Relationship between dGEMRIC and other histochemical markers for cartilage proteoglycan content

M. J. Nissi[1], P. Ek[2], M. T. Nieminen[3], and J. S. Jurvelin[1]

[1]Department of Physics, University of Kuopio, Kuopio, Finland, [2]Laboratory of Analytical Chemistry, Åbo Akademi, Turku, Finland, [3]Department of Diagnostic Radiology, Oulu University Hospital, Oulu, Finland

INTRODUCTION

The quantification of a charged contrast agent or a dye can be utilized as a means of indirect quantification of the negatively charged proteoglycans in articular cartilage. For MRI, the application of Gd-DTPA^{2-} and T_1 relaxation time measurements has been shown to enable the indirect imaging of the proteoglycan (PG) content of articular cartilage, know as the dGEMRIC technique [1]. On the other hand, the concentration of the gadolinium chelate, can be quantified from microscopical sections using inductively coupled plasma mass spectrometry (ICP-MS) [2]. Histologically, the cartilage PG content has been determined by digital densitometry (DD) of safranin-O, a positively charged dye that binds stoichimetrically to PGs [3]. The aim of the present study was to compare the dGEMRIC technique to ICP-MS of gadolinium and digital densitometry of sarfanin-O –stained sections.

MATERIALS AND METHODS

Four different locations of a bovine knee were sampled for this study: upper lateral patella (PAT n=7), medial femoral condyle (MC n=4), lateral patellar groove (LPG n=4) and medial tibial plateau (MTP n=4). Full-thickness cartilage samples (dia.=4mm) were harvested and immersed in phosphate buffered saline (PBS) prior to the measurements. T_1 relaxation time was mapped using saturation recovery sequence first in PBS ($T_{1,0}$: TE=14ms; TR=200, 400, 700, 1400, 2600, 5000ms, in plane resolution of 39µm, 1mm slice thickness) and again after balancing of 2.5 hours in 1mM Gd-DTPA^{2-} solution ($T_{1,Gd}$: TR=100, 170, 300, 500, 900, 1500ms). Depth-wise relaxation time profiles were calculated by averaging columns from relaxation time maps, which were calculated using monoexponential fit. Gd-DTPA^{2-} concentration was calculated from the equation $[\text{Gd-DTPA}^{2-}] = (1/R)(1/T_{1,Gd} - 1/T_{1,0})$ using the relaxivity value $R=3.121\ m^{-1}s^{-1}$ in the calculation [4]. Both $[\text{Gd-DTPA}^{2-}]$ and $T_{1,Gd}$ served as surrogate markers for PG content of cartilage [1].

Following the MRI experiment, the samples remained at 1mM Gd-DTPA^{2-} solution and were frozen. From the centers of the samples, 100µm-thick sections were freeze-cut for ICP-MS. The depth-wise concentration of Gd-DTPA^{2-} was measured using localized laser ablation and subsequent ICP-MS analysis of the generated gas. The system was calibrated using an agarose sample containing 5mM Gd-DTPA^{2-}. Digital densitometry of PGs was measured from 3-µm thick microscopic sections and depth-wise profiles were determined, as described earlier [5]. The dGEMRIC ($[\text{Gd-DTPA}^{2-}]$ and $T_{1,Gd}$), $T_{1,0}$, ICP-MS and DD profiles were resampled for a pixel-wise comparison. Profiles from each topographical location were averaged.

RESULTS

The concentrations of the contrast agent varied between the sampled locations (Figure 1a and 1b). Optical density (figure 1c) shows the increasing light absorption towards deep tissue and shows an inverse relation as compared to content profiles of concentration agent (MRI and ICP-MS). Samples from medial tibial plateau showed the lowest PG content (highest $[\text{Gd-DTPA}^{2-}]$) with all techniques. For the other locations, the techniques differently revealed the PG content. Bulk values were calculated for each sample by taking depth-wise average and these values (n=19) and were correlated with each other (Table 1). The highest correlation was observed between optical density and Gd-DTPA^{2-} concentration as measured by dGEMRIC (r=-0.840, p<0.01). Also, $T_{1,0}$ showed significant correlations with the parameters that reflect the PG content of cartilage.

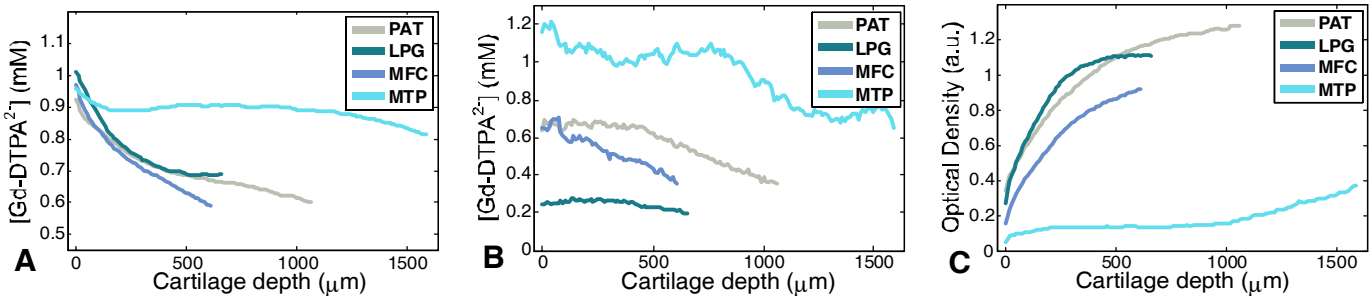

Figure 1. For different topographical locations, average depth-wise profiles for different surrogate markers for PG content: a) dGEMRIC, b) ICP-MS and c) mean optical density.

Table 1. Correlation coefficients for bulk values of all data pooled. $[\text{Gd-DTPA}^{2-}]_{MRI}$ and $[\text{Gd-DTPA}^{2-}]_{ICP}$ denote the contrast agent concentrations as determined by dGEMRIC and ICP, respectively.

	$T_{1,Gd}$	$T_{1,0}$	OD	$[\text{Gd-DTPA}^{2-}]_{ICP}$
$T_{1,0}$	-0.722**	--		
OD	0.791**	-0.800**	--	
$[\text{Gd-DTPA}^{2-}]_{ICP}$	-0.358	0.671**	-0.473*	--
$[\text{Gd-DTPA}^{2-}]_{MRI}$	-0.952**	0.792**	-0.840**	0.471*

(correlation significant at level: *p<0.05, ** p<0.01)

DISCUSSION

The contrast agent concentrations as measured by MRI and ICP-MS are in the same range, although differences between techniques are observed. The concentrations measured using dGEMRIC show a typical decrease from superficial to deep tissue; whereas the concentration profiles obtained with ICP do not change as much. ICP-MS concentrations higher than that of the equilibrating concentration indicate a calibration bias or some other uncertainty in the ICP-MS measurement. A high agreement was observed between dGEMRIC and DD. Yet the techniques show differences in differentiating tissue originating from different topographical locations. These are likely due to fundamental differences in the physicochemical nature of the three techniques that have to be further investigated.

REFERENCES

[1] Bashir A et al. Magn Reson Med 1999;41:857-865; [2] Hattendorf B et al. Anal Chem 2003;75:341A-347A; [3] Panula HE et al. Ann Rheum Dis 1998;57:237-245; [4] Nissi MJ et al. Proc Intl Soc Mag Reson Med 2005;13:203; [5] Király K et al. Histochem J 1996;28:99-107