

Non-surgical stem cell delivery strategies and in vivo cell tracking to injured myocardium

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Abstract Heart failure is a major economic and public health problem. Despite the recent advances in drug therapy and coronary revascularization, the lost cardiomyocytes due to necrosis and apoptosis are not replaced by new myocardial tissue. Cell therapy is an interesting therapeutic option as it potentially improves contractility and restores regional ventricular function. Early clinical data demonstrated that cell transplantation, mainly delivered through non-surgical methods, is safe and feasible. However, several important issues need to be elucidated. This includes, next to determining the best cell type, the optimal delivery strategy, the biodistribution and the survival of implanted stem cells after transplantation.

In this view, pre-clinical animal experiments are indispensable. Reporter genes, magnetic or radioactive labeling of stem cells have been developed to observe the fate and the distribution of transplanted cells using non-invasive imaging techniques. Several studies have demonstrated that these direct and non-direct labeling techniques may become an important tool in cell therapy. Integration of cell delivery and cell tracking will probably be a key for the success of cell therapy in patients. This review will provide a comprehensive overview on the various cell tracking and non-surgical cell delivery techniques, which are highly important in view of experimental and clinical studies.

Keywords Stem cells · Cell delivery · Magnetic resonance imaging · Nuclear imaging

Abbreviations

BM-MNC	Bone marrow mononuclear cells
^{18}F -FDG	^{18}F -Fluorodeoxyglucose
In ¹¹¹	Indium ¹¹¹
MRI	Magnetic resonance imaging
MSC	Mesenchymal stem cells
MI	Myocardial infarction
PET	Positron emission tomography
RPG	Reporter gene
SPECT	Single photon emission computer tomography
SPIO	Superparamagnetic iron oxide
^{99}Tc	^{99}Tc Technetium

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Introduction

Coronary heart disease is a major public and economic health problem leading to more than 7 million deaths world wide each year [1, 2]. Optimal pharmacologic treatment and coronary reperfusion therapy have led to improved survival of patients with coronary artery disease. Clearly, current therapies can not replace dysfunctional or lost cardiomyocytes which finally lead to heart failure. A structural solution may be provided by cell therapy which has emerged as a potential new therapeutic strategy. Cell therapy is considered in the setting of acute myocardial infarction (MI) and chronic ischemic heart failure. The ultimate goals of cell therapy are myocardial regeneration and revascularization, thereby re-establishing synchronous contractility and bioelectrical conductivity to achieve overall clinical improvement of cardiac function without severe adverse effects. Transplantation strategies include percutaneous, surgical and systemic delivery of various types of stem cells [3–7]. To monitor the efficiency of implanted stem cells, most small animal studies use post mortem histology as a gold standard [8, 9]. For in vivo detection of cell retention, sophisticated imaging techniques are necessary. Additionally, non-invasive imaging is preferred to determine the effect of cell therapy on cardiac function (e.g. volume, mass and pressure). Nowadays it is possible to track and quantify transplanted stem cells

by direct and non-direct labeling techniques using (1) nuclear imaging [positron emission tomography (PET) or single photon emission computer tomography (SPECT)] and (2) magnetic resonance imaging (MRI). Various clinically approved radiomarkers are suggested to be useful in cardiac cellular therapies like ^{18}F -fluorodeoxyglucose (^{18}F -FDG) for PET scan, indium 111 (In^{111}) for SPECT and superparamagnetic iron oxide (SPIO) for MRI [10–12].

It is important to further optimize delivery strategies in view of ongoing (pre-) clinical studies for regenerative therapy. To this end, state-of-the-art cell tracking is highly necessary. This review will provide a robust update of available in vivo cell tracking strategies and non-surgical delivery techniques that will guide experimental set up of pre-clinical stem cell research.

Part 1: in vivo cell tracking strategies

In the following section the contrast agents and detectors that have been proposed for non-invasive cell tracking will be discussed. Thereafter, we will review the advantages and disadvantages of each imaging strategy and suggest future directions for research. Figure 1 and Table 1 will provide an overview of all available direct and non-direct labeling techniques.

Fig. 1 Different methods for non-invasive cell tracking. **a** MRI magnetic resonance imaging, SPIO super paramagnetic iron oxide; **b** SPECT single photon emission computer tomography, indium 111 In^{111} , ^{99}Tc 99Technetium, PET positron emission tomography, ^{18}F FDG 18F-fluorodeoxyglucose; **c** RPG reporter gene

