MR Tracking of Transplanted Cells With “Positive Contrast” Using Manganese Oxide Nanoparticles

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Abstract

Rat glioma cells were labeled using electroporation with either manganese oxide (MnO) or superparamagnetic iron oxide (SPIO) nanoparticles. The viability and proliferation of SPIO-labeled cells (1.9 mg Fe/ml) or cells electroporated with a low dose of MnO (100 μg Mn/ml) was not significantly different from unlabeled cells; a higher MnO dose (785 μg Mn/ml) was found to be toxic. The cellular ion content was 0.1−0.3 pg Mn/cell and 4.4 pg Fe/cell, respectively, with cellular relaxivities of 2.5−4.8 s−1 (R1) and 45−84 s−1 (R2) for MnO-labeled cells. Labeled cells (SPIO and low-dose MnO) were each transplanted in contralateral brain hemispheres of rats and imaged in vivo at 9.4T. While SPIO-labeled cells produced a strong “negative contrast” due to the increase in R2, MnO-labeled cells produced “positive contrast” with an increased R1. Simultaneous imaging of both transplants with opposite contrast offers a method for MR “double labeling” of different cell populations.

Keywords

manganese oxide; iron oxide; cellular imaging; contrast agent; transplantation; nanoparticles

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Cells for transplantation have been labeled with MR contrast agents since the beginning of the 1990s. Superparamagnetic iron oxide (SPIO) nanoparticles have been most widely used due to their strong signal attenuation properties. Initially, cells were labeled with SPIO particles conjugated to lectins (1), with a viral envelope containing iron oxide particles (2), or by dextran-coated SPIOs conjugated to monoclonal antibodies (3). Over time, techniques were developed to make the labeling procedure more straightforward and widely applicable, independent of cell type (4), including the use of dendrimers (5), the human immunodeficiency virus (HIV) tat peptide (6), transfection agents (7), and micron-sized particles (8). Recently, electroporation (9,10), in which the cell membrane is permeabilized by a short electrical pulse, was demonstrated to be efficient in labeling cells with SPIO.

MRI cell tracking using SPIOs has now been widely used for tracking transplanted cells in various organs, and has recently also entered the clinic (11). However, in many cases, it is difficult to distinguish labeled cells from other hypointense regions on $T_2$/$T_2^*$-weighted MR images. These hypointensities can have a physiological origin, such as hemoglobin in blood, or a pathological origin, such as blood clots or experimental, traumatic procedures (e.g., caused by cell injections). One attempt to differentiate iron-labeled cells from blood vessels was to alter the inhaled oxygen levels to reduce the BOLD effect (12). Nevertheless, hypointensities on MR images remain a major obstacle in the attempt to increase the specificity of cell tracking, preventing this method from being used in certain applications, particularly those that involve trauma and hemorrhage. Therefore, an alternative method of detecting cells is to use contrast agents that create an opposite contrast.

Other than iron oxide labels, gadolinium complexes have been suggested for labeling fibroblasts with direct gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA) bovine serum albumin (BSA) incubation (13), or incubation with Gd-DTPA/fatty acid complex (14). In these cases, “positive” contrast can be observed. On the other hand, when internalized into cells, gadolinium-based agents may exhibit reduced $T_1$ relaxivity as compared to their unbound counterparts in solution. Therefore, in certain cases, $T_2$-weighted imaging and hypointense, magnetic susceptibility-based cell detection may still be preferred when using paramagnetic contrast agents (15).

Next to gadolinium, manganese is probably the second most used “positive” $T_1$ contrast agent. Increasingly, manganese-enhanced MRI is used for anatomical MRI, for the study of neuronal activity, to monitor neuronal tracts (16), to study neuronal connectivity in animal models of disease (17), and to monitor liposomal drug delivery (18,19). As for MRI cell tracking, manganese salt (MnCl$_2$) has been used for efficient labeling of lymphocytes in vitro, and a decrease in $T_1$ was observed for the first 24 h (20). Recently, some of us demonstrated that manganese oxide (MnO) nanoparticles can be used as $T_1$ MR contrast agent for various body organs, depicting fine anatomic structures (21). Here, functionalized MnO nanoparticles prepared by conjugation with a tumor specific antibody were also used for selectively imaging breast cancer cells in the metastatic brain tumor.

