validated mediator of inducing PG catabolism [28–31], and the right knees acted as the control side. On the 1st, 4th, and 7th day of the

![Fig. 3 T₁ρ pseudo-color images of the FCC on experimental side in Group E and Group a-d after T₁ρ fitting. Compared with Group E, the color of FCC in Group a-d gradually became darker, i.e., progressive changes from pale green to dark green were noted.](image)

![Fig. 4 Shown were sections of H&E stained (×100) FCC from Group e, and the experimental sides of Group a-d. The normal morphology of chondrocytes in Group a-d gradually disappeared, and fibrosis of the ECM was observed, indicating early degeneration in the FCC of the experimental side.](image)
experiment, the twenty-four rabbits of experimental groups were administered an injection of 0.5 ml of 1.6 % papain solution into the joint cavity of the left knee by using a 16-gauge hypodermic needle, and a control injection of the same amount of solution without papain in the right knee. The rabbits were sedated via ear vein injection of Ketamine (10 mg/kg) and Midazolam (1 mg/kg) while being monitored by an anesthesiologist for the period of the surgical procedure. Postoperatively, the rabbits were housed in individual cages without restriction of joint movement.

MRI Protocol

T1ρ and T2-weighted images of the bilateral knee joints were obtained for 32 rabbits by using a clinical 3.0 T MRI scanner (GE discovery MR750; the First Affiliated Hospital of Jinan University, Guangzhou, China) and human 8ch knee array coil. Group A, B, C, and D were scanned in sequence at 1, 2, 3 and 4 weeks post-operation (from the last injection of papain) respectively, and rabbits in the subgroups (E_A, E_B, E_C, and E_D) underwent MRI in the same period for comparison. For the duration of the imaging session (approximately 15 mins), each rabbit was under general anesthesia with intravenous Ketamine (8 mg/kg) and Midazolam (0.8 mg/kg), and the protocol included 2 sequences: the first, conventional T2-weighted imaging (T2WI) for morphological evaluation were acquired using a fast spin-echo (FSE) imaging on sagittal view, with the following parameters: repetition time/echo time (TR/TE) = 2000 ms/85 ms, slice thickness/spacing (thn/spa) = 2 mm/0 mm, field of view (FOV) = 12 × 12 cm, acquisition matrix = 352 × 224, NEX = 2; the second MRI pulse sequence used for a series of T1ρ-weighted sagittal images was 3D Magnetization-Prepared Angle-Modulated Partitioned k-Space Spoiled Gradient Echo Snapshots (3D MAPSS) with a spin-lock pulse amplitude of 500 Hz, under the following parameters: time of spin-lock pulse (TSL) = 0, 10, 40, or 80 ms, TR/TE = 7.8 ms/3.7 ms, thn/ spa = 12 mm/0 mm, FOV = 12 × 12 cm, matrix = 256 × 128, NEX = 1, scan time = 3 min 33 s, and the B1 of spin-lock radio frequency (RF) was 0.145 Gauss.

Histology

Immediately after imaging, rabbits in each group were euthanized by intravenous pentobarbital (100 mg/kg), and both knees were harvested, formalin-fixed, and decalcified. Tissue blocks of 0.5 cm in thickness were harvested from the medial and lateral femoral condyle cartilage (MFCC and LFCC) on the sagittal plane, and made into paraffin-embedded tissue sections. Morphology of the chondrocytes was observed after hematoxylin and eosin (H&E) staining, and the PG content was determined by Safranin-O and Fast Green staining [12, 32].

T1ρ quantification

Sun Advantage Workstation 4.5 (GE) was used for the post-processing of T1ρ images. With an exponential decay, T1ρ maps were reconstructed on a pixel-by-pixel basis by fitting a linear regression of the image intensity data to the following equation:

\[
S(\text{TSL}) = S_0 \times \exp(-\text{TSL}/T_{1\rho}),
\]

where S is the signal intensity on T1ρ images with the given TSL.

