

## In Vivo Stem Cell Imaging

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**Abstract:** In recent years, the emerging and advances of non-invasive *in vivo* stem cell imaging has significantly contributed to the real-time tracking of transplanted stem cells as well as monitoring their proliferation, migration and persistence in live animals and ultimately possibly in humans. This review summarized the different *in vivo* imaging modalities for imaging stem cell, especially for its monitoring viability, death and proliferation; and discussed the strategies of combined multimodality approaches for monitoring of the fate of transplanted stem cell by offering the opportunity to distinguish different biological and biochemical processes.

**Keywords:** Stem cell, molecular imaging, magnetic resonance imaging, positron emission tomography, fluorescent probes.

### INTRODUCTION

Stem cells can be classified as embryonic stem (ES) cells, fetal multipotent stem cells or adult stem cells (found in developed tissue, including brain, fat, skin, kidney, peripheral blood, bone marrow) and progenitor cells based on their origin during development or their location in the body [1]. Being with the potential of differentiation, stem cell has been increasingly attractive in regenerative therapies such as the treatment of Parkinson's disease, Alzheimer's disease, myocardial infarction, leukemia, diabetes, and other degenerative disorders [2, 3]. After the systemic or local transplantation, stem cells and progenitor cells may be able to migrate and repopulate in pathologic sites to bring tremendous therapeutic effect [2]. However, risk may happen for the misbehavior following the stem cell transplantation, for example, teratoma formation by undifferentiated ES cell in case of human ES cell transplantation [4-6]. Therefore, *in vivo* tracking the fate of the transplanted stem cells over time is a vital step in determining the efficacy of the implant. For a long time, studies on stem cell mobility conventionally require histological investigation to determine viable engraftment of the transplanted cells. However, the inability to visualize cell populations in the same animal over time has been a major bottleneck in the *in vivo* evaluation of stem cell therapies because groups of animals have to be sacrificed at different time point for histology purpose [7].

In recent years, the emerging and advances of non-invasive *in vivo* stem cell imaging has significantly

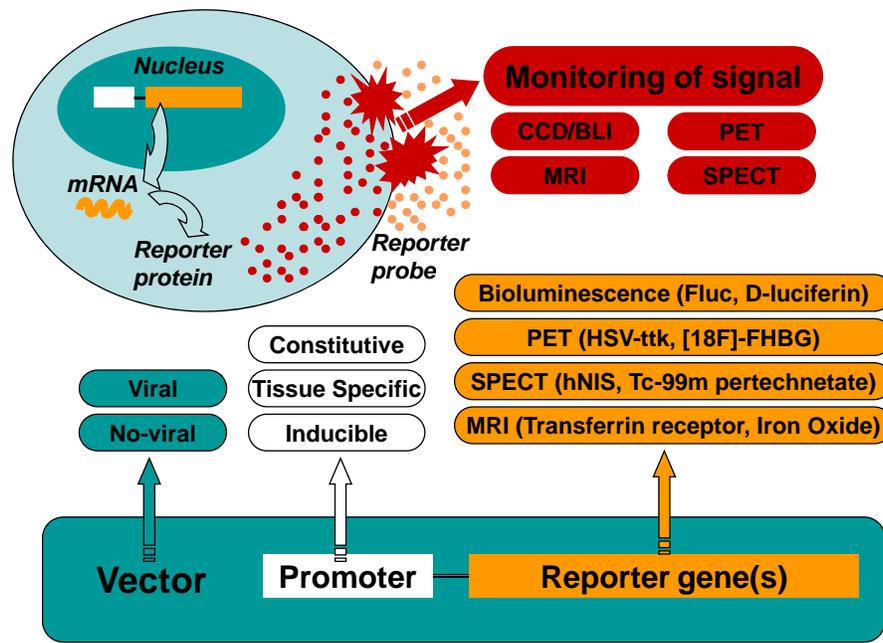
contributed to the real-time tracking of grafted stem cells as well as monitoring their proliferation, migration and persistence in live animals and ultimately possibly in humans [8, 9]. Usually, investigators manipulate cells *ex vivo* either by incorporating different exogenous imaging contrast agents or by transfecting different reporter genes. Stem cells carrying contrast agents (direct labeling) can be then detected by optical, magnetic resonance imaging (MRI) or radionuclide imaging methods, such as single photon emission computed tomography (SPECT) and positron emission tomography (PET) [10-15]. In contrast to direct labeling techniques, reporter gene techniques for stem cell labeling offer an attractive alternative because imaging signals are generated only from viable cells of interest. By sensitive imaging devices such as the optical coupled device, MRI, SPECT or PET, stem cells with the stably transfected imaging reporter genes can be detected with suitable imaging probes to visualize their distribution, to longitudinally monitor the cell survival and even to follow the successful differentiation of stem cells to mature functional cells in stem cell therapies [5, 9, 16]. Fig. (1) shows a schematic overview of stem cell imaging with reporter gene strategies.

