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Tracking and Quantification of Magnetically Labeled Stem Cells using Magnetic Resonance Imaging

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Abstract

Stem cell based therapies have critical impacts on treatments and cures of diseases such as neurodegenerative or cardiovascular disease. In vivo tracking of stem cells labeled with magnetic contrast agents is of particular interest and importance as it allows for monitoring of the cells' bio-distribution, viability, and physiological responses. Herein, recent advances are introduced in tracking and quantification of super-paramagnetic iron oxide (SPIO) nanoparticles-labeled cells with magnetic resonance imaging, a noninvasive approach that can longitudinally monitor transplanted cells. This is followed by recent translational research on human stem cells that are dual-labeled with green fluorescence protein (GFP) and SPIO nanoparticles, then transplanted and tracked in a chicken embryo model. Cell labeling efficiency, viability, and cell differentiation are also presented.

Keywords

MRI; stem cell; SPIO; tracking; chick embryo

1. Introduction

Stem cell based therapies increasingly play critical roles in treating patients with diverse diseases such as diabetes, neurodegenerative, and cardiovascular diseases.^[1] For example, a search in the National Institutes of Health public clinical trials database (<https://>

www.clinicaltrials.gov/) revealed about 172 studies worldwide by using certain keywords “stem cell therapy”, 292 open studies by “mesenchymal stem cell”, and 1922 open studies by simply “stem cell” (as of October 2015). However, the success and advancement of stem cell therapies are often limited and better means of predicting therapeutic outcomes require further investigation. For example, a recent review indicates that both pre-clinical and clinical studies present mixed results for mesenchymal stem cells therapy, and the discrepancy between expected and actual efficacy of mesenchymal stem cells in various diseases has evoked a sense of discouragement.^[2] Additionally, clinical trials regarding adoptive cellular immunotherapy could not consistently show the benefit due to multi-factors in complex immunosuppressive microenvironment *in vivo* compared to that of *in vitro*.^[2] At the most basic level it is still unclear what conditions are favorable for a transplanted stem cell to survive and integrate with the host tissues. When stem cells engraft, careful investigation is required to determine whether they undertake the proper cellular differentiation path that leads to a desired therapeutic outcome.

The applications of imaging technologies in cellular imaging, such as optical methods including fluorescence, bioluminescence, and 2- or 3-photon microscopy, magnetic resonance imaging (MRI), and nuclear imaging including single-photon emission computed tomography (SPECT) and positron emission tomography (PET) have proven particularly useful in cell therapy preclinical research and clinical applications.^[3] Longitudinal *in vivo* imaging allows one to monitor the stem cells’ interactions with host tissues or recipient’s cells and their pathways following transplantation, as well as cells’ biodistribution, viability, or other physiological responses, ideally in a non-invasive manner.

Over the past two decades there have been numerous studies and reviews in the literature on cell labeling and tracking, covering a variety of contrast agents and imaging technologies.^[4–8, 9, 10] This feature article intends to focus on a brief review of tracking and quantification of magnetic nanoparticles-labeled cells with MRI only. We will review recent advances, including basic principles of MRI, labeling, tracking, and quantification of cells labeled with superparamagnetic iron oxide (SPIO) nanoparticles in preclinical applications. The second part will present our recent work on dual-labeling of human stem cells with green fluorescence protein (GFP) and SPIOs, and then transplanting and tracking these stem cells in a chicken embryo model.

2. Review of MRI Contrast Agents and Labeling, Tracking, and Quantification of Cells

2.1. Magnetic Resonance Imaging

MRI is an imaging modality that measures the interaction between an external magnetic field and the magnetic moment of protons in certain nuclear species, e.g. hydrogen (^1H), phosphorus (^{31}P), or fluorine (^{19}F). When measuring certain properties of protons in the hydrogen, MRI can display anatomical structures of living human or animal bodies with high spatial resolution. During the past few decades, preclinical and clinical applications of MRI, including cell tracking, have been rapidly expanding, thanks to many earlier pioneering works.^[11] The protons become magnetized in the presence of an external

magnetic field B_0 . The magnitude of the equilibrium magnetization (M_0) is defined as $M_0 = \chi B_0$, where χ is known as the bulk magnetic susceptibility. The protons spin along the magnetic field B_0 with a Larmor frequency, as defined by $\omega_0 = \gamma B_0$, where γ is gyro-magnetic ratio of water protons.

MRI measures changes in the magnetization by applying short radiofrequency (RF) pulses. When an RF pulse is applied, the protons absorb the RF energy and are excited by rotating away from its equilibrium state. After the short RF excitation pulse is turned off, the absorbed energy is released to the surroundings. This process is illustrated by two simultaneous mechanisms, i.e., the recovery of the longitudinal magnetization component toward the equilibrium state, governed by a spin-lattice or longitudinal relaxation, and the dephasing (loss of coherence among rotating protons) of the transverse magnetization component, governed by a spin-spin or transverse relaxation. The rate of each relaxation is determined by the relaxation times. The longitudinal relaxation time, T_1 , is defined as the time required to recover to 63% of its original longitudinal magnetization (Figure 1). The process of T_1 relaxation is described as $M_z(\tau) = M_0 (1 - e^{-\tau/T_1})$, where $M_z(\tau)$ is the longitudinal magnetization, and τ is the time following an RF pulse.

The transverse relaxation time T_2 is defined as the time required after excitation for the transverse magnetization to be reduced to 37% of its equilibrium magnetization, described by $M_{xy}(\tau) = M_0 e^{-\tau/T_2}$, where $M_{xy}(\tau)$ is the transverse magnetization. Because the main magnetic field B_0 is not perfectly homogenous, dephasing of individual magnetizations of protons occurs, resulting in more rapid loss of transverse magnetization, referred to as T_2^* relaxation.

Relaxation is fundamental to the contrast in an MR image because tissues have different relaxation characteristics. MR images can be created with weighting on different properties of tissues, such as proton density, T_1 and T_2 relaxation rates, blood flow, and water molecule diffusion. Tissues with short T_1 relaxation time give increased MR signals, resulting in bright images or positive contrast, e.g. subcutaneous or visceral fat appears brighter than muscles in T_1 -weighted MRI images. Tissues with short T_2 relaxation time produce reduced MR signals, resulting in dark images or negative contrast, e.g. cortical bones look darker than surrounding tissues in T_2 -weighted MRI. In some cases, the difference in relaxation rates between normal and diseased tissues, or between transplanted cells and host tissues, is too small to be detected, resulting in no significant tissue contrast in MR images. To enhance tissue contrast or visibility of transplanted cells, contrast agents or tracers can be administered.

2.2. MR Contrast Agents

Contrast agents are generally used to enhance MR sensitivity by manipulating relaxation times of the protons in water molecules. Labeling a population of a certain type of cells is usually conducted with a suitable MR contrast agent in cell culture. Depending on the physical properties of the contrast agents and contrast mechanism, they can be divided into different categories. These include paramagnetic, super-paramagnetic, chemical exchange saturation transfer agents, and hetero-nuclear agents, e.g., ^{19}F -MRI [12], ^{129}Xe -MRI [13], and ^{13}C -MRI [14]. Particularly, ^{19}F agents have been used to label a variety of cells including

umbilical cord blood derived CD34⁺ cells [15], mesenchymal stem cells [16], and neural stem cells [17]. However, this paper will focus on contrast agents that possess magnetic properties, i.e., paramagnetic and super-paramagnetic agents.

2.2.1. Paramagnetic Agents—Paramagnetic agents have been widely used in preclinical and clinical MRI. Gadolinium (III) (Gd³⁺) chelates are the most effective agents due to the presence of seven unpaired electrons and the symmetric S-state closely in tune with the proton's frequency. [18] The unpaired electrons create a magnetic moment that shortens the T_1 and T_2 relaxation times (or equivalently, increase the relaxation rates $R_1=1/T_1$ and $R_2=1/T_2$) by spin-lattice and spin-spin interactions. However, these agents usually pose relatively larger effect on shortening of the T_1 relaxation time; they are therefore often referred to as T_1 -weighted contrast agents. The T_1 -weighted agents can generate hyperintense or 'positive' contrast on traditional T_1 -weighted spin-echo or gradient-echo MR images. As a preclinical or research tool, gadolinium has been used to label different stem cells. For example, Gd₂O₃ nanoparticles were used to label and monitor hematopoietic cells. [19] Another study enabled monitoring of the anatomical migration and the survival period of transplanted stem cells by using gadolinium for up to 1 month. [20] They reported that gadolinium in the cells was rapidly eliminated from the site and excreted upon cell death, and endothelial progenitor cells transplanted into the femoral muscle of rats migrated distally through the knee after hind limb ischemia. In a recent study gadolinium-chelate nanoparticles were prepared and entrapped into human mesenchymal stem cells (hMSCs) by the photochemical-internalization (PCI) method for cancer diagnosis via the cancer homing property of hMSCs. The internalization efficiency of Gd in hMSCs was significantly increased to 98 ± 4 pg Gd per cell from 32 ± 2 pg Gd per cell. The Gd-entrapped hMSCs revealed a low exocytosis ratio of gadolinium-chelate nanoparticles during cell division *in vitro* and a high cellular labeling efficiency for at least 21 days *in vivo*. The stem cells detected an early tumor (~ 3 mm³) within 2 h using 4.7 T MR and optical imaging. [21] In another study three different kinds of transfection reagents were used to mediate the transfection of gadolinium-diethylenetriamine penta-acetic acid (Gd-DTPA) into human umbilical-cord-derived mesenchymal stem cells. The proliferation and differentiation abilities of the Gd-DTPA-labeled stem cells were not affected. A minimum of 5×10^4 Gd-DTPA-labeled stem cells could be traced with MRI *in vitro* and produced a high MRI signal compared to the surrounding tissue. The trace duration time *in vitro* was about 12 days. [22]

However, low sensitivity and safety of the contrast agents and low uptake by cells poses a big challenge and remains a main barrier to cell labeling. In order for cells to efficiently uptake more gadolinium chelates, researchers have attempted different strategies. Transfection agents including lipofection (Lipofectin and Lipofectamine) and calcium phosphate transfection agents were applied for increased uptake of paramagnetic particles Gd-DTPA in stem cells. [23] Using this approach, embryonic stem cells and neural stem cells have been labeled with an intracellular labeling efficiency of up to 83% with the transfection protocols used in this study. Neither the proliferation capacity nor the differentiation efficiency were affected, and identical differentiation of labeled and unlabeled embryonic and neuronal cells was observed. In another study, a paramagnetically labeled gadolinium-tetraazacyclododecanetetraacetic acid (DOTA) chelator was shown to be efficiently

internalized into mammalian cells. Intracellular concentrations were readily detectable by MR imaging using both gadolinium and dysprosium chelates. [24] Another major limitation to the use of Gd^{3+} contrast agent is the concern of toxicity or safety, as Gd^{3+} ion is highly toxic after administration as a free ion at low doses. The toxicity is imposed either to the labeled cells themselves or to the surrounding host tissues. Precaution should be exercised when using contrast agents containing gadolinium because of nephrogenic systemic fibrosis reported in patients with impaired kidney function or renal failure. [25] In order to improve biocompatibility, the Gd^{3+} ion needs to be complexed with chelating ligands, e.g. cyclic or linear polyaminocarboxylic acids, forming stable complexes in the design and development of clinical MRI contrast agents. [18]

In addition to Gd^{3+} , manganese II (Mn^{2+}) is another potentially useful paramagnetic agent for cell labeling as it has five unpaired electrons. Because Mn^{2+} ions enter cells through Ca^{2+} channels, Mn^{2+} -enhanced MRI was used to study neuronal activity and visualize neuronal connectivity in several animal models. [26] Manganese oxide nanoparticles were also used to detect cells, with the advantage of duo-labeling when combined with superparamagnetic iron oxide to detect two populations of cells at the same time. [27] Furthermore, manganese oxide nanoparticles coated with silica were also developed for labeling and tracking of mesenchymal stem cells (MSC). [28] Both Gd^{3+} and Mn^{2+} nanoparticles are proven contrast agent suitable for stem cell tracking, but concerns of the nanoparticle's biocompatibility will likely hamper its use in clinical settings.

