Improved Specificity of Cartilage Matrix Assessment Using Multiexponential T2 Parameter Maps with Validation by Fourier-Transform Infrared Spectroscopic Imaging

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Introduction: Detection of cartilage matrix constituents with MRI is an active area of investigation (1), with application to the early diagnosis and potential development of therapeutics for degenerative cartilage disease. Of particular interest is the assessment of proteoglycan (PG) content and distribution. This macromolecule is important in maintaining tissue hydration (2) and mechanical function, and is progressively depleted with tissue degradation (3). Recently, MR methods have been developed that exhibit improved specificity to PG such as dGEMRIC, CEST, and 23N. These require use of contrast agents, specialized pulse sequences, or nonstandard hardware. Here we describe a method for detecting and mapping PG which utilizes a readily-implemented CPMG sequence, already in widespread use for mapping cartilage T_2 values. Sequence design relies upon careful selection of parameters as determined by simulations to permit accurate application of multiexponential analysis to the acquired dataset. Results can then be interpreted in terms of water components associated with matrix macromolecules. We have previously demonstrated improved specificity of this multiexponential T_2 analysis to cartilage matrix composition in bovine nasal cartilage (BNC) (4). Using non-localized CPMG measurements, which permit short TEs (~600 μs), multiple water compartments were identified and assignments were made to compartments which closely reflect water tightly bound to PG and water loosely associated with PG. Unlike the more rapidly-relaxing collagen-associated components identified, these PG-associated fractions are detectable using TEs on the order of ~10 ms, as with standard imaging sequences. Therefore, the goal of the present work is to extend this bulk analysis of cartilage to an imaging experiment allowing for spatially resolved multiexponential T_2 analysis. We applied this approach to BNC taken from animals of different ages and presenting different macromolecular composition. The PG bound water fraction (w_{PG}) was directly compared to results obtained with Fourier-transform infrared spectroscopic imaging (FT-IRIS), which produces quantitative maps of PG content (5).

Table 1. FT-IRIS and MRI

	FT-IRIS PG	FT-IRIS collagen	FT-IRIS PG_{ww}	MRI $T_{2,mono}$	MRI w_{PG}
Young (n = 5)	2953 ± 100	2688 ± 37	3329 ± 155	85 ± 11	0.22 ± .06
Mature (n = 3)	2889 ± 78	3180 ± 79 *	3698 ± 72 *	65 ± 6 *	0.31 ± .04 *

* indicates $p < 0.05$.

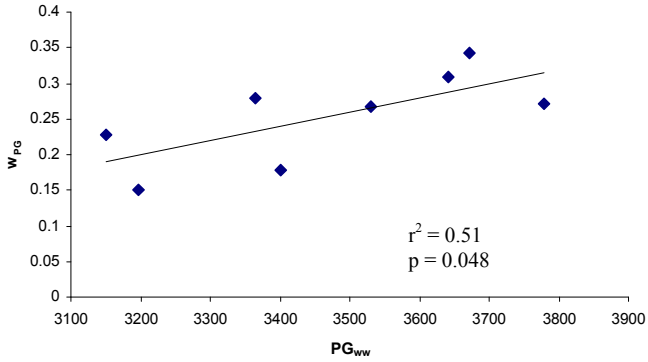

Fig. 1. Magnetization fraction associated with PG bound water (w_{PG}) plotted against FT-IRIS derived PG content normalized using biochemically derived water content (PG_{ww}) for aggregate sample values.

Materials and Methods: *Cartilage Sample Preparation.* BNC plugs (diameter = 6mm) were excised from the nasal septa of young (~3 months old) and mature (~2 years old) cows (Green Village Packing, Green Village, NJ). *T_2 Measurements.* Relaxation data were measured using a single slice multi-echo CPMG imaging sequence with the following acquisition parameters: TE/TR = 5.5ms/5s, 128 echoes, slice thickness = 2mm, FOV = 3x3cm, matrix = 128x128, NEX = 32. T_2 distributions were resolved using a nonnegative least squares (NNLS) method similar to that described in (6), and compared to monoexponential fits of T_2. All fits were performed pixel-by-pixel permitting the generation of parameter maps. *FT-IRIS.* Great care was taken to compare coinciding FT-IRIS sections and MR image slices from each BNC plug. FT-IRIS data consisted of a complete mid-IR spectrum (800-4000cm^{-1}) where PG content was determined from the area under the sugar band from 960-1184cm^{-1}. Since FT-IRIS is performed on histologically prepared tissue sections, PG images from each sample were normalized to their respective biochemically derived water content (PG_{ww}) to permit direct comparison with MR results.

