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MRI rotating frame relaxation measurements for articular cartilage assessment

Jutta Ellermann^{1,*}, Wen Ling¹, Mikko J. Nissi^{1,2}, Elizabeth Arendt², Cathy S. Carlson³, Michael Garwood¹, Shalom Michaeli¹, and Silvia Mangia¹

¹Center for Magnetic Resonance Research and Department of Radiology, University of Minnesota, Minneapolis, USA

²Department of Orthopaedic Surgery, University of Minnesota, Minneapolis, USA

³Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, USA

Abstract

In the present work we introduced two MRI rotating frame relaxation methods, namely adiabatic $T_{1\rho}$ and Relaxation Along a Fictitious Field (RAFF), along with an inversion-prepared Magnetization Transfer (MT) protocol for assessment of articular cartilage. Given the inherent sensitivity of rotating frame relaxation methods to slow molecular motions that are relevant in cartilage, we hypothesized that adiabatic $T_{1\rho}$ and RAFF would have higher sensitivity to articular cartilage degradation as compared to laboratory frame T_2 and MT. To test this hypothesis, a proteoglycan depletion model was used. Relaxation time measurements were performed at 0 and 48 hours in ten bovine patellar specimens, 5 of which were treated with trypsin and 5 untreated controls were stored under identical conditions in isotonic saline for 48 hours. Relaxation times measured at 48 hours were longer than those measured at 0 hours in both groups. The changes in T_2 and MT relaxation times after 48 hours were approximately 3 times larger in the trypsin treated specimens as compared to the untreated group, whereas increases of adiabatic $T_{1\rho}$ and RAFF were 4 to 5 fold larger. Overall, these findings demonstrate a higher sensitivity of adiabatic $T_{1\rho}$ and RAFF to the trypsin-induced changes in bovine patellar cartilage as compared to the commonly used T_2 and MT. Since adiabatic $T_{1\rho}$ and RAFF are advantageous for human applications as compared to standard continuous-wave $T_{1\rho}$ methods, adiabatic $T_{1\rho}$ and RAFF are promising tools for assessing cartilage degradation in clinical settings.

Keywords

adiabatic pulses; cartilage; magnetization transfer; rotating frame relaxation; RAFF; trypsin

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*Corresponding author: Jutta Ellermann, MD. Center for Magnetic Resonance Research, University of Minnesota, 2021 Sixth Street SE, Minneapolis, MN 55455, USA, Tel. +1612-626-2001, FAX: +1612-626-2004, eller001@umn.edu.

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INTRODUCTION

Despite the potential of MRI to probe compositional change of cartilage in early stages of osteoarthritis (OA), the current clinical imaging protocols still have limited capability to provide this information. Several MR techniques have been used to assess biochemical composition of cartilage, including sodium MRI [1,2], delayed gadolinium enhanced MRI contrast [3], magnetization transfer (MT) [4], detection of glycosaminoglycan via chemical exchange dependent saturation transfer [5–7], T_2 relaxometry [8,9], and continuous-wave (cw) rotating frame $T_{1\rho}$ relaxometry (cw- $T_{1\rho}$) [10]. In the latter method, the rotating frame $T_{1\rho}$ (and $T_{2\rho}$) defines relaxation during continuous wave RF irradiation when the magnetization vector is aligned along (and perpendicular to) the direction of the effective magnetic field, here expressed as ω_{eff} , which depends on both the applied RF amplitude (ω_1) and the frequency offset (ω).

Free precession T_2 relaxometry is currently used in clinical practice to assess collagen matrix integrity [11]. The results from the OA Initiative, however, suggest that it has relatively low specificity [12]. On the other hand, cw- $T_{1\rho}$ relaxometry has been shown to have utility for quantitative monitoring of macromolecular status in early OA [13]. cw- $T_{1\rho}$ has been applied on bovine cartilage [14–16] and shown to reveal cartilage changes between healthy and OA patients [17,18], meniscus pathology in OA patients [19], and cartilage abnormalities in patients with anterior cruciate ligament injury [20]. Laboratory frame relaxation parameters such as T_1 and T_2 are sensitive to magnetic fluctuations that are generated as a result of molecular motions occurring at frequencies near the Larmor frequency (ω_0) in the MHz range, whereas the rotating frame relaxation $T_{1\rho}$ (and $T_{2\rho}$) is additionally sensitive to much slower fluctuations occurring at frequencies near the effective frequency (ω_{eff}), which generally falls in the kHz range. The superior sensitivity of cw- $T_{1\rho}$ to detect degeneration of articular cartilage results from this inherent sensitivity of rotating frame relaxation methods to slow molecular motions, relevant in the cartilage system.

We have introduced adiabatic rotating frame relaxation methods, which allow measurement of $T_{1\rho}$ and transverse relaxation, $T_{2\rho}$, during adiabatic rotation. These methods utilize adiabatic full passage (AFP) pulses [21,22]. The parameters which characterize the AFP pulses used for the measurements are critical in determining the values of adiabatic $T_{1\rho}$ and $T_{2\rho}$ [23]. Relaxation dispersions of adiabatic $T_{1\rho}$ and $T_{2\rho}$ are indeed observed when changing the pulse modulation functions, when using different peak power of the AFP pulses, i.e. ω_1^{max} , or when implementing different amplitudes of the frequency sweep of the pulses [24]. The generation of relaxation dispersion via the parameters of the pulses is realized through the dependency of the instantaneous contribution to the relaxation rate constants on the time-dependent effective frequency, $\omega_{\text{eff}}(t)$, and on the change of its orientation in the first rotating frame with the angular velocity, $d\alpha(t)/dt$ [23]. The creation of a broad range of effective frequencies ($\omega_{\text{eff}}(t)$) during the adiabatic pulse constitutes a marked difference of adiabatic rotating frame relaxation methods compared to cw rotating frame relaxation methods, where ω_{eff} is instead constant. Therefore, the sensitivity of relaxation during the adiabatic pulse is extended to many (rather than just one) frequencies of molecular fluctuations defined by $\omega_{\text{eff}}(t)$. Based on this feature, adiabatic relaxation methods inherently are sensitive to a broader range of slow molecular motions as compared

