Published in final edited form as:

Cytotherapy. 2008 ; 10(6): 575–586. doi:10.1080/14653240802165699.

Cellular magnetic resonance imaging: potential for use in assessing aspects of cardiovascular disease

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Abstract

There is rapidly increasing interest in the use of magnetic resonance imaging (MRI) to track cell migration *in vivo*. Iron oxide MR contrast agents can be detected at micromolar concentrations of iron, and offer sufficient sensitivity for T2*-weighted imaging. Cellular MRI shows potential for assessing aspects of cardiovascular disease. Labeling *in vivo* and tracking macrophages using iron oxide nanoparticles has been a goal for cellular MRI because macrophages play a pivotal role in the pathophysiology of many human diseases, including atherosclerosis. Cellular MRI has also been used to track transplanted therapeutic cells in myocardial regeneration. This review looked at iron oxide nanoparticles, methods of cell labeling, image acquisition techniques and limitations encountered for visualization. Particular attention was paid to stem cells and macrophages for the cardiovascular system.

Keywords

atherosclerotic plaque; cellular MRI; myocardial regeneration; superparamagnetic iron oxide nanoparticle

Introduction

There is increasing interest in using cellular magnetic resonance imaging (MRI) to monitor the behavior and physiologic function of superparamagnetic iron oxide nanoparticle (SPIO)-labeled cells *in vivo*. While it lacks a universal definition, cellular imaging can be described as the ‘non-invasive and repetitive imaging of targeted cells and cellular processes in living organisms’ [1]. SPIO have been used to improve image contrast in MRI for more than 10 years [1]. More recently, SPIO have been shown to be sequestered within cells and the labeled cells could be monitored for an order of weeks [1-4], which is clinically relevant. As different cell types have varying levels of uptake of iron oxide, the use of these agents can allow for differing MR contrasts between dissimilar tissues [4,5].

The SPIO sequestered within cells can generate a large magnetic moment that creates substantial disturbances in the local magnetic field. This leads to rapid dephasing of protons, including those surrounding the targeted cell [6,7]. MRI techniques such as gradient echo

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

(GRE) sequences can provide substantial sensitivity for detecting the presence of iron particles as apparent signal voids [7,8]. Although the current limits of spatial resolution preclude direct visualization of individual unlabeled cells, signal amplification with the contrast media uptake within the cells increases the ability of MRI to detect individual and small numbers of SPIO-loaded cells.

Cellular MRI can provide means to visualize SPIO-targeted cells and monitor cell therapy directly in myocardial regeneration [1,5]. The ability to non-invasively track cell migration, cell homing and cellular fate *in vivo* is of pivotal importance for understanding the complex roles of transplanted cells in pre-clinical trials [8,9]. In particular, the ability to dynamically and non-invasively follow cell trafficking and survival over longer periods of time has contributed to the understanding of the potential mechanism of therapeutic cells for myocardial regeneration. MRI has successfully detected and tracked intramyocardial injection of millions of iron-labeled stem cells after myocardial infarction in a swine model [10,11].

Cellular MRI can visualize *in vivo* the composition of the plaque, such as the fibrous cap and the lipid-rich core [12,13]. However, it is difficult to monitor plaque inflammation with conventional MR techniques, which could have an impact on the classification of plaque as stable or unstable. Recent applications of ultrasmall SPIO-based cellular MRI have revealed novel opportunities for specific early detection of atherothrombotic processes, such as angiogenesis and accumulation of macrophages within atherothrombotic plaques [14,15].

This review addressed the iron oxide particles, methods of cell labeling, image acquisition techniques and limitations of the technology. Particular attention was paid to stem cell tracking for myocardial regeneration and macrophage detection in atherosclerotic plaque.

Iron oxide contrast agents

The basic structure of iron oxide MR contrast agents is an iron oxide core coated with a dextran or carboxy-dextran polymer. The core is a single domain iron oxide crystal and thus has superparamagnetic properties, meaning that the magnetic moment of the particle strongly aligns with an external magnetic field. Coating of the core is necessary to prevent aggregation of the nano-scale particles and improve biocompatibility. The iron oxide agents most commonly used for cellular imaging are classified as SPIO and ultrasmall superparamagnetic iron oxides (USPIO). The iron oxide core size is less than 10 nm for both SPIO and USPIO; however, the coating thickness is greater for SPIO, resulting in a larger hydrodynamic size [16]. Table 1 categorizes the common SPIO and USPIO and their associated manufacturers.

