Does Endocytosis of Perfluorocarbon Nanoparticles by Progenitor/Stem Cells Alter 19F Relaxation at 11.7T?

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Introduction

We have reported previously that a unique MRI signal source, fluorine, which is abundant in liquid perfluorocarbon nanoparticles (PFC NP), can be used for molecular and cellular imaging to provide simultaneous readouts of the ^{19}F and ^{1}H signals at 1.5T and 4.7T when gadolinium chelates are incorporated into the lipid membrane of the PFC NP (1, 2). Furthermore, simultaneous optical imaging of fluorescent molecules can be achieved by formulating molecules such as rhodamine into the particle, making these multispectral PFC NP capable of at least three independent signal readouts. The proposed use of PFC NP as unique labeling agents for stem cells depends on endocytosis of particles by stem/progenitor cells. However, whether the ^{19}F relaxation parameters could vary as a result of either cellular endocytosis or the incorporation of Gd-chelates or fluorophores into the PFC NP is not known. Evaluation of these parameters is critical for optimization of the labeling and imaging processes required to track stem cells with ^{19}F MRI, especially at higher field strengths (11.7T) that are advantageous for imaging mice. Accordingly, we characterized ^{19}F T_1 and T_2 relaxation before and after stem cell labeling, and elucidated the consequences of incorporating different molecules into the lipid layer of PFC NP in phantoms and labeled progenitor/stem cells at 11.7T.

Methods

Nanoparticle Preparation: PFC NP consist of 40% (v/v) perfluoro-15-crown-5 ether (CE), 2% (w/v) surfactant commixture, 1.5%(w/v) lecithin and water for balance. It was formulated by a liquid CE core encapsulated by a lipid-surfactant monolayer with a nominal diameter of 250 nm. Paramagnetic particles were produced by incorporating Gd-DTPA to the lipid monolayer, resulting in approximately 50,000 Gd molecules per particle. Similarly, fluorescent labeled PFC NP emulsions were prepared by incorporating rhodamine to the lipid monolayer. All formulations contained equivalent final concentrations of fluorine (12.1 M).

Cells Preparation: Stem/progenitor cells were harvested by density gradient centrifugation from human umbilical cord blood and grown under proendothelial conditions (Clonetics EGM-2 + 20% FBS) on fibronectin-coated plates. After 7-14 days, cells were incubated for 12 hours with a 30 pM concentration of rhodamine-labeled PFC NP. After loading, cell pellets were prepared by removing free nanoparticles with PBS washing, detaching adherent cells from the surface, and preserving samples with 2% paraformaldehyde fixation for 30 minutes.

^{19}F MRS/MRI: All experiments were performed on a Varian 11.7T scanner at room temperature (~23° C). A custom-built 0.5cm, 4-turn solenoid RF coil was used. To measure T_1 values, an inversion recovery sequence was used. Eight Ti values that exponentially increased from 3 ms to 3 s were used (TR=5.7s). T_2 measurement was performed with a standard spin echo sequence. Eight TE values that exponentially increased from 13 ms to 500 ms were used (TR=5s).

Data Analysis: ^{19}F spectra were analyzed using NUTS NMR data processing software (Acorn NMR Inc, Livermore, CA) for phasing and integration. A single exponential curve was fitted to the data using a least squares method to estimate T_1 and T_2. ^{19}F images were processed using MATLAB (Mathworks, Natick, MA) to perform a pixel-by-pixel fitting of T_2 values.

Results and Discussion

The measured T_1 and T_2 from selected formulations are shown in Figure 1. T_1 relaxation time of ^{19}F was approximately 700ms for all samples, indicating that the incorporated molecules on the lipid layer of PFC NP have no effect on ^{19}F T_1 relaxation. However, T_2 relaxation time of ^{19}F showed large variance. Specifically, it was longest for pure CE (250ms), was about 100ms for CE NP and CE NP with rhodamine, and was shortest for CE NP that incorporated with Gd-DTPA (28ms). These data suggested that Gd-DTPA is a T_2, but not a T_1 shortening agent for ^{19}F MRI. It also showed that ^{19}F T_1 and T_2 relaxation time of PFC NP were not changed following endocytosis of nanoparticle, indicating that cell internalization exerts no significant influence on ^{19}F relaxivity of PFC NP.

Quantitative analysis using T_2 weighted images confirms the spectroscopy measurement. T_2 was 100.0 ± 1.0ms for CE NP in cells, which was similar to 103.3 ± 3.0ms which was the T_2 for CE NP in emulsions. Both T_2 relaxation times were appreciably longer than that of Gd-DTPA incorporated PFC NP (33.6 ± 2.8 ms).

Conclusions

The current study presents the first quantitative characterization of the ^{19}F T_1 and T_2 relaxation times of PFC NP under conditions of stem cell endocytosis at 11.7T. No significant changes in relaxation times were observed for PFC NP after being endocytosed by cells, suggesting that ^{19}F imaging of PFC NP could serve as a reliable cell tracking method to characterize the behavior of implanted stem cells. However, the data indicate that simultaneous incorporation of Gd into PFC NP for dual ^{1}H and ^{19}F imaging might limit the potential for ^{19}F imaging because of the T_2 shortening properties of Gd. Accordingly, the dual imaging potential for the agent is likely better achieved with fluorine MRI and fluorescence optical imaging.

References

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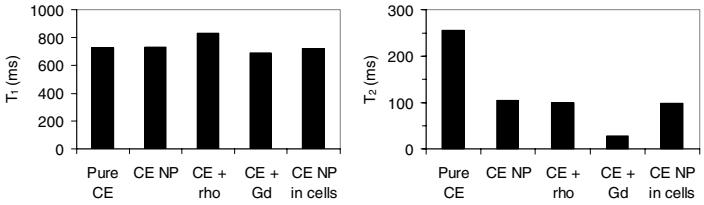

Figure 1. T_1 (left) and T_2 (right) for different CE NP in emulsion and in cells. CE + rho, CE NP with rhodamine; CE + Gd, CE NP with Gd-DTPA.