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Imaging approaches for the study of cell based cardiac therapies

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Abstract

Despite promising preclinical data, the treatment of cardiovascular diseases using embryonic, bone-marrow-derived, and skeletal myoblast stem cells has not yet come to fruition within mainstream clinical practice. Major obstacles in cardiac stem cell investigations include the ability to monitor cell engraftment and survival following implantation within the myocardium. Several cellular imaging modalities, including reporter gene and MRI-based tracking approaches, have emerged that provide the means to identify, localize and monitor stem cells longitudinally *in vivo* following implantation. This Review will examine the various cardiac cellular tracking modalities, including the combinatorial use of several probes in multimodality imaging, with a focus on data from the last five years.

Introduction

Despite nearly a decade of study and promising preclinical data, the role of cell-based therapy for the treatment of cardiac diseases, using various cell types, remains controversial and thus far has failed to translate into mainstream clinical practice. Findings from the BOOST Trial¹ initially offered hope that intracoronary infusion of autologous bone marrow cells (BMCs) would improve left ventricular ejection fraction in patients with ST-segment elevation myocardial infarction (STEMI). By contrast, another well-publicized study (Autologous Stem cell Transplantation in Acute Myocardial Infarction, or ASTAMI) found no benefit from intracoronary BMC infusion in patients with STEMI,² whereas a larger randomized multicenter study (Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction, or REPAIR-AMI) that was published at the same time suggested that intracoronary infusion of autologous bone-marrow derived mononuclear cells provided short-term improvement in left ventricular function when compared to those who received placebo treatment.³ The REPAIR-AMI investigators concluded that although results favored stem cell therapy, their study was not powered sufficiently, and a larger prospective, randomized-controlled trial was still needed. Adding to the uncertainty, a 5-year follow-up study from the BOOST Trial reported that initial (6 and 18 months after treatment) improvements in left ventricular function were not sustained after 5 years.⁴

A fundamental problem in developing cell-based therapies has been identifying the ideal stem cell types, a quest that has been hampered by inefficient engraftment and poor survival of

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Competing interests

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transplanted cells. The conflicting clinical data underscores the need for suitable techniques that can monitor cell-based treatment trials.^{1–3} Cellular imaging techniques are currently under investigation for their ability to identify, localize, and monitor stem cells *in vivo* longitudinally following implantation. These imaging modalities are likely to provide greater insight into the fundamental mechanisms underlying stem cell fate, migration, and survival.

This Review will focus on current *in vivo* cellular imaging approaches that have the greatest potential for translating stem cell applications to the treatment of cardiovascular diseases. MRI-based tracking approaches provide detailed morphological and functional information, and have been the focus of active investigation. Other techniques for monitoring stem cells, such as the use of reporter genes with radioactive probes, are also being tested in cardiovascular disease models and could, ultimately, find their way into clinical evaluation. Cellular imaging modalities that have less apparent clinical potential, including optical or bioluminescent imaging, have been reviewed elsewhere, and interested readers are referred to these articles for further inquiry.^{5–6}

MRI-based assessment of stem cells

In vivo imaging of stem cells using MRI, after transplantation into cardiac tissues, has increased our understanding of stem cell fate and has been the focus of intense investigation. MRI provides excellent spatial and temporal resolution for imaging cardiac anatomy and function, allowing detailed delineation of cardiac and surrounding soft tissues; techniques for evaluating the heart can have been reviewed previously.⁷ Cardiac MRI using gadolinium contrast has been used extensively as a noninvasive tool for the characterization of coronary plaques,⁸ to distinguish myocardial viability,⁹ and to characterize infiltrative diseases of the myocardium.¹⁰ However, intravenous gadolinium contrast agents do not provide cell-specific resolution that would be useful for *in vivo* monitoring of specific cell populations, such as stem cells, after transplantation.

The characteristics of the ideal MRI contrast agents used to label stem cells are listed in Box 1. These specific contrast agents have been incorporated into stem cells by various methods, including endocytosis or using mechanical approaches.^{11–12} Currently, two groups of MRI contrast agents—the superparamagnetic iron oxide nanoparticles (SPIOs) and, to a lesser extent, the gadolinium chelates—improve sensitivity in visualizing a small population of cells, and have been characterized extensively in both preclinical and clinical studies.^{12–17}

