Cellular Magnetic Resonance Imaging: Nanometer and Micrometer Size Particles for Noninvasive Cell Localization

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Summary: The use of nanometer and micrometer-sized superparamagnetic iron oxide particles as cellular contrast agents allows for the noninvasive detection of labeled cells on high-resolution magnetic resonance images. The development and application of these techniques to neurologic disorders is likely to accelerate the development of cell transplantation therapies and allow for the detailed study of *in vivo* cellular biology. This review summarizes the early development of iron oxide–based cellular contrast agents and the more recent application of this technology to noninvasive imaging of cellular transplants. The ability of this technique to allow for the noninvasive detection of *in vivo* transplants on the single-cell level is highlighted. **Key Words:** Stem cells, magnetic resonance imaging, single-cell imaging, superparamagnetic contrast agents, iron oxide, brain.

INTRODUCTION

Several promising cellular transplantation therapies for CNS diseases and injury are currently entering human clinical trials. Such experimental therapies include neural stem cells, olfactory ensheathing cells, Schwann cells, and activated macrophages. For example, activated macrophages were recently tested in a Phase II clinical trial by direct injection into the spinal cord of humans with acute spinal cord injury (ProCord; Proneuron Biotechnologies, Los Angeles, CA).[1] Even as promising cellular treatments for CNS injury and disease rapidly move forward, however, there are no noninvasive, objective methods in current clinical use that allow for the identification and tracking of such cells once they have been transplanted.

There have been rapid advances in the field of imaging technology in the past decade, especially in the areas of cellular and molecular imaging research. One particularly exciting concept uses nanometer- and micrometer-sized iron oxide particles to label individual cells *in vitro* for subsequent *in vivo* MRI. This concept is founded on the principle that nanometer-sized crystals of iron oxide are superparamagnetic and, as such, possess extremely high molar relaxivity. This results in hypointensity on MRI images, creating contrast that extends to greater than the physical dimensions of the particles themselves. The bulk of the studies reported have focused on the use of nanometer-scale iron oxide particles.[2–11] Such particles have been classified as 1) ultrasmall superparamagnetic iron oxide particles or USPIOs (30–50 nm in diameter)[12]; 2) small superparamagnetic iron oxide particles or SPIOs (50–150 nm in diameter); and 3) monocrystalline iron oxide nanocompounds or MIONs (100–200 nm in diameter).[13]

Nanometer-scale particles have been used in experimental paradigms to label and track transplanted human mesenchymal stem cells, neural stem cells, hematopoietic cells, Schwann cells, olfactory ensheathing cells, and oligodendrocyte precursors, among others.[2,11–14] These methodologies have been applied to MRI of experimental disease models such as the trafficking of labeled T cells in a mouse model of multiple sclerosis,[3] the recruitment of diabetogenic CD8+ T cells in NOD mice,[14] and the *in vivo* visualization of SPIO-labeled transplanted pancreatic islets.[15]

The goal of this article is to review the literature surrounding the use of nanometer- and micrometer-scale particles for noninvasive MRI visualization of transplanted and host cell populations.

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BACKGROUND

To identify and track single cells and transplanted cell populations, most investigations have focused on techniques using a contrast agent that can alter the T_1, T_2, and T_2* relaxivities of voxels containing cells of interest. This has been achieved through the application of existing MRI contrast agents,[16] and also through the application or development of novel contrast agents. Materials such as iron, nickel, cobalt, and gadolinium possess permanent magnetic moments that can produce intense local magnetic fields,[17] and such substances have been explored as cellular contrast agents.

Iron oxide–containing particles are superparamagnetic and are commonly used as cell-labeling contrast agents. When superparamagnetic particles are placed in an external magnetic field, the magnetic dipoles orient to produce local disruptions in the main magnetic field. This effect, manifested as spin dephasing, influences neighboring protons at a distance equivalent to thousands of times the actual size of the particles themselves. MRI protocols that are sensitive to these dephasing effects elicit T_2* contrast and can be used to detect these particles.[17–19]

LABELING CELL POPULATIONS WITH IRON OXIDE COMPOUNDS

To distinguish single cells or cellular transplants from host tissue, cells of interest can be tagged or loaded with intracellular magnetic resonance (MR) contrast agents through a variety of means. Initial efforts loaded T-lymphocytes with superparamagnetic particles through an endocytosis-mediated mechanism.[20] One published technique resulted in 0.11 ng Fe/cell and allowed for the detection of $\sim 2 \times 10^6$ cells per 30 μL of fluid.[21] Enhanced uptake of superparamagnetic material by T cells was later achieved by coating the superparamagnetic particles with the transactivator (Tat) protein of the human immunodeficiency virus, which enhanced cellular uptake.[10,11]

Other strategies for labeling cells of interest have relied on the synthesis of mAbs linked to superparamagnetic particles. When the superparamagnetic agent MION-46L was linked to a mAb targeted to the transferrin receptor, enhanced MION-46L uptake could be achieved in a population of oligodendrocyte progenitor cells.[9] These early efforts suffered from the relative inefficiencies of the labeling processes, the cell-type-specific nature of the Tat protein or mAb techniques, and the complex schemes required to synthesize such labeling agents.

