Comparison of 2 different tumor cell labeling techniques for in vitro MRI characterization with new iron oxide particles

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Purpose: Iron oxide particles (SPIO and USPIO) are widely used for MRI-based cell labeling and tracking because they can be loaded on cells by simple protocols and provide high image contrast due to their large susceptibility effect [1,2]. Many investigators are using varying cell labeling models (e.g. AGAR [3] or Ficoll model [4]) for in vitro MRI characterization. Because of the current widespread availability of clinical 1.5 and 3T MR scanners in worldwide clinical centers there also is a big interest in exploring dose depending susceptibility effects at varying field strengths. Therefore, the purpose of this study was to evaluate 2 different cell labeling models in human KB cells for comparison at clinical 1.5 and 3T.

Materials and Methods: Two iron oxides with superparamagnetic properties of different sizes were compared: ferumoxtran-10 (Sinerem®, Guerbet, France) and ferumoxides (Endorem®, Guerbet, France) with a mean hydrodynamic diameters of 30 nm and 150 nm respectively.

Tumor cells and incubation procedure-AGAR technique (Fig. 1):

KB cells were labelled with a saturating concentration of Endorem (2 mM in iron) for 24 hours at 37°C. After the uptake, cells were collected and dispensed as a pellet of 2.10^6 cells/tube. Pellets were then covered up by an agar solution (0.3%) and tubes were disposed into a support filled up with the same agar solution.

Tumor cells and incubation procedure-Ficoll technique (Fig. 2,3):

Samples of 10^6 human KB cells were incubated at 37°C with increasing doses at 8.5 µg Fe/ml, 17 µg Fe/ml, 34 µg Fe/ml and 68 µg Fe/ml of ferumoxtran-10 and at 4.25 µg Fe/ml, 8.5 µg Fe/ml, 17 µg Fe/ml and 34 µg Fe/ml of ferumoxides in 2 ml RPMI media-folate free media (AMIMED) for 24 hrs. After incubation with the contrast agents, the cells were washed 3 times with PBS by sedimentation (10 min, 1500 rpm, 20°C). Additionally we did a dilution series with 3.0- 0.05 × 10^7 human KB cells that we incubated for 4 and 12 hrs with ferumoxides at a dose of 68 µg Fe/ml and Sinerem at a dose of 136µg Fe/ml. Cell viability was determined by the trypan blue exclusion test. We imaged all cells in 0.5 ml isotonic Ficoll solution prepared in Eppendorf test vials at clinical 1.5T and 3T MR scanners with clinical MR head coils.

MRI: AGAR model: MRI imaging of the KB cells was performed using a 2.35T MR scanner (Bruker BioSpec). Images were obtained with a FLASH sequence (TR = 100 ms, FOV = 12.2x9.8 cm, thickness = 2 mm, matrix = 196x196, NA = 16, angle = 30°) with increasing TE (7, 20, 30 and 50 ms).

Ficoll model: MR imaging of the KB cells was performed using 1.5T and 3T MR scanners (Avanto and Allegra, Siemens Medical Systems, Erlangen, Germany) and standard circularly polarized head coils (Siemens Medical Systems, Erlangen, Germany). For measurements of T1-relaxation times sagittal 2D spin echo sequences were obtained through the cell samples with multiple TR values of 100, 200, 300, 400, 500, 750, 1000, 2000 ms, a TE of 11 ms, a resolution of 0.47x0.47x5 mm^3, matrix size 256x104, a slice thickness of 5 mm; for T2 measurements a multi-contrast 2D spin echo sequence was acquired with same resolution, TR 4000ms and 32 contrast with echo spacing of 18ms (Fig. 2). Conventional proton-density weighted and T2-weighted turbo spin echo sequences were acquired for qualitative assessment. For comparison, iron oxide concentrations within the cell samples were measured by ICP-MS. Data analysis: MR images were transferred to a separate PC and processed. ROI were drawn inside the tubes, the mean signal intensity of each ROI was calculated for each value of TR and TE and nonlinear least squares fitting to the characteristic curve of the spin-echo signal was applied for relaxation time calculation. The relaxation times of the cells were converted to R1 and R2 relaxation rates (s^{-1}).

