Correlations of low-field NMR and variable-field NMR parameters with osteoarthritis in human articular cartilage under load

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Abstract

NMR experiments carried out at magnetic fields below 1 T provide new relaxation parameters unavailable at conventional clinical scanners. Contrast of T_1 generally becomes larger towards low fields, as slow molecular reorientation processes dominate relaxation at the corresponding Larmor frequencies. This advantage has to be considered in the context of lower sensitivity and frequently reduced spatial resolution. The layered structure of cartilage is one example where a particularly strong variation of T_1 across the tissue occurs, being affected by degenerative diseases such as osteoarthritis (OA). Furthermore, the presence of ^{1}H-^{14}N cross relaxation, leading to so-called quadrupolar dips in the ^{1}H relaxation time dispersion, provide insight into concentration and mobility of proteoglycans and collagen in cartilage, both being affected by OA.

In this study, low-field imaging and variable-field NMR relaxometry were combined for the first time for tissue samples, employing unidirectional load for probing the mechanical properties. 20 human knee cartilage samples were placed in a compression cell, were studied by determining relaxation profiles without and with applied pressure (0.6 MPa) at 50μm in-plane resolution, and were compared to volume-averaged T_1 dispersion. Samples were subsequently stored in formalin, prepared for histology and graded according to the Mankin score system.

Quadrupolar dips and thickness change under load showed the strongest correlation with Mankin grade. Average T_1 and change of maximum T_1 under load, as well as its position, correlate with thickness and thickness change. Furthermore, $T_1(\omega)$ above 25 mT was found to correlate with physiological properties. While volume-averaged T_1 is not a suitable indicator for OA, its change due to mechanical load, and its extreme values are suggested as biomarkers being available in low-field MRI systems. The shape of the dispersion $T_1(\omega)$ represents a promising access to understanding and quantifying molecular dynamics in tissue, pointing toward future *in vivo* tissue studies.

Keywords: relaxometry; low field NMR; cartilage; mechanical load, quadrupolar dips; osteoarthritis

Abbreviations

GAG: glycosaminoglycan

ILT: Inverse Laplace Transform

MOUSE: Mobile Universal Surface Explorer ™

OA: osteoarthritis

OARSI: Osteoarthritis Research Society International

SNR: signal-to-noise ration

TZ: Transitional Zone

Introduction

Clinical MRI investigations of cartilage tissue in joints and spine has seen growing importance in the recent decade and has become a standard and routine procedure for a number of health issues; however, the main approach of clinical studies rests on topological information, such as distances between bone surfaces, and peripheral tissue such as ligaments and tendons. Common to the tissue of interest is the comparatively short transverse relaxation time, which limits the use of conventional imaging sequences and potentially compromises the theoretical spatial resolution. This leads to the fact that cartilage tissue, with its typical thickness of 2-3 mm, is resolved only into a few pixels at most. Nevertheless, a wealth of information has been derived from clinical [1] and ex-vivo studies, in particular concerning one of the most common diseases, osteoarthritis (OA). Among others, the value of T_2 and its orientational dependence [2,3,4,5],the analysis of $T_{1\rho}$ [6,7,8], the diffusion coefficient and its anisotropy [2,9] and the change of these parameters under mechanical load were investigated, and correlations with the severity of the disease were established. A recent book gives a summary of the state-of-the-art of MRI studies on cartilage [10]. The vast majority of these studies were carried out at either typical clinical field strength of 1.5-3T, or on dedicated high-field scanners with even stronger magnetic field gradients and much higher spatial resolution.

In recent years, a new generation of low-field solutions, either whole-body or extremity scanners, have entered the market; they combine the reduction in cost with larger flexibility, for instance by allowing to tilt the patient together with the detection system in order to compare the state in joints with and without pressure [11], where the effect of the actual body weight provides more realistic conditions than the use of particularly designed devices that attempt to compress, for instance, the lower extremities [12]. While low magnetic field strengths inevitably lead to a loss of SNR, and consequentially of spatial resolution, measurements at lower fields often experience larger contrast and potentially hold extra information not available at typical clinical field strengths. In an early work, the superiority of T_1 vs. T_2 contrast in cartilage imaging at 0.15 T was already pointed out [13]; dedicated devices for extremities have been presented [14], and routines for osteoarthritis prediction at 0.18 T were discussed [15]. Different aspects of low-field and high-field cartilage imaging are reviewed in [16,17].

