Assessing degeneration of human articular cartilage with ultra-short echo time (UTE) T2* mapping

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SUMMARY

Objective: To examine the sensitivity of ultra-short echo time (UTE) T2* mapping to collagen matrix degeneration in human articular cartilage.

Methods: Magnetic resonance imaging (MRI) UTE-T2* maps and standard T2 maps were acquired on four human tibial plateau explants. Thirty-three osteochondral cores were harvested for polarized light microscopy (PLM), and composition analyses. Collagen matrix integrity was evaluated from PLM and histological images. Matrix integrity and composition was compared to standard T2 values and UTE-T2* values on a spatially registered basis.

Results: UTE-T2* values varied with matrix degeneration (P < 0.001) and were lower in severely degraded cartilage compared to healthy tissue (P = 0.012). A trend for higher UTE-T2* values in healthy tissue compared to mildly degenerate tissue (P = 0.051) was detected. Standard T2 values were not found to vary with matrix degeneration (P = 0.13) but tended to be higher in severely degraded cartilage compared to healthy tissue. UTE-T2* value variations were independent of type-II collagen and glycosaminoglycan contents. UTE-T2* mapping of deep cartilage, adjacent to subchondral bone, was more robust than standard T2 mapping in this zone.

Conclusion: UTE-T2* mapping of articular cartilage is sensitive to matrix degeneration and detects short-T2 signal, particularly in deep tissue, that is not well captured by standard T2 mapping. Correlation of UTE-T2* values and PLM indices supports the hypothesis that both may be sensitive to collagen microstructure. Further exploration of UTE-T2* mapping as a non-invasive tool to detect early articular cartilage degeneration is warranted.

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Introduction

As the incidence of osteoarthritis continues to increase, there is a tremendous need for non-destructive diagnosis and staging of cartilage degeneration prior to the onset of irreversible changes. Standard clinical imaging modalities used to diagnose articular chondrosis may not be sensitive to subtle articular matrix alterations which occur in the early stages of degeneration. Ultra-short echo time (UTE) T2* mapping is an emerging imaging technology with the potential to detect early degenerative changes in cartilage appearing grossly intact upon visual inspection.

Although magnetic resonance imaging (MRI) has proven to be essential in the diagnosis of articular joint diseases, standard MRI sequences have limited sensitivity to changes in the deep and calcified zones where highly organized collagen fibrils contribute to the very short T2 relaxation found there and where increased stresses in the tissue lead to early matrix deterioration. UTE-T2* mapping, in which T2* values are calculated pixel-by-pixel from a series of MRI images with varying echo times (TEs) including an TE of 0.5 ms or shorter, is sensitive to changes in short-T2 signal (T2 < 10 ms) and to intravoxel dephasing from local field inhomogeneities. UTE imaging (TE ~ 0.5 ms or shorter) seeks to minimize short-T2 signal decay and thus to reveal short-T2 contrast in tissues where the macromolecular structure restricts proton mobility and causes rapid T2 relaxation.

UTE imaging of the anisotropic collagen matrix found throughout articular cartilage and particularly in the deep layers may provide an improved sensitivity to subtle matrix alterations that are not well captured by long TE (>10 ms) sequences. Previously, UTE imaging has been shown to delineate articular cartilage lesions better than gradient echo and magnetization transfer sequences. A recent spectroscopic UTE imaging (UTESI)
study estimated $T_2^*$ values in deep and calcified cartilage to be between 1 and 2 ms$^7$.

Polarized light microscopy (PLM) exploits the optical properties of materials to reveal information about their composition and structure. Birefringence, the optical property of anisotropically oriented macromolecules to alter the plane of polarized light, is found in the superficial and deep zones of healthy cartilage where collagen fibrils are highly oriented$^{16,17}$. Loss of birefringence as assessed by PLM has been seen following collagenase-induced matrix disruption$^{18}$.

