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Stem cell labeling for noninvasive delivery and tracking in cardiovascular regenerative therapy

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Abstract

Clinical and basic scientific studies of stem cell-based therapies have shown promising results for cardiovascular diseases. Despite a rapid transition from animal studies to clinical trials, the mechanisms by which stem cells improve heart function are yet to be fully elucidated. To optimize cell therapies in patients will require a noninvasive means to evaluate cell survival, biodistribution and fate in the same subject over time. Cell labeling offers the ability to image distinct cell lineages *in vivo* and investigate the efficacy of these therapies using standard noninvasive imaging techniques. In this article, we will discuss the most promising cell labeling techniques for translation to clinical cardiovascular applications.

Keywords

cardiac regenerative therapy; cell delivery; labeling; microencapsulation; MRI; stem cells; tracking

Despite great advances in medical therapy and surgical procedures, ischemic heart disease remains the number one cause of heart failure and cardiovascular mortality in developed countries. Owing to the limited intrinsic self-regenerative capacity of the heart, exogenous cell therapies have been explored as a novel treatment strategy to enhance cardiac function and reverse the detrimental remodeling process after myocardial infarction (MI). There is increasing recognition that adult tissues, including bone marrow, adipose tissue and the myocardium itself, harbor stem cell populations with the potential to differentiate into cardiomyocytes, endothelial cells, vascular smooth muscle cells and other terminal cell populations and release cytokines with favorable paracrine effects [1–4]. Recent meta-analyses of clinical trials indicate that intracoronary infusion of bone marrow-derived stem cells after MI is safe and can provide short-term benefit to patients [5–10]. However, improvements in ejection fraction have been small – on the order of less than 3% – and seldom sustained [11–15]. Furthermore, patients with the most severely impaired left ventricular ejection fraction appeared to benefit the most from cellular therapy [11,15]. Whether the lack of long-term benefit is related to an insufficient therapeutic dose, timing of cellular delivery, poor cellular survival and engraftment, or other reasons cannot be determined without a method to monitor cell delivery and track cell retention.

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In this article, we will describe recent advances in stem cell labeling methods with an emphasis on techniques that show the most promise for clinical translation to cardiovascular cellular therapy. The advantages and disadvantages of these stem cell labeling techniques will be discussed. Finally, the potential directions for future development in stem cell labeling and imaging will be highlighted.

Stem cell labeling

Stem cells are undifferentiated cells that are self-renewing, clonogenic and multipotent [16]. Stem cells are present in the embryo (embryonic stem cells) and most adult tissues, such as the skin, skeletal muscle, fat and bone marrow. Mesenchymal stem cells (MSCs) are adult stem cells found primarily in bone marrow that have been shown to participate in vasculogenesis and myogenesis, and are a rich source of angiogenic growth factors [17]. Recent studies have shown that under appropriate chemical and physical stimuli, stem cells from different origins can differentiate into a wide range of cell types, including cardiomyocytes and endothelial cells [18]. In line with these findings, stem cell-based approaches to cardiac repair were developed to assist the limited regenerative capacity of the heart after an ischemic event. Promising improvements in cardiac function in a limited number of animal studies using histopathological analysis propelled rapid translation of stem cell therapies into cardiovascular clinical studies [19]. However, early clinical results were not as impressive as preclinical studies, prompting the development of stem cell labeling techniques that could be combined with noninvasive imaging to determine cell delivery success, engraftment potential and cellular fate. The ideal labeling technique would need to be biocompatible, highly specific to the target cells, and sensitive for detection of a single or few cells. Traditionally, there are three methods for cellular labeling: receptor-based techniques, reporter gene labeling and direct labeling techniques. Due to the lack of specific cellular markers that are retained on stem cells as they differentiate into a mature phenotype, receptor-based labeling techniques are not for the most part amenable to cardiovascular stem cell therapy. Moreover, due to safety concerns regarding genetic cell manipulation, reporter gene labeling is unlikely to be adopted for clinical translations in cardiovascular trials in the near future. Thus, direct labeling techniques have formed the bulk of both preclinical and clinical methods for cardiovascular applications.