to *cw* methods. This feature is critical for tissue characterization, since a variety of molecular motions is present, especially *in vivo*. Importantly, the instantaneous intrinsic relaxation time constants can vary greatly during the course of an AFP pulse. Therefore, within multi-site exchange spin systems the exchange condition also can vary significantly during the pulse (for more details refer to [24]), eventually spanning from fast to slow exchange regimes. These characteristics of adiabatic rotating frame measurements lead to the opportunity to assess intermediate-slow exchange regimes in living tissue. Recent studies have demonstrated high sensitivity of adiabatic $T_{1\rho}$ to differentiate pathological from the normal state in monitoring of rat glioma gene therapy [25], detection of neural degeneration and iron loads in the brain of Parkinson's patients [26], in animal models of neural degeneration [27], and in stroke [28]. Remarkably, adiabatic rotating frame relaxation methods are robust and less sensitive to B_0 and B_1 inhomogeneities as compared to conventional *cw*- $T_{1\rho}$ and $T_{2\rho}$ relaxation mapping methods.

For human applications, the generation of relaxation dispersions, and thus of MRI contrast, based on the peak power or bandwidth of the AFP is problematic due to the limitations imposed on the specific absorption rate (SAR). To minimize SAR, image acquisition using different pulse modulation functions has been suggested as a way to create contrast with adiabatic $T_{1\rho}$ and $T_{2\rho}$ methods [23]

To further alleviate SAR concerns for human applications, and to extend the sensitivity of relaxation to even slower molecular motions, our group has recently developed a novel rotating frame relaxation technique called relaxation along a fictitious field (RAFF) [29,30]. This method utilizes frequency-swept pulses operating non-adiabatically, and therefore generally requires less peak power than adiabatic pulses of similar pulse-length. During RAFF, the relaxation occurs in the presence of fictitious magnetic field components which function as “locking” fields, with a resultant effective field that is substantially larger than the applied RF field. Because the effective field is instantaneously flipped during the RAFF pulses, rotary echoes are formed in a doubly-rotating frame [31]. Thus, signal intensity (SI) decay during RAFF is governed by two mechanisms: 1) relaxation in the rotating frame of rank $n=2$; and 2) repetitive refocusing of spins in rotary echoes. Notably, relaxation dispersion can be generated also with RAFF, for example by changing the orientation of the effective field [29] or by generating locking fictitious fields in rotating frame of rank $n>2$.

Recently an inversion-prepared MT protocol was developed by Mangia et al [32], allowing a markedly larger dynamic range for sampling the signal evolution during off-resonance RF irradiation. With this MT protocol, two consecutive measurements of the SI evolution during progressive RF irradiation are performed. First, the magnetization M is not perturbed and initially oriented along $+Z$ of the laboratory frame; second, M is initially inverted to $-Z$. With both acquisitions, M evolves to the same steady state (SS) and the formation of the SS is taken into account during curve-fitting.

The overall aim of this study was to characterize the sensitivity of measurements performed with adiabatic $T_{1\rho}$, RAFF, and inversion-prepared MT for detecting changes in trypsin treated bovine articular cartilage matrix *in vitro*. Relaxation dispersion was generated for adiabatic $T_{1\rho}$ measurements using two different AFP pulses of the hyperbolic secant (HS)

family. Previously, similar studies have been performed using $cw-T_{1\rho}$ measurements on trypsin digested cartilage [14] and T_2 measurements on chondroitinase treated cartilage [33]. More recently, studies by Spencer et al. on bovine nasal cartilage have used almost identical degradation conditions (1 mg/mL trypsin for 18~20 hours) to study T_2 and MT methods [34–36]. We compared our new methods to T_2 –relaxation time measurements, which is the most readily available and utilized sequence for quantitative mapping on standard clinical and research MR systems [16,37–39]. Specifically, we hypothesized that our novel relaxation methods would provide greater sensitivity than laboratory frame T_2 and inversion-prepared MT to detect relaxation changes induced by treatment with trypsin. To test this hypothesis, we obtained relaxation measurements on enzymatically treated and intact bovine articular cartilage specimens. In addition, we evaluated the robustness of the various relaxation methods by estimating the between-sample variability of the measurements obtained before enzyme or sham treatment.

MATERIALS AND METHODS

Sample preparation

Ten fresh bovine patellar cartilage specimens were collected at a slaughterhouse from 14-month-old cattle and stored frozen at -40°C until they were evaluated. From each bovine patella, a plug was prepared in a standardized fashion ($n = 10$) (Figure 1). Cartilage-bone blocks from the central area ($20\text{--}30\text{ mm} \times 25\text{ mm}$) of the patella with full thickness articular cartilage and approximately 2–5 mm of subchondral bone were collected. The natural margins were removed because of potential differences in zonal structure of the tissue compared to more centrally-located areas [40], and the specimens were placed into a fixed-sized container. All specimens were placed into the magnet so that the surface normal of the samples was at an angle of $60 \pm 3^{\circ}$ with respect to B_0 . Half of the samples ($n = 5$) were treated with trypsin in buffer and half ($n = 5$) were treated in buffer only (controls), in order to compare the MRI results between trypsin-digested (TD) and non-digested (ND) articular cartilage. Following the initial scanning (0 hour) of all patellae, the specimens in the TD group were immersed in 0.8 mg/mL trypsin (Sigma-Aldrich, St. Louis, MO, USA) in phosphate-buffered saline (Sigma-Aldrich) for 24 hours at 20°C [34,35,41] while those in the ND group were immersed in phosphate-buffered saline containing no trypsin under the same conditions. The specimens were then stored in protease inhibitor (Sigma-Aldrich) solution at 20°C for 24 hours before undergoing post-digestion scans (48 hours after the first scan). After the MRI measurements, all specimens were placed in 10% neutral buffered formalin at 20°C for subsequent histological processing and evaluation.