The long blood circulating time and progressive macrophage uptake in inflammatory tissues of USPIO particles are two properties of major importance for MRI pathologic tissue characterization [16]. The properties of USPIO have been exploited for atherosclerosis imaging [17]. Once sequestered within cells, USPIO no longer exhibit this property and are considered T2 or T2* agents for cellular imaging [18].

SPIO are appropriate for exogenous cell labeling because the half-life in the blood pool is not a concern. Often SPIO are supplemented with antibodies, peptides or transfection agents to increase labeling efficiency *in vitro* and thus improve MRI sensitivity [19-21]. Applications of exogenously labeled cells include myocardial regeneration [22], anti-cancer therapy [23], muscular dystrophy [24] and Parkinson's disease [1]. Considerations of non-phagocytic labeling, especially for myocardial regeneration, will be discussed in greater detail in later sections.

In summary, the primary difference between (U)SPIO is size, which significantly influences their physiochemical and pharmacokinetic properties and thus clinical applications [25,26].

The advantage of larger SPIO is greater labeling efficiency; the disadvantage is rapid clearance from the blood pool by the reticuloendothelial system (RES). Therefore SPIO are better suited for RES imaging or exogenous labeling (i.e. myocardial regeneration) while USPIO are more appropriate for *in situ* labeling of phagocytic cells, such as macrophage (i.e. atherosclerosis imaging) [27,28]. The materials used for surface coating of the magnetic particles must not only be non-toxic and biocompatible but also allow a targetable delivery with particle localization in a specific area. By binding the targeting molecules, such as proteins and antibodies, to particle surfaces, they may be directed to targeted cell tissues in the body [29].

Cellular MR imaging techniques

MRI is the most utilized modality for *in vivo* stem cell tracking because of its safety and three-dimensional (3D) capabilities [30]. Contrast is generated via T1, T2/T2* or spectroscopic mechanisms [30,31]. For iron-labeled cells of interest to be distinguished from complex background signals, they must contain a label capable of producing significant positive or negative contrasts when imaged with the appropriate pulse sequence.

The effect from iron nanoparticles is seen as hypointensity or negative contrast on T2- and T2*-weighted images because of the shortening of T2 and T2* relaxation times. Alternative approaches have been developed that exploit a frequency shift in the region near the particle, which can produce positive contrast or hypointensity in that region [31,32]. Positive-contrast MRI with off-resonance techniques of cells labeled with SPIO has been achieved using spectrally selective radiofrequency (RF) pulses to excite and refocus water off-resonance in regions near the labeled cells [31].

Both the negative and positive contrast effects act to extend the signal change well beyond the particle or cell. This form of signal amplification increases sensitivity in detecting the labeled cells within a complex image back-ground. With the use of signal amplification, potential future applications of (U)SPIO include ‘doping’ of therapeutic cell preparations with a small fraction of labeled cells, to allow cell tracking without altering the majority of cells. It would allow for better delineation and identifying of labeled cells by both techniques. The challenge in both techniques is the difficulty of quantification of labeled cells *in vivo* because of the susceptibility artifact produced by the iron nanoparticle itself.

3D MR sequences have advantages for cellular MRI. 3D cellular MRI yields excellent signal-to-noise ratio (SNR) and soft-tissue contrast and allows identification of subtle anatomical structures. Pronounced developmental alterations of the morphology are observed during metamorphosis. The feasibility of 3D MRI at micrometer resolution, together with the use of suitable contrast agents, means this approach may provide new ways for studying cell therapies and diagnosis diseases at early stages at cellular and molecular levels. For example, the use of isotropic or near-isotropic spatial resolution with linear voxel dimensions of about 25–50 μm turns out to be a prerequisite for minimizing partial volume effects and thus enhancing the contrast of the subtle anatomical structures. Also, using a 3D volume set means that the original data set can be manipulated on the computer software to show the axial, sagittal and coronal planes.

