We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Current Perspectives on Molecular Imaging for Tracking Stem Cell Therapy

Lingling Tong, Hui Zhao, Zuoxiang He and
Zongjin Li

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/53028

1. Introduction

The main objective of stem cell therapy is to repair the tissue with functional cells differentiated from stem cells, and contribute to the lost organ function together with the remaining functional native cells. Nevertheless, there also remain important questions unanswered, regarding the engraftment, viability, biology and safety of transplanted stem cells, as well as interaction with the environment. Consequently, novel molecular imaging techniques are necessary for investigating the behaviors and the ultimate feasibility of cell transplantation therapy of stem cell.

Along with the rapid development of sensitive, noninvasive technologies, several molecular imaging approaches have been implicated to track the fate of stem cells *in vivo* [1-4]. Continuously observing the process of tissue regeneration after stem cell transplantation would markedly improve knowledge about the underlying cellular mechanisms and analysis of the molecular pathways that control this process. Although, this is inconsistent with the most current studies, in which the cells are observed in hours or days time point without definite cell population. So far, there is no single imaging modality that is ideal to observe all the relevant aspects of stem cell therapy with a continuous manner [5]. The new development of molecular imaging technology push the researches of stem cell therapy much closer to the single-cell level, and make the observation of different types of stem cells more continuous and comprehensive.

2. Promises, challenges, and needs for molecular imaging of stem cell therapy

Molecular imaging is a rapidly emerging biomedical research discipline that provides integrated information on specific molecules of interest within the cells of *living* subjects and

thus holds great promise as an effective way to track certain cellular and subcellular events of the transplanted cells [2, 6-8]. The visual representation, characterization, and quantification of biological processes within intact *living* organisms obtained from molecular imaging techniques is particularly helpful in evaluations of the functional outcomes of cell engraftment and may shed light on the mixed findings regarding stem cell therapy. In recent years, a variety of imaging technologies is being investigated as tools for evaluating stem cell therapy in living subjects. Molecular imaging modalities include optical bioluminescence, optical fluorescence, targeted ultrasound, molecular magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), single-photon-emission computed tomography (SPECT), and positron emission tomography (PET) [9]. Moreover, many hybrid systems that combine two or more of these modalities are already commercially available [9]. The use of noninvasive, longitudinal, and quantitative imaging of the fate of stem cells can facilitate preclinical experimental studies in animal models and can help in human stem cell therapy trials as well.

A wide variety of stem or progenitor cells, including adult bone marrow stem cells, endothelial progenitor cells, mesenchymal stem cells (MSCs), resident cardiac stem cells, and embryonic stem cells, have been shown to have positive effects in preclinical studies and therefore hold promise for treating and curing debilitating and deadly diseases. Several of these types of stem cells have been tested in early-stage clinical trials, such as MSCs [10], human embryonic stem (hES) cells [11]. However, to realize the full therapeutic potential of stem cell technology, it will be necessary to develop novel and improved quality assessments that can be used readily to determine the exact cellular state of the transplanted cells.

After the systemic or local administrating, stem cells may be able to proliferate, migrate and repopulate in pathologic sites to bring tremendous therapeutic effect. However, the transplantation success is companied with risks of the stem cell misbehavior after delivered, especially embryonic stem cells [6, 12]. Consequently, real-time visualization of the fate of the transplanted cells over time *in vivo* is a vital step to determine the efficiency of the implantation. By tracking the optimal number of transplanted cells, researchers can define therapeutic windows and monitor cells growth and possible side effects for regenerative therapies [13].

The ability to label and track stem cells in humans would provide a method to answer some of the ongoing, unsolved issues in the field. The most efficacious route of delivery, the appropriate choice of stem cell type(s), the optimal cell population for treatment in a chronic setting and the favorable time-point of cell delivery, however, is still unknown and requires further study. A safe, noninvasive, and repeatable imaging modality that could identify injected stem cells would be able to answer questions about cell viability and retention in future clinical trials of stem cell therapies, as well as provide the ability to adjust the assessment of bioactivity on the basis of actual delivered doses of cells. With the desire to monitor stem cells long-term continuously with high temporal resolution and good biocompatibility, which have the properties of differentiation and self-renew over long periods of time, stem-cell-derived regeneration still faces in its efforts to improve.

Despite significant progress in molecular imaging, no single technique meets all the stem cell tracking criteria. Combined imaging modalities, such as PET-CT, are already well accepted and offer high sensitivity and anatomical detail [14]. Multimodality imaging approaches are likely to play an important role in illuminating different aspects of stem cell biology *in vivo* and elucidating the mechanisms of tissue repair and regeneration. In brief, noninvasive imaging stem cell therapy could provide greater insight into not only the therapeutic benefit, but also the fundamental mechanisms underlying stem cell fate, migration, survival and engraftment *in vivo*.

