

# Investigating Magnetic Susceptibility of Human Knee Joint at 7 Tesla

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**Purpose:** To evaluate the magnetic susceptibility properties of different anatomical structures within the knee joint using quantitative susceptibility mapping (QSM).

**Methods:** A collagen tissue model was simulated and ex vivo animal cartilage experiments were conducted at 9.4 Tesla (T) to evaluate the  $B_0$  orientation-dependent magnetic susceptibility contrast observed in cartilage. Furthermore, nine volunteers (six healthy subjects without knee pain history and three patients with known knee injury, between 29 and 58 years old) were scanned using gradient-echo acquisitions on a high-field 7T MR scanner. Susceptibility values of different tissues were quantified and diseased cartilage and meniscus were compared against that of healthy volunteers.

**Results:** Simulation and ex vivo animal cartilage experiments demonstrated that collagen fibrils exhibit an anisotropic susceptibility. A gradual change of magnetic susceptibility was observed in the articular cartilage from the superficial zone to the deep zone, forming a multilayer ultrastructure consistent with anisotropy of collagen fibrils. Meniscal tears caused a clear reduction of susceptibility contrast between the injured meniscus and surrounding cartilage illustrated by a loss of the sharp boundaries between the two. Moreover, QSM showed more dramatic contrast in the focal degenerated articular cartilage than  $R_2^*$  mapping.

**Conclusion:** The arrangement of the collagen fibrils is significant, and likely the most dominant source of magnetic susceptibility anisotropy. Quantitative susceptibility mapping offers a means to characterize magnetic susceptibility properties of tissues in the knee joint. It is sensitive to collagen damage or degeneration and may be useful for evaluating the status of knee diseases, such as meniscal tears and cartilage disease. **Magn Reson Med** 000:000–000, 2017. © 2017 International Society for Magnetic Resonance in Medicine.

**Key words:** quantitative susceptibility mapping; cartilage; knee

## INTRODUCTION

Magnetic susceptibility is an intrinsic physical property of a material and quantifies the strength of magnetization induced in that material when placed in an external magnetic field, such as the static magnetic  $B_0$  field in MRI. Quantitative susceptibility mapping (QSM), solving the ill-posed inverse problem from field to susceptibility, allows extraction of the spatial distribution of the bulk magnetic susceptibility (1–6). Quantitative susceptibility mapping has been applied to assess iron deposits (7,8); probe the anisotropic structure of white matter in the brain (9–11), tubules in the kidney (12), and myofibers in the heart (13,14); distinguish diamagnetic calcium from paramagnetic iron (15,16); and image the cytoarchitecture of the whole mouse brain (17). Early studies have shown that QSM does show promise in the analysis of joint tissues (18–20) and cartilage canals (21,22). However, it has not been successfully applied for detecting human knee diseases. Previous studies (23) have shown that  $T_2^*$  mapping (that is influenced both by the transverse relaxation ( $T_2$ ) and by local susceptibility fields) can be used to assess the articular cartilage, which indicates a potential application of QSM in the knee diseases.

Various MRI techniques that are sensitive to collagen structure, water content, and proteoglycan content have been previously reported, such as diffusion tensor imaging (24),  $T_2^*$  mapping (23), and  $T_{1\rho}$  ( $T_1$  relaxation time in the rotating frame) quantification (25). Although these techniques have shown promise for characterizing the cartilage matrix composition, knee disease is a multisystemic disease (26), stemming from not only diseased articular cartilage, but also from disease in any type of joint tissues, including the subchondral bone, capsule, menisci, and even sciatic nerves. Each of these components has a unique cellular composition and microstructure, and thus may exhibit varying magnetic susceptibility. Quantitative susceptibility mapping is particularly sensitive to molecular content, cellular arrangement, and tissue microstructure (27–29), and therefore may be suitable to evaluate the microstructure changes throughout the knee joint. However, application of QSM in the knee is faced with an additional technical challenge not critical in the brain—the chemical shift caused by fatty tissues. This chemical shift affects the complex MRI signal, particularly the phase signal, and further the QSM quantification (19,20,30,31).

The purpose of this work is to evaluate the susceptibility properties of different anatomical structures of the knee, and to test QSM on patients with knee disease. We demonstrate that (i) intricate knee structures exhibit different magnetic susceptibility properties and values; (ii)  $B_0$  orientation-dependent collagen contrast exists in

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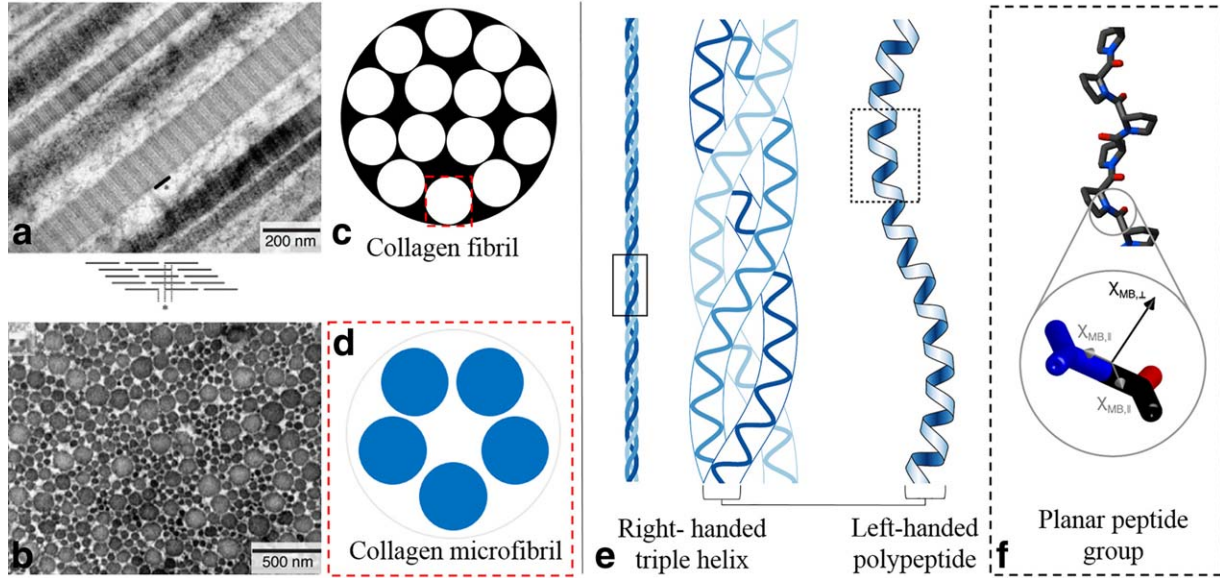


FIG. 1. Microstructural basis of the collagen model. (a, b) Electron microscopy of longitudinal and axial sections of human Achilles tendon fibrils (images used with permission from Magnusson (51)). The black marker (\*) in (a) corresponds to the length of one repeating collagen fibril. (c) A collagen fibril consists of 14 microfibrils. (d) A zoomed-in view of the dashed red-line rectangle from (c) shows the collagen microfibrillar structure that consists of five right-handed triple helices. (e) Right-handed triple helix and left-handed polypeptide structure. A zoomed-in view of the solid rectangle shows the detailed structure of the right-handed triple helix structure. (f) Magnification of the area outlined in black dotted rectangle on the left-handed polypeptide shows the detailed planar peptide groups, which have an out-of-plane susceptibility (black arrow) that is more diamagnetic than in-plane susceptibility (gray arrows).

the cartilage; (iii) QSM is able to reveal the multilayer ultrastructures of articular cartilage; and (iv) QSM may have the potential to provide assessment of meniscal and cartilage degeneration.

