

# Novel perfluorocarbon nanoemulsion for $^{19}\text{F}$ MRI cell tracking of two cell populations *in vivo*

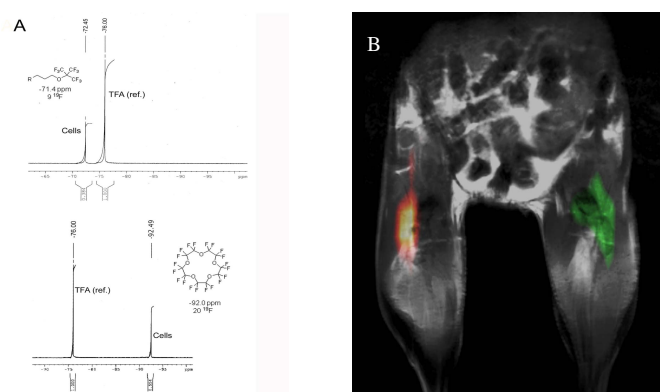
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**Introduction** - Tracking multiple cell populations *in vivo*, such as interacting immune cell subsets, may provide a powerful way to visualize disease process in model systems. In this work, we describe a novel, non-toxic  $^{19}\text{F}$  tracer 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  $^{19}\text{F}$  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<sup>1, 2, 3, 4</sup>. This large chemical shift can be exploited to image two cell populations simultaneously when inoculated into a subject using spectroscopically-resolved  $^{19}\text{F}$  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  $^{19}\text{F}$  reagent for *in vivo* cell tracking.

**Methods** - PFTE was synthesized using the previously published Mitsunobu synthetic protocol<sup>5</sup> with some modifications. The resulting clear oil was formulated into a nanoemulsion by sonication. Fetal skin-derived dendritic cells (FSDCs) cells<sup>6</sup> were incubated with either PCE or PFTE nanoemulsion using protocols described elsewhere<sup>1, 2, 3</sup>. 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  $^{19}\text{F}$  NMR of cell pellets at 470 MHz<sup>1, 2, 3</sup>; trifluoroacetic acid (TFA) in the NMR tube was used as a reference. FSDCs (~10<sup>6</sup>) 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  $^1\text{H}$  or  $^{19}\text{F}$ . High-resolution  $^1\text{H}$  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).  $^{19}\text{F}$  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  $^1\text{H}$ . The  $^{19}\text{F}$  images were rendered in pseudo-color, with PCE in hot-iron and PFTE in green, and superimposed onto the grayscale  $^1\text{H}$  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  $^{19}\text{F}$  NMR labeling results in cell pellets (1A) and after inoculation, detected by *in vivo*  $^{19}\text{F}$  MRI. These data show the feasibility of multi-spectral  $^{19}\text{F}$  MRI using PCE and PFTE to visualize two cell populations *in vivo*. The PFTE nanoemulsion has the advantage of having a single  $^{19}\text{F}$  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  $^{19}\text{F}$  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  $^{19}\text{F}/^1\text{H}$  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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