Techniques available for *in vivo* molecular imaging so far include MR imaging [17], fluorescence and bioluminescence imaging [18, 19], SPECT and PET [20-22] based on their different principle (Table 1). These imaging modalities may provide distinctive, usually complementary information to the others. In this review, we will review mainstream stem cell imaging approaches on their characteristic aspects.

### MAGNETIC RESONANCE IMAGING

MRI can provide high spatial resolution (ranging from 50um in animal and up to 300um in whole body clinical scanners), high temporal resolution and extract physiologic and anatomic information simultaneously [12, 16]. Labeling

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**Fig. (1).** Schematic overview of molecular imaging with reporter gene strategies. A vector containing a DNA reporter construct with the reporter gene(s) driven by a specific promoter. Transcription and translation lead to the expression of mRNA and reporter protein, respectively. After administration of a corresponding reporter probe systemically, the reporter probe will be catalyzed by specific cells that have the reporter proteins. The signals occurred in this amplification process can be detected by a sensitive imaging device. Examples of reporter genes and their specific reporter probes are delineated by imaging modality. Fluc, Firefly luciferase; PET, positron emission tomography; HSV-ttk, herpes simplex virus truncated thymidine kinase; [18F]-FHBG, 9-(4-[18F]-fluoro-3hydroxymethylbutyl) guanine; SPECT, single photon emission computed tomography; hNIS, human sodium/iodide symporter; MRI, magnetic resonance imaging; CCD, charged coupled device; BLI, bioluminescence imaging.

**Table 1.** Imaging Modalities of Stem Cell Trafficking

Imaging Modality	Spatial Resolution	Temporal Resolution	Sensitivity	Depth	Animal Use	Clinical Use	Cost	Application	Advantages	Disadvantages
MRI	1mm	Minutes to hours	$10^{-3}$ - $10^{-5}$ M	No limit	Yes	Yes	Expensive	Anatomical, functional, molecular	High spacial resolution, no radiation	Low sensitivity, signal may not reflect viable cells
Bioluminescence	3-5mm	Minutes	$10^{-15}$ - $10^{-17}$ M	cm	Yes	No	Cheap	Molecular	High sensitivity	Not clinically applicable
Fluorescence	2-3mm	Minutes	$10^{-9}$ - $10^{-12}$	cm	Yes	No	Cheap	Molecular	High sensitivity	Not clinically applicable
SPECT	1-2mm	Minutes to hours	$10^{-10}$ - $10^{-12}$ M	No limit	Yes	Yes	Expensive	Functional, molecular	High translational capacity	Sensitivity 10-100 times lower than PET, radiation exposure
PET	1-2mm	Minutes to weeks	$10^{-11}$ - $10^{-12}$ M	No limit	Yes	Yes	Expensive	Anatomical, functional, molecular	High sensitivity, high biological specificity	Radiation exposure

stem cells with magnetic agents for MRI is a suitable approach to monitor the distribution, migration, survival and differentiation when transplanted into animals over weeks *in vivo* [1, 12, 13, 23-25]. By tracking labeled stem cell with MRI, it is helpful to establishing the optimal number of transplanted cells, define therapeutic windows and monitor cell growth and possible side effects for regenerative therapies [1]. MRI can also provide information about the surrounding tissue (edema, lesion size, inflammation) in addition to the information about grafted cell [1].