Superparamagnetic iron oxide nanoparticles

SPIOs vary in size from 30–180 nm and are internalized into cells using simple incubation or in combination with transfection techniques.¹² Current MRI protocols require direct labeling of stem cells with these contrast agents before transplantation. Once within cells, SPIOs can induce decreased signal intensity on T2-weighted and T2*-weighted images, which is described as the ‘blooming effect’, and can often generate notable signal loss on MRI at a concentration within the picogram range.¹¹

In vivo MRI-based tracking of SPIOs in stem cells in the heart is currently in preclinical development. Although trials of cardiac stem cell therapy have used intracoronary or intravenous administration of cells, most MRI–SPIO stem cell studies performed thus far have been limited to direct intramyocardial delivery of cells via percutaneous catheters.¹⁸ Nevertheless, from the clinical perspective, the promise of MRI-based studies using SPIOs has been exemplified by those that have used tracking of stem cells *in vivo* following stroke^{19,20} and myocardial infarction^{21 22 23 24 25} and immune cell migration following heart transplantation rejection.²⁶ Uptake of SPIOs varies considerably in different stem cell types,

including embryonic,^{27–28,29} hematopoietic,^{30,31} mesenchymal,^{32–33} and immunological lineages.^{13,14,34}

Two proof-of-concept studies demonstrated that SPIOs incorporated into embryonic stem cells (ESCs) can be tracked effectively after implantation.^{35,27} Ebert and colleagues implanted SPIO-labeled mouse ESCs directly into the myocardium after infarction.³⁵ The researchers were able to identify the persistence of signal loss in the infarct zone 28 days after implantation, indicating that SPIO labeled ESCs integrated successfully and remained within the infarcted myocardium. A trend towards reduced cardiac remodeling (as manifested by a smaller change in left ventricular end-diastolic and end-systolic volumes) in animals that received ESCs was noted, although there were no improvements in ejection fraction. In the study conducted by Mani and colleagues,²⁷ mouse ESC-derived cardiac precursor cells (ES-CPCs) were labeled with ferumoxides (Feridex, Berlex Laboratories, Bayer Healthcare, Wayne NJ), a type of SPIO that has received FDA approval for clinical use in liver imaging applications. Approximately 500,000 labeled ES-CPCs were delivered to the infarction border zone via direct intramyocardial injection. T2*-weighted MRI performed 24 h and 1 week after implantation showed areas of signal loss that correlated well with iron deposition in postmortem histological samples²⁷ (Figure 1).

Other types of stem cells have also been labeled with SPIOs and have proved successful as *in vivo* tracking agents. Mesenchymal stem cells (MSCs), either from rat³² or swine,²¹ were implanted directly into infarcted myocardium and retention of stem cells in the infarct zone could be detected 1 month and 21 days, respectively, after implantation. In a study performed by Amado and colleagues, direct intramyocardial delivery of SPIO-labeled bone-marrow-derived stromal cells (BMSCs) into infarcted tissue in a pig model led to substantial ($\sim 42.4 \pm 15\%$) cell retention at the end of 8 weeks, as detected by MRI.³⁷ MSC-treated animals were also found to have near-normalization of cardiac function, as demonstrated by improved hemodynamic measurements, compared with placebo.³⁷ By contrast, a study conducted by Carr and colleagues³⁸ demonstrated that the homing and persistence of BMSCs (implanted either directly or via intravenous injection) into infarcted myocardium in a rat model did not translate to improved cardiac function. Table 1 outlines several studies examining *in vivo* tracking of stem cells using MRI.^{21–25,27,28,32–35,37–39}

In vivo tracking using cells labeled with iron oxide has several limitations. First, signal loss generated by SPIO-labeled contrast agents can be difficult to distinguish from endogenous sources of signal attenuation. Factors such as perivascular effects, partial volume averaging, motion artifacts and the presence of hemosiderin (such as areas of increased red blood cell density, including hemorrhages) can also induce signal loss on T2 or T2*-weighted MRI.⁴⁰ Second, the iron oxide is diluted with each subsequent cell division. Furthermore, imaging with SPIOs tracks the locations of iron nanoparticles, but not necessarily specific cell types. Following apoptosis and cell death, SPIOs might be phagocytosed by non-stem cells such as macrophages, that have been shown to be present histologically.^{32,41} Other studies showing macrophage uptake suggest that changes in MRI signal intensity from stem cells to macrophages would represent a minimal amount of total iron oxide originally infused in the cells.^{42,43}