The ability to detect early degeneration, when potentially reversible changes are occurring, could lead to early institution of treatment to prevent or delay the onset of osteoarthritis. Therefore, the aim of this study is to examine the sensitivity of UTE-$T_2^*$ mapping to collagen matrix degeneration in human articular cartilage. We hypothesize that UTE-$T_2^*$ maps will discriminate between normal and abnormal collagen architecture as observed by PLM.

**Materials and methods**

**Sample preparation**

Thirty-three osteochondral cores were harvested from four human tibial plateaus. Tibial plateaus were collected from intact cadaveric specimens ($n=3$; 18-year-old male; 76-year-old male; elderly female, age unknown) and from total knee replacement (TKR) surgery ($n=1$, 77-year-old female). Tissue samples were obtained in accordance to protocols approved by the Committee for the Oversight of Research for the Dead (CORID) and the Institutional Review Board (IRB).

Tibial plateau explants were dissected and mounted on an acrylic adapter plate using quick-set epoxy. Prior to MRI scans, a registration plate with MRI lucent fiducial markers was rigidly fixed to the acrylic adapter plate with nylon screws. A pattern of precisely located wells filled with 4% agar doped with 2 mM Gd-DTPA$^2$ (Magnevist, Berlex Imaging, Wayne, New Jersey) embedded in the registration plate served as an external reference frame for spatially matching small regions of interest (ROI) on MR images to tissues removed as osteochondral cores (Fig. 1).

**Osteochondral core preparation**

Following MRI scans osteochondral cores were removed from the tibial plateau explants and processed for histologic and matrix composition evaluation. Two orthogonal linear translation stages embedded in the registration plate markers were determined to permit spatial registration of MRI, PLM, and composition evaluations. Unique MRI, PLM, and compositional measurements were computed for each osteochondral core.

**Quantitative MRI methods**

All MR images were collected on a 3 T clinical scanner (MAGNETOM Trio Tim, Siemens Medical Solutions, Erlangen, Germany) using an 8 channel knee coil (Invivo Inc., Gainesville, Florida, USA). Tibial plateau explants were oriented in the bore so that the articular surface was parallel to the main magnetic field ($B_0$). Acquisition of axially oriented images (relative to the magnet bore) produced coronal cross-sections (relative to the tibial plateau). This orientation served to minimize magic angle effects in the UTE-$T_2^*$ maps as the primary orientation of the collagen fibrils was perpendicular to $B_0$.

Free induction decay (FID) images for UTE-$T_2^*$ mapping were acquired using a home-developed, fast, three-dimensional (3D), UTE sequence (Acquisition-Weighted Stack of Spirals, AWSOS). A detailed description of the AWSOS sequence has previously been published$^{14}$. Briefly, AWSOS utilizes a hard radio frequency (RF) pulse to excite the entire cartilage explant. The excitation is then partitioned into thin slices using variable-duration phase encodings to minimize TE and signal decay caused by short-$T_2$ relaxation. Spiral trajectories are used for fast in-plane data acquisitions with high spatial resolution. The k-space data collection starts immediately (except 0.04 ms delay for hardware safety) after the slice encoding gradient and the FID signal is encoded by spiral gradients. There is no spin-/gradient-echo used and no contrast agents needed. Total scan time is defined by the product of slice number, in-plane spiral-interleaf number and repetition time (TR). For UTE-$T_2^*$ mapping in this study on explants, FID images were acquired using a hard RF pulse of duration 0.4 ms at eleven TE's ranging from 0.5 to 40 ms to cover both short- and long-$T_2^*$ relaxations. Fifty slices were collected across a 100 mm field of view (FOV) with a $256 \times 256$ matrix for $391 \times 391$ $\mu$m in-plane resolution and 2 mm section thickness. Sixty-four spiral interleaves were applied with readout time $= 5.28$ ms, TR $= 80$ ms, and flip angle (FA) $= 30^\circ$. Scan time was 4.27 min per TE image and 50 min for all 11-TE images. Fat saturation was achieved by using the scanner’s standard fat-saturation blocks (VB15 platform on Tim Trio 3 T, Siemens, Erlangen, Germany) to suppress all fat signals in the explants.

