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# Mechanism of Disease in early Osteoarthritis: Application of modern MR imaging techniques – A technical report

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## Abstract

The application of biomolecular magnetic resonance imaging becomes increasingly important in the context of early cartilage changes in degenerative and inflammatory joint disease before gross morphological changes become apparent. In this limited technical report, we investigate the correlation of MRI T1, T2 and T1<rho> relaxation times with quantitative biochemical measurements of proteoglycan and collagen contents of cartilage in close synopsis with histologic morphology. A recently developed MR imaging sequence, T1<rho>, was able to detect early intracartilaginous degeneration quantitatively and also qualitatively by color mapping demonstrating a higher sensitivity than standard T2-w sequences. The results correlated highly with reduced proteoglycan content and disrupted collagen architecture as measured by biochemistry and histology. The findings lend support to a clinical implementation that allows rapid visual capturing of pathology on a local, millimeter level. Further information about articular cartilage quality otherwise not detectable in-vivo, via normal inspection, is needed for orthopedic treatment decisions in the present and future.

### Keywords

cartilage; osteoarthritis; degeneration; MRI; histology

## Introduction

Osteoarthritis (OA) of the knee is a multifactorial disease [1–3]. In this investigation we 1) focus on focal degenerative processes that primarily involve the cartilage; and 2) discuss the potential role of a novel MRI technique (T1ρ) in the investigation of early biochemical changes in cartilage when gross morphological changes are not yet apparent. Once the cartilage surface starts to degenerate through non-physiologic repetitive loading, followed by cellular, biochemical and finally macroscopic morphological changes, a vicious cycle of secondary intermittent inflammatory processes and an altered motion-loading situation progresses to disrupt a fine balanced joint mechanism [4].



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**Conflict of interest**
The authors state no conflict of interest.

Hyaline cartilage consists of four main components: Chondrocytes, type II collagen, negatively charged proteoglycans (PG) made of glycosaminoglycans (GAG) and hyaluronic acid and a significant amount of water - altogether maintaining an optimal degree of osmotic pressure, resilience and stiffness [1]. Joint cartilage consists of four continuous layers where each of its four components are present in varying concentrations and orientations resulting in different biomechanical functions. Interestingly, with regards to MR signal detection, the cartilage water concentration has an inverse relation to the present hydrophilic GAG [5]. A number of MRI parameters have been investigated to follow any of the processes and conditions described above. Among them, T2 and T1<rho> have both demonstrated sensitivity to molecular structure and concentration in cartilage to varying degrees [6–9]. Menezes et al. investigated T1 and T2 in articular cartilage systems ex-vivo to show general sensitivity to pathology but also its limitations at the time [10]. The degree of specificity for cartilage pathology is yet undecided. The understanding of interactions between the cartilage components and the underlying patho-mechanisms that lead to primary changes in cartilage composition are of great interest in the development of non-invasive diagnostic procedures to detect early –, and possibly reversible changes – and future treatment options that intervene with cartilage metabolism [11–18].

## Case report

We extensively investigated degenerative cartilage disease in one fully-preserved cadaveric knee from a 47-year-old woman with no known history of trauma to the knee or disease of bones/joints. The cadaver knee was obtained through NDRI (shipped frozen). The protocol in this report for all methods is identical to a study of a larger osteoarthritis cohort recently published by Li et al. [19].

All exams were performed in accordance with the rules and regulations from the Human Research Committee of our institute and comply with the principles outlined in the Declaration of Helsinki.

### MRI

Prior to invasive examination or dissection of the specimen, the entire unopened specimen was scanned at room temperature using a quadrature knee coil in a 3.0 T GE MR scanner (GE Healthcare, Milwaukee, WI, USA). Sagittal 3D SPGR and T1ρ-weighted images (3mm slice thickness) where acquired using a protocol previously described for an in vivo OA study [20]. Three-dimensional T1ρ mapping was accomplished based on SPGR sequence [21] (in-plane resolution = 0.55 × 0.55 mm, slice thickness = 3 mm, time of spin-lock (TSL) = 0/10/40/80 ms, frequency of spin-lock = 500 Hz). The T1ρ map was reconstructed by fitting the T1ρ weighted images pixel-by-pixel to the equation $S(TSL) = S0 * \exp(-TSL/T1\rho)$. After reconstruction, T1ρ color maps representing the local relaxation times were generated. After MRI image acquisition was complete, the knee capsule was opened using a median parapatellar approach, and the specimen was carefully examined for cartilage surface changes. A 5×5 mm focal soft area with an intact surface was palpable in the medial posterior femoral condyle (MPFC). This soft ‘lesion’ is referred to as the region of interest (‘ROI’; Fig. 1). This particular region was separately prepared and re-scanned using a smaller wrist coil with similar MR sequences but at higher spatial resolution (0.31 × 0.31 mm, slice thickness 1 mm).

