Effect of Charge on Transport and Distribution of Contrast Agents in Cartilage

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Introduction
An MRI technique has been developed for imaging glycosaminoglycan (GAG) in cartilage, based on the fact that GAGs have abundant charged groups that confer a fixed negative charge to the matrix. A negatively charged contrast agent (i.e., Gd-DTPA\(^2\)) will therefore distribute within cartilage inversely to the GAG concentration\(^2\). The technique relies upon the assumption that the Gd-DTPA\(^2\) has had time to penetrate the cartilage matrix. In order to investigate the effects of charge on the penetration of contrast agent into cartilage and on the final distribution of contrast, we: (1) measured the diffusion coefficient of Gd-DTPA\(^2\) (Magnevist, Berlex) and the nonionic gadoteridol, Gd(HPD03A) (ProHance, Bracco) in control (GAG rich, hence charged) cartilage and (2) monitored the kinetics of contrast agent wash-in and final concentration in control and GAG depleted cartilage.

Methods
For diffusivity measurements, cylindrical plugs (3 mm diameter and 2 mm thick) from calf cartilage were obtained and hydrated in Hank's saline. Each core was placed in a holder to minimize axial diffusion, which was placed in a 20 mm NMR tube with gadolinium solution. Two solutions were tested; Gd(HPD03A) (molecular weight 559), and Gd-DTPA\(^2\) (molecular weight 548). Images were obtained about every 20 minutes for 4 hours. T1 images were obtained using a saturation recovery sequence on an 8.5 Tesla unit (Bruker). The T1 values were used to obtain a, diffusion equations for a cylindrical sample with radial diffusion\(^2\). The value of the concentration at infinite time was taken as the value at which the experimental data leveled off. The data were then fit to obtain a value for D.

Evaluation of degraded versus intact cartilage with respect to diffusion of MRI contrast was performed on a sample of bovine tibial plateau. Half of the sample was trypsinized in order to proteolyse the GAG in this region of the cartilage. The entire sample was then equilibrated serially in (1) Hanks, (2) 1 mM Gd(HPD03A), (3) Hanks and (4) 1 mM Gd-DTPA\(^2\). Images were obtained before the start and at 11 minute intervals through each of the gadolinium equilibration periods. Saturation recovery images were obtained using a 2 Tesla unit (Bruker Biospec). T1 maps were calculated from the images and used to assess transport kinetic differences and final contrast concentration in the trypsin-degraded and control bovine tibial plateau.

Results
A sample plot of one of the Gd DTPA\(^2\) diffusion data sets along with the fitted model for the data is shown in the figure below.

![Graph showing diffusion data and fitted model](image)

The value for D was found to be 1.98 x 10\(^{-6}\) +/- 0.23 x 10\(^{-6}\) cm\(^2\)/sec for Gd-DTPA\(^2\) (n=6) and 1.45 x 10\(^{-6}\) +/- 0.21 x 10\(^{-6}\) cm\(^2\)/sec for Gd(HPD03A) (n=5). The final concentration in the cartilage was the same as the bath for Gd(HPD03A), but was about half of the bath concentration for Gd-DTPA\(^2\).

In the in vivo bovine tibial plateau experiments, the trypsin-degraded cartilage allowed more rapid diffusion of both ionic and nonionic contrast agents. The final concentration of Gd(HPD03A) was the same in each portion, but the final concentration of Gd-DTPA\(^2\) varied between regions. The trypsinized portion, with less GAG, equilibrated to a higher concentration of Gd-DTPA\(^2\).

Discussion
Transport issues: The diffusivity of Gd(HPD03A) is slightly lower than that of Gd-DTPA\(^2\) in control (negatively charged) cartilage. While the source of the difference is not clear, it is presumably not due to charge, as both the cartilage and Gd-DTPA\(^2\) are negatively charged. Trypsin-degraded cartilage allowed similar or slightly faster diffusion of both contrast agents than in the control cartilage.

Final Concentration: Gd(HPD03A) had the same final concentration in cartilage as in the surrounding fluid in control cartilage, and had the same concentration in control and trypsinized cartilage. Therefore, the final concentration of Gd(HPD03A) was not affected by the state of the cartilage.

Gd-DTPA\(^2\) was in lower concentration in cartilage than in the surrounding fluid, and was in lower concentration in control cartilage than trypsinized cartilage. Both of these reflect the negatively charged contrast agent distributing in lower concentration in the negatively charged cartilage tissue.

The measured diffusivity of Gd-DTPA\(^2\) in cartilage can be used to calculate the time required for equilibration of a sample in the contrast agent before applying the Donnan model to calculate GAG. For the in vivo case, given that the blood level of Gd-DTPA\(^2\) is varying over time after intravenous injection, this value of D needs to be combined with pharmacokinetic modeling to examine Donnan equilibrium within cartilage. These studies are currently underway.

Conclusions
The diffusion of contrast into cartilage is slightly dependent upon the charge of the contrast agent and the GAG content of the cartilage, with faster penetration into GAG depleted areas. The final distribution of an uncharged contrast agent will not be dependent upon GAG content; the final distribution of a charged contrast agent will reflect the GAG distribution in the tissue.

References