In this study, we evaluated the potential of using these MnO nanoparticles as a novel $T_1$ contrast agent for cell labeling. To this end, we have compared MnO-labeled cells to cells labeled with the SPIO formulation Feridex®. We report here that MnO can be successfully used to detect cells with positive contrast in vivo, and that two cell populations, one labeled with MnO and the other one with SPIO, can be detected simultaneously with opposite cell contrast.
MATERIALS AND METHODS

Cell Culture and Labeling

The rat glioma cell line 9L was cultured in Roswell Park Memorial Institute (RPMI) medium, supplemented with 10% fetal bovine serum (FBS) (Sigma), 2 mM L-glutamine (Invitrogen), 1 mM sodium pyruvate, 1:100 amphotericin B solution (Sigma), 0.06 mg/ml gentamicin (QBI), and 175 nM 2-mercapto-ethanol (Sigma).

Electroporation (9,10) was used to label cells with MnO and SPIO particles. MnO nanoparticles were prepared as described (21), with the nanoparticles having an average diameter of 7 nm. Cells were cultured in 80-cm^2 flasks overnight to 80% to 90% confluence. The next day, cells were suspended using trypsin-ethylenediamine tetraacetic acid (EDTA), washed with phosphate buffered saline (PBS) (without Ca^{2+} and Mg^{2+}), and counted. Cells were resuspended in PBS and transferred to sterile 0.4-cm gap electroporation cuvettes (Gene Pulser®; Bio-Rad); each cuvette contained 2 × 10^6 cells in 580 μl of PBS. Metal oxide nanoparticles were added at 1.9 mg Fe/ml for SPIO (Feridex®; Berlex Imaging) and 100 or 785 μg Mn/ml for MnO nanoparticles. Unlabeled cells that were treated with electroporation without contrast agent were used as controls; cells that were incubated with MnO but not electroporated were also included. PBS was added to each cuvette to a final volume of 700 μl. The cuvettes were kept on ice for 1 min, and then electroporated using a BTX electroporation system (ECM830; Harvard Apparatus). The following conditions were used: pulse strength = 100 V; N pulses = 5; pulse duration = 5 ms; and pulse interval = 100 ms. After 30 s, cells were transferred to ice for 2 min, suspended in culture medium, and transferred to 10-cm culture dishes.

In Vitro Toxicity Tests

After electroporation, 5 × 10^3 cells out of the 2 × 10^6 cells were transferred in triplicate to 96-well plates, with the remaining cells plated into the culture dishes. After 24 h, the cells in the 96-well plates were assayed for viability. At the same time, cells in the 10-cm culture dish were washed with PBS, suspended using trypsin-EDTA, and replated in 96-well plates (5 × 10^3 cells per well). These cells were assayed again after 24 h (i.e., a total of 48 h after electroporation).

For assessment of cell viability, a Calcein-acetoxymethyl (AM) enzyme assay was used (4892 −010-K; Trevigen Inc.). This assay is based on hydrolysis of Calcein-AM by intracellular esterases that produce calcein only in viable cells. Cells were washed once with 100 μl of Calcein-AM buffer, and 100 μl of Calcein-AM solution was added. Cells were incubated for 30 min at 37°C in a humidified 5% CO_2 atmosphere. The fluorescence was recorded using a 490-nm excitation filter and a 520-nm emission filter, with the fluorescence intensity being proportional to the number of viable cells.

