Quantification of Iron Oxide Nanoparticles in Cellular MRI: Assessment of Free vs. Cell-Internalized Fraction

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Introduction: Iron oxide nanoparticles (IONPs) are widely used in cellular MRI due to their strong magnetic properties. However quantification of absolute concentration still remains a challenge because relaxivities depend on IONP distribution within a given sample, through variable water access conditions and susceptibility contrast mechanism [1,2]. T_1 and T_2 effects are strongly reduced when IONPs undergo cell-internalization [3]. T_2 and T_2* relaxivity variations are typically understood through a diffusion mediated contrast mechanism that depends on the size of the IONPs (or IONP clusters) [1,2]. The motional narrowing regime is satisfied for small IONPs; in this case r_2 tends to equal r_2*. On the other hand, large IONPs (or IONP clusters) fulfill the static dephasing regime (SDR) theory, according to which r_2*- r_2 is large and its value predictable [2]. Following this concept, Kuelpeter et al. [4] have recently shown that cell-internalized IONPs can be differentiated form free-spread IONPs by using joint T_2 and T_2* mapping. In their case however, IONP concentration and fractions of free vs. internalized IONP were not quantified. Recent works on susceptibility mapping [5,6] have suggested that susceptibility measurements could be used for IONP quantification. Here we study IONP samples that contain a mixture of free- and cell-internalized- IONPs. We investigate multiple MR characteristic parameters (T_1, T_2, T_2* and χ) in order to extract absolute IONP concentration as well as free vs. internalized IONP fractions.

Materials and methods: Cell labeling: Endometrial regenerative stem cells (ERCs) cells were considered in this study. ERCs were labeled by adding 100ugFe/mL of ferumoxides (Feridex, Bayer HealthCare Pharmaceutical Inc., NJ, USA) to each flask containing 15 mL of media. The flasks were then incubated for 24 hours at 37ºC. Post-incubation, the media was aspirated and each flask was washed three times with 10 mL PBS to remove free iron. Cells were removed from the flask by incubating with 3 mL trypsin for 10 minutes, and then quenching with fresh media. Cells were collected and cell count and viability were performed. Sample preparation: Four sample groups were considered: 1) 100% free IONPs; 2) 67% free and 33% cell-internalized IONPs; 3) 33% free and 67% cell-internalized IONPs; and 4) 100% cell-internalized IONPs. For each group serial dilutions (0, 0.03, 0.06, 0.13, 0.25, 0.5, 0.75, and 1mM of Fe) were prepared in 2% agarose gel using mother-solutions of cell-internalized IONPs as well as free ferumoxides. Samples were stocked in 1 mL tuberculin syringes IONP control quantification: All samples were characterized by ICP-MS to obtain accurate IONP concentration. To control retrospectively for the actual Free vs. Internalized IONP percentage of the two mixed groups, samples of the cell-internalized and free IONP mother solutions were characterized by ICP-MS as well. MRI/MRS: MR experiments were performed at room temperature on a Sigma HDx 3T scanner (GE Healthcare, Milwaukee, WI, USA). T_1, T_2 and T_2* were measured by imaging with inversion-recovery fast spin echo (TE=9.9ms, TR=3s, ETL=8,BW=±15.6kHz, FOV=13cm, Mx=160x160, NEX=1, 2mm slice, and 20 TIs ranging from 50ms to 2.5s), multi-echo spin echo (TR=1.5s, FA=50º, BW=±31.3kHz, FOV=13cm, Mx=160x160, NEX=1, 2mm slice, and 8 evenly spaced TEs ranging from 6.9ms to 55.6ms) and multi-echo gradient echo sequences respectively (TR=500ms, BW=±31.3kHz, FOV=13cm, Mx=256x256, NEX=2, 0.5mm slice, and 16 evenly spaced TEs ranging from 4.2ms to 71.7ms). Corresponding relaxivities were extracted by regression analysis, using the usual linear relaxivity assumption $R_i=R_{i0}+r_i.[IONP]$. Magnetic susceptibility χ was measured using MR spectroscopy. Two slice-selective FIDs were acquired on the central slice of each 1 mL tuberculin syringes, with their main axis oriented at 0º and 90º with respect to B_0. Corresponding central frequencies were measured by Fourier transform. χ was extracted from these 0º and 90º frequencies using analytical formula corresponding to infinitely long cylinders [7]. Free vs. internalized IONP fraction estimation: Using 100% free and 100% cell-internalized groups as references, the free IONP fraction was evaluated considering the r_2'relaxivity ($r_2' = r_2^* - r_2$) as follows: Free IONP fraction = $100.(r_2' - r_{2\,100\%\,\text{internalized}}')/(r_{2\,100\%\,\text{free}}' - r_{2\,100\%\,\text{internalized}}')$. The SDR prediction of $r_{2\,100\%\,\text{internalized}}'$ was calculated from our susceptibility data and used as an alternative reference value to test its reliability for fraction extraction.

