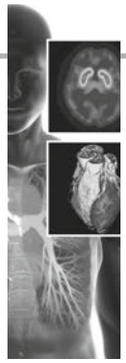

Medical imaging for cardiac regeneration using resident cardiac stem cells

Resident progenitor/cardiac stem cells (CSCs) are known to improve cardiac function through differentiation into cardiomyocytes and vascular lineages. To evaluate the effects of transplanted CSCs, appropriate imaging analysis modalities (e.g., optical imaging, MRI and radionuclide imaging) are required. However, the medical imaging modalities used for *in vivo* monitoring of transplanted CSCs may have distinct advantages and disadvantages. In this article, we will review the classification of CSCs and the imaging analysis methods developed thus far for use in CSC experiments. We will also discuss the direction of future medical imaging analysis strategies for CSC biology.

KEYWORDS: MRI ■ optical imaging ■ radionuclide imaging ■ resident progenitor/cardiac stem cell

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Myocardial infarction accelerates cardiomyocyte loss as cardiomyocyte apoptosis and necrosis are caused by a lack of nutrients and oxygen supply brought about by ischemic conditions [1]. Although commonly used methods, such as drug treatment, cardiovascular intervention and heart transplantation, can reduce mortality in cardiac disease patients, these therapies cannot regenerate the lost cardiomyocytes [2]. Diverse research has been carried out to overcome limitations in the regeneration of damaged cardiomyocytes. Stem cell-based therapy has been considered a novel therapeutic strategy for cardiomyocyte regeneration since the early 2000s [3]. Stem cells from various sources, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and adult stem cells (ASCs) have shown therapeutic potential in the regeneration of cardiomyocytes [2].

Until now, stem cell therapy for cardiac regeneration has been actively explored using a variety of different cell types. Cardiac stem cells (CSCs) could be considered as an obvious cell source for regeneration of damaged cardiomyocytes. These cells pre-exist in the adult heart, coexpress early cardiac transcription factors and differentiate toward cardiac lineages, which together indicate an ability to improve cardiac regeneration potential compared with other stem cells [4]. A number of experiments have demonstrated that CSCs may regenerate the myocardium by differentiating into cardiomyocytes and vascular lineages (e.g., endothelial cells and vascular smooth muscle cells) [5]. Some clinical trials using CSCs are currently underway [6–8], but the efficiency of engraftment and the *in vivo* survival of transplanted stem cells remain challenges that need to be overcome. Therefore, advanced techniques for measuring the efficacy and survival of transplanted stem cells used in clinical applications, including the tracking of injected exogenous CSCs, are required to achieve a better understanding of organ-regeneration mechanisms and the development of stem cell therapy. Imaging approaches not only play important roles in the judgment of clinical availability, including CSC therapy, but also broaden our knowledge regarding stem cell biology [9,10]. There is accumulating evidence that CSCs transplanted into damaged cardiovascular tissues can regenerate, following analyses by medical imaging methods, such as optical imaging, MRI, CT, SPECT and PET [10,11]. However, the tracking of transplanted CSCs in live organs is incredibly difficult and, until now, known imaging analysis methods cannot perfectly trace transplanted exogenous stem cells. Therefore, more advanced medical imaging approaches should be developed for accurate evaluation of implanted CSCs' effects *in vitro* and *in vivo*.

In this article, we present the classification of CSC sources and the most commonly used medical imaging techniques for CSCs for cardiac regeneration. We also discuss the direction of future research for medical imaging of stem cells for cardiac regeneration.

Stem cell sources for cardiac regeneration

Damaged cardiomyocytes have been shown to regenerate when diverse stem cells, including

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ESCs, iPSCs and ASCs are applied [2,12]. ESCs are an important source of stem cells for cardiomyocyte regeneration and they can differentiate into diverse cellular lineages, including cardiomyocytes, under specific culture conditions. Some experiments have shown that ESC-derived cardiac cells and cardiomyocytes play crucial roles in regenerating damaged cardiomyocytes through interaction with the host heart tissue [13,14]. Despite the positive effects demonstrated by these studies, typically only a few differentiated cardiomyocytes have innate contractile ability [15], and the mechanisms of differentiation into cardiomyocytes have not been completely demonstrated [16]. In addition, ESC transplantation may have ethical implications and undesirable adverse effects such as teratoma development or immune rejections. In view of these disadvantages, clinical trials using ESCs have not yet been conducted.

