Nanoparticles and stem cells for drug delivery to the brain
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Chapter 4

Ex vivo labeling of neural stem cells for Magnetic Resonance Imaging using cationic lipid- and polymer-mediated uptake of iron oxide nanoparticles

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Abstract

Neural stem cells are promising candidates for the treatment of brain-related diseases because of their differentiation and migration potential. To be able to better predict their therapeutic efficacy, and as such to limit the resources that are needed for optimization of treatment protocols, it is important to study the in vivo behavior of neural stem cells following transplantation. Prior labeling of stem cells, using iron oxide nanoparticles, allows for their visualization by magnetic resonance imaging (MRI). Through the optimization of nanoparticles’ characteristics, such as size and surface charge, efficient and non-toxic cell labeling procedures may be achieved. Here we show that C17.2 neural stem cells that are labeled with large-sized, i.e. 500 nm, iron oxide particles provide a better signal in MRI than cells labeled with small particles of 100 nm in diameter. The cellular uptake of large-sized iron oxide particles, which by itself is limited, can be enhanced through the use of positively charged lipids and polymers, such as lipofectamine and JetPEI, respectively. The approach of cationic vector-enhanced cell labeling substantially improves the imaging quality of neural stem cells, and may find a wider application in studying cell transplantation in general.
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Introduction

Stem cell therapy is a promising strategy for the treatment of human disease. Currently, the best known approved therapy in this regard is bone marrow transplantation, i.e., the transplantation of haematopoietic stem cells to treat leukemia, and other types of cancers and blood disorders [1-3]. In the future, it is anticipated that stem cell therapy may be further expanded to the treatment of a variety of other diseases, including cancer, and neurodegenerative diseases, such as Parkinson’s disease, and multiple sclerosis [4-6]. For the treatment of brain-related diseases, neural stem cells are the preferred treatment modality as they have the intrinsic potency to differentiate into all three brain cell types, i.e. neurons, astrocytes, and oligodendrocytes. Moreover, neural stem cells have the capacity to migrate to the brain pathology following systemic administration or brain implantation [7, 8]. To investigate the (migratory) behavior of stem cells after transplantation in vivo, noninvasive methods that allow for the real-time visualization and tracking of transplanted stem cells, including positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), and optical microscopy are being developed [9, 10].

However, laborious and time-consuming experiments are commonly needed to establish a correct treatment protocol for each disease. For example, the proper timing of stem cell application, the route of administration, the adequate number of cells, need to be determined. A common way to assess the efficacy of a therapy is by the evaluation of symptom relief. However, knowledge on earlier steps in therapy, including the body distribution of stem cells, can provide great help in speeding up the development and hence the efficiency of such a treatment.

Magnetic Resonance Imaging (MRI) is widely used in hospitals as a noninvasive technique to obtain three-dimensional images inside the human body. Because MRI provides good contrast between the different soft tissues, it is an ideal approach for imaging the brain [11]. In addition, lack of irradiation exposure to stem cells, thus avoiding potential radiation damage, makes MRI the favorable imaging method compared to nuclear medicine techniques like positron emission tomography and single photon emission computed tomography.

In MRI, the electromagnetic flux that is produced by the relaxation of proton spins in a strong electromagnetic field, is measured and translated into images [12]. There are two types of proton relaxation pathways, resulting in two types of MR images, i.e., T1- and T2-weighted. T1-weighted MRI is based on the longitudinal relaxation time of the excited protons, whereas T2-weighted MRI is based on the transverse relaxation time. The use of MRI contrast agents may further improve MR images.
contrast agents, mostly gadolinium complexes, enhance the signal in T1-weighted MRI, whereas superparamagnetic contrast agents like iron oxide particles reduce the T2 signal [13, 14]. It has been proposed that an increase in the diameter of iron oxide particles will improve MRI contrast, because of the larger magnetic core within large particles compared to small particles [15]. However, in general larger-sized particles become less efficiently internalized by cells [15-17]. To overcome this drawback, we investigated whether commonly used transfection agents, like those based on cationic lipids and polymers for introducing plasmids into cells, could similarly facilitate and thus improve the delivery of negatively charged 100 nm and 500 nm iron oxide particles into C17.2 neural stem cells. The short term toxicity of particles was assessed by MTT assay, while the long term stability of labeling was investigated in dividing and non-dividing cell populations, and upon cell differentiation. The effect of nanoparticle iron core size on MRI signal of labeled neural stem cells was investigated using a clinically available MRI scanner.