For assessment of proliferation, a MTS ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulophenyl)-2H-tetrazolium, inner salt) was used (Cell Titer 96® Aqueous, G3582; Promega). The assay is based on mitochondrial assimilation and conversion of substrate. A total of 20 μl of Cell Titer 96® Aqueous One Solution Reagent was added per well. Cells were incubated for 2 h at 37°C in a humidified 5% CO_2 atmosphere. The absorbance was recorded at 490 nm using a 96-well plate reader. For additional study of cell proliferation, CellTiter-Blue®, Cell Viability Assay (G8080; Promega) was used. The assay is based on the ability of living cells to convert a redox dye (resazurin) into a fluorescent end product (resorufin): 20 μl of Cell Titer Blue® reagent was added per well. The cells were incubated for 2 h at 37°C in a humidified, 5% CO_2 atmosphere. The fluorescence was recorded using a 96-well plate reader (excitation at 560 nm and emission at 590 nm). Results are...
expressed from two independent experiments \( (N = 6) \) for MTS and Calcein-AM, and from one experiment for Cell Titer Blue \( (N = 3) \).

**MR Phantom Preparation**

At 24 h after electroporation, cells were washed twice with PBS, harvested using trypsin, and counted. For the cell pellet phantom, \( 3.15 \times 10^6 \) cells suspended in \( 100 \mu l \) PBS were transferred to 0.2 ml polypropylene tubes (VWR International) and centrifuged for 6 min at 1200 rpm. The supernatant was aspirated and cells were resuspended in 20 \( \mu l \) of PBS. For the gelatin phantom, \( 4 \times 10^6 \) cells suspended in 50 \( \mu l \) PBS were transferred to 0.2 mL polypropylene tubes and mixed with 100 \( \mu l \) of 6% gelatin in PBS. Thus, the final cell concentration was \( 2.7 \times 10^4 \) cells/\( \mu l \) in 4% gelatin. Control samples consisted of PBS and 4% gelatin in PBS.

**Animal Studies**

Animal experiments were performed in accordance with protocols approved by our institutional Animal Care and Use Committee. At 24 h after electroporation, cells (labeled with 1.9 mg Fe/ml for SPIO, 100 \( \mu g \) Mn/ml for MnO nanoparticles or unlabeled) were washed twice with PBS, suspended using trypsin-EDTA, and counted. The cells were centrifuged at 1000 rpm for 5 min and diluted to the appropriate concentration. Male Fisher rats (weight 250 – 350 g) were anesthetized with ketamine/acepromazine (100/5 mg/kg) and positioned in a stereotaxic device (Stoelting Lab Standard). A small midline skin incision was made to expose the skull. Using a 10-\( \mu l \) Hamilton syringe with an attached 31G metal needle (Hamilton Co.), \( 2 \times 10^5 \) cells in 2 \( \mu l \) PBS each were injected bilaterally into each striatum \( (\text{AP} = 0.0, \text{ML} = 3.0, \text{DV} = 5.0) \). Cells were injected slowly over 4 min, and the needle was left in place for 1 min before being withdrawn. The incision was sutured and postoperative analgesia was provided (ketoprofen, 2 mg/kg). Rats were anesthetized with 1.5% to 2% isoflurane and imaged at 24 h \( (N = 7; \text{five with MnO- and SPIO-labeled cells, and three with SPIO- and unlabeled cells}) \), at 48 h \( (N = 1) \), and at 72 h \( (N = 1) \) after cell transplantation.

**MRI and Analysis**

MR images were acquired on a Bruker 9.4T horizontal bore magnet equipped with a 30-mm Sawtooth resonator (Bruker) using a multispin multiecho (MSME) pulse sequence.

**In Vitro Cell Pellet Phantoms**—For \( T_1 \) measurements, the following parameters were used: \( \text{TE} = 14.1 \text{ ms}, \) and \( \text{TRs} = 0.2, 0.3, 0.5, 0.8, 1, 1.5, 2, 4, 6, \) and \( 10 \text{ s} \). For \( T_2 \) measurements: \( \text{TR} = 2 \text{ s}, \) and \( \text{TEs} = 14, 20, 30, 40, 50, \) and \( 60 \text{ ms} \). The field of view was \( 26 \times 26 \text{ mm} \) with matrix size of \( 64 \times 64 \text{ pixels} \) and a 1.0-mm slice thickness.