Significantly higher T1ρ relaxation times were found in the ROI. Values in the ROI were ~4x higher (−120ms ± 11.8 ms) (Fig. 2b) than in the surrounding unaffected cartilage (30–50ms ± 8 ms ).

### Biochemistry

Multiple cartilage punch biopsies (weighing approximately 50 mg each) were acquired from the ROI and from the healthy contralateral side for quantitative biochemistry (PG + COL) measurements using a DMMB based assay. PG content in the ROI was reduced by 47% to 1.9 wt% compared to the healthy-appearing condyle. No significant difference in the collagen content was found (COL content →) between the ROI compared to the control side (12wt% vs. 11 wt%)

### Histology

4μm-thick histologic sections were taken through the ROI directly adjacent to the punch biopsy sites for biochemical analysis. The tissue was fixed and paraffin embedded. The tissues were stained using Hematoxylin and Eosin (H&E), Safranin-O for assessment of proteoglycan content and Sirius-red for visualization of collagen content and orientation under polarized light. Chondrocytes in the ROI appeared small, pycnotic and reduced in number. Safranin-O staining revealed a very localized reduction in proteoglycan content throughout all cartilage layers. Above the center of the lesion, the superficial cartilage layer was intact and smooth but the superior and inferior ROI borders showed minor superficial fibrillation. In addition, a horizontal cleft in the intermediate layer could be observed. Collagen fiber orientation under polarized light in normal cartilage showed an inverse correlation to proteoglycan and collagen content with regards to the depth of the cartilage layer (deep zone: high PG and low collagen) [22]. Collagen content in the ROI appeared increased without polarized light and disorganized using polarized light microscopy.

## Discussion

The hyaline joint cartilage investigated in this report did not show any signs of disease by simple visual observation, however direct physical examination revealed a slight decrease in focal resilience suspicious for underlying cartilage pathology. This was a rare occasion to study early degenerative cartilage disease in a human. In contrast to ex-vivo conditions, pathophysiological processes in cartilage degeneration is a continuous cycle with overlapping stages not limited to one component at one time point which may explain many of the inconsistencies in studies investigating cartilage ‘specific’ parameters [10;23]. T1<rho> and T2 mapping are influenced by the main cartilage specific components: water hydration, COL architecture, PG content and to a lesser extend – cells - due to their small number [24]. Which component is dominantly reflected in each MR parameter is controversial. Therefore only a multi-methodological investigative approach, as presented here, may differentiate the influence of each component.

Clinically applied, standard T2-w image sequences did not detect significant changes in cartilage signal intensity, which underlines results from studies showing T2 insensitivity to proteoglycan depletion but a stronger influence of collagen architecture [23;25]. T1<rho>, previously applied in a larger cohort in human cartilage ex-vivo specimen [19], has been shown to predict PG loss across all samples in reference to histology. Also, previous ex-vivo experiments with enzymatic degradation of cartilage have shown changes in T1<rho> relaxation times [26]. Here, in a morphologically intact and unaltered human cartilage, T1<rho> MRI was able to detect biochemical changes in a small region of cartilage ($5^2$mm), under in-vivo equivalent conditions. The size of the lesion was large enough to be detected by thorough palpation but without visible surface irregularities as it would have been performed via arthroscopy view.

The histological and biochemical investigation revealed five main pathologies in the ROI: 1.) chondrocyte necrosis 2.) reduced proteoglycan content and chondrocyte death (Fig. 5b)

3.) disorganized collagen fiber architecture 4.) initial microscopical superficial fibrillation, and a 5.) horizontal cleft (Fig. 4).

As a pathophysiological principle this means, once the elastic forces of the collagen network are disrupted, GAG, though fewer in quantity, will be able to achieve a higher degree of hydration ($H_2O \uparrow$) [27]. This is sometimes referred to as 'swelling' although a significant difference in cartilage height is rarely observed (Fig. 3 ) [28;29]. The initial local disruption of the superficial layer gives rise to deeper fissures (and less frequent horizontal clefts; Fig. 4) due to high shearing strain.

