Dynamics of contrast agent enhancement of intact and enzymatically degraded articular cartilage

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INTRODUCTION

The delayed Gadolinium Enhanced MR Imaging (dGEMRIC) method [1] is based on the distribution of anionic contrast agent (gadopentetate, Gd-DTPA^{2-}) into articular cartilage in inverse relation to the proteoglycan (PG) content. The method has been shown to be sensitive in detecting PG depletion both in vitro [2-4] and in vivo [5,6]. In clinical practice, a delay of 90-120 minutes between contrast agent administration and imaging is typically used in order to allow full contrast agent penetration [7,8]. In this study, the effect of experimental degradation on diffusion kinetics of gadopentetate contrast agent was investigated.

MATERIALS AND METHODS

From visually intact bovine patellae (N = 3), a cartilage-bone plug (d = 25 mm) was prepared and cut in two halves. The first half was enzymatically degraded by immersing the sample in 1 mg/ml trypsin while the other served as a control and was immersed in phosphate buffered saline (PBS) containing inhibitors of proteolytic enzymes (5 mM EDTA and 5 mM Benzamidine HCl). Both halves were incubated for 2.5 hours at 37°C. Subsequently, full-thickness cartilage plugs (d = 4 mm, mean cartilage thickness 2.3 ± 0.4 mm) were prepared from both halves and frozen at -20°C in PBS containing the enzyme inhibitors. Prior to the MRI measurements, the samples were thawed and placed surface up inside a custom-made sample holder which allows the contrast agent penetration only through the cartilage surface. T_1 relaxation time was measured at 9.4 T using saturation recovery 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, 1-mm slice thickness, total imaging time per T_1 map 6 min 4 sec). Baseline T_1 was measured in PBS. After baseline measurement the immersion solution was changed to 1 mM solution of gadopentetate (Gd-DTPA^{2-}, Magnevist, Schering, Berlin). T_1 measurement was repeated immediately after contrast agent immersion and further continued over a time period of 18 hours, yielding a total of 177 T_1 maps. Depth-wise T_1 profiles were averaged over 1.5 mm wide ROIs at the centre of each sample, converted to gadopentetate concentrations ($C=1/R[1/T_{1\_Gd} - 1/T_{1\_PBS}]$, where R = 3.7 $mM^{-1}s^{-1}$ [3]) and further averaged to bulk concentration values. To determine the bulk diffusivity (D) of gadopentetate, 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 measured and simulated concentrations.

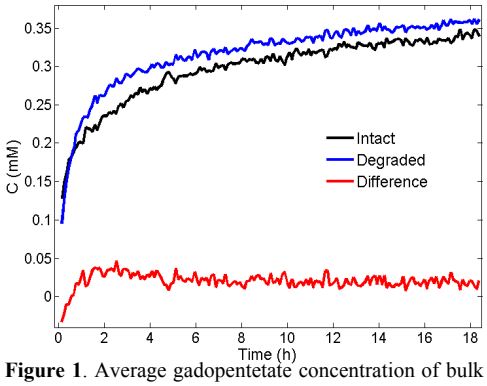

Figure 1. Average gadopentetate concentration of bulk cartilage over time for intact and degraded samples (N = 3). The contrast agent uptake in degraded cartilage is slightly higher.

RESULTS

An exponential increase of the bulk contrast agent concentration as a function of the immersion time was observed both in the intact and enzymatically degraded samples (Fig. 1). Although the bulk uptake of the contrast agent was increased in the degraded samples, the difference was relatively small (Fig.1, Table 1). The concentration difference reached its peak value at approximately two hours, after which the difference remained relatively constant (Fig. 1). Spatio-temporal gadopentetate assessment (Fig. 2) displayed an increased contrast agent accumulation in superficial, PG-depleted areas (Fig. 2B). In degraded cartilage, change in the concentration over time in the superficial layer remained within 10 % after 60 minutes of immersion, whereas in intact cartilage the change was almost 20 % after 60 minutes. The spatial concentration maps also indicated a continuous uptake of gadopentetate in the deeper parts of tissue up to 18 hours in both intact and degraded samples. The calculated diffusivities (Table 1) were 24 % larger for intact cartilage, although significant variation was seen between the samples.

DISCUSSION

Relatively small difference between the contrast agent diffusion in intact and degraded cartilage was observed. Slightly larger bulk uptake of gadopentetate was observed in the degraded samples, indicating increased uptake of the contrast agent after PG depletion. Initial experiments with trypsin degradation indicated significant PG depletion in cartilage; however, this seemed to minimally affect the diffusion kinetics of gadopentetate. This suggests that also other factors than FCD, such as the collagen fibril network and macromolecular content, are likely to affect the diffusion of the contrast agent into cartilage. The largest difference in bulk contrast agent concentration between intact and degraded samples was seen at approximately two hours after immersion. However, the difference was more notable in superficial parts of the tissue, suggesting that visualization of superficial changes may be possible shortly after contrast agent administration. Significant variation in the diffusivity values of individual samples was observed. This is likely to explain the unexpected result of larger diffusivities in intact samples, an issue warranting further investigation. In conclusion, larger concentration of the contrast agent is evident in PG-depleted samples, and the maximum contrast agent concentration difference between intact and degraded cartilage is reached well before the full equilibrium is achieved.

Table 1. Gadopentetate concentrations at several equilibration times and diffusivities of bulk cartilage (mean ± SD, N = 3). The concentration values are higher in degraded cartilage, whereas the diffusivity is higher in intact cartilage.

	C (mM) 1.5 h	C (mM) 2.5 h	C (mM) 18 h	D (μm^2/s)
Intact	0.22 ± 0.03	0.25 ± 0.03	0.34 ± 0.08	656.2 ± 493.6
Degraded	0.26 ± 0.05	0.28 ± 0.06	0.36 ± 0.03	496.4 ± 133.2

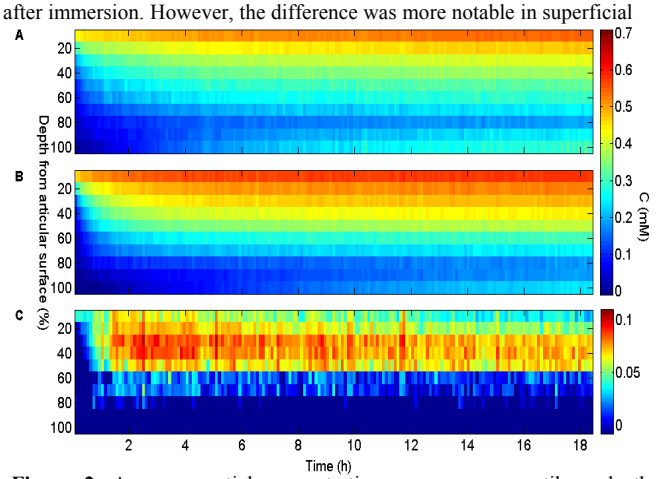

Figure 2. Average spatial concentration maps across cartilage depth (vertical direction) over time (horizontal direction) for intact (A) and degraded (B) samples (N = 3). The concentration difference between intact and degraded is shown in (C). In superficial layers of degraded cartilage the contrast agent concentration is slightly higher.

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