In summary, recent achievements demonstrate the potential of MRI for cell tracking, with obvious applications to the study of trafficking of stem cells, cancer cells and immune cells (i.e. macrophages).

Phagocytic cell imaging with applications for studying atherosclerotic plaque

Atherosclerosis is the major underlying pathologic cause of heart, cerebrovascular and peripheral arterial diseases. Monocytes from the peripheral circulation migrate to sites of

endothelial injury and differentiate to macrophages. A large amount of evidence implicates macrophages in the formation, progression and destabilization of atherosclerotic plaques [33-35]. In one instance, Mauriello *et al.* [33] characterized the plaque composition of patients who died of acute myocardial infarction (AMI) and compared it with patients who died of non-cardiovascular causes. Macrophages were identified by CD69⁺ staining and the results were combined with staining for T lymphocytes as a metric of 'inflammatory infiltration'. The results indicated that plaques of AMI patients had significantly greater inflammatory infiltrate versus other patients. Also, within AMI patients there was greater inflammatory infiltrate in culprit and vulnerable plaques than in stable plaques. These results and others indicate that assessment of macrophage density *in vivo* has the potential to risk-stratify individual atherosclerotic lesions, or perhaps more generally identify patients who are especially vulnerable to acute events [36-39].

Cellular MRI allows for evaluation of plaque composition at a cellular level. Following intravenous administration, circulating USPIO can accumulate in macrophages of atherosclerotic plaque. Two theories have been proposed regarding the mechanism by which USPIO accumulates in the plaque. One is that circulating monocytes are labeled prior to migration to the lesion. The second is that macrophages within the plaque engulf the iron oxide particles. Regardless of the mechanism, if USPIO accumulation within plaque is sufficient, labeled cells can generate a dipolar field inhomogeneity in the vicinity of the cells. Field inhomogeneities cause dephasing, which leads to signal loss on gradient echo (GRE) images. Two basic types of GRE-based techniques have been used for USPIO-labeled plaque imaging: T2*-weighted GRE and T1-weighted fast GRE imaging. The former method has been used to detect USPIO accumulations in human abdominal aorta [40] and carotid arteries [28]. USPIO-based cellular MRI could therefore be used to risk-stratify patients recommended for carotid endarterectomy [40].

Dark blood T2*-weighted imaging is generally acceptable in large arteries because the size of the vessel, and the plaque, allows resolution of intra-plaque components. However, potential difficulties may arise when the vessel wall is thin or the USPIO accumulate at the luminal edge of the plaque. In such instances it may be difficult to distinguish signal loss because of the USPIO from the dark vessel lumen. This may especially be true in the coronary arteries, where delineation of intraplaque components is limited by motion and size of the vessels. Rabbit models of atherosclerosis are a valuable tool in evaluating relevant imaging techniques because the size of the rabbit aorta closely approximates the size of human coronary vessels [28,41].

Previously reported rabbit studies of iron particles have taken a bright blood angiographic approach to detect luminal encroachment of signal voids originating from USPIO-labeled macrophages in the vessel wall. Several investigations have used 3D T1-weighted fast GRE imaging, with and without spoiling. Luminal T1-shortening can be enhanced by low concentrations of USPIO remaining in the blood pool days after contrast administration [41, 42] and studies have shown that luminal signal-to-noise ratio (SNR) in WHHL rabbits peaks 5 days after intravenous administration of USPIO [41,42]. Thus these and other rabbit studies have focused on imaging on day 5 post-injection to optimize contrast between the lumen and USPIO-induced signal voids in the vessel wall [43-46].