## METHODS

### Microstructure Model of a Collagen Fibril with Susceptibility Anisotropy

A microstructural model of collagen was developed to evaluate the contribution of the diamagnetic anisotropy of the peptide bonds on the  $B_0$  orientation-dependent magnetic susceptibility in cartilage, as shown in Figure 1. Collagen II is the major collagen in cartilage and comprises polypeptide chains in the form of left-handed polyproline II-type helices. These peptide group planes orient at approximately  $45^\circ$  to the fibril axis (32). Three parallel polyproline II-type chains wind together into a right-handed collagen-typical triple helix that is approximately 1 to 1.5 nm in diameter (33). The staggered assembly of five triple helices yields a microfibrillar segment (34). A structure of 14 microfibrils (a ring of 10 microfibrils surrounding a core of 4 microfibrils, or  $10 + 4$  for short) make up a complete collagen fibril (34).

A  $64 \times 64 \times 64$  voxel array with 0.3-nm isotropic resolution was simulated inside a volume. This resolution selection is based on axial spacing between peptides in the left-handed II-type helices (35). The collagen microfibrils with diameter of 4 nm are simulated and the  $10 + 4$  model was constructed, producing a collagen fibril. The diameter of the fibril was 15 nm, and the center-to-center microfibril spacing was 4 nm.

The simulation for calculation of the macroscopic susceptibility anisotropy with respect to  $B_0$  was conducted following the procedure proposed by Dibb et al (13). Each voxel within the volume that contains a peptide bond was assigned the same relative susceptibility tensor,

$$\chi = \chi_I I + \begin{bmatrix} \chi_A & 0 & 0 \\ 0 & -\chi_A/2 & 0 \\ 0 & 0 & -\chi_A/2 \end{bmatrix} \quad [1]$$

where  $\chi_A$  and  $\chi_I$  represent the anisotropic and isotropic susceptibility values, respectively. Each of these tensors was rotated according to its position in the right-handed triple helix. Using the theoretical molar susceptibility anisotropy value calculated by Pauling,  $-5.36 \times 10^{-6} \text{ cm}^3/\text{mol}$  (CGS units) (36) and the simulated volume size. The model-specific volume susceptibility anisotropy of each voxel containing a peptide group was calculated as  $\Delta\chi = \chi_{11} - (\chi_{22} + \chi_{33})/2 = -4.14 \text{ ppm}$  (SI units). The anisotropic susceptibility component (ie, the magnetic susceptibility normal to the helix axis) was calculated as  $\chi_A = \Delta\chi \times 2/3 = -2.76 \text{ ppm}$ . The isotropic susceptibility value of the peptide bond was calculated by fitting the simulation model to the measured apparent susceptibility values as described in the ‘‘Ex Vivo Experiments’’ section. According to the angle ( $\theta$ ) between the simulated fibrils orientation and the static  $B_0$  field ( $\hat{H}$ ), 3D frequency maps for the model array ( $\Delta f$ ) were simulated using tensor formulation of the susceptibility-frequency equation in the subject frame of reference (10),

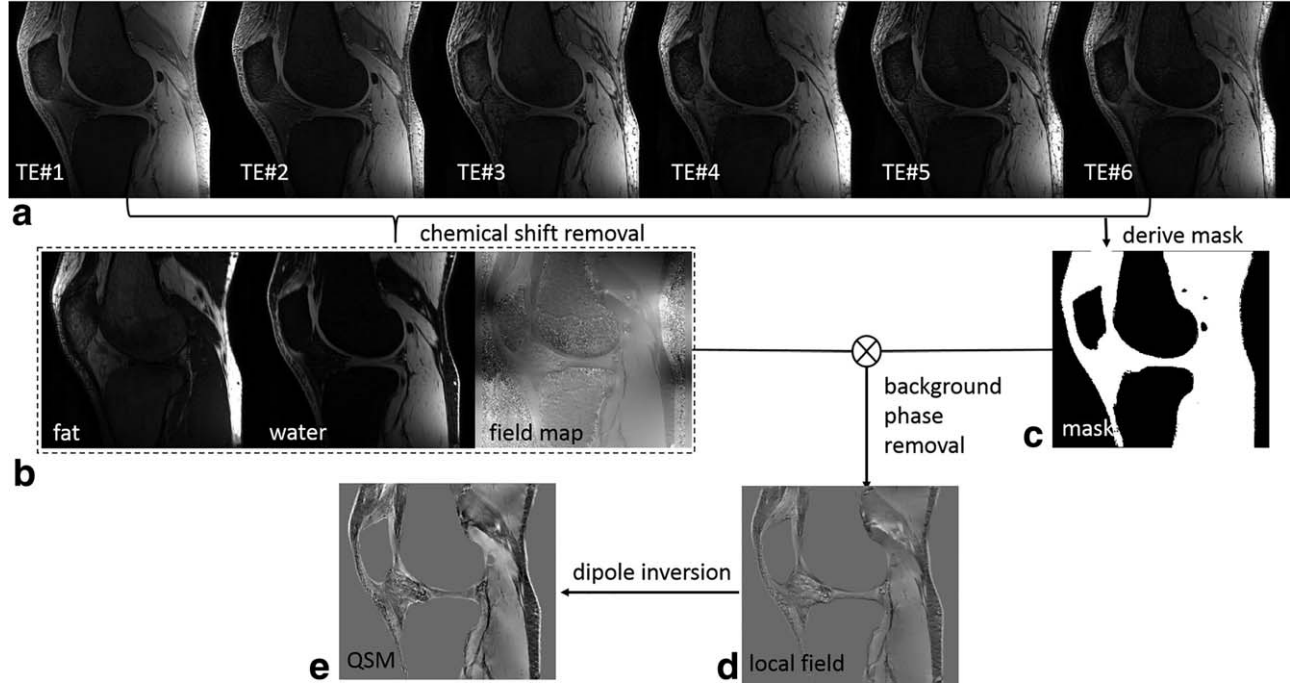


FIG. 2. Processing pipeline to estimate the susceptibility map of knee. Complex multi-echo images (a) are used to estimate the  $B_0$  field map, water, and fat images (b). A knee mask (c) is derived from the magnitude image. The local field (d) is obtained by removing the background field using PDF or other methods. The QSM (e) was reconstructed by using STAR-QSM (streaking artifact reduction for QSM) method.

$$\Delta f = FT^{-1} \left\{ \left( \frac{1}{3} \hat{\mathbf{H}}^T FT\{\chi\} \hat{\mathbf{H}} - \hat{\mathbf{H}}^T \mathbf{k} \frac{\mathbf{k}^T FT\{\chi\} \hat{\mathbf{H}}}{k^2} \right) FT\{\chi\} \right\} \gamma \mu_0 H \quad [2]$$

Finally, a complex average of uniformly distributed spins across the entire 3D frequency map yield a complex signal. An estimate of the bulk susceptibility of a voxel containing collagen fibril tissue,  $\chi$ , was calculated using the theoretical relationship  $\Delta f/f_0 = \chi/3$  with the spherical inclusion correction (37), in which  $f_0$  is the resonance frequency. The simulation was then repeated for a range of collagen fibril orientations ( $0-90^\circ$ ) with respect to  $\hat{\mathbf{H}}$  to model susceptibility anisotropy as a function of echo time (TE). Within a simulated volume, we calculated the spatially varying frequency perturbation and signal magnitude of spins within and outside the collagen fibril in the regions contained by the solid gray and dashed gray rectangle boxes respectively, shown in Figure 3a.