On the intermediate layer of the T1ρ sagittal image, three regions of interest (ROIs) were placed from anterior to posterior on the images of MFCC and LFCC for...
Table 1  

| T1ρ (ms) | Group A (1 week) | Group B (2 weeks) | Group C (3 weeks) | Group D (4 weeks) | Group E (Blank control) |
|----------|------------------|------------------|------------------|------------------|------------------------|
|          | Experimental side | Control side | Experimental side | Control side | Experimental side | Control side | Experimental side | Control side | Experimental side | Control Side | Experimental side | Control Side | Experimental side | Control Side | Experimental side | Control Side | Experimental side | Control Side |
| LFCC     | 43.89 ± 4.87     | 37.69 ± 6.73     | 35.41 ± 6.99     | 38.45 ± 5.83     | 68.74 ± 6.40     | 39.10 ± 5.77     | 79.63 ± 12.26     | 49.67 ± 8.46     | 39.11 ± 4.51     | 41.77 ± 8.05    | 39.38 ± 7.6     | 45.33 ± 12.42   |
| 95% CI   | 42.75-45.04      | 36.11-39.28      | 34.44-57.38      | 36.81-40.08      | 66.47-71.01      | 37.05-41.15      | 73.54-85.73      | 45.46-53.88      | 36.79-41.44      | 37.31-46.23     | 33.53-45.23    | 14.48-76.18     |
| MFCC     | 44.53 ± 5.10     | 37.53 ± 7.57     | 55.33 ± 5.98     | 38.72 ± 5.14     | 69.54 ± 6.39     | 39.76 ± 5.62     | 74.91 ± 11.6     | 47.36 ± 8.44     | 38.50 ± 6.48     | 40.77 ± 5.43    | 41.43 ± 6.42    | 35.08 ± 4.88     |
| 95% CI   | 43.3-45.73       | 35.75-39.31      | 53.64-57.02      | 37.27-40.16      | 67.26-71.8       | 37.76-41.75      | 69.14-80.68      | 43.16-51.56      | 35.17-41.80      | 37.75-43.78     | 36.49-46.37    | 22.94-47.22     |

There was no significant difference in T1ρ values between the LFCC and MFCC, within the bilateral knees of Group A, B, C, and D (P > 0.05)

Between the control side of Group A and the subgroup E, no difference in T1ρ values of LFCC existed (t = -0.98, P = 0.33), and also no difference in T1ρ values of MFCC existed (t = -1.05, P = 0.30)

Between the control side of Group B and the subgroup E, no difference in T1ρ values of LFCC existed (t = -1.12, P = 0.27), and also no difference in T1ρ values of MFCC existed (t = -1.51, P = 0.14)

Between the control side of Group C and the subgroup E, no difference in T1ρ values of LFCC existed (t = -1.30, P = 0.20)

The T1ρ value of LFCC on control side in Group D was significantly higher than in subgroup E, (t = 2.49, P = 0.02), and the T1ρ value of MFCC was obviously greater than in subgroup E, too (t = 4.70, P = 0.00)

*No difference of T1ρ values existed in Group E (P > 0.05)
measurement of the $T_{1\rho}$ values. Each ROI was drawn using a circular tool by the radiologist with more than 10 years of the professional experience of MRI, and in order to reduce the influence of partial volume effect on results, the ROIs were not allowed to be set on the adjacent tissue. Average values of the three ROIs were calculated and recorded as the $T_{1\rho}$ values of MFCC and LFCC, which were statistically analyzed.

Image interpretation
The interpretations of images acquired were determined by two radiologists with more than 10 years of experience, which blinded to all experimental information and the final histological diagnosis, and independently readed the T1$\rho$ original and T2-weighted images of the 32 rabbits. Each observer performed the same interpretation twice, with a delay of two weeks, to assess intraobserver agreement. The image interpretation results were determined according to the agreement and disagreement between the two observers as follows: the same results and the final results after discussion.

