

## Original Article

# Associations between the properties of the cartilage matrix and findings from quantitative MRI in human osteoarthritic cartilage of the knee

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**Abstract:** The aim of this study was to investigate the associations between the properties of the cartilage matrix and the results of T2 mapping and delayed gadolinium-enhanced magnetic resonance imaging (dGEMRIC) in human knee osteoarthritic cartilage. Osteochondral samples were harvested from the middle part of the femoral condyle and tibial plateaus of 20 patients with knee osteoarthritis (OA) during total knee arthroplasty. Sagittal T2 mapping, T1pre, and T1Gd were performed using 7.0T magnetic resonance imaging (MRI). Glycosaminoglycan (GAG) distribution was evaluated by OARSI, collagen anisotropy was assessed by polarized light microscopy (PLM), and biochemical analyses measured water, GAG, and collagen content. Associations between properties of the cartilage matrix and T2 and  $\Delta R1$  ( $1/T1Gd-1/T1pre$ ) values were explored using correlation analysis. T2 and  $\Delta R1$  values were significantly correlated with the degree of cartilage degeneration (OARSI grade;  $P = 0.53$  and  $0.77$ ). T2 values were significantly correlated with water content ( $r = 0.69$ ;  $P < 0.001$ ), GAG content ( $r = -0.43$ ;  $P < 0.001$ ), and PLM grade ( $r = 0.47$ ;  $P < 0.001$ ), but not with collagen content ( $r = -0.02$ ;  $P = 0.110$ ).  $\Delta R1$  values were significantly correlated with GAG content ( $r = -0.84$ ;  $P < 0.001$ ) and PLM grade ( $r = 0.41$ ;  $P < 0.001$ ). Taken together, T2 mapping and dGEMRIC results were correlated with the properties of the cartilage matrix in human knee osteoarthritic cartilage. Combination T2 mapping and dGEMRIC represents a potential non-invasive monitoring technique to detect the progress of knee OA.

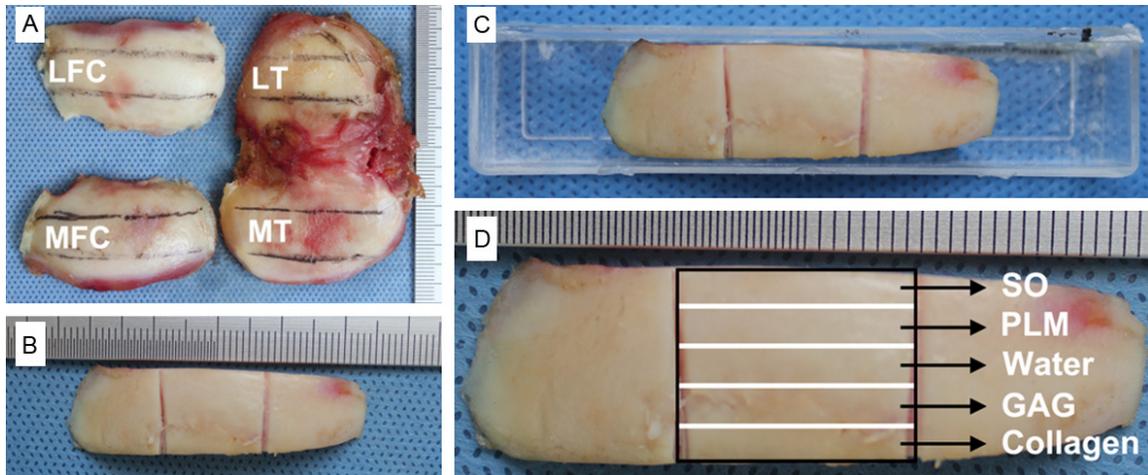
**Keywords:** T2 mapping, dGEMRIC, cartilage matrix, human osteoarthritic cartilage, 7.0T

## Introduction

According to the United Nations (UN), the proportion of people aged 60 years and over will triple during the next 40 years, and will account for more than 20% of the world's population by 2050 [1]. Osteoarthritis (OA) is a musculoskeletal disorder and a major disabling condition among the elderly population. Latest statistics indicate that OA of the knee affects an estimated 9.3 million U.S. adults [2]. As such, it is advantageous for OA patients to be diagnosed at an early stage so timely treatment can be initiated to prevent, slow down, or reverse the process of cartilage degeneration.