MRI measurements

All experiments were performed on a 31 cm bore 9.4T scanner equipped with INOVA console (Varian, Palo Alto, CA, USA), using a volume single loop coil. T_2 maps were obtained using a multi-echo sequence with $\text{TR} = 5\text{ s}$, 16 echoes, and echo time spacing = 14 ms (first echo 14 ms). Imaging time was about 23 minutes. The rotating frame relaxation and MT measurements were obtained by applying a module of preparation pulses prior to fast spin echo (FSE) imaging readout with matrix size 512×256 , slice thickness 2 mm, FOV approximately $25\text{ mm} \times 15\text{ mm}$, $\text{TR} = 5\text{ s}$, receiver bandwidth = 100 kHz. The echo

spacing of the readout was 14 ms, with centric k-space ordering and 4 lines collected per preparation. In the case of adiabatic $T_{1\rho}$, the preparation module consisted of pulse trains of 0, 8, 16, 32, and 64 hyperbolic secant (HS) pulses designed with different “stretching” factors (HSn: n= 1 and 4) (Figure 2a) [21]. Pulse duration was set to 4 ms for both HS1 and HS4 pulses, while maximum RF amplitude $\omega_1^{\max}/(2\pi)$ was set in order to guarantee adiabaticity, i.e., 2000 Hz and 1800 Hz for HS1 and HS4, respectively. Imaging time was about 26 minutes for each measurement of $T_{1\rho\text{-HS1}}$ and $T_{1\rho\text{-HS4}}$. For RAFF [30], *sine/cosine* pulse trains of 0, 8, 16, 24, and 32 pulses were implemented with a pulse duration = 4.526 ms and $\omega_1^{\max}/(2\pi) = 625$ Hz (Figure 2b). For MT, hard pulses with amplitude $\omega_1^{\max}/(2\pi) = 200$ Hz and duration = 0, 0.3, 0.6, 0.9 and 1.2 s were irradiated at -10000 Hz offset. The longitudinal relaxation time during the off-resonance irradiation (i.e., T_{Isat}) was thus measured, and from hereon we will refer to T_{Isat} as the MT measurement (Figure 2c). With RAFF and MT, two measurements with and without an inversion prior to the preparation train were performed [30,32] to enhance the dynamic range of the measurements. As a result, total imaging time for RAFF or MT was about 53 minutes. The total time of each experimental session was about three and a half hours.

Histology

Specimens from the TD and ND groups were examined histologically. Immediately after completion of the MRI scans, the specimens were fixed in 10% neutral buffered formalin, decalcified in 10% ethylenediaminetetraacetic acid (EDTA), dehydrated, processed, and embedded in paraffin blocks. Serial sections were cut at $5\mu\text{m}$ and were stained with hematoxylin & eosin (H&E) stain for routine morphological evaluation and Safranin-O staining for evaluation of matrix proteoglycan content. The Safranin-O stained sections were counterstained with fast green. All sections were examined by a board-certified veterinary pathologist with expertise in orthopedic pathology (C.S.C.).

Data Processing

All data were processed using routines written in MATLAB Matlab 7.3 (Mathworks, Inc., Natick, CA, USA). Relaxation time maps were generated using pixel-by-pixel least-square non-linear curve-fitting. Signal intensities (SIs) of T_2 , $T_{1\rho\text{-HS1}}$, $T_{1\rho\text{-HS4}}$ were fitted into a noise-corrected mono-exponential decay function. The curve fittings of RAFF and MT measurements were performed using the equation $M_{\pm Z} = M_{0,\pm Z} * \exp(-t/T_{SS}) - M_{SS} * (1 - \exp(-t/T_{SS}))$ [30,32] for decaying and rising signal intensities. Briefly, $M_{\pm Z}$ are the measured signal intensities without and with the inversion, M_0 is the initial signal intensity, M_{SS} is the steady state level and T_{SS} is the relaxation time constant, i.e. T_{Isat} and T_{RAFF} , for MT and RAFF measurements, respectively.

In order to compare cartilage with and without trypsin digestion, rectangular-shaped region of interests (ROIs) were delineated on co-registered high resolution anatomical images according to the following criteria: ROIs include intermediate zone, radial zone and calcified zone, whereas the superficial zone was excluded (15–20% of total thickness). The reason for this was that proteoglycan degradation occurred in both TD and ND groups (Figure 3b), likely due to natural leakage in the buffer. The width of the ROIs included the entire cartilage, excluding 2–3 mm margins bilaterally. The ROIs were manually co-registered

between maps from the scan at 0 and those at 48 hours by the same user (W.L.) and the same ROIs were used for all maps in the same MRI session.

Paired t-tests on relaxation measurements performed at 0 and 48 hours were performed to assess the significance of the observed changes separately for each parameter in both treated and non-treated groups. Sensitivity of the different methods to trypsin-induced degradation was evaluated as the ratio of the average percent change in relaxation times measured in the TD group and the ND group. Finally, to quantify the robustness of the different methods, the between-samples variability was calculated as average SD/mean of relaxation measurements obtained in both TD and ND group at 0 hours.