2.2.2. Superparamagnetic Agents—The second category of contrast agents is based on SPIOs or ultra-small SPIOs, developed in the 1980s and 1990s. [29] Mono-dispersed superparamagnetic nanoparticles of Fe_3O_4 , $CoFe_2O_4$ and $MnFe_2O_4$ with tunable diameters (3 to 20 nm) have been synthesized through the reaction of metal acetylacetonate and 1,2-hexadecanediol. [30] Detailed lists of various SPIOs have been reported in many earlier reviews. [5]

The SPIOs usually consist of iron-oxide cores, while the cores are covered by various layers, such as dextran, [31] siloxan, [32] or polymer [33]. Depending on the particle size, they are divided into different groups, i.e., ultra-small SPIOs (10–50 nm in diameter), SPIOs (50–150 nm), and micrometer-sized iron oxide (1 μm or up). The core usually consists of several thousands of iron atoms, which significantly increases the local iron concentration. In the presence of an applied external magnetic field B_0 , individual magnetic moments are aligned along the field resulting in a formation of single spins that has a net moment of several orders higher than a comparable ensemble of paramagnetic spins. This much higher moment generates very large microscopic magnetic field gradients that affects nearby spinning protons. As a result, it shortens the T_2 relaxation time to a dramatic degree, which is far beyond the conventional dipole-dipole relaxation mechanism. Owing to the much shortened T_2 effect, these agents generate hypointense or 'negative' contrast on traditional spin-echo and gradient-echo MR images. It is noted that on gradient-echo MR images, these agents induce an even larger hypointense contrast due to dominant T_2^* effects (a combination of intrinsic spin-spin relaxation and local magnetic field inhomogeneity). A signature representation of the presence of these agents in the region of interest is a flowering-like

‘blooming’ effect. This is due to the fact that microscopic inhomogeneities of the magnetic field surrounding protons induce a rapid dephasing among diffusing protons.

Compared to gadolinium, iron is naturally present in the human or animal body, and consequently more biocompatible with the labeled cells and host tissue.^[34] For example, iron is central to mitochondrial function, and mammalian cells are well adapted to regulation of iron homeostasis. Cytotoxicity and cell function studies indicate no adverse effects when cells are loaded with less than 10 pg Fe.^[7] SPIO based contrast agents can be well tolerated and safely degraded by living organisms demonstrating the biocompatibility of SPIO contrast agents.^[35] The proton relaxation per atom due to iron is much greater than that attributed to paramagnetic elements (e.g. gadolinium), resulting in a lower amount of SPIO contrast agents applied. Various stem cell types labeled with SPIOs have been tracked after transplantation into animal models. Human embryonic stem cells (hESC) were effectively labeled by simple incubation with ferumoxides SPIOs before or after differentiation into cardiomyocytes.^[36] Labeled cells retained the iron and normal cellular function after differentiation to cardiomyocytes (hESC-CM). The results showed that hESC-CM labeled before differentiation demonstrated significant MR effects, iron uptake, and preserved function, while hESC-CM labeled after differentiation showed no significant iron uptake or change in MR signal ($P < 0.05$). To study labeling efficacy of different SPIOs, human neural stem cells (hNSCs) were labeled with ferumoxides, monocrystalline iron oxide, cross-linked iron oxide (CLIO)-NH(2), and tat-CLIO, and cellular proliferation and viability were compared among the three types of SPIOs.^[37] The incorporation of SPIOs into the hNSC did not affect the cellular proliferations and viabilities, while the hNSCs labeled with tat-CLIO showed the longest retention, up to 72 hr. SPIO nanoparticles that were stably incorporated into endosomal vacuoles of stem cells and allowed longitudinal tracking implanted cells for months generated stable labeling and enhanced biocompatibility.^[38, 39] The infarct size resulting from a stroke model as well as fate and function of grafted neural stem cells (NSCs) were tracked in real time for 3 months using MRI and PET. It was found in animal studies that the grafted NSCs reduced small infarct (less than 0.1 cm³) in a dose-dependent manner, while larger stroke was not amenable to such beneficial effects.^[38] A variety of stem cells are amenable to SPIO labeling, including MSC and NSC, and SPIO labeling has enabled noninvasive stem cell tracking of various cell types after transplantation.^[40] Overall, both paramagnetic and super-paramagnetic contrast agents have been used extensively in preclinical or clinical applications.^[18, 30, 41]

2.3. Labeling of Cells

In order to effectively track cells with high contrast, cells are usually labeled with different contrast agents or tracers to facilitate the tracking. The labeling relies on transferring of the contrast agent from the extracellular environment to the intracellular compartments. Labeling efficiency also depends on the cell lines considered and the chemical and physical properties of the nanoparticles. Labeling can be carried out by two approaches, direct labeling and indirect labeling.^[4] Similarly, labeling markers can also be divided into continuous markers and finite markers.^[10] In direct labeling, cells usually incorporate contrast agents when cultured *in vitro* before they are delivered into a living body. This approach does not require genetic modification of the cells and it is straightforward and

simple. However, contrast agents can be diluted when cells divide, and subsequently asymmetrically distributed to daughter cells or released by host cells.^[6, 7, 42] The magnetic field inhomogeneity generated by SPIO nanoparticles induces a negative contrast (signal loss) in MR images, which is different from a signal enhancement generated by nuclear medicine tracers such as ¹¹¹In-oxyquinoline.^[42] In addition, signals from surviving cells are not differentiated by the direct labeling technique from either dead cells or from macrophages that phagocytose dead cells. Many factors, including dilution of contrast agents, asymmetrical distribution, location, and different mechanism in tissue development all contribute to the limited time available for observation of directly labeled cells, e.g. from a few days to several weeks.^[5, 6, 43]

Alternatively, in indirect labeling, a reporter gene is usually introduced into a cell and then translated into either enzymes or receptors.^[4] For example, two genetic reporter systems were developed for multimodality reporter gene imaging (fluorescence, bioluminescence, and nuclear imaging techniques) of different molecular-genetic processes.^[44] The triple-fusion reporter gene was reported to preserve the functional activity of its subunits, and this was very effective for multi-modality imaging. Indirectly labeled cells can be tracked over a long time as long as the expression of the reporter gene is stable and not altered. Because the reporter gene can be passed on to daughter cells, it becomes possible to track growing cell population and differentiate viable cells from dead ones. However, sensitivity of imaging the indirectly labeled cells, particularly MRI-based indirect labeling, has not been studied in detail.^[4] To improve sensitivity, a triple-labeling strategy was suggested that likely has the potential for monitoring short-term cell homing with ¹¹¹In-oxyquinoline into infarcted myocardium of rats for monitoring long-term cell survival with reporter genes and for defining anatomy and assessing migration of the cells with magnetic particles.^[42]

In addition to ex vivo labeling, a recent study demonstrated that intravenous ferumoxytol can be used to label mesenchymal stem cells *in vivo* and can be used for tracking of stem cell transplants, eliminating risks of contamination and biologic alteration of cells associated with ex vivo-labeling procedures.^[45]

2.4 In Vivo Tracking of Cells

Tracking of stem cells *in vivo* has posed a big challenge as it has been hampered by the difficulty of identifying an appropriate imaging technique for obtaining cell dynamic information and cellular resolution days or weeks post transplantation or post delivery of stem cells. There have been different methods to conduct cell tracking, including optical, radionuclear, and magnetic resonance imaging.^[4] Optical methods can track cells in vivo with subcellular to cellular resolution close to the surface of tissue and with lower sensitivity in deeper tissues. The ability to temporally resolve biological processes with high resolution has uncovered the inflammatory homing cascade and advanced understanding of other regenerative processes.^[46] Approaches for single cell resolution imaging primarily use genetic modification of cells for fluorescent protein expression, or stains to label cells prior to administration. Isolation of a stem cell population from transgenic mice or genetic modification of cells are robust methods for fluorescence protein expression, and both have yielded interesting results that demonstrate a wide range of functional activity including the

integration of stem cells to regenerate the hematopoietic system, to heal bone defects, and to improve outcomes in stroke models.^[47] Fluorescent protein expression is a powerful tool for long term tracking of stem cell engraftment and behavior, but requires permanent modification of a stem cell population for analysis of non-mouse species. In addition, the most commonly used fluorescent proteins have excitation and emission in shorter wavelengths with limited tissue penetration and high autofluorescence, especially with single photon excitation. To overcome some of these limitations, use of advanced imaging strategies like 2-photon microscopy allows excitation of fluorescent proteins in the near-IR tissue window at 800nm-1000nm wavelength range, which reduces autofluorescence and has greater tissue penetration depth.^[48] Recently, this principle has been applied to the second tissue window at 1700 nm, where 3-photon excitation with long integration times extended imaging depth of red fluorescent protein to greater than 1mm in the intact mouse brain.^[49]

An alternative to fluorescent protein expression is staining of stem cells with fluorescent dyes. Some of the most widely used are a series of bright lipophilic membrane dyes that are available in green (DiO), red (DiI), far-red (DiD), and near-infrared (DiR) emission. These tracking dyes minimally alter stem cell homing and engraftment, and can longitudinally monitor cell location for 24–48 hours after transplantation for large populations of transplanted stem cells like MSCs.^[49, 50, 51] In rare cells like hematopoietic stem cells, tracking of a single cell has been performed up to 30 days after transplantation with DiD staining, and single cells dividing into clusters of 2–4 cells monitored by multiple imaging sessions on consecutive days.^[51, 52] The use of longer wavelength dyes like DiD and DiR that emit in the far red and near-IR reduces autofluorescence and improves imaging depth. However, optical detection remains largely limited to a few hundred microns below the tissue surface.^[4, 53]

To detect deeper events, optical and radionuclear techniques that do not allow single cell resolution are viable alternatives. By transfecting stem cells to carry a reporter gene for luciferase, a naturally occurring chemiluminescent protein found in fireflies and other organisms, bioluminescence can identify cell location throughout the body. Since there is no excitation source to cause background signal, long integration times enable detection of cells even in deep organs in mice. Bioluminescence is non-invasive and poses no ionizing radiation hazard and has been used for a variety of cell engraftment studies, such as tracking MSC distribution and engraftment after transplantation and evaluating hematopoietic stem cell bone marrow reconstitution.^[54] In addition, the survival of therapeutic stem cells has been investigated with a bioluminescence approach after local transplantation for tissues such as bone, heart, and muscle.^[55] However, it is not quantitative and a large number of cells are required for detection in deep tissues. Radionuclear methods, including single-photon emission computed tomography (SPECT) and positron emission tomography (PET), are extremely sensitive and can detect pico-molar (or even lower) concentrations of radiolabels.^[4, 56] Their high sensitivity makes it possible to use a very small amount of probes that do not stimulate biological reactions, yet these methods expose the patient to ionizing radiation and cannot be used longitudinally.