OA is a common degenerative disease of the articular cartilage. Articular cartilage extracellular matrix (ECM) composition and structural changes represent core pathological changes [3]. In early stage disease, massive loss of proteoglycans (PGs) from the articular cartilage ECM leads to an increase in the proportion and mobility of free water molecules; in late stage disease, there is a progressive disorganization of collagen fibers, a decrease in water content, and glycosaminoglycan (GAG) depletion [4].

Currently, sufficiently validated biomarkers of early OA are absent [5]. However, the use of magnetic resonance imaging (MRI) to study OA



**Figure 1.** Gross view of osteochondral specimen after total knee arthroplasty. A. Osteochondral specimens of the tibia plateau containing the medial tibia (MT), lateral tibia (LT), and medial/lateral femoral condyle (MFC/LFC) were collected. B. The middle area of each sample was divided into three subsections using a scalpel blade. Each subsection represented an ROI. C. The specimen was placed into a plastic container and immersed in PBS for ex vivo MRI. D. After MRI, the middle subsection of each osteochondral specimen was evenly divided into five segments for evaluating histology and water, GAG, and collagen content.

has blossomed, and quantitative MRI (qMRI) of articular cartilage as a noninvasive tool in cartilage and OA research shows great promise for diagnosing joint pathology [6]. Such qMRI technology includes T2 mapping, which predominantly measures changes in the collagen component and hydration in the ECM [7], and delayed gadolinium-enhanced magnetic resonance imaging in cartilage (dGEMRIC), which has been correlated with the loss of PG macromolecules during OA [8]. T2 mapping and dGEMRIC are sensitive enough to detect denaturation at an early stage of OA, prior to morphological alteration, as they can provide information about compositional changes in articular cartilage [6].

The aim of this study was to investigate the associations between the properties of the cartilage matrix (water, GAG, and collagen content) and results of qMRI (T2 mapping and dGEMRIC) in human knee osteoarthritic cartilage after total knee arthroplasty (TKA). We hypothesized that T2 and dGEMRIC values may reflect cartilage degeneration, which may represent a non-invasive imaging biomarker for the progress of knee OA.

## Materials and methods

### Patients and cartilage samples

This study was approved by our institutional review board and complied with the ethical

committee's standards. Written informed consent was obtained from all subjects before their enrollment in the study. From May to October 2013, a total of 20 knees (right/left, 10/10) of 20 patients (8 males and 12 females) with a mean age of  $76.5 \pm 4.1$  years (range, 65-87 years) and Kellgren-Lawrence (KL) score of 3-4 were included.

After unilateral TKA, osteochondral specimens of the tibia plateau containing the medial tibia (MT), lateral tibia (LT), and medial/lateral femoral condyle (MFC/LFC) were collected (**Figure 1A**). A total of 60 specimens (15 in each site) from the side with less advanced degeneration were used for imaging, histological, and biochemical analyses.

### Ex vivo MRI measurements

A 10 mm area in the middle of each specimen was included in the analyses. The area was divided into three subsections using a scalpel blade. Each subsection represented a region of interest (ROI) (**Figure 1B**). Each sample included 3 ROIs, providing a total of 180 ROIs. The samples were wrapped in Ringer's lactate-soaked gauze and stored at 4°C for less than 24 h before ex vivo specimen MRI. No freezing was performed before or during the whole procedure to avoid potential effects of freezing on cartilage matrix biochemistry.