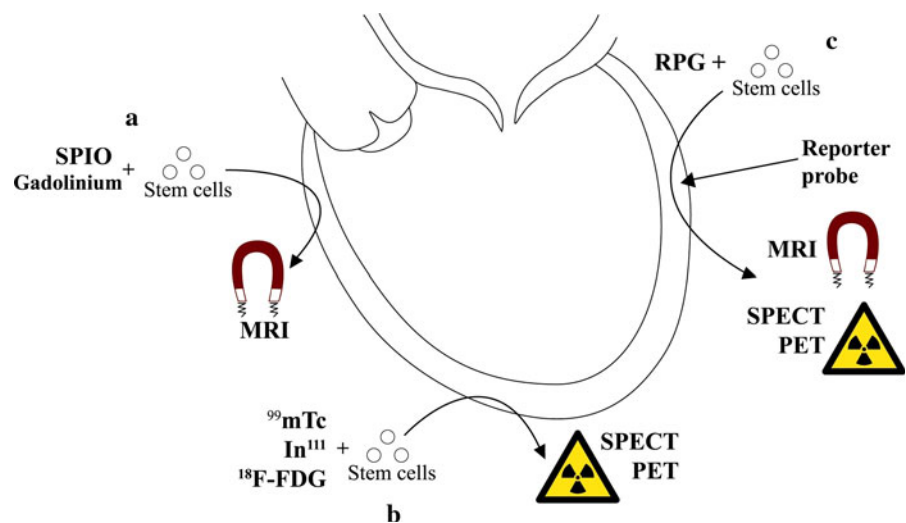


Table 1 Methods of direct and non-direct stem cell tracking

Method	Label	Advantages	Disadvantages
Direct labeling			
MRI	Gadolinium	Simple method	Bio-incompatible Cytotoxic in unchelated form Low relaxivity
		SPIO	Biocompatible Cell friendly High resolution Stem cell imaging and anatomical function can be assessed simultaneously
	SPECT	In^{111} ^{99}Tc	High sensitivity Stem cell imaging and perfusion can be assessed simultaneously
PET	^{18}F -FDG	High spatial resolution No cytotoxicity Stem cell imaging and myocardial vitality can be assessed simultaneously	Radiation exposure to patients Signal may not reflect living cells Signal loss due to radioactive decay
Non-direct labeling			
RPG	Reporter genes/ probes	Detection of viable cells Observation of cell differentiation	Cellular dysfunction or death Immunogenicity of gene products Potential risk of uncontrolled growth and malignancy Costs Not used in patient studies

MRI magnetic resonance imaging, *SPIO* super paramagnetic iron oxide, *SPECT* single photon emission computer tomography, In^{111} indium¹¹¹, ^{99}Tc technetium, *PET* positron emission tomography, ^{18}F -FDG ^{18}F -fluorodeoxyglucose, *RPG* reporter gene

MRI

For MRI, Gadolinium- and iron-based contrast agents can be used for direct labeling of stem cells. Gadolinium is bio-incompatible, cytotoxic in unchelated form and has a low relaxivity; therefore it is an unattractive agent for stem cell imaging. However, novel Gadolinium-based particles are being investigated for this purpose, albeit not yet in the heart [13].

In 1996, SPIO's (30–200 nm) were approved as iron-based contrast agents for clinical use by the US Food and Drug Administration (Feridex, Guerbet, France). SPIO's are composed of an iron oxide core that is coated with a polymer shell to prevent aggregation. The polymer may contain dextran, polyethylene glycol or starch. The iron is biocompatible and can

be recycled by cells using regular biochemical pathways. Labeling of targeted cells is accomplished by endocytosis. In addition, efficiency can be improved by using peptides/antibodies [14], magnetodendrimers [15] or transfection agents [16]. Labeled cells appeared to be hypo intense in T2*- and T2-weighted images.

Numerous studies have shown that mesenchymal stem cells (MSC) can be labeled without affecting in vitro cell viability, proliferation and differentiation into adipogenic and osteogenic lineages by iron contrast agents [10, 16, 17]. Recently, pre-clinical studies were able to detect a minimum of about 10^5 pig MSC using different sized iron particles with a conventional cardiac MRI [10, 12]. Figure 2 shows an example of cell tracking by cardiac MRI using SPIO labeled MSC from our own laboratory. Detection of

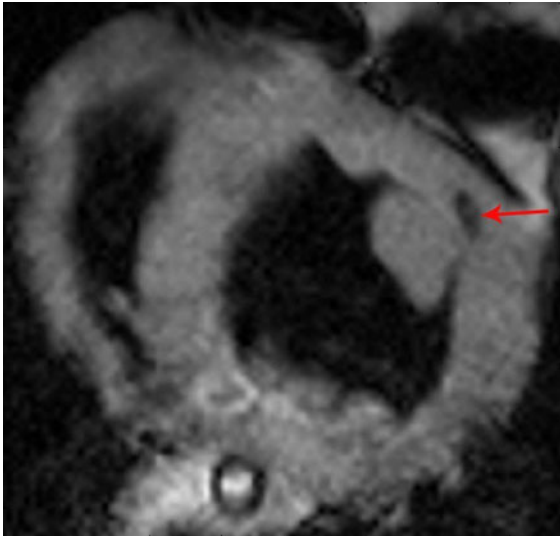


Fig. 2 T2* image of SPIO labeled MSC (arrow) after transepical injection in healthy myocardium

stem cells mainly depends on (1) magnetic field strength, (2) number of cells injected, (3) labeling efficiency and (4) cell size.

