# Optimum Methods for the Preservation of Cartilage Samples in MR Microimaging Studies

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**Introduction** Magnetic resonance imaging (MRI) is widely used as part of complex multi-modality studies of cartilage metabolism and structure. Besides measurements of MRI parameters such as $T_2$, pre- and post-Gd $T_1$, and magnetization transfer (MT) rates, studies may incorporate, for example, gene expression, histology, immunohistochemistry, Fourier transform infrared spectroscopy and imaging, polarized light microscopy, and mechanical testing. Sample handling in such studies can be complex, potentially requiring transportation between facilities and significant periods of storage in addition to lengthy MRI experimental times. While it is of the utmost importance that samples remain stable through the course of these experiments, there is remarkably little published data concerning sample handling to achieve this. Accordingly, this study compares methods intended to stabilize cartilage matrix for MRI studies. We hypothesized that: 1. Long-term storage of cartilage explants in phosphate buffer saline (DPBS) alone will result in substantial changes in $T_2$ and MT, and these changes will be accompanied by matrix compositional changes as determined by biochemical analysis; 2. Addition of protease inhibitors with refrigeration will retard this degradation; and 3. Multiple episodes of freezing and thawing a given sample will further lead to significant changes in MR parameters and biochemical composition.

**Materials and Methods** *Samples:* Four sets of three samples of 8 mm-diameter bovine nasal cartilage (BNC) plugs were inserted into a homebuilt 4-well holder (Fig. 1) for storage and for MRI studies. Samples (N=3 per group) were immersed in one of four solutions (5 ml, pH 7.5): 1. DPBS (diphosphate buffered saline) only; 2. DPBS + protease inhibitor (PI; Sigma); 3. DPBS + 12 mM GM6001 (Chemicon), a potent specific inhibitor of matrix metalloproteases (MMP's), and 4. DPBS + PI + GM6001. One 4-well holder was stored and scanned at +4° C. One 4-well holder was stored at -20° C and scanned, after thawing prior to each MRI examination, at +4° C. MRI scans were repeated periodically over 4 months. Solutions were not replaced between measurement time points. Plugs corresponding to each of these protocols were analyzed with standard biochemical techniques to determine matrix composition.

Fig. 1

*MRI Methods:* All MRI data were acquired at 9.4 T using a Bruker DMX400 spectrometer equipped with 1000 mT/m three-axis microimaging gradients and a 30 mm diameter $^1$H birdcage resonator (Bruker). Geometric parameters included FOV = 3 cm X 1.5 cm, slice thickness = 0.5 mm, 256 X 256 matrix, with read direction parallel to $B_0$ and perpendicular to the cartilage surface. $T_2$, MT ratio, and MT rate were obtained by non-linear least squares fitting of average pixel intensities for each sample in a sequence of weighted images. Minimum TE was 12 ms with 64 echoes collected, TR = 15 sec, and saturation times for MT ranged from 1ms to 4.6 ms in 12 increments with a 6 kHz off-resonance pulse. All 12 samples were scanned simultaneously.

*Statistics:* Data are expressed as percent of initial $T_2$, MT rate ($k_m$), and MT ratio. For each storage protocol, at each time point, $T_2$, $k_m$, and MT ratio results were compared with initial (pre-storage) values via a paired t-test with the Bonferroni correction, with significance taken as $p < 0.05$.

