**Prediction of cartilage compressive modulus using multiexponential analysis of T$_2$ relaxation data and support vector regression**

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Prediction of Cartilage Compressive Modulus using Multiexponential Analysis of $T_2$ Relaxation Data and Support Vector Regression

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Abstract

Evaluation of mechanical characteristics of cartilage by magnetic resonance imaging would provide a noninvasive measure of tissue quality both for tissue engineering and when monitoring clinical response to therapeutic interventions for cartilage degradation. We use results from multiexponential transverse relaxation analysis to predict equilibrium and dynamic stiffness of control and degraded bovine nasal cartilage, a biochemical model for articular cartilage. Sulfated glycosaminoglycan concentration/wet weight (ww), and equilibrium and dynamic stiffness, decreased with degradation from 103.6 ± 37.0 μg/mg ww, 1.71 ±1.10 MPa, and 15.3±6.7 MPa in controls to 8.25±2.4 μg/mg ww, 0.015±0.006 MPa and 0.89±0.25MPa, respectively, in severely degraded explants. Magnetic resonance measurements were performed on cartilage explants at 4°C in a 9.4T wide-bore NMR spectrometer using a Carr-Purcell-Meiboom-Gill (CPMG) sequence. Multiexponential $T_2$ analysis revealed four water compartments with $T_2$’s of approximately 0.14 ms, 3 ms, 40 ms and 150 ms, with corresponding weight fractions of approximately 3%, 2%, 4% and 91%. Correlations between weight fractions and stiffness based on conventional univariate and multiple linear regressions exhibited a maximum $r^2$ of 0.65, while those based on support vector regression (SVR) had a maximum $r^2$ value of 0.90. These results indicate that i) compartment weight fractions derived from multiexponential analysis reflect cartilage stiffness, and ii) SVR-based multivariate regression exhibits greatly improved accuracy in predicting mechanical properties as compared to conventional regression.

Graphical abstract

We performed multiexponential transverse relaxation analysis on bovine nasal cartilage, and detected four water compartments with weight fractions of approximately 3%, 2%, 4% and 91%. Correlations between weight fractions and mechanical stiffness based on conventional univariate and multivariate linear regressions exhibited a maximum $r^2 = 0.65$, while those based on support vector regression had a maximum $r^2 = 0.90$. These results indicate that i) compartment weight fractions derived from multiexponential analysis reflect cartilage stiffness, and ii) SVR-based multivariate regression exhibits greatly improved accuracy in predicting mechanical properties as compared to conventional regression.
vector regression had a maximum \( r^2 = 0.90 \). This indicates that support vector regression analysis using underlying tissue component fractions as input parameters may be useful for the noninvasive determination of cartilage mechanical quality.

**Keywords**

biomechanical stiffness; multiexponential T2 relaxation; cartilage; water compartments

**Introduction**

Cartilage matrix is composed primarily of type II collagen, proteoglycans (PG), and interstitial water (1). Its mechanical properties are determined by the structure and composition of these macromolecules (2). Hydrostatic forces between the polyanionic glycosaminoglycan (GAG) molecules and swelling pressure caused by matrix hydration regulate mechanical stiffness (3,4). Mechanical integrity is critical in enabling articular cartilage to perform its functions of load bearing and shock absorption.

Degenerated cartilage, as seen in osteoarthritis (OA), is characterized by alterations in the structure and composition of matrix components, which affect mechanical properties (5,6). In native cartilage, dynamic and equilibrium compressive stiffness have been shown to be highly correlated with proteoglycan concentration and, to a lesser extent, with collagen content (7).

Magnetic resonance (MR) imaging is used widely to evaluate cartilage matrix properties related to macromolecular status (8–11). Few studies have probed the relationship between MR parameters and mechanical properties. In native and engineered cartilage, \( T_1 \), \( T_{1p} \), and \( T_2 \) relaxation times, and ADC were found to correlate with loading-induced matrix deformation (10,12–16). However, these MR parameters provide only a general assessment of matrix quality and lack the sensitivity and specificity of more invasive techniques such as histology (17) and Fourier transform infrared spectroscopy (18) which can more directly.
evaluate specific matrix components. None of these techniques directly assess biomechanical properties of tissue.

Water molecules associated with particular macromolecules in tissue would be expected to exhibit different relaxation times. Previous studies have exploited this concept to demonstrate that multiexponential analysis of T_2 relaxation greatly improves specificity of MR measurements as compared with conventional monoexponential T_2 analysis (19–23). In native and engineered cartilage, relaxation times and fractional weights of T_2 components change in response to matrix growth and enzymatic degradation, demonstrating their sensitivity to matrix composition (24,25). Therefore, we hypothesized that multiexponential T_2 analysis may be used to assess the mechanical quality of cartilage.

We previously introduced multivariate MRI analysis to evaluate cartilage degradation (26,27). Support vector regression (SVR) is a technique based on the support vector machine (SVM) approach to classification. The SVM provides an adjustable assignment of samples to groups based on measured parameters (28,29); it is a highly flexible nonlinear extension of classic linear discriminant analysis. Similar to the SVM, support vector regression does not require a linear relationship between the dependent and independent variables. The separating hyperplane in SVR and the SVM is based on a subset of data points, known as support vectors, which are farthest from the group means in parameter space, i.e. least characteristic of the group, but still are correctly classified. In both SVM and SVR analyses, data points are transformed from native parameter space into a higher dimensional feature space via a nonlinear kernel function prior to classification or regression.

