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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 $\mu\text{g}/\text{mg}$ ww, 1.71 ± 1.10 MPa, and 15.3 ± 6.7 MPa in controls to 8.25 ± 2.4 $\mu\text{g}/\text{mg}$ ww, 0.015 ± 0.006 MPa and 0.89 ± 0.25 MPa, 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

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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 , $T_{1\rho}$, 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 T₂ relaxation times, and the same data were used for multiexponential T₂ analysis.

Fitting of T₂ Relaxation Data

Regularized non-negative least squares (NNLS) was used for the multiexponential T₂ analysis with 80 T₂ bins logarithmically spaced over the interval [0.1, 3000] ms using MATLAB (MathWorks, Natick, MA) as previously described (19). This method makes no *a priori* assumptions about the number of relaxation components to be fit. The NNLS finds a histogram of amplitudes associated with each T₂ value. T₂ distributions were regularized to achieve a chi-squared of 101% of the non-regularized fit (35) using a minimum energy constraint. Component T₂ 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 × 5% strain and 2 × 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:

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

where $y(n*TE)$ represents the amplitude of the n th echo measured at $n*TE$, 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 σ . The signal-to-noise ratio (SNR) was defined as y_0/σ , 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 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 ($\alpha w_1 + \beta w_2 +$

$c, \alpha w_1 + \gamma w_3 + c, \alpha w_1 + \delta w_4 + c, \beta w_2 + \gamma w_3 + c, \beta w_2 + \delta w_4 + c, \gamma w_3 + \delta w_4 + c, \alpha w_1 + \beta w_2 + \gamma w_3 + c, \alpha w_1 + \beta w_2 + \delta w_4 + c, \beta w_2 + \gamma w_3 + \delta w_4 + c, \alpha w_1 + \gamma w_3 + \delta w_4 + c, \alpha w_1 + \beta w_2 + \gamma w_3 + \delta w_4 + c$), 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 $\pm$ standard deviation. Biochemical data (sGAG), MR parameters (component T_2 s 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 $\mu\text{g}/\text{mg}$ ww in non-degraded explants to 54.3 ± 21.6 $\mu\text{g}/\text{mg}$ ww ($p < 0.0001$) and 8.25 ± 2.4 $\mu\text{g}/\text{mg}$ 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 ± 0.70 MPa in mildly degraded, explants. In addition, equilibrium stiffness decreased by 99% ($p < 0.0001$) to 0.015 ± 0.006 MPa 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 ± 3.5 MPa 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 $[w_1, w_2, w_3]$, with $r^2 = 0.62$ and $r^2 = 0.71$, respectively. Models constructed from $[w_2, w_3]$, $[w_2, w_4]$, 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 $[w_2, w_3]$ ($r^2=0.6$, slope =0.5), and $[w_3, w_4]$ ($r^2=0.52$, 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 multiexponential 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.

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Abbreviations

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

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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

Treatment	sGAG, µg/mg ww	Water, %	Equilibrium modulus, MPa	Dynamic modulus, MPa
Control	103.6±37.0	77.9±6.3	1.71±1.10	15.3±6.7
Mild degradation	54.3±21.6 ^a	84.1±2.6 ^a	1.05±0.70 ^a	10.2±3.5 ^a
Severe degradation	8.25±2.4 ^{a,b}	89.5±1.2 ^{a,b}	0.015±0.006 ^{a,b}	0.89±0.25 ^{a,b}

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.

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

^b 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 T₂ values and fraction sizes as input values

		T _{2,1} (ms)	T _{2,2} (ms)	T _{2,3} (ms)	T _{2,4} (ms)	w ₁	w ₂	w ₃	w ₄
Control	Input	0.14	2.6	33.9	128.1	0.031	0.028	0.04	0.896
	Accuracy, %	-7.0	-3.0	-7.8	-0.2	8.1	-0.5	-10.3	0.5
	Precision, %	3.5	0.4	0.3	0.006	4.7	0.2	0.3	0.2
Mild degradation	Input	0.15	3.2	38.8	148.8	0.029	0.025	0.039	0.910
	Accuracy, %	-4.9	-1.4	-2.8	-0.07	3.2	-0.5	-4.4	-0.1
	Precision, %	6.4	0.5	0.4	0.0001	5.3	0.3	0.6	0.2
Severe degradation	Input	0.136	3.2	46.9	194.3	0.025	0.012	0.034	0.931
	Accuracy, %	-6.2	-3.1	-4.1	-0.03	5.8	0.8	-6.4	-0.1
	Precision, %	7.8	1.3	0.5	0.008	9.4	0.7	0.6	0.2

Accuracy describes the percent difference between fitted and simulated T₂ values and weights, while precision describes the coefficient of variation of the fitted T₂ 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 T₂ analysis of control, mildly degraded and severely degraded bovine nasal cartilage showing mean fraction sizes and relaxation times of each water compartment

	w ₁	T _{2,1} (ms)	w ₂	T _{2,2} (ms)	w ₃	T _{2,3} (ms)	w ₄	T _{2,4} (ms)
Control	0.032±0.007	0.14±0.03	0.029±0.007	2.6±1.1	0.040±0.01	33.9±7.9	0.896±0.03	128.1±41.7
Mild degradation	0.029±0.007	0.15±0.03	0.025±0.005	3.2±0.8	0.039±0.01	39.3±4.7 ^a	0.910±0.02	148.1±19.4
Severe degradation	0.025±0.005 ^a	0.14±0.02	0.012±0.006 ^a	3.2±2.9	0.034±0.02	46.8±6.8 ^{a,b}	0.931±0.03 ^a	194.3±47.1 ^{a,b}

T_{2,4} (with w₄) was assigned to the bulk water fraction, T_{2,3} (with w₃) and T_{2,2} (with w₂) to the PG-associated compartment, and T_{2,1} (with w₁) 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, w₁ and w₂ decreased while w₄ increased with severe degradation.

^a significantly different from control (non-degraded) explants;
^b significantly different from mildly degraded explants; p<0.05