Stem cells should be labeled with contrast agents in advance before tracking with MRI. MR contrast agents contain metal ions, which define their relaxation properties. One type of contrast agents is the agent containing gadolinium-diethylenetriamine penta-acetic acid (Gd-DTPA), and the other type is the agent containing superparamagnetic iron oxide (SPIO) [26-30]. Paramagnetic metals such as gadolinium, iron and manganese mainly affect T1 relaxation. Whereas SPIO nanoparticles

predominantly reduce T2 and T2\* (due to the induction of strong field inhomogeneities) relaxation [31].

Contrast agents can be labeled to stem cell either by bounding to the external surface of the cell membrane by immunomagnetic linking to an antibody [1] or by internalizing into the cytoplasm through facilitated transmembrane uptake [32]. In the instance of immunomagnetic labeling, the contrast agent are not internalized into the cells, so they do not affect cell viability. However, the particles attached to the outer cell membrane are likely to interfere with cell-surface interactions and may easily detach from the membrane or be transferred to other cells [1]. Modified iron oxide particles such as dextran-coated SPIO nanoparticles, polycation-bound superparamagnetic iron oxide (PC-SPIO) and D-mannose-modified iron oxide nanoparticles are commonly used for intracellular magnetic labeling. These particles can provide efficient internalization with minimal deleterious effects on cellular functions [1, 32]. A study using human neural stem cells demonstrated that the biology of SPIO labeled stem cells, such as survival, migration, integration, and differentiation after transplantation is not altered *in vitro* and *in vivo*, making it possible to monitor the fate of the labeled stem cells *in vivo* under a variety of transplant conditions over time [25]. However, the influence of SPIO labeling on stem cell is still disputable because the impairment on the differentiation of SPIO labeled mesenchymal stem cells has also been found [33].

Combination of dextran-coated SPIO and transfection agent is a promising approach for stem cell labeling because transfection agents can effectively transport nanoparticles into cells through electrostatic interactions [1]. Other intracellular magnetic labeling strategies include the utilization of viral protein cages [34] and the use of internalizing monoclonal antibodies [26]. Compared to intracellular labeling, the average iron content per cell by immunomagnetic labeling was much lower (by two orders of magnitude) although sufficient MR contrast could be obtained [1]. Cells in suspension can be labeled using magnetoelectroporation [35]. Since exogenous markers such as SPIO particles are often degraded, diluted, and excreted as cell populations divide, it is not easy to assess the quantification and proliferation of stem cells *in vivo* by MRI [8].

Transferrin receptor (TfR) gene has been developed as a MR reporter gene for *in vivo* stem cell tracking. TfR can be highly expressed on the target cell membrane after transferring TfR gene to target cells. Binding with Tf protein, ultra small superparamagnetic oxides can enter cells through TfR and be detected by MRI as imaging probes [16]. Although efforts to develop suitable reporter genes for MRI have been done for years, the field is still in its early stage [36].

Stem cells labeled with Gd-DTPA can be monitored for only up to seven days after injection. Accordingly, Gd-DTPA labeled stem cell is not suitable for long term monitoring [37]. Whereas SPIO labeled stem cells are more appropriate for long-term *in vivo* monitoring because of the stability and high contrast of SPIO [26, 38]. However, MRI cannot specifically distinguish viable from nonviable cells or proliferating from nonproliferating cell populations, also cannot distinguish iron-labeled cells from free iron released

upon cell death [39]. Therefore, iron particle labeling should better be looked as a marker for high-resolution detection of cell location other than that for monitoring cell viability in MRI stem cell tracking [39].

SPIO labeled stem cells particles can be identified as hypointensities on T2-weighted spin echo and 3D gradient-echo MR images as the iron shortens transverse proton relaxation times. However, hypointense signal has the fundamental drawback of the difficulty in distinguishing iron-labeled cells from the surrounding air, hemorrhage, necrosis, and macrophages [25].

A number of factors, such as the SPIO concentration per cell, the cell density once the cells integrate into the host, and intrinsic MRI parameters such as field strength, signal to noise ratio, pulse sequence, and acquisition parameters, affects the detection threshold of SPIO-labeled cells [37, 40]. Among them, dilution of SPIO during cell division and reduction of cell density during migration and integration are the most important cellular factors determining the detection thresholds of MRI and limit long-term observation of SPIO-labeled stem cells [25].