Statistical analysis
Interobserver and intraobserver reliability of image interpretation was evaluated with the interclass and intraclass correlation coefficient (ICC): poor ($< 0.40$), fair (0.40 – 0.59), good (0.60 – 0.74), and excellent (0.75 – 1.00). The mean ± standard deviation of measurements were calculated for the $T_{1\rho}$ values of MFCC and LFCC. A normal distribution of our data was shown by using the normality test, and then the differences in the $T_{1\rho}$ values of LFCC and MFCC within experimental groups were compared using one-way analysis of variance (ANOVA). Comparison of the $T_{1\rho}$ values in the control side between Group E and experimental groups was conducted with independent sample T-test. A P value of < 0.05 was considered statistically significant. Statistical analyses were performed with SPSS statistics software package, version 10.0 (SPSS Inc., Chicago, Illinois, USA).

Results
Analysis of images
Two observers agreed perfectly that there was no imaging change of the femoral condyle cartilage (FCC)
in the acquired $T_{1p}$ original and $T_2$-weighted images of the 32 rabbits (Figures 1 and 2), in addition, there was no difference in images analysis between the first and the second interpretation by the same observer. Interclass and intraclass correlation coefficient were excellent, with $k$ values of 1.00. $T_{1p}$ pseudo-color images showed relatively significant chromatic aberration in the experimental sides compared with the control sides and Group E, and the color of the experimental sides were darker. Progressive changes from pale green to dark green were observed in the experimental sides of Group A-D (Fig. 3), which indicated the $T_{1p}$ value increased gradually, and the process was consistent with the histological finding (Figs. 4 and 5).

**$T_{1p}$ value differences of intra- and inter-group**

$T_{1p}$ values of LFCC and MFCC in the bilateral knees of rabbits are shown in Table 1: $T_{1p}$ values of LFCC and MFCC on the experimental side (left side) in Group A were $43.89 \pm 4.87$ (95 % CI, 42.75 – 45.04) and $44.53 \pm 5.10$ (95 % CI, 43.3 – 45.73), and $T_{1p}$ values on the control side were $37.69 \pm 6.73$ (95 % CI, 36.11 – 39.28) and $37.53 \pm 7.57$ (95 % CI, 35.75 – 39.31), respectively; $T_{1p}$ values of LFCC and MFCC on the experimental side in Group B were $55.41 \pm 6.99$ (95 % CI, 53.44 – 57.38) and $55.33 \pm 5.98$ (95 % CI, 53.64 – 57.02), and $T_{1p}$ values on the control side were $38.45 \pm 5.83$ (95 % CI, 36.81 – 40.08) and $38.72 \pm 5.14$ (95 % CI, 37.27 – 40.16), respectively; $T_{1p}$ values of LFCC and MFCC on the experimental side in Group C were $68.74 \pm 6.40$ (95 % CI, 66.47 – 71.01) and $69.54 \pm 6.39$ (95 % CI, 67.26 – 71.8), and $T_{1p}$ values on the control side were $39.10 \pm 5.77$ (95 % CI, 37.05 – 41.15) and $39.76 \pm 5.62$ (95 % CI, 37.76 – 41.75), respectively; $T_{1p}$ values of LFCC and MFCC on the experimental side in Group D were $79.63 \pm 12.26$ (95 % CI, 73.54 – 85.73) and $74.91 \pm 11.6$ (95 % CI, 69.14 – 80.68), and $T_{1p}$ values on the control side were $49.67 \pm 8.46$ (95 % CI, 45.46 – 53.88) and $47.36 \pm 8.44$ (95 % CI, 43.16 – 51.56), respectively. In the experimental groups, the $T_{1p}$ values of the experimental side were significantly higher than the control side ($P < 0.05$, Fig. 6). Fig. 7 showed that $T_{1p}$ values of the experimental side increased gradually with the passage of post-operation time ($P < 0.05$), and there was no significant difference in $T_{1p}$ values of the control side among Group A, B, and C ($P > 0.05$), while the $T_{1p}$ values in Group D were obviously greater than in Group A, B, and C ($P < 0.05$). There was no difference in $T_{1p}$ values on control side between Group A, B, C and the subgroup $E_A$, $E_B$, and $E_C$ ($P > 0.05$, Table 1), while the $T_{1p}$ values of LFCC and MFCC on the control side in Group D were obviously greater than in subgroup $E_D$ ($P < 0.05$, Table 1). There was no significant difference in $T_{1p}$ values between the LFCC and MFCC, within the bilateral knees of Group A-D ($P > 0.05$, Table 1). Finally, No difference of $T_{1p}$ values existed in Group E ($P > 0.05$).