Direct stem cell labeling

Direct labeling is the most straightforward and widely used method, and is accomplished by introducing a marker into the cells or onto the cell surface before implantation. The simplest method is direct incubation of cells with the markers. For example, stem cells have been effectively labeled with fluorescent probes for optical imaging [20], superparamagnetic iron oxide (SPIO) for MRI [21], and radiotracers for radionuclide imaging [22].

Fluorescent probe direct labeling

Direct cell labeling with fluorescent probes (fluorescent dyes and nanoparticles) forms the basis from which many other direct labeling techniques were developed. Typically, either the cell nuclei (e.g., 4',6-diamidino-2-phenylindole [DAPI]) or cytoplasm/cell membrane is labeled with a fluorescent probe (e.g., chloromethyl-benzamido octadecyl indocarbocyanines [CM-DiI]). Due to their stability and nontoxic properties, CellTracker™ fluorescent probes have gained popularity for stem cell labeling. These probes contain a chloromethyl or bromomethyl group that reacts with thiols and, thus, are transformed into a cell-impermeant, fluorescent dye–thioether adduct. Fluorescent probe labeling offers direct visualization of stem cell monolayer and spheroids with optical imaging at high spatial resolution [23]. As such, it is often used with other cellular labels, such as radionuclide tracers, for postmortem histology studies to validate the noninvasive imaging results.

Quantum dots—A new class of fluorescence probes, quantum dots (QDs), have been recently adopted for *in vitro* and *in vivo* stem cell labeling and bioimaging [24–27]. QDs are multifunctional, inorganic, fluorescent, semiconductor nanoparticles. Compared to organic fluorescent dyes, QDs provide unique optical advantages, including narrower band emission and broader band excitation with a high quantum yield, exceptional photostability and resistance to chemical and metabolic degradation [28]. These properties not only make QDs suitable for cell labeling, but provide multicolor optical imaging potential. Both MSCs and embryonic stem cells have been effectively labeled with QDs using passive incubation or peptide-mediated uptake (QTracker) [24,26,29]. *In vitro* studies have demonstrated biocompatibility at low concentrations and functional integration of QD-labeled MSCs in cardiac myocyte coculture systems [26]. A few studies have also demonstrated efficient labeling without alterations to stem cell viability, proliferation and differentiation capacity [24,30]. In addition, QDs did not transfer to adjacent stem cells due to their large size (~10 nm) relative to gap junctions (~1 nm) [29]. *In vivo*, QD-labeled stem cells could be detected with multiplex optical imaging up to 2 weeks after injection in mice, with sensitivity of approximately 1×10^5 cells [24]. For cardiovascular applications, human MSCs were first labeled with intracellular QDs and transplanted into the hearts of dogs and rats [29]. QD-labeled human MSCs were retained for at least 8 weeks after delivery, as shown by postmortem histology [29]. However, the long-term effects of QDs on stem cell differentiation and proliferation remain largely unknown. Moreover, the nonspecific binding of QDs to multiple cells and clearance mechanism from the body of the highly toxic cadmium base of QDs may hamper translation into the clinic.

Near-infrared probes—Because of light absorption and scattering in tissue, all visible light-based optical imaging techniques have an inherent limited depth of penetration. New emerging fluorochromes that have long tissue penetration depth may be able to overcome these problems. From this perspective, near-infrared (NIR; 700–1000 nm wavelength) fluorophores have potential for clinical application. Intracoronary delivery of IR-786 (a NIR fluorophore)-labeled bone marrow-derived MSCs has been successfully tracked *in vivo* 90 min after injection in a swine model of MI [31]. Due to the practical penetration depth of NIR fluorophores of 4–10 cm, cardiac imaging in humans will still present a formidable challenge.

Magnetic labeling

Paramagnetics—Stem cell labeling with magnetic contrast agents enables noninvasive tracking in 3D using MRI. Cell labeling with paramagnetic metals, such as gadolinium and manganese, enables the visualization of cells on T₁-weighted MRI (i.e., hyperintense contrast). However, internalized paramagnetic agents often have less access to extracellular water and, thus, have reduced efficiency at altering image contrast [32]. Because unchelated paramagnetic metals are toxic, clinical formulations, such as gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) and gadolinium tetraazacyclododecanetetraacetic acid (Gd-DOTA), are always chelated for biocompatibility. However, the potential for metal dechelation of paramagnetically labeled cells that fail to engraft and are removed from the body has hampered clinical utilization of these agents.