3. Approaches and implications of stem cell imaging

A number of methods are available to track stem cells by molecular imaging. In general, there are two methods to label the cells: [1] direct labeling method, which physically introduce marker(s) into the cells before transplant; [2] indirect labeling method, which genetically introduce reporter gene(s) into the cells before transplant. The current noninvasive imaging approaches for tracking stem cells *in vivo* include imaging with magnetic particles, radionuclides, quantum dots (QDs) and reporter genes (Figure 1).
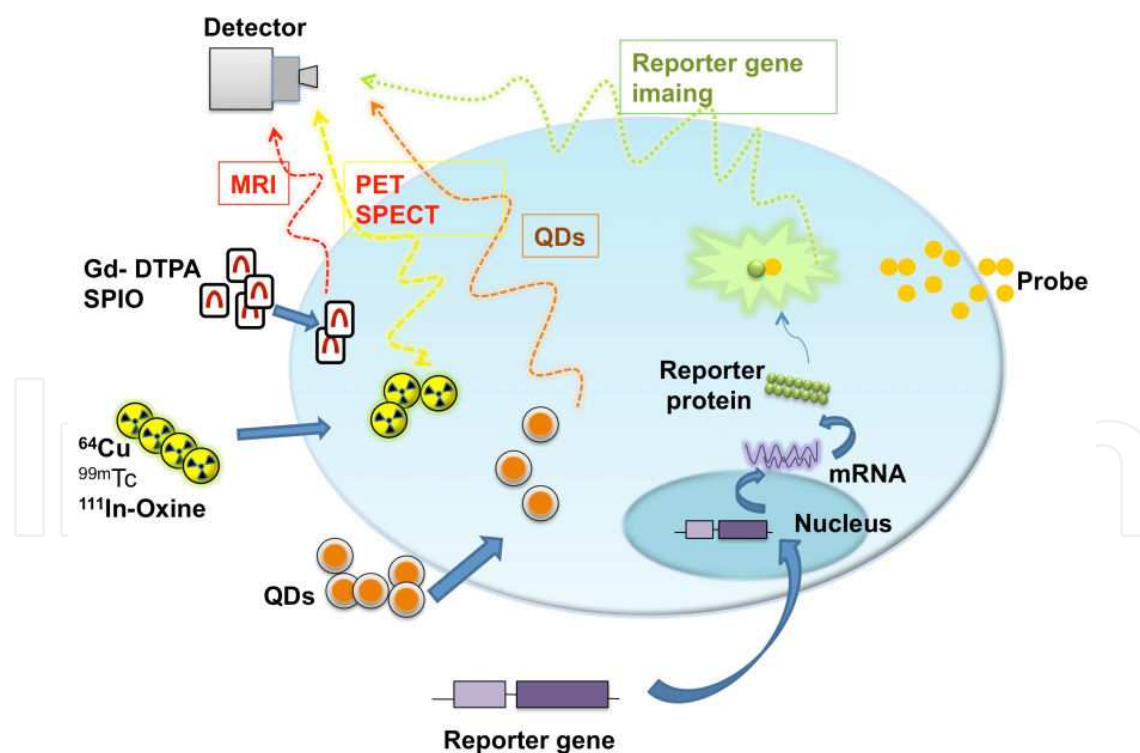
Figure 1. Conceptual basis for noninvasive imaging of transplanted stem cells in living animals. It shows imaging techniques including magnetic resonance imaging, radionuclide imaging, quantum dots imaging and reporter gene imaging. Abbreviations: Gd-DTPA, gadolinium-diethylenetriamine penta-acetic acid; SPIO, superparamagnetic iron oxide; ^{99m}Tc, ^{99m}Tc-hexamethylpropylene amine oxime; ^{111}In-Oxine, ^{111}In-oxyquinoline.

Imaging of stem cell therapy requires the selection of a molecular target, an imaging probe, and an imaging system. Specific molecular targets along with advances in imaging modalities increase the sensitivity and specificity of stem cell imaging. The commonly used labeling methods are discussed below.

3.1. Magnetic particle labeling

Possessing the advantages of high spatial resolution (ranging from 50μm in animal and up to 300μm in whole body clinical scanners) and high temporal resolution, magnetic resonance imaging (MRI) is widely used for *in vivo* cell tracking in preclinical and clinical studies. The fundamental principle underlying MRI is magnetic dipoles (such as hydrogen atoms in water and organic compounds), which align themselves when placed into a magnetic field. To be tracked in ischemic tissues, stem cells need to be enriched with a contrast agent that produces a sufficient positive or negative signal to distinguish them from the background. One type of contrast agents is the agent containing gadolinium-diethylenetriamine penta-acetic acid (Gd-DTPA), and the other type is the agent containing super paramagnetic iron oxide (SPIO). At present, SPIO are the preferred agent for short-term stem cell tracking. With the high spatial and temporal resolution, MRI allows the location of iron-labeled donor cells to be monitored noninvasive over several weeks *in vivo* [3, 15]. However, it is difficult to distinguish iron-labeled cells from the surrounding air, hemorrhage, necrosis, and macrophages. To address these problems, off-resonance (OR) MRI has been developed for imaging iron-labeled hES cells to generate positive contrasts through enhancement of signal and suppression of background tissue [3].

3.2. Radionuclide labeling

Radionuclide imaging techniques, including positron emission tomography (PET) and single-photon emission computed tomography (SPECT), allow the imaging of radiolabeled makers and their interaction with biochemical processes in *living* subjects. Current clinical molecular imaging approaches primarily use PET or SPECT-based techniques. Compared with MRI, PET and SPECT provide high intrinsic sensitivity ($<10^{-11}$M) and can use a variety kind of imaging agents. With the improvements in spatial resolution (1-2mm), radionuclide imaging has been made particularly suitable for cell tracking. Direct labeling of cells with radiometals in clinical practice has used ^{111}In-oxyquinoline and ^{99m}Tc-hexamethylpropylene amine oxime. Imaging plays a role in monitoring short-term cell tracking, long-term cell survival and function, and as a surrogate marker of implant efficacy. Labeling implanted cells with relatively long-lived isotopes, such as ^{111}In-Oxine for SPECT and ^{64}Cu for PET, allows shorten, real-time cell tracking, to determine biodistribution and availability in the target organ [16].

However, these cell-labeling techniques have some significant disadvantages. They are limited by concerns such as the potential transfer of radiotracer to nontargeted cells and potential adverse effects of the radiotracer on stem cell viability, function, and differentiation capacity. Thus, the effects of labeling on the capacity to differentiate stem cells of various origins are needed to be studied.