Materials and Methods

Cell line
The C17.2 murine neural stem cell line, kindly provided by Dr. Evan Snyder (Harvard Medical School, Boston, MA), was grown in high glucose Dulbecco's modified Eagle's medium (Invitrogen, the Netherlands) supplemented with 10% fetal calf serum (Bodinco B.V. The Netherlands), 5% horse serum (Invitrogen, the Netherlands), 100 mg/L ampicillin (Invitrogen, UK), and 100 mg/L streptomycin (Invitrogen, UK) at 37°C under a 5% CO2 atmosphere, and passaged twice a week (1:10 dilution).

Superparamagnetic nanoparticles
100 nm fluidMAG-UC containing 66 wt% of iron (100 nm iron oxide particles), 500 nm screenMAG-Hydroxyl containing 23 wt% iron (500 nm silica particles loaded with iron oxide) and 500 nm screenMAG-PEI beads containing 23 wt% of iron (PEI-functionalized silica particles loaded with iron oxide) were purchased from Chemicell, Germany.

Quantification of cellular viability by MTT assay
The viability of C17.2 cells that had been exposed to increasing amounts of iron oxide particles was evaluated by performing a 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl
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tetrazolium bromide (MTT) assay (Sigma). In short, cells were plated at 40,000 cells / well in a 48-wells plate. One day later cells were treated in triplicate with: 1, 4 and 8 μg of 100 nm iron oxide particles; 3, 10 and 20 μg of 500 nm iron oxide particles, and 3, 10 and 20 μg of PEI iron oxide particles in 0.2 mL complete feeding medium, i.e., corresponding to 5, 20 and 40 μg 100 nm iron oxide particles/mL and 15, 50 and 100 μg 500 nm and PEI iron oxide particles/mL, for 4 h, washed twice with HBSS and incubated in complete feeding medium over the next 20 hours. Alternatively, the cells were exposed to the iron oxide particles during 24 h. Untreated cells were used as a control. During the final 3 hours of the incubation, the samples were exposed to 50 μL MTT solution (5 mg/mL in PBS). Thereafter the plate was centrifuged for 15 min at 800 g (RT), the medium was removed, and the formazan crystals were dissolved in 0.5 mL of DMSO. The optical density of each well was measured using a multiwell plate reader (uQuant, Bio-Tek Instruments, Inc, USA) at 520 nm.

**Loading of C17.2 cells with iron oxide particles**

Cells were plated at 0.3 x 10^6 cells /well in twelve wells plates. The next day, cells were incubated for 4 h and 24 h with 20 μg / well of 100 nm iron oxide particles (13.2 μg iron /mL), 50 μg / well of 500 nm iron oxide particles (11.5 μg iron/mL), and 50 μg / well of PEI iron oxide particles (11.5 μg iron/mL). In addition, prior to the incubation with the cells, 100 nm and 500 nm iron oxide particles were complexed with Lipofectamine 2000 (Invitrogen, UK) and JetPEI (Polyplus transfection, France) transfection reagent. Next, the cells were washed two times with HBSS and left for an additional 24 hours in complete medium. Cells were counted at this point to examine the influence of the transfection agents on cell proliferation. The ratios of iron oxide particles and transfection agents that showed the optimal cell labeling without affecting cell proliferation (Table 1) were used in further experiments.

**Qualitative measurement of intracellular iron content by Prussian blue staining**

Following an incubation of the C17.2 cells with the different types of superparamagnetic iron oxide particles, a Prussian blue staining was performed to visualize the amount of iron oxide within the cells. Cells were seeded on coverslips in 12 wells plates and incubated with iron oxide particles for 4 h and 24 h. Cells were washed three times with HBSS to remove unbound iron oxide particles, and fixed with 4% PFA. Thereafter, cells were incubated for 30 min with a 1:1 mixture of fresh 5%
solution of potassium hexacyanoferrate (II) trihydrate (Sigma – Aldrich, Steinheim, Germany) and 5% hydrochloric acid (VWR Prolabo, Briare, France). After 3 times washing in distilled water, the cells were counterstained for 10 min with nuclear fast red (Sigma – Aldrich, Steinheim, Germany).