Gadolinium chelates

Gadolinium chelates differ from SPIOs in their ability to shorten longitudinal (T₁) relaxation times and thus generate increased MRI signal intensity on T1-weighted sequences.⁴⁴ The most widely used example of such a contrast agent is gadopentate dimeglumine (Gd-DTPA, Magnevist, Bayer Healthcare, Wayne, NJ), which was the first FDA approved MRI contrast agent, and has been used extensively in clinical MRI applications such as noninvasive

angiography.⁴⁵ Gd-DTPA shows decreased signal intensification in areas of reduced perfusion in the heart in conditions such as myocardial ischemia or necrosis.^{46,47} Gadofluorine M (GdFM) has also been used to label transplanted ESC-derived cardiac progenitor cells *in vivo*⁴⁸ (Figure 2). GdFM-labeled cells were identified 2 weeks after injection in both infarcted and noninfarcted mice. GdFM contained a rhodamine tag, allowing correlation between MRI and histopathological images.

Despite their widespread use in clinical MRI applications, gadolinium chelates such as Gd-DTPA have less potential as compared to superparamagnetic iron oxide nanoparticles such as ferumoxides as cell-tracking agents. Intracellular gadolinium has much lower T1 relaxivity, which limits its use in cellular tracking studies because of weakened signal intensification.¹⁵ In addition, current gadolinium formulations do not give signal intensification comparable with iron-based compounds, partly because of the limited amounts of gadolinium that can be taken up by cells. Moreover, gadolinium chelates have been shown to be toxic when used in neural stem cell tracking in a stroke study.⁴⁹ Other gadolinium-based nanoparticles are being evaluated to determine whether they can be used for labeling stem cells for cellular MRI.^{50,51}

Radionuclide approaches

The major advantages of using radionuclide probes for stem cell surveillance include the inherently high signal-to-noise ratio (because the body does not have inherent signals that would interfere with cells tagged with radioprobes), the ability to quantify levels through counts per voxel, and the correlation with cell viability in combination with reporter genes. Several radionuclide and positron emitting tracers have been used in preclinical and clinical studies to directly label cells to monitor migration of cells into the heart.

Indium-111 (¹¹¹In)-oxine and technetium-99 (^{99m}Tc)-hexamethylpropylene amine oxine (HMPAO) are lipophilic agents that are imaged with single-photon emission CT (SPECT). Fluorine 18-fluorodeoxyglucose (¹⁸F-FDG) is used in PET imaging, with various degrees of labeling efficiency ranging from 10–90%,⁵² and seems to depend on cell type or on preparation techniques (that is, incubation time and isotope concentration).⁵³ Stem cells can be labeled with radionuclide or PET agents by direct co-incubation of cells with the probes.

Depending on the route of administration of ¹¹¹In-oxine-labeled stem cells, 1–5% of cells have been detected in the heart in experimental studies.^{33,54–55} ¹¹¹In-oxine-labeled peripheral blood mononuclear cells (PBMCs; 10⁷) have been delivered via intracoronary, intramyocardial, or interstitial retrograde (via coronary venous sinus) approaches in a postinfarction model—less than 10% of delivered stem cells were found to be retained in the heart.^{56,57} A substantial number of cells that were delivered via intravenous injection were subsequently trapped in the lungs, perhaps suggesting their ultimate localization in these models.³³ Furthermore, although stem cell implantation via direct intramyocardial injection has resulted in highly efficient localization of cells within the heart, direct injection has not guaranteed efficient delivery of labeled PBMCs to infarcted cardiac tissues. Another disadvantage of infusing radiolabeled cells is that they might have a negative effect on tissues or organs that were not the intended target.⁴³

Improvements in SPECT imaging might enable the detection of labeled cells at the same resolution presently found with PET. However, PET still tends to be regarded as having higher temporal and spatial resolution compared to SPECT. Nevertheless, both techniques allow for quantification of radiotracer levels, and therefore provides for the ability to determine numbers of labeled cells within a region of interest.⁵⁸ ¹⁸F-FDG has been used for labeling and imaging of BMCs in clinical^{59,60} or experimental studies.⁶¹ The stem cell labeling efficiency of ¹⁸F-FDG following incubation has been reported to vary between 40% and 100%.^{52,60} ¹⁸F-FDG

can be retained in the cell through enzymatic conversion by glucose-6-phosphate, or by receptor-mediated binding.⁵⁸