3.3. Nanoparticle labeling

QDs are emerging as an important class of fluorescent agents. Luminescent colloidal QDs are inorganic semiconductor particles with physical properties that enable them to emit fluorescent light from 525 to 800 nm. The nanoparticles are consisting of an inorganic core, a shell of metal and an outer organic coating. The total diameter of quantum dots is 2–10 nm [17], depending on the physical properties of the material due to the quantum nature of QDs. With the capability of being excited by one single wavelength and emission light of different wavelengths, QDs are ideal probes for multiplex imaging. By contrast, conventional organic labeling agent cannot be easily synthesized to emit different colors and have narrow excitation spectra and broad emission spectra, making it difficult to use these dyes for multiplexing. Due to their extreme brightness and resistance to photobleaching [18], QDs are appropriate for live stem cells imaging, which requires long-term observation under the excitation light source. The approaches of QDs entering stem cells include passive loading, receptor-mediated endocytosis or transfection. Passive loading has been found to be the most effective method owning to the high label efficient and limited damage to surrounding cells. QDs are capable of single quantum dot tracking, multiplex imaging, and 3-D imaging reconstruction.

Unquestionable, quantum dots represent a novel strategy to tracking stem cells *in vivo*. However, the effects of QDs on stem cell biology remain unclear because mixed results have been reported using different stem cells or experimental protocols [19, 20]. Moreover, light scattering limits the applicability of this approach, especially to the brain in humans, making it difficult to use in 3D localization or quantitative estimation of cell survival. Last, several other obstacles, including nonspecific binding to multiple molecules and the tendency for aggregation of QDs in the cytosol must be overcome before clinical application with their full potential.

3.4. Reporter gene labeling

Reporter gene imaging has been commonly applied to the non-invasive imaging of stem cell therapy by studying the survival, localization, and functional effects of exogenously administered stem cells. Imaging of gene expression in *living* subjects can be directed either at genes externally transferred into cells of organ systems (transgenes) or at endogenous genes, and the former one is used in most applications of reporter gene imaging at present. The principle of reporter gene imaging is relatively simple. In general, reporter genes are DNA sequences that encode for easily assayed proteins. In the case of imaging, reporter genes encode for a reporter protein that, when exposed to an imaging probe, produces an analytic signal which can be generates some form of signal that can be captured and quantified by an imaging modality such as MRI(21), PET(22), SPECT(16), or an optical charge-coupled device [6]. Reporter genes can be linked to a gene of interest (*i.e.*, creation of a promoter construct) such that the reporter protein provides a surrogate marker of that gene's activity.

In the fields of stem-cell-driven regeneration, some conventional reporter genes, such as green fluorescent protein (GFP) [7], firefly luciferase [23], renilla luciferase [24], and HSV1-tk [25] allow for localization in some small *living* animals. Stable transfection or transduction

with reporter genes is useful in assessing kinetic survival status of the implanted cells because the reporter genes can be expressed as long as the cells are alive. However, the reporter gene approach in cell tracking requires genetic manipulations of the cells, which may lead to insertional mutagenesis. The advances in site-specific chromosomal integration mediated by phiC31 integrase may cast a new light in overcoming this obstacle [2]. Although small animals or animals transparent to light can be imaged with a cooled charged coupled device (CCD) camera, these imaging techniques are somewhat limited because of their lack of generalizability and detailed tomographic resolution.

4. Advances in molecular imaging for tracking stem cell therapy

Stem cell therapies offer enormous potential for the treatment of a wide range of diseases and injuries including neurodegenerative diseases, cardiovascular disease, diabetes, arthritis, spinal cord injury, stroke, and burns. More research teams are accelerating the use of other types of adult stem cells, in particular neural stem cells for diseases where beneficial outcome could result from either in-lineage cell replacement or extracellular factors. At the same time, the first three trials using cells derived from pluripotent cells have begun [12]. These early trials are showing roles for stem cells both in replacing damaged tissue as well as in providing extracellular factors that can promote endogenous cellular salvage and replenishment [26].

Clinical trials have demonstrated that stem cell therapy can improve cardiac recovery after the acute phase of myocardial ischemia and in patients with chronic ischemic heart disease [10]. Nevertheless, some trials have shown that conflicting results and uncertainties remain in the case of mechanisms of action and possible ways to improve clinical impact of stem cells in cardiac repair [27]. The public clinical trials database http://clinicaltrials.gov shows 238 clinical trials using MSCs for a very wide range of therapeutic applications. Although early clinical trials of stem cell therapy have showed positive effect, there remains much controversy about which cell type holds the most promise for clinical therapeutics and by what mechanism stem cells mediate a positive effect, and further research should be able to answer these questions.

4.1. Imaging of embryonic stem cells driven regeneration

Embryonic stem cells (ESCs) are pluripotent stem cells capable of self-renewal and differentiation into virtually all cell types [28]. Various lineages have been derived from human and mouse ESCs, including cardiomyocytes, neurons, hematopoietic cells, osteogenic cells, hepatocytes, insulin-producing cells, keratinocytes, and endothelial cells. Given their unlimited self-renewal and pluripotency capacity, ESCs have been regarded as a leading candidate source for novel regenerative medicine therapy. So far, ESCs transplantation has been widely investigated as a potential therapy for cell death-related heart disease, ischemic diseases, CNS disorders and diabetes. However, the bottleneck of application of ESC driven regeneration is high risk of teratoma formation *in vivo [3, 6].*