Here, we apply SVR to multiexponential MRI data obtained on degraded cartilage to estimate tissue mechanical properties. Bovine nasal cartilage (BNC) was used as a model for the macromolecular contribution to mechanical properties (30–32). BNC is an incomplete model for the mechanical properties of articular cartilage due to its lack of organized collagen structure. However, it has been used (33) and validated (34) as a longstanding model for investigating the relationship between cartilage macromolecules and mechanical properties without the further complexities of ultrastructural organization. We therefore used BNC as an ideal model preparation for prediction of cartilage mechanical properties from multivariate SVR analysis of water component weight fractions.

**Materials and Methods**

**Tissue Preparation**

Sixty 3.5-mm diameter, 3-mm thick, cylindrical cartilage plugs were excised from nasal septa of freshly slaughtered 1 year-old calves (Green Village Packing, Green Village, NJ). The explants were subjected to mild and severe degradation by incubation in one of three enzymes at 37°C for 5–24 hours: 1mg/ml of trypsin (Sigma-Aldrich, St. Louis, MO), 0.1U/ml of chondroitinase AC (Sigma), or 30U/ml of collagenase (Worthington Biochemical). Samples were then washed in Dulbecco’s phosphate buffered saline (DPBS) solution, followed by MRI and mechanical measurements.
Magnetic Resonance Measurements

MR data were acquired at 4°C with a 5-mm solenoid coil using a 9.4T/105mm Bruker Avance III NMR spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany). Samples were placed in a 5-mm diameter glass tube filled with Fluorinert FC-77 (3M Corp., St. Paul, MN), a proton-free susceptibility matching fluid, and sealed with a Teflon lid. Teflon screws were used to accurately position each sample along the length of the tube at the center of the RF coil. Relaxation data were acquired using a non-localized Carr Purcell Meiboom Gill (CPMG) sequence with echo time TE = 0.09 ms, interpulse delay TR = 10s, 8192 echoes and 64 signal averages. Even echoes were used, resulting in an effective echo time of 0.18 ms. The total time for the acquisition of each transverse decay curve used for multiexponential analysis was ~12 minutes. Intensities were fit to a three-parameter monoexponential function to obtain conventional T\textsubscript{2} relaxation times, and the same data were used for multiexponential T\textsubscript{2} analysis.

Fitting of T\textsubscript{2} Relaxation Data

Regularized non-negative least squares (NNLS) was used for the multiexponential T\textsubscript{2} analysis with 80 T\textsubscript{2} bins logarithmically spaced over the interval [0.1, 3000] ms using MATLAB (MathWorks, Natick, MA) as previously described (19). This method makes no \textit{a priori} assumptions about the number of relaxation components to be fit. The NNLS finds a histogram of amplitudes associated with each T\textsubscript{2} value. T\textsubscript{2} distributions were regularized to achieve a chi-squared of 101% of the non-regularized fit (35) using a minimum energy constraint. Component T\textsubscript{2} values and fractional weights were determined respectively from first moments and integrated areas of the histogram peaks, with the sum of the weights constrained to equal unity by NNLS.

Mechanical Testing

Explants were tested in unconfined compression on a Dynastat mechanical spectrometer (Dynastatics, Albany, NY) using a 250g load cell. To obtain the equilibrium modulus, a ramp displacement of 5% strain was applied to each sample over one minute; this strain was held for 4 minutes until equilibration of reaction force. Ramp displacements of 1 \times 5\% strain and 2 \times 2.5\% strain, each followed by a 4 minute hold, were then applied, resulting in a total strain of 15\%. Stresses were calculated by dividing the measured forces by the sample cross-sectional area. A stress-strain curve for the tissue was obtained based on the magnitude of the equilibrium stress measured after each ramp-and-hold at 10\%, 12.5\%, and 15\% strain.

To measure the dynamic stiffness, the plugs were held at 15\% compression, and a 1\% sinusoidal compressive strain was applied at frequencies 0.005–2Hz. Dynamic stiffness was calculated as the amplitude of dynamic stress divided by dynamic strain at each frequency.

Biochemical Quantification

After mechanical testing, cartilage plugs were blotted, weighed before and after lyophilization, and digested in 0.5 mg/ml of proteinase K in digestion buffer (50 mM Tris hydrochloride, 5 mM calcium chloride). Sulfated glycosaminoglycan (sGAG) content was
quantified from the tissue digests using the colorimetric 1,9 dimethylmethylene blue (DMMB) binding assay with a shark cartilage chondroitin sulfate standard (36).

