Diffusivity and kinetics of gadopentetate in articular cartilage *in vitro*

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INTRODUCTION

In the dGEMRIC method [1], gadopentetate (Gd-DTPA^{2-}) contrast agent is assumed to distribute into articular cartilage in inverse relation to the fixed charge density (FCD) induced by the negative side chains of the proteoglycan (PG) molecules. At equilibrium, the PG content may then be quantified by estimating the contrast agent concentration from T_1 relaxation time measurements. Typically, equilibration times of few hours *in vitro* [1-3] and 1.5-2 hours *in vivo* [4] have been used. However, in recent contrast-enhanced computed tomography (CECT) studies, significantly longer equilibration times were reported [5,6]. The aim of this study was to investigate the kinetics and diffusivity of gadopentetate contrast agent through the articular surface and deep cartilage *in vitro* using high-resolution MRI.

MATERIALS AND METHODS

From lateroproximal part of visually intact bovine patellae (N = 6), two adjacent full-thickness cartilage disks without subchondral bone (N = 12, d = 4 mm, mean cartilage thickness 1.5 ± 0.4 mm) were prepared and frozen. Prior to MRI measurements, the samples were thawed and placed inside a sample holder which allows the contrast agent penetration only from one direction. For the adjacent samples, diffusion was allowed from either (i) superficial to deep (N = 6) or (ii) deep to superficial cartilage (N = 6). T_1 relaxation time was mapped at 9.4 T using a fast spin echo (FSE) sequence (ETL = 4, TE_{eff} = 10 ms, 9 TRs between 44 – 5120 ms, imaging matrix = 256 x 64, FOV = 20 x 20 mm, 78-µm depth-wise resolution, 1-mm slice thickness and total imaging time 6 min 4 sec per T_1 map). Baseline T_1 was measured in PBS, and subsequently the samples were immersed in 1 mM solution of gadopentetate. Post-contrast T1 mapping was started immediately after replacing the PBS with contrast agent and repeated every six minutes up to 18 hours. Depth-wise T_1 relaxation time profiles were calculated for 1.5 mm wide ROIs at the centre of each sample and converted to concentration profiles according to the equation $C = 1/R(1/T_{1\_Gd} - 1/T_{1\_PBS})$, where R = 3.7 mM^{-1}s^{-1} [3]. To determine the bulk diffusivity (D), a one-dimensional finite element (FE) model was fitted to the bulk contrast agent concentration at each time point by minimizing the mean square error between the experimental and simulated concentrations.

RESULTS

The mean contrast agent concentration increased exponentially over 18 hours for both diffusion directions, indicating a continuous diffusion of gadopentetate (Fig. 1). Diffusion through deep cartilage resulted in considerably lower concentration at all time points, indicating slower diffusion of contrast agent (Fig.1, Table 1). The depth-wise concentration profiles (Fig. 2) revealed significant variation over diffusion direction and immersion time. The distribution profiles differed notably even after 18 hours of immersion, indicating incomplete equilibration. However, cartilage layers closest to contrast agent bath (superficial or deep cartilage depending on the diffusion direction) reached near-equilibrium relatively quickly: after 1 hour, the gadopentetate concentration in the superficial cartilage remained within 15 % of the near-equilibrium concentration at 18 hours. Calculated gadopentetate diffusivities (Table 1) were 44 % smaller when diffusion occurred from deep to superficial cartilage. Marked variation in the diffusivities was seen between the samples.

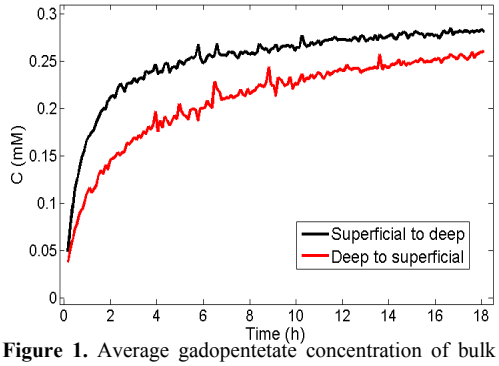

Figure 1. Average gadopentetate concentration of bulk cartilage over time for different diffusion directions (N = 6). Concentration increased exponentially over time for both directions, indicating continuous contrast agent diffusion.