The concurrent development of accurate, sensitive, and noninvasive technologies capable of monitoring ESCs engraftment *in vivo* has greatly accelerated basic research prior to future clinical translation. Numerous imaging modalities have analyzed the behavior of embryonic stem cells that have been transplanted to regenerate tissues, which include MRI, bioluminescence imaging (BLI), fluorescence, PET, and multimodality approaches. Two main PET strategies for embryonic stem cell has been used——direct imaging [29] and indirect imaging [30]. Although the value of PET lies in its easy accessibility and high-sensitivity tracking of biomarkers, potential disadvantages of PET include repeated injection of radioactive substances into an organism with the potential to radiation accumulation [31] and adverse effect on ESCs viability and pluripotency capacity [32]. Additionally, the short half-lives of most current available radiotracers have limited their use for long-term tracing [33]. MRI has been used for tracking mouse ESCs [34] in the heart, hind limbs, brain, lung and kidney. Meanwhile, MRI is accessible for tracking ESCs engraftment, providing detailed morphological and functional information. Drawbacks of MRI include low sensitivity and being unable to quantify cell population. However, the use of CLIO-Tat peptides [35]is promising to overcome some of these limitations. Holding the significant advantage of high sensitivity (100-1000 cells, for more superficial anatomical sites), safety, low cost and the repeated tracking of small numbers of labeled cells in whole body distribution without background signal, BLI is widely used in this field. Zongjin Li, et al. have compared of BLI and MRI for tracking fate of hESCs and hESC derived endothelial cells (hESC-ECs) in animals, data of which prove that reporter gene of BLI is a better marker for monitoring ESCs and ESC-ECs viability and MRI is a better marker for high-resolution detection of cell location. Nevertheless, at present, bioluminescence imaging still lacks adequate tomographic resolution because of attenuation of photons within tissues [3]. An innovative approach to combine the strengths of optical fluorescence, bioluminescence, and PET is the creation and use of a fusion reporter [36] construct composed of RFP, Fluc, and HSV-tk. This fusion reporter construct has been adapted to research the spatio-temporal kinetics of hESC engraftment and proliferation in living subjects, without significant adverse effects on mouse ESC viability, proliferation, differentiation, or proteomic expression [37].

4.2. Imaging of mesenchymal stem cells driven regeneration

Mesenchymal stem cells (MSCs) are a heterogeneous subset of stromal stem cells that can differentiate into cells of the mesodermal lineage, such as bone, fat and cartilage cells, but they also have endodermic and neuroectodermic differentiation potential. The use of MSCs for clinical purposes takes advantage of their poor immunogenicity *in vitro*. Preclinical [38] and clinical [39, 40] studies have supported the possible use of MSCs obtained from allogeneic donors in the clinic. In preclinical researches, MSCs have been applied in tissue regeneration, including haematopoietic organs, heart, CNS, skin, kidney, liver, lung, joint, eye, pancreas and renal glomeruli. The current data indicate that bone-marrow-derived MSCs were first proposed for therapeutic purposes in regenerative medicine on the basis of their stem-cell-like qualities [41].

The versatility of the molecular imaging method could allow cellular tracking using single or multimodal imaging modalities. These single methods include direct labeling of cells for transplantation with iron oxide particles for MRI, with ^{18}F-hexafluorobenzene or ^{18}F-FDG for PET, or with ^{111}In-oxine or ^{111}In-tropolone for SPECT. Noninvasive MRI is fast becoming a clinical favorite, though there is scope for improvement in its accuracy and sensitivity. In that, use of superparamagnetic iron-oxide nanoparticles (SPION) as MRI contrast enhancers may be the best select for tracking MSC treatment delivery and monitor outcome [42, 43]. Indirect labeling relies on the expression of imaging reporter genes transduced into cells before transplantation. A classic example of using reporter gene tracing MSCs transplantation is the research by Zachary Love, et al. They have used a triple-fusion reporter system (fluc-mrfp-ttk) for multimodal imaging to monitor hMSCs transplanted into NOD-SCID mice. Signals from the cubes loaded with reporter-transduced hMSCs were visible by BLI over 3 mo, meanwhile, PET data provided confirmation of the quantitative estimation of the number of cells at one spot (cube) [44]. The reporter gene approach resulted in a reliable method of labeling stem cells for investigations in small-animal models by use of both BLI and small-animal PET imaging.

4.3. Imaging of neural stem cell therapy

Neural stem cells (NSCs)-driven regeneration has been proposed as a promising potential treatment option for CNS-related disease processes, including everything from cerebrovascular disease to traumatic brain injury to degenerative diseases of the CNS. Grafted NSCs differentiated into neurons, into oligodendrocytes undergoing remyelination and into astrocytes extending processes toward damaged vasculatures [45]. At present, Applications of NSCs therapy of neurological diseases, including Alzheimer's disease, Huntington disease, stroke, spinal cord injuries in preclinical researches have raised intense interest and the hope of radical new therapies in clinical.

In contrast to most tissues in adults, the central nervous system has a low regenerative activity, and neural stem cells reside in regions of the adult brain that are difficult to access by most imaging modalities [5] owning to tissue depth and the blood-brain barrier(BBB). The most promising techniques for monitor the fate of NSCs *in vivo* are MRI, PET and optical imaging. MRI has been used in clinical practice for the past 30 years to diagnose brain lesions and is therefore already a standard clinical adjunct for neuropathologies. Other than SPIO, which is the most studied and preferred contrast agent of MRI, magnetic resonance reporter genes [46] are another possible means of magnetic resonance labeling NSCs. However, this technique is still in its infancy, further study into the possibility of magnetic resonance reporter genes is needed before this technology can be used for NSCs. MRI has been used in tracing neural stem cells labeled with SPIO in patient with brain trauma [47]. The hypointense signal generated by the cells demonstrated cell trace from the implantation site to the periphery of the lesion the first week, and then disappeared by the seventh week, which the group attributed to NSC proliferation. PET has been used clinically for the past 20 years to assess for neurotransmitter

changes in a wide variety of disease processes, including Parkinson's disease, Alzheimer's disease, Huntington's disease and psychiatric illnesses. *In vivo* PET imaging for NSCs requires the potential radiotracers in the brain owning the capacity of crossing BBB. A solution to the BBB problem could be the use of the xanthine phosphoribosyl transferase reporter enzyme PET system, which employs xanthine reporter probes that can cross the BBB [48]. With regard to *in vivo* NSCs imaging, bioluminescence is the most studied of the optical imaging techniques and has been employed in numerous small animal studies. Improvement of the existing imaging modalities, assessment of the effect of imaging modalities on cellular biology, and development of new techniques for *in vivo* NSC imaging, would open up the window of the use of NSCs for various neuro-pathologies.