In 2006, Yamanaka and Takahashi introduced iPSCs, developed from somatic cells, which could differentiate into diverse cellular lineages [17]. iPSCs have rapidly emerged as a novel stem cell source for cardiovascular regeneration, which overcomes the disadvantage of the ethical discussion regarding ESCs. Zhang *et al.* have shown that human iPSCs can differentiate into functional cardiomyocytes under specific conditions [18]. Nelson and colleagues have reported that transplanted mouse iPSCs could improve left ventricle function in animal models of ischemic heart disease [19]. Although iPSCs or iPSC-derived cells have shown positive effects on cardiovascular regeneration, the efficacy of cardiomyocyte differentiation is extremely low, and these cells have the same limitations as ESCs, such as teratoma generation, when they are transplanted *in vivo* [20]. Furthermore, the generation of iPSCs, which includes four reverse transcription factors (e.g., *Sox2*, *Klf4*, *c-Myc* and *Oct3/4*) via retrovirus transfection, makes them inappropriate, in terms of safety, for clinical application in cardiovascular regeneration. However, these limitations have been continuously overcome by the generation of iPSCs without the use of viral vectors and the *c-Myc* gene, as evidenced by Okita *et al.* [21] and Nagakawa *et al.* [22].

Recently, an interesting study reported that fibroblasts and cardiac fibroblasts can be transdifferentiated into cardiomyocyte-like cells using direct reprogramming techniques [23,24]. Despite the fact that direct reprogramming techniques offer novel strategies for cardiovascular regeneration without stem cell transplantation, there are still limitations, in terms of safety, as retrovirus vectors are also used for genetic modulation.

ASC-based therapy is more likely to flourish than other stem cell-based therapies using ESCs or iPSCs, since ASCs (e.g., bone marrow-derived stem cells [BMSCs], myoblasts [MBs], mesenchymal stem cells [MSCs] and CSCs) reside in diverse organs, including the mammalian heart, and these cells can be easily isolated [12].

Owing to the strong resistance of MBs to ischemic conditions and their potential to differentiate into myotubes in tissue, the first study on stem cell therapy for cardiac regeneration was conducted with transplantation of MBs into ischemic heart tissue [25,26]. Some experiments have shown that transplantation with MBs improved left ventricle function [25]. However, transplanted MBs are unable to differentiate into cardiomyocytes [27], and they have also been associated with serious side effects, such as triggering arrhythmias [28].

BMSCs are one of the best-characterized cells for cardiac regeneration. Diverse studies have demonstrated that the reduction in cardiac function in ischemic heart disease could be recovered after transplantation with BMSCs [29–31]. Despite these positive results, the use of BMSCs is still controversial owing to the lack of definite knowledge to determine the core cells. Hematopoietic stem cells [32–34] and endothelial progenitor cells, which are different types of BMSCs, have the potential for cardiac regeneration via their endothelial lineages and differentiation potentials [35,36]. Despite the positive results from these cell therapies, cell differentiation into cardiomyocytes remains debatable [37–39].

In addition, MSCs are an advantageous stem cell therapeutic resource in adults as they can be isolated from diverse tissues, such as bone marrow, adipose tissue and umbilical cord blood. MSCs can also differentiate into diverse cellular lineages including cardiomyocytes, osteocytes, chondrocytes and adipocytes [40–42]. However, these cells showed a lower rate of survival and differentiation into cardiomyocytes *in vivo* after transplantation [43]. Another disadvantage is that MSCs often show different characteristics depending on their source or donor owing to the lack of characteristic markers.

As described above, CSCs can regenerate cardiomyocyte populations in the adult heart and, thus, various types of CSCs have been identified by diverse research groups [2].