A caveat with respect to the T1-weighted GRE method of detecting USPIO-labeled cells is that contrast kinetics are dependent on the contrast agent and animal model. For instance, Herborn *et al.* [42] compared luminal enhancement over time with two different types of USPIO: ferumoxtran-10 and ferumoxytol (Advanced Magnetix, Rochester, IN, USA). Their study demonstrated that luminal signal enhancement peaked at day 3 with ferumoxytol, and therefore cleared the blood pool more quickly than ferumoxtran-10, which peaked on day 5. Yancy *et al.* [44] performed a similar experiment, with a balloon-injury model in NZW rabbits

instead of the WHHL model. They also found that ferumoxytol cleared more quickly than ferumoxtran-10. However, luminal signal enhancement did not peak on day 5 following injection of ferumoxtran-10 [44]. Furthermore, signal voids along the vessel wall were observed with ferumoxtran-10 but not with ferumoxytol. In summary, the choice of animal model and contrast agent species can influence the success of the imaging study, especially when contrast is dependent on USPIO kinetics to produce luminal signal enhancement. Table 2 summarizes the models, contrast agents, field strengths, sequences and outcomes of previously reported USPIO-labeled plaque studies in rabbits.

Thus far most *in vivo* rabbit models with USPIO-labeling have focused on demonstrating the feasibility of the technique and evaluating the appropriate contrast agent and imaging conditions. For USPIO-labeled plaque imaging to be of clinical utility, it may be necessary to develop methods to quantify the effect of USPIO on image characteristics. Hyafil *et al.* [46] attempted to quantify USPIO-accumulation by measuring the reduction in luminal area caused by the magnetic susceptibility artifacts (MSA) originating from USPIO-labeled macrophages in the vessel wall. Their results demonstrated that reduction in luminal area correlated with area of iron and macrophage staining on histologic slices. These results highlight a fundamental assumption with USPIO-labeled plaque imaging to assess vulnerability, that iron content within plaque is proportional to macrophage content. Trivedi *et al.* [28] describes the findings of an *in vivo* human study evaluating Sinerem-enhanced MRI to identify inflammation within atherosclerotic plaques with histologic correlation. The histologic studies show that macrophages are in the shoulder regions of the plaque (Figure 1). Quantification of macrophage content is challenging because the amount of labeling is variable within cells and within plaque. Several factors can contribute to cell loading, including contrast agent concentration, duration of exposure to the agent and cellular phenotype [47-49]. An additional complicating factor for quantification is the ambiguous geometry of the atherosclerotic lesion. As a result, absolute quantification of the number of macrophages is not feasible with current technology. Instead, it may be more appropriate to take a semi-quantitative approach to distinguish high macrophage content in vulnerable plaque from low macrophage content in stable plaque [33-35].

In summary, USPIO-based cellular MRI has the potential to non-invasively provide information regarding the inflammatory activity of atherosclerotic plaque. Inflammation may be associated with increased risk of rupture, therefore the imaging technique could be used to risk-stratify atherosclerotic lesions, or perhaps more generally identify individuals who are especially vulnerable to acute events. Results of initial experiences in animal models and patients indicate that USPIO-labeled plaque imaging is feasible but faces several challenges. The optimal contrast agent, imaging sequence, procedural timing and analysis method have yet to be established. It is expected that ultimately cellular MRI of iron oxide-labeled macrophages in atherosclerotic plaques may provide a new measure of clinical cardiovascular risk, and contribute to new strategies of plaque assessment and therapy.

Stem cell imaging with application to regenerative myocardial therapies

Animal studies have shown some success in the use of stem and progenitor cells of diverse origins to treat heart failure and ventricular dysfunction secondary to ischemic injury [50,51]. Currently, traditional pathology-based methods are used for cell detection from tissue biopsy in clinical settings. However, biopsy is not possible in all applications. The clinical use of these cells is, therefore, promising. In order to develop effective cell therapies, the location, distribution and long-term viability of these cells must be evaluated in a non-invasive manner. MRI of cells labeled with SPIO after either direct injection or local or intravenous infusion has the potential to fulfill this goal. MRI is capable of generating images with near cellular (15–50 μm) resolution. These resolutions are not feasible in humans with whole-body systems because of long scanning times. Nevertheless, in animal studies MRI has been used to monitor

the presence of iron-loaded cells *in vivo*. However, most therapeutic cells lack substantial phagocytic capability. The cells must be pre-labeled with intracellular MR probes. Recent advances in probe technology facilitate the labeling of stem cells with (U)SPIO.

