

Novel perfluorocarbon nanoemulsion for ¹⁹F MRI cell tracking of two cell populations in vivo

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Introduction – Tracking multiple cell populations *in vivo*, such as interacting immune cell subsets, may provide a powerful way to visualize disease process in model systems. In this work, we describe a novel, non-toxic ¹⁹F tracer reagent, perfluorotertbutylether (PFTE) nanoemulsion, suitable for *ex vivo* cell labeling and imaging *in vivo*. The PFTE molecule has a single NMR resonance from nine equivalent ¹⁹F nuclei, and a chemical shift separated by ~20 ppm compared to perfluoro-15-crown-5-ether (PCE) and linear perfluoropolyether (PFPE), both previously used in cell tracking applications ^{1, 2, 3, 4}. This large chemical shift can be exploited to image two cell populations simultaneously when inoculated into a subject using spectroscopically-resolved ¹⁹F MRI. As proof-of-principle, we labeled mouse dendritic cells (DCs) with PCE and PFTE, and implanted these in the quadriceps of mice. Our data show that it is possible to simultaneously visualize separate cell populations labeled with PCE and PFTE in the same animal *in vivo*. The PFTE nanoemulsion formulation is a sensitive, new ¹⁹F reagent for *in vivo* cell tracking.

Methods – PFTE was synthesized using the previously published Mitsunobu synthetic protocol⁵ with some modifications. The resulting clear oil was formulated into a nanoemulsion by sonication. Fetal skin-derived dendritic cells (FSDCs) cells⁶ were incubated with either PCE or PFTE nanoemulsion using protocols described elsewhere ^{1, 2, 3}. The cell viability and proliferation were assessed using the Cell-Titer-Glo assay (Promega, Madison, WI), and cellular uptake of PCE and PFTE was quantified by ¹⁹F NMR of cell pellets at 470 MHz ^{1, 2, 3}; trifluoroacetic acid (TFA) in the NMR tube was used as a reference. FSDCs (~10⁶) cells labeled with PCE and PFTE were injected into the right and left quadriceps muscles, respectively, in Balb/c mice (Jackson laboratories, Bar Harbor, ME). Imaging was performed in anesthetized mice using an 11.7 T microimaging system with a birdcage volume resonator that could be tuned to either ¹H or ¹⁹F. High-resolution ¹H images were obtained using a standard spin-echo sequence (TR/TE = 1000/15 ms, 512 x 256 matrix, FOV = 4 x 4 cm, slice thickness = 3 mm). ¹⁹F images for PCE and PFTE were obtained serially using a narrow bandwidth, RARE sequence (RF pulse BW= 2.7 kHz, Effective spectral width (read) = 10 kHz, rare factor = 8, TR/TE = 1500/10 ms, 32 x 32 matrix) with the same slice geometry as the ¹H. The ¹⁹F images were rendered in pseudo-color, with PCE in hot-iron and PFTE in green, and superimposed onto the grayscale ¹H image.

Results – The PFTE nanoemulsion was stable in 10% serum media, with a particle size of 150-180 nm and a polydispersity index 0.15-0.20. *In vitro*, the PFTE nanoemulsion appeared to be non-toxic and did not affect cell proliferation, as seen by the Cell-Titer-Glo assay. Figure 1 shows the ¹⁹F NMR labeling results in cell pellets (1A) and after inoculation, detected by *in vivo* ¹⁹F MRI. These data show the feasibility of multi-spectral ¹⁹F MRI using PCE and PFTE to visualize two cell populations *in vivo*. The PFTE nanoemulsion has the advantage of having a single ¹⁹F NMR peak, thereby maximizing sensitivity, and a large (~ 20 ppm) chemical shift with respect to PCE and PFPE, thus simplifying pulse sequence design. The PFTE nanoemulsion opens up new opportunities for studies of immune cell-cell interactions *in vivo*.

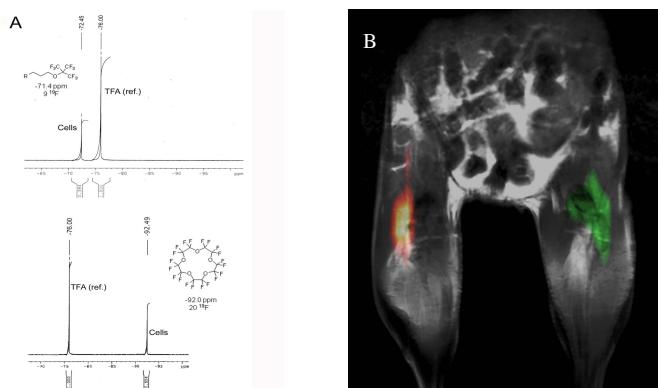


Fig 1. Multi-spectral ¹⁹F MRI of DCs labeled with PCE and PFTE. (A) Shows the structures and spectra of PCE (-92 ppm), and PFTE (-71.4 ppm) in labeled DC pellets, along with the TFA spectra at -76 ppm. (B) A composite ¹⁹F/¹H image of DCs labeled with PCE (hot iron) and PFTE (green) injected into the right and left quadriceps muscles, respectively.

References

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Acknowledgements

This work was funded by NIH grants R01-EB003453, P01-HD047675 and P41-EB001977.