**Histodiagnosis**

This study performed the injections of papain on rabbits to produce a decline in PG content for setting up the animal models of early articular cartilage degeneration, and the histodiagnosis substantiated the occurrence of biochemical changes in the FCC, which with little change in morphology. Figure 8 showed that the LFCC and MFCC of the specimens were smooth and devoid of morphologic alteration upon histological inspection. Figure 4 revealed early degenerative changes in FCC on the experimental side because of the gradually disappearing of normal chondrocytes and the fibrosis of ECM. And there was a visible reduction in histological staining of PG by Safranin-O for papain-treated FCC as compared with control FCC, and the loss of PG was proportional to the degree of cartilage degeneration, meanwhile the range of Fast Green staining of the collagen fibers gradually increased (Figs. 4 and 5). However, there were no significant change in the FCC on control side (except in Group D) and Group E, there was mild degeneration in the FCC on control side of Group D. Histological verification was determined by the observer with the professional experience of pathology.

**Discussion**

In the present study, the primary purpose was to investigate whether $T_{1p}$ MRI could be used as a means to detect biochemical changes prior to the morphologic alterations in articular cartilage with histology. Out of the conventional MRI methods, $T_2$WI is commonly used to depict the morphological changes in cartilage, such as fibrillation, fissure, and partial- or full-thickness defect, which are preceded by biochemical changes of ECM [12, 33, 34]. Even though it can clearly show morphological abnormalities of cartilage, and has relatively
high detection rates for the late cartilage degeneration, which mostly indicate the progressed stage of OA with irreversible variation of the cartilage, it was limited in detecting ultrastructural changes associated with early cartilage degeneration [2, 35, 36]. In this study, T2-weighted images were used to assess whether the morphologic alterations had occurred in the FCC of rabbit models or not, and were taken as a representative of conventional MRI to contrast with T1ρ images on the capability for characterizing the micro-changes in the prophase of cartilage degeneration. The histological examination demonstrated that early cartilage degeneration had already existed in the experimental sides of Group A-D, however, T2WI showed there was no obvious imaging change of the cartilage, and T1ρ was highly sensitive to reflecting the biochemical micro-changes due to the early stages of FCC degeneration. It had suggested that T1ρ MRI was a physiological imaging technique for detecting the biochemical state and micro information of tissue at the molecular level, and can identify early lesions which cannot be identified by routine MRI sequences.

By mean of an animal model, this study aimed to measure the T1ρ value of FCC for quantitative reflection of PG depletion, which was the earliest finding of early cartilage degeneration. Toward this end, we successfully established a rabbit model of PG degradation inducing FCC degeneration. Histodagnosis verified that the model highly replicated the pathological process of early articular cartilage degeneration, and

Group A, B, C, and D were at different stages of early degeneration, which ensured the reliability of the results. In our experiment, to select the rabbit as a model was for the reason that there was a high degree of similarity between the biochemical properties of articular cartilage in a rabbit and a human, such as the main composition of ECM, the morphology and alignment of chondrocytes, and the pathological mechanism of cartilage degeneration in rabbits also resembled human being [37, 38]. We chose to focus on the FCC mostly because that was readily identified by using surface coil for generating images, and easy to dissect from the knee joint thereby histological examination.