**Quantification of intracellular iron content by Tiron assay**

The iron content of the cells after loading with iron oxide particles was measured using a chelating agent, Tiron (4,5-Dihydroxy-1,3-benzenedisulfonic acid, disodium salt monohydrate, Acros Organics, Geel, Belgium). A reaction of Tiron with Fe$^{3+}$ gives a colored product that can be spectrophotometrically measured at 490 nm (modified method by Yoe and Jones [18]). Following incubation with iron oxide particles, cells were trypsinized and resuspended in 1 mL HBSS. A 50 μL sample was prepared for cell counting and the rest of the suspension was digested overnight with an equal volume of analytical grade nitric acid (65% solution in water, Acros Organics, Geel, Belgium) to disrupt the cells plus iron oxide particles. A standard curve ranging from 0 – 20 μg / mL Fe$^{3+}$ was prepared from a 1 mg / mL Fe$^{3+}$ stock solution. Similar to the cell samples, the iron stock solution and standard curve samples were prepared in 32% nitric acid. A 0.5 mL sample of either the cell lysate or standards was mixed with 0.5 mL 10 M NaOH, 0.1 mL 1 % Tiron and 1 mL 0.2 M sodium phosphate buffer, pH 9.5. Absorption at 490 nm was read within 30 min in a multiwell plate reader (uQuant, BioTek Instruments, Inc, USA).

**Stability of cell labeling with iron oxide particles**

A cell label can be lost simply by dilution due to cell division and/or by active cellular exocytosis. Since cell division is expected to be low during migration in vivo, the stability of cell labeling was measured in non-dividing C17.2 cells, i.e., in order to determine active cellular exocytosis of iron oxide particles. Cells were plated in 12-wells plates at 0.4x10$^6$ cells per well in 1 mL of complete medium. The next day cells were incubated for 4 h with iron oxide particles at the conditions shown in Table 1. After two times washing with HBSS, cells were incubated in the absence and presence of the proliferation inhibitor TPI (tubulin polymerization inhibitor II, Calbiochem, San Diego, USA) at a concentration of 0.05 μM in complete medium for 24 and 72 hours [19]. At the end of the incubation period, the cell number was counted using a counting chamber and the cellular iron content was determined by the Tiron assay. The optimal concentration of TPI at which a non-dividing cell population was established without cellular toxicity was determined in a separate experiment.
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**Stability of iron labeling during cell differentiation**

For differentiation studies the cells were plated and loaded with particles on coverslips in 12 wells plates. 24 hours after particle loading complete feeding medium was replaced with differentiation medium, i.e., DMEM/F12 medium (GIBCO) supplemented with 25μg/mL N2 (GIBCO). The samples were fixed in 4% paraformaldehyde 5 days later and immunostained for the neuronal marker anti-βIII tubulin (TuJ1; monoclonal, 1:500, Covance, Richmond, CA).

**MRI of C17.2 cells loaded with iron oxide particles**

C17.2 neural stem cells were loaded for 4 h with 100 nm and 500 nm iron oxide particles complexed with Lipofectamine 2000, and 500 nm PEI iron oxide particles as described under *Loading of C17.2 cells with iron oxide particles*. Alternatively the cells were loaded with half of the amount of prepared complexes. The next day the cells were suspended in 2% agarose at three concentrations: $10^4$ cells/μL, $10^3$ cells/μL and $10^2$ cells/μL. 0.5 mL tubes were filled with 100 μL of each cell suspension and covered with 200 μL of 2% agarose. Tubes filled with non-labeled cells were prepared as well. In order to avoid artifacts in MRI from surrounding air the tubes were placed in a plastic container filled with 2% agarose gel. MR imaging was performed using a clinical 3T Philips Intera MR-scanner with SENSE head-coil (Royal Philips Electronics, Amsterdam, The Netherlands). The phantom was imaged by T2W-TSE sequence using CLEAR (Constant LEvel AppeaRance) coil uniformity correction technique. Imaging parameters were as follows: repetition time (TR) 3000 ms, echo time (TE) 120 ms number of slices 40, field of view (FOV) 150 mm, voxel size 0.29x0.29x1.1mm, scan time 72s.

The data were analyzed by AMIDE software [20] upon conversion of the raw data into Analyze format. The signal intensity (si) of the cells was measured by placing a 3D region of interest (ROI) in the suspension of cells labeled with iron oxide particles (B) or control cells (C). All ROIs were > 70 voxel. The signal intensity ratio (contrast) of iron-labeled cells was calculated from the measured signal intensities using the formula: $(si_B – si_C)/(si_B + si_C)$ [21].
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Results