Improved contrast is mainly expected based on T_1, which is much more susceptible to tissue variations at lower fields, and the existence of cross-relaxation phenomena between ^1H and ^{14}N of nitrogen-containing compounds such as proteins and collagen [18]. However, studies in this field have been rather limited to this date. In [19] it was shown that the variation of ^1H T_1 over the cross-section of mammalian cartilage can amount to a factor of 3-5 at a field of 0.27 T, similar if not larger than the variation in T_2 at any field strength. T_1 is more robust to measure and is independent of orientation; like T_2, it allows, when measured at low fields, for the distinction of the three zones of cartilage (superficial, transition, radial) which are all affected by disease. This information is available in dedicated low-field scanners of high spatial resolution, like the NMR-MOUSE (Magritek, Aachen, Germany) providing resolution mainly along one dimension [20], which however is suitable for materials with layered structure such as cartilage. Measurement of cross-relaxation, the so-called quadrupolar dips in relaxation, on the other hand, require hardware with variable magnetic field strength in the region of these features, i.e. 50-80 mT. So far, only one group has presented spatially resolved data within this field regime [21,22,23], but with resolutions insufficient to separate the details within cartilage tissue. An alternative approach suggests the use of an additional field coil

that is being switched on for a defined period of time before the actual imaging unit. With this approach, the frequency dependence of T_1 is determined by obtaining relaxation parameter images at two similar, but different field strengths; the difference in T_1 is then approximated by a gradient dT_1/dB_0 and employed for tissue characterization [24,25]. To this date, however, volume-averaged studies on field-cycling relaxometers remain to be considered the standard tool for investigating frequency dependences in tissue [26].

In this contribution, the aim is first to quantify the presence or absence of correlations between a number of low-field and variable-field parameters that are accessible with two different commercial hardware units; and secondly, to identify such parameters that are related to OA and can be suitable for low-field MRI studies with a limited spatial resolution. To this end, a total of 20 human knee cartilage samples were studied and graded according to the Mankin score systems in order to allow a quantitative comparison with the state of OA.

Methods

A total of 20 osteochondral plug samples of 6-mm diameter were extracted from human tibial plateaus from patients undergoing total knee arthroplasty, and were stored frozen at -20°C in tubes filled with phosphate buffer solution [27]. The experiments were approved by the Ethical Committee of the Northern Ostrobothnia Hospital District, Oulu, Finland (191/2000).

Each sample was allowed to equilibrate for 24 h at +6°C before being exposed to room temperature, and was then placed in a tightly fitting cylindrical container that allowed the application of mechanical load in the vertical direction via a hydrostatic pressure cell, with small holes drilled in order to release excess water. This cell was mounted on top of an NMR-MOUSE single-sided scanner (Magritek, Aachen, Germany) operating at a ^{1}H Larmor frequency of 11.7 MHz, and the relaxation times T_2 and T_1 of the tissue were determined with a one-dimensional resolution of 50 μm, averaging over the cylinder diameter of 6 mm. The thickness of the cartilage was defined between the surface (0 μm) and the onset of constant T_1 in the calcified zone. The same experiment was repeated under constant vertical pressure of 0.6 MPa immediately afterwards. Note that for practical reasons, a resolution of 50 μm was considered fully sufficient and feasible with respect to typical layer thickness and total experimental duration, while the actual limit of the hardware has been determined to be 20 μm for aqueous systems [28]. From these experiments, the following parameters were derived: maximum value of T_1 and T_2, respectively, within the tissue with/without pressure, and their percentile difference; position of the same, in μm from surface and as percentage relative to the thickness; ratio of maximum and minimum relaxation times without pressure; peak signal intensity with/without pressure and percentile difference; ratio of integrated signal with/without pressure; thickness with/without pressure and percentile difference.