For standard $T_2$ mapping, a multi-contrast spin echo sequence (se_mc, VB15, Siemens, Erlangen, Germany) was used to acquire multislice coronal 2D images with seven echoes ranging from 10 to 80 ms, and TR $= 1800$ ms at bandwidth 326 Hz/pix. Twenty slices were collected with $417 \times 417$ $\mu$m in-plane resolution and 2 mm section thickness. Chemical shift artifact was 1.3 pixels. Total standard $T_2$ scan time was 12 min.

Standard $T_2$ maps and UTE-$T_2^*$ maps were generated on a pixel-by-pixel basis with a mono-exponential fitting routine using MRI Mapper software (© Beth Israel Deaconess and MIT 2006). Full-thickness cartilage ROI were manually segmented, 10–13 pixels wide by full tissue depth. The ROIs were also further subdivided into superficial and deep halves of equal thickness. Mean UTE-$T_2^*$ and standard $T_2$ values were calculated for each full-thickness, superficial and deep ROI by averaging across all pixel values within the ROI. ROI locations were determined relative to registration plate fiducial markers and were sized and positioned to correspond to regions of tissue removed during osteochondral coring.

**Histology and extracellular matrix composition**

Following MR imaging, osteochondral cores were harvested and bisected. One half of each core was processed for matrix
composition assessment; the other half was processed for histologic assessment. For composition analysis, half-cores were further bisected, and the dry-weight of each quarter-core was determined. Osteochondral quarter-cores were air-dried at room temperature under a tissue-culture hood and massed daily. The sample dry-weight was taken as the mass of the quarter-core when the daily mass ceased to change within the sensitivity of the scale (approximately 0.001 g).

Glycosaminoglycan (GAG) content was determined for one quarter of each osteochondral core by placing the sample in 0.5 M NaOH at 4°C for 48 h. GAG extracts were diluted (1:175 saline), assayed (dimethy-methylene blue)19, and GAG contents normalized by corresponding dry-weights. Type-II collagen content was determined for the remaining quarter of each osteochondral core by freezing the tissue in liquid nitrogen, crushing the frozen sample to form a powder, then incubating the powder in pepsin (10 ng/ml dissolved in 0.05 M acetic acid) at 4°C for 48 h. Pepsin incubations were repeated a total of three times, then pancreatic elastase was added to the solution (1 mg/ml dissolved in tris buffered saline) at 4°C for 12 h. Extracts were further diluted as needed (between 1:25 and 1:200 in saline), assayed with ELISA (MD Biosciences), and type-II collagen contents normalized by corresponding dry-weights20. Each normalized GAG and type-II collagen measurement was further standardized to the average measurement per plateau to allow relative compositions to be compared across multiple tibial plateaus.

For histology, core halves were decalcified (Surgipath Decalcifier I, Surgipath Medical, Richmond, IL), fixed, processed, paraffin-embedded, vertically sectioned and stained with hematoxylin/eosin (HE) and picrosirius red (PSR) using standard techniques21. Hemi-cores were sectioned in the coronal plane, parallel to coronal MRI slices. Coronal sectioning orientation was achieved by making a mark on each core at the 12 o’clock position (aligned with the anterior/posterior axis of the plateau, where the 12 o’clock pointed posteriorly). Histologic sections were then sliced orthogonally to the anterior/posterior axis. Histologic sections were approximately 6 μm thick.

