In Vivo Imaging of Stem Cells and Beta Cells Using Direct Cell Labeling and Reporter Gene Methods

Dara L. Kraitchman, Jeff W.M. Bulte

Abstract—Cellular transplantation therapy offers a means to stimulate cardiovascular repair either by direct (graft-induced) or indirect (host-induced) tissue regeneration or angiogenesis. Typically, autologous or donor cells of specific subpopulations are expanded exogenously before administration to enrich the cells most likely to participate in tissue repair. In animal models of cardiovascular disease, the fate of these exogenous cells can be determined using histopathology. Recently, methods to label cells with contrast agents or transduce cells with reporter genes to produce imaging beacons has enabled the serial and dynamic assessment of the survival, fate, and engraftment of these cells with noninvasive imaging. Although cell tracking methods for cardiovascular applications have been most studied in stem or progenitor cells, research in tracking of whole islet transplants and particularly insulin producing beta cells has implications to the cardiovascular community attributable to the vascular changes associated with diabetes mellitus. In this review article, we will explore some of the state-of-the-art methods for stem, progenitor, and beta cell tracking. (Arterioscler Thromb Vasc Biol. 2009;29:00-00.)

Key Words: stem cells ■ beta cells ■ transplantation ■ cell labeling ■ reporter gene ■ MRI ■ SPECT ■ PET

The human body is dependent on progenitor and stem cells for normal organ repair. Recently, several studies have shown that the ability of endogenous stem cells to home to ischemic tissue and perform restorative functions are diminished in patients with diabetes and cardiovascular disease.1–3 In patients with type I diabetes mellitus, destruction of the beta cells leads to insulin dependence for glucose homeostasis. Thus, in many cardiovascular patient populations, the administration of autologous cell therapies may provide suboptimal building blocks for tissue and vessel repair. Because of immunorejection and the hostile engraftment environment, allogeneic cell therapies are likely to lead to increased cellular destruction and a poor therapeutic response. Although cell fate can be determined in animal models of cardiovascular disease, and diabetes using histopathologic examination of tissue, noninvasive methods for assessing cell survival and engraftment will be needed to assess therapeutic efficacy in patients. Like detecting cells microscopically, cell labeling for noninvasive imaging relies on targeting contrast agents to stem or progenitor cells to increase their conspicuity relative to native tissue.

Direct Cell Labeling

Many of the techniques for cell labeling for detection by noninvasive imaging were developed based on methodologies developed for histopathologic cell labeling. The simplest method is to incubate cells with a contrast agent that is taken up by cells similar to 1,1’-Diocetyl-3,3′,3′-tetramethylindocarbocyanine methanesulfonate (DiI) staining, where the fluorescent stain strongly binds cell membranes. Unlike methods that use antibodies to target antigens on the cell for specific binding, such as monoclonal antibodies for cardiac markers used in histological staining, these direct labeling techniques are not species specific, relatively simple to perform, and inexpensive.

Radiotracers for Direct Cell Labeling

The earliest direct cell labeling techniques for clinical use were performed using radionuclide labels. Indium-111 oxine is a radiotracer with a relatively long half-life of ≈2.8 days, which enables serial tracking over 5 to 7 days of cells using single photo electron computed tomography (SPECT) imaging. Since Indium-111 oxine was approved for clinical use for labeling white blood cells to track sites of inflammation more than 20 years ago,4,5 it was a natural extension to label stem cells for noninvasive biodistribution studies.6–10 Cells are labeled by direct incubation with the tracer. In the case of Indium-111 oxine, it passively diffuses into cells, dissociates, and the Indium-111 is subsequently bound to cytoplasmic components.11 However, this binding is somewhat reversible, which can allow leakage of the radiotracer from the cell.6,7,12 Copper-64-pyruvaldehyde-bis(N4-methylthiosemicarbazone; 64Cu-PTSM) is another attractive radiotracer for positron emission tomographic (PET) imaging for cell tracking and biodistribution studies because of the relatively long half-life of 12.7 hours.13

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Like Indium-111 oxine, efflux of 64Cu-PTSM occurs over time. Tracer leaking from the cell is a common problem of direct labeling schemes whereby the detection of the label may not always represent the location of the cells of interest. Nonetheless, radiotracers have been used to serially study the redistribution of a variety of stem cells, eg, mesenchymal stem cells, endothelial progenitor cells, and hematopoietic progenitor cells (Figure 1).6–10 One of the major benefits of exogenous direct cell labeling with radiotracers over direct labeling with MRI contrast agents is the high sensitivity to a small number of cells because of the lack of a preexisting, endogenous background signal. Minimum detection limits using direct radiotracer labeling range from 6250 to 25,000 cells depending on the radiotracer and cell type.6,10,13,14

