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Detection and Quantification of Magnetically Labeled Cells by Cellular MRI

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Abstract

Labeling cells with superparamagnetic iron oxide (SPIO) nanoparticles, paramagnetic contrast agent (gadolinium) or perfluorocarbons allows for the possibility of tracking single or clusters of labeled cells within target tissues following either direct implantation or intravenous injection. This review summarizes the practical issues regarding detection and quantification of magnetically labeled cells with various MRI contrast agents with a focus on SPIO nanoparticles.

Keywords

MRI; cell labeling; SPIO nanoparticles; gadolinium; perfluorocarbon

INTRODUCTION

Advances in stem cell research have provided important understanding of the cell biology and microenvironment in tissues and offered great promise for developing novel repair, replacement and treatment strategies. However, there are several research questions that need to be addressed to translate experimental results to the clinic. For example, there is no long term noninvasive approach for determining the trafficking and biodistribution of the therapeutic cells after delivery and whether the transplanted cells are functioning or have differentiated into the desired cell type in the pathological process. The development of noninvasive imaging techniques to track labeled cells may provide a means of monitoring whether the stem cells reach the target and how long they reside there. Furthermore, tracking of labeled stem cells can be used to ensure the appropriate route of delivery, provide feedback into the preferred sites of engraftment and aid in determining the optimal dosing schedule and numbers of cells to be used to achieve the desired therapeutic outcome [1,2].

MRI is the most commonly used imaging modality for in vivo tracking of labeled stem cells, because it is noninvasive, generates high-resolution images, and does not rely on radioactive isotopes, which may be an important advantage for longitudinal studies [1–5]. Cellular MRI combines the ability of MRI with contrast agents for labeling cells providing dynamic assessment of cell migration into target tissues [6,7]. MRI contrast agents in nanomolar to micromolar concentrations can alter the relaxation rates of many nearby tissue water protons thereby making them conspicuous on post contrast enhanced MRI [8,9].

Labeling cells with superparamagnetic iron oxide (SPIO) nanoparticles [3–5,10–12], paramagnetic contrast agent (gadolinium) [13–19] or perfluorocarbons [20–22] allows for the possibility of detecting single or clusters of labeled cells within target tissues following either

direct implantation or intravenous injection. This review will summarize the practical issues regarding detection and quantification of magnetically labeled cells with various MRI contrast agents with a focus on SPIO nanoparticles. The readers are encouraged to examine several excellent reviews for more details regarding cellular and molecular MRI [1–5,23].

Labeling Cells with SPIO Nanoparticles

Various approaches have been developed that use both experimentally and clinically approved MRI contrast agents to label cells. Endocytosis is a global term used to describe the mechanisms that cells internalize macromolecules and particles within vesicles in the cytoplasm. Endocytosis by definition includes phagocytosis, pinocytosis, clathrin-mediated (also known as receptor mediated) endocytosis, caveolin mediated endocytosis, and clathrin and caveolin independent endocytosis [24]. SPIO nanoparticles consist of magnetite (iron oxide) cores represented by iron oxide crystalline structures, which are coated with dextran or siloxanes encapsulated by a polymer, or further modified to facilitate internalization through endocytosis. Initial studies performed to label nonphagocytic cells such as lymphocytes, C6 glioma cells and progenitor stem cells [25–28] with SPIOs were based on the principles used to label phagocytic cells such as neutrophils, macrophages and monocytes [27,29–32]. Cells can incorporate dextran coated SPIO nanoparticles through endocytosis, however, high concentrations of the label are needed with long incubation times and the labeling efficiency using this approach is not as high as with other techniques [33–35]. Mechanical methods have also been employed to magnetically label cells with contrast agents. Electroporation is used to introduce plasmids and DNA into cells without the use of transfection agents and this technique has recently been applied to labeling cells with SPIO nanoparticles[36]. Magnetofection is another mechanical approach that combines gene vectors with coated SPIO nanoparticles and uses an external magnetic field to target the DNA-SPIO complex into cells [37,38]. By encapsulating SPIOs in viral shells or liposomes or modifying the nanoparticles coating by covalently attaching monoclonal antibodies, HIV-transactivator transcription (Tat) proteins, peptides or polycationic macromolecular transfection agents or by changing the coating of the iron oxide crystal has facilitated the endocytosis of the agents into endosomes in cells[1–5, 11,12,39,40]. It is important to note that the use of MRI contrast agents for magnet labeling of cells is considered an off-label use of the agent and at this time none of the superparamagnetic or paramagnetic agents have been approved by regulatory agencies for use to label cells. A complete discussion of the various mechanisms of cell uptake of MRI contrast agents can be found in several excellent reviews[2,3,5].

