In Vivo Tracking in Cardiac Stem Cell-Based Therapy

Kozo Hoshino, Hung Q. Ly, John V. Frangioni, and Roger J. Hajjar

Cell-based therapy has been heralded as a promising, novel therapeutic strategy for cardiovascular diseases. Despite a rapid transition from animal studies to clinical trials, there remain numerous unresolved, and at times, controversial issues with respect to underlying molecular mechanisms. In parallel, recent advances in the field of molecular imaging has provided a means to bridge the gap in knowledge through in vivo stem cells tracking. Herein, we review current in vivo imaging techniques and future directions for tracking the effects of cell-based therapy.

Despite recent advances in pharmacotherapy and interventional treatment strategies, ischemic heart disease (IHD) remains a leading cause of mortality in the Western world. After the preliminary reports alluding to the potential of bone marrow cells to induce cardiac regeneration, cardiac stem cell–based therapy has been the focus of numerous preclinical studies with controversial, albeit encouraging, findings. Subsequently, initial (phase I) clinical trials have proven the safety and feasibility of such an approach. Intracoronary or intramyocardial injections of endothelial progenitor cells or bone marrow–derived stem cells in patients with ST-elevation myocardial infarction have been reported to improve myocardial contractile function, ventricular remodeling, and myocardial perfusion. Nevertheless, numerous unresolved and controversial issues remain to be answered, more specifically, with regard to the biological fate of injected stem cells within injured myocardium.

In light of these issues, visualization of injected stem or progenitor cells (SPCs) offers further insight into the underlying mechanisms for the aforementioned functional benefits. Although current imaging modalities provide noninvasive morphological as well as functional data, they lack the ability to assess and track in vivo biological phenomenon, a pivotal link for greater mechanistic understanding following cell-based intervention IHD. This review will therefore discuss currently available in vivo imaging modalities.

Roles of In Vivo Imaging Technology for Cardiac Cell-Based Therapy

Unresolved issues in cardiac stem cell-based therapy are listed in Table 1. In vivo imaging technology will hopefully provide specific information for these questions. Visualization and quantification of injected cells within the injured myocardial tissue represent essential components to understanding and comparing key processes (within or between cell populations) of stem cell engraftment. In addition, serial assessments of biological consequences of the various routes used for stem cell delivery are crucial to further the burgeoning field of cardiac regenerative medicine. Whole body imaging will serve to both elucidate biodistribution of injected cells and help reveal undesired seeding in nontarget organs. Finally, with biodistribution of injected cells better defined, the opportunity

From the Massachusetts General Hospital, Harvard Medical School, Boston, MA, Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan, and Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA. Address reprint requests to Roger J. Hajjar, MD, Cardiovascular Research Center, Massachusetts General Hospital, 149 13th St, CNY-4, Boston, MA 02129. E-mail: hajjar@cvcrg.mgh.harvard.edu

0033-0620/$ - see front matter
© 2007 Published by Elsevier Inc.
doi:10.1016/j.pcad.2007.02.005
to track cells in vivo, characterize their intramyocardial distribution, and isolate them will be increasingly feasible.

**Ideal In Vivo Imaging Agent for Cardiac Cell-Based Therapy**

Ideal, noninvasive in vivo imaging technologies should have capabilities of (1) real-time visualization of injected cells either in the target area or throughout the body; (2) SPC quantification; (3) long-term, serial traceability; (4) single cell sensitivity in any location; and (5) reduced false-positive imaging. Ideal contrast agents are one that exerts a sufficient signal that can be detected and quantifiable by imaging hardware. Most currently available contrast agents for in vivo imaging techniques (such as near-infrared [NIR] fluorophores for optical fluorescence imaging or superparamagnetic iron oxide [SPIO] nanoparticles for magnetic resonance imaging [MRI]) require a direct labeling technique, by which a contrast agent pass through the cell membrane by either a mechanism of endocytosis or pinocytosis. Potential drawbacks of these modalities are leakage of contrast agent toward neighboring cells and potential transfer to non–stem cells, such as macrophages after cell death. These issues could lead to false-positive imaging and obscure understanding of mechanisms relating to stem-progenitor cell transfer and engraftment. Needless to say, prerequisites for clinical applicability of contrast agents for cell labeling are biocompatibility, safety, and nontoxicity for cardiac and noncardiac tissues.

