Multiexponential T_2 Analysis in Cartilage Degraded Using Different Enzymatic Protocols

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Introduction: Osteoarthritis (OA) is a highly prevalent and potentially debilitating disease involving degradation and loss of articular cartilage. MR is increasingly used as a sensitive diagnostic modality for OA, but suffers from lack of specificity for matrix components [1]. The initial phases of OA are characterized by disruption and loss of collagen and proteoglycan (PG), the two major matrix components of cartilage. These macromolecules are associated with water within compartments of varying size and mobility. The noninvasive identification of these compartments in normative tissue and in OA remains incomplete, with attempts to interpret monoexponential T_2 values in terms of specific matrix components being inconclusive. Therefore, we sought to improve the specificity for matrix degradation through multiexponential analysis of T_2 relaxation, which has the potential to delineate compartments with varying water fraction and mobility [2]. We applied this approach to cartilage that was degraded by three different enzymatic treatments, targeting different matrix components: trypsin, acting primarily on attachment sites of PG macromolecules; collagenase, acting primarily on collagen, and accordingly also known to permit PG loss; and chondroitinase (Ch AC), acting primarily on glycosaminoglycan (GAG) chondroitin-sulfate side-chains.

Materials and Methods: *Cartilage Sample Preparation.* Bovine nasal cartilage (BNC) disks (diameter = 8 mm) were excised from the nasal septa of 5-6 month-old calves (Green Village Packing, Green Village, NJ). Samples were randomly assigned to control or treatment categories. Treated samples were incubated in either: 1 mg/ml trypsin (Sigma-Aldrich, St. Louis, MO), 30 units/ml collagenase type II (Worthington Biochemical Corp., Lakewood, NJ), or 0.1 units/ml Ch AC (Seikagaku Corp., Tokyo, Japan) at 37 ºC. *T_2 Measurements.* NMR measurements were performed using a 9.4 T Bruker DMX spectrometer with a spectroscopic CPMG pulse sequence with sampling of each echo maximum. Acquisition parameters included: TE/TR = 600μs/10s, 2048 echoes, and NEX = 64. T_2 distributions were resolved using a nonnegative least squares (NNLS) method similar to that described in [3, 4], and compared to monoexponential fits of T_2.

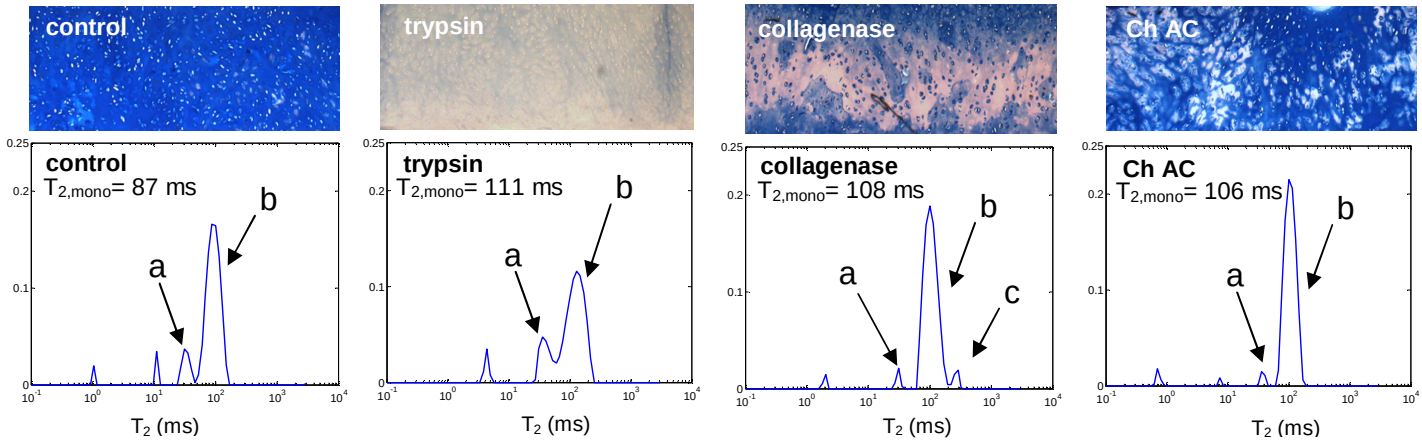

Fig. 1. Upper row: representative alcian blue staining for GAG in control and degraded cartilage. Lower row: corresponding T_2 distributions. Note that the T_2 values of the degraded sample sets were very similar, but the multicomponent T_2 distributions vary substantially.

Results and Discussion: From the results of alcian blue staining (Fig. 1) and biochemical analysis (Table 1), it is evident that each enzyme had a varied effect on GAG content and distribution. While trypsin produced the greatest loss of this macromolecule, collagenase-digested samples also showed regions of moderate GAG loss accompanied by collagen disruption. Monoexponential T_2 values increased comparably for all three degradation protocols (Fig. 1; Table 1), providing very limited specific information related to macromolecular composition. More specific information is, however, provided by the T_2 distribution results, reflecting in water compartment size and mobility. Compartments a and b (Fig. 1) have previously been assigned to water tightly bound to and loosely associated with GAG, respectively. The position and fractional amounts of these components vary substantially with degradation protocol. In Ch AC- and collagenase-treated samples, where GAG loss was relatively moderate and non-uniform, the relative fractional amount of water tightly associated with GAG decreased. However, in trypsin-treated samples, for which GAG loss was greater and spatially uniform, the relative fractional amounts of compartments a and b did not change, while their overall mobility, reflected by increased T_2 values, increased. Furthermore, a new compartment, labeled c, was seen in collagenase samples. The large T_2 of this component is indicative of the emergence of a less restricted water compartment with a relaxation rate more closely resembling that of bulk water in cartilage in which both collagen and PG disruption occurs.

Sample condition	$T_{2,mono}$ (ms)	$T_{2,a}$ (ms)	$T_{2,b}$ (ms)	w_a/w_b	$T_{2,c}$ (ms)	w_c	GAG (mg GAG/ mg w.w.)
Control (n = 4)	82 (5)	31 (1)	86 (7)	0.13 (0.02)	-----	-----	0.161 (0.056)
Trypsin (n = 12)	120 (16)	41 (9)	132 (15)	0.16 (0.07)	-----	-----	0.007 (0.005)
Collagenase (n = 12)	99 (15)	28 (10)	101 (14)	0.05 (0.06)	440 (225)	0.02 (0.02)	0.112 (0.037)
Ch AC (n = 16)	109 (11)	33 (6)	111 (12)	0.03 (0.02)	-----	-----	0.117 (0.026)

Table 1. Multicomponent results for each sample group.

Conclusions: Multiexponential T_2 analysis of control cartilage and of cartilage subjected to three enzymatic degradation protocols showed distinct patterns, consistent with the known actions of the degradative enzymes used. In contrast, while monoexponential T_2 values increased with degradation, there was no enzyme-specificity in this increase. Therefore, multiexponential T_2 analysis demonstrates greatly improved specificity over standard monoexponential T_2 analysis to changes in cartilage matrix, indicating the diagnostic potential of such analysis for detecting cartilage disease.

References: 1. Menezes, N.M., Magn. Reson. Med. 2004; 2. Saab, G., Magn. Reson. Med. 1999; 3. Whittall, K.P., J. Magn. Reson. 1989; 4. Graham, S.J., Magn. Reson. Med. 1996.