These studies have provided insights into the trapping of these cells in nontarget organs after intravenous and intraventricular injections (Figure 1).10,15 As expected, mesenchymal stem cells, which are much larger than endothelial or hematopoietic progenitor cells, have shown a larger percentage of trapping in the pulmonary vasculature after intravenous administration,10,15 in addition, studies in both large and small animals of cardiovascular disease have demonstrated the poor engraftment of stem cells in the heart regardless of the route of administration or cell type despite the administration of millions of cells.10,15 Furthermore, using 2-[18F]-Fluoro-2-deoxy-d-glucose (FDG), a radiotracer with a much shorter half-life than Indium-111 oxine, a recent study has shown that intracoronary injection of a single bolus of bone marrow mononuclear cells (BMMNCs) results in a higher percentage of cardiac engraftment than multiple smaller bolus injections16–18—suggesting that future clinical trials using a single bolus intracoronary infusion may be preferable to multiple short occlusive injections.

Because FDG is more readily available than In-111, a few cardiac cellular patient studies have been performed with FDG-labeled cells. Most notably is the comparison of CD34-positive FDG-labeled BMMNCs showed higher retention in the myocardium after intracoronary injection than nonselected BMMNCs.17 Radiotoxicity and impairment of lymphocyte function after Indium-111 oxine labeling has lead to fears that radiolabeled stem cells may also be impaired. Several detailed studies have demonstrated that the proper titration of the radiation dose can minimize alterations to metabolic function and proliferation in several stem cell lineages,10,12,18

Similarly, FDG labeling of islet cells has been used to demonstrate a high liver engraftment after intraportal delivery in both mice and swine.19,20 However, using a large animal model, early damage of up to 50% of the islet also occurred resulting in release of the radiotracer.20 Fortunately, the released radiotracer is phosphorylated, which should limit uptake by other cells in vivo.20 Nonetheless, nonradioactive methods of stem and islet labeling have gained favor, perhaps in part because of the complications involved in handling radioactive substances in the context of interventional catheterization delivery.

MRI Contrast Agents for Direct Cell Labeling

Unlike radiotracers, most stem and progenitor cells do not readily take up clinically approved MRI contrast agents. On the other hand, macrophages and monocytes will readily phagocytize commercially available iron oxide nanoparticles. This has lead to their limited use to identify phagocytic cells in the atherosclerotic plaque after intravenous infusion of iron oxide nanoparticles.21–23 Similarly, gadolinium-based compounds have been developed that are selectively taken up by the components of the atherosclerotic plaque.24 One notable exception is the uptake of gadolinium HPDO3A, a commercially available MRI contrast agent, which shows some affinity for uptake by stem cells.25,26 A recent review by Nahrendorf et al of promising molecular agents for the atherosclerotic plaque covers these agents in more extensive detail.25 In a like manner, whole islets when incubated with iron oxides show an uneven distribution of the label because of the avidity for uptake by the monocytic lineages within the islet.28 This will vary based on a number of variables: (1) the purity of the islet preparation; (2) the specific preparation of iron oxides used; and (3) the length of iron oxide incubation time. Therefore, it is not surprising that different labeling results have been reported among different investigators. Immunejection of transplanted hearts has also shown promise by monitoring macrophage infiltrates, which will engulf intravenously administered iron oxide compounds as well.29

The addition of a transfection agent (TA), greatly facilitates the uptake of iron oxide nanoparticles by stem and progenitor cells resulting in stable intracellular incorporation in endosomes with minimal toxicity.30,31 Because of the simplicity of TA—iron oxide labeling, these techniques have been used extensively in both small and large preclinical animal models of cardiovascular disease.32–45 Typically, T2*-weighted MR imaging is performed to detect iron oxide–labeled cells as hypointensities. Because the underlying anatomy is obscured using these imaging methods, gadolinium-based compounds, which appear as hyperintensities on T1-weighted MRIs, have been explored by several groups for cellular labeling.36,47 However, once inside cells, the ability of gadolinium (Gd)-based compounds to affect tissue water is severely restricted, and the sensitivity is thus markedly reduced.25,47,48

Also, there are concerns about the dechelation of lanthanide compounds, such as gadolinium, and its subsequent systemic redistribution and potential toxicity after death of transplanted cells. On the other hand, sufficient sensi-
tivity to $10^7$ iron oxide–labeled cells by cardiac MRI$^{14,49}$ can be achieved with picograms of iron per cell, and on cell death this free iron can be easily recycled into the normal iron pool. But there has been considerable controversy, as with direct radiolabeling techniques, about the ability to discriminate iron released from exogenously labeled cells, eg, iron taken up by phagocytic cells in vivo or extracellular iron debris, from the original exogenously labeled cells.$^{42,50–52}$ Another concern is the ability to discriminate hypointensities from iron oxides from other causes of hypointensities such as calcium, air, and hemorrhage. Direct cellular labeling with fluorine, a compound which is not naturally occurring in the body, may provide a means to circumvent these problems,$^{53–55}$ but requires specialized hardware and expertise to image nonproton species with MR imaging and spectroscopy. A relatively new approach is the use of PARACEST agents, which contain lanthanide inducing large chemical shifts of protons. Using a specific off-resonance pulse, these protons can be saturated. After chemical exchange with the water pool, a reduction of signal can be obtained that can be turned “on” and “off.” Initial experiments appear promising,$^{56}$ although the in vivo sensitivity still needs to be determined.