Samples were then taken out of the cell and the cartilage was separated from the bone and calcified tissue. The cartilage samples were subsequently measured in a SpinMaster 2000 Fast Field Cycling relaxometer (Stelar, Mede, Italy), and the T_1 dispersion was obtained in the frequency range 10 kHz … 20 MHz with particular emphasis on the region of quadrupolar dips between 1.5 and 4 MHz where sampling took place with higher density. The area under the quadrupolar peaks, expressed in relaxation rate $R_1 = 1/T_1$, were determined by subtracting the background dispersion by means of a polynomial best fit. All signals were acquired at a detection field corresponding to a Larmor frequency of 16.7 MHz, following a single 90° pulse and integrating the FID. The signal decays were fitted into an exponential function; no significant deviation from monoexponential behavior was observed within the accuracy of these experiments. All NMR experiments were carried out at room temperature. From these experiments, the following parameters were derived: power-law exponent for frequencies below and above 1 MHz, respectively; area of the quadrupolar peaks in R_1; average T_1 at the same field as with NMR-MOUSE, corresponding to 11.7 MHz; T_1 at lowest accessed field, corresponding to 10 kHz.

Following the NMR protocol, samples were stored in a 10% formalin solution, then cut, stained and categorized according to the Mankin [29]and OARSI [30] grading system by three individuals, the results of which were averaged. It has been shown before that both grading systems show a strong correlation [31]; we find a similarly high correlation coefficients between both grades of 0.95. It is thus justified to present only one of them and we restrict our discussion on the Mankin grade. Pearson and Spearman (rank) correlation coefficients of all determined parameters with the averaged grade and among themselves, respectively, were computed. Both coefficients were found to be similar throughout all parameters, so we restrict ourselves to the discussion of Pearson coefficients. Note that for a sample size of 20, all correlation coefficients magnitudes larger than 0.5 were considered significant, which corresponds to $p<0.025$ (in fact, the generally accepted value of $p<0.05$ corresponds to $r>0.45$). As a further approach to investigate correlations of measured parameters with disease

state, the samples were divided into two groups of „mild OA“ and „severe OA“ by a cutoff value of 4.5, resulting in a set of 7 specimens „mild OA“ (average Mankin grade 2.38) and 13 specimens „severe OA“ (average 7.92).

Results

A total of 24 parameters were defined that could be obtained directly, or by comparison between two experiments with and without mechanical load, from the experimental data. Five of them were derived from relaxometry data and the remainder from analysis of the profiles obtained at 0.27 T. These data are listed in Table 1 along with the device on which they were measured.

A two-set t-test statistical analysis was carried out on all these parameters with respect to compare the two groups “mild OA” and “severe OA”, assuming a confidence interval of 0.95. Six out of these parameters showed statistically significant correlations: the maximum value inside the tissue for both T_1 and T_2 under load of 0.6 MPa; the position of the former in the absence of pressure; the sample thickness, measured from the outer sample surface (defined as zero) to the calcified zone surface, with and without load; and the area of the quadrupolar peaks. Their respective averages and correlations are shown in Table 2. All other parameters showed correlations outside of the desired confidence interval.

Rather than distinguishing the tissue grade into two relatively arbitrary groups, we further attempted to determine correlations between all measured parameters, including, but not being restricted to, the Mankin grade. Since this grade must necessarily be a descriptive parameter and cannot be assumed to behave linearly, the conditions for a regression analysis are only approximately met. Cross-correlation of other parameters is assumed to highlight relations that can be exploited by employing different low-field NMR hardware.

Figures 1 and 2 show typical properties of spatially resolved $T_1$and the corresponding integrated signal intensity, being approximately equivalent to water content, obtained with the NMR-MOUSE at 0.27 T (11.7 MHz ^{1}H resonance frequency). Note that the experiment yields relaxation times in a thin, planar slice; curvature of the sample itself will result in some averaging over neighboring regions. This behavior has been thoroughly discussed in [28]. For samples lacking sharp edges or discontinuities in distributions, the effect of such curvature is considered minor, and it certainly does not affect significantly the extreme values and the thickness.