A practical drawback of iron-based contrast agents is that labeling is not permanent and self-replicable. Dilution of the contrast due to cellular fragmentation, fusion, division and migration also limits the use for follow-up after cell delivery. Also, variation in labeling efficiency among different cell types is present. For instance, SPIO-registered MR signals are still detectable in embryonic stem cells [18] 5 weeks after transplantation and 4–16 weeks for skeletal myoblasts [19] and MSC in murine models [20], respectively. Very little is known about the long-term survival after cell delivery in both pre-clinical models and humans. Furthermore, iron particles may still remain in situ and can be taken up by phagocytotic cells (e.g. cardiac macrophages) after cellular death [21]. Thus, MRI signal is still present leading to overestimation of the outcome of cellular survival ('false positive' results). Another potential drawback is negative image contrast artifacts due to air or hemorrhage after cell injection. Finally, patients with an intracardiac defibrillator or pacemaker are no candidates for MRI.

Magnetic resonance imaging has become an appropriate imaging modality for stem cell tracking and therapeutic efficacy, without ionic radiation, high spatial resolution and detailed anatomical function.

Nevertheless, at present this method is only useful for establishing initial retention of cells as it provides little evidence for long-term viability or functionality of transplanted cells. None of the MRI contrast agents have been used in the clinical field to monitor cellular survival. More information about long-term cell tracking and effects on cell behavior (e.g. differentiation and proliferation) in large animal studies is mandatory before applying this technique to clinical stem cell trials.

SPECT

Several radioisotopes are available for stem cell tracking in the heart, Technetium (^{99}Tc) ($T_{1/2}$ 6 h) and In^{111} ($T_{1/2}$ 2.6 days). Labeling is based on established clinical protocols for white blood cells and performed by chelating agents that carry the radionuclides into the cell. Radioactivity is measured by a Gamma camera composing a 3D image.

In vitro studies have shown that cell integrity of both human and canine MSC, and endothelial progenitor cells (EPC) were unaffected after In^{111} labeling with 0,14-30 Becquerel per cell [22–24]. However, radiation induced cell damage was found after labeling hematopoietic progenitor cells (HPC) with In^{111} [25, 26]. In addition, low cellular retention after labeling was observed in all cell types [26–28]. Penicka et al. [29] observed high retention of ^{99}Tc inside bone marrow mononuclear cells (BM-MNC) and no altered proliferation pattern after labeling. Cell viability of MSC was also not influenced by ^{99}Tc [30]. The effect on cell differentiation was not determined in these studies. The use of SPECT is accompanied by a low detection threshold of about 10^4 cells [24] and therefore it is an attractive tool to determine in vivo biodistribution.

Both isotopes have been studied in various large animal models to determine cellular homing after surgical, intramyocardial (IM) and intravenous (IV) delivery [12, 27, 31, 32]. It was shown that a low number of cells accumulate in the heart after injection. However, when injecting cells into healthy myocardium 1/3 of the total radioactivity was still located in the heart [33]. Figure 3 shows a typical example of cellular retention of radioactive labeled stem cells after surgical injection in one of our experiments. Zhou et al. [34] showed that it is possible to simultaneously assess stem cell imaging

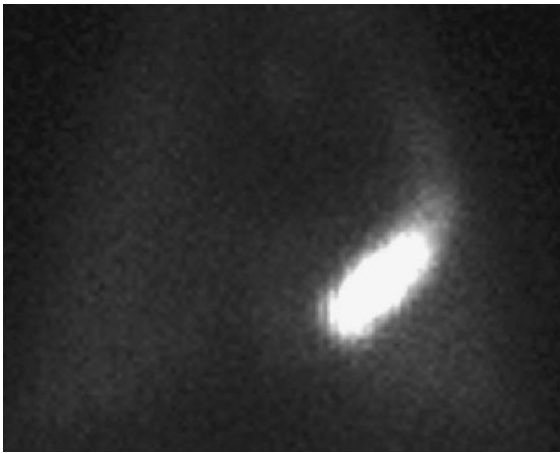


Fig. 3 SPECT image of indium¹¹¹ labeled MSC in the heart after surgical injection in the left ventricle wall in a healthy porcine model

and perfusion in a rat model using dual isotope SPECT by combining both In¹¹¹ (for cell imaging) and ⁹⁹Tc (for perfusion study). This interesting finding should be confirmed in a pre-clinical model.

In humans, SPECT was employed to study the kinetics of ⁹⁹Tc or In¹¹¹ labeled progenitor cells after intracoronary (IC) delivery in a small number of patients with ischemic heart disease. In general, low retention rates of progenitor cells (<10%) to the infarcted myocardium were found 1–2 h after injection [29, 35, 36]. Signal loss due to reduction in activity also limits the use of radioisotopes for long-term follow-up.

Single photon emission computer tomography is an attractive approach to determine delivery efficiency. In Table 3, all studies on cell delivery efficiency are summarized per strategy. Animal studies have shown that SPECT imaging is a promising tool to visualize *in vivo* migration patterns and to assess functional effects of transplanted stem cells. However, the negative effect of radioisotopes on cell behavior (e.g. radiation induced cell damage, possible reduced differentiation rates) can not be neglected in view of clinical use.