Simulation of T2 Relaxation Data

Analysis of simulated data based on the four compartments detected experimentally was performed to ensure the reliability of our results (19). Data were simulated using average experimental values for component T2s and fractions with TE = 0.18 ms and 4096 echoes using the expression:

\[ y(n \times TE) = B + y_0 \sum_{m=1}^{M} w_m e^{-(n \times TE) / T_2m} + N(0, \sigma) \]

where \( y(n \times TE) \) represents the amplitude of the nth echo measured at \( n \times TE \), \( B \) is the baseline offset, \( y_0 \) is the overall signal amplitude, \( w_m \) is the fraction size of the \( m \)th T2 component, and \( N(0,\sigma) \) is additive Gaussian random noise with mean 0 and standard deviation \( \sigma \). The signal-to-noise ratio (SNR) was defined as \( y_0 / \sigma \), with values used in the simulations based on the average experimental SNR of 11272, 10596, and 8824 for control, mildly degraded and severely degraded groups respectively. Reliability was defined as the percentage of simulation trials resulting in the correct number of T2 components; accuracy was defined as the percent difference between input T2 values and fractions, and T2 values and fractions derived from NNLS, and precision was defined as the coefficient of variation (CV) of the T2 values and fractions. These metrics were evaluated over 100 trials with different noise realizations. Admissibility was defined as i) reliability of \( \geq 90\% \), i.e., analyses of at least \( 90\% \) of the simulations identified four T2 components; ii) accuracy \( < 10\% \); and iii) CV \( < 10\% \).

Univariate regression correlations and models

Univariate linear regression analysis was performed with MR-derived component fractions as independent variables and experimentally-measured biomechanical parameters as dependent variables to determine the ability of individual MR parameters to predict cartilage mechanical stiffness.

In addition, univariate regression models were constructed to establish a relationship between predicted and measured mechanical stiffness. The entire data set was randomly divided into 5 groups, of which 4 were used as a training set to establish a linear regression between component fractions and the biomechanical parameters. This cross-validation procedure was performed 5 times, each with a different group excluded from the analysis (23). The slopes and offsets from these analyses were averaged to produce a regression model which was then used to calculate mechanical stiffness from component fractions.

Multivariate Analyses using Multiple Linear Regression

Two analyses were performed on the data using linear combinations of the four component fractions. First, multiple linear regression (MLR) was performed on the entire data set with all eleven possible component fraction combinations as independent variables \( (\alpha w_1 + \beta w_2 + \gamma w_3 + \delta w_4) \), followed by a second analysis using only the four component fractions that best predicted mechanical stiffness.
c, αw1 + γw3 + c, αw1 + δw4 + c, βw2 + γw3 + c, βw2 + δw4 + c, αw1 + βw2 + γw3 + c, αw1 + βw2 + δw4 + c, αw1 + βw2 + γw3 + δw4 + c), and the experimentally-measured dynamic and equilibrium stiffness as dependent variables. Second, the regression coefficients and offset terms derived from the MLR analysis were used to calculate the dynamic and equilibrium stiffness of the samples from the MR component fractions. Correlations were then established between calculated and experimentally-measured dynamic and equilibrium stiffness. This analysis indicates the ability of a combination of MR parameters to predict biomechanical parameters via a linear model.

**Multivariate Analysis via Support Vector Regression**

SVR analysis was performed (23) using MATLAB scripts developed in-house and the e1071 package (37), with eleven possible combinations of the four distinct component weights as independent variables and the measures of experimentally-determined mechanical stiffness as dependent variables. The SVR model was constructed from the entire data set of component fractions and corresponding mechanical stiffness values for each sample, using 5-fold cross-validation. The model was subsequently applied to describe the relationship between experimentally-measured and calculated mechanical stiffness for the entire data set.

**Statistical Analysis**

Experimental values are reported as mean ± standard deviation. Biochemical data (sGAG), MR parameters (component T2s and fractional weights, w), and biomechanical properties (static and dynamic stiffness) were analyzed using one-way ANOVA with appropriate post hoc testing to detect pairwise differences.

**Results**

**Biochemical composition and Biomechanical properties**

sGAG concentration per wet weight (ww) decreased significantly from 103.6±37.0 μg/mg ww in non-degraded explants to 54.3 ±21.6 μg/mg ww (p<0.0001) and 8.25±2.4 μg/mg ww (p<0.0001) in mildly and severely degraded explants respectively (Table 1).

A representative stress-strain relationship for control cartilage is shown in Figure 1; the close linear relationship between stress and strain found in all samples permitted a meaningful calculation of elastic moduli.

Loss of sGAG resulted in decreased equilibrium and dynamic stiffness in these samples (9,17,38). Equilibrium stiffness decreased (p=0.003) by 51% from 1.71±1.10 MPa in non-degraded, to 1.05 ± 0.70MPa in mildly degraded, explants. In addition, equilibrium stiffness decreased by 99% (p<0.0001) to 0.015±0.006MPa in severely degraded explants. Similarly, dynamic stiffness decreased significantly by 44% (p=0.0002) from 15.3±6.7 MPa in non-degraded explants to 10.2±3.5MPa in mildly degraded plugs and by 94% (p<0.0001) to 0.89±0.25 MPa in severely degraded explants.
Figure 2 shows the relationship between the mechanical properties of the cartilage matrix and its components. As expected, dynamic and equilibrium compressive stiffness correlated negatively with water content, with $r^2$ of 0.91 and 0.77 respectively, while they correlated positively with sGAG concentration, with $r^2$ of 0.89 and 0.82 respectively (7,39).