## Associations between cartilage matrix and qMRI in human osteoarthritic knee

The osteochondral specimens were scanned within 24 h after surgery on a 7.0T MRI system with a maximum gradient of 360 mT/m (Bruker PharmaScan; Bruker BioSpin, Karlsruhe, Germany) using a volume 38 mm coil. Before the MR scan, the osteochondral specimens were put into a plastic box filled with phosphate-buffered saline (PBS) pH 7.4 for 4 h at room temperature [9] (**Figure 1C**). Simethicone was applied to the cartilage surface to minimize biochemical exchanges between the specimens and solution, and to help reduce accumulation of small air bubbles on the tissue surface. All specimens were scanned in their respective physiological positions to simulate them in vivo orientation and position. Each specimen was evaluated in one session that consisted of morphological sequences, T2 mapping sequence, and dGEMRIC sequence.

T2 mapping was performed as previously described [10]. Briefly, T2 relaxation times were obtained from T2 maps that were reconstructed using an MSME acquisition (TR = 2889 ms; TE = 11.0 ms, 22.0 ms, 33 ms, up to 165 ms and 176 ms; FOV = 40 × 40 mm<sup>2</sup>; pixel matrix = 256 × 256 mm; flip angle = 180°; bandwidth = 156 Hz/pixel; ST = 1 mm, TA = 9 m 14 s). T2 relaxation times of the middle subsection were calculated using the Bruker ParaVision 5.0 system.

dGEMRIC was obtained with pre-contrast and post-contrast T1 mapping evaluation. For T1 mapping, a RARE sequence was performed at six different non-equidistant TI times: 5000 ms, 2000 ms, 1500 ms, 1000 ms, 600 ms, and 441 ms; TR = 1000 ms; TE = 11 ms; pixel matrix = 256 × 256 mm; FOV = 40 × 40 mm<sup>2</sup>; ST = 1mm; FA = 180°; width = 156 Hz/pixel; TA = 15 m 52 s. All specimens were scanned in sagittal planes. Post-contrast T1 measurements (T1Gd) were performed after pre-contrast measurements (T1pre). The samples were equilibrated in 1 mM contrast agent solution (gadopentetate dimeglumine; Gd-DTPA; Bayer/Germany) for 4 h [9]. The delta relaxation rate  $\Delta R1$  ( $1/T1Gd - 1/T1pre$ ) of the middle subsection was calculated using the Bruker ParaVision 5.0 system.

### *Histological and biochemical analysis of osteochondral specimens*

Following T2 mapping and dGEMRIC, the middle subsection of each osteochondral specimen was used for histological and biochemical

analysis. Each sample was evenly divided into five segments for evaluating histology and the water, GAG, and collagen content (**Figure 1D**).

Osteochondral specimens were fixed in 10% neutral buffered formalin at room temperature for 24 h, dehydrated through graded alcohols, cleared with a xylene substitute, and embedded in paraffin. Sections (4  $\mu$ m in thickness) were separately stained with Safranin-O/Fast-Green (SO) and picrosirius red (Sigma Aldrich). The histological results were classified from 1 to 4 according to the Osteoarthritis Research Society International (OARSI) assessment system [11]. The collagen fibril structure and anisotropy were graded from 1 to 4 by polarized light microscopy (PLM) [7].

Biochemical analyses were performed as previously described [10]. Briefly, the weights of cartilage samples were recorded after the surface moisture was removed and after they had been dried in an oven at 65°C for 2 days. Water content was calculated as (wet-dry weight)/wet weight. The cartilage segments were digested with papain and GAG content was measured using a Blyscan™ sGAG assay kit (Biocolor, Newtonabbey, UK) according to the manufacturer's instructions. Collagen content in the cartilage samples was determined using a Sircol collagen assay kit (Biocolor) according to the manufacturer's instructions.

