Multiparametric MRI Characterization of Degradation in Bovine Nasal Cartilage

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Introduction: A variety of MR parameters, including T_1, T_2, $T_{1\rho}$, magnetization transfer (MT) ratio and apparent rate (k_m), self-diffusion coefficient, and T_1 in the presence of gadolinium, have been applied to the characterization of cartilage [1]. These investigations have all met with some limited success, in that each parameter was found to be somewhat sensitive to cartilage pathology and, in certain cases, also exhibited some specificity for particular cartilage matrix components. However, there is a large degree of overlap in mean values of each parameter for e.g. normal and osteoarthritic cartilage. We sought to extend these mono-parametric analyses by examining changes in *pairs* of MR parameters upon pathomimetic degradation of large groups of cartilage samples. Therefore, rather than evaluating mean parameters individually, we represented the MRI data for the ensemble of samples as a cluster of points in two-dimensional space, with axes consisting of T_1, T_2, and k_m taken pairwise. Each point cluster was characterized by an *error ellipse* with calculated values of centroid position, area and orientation. Finally, we examined the translational and rotational motion and deformation of each cluster and its corresponding ellipse resulting from digestion of tissue collagen and proteoglycan components by collagenase and trypsin, respectively.

Materials and Methods: *Sample Preparation*. Bovine nasal cartilage (BNC) disks of diameter 8 mm were excised from the nasal septa of 5-6 month-old calves. Samples were first imaged in DPBS buffer at pH 7.5 ± 0.1. Degradation was then performed by incubating two different sets of samples for 24 hours with 1mg/ml trypsin (N = 84) or 30 units/ml collagenase type II (N = 66) at 37 ºC in a 5% CO_2 atmosphere. *MRI Measurements*. Imaging was performed with a 9.4T/105-mm Bruker DMX spectrometer at a sample temperature of 4.0 ± 0.1 ºC. MRI parameters included BW = 50 kHz, NEX = 2, FOV = 4.0 × 1.5 cm, 0.5 mm slice thickness, and 256 × 128 matrix size. A 64-echo CPMG pulse sequence with TE/TR = 12.8 ms/5s was used for T_2 measurements. A progressive saturation spin-echo pulse sequence with TE = 12.8 ms and TR varying from 100 ms to 15 s was employed to measure T_1 values. MT data were obtained using the spin-echo sequence (TE/TR = 12.8 ms/5 s) preceded by a 6 kHz off-resonance saturation pulse of amplitude B_1 = 12 μT and width incremented from 0.1 to 4.6 s. *Data Analysis:* For each weighted image, signal intensity was averaged over all pixels in a region of interest (ROI) covering each entire BNC disk. These average pixel intensities were fit to three-parameter monoexponential functions to yield T_1, T_2, MT ratio (MTR), T_{1sat} and $k_m = MTR/T_{1sat}$ [2]. Prior to plotting, MRI data were normalized to unit variance, and the error ellipse, a contour defined by an increase in χ^2 of one unit from its minimum, was calculated from the covariance matrix for each pair of variables using MATLAB.

Results: Upon either trypsin or collagenase digestion, mean T_1 and T_2 increased while k_m was diminished (Table 1). The bivariate scatter plots showing point clusters and their error ellipses are presented in Fig. 1. Figs. 1A and 1B illustrate the results for bivariate analysis using T_1 and k_m. Fig. 1A (trypsin): The angle between the horizontal axis and the semimajor axis of the error ellipse was 158° initially and decreased to 135° with degradation; this rotation was accompanied by a 41% reduction in cluster area. Fig. 1B (collagenase): The error ellipse rotated from an angle of 152° to 135° with an increase in area by 10% upon collagenase treatment. Figures 1C and 1D illustrate the results for analysis using T_2 and k_m. Fig. 1C (trypsin): The error ellipse rotated from 154° to 108° and lost 37% of its area. Fig. 1D (collagenase): The error ellipse rotated by 23°, from 152° to 129°, and lost 19% in area. Figures 1E and 1F illustrate the results for analysis using T_1 and T_2. Fig. 1E (trypsin): The error ellipse rotated from 72° to 82°, with a gain of 38% in area. Fig. 1F (collagenase): The error ellipse rotated from 65° to 50°, with a gain in area of 24%.