**In Vitro Gelatin Phantoms**—For \( T_1 \) measurements, the following parameters were used: \( \text{TE} = 14.1 \text{ ms}, \) and \( \text{TRs} = 0.2, 0.3, 0.5, 0.8, 1, 1.5, 2, 4, 6, \) and \( 10 \text{ s} \). For \( T_2 \) measurements: \( \text{TR} = 2 \text{ s}, \) and \( \text{TEs} = 15, 30, 45, 60, 75, 90, 105, \) and \( 120 \text{ ms} \). The field of view was \( 27 \times 18 \text{ mm} \) with a matrix size of \( 128 \times 64 \) and a 1.0-mm slice thickness. The \( R_1 \) and \( R_2 \) were calculated by fitting of the data to \( \text{Ln} \left(1 - \left[I/I_0\right]\right) = -R_1^*\text{TR} \) and \( \text{Ln} \left(I\right) = -R_2^*\text{TE}+A, \) respectively.

**In Vivo Animal Studies**—For \( R_1 \) maps, the following parameters were used: \( \text{TE} = 14.1 \text{ ms}, \) and \( \text{TRs} = 0.2, 0.3, 0.5, 0.8, 1, 1.5, 2, 3, 4, \) and \( 6 \text{ s} \). For the \( R_2 \) maps: \( \text{TR} = 2 \text{ s}, \) and \( \text{TEs} = 14, 20, 30, 40, 50, \) and \( 60 \text{ ms} \). The field of view was \( 30 \times 30 \text{ mm} \) and the matrix size was \( 128 \times 128 \) and \( 64 \times 64, \) for anatomical images and \( R_1/R_2 \) maps, respectively. The slice thickness was 0.8 mm. \( R_1 \) and \( R_2 \) maps were generated by pixel-by-pixel fitting using Matlab 6.5.1 (The MathWorks, Inc., USA). In vivo \( R_1 \) and \( R_2 \) maps were generated by fitting to \( I = I_0^*(1-e^{-\text{TR}^*R_1}) \) and \( \text{Ln} \left(I\right) = -R_2^*\text{TE}+A, \) respectively.
Measurement of Intracellular Manganese and Iron Content and Cellular Relaxivity

After the in vitro phantom imaging, all samples were kept frozen at −20°C. Samples were assayed for Mn and Fe using inductively coupled plasma (ICP) analysis (Varian Vista Pro ICP; Microbac Laboratories, Inc., Baltimore, MD, USA). The sample detection threshold was 2.5 μg of Fe and 0.25 μg of Mn. The cellular iron concentration was determined by dividing the total metal content of each sample with the number of cells. Cellular relaxivity was determined using the slope of relaxation enhancement per unit concentration of intracellular manganese. The relaxivities were calculated as follows: \( r_1 = \frac{R_{1\text{MnO}} - R_{1\text{Control}}}{[\text{Mn}]} \) and \( r_2 = \frac{R_{2\text{MnO}} - R_{2\text{Control}}}{[\text{MnO}]} \).

RESULTS

To compare the two contrast agents, 9L cells were labeled with MnO, SPIO, or not labeled (but still electroporated as control). A total of 2 × 10^5 cells was inoculated in the ipsilateral and contralateral hemisphere of Fisher rats. Figure 1a and b show representative images of both SPIO- and MnO-labeled cells in the same imaging plane. While the SPIO-labeled cells appear as a hypointense region (dark spot or negative contrast), the MnO-labeled cells appear as a hyperintense region (bright spot). The hyperintense region was found to be induced by the MnO and not a result of edema following cell injection, as hemispheres that were injected with unlabeled cells did not show any contrast (Fig. 1c).

MnO-labeled cells could be best detected on \( R_1 \) maps (Fig. 1d–f). In contrast, SPIO-labeled cells were seen most clearly on the \( R_2 \) maps (Fig. 1g–i), while MnO-labeled cells were barely visible on the \( R_2 \) map. Figure 1j–l shows the merged images of the \( R_1 \) and \( R_2 \) maps, demonstrating a clear proof-of-principle that opposite contrast can be obtained simultaneously using differentially-labeled cell populations.