Myocardial accumulation of ^{18}F -FDG-labeled circulating progenitor cells was demonstrated immediately after balloon occlusion and cell delivery into the coronary artery, accompanied by a rapid decrease in activity, consistent with first-pass kinetics for the cells. About 8% of the total injected activity from ^{18}F -FDG in cells could be localized to the myocardium 1 h after cell delivery in a swine infarct model.⁶¹ High labeling efficiencies (>90%) with ^{18}F -FDG of hematopoietic stem cells or BMCs have been demonstrated in three clinical studies. Kang and colleagues reported that, 2 h after intracoronary injection of PBMCs in 17 patients with myocardial infarction, less than 5% of injected activity was located in the heart, whereas high activity was found in the spleen, liver, bladder and brain.⁶⁰ By 20 h after injection of cells, PET scans revealed only 1.5% of injected activity in the myocardium. However, no activity could be detected in the hearts of three patients who received intravenous injection of the ^{18}F -FDG-labeled cells. Intracoronary infusion of ^{18}F -FDG-labeled enriched hematopoietic stem cells for CD34+ surface marker in patients 5–10 days after stent placement for STEMI, revealed that up to 39% of the total injected dose was detected preferentially in the border zone around damaged area of the heart.⁵⁹ However, PET showed retention in the area around the myocardial infarction of approximately 2% of the total injected dose of intravenous ^{18}F -FDG-labeled CD34+. Despite its promise and preclinical utility, the inability to track labeled cells over prolonged periods of time limits the usefulness of these nuclear medicine and PET approaches in monitoring the ultimate fate of transplanted cells in the treatment of myocardial infarction.

Reporter gene approaches

Reporter genes have been used in numerous studies investigating the fate, proliferation, and migration of multiple cellular subsets, including stem cells. Reporter genes can be introduced into stem cells before implantation via transfection of cDNA plasmids or via transduction into host cell genome with viral vectors.⁶² Expression of reporter genes is driven constitutively by endogenous or exogenous gene promoters (such as the viral promoter from the lentivirus species) and needs to be stably transduced into cells. In comparison to direct labeling of cells with radionuclides or MRI-based modalities using SPIOs and gadolinium chelates (where both viable and nonviable cells could emit signals), gene transcription and translation of reporter probes require intact, viable cells, therefore allowing for the assessment of cell survival following stem cell transplantation. After stable transduction into host cell genome, reporter genes are passed onto daughter cells during cell division, permitting longitudinal time-course assays of stem cell populations.

Two reporter gene approaches—enzyme-based and receptor and transporter-based—have been used in stem cell tracking studies. Enzyme-based reporter gene approaches, of which the herpes simplex virus 1-thymidine kinase (HSV1-tk)⁵² or its mutant form HSV1-sr39tk41·63·64 are the most frequently used, have been used in cell tracking studies because of the signal amplification that occurs as a result of imaging probe trapping and accumulation. This signal amplification is generally not generated by receptor-based and transporter-based reporter systems.

A wide variety of pyrimidine analogues and acycloguanosine derivatives have been investigated as reporter probes for HSV1-tk for PET imaging studies.⁵² Preclinical evaluation of *HSV1-tk* genes inserted into stem cells has been performed using 9-(4- ^{18}F -fluoro-3-hydroxymethylbutyl) guanine ^{18}F -FHBG or similar agents. Cao *et al.* implanted 1×10^7 mouse ESCs transduced with the HSV1-tk gene and firefly luciferase gene into immunoincompetent rats with normal myocardium, and were able to assess stem cell viability and proliferation *in vivo* over 4 weeks using a combination of PET ^{18}F -FHBG imaging and bioluminescent

imaging.⁶³ Quantification of the cell signal activity showed excellent correlation between bioluminescent photon flux and PET signal intensity with a marked increase in signal 2–4 weeks after implantation, corresponding to intracardiac and extracardiac teratomas on histology.⁶³ Rats treated with the antiviral agent ganciclovir after implantation of ESC-HSV1-tk cells did not develop teratomas.⁶³ Ganciclovir is phosphorylated in cells that have been transduced with the *HSV1-tk* gene, and the phosphorylated analogue interferes with DNA synthesis, ultimately resulting in cell death. These results demonstrate the value of using *HSV1-tk*, which can serve as a suicide-gene approach to minimize or eliminate transformed cells. Willmann and colleagues reported initial difficulty with the detection of tk-engineered human MSCs following direct open-chest implantation into pig heart (despite direct injection of 10^8 cells in phosphate buffered saline) using standard clinical PET and CT, highlighting the difficulty in translating this technology into clinical practice.⁶⁵ The researchers were, however, able to detect tk-hMSC in the pig heart after direct implantation of the stem cells contained in a gel matrix and a possible future approach of localizing cell based therapies to a specific region of myocardium (Figure 3).