#### Assessing Susceptibility Anisotropy Using Animal Model

All animal preparation protocols were approved by the Duke University Institutional Animal Care and Use Committee. Fresh samples of two adult pig knee joints were obtained from a local abattoir. Pieces of articular cartilage were removed from the femoral cartilage using a surgical knife. The animal experiments were performed using a 9.4 Tesla (T) 8.9-cm vertical bore Oxford magnet controlled by an Agilent VnmrJ 4.0 console. Each cartilage specimen was firmly affixed in an 11-mm cylindrical polyethylene cartridge filled with Galden (perfluoropolyether; Solvay Specialty Polymers, Bruxelles, Belgium) to provide a background without MR signal and susceptibility properties

similar to that of water. The specimen cartridge was placed inside a sphere, allowing for an arbitrary specimen orientation inside the coil (12). Magnitude and phase data were acquired using a 3D gradient-echo (GRE) sequence with 18 echoes ( $TE_1/\Delta TE/TE_{18} = 3/2/37$  ms), repetition time (TR) = 150 ms, flip angle =  $35^\circ$ , matrix size =  $150 \times 150 \times 150$ , isotropic voxel size =  $100 \mu\text{m}$ , total scan time per orientation = 40 min. The specimen was repositioned in a new orientation before every image acquisition. To assess the  $B_0$  orientation dependence of the magnetic susceptibility, three image orientations were acquired for each specimen. The  $\hat{\mathbf{H}}$  vectors for the three acquisitions were  $(0, 0, 1)$ ,  $(-1, -0.05, 0.07)$  and  $(-0.7, -0.6, 0.36)$ , which corresponds to  $0^\circ$ ,  $86^\circ$ , and  $69^\circ$  referenced to the normal of the articular surface, respectively. The raw data were unwrapped using Laplacian-based phase unwrapping (38). The background phase was removed using projection onto dipole fields (PDF) (39) and the magnetic susceptibility was determined by two-level STAR-QSM (streaking artifact reduction for QSM) algorithm (40).

Two small regions of interest (ROIs) were manually selected from the superficial layer (black rectangle in Fig. 4b) and deep layer (red rectangle in Fig. 4b) of the cartilage using the ITK-SNAP software (41). Within each ROI were tissue voxels with similarly orientated collagen fibrils, and the mean collagen fibril direction acquired at orientation  $[0 \ 0 \ 1]$  were approximately perpendicular and parallel to  $B_0$  field for superficial zone ( $\chi_{90}$ ) and deep zone ( $\chi_0$ ), respectively. The mean apparent magnetic susceptibility within the ROIs was calculated. Then the isotropic susceptibility value of the peptide bond tensor,  $\chi_I$ , was calculated with the simulated model to fit the apparent susceptibility of specimen, using the mean

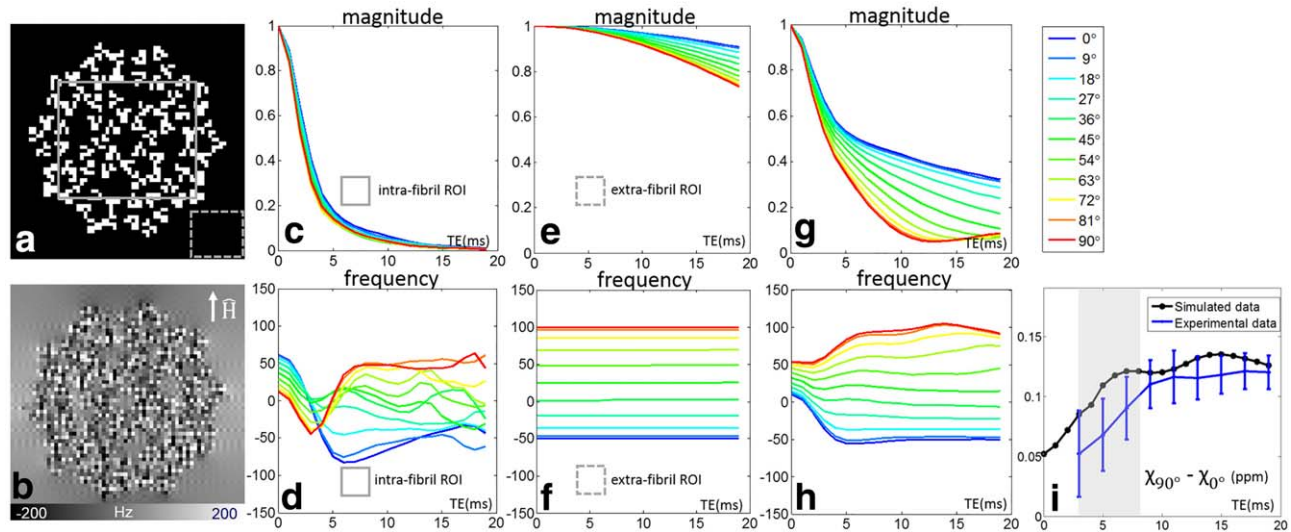


FIG. 3. (a) Cross section of 10+4 collagen model represented by a binary mask. (b) A simulated frequency map at 9.4 T calculated with collagen fibrils orientation perpendicular to  $\vec{H}$ . Mean apparent frequency and magnitude within intrafibril ROI represented by solid-gray rectangle (c, d), within extrafibril ROI represented by dashed-gray rectangle (e, f), and within the simulated volume (g, h) at different fibril orientations relative to  $B_0$  field are plotted over echo time. (i) Comparison of the susceptibility anisotropy between simulated data and experimental data. The experimental data were acquired on the ex vivo animal specimen at 9.4 T. Gray area in (i) indicates the TE range used in the in vivo human knee 3D GRE scans. The experimental data are displayed as mean  $\pm$  standard deviation.

squared error minimizing algorithm that was weighted by the inverse of the standard deviation within each ROI (step size = 0.01 ppm).

#### Human Imaging Protocols and Study Participants

The MR imaging was performed on the knee of nine volunteers (seven men and two women, aged between 29 and 58 years; six volunteers had no history of knee pain or surgery in either knee; three patients had meniscal tear or cartilage degeneration). Imaging was carried out with approval of the institutional review board and informed consent from the subjects, and in compliance with the Health Insurance Portability and Accountability Act. All images were

acquired with a 7T MRI scanner (Siemens Healthcare, Erlangen, Germany) located at UNC Biomedical Research Imaging Center using a dedicated 28-channel knee coil. Automated high-order shimming preceded all acquisitions. A sagittal 3D fast low-angle shot (FLASH) sequence was used to acquire images with the following parameters:  $TE_1/\text{spacing}/TE_4 = 2.12/2.2/8.72$  ms,  $TR = 35$  ms, flip angle =  $15^\circ$ , bandwidth =  $\pm 651$  Hz/pixel, matrix size =  $380 \times 384 \times 88$ , voxel size =  $0.4 \times 0.4 \times 1.6$  mm<sup>3</sup>, frequency encoding in the superior-inferior direction. A parallel imaging method (GRAPPA) was used with a reduction factor of three, and the total acquisition time was under 6 min per scan. The scan was repeated two more times with  $TE_1$  increased by 0.3 ms between acquisitions, giving a total of

