Unique and Rapid 19F Imaging of Stem Cells Labeled with Perfluorocarbon Nanoparticles at 11.7T and 1.5T

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ABSTRACT
Stem cell tracking is rapidly developing as a new application of magnetic resonance imaging because it allows for precise anatomical localization of the cells as they migrate in living tissue. While most current labeling methods have centered on the use of iron oxide particles to produce "negative" contrast, we have developed a liquid perfluorocarbon nanoparticle that is readily endocytosed by stem/progenitor cells and allows for ^{19}F imaging for tracking. In this work, we demonstrate the use of these particles for imaging of labeled cells *in vitro* at both 11.7 T and 1.5T, and in mouse skeletal muscle at 11.7 T.

INTRODUCTION
Endothelial progenitor cells are postulated to localize to areas of injury or ischemia to facilitate vasculogenesis (1). However, longitudinal tracking of their location and migration in the body is needed to define their fate *in vivo*. Current methods for tracking stem cells have used iron oxide particles that are endocytosed by the cells, so that they are then apparent on MR images as a signal void due to magnetic susceptibility effects on proton signals. In contrast, we have developed a contrast agent composed of liquid perfluoro-15-crown-5 ether surrounded by a lipid monolayer that allows direct visualization by imaging the fluorine nuclei (2). Upon uptake, the cells are imaged using ^{19}F MRI, which allows for definitive localization since the only detectable fluorine in the tissue is contained within the particles. Because there is no tissue background signal from endogenous fluorine, the high contrast to noise ratios permit definitive localization of the labeled cells.

MATERIALS and METHODS
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 crown ether nanoparticles created in our lab with previously described techniques (3). 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 fixed and pelleted cells (~1 million) were imaged at 1.5 T using a clinical scanner (Philips Intera CV, Philps Medical Systems; Best, Netherlands) fitted with a dedicated ^{19}F channel using a 7-cm square surface coil tuned to 60.1 MHz for transmit and receive. ^{19}F projection images of the cells were acquired using a balanced FFE sequence (TE 5ms, TR 10 ms, 512 signal averages, 0.5x0.5x25 mm resolution, 60º flip angle, *~9 min total scan time*). Matching ^{1}H images were acquired for comparison using the quadrature body coil for transmission and 6 cm diameter surface coil for receive (spin echo sequence, TE 15ms, TR 500ms, 2 signal averages, 0.5x0.5x5 mm resolution, 70º flip angle). For *in situ* imaging, an adult C57/BL6 mouse was injected with in the right thigh with approximately one million labeled stem/progenitor cells diluted in 50 uL PBS. ^{1}H and ^{19}F MR imaging were performed on a Varion 11.7 T Inova console using a 3 cm surface coil tuned to either ^{1}H or ^{19}F frequency and a multi-slice gradient echo sequence (^{19}F parameters: image matrix 64x64, FOV 3x3 mm, slice thickness 2mm, TR 50ms, TE 3ms, number of averages 128, ~7 minute total acquisition time; ^{1}H parameters: image matrix 256x256, FOV 3x3 mm; slice thickness 2mm, TR 300 ms, TE 3ms, number of average 2, *~2.5 min acquisition time*).

RESULTS and DISCUSSION
Rhodamine-labeled nanoparticles were endocytosed readily by the cells, as shown in the confocal microscopy image in Figure 1. Furthermore, their level of uptake was high enough that upon dilution and injection into a mouse leg, sufficient ^{19}F was present to allow acquisition of an image at 11.7 T. The fluorine signal co-localized with an air bubble introduced during the injection (indicated by the signal void in the muscle), verifying that the location of the signal is correct. In addition, all images were acquired in a short amount of time (~7 minutes), rendering this a viable technique for tracking stem cell migration *in vivo*. The pelleted cells were also imaged prior to injection with a 1.5 T clinical scanner. As illustrated in Figure 3, the fluorine signal is specific for imaging cellular uptake of the nanoparticles and not due to non-specific accumulation. The ^{19}F imaging sequence generated a signal-to-noise ratio of 21 in under 10 minutes at 1.5 T.

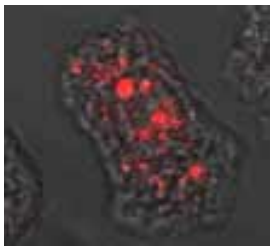

Figure 1: Confocal image showing the rhodamine (red) labeled particles inside of a single stem/progenitor cell.

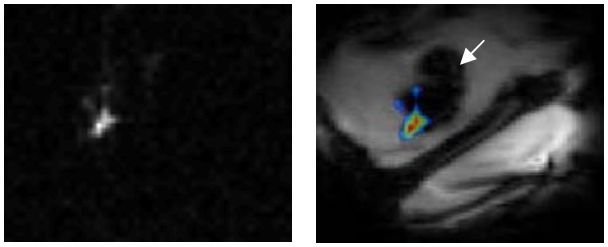

Figure 2: 11.7T imaging: *In situ* imaging of nanoparticle-loaded stem/progenitor cells injected into a mouse leg. Left: ^{19}F image. Right: ^{19}F image overlaid onto the ^{1}H image. The signal void in the muscle is susceptibility induced by an air bubble (arrow) introduced during injection (note: artifact not observable in ^{19}F image).

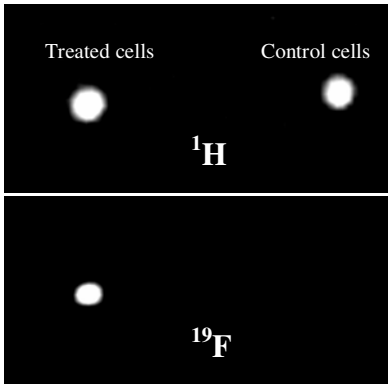

Figure 3: 1.5T imaging: *In vitro* imaging of nanoparticle loaded stem/progenitor cells at 1.5 T. "Treated cells" were incubated with nanoparticles for 12 hours, while "control cells" were incubated for ~10 minutes and then washed.

CONCLUSIONS
The following novel observations were made: 1) fluorinated nanoparticle contrast agents are efficiently endocytosed by stem/progenitor cells, allowing intracellular accumulation of a high concentration of fluorine, 2) the ^{19}F in the particles generates a measurable signal with MRI at both 1.5T and 11.7T in very short scan times compatible with patient imaging requirements, and 3) labeled stem cells can be visualized at 11.7 T in biological tissue. We propose that this technique will facilitate the tracking of stem cell fate after injection into a live animal.

REFERENCES

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