Investigators have also used the dopamine type 2 receptor⁶⁶ or sodium iodide symporter (NIS)⁶⁷ as reporter genes in stem cell tracking studies. Both are nonimmunogenic because they are expressed in mammalian cells. Furthermore, endogenous expression is extracardiac, thereby limiting background signal. For instance, NIS is highly expressed in thyroid epithelial cells, and to lesser degree within the mammary and salivary glands, stomach and colon.⁶⁷

ES or progenitor cells transduced with the NIS reporter gene can be detected by PET using ^{124}I , and by SPECT through infusion of $^{99\text{m}}\text{Tc}$ pertechnatate or ^{123}I .⁶⁷ SPECT ($^{99\text{m}}\text{Tc}$ pertechnatate) and PET (^{124}I) scans have been used in feasibility studies to localize and determine the viability of cardiac-derived stem cells transduced with the NIS reporter gene that was directly injected into the heart in an established rat model of myocardial infarction.⁶⁷ These results demonstrated the versatility of using NIS reporter expression for monitoring transplanted stem cells cardiac disease models. As observed with other reporter gene approaches for tagging stem cells, limitations include the efficiency and duration of reporter gene expression in primary cells, and whether uptake of the radioprobe by other tissues that express the NIS gene is likely to occur.⁵³

Limitations of reporter gene use

Despite the promise of reporter gene technology in studying trafficking of cardiac stem cell, several disadvantages currently limit the utility of these approaches in clinical practice. First, manipulation of the host cell genome by insertion of one or more copies of the reporter gene is required. Further, levels of reporter gene proteins could be variable, given that the number of gene copies inserted into the host cell after transfection or transduction cannot be controlled. Consequently, nonphysiological overexpression of reporter gene proteins could occur within stem cell populations and might perturb endogenous cellular functions. In addition, immune reactions could also be elicited in response to genetically manipulated stem cells, leading to a loss of signal from cells containing the reporter gene.^{52,53,68} Most importantly, malignant transformation of the transplanted stem cells could occur, depending on the number cDNA copies inserted and the insertion sites of the reporter.⁶⁹ Hence, despite the promising results from preclinical trials, reporter gene-based approaches are unlikely to translate into clinical practice in the near future. Protocols using reporter gene imaging to monitor stem cell transplantation are still under development. Reporter gene probes that are suited for clinical trial use will require that there is no foreign antigen presentation on the cell surface that could stimulate an immune reaction and that the inserted reported gene remains active over time.^{12,58} . . Further work is need to improve signal strength originating from the implanted cells, and

cPET (combined with CT or MRI) might provide the increased sensitivity and resolution particularly when cells are dual-labeled for tracking in early phase clinical trials.

Multimodality approaches

Multimodalities approaches such as PET or SPECT in combination with CT or MRI have been used to monitor the temporal and spatial migration of dual labeled stem cells following either intravenous or intramyocardial injection. Kraitchman and colleagues dual-labeled allogenic MSCs with ferumoxides and In¹¹¹-oxine, intravenously infused approximately up to 2×10^8 stem cells in a canine reperfusion myocardial infarction model, and demonstrated focal uptake with SPECT and CT within the first 24 h after injection of stem cells that persisted for up to 7 days.³³ In addition, the In¹¹¹-oxine-labeled stem cells redistributed in the lungs and liver within the first 48 h after injection. Approximately 80,000 In¹¹¹-oxine-labeled MSCs were localized in the infarcted heart 1 week after infusion. The researchers detected MSCs positive for Prussian blue staining in the infarct and peri-infarct region in the heart; however, *in vivo* and *ex vivo* MRI did not detect the presence of ferumoxides-labeled cells.

Other approaches, such as dual-tracer multi-pinhole SPECT and CT, have been shown to simultaneously assess the location of implanted In¹¹¹-oxine-labeled stem cells, myocardial perfusion, and viability by injection of ^{99m}Tc sestamibi, and to monitor the viability of cells and well as changes in the area of infarction.⁷⁰ Blackwood *et al.* monitored In¹¹¹-tropolin-labeled bone marrow mononuclear cells in a canine myocardial infarction model, and detected viable cells using SPECT and CT up to 7 days after implantation.⁷¹ However, the mathematical modeling of signal decay, and acquisition times approaching 180 s/projection, might limit the clinical usefulness of this approach for tracking stem cells implanted within the heart.⁷¹