PET

Positron emission tomography is a well known method to determine myocardial viability and perfusion by injecting ¹⁸F-FDG. It is possible to label stem cells with ¹⁸F-FDG to monitor homing and

biodistribution (see Table 3). No cytotoxicity, or impaired stem cell differentiation were documented after ¹⁸F-FDG labeling. This could be due to the radioactive properties of ¹⁸F-FDG, that emits a long range beta particle and thereby prevents radiation injury inside the cell. Although PET imaging offers high spatial resolution, the short half lifetime remains an obstacle for long-term cell tracking.

In a porcine MI model, dynamic cell tracking of percutaneous implemented ¹⁸F-FDG labeled circulating progenitors cells was demonstrated: only 8–18% of myocardial activity was retained 1 h after IC delivery [37]. Similar results were obtained when autologous BM-MNC's were infused to the heart [38]. In addition, ¹⁸F-FDG was used to label and determine myocardial homing and biodistribution of BM-MNC after IC and IV delivery in post-acute MI patients. Low amount of BM-MNC activity was detected in the infarcted myocardium after injection (less than 3%) [11]. Both studies demonstrate the importance of metabolic myocardial imaging to determine cellular survival and a potential effect on scar tissue. However, larger (pre) clinical randomized studies on this topic are required to establish early and late biodistribution after cell delivery. Furthermore, a metabolic isotope with a longer half lifetime is necessary for chronic cell tracking.

Reporter genes

To solve limitations in traditional cardiovascular imaging (i.e. false positive findings after cell death and cell toxicity), reporter genes (RPG) may be an attractive alternative. In short, a genetically engineered gene (the RPG) is incorporated into the genome of a cell prior to transplantation. The gene product should only be expressed by engrafted and still viable cells. Next, cells can be visualized after IV injection of an imaging tracer that targets the gene product. By its presence, the survival of the graft is certain because expression of the RPG and activity of the gene product depends on the viability of transplanted cells. Enzyme, transport and receptor based gene products are available for molecular imaging.

This strategy is particular well suited to overcome dilution effects which ensure long-term serial imaging of living transplanted stem cells. Also, repetitive imaging is possible and does not depend on decay of the radioisotope. Potential disadvantages

include (1) costs, (2) cellular dysfunction or death, (3) immunogenicity of gene products, (4) potential risk of uncontrolled growth and malignancy; these aspects preclude clinical application in patients at this time. Several RPG's (transferrin receptor (TR), herpes simplex virus type 1 thymidine kinase (HSV1) and human sodium/iodide symporter) have been developed for non-invasive imaging in living animals [39–41]. The transferrin receptor has been proposed as a RPG for MRI [42]. High expression of TR on the cell membrane leads to increased iron uptake that is detectable by MRI and does not depend on intracellular iron concentration. Moreover, detection may be improved by covalent binding with iron nanoparticles [42]. However, accumulation of iron may lead to high levels of intracellular iron and diminished cellular function. Furthermore, not much is known about efficacy and safety of TR in large animal models and humans.

Herpes simplex virus type 1 thymidine kinase is being used for nuclear imaging [40]. Radioisotopes analogous to thymidine and guanosine are used as tracers. After metabolizing, the substrate is trapped intracellularly. Free radioactivity is detectable by PET or SPECT. In 2003, feasibility was tested to monitor survival of cardiomyoblasts after IM delivery using HSV1 thymidine kinase RPG. It was shown that optical imaging was more sensitive for detecting cardiomyoblasts (5×10^5) than PET (3×10^6) [40, 43]. Furthermore, HSV1 thymidine kinase can be transduced in human MSC and visualized in a clinical relevant swine model with healthy myocardium [44]. In 2008, Gyöngyösi et al. [45] demonstrated the feasibility of PET and optical imaging of the stable expressed of the trifusion gene protein (luciferase) for in vivo non-invasive tracking of IM injected MSC in a relevant animal model with survival up to 10 days after injection. Data on HSV1 thymidine kinase and long-term follow-up are currently not available.

Human sodium/iodide symporter controls the membrane conductance of sodium and iodine. It is mainly expressed in the thyroid gland, and it is absent in cardiac cells [46]. Therefore, isotopes for both PET and gamma camera can be used to image cells that express this gene. More detailed information about the effect of sodium influx on cardiomyocytes is required before entering the clinical field.

So far, the available data is limited to reveal the role of RPG in cellular tracking. Up till now, just one study attempted to initiate RPG imaging in an ischemic large animal model. Before human administration, a safe and stable RPG with no effect on cell behavior has to be developed. In parallel, optimal detection signal and more efficient delivery routes have to be established. Nevertheless, in our view RPG is a promising concept for reliable cell tracking with respect to pre-clinical studies that address optimal cell delivery strategies and chronic long-term follow-up.