Figures 1a, 2a demonstrates qualitatively the behavior of healthy tissue. While the thickness is reduced by only about 10%, T_1 remains apparently unchanged. In contrast, the diseased sample (Mankin grade 11, Figure 1b, 2b) shows an overall shrinkage of 50% and clearly distorted T_1 profile under pressure: not only is the shape changed, but also the actual value of T_1 is decreased throughout the tissue, reducing the dynamic range between maximum to minimum approximately from 3 to 2. This is important in situations when spatial resolution is not available, but the distribution of T_1 relaxation times can be accessed as an additional measure of sample characterization (see Discussion). The profiles of T_2 behave accordingly (data not shown), but with larger error bars due to the probably inherent non-exponentiality of the decays due to spatial structural heterogeneity when averaged over the slice [32].

Figure 3 shows typical dispersion curves for cartilage samples. Common features are an approximate power-law behavior $T_1 \sim \omega^{\alpha}$ in the lower field region, transiting to a steeper dependence up to the highest field accessed in this experiment (0.47 T corresponding to 20 MHz ^{1}H Larmor frequency), which can tentatively also be approximated by a power law and which is continuing towards the longer T_1 known from clinical field strengths. In between are the quadrupolar dips, i.e. the effect of additional ^{1}H-^{14}N cross-relaxation of mobile water in contact with the amino acids of glycosaminoglycan (GAG) and collagen. The frequencies of these dips depend on the position of the nitrogen nucleus inside the molecule and are close to 2.1 MHz and 2.8 MHz, respectively, for amide groups, with a third dip at 0.7 MHz frequently becoming inconspicuous relative to the background relaxation rate. In this study, the area of the upper two peaks of R_1 were determined in order to provide a quantifiable parameter [33,18,34].

Table 3 provides a summary of all correlation coefficients obtained for the investigated samples. In the following, a number of particularly relevant correlations will be highlighted.

First of all, in Figure 4, the dependence of quadrupolar peak area on Mankin grade, as obtained from the relaxometry dispersion measurements, is shown. A negative correlation coefficient is observed; the same finding has been reported in [18] on another study of human cartilage samples. The other quantities obtained from the dispersion curves are the power-law exponents themselves, according to

the relation $T_1 \sim \omega^{\alpha}$. Figure 5a demonstrates the absence of any correlation between the two exponents in the lower and higher frequency regions, respectively. While dispersion is essentially unchanged below 1 MHz, remaining in a narrow range of $\alpha \approx 0.27 \pm 0.01$, only the slope above 1 MHz correlates with any of the other parameters. The most obvious correlation was found with thickness change as a consequence of mechanical load which, in turn, is inversely proportional to Young's modulus (see Figure 5b). Correlation with the Mankin grade, however, is slightly below the significance level.

Tissue thickness is a parameter that is accessible in clinical scanners and therefore has been investigated statistically before. In Figure 6, thickness with and without load, and in particular thickness change between these two conditions, is shown to possess significant correlation with Mankin grade: diseased tissue is, on average, thinner and more compressible. Since thickness change is related to the E modulus, this corresponds to a corroboration of existing reports which show a strong relationship of modulus and OA [35,36,37].

Finally, the change of thickness also correlates with the variation of the maximum value of T_1 at 11.7 MHz, a quantity that is, at least in principle, accessible also in low-resolution systems where the thickness itself cannot be determined. This connection is shown in Figure 7.

Discussion

The use of low-field and variable field devices opens up the possibility to obtain parameters that are not commonly accessible in clinical scanners. On the other hand, both low- and high-field modalities have been combined with imaging equipment so that, allowing for certain restrictions in resolution, in vivo studies of cartilage are becoming realistic, despite the fact that no large-scale statistical studies on OA have been reported yet.