PLM analysis of the collagen network was performed using a Nikon Eclipse TE2000-U polarized light microscope (Nikon, Chiyoda-ku, Tokyo) with the two polarizers set orthogonally to each other. PSR stained sections were placed between the polarizers and rotated in the x–y plane of the stage so that the polarizers were arranged 45° against the tissue superficial zone. PLM images were recorded and digitized with an Olympus DP-71 camera and Olympus DP2-BSW software (Olympus, Center Valley, PA).

**PLM cross-polarized light transmission analysis**

PLM images were saved as 8-bit grayscale images and depthwise cross-polarized light transmission profiles were created by averaging pixel intensities parallel to the cartilage surface (ImageJ, NIH, USA). To compare cross-polarized light transmission profiles across tissue samples with different tissue thicknesses, profiles were plotted against normalized tissue depth where 0 represented the tissue surface and 1 represented the bone/cartilage interface (Matlab, TheMathWorks, Natick, MA).

**Qualitative cartilage matrix evaluation**

HE and PLM images were qualitatively assessed in accordance with a scale developed by David-Vaudey et al22. Table 1 describes histologic and birefringence characteristics and the corresponding matrix grade of the David-Vaudey (DV) scale.

**Statistical analyses**

Osteochondral cores (n = 33) constitute the unit of analysis for this study. Unique values for UTE-T2*, standard T2, DV matrix grade,
PLM profile, type-II collagen content and GAG content were calculated for each osteochondral core. The distributions of study metrics were examined both graphically and statistically (via Shapiro–Wilk tests) prior to statistical testing and the results indicated that assumptions of normally distributed data were met. Full-thickness $T2^*$ ROI means and full-thickness standard $T2$ ROI means were binned according to DV matrix grade. Mean $UTE-T2^*$ and standard $T2$ values, ± standard error of the mean (SEM), for each cartilage matrix grade were calculated. In order to account for the degree of dependency between different cores harvested from the same explant, a mixed model analysis in which the explants were treated as random effects and the DV matrix grades were treated as fixed effects was employed to assess if the full-thickness $UTE-T2^*$ or standard $T2$ values varied with DV matrix grade. Post hoc tests were performed with Bonferroni adjustment for multiple comparisons to compare the mean full-thickness $UTE-T2^*$ and full-thickness standard $T2$ values between samples with different degrees of matrix degeneration. Pearson correlation coefficients were used to test for correlation between type-II collagen and GAG contents and full-thickness $UTE-T2^*$ values. A mixed model analysis was also used to assess the variation of type-II collagen and GAG contents with DV matrix grades. All statistical analyses were performed using Excel (Microsoft, Seattle, WA) and SPSS (SPSS Inc, Chicago, IL). Statistical significance was accepted for $P < 0.05$.

Results

$UTE-T2^*$ map appearances vary with collagen matrix organization; areas of cartilage damage tend to exhibit relatively low $UTE-T2^*$ signal and typically low $UTE-T2^*$ standard $T2$ values, ± standard error of the mean (SEM), for each cartilage matrix grade were calculated. In order to account for the degree of dependency between different cores harvested from the same explant, a mixed model analysis in which the explants were treated as random effects and the DV matrix grades were treated as fixed effects was employed to assess if the full-thickness $UTE-T2^*$ or standard $T2$ values varied with DV matrix grade. Post hoc tests were performed with Bonferroni adjustment for multiple comparisons to compare the mean full-thickness $UTE-T2^*$ and full-thickness standard $T2$ values between samples with different degrees of matrix degeneration. Pearson correlation coefficients were used to test for correlation between type-II collagen and GAG contents and full-thickness $UTE-T2^*$ values. A mixed model analysis was also used to assess the variation of type-II collagen and GAG contents with DV matrix grades. All statistical analyses were performed using Excel (Microsoft, Seattle, WA) and SPSS (SPSS Inc, Chicago, IL). Statistical significance was accepted for $P < 0.05$.