To improve the efficiency of endocytosis and to facilitate longer tracking of labeled cells *in vivo*, different strategies have been developed. In an early study performed by Arbab *et al.* [19], liposomes containing dextran-coated iron oxide nanoparticles were used for labeling cells. For efficient incorporation, transfection agents were used to complex with SPIO or USPIO in the labeling solution [52,53].

Transfection agents include proteins, lipids, dextrans, phosphonates and dendrimers. The agents are toxic to cells [19,53]. Therefore the ratio of (U)SPIO to the agents is crucial to provide a stable, non-toxic complex that can be internalized efficiently [53]. Results have shown that complexing SPIO with a 1- μ g/mL concentration of poly-L-lysine provides the highest level of intracellular SPIO labeling [54]. The same group has shown long-term (28-day) viability of cells transfected with two FDA-approved agents (Feridex and protamine sulfate) in stem cells, lymphoblast cells and splenocytes [55]. In other previous studies, Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA) was used with Endorem (SPIO; Guerbet, Roissy, France) to label pig myoblasts and human umbilical vein endothelial cells (HUVEC) [56,57]. Light microscopy and electron microscopy results showed that cells uptake SPIO by endocytosis and subsequently store the particles in vacuoles (Figure 2a, b). Each vacuole size and iron particles were shown on electron microscopic images (Figure 2c, d). The results also showed that 100% labeling was achieved when a transfection agent was used, in comparison with only 70% labeling in the absence of the agent [56]. Another transfection that has been used for this purpose is Dendrimer (Superfect™, Valencia, CA, USA), which showed the same results [58].

Weissleder's group [59-61] utilized Tat peptides to improve significantly the cell loading of their iron nanoparticles. Walczak *et al.* [62] recently demonstrated the use of magnetoelectroporation to label cells instantly. The labeling was demonstrated for stem cells from mice, rats and humans; the uptake of iron was in the picogram range and comparable with values obtained using transfection agents. Magnetoelectroporation-labeled stem cells exhibited an unaltered viability, proliferation and mitochondrial metabolic rate. The labeled cells proliferated normally following intrastriatal transplantation and were readily detected by MR imaging *in vivo* [62]. However, Suzuki *et al.* [63] recently reported a longitudinal *in vitro* evaluation of cellular viability, apoptosis, proliferation and cardiac differentiation of magnetically labeled stem cells, and cardiac differentiation was most attenuated by the electroporation labeling technique. Regardless of the labeling method, cell viability, proliferation and differentiation are always a concern, and should be thoroughly investigated for every application.

Thus, despite the spatial resolution demands of visualizing single cells or small numbers of cells, the use of iron nanoparticle makes it more feasible. Moreover, SPIO within each cell are sequestered in multiple vacuoles, causing each cell to act as basic unit of contrast agent. The dimension of a basic contrast agent unit then becomes the size of cells, ranging from 20 to 35 μ m in diameter. In turn, the MRI-detectable effective radius of an iron-laden cell could approach up to 1–1.7 mm in diameter, well within the resolution range of *in vivo* MRI [64].

MR studies using iron-labeled stem and progenitor cells injected into porcine and rat myocardium have been performed *in vivo* [22,24]. In a pig myocardial infarction model, 400 million SPIO-labeled mesenchymal stromal cells (MSC) were intramyocardially injected under X-ray fluoroscopy [10]. Serial MRI verified hypo-enhancing regions of infarct zones (Figure 3). Amado *et al.* [65] implanted feruxomide-labeled stem cells into myocardial

infarction in a pig model and were able to show improvements in cardiac function by MRI and track the cells over a 8-week period. Table 3 summarizes previously published pre-clinical studies of iron-labeled cells in myocardial regeneration.

To date, most MR cell-tracking studies have been applied in heart that is characterized by local cell grafting without extensive, widespread cell distribution. Moreover, acute myocardial infarction is treated by reperfusion therapy, aggravating microvascular obstruction and causing hemorrhage. Hemoglobin degradation products, such as methemoglobin and hemosiderin, have strong magnetic susceptibility effects, which may mimic the signal voids caused by iron-labeled cells [66]. All of these factors can limit MRI application in myocardial regeneration. With MR new sequence development, it may be possible to track therapeutic stem cells in heart by positive-contrast MRI [67].