Superparamagnetic iron oxides—Superparamagnetic iron oxide nanoparticles are the most widely used stem cell labeling agents, and at picogram concentrations impart negative contrast (hypointensities) in T₂*-weighted images [33]. Stem cells can be directly labeled with SPIOs with no or minimal side effects on cell viability, proliferation and differentiation capacity by two techniques: magnetofection [21,34] or magnetoelectroporation [35].

Magnetofection uses a cationic transfection agent, such as poly-L-lysine or protamine sulfate, to coat the anionic SPIOs via electrostatic interaction [21,34]. The cells are then incubated with the transfection agent–SPIO complex in culture for approximately 18–48 h resulting in stable incorporation of the SPIO endosomes [36]. Owing to particle clustering of SPIOs within endosomes, T2* effects are more pronounced and, hence, the signal hypointensifies [32].

Since SPIO-labeled neural stem cells have been tracked using MRI in patients with brain trauma [37], clinical translation to cardiovascular applications appears possible. For preclinical cardiovascular applications, bone marrow-derived MSCs were labeled with a clinical formulation of SPIOs (Feridex, Berlex Inc., NJ, USA) using magnetofection and tracked for up to 3 weeks *in vivo* using a clinical 1.5 T MRI scanner after transmural injection in a swine model of acute reperfused MI (Figure 1) [38]. Subsequent studies have shown tracking of direct intramyocardial injections of stem cells over 2 months after administration in large animals [33,39]. The power of MRI to distinguish viable from infarcted myocardium has been used to target stem cells to the peri-infarcted region using MRI-visible catheters and delayed contrast-enhanced viability maps in large animal models of ischemic heart disease (Figure 2) [33,39,40].

Alternatively, fusion of MRI viability maps with live x-ray fluoroscopic images may provide another means to target stem cells and determine delivery success [41]. Although many studies have shown that SPIO labeling does not affect stem cell metabolism and viability at clinically relevant labeling doses [42,43], *in vitro* osteogenesis and chondrogenesis but not adipogenic differentiation of MSCs was reduced after SPIO labeling in a dose-dependent manner [44,45]. Since no *in vitro* assay exists for MSC differentiation down a cardiac lineage, the consequences of SPIO labeling for use in cardiovascular applications remain unknown. One of the advantages of magnetofection is that nonphagocytic cells will not readily take up the SPIO [46]. Thus, when exogenously SPIO-labeled cells fail to engraft, there is little concern that the endogenous myocytes or endothelial cells will take up the SPIO left behind by dying cells. Moreover, there is evidence that uptake of SPIOs by resident macrophages shifts these cells towards a more anti-inflammatory phenotype with decreased production, which may have an added benefit in ischemic heart disease [47]. However, the relatively long incubation time required for magnetofection may limit clinical applicability in acute ischemic applications using autologous cells.

Magnetoelectroporation, a technique using low pulse voltage to induce endocytosis of SPIOs, provides a rapid method to label stem cells with MRI contrast agents without requiring transfection agents. A large number of stem cells from different species (e.g., mice, rats and human) can be efficiently labeled with SPIOs in a few minutes without loss of cell viability and alteration of cell metabolism [48]. As no transfection agents are needed, the potential regulatory hurdles for clinical acceptance are also reduced. Although no clinical trials using magnetoelectroporation have been initiated for cardiac stem cell therapy, animal studies using bone marrow-derived stem cells labeled with SPIO have been investigated in a rabbit model of peripheral arterial disease [49]. Furthermore, these studies have used a special method of MRI using a hyperintense signal rather than a hypointense signal, which enabled easy visualization of SPIO-labeled cells from other potential metallic image artifacts (Figure 3) [50]. Follow-up MRI angiography at 1 week post-MSC injection showed the development of collateral vasculature from the deep femoral artery (Figure 4) [32].

While SPIO labeling has been used to view single cells in the brain and fixed tissue by high-field MRI [51,52], cardiac and respiratory motion imposes detection limits on clinical scanners on the order of a minimum of 10^4 – 10^5 cells [33]. Absolute quantification of SPIO-

labeled cells is also problematic since the cellular division will alter the amount of label per cell, and cell migration will also alter the spatial distribution. As only 3–5% of the exogenously administered stem cells are anticipated to survive long term in the heart [53], an ongoing debate is whether MRI at later time points represents live transplanted stem cells or residual iron in the extracellular space or within macrophages [54–57].

