

Efficacy of different lipid-coated nanoclusters of iron oxide for image-based detection of labeled cells

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Introduction

There is growing interest in the development of nanoparticles that allow in vivo tracking of cells for monitoring of disease pathophysiology (e.g., leukocyte invasion) or cell-based treatment strategies (e.g., stem cell delivery) [1]. Preferably, such nanoparticles should allow incorporation of high amounts of contrast material for optimal detection, without affecting cell viability and function. Lipid-coated iron oxide formulations may provide an ideal platform because of their relative ease of synthesis and potentially high pay-load of contrast material [2]. The goal of this study was to develop an efficient and safe nanoparticle platform for detection of labeled cells with MRI and fluorescence microscopy. To that aim we compared labeling efficiency, intracellular localization, biocompatibility and detectability of three different lipid-coated nanoclusters of iron oxide in murine macrophages.

Materials and methods

Fluorescently labeled lipid-coated iron oxide nanoclusters were prepared using a solvent evaporation technique, as described by Jarzyna et al. [2]. Each nanoparticle consisted of a PEG2000-DSPE/DSPC/Rhodamine-PE lipid monolayer, encapsulating multiple oleic acid-coated iron oxide crystals only or dispersed in soybean oil or medium-chain triglycerides (MCT). Nanoparticle size and morphology were studied with dynamic light scattering and transmission electron microscopy (TEM). MR relaxivities r_1 and r_2 were acquired at 9.4T (Varian Inc.) using multi-echo spin echo and look-locker EPI sequences for T_2 and T_1 measurements, respectively. Murine macrophage cells (RAW 264.7) were incubated with each nanoparticle at a final concentration of 200 $\mu\text{g Fe/ml}$ for 3 h at 37 °C. Incubations were performed 1 day after nanoparticle preparation and particles were stored at 4 °C under nitrogen, unless stated otherwise. Following incubation, cells were extensively washed in PBS, and cellular iron content was determined with a 1,10-phenanthroline colorimetric assay. Cell viability was assessed with an MTT assay for mitochondrial activity. Nanoparticle localization was determined at the (sub)cellular level using TEM as well as fluorescence- and light microscopy. For light microscopy, cells were additionally stained for iron with Prussian Blue (PB). T_2^* -weighted MR images of homogeneously distributed labeled cells in 0.4% agarose were obtained with a 9.4T horizontal bore MR system (Varian Inc.), using a gradient echo sequence (TR/TE = 100/15 ms, $\alpha = 35^\circ$, 16 averages and 100x100x100 μm^3 voxel resolution).

Results

The solvent evaporation procedure described above resulted in lipidic nanoparticle aggregates of approximately equivalent size (Table). Each of these nanoparticles showed a distinct distribution of iron oxide crystals within the core of the lipid monolayer (Fig. A), giving rise to differences in relaxivities r_1 and r_2 (Table). Microscopy of labeled macrophages revealed comparable compartmentalization of iron oxide (Fig. C; blue) and fluorescence (Fig. D; red) throughout the cell, indicative of intact nanoparticle uptake. These results were corroborated by TEM images of labeled cells, where iron oxide within endosomal/lysosomal compartments resembled iron oxide distribution within the nanoparticles prior to labeling (Fig. B). Macrophage labeling efficiency was highest in pure iron oxide-containing lipid nanoparticles and lowest in soybean-containing preparations (Table). T_2^* -weighted contrast increased accordingly (Fig. E; right), and clearly allowed the discrimination of single cells with MRI in case of labeling with pure iron oxide and MCT-containing nanoclusters, with uptake of ≥ 2.61 pg Fe/ml (Fig. E; left). Cell viability was largely maintained after labeling with nanoparticles without oil, whereas incubations with MCT and soybean-containing nanoparticles reduced cell viability (Fig. F). In case of pure and MCT-containing lipid-coated nanoclusters of iron oxide this was unaffected by long-term storage at 4 °C, whereas the inclusion of soybean oil induced a partially oxygen-dependent reduction in cell viability with storage time (Fig. F; compare nitrogen and air).

Conclusions and discussion

Lipid-coated nanoclusters of iron oxide allowed the detection of single cells with MRI and optical imaging techniques. Nanoparticles that did not contain an oil phase exhibited superior properties in terms of relaxivity (r_2), labeling efficiency, biocompatibility and shelf-life, while the inclusion of soybean oil resulted in reduced biocompatibility when labeling cells following weeks of nanoparticle storage. Differences observed between ‘nitrogen’ and ‘air’ storage suggest the latter effect is due to lipid peroxidation within the oil phase, which, most probably, is catalyzed by the dispersed iron.

In conclusion, lipid-coated nanoclusters of iron oxide represent an attractive, potent and flexible platform for cell labeling that, even though composed of biocompatible compounds such as phospholipids, iron oxide and vegetable oils, should be carefully designed and evaluated, as the combination of biocompatible building blocks may result in decreased biocompatibility.

References

- [1] Bulte J.W., NMR Biomed, 2004
- [2] P. Jarzyna et al., Biomaterials, 2009

	r_1 ($\text{mM}^{-1} \cdot \text{s}^{-1}$)	r_2 ($\text{mM}^{-1} \cdot \text{s}^{-1}$)	Diameter (nm)	Iron content (pg Fe/cell)
Iron/Soybean	0.29 ± 0.06	268.89 ± 17.80	76.2 ± 8.6	0.98 ± 0.05
Iron/MCT	0.55 ± 0.08	267.19 ± 19.25	78.9 ± 5.7	2.61 ± 0.35
Iron	0.62 ± 0.12	402.48 ± 8.82	62.4 ± 4.1	5.28 ± 0.67

