

Tracking stem cells for cardiovascular applications *in vivo*: focus on imaging techniques

Despite rapid translation of stem cell therapy into clinical practice, the treatment of cardiovascular disease using embryonic stem cells, adult stem and progenitor cells or induced pluripotent stem cells has not yielded satisfactory results to date. Noninvasive stem cell imaging techniques could provide greater insight into not only the therapeutic benefit, but also the fundamental mechanisms underlying stem cell fate, migration, survival and engraftment *in vivo*. This information could also assist in the appropriate choice of stem cell type(s), delivery routes and dosing regimes in clinical cardiovascular stem cell trials. Multiple imaging modalities, such as MRI, PET, SPECT and CT, have emerged, offering the ability to localize, monitor and track stem cells *in vivo*. This article discusses stem cell labeling approaches and highlights the latest cardiac stem cell imaging techniques that may help clinicians, research scientists or other healthcare professionals select the best cellular therapeutics for cardiovascular disease management.

KEYWORDS: cardiovascular disease ■ cell labeling ■ cell tracking ■ computed tomography ■ MRI ■ optical imaging ■ radionuclide imaging ■ stem cells

Coronary heart disease is usually a result of atherosclerotic progression, which is characterized by the narrowing of small blood vessels that supply blood and oxygen to the heart. Despite recent advances in pharmacotherapy and interventional procedures, coronary heart disease remains the leading cause of heart failure in the western world [1]. Owing to the limited regenerative capacity of cardiac cells and subsequent detrimental ventricular remodeling after myocardial infarction, heart transplantation is currently the only definitive treatment for end-stage cardiovascular disease. Yet, the lack of suitable organs and high cost limit the number of heart transplants that are performed. Stem and progenitor cells possess the capability of self-renewal and can differentiate into organ-specific cell types with the potential to reconstitute damaged organ systems. Thus, many types of stem cell therapeutics have emerged for cardiac repair with rapid translation to clinical trials, sometimes without extensive preclinical testing.

Preclinical cardiovascular stem cell studies in animal models of myocardial infarction have shown encouraging results ranging from restoration of ventricular function to improved myocardial perfusion [2]. The results from clinical trials, however, have been mixed (TABLE 1). The BOOST trial demonstrated the safety and feasibility of intracoronary infusion of autologous bone marrow cells and early improvements of left ventricular function [3], but the improvements were not sustained after 5 years [4]. In

addition, other double-blinded, randomized and placebo-controlled trials showed little or no long-term benefits [5–7]. Each clinical trial has involved a relatively small number of patients with a primary end point of safety. However, meta-analyses of these trials have generally shown a slight positive trend towards improved cardiac function [8–12].

Noninvasive imaging, such as echocardiography, MRI, PET, SPECT and CT, can play a pivotal role in tracking stem cell engraftment and expand these imaging modalities beyond merely assessing cardiac function. This article will provide an overview of the fundamentals of stem cell labeling techniques and discuss the advantages and disadvantages of each imaging modality with a focus on those with the greatest potential for clinical translation.

Stem cell labeling

Embryonic stem cells (ESCs), adult stem cells and induced pluripotent stem cells are the three major types of stem/progenitor cells that possess the ability to provide the building blocks for cardiovascular system repair. Owing to the ethical issues and the potential danger of teratoma formation of undifferentiated ESCs and the low reprogramming efficiency of induced pluripotent stem cells, adult stem cells (e.g., bone marrow-derived mesenchymal stem cells, skeletal myoblasts and cardiac/endothelial progenitor cells) have dominated current clinical investigations (TABLE 1).

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Table 1. Clinical trials using stem cells for the treatment of cardiovascular disorders.