Effect of PEI iron oxide particles on the viability of NSCs

In order to verify whether iron oxide particles might cause cellular toxicity, the C17.2 neural stem cells were exposed to increasing concentrations of these particles up to 24h. Specifically, cells were incubated with 5, 20 and 40 μg/mL of particles with a diameter of 100 nm, 15, 50 and 100 μg/mL of particles with a diameter of 500 nm, and with PEI-coated iron oxide particles (500 nm). Following incubation for 4h (Fig 1A) or 24h (Fig. 1B) with low, medium, and high concentrations of 100 nm and 500 nm iron oxide particles, the cellular viability remained virtually unaffected. Only at the highest concentration of the PEI coated particles (100 μg/ml) a minor but statistically significant decrease in cell viability after 24h of incubation (p < 0.05 [p=0.015]) was apparent, suggesting that these high concentration represented the limit of tolerance. Therefore, in the next experiments, C17.2 neural stem cells were incubated with the medium concentration of all three types of iron oxide particles, i.e., 20 μg/mL of 100 nm, and 50 μg/mL of both 500 nm and PEI-coated iron oxide particles.

**Figure 1:** Cellular toxicity of 100 nm, 500 nm and PEI iron oxide particles. To check for toxicity of particles, cells were exposed to 5, 20 and 40 μg of 100 nm particles/mL and 15, 50 and 100 μg of 500 nm and PEI particles/mL during 4h (a) or 24 h (b). The results were evaluated by MTT assay. Concentrations of particles were marked as low (5-15 μg/mL), medium (20-50 μg/mL) and high (40-100μg/mL). The results are presented relative to control cells. Student t-test was performed for each condition against control. * - p<0.05.
Efficiency of NSCs labeling, as accomplished with 100 nm and 500 nm iron oxide particles

C17.2 NSCs were incubated with 100 nm and 500 nm iron oxide particles for 4h and 24h. After 4h of incubation, the cells showed an efficient uptake of the 100 nm iron oxide particles, as was visualized by Prussian blue staining (Fig. 2a). After another 20 h of incubation (total 24h), no visually distinguishable additional increase in the cellular uptake of the 100 nm particles could be observed (Fig. 2b), as confirmed by quantitative measurements of the Fe content (Fig.2e). Incubation of the cells with 500 nm iron oxide particles for 4h resulted in a substantially lower cellular iron content than following an incubation with 100 nm particles (Fig.2c vs. 2a), consistent with quantification of the Fe uptake by the cells (Fig.2e). However, after 24h the cells showed a pronounced increment in the uptake of the 500 nm particles (Fig. 2d vs. 2c) and quantitatively, the iron content of the cells was two-fold higher than in the case of an incubation with the 100 nm particles (Fig. 2e).
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Transfection agents improve cell labeling with iron oxide particles

To optimize iron oxide labeling conditions in terms of speed concerning particularly 500 nm particles, we next investigated whether the complexation of the (negatively charged) iron oxide particles with positively charged transfection agents may accelerate iron oxide internalization by NSCs. Here, complexation with the transfection agents Lipofectamine 2000 (LF2000) and JetPEI was used for charge neutralization of the iron oxide particles, using the optimized labeling conditions as shown in Table 1. In addition, we compared these data with commercially available PEI-coated iron oxide particles. As can be seen in Figure 3, relative to non-coated iron oxide particles (Figs 3a and d), the complexation of 100 nm and 500 nm particles with LF2000 and JetPEI

Figure 2. Uptake of 100 nm and 500 nm iron oxide particles by C17.2 neural stem cells. Cells were incubated for 4 h and 24 h with iron oxide particles and subsequently stained with Prussian blue: A. 100 nm particles 4h incubation; B. 100 nm particles 24h incubation; C. 500 nm particles 4h incubation; D. 500 nm particles 24h incubation. Quantification was performed using Tiron assay (E).
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significantly increased internalization by the NSCs (Fig. 3 a-c (100 nm) and d-f (500 nm). The commercially available 500 nm PEI iron oxide particles were also readily internalized (Fig. 3 g). Since the Prussian blue staining reaction may not properly reflect quantitative aspects of the extent of particle internalization, staining being influenced by the particle’s surface composition, a quantitative analysis of the cellular iron contents was performed using the Tiron assay. As shown in Fig. 3h, the cellular iron content as determined by the Tiron assay, confirms that both transfection agents improve the labeling of C17.2 NSC with iron oxide particles. Thus, compared to the bare iron oxide particles, JetPEI increased the cell labeling with 100 nm and 500 nm particles with a factor of approximately 2 and 4, respectively, while Lipofectamine 2000 increased the cell labeling with 100 nm particles approximately 1.5 times, and that of 500 nm particles approximately 3 times. Cell labeling with the commercially available PEI particles (500 nm) gave similar results as the labeling with 100 nm and 500 nm particles, complexed with JetPEI (Fig. 3h). Furthermore, it is noteworthy that the relative uptake of the added dose was fairly efficient, in that labeling with JetPEI-derivatized particles resulted in a cellular uptake of 76.3 ± 6.8 % of the administered dose in case of 100 nm iron oxide particles and 77.3 ± 9 % dose in case of 500 nm iron oxide particles.