### *Statistical analysis*

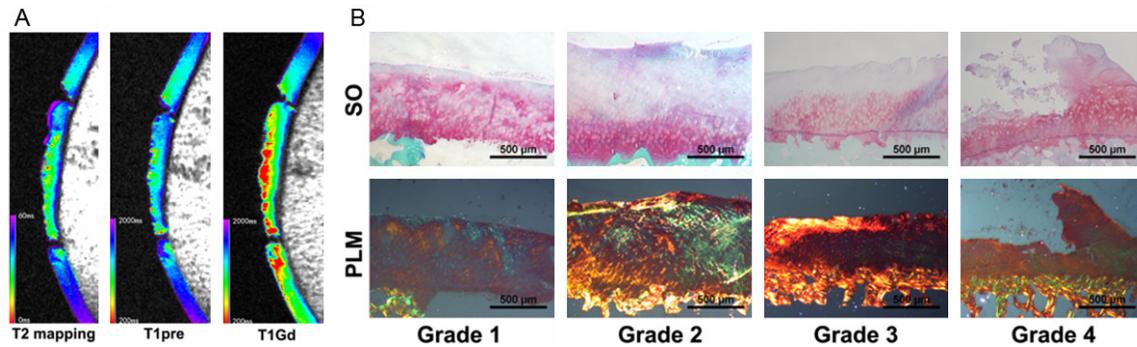
Associations between the T2 and  $\Delta R1$  values, water, GAG, and collagen content, and percentage of denatured collagen were determined using Pearson's correlation analysis. Spearman's rank correlation test was performed to study the relationships between the histological evaluation (OARSI and PLM grades) and the T2 and  $\Delta R1$  values, water, GAG, and collagen content, or percentage of denatured collagen. One-way analysis of variance (ANOVA) and Student-Newman-Keuls test were used to assess differences in T2 and  $\Delta R1$  values and ECM composition among the different OARSI and PLM grades. All statistical tests were performed using the SPSS 13.0 Statistics software package (SPSS Inc., Chicago, IL, USA).

## Results

### *qMRI and histological grades*

A total of 60 specimens including 15 MFC, 15 LFC, 15 MT, and 15 LT were scanned with T2

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**Figure 2.** Quantitative MRI and histological grades. A. Specimens were scanned with T2 mapping, T1pre, and T1Gd. B. SO and picrosirius red staining were graded from 1-4 by OARSI and PLM, respectively.

**Table 1.** Associations between OARSI grade and T2 values,  $\Delta R1$  values, water content, GAG content, collagen content and PLM grade<sup>#</sup>

	OARIS grade				P value	P
	1 (n=44)	2 (n=53)	3 (n=63)	4 (n=20)		
T2 values (ms)	29.07±0.75	30.73±0.27*	32.62±0.24* <sup>Δ</sup>	35.03±0.19* <sup>Δ,☆</sup>	<0.001	0.53
$\Delta R1$ (1/s)	1.50±0.21	1.86±0.20*	2.24±0.28* <sup>Δ</sup>	2.99±0.10* <sup>Δ,☆</sup>	<0.001	0.77
Water content ratio	0.69±0.03	0.74±0.04*	0.75±0.03*	0.77±0.02* <sup>Δ,☆</sup>	<0.001	0.35
GAG content (µg/mg)	48.96±6.84	41.54±5.04*	35.46±5.76* <sup>Δ</sup>	23.96±3.62* <sup>Δ,☆</sup>	<0.001	-0.62
Collagen content (µg/mg)	56.98±2.49	52.64±1.63	51.63±1.47*	50.09±2.70	0.129	-0.03
PLM grade	1.6±0.6	2.3±0.4	3.3±0.4	3.8±0.2	<0.001	0.82

The values represent the mean ± SD. GAG: glycosaminoglycans; PLM: polarized light microscope. <sup>#</sup>Spearman's rank correlations test; \*Versus grade 1; <sup>Δ</sup>Versus grade 2; <sup>☆</sup>Versus grade 3, determined by ANOVA with Scheffe's test.

mapping, T1pre, and T1Gd (**Figure 2A**). SO staining of all sections was graded from 1-4 according to the OARSI assessment system; picrosirius red staining of all sections was graded from 1-4 by PLM (**Figure 2B**).