4.4. Imaging of hematopoietic stem cell transplantation

Hematopoiesis is described to be the production of all types of fully differentiated daughter blood cells from ancestral great-grandmother hematopoietic stem cells (HSCs). HSCs studies and clinical applications have historically been ahead of other tissue stem cells and have generated most stem cell biology models. However, hematopoiesis is arguably among the most difficult of the mammalian stem-cell systems to image real-time *in vivo* [5]. In homeostatic conditions, the different short-lived cell types of blood are regenerated from a small population of HSCs [49], while a significant proportion of HSCs with long-term reconstitution potential is predominantly quiescent or divides infrequently. The HSC niche is most likely a complex, multi-component microenvironment of which the osteoblast is just one of the major constituents identified so far. Thus, non-invasive long-term imaging is more challenging in the bone marrow. Hematopoiesis is better understood than other stem-cell systems and has important clinical significance, but despite intensive research in the past decade, many basic questions are still unresolved [50]. MRI [51], bioluminescence imaging [52], and multiphoton fluorescence microscopy [53] had been applied in continuous observation of cellular behavior of HSCs. Mentionable, continuous observation of hematopoietic progenitor cells in the bone marrow was achieved at single-cell resolution by using multiphoton fluorescence microscopy after the transplantation, filling the gap of low single cell sensitivity and resolution of the first two modalities. The transplantation option of multiphoton fluorescence microscopy is clinically relevant because HSC transplantation is used to treat patients with hematological malignancies such as leukemia and multiple myeloma. Multiphoton fluorescence microscopy was also used to observe the homing of normal and malignant hematopoietic progenitor cells in the bone marrow and to characterize the specialized niches of these cells [54].

4.5. Imaging of endothelial progenitor cell therapy

Endothelial progenitor cells (EPCs) recruitment is often involved in the tissue injury triggered reparative processes, and contribute to healing ischemic tissues. Transplantation of

EPCs offers the potential for targeted treatment of ischemic diseases such as myocardial [55], hind-limb ischemia [56], and renal injury [57]. Considerable efforts have been made to monitor the fate of endothelial progenitor cells fate *in vivo* using the *in vivo* molecular imaging modalities, such as PET [58], computed tomography(CT) [59], MRI [56], BLI [60]. Micro-CT has been applied in studies of EPCs in rat, pig and human beings. In the research of homing and renal repair function of EPCs in renovascular disease [59], renal hemodynamics and function were assessed in pigs by multidetector computed tomography, showing that EPCs are renoprotective as they attenuated renal dysfunction and damage in chronic atherosclerotic renal artery stenosis, and consequently decreased the injury signals. Based on the previous research, maybe CT is promising in clinical application of endothelial progenitor-driven regeneration.

5. Ideal imaging modalities for stem cell therapy

Currently, none individual imaging modalities can fill the bill of flawless, without limitations in the spatial and/or temporal resolution or the time span and/or volume that can be observed in a single experiment. The use of direct labeling, with labeling agent such as SPIO or ^{18}F-FDG is hindered by signal decrease, as a result of radio-decay or cell division or cell dispersion. Additionally, the label may become dissociated from the exogenous stem cell. Thus, direct labeling may not be a reliable means of monitoring long-term cell viability. On the contrary, this approach is a valid method to observing the stem cell delivery and homing properties. Meanwhile, Reporter gene imaging offers unique capabilities for noninvasive and longitudinal measurement to determine cell biology and cell viability.

Fundamentally, the choice of modality depends on the questions being addressed (**Table 1)**. If the objective of the research is to image the delivery and early cell localization and homing of stem cells in different organs, a direct labeling approach may be the answer, even though potential toxicity must be taken into account. MRI is among the least invasive of available imaging technologies, equipped with expensive new experimental machines, which provide almost the highest spatial and high temporal resolution for continuous single-cell delivery imaging. But the molecular sensitivity of MRI is lower than other modalities such as radionuclide imaging. Radionuclide imaging modalities (PET, SPECT) have been successfully and extensively used with high intrinsic sensitivity, although they may not provide sufficient spatial resolution. On the other hand, if observation of stem cell biology and interaction with microenvironment or long-term monitoring of cell viability is the goal, reporter gene imaging using optical imaging (bioluminescence, fluorescence), PET/ SPECT imaging, MR imaging appears to be a better option. For example, if a study is about ESCs derived myocyte, use of a reporter gene that is driven by a promoter that will only be activated when the cell has the features of an adult myocyte (*i.e.*, expresses the sarcomeric protein Troponin T) can provide the information of stem cell differentiation into goal histiocyte.

