Contrast agent diffusion in dGEMRIC: exploring Donnan equlibrium *in vitro* and *in vivo*

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INTRODUCTION: Delayed gadolinium enhanced MRI of cartilage (dGEMRIC) is used to assess GAG content in articular cartilage [1]. Currently clinical measurements are made 90-120 mins after an intravenous injection of Gd- DTPA^{2-} at which time point the anionic contrast agent is assumed to have reached equilibrium. However, recent results [2] show that the maximum Gd-DTPA concentration within knee joint cartilage is reached at different times at different depths within the tissue. In addition, synovial fluid Gd-DTPA concentration is not well known and is routinely not assessed clinically. The aim of this study was to compare the results of T1Gd measurements *in vitro*, in which Gd-DTPA has equilibrated (saturated) within the tissue, with results of *in vivo* cartilage T1Gd measurements in which contrast concentration has been theoretically modeled to estimate the fully non-equilibrium case.

METHODS: *In Vitro Experiments:* Human femoral heads (n=8, age: 79±7, range 65-88) were harvested from patients undergoing hip replacement surgery due to hip fracture. Visually intact osteochondral plugs (4mm diameter) were detached from weight-bearing regions and soaked in PBS. A 200MHz (4.7T) spectrometer (Bruker Avance-II) was used to obtain profile images from the samples in the depth-direction. First, T1 relaxation time was measured without contrast agent (inversion recovery sequence, TR=5 s, 16 TI's between 1 and 1000 ms, depth-wise resolution of 20 µm). Subsequently, the samples were exposed to 2mM solution of Gd-DTPA^{2-} MRI contrast agent (Magnevist, Bayer Schering Pharma AG, Berlin, Germany). Due to test tube geometry the contrast agent could transport into the cartilage through the surface only. After this, T1 was measured at 25 minutes intervals until 10 hours. *In Vivo Experiments:* T_1 measurements (1.5 T) were performed in femoral knee cartilage of 23 healthy volunteers. The weight-bearing central cartilage was analyzed before contrast and at eight time points between 12 and 240 minutes after an intravenous injection of Gd-DTPA^{2-}:12-60 minutes (4 volunteers) and 1-4 hours (19 volunteers). Three regions of interest were segmented manually: deep, middle and superficial. Estimated gadolinium concentrations were calculated using the formula: $[Gd] = (1/T_{1Gd} - 1/T_{1pre})/r_1$, where T_{1Gd} is the T_1 value at a certain time point after contrast agent injection, T_{1pre} is the T_1 value before Gd-DTPA^{2-} injection, and r_1 is the relaxivity of Gd-DTPA^{2-}, for which the values 4.1 $s^{-1}mM^{-1}$ (1.5T) and 3.8 $s^{-1}mM^{-1}$ (4.7T) were used.

Numerical modeling of Gd-DTPA^{2-} diffusion - In Vitro Studies: The time and space profile of $c \equiv$ [Gd-DTPA^{2-}] in cartilage is governed by electrodiffusion (ED), $\partial_t c = D[\partial_x^2 c - z\,\partial_x(cE/V_T)]$, where D is diffusivity of Gd, z is Gd valence (−2), and E/V_T is the local intra-tissue electric field generated by depth-dependent gradients in tissue fixed charge density (FCD) as well as time- and space-dependent gradients in all mobile ion concentrations [3]. Electrodiffusion of Gd-DTPA^{2-} was numerically simulated using MATLAB. First, the final measured equilibrium Gd concentration (t = 10 hour) was used to compute FCD for each cartilage plug assuming Donnan equilibrium, using previously published methods [1]. The ED model was then used to calculate the space-time evolution of Gd transport into the cartilage plugs from t=0 up until final equilibrium (t=10hrs) using the calculated FCD, the known diffusivities of Na, Cl and Gd, and the known concentration of these ions in the external bath. *In Vivo Studies:* While the Gd concentration of the bath was known for *in vitro* experiments, the *in vivo* synovial fluid concentration of Gd was not known; To test the accuracy of the Donnan assumption (that Gd concentration is in true equilibrium within the knee cartilage *in vivo*) we first used a simplifying linear profile of FCD, varying from -0.05 M to -0.15 M with depth. We then used the in vitro-validated ED theory to compute the non-equilibrium transport of Gd into cartilage by combining the clinical dGEMRIC data and modeling the unknown synovial [Gd-DTPA^{2-}] as a sum of decaying exponentials to simulate uptake and outflow of Gd-DTPA in the synovial fluid.