Various types of resident CSCs used for cardiac regeneration

CSCs in adult hearts can be isolated from the myocardium and epicardium by diverse methods, such as conjugation with specific antibodies and vital dye-exclusion methods. CSCs can thus be classified as described below.

■ Sca-1-positive cells

Sca-1 is a well-defined cell surface marker expressed on hematopoietic stem cells [44]. Oh *et al.* suggested that Sca-1-positive cells isolated from the mouse adult heart can differentiate into GATA4 and Nkx2–5-positive cardiomyocytes following treatment with 5-azacytidine [45]. Matsuura *et al.* reported that mouse Sca-1-positive CSCs can differentiate into beating cardiomyocytes following treatment with oxytocin [46]. Huang *et al.* recently reported that Sca-1-positive mouse CSCs protect the myocardium via their paracrine effects in ischemic/reperfusion injury models [47]. Furthermore, some groups reported that Sca-1-positive CSCs isolated from the adult human myocardium also improved cardiac function following transplantation [48,49]. Unfortunately, there is no evidence from clinical trials using Sca-1-positive CSCs.

■ c-kit-positive cells

c-kit is also a well-defined stem cell surface marker expressed on diverse stem cells, including CSCs. Beltrami and colleagues first identified c-kit-positive CSCs in the adult rat myocardium and showed that c-kit-positive CSC injection improved cardiac functions following differentiation into cardiomyocytes [50]. Another research group reported that c-kit-positive CSCs isolated from the adult human myocardium can improve cardiac function when they are transplanted [51]. Recently reported research showed that treatment with ephrin A1 enhanced migration of c-kit-positive CSCs and improved left ventricle function [52]. More recently, SCIPIO clinical trials first reported clinical trials of CSCs, which have shown that implantation with c-kit-positive human CSCs can improve the cardiac function [6,7].

■ Isl-1-positive cells

Isl-1 plays crucial roles in fetal organ development [53]. Laugwitz *et al.* first identified Isl-1-positive CSCs in the mammalian fetal heart and found that the cells can differentiate into functional cardiomyocytes *in vitro* [54]. Moretti *et al.* reported that embryonic-derived Isl-1-positive CSCs can differentiate into cardiomyocytes and vascular lineages [55]. Although there is diverse evidence showing the positive effects of Isl-1-positive CSCs [56–58], it is still unclear whether these cells can be classified as ASCs since Isl-1 is predominantly expressed in the fetal heart tissue, rather than adult tissue [59]; moreover, thus far, there is no evidence from clinical trials using Isl-1-positive CSCs.

■ Cardiac side population cells

Side population cells can be found in diverse organs, including the skeletal muscle, bone marrow and adipose tissue [60,61]. Cardiac side population cells (CSPCs) can also be isolated from the adult myocardium using an exclusion assay with vital dyes (Hoechst 33342 or Rhodamine 123). Martin *et al.* first identified CSPCs in the developing adult heart, with these cells expressing Abcg2 [60]. Oyama *et al.* demonstrated that CSPCs express Bcrp-1 and can differentiate into diverse lineages, including cardiomyocytes [62]. More recently, a high frequency of Brcp-1-positive CSPCs was detected in ischemic human myocardium [63]. In addition, Yoon *et al.* reported that transplanted CSPCs improved blood perfusion in a hind limb ischemia model [64]. However, the differentiation mechanisms of these cells are yet to be elucidated.

■ Cardiosphere-derived stem cells

Messina *et al.* were the first to demonstrate that CSC populations can form an aggregated spheroid form in culture (i.e., a cardiosphere) [65]. Cardiosphere-derived stem cells (CDSCs) can also differentiate into cardiomyocytes, endothelial cells and vascular smooth muscle cells both *in vitro* and *in vivo* [66]. Diverse animal experiments have been conducted and have shown that transplanted CDSCs improve cardiac function [67,68]. Recently, a clinical trial of transplanted human CDSCs (CADUCEUS), carried out by Makkar *et al.*, demonstrated that implantation of human CDSCs improved ventricle wall thickness but did not show ejection fraction improvement [8]. CDSCs may have disadvantages, such as contamination with cardiac fibroblasts, but this disadvantage can be overcome by using stem cell markers.