Histological sections showed that there was a visible reduction in staining intensity of PG in papain-treated cartilage as compared with control cartilage, and the red of staining in Group A-D gradually faded, while the normal morphology of chondrocytes gradually disappeared with the emergence of fibrosis in ECM over time. All of that indicated the degree of early cartilage degeneration progressively increased from Group A to Group D, and PG depletion was the characteristics of early cartilage degeneration, its loss was proportional to the degree of cartilage degeneration. With comprehensive analysis of T1ρ imaging, a strong correlation was revealed between changes in PG content and T1ρ value. The result of this study showed a obviously difference in T1ρ values of FCC between experimental side and control side, the T1ρ values on the experimental side were significantly higher than the control side. Moreover, there was a trend of gradual increase in T1ρ values with the passage of post-operation time (after the last papain injection). This result was consistent with the loss of PG determined by the histodagnosis, which suggested that a decreased PG content induced the increase of T1ρ value, and the increased T1ρ value reflected the decreased PG content. However, there was no significant difference in T1ρ values between the control sides of Group A-C and the blank group (Group E), and histological examination showed that there was no cartilage degeneration in the control sides (except Group D) and Group E, which indicated the scientific validity of the case–control design and the objective presence of T1ρ relaxation time extension in the experimental-side cartilage. Therefore, it can be clearly demonstrated based on the above analyses that the results of the present study were supported by the histological evidence, the increased T1ρ value suggests early cartilage degeneration, and was positively correlated with the degree of cartilage degeneration. In addition, T1ρ values increased in the control sides of Group D, and the phenomenon was consistent with the histological result.

We speculated that this result was caused by the compensatory overload in the control-side cartilage due to cartilage damage of the experimental side, and such speculations surely warrants further investigations. Furthermore, we also found that 1 week after the last injection of papain, there was a significant difference in T1ρ values of Group D between the experimental side and control side prior to cartilage degeneration shown by histological examination. This showed that the sensitivity of T1ρ imaging may exceed that of histological examination for ultra-early low-level cartilage lesions, however, which should be verified by further research.

**Conclusions**

In conclusion, the main contribution of this study lay in the investigation of T1ρ imaging to quantitatively reflect biochemical changes in the initial phase of cartilage degeneration. The results suggested that T1ρ value was a sensitive indicator for accurately detecting early articular cartilage degeneration, its increasing was closely related to the degenerative change. Therefore, T1ρ MRI could be potentially used for the prospective and quantitative evaluation of early cartilage degeneration, and might allow intervention of cartilage degeneration at the earliest stages. However, T1ρ technique was still available with only a few types of MR scanners (minimum usable field
strength of 3.0 T) with specialized modified pulse sequences on a research basis, and it had not been widely applied [2, 25, 26]. The present study is also a preliminary exploration of the T<sub>1rho</sub> imaging in the evaluation of articular cartilage, and there were some difficulties with the T<sub>1rho</sub> technique, such as the repeatability of T<sub>1rho</sub> imaging, the setting of scanning parameters, the design of the T<sub>1rho</sub> image reconstruction program and the quality of the imaging. But despite all this, with the improvement of T<sub>1rho</sub> technique and optimization of the scanning sequence, T<sub>1rho</sub> MRI may be a useful tool for detecting early cartilage degeneration in clinical practice.

Abbreviations
ECM: extracellular matrix; FCC: femoral condyle cartilage; FOV: field of view; LFCC: lateral femoral condyle cartilage; MFCC: medial femoral condyle cartilage; MRI: Magnetic resonance imaging; OA: Osteoarthritis; PG: proteoglycan; ROI: region of interest; T<sub>1rho</sub>WI: T<sub>1rho</sub>-weighted imaging; TE: echo time; TR: repetition time; TSL: time of spin-lock pulse.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
Si Shen and Hao Wang contributed equally to this work, as the same first author. Si Shen: Study concept and design; preparation of animal model; reading the MRI scans and acquisition of data; histodiagnosis; analysis and interpretation of data; statistical analysis; revising manuscript critically for important intellectual content; final approval of the version to be published. Hao Wang: Study concept and design; preparation of animal model; statistical analysis; drafting of the manuscript; final approval of the version to be published. Jing Zhang: Preparation of animal model; reading the MRI scans and acquisition of data; final approval of the version to be published. Fei Wang: Analysis and interpretation of data; final approval of the version to be published. Meng Chen: Histodiagnosis; final approval of the version to be published. All authors read and approved the final manuscript.