Trials	Conditions treated	n	Cell types	Delivery routes	Imaging modality					Ref.
					Angio	Echo	SPECT	PET	MRI	
BOOST	AMI	60	Autologous BMC	Intracoronary	X	X			X	[3,4,102–105]
COMPARE-AMI	AMI	14	CD133+ enriched BMC	Intracoronary			X		X	[106]
REPAIR-AMI	AMI	204	BM progenitors	Intracoronary	X					[107]
REGENT	AMI	200	Selected (CD34+CXCR+) BMC, unselected BMC	Intracoronary	X				X	[108]
PROTECT-CAD	CAD	28	Autologous BMC	Endomyocardial	X		X		X	[109]
Chan <i>et al.</i> (subgroup of PROTECT-CAD)	CAD	12	Autologous BMC	Endomyocardial					X	[110]
Brehm <i>et al.</i>	CMI	18	Autologous BMC	Intracoronary	X	X	X	X		[111]
Fernandez-Aviles <i>et al.</i>	CMI	20	Autologous BMC	Intracoronary	X	X			X	[112]
Fuchs <i>et al.</i>	CMI	10	Autologous BMC	Transendocardial		X	X			[113]
Galinanes <i>et al.</i>	Transmural MI	14	Autologous BMC	Transmyocardial		X				[114]
Hamano <i>et al.</i>	CMI	5	BMC	Transmyocardial	X	X				[115]
Strauer <i>et al.</i>	Heart failure	391	BMC	Intracoronary	X					[116]
IACT	CAD	18	Autologous BMC	Intracoronary	X		X	X		[117]
Janssens <i>et al.</i>	CMI	67	Autologous BMC	Intracoronary		X		X	X	[6]
Kuethe <i>et al.</i>	AMI	5	BMC	Intracoronary	X	X				[118]
Lunde <i>et al.</i>	STEMI	100	Mononuclear BMC	Intracoronary	X	X	X		X	[119]
Perin <i>et al.</i>	CMI	21	Mononuclear BMC	Transendocardial		X	X			[120,121]
Silva <i>et al.</i>	Heart failure	5	Mononuclear BMC	Transendocardial		X	X			[122]
Arnold <i>et al.</i>	STEMI	37	Mononuclear BMC	Intracoronary	X	X				[123]
van Ramshorst <i>et al.</i>	CMI	50	BMC	Transendocardial			X		X	[124]
Strauer <i>et al.</i>	AMI	20	Mononuclear BMC	Intracoronary	X	X				[125]
FOCUS	CAD	87	Mononuclear BMC	Intramyocardial			X			[126]
Assmus <i>et al.</i>	CMI	75	CPC/BMC	Transcoronary	X	X			X	[127]
TOPCARE-AMI	AMI	20	CPC/BMC	Intracoronary	X	X		X	X	[128–130]
Bartunek <i>et al.</i>	AMI	35	CD133+ BMC	Intracoronary	X			X	X	[131]
Goussetis <i>et al.</i>	CMI	8	CD133+ BMC/CD133- CD34+ BMC	Intracoronary	X	X	X			[132]
Stamm <i>et al.</i>	AMI	12	CD133+ BMC	Transendocardial	X	X				[133,134]
Losordo <i>et al.</i>	CMI	24	Autologous CD34+, G-CSF mobilized PBC	Transendocardial			X			[135]
TOPCARE-CHD	CMI	75	PBC, BMC	Intracoronary	X		X		X	[127]
Choi <i>et al.</i>	AMI	73	G-CSF mobilized PBC	Intracoronary	X	X			X	[136]
MAGIC Cell-DES	AMI	96	G-CSF mobilized PBC	Intracoronary	X				X	[137]
Chachques <i>et al.</i>	MI	20	Autologous skeletal myoblast	Intramyocardial		X				[138]
Dib <i>et al.</i>	MI	30	Autologous skeletal myoblast	Epicardial		X		X	X	[139]

AMI: Acute myocardial infarction; Angio: X-ray angiography; BMC: Bone marrow cell; CAD: Coronary arterial disease; CMI: Chronic myocardial infarction; CPC: Circulating blood-derived progenitor cell; Echo: Echocardiography; EPC: Endothelial progenitor cell; G-CSF: Granulocyte colony stimulating factor; MI: Myocardial infarction; MSC: Mesenchymal stem cell; PBC: Peripheral blood cell; STEMI: ST-elevation myocardial infarction.

Table 1. Clinical trials using stem cells for the treatment of cardiovascular disorders (cont.).

Trials	Conditions treated	n	Cell types	Delivery routes	Imaging modality					Ref.
					Angio	Echo	SPECT	PET	MRI	
Herreros <i>et al.</i>	CAD	12	Autologous skeletal myoblast	Intramyocardial		X		X		[140]
Ince <i>et al.</i>	MI	12	Autologous skeletal myoblast	Intramyocardial		X				[141]
Menasche <i>et al.</i>	MI	10	Autologous skeletal myoblast	Epicardial		X		X		[142]
POZNAN	Heart failure	10	Autologous skeletal myoblast	Intramyocardial		X				[143]
Siminiak <i>et al.</i>	AMI	10	Autologous skeletal myoblast	Intramyocardial		X				[144]
Smits <i>et al.</i>	Heart failure	5	Autologous skeletal myoblast	Intramyocardial	X	X			X	[145]
MAGIC	CMI	97	Autologous skeletal myoblast	Epicardial	X					[5]
Chen <i>et al.</i>	AMI	69	Autologous MSC	Intracoronary	X	X	X			[146]
Chen <i>et al.</i>	CMI	55	Autologous MSC	Intracoronary	X					[147]
Hare <i>et al.</i>	AMI	53	Allogeneic MSC	Intravenous		X			X	[148]
Katritsis <i>et al.</i>	AMI	22	EPC/MSC	Transcoronary			X			[149]
Lasala <i>et al.</i>	Angina pectoris	10	EPC/MSC	Intracoronary	X	X				[150]

AMI: Acute myocardial infarction; Angio: X-ray angiography; BMC: Bone marrow cell; CAD: Coronary arterial disease; CMI: Chronic myocardial infarction; CPC: Circulating blood-derived progenitor cell; Echo: Echocardiography; EPC: Endothelial progenitor cell; G-CSF: Granulocyte colony stimulating factor; MI: Myocardial infarction; MSC: Mesenchymal stem cell; PBC: Peripheral blood cell; STEMI: ST-elevation myocardial infarction.

For noninvasive tracking of stem cells, the label should ideally be [13]:

- Inert and/or biocompatible
- Highly specific to target cells
- Detected at the level of a single or few cells

At present, there is no single label/probe that meets all these requirements in combination with a single imaging technique. However, each imaging modality has desirable characteristics that, in part, may drive the choice of stem cell labels (TABLE 2). In general, stem cell labeling falls into two primary methods: direct/physical labeling and indirect/genetic labeling.