Qiao and colleagues demonstrated the value of dual-modality imaging using MRI and PET by transducing mouse ESCs with mutant *HSV1-sr39tk*.⁴¹ These cells were then labeled with SPIOs, and $1-5 \times 10^6$ cells were injected directly into the border zone of infarcted myocardium in a well-established rat model. Early localization of implanted cells was detected as hypointense region in the left ventricle consistent with the presence of SPIO-labeled cells, whereas PET did not detect the presence of implanted cells for the first few days after transplantation. However, after 4 weeks, using the reporter gene *HSV1-sr39tk*, ¹⁸F-FHBG–PET demonstrated survival and proliferation of cells in areas where MRI had confirmed cell implantation. MRI provided detailed information on myocardial wall function and contractility. In addition, ¹⁸F-FHBG activity corresponded to the number of cells implanted in normal or infarcted myocardium. Histological examination revealed that less than 0.5% of implanted ESCs were cardiomyocytes and that most of the cells positive for Prussian blue staining were macrophages, which suggests uptake of the SPIO from either dead cells or rapidly proliferating cells that released particles into the extracellular space.⁴¹ These results indicate that complementary information can be obtained when reporter gene approaches are combined with SPIO nanoparticle labeling to determine cell survival and proliferation.

Translation to the clinic

Translation of cell-tracking technologies into clinical settings will require a substantial amount of preparation and perseverance. A variety of imaging modalities show promise. Among the possible cellular MRI labels agents that could be tested in a phase I clinical trial, SPIOs should be considered as the first choice, given the extensive studies conducted on these agents. SPIO cell labels have been used in clinical trials outside the US, although not for cardiac applications. 1572–74 Labeling cells with SPIOs for cellular MRI does not fall under the auspices of the FDA approved exploratory investigative new drug or phase 0 trials. All agents used will, therefore, need to be clinical-grade ancillary products or made with good manufacturing practice (GMP).¹⁶ Unfortunately, the US Pharmacopeia (USP) grade material, ferumoxides

(Feridex IV or Endorem in Europe/Asia), is no longer available. Although alternative SPIO agents for cell labeling are available from various companies, none are FDA-approved or made in a clinical GMP facility. Promising new agents, such as ferumoxytol, are being evaluated as MRI contrast agents, but their effectiveness in cell tracking remains unknown.⁷⁵ Ultimately, more rigorous preclinical studies are required before clinical studies with SPIOs can proceed.

Cell tracking will require a multidisciplinary team of clinicians and imaging scientists working together to label cells in approved clinical GMP facility and demonstrate the utility of noninvasive cell trafficking imaging studies in the evaluation of cellular therapies. Although significant challenges remain, overcoming these hurdles will require a critical component of unlocking the potential of cell-based technologies for cardiovascular disease. Guidelines for researchers who want to use non-USP-grade material to label cells for clinical studies can be found in Box 2. FDA guidelines for evaluation of SPIO-labeling of cells for cellular MRI have not yet been published, and further preclinical studies will be required before these techniques can be implemented in clinical practice.

Conclusions

Over the past decade, there has been significant progress made in experimental and clinical Nuclear Medicine and MRI scanners along with novel methods of labeling stem cells that have allowed for the in vivo tracking of cells. The development of labeling techniques that are nonspecific for cell type has allowed for the tagging of cells with contrast agents that were clinically approved for other indications (i.e., ¹⁸F-FDG, In111 oxine, Ferumoxides, and Gadolinium Chelates). In addition, improvements in scanner hardware have also allowed for the improved sensitivity and ability to localize signals from labeled cells with higher resolution scans and may allow in the future for the quantification of numbers of viable cells following transplantation. Reporter gene approaches to tag stem cells are still in its infancy and will require further development to ensure that labeled cells are not immunogenic, that gene silencing does not occur and that there is no risk of malignant transformation. Future research will require the development of novel agents for labeling cells that will allow for non-invasive imaging approaches to determine if transplanted cells survived infusion or transplantation, differentiated and functionally integrate resulting in improvement in cardiac disease. Ultimately cellular imaging techniques will be directly linked to the success of cell based therapies in clinical trials of heart disease. Whether small molecule approaches derived from stem cells or manipulated and/or expanded specific cell populations will be used in treating heart disease, non-invasive imaging in conjunction with cell labeling should play a key role in designing novel treatment trials and developing strategies to optimize cell or drug dosage to maximize therapeutic response.