Imaging Cells Labeled with SPIO Nanoparticles

SPIO nanoparticles, such as the clinically approved formulations ferumoxides, ferucarbotran or ferumoxtran 10, perturb the static magnetic field out to a distance many times of its diameter, resulting in a dramatic reduction in T2 and T2* of the nearby water molecules[41]. SPIO nanoparticles are by far the most widely used contrast agents for the detection of implanted cells in vivo because of the following properties[42]: (1) their contrast effect is stronger than that of the paramagnetic particles; (2) their magnetic properties can be manipulated by controlling the size of core and coating surface; (3) SPIO nanoparticles coated with dextran show improved biocompatibility and biodegradability.

The detection of cells labeled with SPIOs has primarily been accomplished by using various types of T1, T2 and T2* weighted imaging [33,39,43–45]. SPIO nanoparticles' relaxation rates differ substantially when compartmentalized within cells compared with when they are within regions of freely diffusible water. As a result, T2* weighted gradient echo acquisitions provide the greatest sensitivity to the presence of intracellular SPIO nanoparticles [46]. The susceptibility effect on the SPIO nanoparticle label extends well outside the volume occupied by the cell, and this extension augments its detectability. Gradient echo T2* weighted measures,

however, are sensitive to background field inhomogeneities induced by imperfect shimming, blood, and endogenous ferritin deposits and thus have poorer specificity for iron particles. Conversely, T2 weighted spin echo acquisitions can be much less sensitive to iron labeled cells, than T2* measurements[46]. Balanced steady state free precession (b-SSFP) sequence (also known as FIESTA or True-FISP) imaging method has been shown to provide similar sensitivity as gradient echo imaging and a spin echo like insensitivity to background magnetic field inhomogeneities[47,48].

T2 and T2* based imaging methods depict SPIO labeled cells as pronounced local signal voids or hypointense regions. Deoxyhemoglobin in small vessels also generates hypointensities similar to the SPIO labeled cells, making it potentially difficult to differentiate between transplanted cells and slow flowing blood. Administration of paramagnetic contrast agent prior to imaging could improve the detection of labeled cells[49]. Suppression of the hypointensities from small vessels can also be achieved by the use of carbogen (95% O₂ and 5% CO₂) for inhalation, instead of air, as vasodilation by CO₂ causes an increase in blood flow [50]. Nevertheless, differentiation between the signal loss caused by the intracellular nanoparticles and native low signals, for example those from artifacts or imaging metals such as calcium, is challenging, especially when a limited number of cells are present at the sites. Furthermore, the detection of labeled cells is limited by partial volume effects, in which void detection is dependent on the resolution of the image. Mills et al. proposed a post-processing algorithm based on phase map cross-correlation for identification of SPIO labeled cells and showed this method to be robust in very low signal-to-noise ratio images [51]. But further evaluation is needed for in vivo applications with more complicated geometry. To improve the sensitivity and specificity to track magnetically labeled stem cells or other cells, several positive contrast or white marker techniques have been developed[52–57].

