^{19}F-MRI applications of PERFECTA at 7T: characterization studies on phantoms and on *in vitro* fibroblasts and T cells.

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Target Audience

This work is useful for radiologists, physicists, engineers and biologists that are involved in ^{19}F MRI studies for cell tracking and molecular imaging.

Background and Purpose

^{19}F MRI is gaining an important role in molecular imaging to perform *in vivo* cell tracking. Fluorinated compounds were extensively used to label dendritic cells (DC), T cells, hematopoietic stem cells and neuronal stem cells. Recently, a novel superfluorinated emulsion called PERFECTA[1], composed by a molecule bearing 36 equivalent ^{19}F atoms, was developed and effectively applied for labeling DCs. This new ^{19}F MRI contrast agent has shown promising results in terms of sensitivity and biocompatibility. In this work, a further characterization of PERFECTA was performed in terms of relaxation rates, sensitivity and linearity in different biological fluids. Furthermore, a preliminary magnetic characterization was also carried out in *in vitro* labeled cells (fibroblasts and T cells).

Methods

MRI and MRS experiments were performed using a BioSpec USR 70/30 (Bruker BioSpin, Ettlingen, Germany) 7T preclinical imaging system. MRI acquisitions were performed using a custom-built Radio Frequency (RF) linear volume coil ^{1}H/^{19}F having an inner diameter of 7 cm. *Phantoms experiments*:^{19}F MRI was performed on phantoms containing PERFECTA diluted in water and biological fluids (DMEM, RPMI) at different concentrations (10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1 mg/mL).*In vitro experiments*: BHK-21 cells and primary rat CD4+ T cells were labeled with PERCFECTA in DMEM at different concentrations (10 mg/mL, 5 mg/mL, 2.5 mg/mL) and different incubation times (2 h, 4 h, 8 h, 16h). After the incubation time, the cells were washed for three times in PBS and fixed in PFA 4%. ^{19}F MRI and MRS were then performed. Sensitivity studies *in vitro* were carried out on phantoms containing different number of cells (10^6 to 10^4). In order to estimate the amount of ^{19}F atoms in the phantoms, ^{19}F MRS was performed to compare the ^{19}F spectra to an external reference (Trifluoroethanol (TFE) in water, 1mL (50 mg/L)). A single pulse sequence was used with the following parameters (TR=20s; NA=15; pulse width; Spectral width (SW)=100 kHz; FID Acquisition Time=40.96; Number of points=4096, Dwell time 5 us, Spectral Resolution=12.21 Hz). For estimating T_1 relaxation times, a saturation recovery (RAREVTR) sequence was used with the following parameters (FOV4.2*4.2 cm^2; Matrix=32*32; Slices=14; ST=0.7 mm; TE=11 ms; TR=10000, 5000, 2500, 1500, 800, 400, 200, 100 ms; Rare Factor=2; BW=15 kHz; NA=50; Dummy Scans (DS)=0). For estimating T_2 relaxation times a Multi Slice Multi Echo (MSME) sequence was performed with the following parameters (FOV=4.2*4.2 cm^2; Matrix=32*32; Slices=1; Slice Thickness=10 mm; TE=11 ms; TR=5000 ms; Number of echoes=40; BW=15 kHz; NA=100; DS=0).

Results

PERFECTA exhibits a single sharp resonance peak (frequency 282.5733 MHz) in all the biological fluids and an optimal linearity at different concentrations (r^2>0.99). The linearity does not change significantly in different biological environments. The sensitivity of PERFECTA, evaluated in 1hr of acquisition time, had an average value of $2.34*10^{16}\pm 5.75*10^{15}$^{19}F atoms per voxel. T_1 relaxation time had an average value of 587 ± 22 ms while T_2 had a value of 181 ± 8 ms. No significant difference was highlighted between different environments. Fibroblasts and T cells were effectively labeled using PERFECTA. The amount of ^{19}F atoms per cell varied in a range of $1*10^{11}$ - $1*10^{12}$for BHK-21 cells and in a range of $2*10^{12}$ – $2*10^{13}$for T cells. Obviously, the labeling efficacy is affected by the concentration of the emulsion and by the incubation time. Interestingly, a short acquisition time (about 2 hr) is sufficient to detect both cell types. BHK-21 cells exhibited an average T_1 of 548 ± 17 ms and an average T_2 of 163 ± 5 ms, while T cells exhibited a T_1 value of 567 ±18 ms and a T_2 value of 167 ± 12 ms. The minimum number of cells detectable by ^{19}F MRI was about 10^4 cells per voxel in 1hr of acquisition.

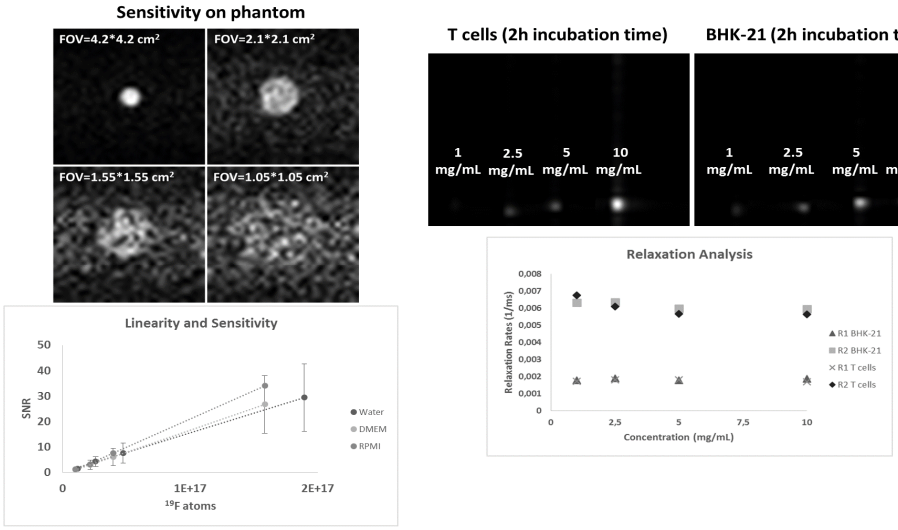

Figure 1. Left panels: Sensitivity and linearity assessment on phantom containing PERFECTA 1 mg/ml in RPMI. Different FOVs and resolution were considered to determine the sensitivity threshold. A SNR of 3.5 was considered as minimum detection limit. The left bottom figure shows the SNR vs. the amount of ^{19}F atoms per voxel for all the biological fluids.
Right panels: Evaluation of the efficacy of labeling in T cells and BHK-21 cells and relaxation analysis in vitro. In the upper panel cells were incubated for 2h and different concentrations of PERFECTA were used. Relaxation rates are displayed in the bottom panel

Discussion and Conclusion

The new superfluorinated probe PERFECTA was effectively diluted in different biological fluids and was characterized on a 7T scanner. Its magnetic properties (spectra, T_1, T_2) remained unchanged in different environments. This aspect is crucial to optimize image sequence and increase sensitivity[2]. Moreover, ^{19}F MRI applications on phantoms have shown a valuable linearity and sensitivity in relatively short acquisition times. Fibroblasts and T cells were effectively labeled and different labeling protocols were evaluated. ^{19}F MR images confirm the efficacy of labeling procedure. Furthermore, the sensitivity and relaxation rates provided by this preliminary work are promising for future cell tracking experiments. In conclusion, the MRI performances of PERFECTA, in terms of spectral properties, relaxation times, and sensitivity, and its ability to label different cell types, strongly indicate that the new ^{19}F-MRI probe is suitable for *in vivo* applications.

References

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