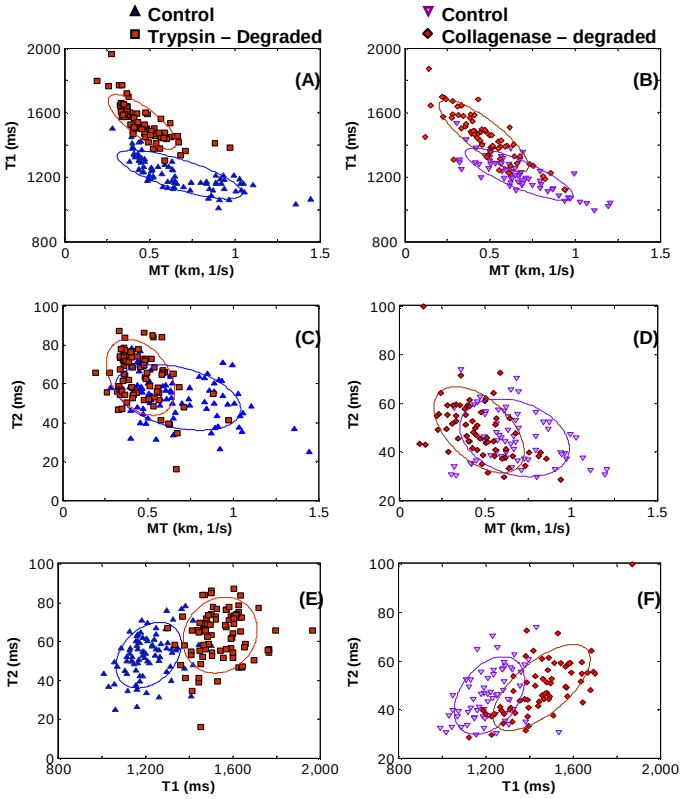

Figure 1. Scatter plots of MR parameter distributions and corresponding error ellipses before and after enzymatic treatments.

Discussion: As expected, both trypsin-induced PG depletion and collagenase-induced collagen damage resulted in increases in mean T_1 and T_2 values and a decrease in mean k_m [3]. A more complete account of parameter changes was provided by the multivariate covariance analysis. An initial observation was that the error ellipses maintained their general shapes and orientations upon digestion with either enzyme. Additionally, while cluster motion upon digestion was similar for the two enzymes, certain differences were apparent. In the T_1 vs k_m plots, treatment with trypsin resulted in a denser cluster, while collagenase treatment led to dispersion of the cluster points and an increase in error ellipse area. We note that the pre- and post-digestion clusters were well-separated for trypsin but exhibited extensive overlap for collagenase digestion. The T_2 vs k_m plots indicated that trypsin resulted in a larger rotation of the error ellipse, accompanied by area reduction, while the primary effect of collagenase was to translate the cluster along the k_m dimension, with only a small change in orientation. For both enzymes, there was a substantial overlap between the pre- and post-digestion clusters. In the T_1-T_2 scatter plots, collagenase digestion led to a more elongated cluster morphology than did trypsin treatment. As was the case for T_1 vs k_m, trypsin digestion yielded a more complete separation of clusters than did collagenase. We conclude that examination of bivariate clusters in terms of shape, size, and orientation distinguishes between the selective degradation effects of trypsin and collagenase, and permits analysis of pathomimetic effects in cartilage superimposed upon underlying sample heterogeneity.

Table 1. The averages of MR parameters in non- and digested BNC samples

	Trypsin		Collagenase	
	Control	Degraded	Control	Degraded
T_1 (ms)	1216 ± 100	1543 ± 112	1218 ± 107	1457 ± 147
T_2 (ms)	52.9 ± 11.2	63.2 ± 13.0	45.7 ± 10.6	49.0 ± 11.7
MT (k_m, s^{-1})	0.67 ± 0.24	0.46 ± 0.13	0.67 ± 0.21	0.46 ± 0.17

References: 1. Mosher TJ, et al. Semin Musculoskelet Radiol 2004; 8:355-368; **2.** Hajnal JV, et al. J Comput Assist Tomogr 1992;16(1):7-18; **3.** Laurent D, et al. Magn Reson Imaging 2001;19(10):1279-1286.