The main advantage of “positive contrast labeling” would be the ability to distinguish cells from blood/hemosiderin-associated hypointense regions. Although some pathologies, such as tumor and stroke-related edema, can appear as hyperintense regions on \( T_1 \)-weighted and spin-density/\( T_2 \)-weighted images, they can be easily distinguished from labeled cells on \( R_1 \) maps: the former results from an increase in tissue water content, which is characterized by shortening of \( R_1 \), while MnO-labeled cells result in an increase of \( R_1 \). A histogram of the pixel distribution (Fig. 2) reveals that the pixels from the MnO-labeled transplanted cells have a higher \( R_1 \) but not \( R_2 \), whereas the pixels from the brain tissue surrounding the transplanted cells have \( R_1 \) values similar to brain and to unlabeled transplanted cells. After 2–3 days, it became difficult to detect the MnO-labeled cells, although they were detectable on the \( R_1 \) maps (Fig. 3). The reduction in the \( R_1 \) values could possibly be related to dilution of the contrast agent following multiple cell divisions.

To characterize the relaxation properties of the labeled cells in vitro, the \( T_1 \) and \( T_2 \) relaxation for cell pellets and cells suspended in 4% gelatin were measured 24 h after electroporation (Fig. 4). The loss of signal from SPIO-labeled cells was not beyond the background noise, thus preventing accurate measurements. For cell pellets, the \( R_1 \) was 0.80 s\(^{-1}\) for unlabeled cells and 4.92 s\(^{-1}\) for cells labeled with MnO. For the cell suspensions embedded in gelatin, the \( R_1 \) increased from 0.34 s\(^{-1}\) for unlabeled cells and 0.41 s\(^{-1}\) for gelatin only to 0.50 s\(^{-1}\) for cells labeled with MnO. The \( R_2 \) increased from 21 s\(^{-1}\) and 4.8 s\(^{-1}\) for unlabeled cells to 94 s\(^{-1}\) and 7.7 s\(^{-1}\) for MnO-labeled cells in phantoms of cell pellets and cells suspended in gelatin, respectively. Even though in vitro labeled cells exhibit both the \( R_1 \) and \( R_2 \) effect, in vivo the \( R_1 \) effect was more pronounced (Fig. 1).

For all phantoms, the manganese and iron content was measured with ICP. The Mn concentration was 47.5 μg Mn/ml for the phantom with the MnO-labeled cell pellet, which

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corresponds to 0.3 pg Mn/cell. For the phantom with MnO-labeled cell suspensions in gelatin, the Mn concentration was 3.53 μg Mn/ml, which corresponds to 0.13 pg Mn/cell. No Mn was detected in control, unlabeled samples or 4% gelatin samples. The iron content in the SPIO-labeled cell pellet was 4.4 pg Fe/cell. Iron was not detected in any of the other samples. Thus, the $R_1$ was $4.77 \text{ s}^{-1}\text{mM}^{-1}$ and $2.49 \text{ s}^{-1}\text{mM}^{-1}$ and the $R_2$ was $84.53 \text{ s}^{-1}\text{mM}^{-1}$ and $45.18 \text{ s}^{-1}\text{mM}^{-1}$ for cell pellets and cells suspended in gelatin, respectively.

To assess any potential toxic effects of MnO labeling using electroporation, five independent cell labeling experiments were performed. 9L cells were electroporated under conditions identical to those that were used for the in vitro and in vivo MRI. Cell viability was determined by testing the mitochondrial activity (MTS) and cellular enzymatic activity (Calcein-AM and Cell Titer Blue), both of which can be correlated with the number of viable cells.

After 24 h, a reduction in the number of viable cells was observed for cells labeled with both high and low MnO concentrations ($t$-test, unpaired, two-tailed, $P < 0.05$), but not those labeled with SPIO (Fig. 5). Notably, cells that were incubated with MnO without electroporation showed a similar degree of toxicity (Fig. 5a), indicating that the contrast agent and not the electroporation itself leads to cellular impairment.