Multiexponential $T_2$ Simulation Results

The accuracy and precision for compartment fractions and relaxation times are shown in Table 2. Admissibility criteria were met for all component weights and relaxation times. Reliability was 100%, 96%, and 98% for control, mildly and severely degraded groups respectively. All simulated component fractions and relaxation times were accurate to within approximately 10% of the input value. Precision in parameter determination was also within 10%.

Multiexponential $T_2$ Experimental Results

Four compartments were consistently detected in the non-degraded and degraded explants with approximate $T_2$s of 0.14 ms, 3 ms, 40 ms and 150 ms and fractions of 3%, 2%, 4% and 91% (Table 3). The most rapidly relaxing component was provisionally assigned to collagen-associated water, the two intermediate components to subpopulations of proteoglycans, and the most slowly relaxing component to the bulk water fraction (23). The accuracy of estimating the rapidly relaxing component’s $T_2$ value and weight fraction is potentially limited by the minimum effective echo time of 0.18 ms. However, the detailed simulation results indicate that these values ($T_{2,1}, w_1$) are reliable and admissible.

$T_{2,1}$ remained fairly constant with degradation while $w_1$ decreased ($p=0.005$) from 3.2% in non-degraded samples to 2.5% in the severely degraded samples. Mildly degraded samples had an intermediate weight fraction of 2.9%, which was not different from values in the non-degraded samples ($p=0.2$), but differed from values found in severely degraded samples ($p=0.06$). These results are consistent with our previous findings in which changes in compartment sizes were not necessarily associated with changes in relaxation times (23).

Similar to the case for $T_{2,1}$ and $w_1$, the 23% change in $T_{2,2}$, from 2.6 ms in non-degraded samples to 3.2 ms, was proportionally smaller than the 62% change in $w_2$, from 2.9% in non-degraded samples to 1.1% after severe degradation. Mildly degraded explants also showed a 13% decrease in $w_2$ ($p=0.07$) when compared to control levels, with an average weight fraction of 2.5%. This trend towards a decrease in size of this proteoglycan-associated water compartment with degradation is consistent with our biochemical results showing loss of sGAG after enzymatic degradation.

In contrast, the relaxation time of the more slowly-relaxing PG compartment, $T_{2,3}$, increased by 38% with degradation ($p<0.0001$) from 33.9±7.9 ms, to 46.8±6.8 ms in severely degraded explants. Furthermore, relaxation times for this compartment in mildly degraded explants increased by 16% ($p=0.01$) over non-degraded explants to 39.3±4.7 ms. These increases in $T_{2,3}$ are consistent with increased mobility of the matrix macromolecules after enzymatic digestion (19). However, there was no significant decrease in the size of the corresponding weight fraction, $w_3$. This observation illustrates the complex relationship between compartment weight fractions and relaxation times, which depend on multiple
factors related to the macromolecular environment. Moreover, proton exchange between compartments, and diffusion, further enhanced by enzymatic digestion of the matrix components, complicate this already complex relationship.

The compartment provisionally assigned to bulk water had the longest $T_2$, $T_{2,4}$, and the largest weight fraction, $w_4$. $T_{2,4}$ increased ($p=0.0002$) by 51% from 128.1±41.7 ms in the non-degraded explants to 194.3±47.1 ms in the severely degraded explants. This compartment relaxation time also increased ($p=0.03$) with moderate degradation, to 148.1±19.4 ms. This increase in molecular mobility is consistent with fewer PG aggregates in the matrix (23). Moreover, water molecules associated with freely-tumbling PG fragments, created from enzymatic degradation, have a much longer relaxation time than water associated with the more static and rigid pre-degradation aggregates. In addition, the corresponding weight fraction, $w_4$, increased significantly ($p=0.002$) from 89.6% in the non-degraded explants to 93.1% in the severely degraded explants. However, there was no significant difference between the bulk water pool sizes in non-degraded and mildly degraded explants.

Further analysis with component relaxation times was not undertaken; instead, component weight fractions were used for MLR and SVR as more direct measures of macromolecular content.

**Univariate and Multivariate Models Relating MR Parameters to Biomechanical Properties**

Univariate regressions showed that $w_2$, assigned to PG-bound water, correlated positively with both dynamic and equilibrium compressive stiffness with $r^2$ values of 0.63 and 0.55 respectively (Figure 3), while the bulk water fraction, $w_4$, exhibited negative but weak correlations, with $r^2$ values of 0.16 and 0.14 respectively. These findings are consistent with increasing stiffness with an increase in macromolecular content and a concomitant decrease in hydration. However, neither $w_1$, provisionally assigned to collagen, nor $w_3$, assigned to a different proteoglycan fraction, correlated with dynamic stiffness or equilibrium stiffness. Moreover, the two PG fractions, $w_2$ and $w_3$, were only weakly correlated ($r^2 = 0.24$, data not shown), suggesting that they represent different PG moieties with distinct configurations.

Matrix proteoglycan concentration is known to modulate compressive stiffness while collagen influences the tensile stiffness of the cartilage matrix (40). The collagen-bound water fraction, $w_1$, would therefore not be expected to correlate significantly with compressive stiffness. However, we interpret the lack of correlation between $w_3$ and mechanical stiffness to be due to the structure of the macromolecules in this compartment.

