Cell labeling using superparamagnetic iron oxide particles: Impact of particle size, surface-coating and lipofection on labeling efficiency

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The ability to track mammalian cells in vivo would have significant implications for both research and clinical applications such as cell based therapies. Various approaches for cell tagging have been explored ranging from simple incubation techniques, receptor-targeting of iron oxides to sophisticated methods of iron oxide surface modification with membrane translocation signals such as the HIV derived tat-peptide or magneto-dendrimers (1-4).

The aim of this study was to analyze the impact of lipofection, hydro-dynamic diameter and surface-coating of different clinically approved superparamagnetic iron oxide particles (SPIOs) on the labeling efficiency in the presence or absence of a polycationic-transfection medium (TM).

MATERIAL AND METHODS:

Different cancer cell lines (CLL-185, HTB-56, DU-4475, HT-1080) were tagged using carboxydextran-coated SPIOs of different diameters (65, 46, 21, 17 nm) and a dextran-coated SPIO (diameter: 150 nm). Cells were incubated in iron containing medium (0.01-1.00 mg iron/ml), with or without TM. Iron-uptake was visualized by light-microscopy after Prussian-Blue-staining and quantified by Atomic Emission Spectroscopy (AES). MR-signal characteristics and the number of detectable cells were analyzed at 1.5- and 3.0-Tesla using T2-weighted Gradient-Echo- and Turbo-Spin-Echo-sequences.

RESULTS:

All experiments demonstrated cellular iron-uptake verified by light-microscopy (Fig. 1) and AES (Fig. 2).

Presence of TM significantly increased the iron load of cells up to 2.9-fold ($p < 0.05$) (Fig. 2).

Improved cellular uptake was achieved using larger SPIOs and higher iron-concentrations (e.g.: [1.00 mg Fe/ml]: 65nm: 4.37 ±0.92 µg/100,000 cells; 17nm: 2.23 ±0.50 µg/100,000 cells; $p < 0.05$) (Fig. 2b). Cellular uptake of dextran-coated particles was virtually identical to large carboxydextran-coated particles (e.g.: [1.00 mg Fe/ml]: 65 nm: 4.37 ±0.92 µg/100,000 cells; 150 nm: 3.81 ±0.39 µg/100,000 cells; $p > 0.05$) (Fig. 2b).

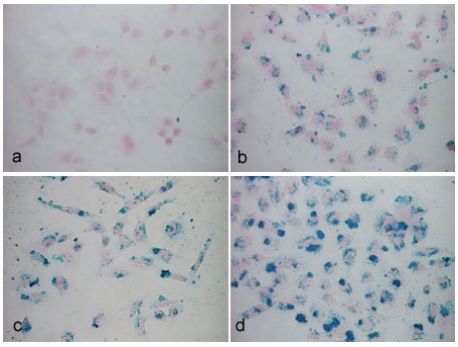

Figure 1: Light-microscopy after Prussian-blue staining The blue precipitate within the cells represents the incorporated iron-particles: CLL-185-cells labeled with iron particles (65 nm) in the presence of transfection medium: a) native cells b) 0.01 c) 0.10 d) 1.00 (mg Fe/ml in incubation medium).

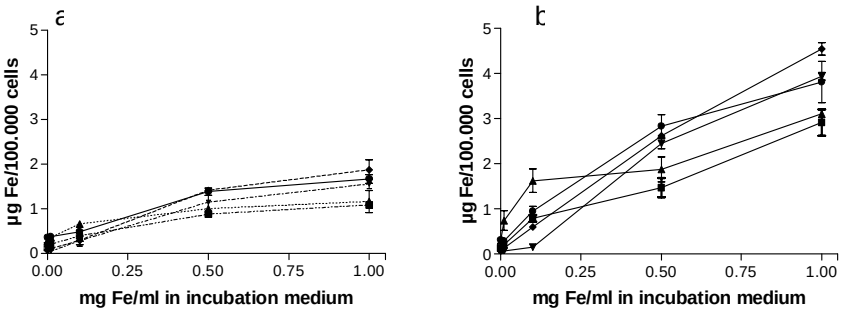

Figure 2: Quantitative iron determination by AES
Cells incubated with SPIOs in the absence (a) and presence (b) of transfection medium:
● 150 nm ♦ 65 nm ▼ 46 nm ▲ 21 nm ■ 17 nm

As little as 10,000 cells were readily detectable with clinically available MR-techniques. Using the optimized cell tagging protocol all other cell types could easily be tagged with iron oxides. Tagged cells were readily visible by MRI using SE- and GRE- sequences.

DISCUSSION:

Lipofection based cell tagging is a simple method for efficient cell labeling with clinically approved iron oxide based contrast agents. While large particle size is preferable for cell-tagging, surface coating might be less important in lipofection based cell tagging methods.
Additional experimental studies are warranted to evaluate the potential of this method for in-vivo cell trafficking-studies.

LITERATURE:

1. Bhorade R, Weissleder R, Nakakoshi T, Moore A, Tung CH. Macrocyclic chelators with paramagnetic cations are internalized into mammalian cells via a HIV-tat derived membrane translocation peptide. Bioconjug Chem 2000;11(3):301-5.
2. Bulte JW, Laughlin PG, Jordan EK, Tran VA, Vymazal J, Frank JA. Tagging of T cells with superparamagnetic iron oxide: uptake kinetics and relaxometry. Acad Radiol 1996;3 Suppl 2:S301-3.
3. Bulte JW, Zhang S, van Gelderen P, et al. Neurotransplantation of magnetically labeled oligodendrocyte progenitors: magnetic resonance tracking of cell migration and myelination. Proc Natl Acad Sci U S A 1999;96(26):15256-61.
4. Lewin M, Carlesso N, Tung CH, et al. Tat peptide-derivatized magnetic nanoparticles allow in vivo tracking and recovery of progenitor cells. Nat Biotechnol 2000;18(4):410-4.