Compared with the optical and radio-nuclear methods, MRI has become a well-studied and accepted technique for tracking stem cells. Among different clinical imaging modalities such

as computed tomography (CT) or ultrasound, MRI offers numerous advantages such as high spatial resolution (near-cellular or tens of microns), non-ionizing radiation, and whole-body scanning capability. Therefore, MRI is amongst today's most used imaging modalities for cellular labeling and tracking. MRI based cell tracking with SPIO nanoparticles is capable of tracking a few hundred cells, varying by magnetic field strength. An *in vivo* detection of about five hundred cells implanted in a rat brain was demonstrated on a 7 T scanner.^[57] A detection limit of approximate 500–600 cells was reported on a 4.7 T scanner.^[58] Feasibility was also demonstrated to detect a small number of cells or even single cells. SPIO-labeled sensitized T-cells were injected into tumor antigens, and as few as 3 cells were detected migrating into the tumor.^[59] Single mammalian cells labeled with SPIO nanoparticles in a cell phantom were imaged on a 1.5 T scanner.^[60] Resolution of cell numbers detected in the above studies may be attributed to many factors, including cell type, rate and level SPIO nanoparticles uptake in cells, MR system, and potentially other factors yet to be determined.

While the amount and type of contrast agents that cells uptake usually determine the sensitivity of MRI-based cell-tracking methods, MR acquisition approaches, i.e. pulse sequences, also play important roles. Owing to the much shortened T_2 (or T_2^*) effect, SPIO nanoparticles generate hypointense or 'negative' contrast on traditional spin-echo and gradient-echo MR images. To mitigate these negative effects, various approaches have been proposed to generate 'positive' contrast to improve effective detections of SPIO nanoparticles and other materials with extremely short T_2 in various applications. These techniques can be divided into two groups. The first group of techniques makes use of modified pulse sequences to directly or indirectly produce "positive" contrast, such as the white marker technique^[61], GRASP^[62], spectrally selective RF pulses to excite water off-resonance^[63] or to saturate water on-resonance^[64], adiabatic pulse preparation^[65], ultra-short echo time imaging^[66], sweep imaging with Fourier Transform^[67, 68], off-resonance saturation^[69], and zero echo time imaging^[70]. The second group of techniques employs post-processing methods to generate "positive" contrast from data acquired from regular gradient echo sequences. An early study showed that susceptibility-induced gradients result in an echo-shift in k-space.^[71] Another study showed that the signal loss resulting from susceptibility along the slice selection direction could be reduced by acquiring a 3-D image.^[72] More recently, susceptibility gradient mapping and phase gradient mapping were proposed to calculate and visualize susceptibility gradients and phase gradients in a parametric map directly from regular gradient-echo images.^[73, 74]

2.5. MRI based Quantification of SPIO Nanoparticles and Labeled Cells

Quantification of SPIO nanoparticle cellular uptake is important because it provides a way to estimate the number of magnetically labeled cells that are being tracked, and potentially offer an effective approach to monitor and optimize cellular therapies. MRI based quantification of SPIO nanoparticles can generally be divided into two categories, relaxometry methods and model-based methods. The relaxometry methods use multiple complex-valued MR images (magnitude information only) and quantify the concentration of SPIO nanoparticles by mapping the relaxation rate of R_2 . A linear relationship between R_2 and iron content was obtained from marmosets with hemosiderosis.^[75] A linear correlation was also observed between R_2 and the number of labeled cells for several cell types.^[76] For

higher iron concentration, a modified algorithm was used to identify on-resonance acquisitions. Homogeneous R_2 maps and linear R_2 calibration curves were created up to 100 $\mu\text{g mL}^{-1}$ of iron concentration, despite substantial B_0 inhomogeneity.^[77]

The effective transverse relaxation rate, denoted $R_2^*(=1/T_2^*)$ of SPIO labeled cells satisfies the static dephasing regime theory.^[78, 79] Since the value of R_2 for SPIO nanoparticles is approximately two orders smaller than R_2^* ^[79], most relaxometry methods attempt to measure R_2^* over R_2 .^[76, 80] A general assumption is that R_2^* varies linearly with SPIO concentration, described as $R_2^*=R_{20}^*+r_2^*c$, where R_{20}^* denotes the intrinsic relaxation rate (e.g. no presence of SPIO), r_2^* denotes the relaxivity of the contrast agent, and c denotes the SPIO concentration. In addition to R_2 , some relaxometry methods can also quantify the SPIO concentration by measuring R_1 .^[81] Recent advances in the relaxometry-based methods have utilized multiple acquisition pulse sequences that reduce the total number of scans.^[82] Another study used TurboSPI, a multi-echo single point imaging sequence, for the quantification of labeled cells containing moderate to high concentrations (20–120 $\mu\text{g}\cdot\text{mL}^{-1}$) of SPIO nanoparticles.^[83] A variable flip angle sweep imaging with Fourier Transform was also used for quantification of high concentration (1–7 mM Fe) of SPIO nanoparticles.^[68]

However, accuracy of relaxometry based quantification of labeled cells, particularly for in vivo applications, remains to be investigated and verified, especially for longitudinal studies.^[9] First, R_2^* or R_2 relaxation rates are affected by not only SPIO nanoparticles in labeled cells but also by macroscopic susceptibility differences, e.g. air-tissue interfaces, leading to overestimation of the relaxation rates or obscuring low concentration labeled cells. Second, existence of free or extracellular non-compartmentalized SPIO nanoparticles may decrease the quantification accuracy. It is very difficult to differentiate extracellular iron in the microenvironment from the labeled cells. Free iron was found at transplanted sites where hemorrhage and labeled cells are present.^[76] Cell death, cell migration, and cell division may further complicate the quantification process. Various intravoxel SPIO-labeled cells, SPIO nanoparticles, and micron-sized iron oxide particle distributions were studied by mimicking biological processes such as compartmentalization, migration, division, and cell death, and it was found that relaxometry does not allow labeled cell quantification when multiple physiological processes (e.g., cell division and migration) coexist.^[84]

Model-based quantification methods depend on analytic models of simple geometries and estimate magnetic field inhomogeneities generated by the SPIO nanoparticles using phase information of complex MRI images. In earlier studies, the concentration of SPIO nanoparticles was quantified by modeling distributions of iron as spherical dipoles^[85, 86] or by modeling the magnetic field inhomogeneity as simple geometries such as a sphere^[87] or infinite cylinder^[88]. This is followed by fitting the magnetic field of the model to the magnetic field inhomogeneity acquired experimentally. One major limitation of the simple geometry-based quantification method is that it usually fails when a complex geometric shape is encountered.^[85] This limitation can be dealt with by applying different numerical techniques to take into account the complex geometry and then calculate the magnetic field inhomogeneity. These methods include finite element or finite difference methods,^[89] those

that utilize boundary conditions, [90] approximation methods, [91] and Fourier-based methods. [91, 92]

Different from regular magnitude images, phase images of complex MRI data carry critical information regarding magnetic field inhomogeneity. Therefore, they can be employed to quantify SPIO nanoparticles to deal with a complex geometrical case. A phase map cross-correlation Detection and Quantification (PDQ) method was proposed that identifies localized deposits of SPIO that are then used to detect and characterize SPIO-labeled magnetocapsules implanted in porcine liver. [93] Another computational approach, referred to as phase gradient mapping (PGM) [74], can bypass the phase unwrapping step and has found many applications in the quantification process. Modified finite perturber methods have been used to model the magnetic field for complex geometries. [91, 94] Quantification of SPIO nanoparticles was carried out in two steps: first, calculating the phase gradient map of the acquired MR data; second, fitting the phase gradient map to the gradient generated by the theoretical model, a distribution of spherical dipoles occupying the geometry of the object under consideration. [94] Assume that the field map of a given image displays the z -component (parallel to B_0 , the main magnetic field in MRI) of magnetic field inhomogeneities, then its partial derivative, e.g. x -gradient is calculated as

$$\nabla_x(\Delta B(x, y, z)) = -\frac{\nabla_x \Psi(x, y, z)}{\gamma \cdot TE}$$
, where γ denotes the gyromagnetic ratio, and TE denotes the echo time when the phase map $\Psi(x, y, z)$ was taken. [95] The finite perturber method models each voxel as a spherical dipole. To calculate the partial derivative, for example the x -component of the magnetic field for the spherical dipole in Cartesian

coordinates, one can derive $\nabla_x B_{\text{voxel}}(x, y, z) = -xm\mu_0 R^3 \frac{4z^2 - x^2 - y^2}{(x^2 + y^2 + z^2)^{7/2}}$. [94] Following calculation of its partial derivative, the total magnetic field gradient is determined by a contribution from all involved voxels, i.e.,

$$\nabla B(x, y, z) = \sum_{m=1}^{N_x} \sum_{n=1}^{N_y} \sum_{p=1}^{N_z} \nabla(B_{\text{voxel}}(x-m, y-n, z-p)) \beta(m, n, p),$$

where N_x , N_y , and N_z denote the total number of voxels along the x , y , and z axes of the region, and $\beta(m, n, p)$ is a mask. The magnetization within the geometry is obtained by calculating the value that minimizes the squared error between the components of ∇B and $\nabla(B)$. [94] Figure 2 presents magnitude and phase images of a phantom consisting of three vials of Ferumoxides concentrations (labeled 1–3, $160 \mu\text{g ml}^{-1}$, $80 \mu\text{g ml}^{-1}$, and $40 \mu\text{g ml}^{-1}$) at high SNR and low SNR environments and a cross section image of a mouse subcutaneously implanted in the flanks with 10^5 Ferumoxides-labeled and 10^6 unlabeled C6 glioma cells. The implanted tumor was labeled with a circle. Magnetic field inhomogeneities induced by various concentrations of SPIO nanoparticles or SPIO-labeled cells generated significant magnetic dipole effects and flowering artifacts were clearly seen in the phase images. [94]