In contrast to direct labeling techniques, reporter gene techniques for SPC labeling offer an attractive alternative because imaging signals are generated only from viable cells of interest. As the signals from reporter gene probes emanate only from the reporter gene product expression, the imaging signal will be directly dependent on the viability of transplanted SPC. This genetic modification of transplanted cells enables more specific tracking in vivo with less false-positive findings. However, certain problems with current reporter gene labeling techniques still limit clinical efficacy and applicability: gene modification adds additional cost and could potentially induce genetic instability including malignant transformation.

**Imaging Modalities for In Vivo Stem-Progenitor Cell Tracking**

Prior studies have reported on various in vivo imaging modalities used either in animal or clinical studies. Currently, there exists no modality that fulfills all the characteristics of an ideal in vivo imaging system as outline above. Advantages and disadvantages of currently available in vivo imaging modalities used to monitor biological fate and therapeutic effect of cell-based therapy in IHD will now be discussed.

**X-Ray–Based Methods**

Although plain films and computed tomography (CT) are the most readily available clinical imaging modalities, x-ray–based methods are not suitable for stem cell tracking at present. These modalities require extremely high concentrations of contrast agents, such as iodine, gadolinium, or metals. The need for long-term data in the field of regenerative cardiovascular medicine would render use of such agents prohibitive due to potential untoward effects resulting from such high concentrations.

**Ultrasound**

Echocardiography is the most widely used cross-sectional imaging modality. Because of its

As the signals from reporter gene probes emanate only from the reporter gene product expression, the imaging signal will be directly dependent on the viability of transplanted SPC. This genetic modification of transplanted cells enables more specific tracking in vivo with less false-positive findings. However, certain problems with current reporter gene labeling techniques still limit clinical efficacy and applicability: gene modification adds additional cost and could potentially induce genetic instability including malignant transformation.

**Imaging Modalities for In Vivo Stem-Progenitor Cell Tracking**

Prior studies have reported on various in vivo imaging modalities used either in animal or clinical studies. Currently, there exists no modality that fulfills all the characteristics of an ideal in vivo imaging system as outline above. Advantages and disadvantages of currently available in vivo imaging modalities used to monitor biological fate and therapeutic effect of cell-based therapy in IHD will now be discussed.

**X-Ray–Based Methods**

Although plain films and computed tomography (CT) are the most readily available clinical imaging modalities, x-ray–based methods are not suitable for stem cell tracking at present. These modalities require extremely high concentrations of contrast agents, such as iodine, gadolinium, or metals. The need for long-term data in the field of regenerative cardiovascular medicine would render use of such agents prohibitive due to potential untoward effects resulting from such high concentrations.

**Ultrasound**

Echocardiography is the most widely used cross-sectional imaging modality. Because of its
convenience, echocardiography remains an attractive option for clinical trials of cardiac stem cell therapy. A variety of contrast agents for echocardiography have been developed such as nanoparticles, liposome, or microbubbles. Technological advances in molecular imaging agents for echocardiography offer the potential to image in vivo cellular morphology and/or characterize pathophysiologic processes. In addition, echocardiography has been reported to have the potential to detect a single cell loaded with a single unit of contrast. Limitations of ultrasound for in vivo cell tracking include lack of accuracy in cell quantification, spatial resolution, and lack of “robust” techniques for intracellular accumulation of the agent. Finally, as this imaging modality remains by and far a transthoracic, 2-dimensional–based technique, anatomical inaccessibility of certain cardiac structures could present an important limitation.

Optical Imaging

Two complementary optical imaging methods, bioluminescence and fluorescence, can be used for stem cell tracking. Bioluminescence uses light generated by the enzyme luciferase. Successful tracking with bioluminescence of the in vivo distribution and engraftment of SPCs have been reported in small-animal studies. Unfortunately, luciferase genes and substrates described to date, which are associated with very high absorption and scatter in living tissue, generate only visible (400-700 nm) light. The need to take cell depth into account (when considering accuracy of detection) represents another important drawback. Taken as a whole, these limitations preclude use of the technique in animals larger than rats with false-negative scanning reported even in mice. Hence, the reliance on nonhuman gene expression and injection of high concentrations of potentially immunogenic nonhuman substrates limit clinical use of this technique. Fluorescence imaging uses organic (eg, green fluorescent protein, small-molecule polymethines) or organic/inorganic hybrids (eg, quantum dots) as exogenous contrast agents for in vivo imaging. Because of diminished absorption and scatter of photons at visible wavelengths, NIR fluorophores show the greatest promise for clinical applicability. In the NIR wavelength window (700-1000 nm), absorbance spectra for all biomolecules reach minima compared with visible wavelengths. The ability to provide real-time imaging combined with high spatial resolution represents an important advantage of in vivo NIR fluorescence imaging. This imaging system is well-suited for the study of large animal models of IHD. Our group has successfully labeled bone marrow–derived mesenchymal stem cells with IR-786 (a NIR fluorophore) and, subsequently, performed in vivo tracking 90 minutes after intracoronary cell injection in a swine model of myocardial infarction (see Fig 1). Potential disadvantages