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References
1. Dieppe PA, Lohmander LS. Pathogenesis and management of pain in osteoarthritis. Lancet. 2005;365:965–73.
2. Choi JA, Gold GE. MR Imaging of Articular Cartilage Pathology. Magn Reson Imaging Clin N Am. 2011;19:249–82.
3. Quaia E, Toffanin R, Guglielmi G, Ukmari M, Rossi A, Martellini B, et al. Fast T2 mapping of the patellar articular cartilage with gradient and spin-echo magnetic resonance imaging at 1.5 T: validation and initial clinical experience in patients with osteoarthritis. Skeletal Radiol. 2008;37:511–7.
4. Hunter DJ, Felton DT. Osteoarthritis. Br Med J. 2006;332:639–42.
5. Zhang Y, Jordan JM. Epidemiology of osteoarthritis. Clin Geriatr Med. 2010;26:55–69.
6. Lawrence RC, Felton DT, Helmsick CG, Arnold LM, Choi H, Deyo RA, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II Arthritis Rheum. 2008;58:26–35.
7. Abramson SB, Attur M. Developments in the scientific understanding of osteoarthritis. Arthritis Res Ther. 2009;11:227.
8. Helmsick CG, Felton DT, Lawrence RC, Gabriel S, Hirsch R, Kwoh CK, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I Arthritis Rheum. 2008;58:15–25.
9. Souza RB, Splendid L, Wyman BT, Helio Le Graverand MP, Li X, Link TM, et al. The effects of acute loading on T1rho and T2 relaxation times of tibiofemoral articular cartilage. Osteoarthritis Cartilage. 2010;18:1557–63.
10. Tchetaia EV. Developmental mechanisms in articular cartilage degradation in osteoarthritis. Arthritis. 2011. doi:10.1155/2011/683970.
11. Wang Y, Guo Y, Zhang L, Niu H, Xu M, Zhao B, et al. Ultrasound biomicroscopy for the detection of early osteoarthritis in an animal model. Acad Radiol. 2011;18:167–73.
12. Wheeler AJ, Dodge GR, Botharka A, Kaneland JB, Schumacher HR, Reddy R. Detection of changes in articular cartilage proteoglycan by T<sub>1rho</sub> magnetic resonance imaging. J Orthop Res. 2005;23:102–8.
13. Regatte RR, Akella SW, Lonner JH, Kaneland JB, Reddy R, T<sub>1rho</sub>, relaxation mapping in human osteoarthritic (OA) cartilage: comparison of T<sub>1rho</sub> with T2. J Magn Reson Imaging. 2006;23:547–53.
14. Glason SS, Askew R, Sheppard B, Carito B, Blanchet T, Ma HL, et al. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. Nature. 2005;434:644–8.
15. Hardingham T. Extracellular matrix and pathogenic mechanisms in osteoarthritis. Curr Rheumatol Rep. 2008;10:30–6.
16. Zarin S, Bolbos R, Piadot JB, Link TM, Li X, Souza RB, et al. Cartilage and meniscus assessment using T1rho and T2 measurements in healthy subjects and patients with osteoarthritis. Osteoarthritis Cartilage. 2010;18:1408–16.
17. Mäkelä HI, Gröhn OH, Kettunen MI, Kauppinen RA. Proton exchange as a relaxation mechanism for T1 in the rotating frame in native and immobilized protein solutions. Biochem Biophys Res Commun. 2001;289:813–8.
18. Blumenkranz G, Majumdar S. Quantitative magnetic resonance imaging of articular cartilage in osteoarthritis. Eur Cell Mater. 2007;13:76–86.
19. Hirose J, Nishihara H, Okamoto N, Oniki Y, Nakamura E, Yamashita Y, et al. Articular cartilage lesions increase early cartilage degeneration in knees treated by anterior cruciate ligament reconstruction: T<sub>1rho</sub> mapping evaluation and 1-year follow-up. Am J Sports Med. 2013;41:2353–61.
20. Jungmann PM, Baum T, Bauer JS, Karampinos DC, Erdle B, Link TM, et al. Cartilage repair surgery: outcome evaluation by using noninvasive cartilage biomarkers based on quantitative MRI techniques. Biomed Res Int. 2014; doi:10.1155/2014/840170.
21. Nestrasil I, Michaeli S, Liimatainen T, Rydeen CE, Kotz CM, Nixon JP, et al. T<sub>1rho</sub> and T<sub>1rho</sub> MRI in the evaluation of Parkinson’s disease. J Neurool. 2010;257:364–8.
22. Zobel BB, Vadala G, Del Vescovo R, Battisti S, Martina FM, Stellato L, et al. T<sub>1rho</sub> magnetic resonance imaging quantification of early lumbar intervertebral disc degeneration in healthy young adults. Spine (Philad Pa 1976). 2012;37:964–70.
23. Menezes NM, Gray ML, Hartke JR, Burstein D, T<sub>1rho</sub> and T<sub>2rho</sub> MRI in articular cartilage systems. Magn Reson Med. 2004;51:503–9.
24. Keenan KE, Besier TF, Pauly JM, Han E, Rosenberg J, Smith RL, et al. Prediction of glycosaminoglycan content in human cartilage by age, T<sub>1rho</sub> and T<sub>2rho</sub> MRI. Arthritis Rheumatol. 2011;617:1–9.
25. Li X, Cheng J, Lin K, Saadat E, Bolbos R, Jobke B, et al. Quantitative MRI using T<sub>1rho</sub> and T<sub>2rho</sub> in human osteoarthritic cartilage specimens: correlation with biochemical measurements and histology. Magn Reson Imaging. 2011;29:232–34.
26. Kim T, Min BH, Yoon SH, Kim H, Park S, Lee HY, et al. An in vitro comparative study of T2 and T2* mappings of human articular cartilage at 3-Tesla MRI using histology as the standard of reference. Skeletal Radiol. 2014;43:947–54.
27. Peers SC, Maerz T, Baker EA, Shetty A, Xia Y, Pujwal S, et al. T<sub>1rho</sub> magnetic resonance imaging for detection of early cartilage changes in knees of asymptomatic collegiate female impact and nonimpact athletes. Clin J Sport Med. 2014;24:218–25.
28. Hadvpuri T, Telhag H. Papain-induced changes in the knee joints of adult rabbits. Acta Orthop Scand. 1977;48:143–9.
29. Liu S, Shen S, Zhu T, Liang W, Huang L, Chen H, et al. Gadolinium-enhanced magnetic resonance imaging of the knee: an experimental approach. Skeletal Radiol. 2010;39:885–90.