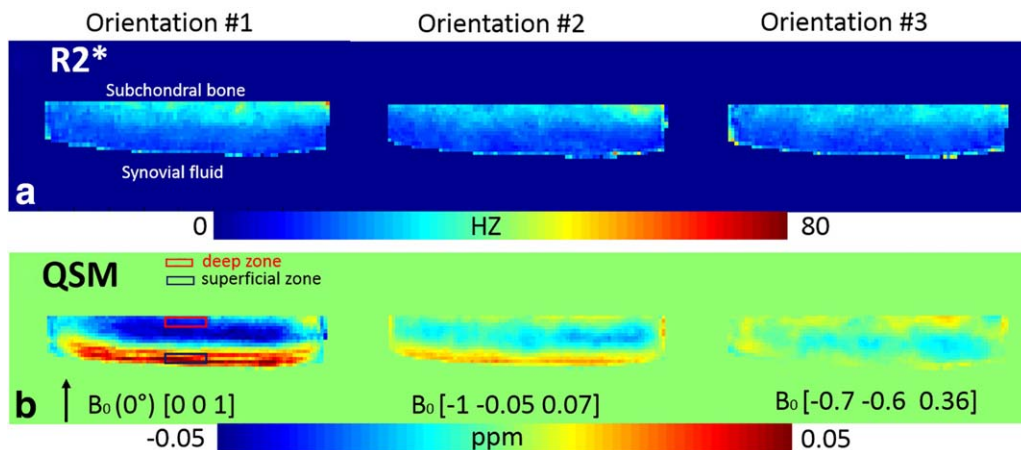


FIG. 4.  $B_0$  field-orientation-dependent modulation of  $R_2^*$  and magnetic susceptibility contrast in the cartilage by the ex vivo experimental data. The susceptibility of the collagen in different cartilage layers varies markedly from one orientation to another. Contrarily, the  $R_2^*$  contrast reveals a relatively small  $B_0$  field-orientation dependence. The  $\vec{H}$  vectors for the three acquisitions were  $(0, 0, 1)$ ,  $(-1, -0.05, 0.07)$  and  $(-0.7, -0.6, 0.36)$ , which corresponds to  $0^\circ$ ,  $86^\circ$ , and  $69^\circ$  referenced to the normal of the articular surface, respectively.

three scans. Then the three scans were combined and used as input for the water-fat separation reconstruction.

The patients (Patient 1, 58-year-old male; Patient 2, 39-year-old male; Patient 3, 30-year-old female) were scanned with two additional sequences. A sagittal 3D proton density (PD) turbo-spin-echo sequence with fat saturation was applied with the following imaging parameters: TR/TE=8170/14 ms, flip angle=180°, bandwidth=±132 Hz/pixel, matrix size=380×384×36, voxel size=0.4×0.4×3 mm<sup>3</sup>. A sagittal T<sub>2</sub> fast spin echo (FSE) sequence with fat saturation was applied with the following parameters: TR/TE=4000/148 ms, flip angle=120°, bandwidth=±766 Hz/pixel, matrix size=380×384×88, voxel size=0.4×0.4×1.6 mm<sup>3</sup>.

### Data Reconstruction

Application of QSM in the knee joint faces a technical challenge: The presence of fat introduces a significant chemical shift that confounds the estimate of B<sub>0</sub> field map. In this study, the B<sub>0</sub> field map is estimated using a chemical shift encoded reconstruction (42). The local susceptibility distribution can be estimated from the measured B<sub>0</sub> field map without contamination by chemical shift. Moreover, we improve susceptibility-map calculations with reduced streaking artifacts using a two-level regularization approach to reconstruct large and small susceptibility values (40,43). A schematic view of QSM reconstruction is shown in Figure 2. First, complex multi-echo images are used to estimate the B<sub>0</sub> field map, water and fat images (Fig. 2b). Second, the sum of squares of the magnitude images are used to obtain the mask (Fig. 2c), covering the knee joint while excluding the bone regions, by using a semi-automatic level-set based segmentation integrated in MIPAV software (NIH, Medical Image Processing, Analysis, and Visualization). This binary mask provides edge information to calculate the local field (Fig. 2d) by removing the background field using PDF (39). The local field map is subsequently input to the two-level STAR-QSM algorithm (40,43) to obtain the QSM maps (Fig. 2e).

### Data Analysis

The anatomical structure in the susceptibility maps were drawn manually using a MATLAB (MathWorks, Natick, MA, USA) based ROI tool integrated in STISuite (<http://people.duke.edu/~cl160/>). Six major tissues were segmented, each labeled with different colors: cartilage (Label 1), muscle (Label 2), tibial nerve (Label 3), fat pad (Label 4), menisci (Label 5), and subcutaneous fat (Label 6). The mean and standard deviation was calculated for each ROI. The one-way analysis of variance with post hoc Tukey procedure was used to evaluate the significance of difference between tissues that have the same susceptibility property (ie, either paramagnetic or diamagnetic). All statistical analyses were performed using SPSS 21 (IBM, SPSS Inc, Chicago, IL, USA), in which  $P < 0.05$  was considered to be statistically significant.

Meniscal tears and abnormal areas of cartilage were compared on PD, T2 FSE, and QSM images. Moreover, the abnormal areas within the medial side cartilage revealed by QSM were compared with normal healthy

volunteers on GRE magnitude, R<sub>2</sub><sup>\*</sup>, and further compared against the normal lateral side cartilage.

## RESULTS

### Simulation Results

Using the mean apparent magnetic susceptibility within selected ROIs (Fig. 4b) calculated from each individual GRE echo image, the simulated model computed a best-fit relative isotropic susceptibility value for the peptide group tensor ( $\chi_I = 0.02$  ppm). Figures 3c and 3e show that signal magnitude is orientation-dependent and decays faster within intrafibril space (within solid gray box) than extra-fibril space (within dashed gray box). Figure 3d shows that the apparent frequency of a complex average of the solid gray box varies with time, and is strongly dependent on the fibril orientation relative to B<sub>0</sub> field. The complex average of distributed spins across the entire simulated volume yielded a TE-dependent frequency map (Fig. 3h). Figure 3i compares the simulated anisotropy data to the experimental data on the ex vivo animal specimen. Both simulated and experimental data show that susceptibility anisotropy increases as TE increases. Susceptibility anisotropy reaches its plateau at TE around 8 ms.

### Ex Vivo Animal Results

The R<sub>2</sub><sup>\*</sup> and QSM images measured at each orientation are shown in Figure 4. The measured susceptibility contrast varies markedly among different orientations. The anisotropy in the cartilage collagen fibrils is clearly evident, and corresponds to the collagen fibril orientation with respect to the B<sub>0</sub> field. Contrarily, this B<sub>0</sub> field-orientation dependence appears to be relatively small from the R<sub>2</sub><sup>\*</sup> maps.

### In Vivo Human Knee Results

An example of a high-resolution 3D GRE magnitude sagittal slice is shown in Figure 5a. Several intricate structures and complex interfaces are visible, including body fat, tendons, cartilages, fat pad, menisci, and muscles. Figures 5b and 5c show a comparison of QSM maps obtained with (Fig. 5b) and without (Fig. 5c) the use of chemical shift removal reconstruction of a healthy volunteer. Overall, complex anatomical structure is better preserved in the QSM map by removing the chemical shift. There is a marked reduction of artifacts between fat and muscle, as indicated by the white arrows. Improved image contrast and clear delineations among prefemoral fat, cartilage, and muscle are observed.

The common peroneal nerve exhibits a diamagnetic susceptibility relative to its surroundings as shown in Figure 5f. The susceptibility value of the peroneal nerve is  $-0.39 \pm 0.06$  ppm. Artery walls (red arrows in Fig. 5i), mostly comprising collagen, show a diamagnetic susceptibility with a value of  $-0.25 \pm 0.14$  ppm, while the blood appears relatively paramagnetic.