Like any model-based quantification method, B_0 inhomogeneities (e.g. generated by air-tissue interfaces) and gradient instabilities may influence the quantification results. Though many efforts have been spent, it still remains difficult to completely correct and/or compensate for gradient instabilities. [94] An assumption that the distribution of SPIO nanoparticles is approximately homogeneous throughout the labeled-region does not

necessarily hold *in vivo*. For more complicated *in vivo* data sets, a method of incorporating inhomogeneous distributions of SPIOs needs to be addressed.^[94]

In addition to geometry- or finite perturber-based quantification methods, quantitative susceptibility mapping (QSM) methods have been studied in recent years, which allows one to estimate the susceptibility of biological tissues and quantification of SPIO contrast agents.^[96, 97] Similar to the finite perturber method, the QSM methods take advantage of the induced phase perturbation as well. However, a common problem of many proposed QSM methods is that they depend on original phase images, which are not continuous and have fringes, since phase can only evolve in the principle range between $[-\pi, \pi)$. One has to unwrap the raw phase images before any further data processing is done. But phase unwrapping algorithms, particularly for a three-dimensional phase image, are usually nontrivial and time-consuming. Instead, a phase gradient based QSM method was used to estimate quantification of SPIO nanoparticles.^[97] In this approach spatial gradients of the phase images were demonstrated to be continuous, smooth, and without any fringe. Compared with the original phase image, the detection specificity of the accumulated SPIO nanoparticles was further improved by using the phase gradient images, as the calculation of the spatial gradients functions as a high-pass filter that can enhance the image contrast of the region where significant phase changes happen.^[74] A spherical mean value (SMV) theorem was used to reduce the phase gradients induced by the external sources. The results showed that the concentration of SPIO nanoparticles can be determined in both phantom and *in vivo* experiments using the MR phase gradients approach.^[97]

3. Labeling and Tracking of Human Stem Cells within the Chicken Embryo

3.1. Human Stem Cells

Human pluripotent stem cells (hPSC) are either induced pluripotent stem cells (iPSC) derived from somatic cell reprogramming or hESC derived from inner cell mass cells of preimplantation embryos.^[98] hPSC are a potential source of neural cell lineages for research and human cell therapies.^[99] hPSCs can proliferate continuously in culture and remain pluripotent, having the ability to give rise to most, if not all, cell types.

Current research indicates that directed differentiation of hPSCs recapitulates many of the same differentiation pathways in human development.^[100] hPSC differentiate into hNSC or human neural progenitors (hNP) cells that give rise to a range of cell types of neural subtypes (Figure 3). For example, the hNP cells used in our SPIO labeling studies described below can be induced to differentiate *in vitro* into neural restricted progenitors and to glial cell restricted progenitors.^[101] Our hNP cells are non-immortalized, proliferative adherent monolayers (>80 passages) circumventing 3-dimensional variability by completely eliminating the typical embryoid body or neurosphere stages of derivation.^[101, 102] As adherent monolayers, our hNP cells allow for uniform imaging, genetic manipulation, and exposure to growth factors and/or morphogens for repeatable and reliable results.^[103–105] These hNP cells are consistent, NESTIN and SOX2 positive, and devoid of OCT4 positive populations across multiple derivations, while maintaining the potential to readily differentiate into multiple neural phenotypes with proven utility in compound screening (Figure 4 and Figure 5).^[102, 104, 106]

Successful clinical use of hPSC therapies is likely to require that most, if not all, neural cells of interest be differentiated beyond the hPSC stage since residual hPSCs could produce teratoma tumors if transplanted into the patient.^[107] This is a serious safety issue for hPSC based therapies. Since our hPSC derived lines of hNP cells are devoid of pluripotent cells and recent successful animal models of stroke indicate a benefit, we, as well as others, are motivated to conduct further translational research.^[102, 108] Questions concerning the best dose, timing, and method of NP cell delivery for treatment must be addressed. Once hNP cells are transplanted into a patient their distribution and therapeutic efficacy must be assessed. In preclinical settings, tracking hNP cell distribution by histological confirmation requires sacrificing animals at multiple time points and/or biopsies, and thus is not well optimized for stem cell tracking, which ideally would be performed longitudinally and non-invasively in animals and humans. Therefore, the application of non-invasive imaging methods in evaluating hNP cell therapies will advance preclinical and clinical safety and efficacy studies.^[109]

Recently we were able to successfully create a stable population of GFP expressing human neural progenitor (GFP⁺ hNP) cells that exhibits proliferation, differentiation, and morphological properties indistinguishable from unmodified parental cells (Figure 6). These labeled cells were generated by random transposition of GFP-expressing *piggyBac*TM transposons into the genome. Importantly, the transduced cells could be propagated for at least 24 passages (equivalent to expansion to 10⁹ cells) without loss of GFP expression or developmental capacity in contrast to cells transduced with plasmid- or virus-based methods.^[110] We observed, by flow analysis, that approximately 96% of the total population of differentiated neural cells expressed MAP2, a major microtubule associated protein in brain tissue, and moreover, over 92% of the MAP2-positive cells were also GFP-positive. This data indicates that we can produce nearly pure differentiated cultures of GFP⁺ neurons. Thus, selection required for genetic modification of hNP cells does not impact the expansion and developmental potential of the engineered cells.

3.2. Cell Labeling

While optical imaging of GFP⁺ hNP cells allows for longitudinal in vitro and limited in vivo studies they are not ideally suited for our ultimate goal of noninvasively tracking transplanted cells in all areas of the central nervous system (CNS) and other tissues for safety and efficacy regenerative cell therapy studies. To this end, we sought to establish a reliable method of tracking hNP cells in vivo with non-invasive MRI based cell-tracking methods. The goal of stem cell tracking in a clinical context is to ensure the transplanted cells do not harm the host after transplantation (e.g. teratoma formation). Of equal importance is the fact that the method of cell tracking must be biocompatible with the cells. In other words, the cell tracking modality must not compromise the integrity of the transplanted stem cells, and therefore, diminish their therapeutic efficacy.

Optimal use of the SPIO contrast agent requires adequate labeling of the cells for cell tracking, and minimal uptake to limit perturbation of cells and to ensure the biocompatibility of the contrast agent. To present a model for assessing the biocompatibility of contrast agents, we developed a method of labeling GFP⁺ hNP cells for in vivo tracking with Molday

Ion Rhodamine B (MIRB) SPIO that allows for multimodal stem cell tracking with fluorescent microscopy and MRI. Together, we believe that in vivo tracking of GFP⁺ and MIRB labeled (MIRB⁺) hNP cells will provide an excellent starting point for pre-clinical research evaluating hPSC derived stem cell treatments.

MIRB readily labels stem cells in their growth medium without the use of transfection reagents and generates robust T_2 contrast upon in vitro MRI analysis.^[111, 112] Here, GFP⁺ hNP cells are labeled in a proliferative monolayer with 20 $\mu\text{g}\cdot\text{ml}^{-1}$ MIRB for 48 hours. After labeling and upon removal of growth factors from the growth medium hNP cells cease proliferation and differentiate into neurons. These differentiating GFP⁺ hNP cultures retained the MIRB label after 14 days of in vitro (DIV) differentiation (Figure 7 A–D). Our labeling method presents an improvement over other documented methods of neural cell labeling with SPIO contrast agents.^[113] Our protocol of labeling cells in a monolayer both provides better uniformity and a faster time to maximal labeling than neurospheres, which is desired for clinical translation.^[113] The viability of the GFP⁺ hNP cells labeled with MIRB was not compromised immediately after labeling (0 DIV) or when subjected to 14 days of in vitro neural differentiation (7 & 14 DIV). Fluorescent microscopy and inductively coupled plasma mass spectroscopy demonstrated that GFP⁺ hNP retain the MIRB label despite the proliferative nature of the hNP cells. The population of GFP⁺ hNP cells increased greater than four-fold over the 14 DIV differentiation, suggesting that the GFP⁺ hNP proliferation slowed considerably and SPIO contrast agent was passed onto all GFP⁺ hNP daughter cells and differentiated cells during the 14 DIV (Figure 7 E–H). This conclusion is contrary to traditional thinking of SPIO labeling of proliferative cells, but this data suggest that the MIRB label is retained over 14 DIV. We postulate that the elevated retention of MIRB is due to enhanced biocompatibility of the dextran surface coating and reduced exocytosis of the nanoparticle.

Next, the effect of MIRB labeling hNP cells on differentiation towards a mature phenotype was evaluated. If in vitro differentiation of labeled hNP cells is impaired, then the therapeutic and regenerative properties of hNP cells would likely be compromised when transplanted in vivo. First, we evaluated the expression of known hNP cell specific proteins.^[101–106, 114] SOX1 and NESTIN were evaluated with immunocytochemistry immediately after labeling and over a 14 DIV differentiation. hNP cell specific protein was not observed in a majority of the differentiating cells (Figure 8 A–G). This observation is in agreement with differentiation of labeled hNP cells derived from various sources.^[38, 101–104, 113, 114] To determine if these differentiated hNP cell cultures gain a neural phenotype we again used immunocytochemistry to evaluate the expression of protein exclusively expressed in neuronal cells: MAP2 and HuC/D. These protein markers increased in a spatially and temporally equal fashion in both MIRB⁺ and unlabeled (MIRB⁻) GFP⁺ hNP cells (Figure 8 H–N). Collectively, the results demonstrate the anticipated transition of the MIRB loaded hNP cell to maturing neural cells similar to results previously obtained in our lab.^[101–106, 114] This evidence demonstrates that MIRB loading does not impair the in vitro neural differentiation process of hNP cells, indicating that in vivo studies employing MIRB⁺ hNP cells are equally likely to be effective when compared to unlabeled hNP cells.

Verification of viability and pluripotency represent the classical criteria for assessing the effects of SPIO labeling.^[111, 113, 115] However, these endpoints do not assess acute damage to neural cellular processes leading to impaired function and diminished therapeutic benefits of hPSC derived cells therapies. Appropriate neurite extension and branching during in vitro differentiation is related to functional neural cell activity and critical determinants of neuronal connectivity.^[116] Moreover, minor changes in neurite structure can be measured and related to altered neural maturation.^[106] Therefore, evaluating neural morphology presents a finer measure of GFP⁺ hNP cell neural differentiation and an established method of measuring neural toxicity. Consequently, we evaluated both MIRB⁺ and MIRB⁻ GFP⁺ hNP cells using a neurite outgrowth assay.^[106, 116] After thorough investigation, no significant alterations in neurite outgrowth or branching were found between unlabeled and labeled populations of GFP⁺ hNP cells (Figure 9). MIRB labeling did not alter the neural maturation of GFP⁺ hNP cells. Our approach to assessing biocompatibility using neural cell functional assays suggests that MIRB labeling does not alter the innate biology of hPSC derived hNP cells.