RESULTS AND DISCUSSION

Histological results in the TD group (Figure 3a) revealed a total loss of Safranin-O staining throughout the full thickness of articular cartilage matrix but there was strong retention of Safranin-O staining within the zone of calcified cartilage. In contrast, the histological results from the ND group (Figure 3b) revealed strong Safranin-O staining in the articular cartilage, with the exception of the most superficial 10–20%. This superficial loss of staining is mainly attributed to the loss of proteoglycans due to buffer alone.

The relaxation time constants T_2 , T_{1sat} , $T_{1\rho-HS1}$, $T_{1\rho-HS4}$, and T_{RAFF} at 0 and 48 hours in both ND and TD are summarized in Table 1. As expected, $T_{1\rho-HS1}$, $T_{1\rho-HS4}$, and T_{RAFF} relaxations are slower than T_2 relaxation, which is an advantage of the rotating frame relaxation methods [22,30], allowing for more robust estimation of the parameters. Notably, adiabatic $T_{1\rho}$ can be modified to provide different contrasts by varying the modulation functions of the AFP pulses. Specifically, in agreement with the observations in other tissues [23,24], the adiabatic $T_{1\rho}$ relaxation time is longer by ~40–50% when using HS1 instead of HS4 pulses (table 1). The fact that most relaxation times differ also in the ND group at 0 and 48 hours is an indication of the apparent sensitivity of the MRI techniques to detect alterations in tissue composition.

The relative increases of T_2 and T_{1sat} between 0 and 48 hours in TD specimens were 3.2 and 3.1 times higher than in ND specimens, respectively. On the other hand, the relative increases of $T_{1\rho-HS1}$ and $T_{1\rho-HS4}$ between 0 and 48 hours in TD were 48% and 53%, respectively, which was 4.8- and 4.1-fold higher than what was observed in the ND specimens (10% and 13%, respectively). The elevated adiabatic $T_{1\rho}$ values observed with trypsin are in line with the observations in previous studies utilizing $cw-T_{1\rho}$ [14]. The relative increase of T_{RAFF} in TD was 52%, which was 4.0-fold higher than in ND, similar to that observed for $T_{1\rho-HS4}$. Importantly, with RAFF this contrast was achieved using lower peak power ($\omega_1^{\max}/(2\pi) = 625$ Hz) than that used for adiabatic $T_{1\rho}$ measurements ($\omega_1^{\max}/(2\pi) = 1800$ or 2000 Hz), since the amplitude of the effective field generated by the RAFF pulse is greater than the peak RF field used in these *sine/cosine* modulated pulses [42]. Overall, these results indicated higher sensitivity of adiabatic $T_{1\rho}$ and RAFF to detect changes induced by trypsin as compared to laboratory frame MT and T_2 , agreeing with our hypothesis. Rotating frame relaxation methods, in fact, provide enhanced sensitivity to a variety of molecular motions in the intermediate and slow regime as compared to

conventional laboratory frame methods [22]. This feature originates from the additional terms of relaxation that depend on the effective field generated by the external RF field (in the kHz range), as opposed to the static magnetic field (in the MHz range) [22]. In particular, the adiabatic $T_{1\rho}$ approach further enhances this general property of rotating frame methods; with this method the effective field changes during the application of the RF pulses, covering a broad range of frequencies, as opposed to *cw* spin-lock approaches where only one value of the effective field is employed. Another stark advantage of adiabatic $T_{1\rho}$ methods is grounded on the observation that, as opposite to *cw*- $T_{1\rho}$, the relaxation time constants are practically invariant within an extensive range of chemical shifts, namely in a large subregion of the bandwidth of the hyperbolic-secant pulse where complete inversion is achieved (for further details, refer to [43]). Therefore, the adiabatic $T_{1\rho}$ approach is very robust even in presence of large B_0 inhomogeneities, a feature that, along with the known reduced sensitivity of adiabatic pulses to B_1 inhomogeneities, makes adiabatic $T_{1\rho}$ a very attractive method for studying tissue *in vivo*.

Figure 4 shows representative relaxation time maps in both TD and ND specimen at 0 hour and 48 hour in absolute units (ms). All maps demonstrated larger differences between the two time points in TD specimens as compared to ND specimens. The most significant relaxation time change occurred in superficial and intermediate zones, which cannot be explained by the loss of PG only; histological results (Figure 3a) indicated a universal loss of PGs in superficial, transitional, and radial layers. In ND, there was a minimal increase of T_2 in the superficial zone, while the increase of T_2 in TD was more pronounced, and occurred in all four zones. Additionally, in TD nearly linear decrease of T_{1sat} from articular surface to bone interface suggested that the degradation of collagen matrix from superficial layer to radial layer was gradually reduced. In ND, the most obvious natural degradation occurred in the superficial zone.

The increase of relaxation times observed in all MRI parameters of TD specimens can be explained by two main processes. First, the increased hydration level after treatment, ie. increase in the free water pool after degradation of proteoglycan macromolecules can contribute to the observed changes. Second, the relatively high concentration and long-time digestion by trypsin degrades also the structure of the collagen matrix, which could lead to an increase of T_2 [44], as well as of the other relaxation times. This is in agreement with previous studies on T_2 and MT on treated bovine nasal cartilage [34,35]. Although patellar cartilage has very different collagen architecture than that of bovine nasal cartilage, the change of 11.5 ms in mean T_2 of the previous study is comparable to the change of 12.2 ms after treatment as observed in the present study. It is worth noting that the samples were oriented with surfaces normal to the magic angle with respect to the main magnetic field, causing the depth-dependent variation of T_2 relaxation time, as well as the layered structure in cartilage to mostly disappear [40,45].

The inversion-prepared MT provided the smallest between-sample-variability (4%) compared to T_2 (17%), $T_{1\rho-HSI}$ (10%), $T_{1\rho-HS4}$ (11%) and RAFF (10%), demonstrating greater robustness of this MT protocol relative to the other methods. The observation of a smaller variability of MT measurements might originate from a lower sensitivity of MT to water hydration level as compared to the other methods [46]. As a result, the inversion-

prepared MT protocol may provide more specific diagnostic information on collagen matrix changes compared to T_2 , whose relaxation is known to be mainly determined by both the collagen matrix and the water hydration level.