![Fig 1. In vivo NIR fluorescence imaging of NIR fluorophore-labeled bone marrow–derived stem cells during intracoronary injection in a swine model of myocardial infarction. Left image shows color video image of the infarcted heart during cell injection. Right image shows the simultaneous NIR fluorescence image of the left image.](image)
of SPC tracking with NIR fluorescence imaging are (1) imaging capacity limited to only 4 to 10 cm of tissue depth \(^25,26\) (a common limitation of light-based imaging), which would thus limit the clinical use of NIR fluorescence to near-surface applications, such as in the setting of intraoperative imaging \(^22,27,28\); (2) the dilution of the agent with each cell division; and (3) the possibility of uptake by non–stem cells after stem cell death. A novel, complementary technique uses a “stealth” NIR fluorescent probe, \(^29\) which brightens and becomes unquenched after enzymatic hydrolysis. A net advantage of this technique is that deep tissue targets (such as the aorta) would then have improved in vivo visualization.

**Single-Photon Emission CT**

Single-photon emission CT (SPECT) detects high-energy \(\gamma\) rays emitted by radioactive as \(^{99m}\)Tc, \(^{111}\)In, and \(^{123}\)I by rotating a collimated gamma camera around the subject and reconstructing 3-dimensional images. Three strategies for in vivo SPCs detection have been described: direct loading with a radiometal, \(^30-33\) enzymatic conversion with retention of a radioactive substrate, \(^34\) and receptor-mediated binding. \(^34,35\) Direct loading of stem cells with a radiometal enables tracking of stem cells and their body distribution after injection in myocardial infarction. Bone marrow–derived mesenchymal stem cells labeled with \(^{99m}\)Tc were successfully visualized up to 4 hours after cell infusion in a rat model of myocardial infarction. \(^32\) Interestingly, most of the cells were detected in the lungs (over 50%), and less than 1% of the injected mesenchymal stem cells were actually detected in the infarcted area. \(^32\) An important limitation with the direct loading approach is the trade-off between half-life and long-term exposure to ionizing radiation. A longer half-life would entail greater long-term exposure to radiation. The latter problem notwithstanding, the potential of transfer of the radiometal to non–stem cells must also be taken into consideration.

Enzymatic conversion and retention have been used for both SPECT and positron emission tomography (PET) substrates. This technique uses introduction of the enzyme through a transgene. \(^36\) This approach was first developed for the purpose of transgene imaging, initially using visible light reporters and subsequently PET probes. Significant advantages of this strategy include the ability to follow SPCs indefinitely after stable integration of the transgene as well as the absence of dilution by cell division. Reporter-mediated targeting is however conditional on stable expression of receptor not found elsewhere in the body and intravenous injection of the radioactive receptor ligand. \(^35,36\) SPECT enables not only cell quantification but also yields a high degree of sensitivity compared with optical imaging and MRI. \(^36\) Disadvantages of this strategy include ex vivo genetic manipulation of SPCs and the need to administer a substrate intravenously for each imaging session.

**Positron Emission Tomography**

Positron emission tomography uses coincident detection of 2 antiparallel \(\gamma\) rays emitted after positron annihilation. Positron emission tomography has a higher sensitivity than SPECT and permits more accurate quantification of cell number. The 3 strategies mentioned above for SPECT can be readily used for cell tracking with PET.

Fluorine 18 2-fluoro-2-deoxy-d-glucose (\(^{18}\)F-FDG) is an attractive tracer for PET imaging. Compared with other PET tracer, \(^{18}\)F-FDG has a longer positron range of the emitted \(\beta\)-particle (\(\approx 0.5\) mm), which results in a radiation dose deposition mostly outside the labeled cells. \(^37\) Initial clinical experience with \(^{18}\)F-FDG and PET imaging in the field of cardiac stem cell–based therapy was reported in a study that monitored myocardial homing and biodistribution of bone marrow cells. \(^37\) In the study, unfractionated bone marrow cells were labeled with \(^{18}\)F-FDG and delivered by either intracoronary or intravenous route in the infarcted myocardium. Using this imaging modality, only 1.3% to 2.6% of the cells were detected around the infarct border zone within 1 to 1.5 hours after intracoronary injection, whereas only background activity was detected in the infarcted myocardium after intravenous injection. A limitation with \(^{18}\)F-FDG remains the short physical half-life (110 minutes) of fluorine. This imposes a time window for tracking radiolabeled cells of a few hours (at best, a few days) after any therapeutic
intervention. Problems of direct labeling technique (such as dilution, leakage, or undesired transfer to nontarget cells) are also applicable to the use of $^{18}$F-FDG for cell tracking.