■ Epicardium-derived cells

CSCs exist not only in the adult mammalian myocardium but also in the epicardium. Some of the epicardial cells covering the primitive heart acquire migration abilities by epithelial–mesenchymal transition [69]. Limana *et al.* demonstrated that c-kit$^+$/CD34$^+$/CD45$^-$ populations exist in human

and murine epdicardiums and can differentiate into cardiomyocytes [70]. In addition, Zhou *et al.* reported that Wt1-positive epicardium-derived cells (EPDCs) also differentiate into cardiomyocytes [71]. Furthermore, some evidence suggested that thymosin-β4 improved migration of EDPCs and Wt1-positive EDPC populations primed with thymosin-β4 differentiated into cardiomyocytes [72,73]. However, the differentiation potential of EDPCs is still controversial. Thus, further development of cellular surface markers to isolate EDPCs may be required.

Medical imaging of resident CSCs

As described above, CSC-based cell therapies have shown improvement of cardiac function in animal experiments [74]; however, some clinical evidence has shown that cell-therapeutic effects are rather weak since stem cell therapy has fundamental limitations [6–8], such as inefficient long-term engraftment and low survival rates of stem cells transplanted into target organs [2,12]. To overcome these limitations, stem cell research requires a more suitable and advanced technology for monitoring the state of transplanted stem cells. Medical imaging technology is rapidly developing and could provide wider insights into the understanding of stem cell fate, migration and survival *in vivo*.

■ Optical imaging analysis

Optical imaging analysis has been widely used in the monitoring and tracking of transplanted stem cells *in vivo*. To monitor transplanted stem cells, diverse probes, such as chemical (e.g., fluorescence dyes or quantum dots [Q-dots]) or biological reporters (e.g., fluorescence proteins), are used to label the stem cells, and these methods have been previously well demonstrated (Table 1). Smith and colleagues showed that 1×10^5 of Dil-labeled human CDSCs could be traced in mouse-infarcted myocardium even at 3 weeks after their transplantation [68]. Furthermore, Bearzi *et al.* reported that 8×10^5 of Q-dot-605-labeled human c-kit-positive CSCs could be detected in mouse- and rat-infarcted myocardium 2 weeks after transplantation [51]. These chemical reporter-labeled techniques are easily applicable to post-transplant stem cell tracking; however, they have some limitations. Fluorescence intensity measured using chemical reporters could be diluted by cell division and detected *in vivo* regardless of cell survival [10].

The limitations of chemical reporter-labeled stem cell tracking techniques can be overcome using biological reporters such as fluorescence proteins and luciferase. Some evidence has shown that green fluorescent protein-labeled human CDSCs can be detected in infarcted rat myocardium until 3 weeks after transplantation [75]. More recently, Gorbunov and colleagues reported that the homing capability of rat c-kit-positive CSCs was enhanced by resveratrol priming, which could be traced long term (4 months) using a green fluorescent protein-labeling technique [76]. Furthermore, in 2009, Li *et al.* reported that mouse Sca-1-positive CSCs could be traced using diverse imaging analysis methods. This study showed that mouse Sca-1-positive CSCs, labeled using firefly luciferase transfection, were successfully detected 8 weeks after implantation by live imaging [77]. Cells transfected with fluorescent protein gene and luciferase are available for transplanted cell tracking. Most of the accumulating evidence suggests that diverse sources of CSCs have been traced effectively after *in vivo* transplantation using green fluorescence labeling methods (Table 1). Although stem cell labeling using fluorescence protein has been shown to appropriately overcome the disadvantages of chemical dyes or Q-dots, this technique has disadvantages. Most

Table 1. Optical imaging analysis in cardiac stem cells.