The limitations of the present study are that our measurements were conducted on a small cohort ($n=5$), and no quantitative biochemical measurements were available for both PG and collagen. The goal of current study, however, was to investigate the feasibility of our methods for cartilage research. Hence, our experiments were conducted under previously established conditions, and the loss of PG was confirmed histologically. In addition, $cw-T_{1\rho}$ measurements were not performed in this study; however our measurements of T_2 and adiabatic $T_{1\rho}$ were consistent with previously reported T_2 and $cw-T_{1\rho}$, which were conducted on larger cohorts and with quantitative biochemical measurements for PG [14,34,35]. Moreover, comprehensive studies on larger cohorts comparing a large number of cartilage mapping techniques, including those presented here, are underway [47].

One potential disadvantage of adiabatic $T_{1\rho}$ methods is that peak power requirements can be quite high for achieving adiabaticity during the AFP pulses. In the present study, 4-ms HS1 and HS4 pulses were used with peak power of 2000 and 1800 Hz, respectively, which in fact would not be achievable with clinical hardware and would not be compatible with the SAR limits in humans. The pulse duration and the bandwidth of the adiabatic pulses can nonetheless be adjusted to substantially mitigate the peak power requirements and to overcome the SAR limitations [23]. As a matter of fact, adiabatic $T_{1\rho}$ was successfully used in a number of human studies at high magnetic field 4T [23,24,26,48,49]. Notably, the RF power deposition of RAFF is always inherently reduced as compared to $cw-T_{1\rho}$ [30]. Overall, reduced power requirements and high sensitivity to PGs makes these methods very promising candidates in clinical settings to study cartilage integrity. Further research which will include the comparisons with T_2 and $cw-T_{1\rho}$ methods is now warranted to specifically assess the sensitivity of these novel rotating frame approaches to cartilage degeneration in humans.

Finally, the measurements were performed at a sample orientation minimizing the confounding factors introduced by the magic angle effect. For clinical implementation, the sensitivity to degeneration of different techniques is to be determined at varying angles. Additionally, the scanning times of the present study would not be feasible for clinical situations; possible strategies for reducing scan time include giving up full relaxation conditions by using shorter TR [50], using parallel acquisition and other ways of reducing the readout duration or using alternative, faster readout schemes.

In conclusion, we report our initial experience on the application of adiabatic $T_{1\rho}$ RAFF and inversion-prepared MT methods in trypsin-treated bovine patellar cartilage and compare them to the widely available and clinically used T_2 -mapping method. Our initial data support the hypothesis that the rotating frame methods, adiabatic $T_{1\rho-HS1}$, $T_{1\rho-HS4}$, and RAFF have higher sensitivity to trypsin induced articular cartilage degradation as compared to laboratory frame T_2 and MT, similar to what has been shown by others for $cw-T_{1\rho}$. When comparing the laboratory frame techniques, the inversion-prepared MT protocol was also shown to be particularly robust, especially relative to T_2 relaxometry. Furthermore, our

results show that the presented rotating frame relaxation and inversion-prepared MT provide several other advantages as compared to T_2 relaxometry. RAFF provided similar contrast as $T_{1\rho}$ but with less peak power, which is beneficial at high fields and for clinical applications in general. In essence, these properties are encouraging; they suggest a need for further exploration of articular cartilage pathologies using these techniques in early stages of disease, and for follow-up studies on subtle changes subsequent to therapeutic intervention in controlled models or in specific patient populations.

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Abbreviations

AFP	adiabatic full passage
cw	continuous wave
RAFF	relaxation along a fictitious field
HS	hyperbolic secant
MT	magnetization transfer
ND	non-digested
OA	osteoarthritis
PG	proteoglycan
ROI	region of interest
SAR	specific absorption rate
SS	steady state
TD	trypsin-digested

References

1. Lesperance LM, Gray ML, Burstein D. Determination of Fixed Charge-Density in Cartilage Using Nuclear-Magnetic-Resonance. *Journal of Orthopaedic Research*. 1992; 10:1–13. [PubMed: 1309384]
2. Borthakur A, Mellon E, Niyogi S, Witschey W, Kneeland JB, Reddy R. Sodium and T1rho MRI for molecular and diagnostic imaging of articular cartilage. *NMR Biomed*. 2006; 19:781–821. [PubMed: 17075961]
3. Burstein D, Bashir A, Gray ML. MRI techniques in early stages of cartilage disease. *Invest Radiol*. 2000; 35:622–38. [PubMed: 11041156]
4. Laurent D, Wasvary J, Yin J, Rudin M, Pellas TC, O'Byrne E. Quantitative and qualitative assessment of articular cartilage in the goat knee with magnetization transfer imaging. *Magn Reson Imaging*. 2001; 19:1279–86. [PubMed: 11804755]