While there were significant increases in T1<rho> in the pathologic ROI compared to the healthy contralateral regions, T2-weighted MRI did not show these changes which may be mainly due to the fact that histopathology did not reveal significant changes in collagen content. In fact, degraded cartilage in OA may show no change in collagen concentration or even increased levels [28;30].

The histopathology and biochemistry confirmed that the changes observed in T1<rho> are correlated with the loss of PG, increased water content and disorganized molecular structure [9]. Previously, T1ρ has been shown to detect depletion of PG in artificially degraded bovine cartilage [6;31;32] but in-vivo data on local intrasubstance changes due to degradation with reference histology and biochemistry are still missing. An in-vivo study by Li et al. [19] was a step forward towards a clinical implementation but time consuming segmentation and quantification is not always feasible in a clinical setting. There is a growing request for image protocols for clinical use, capable of detecting focal changes in cartilage composition before gross structural changes appear. Arthroscopists and musculoskeletal radiologists are well aware of the discrepancies of their tools and modalities. T1<rho> and other cartilage specific sequences [33;34] may build a new bridge between these two specialties. Musculoskeletal pathologists and histology as the standard of reference, although time consuming, should play a pivotal role in validation studies of newly developed MR-sequences [19;24;25;35–45], in particular with regard to interaction between tissue architecture and MR image acquisition [25;43;46–48].

### Conclusion

T1ρ, in contrast to clinically applied T2-w sequence was capable of detecting subtle changes in biochemical composition ($H_2O \uparrow$ + PG $\downarrow$+ COL content $\rightarrow$ ) in early OA of the human knee. This study introduces new insights into pathophysiological changes in human cartilage microstructure depicted by sensitive MR signal and their potential for clinical application.

Further improvements in spatial resolution, protocols and image acquisition will significantly change the way we investigate cartilage pathology in the future.

## Acknowledgments

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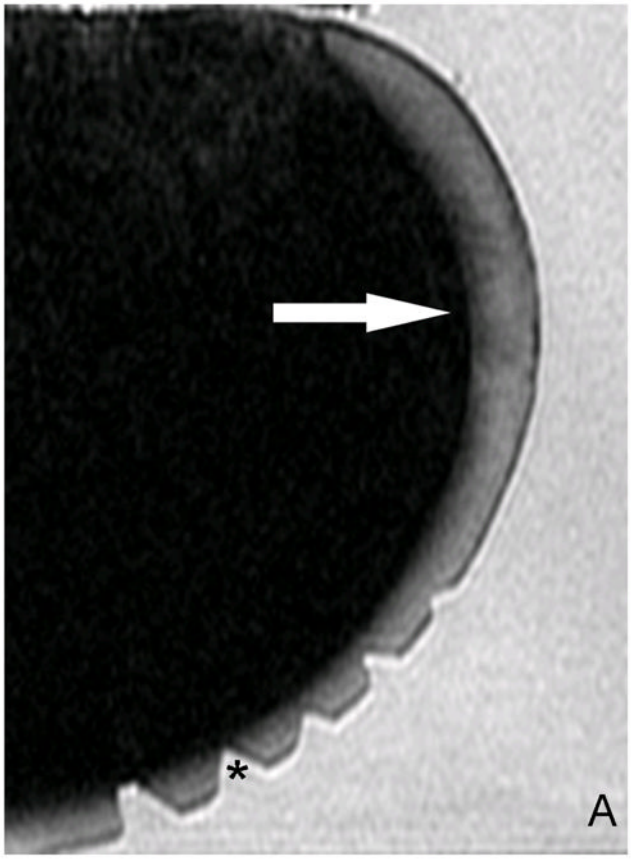
**Fig. 1.**
Human knee cadaver with visually intact femoral condyles. Forceps tip pointing to a 5×5mm soft region (ROI) that was examined in sagittal sections histologically, biochemically and with MR.



