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

G. A. van Tilborg[1], W. J. Mulder[2], S. M. van der Pol[3], L. van Bloois[4], A. van der Toorn[1], G. Storm[4], H. E. de Vries[3], and R. M. Dijkhuizen[1]
[1]Image Sciences Institute, University Medical Center Utrecht, Utrecht, Utrecht, Netherlands, [2]Translational and Molecular Imaging Institute, Mount Sinai School of Medicine, New York, New York, United States, [3]Department of Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, Netherlands, [4]Department of Pharmaceutics, Institute for Pharmaceutical Sciences, Utrecht, Utrecht, Netherlands

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[1]. 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 nanoparticulate 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 $r_1 = 0.5\ mM^{-1}s^{-1}$ and $r_2 = 408.7\ mM^{-1}s^{-1}$ at 9.4T.
Cell labeling: Murine macrophages (RAW) were incubated with the contrast agent in complete RMPI 1640 medium containing final concentrations of 0, 50, 100 and 200 µg Fe/ml, respectively. After 3 hours of incubation, at 37ºC in a humified atmosphere and 5% CO_2, 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-phenantroline colorimetric assay for iron was performed to quantify cellular uptake of the nanoparticles.
MRI: Washed cells were harvested and resuspended to $1x10^4$ and $0.5x10^4$ cells/µl 0.8% agarose-containing PBS. MRI was conducted on a 9.4T horizontal bore MR system (Varian Inc.). T_2-weighted spin echo images were obtained using the following parameters: TR/TE = 5000/15 ms, 256x256 matrix, 3x3 cm^2, 1 mm slice thickness and 4 averages. T_2 values were obtained with a multi-slice multi-echo sequence with: TR/TE = 5000/9.4 ms, 256x256 matrix, 3x3 cm^2, 1 mm slice thickness, 4 averages and 60 echoes.

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 T_2-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[2]. However, this was compensated for by the relatively high transverse relaxivity r_2, which was approximately 2-3 times higher than the r_2 of commercially available SPIO[3]. 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.

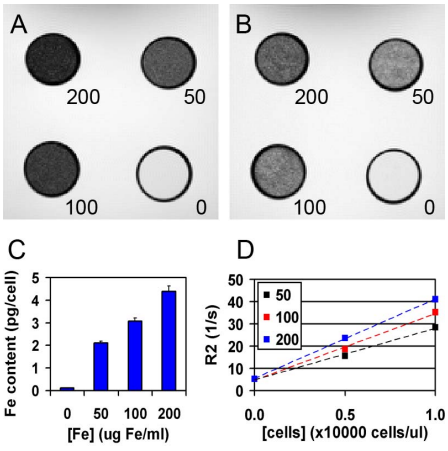

Figure 1: (A,B) T_2-weighted spin echo images of $1x10^4$ (A) and $0.5x10^4$ (B) cells/µl agarose after 3 hours of incubation with 0, 50, 100 or200 µg Fe/ml, respectively. (C) Cellular iron content for the different incubation concentrations (n=3). (D) Transverse relaxation rate R_2 versus labeled cell density in agarose for the different incubation concentrations (n=1).

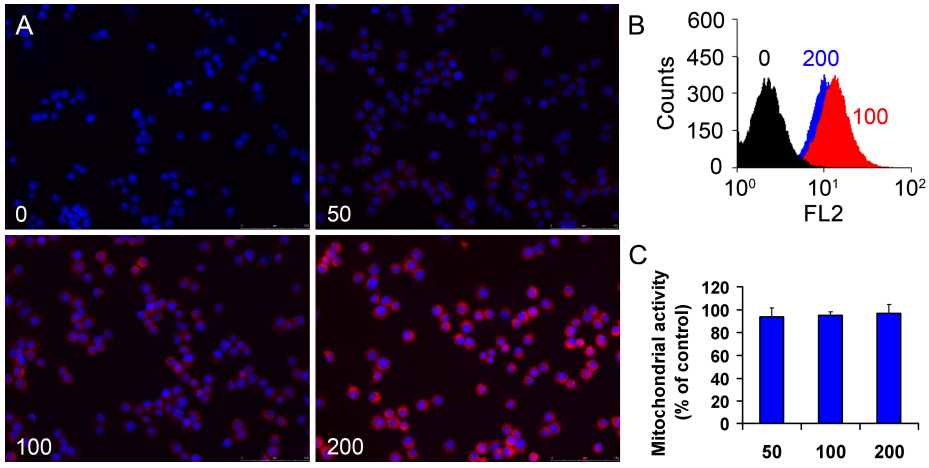

Figure 2: RAW cells after 3 hours of incubation with 0, 50, 100 and 200 µg Fe/ml, respectively. (A) Fluorescence microscopy images (200x) showing nuclei (blue) and contrast agent (red). (B) Flow cytometry of labeled cells. (C) Mitochondrial activity, obtained with an MTT assay, to assess nanoparticle-induced toxicity (n=3).

References:

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