After removing the chemical shift effect, multilayered structures of articular cartilage are revealed by QSM (Fig. 6c) and compared with the R<sub>2</sub><sup>\*</sup> map (Fig. 6a). The susceptibility properties appear more diamagnetic (colored by



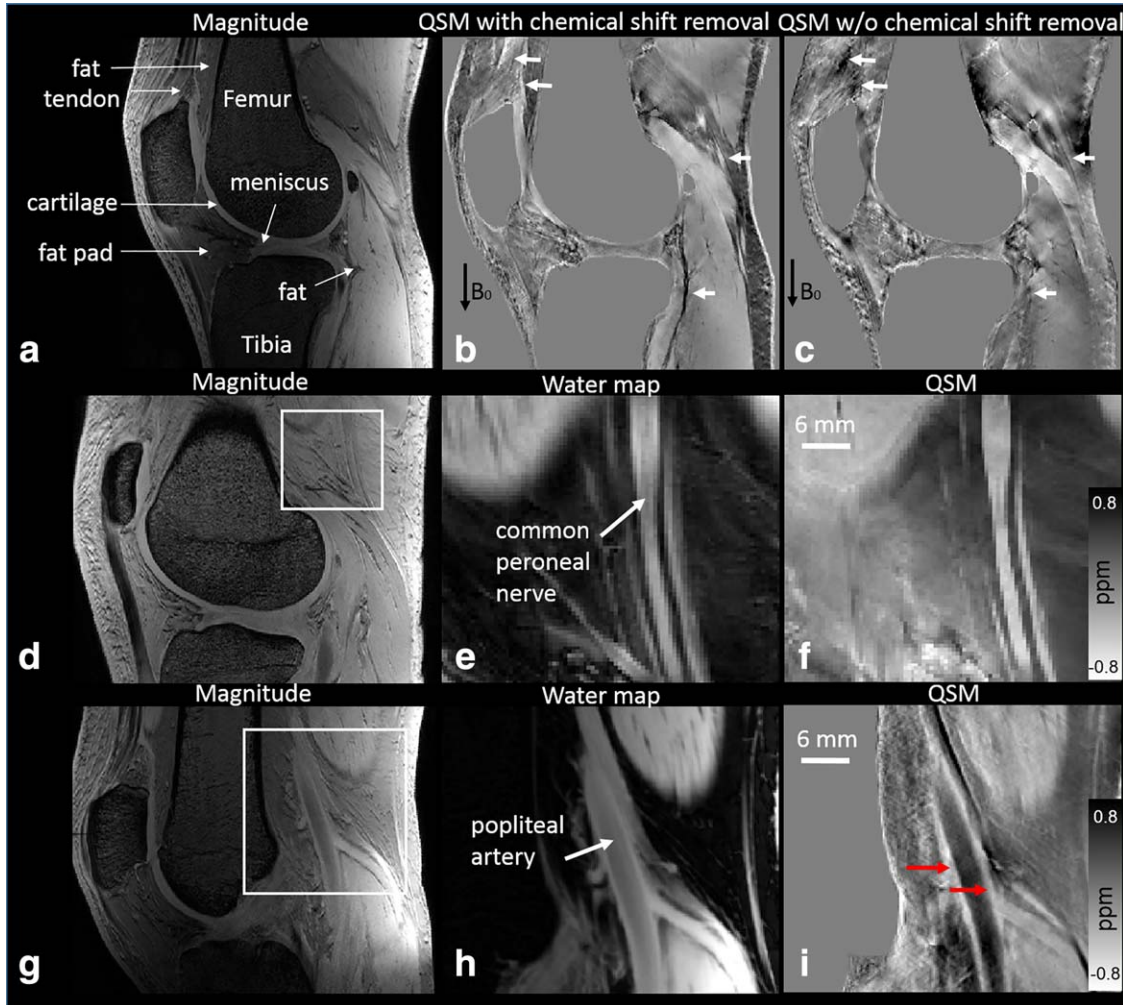


FIG. 5. Sagittal GRE images from the knee in healthy 38-year-old male. Magnitude (a), QSM with (b) and QSM without (c) chemical shift removal. Estimated water (e) and reconstructed (f) susceptibility maps corresponding to the area outlined in the white box on the magnitude image (d). Both water and QSM maps identify a portion of the common peroneal nerve just behind the knee. Magnitude (g), estimated water (h), and QSM (i) map show the structure of the popliteal artery. Red arrows indicate the artery wall revealed by QSM.

blue) in the deep zone with a value of  $-0.07 \pm 0.01$  ppm, relatively less diamagnetic with a value of  $-0.01 \pm 0.03$  ppm in the middle zone, and relatively more paramagnetic with a value of  $0.08 \pm 0.02$  ppm in the superficial zone (Fig. 6d).

Examples of the six ROIs, and their associated mean susceptibility values in the healthy volunteers, are given in Figures 7a and 7b. Generally, the cartilage, muscle, and tibial nerve are more diamagnetic than fat pad, menisci and subcutaneous fat, which are relatively paramagnetic. The mean susceptibility values in all the volunteers are given in Supporting Table S1. The tibial nerve is significantly more diamagnetic than the muscle ( $P < 0.001$ ), and the muscle is significantly more diamagnetic than cartilage with the  $P < 0.001$ . No significant difference was found between the fat pad and the menisci.

In Patient 1, a posterior meniscal tear is detected by a hyperintensity on PD images as indicated by white arrow (Fig. 8d). This area is also visible in  $T_2$  FSE image (Fig. 8e), although the increased signal intensity is less clear than that of the PD image. Quantitative susceptibility mapping

imaging consistently shows a contour abnormality (more diamagnetic) in the same area compared with the surrounding meniscal tissue (Fig. 8f) as a result the contour of the triangle missing, compared with that of the healthy volunteer (Fig. 8c). Similar results are observed in Patient 2, in which the anterior meniscal tear was detected. The more diamagnetic susceptibility caused by the tears may be related to the collagen-orientation change within the menisci. Most of the collagen in the normal menisci are coherently orientated perpendicular to the  $B_0$  field, thus exhibiting paramagnetic susceptibility. The tears/damage can disrupt these well-oriented collagen fibers and lead to a relatively random collagen fiber orientation, which causes susceptibility changes compared with the normal meniscal regions.