Goal	Strategy	Imaging modality	Spatial resolution	Depth	Sensitivity (mol/L)	Quantitative degree	Clinical	Advantages	Disadvantages
Cell delivery	Direct visualization	Ultrasound	50–500μm	mm to cm	Not well characterized	+	Yes	Real-time, low cost	Limited spatial resolution
		MR fluoroscopy	1mm	No limit	10^{-3}–10^{-5}	++	Yes	3D datasets, soft-tissue contrast	Low spatial resolution
Early cell localization and homing	Direct cell labeling	QDs	100–400μm	<3cm	10^{-3}–10^{-9}	+	No	High sensitivity, multiplexing	Undefined effects on stem cell biology
		MRI (e.g.SPIO)	50–300μm	No limit	10^{-3}–10^{-5}	++	Yes	Combines morphological and functional imaging	Long scan and postprocessing time
		PET (e.g., ^{64}Cu, ^{18}F-FDG)	1–2mm	No limit	10^{-11}–10^{-12}	+++	Yes	High sensitivity, quantitative translation	Low spatial resolution, radiation to subject
		SPECT (e.g., ^{111}In-Oxine)	1–2mm	No limit	10^{-10}–10^{-11}	++	Yes	Can image multiple probes simultaneously	Low spatial resolution
Stem cell viability and biology	Reporter gene imaging	Bioluminescence (Flu, Rlu)	3–5mm	1–2 cm	10^{-15}–10^{-17}	+ ~ ++	No	Highest sensitivity, low-cost, high-throughput	2D imaging, limited translational research
		Fluorescence (RFP, GFP)	2–3mm	<1 cm	10^{-9}–10^{-12}	++	Yes	Detects fluorochrome in live and dead cells	Low spatial resolution, surface-weight
		MRI (e.g., Transferrin)	50–300μm	No limit	10^{-3}–10^{-5}	++	Yes	Combines morphological and functional imaging	Long scan and postprocessing time
		PET (e.g., HSV1-tk)	1–2mm	No limit	10^{-11}–10^{-12}	+++	Yes	High sensitivity, quantitative translational research	Low spatial resolution, radiation to subject
		SPECT (e.g., NIS, ^{99m}Tc)	1–2mm	No limit	10^{-10}–10^{-11}	++	Yes	Can image multiple probes simultaneously	Low spatial resolution

Table 1. The different imaging strategies of stem cell trafficking and guide to finding the appropriate molecular imaging modalities

6. Conclusions

Stem-cell-driven regeneration offers tremendous approach for the treatment of intractable diseases. Tracking the fate of implanted cells is vital to monitor the delivery and viability of the grafts over extended periods of time. Molecular imaging represents one such tool that can provide insight into cell survival and proliferation following transplantation into the tissue. The greatest potential for optimizing imaging approaches for regeneration research probably lies in applying new insights from stem-cell biology and the development of molecular imaging. As experimental techniques and molecular imaging technologies progress, the potential benefits of regenerative medicine should be a strong motivation to continuously improve imaging technology that will enable stem-cell-driven regeneration in mammals to be more understood. Efforts now should focus on the development of novel labeling agent and multimodality approaches to increase perception of regenerative medicine, and promote the clinical translation of these techniques.

Acknowledgment

The authors indicate no potential conflicts of interest.

Author details

Lingling Tong[1], Hui Zhao[2], Zuoxiang He[3] and Zongjin Li[1]

*Address all correspondence to: zongjinli@nankai.edu.cn

1 Department of Pathophysiology, Nankai University School of Medicine, Tianjin, China

2 Tianjin Key Laboratory of Food Biotechnology, School of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin, China

3 Department of Nuclear Medicine, Cardiovascular Institute and Fuwai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

References

[1] Gu E, Chen WY, Gu J, Burridge P, Wu JC. Molecular imaging of stem cells: tracking survival, biodistribution, tumorigenicity, and immunogenicity. Theranostics. 2012;2(4):335-45.

[2] Li Z, Han Z, Wu JC. Transplantation of human embryonic stem cell-derived endothelial cells for vascular diseases. Journal of Cellular Biochemistry. 2009;106(2):194-9.

[3] Li Z, Suzuki Y, Huang M, Cao F, Xie X, Connolly AJ, et al. Comparison of Reporter Gene and Iron Particle Labeling for Tracking Fate of Human Embryonic Stem Cells and Differentiated Endothelial Cells in Living Subjects. Stem Cells. 2008;26(4):864-73.

[4] Huang NF, Okogbaa J, Babakhanyan A, Cooke JP. Bioluminescence imaging of stem cell-based therapeutics for vascular regeneration. Theranostics. 2012;2(4):346-54.

[5] Schroeder T. Imaging stem-cell-driven regeneration in mammals. Nature. 2008;453(7193):345-51.

[6] Su W, Zhou M, Zheng Y, Fan Y, Wang L, Han Z, et al. Bioluminescence reporter gene imaging characterize human embryonic stem cell-derived teratoma formation. Journal of Cellular Biochemistry. 2011(112):840–8.

[7] Li Z, Lee A, Huang M, Chun H, Chung J, Chu P, et al. Imaging survival and function of transplanted cardiac resident stem cells. Journal of the American College of Cardiology. 2009;53(14):1229-40.

[8] Weissleder R, Moore A, Mahmood U, Bhorade R, Benveniste H, Chiocca EA, et al. *In vivo* magnetic resonance imaging of transgene expression. Nat Med. 2000;6(3):351-5.

[9] Cai W, Chen X. Nanoplatforms for Targeted Molecular Imaging in Living Subjects. Small. 2007;3(11):1840-54.

[10] Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. Journal of the American College of Cardiology. 2009;54(24):2277-86.

[11] Guenou H, Nissan X, Larcher F, Feteira J, Lemaitre G, Saidani M, et al. Human embryonic stem-cell derivatives for full reconstruction of the pluristratified epidermis: a preclinical study. Lancet. 2009;374(9703):1745-53.