Direct cell labeling typically requires incubation of a cell with the label of interest. The label may then be bound on the cell surface or internalized by the cell. As such, direct labeling is the simplest and most straightforward method that can be performed with a variety of probes to enable visualization of stem cells by noninvasive clinical imaging techniques [14]. For example, direct stem cell labeling using superparamagnetic iron oxide particles (SPIOs) and radioactive tracers (e.g., ¹¹¹In and ¹⁸F) has been widely used for MRI [15–17] and radionuclide imaging [18,19], respectively. Optical imaging of stem cells labeled with fluorescence probes

(e.g., near-infrared fluorophores and quantum dots) has been reported in animal models [20,21]. The primary disadvantages of direct labeling are label dilution/loss with cell division or cell death and inability to differentiate viable from dead cells due to retained label *in situ* or native cell uptake of label from dead cells. Thus, direct cell labeling is best for confirmation of cell delivery success and short-term localization of cells after delivery.

In contrast to direct stem cell labeling, genetic labeling of stem cells with reporter genes involves insertion of genetic material that encodes for an enzyme, receptor or protein that can then be imaged directly or interacts with a reporter probe to enable noninvasive visualization of the cell. Because only live cells can produce the reporter gene product, this technique is better at discriminating live from dead cells and, thus, can provide longitudinal imaging of cell survival and engraftment *in vivo*. Several reporter genes, such as firefly luciferase (*Fluc*, bioluminescence imaging reporter) [22], herpes simplex virus thymidine kinase (*HSVtk*, PET reporter) [22] and ferritin (MRI reporter) [23] have been constructed for stem cell tracking on different imaging platforms. However, safety concerns regarding genetic cell manipulation have limited reporter gene labeling clinical adoption.

Table 2. Advantages and disadvantages of labeling techniques by imaging modality.

Modality	Cell labels	Advantages	Disadvantages
US	Microbubbles Liposomes Microcapsules Perfluorocarbons	High potential of real-time interactivity No ionizing radiation Inexpensive Readily available	Limited acoustic windows Highly operator dependent Limited resolution Acoustic artifacts may compromise the image
MRI	Gd chelates Iron oxides Nonproton Fluorine Sodium Carbon Microcapsules Reporter genes Ferritin Lysine-rich protein	High spatial resolution High anatomic detail No ionizing radiation Permits medium-term tracking Moderate real-time fluoroscopy	Low sensitivity Lack of MRI-compatible devices for interactivity Not compatible for patients with implants Expensive Acoustic noise
SPECT/PET SPECT	Radionuclides ¹¹¹ In ^{99m} Tc ¹⁸ F Reporter genes HSV1-srtk hNIS D2R	High sensitivity Clinically translated or highly amenable to clinical translation Longitudinal tracking with reporter genes and some radiotracers	Poor anatomic detail Poor interactivity Ionizing radiation Short-term serial imaging (due to radioactive decay) Biohazardous labels Expensive Limited availability of scanners
Optical Imaging	Fluorophores GFP Near-infrared probes Quantum dots Reporter genes Luciferase	Permits longitudinal monitoring Low background/high sensitivity No excitation light required Potential to use several labels simultaneously for 'multicolor'	Limited spatial resolution No clinical imaging platforms to date Potential for autofluorescence Photon attenuation issues Immunoreactivity of nonmammalian proteins Toxicity concerns Seldom 3D
X-ray/CT	Microcapsules Gold nanoparticles	High sensitivity Real-time interactivity	Ionizing radiation Lacks soft tissue detail

D2R: Dopamine type 2 receptor; Gd: Gadolinium; GFP: Green fluorescent protein; hNIS: Human NIS; HSV1-srtk: Herpes simplex virus-1 thymidine kinase (mutant form); US: Ultrasound.

Echocardiography

Owing to its wide availability, low cost and lack of ionizing radiation, echocardiography is routinely used to assess cardiac function, diagnose

pericardial disease and evaluate stem cell therapy efficacy. However, few studies have explored using echocardiography to track cells. In part, this is due to the low spatial resolution and lack

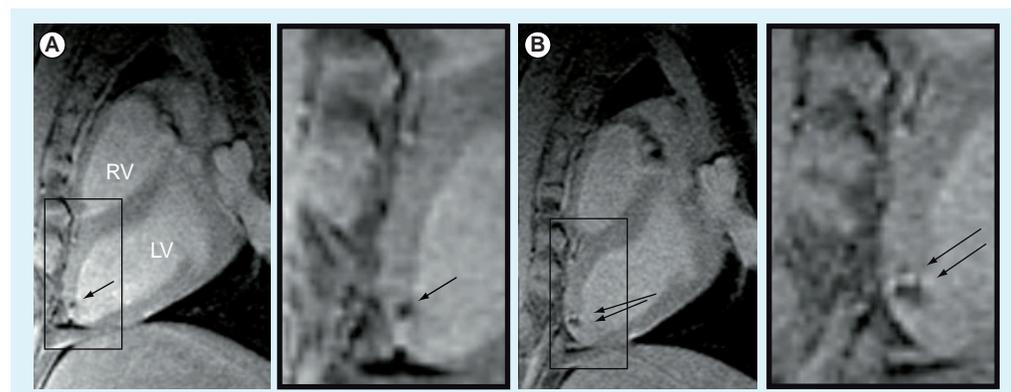


Figure 1. Long-axis MRIs showing hypointense lesions (arrows) caused by magnetic resonance-labeled mesenchymal stem cells acquired within (A) 24 h and (B) 1 week of injection. Insets demonstrate expansion of lesion over 1 week. Reproduced with permission from [43].