### *Associations between OARSI grade and T2 values, $\Delta R1$ values, water content, GAG content, collagen content, and PLM grade*

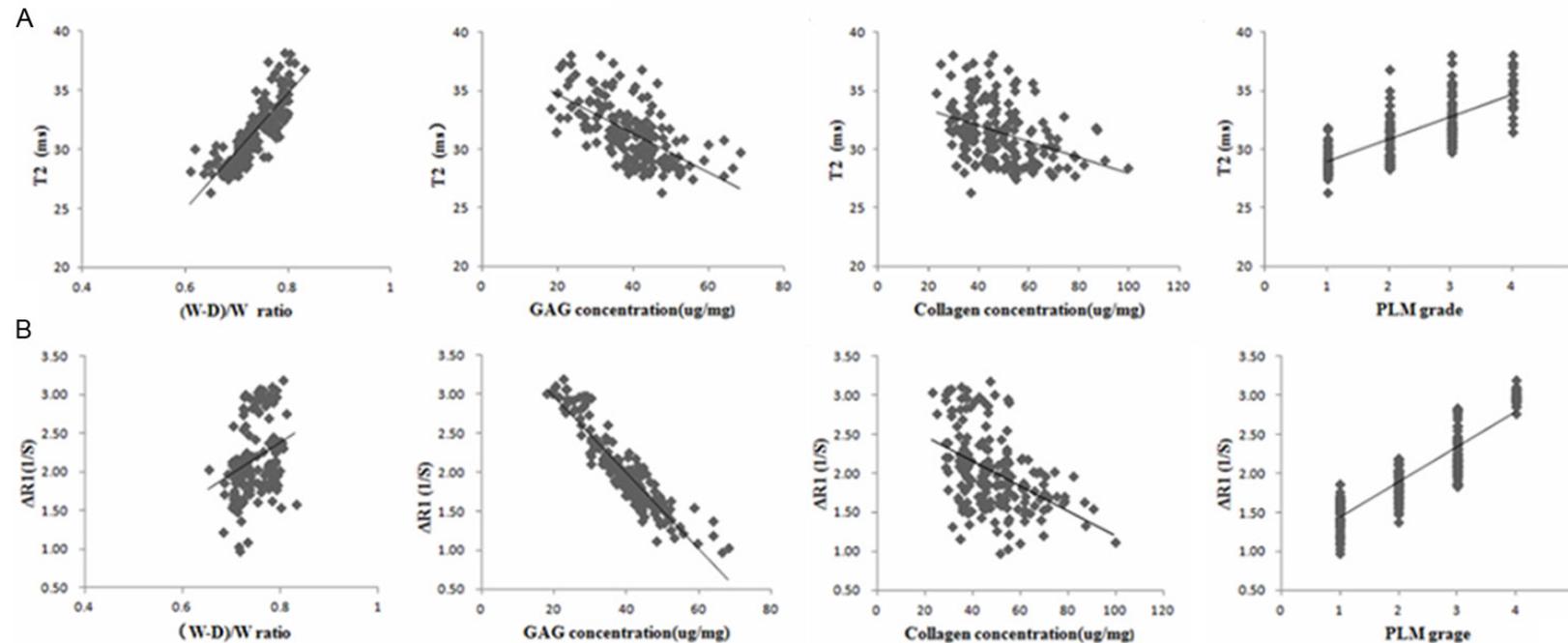
The mean T2 value was 31.46 ms (range, 26.4 to 38.2 ms). Mean T2 values significantly increased from 29.07±0.75 ms for OARSI grade 1 samples to 35.03±0.19 ms for OARSI grade 4 samples. Mean T2 values were positively correlated with OARSI grade ( $P = 0.53$ ;  $P < 0.001$ , **Table 1**). The mean  $\Delta R1$  value was 2.03 ms (range, 0.98 to 3.20 ms). Mean  $\Delta R1$  values significantly increased from 1.50±0.21 ms for OARSI grade 1 samples to 2.99±0.10 ms for OARSI grade 4 samples. Mean  $\Delta R1$  values were positively correlated with OARSI grade ( $P = 0.77$ ;  $P < 0.001$ , **Table 1**). Mean water content (0.73; range, 0.61 to 0.83) significantly increased from 0.69 in OARSI grade 1 samples

