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Load-dependent NMR low-field profiling and relaxation dispersion study of osteoarthritic articular cartilage

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ABSTRACT

At low magnetic fields, T_1 variation within cartilage represents a robust parameter that is employed to quantify the layered structure in the tissue and is sensitive to factors such as enzymatic degradation, external load, and degeneration such as osteoarthritis. Variable-field relaxometry, on the other hand, provides access to the quadrupolar dips, i.e. enhanced relaxation rates of ^{1}H particularly at field strengths between 50 and 70 mT, that probe proton-nitrogen interaction and thus the content and local order of macromolecular constituents, namely glycosaminoglycans and collagen. At the same time, an strong overall dispersion of T_1 is observed over the whole accessible range of magnetic fields upward from 0.25 mT.

In this study on 20 human cartilage samples, low-field and variable-field techniques were combined for the first time to correlate corresponding NMR parameters and the response to load with the severity of osteoarthritis. The magnitude of the quadrupolar dips, as well as cartilage thickness obtained from profile measurements, is found to correlate with the severity of osteoarthritis. At the same time, a significant correlation was identified for relaxation time variation before and after uniaxial compression at 0.6 MPa, a typical value for forces appearing in the human knee and hip joint. This finding is of importance since the spatial resolution of 50 μm obtained with the single-sided scanner is about one order of magnitude better than the one in clinical high-field or low-field scanners, thus allowing a much more detailed investigation and yet providing constraints for the interpretation of averaged values obtained with whole-body scanners.

1. Introduction

Clinical investigations of joint tissue has become of increasing importance during the last decade and have been developed into a standard and routine procedure for a number of health issues, osteoarthritis being one of the most important ones. However, clinical studies usually rely on spatial information, such as measuring the distance between bone surfaces, and frequently do not attempt to quantify properties such as NMR relaxation times. As far as transverse relaxation is concerned, cartilage possesses some of the shortest values in the body apart from its solid components, which limits the application of imaging sequences if one assumes conventional, commercially available gradient systems. This particularly restricts also the spatial resolution of the cartilage tissue with its typical thickness of 2–3 mm to a few pixels at most. While T_2 relaxation contains significant information and is strongly affected by the layered structure of the collagen network with distinctly different order parameters [1–3] which suggest correlation with OA [4], T_1 remains – without the addition of contrast agents – less affected by the degeneration, but also not prone to the magic angle effect.

The observations above are made at typical magnetic field strengths found in clinical systems. At lower fields, however, T_1 varies in a similar fashion as T_2, possibly with an even larger range of values [5]. A further variable that introduces potential changes in the relaxation characteristics of cartilage is mechanical load: if the

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joint is subjected to "realistic" conditions, i.e. by applying the body weight onto the knee joint, the water distribution as well as arrangement of macromolecules and, consequentially, its relaxation properties are affected. While normal, healthy tissue would react in a well-defined way, by water being expelled from the cartilage tissue during loading and re-entering within several seconds after un-loading, the osteoarthritic tissue has much less capacity in resorbing water due to reduced swelling pressure, a fact which is confirmed by the reduced mechanical modulus found in diseased tissue [6–8]. This has led to the desire to compare MRI images with and without loading; apart from mechanical devices that apply pressure on the extended leg, the alternate approach is by putting the patient into a natural, upright position and acquiring images in two positions, with and without load. To this end, low-field scanners have become available that are able to rotate the magnet system with the patient fixed inside [9].

