Heteronuclear proton MRI: In vivo detection of Tm-DOTMA labeled HT-1080 cells using ultra-short echo time imaging (UTE)

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Introduction: Proton MRI of labeled cells normally employs Gd-based contrast agents (e.g. Gd-DTPA) or iron oxide nano-particles, which both alter the MR relaxation in their vicinity, and allow achieving either positive or negative contrast (1). Unambiguous identification of the labeled cells is often hampered by "false positive" background signal. To overcome this problem ^{19}F may be used, which has no natural background, but requires dedicated hardware. An alternative approach to avoid background signal is the use of lanthanide complexes of 1, 4, 7, 10-tetramethyl-1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetate (DOTMA) that have recently been used in temperature monitoring (2-4). Lanthanide-DOTMA has 12 equivalent methyl protons, which show a chemical shift of up to ~100 ppm (Fig. 1) and can be imaged (2,3). The advantage of this largely shifted signal is that water background is low. Here, we explore the feasibility of imaging Tm-DOTMA labeled HT-1080 cells (human fibrosarcoma) in vivo, using the shifted methyl protons of Tm-DOTMA.

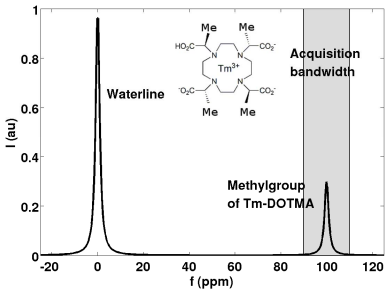

Figure 1. Scheme of the acquisition frequency and bandwidth used to image the methyl (Me) protons of Tm-DOTMA.The signal of the methyl protons is enlarged by a factor of ~10000.

Materials and Methods: *Cell labeling:* A cell suspension of ~7 x 10^6 HT-1080 cells was incubated with 50 µmol Tm-DOTMA (Macrocyclics, USA) under the use of Roti-Fect (Carl Roth, Karlsruhe, Germany) as a polycationic transfection agent. *Fluorescence*: The fluorescence absorption and emission of Tm-DOTMA was characterized using a fluorescence spectrophotometer (Hitachi, Japan). Labeled cells were visualized with a light microscope (Nikon Instruments Europe, England) using broad band UV excitation and emission at 460 nm. *Mouse model*: The animals received a subcutaneous injection of 200 µL of 5 x 10^6 Tm-DOTMA labeled HT-1080 cells in the right flank. For MRI measurements, the animals were anaesthetized with isofluorane. *MRS:* Non-localized spectroscopy on phantoms filled with labeled cells was used to assess the Tm-DOTMA load of the cells. *MRI:* MR images were obtained on a BioSpec 94/20 (Bruker, Germany) equipped with a 1 T/m gradient system and a 35 mm birdcage coil. On phantoms an ultra-short echo time (UTE) sequence and a FLASH sequence were optimized for best SNR-efficiency of the methyl protons of Tm-DOTMA. Animals were investigated using the optimized 3D UTE sequence with radial acquisition and a 365 µs gauss pulse excitation (field of view (FOV) = 4 x 4 x 8 cm^3, Matrix = 32 x 32 x 32). Anatomical reference images were also acquired using FLASH and RARE sequences.

Results and discussion: Fluorescence microscopy (Fig. 2) and non-localized MR spectroscopy showed that labeling of the HT-1080 cells with Tm-DOTMA was successful. No comparable fluorescence was detected in non-labeled cells. Phantom measurements showed that the HT-1080 cells were loaded with an average of about 10^{11} Tm-DOTMA molecules per cell. Critical parameter in the MR measurement was the acquisition bandwidth. Optimum SNR-efficiency was obtained with 25 kHz for UTE and 20 kHz for FLASH. At these bandwidths background signal from the flank of the water line was minimized, while TE and TR could still be kept reasonably low. Phantom measurements showed a 2.5 times higher SNR-efficiency for the optimized 3D-UTE sequence, as compared to the optimized FLASH sequence. In vivo, subcutaneously grafted Tm-DOTMA labeled HT-1080 cells were observed (Fig. 3) within 2 hour scan time with 3D UTE. Cells were detected with SNR values ranging from 7 to 20, showing that water background is negligible with the chosen imaging parameters.

Conclusion: Virtually background-free positive signal of HT-1080 cells labeled with Tm-DOTMA can be obtained, using 3D UTE to detect the shifted methyl protons of the contrast agent. This approach of heteronuclear proton MRI may provide a versatile tool to follow labeled cells in vivo and thus facilitate the application of molecular MRI, without the need for extra hardware.

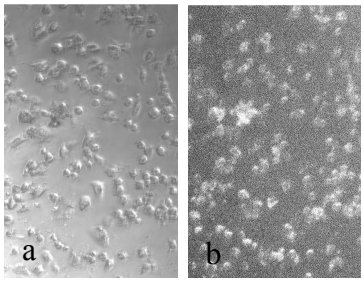

Figure 2. Microscopy of HT-1080 cells labelled with Tm-DOTMA (× 10) **a)** visible light image **b)** fluorescence image

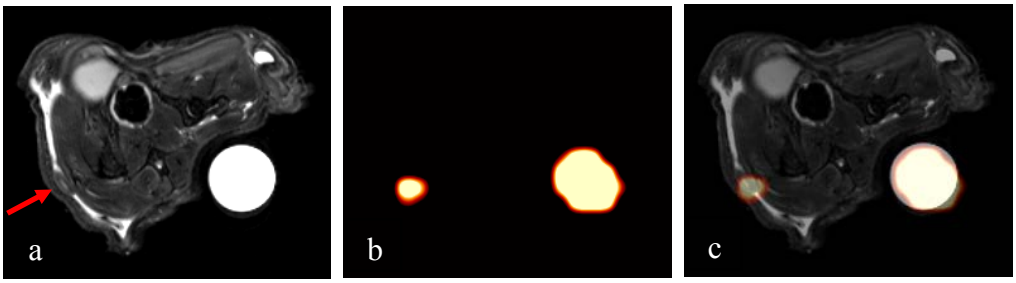

Figure 3. a) Axial anatomical RARE image of a mouse, showing the grafted cells (red arrow). A tube containing an aqueous solution of 0.25 mM Tm-DOTMA was placed at the left flank of the animal as reference. b) Axial 2.5-mm slice from a 3D UTE data set of the methyl proton signal of Tm-DOTMA (SNR=7 here). The slice position is the same as in the RARE image. c) Image fusion of a) and b).

References: 1. Rogers, W.J., et al. 2006, *Nat Clin Pract Cardiovasc Med* **3**: 554-562. 2. Aime, S., et al. 1996. *Magn Reson Med* **35**: 648-651. 3. Hekmatyar, S.K., et al.2005. *Magn Reso Med* **53**: 294-303. 4. Coman, D., et al. 2010. NMR Biomed **23**: 277-285.