Results: Average iron load of about 60pg/cell was derived from ICP-MS measurements and cell count data. Control free IONP fraction determined retrospectively by ICP-MS calibration is given in Table 1. All samples yield measurable T_1s, T_2s, and χs using described methods. Conversely, T_2*s shorter than ~ 3.5ms were not accurately measurable using our TE set, and were then ignored for further analysis. Linear regression analysis correctly fitted experimental data, as testified by a minimum R^2 value of 0.976. We observed IONP relaxivity variations with IONP spatial distribution, consistent with published studies [2,3,4] (see Fig.1). Relaxivities r_1 and r_2 are strongly reduced for internalized IONPs as compare to free IONPs, whereas r_2* behaves in the opposite way. Interestingly, magnetic susceptibility was found to be constant, regardless of IONP spatial distribution. The average susceptibility among all samples was found to be 1.77 ± 0.05 ppm.mM^{-1}, in good agreement with literature (1.81 ppm.mM^{-1} from [5], assuming identical IONP magnetization at 1.5T and 3T). Using this value we estimated a local magnetic dose (LMD) of 53.1 mG/mM and then a SDR prediction of 573 s^{-1}.mM^{-1} for r_2' [2], consistent with measured r_2' for the fully internalized group ($r_2' = 587 \pm 21$ s^{-1}.mM^{-1}). Estimated IONP fractions are in good agreement with control ones when the r_2' SDR prediction is used (see Table 1).

Group #	1	2	3	4
ICP-MS Control	100%	73.0%	27.7%	0%
Measured r_2'	100%	73.1%	34.2%	0%
SDR r_2' prediction	100%	72.1%	31.8%	-3.7%

Table 1: Free IONP fraction extracted using measured r_2' and SDR prediction

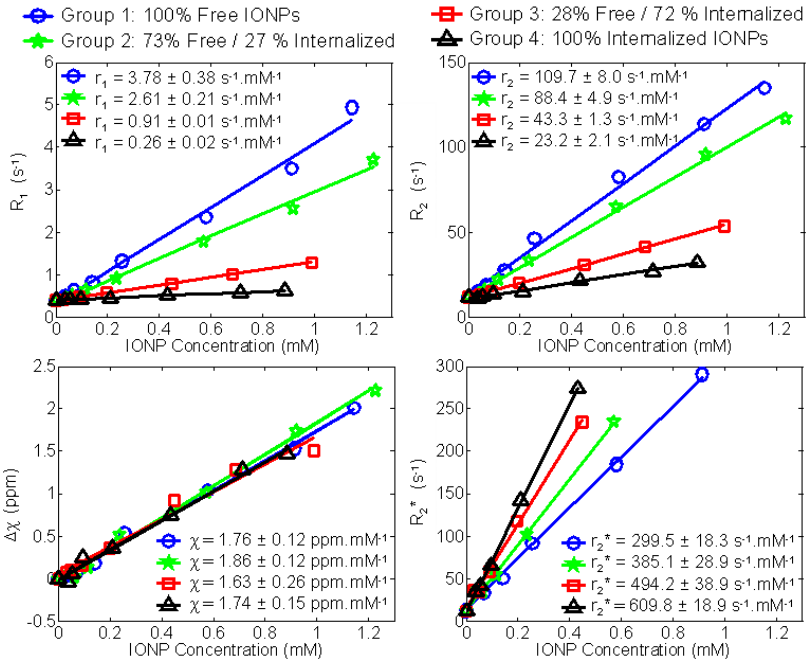

Figure 1: IONP relaxivities and χ as a function of spatial distribution. $\Delta\chi=\chi_{[IONP]}-\chi_{gel\ only}$

Discussion and Perspectives: The ability to extract free and internalized IONP fractions from relaxation measurements relies on IONP concentration estimation and necessitates free and internalized reference relaxivity values. The SDR theory correctly predicts r_2' in the case of cell-internalized IONP, which makes the corresponding calibration step dispensable. Presented susceptibility measurements show good promise for IONP robust concentration estimation. In our experiment, spectroscopic data used to extract χ were measured inside the solution of interest (different from external phase measurements such as in [2]), in order to probe the local magnetic fields induced by IONPs. Imaging based susceptibility mapping techniques that rely on the local phase information [5,6] may be independent of IONP distribution as well. Combined with T_2- and T_2*-maps, this could be an efficient way to measure both IONP concentration and free vs. internalized IONP fraction for samples of arbitrary shape. Further studies will address this question.

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References: [1] Gillis P et al., *MRM*, 2002; 47: p.257 [2] Bowen CV et al., *MRM*, 2002; 48: p.52 [3] Billotey C et al., *MRM*, 2003; 49: p646 [4] Kuhlpeter R et al., *Radiology*, 2007; 245(2): p.449 [6] De Rochefort L et al., *MRM*, 2008 ; 60: p1003 [6] De Rochefort L et al., *MRM*, 2010 ; 63: p194 [7] Chu S et al., *MRM*, 1990 ; 13 : p239