Customizable PLGA-encapsulated perfluorocarbon particles for in vivo 19F MRI

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Introduction
Magnetic Resonance Imaging (MRI) using 1H spins from water is a major in vivo imaging modality. With 19F MRI, cells labeled with fluorinated compounds can be visualized in the body without interference of the large H background from mobile water in tissues. Importantly, it also allows quantification of the number of cells directly from the image data [1, 2]. However, the perfluorocarbons (PFCs), typically used as 19F labels are unstable in aqueous environments and often toxic for direct injection in humans. Here we report on the use of a biocompatible polymer currently in clinical use, poly(D,L-lactide-co-glycolide), to entrap various 19F compounds. These particles can be customized in terms of their content, size and surface coating, including targeting antibodies. We encapsulate a range of clinically-relevant perfluorocarbons and test the particles for labeling primary human dendritic cells (DCs) and in vivo imaging using 19F MRI.

Methods
Primary human DCs were cultured, as per standard protocols for DC vaccination trials [3]. Cells were incubated with the 19F label, washed and studied further. PLGA (Boehringer Ingelheim, Ingelheim am Rhein, Germany) particles were formulated with PFCs using a single emulsion technique, with or without the addition of carboxyfluorescein. In vivo MRI was done on a 7T Clinscan Bruker system, with a mouse injected with 10 mg of particles in one footpad. The false color 19F GRE image (TR/TE=200/2.82ms, 20° flip angle, 1.88×0.94×2mm voxels, 512 averages, 27min) was overlaid on the grayscale 1H image (TR/TE=1500/14ms, 0.12×0.6×2mm voxels, 8 averages). Particle morphology was studied by SEM on a Jeol JSM-6310 (Jeol Inc, Peabody, MA, USA). Dynamic light scattering (DLS) measurements on the particles were performed on an ALV light-scattering instrument equipped with an ALV5000/60X0 Multiple Tau Correlator and an Oxxius SLIM-532 150mW DPSS laser operated at a wavelength of 532nm (Langen, Germany).

Results and Discussion
Nanospheres in the size range 200-300nm with a monodisperse distribution, encapsulating various PFCs in clinical use were synthesized [Fig. A: SEM image of the particles; scale bar is 100 nm]. Primary human DCs were labeled with these particles, resulting in a loading of 10^13 F's per cell with minimal toxicity, with an intracellular distribution [Fig. B: Confocal micrograph with the cell surface in blue and the particles in green]. The particles showed no toxicity with the DCs, even at concentrations as high as 20 mg of PLGA per 10^6 cells, or over 2000 µg PFC [Fig. C: Plot showing the viability of the DCs with increasing concentration of particles added, shown as mg of PLGA, and the corresponding viability, shown as the percent of live cells relative to the non-labeled cells]. Furthermore, we did not observe any effect of labeling on cell function, in terms of migration or expression of maturation markers. The 19F loading per cell is also similar to previous work using DCs labeled with PFC emulsions and transfection agents [1]. However, the PLGA particles in our study bypassed the need for transfection agents to achieve sufficient 19F loading. This is a major advantage as transfection agents are generally not clinically applicable. The MR image demonstrates the detection of the particles in vivo [Fig. D: 2mm thick coronal slice of the footpads of a mouse injected with the particles, showing the 19F image in false color overlaid on the grayscale 1H image].

Conclusion
In our studies, we focused on cell labeling for MRI. However, the particles can be adapted for the experimental system, depending on the PFC encapsulated and the particle formulation. The addition of a fluorescent dye to the particles may also allow in vivo optical imaging, and flow cytometric and histological analyses of labeled cells after transfer to the subject. Another major advantage is that the PLGA particles present a stable surface for the covalent addition of targeting agents, such as antibodies. We suggest that PLGA encapsulation is a suitable method for stabilizing perfluorocarbons in aqueous environments for a myriad of in vivo imaging and targeting applications.

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References