Resolving Multiple T_2 Compartments in Cartilage with MRI

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Introduction: T_2 measurements have been used extensively to characterize properties of cartilage due to its sensitivity to macromolecular concentration and structure. T_2's are typically estimated by fitting NMR relaxation data to a monoexponential function. An increase in cartilage T_2 after enzymatic degradation has been consistently reported, although attempts to associate changes in average values of T_2 to loss of specific tissue components have been inconclusive [1]. Cartilage is composed of various macromolecular components that constitute compartments of water with differing fractions and mobility. Thus, transverse relaxation decay of cartilage is poorly described by a single exponential [2]. Multiexponential analysis of T_2 relaxation has typically been performed using spectroscopic data with good SNR and closely spaced echoes. In imaging experiments, the ability to achieve these conditions is much more constricted, limiting the ability to accurately resolve multiple T_2 components. It has been demonstrated in several biological tissues (i.e. brain, breast, and muscle), that multiple T_2 components can be resolved at SNR, TE, and number of echoes (N) attainable using imaging methods [3]. Therefore, we sought to identify the acquisition parameters (i.e. SNR, TE, and N) required for resolving multiple T_2 relaxation components in cartilage with typical component T_2 values and associated fractions. We applied this analysis to characterize multicomponent T_2 relaxation in intact and enzymatically degraded cartilage using two different enzymes acting primarily on collagen and proteoglycan (PG) matrix components.

Materials and Methods: *Cartilage Sample Preparation.* Bovine nasal cartilage (BNC) disks (diameter = 8 mm) were excised from the nasal septa of 5-6 month-old calves (Green Village Packing, Green Village, NJ) [4]. Samples were threaded onto a hollow tube and inserted into a four-well sample holder filled with DPBS buffer adjusted to pH 7.5 ± 0.1 for initial T_2 measurements. 1 mg/ml trypsin (Sigma-Aldrich, St. Louis, MO) or 30 units/ml collagenase type II (Worthington Biochemical Corp., Lakewood, NJ) was then added to the buffer, with T_2 measurements repeated after 24 hours of incubation at 37 ºC in the degradative enzyme. *MRI T_2 Measurements.* MRI experiments were performed using a 9.4 T Bruker DMX spectrometer (Bruker Instruments, Billerica MA) using a CPMG imaging pulse sequence with: TE/TR = 12.8ms/5s, N = 64, NEX = 2, FOV = 4×1.5cm, 0.5mm slice thickness, and 256×128 matrix size. Spin-echo amplitude at each echo time in the relaxation decay was quantified by averaging the pixel intensities from a region of interest (ROI) in the sample. *T_2 Relaxation Simulation.* Relaxation data were simulated from the following expression:

$$y(n\cdot TE) = B + y_0 \sum_{m=1}^{M} w_m \cdot e^{-(n\cdot TE)/T_{2,m}} + \varepsilon(0,\sigma),$$

where $y(n\cdot TE)$ represents the signal amplitude of the n^{th} echo, B represents a baseline offset, y_0 represents the initial signal amplitude, w_m represents the fractional weight of the m^{th} T_2 component, and $\varepsilon(0,\sigma)$ represents the addition of Gaussian random noise with a mean 0 and standard deviation of σ. Simulation input values for T_2's and relative fractions (w) were selected based on preliminary work from out lab which agree well with previously reported values [5]: $T_{2,fast}$ (7.7 ms) , $T_{2,slow}$ (66 ms), w_{fast} (0.75), and w_{slow} (0.25). Simulations were performed with SNR ranging from 100 to 2000, TE ranging from 4 to 14 ms, and N ranging from 32 to 128. 100 trials were run for each condition. The multiexponential fit of simulation and experimental data was obtained using a nonnegative least squares (NNLS) method similar to that described in [6].

Results and Discussion: Figure 1 shows the minimum SNR required for each combination of TE and N to obtain admissible fits in 95% of the runs. Admissible fits were defined as having two components with T_2's and fractional weights resolved to within 10% of the input simulation value. These results indicate the optimum TE for a given number of echoes for reliable biexponential T_2 relaxation measurements in cartilage. Figure 2 shows precision of T_2 and w as it relates to SNR under similar imaging conditions used in the cartilage degradation experiment. Analysis of relaxation data from an imaging experiment is typically performed on an ROI as opposed to pixel by pixel; averaging over a large ROI results in increased SNR. In these experiments, ROI's were chosen such that SNR was ~1500, resulting in T_2 and fractional weight precision within 1 ms and 2% respectively. Both enzymatic treatments resulted in a significant shift in the fractional amounts and T_2 components (Fig. 3), while maintaining (w_{fast}) > (w_{slow}). The importance of this work is to detect changes in cartilage matrix components as they relate to degradation. Cartilage matrix consists primarily of collagen and PG. Previous work has demonstrated that water compartments are associated with these macromolecules and that their T_2 decay is faster than that of bulk water [2, 7]. The T_2 components and associated weights of these compartments have been documented as changing with maturation of cartilage and have been associated with an increase in collagen density [5]. Our results revealed two compartments and demonstrated a shift in water fraction from $T_{2,fast}$ to $T_{2,slow}$ with enzymatic degradation which can be interpreted as a loss of macromolecular-associated water fraction to bulk water. This is the first work to demonstrate the diagnostic potential of multiexponential T_2 analysis of MR images in cartilage.

Conclusions: Simulations were performed to define the conditions under which multiple T_2 relaxation components could be reliably performed in cartilage. Experimental results were sensitive to the matrix changes presumed to result from enzymatic degradation. Further work will be directed at achieving measurements of more diagnostic potential by more extensive multiexponential analysis of relaxation time data.

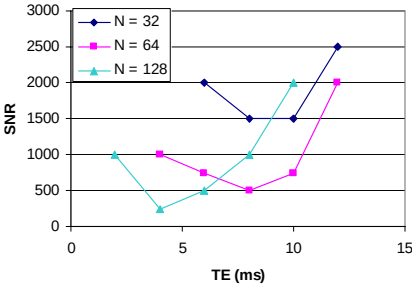

Figure 1. Minimum SNR required for a given TE and N. Admissible solutions are based on the following: 95% of the trials properly demonstrated two components, and the T_2 components and fractional weights w were resolved to within 10% of the input value.

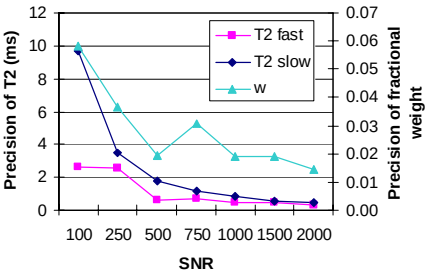

Figure 2. Precision of T_2 and fractional weight w determination as a function of SNR using parameters appropriate for degraded cartilage (TE = 12 and N = 64).

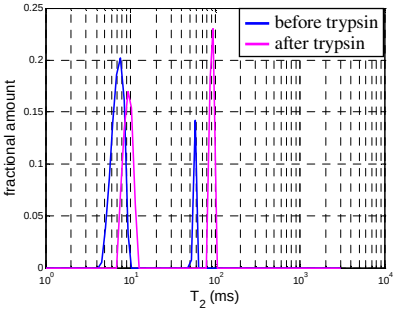

Figure 3. Representative T_2 histogram before and after trypsin treatment using NNLS.

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