5. Ling W, Regatte RR, Navon G, Jerschow A. Assessment of glycosaminoglycan concentration in vivo by chemical exchange-dependent saturation transfer (gagCEST). *Proc Natl Acad Sci U S A*. 2008; 105:2266–70. [PubMed: 18268341]
6. Ling W, Eliav U, Navon G, Jerschow A. Chemical exchange saturation transfer by intermolecular double-quantum coherence. *J Magn Reson*. 2008; 194:29–32. [PubMed: 18571444]
7. Schmitt B, Zbyn S, Stelzeneder D, Jellus V, Paul D, Lauer L, Bachert P, Trattnig S. Cartilage quality assessment by using glycosaminoglycan chemical exchange saturation transfer and ²³Na MR imaging at 7 T. *Radiology*. 2011; 260:257–64. [PubMed: 21460030]
8. Nieminen MT, Rieppo J, Töyräs J, Hakumäki JM, Silvennoinen MJ, Hyttinen MM, Helminen HJ, Jurvelin JS. T2 relaxation reveals spatial collagen architecture in articular cartilage: a comparative quantitative MRI and polarized light microscopic study. *Magn Reson Med*. 2001; 46:487–93. [PubMed: 11550240]
9. Nissi MJ, Rieppo J, Töyräs J, Laasanen MS, Kiviranta I, Jurvelin JS, Nieminen MT. T(2) relaxation time mapping reveals age- and species-related diversity of collagen network architecture in articular cartilage. *Osteoarthritis Cartilage*. 2006; 14:1265–71. [PubMed: 16843689]
10. Wheaton AJ, Casey FL, Gougoutas AJ, Dodge GR, Borthakur A, Lonner JH, Schumacher HR, Reddy R. Correlation of T1rho with fixed charge density in cartilage. *J Magn Reson Imaging*. 2004; 20:519–25. [PubMed: 15332262]
11. Mosher TJ, Smith HE, Collins C, Liu Y, Hancy J, Dardzinski BJ, Smith MB. Change in knee cartilage T2 at MR imaging after running: A feasibility study. *Radiology*. 2005; 234:245–9. [PubMed: 15550376]
12. Stehling C, Liebl H, Krug R, Lane NE, Nevitt MC, Lynch J, McCulloch CE, Link TM. Patellar cartilage: T2 values and morphologic abnormalities at 3.0-T MR imaging in relation to physical activity in asymptomatic subjects from the osteoarthritis initiative. *Radiology*. 2010; 254:509–20. [PubMed: 20019141]
13. Borthakur A, Gur T, Wheaton A, Corbo M, Trajanowski J, Lee V, Reddy R. I Vivo Measurements of Plaque Burden in a Mouse Model of Alzheimer's Disease. *J Magn Reson Imaging*. 2006; 24:1011–7. [PubMed: 17036339]
14. Akella SV, Regatte RR, Gougoutas AJ, Borthakur A, Shapiro EM, Kneeland JB, Leigh JS, Reddy R. Proteoglycan-induced changes in T1rho-relaxation of articular cartilage at 4T. *Magnetic Resonance in Medicine*. 2001; 46:419–23. [PubMed: 11550230]
15. Akella SV, Regatte RR, Wheaton AJ, Borthakur A, Reddy R. Reduction of residual dipolar interaction in cartilage by spin-lock technique. *Magn Reson Med*. 2004; 52:1103–9. [PubMed: 15508163]
16. Regatte R, Akella S, Lonner J, Kneeland J, Reddy R. T1r Relaxation Mapping in Human Osteoarthritis (OA) Cartilage: Comparison of T1r with T2. *J Magn Reson Imaging*. 2006; 23:547–53. [PubMed: 16523468]
17. Stahl R, Luke A, Li X, Carballido-Gamio J, Ma CB, Majumdar S, Link TM. T1rho, T2 and focal knee cartilage abnormalities in physically active and sedentary healthy subjects versus early OA patients—a 3.0-Tesla MRI study. *Eur Radiol*. 2009; 19:132–43. [PubMed: 18709373]
18. Carballido-Gamio J, Stahl R, Blumenkrantz G, Romero A, Majumdar S, Link TM. Spatial analysis of magnetic resonance T(1 rho) and T(2) relaxation times improves classification between subjects with and without osteoarthritis. *Medical Physics*. 2009; 36:4059–67. [PubMed: 19810478]
19. Bolbos RI, Link TM, Ma CB, Majumdar S, Li X. T1rho relaxation time of the meniscus and its relationship with T1rho of adjacent cartilage in knees with acute ACL injuries at 3 T. *Osteoarthritis and Cartilage*. 2009; 17:12–8. [PubMed: 18602280]
20. Li X, Kuo D, Theologis A, Carballido-Gamio J, Stehling C, Link TM, Ma CB, Majumdar S. Cartilage in anterior cruciate ligament-reconstructed knees: MR imaging T1{rho} and T2-initial experience with 1-year follow-up. *Radiology*. 2011; 258:505–14. [PubMed: 21177392]
21. Garwood M, DelaBarre L. The return of the frequency sweep: designing adiabatic pulses for contemporary NMR. *J Magn Reson*. 2001; 153:155–77. [PubMed: 11740891]
22. Michaeli S, Sorce DJ, Garwood M. T-2 rho and T-1 rho adiabatic relaxations and contrasts. *Current Analytical Chemistry*. 2008; 4:8–25.