Positive Contrast (White Marker) Techniques—The signal intensities on MRI surrounding the SPIO nanoparticles have a characteristic barbell pattern and have been theoretically derived for a single dipole in a homogeneous magnetic field [8]. Cunningham et al. proposed to improve the detection of SPIO labeled cells by selecting the off resonance signals originating in tissues caused by the labeled cells[52]. Stuber et al. used inversion recovery on-resonance water suppression (IRON) to pre-saturate on-resonance water generating voxels with hyperintensities from off-resonance regions near SPIO labeled cells [56]. Zurikya and Hu used a diffusion-mediated off-resonance saturation method to obtain images with positive contrast[57]. Farrar et al. later has demonstrated that the optimal performance of the off-resonance based positive contrast approaches will likely be seen at low fields, with moderate iron concentrations[58]. A limitation of all the off-resonance positive contrast techniques is that voxels with hyperintensities can be seen arising from endogenous areas (e.g. lipids) that are also shifted in frequency from the water protons. Moreover, off-resonance based positive contrast techniques are very sensitive to large-scale B₀ field inhomogeneities.

Alternatively, the “White Marker” technique proposed by Seppenwoolde et al. achieved positive contrast by dephasing the background signal with a slice gradient while in the region near the paramagnetic marker, the signal was conserved because the induced dipole field compensated for the dephasing gradient[55]. A similar approach has been used for imaging of SPIO labeled cells and is known as gradient echo acquisition for superparamagnetic particles (GRASP)[54]. However, the White Marker technique or GRASP only compensates for the susceptibility gradients along the slice select direction.

Positive contrast images can also be derived from the magnetic field map by applying different post-processing techniques[59,60]. The local magnetic gradients induced by magnetic susceptibilities lead to echo-shifts in k-space with gradient echo imaging. Bakker et al.

exploited the echo-shift by applying a shifted reconstruction window in k-space[61]. Recently, a susceptibility gradient mapping (SGM) technique has been developed that calculates the positive contrast images from a regular complex gradient echo dataset [53,62]. The SGM method generates a parameter map of the 3D susceptibility gradient vector for every voxel by computing the echo-shifts in all three dimensions.

The SGM positive contrast technique was compared with the White Marker and the IRON techniques in an in vivo experimental model[62]. Liu et al. subcutaneously implanted in both flanks of nude rats with 1×10^6 ferumoxides and protamine sulfate (FePro) labeled or unlabeled (control) C6 glioma cells. The SGM, White Marker and IRON positive contrast images were acquired when the labeled tumors were approximately 5 mm (small), 10 mm (medium) and 20 mm (large) in diameter along the largest dimension to evaluate their sensitivity to the dilution of the SPIO nanoparticles as the tumor cells proliferated (Figure 1). In vivo MRI has demonstrated that all three positive contrast techniques can produce hyperintensities in areas around the labeled flank tumors against a dark background. The number of positive voxels detected around small and medium tumors was significantly greater with the SGM technique compared to the White Marker and IRON techniques. For large tumors, SGM resulted in similar number of positive voxels as the White Marker technique while the IRON approach failed to generate positive contrast on the images.

The hyperintense regions on positive contrast images originating from SPIO labeled cells can be easily differentiated from other signal voids on T2 or T2* weighted images, therefore providing a greater degree of certainty in the determination of labeled cells. Moreover, the hyperintensities appeared to provide a greater sensitivity than the dark spots on regular MR images. However, positive contrast imaging approaches do not provide sufficient anatomical information and therefore it is necessary to combine positive contrast techniques with conventional gradient echo or spin echo imaging. Both the White Marker and IRON techniques require special pulse sequence design; therefore extra scans are required to obtain the anatomic dataset. In contrast, the SGM method is a post-processing approach and the positive contrast images can be derived directly from the T2* weighted images.

Detection Threshold for SPIO Labeled Cells—The detection threshold for SPIO labeled cells is affected by a number of factors, including field strength, signal to noise ratio, pulse sequence, and acquisition parameters etc. Following direct injection of a mixture of ferumoxides labeled and Indium 111 oxine labeled dendritic cells in the lymph node of patients with melanoma, de Vries et al. [63] were able to demonstrate the migration of labeled cells by single photon emission computerized tomography (SPECT) and 3T MRI through 4 different inguinal lymph nodes in the pelvis. By mixing two populations with two different labels, these authors reported detecting approximately 2000 ferumoxides labeled dendritic cells per voxel on MRI based on corresponding measurements by SPECT. Verdijk et al. found that 1000 cells/mm³ could be detected in patients treated with SPIO labeled therapeutic cells at 3T [64]. With both theoretical and experimental analyses, Heyn et al. [47] estimated that femtomole quantities of SPIOs could be detected under typical micro-imaging conditions and concluded that under certain conditions, MRI cell tracking with iron oxide particles could actually be more sensitive than nuclear techniques, which have a sensitivity of a few hundred cells. A number of studies have confirmed the detection of a few hundreds cells with SPIO labeling.