Correlations between predicted and measured stiffness were established from the univariate regressions (Figure 4). The best univariate predictors of stiffness were $w_2$, with $r^2=0.55$ for equilibrium stiffness ($p<0.01$) and $r^2=0.6$ for dynamic stiffness ($p<0.01$). The substantial deviation of the slopes from unity and the large intercepts indicate the limited ability of the univariate regression to accurately predict matrix mechanical stiffness.

The MLR analysis performed better than the univariate regression analysis in predicting the samples’ stiffness (Fig. 5). The most accurate four models (all $p<0.01$) were formed from
the following variable sets: \([w_2, w_3], [w_2, w_4], [w_1, w_2, w_3] \) and \([w_1, w_2, w_4] \). The MLR model combining all 4 weight fractions was excluded due to the constraint \(w_1 + w_2 + w_3 + w_4 = 1 \). The best predictor of both equilibrium and dynamic stiffness was the model constructed from \([w_1, w_2, w_3], [w_2, w_4], \) and \([w_1, w_2, w_4] \) to predict equilibrium stiffness had \(r^2 \) values within 4\% of that for \([w_1, w_2, w_3] \). We interpret these small differences as indicating that all four models have similar abilities in predicting equilibrium stiffness. Likewise, the models constructed from \([w_2, w_3], [w_2, w_4], \) and \([w_1, w_2, w_4] \) to predict dynamic stiffness had \(r^2 \) values within 4\% of that for \([w_1, w_2, w_3] \). These results indicate that the addition of \(w_1 \) in \([w_1, w_2, w_3] \) or in \([w_1, w_2, w_4] \) did not significantly improve the predictive abilities of the linear regression models, \([w_2, w_3] \) and \([w_2, w_4] \). We interpret these results, which indicate that all four models have similar ability to predict equilibrium and dynamic stiffness, as reflecting a complex non-linear relationship between compartment sizes and mechanical stiffness.

SVR results are shown in Fig. 6. The parameter combination \([w_2, w_3] \) was the best predictor of dynamic stiffness with \(r^2 = 0.8 \) and slope 0.85 (p<0.01). However, the parameter combination with the highest prediction accuracy for equilibrium stiffness was \([w_1, w_2, w_3] \) with \(r^2 = 0.90 \) and slope 0.910 (p<0.01). These slopes were considerably closer to unity and the intercepts closer to zero than those obtained from univariate regression and the MLR analyses, indicating the substantial improvement resulting from use of SVR.

**Discussion**

Previous studies have shown that multiexponential transverse relaxation analysis can be used in conjunction with support vector regression analysis to predict proteoglycan content and degree of maturation of engineered cartilage (23,25). Cartilage proteoglycans largely consist of a core protein with two predominant glycosaminoglycan side chains, chondroitin sulfate (CS) and keratan sulfate (KS) (4), with a larger proportion of CS than KS(41). The greater length of CS chains as compared to KS would be expected to result in comparatively greater mobility (4,42) and therefore longer transverse relaxation times of associated MR-visible resonances. Based on these mobility considerations and the relatively greater weight fraction found for w_3, we previously provisionally assigned the proteoglycan fractions w_2 and w_3 to KS-enriched and CS-enriched PGs respectively (23). The results of the current study are consistent with this, with w_3 having been found to be larger than w_2 and T_{2,3} significantly longer than T_{2,2}. The differences in the post-degradation behavior of w_2 and w_3, with w_2 but not w_3 decreasing significantly with severe degradation, further indicates a structural difference between the corresponding macromolecules. The diffusion of CS from the extracellular matrix after enzymatic treatment may be hampered by greater steric hindrance than for KS, resulting in a relatively greater preservation of weight fraction. Moreover, the water binding capacity of PG moieties increases with the length of GAG chains, consistent with the relatively insignificant decrease in w_3 with degradation. Nonetheless, for either moiety, detachment of the PG monomer from the hyaluronic acid backbone by trypsin would be expected to decrease matrix stiffness, even without changes in compartment water fractions. The lack of correlation between w_3 and mechanical stiffness may therefore result from the complex relationship between macromolecular concentration and structure.
Contradictory data has been published regarding the presence of multiple relaxation components in cartilage. Xia et al. (43) found monoexponential transverse relaxation in BNC. Further work attributed this result to the specifics of sample handling, and in particular the analysis by Xia et al. of frozen-then-thawed samples; this and related considerations regarding the presence or absence of multiexponential decay in BNC has been extensively discussed (44).

As expected, experimentally-measured total water content correlated significantly with both dynamic and equilibrium stiffness (Fig. 2). There was a weak but significant correlation \( r^2 = 0.13, p<0.01 \) between the experimentally-measured water fraction and the MR-derived mobile (bulk) water fraction, \( w_4 \) (data not shown). This is likely due in part to inter-compartmental chemical exchange modulating apparent fraction size. Consistent with this decoupling of \( w_4 \) and biochemically-determined water, MR-determined mobile water fraction alone, \( w_4 \), was a poor predictor of mechanical stiffness (Figure 3). Nonetheless, SVR analysis showed that the parameter combination \([w_2, w_4]\) \( r^2=0.803, \) Figure 6) performed considerably better than \( w_2 \) alone \( r^2=0.63, \) Figure 4) in predicting dynamic stiffness. These results suggest that although \( w_4 \) may not directly reflect matrix water content, it does play a role in determining the dynamic stiffness of BNC.

