Labeling and Imaging Stem/Progenitor Cells with Multiple Unique Nanoparticulate Fluorine Markers: The Potential for Multispectral Stem Cell Detection with 19F MRI

J. Chen[1], K. Crowder[1], J. Brant[1], A. M. Neubauer[2], S. D. Caruthers[3], G. M. Lanza[1], S. A. Wickline[1]

[1]School of Medicine, Washington University, St. Louis, MO, United States, [2]Biomedical Engineering, Washington University, St. Louis, MO, United States, [3]Philips Medical System, St. Louis, MO, United States

Introduction

Current methods for non-invasive imaging of stem/progenitor cells *in vivo* entail the use of paramagnetic gadolinium compounds or super-paramagenetic iron oxides. However, the prominent ^{1}H background from tissue and the loss of contrast agent from stem cells during migration may thwart unambiguous detection of labeled cells. We and others have shown previously that ^{19}F MRI of liquid perfluorocarbon (PFC) nanoparticles may present a unique and sensitive cell label (fluorine) that cannot be obscured by traditional tissue background proton signal. We now seek to demonstrate the possibility for creating *multiple* unique and differentiable PFC labels for stem cells. Accordingly, perfluoro-15-crown-5 ether (CE) and perfluorooctylbromide (PFOB) were used to formulate nanoparticles that could serve as simultaneous intracellular markers with the use of ^{19}F MRS/MRI at 11.7T.

Methods

Nanoparticle Preparation: Nanoparticles (~250 nm) consist of a liquid CE or PFOB core surrounded by a lipid monolayer. Microemulsification of particles was performed with 40% (v/v) CE or PFOB, 2% (w/v) surfactant commixture, 1.5%(w/v) lecithin and water. The final concentration of ^{19}F was 12.14 M for CE emulsions and 13.15M for PFOB emulsions.

Cell Culture: 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 CE or NBD labeled PFOB nanoparticles. Control cultures were treated with the same concentration of particles for ~10 minutes before washing. 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. The cells were centrifuged at 100G (5 minutes) for MR characterization. A total of 372,000 CE labeled cells and 490,000 PFOB labeled cells were prepared.

^{19}F MRS/MRI: ^{19}F MRS/MRI of labeled stem/progenitor cells was performed on a Varian 11.7T scanner using a 0.5cm 4-turn solenoid RF coil. The signal from labeled cells was compared to an external standard consisting of neat nanoparticle emulsion (CE emulsion was used as a standard for PFOB labeled cells and vice versa). ^{19}F MRS of the cells was performed for qualitative evaluation of intracellular labeling of nanoparticles (TR 1s, 128 averages, 2 min acquisition time). The spectrum of CE and PFOB were further used to determine the offset frequency of RF output for ^{19}F imaging. ^{19}F images of PFOB and CE labeled cells were acquired using a multi-slice gradient echo sequence (3ms TE, 50ms TR, , 20° flip angle, 1x1 cm^2 FOV, 156x156 µm^2 image resolution, , 2 mm slice thickness, 128 averages, ~7 min acquisition time).

Results

The spectrum of CE nanoparticle labeled cells with an external 4uL PFOB nanoparticle emulsion standard is shown in Figure 1A. The spectrum of PFOB nanoparticle labeled cells and 2uL CE nanoparticle emulsion standard is shown in Figure 1B. These data illustrate that the spectrum of CE is comprised of a single ^{19}F peak, whereas the spectrum of PFOB is characterized by multiple peaks that span 60 ppm in the frequency domain. No ^{19}F signal was detected from control stem/progenitor cells. For ^{19}F MRI of PFOB labeled cells, the center frequency of the second PFOB peak from the right was selected as RF output frequency of MR console. The ^{19}F images of both CE and PFOB nanoparticle labeled cells are shown in Figure 2. The intracellular labeling of nanoparticles was confirmed by confocal microscopy imaging (Figure 3).

Conclusion and Discussion

The current study presents the first evidence confirming the feasibility of ^{19}F imaging of stem cells labeled with two MR-differentiable agents. Although the signal intensity of PFOB labeled cells was lower than that of CE labeled cells, ^{19}F MRI suggested that PFOB labeled cells are detectable even at high image resolution, indicating PFOB nanoparticles are readily endocytosed by the stem/progenitor cells. We suggest that these findings will facilitate the in-vivo tracking of implanted therapeutic stem cells to investigate their migration behavior using ^{19}F MRI, as well as provide unique and differentiable signals for simultaneous labeling and tracking of multiple types of stem cells

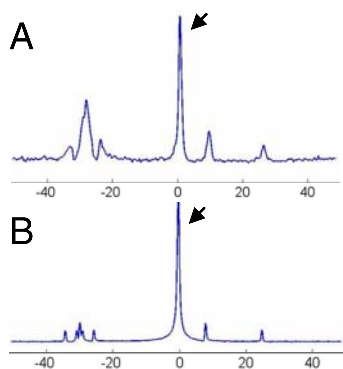

Figure1. (A) Spectrum of a CE labeled cells with 4uL PFOB nanoparticle emulsion standard; (B) Spectrum of PFOB labeled cells and 2 uL CE nanoparticle emulsion standard. Arrows indicate single CE peak. Other peaks are from PFOB and are clearly resolved.

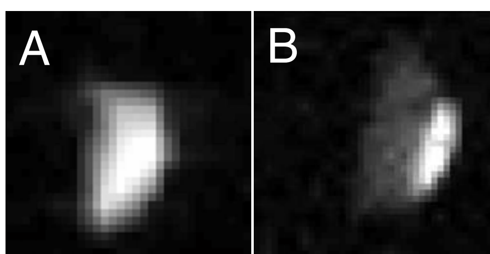

Figure 2. ^{19}F image of CE (A) and PFOB (B) labeled stem/progenitor cells *in vitro*.

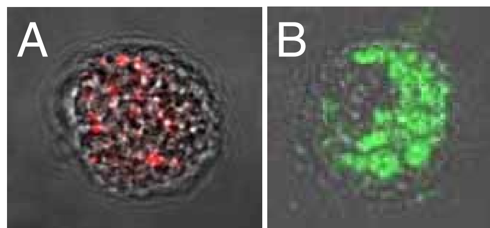

Figure 3. Confocal image showing PFC nanoparticle inside cells. (A) Rhodamine (red) labeled CE nanoparticle inside a single cell. (B) NBD (green) labeled PFOB nanoparticles inside a single cell.