Abnormal areas in both femoral and tibial cartilages were detected by QSM in Patient 1 (54-year-old male), which shows that these areas exhibit more paramagnetic susceptibility (arrows in Fig. 9h) near the deep zone than that of the healthy subject (Fig. 9d). Mean susceptibility values in these areas are  $0.45 \pm 0.06$  ppm (paramagnetic),

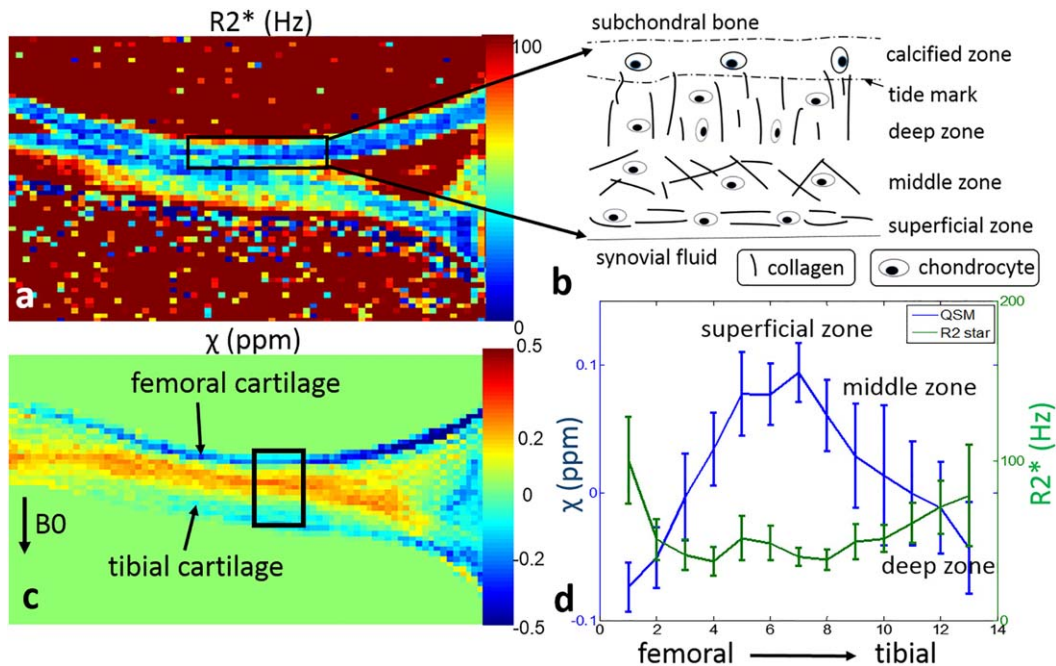


FIG. 6. Color maps of  $R_2^*$  (a) and susceptibility ( $\chi$ ) (c) in articular cartilage. (b) A schematic diagram represents the arrangements of collagen at different depths in the articular cartilage. (d) Susceptibility and  $R_2^*$  values plotted along the vertical positions from femoral to tibial cartilages within the black box in (c). The size of the box is  $13 \times 10$  (row  $\times$  column). The labels on the horizontal axis of (d) represents the voxel (row) number from femur bone surface to tibia bone surface. Error bars represent the standard deviation in each row (across 10 columns).

which is much more paramagnetic compared with those of healthy volunteers (approximately  $-0.1$  ppm) in the deep zone. These areas are also identified on the  $R_2^*$  map, as indicated by red and blue arrows in Figure 9f. The increased  $R_2^*$  values in the focal area are consistent with a lower collagen density or collagen degeneration, as collagen has a shorter  $R_2^*$  relaxation rate (44). The contrast is higher on the QSM image (red arrow in Fig. 9h) than the  $R_2^*$  map (red arrow in Fig. 9f). This suggests that QSM may be more sensitive to the degenerative articular cartilage disease than both GRE magnitude and  $R_2^*$  mapping (Figs. 9e and 9f). These medial abnormal areas are also clearly visible in the coronal orientation (Fig. 10). In addition, layer-specific contrast of the cartilage, which is clearly visible in QSM imaging in the normal lateral side cartilage, is lost in the abnormal medial side (Fig. 10).

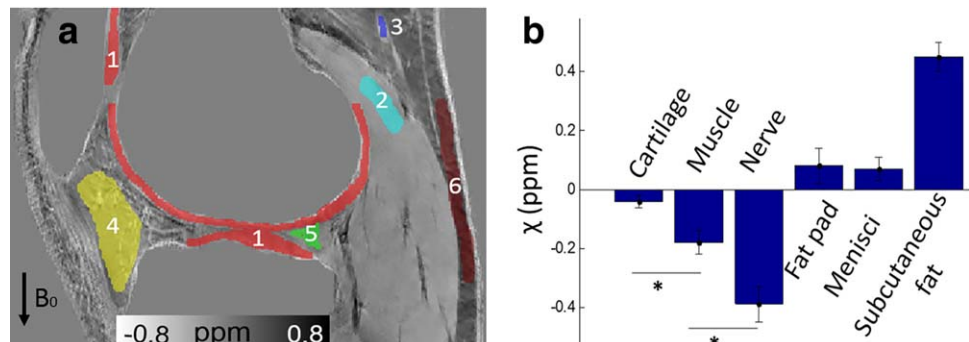
## DISCUSSION

The results presented here indicate that QSM allows in vivo imaging and quantification of the magnetic susceptibility of

the knee at high resolution and shows strong contrast between different tissues. High image quality is obtained as a result of both the chemical shift removal and improved QSM reconstruction algorithm with negligible streaking artifacts. First, a contrast pattern suggestive of an underlying layer structure is observed within the articular cartilage. Second, meniscal tears are detected using QSM. These preliminary findings demonstrate the promise of QSM to serve as a quantitative tool for evaluating various knee diseases.

One source of diamagnetic susceptibility may not act alone in producing the  $B_0$  orientation-dependent susceptibility contrast in the cartilage. Other potential sources of anisotropy include proteoglycans, noncollagenous proteins, and glycoproteins. Proteoglycans, including aggrecan, decorin, biglycan and fibromodulin, represent the second-largest group of macromolecules in the extracellular matrix and account for 10 to 15% of the wet weight (35). The most abundant by weight is aggrecan, which has more than 100 chondroitin sulfate and keratin sulfate chains. However, because sulfate constitutes such a small fraction of the cartilage relative to collagens, the susceptibility

FIG. 7. Regions of interest and mean susceptibility for different specific tissue structures: 1, cartilage; 2, muscle; 3, tibial nerve; 4, fat pad; 5, menisci; 6, subcutaneous fat.  $*P < 0.05$ .