Study (year)	Experimental specimens	Type of transplanted cells	Cells (n)	Probe	Duration	Ref.
Beltrami *et al.* (2003)	Rat	Rat c-kit-positive CSCs	2×10^5	GFP	3 weeks, 20 days	[50]
Gorbunov *et al.* (2012)	Rat	Rat c-kit-positive CSCs	ND	GFP	4 months	[76]
Smith *et al.* (2007)	Mouse	Mouse CDSCs	1×10^5	GFP	3 weeks	[68]
Li *et al.* (2011)	Mouse	Mouse c-kit-positive CSCs	4×10^5	GFP	6 weeks	[92]
Li *et al.* (2009)	Mouse	Mouse Sca-1-positive CSCs	5×10^5	Luciferase	8 weeks	[77]
Messina *et al.* (2004)	Mouse	Human CDSCs	10 spheres	GFP	18 days	[65]
Li *et al.* (2010)	Mouse	Human CDSCs	2×10^5	GFP	3 weeks	[75]
Dawn *et al.* (2005)	Rat	Human c-kit-positive CSCs	1×10^6	GFP	2–5 weeks	[67]
Bearzi *et al.* (2007)	Mouse and rat	Human c-kit-positive CSCs	8×10^5	GFP	2 weeks	[51]

CSC: Cardiac stem cell; CDSC: Cardiosphere-derived stem cell; GFP: Green fluorescence protein; ND: Not determined.

Table 2. MRI analysis of cardiac stem cells.

Study (year)	Experimental specimens	Type of transplanted cells	Cells (n)	Probe	Duration	Ref.
Johnston *et al.* (2009)	Pig	Porcine CDSCs	3×10^5	Gd	4 weeks	[93]
Williams *et al.* (2013)	Porcine	Human c-kit-positive CSCs	2×10^6	Gd	2–6 weeks	[94]
den Haan *et al.* (2012)	Mouse	Human Sca-1-positive CSCs	2×10^5	Gd–DTPA	14 days	[49]
Terrovitis *et al.* (2008)	Rat	Human and rat CDSCs	5×10^5	Iron oxide	2–21 days	[81]
Carr *et al.* (2011)	Rat	Rat CDSCs	2×10^6	Iron oxide	16 weeks	[82]
Campan *et al.* (2011)	Rat	Swine CDSCs	2×10^5	Ferritin	4 weeks	[83]

CSC: Cardiac stem cell; CDSC: Cardiosphere-derived stem cell; Gd: Gadolinium; Gd–DTPA: Gadolinium with diethylenetriaminepentaacetic acid.

experiments have shown that transplanted stem cells could be detected after sacrificing the animals using an invasive method, but this limitation could be overcome using a luciferase gene-based cell-labeling technique. This technique is needed to appropriately extract the substrate (such as D-luciferin) without sacrificing the animal for cell tracking.

However, these techniques are not suitable for clinical applications, as they are based on genetic manipulation. In addition, the detection of transplanted stem cells using light-based imaging analysis has the fundamental limitation that it can only penetrate tissues with a depth of <10 cm [9].

Furthermore, Goichberg *et al.* have reported that CSCs could be detected 2 weeks after transplantation using *in situ* hybridization using the Y chromosome and human-specific *Alu* sequence. However, these tracking techniques are considered inappropriate for stem cell tracking in clinical trials [52].

■ MRI analysis

MRI provides excellent resolution of images of cardiac anatomy and function [78]. Furthermore, MRI has benefits for *in vivo* tracking of stem cells due to its safety and 3D reconstructive capability [79]. Thus, MRI analysis has been widely used to monitor transplanted CSCs and analyze their functions (Table 2).

Diverse MRI analyses with gadolinium contrast reagents have been used extensively as non-invasive tools for the assessment of myocardial infarct size following CSC transplantation in clinical trials. In the SCIPIO clinical trial, implantation of human c-kit-positive CSCs reduced the infarct sizes to 24 and 30% at 4 and 12 months after transplantation, respectively, and myocardial infarct sizes were properly assessed using gadolinium-based MRI in ischemic myopathy patients (SCIPIO clinical trial) [6,7]. Another clinical trial has reported that injected human CDSCs altered ventricle wall thickness, but did not significantly improve ejection fraction [8]. Although gadolinium-based MRI has been used to effectively assess myocardial infarct sizes with excellent resolution after CSC transplantation, intravenous gadolinium contrast reagents are not sufficient to trace transplanted CSCs as these reagents do not provide cell-specific imaging [80].