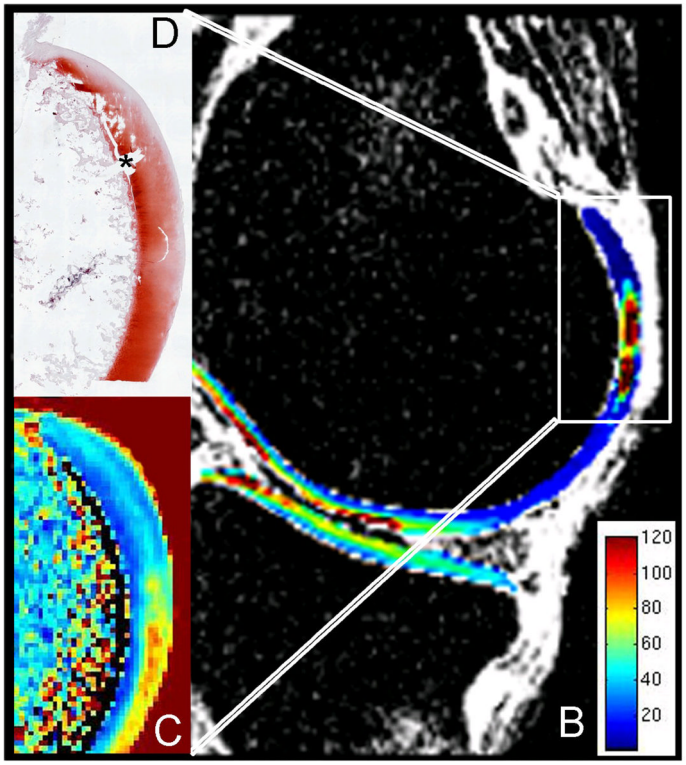



**Fig. 2.**

**A**) Original T2 -w image. Without color mapping the ROI (white arrow) in the human posterior femoral condyle (PFC) presents only subtle structural signal inhomogeneity. *(Cutting artifacts). **B)** SPGR image with fitted T1ρ color map. ROI in the PFC (sagittal image orientation through the knee) displays T1ρ values up to 120ms (red=abnormal, blue=normal cartilage) as an indicator for extended relaxation times due to an increase in cartilage bound water and a reduction in PG related to catabolic cartilage processes. **C)** Magnification of the ROI after repeated scanning of the separated PFC. **D)** Safranin-O stained histo section of a representative sagittal slice through the ROI shows focal PG (red) reduction and a bow-like intrasubstance fissure. The cartilage surface shows only mild irregularities. *(Cutting artifacts)

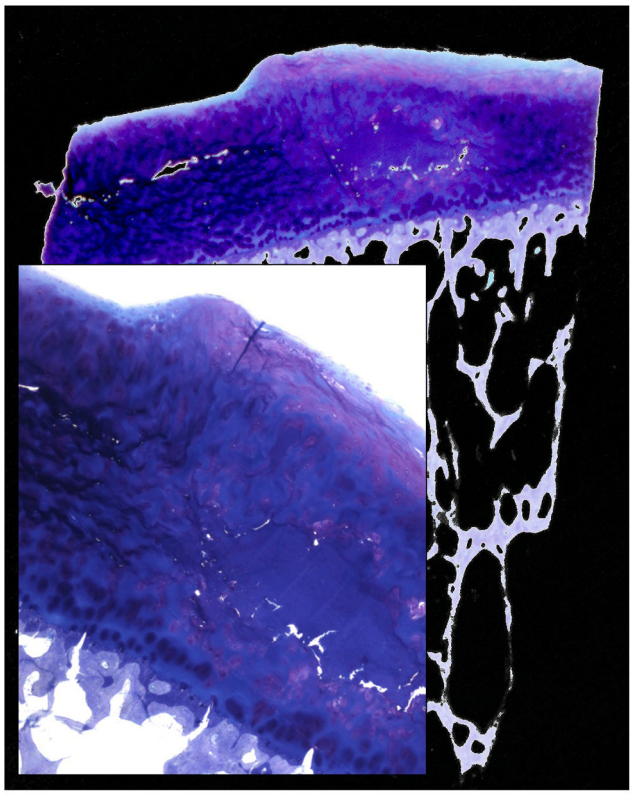


**Fig. 3.**
Giemsa staining (overview and magnification from a different case) presenting rarely observed cartilage swelling with surface height differences in the affected area consisting of an acellular matrix surplus (light blue). Additionally fibrous cartilage can be observed on the top surface.

