# High resolution diffusion tensor imaging can detect molecular and structural cartilage changes after destabilization of the medial meniscus

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INTRODUCTION: Cartilage integrity depends mainly on two extracellular (ECM) components: collagen, which provides tensile strength, and proteoglycans (PGs), whose glysaminoglycan (GAG) chains maintain hydration and compressive resistance. During initial phases of osteoarthritis (OA), cartilage changes occur at molecular levels, characterized by proteoglycan degradation and increased water content, and structural levels, characterized by disorganization of collagen fibers. Various MRI techniques have been employed to examine changes in OA cartilage, but they often fail to capture these microstructural alterations in early disease stages. Diffusion MRI (dMRI) can provide a more comprehensive assessment of cartilage ECM components, including GAG and collagen, and diffusion tensor imaging (DTI), a type of dMRI, has shown sensitivity to collagen fiber orientation and water diffusion properties, enabling evaluation of cartilage degradation (1). DTI metrics such as fractional anisotropy (FA), which may indicate collagen architecture by measuring deviations from isotropic diffusion, along with mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD), which quantify rate and directionality of water movement, may be sensitive to cartilage microstructure changes (2). How these metrics correlate with specific molecular changes in degenerating cartilage, is not well known. Moreover, whether advanced tractography can detect 3D collagen fiber disorganization in OA remains unknown. The aim of this study was to evaluate the sensitivity of dMRI to GAG loss and collagen fiber disorganization in the articular cartilage of rat knees following destabilization of the medial meniscus (DMM) using high-resolution imaging, tractography, and Monte Carlo simulations, with validation from histology.

METHODS: After IACUC approval, DMM surgery was performed on left knees of 8 male rats at 12 weeks old (3); SHAM surgery was performed on right knees. Eight weeks later, animals were perfusion-fixed with a 1:10 mixture of ProHance-buffered formalin. MRI scans were done on all specimens: a 3D diffusion-weighted spin-echo pulse sequence, incorporating undersampling in both phase dimensions, was employed on a 9.4 T scanner. Scan parameters were: matrix size = 420 × 256 × 256, FOV = 18.9 × 11.52 × 11.52 $mm^3$, isotropic voxel size = 45 µm, 43 unique diffusion directions with the b value of 1000 $s/mm^2$ and four non-diffusion-weighted (b0) measurements. Compressed sensing (CS) reconstruction was applied to the under-sampled k-space data as described in previous studies (4). The DTI model was used to calculate the tensor and tractography using DSI studio software. The scalar indices (FA, MD, AD, RD) were also calculated. Deterministic fiber tracking was performed for the articular cartilage. Regions of interest (ROIs) of femoral cartilage and tibial cartilage were manually drawn based on anatomical b0 images using ITK-SNAP for three consecutive slices in the center of medial knee compartment. Differences in metrics were compared using paired two-tailed t-test. Significance was set at $p<0.05$. Histology with Toluidine Blue (TB) and Picrosirius Red (PSR) staining was performed to reflect PG content and collagen network respectively and correlate with our imaging findings. To more directly investigate how DTI findings may correlate with collagen disruption and GAG loss, Monte Carlo simulations were performed using voxelized numerical phantoms with aligned or dispersed collagen fibers, and with and without GAG water compartments. Diffusion signals from $5\times10^5$ random walkers were simulated at b=1000 $s/mm^2$, combined accordingly to physiologic water fractions to calculate DTI metrics.