Recent MRI clinical trials were performed for functional assessment of cardiac performance only; however, some other researchers have demonstrated that transplanted CSCs can be traced by MRI with advanced contrast reagents [6–8]. Terrovitis and colleagues reported that ferumoxide-labeled rat CDSCs can be detected by 3T MRI at 3 weeks post-transplantation, although ferumoxide signaling was also detected in dead CDSCs and macrophages [81]. Furthermore, Carr *et al.* reported that fluorescent micron-sized particles of iron oxide-labeled rat CDSCs could be detected at 16 weeks post-transplantation [82]. This result strongly indicates that MRI with advanced contrast reagents offers benefits in terms of tracking transplanted CSCs *in vivo*. However, iron oxide-based MRI reagents can be diluted by subsequent cell divisions, and this remains a limitation of this technique.

Interestingly, recently reported evidence has shown that transplanted stem cells can be traced using the induction of iron-storage proteins, such as ferritin heavy chain. According to this report, porcine CDSCs transfected with human ferritin heavy chain can be detected at 6 weeks post-transplantation in infarcted border zones using 1.5T MRI [83]. This research indicates that transplanted CSCs can be traced using biological MRI reagents, which overcomes the limitation of reagent dilution brought about by CSC division. However, the fact that MRI analysis is incompatible with pacemaker and implantable cardioverters/defibrillators remains a challenge in cardiology [80].

■ Radionuclide imaging analysis

Radionuclide imaging analyses, including PET and SPECT, have high sensitivities in the

measurement of cell tracking since the body does not emit inherent signals that interfere with radioprobes [11]. Several positron-emitting probes and radionuclides have been used in preclinical studies to study stem cell migration and engraftment (Table 3).

Terrovitis and their colleagues have reported that implanted rat CDSCs could be traced for shor-term engraftment for 1–6 days post-transplantation by labeling with technetium-99 and iodine-124 [84]. This study showed that technetium-99 and iodine-124 uptake increased using sodium–iodine symporter and did not affect cellular viability and proliferation.

Fluorine 18-fluorodeoxyglucose (^{18}F-FDG) is widely used in PET imaging and diverse research groups have demonstrated cellular tracking and cardiac functional analysis using ^{18}F-FDG-labeled CSCs (Table 3). The short-term engraftment of transplanted CSCs has previously been measured using an ^{18}F-FDG labeling protocol. Lautamaki *et al.* have shown that only 20% (400,000 cells) of cells injected into rat myocardium were detected at 1-h post-transplantation, out of the 2 million ^{18}F-FDG-labeled rat CDSCs initially transplanted [85]. Furthermore, Bonios and colleagues measured cellular retention rates following different ^{18}F-FDG-labeled rat CDSC injection protocols, and showed that 12.0, 15.4 and 0.8% of ^{18}F-FDG-labeled CDSCs were detected at 45 min after intramuscular, intracoronary artery and intravenous injections, respectively [86]. These results indicate that radio probe-labeling methods have the advantage of short-term tracking for injected CSCs; however, these methods have limitations in terms of monitoring the long-term engraftment of injected CSCs owing to the short half-life of radio probes. Recently, Lan *et al.* reported that ^{18}F-FDG-labeled human Sca-1-positive CSCs were detected in the mouse myocardium, but the signaling intensities of PET and bioluminescence imaging (BLI) were not matched at 7 days after transplantation (PET: 44.7 ± 3.2%; BLI: 22.7 ± 11.5%) [87]. In addition, the decrement of signal intensity rates for PET and BLI during transplanted cell tracking were also different (PET: -Δ31.5%; BLI: -Δ12.1%). These different results were related to the characteristics of radio probes (short half-life) and bioluminescence (short penetration depth) [87].