PLM

Depthwise PLM cross-polarized light transmission profiles varied greatly between tissue samples. Of the 33 osteochondral cores examined in this study, only seven cores (four from the 18-year-old male; two from the elderly female; one from the 76-year-old male) demonstrated a cross-polarized light transmission profile typical of intact healthy cartilage with relatively high signal intensity observed both in the superficial and deep zones. Each of these seven osteochondral cores was harvested from the submeniscal region of the plateau (four medial, three lateral) and each was graded as healthy or mildly degenerate (DV matrix grade 0 or 1). None of the cores from the TKR explant exhibited normal profiles. Among the 26 osteochondral cores with cross-polarized light transmission profiles not typical of healthy cartilage, the matrices were found, on average, to exhibit more degeneration (average DV matrix grade = 2.3 ± 1.6). Figure 3 depicts depthwise cross-polarized light transmission profiles corresponding to the three tissue samples shown in Fig. 2.

$UTE-T2^*$ values and standard $T2$ values vs DV matrix grading

The $UTE-T2^*$ value of one core from the TKR knee with DV grade 4 could not be evaluated due to poor signal-to-noise ratio in that region of the image. Across the remaining 32 cores from the four explants, $UTE-T2^*$ values measured in full-thickness ROIs were found to differ with DV matrix grade (mixed model analysis, $F = 4.87, P = 0.008, n = 32$), with the mean $UTE-T2^*$ value appearing to decrease with increasing matrix degeneration [Fig. 4(a)]. Standard $T2$ values were not found to differ significantly with DV matrix grade (mixed model analysis, $F = 5.06, P = 0.13, n = 33$), although the mean standard $T2$ value appeared to increase with increasing matrix degeneration for DV matrix grades 0–3 [Fig. 4(b)] Post hoc analysis with Bonferroni adjustment for multiple comparisons to determine pairwise differences between DV matrix grades for $UTE-T2^*$ indicated that $UTE-T2^*$ values in severely degenerate tissue (DV matrix grade 4, $n = 8$) were lower than $UTE-T2^*$ values of healthy tissue (DV matrix grade 0, $n = 9$; estimated marginal mean difference (EMMD) = −9.3 ms, $P = 0.012, 95\%$ confidence interval (CI): −17.0 to −1.6). Post hoc analysis also detected a trend for lower $UTE-T2^*$ values in mildly degenerate tissue (DV matrix grade 1, $n = 10$) compared to healthy tissue (DV matrix grade 0, $n = 9$; EMMD = −5.9 ms, $P = 0.051, 95\%$ CI: −11.8 to 0.2).

Matrix composition

Composition assessments did not detect correlations between either type-II collagen or GAG contents and $UTE-T2^*$ values (Pearson’s $r = 0.12, P = 0.52, 95\%$ CI: −0.45 to 0.24; Pearson’s $r = 0.08, P = 0.68, 95\%$ CI: −0.42 to 0.29, respectively, $n = 31$). Type-II collagen was not found to vary with matrix organization assessed by DV matrix grade (mixed model analysis $F = 1.49, P = 0.26, n = 27$). GAG content, however, was found to vary with matrix organization (mixed model analysis $F = 3.86, P = 0.024, n = 27$). Post hoc analysis with Bonferroni adjustment for multiple comparisons indicated that the GAG content of moderately degenerate tissue (DV matrix grade 3, $n = 5$) was higher than the GAG content of healthy tissue (DV matrix grade 0, $n = 9$; EMMD = 0.52, $P = 0.018, 95\%$ CI: 0.07 to 0.97).

Discussion

$UTE-T2^*$ mapping of articular cartilage is sensitive to the degree of organization (and disorganization) in the extracellular matrix and detects short-$T2$ signal, particularly in deep tissue, that is not well captured by standard $T2$ mapping. In this work, variations in the $UTE-T2^*$ values could not be explained by differences in type-II collagen content suggesting that $UTE-T2^*$ mapping is sensitive to matrix architecture rather than composition.