Comparison of imaging techniques

At present, various direct and non-direct labeling strategies have been investigated for in vivo cell tracking. No technique has emerged as the most optimal tracking method. Fate and biodistribution after IV delivery by colabeling allogenic MSC with In^{111} and SPIO was observed. Migration of low amount of cells to the heart could be detected by SPECT, but not by MRI [12]. A combined approach using SPECT and cardiac MRI was used to determine function and precise visualization of In^{111} labeled stem cells in an ischemic rat model [47]. Simultaneous detection of stem cells and imaging of both perfusion deficit and myocardial function of the ischemic area was done by signal coregistration. Bioluminescence firefly luciferase RPG was more accurate compared to SPIO for long-term cell survival using optical and magnetic imaging [48].

In patients, imaging is mainly performed to determine the effect of cell therapy on myocardial function and perfusion. To the best of our knowledge, no direct clinical comparison between imaging techniques has been performed to observe homing and distribution of transplanted human stem cells.

In summary, nuclear imaging is more sensitive than MRI for short-term cell tracking. For high spatial resolution and evaluation of cardiac function MRI is more appropriate. In case of long-term follow-up, iron particles and RPG can play an important role. In our view, a multimodality approach using both magnetic and nuclear radioagents in combination with RPG would provide a solution to current limitations in cell tracking in the near future.

Part 2: non-surgical methods of cell delivery

The main objective of various cell delivery methods is to inject sufficient number of cells into the myocardium and to keep maximum retention of cells within the area of interest. A summary of the different cell delivery routes in clinical and pre-clinical setting will be provided (Fig. 4; Table 2) and also directions for future research are discussed.

Intracoronary delivery

During routine cardiac catheterization, IC delivery is performed through the central lumen of an over-wire balloon catheter that is advanced into the coronary artery of interest. By using transient balloon inflations, the duration of cell delivery is maximized,

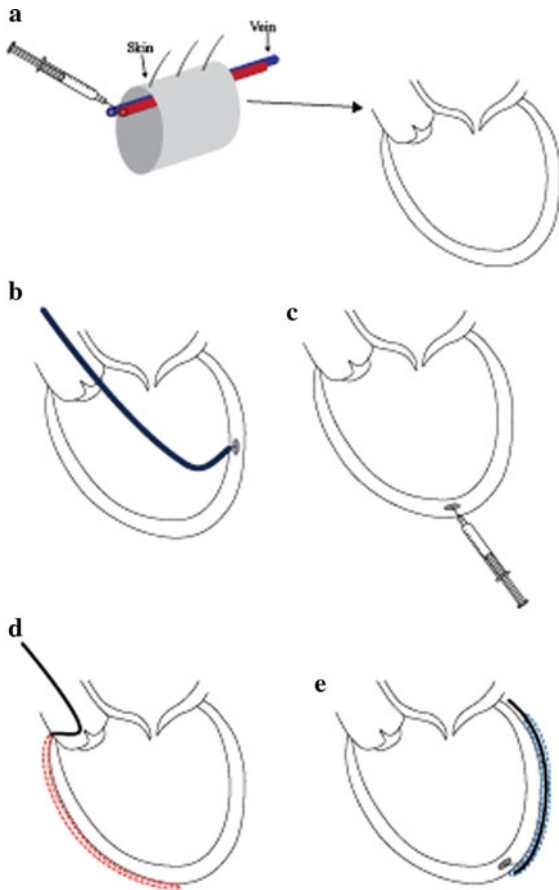


Fig. 4 Schematic overview of the different delivery techniques to the injured myocardium. **a** Intravenous infusion **b** Trans-endocardial delivery **c** Surgical delivery **d** Intracoronary infusion **e** Retrograde coronary transvenous injection

leading to migration of the delivered cells to the infarct related area. A major advantage of IC delivery is direct infusion into the target area using infarct related or a contralateral artery.

Based on animal and patient studies Strauer et al. looked for a non-surgical method for autologous cell therapy [7, 49]. In 2002, IC infusion of autologous BM-MNC appeared to be promising method for cell delivery in ten patients with acute MI [50]. Since then, a number of clinical trials have been conducted [51–60]. These studies showed that IC infusion was a safe delivery strategy and associated with a modest increase in myocardial function in patients with ischemic heart disease. Nevertheless, 5-year follow-up data of cell therapy demonstrated no significant improvement in left ventricle ejection fraction (EF) compared to placebo [61, 62]. In 31 clinical studies performed so far, 22 used IC infusion as delivery strategy in approximately 1,200 patients, despite unresolved issues regarding this transplantation technique [63].

Important drawbacks of IC delivery are known, including the impossibility to access to the area of interest in patients with chronic occlusion. Other potential disadvantages of IC delivery of cells include intimal dissection [64, 65], embolization of these cells from the site of injection to the microvasculature in the heart leading to micro infarctions [66] or abdominal region [6] and in-stent restenosis due to transient balloon inflation [67]. Finally, imprecise localization and systemic delivery to non-cardiac tissues are limitations of IC therapy [68]. This can be explained by inadequate cellular migration into the myocardium during the first transit of coronary reperfusion causing a considerable loss of cells to the systemic circulation. A large portion of these cells are found in non-cardiac tissues, like lungs and liver [68, 69]. It has been shown that approximately 2% of the infused non-enriched BM-MNC home to the target area of cardiac injury in humans [11]. However, a higher retention (14–39%) in the infarcted myocardium was observed when using enriched BM-MNC [11]. This effect may be caused by differences in injected cell numbers. Notably, most clinical trials used non-enriched BM-MNC.