Many strategies of stem cell therapy in heart are based on intracoronary or intravenous administration of cells, as this pathway is least invasive. Few MR imaging studies have addressed the homing potential of stem cells in disease models following systemic (intravenously injected) administration [68]. Intracoronary or intravenous delivery of labeled cells requires a high concentration of cells to be injected. It is not known how many injected cells will home to the target areas. It is a challenge to monitor the homing, distribution and division of transplanted cells in the area.

There are certain limitations that still need to be considered. (U)SPIO labeling for MRI, despite its potential for reducing direct visualization of cells with high spatial resolution, suffers from relatively low contrast agent sensitivity. At present, MRI is limited to visualizing cells that have been injected directly into myocardium [69]. However, there is no clear quantitative relationship between the size of the signal voids on MR images and transplanted cell number, especially viable cell number, delivered to the myocardium. Moreover, the techniques have no specificity. The iron concentration dilutes with cell division. Therefore, the fate of the iron-loaded cells in the long term is not known. So far, most research has paid attention to the target organs, which are imaged because of coil sensitivity constraints. Other organs might shed light on the destination of iron-labeled cells that are leaving the target organs. In addition, the reproducibility of the iron load per cell of the labeling protocol has not been examined systematically.

Cellular imaging methods, such as PET, SPECT, MRI, optical and ultrasound, are used to map the anatomic locations of specific cells of interest within living tissue and have enormous potential as a powerful means to track therapeutic cells [51]. Cellular imaging agents comprise a targeting component that confers localization and a component that enables external detectability with an imaging modality. The advantages and disadvantages of each of these modalities have been discussed with regard to spatial resolution, temporal resolution, sensitivity and cost [24,34,51]. However, a multimodality approach using combined PET or SPECT and MRI agents may ultimately prove most useful in clinical settings.

Conclusions

MR iron oxide contrast agents are commonly used for labeling and tracking cells. In this review we have considered the work to date on the use of cell-labeling studies to visualize macrophages, which play a crucial role in the inflammatory process underlying the formation of atherosclerotic plaque. In addition, we have reviewed the use of MRI to track transplanted labeled therapeutic cells in myocardial infarction, which may provide answers to questions about cell homing, location and viability following transplantation. The primary motivation for advancing MRI as a platform for cellular imaging is the hope that, in the future, MRI can be used to collect anatomic and cellular information simultaneously. MRI is well suited as a

cellular imaging modality for non-invasive cell tracking because of its tissue characterization, excellent imaging quality and high spatial resolution. Although currently limited by adequate sensitivity, in the future high magnetic field clinical scanners combined with developments in contrast agents may allow improved detection capabilities. Further studies should also aim to build a quantitative relationship between cellular image signal intensity and number of labeled cells.

In summary, while much progress has been made to date, many challenges still face cellular MRI aimed at assessment of atherosclerosis and regenerative myocardial therapies. For this technology to be successful, the combined expertise of basic scientists, clinicians and representatives from industry will undoubtedly be essential.

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Figure 1.

Localization of macrophages to fibrous cap. CD68⁺ macrophages (a) accumulating in shoulder regions of the plaque at 4x and high-power view 80x magnifications and (b) showing both intracellular accumulation (white arrowheads) and extracellular location (black arrowheads) of USPIO particles. Adopted from [28].

Figure 2.

(a, b) Optical microscopic view of pig myoblasts stained with Prussian blue demonstrating the uptake of SPIO particles. SPIO particles are visible as blue iron deposits (arrow) around the nucleus (Nu) in (a). No intranuclear or extracellular uptake of SPIO could be detected. (c, d) Electron microscopy images (14 000x and 20 000x, respectively) with a conglomeration of SPIO within membrane-bound vacuoles (arrow). The particle size of a single SPIO is 150 nm (iron oxide core of 4 nm). The size of the organelle, of the order of 2 μm, demonstrates a high particle density. Adopted from [57].

Figure 3.