Besides being a low-cost and facile method to label cells, iron oxide labeling when combined with interventional MRI techniques offers a method to guide cellular delivery to specific portions of the heart or vasculature. Several groups have used conventional percutaneous techniques to deliver iron oxide–labeled stem cells in large animal models and demonstrated that the success of the transplantation and engraftment can be assessed without the use of ionizing radiation (supplemental Figure I).$^{32–35,57}$ Because the number of interventional cardiologists and radiologists with interventional MRI expertise is limited, a recently proposed alternative is to combine MRI angiograms or cardiac viability maps with conventional X-ray fluoroscopic images to guide delivery and limit overall radiation exposure (supplemental Figure II).$^{58}$

Another issue with direct labeling techniques is that after a certain number of divisions, the label is sufficiently diluted to be undetectable by MRI.$^{59}$ Embryonic stem cells (ESC), which may replicate rapidly in vivo, are especially prone to these problems.$^{53–55}$ But requires specialized hardware and expertise to image nonproton species with MR imaging and spectroscopy. A relatively new approach is the use of PARACEST agents, which contain lanthanide inducing large chemical shifts of protons. Using a specific off-resonance pulse, these protons can be saturated. After chemical exchange with the water pool, a reduction of signal can be obtained that can be turned “on” and “off.” Initial experiments appear promising,$^{56}$ although the in vivo sensitivity still needs to be determined.

**Reporter Gene Methods**

Like direct labeling, reporter gene methods were originally developed for postmortem tissue analysis of cell fate. For noninvasive reporter gene imaging, a cell is transduced with a reporter gene to produce a nonnative enzyme, receptor, or protein that accumulates and can be detected by the administration of a reporter probe. One of the greatest advantages of reporter gene imaging is that only viable cells will produce the reporter product. Although reporter genes have been developed for MRI,$^{60–64}$ the most widely used reporter genes have been developed for radionuclide imaging.$^{65–74}$ At present, only the artificial lysine-rich protein$^{66}$ has shown to be a suitable MRI reporter gene for cell tracking. The problem with the other MRI reporter genes is that they rely on metals (eg, gadolinium and iron), which induce long-term background contrast in the surrounding cellular environment, regardless of whether or not the reporter is active (ie, the cell is alive).$^{64}$ The best known reporter gene for radionuclide imaging is the herpes simplex virus type 1 thymidine kinase (HSV1-tk) gene. The wild-type HSV1-tk can be used in combination with the reporter probe, Iodine-124–labeled FIAU, for PET imaging; the mutant HSV1-tk is used in combination with Fluorine-18-labeled FIAU or FHGB for PET imaging. Wu and colleagues have used reporter gene imaging to demonstrate teratoma formation after intramyocardial injection of undifferentiated embryonic stem cells in a murine model of myocardial infarction (supplemental Figure III).$^{65}$ Initially developed for oncological applications (eg, gene therapy for gliomas via “the bystander” effect),$^{75}$ there is the added benefit that the reporter gene can also act as a suicide gene using the HSV1-tk/gancyclovir combination where the thymidine analog, gancyclovir, can be administered to kill transduced cells that proliferate uncontrollably.$^{55}$

For reporter gene imaging of pancreatic beta cells, a few studies have used the HSV-tk and the luciferase reporter gene for monitoring islet cell transplantation.$^{76–80}$ Although still in its infancy, these studies have shown that reporter gene–based imaging may result in a better understanding of overall graft function including its immediate and long-term survival.

Hybrid reporter genes have been developed to allow a combination of optical, radionuclide, or MR imaging techniques.$^{68–70}$ Bioluminescence imaging of stem cells transplanted with a reporter gene that produces luciferase, an enzyme that in the presence of the reporter probe luciferin, creates light similar to the firefly, has been widely used in cardiovascular mice and rat studies. Although clinical translation of bioluminescence imaging is not possible because of the physical constraints of light scatter and poor penetration, these studies can provide high throughput screening of potential cell types that are most beneficial, immunosuppressive drug therapies that would be most successful, or mechanisms of stem cell engraftment and homing.$^{78,71,72}$ Recently, multimodality reporters have been translated to large animal models of myocardial infarction, which lends promise to the use of reporter gene methods in clinical trials in the distant future.$^{73}$ Although fears of gene therapy still persist, most reporter genes are not stably expressed forever. For clinical translation, the silencing of reporter genes may be a blessing in itself, in that fears of introducing a genetically altered product in patients will only need monitoring for several months rather than the patient’s lifetime.