23. Mangia S, Liimatainen T, Garwood M, Michaeli S. Rotating frame relaxation during adiabatic pulses vs. conventional spin lock: simulations and experimental results at 4 T. *Magn Reson Imaging*. 2009; 27:1074–87. [PubMed: 19559559]
24. Michaeli S, Sorce DJ, Springer CS Jr, Ugurbil K, Garwood M. T1rho MRI contrast in the human brain: modulation of the longitudinal rotating frame relaxation shutter-speed during an adiabatic RF pulse. *J Magn Reson*. 2006; 181:135–47. [PubMed: 16675277]
25. Sierra A, Michaeli S, Niskanen JP, Valonen PK, Grohn HI, Yla-Herttuala S, Garwood M, Grohn OH. Water spin dynamics during apoptotic cell death in glioma gene therapy probed by T1rho and T2rho. *Magn Reson Med*. 2008; 59:1311–9. [PubMed: 18506797]
26. Michaeli S, Oz G, Sorce DJ, Garwood M, Ugurbil K, Majestic S, Tuite P. Assessment of brain iron and neuronal integrity in patients with Parkinson's disease using novel MRI contrasts. *Mov Disord*. 2007; 22:334–40. [PubMed: 17149719]
27. Michaeli S, Burns TC, Kudishevich E, Harel N, Hanson T, Sorce DJ, Garwood M, Low WC. Detection of neuronal loss using T(1rho) MRI assessment of (1)H(2)O spin dynamics in the aphakia mouse. *J Neurosci Methods*. 2009; 177:160–7. [PubMed: 19027791]
28. Jokivarsi KT, Niskanen JP, Michaeli S, Grohn HI, Garwood M, Kauppinen RA, Grohn OH. Quantitative assessment of water pools by T 1 rho and T 2 rho MRI in acute cerebral ischemia of the rat. *J Cereb Blood Flow Metab*. 2009; 29:206–16. [PubMed: 18827834]
29. Liimatainen T, Mangia S, Ling W, Ellermann J, Sorce DJ, Garwood M, Michaeli S. Relaxation dispersion in MRI induced by fictitious magnetic fields. *J Magn Reson*. 2011; 209:269–76. [PubMed: 21334231]
30. Liimatainen T, Sorce DJ, O'Connell R, Garwood M, Michaeli S. MRI contrast from relaxation along a fictitious field (RAFF). *Magn Reson Med*. 2010; 64:983–94. [PubMed: 20740665]
31. Solomon I. Rotary spin echoes. *Phys Rev Lett*. 1959; 2:301–5.
32. Mangia S, De Martino F, Liimatainen T, Garwood M, Michaeli S. Magnetization transfer using inversion recovery during off-resonance irradiation. *Magn Reson Imaging*. 2011; 29:1346–50. [PubMed: 21601405]
33. Nieminen MT, Toyra J, Rieppo J, Hakumaki JM, Silvennoinen J, Helminen HJ, Jurvelin JS. Quantitative MR microscopy of enzymatically degraded articular cartilage. *Magn Reson Med*. 2000; 43:676–81. [PubMed: 10800032]
34. Lin P-C, Reiter DA, Spencer RG. Classification of degraded cartilage through multiparametric MRI analysis. *J Magn Reson*. 2009; 201:61–71. [PubMed: 19762258]
35. Reiter DA, Lin PC, Fishbein KW, Spencer RG. Multicomponent T2 relaxation analysis in cartilage. *Magn Reson Med*. 2009; 61:803–9. [PubMed: 19189393]
36. Reiter DA, Roque RA, Lin PC, Irrechukwu O, Doty S, Longo DL, Pleshko N, Spencer RG. Mapping proteoglycan-bound water in cartilage: Improved specificity of matrix assessment using multiexponential transverse relaxation analysis. *Magn Reson Med*. 2011; 65:377–84. [PubMed: 21264931]
37. Mlynarik V, Trattnig S, Huber M, Zembsch A, Imhof H. The role of relaxation times in monitoring proteoglycan depletion in articular cartilage. *J Magn Reson Imaging*. 1999; 10:497–502. [PubMed: 10508315]
38. Jobke B, Bolbos R, Saadat E, Cheng J, Li X, Majumdar S. Mechanism of disease in early osteoarthritis: application of modern MR imaging techniques - a technical report. *Magn Reson Imaging*. 2013; 31:156–61. [PubMed: 22902064]
39. Keenan KE, Besier TF, Pauly JM, Han E, Rosenberg J, Smith RL, Delp SL, Beaupre GS, Gold GE. Prediction of glycosaminoglycan content in human cartilage by age, T1rho and T2 MRI. *Osteoarthritis Cartilage*. 2011; 19:171–9. [PubMed: 21112409]
40. Xia Y, Moody JB, Alhadlaq H, Hu J. Imaging the physical and morphological properties of a multi-zone young articular cartilage at microscopic resolution. *J Magn Reson Imaging*. 2003; 17:365–74. [PubMed: 12594728]
41. Bartholomew JS, Handley CJ, Lowther DA. The effects of trypsin treatment on proteoglycan biosynthesis by bovine articular cartilage. *Biochemical Journal*. 1985; 227:429–37. [PubMed: 4004772]

42. Liimatainen, T.; Nissi, MJ.; Nieminen, MT.; Michaeli, S.; Garwood, M.; Gröhn, OHJ. Book of abstracts: Eighteenth Annual Meeting of the International Society for Magnetic Resonance in Medicine. Vol. 18. Stockholm, Sweden: ISMRM; 2010. Relaxation along Fictitious Field (RAFF) Contrast in Bovine Articular Cartilage; p. 836
43. Mangia S, Liimatainen T, Garwood M, Tkac I, Henry PG, Deelchand D, Michaeli S. Frequency offset dependence of adiabatic rotating frame relaxation rate constants: relevance to MRS investigations of metabolite dynamics in vivo. *NMR Biomed.* 2011; 24:807–14. [PubMed: 21264976]
44. Moody HR, Brown CP, Bowden JC, Crawford RW, McElwain DLS, Oloyede AO. In vitro degradation of articular cartilage: does trypsin treatment produce consistent results? *Journal of Anatomy.* 2006; 209:259–67. [PubMed: 16879604]
45. Xia Y, Farquhar T, Burton-Wurster N, Lust G. Origin of cartilage laminae in MRI. *J Magn Reson Imaging.* 1997; 7:887–94. [PubMed: 9307916]
46. Welsch GH, Trattnig S, Scheffler K, Szomonyi P, Quirbach S, Marlovits S, Domayer S, Bieri O, Mamisch TC. Magnetization transfer contrast and T2 mapping in the evaluation of cartilage repair tissue with 3T MRI. *J Magn Reson Imaging.* 2008; 28:979–86. [PubMed: 18821633]
47. Salo, E-N.; Nissi, MJ.; Liimatainen, T.; Michaeli, S.; Mangia, S.; Ellermann, JM.; Nieminen, MT. Book of abstracts: Twentieth Annual Meeting of the International Society for Magnetic Resonance in Medicine. Vol. 20. Melbourne, Australia: ISMRM; 2012. Multi-parametric MRI characterization of enzymatically degraded articular cartilage; p. 279
48. Nestrasil I, Michaeli S, Liimatainen T, Rydeen CE, Kotz CM, Nixon JP, Hanson T, Tuite PJ. T1rho and T2rho MRI in the evaluation of Parkinson's disease. *J Neurol.* 2010; 257:964–8. [PubMed: 20058018]
49. Tuite PJ, Mangia S, Tyan AE, Lee MK, Garwood M, Michaeli S. Magnetization transfer and adiabatic R 1rho MRI in the brainstem of Parkinson's disease. *Parkinsonism Relat Disord.* 2012; 18:623–5. [PubMed: 22265140]
50. Borthakur A, Wheaton A, Charagundla SR, Shapiro EM, Regatte RR, Akella SV, Kneeland JB, Reddy R. Three-dimensional T1rho-weighted MRI at 1.5 Tesla. *J Magn Reson Imaging.* 2003; 17:730–6. [PubMed: 12766904]