The parameters most easily determined are average relaxation times, and indeed positive correlations with OA have been found for T_2 and $T_{1\rho}$ [2,4,5,6,7,8], but not for T_1 [38]. At the same time, it has been reported that T_2 is frequently non-exponential even when averaged over rather small volumes of the tissue, and that careful analysis of the different components, as well as their orientation dependence with respect to the axis of the magnetic field, is required to establish and justify such correlations [32]. A superficial explanation for these findings is the enhanced water content known for OA tissue [39] – all other components kept constant, this would increase the amount of "free" water not being affected by any surface, and hence averaging due to fast molecular exchange would lead to longer relaxation times [40]. The situation must be, however, more complicated, since the depletion of GAG also removes relaxation sinks for water protons, and the rearrangement of water in the weaker OA tissue under compression changes the relative weight of "free" water.

In this study, no correlation with degeneration state was found for the averaged values, but for the maximum value of both T_1 and T_2 under conditions with and without load. These maxima are identified at a spatial resolution of 50 μm, while one needs to keep in mind that averaging already takes place over the 6 mm diameter of the cartilage plug. Although this resolution is accessible, and even surpassed, at dedicated microimaging systems in high fields, it is out of reach for clinical scanners. These very same maximum values would therefore not be directly available, but could be extracted by a detailed analysis of the signal decay, in particular applying Inverse Laplace Transform (ILT) or related techniques to the data. ILT is established for broad distributions of relaxation times but so far sees only limited use in biomedical studies. Nevertheless, it appears to be a promising tool insofar as it can provide the extreme values of a distribution, or its width as an alternative fitting parameter. Corresponding results on biological samples will be discussed in a forthcoming paper.

The maximum of T_2 typically occurs in the Transitional Zone (TZ) of cartilage and is connected to the anisotropy of collagen fibrils being highest in this range [4] in fact, the gradient of relaxation times variation outside this maximum has been used for a precise definition of the boundaries between layers [41,42,43]. From earlier studies with bovine articular cartilage, but also from results within this study, it was found that the position of the maximum of T_1 generally occurs in the vicinity of that of T_2, while it can be assigned much more easily due to the often multiexponential behaviour of T_2 which is not observed for T_1. It can thus be assumed that parameters describing the actual maximum value of T_1 are predominantly related to morphological changes in the TZ, possibly changes of local water content, while variations of its position include changes in the remaining zones as well.

In this study, no correlation of $T_{1,max}$ with any other parameter, apart from the trivial case of the average T_1 at 11.7 MHz, was observed in the uncompressed sample. This was somewhat unexpected, considering the general increase of relaxation contrast towards lower

magnetic field strengths. However, significant correlation were found for $T_{1,max}$under load, such as thickness and thickness change, Mankin grade, maximum signal intensity, and also the corresponding value $T_{2,max}$. The combination of these findings indicates that the mobility and content of water in cartilage, in particular within the TZ, is a suitable indicator for OA severity.

The position of $T_{1,max}$, on the other hand, measured as the distance from the tissue surface, correlates with Mankin grade, Q-dip area, cartilage thickness and thickness change, where the latter two parameters may possibly not be fully independent of each other. However, when normalizing the position within the cartilage, i.e. dividing the value by the thickness measured under the same conditions, the correlation with thickness change still remains but is somewhat weaker (-0.53 compared to -0.60). This corresponds to the finding that the T_1 profile across cartilage is not deformed affinely under pressure, but the position of $T_{1,max}$, in absolute as well as relative scale, moves towards the surface. Note that this observation is at variance to a high-field study where the weakly pronounced T_1 variation sees a maximum closer to the top of the cartilage, which then moves inward after the application of mechanical load [44]. This, however, is not a contradiction since T_1 obtained at 7 T and at 0.27 T probe entirely different molecular dynamics. In [45,46], variations of T_2 and zone thicknesses are reported for canine cartilage tissue, and were found to strongly depend on sub-cartilage tissue and the amount of compression.

Next to relaxation times come the geometric properties which are routinely analyzed, at least indirectly, in clinical scans, frequently via the distance between bone surfaces on both sides of a joint [47,48]. Thinning of cartilage during OA is therefore a well-known phenomenon. The negative correlation of tissue thickness with Mankin grade is confirmed in this study. Moreover, the relative thickness change under pressure correlates positively, an observation that is in agreement with earlier studies at high field [49]. For hardware without sufficient spatial resolution, it is interesting to look at the cross-correlation coefficients with other observables: in particular, these are the parameters derived from variable-field measurements such as power-law and q-dip area (see below), but also $T_{1,max}$ at 0.27T and its change. It appears intuitively obvious to assume that “softer” tissue, which contracts more under load, will lead to a larger change in water content, hereby also affecting the distribution of T_1 and its maximum value. It is also relevant to mention that a weak correlation with the average T_1 was only found for the thickness without load, but not with load, or any other directly disease-related quantities.