Hoehn et al. demonstrated in vivo detection of 500 cells implanted in the rat brain at 7T [65]. Jendelova et al. reported a detection limit of 625 cells on a 4.7 T magnet [33]. Similarly, Magnitsky et al. reported detection of 500 cells at 4.7 T [66]. Dahnke and Schaeffter predicted the detect limit of 120 cells/mm³ in the brain and 385 cells/mm³ in the liver on a 3T whole body MR scanner[67]. The variance in the number of detectable cells in the above studies could be attributed to the differences in hardware, resolution of acquired images and uptake of SPIO

nanoparticles in cells. Recent studies have demonstrated the feasibility to detect a small number of cells or even single cells. Kircher et al. reported that following injection of SPIO labeled sensitized T-cells to tumor antigens they were able to detect as few as 3 cells migrating into the tumor [68]. Foster-Gareau et al. showed that single cells labeled with SPIO nanoparticles could be imaged with a 1.5T clinical scanner [69]. No other modality with whole body imaging capability has shown in vivo single cell tracking ability, according to Heyn et al. [47].

Quantification of SPIO Labeled Cells—To date, qualitative assessment of the hypo or hyperintense voxels in tissues following infusion or implantation of magnetically labeled cells has been made in cellular MRI studies. Quantification of the approximate number of magnetically labeled cells within a voxel may provide an effective and efficient method to monitor and optimize cellular therapies. Bulte and colleagues demonstrated a linear relationship between the R2 (i.e. $1/T_2$) relaxation rate and iron content obtained from marmosets with hemosiderosis [70]. Rad et al. observed a strong linear correlation between R2 values and labeled cell numbers with different regression lines for different cells types [71]. Because R2 relaxation rate is sensitive to both iron concentration and distribution of the nanoparticles within a voxel [46,71], it is not suitable for use in quantification of SPIO labeled cells by itself. A simple linear relationship, however, exists between the iron concentration and R2* (i.e. $1/T_2^*$) change for cell suspensions where the magnetic material is distributed in clusters [46]. Using a multiple readout gradient echo pulse sequence, R2* relaxation rates can be determined for the labeled cells in tissues, therefore come one step closer toward quantification of nanoparticle distributions. Bos et al. demonstrated that the changes of R2* in the liver corresponded to the approximate number of mesenchymal stem cells (MSCs) injected in the portal vein in a rat model [72]. Using a standard calibration curve, quantitative prediction of the number of labeled cells in a given region was therefore obtained within the brain of transplanted EAE mice [73].

However, R2* based quantification of the number of labeled cells in tissues on in vivo MRI remains inexact, especially when comparing changes across longitudinal studies. First, the R2* relaxation rate is not only influenced by SPIO nanoparticles in labeled cells, but also by macroscopic susceptibilities that arise from air-tissue interface. These susceptibility artifacts lead to overestimation of the relaxation rates or obscure low concentration labeled cells. Several methods have been proposed to correct for the macroscopic magnetic susceptibility influence such as increasing the spatial resolution [74], altering the slice selection gradient [75], or utilizing B₀ inhomogeneity correction to compensate for magnetic field susceptibilities from tissues that do not contain magnetically labeled cells [67]. Second, quantification of SPIO labeled cells in vivo can be complicated by the existence of free or extracellular non-compartmentalized iron oxide. It is difficult to completely separate extracellular iron in the microenvironment from the labeled cells. Free iron could also be found at injected sites where hemorrhage and labeled dead cells are often present [71]. Because intracellular SPIO nanoparticles have much smaller R2 than nanoparticles freely suspended in the extracellular space, measuring both R2 and R2* relaxation rates could reduce the interference from this iron pool and lead to a more accurate quantification of the number of intracellular SPIOs [76]. Finally, it should be noted that MRI quantification of SPIO labeled cells is an indirect technique. As such, signal change is due to the concentration of SPIO nanoparticles and not the total number of cells. As cells proliferate and the iron is divided symmetrically or asymmetrically between daughter cells, the total iron content and the signal from each cell decreases [77]. Furthermore, the iron from cells undergoing apoptosis or cell lysis can be internalized by macrophages resident in local tissue, resulting in signal attributable to cells [78]. However, Pawelczyk et al. recently have shown that the amount of iron transferred from a labeled stem cell to activated macrophages is less than 10% of the total iron load injected into the tissue and therefore may not contribute significantly to the R2* changes on MRI [79].