Based on the above experiments, the radio probe-based CSC labeling technique is an appropriate method for the evaluation of the short-term proliferation of transplanted CSCs, but this application may have some limitations in terms of monitoring the differentiation of injected CSCs, owing to the short half-life of radio probes. Although long half-life radio probes may be useful for evaluating the differentiation potential of injected CSCs, they may not be appropriate for clinical applications owing to the safety issues related to radiation accumulation. Thus, advanced radio probe-based cell monitoring techniques should be developed for the long-term tracking of injected CSCs.

■ Other imaging modalities: echocardiography

Echocardiography is widely used to assess cardiac function and evaluate stem cell therapy efficacy. In addition, echocardiography could be an attractive modality of stem cell tracking owing to the low cost and lack of radiation toxicity. Despite these attractive qualities, echocardiography has some disadvantages, such as low anatomical resolution and low stem cell qualification accuracy. For these reasons, there is not yet evidence of CSC tracking using echocardiography. However, only a few studies have reported tracking of MSCs and EPCs using microbubbles [88,89] and CliniMACS nanoparticles [90,91] by echocardiography.

Table 3. Radionuclide imaging analysis of cardiac stem cells.

Study (year)	Experimental specimens	Type of transplanted cells	Cells (n)	Probe	Duration	Ref.
Terrovitis *et al.* (2008)	Rat	Rat CDSCs	2×10^6	^{18}F-FDG	5 min	[84]
Terrovitis *et al.* (2009)	Rat	Rat CDSCs	$1–4 \times 10^6$	^{99m}Tc and ^{124}I	1–6 days	[95]
Bonios *et al.* (2011)	Rat	Rat CDSCs	im.: 2×10^6 ica.: 1×10^6 iv.: 5×10^6	^{18}F-FDG	45 min	[86]
Lautamaki *et al.* (2011)	Rat	Rat CDSCs	2×10^6	^{18}F-FDG	1 h	[85]
Li *et al.* (2009)	Mouse	Mouse Sca-1-positive CSCs	5×10^6	^{18}F-FDG	2–56 days	[77]
Lan *et al.* (2012)	Mouse	Human Sca-1-positive CSCs	1×10^6	^{18}F-FDG	28 days	[87]

^{18}F-FDG: Fluorine 18-fluorodeoxyglucose; ^{99m}Tc: Technetium-99; ^{124}I: Iodine-124; CSC: Cardiac stem cell; CDSC: Cardiosphere-derived stem cell; ica.: Intracoronary artery; im.: Intramuscular; iv.: Intravenous.

Table 4. Advantages and disadvantages of cardiac stem cell-labeling techniques.

Modality	Cell labeling	Advantages	Disadvantages
Optical imaging	Q-dot	Longitudinal monitoring available Potential to use multilabeling simultaneously	Photon attenuation Fluorescence dilution during cell division Signal persists after cell death
	GFP Luciferase	Repeated imaging over long period Cell-specific detection for living cells Nondiluent fluorescence during cell division Potential for imaging of subcellular event	Risk of genetic manipulation using viral vector Immunoreactivity of non-mammalian proteins (luciferase)
MRI	Gd chelates Iron oxide Ferritin	High anatomical details through high resolution Evasion of radiation toxicity	Low sensitivity than radioisotope No linear relationship between signal and cell number Signal persists after cell death Risk of genetic manipulation using viral vector (ferritin) Acoustic noise Incompatible with implant patients
SPECT–PET SPECT	^{124}I ^{99m}Tc ^{18}F-FDG	High sensitivity and robust signal Established tracers Clinically applicable	Limitation of signal decomposition during observation period Toxicity in radiolabeling Low anatomical detail Low interactivity Short-term tracking High cost

^{18}F-FDG: Fluorine 18-fluorodeoxyglucose; ^{99m}Tc: Technetium-99; ^{124}I: Iodine-124; Gd: Gadolinium; GFP: Green fluorescence protein; Q-dot: Quantum dot.