Finally, the variable-field study provides parameters not accessible by most conventional instruments. The correlation of quadrupolar dip are, or peak area, with Mankin grade was already mentioned. The origin of these dips is the additional relaxation rate attributed to ^{1}H nuclei in the vicinity of the quadrupolar ^{14}N nuclei. If the Zeeman energy of the ^{1}H is equal to one of the three quadrupolar energies of ^{14}N [50,51,52], magnetization can be transferred, and will always “flow” towards the pool of ^{14}N which equilibrate within a much shorter time; the result is a shortened T_1 of ^{1}H.

These energy levels are defined as:

$$\Omega_{\pm} = \frac{K}{\hbar}(3 \pm \eta),\ \Omega_0 = \frac{2K}{\hbar}\eta,$$

with $K = \frac{e^2 qQ}{4}$, where q is the electric field gradient along the z axis of the principal axes system, Q is the quadrupolar moment, and η is the asymmetry parameter, of the order 0.4 for amide nitrogens. In fact, all amide nitrogens – with a natural abundance of close to 100% - have very similar atomic environment, and thus also nearly identical transition frequencies. The sum of amino acids in GAG and collagen, respectively, gives rise to a particular pattern, but in most biological tissue these dips are in the vicinity of ^{1}H Larmor frequencies of 2.8 MHz, 2.1 MHz and 0.7 MHz, and show only weak temperature dependence [50].

The prominence of these features in cartilage is related to the concentration of GAG and collagen on the one hand, i.e. GAG depletion as a consequence of OA is expected to have a negative effect on the area of the dips, which was indeed observed [18]. In [53] it was shown by using selective enzymes that both the GAG and collagen nitrogens contribute to the dips. On the other hand, mobility of the ^{14}N-containing species and of the water molecules also affect the presence of the features: if the interaction is completely averaged out due to rapid tumbling of the molecule with the ^{14}N attached, such as for single amino acids in solution, no effect will be seen. In fact, the enhanced mobility of proteins due to increased water concentration is a second, important contribution to the suppression of the quadrupolar dips [53]. In fact, both GAG depletion and increasing water concentration are signs of advanced OA [54,55], so that both effects combined can explain the negative correlation between quadrupolar dip area and Mankin grade found in this study and in [18].

Contrary to the quadrupolar cross-relaxation, discussion of the overall shape of the T_1 dispersion, i.e. its dependence on Larmor frequency, must currently remain empirical. While the theory of T_1 dispersion is often well understood for single component systems, such as polymer melts or water in contact with interfaces, the complexity of biological tissue is prohibiting a thorough description. Despite this, the dependence of T_1 on magnetic field strength, particularly at higher fields where conventional MRI studies take place, has been reported before, and databases have been compiled that allow a better comparison of images obtained at different magnetic field

strengths [56]. Attempts have been made to explain dispersion in various types of tissue in a semi-phenomenological way [57]. With cartilage being a rather simple system, it can be generally assumed that the dynamics of water interacting with surfaces such as GAG and collagen is mostly responsible for the observed dispersion, although magnetization transfer and therefore influence of the dynamics of the protons of the solid components will certainly be another source, as has been proven for hydrated proteins in the absence of excess water [58,59,52,60].