To determine whether surviving cells can proliferate at the same rate as the nonlabeled cells, 24 h after electroporation, labeled cells were washed, counted again, and transferred into new culture plates. The cell viability was tested again after 24 h (total 48 h after electroporation) with MTS, Calcein-AM, and Cell Titer Blue. At this time, no significant difference could be observed between any of the conditions, except for cells that were electroporated with the higher MnO concentration ($t$-test, unpaired, two-tailed, $P < 0.05$). Of note is that cells electroporated with SPIO showed an enhanced proliferation, even relative to control cells (Fig. 5b).

The overall findings demonstrate that cells labeled with the lower MnO concentration recover 48 h after labeling (at least in vitro) and show viability similar to control cells.

**DISCUSSION**

In this study, we compared labeling of cells with two different contrast agents, i.e., MnO and SPIO nanoparticles. Both are metal oxides; SPIO nanoparticles have been in use for several years as an effective $T_2/T_2^*$ contrast agent for labeling and tracking cells. This cell tracking approach generates negative contrast on $T_2$-weighted MR images. Here we explored the use of a “positive” contrast agent that can provide opposite contrast on the same type of generated image, using a newly developed MnO nanoparticle preparation (21).

Depending on the specific application, labeling cells with MnO nanoparticles could be a viable alternative for iron oxide labeling. These applications include experimental settings where it is difficult to distinguish iron oxide-labeled cells from blood/hemoglobin-derived hypointense regions, or from tissues and organs that have inherently high concentrations of iron, such as the liver, or certain tumors, such as melanomas. An additional application for using MnO nanoparticles includes tracking two different cell populations simultaneously, where one is labeled with MnO and the other with SPIO nanoparticles. In this study, we have shown this proof-of-principle of MR “double labeling” with opposite contrast (Fig. 1). This method will be most suitable for studies that require tracking of two cell populations that are injected in different locations or for tracking two cell populations that are injected at two different time points.
The negative contrast enhancement of SPIO-labeled cells was found to be more pronounced than the positive cellular contrast induced by the MnO nanoparticles. Aside from the differences in relaxivities and concentrations used, this contrast could also partially result from using a labeling procedure that has been optimized for SPIO labeling (9,10), with the MnO-labeling procedure currently being adapted from it. The rationale was to use a protocol that would provide the maximum labeling for SPIO, serving as a reference for the best labeling, with minimal differences between the labeling procedures. It may be possible that further optimization of the MnO labeling protocol can increase the concentration of intracellular manganese, along with detection of cells over a longer time frame.

One of the drawbacks of labeling cells with exogenous nanoparticles is the loss of signal over time, as shown in Fig. 3. The loss of signal can be attributed to either biodegradation of the contrast agent or, in the case of dividing cells, dilution of label that is amplified in asymmetric cell division (22). One approach to overcome this is to use MRI reporter genes, in which daughter cells retain a constant amount of the contrast-enhancing agent after each cell division (23,24).

We have employed a prototype MnO nanoparticle that has not yet been fully optimized for biocompatibility and biomedical use. We observed a transient toxic effect after 24 h in cells that were electroporated with MnO. However, after another 24 h, the surviving cells showed viability similar to that of controls, as manifested by mitochondrial and cytoplasmic enzymatic activity. It is well known that Mn can be toxic to mammalian cells. However, it has no cellular toxicity at concentrations <2 mM, as reported for lymphocytes (20), and at concentrations <0.8 mM, as reported for human fibroblasts and cancer cells (21). In this study, we used 1.85 mM and 14.5 mM Mn to get maximal loading of the cells; while the high dose was found to be toxic, the low dose of MnO did not induce significant toxicity, in analogy with those studies.