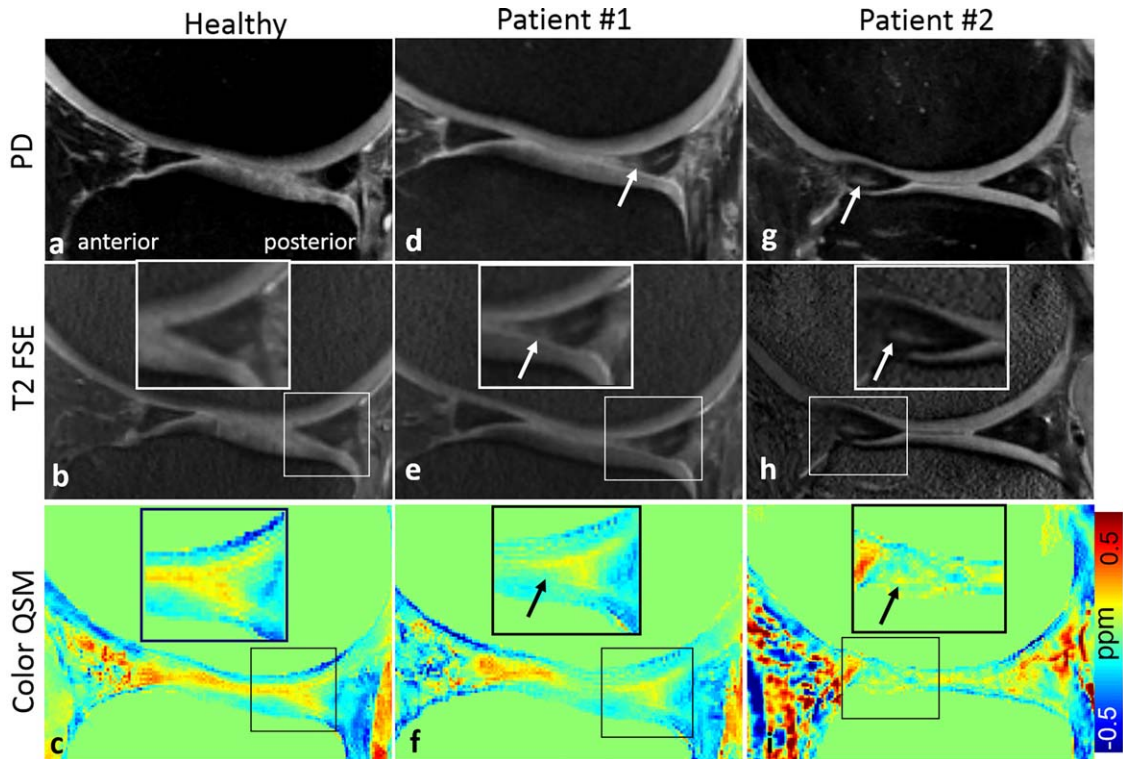


FIG. 8. Representative MRI images show two patients with meniscal tear (d–i) compared with a healthy subject (a–c). (d) Proton density image shows an area of hyperintense signal (arrow). (e)  $T_2$  FSE images also show hyperintensity at the same location. (f) Color QSM maps show findings consistent with PD and  $T_2$  FSE images. The triangle shape of the meniscus appears to be smeared or missing, as indicated by the arrows in zoomed-in view, and the susceptibility values become more diamagnetic compared with the normal meniscal tissue (c). Similar results are found in the anterior meniscus in Patient 2.

anisotropy of the collagen fibers appears to be more dominant.

The TE dependence of magnetic susceptibility anisotropy can be attributed to multiple compartmentalized

contributions to the signal from water pools experiencing different magnetic fields within the measured volume. Figures 3d and 3f show that the apparent frequency of a complex average of intrafibril and extrafibril space is

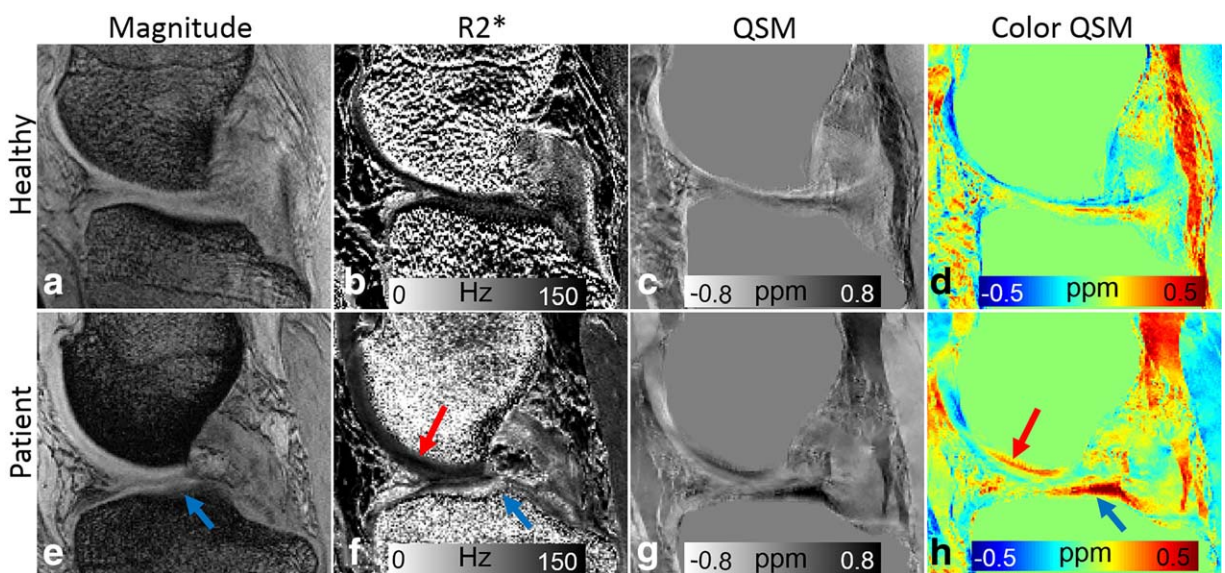
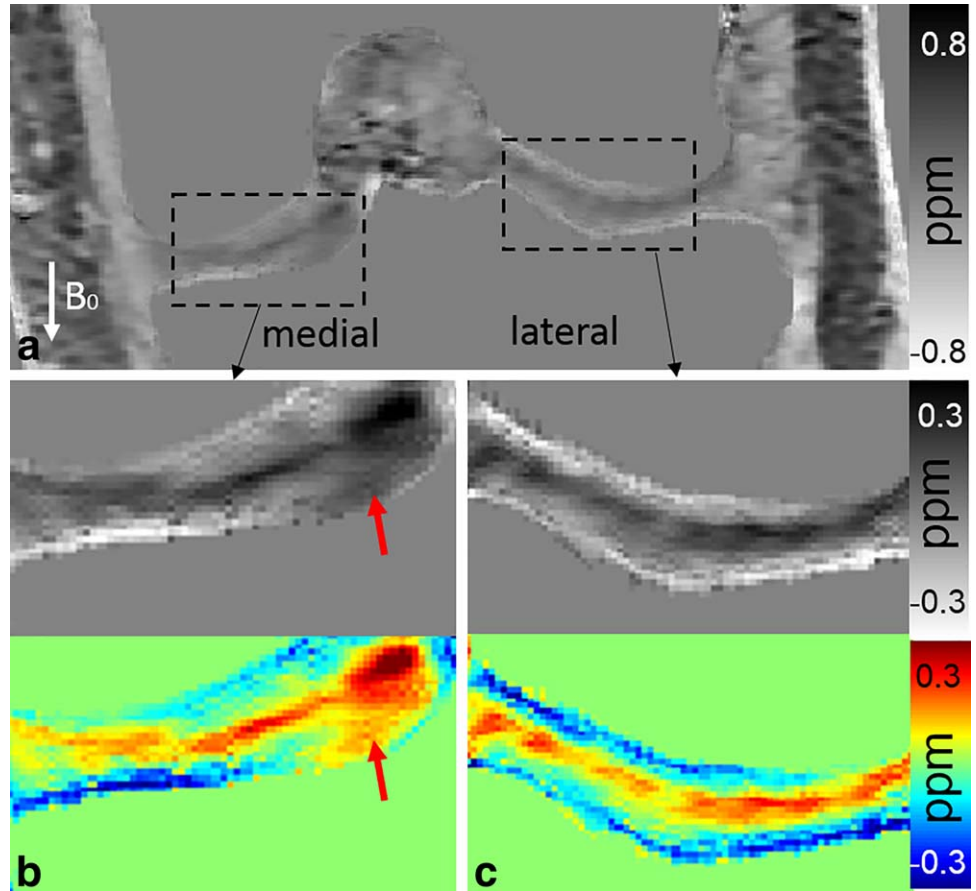


FIG. 9. Representative MRI images show medial cartilage in a healthy subject (a–d) and Patient 1 with collagen damage or degeneration (e–h). (a, e) The GRE magnitude images show morphology of the cartilage. The cartilage appears normal in (a), but shows a decreased signal intensity in the tibial plateau (e). The corresponding  $R_2^*$  (b, f), QSM (c, g) and color QSM (d, h) maps clearly show the difference in the cartilage between the healthy subject and the patient.