DISCUSSION

In the present study, the kinetics and diffusivity of gadopentetate were investigated in vitro at 9.4 T. The results indicate that the time needed for full equilibrium is significantly longer than previously assumed. This may have implications on the accuracy of dGEMRIC technique when the quantification of PG content is sought, as shorter equilibration times are typically used. The present findings are also in accordance with the previous CECT findings [5,6]. Furthermore, a clear difference in 18-hour concentration and diffusivity was observed at different diffusion direction. The diffusion through deep cartilage was significantly slower, which suggests that also other factors than FCD are likely to affect the diffusion of the contrast agent. Cartilage structures in the deeper parts of tissue are likely to restrict the penetration of the contrast agent, which further suggests that the diffusion through cartilage-bone interface in vivo may not be as important as previously believed [7]. Our findings are supported by a previous study reporting slow T1 decrease in deep cartilage in vivo [8]. Cartilage layers closest to the contrast agent bath reach near-equilibrium in a relatively short time. This suggests that visualization of early osteoarthritic changes or other lesions in superficial cartilage may be possible shortly after administration of the contrast agent. This has particular significance for the in vivo application of the technique, as a delay of 1.5 h is typically used. The diffusivities presented in this study are smaller than previously reported [2]. However, the diffusion geometries in these studies are different, which might affect the comparison to previous results. Marked variation in the diffusivities was seen between the samples, indicating large variation in the structure and composition of the patellar cartilage samples. This is in agreement with previous studies, which suggest that diffusivity depends on tissue composition [9].

CONCLUSIONS

The time required for the contrast agent to reach equilibrium in the cartilage was found to be significantly longer than previously assumed. While this may have implications on the accuracy of the dGEMRIC method, visualization of early osteoarthritic changes in superficial cartilage may be possible shortly after contrast agent administration. The diffusion of the contrast agent through deep cartilage was slow, suggesting that the contrast agent uptake through deep cartilage may only have a minor role in the clinical setting.

Table 1. Bulk gadopentetate concentrations at typical equilibration times and diffusivities for both diffusion directions (mean ± SD, N = 6). Both the concentrations and the diffusivities indicate that the contrast agent diffusion is slower through deep cartilage.

Diffusion direction	C (mM) 1.5 h	C (mM) 2.5 h	C (mM) 18 h	D (µm^2/s)
Superficial to deep	0.20 ± 0.06	0.22 ± 0.07	0.28 ± 0.05	161.9 ± 141.7
Deep to superficial	0.13 ± 0.06	0.15 ± 0.07	0.26 ± 0.08	91.4 ± 60.4

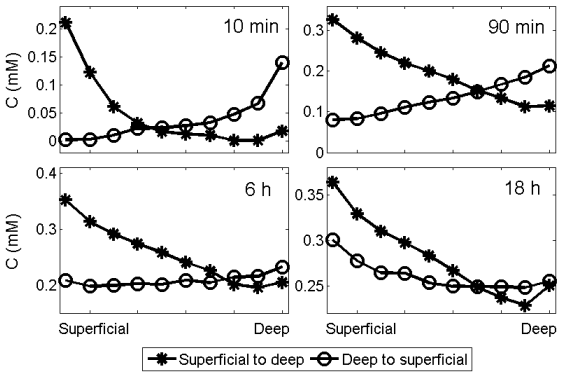

Figure 2. Average concentration profiles across cartilage depth at 10 min, 90 min, 6 h and 18 h after the contrast agent immersion (N = 6). Contrast agent distribution varied significantly over immersion time and diffusion direction. Please note the different concentration scales for each time point.

REFERENCES: [1] Bashir A et al. Magn Reson Med 36: 665-673, 1996 [2] Gillis A et al. Magn Reson Med 48: 1068-1071, 2002 [3] Nieminen MT et al. Magn Reson Med 48: 640-648, 2002 [4] Burstein D et al. Magn Reson Med 45: 36-41, 2001 [5] Silvast TS et al. Phys Med Biol 54: 6823-6836, 2009 [6] Kallioniemi AS et al. Phys Med Biol 52: 1209-1219, 2007 [7] Bashir A et al. Radiology 205: 551-558, 1997 [8] Hawezi Z et al. Proc. ISMRM 17:3963, 2009 [9] Evans RC et al. Arch Biochem Biophys 442: 1-10, 2005