of accuracy in cell quantification of ultrasound. Cardiac stem cell tracking using microbubbles [24,25] or CliniMACS® nanoparticles [26] have very recently been performed. In one study, the engraftment of genetically modified endothelial progenitor cells within a Matrigel™ plug was imaged with contrast-enhanced ultrasound using targeted microbubbles [25]. Two issues with microbubble technology are that the microbubble integrity cannot be maintained over time and the delivery of microbubbles outside the vascular space is challenging.

MRI

MRI is a multipurpose imaging modality that provides excellent soft tissue contrast with high spatial and moderate temporal resolution. Therefore, it has been frequently used in the clinic to assess cardiac anatomy, ventricular function, blood flow and myocardial perfusion. In noncardiac applications, MRI has been used to visualize individual labeled cells against a homogeneous background [27]. Stem cells can be directly labeled with a magnetic resonance (MR) contrast agent (e.g., gadolinium chelates and SPIOs) through endocytosis, magnetofection [28] or electroporation [29,30] approaches, or indirectly labeled with an MR reporter gene (e.g., ferritin) via viral [31] or nonviral transfection [32]. Thus, MRI offers the ability not only to determine the efficacy of stem cells, but to track the engraftment of stem cells based on the local environment using a single imaging modality.

■ Gadolinium chelates

As the first US FDA-approved MR contrast agents, gadolinium-chelated contrast agents have been widely used in off-label approaches to quantify myocardial perfusion and viability [33]. On T₁-weighted MRI, cells labeled with paramagnetic gadolinium chelates appear hyperintense. Recently, a gadolinium-based contrast agent, Cy3-labeled gadofluorine M, has been used to label ESC-derived cardiac progenitor cells [34]. No effect on cell viability was observed *in vitro* and transplanted cells could be imaged *in vivo* 2 weeks post injection in both infarcted and normal mice [34]. Interestingly, this agent overcame the issue of intracellular compartmentalization of gadolinium in labeled cells, which results in smaller decreases in T₁ relaxivity or decreased sensitivity to gadolinium-labeled cells [35]. Nonetheless, since unchelated gadolinium is highly toxic, concerns about clinical utilization of these agents for cell tracking remain.

■ Superparamagnetic iron oxides

Superparamagnetic iron oxide nanoparticles are the most widely used MR contrast agents for

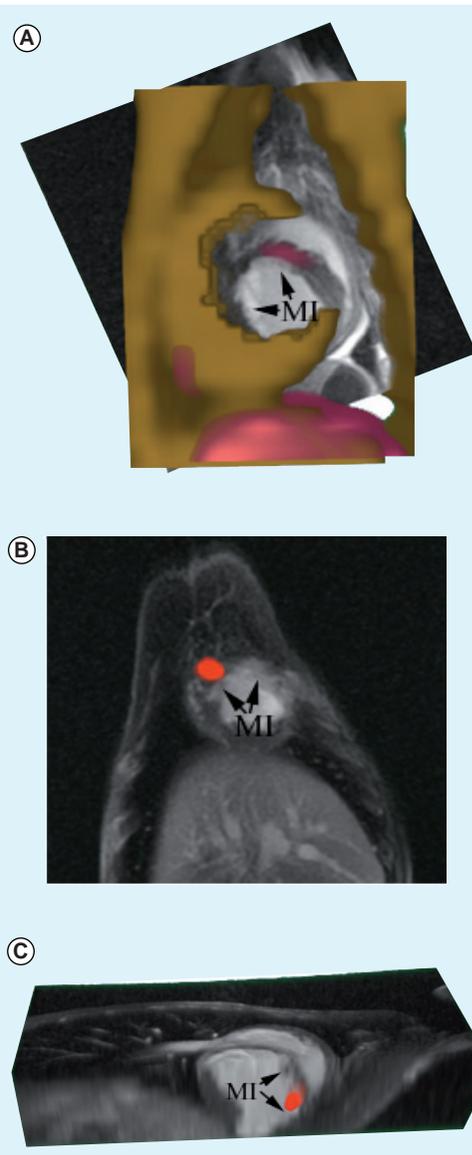


Figure 2. Registration of SPECT/CT with magnetic resonance images of the heart in a dog with a reperfused myocardial infarction receiving ¹¹¹Indium oxine and superparamagnetic iron oxide-labeled mesenchymal stem cells. (A) Short-axis view of alignment of CT (gold) with MRI (grayscale) and SPECT (red) 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 **(B)** short-axis and **(C)** long-axis views. SPECT, due to the higher sensitivity, was able to detect the labeled MSCs whereas MRI failed to detect the superparamagnetic iron oxide particle-labeled MSCs.

MI: Myocardial infarction.