Many cell types have been used to treat MI using IC delivery in the (semi) acute setting. Although initial results were positive, low delivery efficiency remains an obstacle for clinical application.

Table 2 Advantages and disadvantages of stem cell delivery methods

Method	Advantages	Disadvantages
Intracoronary delivery	Direct infusion infarct related or contralateral coronary artery Well known technique by cardiologists	In-stent restenosis No access to occluded coronary artery Embolisation of microvessels, leading to (micro) infarction Intima dissection Vascular access complications Systemic delivery to non-cardiac tissues
Catheterized peripheral vein delivery	Non-invasive and easy method Allows intermittent cell infusion	Microembolism Low cellular migration and differentiation Low delivery efficiency
Trans-endocardial injection	Cell delivery in occluded areas Implementation of high cell concentration in the ischemic region Assess non-viable myocardium before transplantation	Requires training; lengthen time of procedure Expensive method Risk of myocardial perforation Arrhythmias Vascular access complications
Retrograde coronary transvenous injection	Low costs May enter thinned myocardium due its co-axial injection technique	May cause irreversible damage to venous wall Perforation of the vein Only access to the anterior wall along the vein Technical difficult procedure Vascular access complications

In general, this technique can not be used in chronic ischemic heart failure patients with occluded arteries. In addition, most studies related to IC infusion are small and lack of long-term follow-up data. In the future, research should focus on larger, blinded, randomized trials in MI patients with long-term follow-up to investigate the immediate and sustained effect of IC delivery.

Catheterized peripheral vein delivery

Cell delivery can be achieved by direct IV infusion of cells into a catheterized peripheral vein. Although it is an easy and safe method for cell delivery [3, 4], non-cardiac uptake of stem cells after systemic delivery remains a major obstacle for clinical application [27, 69, 70]. Moreover, several studies have shown that no (0% of injected) cells retained in the heart (see Table 3). Additionally, the occurrence of microembolism in non-cardiac organs due to cellular entrapment of cell types with large diameter (e.g.

skeletal myoblasts or MSC) is an important drawback.

In our view, this technique is currently obsolete for clinical cardiac stem cell therapy. In case of future specific cardiac targeting of stem cells for optimal homing and engraftment, this technique can possibly re-enter the research arena.

Intramyocardial delivery

Nowadays, percutaneous injection of cells for cardiac repair directly into the injured myocardium is possible. Two delivery techniques are available for percutaneous IM injections: trans-endocardial injection (TE) and retrograde coronary transvenous (RCV) injection.

Trans-endocardial injection

Five different IM injection catheters are available for clinical use: Steerjet (MicroHeart) [71], Stiletto

Table 3 Comparison of delivery efficiency of unselected stem cells to the heart observed in patient and large animal studies

	Setting/study design	n	Cell type	Label	Labeling efficiency (%)	Cell viability (%)	Imaging method	Cell injection to detection (time)	Delivery efficiency to the heart (%)
Intracoronary delivery									
Hofmann et al. [11]	AMI/observational	3	BM-MNC	¹⁸ F-FDG	>99	92–96	PET	55–75 min	1.3–2.6
Hou et al. [31]	AMI/randomized	5	PBMNC	In ¹¹¹	66	N/A	PET	60 min	1.6
Freyman et al. [69]	AMI/randomized	6	MSC	Iridium particles	N/A	>70	Histology	14 days	6
Doyle et al. [37]	AMI/observational	3	CPC	¹⁸ F-FDG	>90	>98	PET	60 min	8.7
Blocket et al. [97]	AMI/observational	6	HPC	¹⁸ F-FDG	6	N/A	PET	60 min	5.5
Kang et al. [98]	AMI/observational	17	PBCS	¹⁸ F-FDG	72	N/A	PET	120 min	1.5
Schachinger et al. [36]	AMI, OMI/observational	17	CPC	In ¹¹¹	10	90	Gamma camera	60 min	6.9
Caveliers et al. [35]	OMI/observational	2	PBCS	In ¹¹¹	51	88	SPECT	60 min	6.9–8
Qian et al. [38]	AMI/observational	7	BM-MNC	¹⁸ F-FDG	91	97	PET	60 min	6.8
Penicka et al. [29]	AMI, OMI/observational	10	BM-MNC	^{99m} Tc	90	94–99	SPECT	120 min	1–5
Intravenous delivery									
Hofmann et al. [11]	AMI/observational	3	BM-MNC	¹⁸ F-FDG	>99	92–96	PET	50–60 min	0
Kang et al. [98]	AMI/observational	3	PBCS	¹⁸ F-FDG	72	N/A	PET	120 min	0
Freyman et al. [69]	AMI/randomized	6	MSC	Iridium particles	N/A	>70	Histology	14 days	0
Chin et al. [27]	AMI/observational	2	MSC	In ¹¹¹	86	>95	SPECT	<24 h	0
Kupatt et al. [32]	AMI/observational	3	EPC	^{99m} Tc	45–80	>80	SPECT	60 min	0.5
Retrograde coronary transvenous delivery									
Hou et al. [31]	AMI/randomized	5	PBMNC	In ¹¹¹	66	N/A	PET	60 min	3.2
Kupatt et al. [32]	AMI/observational	3	EPC	^{99m} Tc	45–80	>80	SPECT	60 min	2.7
Surgical delivery									
Mitchell et al. [99]	AMI/observational	6	EPC	In ¹¹¹	N/A	N/A	SPECT	40 min	57
Hou et al. [31]	AMI/randomized	6	PBMNC	In ¹¹¹	66	N/A	PET	60 min	11
Trans-endothelial delivery									
Dib et al. [100]	AMI/observational	1	Skelet myoblasts	Iridium particles	N/A	N/A	Histology	120 min	4
Lyingbaek et al. [33]	Healthy/observational	6	MSC	In ¹¹¹	N/A	96	Gamma camera	30 min	35
Mitchell et al. [99]	AMI/observational	7	EPC	In ¹¹¹	N/A	N/A	SPECT	40 min	54
Freyman et al. [69]	AMI/randomized	6	MSC	Iridium particles	N/A	>70	Histology	14 days	3