Table 1. Labeling conditions for C17.2 neural stem cells with 100 nm and 500 nm iron oxide particles. The amount of particles, their iron content, and the amount of transfection agents used for complexation are indicated.

<table>
<thead>
<tr>
<th>Size of beads (nm)</th>
<th>Amount of beads (μg/mL)</th>
<th>Corresponding amount of iron (μg)</th>
<th>Amount of Lipofectamine 2000 (ul)</th>
<th>Amount of JetPEI (ul)</th>
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</thead>
<tbody>
<tr>
<td>100</td>
<td>20</td>
<td>13.2</td>
<td>4</td>
<td>2</td>
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<tr>
<td>500</td>
<td>50</td>
<td>11.5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
Loss of iron label from NSCs is due to cell division

For monitoring iron-labeled NSCs in vivo by MRI, it is important to assess the stability of the label in terms of it properly reflecting the fate of the labeled cell, rather than its fate per se. When NSCs were incubated for 24 h and 72 h following their labeling with iron oxide particles, the iron content expressed per cell decreased (Fig. 4, 24h control and 72h control; cf. with Fig 3h). The seemingly time-dependent decrease in iron content was shown to occur, irrespective of the physical nature of the iron oxide particles applied. Strikingly, the time in which the iron content showed a two-fold decrease, as expressed per cell, exactly correlates with the cell doubling time of C17.2 NSCs, i.e., 16h. Therefore, the apparent decrease in iron content as expressed per cell over time can be ascribed to ‘the natural dilution’ of the iron oxide particles within the
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cells that occurs during cell division. To obtain further support for this notion, a non-dividing cell population was established after labeling of the cells with iron, using the tubulin polymerization inhibitor TPI. When these cells were subsequently incubated over time, the iron content, expressed per cell in this non-dividing culture remained constant (Fig 4).

![Figure 4](image_url)

Figure 4. Stability of cell labeling with iron oxide particles in a non-dividing cell population. C17.2 neural stem cells were loaded with iron oxide particles as described in materials and methods, washed, and incubated for 24 and 72 hours in complete medium (control) or in medium supplemented with 0.05 mM tubulin polymerization inhibitor II (TPI), which generates a non-dividing cell population. At the end of the incubation period the cellular iron content was quantified by Tiron assay.

Stability of cell labeling during differentiation

Another important feature of a neural stem cell marker for in vivo use is that it should not negatively influence stem cell differentiation into various brain cell types. This was examined next. C17.2 NSCs were labeled with iron oxide particles and subsequently allowed to differentiate following their culturing in differentiation medium. Immunostaining of the differentiated cells for Tuj1, a neuronal marker, showed that the amount of C17.2 cells that differentiated into neurons was similar for untreated cells and cells treated with the different types of iron oxide particles. Moreover, all neurons were labeled with iron oxide particles upon differentiation (Fig. 5 a-f arrowheads). The iron oxide particles mostly appeared in the soma. Occasionally, they were found in neurite outgrowths (Fig 5 c, arrow). Apparently, the presence of the iron particles does not interfere with the differentiation of NSCs into neurons. Likewise, the differentiation of NSCs into astrocytes was unaffected by the presence of iron oxide nanoparticles (not shown).
Effect of particle size on ability to visualize labeled cells

Finally, an MRI phantom was prepared as described in Materials and Methods to detect the differences in signal intensities from cells containing equal amounts of iron by labeling with 100 nm and 500 nm iron oxide particles complexed with Lipofectamine 2000 as well as 500 nm PEI particles, using the predetermined conditions. With a clinically available MRI scanner and using the settings as specified in the Materials and Methods, cells labeled with small- and large-sized particles were easily detectable by eye at a number of $10^3$ cells/μL (Fig. 6 a and b). Better contrast came from cells that were labeled with 500 nm particles compared to 100 nm particles regardless the amount of loaded particles (Fig. 6 a and b). This was confirmed by quantification of the data, as shown in Figure 7. At a concentration of $10^2$ cells/μL the contrast enhancement in MRI is negligible for cells labeled with all types of iron particles. In samples with $10^4$ cells /μL the difference in contrast enhancement between 100 nm and 500 nm iron oxide particles cannot be distinguished due to saturation of the signal.
Figure 6. Visualization of iron-labeled cells with MRI (phantom). C17.2 neural stem cells were labeled with 100 nm and 500 nm iron oxide particles complexed with Lipofectamine 2000, and 500 nm PEI beads, at the indicated concentrations. Three different concentrations of labeled cells were suspended in 2% agarose and imaged by T2W-TSE sequence with CLEAR (A). In (B) cells incubated with half the amount of iron oxide particles as compared to cells in (A) are visualized.