FIG. 10. (a) Cropped QSM image of cartilage in coronal orientation of Patient 1. Rectangular boxes with dashed lines correspond to medial and lateral cartilages. (b, c) Magnifications of the area outlined in black boxes from the medial and lateral cartilages, respectively, by both grayscale and color maps. Arrows indicate the deep zone of medial side tibial cartilage (b) with paramagnetic susceptibility compared with normal diamagnetic lateral side (c). The displayed coronal images were obtained by zero padding in k-space to interpolate to an isotropic resolution of  $0.4 \times 0.4 \times 0.4 \text{ mm}^3$ .



strongly dependent on the fibril orientation relative to  $B_0$  field. In addition, the apparent frequency of intrafibril space varies with time. As shown by the signal magnitude decays in Figures 3c and 3e, the intrafibril space has a significant lower  $T_2$  than the extra-fibril space. In this case, spin dephasing and therefore signal attenuation in the intrafibril region are faster, leaving the extrafibril field perturbation to dominate the measured frequency and calculated susceptibility. Moreover, the susceptibility anisotropy in the extrafibril space is larger than that of the intrafibril space, in which the susceptibility anisotropy of collagen microfibrils may cancel each other. As a result, the complex average of distributed spins across the entire 3D frequency maps yielded a TE-dependent magnetic susceptibility anisotropy (Figs. 3h and 3i). The current simulation model does not apply  $T_1$  or  $T_2$  weighting to the intrafibril and extrafibril volume signals. However, the model takes into account the  $T_2$  decays that are inherently modeled as a result of the inhomogeneous magnetic fields within the simulated intrafibril and extrafibril volumes.

The characteristics of  $B_0$  orientation-dependent  $R_2^*$  anisotropy have been investigated in brain white matter (45,46). In this study, an  $R_2^*$  anisotropy contrast weaker than susceptibility anisotropy was observed in the articular cartilage as shown in Figure 4. Because  $R_2^* = R_2 + R_2'$ , one possible explanation for the weaker  $R_2^*$  anisotropy is that the  $R_2$  anisotropy and  $R_2'$  anisotropy have opposing signs and may cancel each other.

Previous studies on orientation-dependent  $T_2$  relaxation in cartilage shows that superficial zone has higher  $T_2$  than deep zone when the normal of specimen surface is parallel to  $B_0$  (47). At this orientation, collagen fibrils are perpendicular and parallel to  $B_0$  within the superficial zone and deep zone, respectively. In contrast, as shown by our simulation results in Figure 3g,  $T_2$  is actually lower when collagen fibril is perpendicular to  $B_0$  than that of parallel to  $B_0$ . Therefore, the  $B_0$ -orientation-dependent  $T_2$  and  $T_2'$  anisotropy do cancel each other, which results in a reduced  $B_0$  field orientation dependence of  $T_2^*$  (or  $1/R_2^*$ ).

Cartilage exhibits a variation of susceptibility values from the superficial zone to the deep zone, with more diamagnetic tissue in the deep zone, approximately zero susceptibility in the middle zone, and paramagnetic tissue in the superficial zone. This spatial variation of the susceptibility as measured in cartilage is consistent with the simulation results as shown by the gray area in Figure 3i. This regional variation on QSM images (Fig. 6d) is caused by the orientation of collagen fibrils within the cartilage (Fig. 6b) relative to  $B_0$  field. When collagen fibrils are mostly parallel to  $B_0$  field, the susceptibility values become more diamagnetic, such as in the deep zone. When collagen fibrils are mostly oriented perpendicular to  $B_0$  field, the susceptibility values are more paramagnetic, such as in the superficial zone. This behavior of susceptibility anisotropy differs from that observed in brain white matter, in which myelinated

axons appear more paramagnetic when parallel to  $B_0$  field. This difference is caused by the right-handed triple-helix structure of the collagen fiber. The planar peptide groups in the polyproline helix of collagen fiber have an out-of-plane susceptibility that is more diamagnetic than the in-plane susceptibilities (32,48).

The susceptibility values reported in Supporting Table S1 indicate a large variation for similar fat tissues (eg, 0.08 ppm in the fat pad and 0.45 ppm in the subcutaneous fat). This difference can be caused by the different portion of fibrous septa contained in the fat pad and subcutaneous fat, as well as their distributed orientations relative to  $B_0$  field, which affect the measured susceptibility value averaged within each ROI. In addition, the shape and orientation of fat lobules, which are separated by these fibrous septa, may also affect the measured magnetic susceptibility.

The tibial nerve has diamagnetic susceptibility in the human knee. This diamagnetic susceptibility is determined by the basic unit of the peripheral nerve–myelination by Schwann cells and collagen supporting structures. Note that not all peripheral nerves are myelinated. Although imaging the peripheral nervous system is difficult, QSM may be useful for identifying and characterizing certain peripheral nerve pathology, such as nerve tumors.

The susceptibility difference between diamagnetic sciatic nerve ( $-0.39$  ppm) and paramagnetic subcutaneous fat (0.45 ppm) is  $-0.84$  ppm, which is comparable with susceptibility difference between white matter and fat tissue in brain reported by prior literature (49,50). However, the blood magnetic susceptibility in the knee artery should not be simply compared with the artery in brain, which it may depend on the vessels orientation relative to the main field and blood oxygen level.

Although our study has demonstrated the feasibility of high-resolution and high-contrast QSM of the knee joint at 7 T, more data are necessary to draw conclusions on its clinical utility in comparison with other techniques (eg,  $T_{1\rho}$  and  $T_2$  mapping). Nevertheless, QSM offers several clear technical advantages: reduced heating, less sensitivity to  $B_1$  and  $B_0$  inhomogeneity, and volumetric coverage at high resolution and throughput. Increased specific absorption rate (SAR) is a major concern at 7 T for both  $T_{1\rho}$  and  $T_2$  mapping because of the use of high-energy radiofrequency pulses such as spin lock pulses and refocusing pulses; however, QSM uses low-power small flip angle excitation pulses, thus dramatically reducing SAR. Both  $B_1$  and  $B_0$  field inhomogeneity increase at 7 T and can substantially affect the quantification of  $T_{1\rho}$  and  $T_2$  relaxation times; in contrast, QSM has limited sensitivity to  $B_1$  inhomogeneity and in fact benefits from increased contrast as a result of the higher  $B_0$  field. Shorter  $T_2^*$  relaxation and higher phase contrast-to-noise ratio at 7 T allows QSM to be acquired rapidly with the robust GRE sequence using a relatively short TR. The entire QSM study protocol can be performed within 17 min at a resolution of  $0.4 \times 0.4 \times 1.6$  mm<sup>3</sup> covering the entire knee.

One limitation of this study is that susceptibility of bone and marrow cannot be depicted. The phase of bone is not accurately available, as the  $T_2^*$  relaxation of the

bone and marrow is much shorter in comparison to surrounding soft tissues at 7 T. In the current study, the bone areas are masked out, to prevent inaccurate estimation of the field map, which would introduce errors caused by the ill-posed inverse problem. Future studies using ultrashort TE pulse sequences can be used for imaging magnetic susceptibility of bone and marrow (20).

## CONCLUSIONS

In this study we have successfully demonstrated the feasibility of quantifying susceptibility of the human knee joint in vivo at 7 T. The multilayered structures of articular cartilage are demonstrated by QSM. Quantitative susceptibility mapping is able to probe meniscal tears and demonstrate a visible contrast change of degenerative cartilage. These results suggest that QSM may be sensitive to early knee disease detection, which is useful for treatment and the evaluation of disease progression.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

**Table S1.** Susceptibility Values (ppm) for Different Specific Tissue Structures Labeled in Figure 6e