Mechanical properties of articular cartilage are determined by both composition and macromolecular structure and organization (45). Using BNC as a model represents a significant simplification, allowing us to investigate the macromolecular compositional contribution to biomechanics. This has enabled us to use multivariate modeling of water fractions to predict mechanical properties in a relatively homogeneous isotropic tissue.

Based on the success of this work, further investigation of articular cartilage using this approach is warranted. Such investigation will extend the present analysis in two important ways. First, the mechanical properties of articular cartilage are highly dependent not only on composition, as is the case for the present analysis of BNC, but also on tissue architecture. Second, the architecture of articular cartilage has a strong effect on transverse relaxation properties themselves. Thus, while the current work demonstrates that our approach can be applied to cartilage tissue, it is clear that the details of the results themselves will in all likelihood differ between BNC and articular cartilage.

We established nonlinear regression relationships using SVR to account for the complex relationship between water component fractions and macromolecular concentration, as well as between these fractions and mechanical properties. Our results indicate that the parameter combination, \([w_2, w_3]\), was the best predictor of dynamic stiffness (Figure 6), consistent with the known influence of cartilage proteoglycans on matrix biomechanics. Furthermore, the finding that \( w_3 \) did not decrease significantly with PG loss from the matrix suggests that \( w_3 \) may be a function of both PG quantity and structure, both of which would influence mobility. Inclusion of \( w_3 \) in the SVM regression parameter set substantially increased the prediction accuracy of the model over the univariate linear model based on \( w_2 \) alone. This is consistent with previous studies showing that mechanical stiffness is a function of both the quantity and configuration of the PG macromolecules (46). Additionally, the parameter combination \([w_2, w_4]\) performed as well as \([w_2, w_3]\) in predicting dynamic stiffness. The
mobile water fraction, \( w_4 \), may be an indirect measure of PG content within the tissue. We found that \( w_4 \), the bulk water fraction, correlated negatively with both PG-bound water fractions, \( w_3 \) and \( w_2 \). In other words, as the proteoglycan content of the cartilage samples decreased, the samples’ water content increased. This finding is consistent with the increase in hydration found in degraded cartilage in vitro and in osteoarthritic cartilage in vivo.

The SVR parameter combinations \([w_1, w_2, w_3]\) and \([w_1, w_3, w_4]\) were found to be the best predictors of equilibrium stiffness (Figure 6), outperforming \([w_2, w_3]\) \( (r^2=0.6, \text{slope}=0.5) \), and \([w_3, w_4]\) \( (r^2=0.52, \text{slope}=0.5) \) (data not shown). Inclusion of \( w_1 \) in both models substantially improved prediction accuracy. These results suggest that \( w_1 \) plays a role in modulating the equilibrium stiffness of the cartilage matrix. Models incorporating \( w_3 \) or \( w_4 \) showed the best prediction accuracy. It is evident that the mobile water fraction, \( w_4 \), like \( w_3 \) is influenced by matrix structure and thus incorporation of this variable improves predictive capability.

While this approach has been shown to be of substantial value, application to clinical research will require significant effort. \( T_2 \) relaxation within human articular cartilage may be too rapid to permit measurement of an adequate number of echoes for multiexponential analysis. A promising alternative may instead be to evaluate \( T_2^* \) \( (47,48) \). Although \( T_2^* \) is shorter than \( T_2 \), the available TE for a \( T_2^* \)-sensitive gradient echo sequence is much shorter than that available for a spin echo sequence. This should permit measurement of a sufficient number of echoes for multiexponential analysis. Efforts are currently underway to develop this on a clinical 3T MR scanner.

Recent work has applied the mcDESPOT sequence \( (49) \) to the measurement of multiexponential \( T_2 \) in the cartilage of normative human subjects \( (50) \). This may be a promising approach to evaluate cartilage status clinically as further developments are undertaken to demonstrate its reliability.

\( T_2 \) exhibits a strong dependence on macromolecular organization and therefore, in the case of human joints, a dependence on orientation with respect to the main magnetic field. As a result of this, as well as compositional differences, relaxation times within articular cartilage are significantly shorter than those in BNC. These differences will render interpretation of the mutiexponential relaxometry results and assignments of the water compartments in articular cartilage more challenging than in BNC.

In summary, we used the results of multiexponential transverse relaxation time analysis as inputs into a multivariate SVR to determine the extent to which the mechanical properties of native cartilage can be predicted noninvasively in control and degraded BNC. Using conventional univariate and multivariate linear regression analysis, macromolecular component fractions were shown to be moderately good predictors of mechanical stiffness, reflecting the well-established relationships between biochemically-derived matrix composition and material properties. However, the prediction accuracy of the models was significantly improved using SVR analysis, reflecting the complex relationship between these water compartments and cartilage material properties. This approach may be of great value in noninvasive determination of the mechanical quality of native and engineered
cartilage during ex-vivo development and, with further development, in the assessment of mechanical integrity of repair tissue in vivo after transplantation.