Stem cell imaging with MRI is still lacking progress. As mentioned above, the low detection sensitivities of the imaging contrast agents and molecular reactions, the undesirable pharmacokinetics of substrates, and difficulties associated with the interpretations of signal changes are main impediments to overcome [16].

## BIOLUMINESCENCE AND FLUORESCENCE IMAGING

Transducing bioluminescent and fluorescent reporter genes to stem cells guarantee the *in vivo* long term optical tracking of the cells because these reporter gene can be expressed in all cell progeny without dilution [8, 41]. Bioluminescent and fluorescent imaging are simpler, cheaper, more convenient, more user friendly than other imaging modalities and high-throughput due to the ease of operation, short acquisition times (usually 10–60 s), and the capability for simultaneous measurement [6, 16].

Bioluminescence imaging (BLI) detects the emission of visible photons at specific wavelengths based on energy-dependent reactions catalyzed by luciferases. The light of bioluminescence typically has very broad emission spectra that frequently extend beyond 600 nm, with the red components of the emission spectra being the most useful for imaging by virtue of easy transmission through tissues [42]. Based on the high sensitivity of liquid cooled CCD cameras and the absence of background light emission caused by external illumination, bioluminescent imaging has great sensitivity to detect low levels of gene expression [16]. Firefly luciferase (Fluc), click beetle luciferase, Renilla luciferase (Rluc) and Gaussia luciferase (Gluc) are commonly used bioluminescence reporter gene for real-time stem cell tracking and Fluc is the most widely used one [16, 42]. By administering nontoxic substrate solution intravenously or intraperitoneally, bioluminescent images can be obtained within minutes [16]. The location, proliferation, migration, magnitude, and survival duration of stem cells of various origins have been monitored noninvasively *in vivo* using bioluminescent reporter genes, such as in brain [43, 44] and heart [5, 45]. A close

relationship has been verified between cell numbers and bioluminescence imaging signals [4, 5, 7, 46]. However, compared with other modalities such as MRI [8] and intravital microscopy (IVM) which can offer higher spatial resolution at a single-cell resolution [41], bioluminescence so far is still limited by a lower spatial resolution and unable to produce 3-dimensional and tomographic images because of attenuation of photons within tissues [39] although efforts in novel system development has been made to improve the accuracy of quantification and spatial resolution [42, 47].

Different from bioluminescence imaging, fluorescence imaging generally uses a fluorescent protein that is excited by an external light source with a wavelength a little shorter than that of the emitted light [16]. Green fluorescence protein (GFP) gene is the most commonly used fluorescence biomarker, which can be easily introduced into virtually any cell type using standard transduction methods and stable cell lines with continuous GFP expression can be produced [42]. Fluorescence imaging offers the possibility to image proteins and mammalian cells expressing them rapidly and inexpensively. However, due to the very low tissue penetrance (1–2 mm) of the excitation and emission wavelengths of GFP which are within the range of 500 nm and unacceptable amount of background signal caused by extrinsic excitation light, called autofluorescence, GFP reporter cannot be used to reliably track *in vivo* characteristics of transplanted stem cells [6, 16, 42, 48]. Many strategies for visualization of GFP *in vivo* have been designed. Fluorescence-mediated tomography (FMT) is an advanced method for acquiring fluorescent images of deeper targets [42]. With a novel system enabling 3-dimensional imaging of fluorescent probes in whole animals and in parallel with a 3-dimensional surface reconstruction algorithm. Garofalakis *et al.* improved the accuracy of quantitative three-dimensional reconstruction of fluorescence distribution and obtained a higher spatial resolution in a GFP-expressing transgenic mouse model [47]. However, in most cases, GFP-expressing tissue must often be exposed surgically for imaging [42]. New fluorescent proteins with long emission of near-infrared (NIR) wavelengths (700–900 nm), which have lower tissue absorption coefficients, also has been developed [49]. Imaging in the NIR spectrum can maximize tissue penetrance in addition to minimizing the autofluorescence from nontarget tissue. Studies with activatable NIR fluorescence optical probes have shown that the fluorescence can be increased over several hundred-fold by enzyme specific activation of probes in an *in vitro* cell culture experiments [50]. However, so far the only available NIR fluorochromes for stem cell tracking are quantum dots or molecule chemicals, by intracellular labeling [51] and by bounding to the external surface of the cell membrane [52].