3.3. Cell Tracking by MRI

As discussed in Section 2.3, success of clinical trials involving stem cell therapies will hinge upon reliable cell tracking to access dose and efficacy of the treatment.^[109] To demonstrate in vivo tracking, we transplanted 2×10^5 MIRB⁺ GFP⁺ hNP cells (<10 pg of iron per cell by inductively coupled plasma mass spectrometry measurement) into the Hamburger-Hamilton stage 15 chicken embryo.^[117] All MRI experiments were carried out on a 7 T Varian Magnex small animal scanner with a maximum gradient strength of 440 mT m⁻¹. After transplantation, the chicken embryos were scanned with MRI at multiple time points (Embryonic Day 3, 6, and 10 of chick development). The resulting T₂ and T₂^{*}-weighted anatomy image acquired on Day 3 clearly depicted hypointense regions indicative of typical SPIO T₂ contrast agents (**Figure 12 G–H**). The anatomical location of the MIRB⁺ GFP⁺ hNP was confirmed with florescent microscopy (Figure 10 A–F). T₂-weighted and T₂^{*}-weighted anatomical images acquired on Day 6 and Day 10 are presented in Figure 11, together with T₂^{*}-weighted phase images. SPIO nanoparticles carried by labeled cells exhibited strong magnetic dipole effects (flowering artifact), shown in both anatomical and phase images (Figure 10 G–I). This successful in vivo tracking of transplanted MIRB⁺ GFP⁺ hNP cells demonstrates that hPSC derived hNP cells can be tracked with clear anatomical resolution. We expect that GFP⁺ hNP cell tracking with MIRB is possible over a time span of several weeks to possibly months since this was demonstrated using MIRB in other NSC lines.^[118] All together our in vitro evaluation and in vivo tracking of MIRB⁺ GFP⁺ hNP cells indicates that this method of cell tracking provided a reliable means of tracking hPSC derived progenitor cells during pre-clinical studies.

4. Discussion and Conclusions

Accurate quantification of SPIO labeled cells remains a significant challenge. It should be noted that quantification of labeled cells by MRI is an indirect approach, because MR signal changes are most often due to the concentrations of SPIO nanoparticles, but not the number of cells present. As labeled stem or progenitor cells proliferate, SPIO nanoparticles are most

often asymmetrically divided among daughter cells, the total amount of iron measurable, and the MR signal from each cell may decrease.^[119] Additionally, the SPIO nanoparticles from cells undergoing apoptosis or cell lysis are usually internalized by macrophages present in the host tissues, resulting in MR signals that falsely attribute to transplanted cells.^[120]

This paper focused on tracking and quantification of SPIO labeled neural stem cells using MRI. MR imaging technologies have been, and will continue to be, developed to improve its sensitivity and specificity in cell tracking and quantification. As stem cell based therapy progresses, qualitative assessment of presence or extent of stem cell migration is inadequate and cannot meet the requirement of preclinical research or clinical practice. Quantitative analysis using either the relaxometry-based or model-based approach is much preferred to reveal presence of SPIO labeled cells that are usually sparsely mixed with host cells throughout the host tissues. To further increase sensitivity and specificity of MRI, improved SPIO labeling efficiency, MR spatial resolution, stability of MRI system including gradient, eddy currents, B_0 drift, B_1 inhomogeneity will be required. Also it should be noted that labeling cells with a SPIO contrast agent cannot be employed to interrogate viability of transplanted cells, their function or ability to differentiate into desired phenotypes. On the other hand, it is important to be cognizant of effects that both indirect and direct labeling modalities may have on stem cell behavior and fate. Therefore, functional validation of labeled cells using developmental assays such as the chick embryo model presented in this work or reconstitution assays such as has been done for labeled hematopoietic cells provide critical endpoints.^[121] Current efforts by a number of groups are working to integrate this type of functional readout to both direct and indirect labeling approaches, but more work remains to combine simultaneous stem cell activity and tracking. To determine the functional status of transplanted cells and their subsequent therapeutic effect, a multimodality imaging approach with both direct and indirect labeling may turn out to be the most useful. For preclinical applications in small animal models, integrating MRI with fluorescent, bioluminescent, 2- or 3- photon microscopy imaging might be helpful. For human whole-body scans, MRI and nuclear imaging (e.g. PET) may play a central role. Many clinical studies of in vivo MRI cell tracking have been reported.^[7] Because MRI and PET are complementary, integration of the two technologies has been implemented.^[122]

The potential of cell therapies for degenerative diseases and injuries to the central nervous system is vast. Currently several stem cell clinical trials are underway for neural injury and disease, ranging from spinal cord injury to stroke, and up to date information can be found in a searchable database at www.clinicalTrials.gov, a National Institute of Health (USA) service. Numerous pre-clinical studies indicate that hPSC derived stem and progenitor cell treatments will provide a basis for therapies in the future and several hPSC derived cell therapies are in or nearing clinical trials.^[123] However, the challenges of assessing the preclinical safety and efficacy of these treatments still remains and progression to clinical trials is often slowed by a lack of robust preclinical data that address the engraftment and what these cells ultimately differentiate into if they do engraft. Here we presented potential methods for in vivo tracking of stem cells transplanted for therapeutic benefits. Using hNP cells we show how to develop biocompatible MR labeling techniques in an optically traceable GFP cell that will not compromise the integrity of the stem cells. Combining in vivo optical and MR imaging could provide more reliable preclinical safety and efficacy

data. Overall, the field of hPSC therapies will continue to grow, but its success depends on methods of in vivo cell tracking with non-invasive methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH. *Nat Med*. 2000; 4:1313. McKay R. *Nature*. 2000; 406:361. [PubMed: 10935622]
2. Kim N, Cho SG. *Int J Stem Cells*. 2015; 8:54. [PubMed: 26019755] Shi H, Qi X, Ma B, Cao Y, Wang L, Sun L, Niu H. *Chinese Journal of Cancer Research*. 2015; 27:128. [PubMed: 25937774]
3. Bengel F, Schachinger V, Dimmeler S. *European Journal of Nuclear Medicine and Molecular Imaging*. 2005; 32:S404. [PubMed: 16205898] Shah K, Weissleder R. *NeuroRx*. 2005; 2:215. [PubMed: 15897946]
4. Kircher MF, Gambhir SS, Grimm J. *Nat Rev Clin Oncol*. 2011; 8:677. [PubMed: 21946842]
5. Hachani R, Lowdell M, Birchall M, Thanh NT. *Nanoscale*. 2013; 5:11362. [PubMed: 24108444]
6. Bulte JW, Duncan ID, Frank JA. *J Cereb Blood Flow Metab*. 2002; 22:899. [PubMed: 12172375]
7. Bulte JW. *AJR Am J Roentgenol*. 2009; 193:314. [PubMed: 19620426]
8. Srivastava AK, Kadayakkara DK, Bar-Shir A, Gilad AA, McMahon MT, Bulte JW. *Dis Model Mech*. 2015; 8:323. [PubMed: 26035841] Cromer Berman SM, Walczak P, Bulte JWM. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*. 2011; 3:343. [PubMed: 21472999] Rogers WJ, Meyer CH, Kramer CM. *Nat Clin Pract Cardiovasc Med*. 2006; 3:554. [PubMed: 16990841] Ahrens ET, Bulte JW. *Nat Rev Immunol*. 2013; 13:755. [PubMed: 24013185] Korchinski DJ, Taha M, Yang R, Nathoo N, Dunn JF. *Magn Reson Insights*. 2015; 8:15. [PubMed: 26483609]
9. Liu W, Frank JA. *Eur J Radiol*. 2009; 70:258. [PubMed: 18995978]
10. Edmundson M, Thanh NT, Song B. *Theranostics*. 2013; 3:573. [PubMed: 23946823]
11. Lauterbur PC. *Nature*. 1973; 242:190. Damadian R, Goldsmith M, Minkoff L. *Physiological Chemistry and Physics*. 1977; 9:97. [PubMed: 909957]
12. Ahrens ET, Zhong J. *NMR Biomed*. 2013; 26:860. [PubMed: 23606473] Srinivas M, Boehm-Sturm P, Figdor CG, de Vries IJ, Hoehn M. *Biomaterials*. 2012; 33:8830. [PubMed: 22959182]
13. Klippel S, Freund C, Schroder L. *Nano Lett*. 2014; 14:5721. [PubMed: 25247378]
14. Wilson DM, Kurhanewicz J. *J Nucl Med*. 2014; 55:1567. [PubMed: 25168625]
15. Duinhouwer LE, van Rossum BJ, van Tiel ST, van der Werf RM, Doeswijk GN, Haeck JC, Rombouts EW, Ter Borg MN, Kotek G, Braakman E, Cornelissen JJ, Bernsen MR. *PLoS One*. 2015; 10:e0138572. [PubMed: 26394043]
16. Gaudet JM, Ribot EJ, Chen Y, Gilbert KM, Foster PJ. *PLoS One*. 2015; 10:e0118544. [PubMed: 25767871]
17. Boehm-Sturm P, Mengler L, Wecker S, Hoehn M, Kallur T. *PLoS One*. 2011; 6:e29040. [PubMed: 22216163]
18. Caravan P, Ellison JJ, McMurry TJ, Lauffer RB. *Chem Rev*. 1999; 99:2293. [PubMed: 11749483]
19. Hedlund A, Ahren M, Gustafsson H, Abrikosova N, Warntjes M, Jonsson JI, Uvdal K, Engstrom M. *Int J Nanomedicine*. 2011; 6:3233. [PubMed: 22228991]