Results: AGAR model: (Fig. 1a,b) The increase of TE amplified the susceptibility effects and caused signal losses which was directly proportional to the intensity of susceptibility effect. As expected, the surface of hyposignal increased with the value of TE and was dependent with the iron concentration assayed in each tube (data not shown). Ficoll model: (Fig. 2,3) Acceptable R2 relaxations rates for ferumoxides and ferumoxtran-10 of the samples could be measured with different contrast agent doses at 1.5 T and 3 T. The best labeling results could be achieved with Endorem-labeled KB cells at decreasing cell numbers after 12 hrs incubation with a concentration of 68 µg Fe/ml (Fig. 3). Ferumoxide labeled cells showed an higher iron uptake than ferumoxtran-10 labeled cells determined by ICP-MS (data not shown).

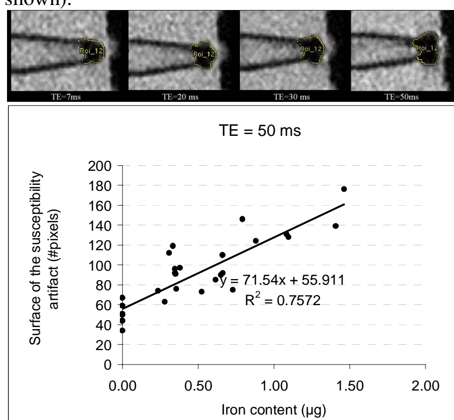
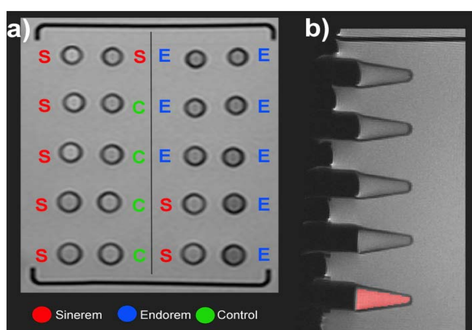


Fig. 1a: The image shows labelled KB cells with saturating concentration of ferumoxides (2 mM of iron, 24 hours, 37°C) in an AGAR-model; 2.10^6 cells/tube. Pellets were covered up by an agar solution (0.3%). A FLASH sequence with increasing TE (7, 20, 30, 50 ms) were applied.



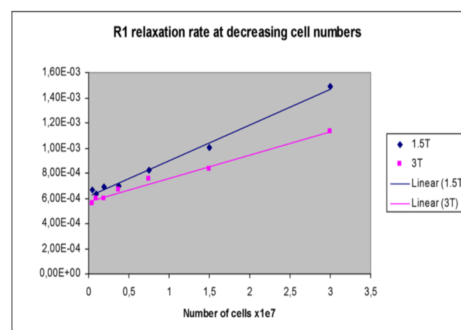


Fig. 1b: Correlation between the surface of signal void with the iron content (µg) for labelled KB cells with saturating concentration of ferumoxides (2 mM of iron, 24 hours, 37°C) for a FLASH sequence with TE=50 ms. Pellets were covered up by an agar solution (0.3%)

Fig. 2a): Labeled KB cells with USPIO (ferumoxtran-10) and SPIO (ferumoxides) at different concentrations in test tubes, incubations time: 24 hrs. **2b)** drawn ROI for T1- and T2 measurements.

Fig. 3: SPIO (ferumoxides)-labeled KB cells at increasing cell numbers after 12 hrs incubation with a concentration of 68 µg Fe/ml at 1.5 and 3T.

Conclusion: Both presented cell labeling methods (AGAR and Ficoll model) are feasible for in vitro MR characterization of KB tumor cells at 1.5, 2.35 and 3T. Ficoll method is easier to perform because it does not need a heating procedure for preparation without air bubbles as compared to AGAR method. Ferumoxides labeled cell samples showed slightly higher R2 relaxations rates than ferumoxtran-10 labeled cell samples at 1.5 T and 3 T. Our continuing investigations focus on improvements of the different MR techniques and labeling models using a variety of T1-weighted and T2 and T2*-weighted pulse sequences to improve unspecific tumor cell labeling methods. R2 quantification might be also useful for in vivo imaging in order to assess the contrast agent uptake of cells.

References: [1]Bulte JW et al., NMR Biomed 2004;7: 484-499, [2]Corot C et al.Adv Drug Deliv Rev 2006; 58:1471-1504,[3]Matuszewski L et al.. Radiology 2005;235:155-161,[4]Simon GH et al. Eur Radiol 2006;16:738-745