Acknowledgments

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Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG</td>
<td>Proteoglycan</td>
</tr>
<tr>
<td>sGAG</td>
<td>sulfated glycosaminoglycan</td>
</tr>
<tr>
<td>T₁</td>
<td>longitudinal relaxation time</td>
</tr>
<tr>
<td>T₂</td>
<td>transverse relaxation time</td>
</tr>
<tr>
<td>ADC</td>
<td>apparent diffusion coefficient</td>
</tr>
<tr>
<td>MR</td>
<td>magnetic resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>TE</td>
<td>echo time</td>
</tr>
<tr>
<td>CS</td>
<td>chondroitin sulfate</td>
</tr>
<tr>
<td>KS</td>
<td>keratan sulfate</td>
</tr>
<tr>
<td>SVM</td>
<td>support vector machine</td>
</tr>
<tr>
<td>SVR</td>
<td>support vector regression</td>
</tr>
<tr>
<td>MLR</td>
<td>multiple linear regression</td>
</tr>
<tr>
<td>BNC</td>
<td>bovine nasal cartilage</td>
</tr>
<tr>
<td>NNLS</td>
<td>non-negative least squares</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>MPa</td>
<td>mega pascal</td>
</tr>
<tr>
<td>kPa</td>
<td>kilo pascal</td>
</tr>
</tbody>
</table>

References


Figure 1.
Representative stress-strain relationship for control cartilage. Note the linearity across the range of measurement, permitting calculation of elastic moduli.
Figure 2.
Relationship between matrix biomechanical stiffness and biochemical components. A) Correlation between dynamic stiffness and water content, B) correlation between equilibrium stiffness and water content, C) correlation between dynamic stiffness and sGAG concentration and D) correlation between equilibrium stiffness and sGAG concentration. As seen, matrix water content correlated negatively, while sGAG concentration correlated positively, with cartilage biomechanical stiffness.
Figure 3.
Linear regression analysis using MR-derived component fractions as independent variables and biomechanical parameters as dependent variables. Correlations between: A) dynamic stiffness and \( w_2 \), B) equilibrium stiffness and \( w_2 \), C) dynamic stiffness and \( w_4 \) and D) equilibrium stiffness and \( w_4 \). As discussed in the text, \( w_2 \) and \( w_3 \) were assigned to PG-associated water fractions, while \( w_4 \) was assigned to the bulk water fraction. As shown in the figure, \( w_2 \) was the best predictor of matrix biomechanical stiffness in cartilage.
Figure 4.
Correlation (all p < 0.01) between predicted biomechanical stiffness parameters obtained from univariate linear regression analysis incorporating the component fractions and experimentally-measured biomechanical stiffness. Correlations between measured and predicted equilibrium stiffness based on A) $w_2$ and C) $w_4$; correlations between measured and predicted dynamic stiffness based on B) $w_2$ and D) $w_4$. $w_2$ was the best predictor of matrix biomechanical stiffness in cartilage.
Figure 5.
Correlations (all $p < 0.01$) between predicted biomechanical stiffness parameters obtained from multiple linear regression analysis incorporating the indicated component fractions, and experimentally-measured biomechanical stiffness. A) Correlation between measured and predicted dynamic stiffness, and B) correlation between measured and predicted equilibrium stiffness based on a linear combination of $w_1, w_2, w_3$. This combination was the best predictor of both biomechanical stiffness parameters in cartilage explants.
Figure 6.
Correlations (all p < 0.01) between predicted biomechanical stiffness parameters obtained from support vector regression analysis incorporating the indicated component fractions, and experimentally-measured biomechanical stiffness. Correlations between measured and predicted dynamic stiffness based on A) \([w_2, w_4]\) and B) \([w_2, w_3]\). Correlations between measured and predicted equilibrium stiffness based on C) \([w_1, w_3, w_4]\) and D) \([w_1, w_2, w_3]\).
As seen, \([w_2, w_3]\) was the best predictor of dynamic stiffness while \([w_1, w_2, w_3]\) was the best predictor of equilibrium stiffness in cartilage explants.
Table 1

Effect of enzymatic degradation on the biochemical composition and biomechanical properties of cartilage explants

<table>
<thead>
<tr>
<th>Treatment</th>
<th>sGAG, μg/mg ww</th>
<th>Water, %</th>
<th>Equilibrium modulus, MPa</th>
<th>Dynamic modulus, MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>103.6±37.0</td>
<td>77.9±6.3</td>
<td>1.71±1.10</td>
<td>15.3±6.7</td>
</tr>
<tr>
<td>Mild degradation</td>
<td>54.3±21.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.1±2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.05±0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.2±3.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Severe degradation</td>
<td>8.2±2.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>89.5±1.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.015±0.006&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.89±0.25&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

sGAG concentration significantly decreased with enzymatic degradation, with the severely degraded explants having the lowest sGAG content. Similarly, both equilibrium and dynamic stiffness decreased significantly with degree of degradation, with severely degraded explants having the lowest equilibrium and dynamic stiffness.