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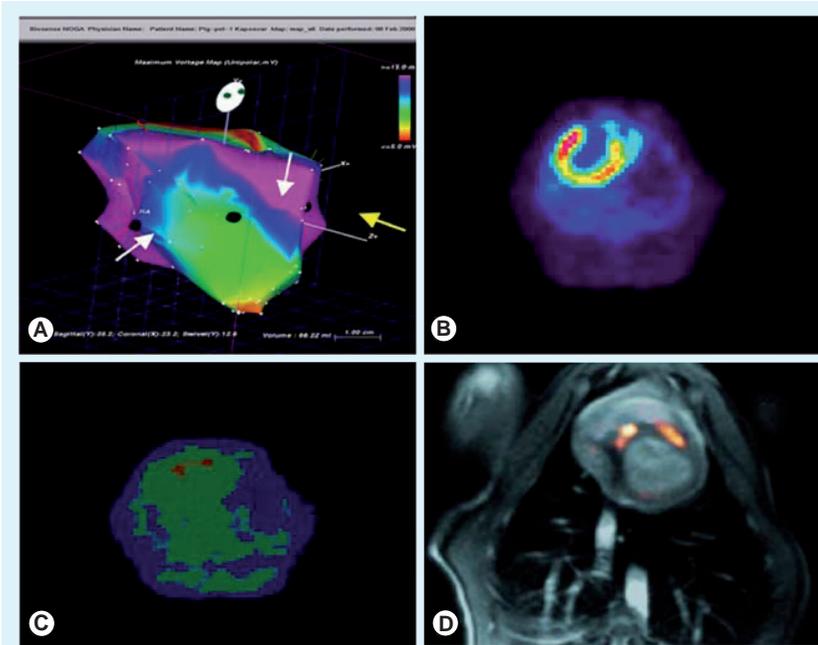


Figure 3. Electromechanical mapping-guided mesenchymal stem cell delivery in a swine myocardial infarction model. (A) Endocardial mapping of a pig heart 16 days after myocardial infarction. Mesenchymal stem cells (MSCs) transfected with a truncated thymidine kinase reporter gene were intramyocardially injected into the border zone of the infarction (white arrows) and unlabeled MSCs were delivered into noninfarcted posterior wall (yellow arrow). (B) ^{13}N -ammonia PET with transmission scan of the pig heart showing perfusion defect in the anterior wall and apex 16 days after myocardial infarction. (C) The location of two injection sites of reporter gene transfected MSCs are demonstrated by the ^{18}F -FHBG PET image of the pig heart 8 h after injection. Unlabeled MSCs could not be detected. (D) Registration of ^{18}F -FHBG PET (hot scale) with MRI (grayscale) demonstrating tracer uptake only at MSCs injection sites. Reprinted with permission from [91].

cellular labeling and typically impart hypointense contrast on T_2^* -weighted images [36]. For the most part, direct labeling of stem cells with SPIOs is performed prior to cell transplantation. Because most stem cells of interest for cardiovascular applications are not phagocytic cells, spontaneous uptake of SPIOs is unlikely to occur. Instead, a positively charged transfection agent, such as poly-L-lysine, cationic liposome or protamine sulfate, is often used to coat the negatively charged iron oxide particles to enable cellular uptake of the transfection agent–SPIO complex through electrostatic interactions. Once within the cells, extremely small quantities of SPIOs (e.g., picograms) can generate high contrast without the toxicity concerns of gadolinium since SPIOs are biodegradable and recycled into the normal iron pool [37].

To date, no clinical trials using SPIO-labeled stem cells have been initiated for cardiac repair. However, several studies with tracking of SPIO-labeled stem cells have now been performed in other diseases [30,38–41]. In preclinical

cardiovascular applications, MR-based tracking of SPIO-labeled ESCs, mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) have been performed in varied animal models [42–44]. In one study of SPIO-labeled mouse ESCs, the hypointensities in the ischemic myocardium were observed 4 weeks after implantation in mice, indicating the successful integration of labeled ESCs with infarcted myocardium [42]. In addition, intramyocardially injected SPIO-labeled MSCs could be detected by MRI up to 3 weeks after infarction in pigs (FIGURE 1) [43]. A similar study carried out by Amado *et al.* demonstrated substantial retention of SPIO-labeled bone marrow-derived stromal cells in infarcted myocardium at 8 weeks [45].

In an effort to take additional advantage of the lack of ionizing radiation with MRI and the ability to see the success of injections immediately, several groups have developed MR-compatible delivery devices often in combination with graphical interfaces for real-time MRI to perform MR-based stem cell delivery interventions [46–48]. At present, the regulatory hurdles for labeling stem cells are sufficiently large such that the addition of an investigational new device for delivery on an MRI platform that is not familiar to interventional cardiologists will probably limit the adoption of these techniques clinically in the near future.

Furthermore, beyond the normal limitations of direct cell labeling, such as possibility of signal dilution with cell replication and detachment of the label from the cell [49,50], the signal void generated by SPIOs may be problematic to distinguish from native tissue hypointensities, such as areas of ischemia, calcification and hemorrhage, motion artifacts and the presence of metallic objects (e.g., stents). To tackle this problem, a variety of positive-contrast MRI techniques or post-imaging processing methods (e.g., IRON, SWIFT and PARTS) [44,51–53] have been developed. However, the biggest hurdle to clinical adoption of these techniques is the removal of commercially available SPIO formulations. Nevertheless, preclinical studies of cardiac SPIO stem cell tracking will continue to be an active field that can be used to help guide clinical trials with respect to dosing, timing and cell choices with the caveat that any direct labeling scheme, such as SPIOs, may become detached from the cell of interest.