Less specific but more efficient cellular labeling strategies were later developed. One approach used a superparamagnetic cell-labeling agent called the magnetodendrimer (MD-100).[4] This compound consists of carboxylated poly(amidoamine) dendrimers encapsulating a superparamagnetic iron oxide core. Such dendrimers have a known affinity for cellular membranes and have been previously used to transport foreign DNA into cells.

It was hypothesized that the dendrimers could also effectively load cells with iron oxide.[22–26] The simple addition of MD-100 to cell-culture media for 48 hours resulted in the intracellular accumulation of iron oxide in a variety of cell types, including the HeLa cell line, 3T3 fibroblasts, and oligodendrocyte progenitor cells.[4,27] This labeling process resulted in a relaxation enhancement five times greater than that achieved using mAbs to the transferrin receptor. This labeling technique was successfully implemented to consecutively monitor the biodistribution of oligodendroglial progenitor transplants into demyelinated rat brain at 4.7 T and 1.5 T, and has been applied to other organ transplants, including muscle.[4,28]

More recent nanometer-scale techniques have used commercially available cellular transfection reagents combined with ferumoxide as the cellular MR contrast agent.[29,30] Ferumoxide (Feridex; Advanced Magnetics, Cambridge, MA) is a commercially available SPIO that readily forms complexes with cellular transfection agents such as poly-L-lysine. Such complexes are nonspecifically taken up into endosomes by many cell types and allow for efficient magnetic labeling.[7,31]

TOWARD SINGLE-CELL IMAGING

As we have noted, cellular detection using intracellular contrast agents has been extensively studied both *in vitro* and *in vivo* over the past 15 years.The majority of this work has focused on imaging of transplanted and host cell populations. More recent work has focused on the ability of this technology to allow for single-cell detection by MRI.

Single-cell detection using MRI has been evaluated both *in vitro* and *in vivo*. Early work with USPIOs evaluated the sensitivity of MRI at the cellular level.[32] This work used dextran-coated USPIO particles with an average diameter of 30 nm to nonspecifically label rat T cells. Although this work was limited by the inefficient uptake of USPIOs by cultured T cells, images of *in vitro* SPIO-labeled T cells on microscope slides were obtained at 7 T. Using colabeling with the cellular membrane dye DiI, colocalization of red fluorescence with areas of decreased MR signal corresponding to single cells was illustrated. Electron microscopy studies revealed that the T cells endocytosed the SPIO particles into larger clusters, creating a localized magnetic field larger than that of the single particles.

The application of USPIO-labeled cells for *in vivo* cellular detection has also been extensively studied. The detection of small numbers of cells and the migration of

those cells in the rat brain was illustrated using USPIO-labeled stem cell transplants.[33] This study used embryonic stems cells transfected with a USPIO contrast agent. Initial *in vitro* studies of labeled cell phantoms showed that as few as 40 cells could be detected at 7 T, and *in vivo* studies of stereotactically placed, labeled cell grafts into rat brain showed that cell grafts of as few as 500 cells could be detected in the rat brain. Additionally, the migration pattern of SPIO-labeled, green fluorescent protein (GFP)–producing embryonic stem cells implanted contralateral to an ischemic hemisphere could be followed with serial MRI scans. The migration of the cell grafts toward the ischemic hemisphere was imaged and correlated with the results from conventional histologic GFP studies.

The application of SPIO to single-cell imaging has not been limited to high field research magnets. Other work has shown that single-cell sensitivity is possible using clinical scanners.[34] SPIO-labeled THP-1 cells were imaged in cell phantoms using a three-dimensional (3D) FIESTA [fast imaging employing steady state acquisition] protocol at a resolution of 100 μm isotropic on a 1.5-T clinical whole-body scanner. Using customized high-power gradient coils, individual cells could be detected on 3D FIESTA sequences and colocalized with fluorescent images.