A purpose of this study was to ascertain whether or not $UTE-T2^*$ values differentiated between healthy and degenerate cartilage in a way consistent with existing metrics (histology, PLM analysis,

<table>
<thead>
<tr>
<th>Grade</th>
<th>Histologic characteristics</th>
<th>PLM characteristics</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>No surface irregularities</td>
<td>Presence of birefringence in the surface and deep zone</td>
</tr>
<tr>
<td>1</td>
<td>Mild surface fibrillation</td>
<td>Minor disruption of birefringence in the surface</td>
</tr>
<tr>
<td>2</td>
<td>Significant surface fibrillation</td>
<td>Disruption of birefringence in the superficial zone</td>
</tr>
<tr>
<td>3</td>
<td>Significant surface degeneration and moderate transitional zone degeneration</td>
<td>Complete breakdown of superficial birefringence</td>
</tr>
<tr>
<td>4</td>
<td>Significant structural degeneration extending well into the radial zone</td>
<td>Breakdown of birefringence in the superficial as well as deep zone</td>
</tr>
</tbody>
</table>

Table I: DV matrix grading scale. Matrix grading scale based on histologic and PLM characteristics to evaluate the progression of osteoarthritis, developed by David-Vaudey et al.22
standard $T_2$ mapping). Beyond confirming that UTE-$T_2^*$ values do indeed discriminate between healthy and severely degenerate tissue, the data suggest several benefits of UTE-$T_2^*$ mapping over existing technologies: (1) UTE-$T_2^*$ mapping is capable of evaluating the deep zone of cartilage which is difficult to examine by other existing non-invasive quantitative methods, and (2) UTE-$T_2^*$ shows greater potential than standard $T_2$ for detecting mild degeneration (UTE-$T_2^*$ nearly discriminated healthy from mildly diseased tissue, $P = 0.051$). Although the ability of UTE-$T_2^*$ mapping to detect the earliest stages of disease (matrix alterations prior to the breakdown of the articular surface) remains to be established, the finding that UTE-$T_2^*$ values varied with PLM-based matrix evaluation of the tissue warrants further exploration of UTE-$T_2^*$ mapping as a non-invasive tool to detect early degeneration.

The ultra short echo times collected in this study permitted the influence of short-$T_2$ components ($<10$ ms) on the $T_2^*$ measurement. Short-$T_2$ components, from fast spin-spin interactions between bound and free water molecules, arise in cartilage tissue where free water closely associates with protons bound in the collagen fibrils. A higher concentration of constrained collagen fibrils in the highly anisotropic deep and calcified zones of cartilage gives rise to shorter $T_2$ times in deep tissue compared to the middle zone. Standard $T_2$ sequences, with typical echo times of 10 ms or longer, are insensitive to short-$T_2$ signals that decay more rapidly than they can be measured. The UTE-$T_2^*$ values measured in this study reflect signal from both long and short-$T_2$ components of cartilage tissue, therefore, UTE-$T_2^*$ mapping has a wider sensitivity to cartilage degeneration than standard $T_2$ mapping.

In contrast to the widely recognized tendency for standard $T_2$ values to increase with matrix damage, UTE-$T_2^*$ values measured in this study were found to decrease with increasing matrix degeneration. The reason for this behavior can not be conclusively determined from this study. However, it may be speculated that a loss of water trapped within collagen fibrils...
Collagen network integrity and tissue hydration.

Increased dominance of grade 4 tissue, suggesting extracellular matrix becomes loosened or damaged. Trapped water may occur in early disease and/or in deep tissue, if collagen network is compromised. The dotted and dashed profiles correspond to tissues from Fig. 2(b, c), respectively. These two samples exhibit a relative lack of birefringence in the deep half of tissue indicative of a chaotic collagen matrix structure.