Combined use of bioluminescent and fluorescent gene can be complementary to each other. Fluorescent marker may serve to determine the efficiency of transduction, and the conjuncted bioluminescent marker, may serve as an *in vivo* cell tracking protein [41, 46]. With these strategies, a study using lentiviral vectors transduced human neural stem cells (hNSCs) expressing fusions of fluorescent and bioluminescent proteins has been performed to study hNSC kinetics and migration to malignant brain tumors by dual bioluminescence imaging and IVM [41].

Because bioluminescent and fluorescent proteins expressed in transduced stem cell are foreign antigens, they may induce immune responses [53, 54]. However, a number of studies have demonstrated that there was no significant adverse effects on the biological properties such as viability, proliferation and differentiation of stem cells transduced with reporter genes including bioluminescence and fluorescence ones [5, 43, 55, 56].

Despite the limitations, molecular bioluminescence and fluorescence imaging may continue to play a key role by allowing efficient, noninvasive, and rapid assessments of transgene expression in preclinical models [57].

## RADIONUCLIDE IMAGING

Radionuclide imaging or nuclear medicine techniques, which include SPECT and PET, allow the imaging of radiolabeled markers and their interaction with biochemical processes in living subjects. Due to their exquisite nanomolar ( $<10^{-9}$  M) sensitivity, SPECT and PET are able to measure biological processes at very low concentrations.

Various clinical applicable radionuclides have been used based on previously established protocols for leukocyte or thrombocyte scintigraphy. For stem cell trafficking with SPECT, three strategies have been used: direct labeling with a radiometal [58, 59], enzymatic conversion with retention of a radioactive substrate [60], and receptor-mediated binding [61].  $^{99m}\text{Tc}$ -HMPAO labeling of mononuclear cells indicated that cardiac engraftment of cells in a dynamic process: the radioactivity uptake by the heart was 5% at 2 hours and 1% at 18 hours after transcortical cell transplantation in a patient with acute myocardial infarction [62]. Although bone marrow-derived mesenchymal stem cells labeled with  $^{99m}\text{Tc}$  were successfully visualized up to 4 hours after cell infusion in a rat model of myocardial infarction [63], limitation of this technique include the trade-off between half-life and long-term exposure to ionizing radiation as well as potential of the radiometal transfer to non-stem cells must be taken into consideration. The technique of enzymatic conversion with retention of a radioactive substrate has been used for both SPECT and PET imaging, which uses introduction of the enzyme through a transgene. The significant advantage of this approach is the ability to follow stem cells indefinitely after stable integration of the transgene as well as the absence of dilution by cell division [64]. Genetically engineered receptor-mediated binding is conditional on stable expression of receptor not found elsewhere in the body and intravenous injection of the radioactive receptor ligand [61]. SPECT provides the advantages of cell quantification, a lower background signals, but also has disadvantage of a lower spatial resolution compared with MRI and optical imaging [65].

PET has a higher sensitivity than SPECT and permits more accurate quantification of cell numbers. The above mentioned three stem cell tracking strategies used in SPECT can be readily used for PET. By direct bone marrow cell (BMC) labeling, the initial clinical study was performed by using  $^{18}\text{F}$ -FDG PET imaging. In this study, unselected BMCs were radiolabeled with  $^{18}\text{F}$ -FDG and infused into the infarct-related coronary artery or injected *via* an antecubital vein. After intracoronary transfer, 1.3% to 2.6% of  $^{18}\text{F}$ -FDG-labeled unselected BMCs were detected in the infarcted

myocardium; the remaining activity was found primarily in liver and spleen. However, only background activity was detected in the infarcted myocardium after intravenous transfer. Unselected BMCs engrafted in the infarct center and border zone [66]. With immunomagnetically enriched CD34-positive cells, 14% to 39% of total injected radioactivity was detected in infarcted myocardium after intracoronary injections, preferentially in the border zone. A similar clinical tracking study using  $^{18}\text{F}$ -FDG labeled peripheral hematopoietic stem cells has also been reported in patients with myocardial infarction [67]. However, due to the limitation of short half-life of  $^{18}\text{F}$ , direct cell labeling with  $^{18}\text{F}$ -FDG could not assess cell trafficking and persistence days after therapeutic application.