Figure 1.

Schematic drawing of bovine patellar cartilage structure and zones. The unmineralized hyaline cartilage is divided into a superficial zone (SZ), intermediate zone (IZ) and radial zone (RZ), based on the architecture of the collagen fibril network [8,9,40]. Deep to the RZ, a zone of calcified cartilage (ZCC) separates the unmineralized hyaline cartilage from the subchondral bone.

Figure 2.

Pulse sequences used to measure adiabatic $T_{1\rho}$ (A), RAFF (B) and magnetization transfer (C). In the adiabatic $T_{1\rho}$, a variable train of adiabatic full passage (AFP) pulses (HS n family, $n=1$ or 4) is placed prior to the imaging readout with MLEV-4 phase cycling. The amplitude and frequency modulation functions of HS1 and HS4 are schematically shown in the inset of A. In the RAFF configuration, a train of RAFF pulses is similarly in front of the imaging readout. Each RAFF pulse is delivered with the same phase, as the MLEV-4 scheme is inherently built within the so-called P-packet ($P P^{-1} P_{\pi} P^{-1} \pi$ inset B). Finally, in the inversion-prepared MT protocol (C), a global on-resonance inversion is either turned off or on prior to the off-resonance continuous wave (cw) irradiation before the readout. In the present study, fast spin echo readout was used.

Figure 3.

Safranin-O stained articular cartilage and subchondral bone from bovine patella processed after the second MRI scanning at 48 hours: (a) specimen from the group of trypsin-digested (TD) bovine cartilage; slide reveals proteoglycan loss involving the superficial, intermediate and deep layer of the cartilage with the exception of the tidemark. Safranin-O stained slides confirm the enzymatic proteoglycan depletion using trypsin; (b) specimen from the group of non-digested (ND) bovine cartilage immersed in PBS (please note that the dark red bands are artifacts due to section folds). Safranin-O stained slides reveal retention of proteoglycans in the intermediate and radial zones with loss of proteoglycans in the superficial zone.

Figure 4. Representative T_2 , $T_{1\text{sat}}$ (MT), adiabatic $T_{1\rho}$ and RAFF relaxation time constant maps of bovine patellar cartilage from the non-digested (ND) specimens shown on the left and trypsin-digested (TD) specimens shown on the right. In each group the relaxation time constant maps were obtained at 0 and 48 hours, respectively.

Table 1

Relaxation times measured in TD (n=5) and ND (n=5) samples

	Time Point (hr) ^a	Mean ± STD (ms) ^b	p-value ^c	% ^d	Ratio (TD vs. ND) ^e
<i>T</i> ₂ TD	0	42.2 ± 8.7	0.00137	29	3.3
	48	54.4 ± 7.2			
<i>T</i> ₂ ND	0	40.0 ± 5.0	0.02937	9	
	48	43.5 ± 3.0			
<i>T</i> _{1sat} TD (MT)	0	464.8 ± 16.7	0.02464	18	3.0
	48	548.6 ± 49.3			
<i>T</i> _{1sat} ND (MT)	0	413.4 ± 21.9	0.12239	6	
	48	437.8 ± 48.6			
<i>T</i> _{1p-HS1} TD	0	125.2 ± 8.5	0.00146	48	4.9
	48	185.9 ± 17.1			
<i>T</i> _{1p-HS1} ND	0	121.8 ± 15.5	0.00002	10	
	48	133.9 ± 16.2			
<i>T</i> _{1p-HS4} TD	0	84.0 ± 7.5	0.00104	53	4.0
	48	128.7 ± 13.7			
<i>T</i> _{1p-HS4} ND	0	82.1 ± 10.8	0.00159	13	
	48	93.1 ± 10.5			
<i>T</i> _{RAFF} TD	0	92.4 ± 9.9	0.00029	52	4.1
	48	140.2 ± 13.4			
<i>T</i> _{RAFF} ND	0	85.9 ± 8.2	0.00035	13	
	48	96.8 ± 8.7			

^a 0 hr and 48 hr represent the time of the scanning before and after digestion (by trypsin or controls), respectively.

^b STD: standard deviation.

^c Two-tailed p-values refer to paired two-side t-test on relaxation measurements performed at 0 and 48 hours for each specimen group and parameter.

^d The change of relaxation time was calculated as: ((M48hr - M0hr) / M0hr) × 100%, where M48hr and M0hr are mean values in the table.

^e The ratio is calculated by dividing the average % relaxation time change observed in the TD group by the average % change observed in the ND group. TD: trypsin-digested; ND: non-digested.