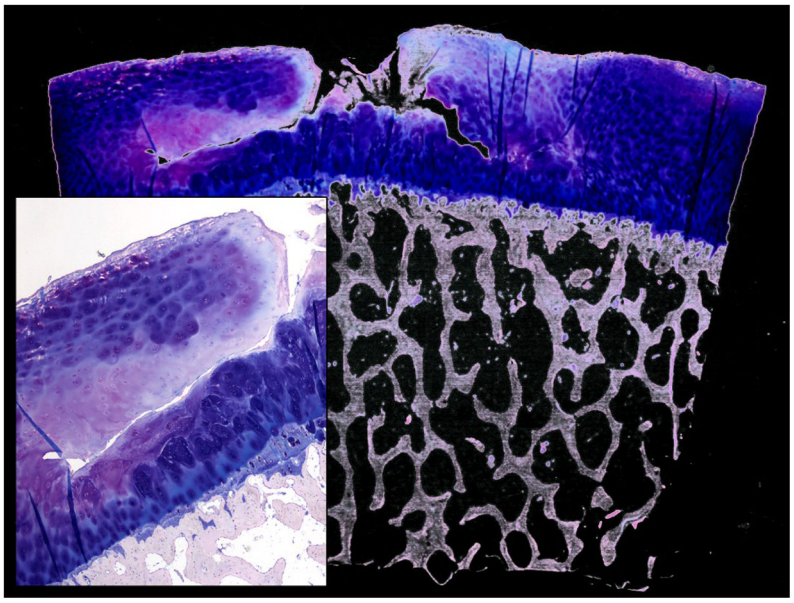


**Fig. 4.**
Giemsa staining (overview and magnification from a different case) presenting a large horizontal cartilage cleft with vertical surface contact as a probable consequence of pre-damaged cartilage and tissue failure due to loading.

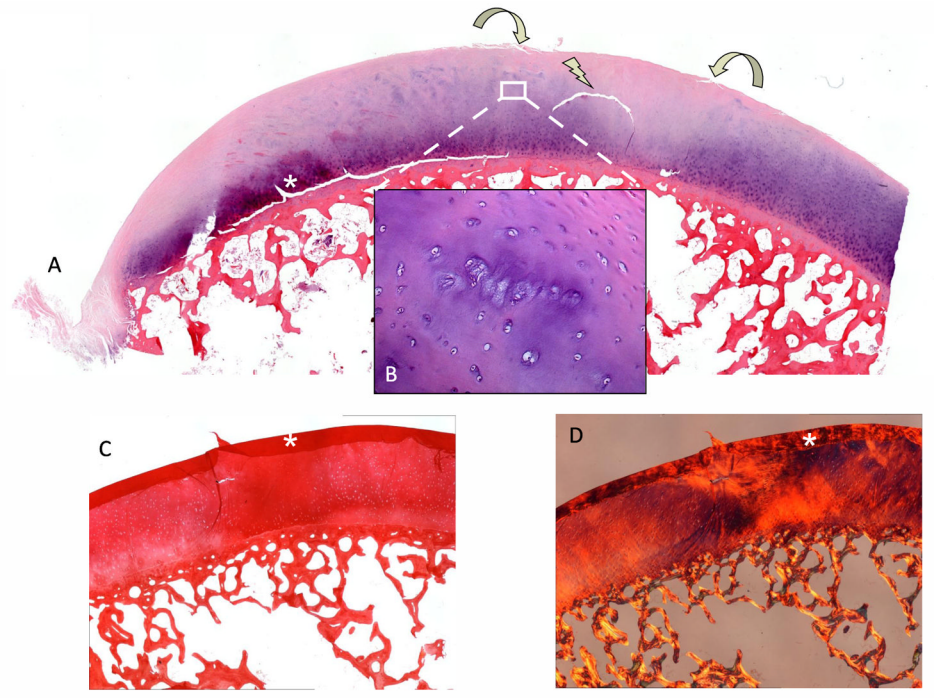



**Fig. 5.**

**A)** Section through the ROI with H&E stain. Focal reduction in staining throughout all cartilage layers with a horizontal cleft is visible (arrow). **B)** CCs within the transitional zone in affected area. A reduced diffusion gradient between synovial fluid and cartilage matrix leads to a decrease in chondrocyte metabolism resulting in chondrocyte death with dissolution of the surrounding matrix, a reduction in pericellular PG and dying CCs (Weichselbaum's lacunae) [1]. With the loss of PG, COL fibers, usually masked by interstitial matrix become visible (image center). **C)** (+Fig. 2d) A reduction in PG Safranin-O and changes COL quality **(D, polarized light)** and orientation Sirius-red (COL) led to a blister-like lesion through hypermobility in the ROI. Biomechanical changes in stiffness and resilience in the transition to normal cartilage led to surface fibrillation (curved arrows).