<sup>a</sup> significantly different from control (non-degraded) explants;

<sup>b</sup> significantly different from mildly degraded explants; p<0.05
Table 2

Accuracy and precision in component relaxation times and fraction sizes determined from simulations performed using experimentally-measured compartmental $T_2$ values and fraction sizes as input values

<table>
<thead>
<tr>
<th></th>
<th>$T_{2,1}$ (ms)</th>
<th>$T_{2,2}$ (ms)</th>
<th>$T_{2,3}$ (ms)</th>
<th>$T_{2,4}$ (ms)</th>
<th>$w_1$</th>
<th>$w_2$</th>
<th>$w_3$</th>
<th>$w_4$</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Input</td>
<td>0.14</td>
<td>2.6</td>
<td>33.9</td>
<td>128.1</td>
<td>0.031</td>
<td>0.028</td>
<td>0.04</td>
<td>0.896</td>
</tr>
<tr>
<td>Accuracy, %</td>
<td>−7.0</td>
<td>−3.0</td>
<td>−7.8</td>
<td>−0.2</td>
<td>8.1</td>
<td>−0.5</td>
<td>−10.3</td>
<td>0.5</td>
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<tr>
<td>Precision, %</td>
<td>3.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.006</td>
<td>4.7</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Mild degradation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Input</td>
<td>0.15</td>
<td>3.2</td>
<td>38.8</td>
<td>148.8</td>
<td>0.029</td>
<td>0.025</td>
<td>0.039</td>
<td>0.910</td>
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<tr>
<td>Accuracy, %</td>
<td>−4.9</td>
<td>−1.4</td>
<td>−2.8</td>
<td>−0.07</td>
<td>3.2</td>
<td>−0.5</td>
<td>−4.4</td>
<td>−0.1</td>
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<td>Precision, %</td>
<td>6.4</td>
<td>0.5</td>
<td>0.4</td>
<td>0.0001</td>
<td>5.3</td>
<td>0.3</td>
<td>0.6</td>
<td>0.2</td>
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<tr>
<td>Severe degradation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Input</td>
<td>0.136</td>
<td>3.2</td>
<td>46.9</td>
<td>194.3</td>
<td>0.025</td>
<td>0.012</td>
<td>0.034</td>
<td>0.931</td>
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<tr>
<td>Accuracy, %</td>
<td>−6.2</td>
<td>−3.1</td>
<td>−4.1</td>
<td>−0.03</td>
<td>5.8</td>
<td>0.8</td>
<td>−6.4</td>
<td>−0.1</td>
</tr>
<tr>
<td>Precision, %</td>
<td>7.8</td>
<td>1.3</td>
<td>0.5</td>
<td>0.008</td>
<td>9.4</td>
<td>0.7</td>
<td>0.6</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Accuracy describes the percent difference between fitted and simulated $T_2$ values and weights, while precision describes the coefficient of variation of the fitted $T_2$ values and weights over 100 different noise realizations. All component relaxation times and fraction sizes met the admissibility criteria. Accuracy and precision values were all within 10.3% of input simulation values.
Table 3

Multiexponential \( T_2 \) analysis of control, mildly degraded and severely degraded bovine nasal cartilage showing mean fraction sizes and relaxation times of each water compartment

<table>
<thead>
<tr>
<th></th>
<th>( w_1 )</th>
<th>( T_{2,1} ) (ms)</th>
<th>( w_2 )</th>
<th>( T_{2,2} ) (ms)</th>
<th>( w_3 )</th>
<th>( T_{2,3} ) (ms)</th>
<th>( w_4 )</th>
<th>( T_{2,4} ) (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.03±0.007</td>
<td>0.14±0.03</td>
<td>0.029±0.007</td>
<td>2.6±1.1</td>
<td>0.04±0.007</td>
<td>33.9±7.9</td>
<td>0.89±0.03</td>
<td>128.1±41.7</td>
</tr>
<tr>
<td>Mild degradation</td>
<td>0.029±0.007</td>
<td>0.15±0.03</td>
<td>0.025±0.005</td>
<td>3.2±0.8</td>
<td>0.039±0.01</td>
<td>39.3±4.7</td>
<td>0.91±0.02</td>
<td>148.1±19.4</td>
</tr>
<tr>
<td>Severe degradation</td>
<td>0.025±0.005</td>
<td>0.14±0.02</td>
<td>0.012±0.006</td>
<td>3.2±2.9</td>
<td>0.034±0.02</td>
<td>46.8±6.8</td>
<td>0.93±0.03</td>
<td>194.3±47.1</td>
</tr>
</tbody>
</table>

\( T_{2,4} \) (with \( w_4 \)) was assigned to the bulk water fraction, \( T_{2,3} \) (with \( w_3 \)) and \( T_{2,2} \) (with \( w_2 \)) to the PG-associated compartment, and \( T_{2,1} \) (with \( w_1 \)) was assigned to collagen. Overall, both \( T_{2,3} \) and \( T_{2,4} \) increased significantly with both mild and severe degradation. For the component fractions, \( w_1 \) and \( w_2 \) decreased while \( w_4 \) increased with severe degradation.

\( a \) significantly different from control (non-degraded) explants;

\( b \) significantly different from mildly degraded explants; \( p<0.05 \)