Fluorinated labels—Fluorinated nanoparticles have also been investigated for direct cell labeling for MRI detection [58,59]. Because ^{19}F atoms are naturally absent in the body, there is no background signal to confound detection of the labeled stem cells and sensitivity is thereby enhanced [58,60]. Anatomical localization can be achieved by conventional proton MRI. However, the requirement for specialized hardware and software to image nonproton species will limit rapid adoption of these techniques.

Radionuclide direct labeling

Similar to ^{19}F MRI, the lack of radioactive isotopes in the body leads to a high sensitivity (10^{-11} to 10^{-12} mol/l) to radiotracers used for direct cell labeling. ^{111}In oxyquinoline (oxine), $^{99\text{m}}\text{Tc}$ and ^{18}F fluoro-2-deoxy-D-glucose (FDG) are widely used in the clinic for positron emission tomographic (PET) or single photoelectron computed tomographic (SPECT) imaging with a spatial resolution of 1–2 mm. Like MRI and optical imaging, stem cells can be labeled by direct incubation with the radioactive agent [61,62] or receptor-mediated binding [63]. However, receptor-mediated methods have the same issues of a lack of a good cell surface marker. Serial tracking of stem cells labeled with radiotracers will be governed primarily by the radioisotope decay, with sensitivity declining beyond detectability after 4–5 half-lives. With the development of combined radionuclide/computed tomography clinical imagers, anatomical localization of radioisotope hot spots has been facilitated.

^{111}In oxine—Among the many potential radioisotopes, labeling cells with ^{111}In oxine has been used most extensively for cardiovascular applications due to its favorable half-life (~2.8 days) and prior US FDA approval for labeling lymphocytes to study infection and inflammation [64]. Direct incubation of stem cells with ^{111}In oxine, which passively diffuses into cells, dissociates and becomes trapped in the cytoplasm [65], can be performed in less than 2 h. Using a canine reperfused MI model, our group has demonstrated the redistribution of ^{111}In oxine- and SPIO-labeled MSCs after intravenous injection from initial localization in the lungs to the liver and spleen within 24–48 h after injection [62]. Focal and diffuse uptake of MSCs in the infarcted myocardium was detectable using a clinical SPECT/CT scan within 24 h after injection and persisted up to 7 days after stem cell delivery. However, MRI, with a much lower sensitivity than SPECT, was unable to detect the SPIO-labeled MSCs in the myocardium (Figure 5) [62]. Depending on the radiotracer used and cell type, minimum detection limits of direct radiotracer labeling vary from 2900 to 25,000 cells [62,66]. Similar observations of radiolabeled stem cells homing to the infarcted heart have been observed after intravenous, intramyocardial, intraventricular, intracoronary and retrograde coronary venous delivery [67–70]. Whilst there is some debate regarding the best delivery method [71,72], all studies with radiolabeled cells have shown poor retention of stem cells in the heart (less than 5% of the injected dose) regardless of the animal model [68–71].

^{18}F -FDG—Due to the ready availability of FDG, it is an attractive alternative to ^{111}In oxine. However, it has a much shorter half-life, which limits serial imaging potential. The first human cardiovascular cellular study using radiolabeled stem cells demonstrated a higher retention of ^{18}F -FDG-labeled CD34⁺ bone marrow mononuclear cells (BMNCs) in the infarcted myocardium than nonselected BMNCs after intracoronary delivery [73]. In

another clinical study involving 20 MI patients, PET imaging indicated that 0.2–3.3% of intracoronary infused ^{18}F -FDG-labeled hematopoietic stem cells accumulated within the infarcted myocardium at 2 h postdelivery [74].

Radiotracer labeling limitations

Major concerns regarding direct radiotracer labeling include the potential radiation damage to the cells and leakage of radiotracers from the cells. Whereas there are some studies showing significant interference of proliferation and function of ^{111}In oxine-labeled hematopoietic progenitor cells [68], high cell viability and retention of cellular function can be achieved by carefully selecting the activity of the radiotracer [62,66]. In addition, cytotoxic effects of ^{111}In on human stem cells may be time-dependent rather than dose-dependent, raising concerns that assessment of stem cell viability immediately after radiolabeling may give a false sense of cell integrity [75].