■ Other MR contrast agents

In addition to ^1H -based contrast labeling agents, other nonproton-based compounds containing ^{19}F , ^{23}Na or ^{13}C have also been explored for MR

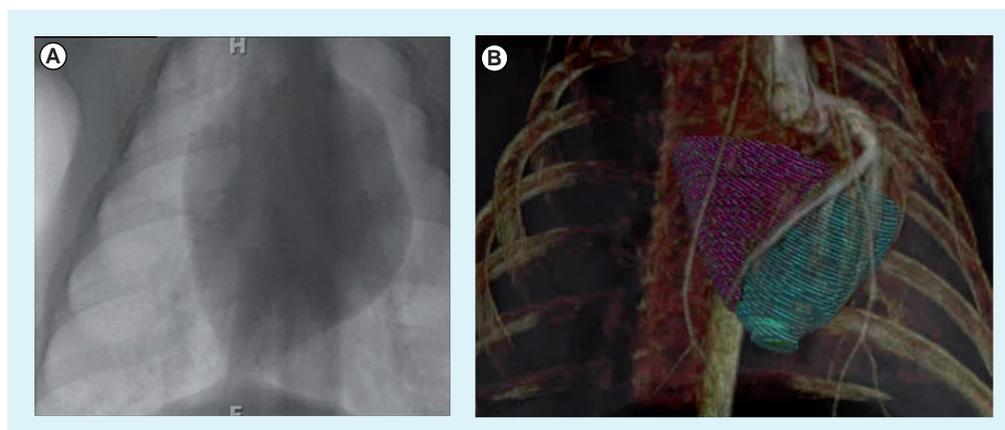


Figure 4. X-ray fused with MRI for targeted myocardial delivery of cellular therapeutics.

(A) Digital fluoroscopic image taken using a conventional flat-panel x-ray angiographic system (Axiom dFA, Siemens AG) demonstrating the lack of ability to see the myocardial borders for transmural delivery of stem cells. **(B)** Live fluoroscopic image overlaid on x-ray fused with MRI from a c-arm CT (syngo dynaCT, Siemens AG) acquired with the same flat-panel angiographic system combined with segmented myocardial borders (blue and pink) from a whole-heart MRI (Espree, Siemens AG) using vendor software (i-pilot). This enhanced visualization of vessels and myocardial wall may enable more precise targeting of stem cell therapeutics.

detection. In particular, ^{19}F -based agents have been used by several investigators for stem cell tracking [54–57]. Because there is essentially no native fluorine in the body, ^{19}F ‘hot spot’ imaging [58] can be achieved with high sensitivity. However, specialized hardware and MRI sequences are often required to perform such studies.

■ MR reporter genes

MR reporter gene labeling may address the problems associated with direct MR contrast labeling. Several MR reporters have been developed, including creatine kinase [59], iron storage proteins (e.g., ferritin, transferrin and transferrin receptor) [23,32,60–63] and artificial proteins (e.g., lysine-rich protein) [64]. Overexpression of the transgenic human ferritin receptor and ferritin heavy chain subunit has been induced in tumor cells [32], neural stem cells [62] and ESCs [60]. Cardiac applications, however, have not yet been explored. Recently, preclinical studies have demonstrated the feasibility of labeling mouse skeletal myoblasts with the MR reporter ferritin. These transgenic cells were successfully detected by MRI *in vitro* and *in vivo* after transplantation into the infarcted mouse heart [23]. Besides safety concerns due to genetic alteration, the primary inherent problem with imaging of MR reporters is whether small numbers of cells can generate sufficient reporter gene products to enable visualization.

Radionuclide imaging

Radionuclide imaging, including PET and SPECT, has the highest sensitivity (PET: 10^{-11} to 10^{-12} mol/l; SPECT: 10^{-10} to 10^{-11} mol/l) and

spatial resolution among all currently used imaging modalities with the ability to quantify radioisotope levels [65]. Clinically, radionuclide imaging has been routinely used to assess cardiac metabolic function, viability, contractile function, as well as cell tracking [66,67]. In general, radioisotopes with a relatively long decay half-life are preferred for cell labeling and tracking.

■ Direct radiolabeling

Direct cell labeling with ^{111}In oxine ($t_{1/2} \approx 2.8$ days) developed for lymphocyte labeling has been adapted for cardiac stem cell imaging. For example, the trafficking of ^{111}In oxine

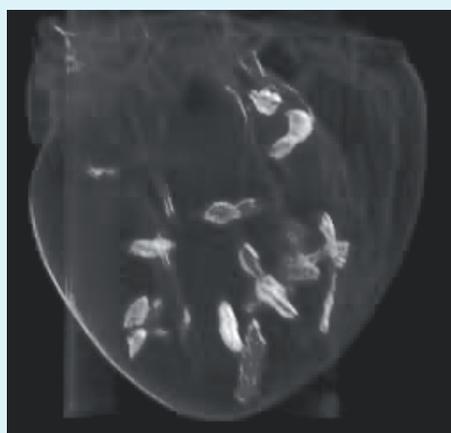


Figure 5. Micro-CT image of an excised swine heart demonstrating multiple injections of barium sulfate microcapsules containing mesenchymal stem cells that appear as bright spots in the myocardium. Adapted with permission from [100].