Review criteria

The Pubmed database was searched for English language papers that appeared in the literature between 2003 and 2009. The search was not limited to full-text articles, and references with a full-text paper available through institutional library subscriptions were used. Searched terms included “cellular imaging” in combination with “cell labeling with contrast agents” and “myocardial imaging”. Papers on specific contrast agents or cellular agents were search in combination with term “cellular imaging”. The scope of imaging studies reviewed was limited to those involving the myocardium.

Key points

- Two groups of contrast agents, superparamagnetic iron oxide nanoparticles (SPIOs) and to a lesser degree the gadolinium chelates enable detection of cardiac stem cell populations by MRI following implantation
- Currently, clinical application of *in vivo* tracking using SPIOs or gadolinium chelates in cardiovascular disease has several limitations
- The ability to correlate cell viability with signals from radionuclide probes and reporter genes is a major advantage, although *in vivo* use of reporter genes is currently limited to animal models
- Multimodality approaches combining both iron-based and radionuclide probes offer increased sensitivity and specificity for stem cell localization and tracking.
- Protocols using MRI and reporter genes to monitor stem cell transplantation are being developed and translation to the clinic will require nonimmunogenic probes that are stably expressed after implantation

Box 1 Characteristics of the ideal MRI contrast agent

- Affects MRI signal intensity without interfering with target cell activity
- Allows for the detection of small numbers of cells, allowing for increased sensitivity
- Remains within cells after transplantation
- Stable for a long enough to allow for time-course studies of stem cell targeting

Box 2 Guidelines for using nonclinical-grade material in clinical trials

Synthesis and labeling

- Good laboratory practice should be used
- Products and instrumentation used for synthesis should be approved for clinical use or received exemption by the FDA
- Description of the manufacturing process, standardization of the product size, coating and long-term stability should be provided for superparamagnetic iron oxide nanoparticles
- Labeling should be performed using good manufacturing practice in an approved facility

Characteristics of the agent

- Does not contain contaminants or generate toxic byproducts
- Wide therapeutic window
- Does not alter cell proliferation and viability, stemness, differentiation capacity or result in an increase in reactive oxygen species or apoptosis of the labeled cells

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Figure 1.

Embryonic stem cell-derived cardiac precursor cells (ES-CPCs) labeled with SPIOs. Images obtained from the murine heart 1 week after implantation of 500,000 Fe-Pro-labelled ES-CPCs. (a) Full view of the conventional T2*-weighted image. (b) Magnified view of the conventional T2*-weighted image. (c) The corresponding GRASP image. (d) Histology. Abbreviations: GRASP, gradient acquisition for superparamagnetic particles/susceptibility; GRE, gradient-recalled echo. Permission obtained from Wiley © Mani, V. *et al.* Serial *in vivo* positive contrast MRI of iron oxide-labeled embryonic stem cell-derived cardiac precursor cells in a mouse model of myocardial infarction. *Magn. Reson. Med.* **60**, 73–81 (2008).

Figure 2.

Evaluation of non-infarcted mice. T1-weighted high resolution MRI scan of noninfarcted mouse one day after injection. (a) Longitudinal image indicating position of axial slice (red line) in top frame. (b) Corresponding histology shows GdFM-Cy3 (red) within the myocardial wall ($\times 5$ magnification; green indicates cardiac troponin T stain; blue indicates DAPI. (c) Frames 1–10 show different frames within cardiac cycle. *Right ventricle; **Left ventricle. Arrows show volume containing GdFM-Cy3 cells. Abbreviations: DAPI, (4',6- diamidino-2-phenylindole); GdFM-Cy3, gadofluorine M. Permission obtained from the American College of Cardiology Foundation © Adler, E., *et al.* Derived cardiovascular progenitor cells using Cy3-labeled gadofluorine M in murine myocardium. *JACC; Cardiovasc. Imaging* **1**, 9 (2009).

Figure 3.

PET-CT imaging of intramyocardial reporter gene expression. **(a)** Transverse nonenhanced PET-CT fusion image reconstructed at the level of the LV after administration of transduced human mesenchymal stem cells. The image was acquired 4 h after intravenous FHBG administration. A distinct imaging signal (small arrows) can be delineated at the intramyocardial injection site of human mesenchymal stem cells. Note the postoperative soft-tissue edema, emphysema and fluid collection in the left chest wall (large arrows). **(b)** Coronal reconstruction of PET data set of the thorax and cranial upper abdomen in the same animal demonstrates high FHBG uptake at the level of intramyocardial injection site (arrows), with low background signal intensity in all other intrathoracic anatomic structures. Note the high tracer accumulation in the liver (white L) due to biliary FHBG excretion. Abbreviations: FHBG, 9-(4- ^{18}F -fluoro-3-hydroxymethylbutyl) guanine; L, left proximal foreleg; LV, left ventricle; R, right proximal foreleg; RV. Permission obtained from the Radiological Society of America © Willmann *et al.* Imaging gene expression in human mesenchymal stem cells: from small to large animals. *Radiology* **252**, 117–127 (2009).