20. Agudelo CA, Tachibana Y, Hurtado AF, Ose T, Iida H, Yamaoka T. *Biomaterials*. 2012; 33:2439. [PubMed: 22206594]
21. Kim KS, Park W, Na K. *Biomaterials*. 2015; 36:90. [PubMed: 25301637]
22. Shuai H-L, Yan R-L, Song H, Chen D-L, Luo X. *Magnetic Resonance Imaging*. 2014; 32:934. [PubMed: 24794127]
23. Rudelius M, Daldrup-Link HE, Heinzmann U, Piontek G, Settles M, Link TM, Schlegel J. *Eur J Nucl Med Mol Imaging*. 2003; 30:1038. [PubMed: 12567250]
24. Bhorade R, Weissleder R, Nakakoshi T, Moore A, Tung CH. *Bioconjug Chem*. 2000; 11:301. [PubMed: 10821645]
25. Thomsen HS. *Eur Radiol*. 2007; 16:2619. Richmond H, Zwerner J, Kim Y, Fiorentino D. *Arch Dermatol*. 2007; 143:1025. [PubMed: 17709661]
26. Silva AC, Bock NA. *Schizophr Bull*. 2008; 34:595. [PubMed: 18550591]
27. Gilad AA, Walczak P, McMahon MT, Na HB, Lee JH, An K, Hyeon T, van Zijl PC, Bulte JW. *Magn Reson Med*. 2008; 60:1. [PubMed: 18581402]
28. Kim T, Momin E, Choi J, Yuan K, Zaidi H, Kim J, Park M, Lee N, McMahon MT, Quinones-Hinojosa A, Bulte JW, Hyeon T, Gilad AA. *J Am Chem Soc*. 2011; 133:2955. [PubMed: 21314118]
29. Kresse M, Wagner S, Pfefferer D, Lawaczek R, Elste V, Semmler W. *Magn Reson Med*. 1998; 40:236. [PubMed: 9702705] Bentzon MD, Vanwonderghem J, Morup S, Tholen A, Koch CJW. *Philos Mag B*. 1989; 60:169.
30. Sun S, Zeng H, Robinson DB, Raoux S, Rice PM, Wang SX, Li G. *J Am Chem Soc*. 2004; 126:273. [PubMed: 14709092]
31. Shen T, Weissleder R, Papisov M, Bogdanov A Jr, Brady TJ. *Magn Reson Med*. 1993; 29:599. [PubMed: 8505895]
32. Jung CW. *Magn Reson Imaging*. 1995; 13:675. [PubMed: 8569442]
33. Shapiro EM, Skrtic S, Sharer K, Hill JM, Dunbar CE, Koretsky AP. *Proc Natl Acad Sci U S A*. 2004; 101:10901. [PubMed: 15256592]
34. Lieu PT, Heiskala M, Peterson PA, Yang Y. *Mol Aspects Med*. 2001; 22:1. [PubMed: 11207374]
35. Josephson L, Lewis J, Jacobs P, Hahn PF, Stark DD. *Magn Reson Imaging*. 1988; 6:647. [PubMed: 2850434] Stark DD, Weissleder R, Elizondo G, Hahn PF, Saini S, Todd LE, Wittenberg J, Ferrucci JT. *Radiology*. 1988; 168:297. [PubMed: 3393649] Weissleder R, Hahn PF, Stark DD, Elizondo G, Saini S, Todd LE, Wittenberg J, Ferrucci JT. *Radiology*. 1988; 169:399. [PubMed: 3174987] Levy M, Luciani N, Alloeyau D, Elgrabli D, Deveaux V, Pechoux C, Chat S, Wang G, Vats N, Gendron F, Factor C, Lotersztajn S, Luciani A, Wilhelm C, Gazeau F. *Biomaterials*. 2011; 32:3988. [PubMed: 21392823]
36. Castaneda RT, Boddington S, Henning TD, Wendland M, Mandrussow L, Liu S, Daldrup-Link H. *Pediatr Radiol*. 2011; 41:1384. [PubMed: 21594541]
37. Song M, Moon WK, Kim Y, Lim D, Song IC, Yoon BW. *Korean J Radiol*. 2007; 8:365. [PubMed: 17923778]
38. Daadi MM, Hu S, Klausner J, Li Z, Sofilos M, Sun G, Wu JC, Steinberg GK. *Cell Transplant*. 2013; 22:881. [PubMed: 23044338]
39. Hinds KA, Hill JM, Shapiro EM, Laukkanen MO, Silva AC, Combs CA, Varney TR, Balaban RS, Koretsky AP, Dunbar CE. *Blood*. 2003; 102:867. [PubMed: 12676779]
40. Chang DJ, Oh SH, Lee N, Choi C, Jeon I, Kim HS, Shin DA, Lee SE, Kim D, Song J. *Exp Mol Med*. 2013; 45:e53. [PubMed: 24232252] Hu SL, Lu PG, Zhang LJ, Li F, Chen Z, Wu N, Meng H, Lin JK, Feng H. *J Cell Biochem*. 2012; 113:1005. [PubMed: 22065605]
41. Tan M, Lu ZR. *Theranostics*. 2011; 1:83. [PubMed: 21547154]
42. Zhou R, Thomas DH, Qiao H, Bal HS, Choi SR, Alavi A, Ferrari VA, Kung HF, Acton PD. *J Nucl Med*. 2005; 46:816. [PubMed: 15872356]
43. de Vries IJ, Lesterhuis WJ, Barentsz JO, Verdijk P, van Krieken JH, Boerman OC, Oyen WJ, Bonenkamp JJ, Boezeman JB, Adema GJ, Bulte JW, Scheenen TW, Punt CJ, Heerschap A, Figdor CG. *Nat Biotechnol*. 2005; 23:1407. [PubMed: 16258544] Himes N, Min JY, Lee R, Brown C,

- Shea J, Huang X, Xiao YF, Morgan JP, Burstein D, Oettgen P. *Magn Reson Med*. 2004; 52:1214. [PubMed: 15508153]
44. Ponomarev V, Doubrovin M, Serganova I, Vider J, Shavrin A, Beresten T, Ivanova A, Ageyeva L, Tourkova V, Balatoni J, Bornmann W, Blasberg R, Gelovani Tjuvajev J. *Eur J Nucl Med Mol Imaging*. 2004; 31:740. [PubMed: 15014901]
 45. Khurana A, Chapelin F, Beck G, Lenkov OD, Donig J, Nejadnik H, Messing S, Derugin N, Chan RC-F, Gaur A, Sennino B, McDonald DM, Kempen PJ, Tikhomirov GA, Rao J, Daldrup-Link HE. *Radiology*. 2013; 269:186. [PubMed: 23850832]
 46. von Andrian UH, Chambers JD, McEvoy LM, Bargatzke RF, Arfors KE, Butcher EC. *Proc Natl Acad Sci U S A*. 2008; 88:7538. Schroeder T. *Nature*. 2008; 453:345. [PubMed: 18480816]
 47. Wagers AJ, Sherwood RI, Christensen JL, Weissman IL. *Science*. 2002; 297:2256. [PubMed: 12215650] Herberg S, Kondrikova G, Hussein KA, Periyasamy-Thandavan S, Johnson MH, Elsalanty ME, Shi X, Hamrick MW, Isales CM, Hill WD. *Tissue Eng Part A*. 2014; 20:3212. [PubMed: 24914464] Rosenblum S, Wang N, Smith TN, Pendharkar AV, Chua JY, Birk H, Guzman R. *Stroke*. 2012; 43:1624. [PubMed: 22535265]
 48. Denk W, Svoboda K. *Neuron*. 2003; 18:351. Zipfel WR, Williams RM, Webb WW. *Nat Biotechnol*. 2003; 21:1369. [PubMed: 14595365]
 49. Horton NG, Wang K, Kobat D, Clark CG, Wise FW, Schaffer CB, Xu C. *Nat Photonics*. 2013; 7:205.
 50. Horton NG, Wang K, Kobat D, Clark CG, Wise FW, Schaffer CB, Xu C. *Nature photonics*. 2013; 7:Mortensen LJ, Levy O, Phillips JP, Stratton T, Triana B, Ruiz JP, Gu F, Karp JM, Lin CP. *PLoS One*. 2013; 8:e78145. [PubMed: 24205131] Levy O, Zhao W, Mortensen LJ, Leblanc S, Tsang K, Fu M, Phillips JA, Sagar V, Anandakumaran P, Ngai J, Cui CH, Eimon P, Angel M, Lin CP, Yanik MF, Karp JM. *Blood*. 2013; 122:e23. [PubMed: 23980067]
 51. Lo Celso C, Fleming HE, Wu JW, Zhao CX, Miake-Lye S, Fujisaki J, Cote D, Rowe DW, Lin CP, Scadden DT. *Nature*. 2009; 457:92. [PubMed: 19052546]
 52. Fujisaki J, Wu J, Carlson AL, Silberstein L, Putheti P, Larocca R, Gao W, Saito TI, Lo Celso C, Tsuyuzaki H, Sato T, Cote D, Sykes M, Strom TB, Scadden DT, Lin CP. *Nature*. 2011; 474:216. [PubMed: 21654805] Lo Celso C, Lin CP, Scadden DT. *Nat Protoc*. 2011; 6:1. [PubMed: 21212779]
 53. Shah K, Weissleder R. *NeuroRx*. 2005; 2:215. [PubMed: 15897946] Ntziachristos V, Ripoll J, Wang LV, Weissleder R. *Nat Biotechnol*. 2005; 23:313. [PubMed: 15765087]
 54. Wang H, Cao F, De A, Cao Y, Contag C, Gambhir SS, Wu JC, Chen X. *Stem Cells*. 2009; 27:1548. [PubMed: 19544460] van der Bogt KE, Schrepfer S, Yu J, Sheikh AY, Hoyt G, Govaert JA, Velotta JB, Contag CH, Robbins RC, Wu JC. *Transplantation*. 2009; 87:642. [PubMed: 19295307] Cao YA, Wagers AJ, Beilhack A, Dusich J, Bachmann MH, Negrin RS, Weissman IL, Contag CH. *Proc Natl Acad Sci U S A*. 2004; 101:221. [PubMed: 14688412] Wang X, Rosol M, Ge S, Peterson D, McNamara G, Pollack H, Kohn DB, Nelson MD, Crooks GM. *Blood*. 2003; 102:3478. [PubMed: 12946998] Lin P, Correa D, Kean TJ, Awadallah A, Dennis JE, Caplan AI. *Mol Ther*. 2014; 22:160. [PubMed: 24067545]
 55. Cosgrove BD, Gilbert PM, Porpiglia E, Mourkioti F, Lee SP, Corbel SY, Llewellyn ME, Delp SL, Blau HM. *Nat Med*. 2014; 20:255. [PubMed: 24531378] akaku Y, Murai K, Ukai T, Ito S, Kokubo M, Satoh M, Kobayashi E, Yamato M, Okano T, Takeuchi M, Mochida J, Sato M. *Biomaterials*. 2014; 35:2199. [PubMed: 24360579] Lepperhof V, Polchynski O, Kruttwig K, Brüggemann C, Neef K, Drey F, Zheng Y, Ackermann JP, Choi Y-H, Wunderlich TF, Hoehn M, Hescheler J, Šari T. *PLoS ONE*. 2014; 9:e107363 EP. [PubMed: 25226590]
 56. Massoud TF, Gambhir SS. *Genes Dev*. 2003; 17:545. [PubMed: 12629038]
 57. Hoehn M, Kustermann E, Blunk J, Wiedermann D, Trapp T, Wecker S, Focking M, Arnold H, Hescheler J, Fleischmann BK, Schwindt W, Buhrle C. *Proc Natl Acad Sci U S A*. 2002; 99:16267. [PubMed: 12444255]
 58. Magnitsky S, Watson DJ, Walton RM, Pickup S, Bulte JW, Wolfe JH, Poptani H. *Neuroimage*. 2005; 26:744. [PubMed: 15955483]
 59. Kircher MF, Allport JR, Graves EE, Love V, Josephson L, Lichtman AH, Weissleder R. *Cancer Res*. 2003; 63:6838. [PubMed: 14583481]