Labeling Cells with Paramagnetic Contrast Agents

Paramagnetic gadolinium (Gd) chelates, such as gadopentate dimeglumine, are the most widely used T1 contrast agent for clinical use. Conventional gadolinium chelates (i.e., DOTA, DO3A, DTPA) are generally not permeable through the cell membrane and thus passive labeling cells with these compounds requires high concentrations and long incubation times [14]. Efficient paramagnetic labeling of cells can be done with cationic liposomes or microemulsions containing a high payload of an amphiphilic paramagnetic moiety. Daldrup-Link et al. labeled stem cells with Gadophrin-2, a fluorescent gadolinium contrast agent and intravenously administered $1 \times 10^6 \sim 3 \times 10^8$ cells into mice. They were able to clearly demonstrate the presence of gadophrin-2 labeled hematopoietic stem cells in the liver spleen and bone marrow by optical imaging. Significant changes in signal intensity on MRI from these same organs could be observed when 1×10^7 gadophrin-2 labeled cells were given to the animals [15]. Giesel et al. labeled MSCs with Gadofluorine M (a bifunctional amphiphilic gadolinium complex with a Cy3.5 as a fluorescent marker) for both MRI and optical imaging [16]. Gadofluorine M has a hydrophilic tail allowing the agent to insert in the cell wall and then it is internalized into cytosol. Gadofluorine M labeled cells implanted intracerebrally in the rat brain was clearly detected on T1 weighted images at 1.5 T.

Recently, gadolinium-based agents that have higher T1 relaxivities have been developed to label cells such as metallofullerenes [80]. Anderson et al. were able to label MSCs with gadolinium fullerenol, having relaxivities up to 10 fold of the conventional gadolinium chelates, and detected an increase in signal intensity on T1 weighted images following direct injection into the rat thigh at 7T [13]. However, gadolinium fullerenol labeling decreased the stem cell proliferation initially suggesting that the agent may be altering mitochondrial function. Brekke et al. used a combination of gadolinium chelates with fluorescent tag to label cells and noted a significant decrease in proliferation and increase in reactive oxygen species following incubation of cells for 24 hours [81]. The transient negative effect of gadolinium based agents on cell proliferation used for cellular MRI requires evaluation to ensure that there is no long term impairment of cellular function.

Imaging Cells Labeled with Paramagnetic Contrast Agents

Gadolinium chelates show a T1 shortening effect through interactions between the electron spins of the paramagnetic center and the nearby proton nuclei thus alter signal intensity in the region near the contrast material [82]. In general, majorities of the gadolinium agents incorporated into cells demonstrated moderate T1 enhancement. In order to maximize image contrast, T1-weighted spin echo imaging is the most suitable sequence. However, Gd^{3+} ions inside large macromolecules as in [18] appeared to act essentially as T2 agents, and T2-weighted spin echo imaging is the most suitable in this case.