to 0.77 in OARSI grade 4 samples. There was a positive correlation between water content and OARSI grade ( $P = 0.35$ ;  $P < 0.001$ , **Table 1**). Mean GAG content (39.27 µg/mg) significantly decreased from 48.96 µg/mg in OARSI grade 1 samples to 23.96 µg/mg in OARSI grade 4 samples. There was a negative correlation between GAG content and OARSI grade ( $P = -0.62$ ;  $P < 0.001$ , **Table 1**). Mean collagen content (48.12 µg/mg) decreased from 56.98 µg/mg in OARSI grade 1 samples to 50.09 µg/mg in OARSI grade 4 samples ( $P = -0.03$ ;  $P = 0.129$ ; **Table 1**). Mean PLM grade increased from 1.6 in OARSI grade 1 samples to 3.8 in OARSI grade 4 samples; PLM and OARSI grades were highly correlated ( $P = 0.82$ ;  $P < 0.001$ , **Table 1**).

### *Associations between T2 and $\Delta R1$ values and properties of the cartilage matrix*

The T2 values and the parameters of ECM composition showed a significant correlation with water content ( $r = 0.69$ ;  $P < 0.001$ ), GAG content ( $r = -0.43$ ;  $P < 0.001$ ) and PLM grade ( $r = 0.47$ ;  $P < 0.001$ ); however, there was no correla-

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**Figure 3.** Associations between T2 and  $\Delta R1$  values and properties of the cartilage matrix. A. T2 values were positively correlated with water content ( $r = 0.69$ ;  $P < 0.001$ ), negatively with GAG content ( $r = -0.43$ ;  $P < 0.001$ ), and positively with PLM grade ( $r = 0.47$ ;  $P < 0.001$ ), but not with collagen content ( $r = -0.02$ ;  $P = 0.110$ ). B.  $\Delta R1$  values were negatively correlated with GAG content ( $r = -0.84$ ;  $P < 0.001$ ) and positively with PLM grade ( $r = 0.41$ ;  $P < 0.001$ ), but not with water ( $r = 0.23$ ;  $P = 0.124$ ) and collagen ( $r = -0.20$ ;  $P = 0.21$ ) content.

tion with collagen content ( $r = -0.02$ ;  $P = 0.110$ ; **Figure 3A**). Statistical analysis revealed no correlations between  $\Delta R1$  values and water content ( $r = 0.23$ ;  $P = 0.124$ ) or collagen content ( $r = -0.20$ ;  $P = 0.21$ ); however, there was a significant correlation between  $\Delta R1$  values and GAG content ( $r = -0.84$ ;  $P < 0.001$ ) and PLM grade ( $r = 0.41$ ;  $P < 0.001$ ; **Figure 3B**).

### Discussion

In the present study, we used T2 mapping and dGEMRIC techniques to detect associations between T2 and  $\Delta R1$  values and properties of the cartilage matrix (water, GAG, and collagen) in human knee osteoarthritic cartilage after TKA. Our results showed that qMRI results were significantly correlated with properties of the cartilage matrix. The main features of knee OA include the loss and biomechanical failure of articular cartilage due to the degradation and loss of matrix components, increase in water content, collagen network remodeling, decreased cellularity, and mechanical wear [12]. In this study, T2 and  $\Delta R1$  values were significantly correlated with the degree of cartilage degeneration. Furthermore, T2 values correlated with water content and PLM grade, but not with collagen content. These observations are in accordance with previously reported results [13, 14].

T2 is the spin-spin relaxation time, which reflects interactions between water protons and between water and the macromolecular concentration and structure of the ECM [15-17]. Chou et al. [14] reported a strong positive correlation between the T2 value and relative water content of articular cartilage. They emphasized the importance of this relationship, as it implies that the percentage increase in actual water content of articular cartilage can be estimated by T2 measurements, and that the T2 value may be useful for early diagnosis of OA. However, Nishioka et al. found no correlation between T2 values and water content [18]. These discrepant results may be explained by the radiologic grading system used for selection of specimens. All studies used the Kellgren-Lawrence grading system, which is heavily dependent on osteophytes for classification of disease, and suffers from several limitations [19].