AMI acute myocardial infarction, OMI old myocardial infarction, N number of animals or patients, SPECT single positron emission computer tomography, In¹¹¹, indium¹¹¹, ^{99m}Tc technetium, PET positron emission tomography, ¹⁸F-FDG ¹⁸F-fluorodeoxyglucose, PBCS peripheral blood stem cells, MSC mesenchymal stem cell, BM-MNC bone marrow mononuclear cell, HPC hematopoietic stem cell, PBMNC peripheral blood mononuclear cell, CPC circulating progenitor cell, EPC endothelial progenitor cell, N/A not available

(Boston Scientific SciMed, Natick, MA) [10, 72], Bioheart Myocath (Santa Rosa, CA), the Helix needle catheter (being developed) (BioCardia, CA) and Biosense Webster Myostar (Diamond Bar, CA) [5]. All above stated devices are developed for cell and gene based therapies.

In general, IM injection of cells requires extensive fluoroscopic guidance to navigate within the ventricle, which is an important drawback for both patient and operator. To overcome this issue, the Myostar catheter is incorporated into a three dimensional electromechanical mapping system (NOGA). The target area can be determined by identifying viable, hibernating and infarcted myocardium, without the need of fluoroscopic guidance. Therapeutic cells can be injected in the region of interest, that is defined as a ‘mismatch’ area, i.e. presence of electrical activity in absence of mechanical movement. The use of the NOGA system was generally proven to be safe and feasible in animal studies and clinical trials for cellular [5, 73] and gene [74, 75] therapy.

Perin et al. evaluated the safety and effect of TE delivered autologous BM-MNC in patients with severe heart failure. They observed an improved regional and global myocardial function compared to controls, without safety issues [5]. These encouraging results initiated a number of new trials [76–79].

Other possible advantages of this technique include: cell delivery in occluded areas and implementation of high cell concentration in the myocardial region of interest. Potential drawbacks of IM delivery are the risk of myocardial perforation due to injection [80]. Furthermore, handling of the NOGA system requires technical training, is time consuming and expensive due to the use of a separate mapping and injection catheter. Another major drawback of TE injection is that direct cell injection may alter the gap junction orientation leading to ventricular arrhythmias [81]. Also, the ischemic environment and needle puncture may lead to a release of inflammatory stimuli which could be a trigger for arrhythmias [82]. Cellular retention ranges from 3 to 54% after TE injection. This wide variety is due to differences in animal model, TE catheter, cell type, imaging method and study design (see Table 3).

Over the past years, TE has rapidly evolved from an experimental technique towards a promising IM delivery technique. In the coming years research should focus on determining the most efficient TE catheter and long-term effects of this strategy.

Retrograde coronary transvenous injection

During a routine transvenous catheterization procedure a roadmap coronary venogram will be performed to gain access to all areas of the heart. Of note: no left-sided catheterization procedure is necessary for this technique. A composite catheter (TransAccess, Menlo Park, California) with a nitinol needle will be inserted into the venous wall under intravascular ultrasound, followed by microinfusion of stem cells by an IntraLume (Trans Vascular Inc.) catheter that will penetrate the myocardium under fluoroscopic guidance [83]. Thompson et al. [83] were the first to demonstrate the safety and feasibility of RCV delivery in a non-infarcted swine model. In addition, retrograde infusion of bone marrow cells induced angiogenesis and improved cardiac function in ischemic pigs compared to controls [84]. It was shown that RCV is a safe and feasible method for myoblast transplantation in patients 3 months after MI [85]. The authors also suggested that the RCV catheter rotates better which may improve target accuracy compared to TE injection. Furthermore, RCV is advantageous in cost, time performance thereby preventing cell loss and may enter thinned myocardium (<5 mm) due its co-axial injection technique [83, 84]. However, possible irreversible damage to the venous wall may occur during the injection procedure [86] and it is technical difficult to implement cells in the coronary venous system. With this technique only access to the anterior wall can be achieved, and only along the veins anatomy. Incorrect position of the needle may cause perforation of the venous wall leading to a pericardial hemorrhage. A small number of studies [83–86] have been conducted, but it is still early to draw a conclusion regarding the efficacy of RCV.