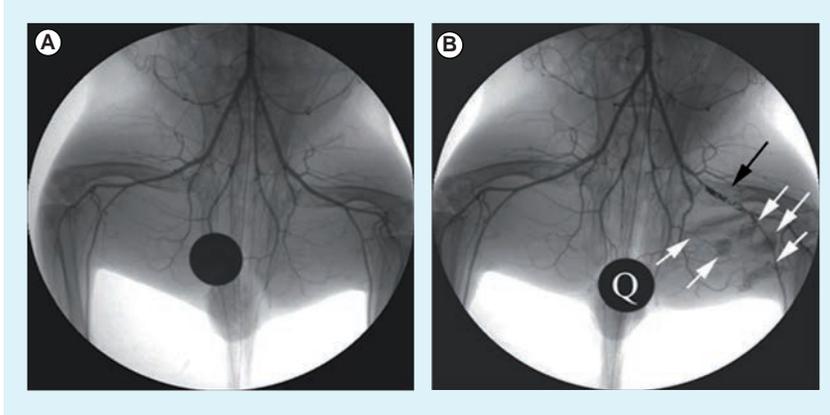


Figure 6. X-ray angiogram of the rabbit hindlimb prior to (A) superficial femoral artery occlusion and (B) post-occlusion via endovascular placement of platinum coils (black arrow). X-ray-visible microencapsulated stem cell injections injected intramuscularly in the medial thigh appear as radiopacities (white arrows). A quarter (Q) is used for reference measurements. Reprinted with permission from [151].

and SPIO-labeled MSCs could be monitored by clinical SPECT/CT up to 7 days in the infarcted canine myocardium, while MRI failed to detect the cells (FIGURE 2) [68]. Several other studies have demonstrated the varied retention of radiolabeled stem cells in the heart depending on the route of delivery, such as intravenous, intramyocardial, intracoronary and interstitial retrograde coronary venous delivery [69–76]. ^{111}In has also been used in patient studies to better understand the trafficking of peripheral blood progenitor cells after intracoronary and intravenous delivery in patients with heart disease [77,78]. ^{18}F -FDG ($t_{1/2} \sim 110$ min) is another attractive radiotracer that is more readily available for stem cell labeling. The first human study demonstrated higher retention of ^{18}F -FDG-labeled CD34-positive enriched bone marrow mononuclear cells in the infarcted myocardium than nonselected bone marrow cells 70 min after intracoronary delivery [79]. In a similar study, PET imaging revealed less than 3.3% of ^{18}F -FDG-labeled HSCs accumulated within the infarcted myocardium at 2 h post-delivery [80]. Similar to MRI, which can provide viability and anatomical location information as well as cell tracking, dual isotope imaging, such as ^{18}F -FDG or $^{99\text{m}}\text{Tc}$ with ^{111}In , may be used to monitor cell migration relative to local tissue perfusion or metabolism [70,81].

Depending on the radiotracer used and cell type, minimum detection limits of direct radiotracer labeling vary from 2900 to 25,000 cells [82]. Major concerns of direct radiotracer labeling include the potential radiation damage to the cells [82–84], leakage of radiotracers over the time course [85], short imaging window due to radioactivity decay and radiotracer detachment from the cells such as direct MRI contrast labeling.

■ PET/SPECT reporter gene labeling

To date, three major PET/SPECT reporter genes, namely enzyme based, receptor based and transporter based, have been developed and applied to cardiac imaging in large animals or human studies. Examples include transporter-based sodium-iodide symporter (NIS) [86–88] for SPECT imaging, receptor-based dopamine type 2 receptor (D2R) [89,90] and the most commonly used enzyme-based herpes simplex virus type 1 thymidine kinase (HSV1-tk) or its mutant form HSV1-sr39tk [22] for SPECT/PET imaging.

The reporter probes for imaging thymidine kinase reporter genes are radiolabeled pyrimidine nucleoside analogs (such as ^{18}F -FHBG and ^{123}I -FIAU/ ^{124}I -FIAU) and acycloguanosine. In a large animal model of myocardial infarction, Gyöngyösi *et al.* demonstrated the first successful translation of PET imaging of the HSV1-tk reporter gene to track cardiac stem cell biodistribution after intramyocardial injection using electromechanical mapping guidance (FIGURE 3) [91]. Enzyme-based PET reporter gene labeling has the advantage of signal amplification. Thus, a very low level of reporter gene expression or small number of transplanted cells can often be detected using radionuclide imaging. The major limitations include potential immune response elicitation to the foreign reporter gene product, limited reporter probe trapping due to rate limited probe transport into the cells and silencing of the reporter gene leading to inability to detect the transplanted cells [92]. In addition, leakage of the reporter probe from cells transfected with the reporter gene has also been reported with the NIS reporter gene [88]. Although radionuclide imaging shows a high sensitivity to a small number of cells, anatomical information is lacking. Thus, CT or MRI is needed to provide localization of cell distribution.