Loss of the radiolabel from viable cells is also variable and may lead to inaccurate reporting of stem cells based on radioactivity that is no longer bound to the exogenously administered cells [66,76]. Thus, excretion of the radiotracer will be important when short-term tracking of stem cell biodistribution is performed. If radiochemistry issues can be overcome, more long-lived radiotracers, such as ^{64}Cu -pyruvaldehydebis(*N*4-methylthiosemicarbazone), show promise as a means to perform tracking over longer time periods. Nonetheless, as with other direct labeling methods, cell division and migration present significant hurdles to detection and absolute quantification of cell numbers at later time points after delivery due to radiodecay and cell division. The impact of radioactivity in nontarget organs may also be a consideration when translating these methods to clinical trials.

In summary, direct labeling provides an initial localization of the implanted stem cells for confirming targeting of cells to an organ of interest. Due to the high sensitivity, radiotracer labeling can provide a means for studying whole-body biodistribution of stem cells after an intravenous infusion. Because of stem cell migration and early stem cell destruction, direct labeling techniques are ideally suited for short-term tracking of infused stem cells before signal attenuation or uptake of the tracer by endogenous cells. The biggest confounding factor with direct cell labeling is that dead cells can still generate signals for days to weeks either due to their presence in cellular debris or uptake by phagocytic cells [77].

Microencapsulation for cell tracking

While direct cell labeling enables the monitoring of cell delivery and engraftment by noninvasive imaging, a large number of cells that are administered fail to engraft. For cardiovascular applications, ischemic injury and lack of a sufficient nutritional supply may contribute to the substantial cell death after delivery [20]. For treatment of acute ischemic disease, it would be desirable to have off-the-shelf cellular therapeutics available such that allogeneic or xenogeneic cell-based therapies could be used. However, immunorejection of these mismatched cellular therapies is a concern. To prevent immunodestruction in islet cell transplantation, microencapsulation was employed to create a semipermeable membrane that would restrict large immunoglobulins and immune cells but permit the free diffusion of insulin and other nutrient and waste products [78]. A recent study has also demonstrated that stem cell retention in myocardium could be increased by coadministering a matrigel scaffold, which provides better cell-to-cell contact and adhesion [79].

Recently, our group has modified the alginate–poly-L-lysine–alginate microencapsulation method to include the addition of contrast agent to enable monitoring of microencapsulated cell delivery [80–86]. In so doing, higher concentrations of the contrast agent could be added to the capsule without affecting cell viability and accordingly imaging sensitivity was

enhanced. The first MRI-visible microcapsules were developed for pancreatic islet cell delivery using SPIO (20% weight volume) [80]. Infusion of these 'magnetocapsules' through the portal vein into the liver could be monitored in real-time using MRI.

Since the vast majority of minimally invasive cardiovascular interventions are performed using angiography, it would be advantageous to monitor cell delivery directly under x-ray fluoroscopy. However, radiopaque contrast agents are highly cytotoxic. By restricting a radiopaque contrast agent to the microcapsule, x-ray-visible cell therapies can be envisioned [81,84]. Initial studies have demonstrated that alginate microcapsules containing barium sulfate remain visible on x-ray fluoroscopy for several months (Figure 6) [81,84]. However, concerns regarding the biocompatibility and eventual elimination of barium sulfate from the body have led to the development of other x-ray-visible formulations. Formulation of the alginate microcapsules has been an area of intense interest with the addition of substances such as perfluorocarbons to increase oxygenation within the microcapsules [87]. A slight modification to this formulation has led to a microcapsule that is potentially visible by ^{19}F MRI, x-ray, CT scan and ultrasound [82,85]. Microencapsulation may also provide an additional benefit of transfixing stem cells to a specific region. In such cases, targeting of the stem cells to the appropriate location by direct visualization of the microcapsule by noninvasive imaging will be important to ensure the optimal therapeutic efficacy. On the other hand, microencapsulated stem cells cannot directly participate in tissue regeneration unless replication of the stem cells or degradation of the capsule occurs. Thus, microencapsulation techniques may be best utilized for using stem cells to provide cytokines to reduce myocardial inflammation and injury and assist with recruiting native cells for repair [88–91]. However, as the first x-ray-visible method for tracking stem cells, microencapsulated x-ray-visible cellular therapy may enhance cell survival using a clinical imaging modality that is currently preferred for cardiovascular interventions and cellular delivery.