60. Foster-Gareau P, Heyn C, Alejski A, Rutt BK. *Magn Reson Med*. 2003; 49:968. [PubMed: 12704781]
61. Seppenwoolde J-H, Viergever MA, Bakker CJG. *Magnetic Resonance in Medicine*. 1988; 50:784. Frahm J, Merboldt K-D, Hänicke W. *Magnetic Resonance in Medicine*. 1988; 6:474. [PubMed: 3380007]
62. Mani V, Briley-Saebo KC, Itskovich VV, Samber DD, Fayad ZA. *Magn Reson Med*. 2006; 55:126. [PubMed: 16342148]
63. Cunningham CH, Arai T, Yang PC, McConnell MV, Pauly JM, Conolly SM. *Magn Reson Med*. 2005; 53:999. [PubMed: 15844142]
64. Stuber M, Gilson WD, Schar M, Kedziorek DA, Hofmann LV, Shah S, Vonken EJ, Bulte JW, Kraitchman DL. *Magn Reson Med*. 2007; 58:1072. [PubMed: 17969120]
65. Harris SS, Mao H, Hu XP. *Magnetic Resonance in Medicine*. 2012; 67:1133. [PubMed: 22213366]
66. Robson MD, Gatehouse PD, Bydder M, Bydder GM. *J Comput Assist Tomogr*. 2003; 27:825. [PubMed: 14600447] Zhang L, Zhong X, Wang L, Chen H, Wang YA, Yeh J, Yang L, Mao H. *Journal of Magnetic Resonance Imaging*. 2011; 33:194. [PubMed: 21182139] Wang L, Zhong X, Qian W, Huang J, Cao Z, Yu Q, Lipowska M, Lin R, Wang A, Yang L, Mao H. *J Magn Reson Imaging*. 2014; 40:1071. [PubMed: 25485347] Tyler DJ, Robson MD, Henkelman RM, Young IR, Bydder GM. *Journal of Magnetic Resonance Imaging*. 2007; 25:279. [PubMed: 17260388]
67. Idiyatullin D, Corum C, Park JY, Garwood M. *J Magn Reson*. 2006; 181:342. [PubMed: 16782371]
68. Wang L, Corum CA, Idiyatullin D, Garwood M, Zhao Q. *Magn Reson Med*. 2013; 70:341. [PubMed: 23813886]
69. Zurkiya O, Hu X. *Magnetic Resonance in Medicine*. 2006; 56:726. [PubMed: 16941618]
70. Weiger M, Brunner DO, Dietrich BE, Müller CF, Pruessmann KP. *Magnetic Resonance in Medicine*. 2013; 70:328. [PubMed: 23776142]
71. Posse S. *Magn Reson Med*. 1992; 25:12. [PubMed: 1593945]
72. Reichenbach JR, Venkatesan R, Yablonskiy DA, Thompson MR, Lai S, Haacke EM. *J Magn Reson Imaging*. 1997; 7:266. [PubMed: 9090577]
73. Liu W, Dahnke H, Jordan E, Schaeffter T, Frank J. *NMR Biomed*. 2007; 21:242. Dahnke H, Liu W, Herzka D, Frank J, Schaeffter T. *Magn Reson Med*. 2008; 60:595. [PubMed: 18727097]
74. Zhao Q, Langley J, Lee S, Liu W. *NMR in Biomedicine*. 2011; 24:464. [PubMed: 20931569] Bakker CJ, de Leeuw H, Vincken KL, Vonken EJ, Hendrikse J. *Phys Med Biol*. 2008; 53:N349. [PubMed: 18723928]
75. Bulte JW, Miller GF, Vymazal J, Brooks RA, Frank JA. *Magn Reson Med*. 1997; 37:530. [PubMed: 9094074]
76. Rad AM, Arbab AS, Iskander AS, Jiang Q, Soltanian-Zadeh H. *J Magn Reson Imaging*. 2007; 26:366. [PubMed: 17623892]
77. Pelot NA, Bowen CV. *Magn Reson Imaging*. 2013; 31:953. [PubMed: 23601361]
78. Yablonskiy DA, Haacke EM. *Magn Reson Med*. 1994; 32:749. [PubMed: 7869897]
79. Bowen CV, Zhang X, Saab G, gareau PJ, Rutt BK. *Magn Reson Med*. 2002; 48:52. [PubMed: 12111931]
80. Kuhlper R, Dahnke H, Matuszewski L, Persigehl T, von Wallbrunn A, Allkemper T, Heindel WL, Schaeffter T, Bremer C. *Radiology*. 2007; 245:449. [PubMed: 17848680]
81. Billotey C, Wilhelm C, Devaud M, Bacri JC, Bittoun J, Gazeau F. *Magn Reson Med*. 2003; 49:646. [PubMed: 12652535]
82. Liu, Wei, Dahnke, Hannes, Rahmer, Juergen, Jordan, E. Kay, Frank, JA. *Magnetic Resonance in Medicine*. 2009; 61:761. [PubMed: 19191285]
83. Rioux JA, Brewer KD, Beyea SD, Bowen CV. *J Magn Reson*. 2012; 216:152. [PubMed: 22364896]
84. Kotek G, van Tiel ST, Wielopolski PA, Houston GC, Krestin GP, Bernsen MR. *Contrast Media Mol Imaging*. 2012; 7:195. [PubMed: 22434632]
85. McAuley G, Schrag M, Sipos P, Sun SW, Obenaus A, Neelavalli J, Haacke EM, Holshouser B, Madacs R, Kirsch W. *Magn Reson Med*. 2010; 63:106. [PubMed: 19953510]

86. Dixon WT, Blezek DJ, Lowery LA, Meyer DE, Kulkarni AM, Bales BC, Petko DL, Foo TK. *Magn Reson Med*. 2009; 61:1132. [PubMed: 19215047]
87. Robson P, Hall L. *AICHe Journal*. 2005; 51:1633.
88. Cantillon-Murphy P, Wald LL, Zahn M, Adalsteinsson E. *NMR Biomed*. 2009; 22:891. [PubMed: 19588450]
89. Li S, Dardzinski BJ, Collins CM, Yang QX, Smith MB. *Magn Reson Med*. 1996; 36:705. [PubMed: 8916021] Collins CM, Yang B, Yang QX, Smith MB. *Magn Reson Imaging*. 2002; 20:413. [PubMed: 12206867] Truong TK, Clymer BD, Chakeres DW, Schmalbrock P. *Magn Reson Imaging*. 2002; 20:759. [PubMed: 12591571]
90. Li L, Wang ZJ. *Med Phys*. 1999; 30:449. Wang ZJ, Li S, Haselgrove JC. *J Magn Reson*. 1999; 140:477. [PubMed: 10497053]
91. Pathak AP, Ward BD, Schmainda KM. *NeuroImage*. 2008; 40:1130. [PubMed: 18308587]
92. Jenkinson M, Wilson JL, Jezzard P. *Magn Reson Med*. 2004; 52:471. [PubMed: 15334564] Li L, Leigh JS. *Magn Reson Med*. 2004; 51:1077. [PubMed: 15122694] Koch KM, Papademetris X, Rothman DL, de Graaf RA. *Phys Med Biol*. 2006; 51:6381. [PubMed: 17148824] Cheng YC, Neelavalli J, Haacke EM. *Phys Med Biol*. 2009; 54:1169. [PubMed: 19182322]
93. Mills PH, Hitchens TK, Foley LM, Link T, Ye Q, Weiss CR, Thompson JD, Gilson WD, Arepally A, Melick JA, Kochanek PM, Ho C, Bulte JWM, Ahrens ET. *Magnetic Resonance in Medicine*. 2012; 67:278. [PubMed: 21656554]
94. Langley J, Liu W, Jordan EK, Frank JA, Zhao Q. *Magn Reson Med*. 2011; 65:1461. [PubMed: 21500271]
95. Haacke, EM., Brown, RW., Thompson, MR., Venkatesan, R. *Magnetic resonance imaging : physical principles and sequence design*. New York: Wiley; 1999.
96. Schweser F, Deistung A, Lehr BW, Reichenbach JR. *Neuroimage*. 2011; 54:2789. [PubMed: 21040794] Li W, Wu B, Liu C. *Neuroimage*. 2011; 55:1645. [PubMed: 21224002] de Rochefort L, Liu T, Kressler B, Liu J, Spincemaille P, Lebon V, Wu J, Wang Y. *Magn Reson Med*. 2010; 63:194. [PubMed: 19953507] de Rochefort L, Brown R, Prince MR, Wang Y. *Magn Reson Med*. 2008; 60:1003. [PubMed: 18816834]
97. Wang L, Potter WM, Zhao Q. *Contrast Media Mol Imaging*. 2015; 10:43. [PubMed: 24764174]
98. Mitalipova M, Calhoun J, Shin S, Wininger D, Schulz T, Noggle S, Venable A, Lyons I, Robins A, Stice S. *Stem Cells*. 2003; 21:521. [PubMed: 12968106] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. *Cell*. 2007; 131:861. [PubMed: 18035408] Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. *Science*. 1998; 282:1145. [PubMed: 9804556]
99. Reubinoff BE, Itsykson P, Turetsky T, Pera MF, Reinhartz E, Itzik A, Ben-Hur T. *Nat Biotechnol*. 2001; 19:1134. [PubMed: 11731782]
100. Galloway JL, Zon LI. *Curr Top Dev Biol*. 2003; 53:139. [PubMed: 12510667] Estes BT, Gimble JM, Guilak F. *Curr Top Dev Biol*. 2004; 60:91. [PubMed: 15094297] Lensch MW, Daley GQ. *Curr Top Dev Biol*. 2004; 60:127. [PubMed: 15094298]
101. Shin S, Mitalipova M, Noggle S, Tibbitts D, Venable A, Rao R, Stice SL. *Stem Cells*. 2006; 24:125. [PubMed: 16100006]
102. Dhara SK, Hasneen K, Machacek DW, Boyd NL, Rao RR, Stice SL. *Differentiation*. 2008; 76:454. [PubMed: 18177420]
103. Dhara SK, Majumder A, Dodla MC, Stice SL. *Methods Mol Biol*. 2011; 767:343. [PubMed: 21822887]
104. Mumaw JL, Machacek D, Shields JP, Dodla MC, Dhara SK, Stice SL. *Microsc Microanal*. 2010; 16:80. [PubMed: 20082731]
105. Shin S, Dalton S, Stice SL. *Stem Cells Dev*. 2005; 14:266. [PubMed: 15969621]
106. Harrill JA, Freudenrich TM, Machacek DW, Stice SL, Mundy WR. *Neurotoxicology*. 2010; 31:277. [PubMed: 20188755]
107. Gordeeva OF. *J Stem Cells*. 2011; 6:51.
108. Kim JH, Auerbach JM, Rodriguez-Gomez JA, Velasco I, Gavin D, Lumelsky N, Lee SH, Nguyen J, Sanchez-Pernaute R, Bankiewicz K, McKay R. *Nature*. 2002; 418:50. [PubMed: 12077607]