A more recent study from the same group using a 1.5-T clinical scanner demonstrated single-cell detection in mouse brain *in vivo*.[35] An intracardiac injection of SPIO-labeled J774 cells was used to deliver cells to the mouse brain. The J774 cell line is a macrophage cell line capable of obtaining an extremely high 60.9 pg Fe per cell after SPIO labeling. After arterial injection of 10,000 cells, 3D FIESTA sequence MRI scans could detect on average 28 signal voids corresponding to labeled cells in brain. This number corresponds with the predicted number of cells expected in the brain after arterial delivery ($<$0.2%). The areas of decreased signal correlated with DiI fluorescence on confocal microscopy. The demonstrated adaptation of clinical scanners for single-cell scanning holds great promise for the ultimate goal of translating single-cell MRI to human applications. The authors noted that the 60 pg of iron per cell was two to three times higher than the amount of iron accumulated in nonphagocytic cell lines following SPIO labeling.[35]

MPIOs AND SINGLE-CELL IMAGING

In contrast to nanometer-sized USPIOs, larger superparamagnetic particles in the micrometer size range (MPIOs) have been developed and evaluated for cellular imaging. Microspheres from Bangs Laboratories (Fishers, IN) consist of magnetite iron oxide with a green fluorescent component embedded in a polystyrene matrix. The application of these particles as cellular contrast agents has been directly compared to nanometer-sized particles.[36] That is, micrometer-sized (0.9 μm average size) superparamagnetic microspheres were directly compared to ferumoxide USPIOs (Feridex: 10 nm crystal core, 150 nm overall size).

It was shown *in vitro* that the MPIOs had increased R_2* relaxivities at an equal iron content, compared with the nanometer size particles (240 mmol/L^{-1}s^{-1} *versus* 356 mmol/L^{-1}s^{-1}).[36] This increased relaxation produced a greater degree of hypointensity on MR images. This study also evaluated the labeling efficiency of hematopoetic and mesenchymal stem cells and showed efficient MPIO labeling with incubations as short as 1 hour. Notably, no effect was found on *in vitro* cellular differentiation assays. Finally, the authors showed that single, labeled cells could be detected in MR images of culture dishes containing live cells at 11.7 T.[36,37]

In addition to single-cell detection *in vitro,* recent work using MPIOs has shown that the disruption in magnetic field homogeneity can be detected even from single MPIO particles.[38] The dark MR signal produced by MPIO particles is robust enough that single MPIOs could be detected at a resolution of 100 μm. Comparison studies of increasing particle sizes from 0.76 μm to 1.63 μm revealed, as expected, increasing contrast with increasing the size of the particles. This study further documented the application of MPIOs to *ex vivo* single-cell detection in whole organisms. Single-cell mouse embryos were injected with MPIOs then re-implanted and allowed to develop. Fixed embryonic day 11.5 embryos were imaged at 7 T. Punctuate dark areas in single cells could be detected and Prussian blue–stained histology sections confirmed the presence of the detected single MPIOs.[38]

Single-cell detection both *in vitro* and *ex vivo* has been demonstrated using cells labeled with micrometer-sized superparamagnetic iron oxide. The application of this technology to obtain images of cells *in vivo* has also been explored. A recent study applied this technology to a model of hepatocyte transplantation.[39] Mouse hepatocytes were labeled with 1.63-μm-diameter MPIOs and injected into the spleen. Labeled hepatocytes that migrated and engrafted into liver were detectable as punctate dark contrast spots on T_2* gradient recalled echo (GRE) images at 7 T. The colabeling of the cells with CM-DiI allowed for detailed histologic studies colocalizing the MPIO particles with CM-DiI-labeled cells.

Additionally, this report included several important control studies examining the fate of MPIO labeling from transplanted cells that died on transplantation and failed to engraft.[39] Heat-killed MPIO-labeled cells were injected and corresponding MR images of the liver were obtained. In comparison to live injected cells, which produced punctate areas of contrast, the contrast ob-

served in the liver after transplantation of heat-killed cells was grainy and less intense. Histologic analyses revealed that some free particles could travel from the spleen to the liver, accounting for the grainy contrast seen on MR images; however, careful analyses of signal intensities revealed that signal decreases from labeled cells could be distinguished from the noise-like signal profiles of free particles. This study concluded that single transplanted hepatocytes that migrated from the spleen to engraft in the liver could be detected on *in vivo* MRI studies and distinguished from residual free label from nonviable cells.[39]

Recent work in mouse brain imaging has revealed that single MPIO-labeled cells can be detected *in vivo* following intravascular delivery of labeled cells to the brain.[35] For this study, the human breast carcinoma MDA-MB-231 cell line was labeled with 0.9-μm MPIOs and then delivered to mouse brain by arterial injection. This cell line has a propensity for the formation of brain metastases. Single, labeled cells could be detected on 3D FIESTA MR images at sites that later formed metastases in the mouse brain. The authors showed that this technique can be used to follow the fate of individual cells in the brain over time.