Conclusion

Previous studies have shown that CSCs exert positive effects on cardiac regeneration, and this has been successfully confirmed by tracking injected CSCs with various medical imaging methods. However, no single imaging analysis is sufficient for tracking injected CSCs as each imaging analysis modality has advantages and disadvantages (Table 4). Therefore, the application of diverse approaches using multiple imaging analyses will provide better insights toward understanding the proliferation, differentiation and migration of injected CSCs *in vivo*. Furthermore, the development of advanced imaging reagents (to overcome existing efficacy and safety limitations) and multifunctional imaging modalities (multi-imaging analysis systems using a single piece of equipment) will lead us toward the next generation of monitoring systems used in the research of transplanted CSCs.

Future perspective

Medical imaging clearly shows benefits in terms of tracking transplanted CSCs *in vivo*, but the advantages and disadvantages of their clinical application must be clearly distinguished. As described above, the diverse probes used to label CSCs have shown different results in terms of tracking transplanted CSCs in the long or short term. Therefore, appropriate imaging probes should be developed for successfully tracing transplanted CSCs. The primary consideration in the development of imaging probes is that these agents should not generate toxic products or contain contaminants. Furthermore, multifunctional image agents must be developed, as tracking transplanted CSCs is extremely complex and most imaging analysis methods are dependent on a single image modality. One final consideration is that agents should not alter the cellular proliferation, stemness and differentiation capability of labeled CSCs.

Financial & competing interests disclosure

This work was supported by grants from the National Research Foundation funded by the Korean goverment (MEST; 2010-0020260, 20120618, 2012M3A9C6049720) and also supplerted by the Brain Busan 21 Project in 2013. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Stem cell sources for cardiac regeneration

- Damaged myocardia could be regenerated by transplantation of different stem cells, including embryonic stem cells, induced pluripotent stem cells and adult stem cells. Although these cells improve cardiac function, each cell type has its distinct advantages and disadvantages.

Classification of cardiac stem cells used for cardiac regeneration

- Resident cardiac stem cells (CSCs) that exist in the myocardium and epicardium could improve cardiac function through their differentiation properties, such as cardiomyocytes and vascular lineages. These cells can be isolated using specific cell-surface markers (e.g., Sca-1, c-kit and islet-1 among others) and the exclusion of vital dyes (e.g., Hoechst 33342 or Rhodamine 123).

Medical imaging in CSC biology

- Medical imaging methods, such as optical methods, MRI and radionuclide imaging, are effective modalities and provide insights to understanding CSC biology.
  - Optical imaging analysis: implanted CSCs can be traced using cells labeled with chemical or biological reporters. However, the use of optical imaging analysis for tracking CSCs has some limitations, such as the possibility of chemical probe dilution during cell division and the low penetration depth of biological reporter genes.
  - MRI analysis: MRI provides excellent imaging of cardiac anatomy and function and monitoring of transplanted CSCs, which can be detected using gadolinium chelates, iron oxide-based contrast reagent, and transfected with iron-storage protein (e.g., ferritin heavy chain) by MRI. However, *in vivo* tracking of CSCs using MRI analysis is not compatible with patients who use pacemakers and implantable cardioverts/defibrillators.
  - Radionuclide imaging analysis: technetium-99-, iodine-124- and fluorine 18-fluorodeoxyglucose-labeled CSCs can be traced using PET and SPECT. However, radionuclide imaging analysis is not suitable for the analysis of long-term engraftments of CSCs as the radio probes used in radionuclide imaging analysis have short half-lives.
  - Other imaging modality: echocardiography is an attractive modality for stem cell tracking owing to the low cost and lack of radiation toxicity. However, this modality has a low anatomical resolution and a low stem cell qualification accuracy.

Conclusion

- Medical imaging analysis clearly provides various approaches for monitoring the effects of transplanted CSCs *in vivo*. However, the existing imaging probes still have limitations in terms of effectively monitoring transplanted CSCs. Thus, new applicable imaging probes for multi-imaging devices should be developed.

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