Optical imaging

Optical imaging techniques, including fluorescence imaging and bioluminescence imaging, can provide high sensitivity for cell tracking with detectability of 10^{-9} to 10^{-12} mol/l and 10^{-15} to 10^{-17} mol/l, respectively [93]. In particular, optical imaging of reporter genes (e.g., green fluorescence protein or luciferase) can be used to monitor stem cell survival, proliferation and cardiac-specific differentiation in small animals [49,94]. However, technical challenges, such as the limited tissue penetration and low energy photon attenuation that restricts visualization of deep structures, such as blood vessels and the heart, limit development of clinical imaging systems [95,96].

Multimodality imaging

Multimodality imaging, such as the combination of CT with SPECT or PET, to obtain high sensitivity and anatomical detail can be expanded to other technologies to enhance stem cell tracking and measurement of cardiovascular function. A solution to some of the disadvantages of MRI, such as lack of MR compatible devices, poor physiological monitoring and limited temporal resolution for real-time interventions, would be to combine MRI with x-ray interventional techniques for stem cell delivery (FIGURE 4). Fusion of myocardial anatomy and viability maps from MRI in a swine infarction model have been used to target injections to the infarct borders using an x-ray fused with a MR registration platform [97].

Although stem cell labeling enables noninvasive visualization of cells in infarcted or normal subjects, a big concern for cardiac stem cell therapy is the significant cell death, which may be attributed, in part, to the ischemic environment and immunodestruction. To overcome early cell death, a hybrid technique whereby cells are encapsulated in a protective barrier (which blocks cell destruction by immunoglobulins and immune-mediated cells) and impregnated with imaging contrast agents is currently being explored [55,98,99]. By moving the labeling agents to the protective capsule rather than within cells, high contrast payloads for enhanced sensitivity

can be used, which would often be cytotoxic. Recently, these imaging-visible microcapsules have been demonstrated in a swine heart (FIGURE 5) [100] and also used to track stem cells in a rabbit model of peripheral arterial disease (FIGURE 6) [101].

While these microencapsulation techniques, like direct labeling schemes, fail to measure cell viability, reporter gene labeling of the cells could be used to overcome this obstacle. At present, these microcapsules remain relatively large (~300–500 µm), therefore eliminating the possibility of direct intramyocardial or intracoronary injection. Furthermore, since the stem cells are trapped within the microcapsules, direct integration of the stem cells is prevented. Therefore, this technique will be most useful if stem cells are used to release cytokines to enhance angiogenesis and recruit native stem cells to differentiate into myocytes.

Conclusion

Stem cell labeling in conjunction with noninvasive imaging provides a powerful tool to aid in the optimization of stem cell type, selection dosing, delivery route and timing of transplantation to guide clinical cardiovascular stem cell trials. Despite significant progress in imaging techniques and label developments, no single labeling technique meets all the cardiac stem cell tracking criteria. A multimodality imaging

Executive summary

Echocardiography

- Cardiac ultrasound is an inexpensive, well-accepted method to measure cardiac function. Ultrasonic cellular labeling techniques are very immature at present with difficulties extending the labels outside the vascular system.

MRI

- Direct labeling with superparamagnetic iron oxides (SPIOs) for MRI is the most widely adopted cellular labeling method. However, US FDA-approved formulations of SPIOs are no longer commercially available, which will limit clinical translation. MRI in cardiovascular patients provides highly reproducible cardiac function metrics without ionizing radiation, but the lack of magnetic resonance-compatible devices, such as stents and pacemakers, and the high cost of MRI will limit more intensive use.

Radionuclide imaging

- Direct labeling techniques with radiotracers have already been performed in clinical cardiac cellular trials. The lack of accessibility to scanners and issues with handling radioactive materials with the potential for cell toxicity are hurdles for larger clinical adoption. Reporter gene methods using radiotracers are the most mature and can provide preclinical data to drive clinical trial design.

Optical imaging

- Optical imaging, although sensitive, has restricted clinical applications due to its low tissue penetration depth.

Multimodality imaging

- Combined imaging modalities, such as PET–CT, are already well accepted and offer high sensitivity and anatomical detail. The trend will be towards increased use of multimodality imaging or fusion of different imaging techniques to enable real-time interactivity with high sensitivity to a small number of cells and high anatomical detail.

Conclusion

- Stem cell labeling for noninvasive imaging has been under development for several decades. The initial clinical trials with stem cells have yielded mixed results, but have demonstrated that most cellular therapies are relatively safe. The ability to better determine the degree of engraftment in combination with measures of cardiac function by noninvasive imaging of labeled stem cells will increase in the coming years and ultimately may lead to personalized cellular therapies for cardiac patients.

approach is likely to play an important role in illuminating different aspects of stem cell biology *in vivo* and elucidating the mechanisms of cardiac repair and regeneration.

Future perspective

For now, stem cell labeling for noninvasive tracking will remain mostly a preclinical tool to obtain FDA approval for new stem cell biologics and to guide the design of clinical trials. Since most interventional procedures are performed with x-ray angiographic systems, fusion of x-ray imaging with CT, MRI, PET or echocardiography appears the mostly likely imaging platform for cardiac stem cell tracking in clinical settings in the next 3–5 years. Ultimately, ultrasound, optical or MRI tracking of stem cells will be adopted for serial tracking of stem cells owing to the lack of ionizing radiation. Thus, long-term, future efforts will

focus on delivery using existing technologies with the development of multimodality imaging approaches to interpret the results from cardiac stem cell trials and assess the long-term effects of stem cell labeling.

Financial & competing interests disclosure

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