In contrast to the injection and subsequent imaging of MPIO-labeled cells, an alternative strategy is to image cells after direct injection of free MPIOs. The premise is that, because single particles can be detected, inefficient labeling schemes could be tolerated and still yield robust detection of cells. This strategy was applied in rodent brains to demonstrate the ability to obtain MR images of *in vivo* cell migration of neural progenitor cells along the rostral migratory stream.[40] Free 1.63-μm-diameter MPIOs were injected into the lateral ventricles of Sprague–Dawley rats and then imaged at 11.7 T or 7.0 T. Signal hypointensity could be detected along the rostral migratory stream beginning at 1 week post injection, and persisting at least 5 weeks post injection. Prussian blue-stained histology and fluorescent immunohistochemistry sections revealed that astrocytes, ependymal cells, migrating neuroblasts, and mature neurons contained MPIOs. It was hypothesized that labeled neuroblasts differentiated into mature granular interneurons accounting for the labeled neurons. Additionally, cells that presumably died along the migratory pathway released MPIO label that could be taken up by surrounding microglia. Overall, the authors concluded that direct *in vivo* labeling of stem or progenitor cells is a technique that would allow for novel studies on cell migration and stem cell behavior.[40]

LIMITATIONS

Superparamagnetic iron oxide is a passive contrast agent, in that the agent will produce contrast in MR images regardless of location, cell type, or cell viability. This leads to the potential for nonspecific findings, because residual label from nonviable cells may not be distinguishable from label contained within viable cells. Residual label can be taken up by macrophages and microglia and may contaminate MR images.[31,40] Careful analysis and control experiments must be applied to ensure that the signal corresponds to and resides within the cell population of interest. Active MRI contrast agents, agents that only produce signal when within viable cells or specific cell types, will greatly improve MR-based cell transplantation studies. Preliminary work has been completed with active agents; however, the sensitivity of these agents for single-cell imaging remains to be established.[41]

Furthermore, cell division can have a negative effect on the ability to track cells. As the implanted cell population divides, the label is diluted when passed to daughter cells. It has been shown that cell proliferation can result in a dilution of the label and a decline in signal intensity.[36] An alternative strategy that may help overcome this limitation is to genetically transduce a cell with a reporter gene that can produce superparamagnetic material. This novel concept has had some preliminary success using the ferritin gene.[42,43] Overexpression of this gene leads to accumulation of endogenous iron. With a viral vector used for ferritin gene delivery, cells overexpressing ferritin could produce contrast on T2 and T2* sequences. This effect was also demonstrated *in vivo* following ferritin gene delivery to the mouse brain.

Many questions remain to be answered with respect to this alternative approach, particularly whether this approach will be useful for imaging of individual transplanted or host cells, and whether genetic alteration significantly affects the behavior of transplanted or endogenous cell populations.

CONCLUSIONS

Progress in developing cellular transplantation strategies for human clinical therapies has been somewhat hampered by the limitations inherent in conventional animal experimental methods. Conventional animal cell transplantation studies rely on the use of labels or tracer agents for cell identification in postmortem analyses. This labeling process can be achieved through the expression of reporter genes such as β-galactosidase, or through the intracellular accumulation of dyes that allow for cell-graft identification during conventional histology. Certain fundamental questions are extremely difficult, if not impossible, to address using these techniques.

For example, to determine the biodistribution of a cell graft, large parts of the target tissue must be sectioned and evaluated histologically. Information concerning quantitative engraftment rates, 3D spatial relationships,

and dynamic information such as migration may be exceedingly difficult to obtain. In addition, within-subject, prospective analyses are of course not possible in studies requiring sacrifice and histology. The field of noninvasive cellular imaging has the potential to obviate many of the limitations posed by conventional histology, and to hasten human application of cellular transplantation therapies.

The application of superparamagnetic iron oxides to cellular imaging has allowed for small numbers and even single cells to be detected noninvasively. Studies using this technology have already begun to elucidate some of the basic mechanisms of transplanted and host cell population biology. This is a relatively young field, and the applications of this technology continue to increase. Further research can continue to expand and refine these techniques for potential human clinical use in studies of transplanted and endogenous cell populations.

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