Expert commentary

Direct labeling of stem cells can be performed with a variety of agents that enable the visualization of stem cells by noninvasive clinical imaging techniques such as PET, SPECT and MRI. Preclinical studies using these techniques have yielded vital information about cell homing, dosing regimes and engraftment efficacy based on delivery route that will guide future cardiovascular clinical cellular therapy trials. Whereas all direct labeling techniques have advantages and disadvantages (Table 1), the ability to immediately determine the success of delivery and short-term tracking of the cells warrants adoption of these techniques in clinical trials. To lessen the impact of potential negative side effects of labeled cells, initial studies should employ a mixed therapeutic strategy using both labeled and nonlabeled cells. As labeling strategies evolve and are enhanced, these techniques offer the potential for not only the production of better cellular therapeutics and dosing schemes, but also the ability to personalize cell therapy for the individual patient with cardiovascular disease and guide treatment and retreatment strategies.

Five-year view

The success of stem cell labeling and tracking strategies will require the use of both sensitive and specific agents to target cells that can be combined with anatomical and functional imaging with sufficient sensitivity and spatial resolution. Each stem cell labeling and imaging technique discussed here has many benefits that are not without drawbacks (Table 1). From the imaging point of view, optical techniques, such as fluorescence and bioluminescence imaging, are the most sensitive imaging modalities with detectability of 10^{-9} – 10^{-12} mol/l and 10^{-15} – 10^{-17} mol/l, respectively [92]. However, the limited tissue

penetration and low energy photon attenuation of optical techniques that prevents visualization of deep structures such as blood vessels and the heart are technical challenges that are unlikely to be resolved in the next 5 years.

Radionuclide labeling, although sensitive (10^{-8} – 10^{-9} mol/l), cannot be used for long-term stem cell tracking due to radioactive decay and lacks the interactivity needed for interventional cardiac procedures. However, familiarity with radionuclide labeling of leukocytes in nuclear medicine will likely result in more clinical trials where a portion of the cellular therapeutic is radiolabeled for short-term tracking.

Magnetic resonance imaging is a tempting choice for clinical translation of stem cell labeling due to the excellent soft-tissue contrast, the potential for interventional delivery and the relative ease of SPIO labeling. However, the low sensitivity (10^{-3} – 10^{-5} mol/l), the issue of patients with devices, such as pacemakers, that are frequently MRI-incompatible, the high cost of imaging, and the recent withdrawal of clinical formulations of SPIOs in the USA will probably limit clinical translation.

Overall, significant hurdles for clinical translation remain until safety issues regarding the manipulation of stem cells by direct labeling or tissue engineering are addressed. As the development of stem cell labeling and imaging gains momentum, smart labeling strategies will enable the optimization of cell therapies with respect to delivery location, dose, timing and cell type. Short-term efforts over the next 5 years will most likely concentrate on the development of stem cell labeling techniques that enable the use of x-ray angiographic suites for interactivity, with visual overlays from MRI, CT and ultrasound for 3D anatomical detail. Microencapsulation techniques offer the first stem cell labeling method that would enable direct x-ray visualization. The potential for multimodality imaging of microcapsules offers a means to perform follow-up imaging without ionizing radiation and has the added benefit of enhanced survival of allogeneic stem cells. Due to the therapeutic size, however, applications for peripheral arterial disease are more likely than cardiac applications in the next 5 years.

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Figure 1. Long-axis MRIs of a swine model of reperfused myocardial infarction showing hypointense lesions (arrows) caused by magnetically labeled mesenchymal stem cells (A) 24 h after mesenchymal stem cell injection. (B) 1 week postinjection. Inserts at right demonstrate expansion of lesion over 1 week. LV: Left ventricle; RV: Right ventricle. Adapted with permission from [38].