Figure 7. Signal intensity ratios (contrast) of neural stem cells labeled with different iron oxide particles. Neural stem cells were labeled with similar amounts of iron by incubation with 100 nm and 500 nm iron oxide particles complexed with Lipofectamine 2000, and 500 nm PEI iron oxide particles. The resulting contrast enhancement in MRI was calculated from the images as shown in Figure 6 (A,B). Reduction of the cellular iron content by incubating the cells with half the amount of iron oxide particles results in a reduction in contrast enhancement.
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Discussion

Labeling of neural stem cells with iron oxide particles allows for their \textit{in vivo} tracking by MRI. Here we show that C17.2 neural stem cells can be labeled with commercially available 100 nm and 500 nm iron oxide particles. The uptake of large-sized (500 nm) particles by NSCs, although initially slower than that of small-sized (100 nm) particles, after 24 h shows a higher total capacity (Fig. 2).

Prior complexation of the iron oxide particles with the transfection agents LF2000 and JetPEI enhances the cellular uptake of the particles. Particularly, the large-sized particles benefit from complexation by transfection agents in the sense that their intrinsic slow internalization rate drastically improves upon neutralization of their anionic charge (cf. Figs. 2e and 3h). In parallel with an enhancement in internalization rate the extent of internalization is improved. For the 100 nm particles, complexation with transfection agents shows a less pronounced effect on rate and extent of internalization than for the larger particles. Importantly, in cells that are labeled with 100 nm and 500 nm iron oxide particles complexed with LF2000 and JetPEI, the iron oxide particles are not present at the cell-surface but localize intracellularly, as can be concluded from their perinuclear distribution (Fig. 3, b,c and e-f). Accomplishment of \textit{intracellular} labeling is a critical requirement since a surface-bound magnetic probe is likely to interfere with cell-tissue interactions and/or may detach from the cell membrane and be taken up by other cells \textit{in vivo} [22]. The latter would result in false positive signals in MRI.

The labeling conditions that result in effective contrast enhancement of the cells in MRI are non-toxic, as was shown by MTT assay (Fig. 1) and unchanged cell division time upon labeling. In addition, the cells retain their differentiation capacity upon labeling with iron oxide particles (Fig. 5). Notably, the iron labeling in C17.2 neural stem cells proved to be stable in a non-dividing cell population. As the stem cells are expected to show little cell division during their \textit{in vivo} migration, no loss of iron signal will occur upon their \textit{in vivo} administration, thus allowing detection of the iron-labeled stem cells by MRI for prolonged periods of time.

Finally, by the use of transfection agents the uptake of large-sized iron oxide particles by C17.2 neural stem cells was made as efficient as the uptake of small-sized particles, as was measured from the cellular iron content (Fig. 3h). Complexation of iron oxide particles with transfection agents, as well as the covalent attachment of PEI to the particles gave similar results. This allowed for a comparative analysis of the MRI signal strength of cells labeled with iron oxide particles with a big magnetic core and a small magnetic core. It turns out that an increase in the magnetic core of the iron oxide particles leads to an increase in MRI contrast enhancement (Fig. 7). Instead of using
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small superparamagnetic iron oxide nanoparticles, efforts should therefore be aimed at optimizing cell labeling with large-sized nanoparticles. The 500 nm iron oxide particles that were used in this study were calculated to have a magnetic core of ~330 nm and a shell of 85 nm. An increase of the magnetic core or, similarly, a reduction of the shell thickness would result in increased iron content per particle, and may further increase MRI signal of labeled cells [23]. Interestingly, it has been shown that although larger particles may have higher relaxivity per particle compared to smaller particles, their relaxivity per iron basis may actually be lower, as caused by differences in domain size and clustering of the iron oxide crystals within the particles [15]. Therefore in the future particular attention should also be paid to the internal composition/structure of large iron oxide particles for MRI purposes.

Acknowledgements

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References


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