

Relationship between Relaxation Component T2 values and Weight Fractions and Mechanical Moduli in Native Cartilage

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Introduction Multiexponential analysis of transverse relaxation data provides more specific information about cartilage matrix composition than is provided by conventional monoexponential T2 measurement. Relaxation components may be interpreted in terms of underlying water, and hence macromolecular, compartments^{1,2,3} associated with proteoglycan (PG) and collagen. We have previously established correlations between component T2 values and weight fractions, and collagen concentration in bovine nasal cartilage² and in engineered cartilage constructs³. In addition, cartilage biochemical content has been shown to correlate with mechanical properties and to be a predictor of mechanical quality⁴. Specifically, matrix proteoglycan and collagen were both shown to be highly correlated with equilibrium and dynamic moduli^{4,5}. Therefore, we hypothesized that individual components derived from multiexponential analysis of transverse relaxation in cartilage would correlate with cartilage mechanical properties. Analysis was performed on bulk T2 relaxation data obtained from bovine nasal cartilage, with identical explants mechanically tested to determine the matrix mechanical properties.

Materials and Methods Cylindrical explants (3.5mm x 3mm) of bovine nasal cartilage (BNC) were excised from two bovine snouts and subjected to mild-to-moderate degradation using one of three enzymes, chondroitinase, trypsin or collagenase, to model degenerative cartilage. *Magnetic Resonance Measurements:* Data were acquired with a 9.4T/105mm Bruker DMX NMR spectrometer at 4°C. Transverse relaxation data were obtained using a non-localized CPMG sequence with a TE/TR of 0.09ms/10s, 4096 echoes and an NA of 64. Multiexponential T2 analysis was performed using the non-negative least squares algorithm, implemented in MATLAB (Mathworks, Natick, MA). *Mechanical testing:* Samples were tested in unconfined static and dynamic compression. To obtain the equilibrium modulus, each sample was subjected to a 5% compression ramp in 1 minute followed by a 4 minute hold. To measure dynamic modulus, each sample was initially held at 15% compression, after which a 1% sinusoidal compression at 1 Hz was applied. *Biochemical quantification:* PG and total collagen contents were determined from the BNC digests using the colorimetric DMMB dye binding assay and the hydroxyproline assay, respectively. *Statistical analysis:* Linear regression analysis was performed to define the relationships between PG and collagen, and the derived MR parameters.

Results and Discussion Our results showed that dynamic and equilibrium moduli correlated significantly and positively with PG ($R^2=0.89$, $p<0.001$ and $R^2=0.40$, $p=0.002$, respectively) and collagen ($R^2=0.78$, $p<0.001$ and $R^2=0.25$, $p<0.03$ respectively) consistent with literature reports on native articular cartilage⁵. Four relaxation components, with T2 values and corresponding fractions denoted (T_{2i} , w_i), were detected in all samples. The most slowly relaxing component (T_{24} - w_4) was assigned to the bulk water compartment, the components T_{22} - w_2 and T_{23} - w_3 , with T2 values ~1 ms and ~39 ms respectively, were assigned to different compartments of PG-bound water, and the most rapidly relaxing component, w_1 - T_{21} , with T2 ~0.15ms was assigned to relatively immobile collagen-bound water. As shown in Fig. 1, equilibrium stiffness correlated positively with w_3 , consistent with an increase in stiffness with increasing macromolecular concentration; this is consistent with previous studies in articular cartilage demonstrating that PG contributes significantly to the equilibrium modulus⁶. In contrast, there was a significant negative correlation between equilibrium stiffness and w_4 , consistent with a decrease in mechanical stiffness with increased water content. Previous work has in fact demonstrated that cartilage stiffness decreases with hydration⁷. w_1 showed essentially no correlation with either dynamic or equilibrium stiffness (Figure 2). Additionally, both of these mechanical moduli showed significant negative correlations with T_{23} and T_{24} (Figure 3) but did not correlate with T_{21} and T_{22} (not shown). These results are consistent with the expected decrease in T2 with increasing PG concentration and decreasing water mobility.

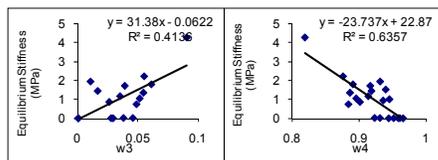


Figure 1: Correlations between equilibrium stiffness and fractions, w_3 and w_4

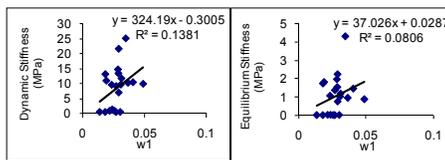


Figure 2: Correlations between dynamic and equilibrium moduli and w_1

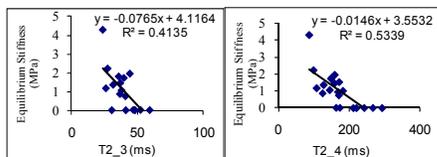


Figure 3: Correlations between equilibrium stiffness and T2 components

Conclusions: Cartilage degeneration is typified by changes in matrix structure and composition which ultimately lead to substantial loss of matrix mechanical integrity. We have shown that T2 components and weight

fractions obtained from multiexponential transverse relaxation analysis correlate with dynamic and equilibrium stiffness. Thus, this method may be of potential use for non-invasive *in vivo* evaluation of degenerative cartilage and of therapeutic interventions.

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