In this study, statistical analyses indicated that T2 values were not correlated with the collagen

content of articular cartilage. These data are consistent with previous studies [20]. It is likely that T2 maps reflect alterations in collagen structure rather than changes in collagen content, as changes in T2 values within the range of the collagen concentrations in articular cartilage are small, and T2 values are more sensitive to disorganization of the collagen network than to collagen content [20].

The relationship between T2 values and GAG content of articular cartilage remains controversial. T2 values are affected by the hydration and the integrity of the collagen matrix. Our results found a correlation between T2 values and GAG content, which is consistent with several previous in vitro studies [21, 22]. Proteoglycan loss in rat patellar cartilage induced by hyaluronidase degradation was associated with significantly increased T2 values [21], and a relationship between T2 values and GAG content was shown using patella cartilage from the knees of human cadavers [22]. However, other studies found that the depletion of PG had minimal effects on T2 values [23-25]. These in vitro studies demonstrate that the biochemical changes associated with cartilage degeneration are related to elevated T2 values; however, the effects of PG concentration on T2 values must be further evaluated. It is reasonable to expect that T2 values can represent GAG concentration as signal intensity on T2-weighted images should be elevated by the increase in water mobility that results from the loss of collagen and PG in degenerating cartilage and the elevation of cartilage water content that accompanies matrix loss [26, 27].

In this study, PLM was used to assess collagen anisotropy because PLM of histologic sections using picrosirius red staining easily distinguishes the architecture and orientation of collagen fibers [28]. Our data demonstrated increases in T2 values in articular cartilage exhibiting signs of collagen disorganization as assessed by PLM. Previous studies using PLM report that T2 values correspond to collagen fiber anisotropy, and that an increase in T2 values reflects a considerable loss of the birefringence signal during PLM evaluation of collagenase-treated cartilage [29]. Taken together, these data indicate that increases in T2 values correlate with the disruption of normal collagen structure and the development of OA [30].

The utility of the dGEMRIC-T1 mapping technique for measuring GAG concentration of car-

tilage has been validated in both in vitro and in vivo studies through comparison to gold standard biochemical and histologic measures of GAG [31, 32]. Evidence suggests dGEMRIC-T1 mapping can also provide valid information on the distribution of GAG in cartilage [6]. It has been reported that  $\Delta R1$ , calculated as  $1/T1(\text{Gd})-1/T1(0)$  (dGEMRIC index), should be more representative of  $\text{Gd}-(\text{DTPA})^2$  concentration than  $T1(\text{Gd})$  for estimating GAG [33-34]. In our study, we demonstrated significant inverse correlations between  $\Delta R1$  values and the GAG content of articular cartilage at 7T. In addition, the results showed that  $\Delta R1$  values were increased and correlated with histological degeneration of cartilage. Regarding collagen, our study showed that the  $\Delta R1$  values were not correlated with collagen content. However, previous reports indicate that the rate and degree of contrast accumulation in cartilage may be influenced by factors other than GAG content, such as collagen content and orientation [35, 36], suggesting the need for additional studies.

There were a few limitations to this study. First, full-thickness measurement of T2 values and cartilage components were used. This may cause potential inaccuracies in our observation, as T2 values increase from the deep cartilage layer adjacent to the cortical bone to the superficial cartilage layer adjacent to the cartilage surface, and the ECM of hyaline cartilage varies with depth from the surface. Second, our study included a relatively small patient population. Our results should be substantiated by a comprehensive assessment with a larger sample size.

In conclusion, the present study demonstrated that qMRI (T2 mapping and dGEMRIC) results were associated with properties of the cartilage matrix in human knee osteoarthritic cartilage. Both in vitro T2 and  $\Delta R1$  values increased with the degree of cartilage degeneration, indicating that combination T2 mapping and dGEMRIC has potential as a non-invasive monitoring technique to detect the progress of knee OA. Both imaging methods are potentially useful for assessing cartilage matrix changes, and may facilitate the initiation of early treatment, monitoring of disease progression, and follow-up of operative cartilage repair and resurfacing.

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### Disclosure of conflict of interest

None.

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