In this study, signal decays were analyzed by monoexponential fitting functions, i.e. the obtained relaxation times represent averages over the total cartilage volume. The dispersion data could be fitted phenomenologically to two different power-law relations $T_1 \sim \omega^{\alpha}$, with a more pronounced frequency dependence in the upper region above 1 MHz. The exponents α were taken as fitting parameters, which are statistically unrelated to each other, i.e. the frequency behavior below 1 MHz was found to be almost identical for all samples, in particular it does not correlate with any other relaxation figure. The exponent above 1 MHz, on the other hand, shows correlations, which is not entirely surprising since this range covers the frequency where the MOUSE measurements were carried out, although a correlation with the actual value of T_1 does not exist. The dependence on Mankin grade remains below the significance level. The observed relation with thickness change and signal amplitude change, both being quantities related to water content, indicate that the latter plays an important role, although in a somewhat counterintuitive fashion: a higher water content, just as well as an increase of mobility of the restricting medium, would generally increase the average of T_1 in the full frequency range. This has been found in enzymatically degraded hydrated collagen and GAG, respectively [28], but is obviously not seen in actual cartilage with OA, suggesting that the origin of T_1 dispersion in this tissue is more complicated. At this stage, a detailed explanation cannot be given; we note, however, that the slope in the upper frequency region is a valuable parameter since it can be obtained from measuring T_1 at two different field strengths about 20 MHz, an approach that is potentially feasible for a number of scanners with limited hardware modification. However, the full range of T_1 dispersion up to clinically relevant fields needs yet to be investigated in order to arrive at a suitable model for elucidating the actual processes acting on a molecular level, including the known observation of T_1 converging at high fields irrespective of OA status.

The behavior in the frequency range between 10 kHz and 1 MHz is actually related to the absence of any correlations found for T_1 at 10 kHz, the lowest frequency that could be used in this work. In the limit of zero frequency, T_1, T_2 and $T_{1\rho}$ become identical, and the same correlations known for T_2 and $T_{1\rho}$ could be expected for T_1 as well. However, such a similarity is not found in this study; it can therefore be tentatively concluded that the origin of correlations of T_2 and $T_{1\rho}$ with OA must be sought in very slow, i.e. below 10 kHz, dynamic processes of the water molecules in interaction with their environment, a finding that appears to be supported by studies employing $T_{1\rho}$ at different locking field strengths where larger deviations were found towards lower locking frequencies [27]. Note that typical locking frequencies of MRI equipment are even one or two magnitudes below the lower limit of the field-cycling device used in this work.

Conclusions

Low-field and variable field NMR was combined, for the first time, into a study of human articular cartilage with varying degree of osteoarthritis. Correlations were found for the area of the quadrupolar peaks in the relaxation profile, as well as the magnitude of the ^1H dispersion in the frequency range between 1 and 20 MHz, expressed by a power-law exponent. Only maximum values of relaxation times within the tissue were found to correlate with OA, but not average values, pointing to the need for developing improved analysis algorithms for non-exponential relaxation decays. In general, applying unidirectional load of 0.6 MPa onto cartilage plugs enhanced the correlation of observed quantities, in particular the corresponding change with/without load in T_1 relaxation time properties is identified as a promising indicator for OA severity.

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Figure Captions

Figure 1:(a) T_1 across healthy (Mankin grade 1) cartilage plug measured at a ^1H Larmor frequency of 11.7 MHz. (b) as in (a), but for a sample with severe OA (Mankin grade 11). Filled symbols represent unloaded tissue, open symbols represent loading at 0.6 MPa. Depth is counted from the tissue surface.

Figure 2: As in figure 1, but with the signal intensity plotted for the same samples.

Figure 3: Relaxation time as a function of Larmor frequency,$T_1(\nu)$, for a typical cartilage sample, indicating the apparent power laws fitted to the data, and showing the region of quadrupolar dips. Lines are drawn as guides to the eye, symbolizing the two distinct regimes.

Figure 4: Correlation between Mankin Grade of cartilage tissues and the area under the quadrupolar relaxation rate peaks (description see text).

Figure 5: (a)Correlation between power-law exponents in the frequency ranges below and above 1 MHz, respectively. (b) Correlation between thickness change with and without load, and power-law exponent in the frequency range between 1 and 20 MHz.

Figure 6: Correlation between (a) thickness without load, (b) thickness under 0.6 MPa load, and (c) relative difference between these two values, with Mankin grade.

Figure 7: Correlation between the change of the maximum T_1 at 11.7 MHz and the change in thickness, comparing tissue with and without load.