Because the change in signal intensity per concentration of gadolinium based contrast agents is less than the effects observed from SPIO nanoparticles, a greater concentration of gadolinium is needed to allow for visualization by in vivo cellular MRI. With presently available field strengths, gadolinium based contrast agents require 50 to 500 $\mu\text{mol/L}$ concentrations of low-molecular-weight Gd^{3+} containing molecules or attachment to macromolecular scaffolds such as dendrimers and dextrans to increase the T1 effect. Daldrup-Link et al. [83] demonstrated that cell pellets labeled with gadopentate dimeglumine liposomes showed increased signal intensity on T1-weighted MR images and decreased signal intensity on T2 weighted MR images. They estimated that 5×10^5 cells labeled with gadopentate dimeglumine liposomes could be depicted at 1.5T. Crich et al. were able to detect 5×10^5 cells labeled with Gd-HPDO3A implanted within matrigel in vivo and found that the minimum detectable number of cells labeled with Gd-HPDO3A was on the order of $2 \sim 3 \times 10^3$ under in vitro conditions [14]. The

in vivo detection limit of cells labeled with gadolinium contrast agents still needs to be investigated.

T1 mapping with inversion recovery or saturation recovery spin echo sequence has been used to access the relativities of various gadolinium contrast agents. By using quantitative R1 (i.e., $1/T_1$) maps, Granot et al. [84] demonstrated the migration of tumor infiltrating fibroblasts into model of ovarian cancer in mice. These authors were able to detect small incremental changes in R1 that corresponded to areas of labeled cells by two-photon confocal microscopy. However, the measurement of R1 of internalized gadolinium contrast agents is dependent on the amount of Gd^{3+} taken up by the cell [14]. This is possibly the result of sequestration, steric changes, and other impediments to water access and favorable motion characteristics of the agent in the intracellular environment. Therefore, it is not straightforward to use T1 mapping alone to quantify the number of gadolinium contrast agent labeled cells in vivo.

Labeling Cells with Perfluorocarbons

1H contrast agents present an inherent challenge that the large background signal from mobile water makes it difficult to unambiguously identify the transplanted cells in vivo, especially if the cell biodistribution is not known a priori. Recently, cationic perfluorocarbons have been introduced as contrast agents for cell labeling [20–22]. The main advantage of ^{19}F MRI is that it allows imaging of the cells without any background, so-called hot-spot imaging, since there are no endogenous fluorine atoms present in the body. Ahrens et al. successfully labeled and tracked dendritic cells with perfluoropolyether (PEPE) which was composed of perfluoro-15-crown-ether (CE) emulsions [20]. The ^{19}F spectrum from pelleted dendritic cells labeled with PEPE nanoparticles showed a single resonance line sufficiently narrow for MRI applications. There was no change in the ^{19}F NMR line shape as a function of time, suggesting no breakdown of the PEPE molecules. More recently, Partlow et al. injected populations of mononuclear cells labeled with perfluoro-octylbromide (PFOB) or CE nanoparticles into the left and right hind limbs of a mouse, and were able to image the two populations separately with selected excitation ^{19}F imaging [21].

Imaging Cells Labeled with Perfluorocarbons

The unique spectral chemical shift from the perfluorocarbons allows for the detection of cells with ^{19}F spectroscopy or ^{19}F imaging. Because the gyromagnetic ratios of ^{19}F and 1H differ by only ~6%, the minimum number of detectable ^{19}F spins per voxel is of the same order of magnitude as that of conventional 1H MRI, which is of the order of 10^8 spins per voxel. Ahrens et al. [20] detected local injection of 4×10^6 labeled cells with in vivo ^{19}F imaging at 11.7T. Partlow et al. [21] found that 4×10^6 locally injected CE labeled cells produced fluorine signals strong enough for detection with ^{19}F imaging at 1.5T. The authors also injected 6×10^6 labeled cells intravenously and were able to localize the cells in the mouse liver with ^{19}F spectroscopy at 11.7T. They demonstrated that about 2000 CE-labeled and 10,000 PFOB-labeled cells were detectable. They estimated the detection limit in a single voxel was 6100 cells with ^{19}F MRI of a cell pellet. With further refinements in sequence design and cell labeling techniques, the cell detection limits can be improved several fold and will certainly be under investigation. Nevertheless, the sensitivity of perfluorocarbon agents is not as high as that of iron oxide agents and to date is limited to large cell numbers. Single cell imaging is not possible with the perfluorocarbon cell labeling approach.