[12] Li Z, Hu S, Ghosh Z, Han Z, Wu JC. Functional Comparison and Expression Profiling of Human Induced Pluripotent Stem Cell- and Embryonic Stem Cell-Derived Endothelial Cells. Stem Cells and Development. 2011;20:1701-10.

[13] Chang GY, Xie X, Wu JC. Overview of stem cells and imaging modalities for cardiovascular diseases. J Nucl Cardiol. 2006;13(4):554-69.

[14] Fu Y, Azene N, Xu Y, Kraitchman DL. Tracking stem cells for cardiovascular applications *in vivo*: focus on imaging techniques. Imaging in medicine. 2011;3(4):473-86.

[15] McClelland R, Wauthier E, Tallheden T, Reid LM, Hsu E. In situ labeling and magnetic resonance imaging of transplanted human hepatic stem cells. Mol Imaging Biol. 2011;13(5):911-22.

[16] Acton PD, Zhou R. Imaging reporter genes for cell tracking with PET and SPECT. Q J Nucl Med Mol Imaging. 2005;49(4):349-60.

[17] Li SC, Tachiki LM, Luo J, Dethlefs BA, Chen Z, Loudon WG. A biological global positioning system: considerations for tracking stem cell behaviors in the whole body. Stem Cell Rev. 2010;6(2):317-33.

[18] Rak-Raszewska A, Marcello M, Kenny S, Edgar D, See V, Murray P. Quantum dots do not affect the behaviour of mouse embryonic stem cells and kidney stem cells and are suitable for short-term tracking. PLoS One. 2012;7(3):e32650.

[19] Yukawa H, Watanabe M, Kaji N, Okamoto Y, Tokeshi M, Miyamoto Y, et al. Monitoring transplanted adipose tissue-derived stem cells combined with heparin in the liver by fluorescence imaging using quantum dots. Biomaterials. 2012;33(7):2177-86.

[20] Ranjbarvaziri S, Kiani S, Akhlaghi A, Vosough A, Baharvand H, Aghdami N. Quantum dot labeling using positive charged peptides in human hematopoetic and mesenchymal stem cells. Biomaterials. 2011;32(22):5195-205.

[21] Iordanova B, Ahrens ET. *In vivo* magnetic resonance imaging of ferritin-based reporter visualizes native neuroblast migration. Neuroimage. 2012;59(2):1004-12.

[22] Min JJ, Gambhir SS. Molecular imaging of PET reporter gene expression. Handb Exp Pharmacol. 2008(185 Pt 2):277-303.

[23] Li Z, Wu JC, Sheikh AY, Kraft D, Cao F, Xie X, et al. Differentiation, Survival, and Function of Embryonic Stem Cell Derived Endothelial Cells for Ischemic Heart Disease. Circulation. 2007;116(11_suppl):I-46-I-54.

[24] Xie X, Cao F, Sheikh AY, Li Z, Connolly AJ, Pei X, et al. Genetic modification of embryonic stem cells with VEGF enhances cell survival and improves cardiac function. Cloning and stem cells. 2007;9(4):549-63.

[25] Pomper MG, Hammond H, Yu X, Ye Z, Foss CA, Lin DD, et al. Serial imaging of human embryonic stem-cell engraftment and teratoma formation in live mouse models. Cell Res. 2009;19(3):370-9.

[26] Trounson A, Thakar RG, Lomax G, Gibbons D. Clinical trials for stem cell therapies. BMC medicine. 2011;9:52.

[27] Sanz-Ruiz R, Gutierrez Ibanes E, Arranz AV, Fernandez Santos ME, Fernandez PL, Fernandez-Aviles F. Phases I-III Clinical Trials Using Adult Stem Cells. Stem cells international. 2010;2010:579142.

[28] Li ZJ, Wang ZZ, Zheng YZ, Xu B, Yang RC, Scadden DT, et al. Kinetic expression of platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) during embryonic stem cell differentiation. J Cell Biochem. 2005;95(3):559-70.

[29] Tarantal AF, Lee CC, Batchelder CA, Christensen JE, Prater D, Cherry SR. Radiolabeling and *in vivo* imaging of transplanted renal lineages differentiated from human embryonic stem cells in fetal rhesus monkeys. Mol Imaging Biol. 2012;14(2):197-204.

[30] Sun N, Lee A, Wu JC. Long term non-invasive imaging of embryonic stem cells using reporter genes. Nat Protoc. 2009;4(8):1192-201.

[31] Frangioni JV, Hajjar RJ. *In vivo* tracking of stem cells for clinical trials in cardiovascular disease. Circulation. 2004;110(21):3378-83.

[32] Narsinh KH, Cao F, Wu JC. Molecular imaging of human embryonic stem cells. Methods Mol Biol. 2009;515:13-32.

[33] Jiang H, Cheng Z, Tian M, Zhang H. *In vivo* imaging of embryonic stem cell therapy. Eur J Nucl Med Mol I. 2011;38(4):774-84.

[34] Hong H, Yang Y, Zhang Y, Cai W. Non-invasive imaging of human embryonic stem cells. Curr Pharm Biotechnol. 2010;11(6):685-92.

[35] Lewin M, Carlesso N, Tung CH, Tang XW, Cory D, Scadden DT, et al. Tat peptide-derivatized magnetic nanoparticles allow *in vivo* tracking and recovery of progenitor cells. Nat Biotechnol. 2000;18(4):410-4.

[36] Wang L, Su W, Liu Z, Zhou M, Chen S, Chen Y, et al. CD44 antibody-targeted liposomal nanoparticles for molecular imaging nd therapy of hepatocellular carcinoma. Biomaterials. 2012;33:5107-14.