RESULTS SECTION: In femoral cartilage, the DMM group had significantly lower FA values (-16.42%, $p<0.01$) and higher MD (+9.30%, $p=0.022$) and RD (+11.24%, $p=0.015$) values (Fig. 1a-d). In tibial cartilage, the DMM group showed a lower FA (-12.76%, $p=0.014$), while the MD (+10.88%, $p=0.011$), AD (+8.54%, $p=0.020$), and RD (+12.29%, $p<0.01$) values were higher compared to the SHAM group (Fig. 1e-h). Through tractography investigations, the fiber tracts in the SHAM group were shown to exhibit a well-organized pattern (Fig. 2a): the tibial collagen fibers were perpendicular to the cartilage surface in the radial zone (green color) and parallel to the cartilage surface in the superficial zone (red color). In contrast, the 3D collagen fiber structure in the DMM group was dramatically disordered (Fig. 2b). On PSR staining, the DMM femoral cartilage (Fig. 2d) displayed some loss of red stain compared to the SHAM (Fig. 2c), but the largest difference was seen in the tibial cartilage where the SHAM cartilage was thicker, cells were more organized and collagen fibers were perpendicular to the surface in the deep and mid zone (black arrows) (Fig. 2e). On TB staining, mainly just the tibial cartilage differed in the DMM as evidence by roughened surface and cracks (green arrow), cellular disorganization (white asterisk), as well as overall TB stain depletion (Fig. 2J). The Monte Carlo simulation demonstrated that loss of GAG mainly led to an increase in MD. Collagen dispersion, on the other hand, mainly resulted in a decrease in FA.

DISCUSSION: These results are consistent with previous FA and MD findings in degenerating cartilage and support the potential of DTI as an imaging-based biomarker for OA (5). When the highly ordered collagenous structure of healthy cartilage is disrupted, water molecules are expected to exhibit increased diffusivity and more isotropic diffusion patterns. Although few studies have examined AD and RD in this context, our findings may be attributed to alterations in water diffusion caused by directionally specific structural damage. AD reflects diffusivity along the primary diffusion axis (i.e., aligned with fiber orientation), whereas RD reflects diffusivity perpendicular to this direction. We observed that RD was more affected than AD following DMM, possibly because disruption of cartilage organization reduces perpendicular restriction more significantly, while diffusion along the fiber orientation is less impacted. The 3D tractography results successfully demonstrated the structural disorganization in DMM articular cartilage and was consistent with microanatomy on 2D histology PSR images. The TB images further validated the DTI metric statistics and further highlighted how degeneration was most severe in the tibial articular cartilage. Only the simulations, however, could uniquely establish a mechanistic basis linking GAG loss and collagen dispersion to FA and MD changes. Limitations of our study are the relative inactivity of post-surgical rats and that we only assessed one time point.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding the microstructural changes in OA progression is essential given the lack of therapies. By linking DTI metrics to specific collagen and GAG alterations, this study provides the first step needed for this, a non-invasive imaging approach for detecting pathological changes of this disease.

REFERENCES: 1. Deng et al. Magnetic Resonance Imaging 2007, 25, 168-171. 2. Ukai et al. BMC Musculoskeletal Disorders 2015, 16, 35. 3. Glasson et al. Osteoarthritis and Cartilage 2007, 15, 1061-1069. 4. Wang et al. Magnetic Resonance in Medicine 2019, 81, 3775-3786. 5. Raya et al. Journal of Magnetic Resonance Imaging 2024, 59, 376-389.

IMAGES AND TABLES:

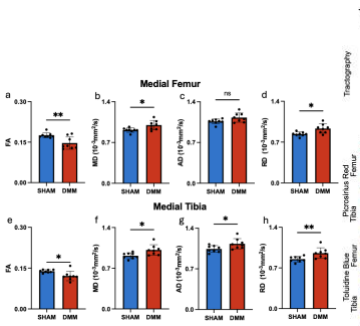


Figure 1

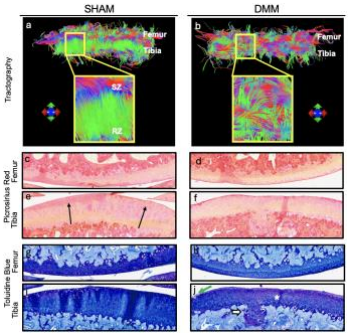


Figure 2

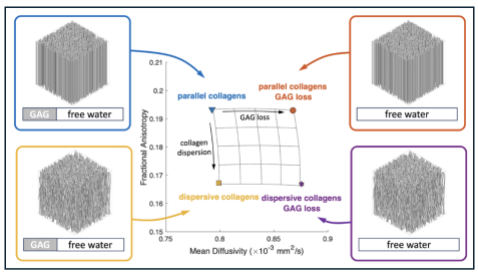


Figure 3