30. Laurent D, Wasvary J, O’Byrne E, Rudin M. In vivo qualitative assessments of articular cartilage in the rabbit knee with high-resolution MR at 3 T. Magn Reson Med. 2003;50:541–9.

31. Kim SB, Kwon DR, Kwak H, Shin YB, Han HJ, Lee JH, et al. Additive effects of intra-articular injection of growth hormone and hyaluronic acid in rabbit model of collagenase-induced osteoarthritis. J Korean Med Sci. 2010;25:776–80.

32. Klohnen A, Wilson DG, Hendrickson DA, Cooley AJ, MacWilliams PS. Effects of potentiated chlorhexidine on bacteria and tarsocrural joints in ponies. Am J Vet Res. 1996;57:756–63.

33. Link TM, Steinbach LS, Ghosh S, Ries M, Lu Y, Lane N, et al. Osteoarthritis: MR imaging findings in different stages of disease and correlation with clinical findings. Radiology. 2003;226:373–81.

34. Cicuttini F, Forbes A, Asbeutah A, Morris K, Stuckey S. Comparison and reproducibility of fast and conventional spoiled gradient-echo magnetic resonance sequences in the determination of knee cartilage volume. J Orthop Res. 2000;18:580–4.

35. Potter HG, Foo LF. Magnetic resonance imaging of articular cartilage. Am J Sports Med. 2006;34:661–77.

36. Gold GE, Mosher TJ. New MRI techniques for osteoarthritis. In: Arthritis in color. Advanced imaging of arthritis. Bruno MA, Mosher TJ, Gold GE, editors. Philadelphia: Saunders; 2009. p. 153–192.

37. Hunziker EB. Biologic repair of articular cartilage: Defect models in experimental animals and matrix requirements. Clin Orthop Relat Res. 1999;367(Suppl):135–46.

38. Borrelli Jr J, Burns ME, Ricci WM, Silva M. A method for delivering variable impact stresses to the articular cartilage of rabbit knees. J Orthop Trauma. 2002;16:182–8.