Other delivery methods

Cell transplantation into the coronary venous system and the pericardial space has been tested in pre-clinical models and may have promising clinical applications in the future. Local intrapericardial delivery can be achieved by transatrial or subxyphoid access [87, 88]. Both techniques were well tolerated without apparent complications. However, to our knowledge no studies have investigated cell injections to the injured heart. Moreover, clinical

experience with this technique is limited. Only one study has been conducted so far [89].

Coronary sinus venous infusion is performed by advancing a single or double balloon catheter via the coronary sinus into the area of interest [31, 90]. Before cell infusion, a detailed anatomical map will be obtained by a coronary sinus venogram. During the procedure infusion pressure should be monitored closely to prevent disruption of the venocapillary system [91]. Studies have shown that it is feasible to access most myocardial segments through the cardiac venous system [92]. Therefore, this technique may be an alternative for patients with a coronary arterial occlusion. Compared to IC delivery brief periods of venous balloon occlusion are unlikely to cause clinical complaints or myocardial ischemia due to the existence of venous anastomoses [92]. The limitations of this approach are similar to RCV injections.

It was demonstrated that coronary venous infusion does not produce hemodynamical changes in a porcine model of myocardial injury. The authors concluded that this strategy was effective because autologous unfractionated bone marrow cells were observed in the myocardium and enhanced angiogenesis [93]. Later, the same research group conducted a prospective study in 14 patients with chronic stable angina. Autologous cell infusion was safe and tolerable. Significant improvement in myocardial perfusion and EF were observed during follow-up. Coronary angiography showed more collateral vessels in 9/14 patients [94]. However, these results do not prove efficacy assessed by a randomized trial.

Comparison of delivery techniques

Hou et al. assessed cell distribution of human mononuclear cells after surgical, IC and coronary venous delivery in an ischemic swine model. Only 11, 2.6 and 3.2% were retained in the heart after surgical, IC and venous delivery, respectively [31]. Although surgical delivery appeared to be the most efficient technique, there was a huge variation in efficiency. The group of Freyman compared allogenic MSC engraftment after IV, IC and IM (Stiletto) delivery in a porcine MI model [69]. They found that IC delivery was associated with significant higher engraftment rates after 14 days compared to IM and IV. However, decreased coronary bloodflow and

greater myocardial injury were observed after IC delivery. This could be due to high cell numbers injected. Perin et al. [6] demonstrated that IM injection (using NOGA technology) of autologous MSC significantly improved left ventricle EF and reduced myocardial ischemia in a canine model. Conversely, no change in the IC group was observed. Another study compared IM and RCV delivery of microspheres and found no significant difference in myocardial retention between these techniques. The authors also suggested that IM injection is superior to RCV in the infarct region, but that RCV is preferred for treatment of the peri-infarct region were to be treated based on differences in target areas of the devices [95]. Recently, it was demonstrated that RCV injection of BM-MNC is better than IC delivery in view of cell retention and tissue penetration in an acute MI model. However, the study is limited by a very small sample size ($n = 2$ per group) [96].

In summary, several large animal studies showed conflicting results in the efficacy of different transplantation strategies. Notably, the optimal transplantation technique also depends on type of model (acute MI vs. chronic heart failure). To provide a definite answer to the most optimal delivery strategy, we believe that a randomized trial in a clinically relevant animal model (porcine) is necessary, using state-of-the-art cell tracking techniques, including determination of biodistribution after the various delivery strategies.

Conclusion

Cell based cardiac repair showed beneficial effect on myocardial function in animal experiments. A number of clinical trials have already been conducted, although important unresolved issues concerning cell therapy are present. Interestingly, the most optimal delivery strategy still needs to be determined. Non-invasive imaging plays an essential role in determining biodistribution, survival and functional effects to the heart, that is of importance for several aspects of cell therapy (e.g. delivery strategy, cell type). Imaging parameters like contractility, perfusion, and viability of myocardium do not grant direct visualization of transplanted cells. New advancements in MRI and nuclear imaging have shown to provide reliable and highly sensitive visualization of

transplanted cells, although mainly performed in animal models. The introduction of molecular cell tracking will contribute immensely to future studies of cellular mechanisms attributable to functional improvement. Until now, a small number of studies compared biodistribution between different delivery techniques in acute MI models. Unfortunately, results are still inconclusive due to differences in cell type, animal model, labeling method and delivery techniques.

In view of clinical trials it is important to determine the most optimal delivery strategy in a pre-clinical MI model using state-of-the-art cell tracking for both biodistribution and long-term survival. Adequate cell tracking is essential to guide molecular approaches to enhance homing, engraftment and survival of transplanted stem cells. Therefore, additional and more focused pre-clinical studies are mandatory before designing new clinical trials.

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