^{19}F NMR MOLECULAR IMAGING: CELL LABELING WITH EMULSIFIED PERFLUORO-15-CROWN-5-ETHER

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Introduction: Monitoring ^{19}F-labelled cells is an important new technique to evaluate in vivo the fate of implanted cells such as in stem cell therapy [1]. The use of ^{19}F-marker substances such as perfluorocarbons (PFC) has been explored by ^{19}F spectroscopy and magnetic resonance imaging (MRI) for cell tracking. We present first results of single volume selective spectroscopy of fibroblasts, which have been labeled with perfluoro-15crown-5-ether (PFCE; ABCR- GmbH/Germany) emulsion [2,3]. The efficacy of these synthetic compounds is highly dependent on the magnetic field strength and on the composition and number of fluorine atoms present in the compound. PFCE has 20 chemically equivalent fluorine atoms, and thus, the ^{19}F spectrum presents a single narrow resonance, enabling imaging applications with relatively high sensitivity and no chemical shift artefacts [4]. However, to ease the transfection of the cells we applied the PFCE 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).) were used for cell labelling. The emulsion 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), 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 an 1.5% agarose (Sigma Aldrich) gel core phantom. The phantom consisted of 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 the single volume selective spectroscopy ^{19}F-PRESS protocol for the spectroscopy with TR: 3000 ms, TE: 11.32 ms, SF: 188.539424 MHz, flip angle: 90° , 64 averages.

Results: The incubation of the cells with the PFCE emulsions did not impair cellular viability. Staining the cells with trypan-blue showed that 90% of the cells were still intact. The labelling procedure was controlled using inverse microscopy (Olympus, 20x), where labelled cells non labelled and labelled incubated cells are shown in figure 1 and figure 2; respectively. Labelled cells (Fig. 1) can be easily identified by their content of intense white spots that are due to PFCE-emulsion while the control of non-labelled cells (Fig 2) did not exhibit any cells with white spots.
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 (fig. 3). The ^{19}F-resonance clearly depicts the fluorine content in the complete cell sample. The single line confirms that PFCE leads to a maximum signal-to-noise (SNR) ratio as compared to most other PFCs as all ^{19}F nuclei are magnetically equivalent and therefore contribute to the signal. The SNR enabled a volume-selective spectroscopy with 64 averages in a volume of 8x8x8 mm^3 which contained about $8x10^5$ labelled cells.

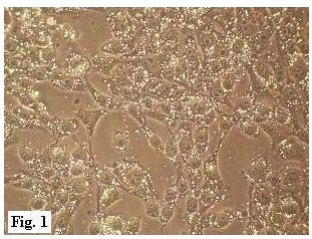
Fig. 1

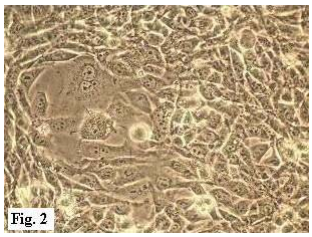
Fig. 2

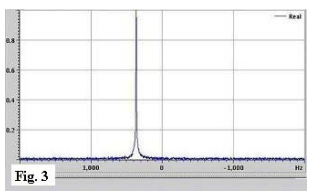
Fig. 3

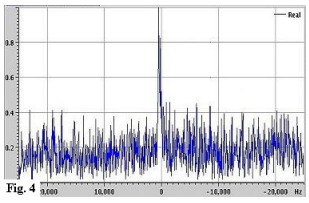
Fig. 4

Conclusion: The study showed that fibroblasts labelled with PFCE emulsion can be successfully detected by ^{19}F-NMR (fig. 4). The single volume selective spectroscopy detected the ^{19}F signal in the PFCE-labelled cells while agarose with non-labelled cells did not exhibit any ^{19}F-signal. PFCE with a single ^{19}F-resonance and the resulting high SNR is therefore very useful to perform 2D MR Imaging of other ^{19}F-labelled cells such as stem cells [5-7].

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