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

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## Introduction

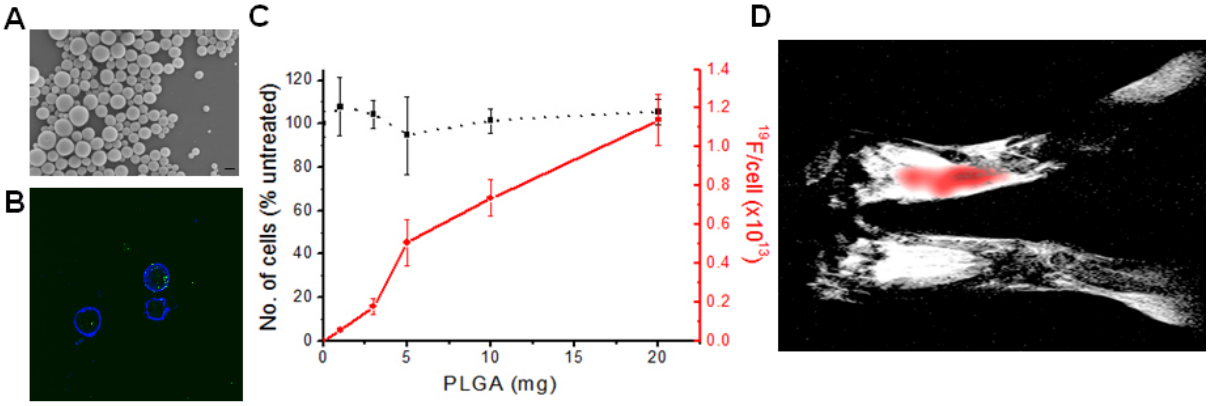
Magnetic Resonance Imaging (MRI) using $^{1}$H spins from water is a major *in vivo* imaging modality. With $^{19}$F MRI, cells labeled with fluorinated compounds can be visualized in the body without interference of the large $^{1}$H background from mobile water in tissues. Importantly, it also allows quantification of the number of cells directly from the image data [1, 2]. However, the perfluorocarbons (PFCs), typically used as $^{19}$F 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 $^{19}$F 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 $^{19}$F MRI.

## Methods

Primary human DCs were cultured, as per standard protocols for DC vaccination trials [3]. Cells were incubated with the $^{19}$F 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 $^{19}$F GRE image (TR/TE=200/2.82ms, 20$^{o}$ flip angle, 1.88×0.94×2mm voxels, 512 averages, 27min) was overlaid on the grayscale $^{1}$H 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 $^{19}$F 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 $^{19}$F 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 $^{19}$F image in false color overlaid on the grayscale $^{1}$H 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

[1] Srinivas M, Morel PA, Ernst LA, Laidlaw DH, Ahrens ET. Fluorine-19 MRI for visualization and quantification of cell migration in a diabetes model. Magn Reson Med 2007; 58:725-734.

[2] Srinivas M, Turner MS, Janjic JM, Morel PA, Laidlaw DH, Ahrens ET. In vivo cytometry of antigen-specific t cells using (19)F MRI. Magn Reson Med 2009.

[3] de Vries IJ, Lesterhuis WJ, Barentsz JO, Verdijk P, van Krieken JH, Boerman OC, Oyen WJ, Bonenkamp JJ, Boezeman JB, Adema GJ, Bulte JW, Scheenen TW, Punt CJ, Heerschap A, Figdor CG. Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy. Nat Biotechnol. 2005;23(11):1407-13.