Detection of delivery of iron-labeled MSC in a swine myocardial infarct (MI) model. Representative hypointense lesions in fast spin echo (A), fast gradient echo (B) and delayed-enhancement MRI (C) of MR-MSC injection sites (arrows) within 24 h of injection. MR-MSC were injected in the infarct (MI, hyperintense region in C). Long-axis MRI showing hypointense lesions (arrows) caused by MR-MSC acquired within 24 h (D) and 1 week (E) of injection, with inset at right demonstrating expansion of lesion over 1 week. Needle tract (arrow) of MR-MSC is demonstrated in histologic section at 1 week after injection with Prussian blue staining (F) as cells with blue iron inclusions (arrowhead) that are excluded from the nucleus (G). Iron inclusion from DAB-enhanced Prussian blue staining (H) matches co-

labeling with DiI (I) and DAPI fluorescent dyes (J) on adjacent histologic sections at 20x magnification after 24 h following MSC injection in another animal, indicating SPIO are still contained within original MSC. LV, left ventricle; RV, right ventricle. Adopted from [10].

Table 1

Classification of common iron oxide contrast agents, including size, coating, trade-names and associated manufacturers

	Application	Hydrodynamic Size	Coating: Dextran Company: Guerbet Group/Advanced Magnetics	Carboxydextran Schering
SPIO	Myocardial Regeneration	60–180 nm	Ferumoxides (Endorem/Feridex I.V.)	Ferucarbotran (Resovist)
USPIO	Atherosclerosis	15–30 nm	Ferumoxtran-10 (Sinerem/Combidx)	SHU 555C (Resovist S)

Summary of previously published studies of USPIO-labeled plaque in rabbit models of atherosclerosis

Table 2

Reference	Rabbit model	Contrast agent	Field strength (T)	Primary sequence	Outcome
43	WHHL	DDM43/34 (Bayer Schering Pharma)	1.5	3D FLASH selective H ₂ O excitation	USPIO-labeled thrombus imaging (not macrophages)
41	WHHL and NZW (control)	Sinerem (Guerbet Group)	1.5	Conventional 3D MRA	Signal voids along wall of WHHL
44	NZW with injury	Combindex and ferumoxytol (Advanced Magnetix)	4.7 <i>in vivo</i> , 9.4 <i>ex vivo</i>	T1-w SE, T1-w GRE, PD-w (9.4 T)	Combindex remained in blood pool longer and had more uptake
42	WHHL and NZW (control)	Sinerem (Guerbet Group) and ferumoxytol (Advanced Magnetix)	1.5	3D FLASH	Ferumoxtran-10 tended to be better
45	WHHL	DDM43/34 (Bayer Schering Pharma)	3.0	3D fast GRE	No enhancement on images; low uptake shown with histology
46	NZW with injury	Sinerem (Guerbet Group)	1.5	3D fast GRE	Measurement of signal voids allows for quantitative analysis

Summary of previously published studies of iron-labeled stem cell in myocardial regeneration

Table 3

Reference	Therapeutic cell type	Contrast agent	Field strength (T)	Animal model	Outcome
11	Myogenic precursors, autologous	Endorem (Guerbet Group)	1.5	Pigs, intramyocardial	Implant sites were visualized
22	MSC, non-autologous	Iron-fluorescent particles (Bangs Laboratories)	1.5	Pigs, intramyocardial	Guided delivery and labeled cell imaging with MRI
68	Embryonic stem cells, non-autologous	Feridex (Advanced Magnetics)	4.7	Mice, intramyocardial	Labeled cells visualized up to 5 weeks post-injection
Baklanov <i>et al.</i> (2004)	Bone marrow cells, autologous	Feridex (Advanced Magnetics) and microspheres (Cenospheres, Tempe, AZ, USA)	1.5	Pigs, transvascular	Microspheres co-localized with labeled spheres
Kustermann <i>et al.</i> (2005)	Cardiac progenitors, non-autologous	Sinerem (Guerbet Group)	7.0	Mice, intramyocardial	T2* imaging was most sensitive
Leor <i>et al.</i> (2006)	Macrophages, non-autologous (human)	Endorem (Guerbet Group)	0.5	Rats, intramyocardial	Improved vascularization, tissue repair and remodeling
65	MSC, non-autologous	Feridex (Advanced Magnetics, Rochester, IN, USA)	1.5	Pigs, intramyocardial	Long-term engraftment; no rejection