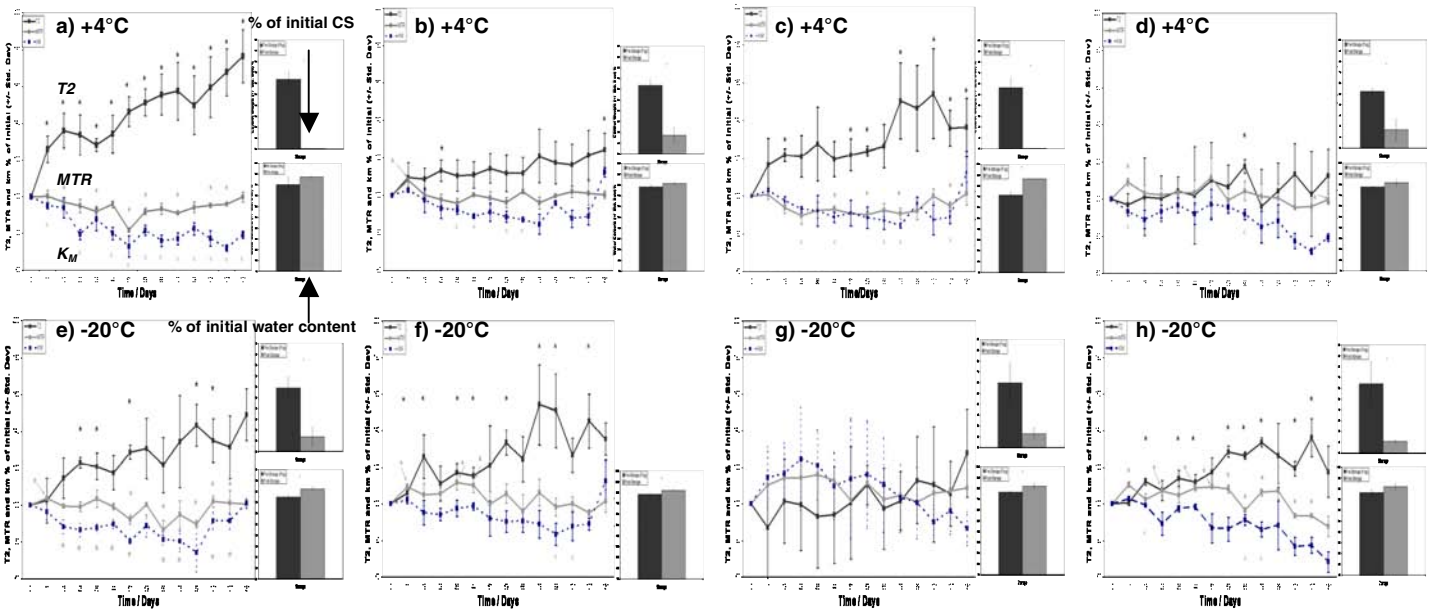


Fig. 2 % Percent of initial $T_2$, MT rate ($k_m$), MT ratio (MTR), Chondroitin Sulfate (CS) and water content – **a),e)** DPBS – **b), f)** DPBS + PI – **c), g)** DPBS + GM6001 – **d), h)** DPBS + PI + GM6001

**Results and Discussion** *Biochemical endpoints:* Proteoglycan content decreased with storage for all bath fluids at both temperatures. Water content was maintained with storage in DBPS at +4°C, DPBS + PI at +4°C, and DPBS + PI + GM6001 at +4°C and at -20°C (Fig. 2).

*MRI endpoints:* With storage at 4°C, $T_2$ increased rapidly with storage time for DPBS and DPBS + GM6001, while MT rate and ratio rapidly decreased. The addition of protease inhibitors at 4°C markedly attenuated changes in initial T2, $k_m$, and MT ratio value. For storage at -20°C, $T_2$ increased rapidly with storage time for DPBS, DPBS + PI, and DPBS+ PI + GM6001, while again MT rate and ratio rapidly decreasd. Samples stored in DPBS + GM6001 at -20°C showed inconsistent results (Fig. 2).

**Conclusions** No storage method prevented loss of proteoglycans upon soaking and refrigeration and/or freezing and thawing. By adding PI at 4°C and GM6001 at -20°C to the storage buffer, however, it is possible to arrest changes in $T_2$. This observation also supports the notion that $T_2$ does not uniquely depend upon proteoglycan content. In addition, the observed increase in $T_2$ with certain storage protocols cannot be explained accounted for by an increase in water content. During short-term storage (up to 2 weeks) at +4°C, $T_2$ changes to a greater extend than does $k_m$, suggesting that proteoglycan loss precedes degradation or loss of collagen. This is consistent with literature observations on the effects of osteoarthritis or pathomimetic enzymatic digestion on cartilage extracellular matrix. The fact that GM6001 alone is not sufficient to stabilize $T_2$ at +4°C suggests that increases in $T_2$ with storage time may be associated with the action of proteolytic enzymes other than MMP's. This is consistent with the interpretation that increases in cartilage $T_2$ mainly reflect proteoglycan loss rather than collagen degradation. In summary, of the protocols investigated, storage of cartilage samples at 4°C with the addition of PI's favors matrix homeostasis.