Quantitative spatial analysis of articular cartilage proteoglycans using $GdDTPA^{2-}$-enhanced T_1 imaging

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Introduction

Proteoglycans (PGs) play a major role in the structure and function of articular cartilage. Negative electrostatic repulsion forces between PG molecules and their hydrophilic nature creates a swelling pressure, which contributes to cartilage stiffness together with the collagen network. PG depletion is related to early stages of degenerative joint disease (osteoarthrosis) [1].

$GdDTPA^{2-}$-enhanced T_1 imaging offers a potential technique for the quantitation of cartilage PGs *in vivo* [2]. The distribution of this anion in cartilage is inversely related to negative glycosaminoglycans (GAGs) and thus enables the indirect estimation of cartilage GAG (and PG) content. In this study, a quantitative μMRI approach was conducted to investigate the spatial sensitivity of $GdDTPA^{2-}$-enhanced T_1 imaging, in reference to quantitative digital densitometry (DD) of GAGs.

Methods

Full thickness cartilage disks without subchondral bone were extracted from bovine patellae (n=5) using a biopsy punch (ϕ=4mm) and a razor blade. A smaller sample was cut from the disk for microscopic analysis. Disks were balanced in phosphate buffered saline (PBS) for 2.5 hours, and placed in a 5mm NMR tube. After T_1 imaging, the samples were immersed in 1mM $GdDTPA^{2-}$ for a further 2.5 hours, and imaged again.

T_1 mapping was performed at 9.4T using a 5mm high resolution spectroscopy probe. A saturation recovery sequence was used with TE=14ms and 6 time points between TR=200-5000ms for PBS immersed samples and TR=100-1500ms for GdDTPA immersed samples (1mm slice thickness, 10mm FOV with 256*64 resolution at 25±1°C). Slice orientation was aligned similarly for both measurements using the small cut in the sample as a landmark. T_1 profiles across cartilage thickness were calculated from a 1mm wide area. Depthwise T_1 and T_{1Gd} profiles were used to calculate spatial $GdDTPA^{2-}$ concentration ($[GdDTPA^{2-}]$), using $[GdDTPA^{2-}]=1/R(1/T_{1Gd}-1/T_1)$ according to Bashir et al [3]. Relaxivity R for GdDTPA in PBS was determined spectroscopically from 10 solutions with 0.2-2mM GdDTPA, giving R=3.79± 0.03 $(mM\ s)^{-1}$.

Cartilage samples were prepared for quantitative DD using formalin fixation, dehydration, mounting in paraffin and staining of GAGs with safranin O. Safranin O is a cationic dye which binds stoichiometrically to GAG polyanions in an orthochromatic fashion, and correlates linearly with fixed charge density (FCD) [4]. For each sample, spatial optical densities (ODs), i.e. absorbance, of 3μm-thick microscopic sections were measured along articular cartilage depth to obtain a quantitative measure of spatial [GAG] [4,5].

Results

By equalizing MR and DD spatial resolution, a quantitative spatial comparison of $GdDTPA^{2-}$-enhanced T_1 profiles and $[GdDTPA^{2-}]$ profiles with OD profiles of each sample could be done (Fig. 1). A significant inverse relation was detected between $[GdDTPA^{2-}]$ and [GAG] in individual samples (Fig. 2), mean r=-0.91±0.02. A correlation of r=-0.89 was obtained after pooling all data points (n=179).

T_{1Gd} alone correlated linearly with [GAG], r=0.87±0.04 in individual samples and r=0.86 in pooled data (n=179).

Discussion

Both techniques used in this study are based on the ionic distribution of a foreign charged chemical. As expected, $[GdDTPA^{2-}]$ followed spatial changes in cartilage [GAG] in inverse relation, as investigated by quantitative DD of GAGs. The results also support the assumption that T_{1Gd} alone can satisfactorily reflect [GAG] [2].

Interestingly, however, $[GdDTPA^{2-}]$ or T_{1Gd} correlated less successfully with OD in the deep tissue layer of some samples (Figs. 1 and 2). It seems that in some samples T_{1Gd} does not reach a plateau in deep cartilage as predicted by OD (Fig. 2), leading to an overestimation of [GAG]. This could indicate an incomplete penetration of $GdDTPA^{2-}$ in comparison to the negative charges within cartilage.

Several reasons for the anomalous behavior of $[GdDTPA^{2-}]$ in deep cartilage can be suggested. Diffusion time could be too short for some samples and would lead to incomplete distribution of the contrast agent in sites with high [GAG]. This may not be the explanation since the samples were extracted from subchondral bone and, thereby, also the deep cartilage layer was open for free diffusion. More likely, the high FCD of deep cartilage, leading to a highly compact matrix with small pore size, together with the densely packed collagen network, could restrict $GdDTPA^{2-}$ penetration into PG rich areas.

$GdDTPA^{2-}$-enhanced T_1 imaging obviously enables the visualization of cartilage PGs. Further studies, however, are needed to solve the question of overestimated [GAG] in the deep cartilage.

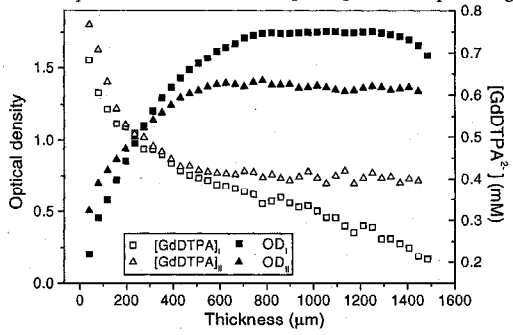

Fig. 1: Optical densities (OD) and $GdDTPA^{2-}$ concentrations as a function of thickness from cartilage surface in two representative samples.

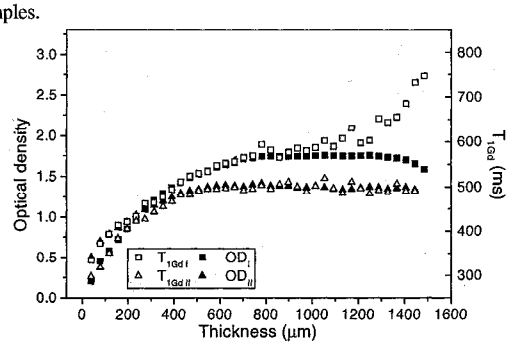

Fig. 2: Optical densities (OD) and T_{1Gd} as a function of thickness from cartilage surface in two representative samples.

References

1. Buckwalter, J.A., Mankin, H.J., *J. Bone Jt. Surg.*, 79-A, 612-32, 1997.
2. Bashir, A. et al., *Magn. Reson. Med.*, 41, 857-65, 1999.
3. Bashir, A. et al., *Magn. Reson. Med.*, 36, 665-73, 1996.
4. Kiraly, K. et al., *Histochem. J.*, 28, 577-90, 1996.
5. Panula, H.E. et al., *Ann. Rheum. Dis.*, 57, 237-45, 1998.

Acknowledgments

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