[37] Wu JC, Cao F, Dutta S, Xie X, Kim E, Chungfat N, et al. Proteomic analysis of reporter genes for molecular imaging of transplanted embryonic stem cells. Proteomics. 2006;6(23):6234-49.

[38] Zheng W, Honmou O, Miyata K, Harada K, Suzuki J, Liu H, et al. Therapeutic benefits of human mesenchymal stem cells derived from bone marrow after global cerebral ischemia. Brain Res. 2010;1310:8-16.

[39] Yamada Y, Nakamura S, Ito K, Sugito T, Yoshimi R, Nagasaka T, et al. A feasibility of useful cell-based therapy by bone regeneration with deciduous tooth stem cells, dental pulp stem cells, or bone-marrow-derived mesenchymal stem cells for clinical study using tissue engineering technology. Tissue Eng Part A. 2010;16(6):1891-900.

[40] Rush SM, Hamilton GA, Ackerson LM. Mesenchymal stem cell allograft in revision foot and ankle surgery: a clinical and radiographic analysis. J Foot Ankle Surg. 2009;48(2):163-9.

[41] Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol. 2008;8(9):726-36.

[42] Tang C, Russell PJ, Martiniello-Wilks R, Rasko JE, Khatri A. Concise review: Nanoparticles and cellular carriers-allies in cancer imaging and cellular gene therapy? Stem Cells. 2010;28(9):1686-702.

[43] Wang HH, Wang YX, Leung KC, Au DW, Xuan S, Chak CP, et al. Durable mesenchymal stem cell labelling by using polyhedral superparamagnetic iron oxide nanoparticles. Chemistry. 2009;15(45):12417-25.

[44] Love Z, Wang F, Dennis J, Awadallah A, Salem N, Lin Y, et al. Imaging of mesenchymal stem cell transplant by bioluminescence and PET. J Nucl Med. 2007;48(12): 2011-20.

[45] Daadi MM, Li Z, Arac A, Grueter BA, Sofilos M, Malenka RC, et al. Molecular and magnetic resonance imaging of human embryonic stem cell-derived neural stem cell grafts in ischemic rat brain. Mol Ther. 2009;17(7):1282-91.

[46] Zurkiya O, Chan AW, Hu X. MagA is sufficient for producing magnetic nanoparticles in mammalian cells, making it an MRI reporter. Magn Reson Med. 2008;59(6): 1225-31.

[47] Zhu J, Zhou L, XingWu F. Tracking neural stem cells in patients with brain trauma. N Engl J Med. 2006;355(22):2376-8.

[48] Doubrovin M, Ponomarev V, Serganova I, Soghomonian S, Myagawa T, Beresten T, et al. Development of a new reporter gene system--dsRed/xanthine phosphoribosyltransferase-xanthine for molecular imaging of processes behind the intact blood-brain barrier. Mol Imaging. 2003;2(2):93-112.

[49] Wilson A, Laurenti E, Oser G, van der Wath RC, Blanco-Bose W, Jaworski M, et al. Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell. 2008;135(6):1118-29.

[50] Schroeder T. Tracking hematopoiesis at the single cell level. Ann N Y Acad Sci. 2005;1044:201-9.

[51] Daldrup-Link HE, Rudelius M, Piontek G, Metz S, Brauer R, Debus G, et al. Migration of iron oxide-labeled human hematopoietic progenitor cells in a mouse model: *in vivo* monitoring with 1.5-T MR imaging equipment. Radiology. 2005;234(1):197-205.

[52] Lin Y, Cheung P, Roth JC, Wilson DL, Gerson SL. Imaging stem cell-derived persistent foci after *in vivo* selection of lentiviral MGMT-P140K transduced murine bone marrow cells. Mol Ther. 2011;19(7):1342-52.

[53] Junt T, Schulze H, Chen Z, Massberg S, Goerge T, Krueger A, et al. Dynamic visualization of thrombopoiesis within bone marrow. Science. 2007;317(5845):1767-70.

[54] Sipkins DA, Wei X, Wu JW, Runnels JM, Cote D, Means TK, et al. *In vivo* imaging of specialized bone marrow endothelial microdomains for tumour engraftment. Nature. 2005;435(7044):969-73.

[55] Yao Y, Li Y, Ma G, Liu N, Ju S, Jin J, et al. *In vivo* magnetic resonance imaging of injected endothelial progenitor cells after myocardial infarction in rats. Mol Imaging Biol. 2011;13(2):303-13.

[56] Agudelo CA, Tachibana Y, Noboru T, Iida H, Yamaoka T. Long-term *in vivo* magnetic resonance imaging tracking of endothelial progenitor cells transplanted in rat ischemic limbs and their angiogenic potential. Tissue Eng Part A. 2011;17(15-16): 2079-89.

[57] Patschan SA, Patschan D, Temme J, Korsten P, Wessels JT, Koziolek M, et al. Endothelial progenitor cells (EPC) in sepsis with acute renal dysfunction (ARD). Crit Care. 2011;15(2):R94.

[58] Higuchi T, Anton M, Saraste A, Dumler K, Pelisek J, Nekolla SG, et al. Reporter gene PET for monitoring survival of transplanted endothelial progenitor cells in the rat heart after pretreatment with VEGF and atorvastatin. J Nucl Med. 2009;50(11):1881-6.

[59] Chade AR, Zhu XY, Krier JD, Jordan KL, Textor SC, Grande JP, et al. Endothelial progenitor cells homing and renal repair in experimental renovascular disease. Stem Cells. 2010;28(6):1039-47.

[60] Fernandez-Ruiz V, Kawa M, Berasain C, Iniguez M, Schmitz V, Martinez-Anso E, et al. Treatment of murine fulminant hepatitis with genetically engineered endothelial progenitor cells. J Hepatol. 2011;55(4):828-37.