Currently, the most advanced strategy for cell tracking with PET imaging is the stable integration of a mutant herpes simplex type 1 thymidine kinase into stem cells and periodic intravenous injection of the thymidine kinase substrate, $^{18}$FHBG, which allows for activation of the tracking agent and thus serial imaging data acquisition. Although it permits tracking and quantification of stem cells over the course of many months, certain prerequisites might hamper immediate clinical applicability of this strategy: ex vivo genetic manipulation of the cells, an infrastructure for $^{18}$F chemistry, a PET scanner, and radiation exposure to the stem cells and subject.

Finally, additional drawbacks with PET (as well as SPECT) imaging include nonspecific uptake of the radiotracer by normal tissues, such as liver or kidney, and nonnegligible tissue photon attenuation.

Magnetic Resonance Imaging

Because of its safety profile and 3-dimensional capabilities, MRI is currently the most used imaging modality for in vivo tracking of labeled SPCs. At present, MRI imaging techniques can be divided into those generating primarily T1 contrast and those generating primary T2/T2$^*$ contrast.

T1 contrast agents are those that use the lanthanide gadolinium (Gd$^{3+}$), which increase the relaxivity of protons from associated water molecules and generate the signal on T1-weighted images. To generate optimal signal for detection by a 1.5-T MRI, a currently clinical standard MRI, intracellular concentrations greater than 50 μmol/L are required. Although there are several reports of the Gd$^{3+}$ agent being used to monitor the effects of cell-based therapy, achieving high enough (while at the same time nontoxic to cells of interest) concentrations within cells are enough to track cells in vivo is problematic.

T2/T2$^*$ contrast SPIO nanoparticle is one of the most frequently used contrast agents for cell labeling in MRI. The SPIO can be loaded on cells within 1 hour. Concentrations of 5 to 10 μmol/L are sufficient to generate detectable signals on T2-weighted images, which is more sensitive than T1-weighted images obtained with Gd$^{3+}$. Based on these characteristics, SPIO has been used in numerous studies to collect in vivo imaging data in stem cell–based therapy. Feridex (a clinical available SPIO)-labeled mesenchymal stem cells has been previously reported to provide optimal in vivo imaging for up to 8 weeks after cell transplantation into a swine model of infarction. Using this modality, it was possible to document a reduction in scar formation and prevention of left ventricular dysfunction after myocardial infarction. With SPIO labeling, adequate quantification of labeled stem cells is still problematic as there is susceptibility for artifact production by the SPIO itself. Furthermore, potential transfer of the contrast to non–stem cells, such as macrophages, after stem cell death adds another source of false-positives during imaging acquisition.

Incorporation of MRI contrast agent did not affect the viability of stem cells or progenitor cells. However, recent reports have alluded to the fact that Feridex loading might block cellular differentiation of mesenchymal stem cells (while not directly affecting viability and proliferation). Although controversial, these data raise concerns over the effect of Feridex on stem cell biology. Finally, an additional but clinical relevant issue with MRI is compatibility with implantable devices, such as pacemakers and implantable cardioverters/defibrillators. Despite recent reports suggesting that patients with pacemakers can be safely scanned at 1.5 T, the controversy persists regarding the use of MRI in patients with such devices, who could likely also be candidates for stem cell–based therapy. Further advances in in-dwelling cardiac devices and MRI technology will help to contribute to increase accessibility and safety for all eligible patients.

Multimodality Imaging

Recent research efforts have focused on the development of multimodality contrast agents, as there is currently no ideal imaging modality. Dual optical/MRI contrast agents have been described using visible wavelength fluorophores and Gd$^{3+}$ chelators conjugated to high-molecular-weight scaffolds such as dextran. Large
nanoparticles generating simultaneous MRI, ultrasound, and fluorescence contrast have also been described\(^4\) and might prove useful for multimodality stem cell tracking.

**Conclusion and Future Directions**

Although each modality presents unique advantages and disadvantages, each offer the possibility to provide specific answers to understand underlying mechanisms in cardiac stem cell–based therapy. No ideal imaging modality is currently available for in vivo stem cell tracking. Emerging molecular imaging technologies are awaited to further this important and exciting field.

**References**