Fluorine Diffusion Measurements Confirm Intracellular Labeling of Perflurocarbon Nanoparticles in Therapeutic Stem/Progenitor Cells as Tracking Agents

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Introduction

The ability to track transplanted therapeutic cells in-vivo is critical for elucidating mechanisms of cell migration and differentiation. Previously, ^{1}H MRI has been used to define the migratory behavior of stem/progenitor cells in *vivo* using contrast agents such as Gadolinium or super-paramagenetic iron oxides. However, the large ^{1}H background from tissue and the loss of contrast agent from stem cell during migration may thwart unambiguous detection of labeled cells. ^{19}F MRI of liquid perfluorocarbon nanoparticles (PFC NP: 250 nm) may offer a unique and sensitive cell marker (fluorine) that cannot be obscured by traditional tissue background proton signal. We now propose ^{19}F diffusion weighted MRS/MRI (DWS/DWI) could be used as a non-invasive method to confirm intracellular labeling of stem/progenitor cells by specifically detecting intracellular PFC NP as its diffusivity decreases dramatically upon successful cell labeling.

Methods

Nanoparticle Preparation: Nanoparticles consist of a liquid CE or PFOB core surrounded by a lipid monolayer. Microemulsification of particles was performed with 40% (v/v) CE, 2% (w/v) surfactant commixture, 1.5%(w/v) lecithin and water. The final concentration of ^{19}F was 12.14 M for CE emulsion. PFC NP emulsion was further diluted to 50%, 5% and 0.5% for diffusion weighted MRS/MRI. Pure CE was also prepared to calibrate the diffusivity of ^{19}F molecules in un-restricted environment.

Cell Culture: Endothelial 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 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. The cells were centrifuged at 100G (5 minutes) in a pellet for MR characterization. A total of 372,000 CE labeled cells were prepared.

^{19}F DWS/DWI: ^{19}F DWS/DWI of emulsions and labeled stem/progenitor cells were performed on a Varian 11.7T scanner using a 0.5cm 4-turn solenoid RF coil. A slice selective spin-echo sequence with diffusion sensitizing gradient was used to acquire diffusion weighted spectra. Eight b-values ranged from 0 to 1.99 s/μm^2 were used. Other acquisition parameters were: TE, 230ms; TR, 2s; Δ, 200 ms; δ, 20 ms; number of averages, 4; acquisition time, 1 minute. A multi-slice spin-echo sequence with diffusion sensitizing bipolar gradient was used to acquire diffusion-weighed images. Six b-values ranged from 0 to 3.05 s/μm^2 were used. Other imaging parameters were: TE, 240 ms; TR, 1.0 s; Δ, 200 ms; δ, 30 ms; FOV, 1.5×1.5 cm^2, slice thickness, 2 mm; image resolution, 234×234 μm^2; number of averages;4; acquisition time, 6 minutes.

Data analysis: Diffusion coefficient was calculated from diffusion weighted spectra and images using a single-component diffusion model that assuming isotropic diffusion inside of the spherical cells.

Results

Figure 1 shows the ^{19}F signal intensity in pure CE, 5% PFC NP emulsion, and PFC NP labeled stem/progenitor cells using DWS. An exponential decay of diffusion weighted ^{19}F signal intensity that associated with increased b-values was observed for pure CE and PFC NP emulsion. However, ^{19}F signal intensity was not significantly changed for PFC NP labeled cells. Specifically, when b-value is 1.99 s/μm^2, ^{19}F signal intensity decreased about 99.99% in pure CE; and decreased 99%, 96% and 72% in 0.5%, 5% and 50% PFC NP emulsions, respectively. In contrast, ^{19}F signal intensity *decreased less than 5%* in PFC NP labeled cells.

Figure 2 shows ^{19}F diffusion coefficients of 5% PFC NP emulsion, 50% PFC NP emulsion and PFC NP labeled cells. At all circumstance, ^{19}F diffusion coefficient of PFC NP was much lower than that of pure CE (203±47 μm^2/s). Figure 3 shows diffusion weighted images of a pellet of stem/progenitor cells that suspended in 5% PFC NP emulsion. ^{19}F signal intensity of 5% PFC NP emulsions decreased as b-value increased. In contrast, ^{19}F signal intensity of PFC labeled cells remains constant for all b-values.

Conclusion and Discussion

The current study presents the first characterization of the diffusion behavior of intracellular PFC NP in stem/progenitor cells using DWI/DWS. The PFC NP (~250 nm diameter) is analogous to a macromolecular structure; and its diffusivity is significantly lower than that of ^{19}F molecules are in pure CE. The diffusivity of PFC NP in solution decreases as its concentration increases, which likely reflects increased viscosity of the oily native material. The diffusivity of PFC NP inside stem cell is close to two orders of magnitude lower than that in neat emulsions, indicating markedly restricted intracellular diffusion. At high b-value, DWI/DWS indicates that the ^{19}F signal from intracellular PFC NP is much greater than that in neat emulsion. Thus, this approach may be able to depict the uptake rate of PFC NP by stem/progenitor cells during cell culture as a way to look at labeling kinetics, while also allowing in-vivo tracking of the migration behavior of implanted therapeutic cells by specifically detecting intracellular PFC NP.

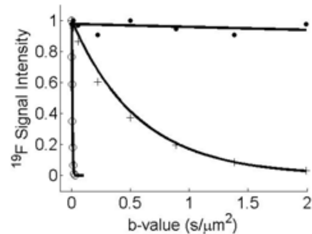

Figure 1. ^{19}F signal of pure CE (o), 5% PFC NP emulsion (+), and PFC NP labeled cells (•) at different b-values using DWS.

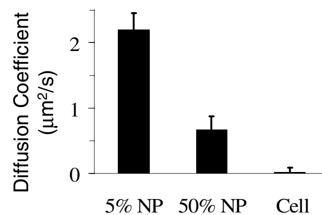

Figure 2. ^{19}F diffusion coefficient in 5% PFC NP emulsion (5% NP), 50 % PFC NP emulsion (50% NP), and PFC NP labeled cells (cell).

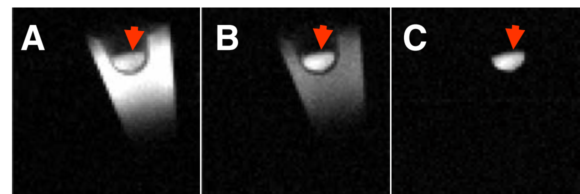

Figure 3. Diffusion weighted images of a pellet of stem/progenitor cells (arrows) that suspended in 5% CE NP emulsion. A. b-value=0 s/μm^2; B. b-value= 0.48 s/μm^2; C. b-value = 3.05 s/μm^2.