**Microencapsulation of Allogeneic/Xenogenic Cells**

Because of the acute nature of many cardiovascular diseases that could be expected to benefit from cellular therapy, the time needed to culture and expand stem cells is frequently not possible. In the case of Type I diabetes mellitus, where the pancreatic beta cells have been destroyed, cadaveric islet transplantation is currently the only option available as an alternative to insulin therapy. However, immunosuppressive therapies to prevent rejection of allogeneic cells have the unwanted side effect of cytotoxicity of the implanted islet cells. Thus, microencapsulation methods were developed to provide a porous coating to the transplanted islets that would allow the free flow of nutrients, such as glucose, oxygen, and insulin, but restrict large substances, such as immunoglobulins or antigen presenting cells. The addition to this capsule of MRI-visible
contrast agents to an alginate microcapsule provides a method for noninvasive imaging of the fate of individual islets. Our group has combined these “magnetocapsules” with intervention MRI techniques to enable the real-time monitoring of the Edmonton procedure, i.e., intraportal delivery of islets to the liver (supplemental Figure IV). Because the MR contrast agent is now within the microcapsule rather than inside cells as occurs with direct labeling, the amount of contrast agent can be increased to enhance sensitivity without increasing cytotoxicity. Fortunately, the addition of MRI contrast agents to the capsule does not have adverse effects on the capsule properties. Recently, we have developed a family of imaging-visible microcapsules that would be amenable for not only MRI but also X-ray and CT imaging applications (Figure 2). For stem cell therapies, microencapsulation offers the prospect of enhanced cell survival in a hostile, ischemic environment, but would not allow direct cellular incorporation of stem cells. Because paracrine mechanisms have been implicated in the therapeutic effects of many cardiovascular cellular therapies rather than direct tissue regeneration, the lack of direct cellular engraftment may not be so problematic. The current wide use of X-ray−based interventiononal techniques and devices should enhance translation of these microencapsulation methods, which may reduce cellular destruction and thereby decrease the number of cells needed to treat a patient.

Conclusions
There has been an increasing interest in cellular therapy for the treatment of cardiovascular disease and diabetes mellitus in the past decade. Noninvasive imaging can provide a means to determine the efficacy of these therapies in patients. Direct labeling of stem, progenitor, and beta cells has provided insights into the underlying mechanisms of action and determining the optimal route, dosing, and cell type. Limited adoption of these techniques has been performed in clinical trials but can be anticipated to increase as these labeling techniques are now reaching maturity. Reporter gene methods offer several advantages over direct labeling techniques but are not as established and more difficult to perform. Thus, these techniques are expected to be adopted but with a longer translational window to the clinical realm. The recent introduction of microencapsulation methods offers an X-ray−visible tracking method that is very attractive because of the widespread use of percutaneous procedures in patients with cardiovascular disease.

Disclosures
None.

References


Figure I. ●●●

Figure II. ●●●

Figure III. ●●●

Figure IV. ●●●
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Supplemental Figure 1:  *In vivo* MR image demonstrating the hypointensities created by two injections (arrows) of ~7x10^6 SPIO-labeled mesenchymal stem cells (MSCs) in a swine model of reperfused myocardial infarction. The injections can be visualized in the infarcted myocardium (MI), which appears hyperintense on delayed contrast-enhanced MRI. Adapted from Kraitchman et al.\textsuperscript{32}
Supplemental Figure 2:  **A**: A combined X-ray angiographic and MR imaging suite with a flat-panel angiographic display and 1.5T clinical MRI scanner (Siemens Medical Systems that allows registered patient transfer between the two imaging modalities).  **B**: A representative fused volume rendering of MR cine images with real-time fluoroscopic image obtained using a combined imaging suite. The left ventricular cavity is shown in red and the myocardium in blue. Three target injection sites are shown in pink, green and yellow.
Supplemental Figure 3: Transplanted embryonic stem cells transfected with the triple fusion reporter gene were serially imaged with bioluminescence (optical) and PET imaging over 4 weeks. Both imaging modalities showed an increase signal, which correlated with cell proliferation over time. Reprinted with permission from Cao et al.65
Supplemental Figure 4: *In vivo* T2*-weighted MR images obtained before (A) and 5 minutes (B) after intraportal infusion of magnetic microencapsulated human cadaveric islet cells in a pig. The microcapsules appear as hypointensities that are distributed throughout the liver. MRI repeated at 3 weeks (C) after infusion shows the persistence of the microencapsulated islets. Reprinted with permission from Barnett et al.82