Reporter gene approaches have many advantages over direct and indirect cell labeling methods. Stable transfection of cells ensure for long term expression of the reporter gene that does not dilute out in proliferating cells. Furthermore, over time accumulated divided cells can generate increased signal that can be detected with repeated imaging [68]. In addition, the signals detected prove the *in vivo* presence of viable cells [69]. Currently, one of the most widely used reporter genes for PET imaging is wild-type herpes simplex type 1 thymidine kinase (*HSV1-tk*) and its *HSV1-sr39tk* mutant. This enzyme efficiently phosphorylates purine and pyrimidine analogs and has been very successfully used with radio-labeled reporter probes such as  $^{124}\text{I}$ -2'-fluoro-2'-deoxy-1- $\beta$ -D- $\beta$ -arabinofuranosyl-5-iodouracil (FIAU),  $^{18}\text{F}$ -2'-fluoro-2'-deoxy-1- $\beta$ -D- $\beta$ -arabinofuranosyl-5-ethyluracil (FEAU) and  $^{18}\text{F}$ -9-(4- $^{18}\text{F}$ -fluoro-3-hydroxymethyl-butyl) guanine (FHBG) [5, 70, 71]. One of the advantages of enzymatic reporter gene systems, such as -tk enzyme, is the signal amplification that occurs as a result of imaging probe trapping and accumulation. This signal amplification is generally not generated by receptor and transporter based reporters. However, the major limitation for successful translation of HSV-tk reporter gene into clinical setting is the immune reaction that the viral protein elicits in humans [69, 72]. Although reporter gene imaging 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  $^{18}\text{F}$  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 non-negligible tissue photon attenuation. Despite bearing a conceptual promise, the use of reporter-gene imaging to monitor cell transplantation is still limited to animal model studies except for one recent clinical report [73]. In order to proceed from bench to bedside, further work is required to develop nonimmunogenic probes, improve transfection stability, and reduce the interference of transfection with the cell function and desired molecular effect. Strength of the imaging signal is critical, and additional work is necessary to establish a robust approach for cell visualization that is also practical for use in the clinical setting [65].

### MULTIMODALITY IMAGING

Combined multimodality approaches are novel strategies that allow the monitoring of the fate of transplanted stem

cells by offering the opportunity to distinguish different biological and biochemical process in a single animal. Various combinations, such as double fusion of bioluminescence and fluorescence reporter gene [5, 55], double fusion of different bioluminescence reporter genes [74-76], co-labeling with reporter gene and iron particles [39], and triple fusion of the fusion of fluorescence, bioluminescence and PET reporter genes [46] have been developed for *in vivo* stem cell tracking. Multimodal systems containing MRI reporter gene might be helpful because the images of MRI reporter gene expression with high resolution may be perfectly complementary to PET or bioluminescent and fluorescent images with high sensitivity [16]. In many respects the techniques are complementary; there is no "all-in-one" imaging modality providing optimal sensitivity, specificity and temporo-spatial resolution. Novel combined multimodality approaches, such as PET-MRI, PET-CT, PET-optical imaging, etc., may be designed in the future as a more suitable approach for monitoring the spatial and temporal kinetics of transplanted stem cells in living subjects *in vivo*.

### CONCLUSION

Reporter gene imaging using PET is a better technique for monitoring long-term cell viability, death, and proliferation, whereas MR imaging is a better technique for high-resolution detection of cell location post-transplantation [39]. Bioluminescent imaging should be seen as the one that is possibly complementary to other modalities such as MRI in which higher resolution is required [8]. To confirm the fate of stem cells *in vivo*, it is crucial to continue the development and further refinement of noninvasive imaging techniques.

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