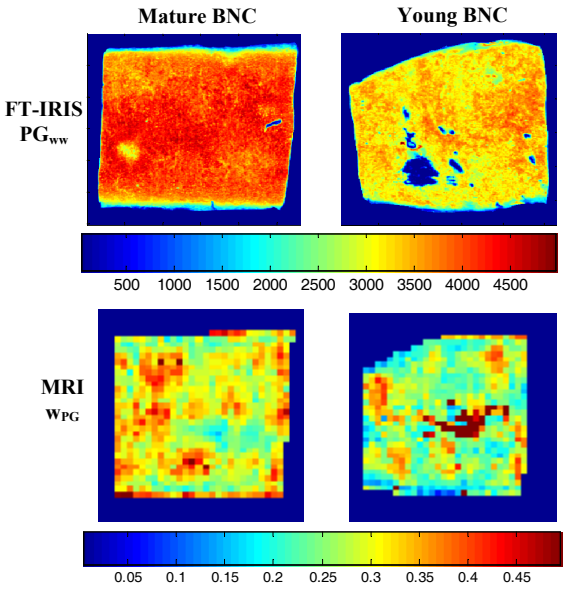

Fig. 2 top - representative FT-IRIS PG_{ww} images; bottom - MRI derived w_{PG} maps; from young and mature BNC.

Results and Discussion: FT-IRIS showed greater PG content (n.s.) and significantly less collagen content in young BNC (Table 1). These relationships were consistent with biochemically derived PG and collagen content per dry weight (data not show). To obtain results that correspond to MRI-derived quantities, FT-IRIS-derived PG content was normalized by water content (PG_{ww}). Results were again consistent with biochemically derived PG content per wet weight (data not shown), with young BNC showing significantly less PG_{ww}, than the tissue from mature BNC. $T_{2,mono}$ was significantly shorter in mature BNC (Table 1), consistent with the lower hydration and collagen per wet weight in these samples (data not shown). Young BNC had significantly lower MR-derived w_{PG}, consistent with FT-IRIS-derived PG_{ww}. Using values averaged over each sample slice, MR-derived w_{PG} showed a significant correlation with FT-IRIS-derived PG_{ww} (Fig. 1), while $T_{2,mono}$ showed no correlation (data not shown). Fig. 2 shows representative PG_{ww} images and w_{PG} maps; results are consistent between these modalities, with both showing less PG in the younger BNC tissue. There is also a spatial correspondence between modalities; in both young and mature samples, regions of greater PG_{ww} also show greater w_{PG}. While the correspondence between these distinct modalities serves to validate our approach to PG mapping using MRI, the correspondence will be improved in further studies through use of thinner MR slices (2mm in Fig. 2) to correspond more accurately to the 6μm slices in the FT-IRIS images. Another factor that limits the correspondence between these two techniques is the difference in sample hydration; sample preparation for FT-IRIS involves histological techniques which affect sample shape and water distribution.

Conclusions: Spatially resolved multiexponential T_2 analysis permits mapping of the PG bound water fraction, w_{PG}, in cartilage, without requiring the use of specialized pulse sequences, hardware, or contrast agents. Results are in good agreement with molecule-specific FT-IRIS-derived PG maps. MR maps of w_{PG} were sensitive to age related variations in PG content, indicating the potential capability of this approach to detect and map PG in degraded cartilage.

References: 1. Nissi M.J., et al. JOR 2004; 2. Urban J. et al., Biorheology 1981; 3. Rippo J. et al., Cells Tissues Organs 2003; 4. Reiter D.A. et al., ISMRM Proc. 2009; 5. Camacho N.P. et al., Biopolymers 2001; 6. Reiter D.A. et al., MRM 2009.