Figure 2. MR images of an infarcted dog showing the decrease in circumferential extent of the infarcted myocardium

(A) Dynamic contrast enhanced MRI (DCE-MRI) of infarcted myocardium (hyperenhancing tissue) before superparamagnetic iron oxide-labeled mesenchymal stem cell transplantation. (B) DCE-MRI at 1 week postinjection. (C) DCE-MRI at 2 weeks postinjection. Viable myocardium (arrow) is present at the injection site at 2 weeks. (D) Fast gradient echo (FGRE) image of the same slice as (C) showing superparamagnetic iron oxide-labeled mesenchymal stem cell injection.

Figure 3. MR images of a rabbit peripheral arterial disease model that received superparamagnetic iron oxide-labeled mesenchymal stem cells

(A) A conventional gradient echo cross-sectional MRI of a rabbit thigh. The hypointensities are hard to distinguish from the diaphysis of the femur and other hypointense artifacts. (B) Positive-contrast MRI of the rabbit thigh. Two injections of superparamagnetic iron oxide-labeled mesenchymal stem cells are visualized as dumbbell-shaped hyperintensities and can be distinguished from a metallic coil (large arrow) that was used to occlude the femoral artery in this rabbit peripheral arterial disease model.

Figure 4. MR images of a rabbit peripheral arterial disease model showing the location of labeled stem cells relative to vasculature

(A) MR angiogram of a peripheral arterial disease rabbit at 24 h postocclusion showing the region of superficial femoral artery occlusion (arrow). (B) Mesenchymal stem cells that were superparamagnetic iron oxide-labeled using magnetoelectroporation were injected into the medial thigh. Two injection sites imaged immediately after injection are shown with positive contrast imaging (arrows) with MR angiogram overlay. Adapted with permission from [32].

Figure 5. Registration of SPECT/CT with MR images of the heart in a dog with a reperfused myocardial infarction receiving 111 -indium oxine and SPIO-labeled mesenchymal stem cells (A) Short axis view of alignment of CT (gold) with MRI (grayscale) and SPECT (red) showing focal uptake in the septal region of the MI in a representative dog. (B) Focal uptake on SPECT (red) in another animal demonstrating localization of the mesenchymal stem cells (MSCs) to the MI in the short-axis (B) and long-axis (C) views. SPECT, due to the higher sensitivity, was able to detect the labeled MSCs whereas MRI was unable to detect the SPIO-labeled MSCs.

MI: Myocardial infarction; SPECT: Single photoelectron computed tomographic; SPIO: Superparamagnetic iron oxide.

Adapted with permission from [62].

Figure 6. X-ray angiograms of a rabbit hindlimb

(A) Before occlusion. (B) After left femoral artery occlusion via a platinum coil (black arrow). X-ray-visible microencapsulated mesenchymal stem cells injected intramuscularly in the medial thigh appear as radiopacities (white arrows). A quarter (Q) is used for reference measurements.

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Table 1
Key features of stem cell direct labeling and imaging

Labeling method	Imaging modality	Probes	Sensitivity	Advantages	Disadvantages
Fluorochrome	Optical	Fluorophores QDs NIR	10^{-9} – 10^{-12} mol/l	High sensitivity Low cost No ionizing radiation Suitable for short-term tracking	Photon attenuation Poor tissue penetration Potential high background due to autofluorescence
MRI contrast agents	MRI	Gadolinium Iron oxides	10^{-3} – 10^{-5} mol/l	High spatial resolution Soft-tissue detail No ionizing radiation Potential for medium-term tracking	Low sensitivity Expensive
Radionuclides	PET/SPECT	^{111}In $^{99\text{m}}\text{Tc}$ ^{18}F -FDG	10^{-8} – 10^{-9} mol/l	High sensitivity Hot spot imaging Clinically approved agents available	Ionizing radiation Expensive Requires CT scan or MRI for anatomical detail Tracking limited by tracer half-life
Microencapsulation	X-ray CT US MRI	BaSO_4 Perfluorocarbon Iron oxides Gold Gadolinium	1–5 microcapsules	High sensitivity due to ability to load a high concentration of contrast agent Little or no ionizing radiation Immunoprotection/enhanced cell survival Suitable for long-term tracking	Large size of capsule restricts cell incorporation

F: Fluorine; FDG: Fluoro-2-deoxy-D-glucose; In: Indium; NIR: Near infrared; PET: Positron emission tomography; QD: Quantum dot; SPECT: Single photoelectron computed tomography; Tc: Technetium; US: Ultrasound.