In vitro measurements of $R_1$ and $R_2$ showed good correlation with the Mn content in labeled cells as measured with ICP. Interestingly, the cellular $T_1$ and $T_2$ relaxivities were different for cell pellet and cells suspended in gelatin, indicating that the microenvironment of the transplanted cells has considerable impact on the relaxivities. This is also true in vivo, in which the relaxation measured from each voxel is composed not only from the transplanted cells but also from a variety of inhabitant cells and the extracellular matrix. The mechanism by which the MnO nanoparticles generate contrast is not fully understood. The dipolar relaxation that underlies $T_1$ contrast must arise from direct interactions between magnetic moments at a short spatial scale. Several possibilities arise that can accomplish this. A first explanation would be the presence of Mn on the outer shell of the crystal or Mn leaching out from the particles. A second would be, if phospholipid-polyethylene glycol (PL-PEG) allows water diffusion across the coating toward the crystal outer surface, that proton relaxation is enhanced after direct contact with the crystal and water diffuses outward. Finally, as the phospholipid shell is directly in contact with the crystal and the total particle is moving slow, there is a rapid dipolar transfer (spin diffusion) between them. The same is true between the phospholipids and PEG. As PEG contains rapidly exchanging OH protons, the paramagnetic relaxation effects may be transferred through exchange to the solvent water. Although we do not have direct evidence to support any of these possible mechanisms we are aiming to elucidate this in future work.

In summary, the present study exemplifies the use of MnO and SPIO nanoparticles for obtaining dual contrast of two different cell populations, which may encourage further investigations into developing and optimizing nanoparticles that can provide positive contrast.

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FIG. 1.
In vivo MRI of labeled 9L cells 24 h after transplantation in the striata of rat brain. a–c: Spin echo image (TR = 1000 ms, TE = 14.1 ms). d–f: $R_1$ maps. g–i: $R_2$ maps. j–l: $R_1/R_2$ merged maps. Shown are representative images of three out of seven rats, two injected with MnO- and FeO-labeled cells (a,b), and one with FeO-labeled cells and unlabeled cells (c; control). Note the simultaneous double contrast in (j–l).
**FIG. 2.**

*R*$_1$ and *R*$_2$ histograms. **a:** The *R*$_1$ of each pixel is presented for two representative rats. A region of interest (ROI) was selected to cover both the transplanted cells and brain tissue. Pixels of unlabeled transplanted cells (green, *N* = 71 pixels) have more uniform and lower *R*$_1$ values, while MnO-labeled transplanted cells (red, *N* = 58 pixels) exhibit a broader distribution, shifting toward higher *R*$_1$ values. The overlapping pixels are from brain tissue surrounding the labeled transplanted cells; the “tail” can be attributed to pixels with a higher *R*$_1$ that represent MnO-labeled cells. The average *R*$_1$ for unlabeled and MnO-labeled cells was 0.52 s$^{-1}$ and 0.63 s$^{-1}$, respectively. **b:** These findings are consistent with the average distribution of *R*$_1$ from MnO-labeled transplanted cells (red, *N* = 5) and unlabeled transplanted cells (green, *N* = 2).
c: The $R_2$ distribution is similar for MnO-labeled transplanted cells (red, $N = 5$) and unlabeled transplanted cells (green, $N = 2$), implying that MnO can be used as dominant $T_1$ positive contrast agent.
FIG. 3.
Spin echo images (TR = 1000 ms, TE = 14.1 ms) (a,b) and R1 maps (c,d) of the same rat brain at 1 (a,c) and 3 (b,d) days after cell grafting. The contrast decreases for both MnO- and SPIO-labeled cells over time. Although it is difficult to see MnO-labeled cells on the spin echo image (b), the cells are still visible on the R1 map (d).
FIG. 4.
In vitro characterization of relaxation enhancement. The relative change in signal $I/I_0$ intensity (mean of region of interest [ROI]) is plotted for a series of TRs and TEs for (a,b) PBS samples (◇), 4% gelatin only samples (△), cells suspended in 4% gelatin labeled with MnO (●), and unlabeled cells (□); (c,d) MnO-labeled cell pellets (●) and unlabeled cell pellets (□).
FIG. 5.
Assessment of cell viability and toxicity at 24 h (a) and 48 h (b) after electroporation with MnO (low: 100 μg Mn/ml) or (high: 785 μg Mn/ml) and SPIO (1.9 mg Fe/ml). Cell viability was tested using three different assays: MTS (black bars); Cell Titer Blue (gray bars); and Calcein-AM (empty bars). All bars represent the average ± SD of the percentage relative to control (electroporation without contrast agent).