The Effect of Paraformaldehyde Fixation on the Delayed Gadolinium Enhanced MRI of Cartilage (dGEMRIC) Measurement

Arushi Dugar1,2, Michelle L. Farley1,2,3, Angeline L. Wang1,2, Mary B. Goldring4, Steven R. Goldring2,5, Benjamin E. Bierbaum5, Bryan H. Swain5, David A. Mattingly5, Geoffrey J. Van Flandern2, Daniel Ward2, Deborah Burstein3,1, Martha L. Gray1,2

1Massachusetts Institute of Technology, Cambridge, MA; 2New England Baptist Bone and Joint Institute, Boston, MA; 3Beth Israel Deaconess Medical Center, Boston, MA; 4Hospital for Special Surgery, New York, NY; 5New England Baptist Hospital, Boston, MA

mgray@mit.edu

Introduction: The delayed gadolinium enhanced MRI of cartilage (dGEMRIC) method allows for both a qualitative and quantitative measurement of the spatial distribution of glycosaminoglycan concentration ([GAG]) and has been used as a non-destructive surrogate for toluidine blue or Safranin-O histological staining. Published studies of the validation and subsequent use of dGEMRIC have used unfixed tissue. The question we address in this study is whether the fixation affects the dGEMRIC image. dGEMRIC imaging involves measuring the MRI relaxation parameter T1 in the presence of the ionic contrast agent, Gd(DTPA)2-. The concentration of contrast agent in cartilage varies in proportion to the fixed charge (and thus the GAG) concentration; in turn, T1_Gd depends predominantly on the concentration of contrast agent, according to the relation: 

\[ T1_Gd = \frac{1}{r} T1_Gd \]  

where T1p, T1_Gd = T1 without and with contrast, and r = relaxivity. The fixation process crosslinks tissue proteins, and as such may have some influence on T1p. Indeed, previous studies of non-cartilaginous tissues have found that paraformaldehyde fixation decreases T1 in the brain, liver and spleen [1,2,3]. Fixation should have minimal effect on sites of fixed charge, and thus if hydration is preserved, should have minimal effect on contrast agent concentration. Since, the effect of fixation on these parameters in cartilage is unknown, we compared T1p, T1_Gd, and dGEMRIC measurements of GAG before and after paraformaldehyde fixation to evaluate whether the standard dGEMRIC protocol can be applied to fixed tissue.

Materials and Methods: Sample Preparation: Bovine nasal cartilage and visually intact human articular cartilage (obtained during total joint surgery) were harvested in accordance with institutional approved protocols. Tissue was immediately frozen to -20°C and later thawed to room temperature for studies. Full depth cartilage was cut into plugs 4 mm in diameter with added fiducial markers to aid in image orientation. The wet weight of each plug was measured.

MR Imaging: Samples were equilibrated overnight at 4°C in Hank’s Balanced Salt Solution (HBSS), imaged, and then equilibrated over the following night in HBSS containing 1 mM Gd(DTPA)2- and imaged again. Plugs were imaged individually in a Bruker 8.5T microimaging system using a T1 weighted sequence series with TRs ranging from 100-5000 for T10 and 100-2700 for T1_Gd, and an image slice thickness of 1 mm. One coronal imaging slice was obtained, and the fiducial marks were used to ensure that repeated images of the same plug yielded the same image slice. Maps of T10 and T1_Gd were generated by curve fitting each T1-weighted image. [GAG] maps were computed from T10 and T1_Gd using a previously validated modified Donnan theory[4,5], assuming a relaxivity of 4.6 mM⁻¹s⁻¹.

Fixation: The samples were fixed for 18 hours in 4% paraformaldehyde solution. All imaging and [GAG] measurements were repeated on the fixed plugs.

Statistics: A paired student t-test was performed on each data set using SigmaStat to assess whether there were significant differences (p<0.05).

Results: Paraformaldehyde fixation had no significant effect on the T1p, T1_Gd, or the measure of [GAG] obtained from dGEMRIC (Fig.1), with ratios near 1 for both bovine cartilage (1.03, 1.01, 1.00) and human cartilage (0.96, 1.04 and 1.09). dGEMRIC images before- and after- fixation were qualitatively similar. (Fig.2)

Discussion: Unlike previous findings for non-cartilaginous tissues, these data indicate that fixation of either bovine or human cartilage had no significant effect on the T1 measurements. The variation in measurements before and after fixation is similar to the variation seen with repeated measurement. This may be explained in part by the fact that hydration was not affected by fixation. Thus, these data suggest that dGEMRIC can be used on previously-fixed samples to assess the spatial distribution of GAG. One of the challenges in evaluating cartilage using histology is the practical inability to routinely evaluate the cartilage everywhere in the joint. This finding offers the opportunity to take advantage of the intrinsic ability of non-destructive imaging to visualize the 3-dimensional volume. By analyzing the whole joint, one might more easily note particular spatial patterns, or perhaps identify regions of particular interest that could be further evaluated using histological approaches.


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