$(T_2 \sim 4 \text{ ms})^{23}$ may result in a relative increase of shorter $T_2$ component intensities in the measured FID decay curve thus leading to a net decrease in $T_2^*$ value which includes contributions from all measured $T_2$ components. In standard $T_2$ mapping, by contrast, a loss of trapped water increases the dominance of long-$T_2$ components on the $T_2$-decay curve resulting in a larger standard $T_2$ value. Loss of trapped water may occur in early disease and/or in deep tissue, if the extracellular matrix becomes loosened or damaged.

Standard $T_2$ maps poorly detected signal from the deepest layer of cartilage adjacent to bone. In fact, the standard $T_2$ data in deep tissue was so noisy that a $T_2$ value could not be determined within the tolerances of the curve-fit algorithm for many voxels. Interpretation of the standard $T_2$ values that were measured in this study is complicated by the fact that $T_2$ is known to be sensitive to both collagen network integrity and tissue hydration.$^{24}$ Collagen network disruption has been shown to reduce $T_2$, while fragmentation of the network accompanied by an increase in hydration, has been shown to increase $T_2$. Since changes to matrix structure and hydration produce competing effects on the $T_2$ parameter, and since both are altered with degeneration, it is difficult to determine cartilage status on the basis of standard $T_2$ alone.

In the current study, special hardware and protocols were employed to spatially match tissue regions and section orientations across imaging modalities. By employing a common reference using MR lucent markers on the registration plate, micrometer driven actuators with 1 μm positioning resolution and laser-guided core location and orientation tracking, the expected spatial registration error between PLM and MRI evaluations in this work is within 2 mm (the thickness of an MRI section). However, the disparity in image scales between the assessment modalities (PLM images are micrometers thick and MRI image are millimeters thick) mean that different quantities of tissue were represented by each measurement. Additionally, the DV matrix evaluation was available only for the actual plane of histologic sectioning. Although the plane corresponding to the MR scan was marked for histological section, it remains possible that the collagen microstructure assessed by the DV matrix grade differed from the macrostructural arrangement assessed by MRI either due to slight mismatch in plane of section or due to the disparity in image scale. Furthermore, the PLM analysis scheme employed in this work did not attempt to measure total birefringence. Rather, it used birefringence intensity within the superficial and deep zones (observed in a single plane, in a single histologic slice, at a single orientation with respect to the polarizers) as a proxy for gross collagen organization.$^{22}$ Despite these limitations, the quantitative spatial agreement between MRI and PLM indices suggests that $T_2^*$ mapping is sensitive to collagen matrix architecture.

Degenerative articular cartilage disease is known to manifest with extensive regional variation across the tibial plateau.$^{29-33}$ In the current work, however, variation in the study metrics is due to a combination of disease-dependant variation and inter-individual variations. Although inter-individual variations could not be effectively assessed due to the small number of tibial plateau explants examined, mixed model analyses were used to account for dependencies between samples harvested from the same explant. After considering the dependency imposed by multiple cores from the same explants, $T_2^*$ was still found to vary with the degree of matrix degeneration. Given that previous in vitro cartilage research has examined multiple samples from the same subject without explicit acknowledgment of such limitations,$^{34-36}$ the field would benefit from appropriate usage of mixed model statistical analyses in the future. Regarding this study, the degree to which inter-individual $T_2^*$ variations depend on disease or injury-induced tissue degeneration relative to inherent variations between healthy individuals requires further examination.

The results of this study showed that $T_2^*$ mapping was sensitive to changes in the sub-surface matrix microstructure of articular cartilage. $T_2^*$ was found to be more robust than...
standard $T_2$ mapping in detecting the deepest layers of cartilage. This study demonstrates a new and promising non-invasive technique for imaging articular cartilage matrix structure which may permit improved diagnosis of articular degeneration.

Conflict of interest
The authors of this work, Assessing degeneration of human articular cartilage with ultra-short echo time (UTE) $T_2^*$ mapping, have no financial or personal relationships to disclose that may constitute a conflict of interest.

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