^{19}F MRI of Fibroblasts and Neuroblastoma cells labeled with emulsified Perfluoro-15-Crown-5 Ether

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Introduction: ^{19}F-labelled cells provide an important new technique to evaluate in vivo the migration of implanted cells such as in stem cell therapy [1]. ^{19}F-marker substances such as perfluorocarbons (PFC) have been used for cell tracking based on ^{19}F spectroscopy **[2]**as well as ^{19}F magnetic resonance imaging (MRI). We present first results of ^{19}F MRI of fibroblasts and neuroblastoma cells labeled with a perfluoro-15crown-5-ether (PFCE; ABCR-GmbH/Germany) emulsion [3,4]. The efficacy of the labelling compounds is mainly dependent on the composition and number of fluorine atoms. Often fluorine compunds exhibit strong chemical shifts reducing the sensitivity as well as introducing additional artifacts in MRI. In contrast PFCE has 20 chemically equivalent fluorine atoms. The ^{19}F spectrum therefore presents a single narrow resonance enabling imaging applications with a high sensitivity but without chemical shift artefacts [5]. To ease the transfection of the cells PFCE was applied as an emulsion.

Materials and Methods: Perfluoro-15crown-5-ether emulsion (40% (v/v) PFCE, 50% (v/v) DMEM-medium- ATCC, 10% (v/v) Acetone) was mixed in a 42 kHz ultrasonic water bath resulting in a total diameter of emulsion particles of about 1-5 µm. 50µl PFCE emulsion and 10 µl transfection reagents METAFECTENE, (Biontex, Germany) were added to fibroblasts of mouse embryo (Mus musculus, L929, CRL 1503 and mouse brain neuroblastoma CCL-131), and incubated for 24h at 37 °C and 5% CO_2. After incubation, the cells were centrifuged at 1200 RPM and washed twice in PBS. Cells were immersed into a 1.5% agarose (Sigma Aldrich) gel phantom. The phantom consisted of a core part (ca.1.5 ml agarose) containing 5x10^5 cell/ml with PFCE-emulsion labelled cells surrounded by an external cylindrically shaped 1.5% agarose phantom to optimize coil load and field homogeneity. Measurements were performed at 4.7 T (BRUKER BIOSPEC, Germany) using a ^{1}H/^{19}F Litz r.f. volume coil Doty scientific, Inc, USA). We used a 2D RARE-8 sequence for ^{1}H/^{19}F MRI, matrix 256 x 256/32 x 32, slice thickness 1/5 mm (other parameters s.. fig. 3/4). To optimize the ^{19}F-NMR parameters with respect to chemical shift, bandwidth, and pulse angles a 0.1 mmol solution of pure PFCE solved in Acetone was measured.

Results: Incubation of the cells with PFCE emulsions did not impair cellular viability. After staining the cells with trypan-blue 90% of the cells were shown to be still intact. Inverse microscopy (Olympus, 20x) served to control the labelling procedure (fig. 1 and fig. 2). Successfully labeled neuroblastoma cells (fig. 1) can be easily identified by the included intense white spots that are due to PFCE-emulsion while the control of non-labeled cells (Fig 2) did not exhibit any cells with white spots.
The ^{1}H image (Fig. 3) shows the agarose phantom with three labeled cells types (L 929, CRL 1503, and CCL 131). The ^{19}F image depicts the different contrast behaviour of the three cell types. While the neuroblastoma cell line CCL 131 exhibits the highest signal intensity, the CRL1503 has only weak signal intensity probably due to a reduced transfection with the ^{19}F emulsion.

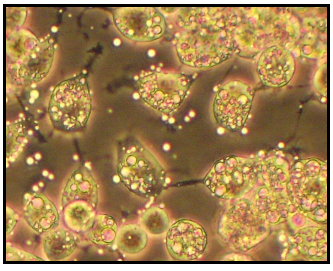

Fig.1

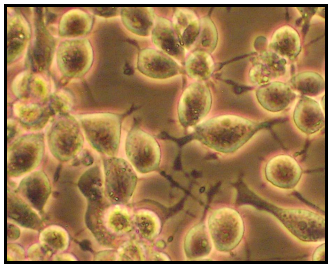

Fig. 2

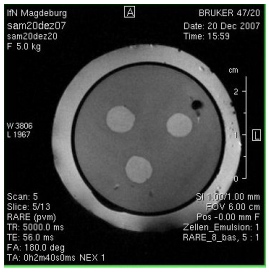

Fig. 3

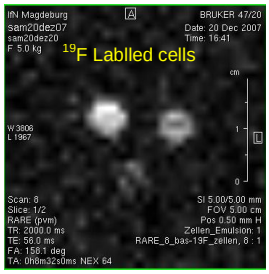

Fig. 4

Conclusion: The results of the present study show that the labelling procedure of fibroblasts and neuroblastoma cells allows a hight transfection of the cells. The concentration of the PFCE emulsion reached high levels and cells can be successfully monitored using ^{19}F-MR Imaging (fig. 4). Further optimizing ^{19}F cell-labeling procedure will improve this technique as a promising tool for positive contrast molecular imaging marker substances [6-8].

References: [1] A Kimura et al. Magn Reson Imag,22, 855-860 (2004). [2] Mulla-Osman et al. Proc. Intl. Soc. Mag. Res. Med. 16, p3237 (2008). [3] E T Ahrens et al., Nat Biotech. 23: 983-987 (2005). [4] JWM Bult et al. Nat Biotech 23. 945-946 (2005). [5] D S Caruthers et al. Inves. Radiologly 41:3, 305-312 (2006). [6] Hoehn M et al., *PNAS* 99: 16267-72 (2002). [7] Stroh A et al., Neuroimage 24:635-45 (2005). [8] Chen et al. Proc. Intl. Soc. Mag. Res. Med. 14, p239 (2006)