EFFICIENCY OF CELL LABELING WITH DIFFERENT PERFLUORO-15-CROWN-5 ETHER NANOPARTICLES FOR ^{19}F MRI

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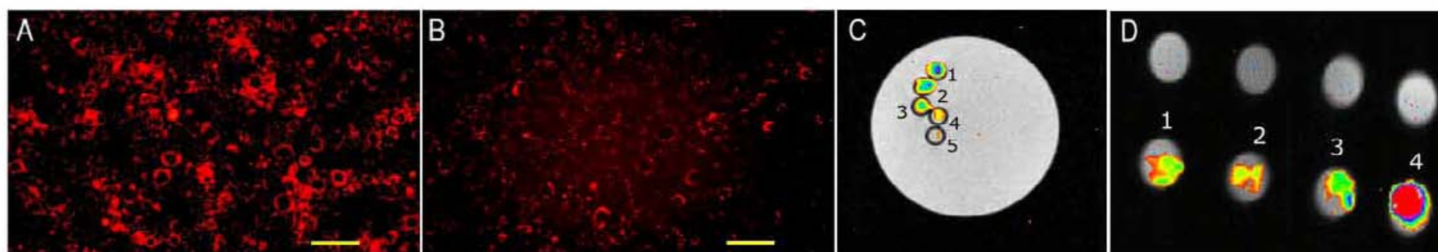
INTRODUCTION: The use of perfluoropolyether nanoparticles has been explored by ^{19}F MRI for cell tracking (1) and this has encouraged new applications. As compared to protons, the lack of mobile endogenous fluorine atoms greatly facilitates interpretation of the images as “hot spot” MRI (2). Fluorine emulsions of perfluorocarbons have been used in many biomedical applications, such as the measurement of partial pressure of oxygen in tissue, and blood substitutes. 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. Perfluoro-15-crown-5-ether (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 artifacts.

METHODS: C17.2 cells were cultured either in conventional polystyrene culture dishes having a carboxylic acid coating, or in modified commercially available dishes coated with both carboxylic acid and amino groups. The cationic nanoparticle (+62.8 mV zeta potential) was formulated with 20% PFCE, and a mixture of lipids, including 15% w/v phosphatidylcholine (PC), 5% w/v cholesterol, 59.9% w/v 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), 20% w/v 1,2-dioleoylphosphatidylphosphoethanolamine (DOPE), and 0.1% w/v rhodamine. The first anionic nanoparticle (-68.7 mV zeta potential) was formulated with 20% PFCE and emulsified in a mixture of lipids (15% PC, 5% cholesterol, 59.9 % dipalmitoyl phosphatidylserine, 20% DOPE, and 0.1 % rhodamine). This nanoparticle was modified to incorporate Gd-DTPA-BOA (15% mol). Cells were incubated with the PFCE nanoparticle from 0.6 mM to 2.4 mM for 4-48 h, and then washed three times. 19F relaxation times of PFCE were measured in 5 mm NMR tubes containing 500 µL, 60.7 mM PFCE. For sensitivity determination, a phantom was prepared with five 1 mm glass capillary tubes containing 60 µL PFCE (60.7-30.3 mM in gelatin 4% w/v). PFCE-labeled cells were suspended in 4% (w/v) gelatin at a density of 10^6 cells/ml. Proton and ^{19}F MRI was performed using a 9.4 T Bruker Biospec spectrometer using multi-slice (10 x 1 mm slices), and FOV=2.5x2.5 cm or 2.0x3.0 cm for all sequences. For ^{1}H, a spin echo sequence (TR/TE 1000/15 ms) with 128x128 matrix size, and for ^{19}F, a fast spin echo sequence with TR/TE: 1080/47 ms, 64 NA and matrix=64x32 were used. For T_1 acquisition, SE saturation recovery images were acquired with TE=15 ms and variable TR (100 ms-5000 ms). T_2 was obtained using a CPMG sequence with 20 echoes, TE=10 or 50 ms, and TR=5000 ms. T_1 and T_2 for fluorine in PFCE nanoparticles were calculated on a pixel-by-pixel basis using a monoexponential decay.

RESULTS: A. Cell labeling. The results displayed in panel A-C corresponds to 4 h incubation with cationic and anionic PFCE nanoparticles. Cationic PFCE nanoparticles (panel A) showed a higher and faster uptake (4h-18h) than the anionic PFCE ones (panel B). For the gadolinium-based nanoparticles, a longer incubation time (>48 h) was required to obtain a comparable labeling. For both types of nanoparticles, the best labeling (maximum cell density and least non-specific labeling) was achieved using surface-modified, polystyrene dishes with both carboxylic and amino groups. Cell viability and proliferation of labeled and unlabeled C17.2 cells were >95% for both the cationic and the two anionic nanoparticles.

B. NMR experiments. The measured T_1 of the cationic and anionic PFCE nanoparticle at 9.4 T (without gadolinium) was 1074±11 (n=5) ms for 4.8 mM dispersions in culture medium. The presence of Gd-DTPA-BOA in the nanoparticle shortened this spin lattice relaxation time to 812±53 ms at the same concentration and magnetic field. The T_2 of the Gd-DTPA-BOA anionic nanoparticle was significantly lower than that of cationic or anionic nanoparticles without Gd-DTPA-BOA (75 ms versus 670 ms, respectively). To evaluate the quantification and concentration limits relevant to MRI in vivo ^{19}F MRI applications, a phantom was prepared with decreasing PFCE concentrations. Panel C shows the T2-weighted ^{1}H image (background) with superimposed ^{19}F MRI images represented in a pseudo-color scale ranging from 60 mM (tube 1) to 30 mM (tube 5). Panel D shows the results with ^{19}F-labeled C17.2 cells after 4 h incubation with anionic (upper row) and cationic nanoparticles (bottom row), with different PFCE concentrations (from 0.6 mM, panel D1, to 2.4 mM, panel D4).



CONCLUSIONS: Cationic PFCE nanoparticles showed excellent labeling at short incubation times (4-18 h), with minimal non-specific residual binding when modified commercial plates were used. Anionic nanoparticles showed a slower and less efficient uptake with longer incubation times (>24h). Addition of gadolinium-DTPA-BOA decreased labeling efficiency. There was a strong ^{19}F T2 relaxation effect for the gadolinium-based nanoparticles, which requires the use of appropriate imaging sequences. A minimum of 30 mM PFPE concentration was required to obtain enough fluorine signal for MR imaging, with cells concentrating the PFCE from the cationic nanoparticle.

References:

1) E. Ahrens et al., Nat. Biotech. 2005, 23: 983-987; 2) JWM Bulte et al., Nat. Biotech. 2005, 23: 945-946.