Table 1

In vivo tracking studies using MRI

Study	Cell type	Species	Delivery method	Contrast agent	Conclusions
Amado <i>et al.</i> 37	MSC	Pig	Direct IM (surgical)	SPIO [§]	Detection of 42.4 ± 15% of the magnetic label after 8 weeks; improvement in hemodynamic profile including percent ejection fraction
Amsalem <i>et al.</i> 32	MSC	Rat	Direct IM (surgical)	SPIO [‡]	Few iron-labeled MSCs survived (engulfed by macrophages) by 4 weeks; however, significant improvement in LVEDS, LVEDA, LVESA, and fractional shortening in animals that received MSCs
Baklanov <i>et al.</i> 39	BMC	Pig	Transvascular	SPIO [§] and Gd	BMCs labeled with SPIO and Gd colocalized to infarcted myocardium
Carr <i>et al.</i> 38	BMSC	Rat	Direct IM (surgical); intravenous	MPIO [*]	Successful engraftment in infarcted myocardial at 10 weeks in both groups; however, no improvement in hemodynamics
Dick <i>et al.</i> 25	MSC	Pig	Direct IM (percutaneous)	Iron-fluorescent particles [*]	Feasibility study that detected labeled MSCs immediately after injection
Ebert <i>et al.</i> 35	ESC	Mouse	Direct IM (surgical)	MPIO [*]	Labeled ESCs still detected after 28 days in ischemic myocardium
Hill <i>et al.</i> 21	MSC	Pig	Direct IM (surgical)	Iron-fluorescent particles [*]	Labeled MSCs detected in infarcted myocardium after 28 days
Himes <i>et al.</i> 28	ESC	Mouse	Direct IM (surgical)	SPIO [§]	Labeled ESCs detected in infarcted myocardium up to 5 weeks
Kraitchman <i>et al.</i> 22	MSC	Pig	Direct IM (percutaneous)	SPIO [§]	Labeled MSCs detected in infarcted myocardium after 3 weeks
Kraitchman <i>et al.</i> 33	MSC	Dog	Intavenous	Ferumoxide (and In ¹¹¹ -oxine)	Postinjection: detection in lungs, liver, and kidney; 24 h post: infarcted myocardium, lung, liver, kidney, spleen, bone marrow; 48 h post: infarcted myocardium, lung, liver, kidney, liver, spleen; 72 h post: No detection
Kustermann <i>et al.</i> 29	EVC	Mouse	Direct IM (surgical)	Ultra-small SPIO [¶]	Labeled cells detected in infarcted myocardium immediately
Leor <i>et al.</i> 34	AHM	Rat	Direct IM (surgical)	SPIO [‡]	Labeled macrophages detected in infarcted myocardium after 4–7 days; improvement in LV fractional shortening, LV diastolic dilatation, and scar tissue repair
Mani <i>et al.</i> 27	ES-CPC	Mouse	Direct IM (surgical)	SPIO [§]	Labeled ESCs detected in infarcted myocardium after 24 hours and 1 week
Suckey <i>et al.</i> 23	BMSC	Rat	Direct IM (surgical)	Iron-fluorescent particles [§]	Labeled BMSCs detected in infarcted myocardium up to 16 weeks
Terrovitis <i>et al.</i> 24	CDCs (human and rat)	Rat	Direct IM (surgical)	SPIO [§]	Signal persisted after 3 weeks in both xenogeneic (human CDCs implanted into rat) and syngeneic models. Histology revealed mostly iron-containing macrophages at injection site

Abbreviations: AHM, activated human macrophage; BMC, bone marrow cell; BMSC, bone marrow stromal cell; CDC, cardiac-derived stem cell; ESC, embryonic stem cell; ES-CPC, cardiac precursor cell; EVC, embryonic ventricular cardiomyocyte; Gd, gadolinium chelates; IM, intramyocardial; IC, intracoronary; LVEDS, left ventricular end systolic diameter LVEDA, left ventricular end diastolic area; LVESA, left ventricular end systolic area MPIO, micron-sized iron oxide particle; SPIO, superparamagnetic iron oxide nanoparticle

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^{//} Sinerem, Guerbet Group.