- Liker MA, Petzinger GM, Nixon K, McNeill T, Jakowec MW. Brain Res. 2008; 971:168. Maegele M, Schaefer U. Minim Invasive Ther Allied Technol. 2008; 17:119. [PubMed: 18465446]
109. Bulte JW, Arbab AS, Douglas T, Frank JA. Methods Enzymol. 2009; 386:275. Feigenbaum GS, Lemberg L, Hare JM. J Am Coll Cardiol. 2009; 54:1627. [PubMed: 19833263]
110. Jane Le CC, Majumder Anirban, Wu Xian, Stice Steve. Society for Neuroscience, Society for Neuroscience. 2014:376.
111. Addicott B, Willman M, Rodriguez J, Padgett K, Han D, Berman D, Hare JM, Kenyon NS. Contrast Media Mol Imaging. 2011; 6:7. [PubMed: 20690161]
112. He T, Wang Y, Xiang J, Zhang H. Curr Gene Ther. 2014; 14:136. [PubMed: 24712550]
113. Shen WB, Plachez C, Chan A, Yarnell D, Puche AC, Fishman PS, Yarowsky P. Int J Nanomedicine. 2013; 8:4593. [PubMed: 24348036]
114. Dhara SK, Gerwe BA, Majumder A, Dodla MC, Boyd NL, Machacek DW, Hasneen K, Stice SL. Tissue Eng Part A. 2009; 15:3621. [PubMed: 19795983] Young A, Assey KS, Sturkie CD, West FD, Machacek DW, Stice SL. J Neurosci Res. 2010; 88:3222. [PubMed: 20857516] Young A, Machacek DW, Dhara SK, Macleish PR, Benveniste M, Dodla MC, Sturkie CD, Stice SL. Neuroscience. 2011; 192:793. [PubMed: 21672611]
115. Chen CC, Ku MC, D MJ, Lai JS, Hueng DY, Chang C. PLoS One. 2013; 8:e56125. [PubMed: 23468856]
116. Radio NM, Mundy WR. Neurotoxicology. 2008; 29:361. [PubMed: 18403021]
117. Hamburger V, Hamilton HL. Dev Dyn. 1992; 195:231. [PubMed: 1304821]
118. Berman SC, Galpoththawela C, Gilad AA, Bulte JW, Walczak P. Magn Reson Med. 2011; 65:564. [PubMed: 20928883]
119. Walczak P, Kedziorek DA, Gilad AA, Barnett BP, Bulte JW. Magn Reson Med. 2007; 58:261. [PubMed: 17654572]
120. Terrovitis J, Stuber M, Youssef A, Preece S, Leppo M, Kizana E, Schar M, Gerstenblith G, Weiss RG, Marban E, Abraham MR. Circulation. 2008; 117:1555. [PubMed: 18332264]
121. Lo Celso C, Fleming HE, Wu JW, Zhao CX, Miake-Lye S, Fujisaki J, Côté D, Rowe DW, Lin CP, Scadden DT. Nature. 2009; 457:92. [PubMed: 19052546]
122. Sauter AW, Wehrl HF, Kolb A, Judenhofer MS, Pichler BJ. Trends Mol Med. 2010; 16:508. [PubMed: 20851684] Pichler BJ, Kolb A, Nagele T, Schlemmer HP. J Nucl Med. 2010; 51:333. [PubMed: 20150252]
123. Sallam K, Li Y, Sager PT, Houser SR, Wu JC. Circ Res. 2015; 116:1989. [PubMed: 26044252] Chen Z, Zhang YA. Sci China Life Sci. 2015; 58:119. [PubMed: 25528254] Schwartz SD, Regillo CD, Lam BL, Elliott D, Rosenfeld PJ, Gregori NZ, Hubschman JP, Davis JL, Heilwell G, Spirn M, Maguire J, Gay R, Bateman J, Ostrick RM, Morris D, Vincent M, Anglade E, Del Priore LV, Lanza R. Lancet. 2015; 385:509. [PubMed: 25458728]

Figure 1.

Left: Longitudinal T_1 relaxation curve displays the regrowth of longitudinal component of magnetization from the initial value $M_z(0)$ to the equilibrium value M_0 . Right: Transverse T_2 relaxation curve shows the decay of the transverse magnetization component from an initial equilibrium value.

Figure 2.

Magnitude images of a phantom consisting of three vials of Ferumoxides concentrations (labeled 1–3) of $160 \mu\text{g ml}^{-1}$, $80 \mu\text{g ml}^{-1}$, and $40 \mu\text{g ml}^{-1}$, respectively, at high SNR and low SNR environments, are shown in (a) and (b), respectively; (c) A cross section magnitude image of a mouse, subcutaneously implanted in the flanks with 10^5 labeled and 10^6 unlabeled C6 glioma cells. The implanted tumor was labeled with a circle. Phase images for the phantom data set (at high SNR and low SNR) and the mouse were shown in (d), (e), and (f) respectively. Magnetic field inhomogeneities induced by various concentrations of SPIO nanoparticles or SPIO labeled cells generated significant magnetic dipole effects. Reproduced with permission.^[94] Copyright 2011, Wiley

Figure 3.

Derivation of terminally differentiated neurons and glial cells from PSC derived NP cells (A) Cells in the inner cell mass of pre-implantation embryos or as reprogrammed somatic cells (B) can be isolated and cultured in vitro as hPSCs. (C) Spontaneous differentiation of hPSCs into all three germ layers. (D) NP cells that are derived from ectoderm cells differentiate into (E) neurons, including motor neurons, and (F) glial cells, including astrocytes and oligodendrocytes. To simplify presentation, the diversity of neuron and glial cell derivatives is not shown.

Figure 4. Derived hNP cell lines. (A) Adherent human NP cells at 95% confluency. (B) hPSC are OCT4 positive, while mouse feeder cells are negative (arrows) and (C) hNP cell lines are OCT4 negative. (D) NP cells are NESTIN+ and (E) hNP cells are SOX2+. Scale Bar: (A) 100 μ m (B) 40 μ m.

Figure 5.

Human NP cells differentiate into multiple disease relevant neuronal and glial phenotypes. Panels: (A) Phase contrast image of differentiated cells. (B) Differentiation into post-mitotic motoneurons (HB9+); (C) cholinergic, (D) dopaminergic and (E) GABAergic neurons; (F) Highly MAP-2 positive. (G&H) Glial cells. GFAP-positive cells. (G) represents the morphological characteristics of astrocytes, whereas (H) represents the structure of radial glia. (I & J) mature oligodendrocytes as defined by positive basic myelin protein staining. Scale Bar: (A) 40 μ m (B–J) 10 μ m.

Figure 6. GFP⁺ Human Neural Progenitors and Neurons. Panels: **A–B**, Clonal hNP cell “C4”, showing 100% GFP expression and its corresponding phase contrast image; **C–D**, GFP⁺ Neurons Differentiated for 18 days, showing GFP expression and its corresponding phase contrast image. Scale Bar= 100 μ m.

Figure 7.

Molday Ion Rhodamine B (MIRB) did not alter GFP⁺ hNP cell proliferation, or viability pre and post differentiation towards neuronal cells. MIRB+ GFP⁺ hNP cells immediately after labeling and after 14 DIV in differentiation medium (DAPI-Blue, GFP-Green, MIRB-Red). A–B. Prussian blue stained 0 and 14 DIV MIRB+ GFP⁺ hNP. C–D. MIRB did not alter GFP⁺ hNP cell viability pre (0 DIV) or during neuronal differentiation, 7 and 14 DIV ($P < 0.05$). E. Iron content significantly decreased from 0 to 7 DIV, but did not fall below 2.47 ± 0.34 pg Fe/cell. F. The total number of GFP⁺ hNP cells increased significantly at each

time point ($P < 0.05$) G. A majority GFP⁺ hNP cells retained the MIRB labeling throughout the 14 day in vitro differentiation. Scale Bar= 100 μ m.

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Figure 8.

MIRB loading does not alter GFP⁺ hNP cell properties. After labeling, GFP⁺ hNP cells (MIRB⁺) maintained NESTIN and SOX1 expression and hNP phenotype, but did not express maturing neuronal proteins MAP2 or HuC/D or phenotype. No significant difference between MIRB⁺ and non-labeled (MIRB⁻) cultures was observed ($p > 0.05$) A–G. After 14 DIV in neural differentiation conditions, MIRB⁺ cells transitioned towards expression of maturing neuronal proteins MAP2 and HuC/D with no significant difference between MIRB⁺ and MIRB⁻ cells ($P > 0.05$). H–N. Scale bars=50 μ m

Figure 9.

MIRB does not alter morphology of 14 DIV GFP+ hNP. MIRB+ and MIRB- populations generated an equal number of neurons. A. After 14 DIV in differentiation medium, analysis of neuronal morphology indicated no significant differences between MIRB+ and MIRB- populations. B–D. When neuronal morphology was evaluated on a per-neurite basis, no significant differences were observed after 14 DIV. E–F. All comparisons were made with a sample t-test ($P > 0.05$).

Figure 10.

MIRB+ GFP+ hNP cells were observed with fluorescent microscopy and MRI co-localize within the chick embryo. Twenty-four hours after transplantation, MIRB+ GFP+ hNP1 cells were observed through windows created at time when cell were injected. (Green - GFP+ hNP, Red - MIRB, inset i demonstrate merged image) **A–F**. Scale bars: 2mm. MR images of T_2 and T_2^* -weighted anatomy (**G and H**) and T_2^* -weighted phase images (**I**) demonstrate MIRB+ GFP+ hNP cells as hypointense regions as well as strong magnetic dipole effects corresponding to the location of injected cells (* and <). MRI protocol: Fast spin echo

sequence, TR/TE=5000/30 ms, matrix 256×256 , FOV=60 mm $\times$ 45 mm, slice thickness=1 mm; Gradient echo sequence, TR=1000 ms, 6 echoes (TE1=3 ms, increment=3.6 ms), FA=30°.

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Figure 11.

In vivo MRIB+ GFP⁺ hNP1TM cell tracking on embryonic day 6 (1st row) and day 10 (2nd row) of chick development. T₂-weighted (1st column) and T₂^{*}-weighted (2nd column) anatomical images are presented, together with T₂^{*}-weighted phase images (3rd column). SPIO nanoparticles carried by labeled cells exhibit strong magnetic dipole effect (like a flowering artifact), shown in both anatomical (pointed by red and yellow arrows) and phase images (orange arrows). MRI protocol: Fast spin echo sequence, TR/TE=5000/30 ms, matrix 256 × 256, FOV=60 mm × 45 mm, slice thickness=1 mm; Gradient echo sequence, TR=1000 ms, 6 echoes (TE1=3 ms, increment=3.6 ms), FA=30°.