The existence of such low-field scanners, typically operating at field strengths well below 0.5 T, has highlighted the shortage of available NMR data on tissue in this respective field range, where it has been shown that in particular T_1 is strongly field-dependent. One further aspect is the occurrence of so-called quadrupolar dips in the relaxation profiles below about 80 mT [5]. These dips correspond to cross-relaxation phenomena of the observed ^{1}H with ^{14}N nuclei of nitrogen-containing compounds such as proteins and collagen. The dips are correlated to the concentration of these substances as well as their mobility, in the sense that freely tumbling molecules would not give rise to the extra relaxation mechanism due to motional averaging. Since osteoarthritis is correlated with a decrease of glycosaminoglycan (GAG) concentration and with an increase of water in the tissue [10], a decrease in the strength of these quadrupolar dips can be expected and has actually been proven in a pilot study [11]. In an investigation of enzymatically degraded tissue and its constituents, it was shown that both GAG and collagen contribute to the presence of quadrupolar dips [12]. However, the identification of these features requires an instrument capable of measuring T_1 at different field strengths; so far, only prototypes have been developed with imaging capability [13], while field-cycling relaxometers without imaging units are commercially available.

The purpose of this study is to combine different methods available at low magnetic field strengths, and to suggest suitable parameters that can be employed for a possible identification of osteoarthritis. To this end, we have combined, for the first time, a detailed study of field-cycling relaxometry with investigations at high spatial resolution in the profiling dimension, which can be provided by a single-sided NMR scanner, the NMR-MOUSE working at a field comparable to commercial MRI systems (0.27 T). The latter allows measurement of the samples under mechanical load and is thus suitable to quantify the structural changes occurring during tissue compression. To this end, a total of 20 human knee cartilage samples have been analyzed, first, for spatially resolved T_1 with an NMR-MOUSE scanner, and subsequently studied with a variable-field relaxometer providing volume-averaged relaxation times.

2. Experimental

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 [14]. 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 same experiment was repeated under constant pressure of 0.6 MPa immediately afterwards. Consequently, samples were 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 T_1 dispersion was measured 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 was performed with higher density. All signals were acquired at a detection field corresponding to the Larmor frequency of 16.7 MHz, followed by a single 90° pulse and integrating the FID. The signal decays were fitted by an exponential function; no significant deviation from monoexponential behavior was observed within the accuracy of these experiments.

Following the NMR protocol, samples were stored in a 10% formalin solution, then cut, stained and categorized according to the Mankin grading system by three individuals, the results of which were averaged. Mankin grading assigns numbers to certain properties of the tissue guided by visual inspection, covering the range between 0 (unaffected) and 14 (highest degree of OA) [15]. Pearson and Spearman (rank) correlation coefficients of all determined parameters with the averaged grade and among themselves, respectively, were computed; both values were mostly identical. Note that for a sample size of 20, all correlation coefficients magnitudes larger than 0.5 were considered significant, with a corresponding $p < 0.05$ value.

3. Results and discussion

T_1 and T_2 relaxation time profiles showed significant variation with increasing cartilage degeneration (Figs. 1 and 2). Furthermore, it becomes apparent that the general shape of the T_1 distribution is only weakly affected by load in the healthy tissue, whereas the reduction of particularly T_1 and the volume change is much more evident in the diseased tissue. Also, after compression the dynamic range of T_1 is reduced by about a factor of 3 compared to the situation before application of pressure. The pattern is similar for T_2, however, here the error bars are significantly larger, particularly for the diseased tissue, a fact that can be attributed to strongly multiexponential relaxation due to tissue heterogeneity within the sensitive region, i.e. a plane of 50 μm thickness. However, due to the limited SNR, a multiexponential analysis was not attempted at this stage. The common interpretation of both relaxation times is a reduction of the free water component with its long T_1 (T_2), due to water being expelled by the external pressure. The fraction of free water, due to pressure, is reduced while some of the residual water in the tissue remains in contact with the proteins and the collagen fibers, which together give rise to shorter relaxation times much as the surface relaxivity of an interface does in a porous medium. Additionally, the shortening of T_2 is also attributed to the realignment of the collagen network that leads to altered magic angle conditions and associated dipolar relaxation effects. Assuming fast exchange conditions, one might approximate the measured relaxation times with the residual water content [10,16]. Note that the relation of different tissue density with corresponding variation of water diffusivity [17] and the restricted diffusion in the matrix [18] have been described before, as well as the connection with relaxation properties of the water protons themselves [19,20]. Due to the complex and unresolved microstructure of the tissue, an attempt

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