^{19}F signals are intrinsically quantitative based on the localized or chemical shift spectroscopic imaging techniques and have the potential to provide accurate concentration of particles in a given region of interest. Ahrens et al. achieved quantitative estimate of the intracellular PEPE concentration with a ^{19}F NMR reference placed adjacent to the test sample [20]. From the ratio of the integrated areas of the PEPE and the reference spectra, it was estimated that each dendritic

cell on average contained 5.2×10^{12} fluorine spins, equivalent to 0.25 ng of PEPE [20]. In vivo quantification was achieved more recently by Srinivas et al. with PEPE labeling in a diabetes model with spin density-weighted ^{19}F MRI [22]. The number of apparent T cells estimated directly from the in vivo ^{19}F imaging was on the order of 2.8×10^4 cells/voxel, approximately 2% of the transferred cells homed to the pancreas after 48 hours.

The ability to differentiate distinct perfluorocarbon signals with ^{19}F spectroscopy could provide additional advantages such as the type and number of cells accumulating at target sites. A variety of perfluorocarbons might serve as core materials for uniquely labeled nanoparticles, which could allow for simultaneous tracking of different transplanted cell populations within tissues[21].

Future Directions

In order for cellular therapies to become an integral part of the treatment of diseases, it will be necessary to develop non-invasive approaches that can provide clinicians feedback as to whether the stem cells or genetically engineered cells or immune cells are present and functioning in target tissues. This review focused on the various methods to magnetically label cells and techniques used to track and quantitate the number of labeled cells in tissues. MR imaging techniques are being developed to improve the sensitivity and specificity to detect the fewest number of labeled cells in pathology. Unfortunately, qualitative assessment of cellular MRI alone to determine the presence or extent of migration of the fewest numbers of magnetically labeled cells is inadequate and will probably require quantitative image analysis approaches to reveal the presence of labeled cells that are sparsely mixed with host cells through-out the target tissue.

In order to use quantitative MRI approaches (i.e., T1, T2, or T2* relaxation properties) to determine the presence of labeled cells in tissues, there will need to be improvements in the hardware stability and reproducibility in order to perform serial MRI studies to track magnetically labeled cells over time. Gradient instabilities, eddy currents, B_0 drifts or B_1 radiofrequency inhomogeneities may all contribute to inaccuracies in quantitative relaxation rate measures of tissue over time. It is also unclear if rapid scanning approaches commonly used with phase array coil technology to shorten scan time or increase resolution will be sufficiently stable and reproducible to perform quantitative MRI. In order to improve MRI sensitivity of magnetically labeled cells in tissues, smaller voxels will be required in order to limit partial volume effects and it may be necessary to move to 7T MRI to increase signal to noise and sensitivity to changes in magnetic susceptibility. Susceptibility weighted imaging approaches [85] may also be useful in localizing magnetically labeled cells within target tissues.

At the present time labeling cells with MRI contrast agents cannot be used to interrogate the transplanted cells' viability, function or ability to differentiate towards a desired phenotype. Multimodality imaging approaches either combining MRI with PET or SPECT for clinical studies or MRI with optical or bioluminescent imaging in experimental models may be useful in determining the functional status of transplanted cells as well as their therapeutic effect. In the future, cellular MRI may be used to track magnetically labeled cells for days to weeks following infusion or direct implantation in tissues and then a combination of nuclear medicine approaches or other MRI techniques (i.e. spectroscopy, magnetization transfer, diffusion imaging) will be used to assess improvements in tissues and organs as a result of the cellular therapy.

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Figure 1.

Top row: Images acquired when the SPIO labeled tumor was approximately 5 mm in diameter, representing highly concentrated SPIO labeled tumor cells (yellow circle). Center row: Images acquired when the SPIO labeled tumor was approximately 10 mm in diameter, representing relatively diluted SPIO nanoparticles (yellow circle). Bottom row: Images acquired when the SPIO labeled tumor was approximately 20 mm in diameter, representing diluted SPIO nanoparticles (yellow circle). The image on the far left is an axial slice through the rat showing the region selected for the zoom views of the labeled tumor with T2* weighted, SGM, White Marker and IRON techniques. The IRON technique failed to generate positive contrast images of the diluted SPIO nanoparticles (bottom row).