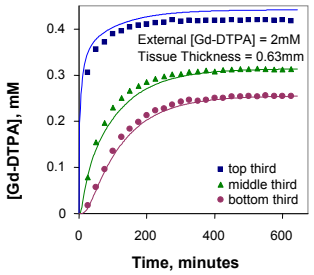

Fig 1: Averaged [Gd-DTPA] from MRI for a 0.63 mm thick cartilage disk specimen bathed in PBS with 2 mM Gd-DTPA. Solid lines: model predictions based on FCD of Fig 2.

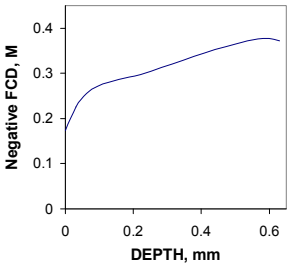

Fig 2: Calculated FCD as a function of depth for a 0.63 mm thick cartilage specimen equilibrated in PBS and 2 mM Gd-DTPA for 10 hours.

RESULTS: *In vitro Studies:* Gd concentration for a typical cartilage plug is shown as the data points of Fig 1. Using the Gd concentration measured at t=10 hours (equilibrium), the calculated depth-dependent FCD for this explant is shown in Fig 2. To validate the model, the predicated transient [Gd-DTPA^{2-}] was averaged over the top, middle, and bottom thirds and compared to measured transient [Gd-DTPA^{2-}] MRI data averaged over the top, middle, and bottom thirds of the same cartilage disk. The predicted space-time distribution of Gd in the cartilage compared well with the MRI-dGEMRIC data using the previously reported value of D [4] and no adjustable coefficients (Fig. 1). These measurements and calculations were repeated for all cartilage plugs, and motivated the use of this non-equilibrium transport model to examine the in vivo dGEMRIC data. *In Vivo Studies:* the calculated Gd concentrations in the top, middle and bottom zones of femoral knee cartilage of the healthy volunteers are shown in Fig 3. The synovial fluid concentration (top solid line, Fig 3) was adjusted to give a best fit of the measured transient tissue Gd concentration when averaged over the top, middle, and bottom thirds (solid lines, Fig 3). Fig 4 compares the GAG-FCD calculated using "instantaneous" Gd-MRI assuming Donnan equilibrium at each instant in time, vs. the true FCD. It is seen that the use of the non-equilibrium Gd concentration can greatly overestimate the true FCD during the initial 2-3 hours of imaging time predominantly in the middle and deep zones, while the error in using the transient measured Gd is less in the superficial zone.

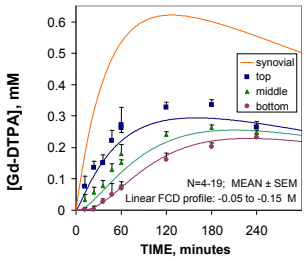

Fig 3: Gd-DTPA concentration in knee femoral condyle cartilage from MRI. Solid lines: model synovial fluid concentration and resulting tissue [Gd-DTPA] for the case of a linear profile of FCD.

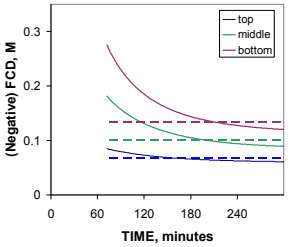

Fig 4: Solid lines - predicted FCD using the instantaneous Gd data of Fig 3 assuming Donnan equilibrium at each point in time. Dashed lines - correct FCD in top, middle, and bottom regions.

DISCUSSION: The temporal and spatial analysis combined with diffusion modeling in this study suggests that Gd concentration is never in true equilibrium within the cartilage *in vivo*. The validity of using the in vivo MRI-measured Gd concentration to calculate FCD quantitatively using the Donnan assumption could be questioned. To improve cartilage *in vivo* examination by dGEMRIC, careful analysis by time and depth should be considered. Specifically, to obtain valid FCD values our study suggests analysis of different depths at different time points. Still it seems that using bulk cartilage T1Gd calculations that predominantly take superficial cartilage into account, gives an overall reasonable estimate of the cartilage quality in group comparisons.

REFERENCES

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