Lipid-coated iron oxide: A versatile, biocompatible and multimodal material for cellular imaging

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Introduction
In recent years, various studies have shown that cell labeling with magnetic material, often commercially available superparamagnetic iron oxide particles (SPIO), enables in vivo monitoring of cellular migration with MRI. With the increasing availability of multimodal imaging settings, which allows for more extensive characterization of the fate of labeled cells, there is a growing interest in multimodal contrast agents. For example, additional fluorescent labeling of MRI contrast agents would enable the specific identification of labeled cells after in vivo MRI, by means of in vitro flow cytometry or fluorescence microscopy.

In this study we propose a novel methodology for simultaneous magnetic and fluorescent cell labeling using a single nanoparticle agent. Murine macrophages were incubated with the contrast agent to explore labeling efficiency, biocompatibility, as well as the detectability of labeled cells with MRI and optical techniques.

Materials and Methods
Contrast agent: Multiple oleic acid-coated iron oxide nanocrystals (~5 nm) were incorporated in a lipid monolayer composed of PEG2000-DSPE/DSPC (1:1 molar ratio) and 1 mol% rhodamine-PE using an adjusted solvent evaporation method. The resulting nanoparticle had a hydrodynamic diameter of 109.4 nm, with a polydispersity index of 0.2. Relaxivities in water were $r_1 = 0.5 \text{ mM}^{-1}\text{s}^{-1}$ and $r_2 = 408.7 \text{ mM}^{-1}\text{s}^{-1}$ at 9.4T.

Cell labeling: Murine macrophages (RAW) were incubated with the contrast agent in complete RPMI 1640 medium containing final concentrations of 0, 50, 100 and 200 μg Fe/ml, respectively. After 3 hours of incubation, at 37°C in a humidified atmosphere and 5% CO₂, cells were extensively washed with PBS to remove free nanoparticles. Labeled cells were characterized with MRI, flow cytometry and fluorescence microscopy. Particle-induced toxicity was evaluated using an MTT assay, while a 1,10-phenanthroline colorimetric assay for iron was performed to quantify cellular uptake of the nanoparticles.

Results
RAW cells were successfully labeled with the lipid-coated iron oxide particles, which allowed their detection with both MRI and optical techniques (Figs. 1 and 2). Signal intensity on the T₂-weighted images visibly decreased with increasing cell density and incubation concentrations (Fig. 1A,B), as a result from an increase in intracellular iron content (Fig. 1C). A linear correlation was observed between the concentration of labeled cells and its corresponding transverse relaxation rate $R_2$, of which the slope increased with increasing intracellular iron content (Fig. 1D). This suggests that the detection limit of labeled cells with MRI is reduced for cells with high nanoparticle content. MRI results were corroborated by fluorescence microscopy images that clearly demonstrated increased cellular uptake of the nanoparticles with increasing incubation concentration (Fig. 2A). Labeling of the cells was additionally confirmed by flow cytometry (Fig. 2B). Importantly, no particle-induced toxicity was observed within the used range of incubation concentrations (Fig. 2C).

Conclusions and discussion
Lipid-coated iron oxide particles allow for simultaneous magnetic and fluorescent labeling of RAW cells, without inducing toxicity. The labeling efficiency was somewhat lower than that of commercially available SPIO in monocytes when using comparable incubation time and concentration. However, this was compensated for by the relatively high transverse relaxivity $r_2$, which was approximately 2-3 times higher than the $r_1$ of commercially available SPIO. The approach is facile and flexible as it allows adjustment of particle size and surface properties, such as charge and PEG content. This can improve nanoparticle uptake by phagocytising as well as non-phagocytic cells without the use of transfection agents. Alternatively, molecules that facilitate nanoparticle uptake, such as the TAT-peptide, can be easily conjugated. In conclusion, our study demonstrates that lipid-coated iron oxide particles represent an attractive, versatile and potent contrast material for simultaneous fluorescent and magnetic cell labeling.

References: