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# Stem Cells for the Heart, Are We There Yet?

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**Key Words**
Stem cells · Myocardial infarction · Delivery · Differentiation · Adverse effects

**Abstract**
Although several repair mechanisms have been described in the human heart, all fall too short to prevent clinical heart disease in most acute or chronic pathological cardiac conditions. Moreover, despite many breakthroughs in cardiovascular medicine, the complications of a myocardial infarction such as chronic heart failure remains a serious worldwide problem.
Bone marrow stem cells could provide for a promising strategy to restore myocardial infarctions and prevent postinfarct congestive heart failure, because there is growing body of evidence that bone marrow stem cells, such as mesenchymal stem cells, can generate new cardiomyocytes in animals and humans.
In this review, we will discuss important issues on stem cell therapy for cardiac regeneration after myocardial infarction, which might be of paramount importance when considering future human trials.



## Introduction

It has been widely accepted that cardiac myocyte hypertrophy is the most important compensation mechanism of the heart to satisfy increased physiological demands and or to adapt to pathological cardiac conditions. On the other hand, there has been a strong belief that the muscle cells of the adult heart cannot divide or replicate under the same conditions [1].

However, recent reports have highlighted evidence on the existence of cardiac cell renewal in either the normal or diseased heart, but the clinical significance of this cell renewal in several pathological conditions in humans remains uncertain [2–4].

In the setting of myocardial infarction, for instance, the compensatory replication of myocytes does not repair the infarct area itself, but occurs at the border and at a distance of the infarcted myocardium, leaving behind a scar lesion whose size is a major determinant of morbidity and mortality [5]. Although we might be able to enhance this proliferative response in the future using pharmacological modulation or genetic manipulations of the cardiac cell cycle, the cardiac regeneration process is likely to be more complex than replicative enhancement as the sole means of obtaining a scarless heart [6]. In addition, cell cycle manipulations are likely to induce apoptosis, tumor genesis and even cardiac myopathies [7, 8].

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Stem cells could provide an alternative biological strategy to repair myocardial infarctions and prevent postinfarct congestive heart failure, because there is a growing body of evidence that bone marrow stem cells can generate new cardiomyocytes in animals and humans [9–12]. However, before we can claim that this regenerative strategy in cardiovascular medicine is truly applicable in humans, many hurdles must be overcome first.

In this review, we will critically discuss important issues on stem cell therapy for cardiac regeneration following myocardial infarction, which might be of paramount importance when considering future human trials. We will focus on the use of bone marrow stem cells, such as hematopoietic or mesenchymal stem cells, which are an accessible autologous source of stem cells and do not seem to pose the ethical or immunological concerns inherent in using embryonic stem cells. (1) The concept of stem cell therapy will be highlighted and from a developmental point of view, we will critically discuss (2) how stem cells can be delivered to the infarct area. (3) We also summarize possible hurdles regarding the transdifferentiation or plasticity of stem cells towards cardiomyocytes once implanted in the heart. (4) Finally, the functional aspects of the reconstituted heart will be considered and (5) potential adverse reactions will be addressed when envisaging stem cells for myocardial regeneration.

A discussion of skeletal myoblasts for myocardial scar repair or cell therapy in chronic heart failure is beyond the scope of this review, and readers are referred to other articles for details [13–16].

## Concept: Enhance Cardiac Regeneration Using Bone Marrow Stem Cells

Several investigators have demonstrated that cardiac injury in rodents (rats, mice) recruits bone marrow stem cells with their migration to the injured area via the blood stream and their subsequent differentiation into cardiomyocytes [9–11]. Most of these models of stem cell plasticity involved animals with experimental myocardial infarctions, so that it is conceivable that stem cell homing to the heart was in response to signals sent by the ischemic and injured cardiac area [17]. This is in accordance with the very low level of incorporation of stem cells in the intact heart as compared to the injured heart [18]. Although bone marrow-derived cardiomyocytes are also present in the adult human heart in certain conditions, the exact phenotype of these regenerating bone marrow cells still remains to be determined and we do not know yet whether such a repopulation of bone marrow-derived cardiomyocytes observed in animals also occurs in the context of myocardial infarction in humans [12].

How bone marrow stem cells are mobilized or traffick to the injured heart is currently not known, but it is likely to be regulated through sequential interactions with adhesion molecules and chemokines such as stromal-cell-derived factor-1 (SDF-1), which is upregulated after infarction [19, 20]. However, many of these mediators are still unknown and some are likely to be expressed transiently [21].

All experimental animal studies confirm that the frequency at which the spontaneous cardiac incorporation and regeneration occurs from migrated bone marrow stem cells is very low and inadequate for a clinically relevant regeneration of the injured heart [10, 11]. Therefore, part of the concept of stem cell therapy must focus on the characterization and augmentation of signals that home stem cells to the heart in order to optimize cellular recruitment and migration to the injured heart. Meanwhile, investigators have isolated and cultured bone marrow stem cells ex vivo such as mesenchymal and hematopoietic stem cells to increase cell dose for augmenting local accumulation of transplanted stem cells in the infarct area, irrespective of the possible quantitatively, spatially or temporally restricted homing/adhesion signals [22, 23]. In addition, the exact number of bone marrow stem cells needed to reconstitute an infarcted heart is not known and before a sufficient graft size can be obtained in the infarct area, these cells must first overcome different biological obstacles (e.g. ischemia, inflammation) following myocardial infarction, which are known to reduce significantly stem cell viability. This may require anti-death strategies to improve stem cell survival/number in the infarcted heart [24]. Although prosurvival strategies have been proven successful, some of them might actually not be suitable for human use [24–26].

## Stem Cell Delivery

Therapeutic stem cells have been transplanted or mobilized into the injured heart in different manners: (1) by intravenous instillation; (2) by myocardial injections either through the epicardium or the endocardium; (3) through an intracoronary delivery system, or (4) using cytokines to stimulate bone marrow [27].

The most appropriate route of stem cell administration offers a high cell concentration in the damaged myocardium and prevents 'adverse' homing to other organs. This

optimal route is currently unknown because dose-graft/effect studies comparing the different delivery modalities are lacking and are difficult to obtain.

*Intravenous Administration of Stem Cells*

The intravenous route of administration seems to be the least effective delivery method, because the coronary blood flow makes up a small part of the normal cardiac output and many circulation passages will be needed to populate the infarct-related area [27]. In addition, adverse homing of stem cells to other organs such as the lungs, brain or spleen would be likely to occur especially when intravenous stem cell delivery is used, because this seems to be the most indirect pathway to the injured heart and because of the lack of knowledge of the exact timing, signals or mechanisms that home the stem cells to the infarcted myocardium.

Some reports even suggest that (considering hematopoietic stem cells) homing molecules might not be organ specific, which also imposes a heart-selective delivery to obtain an optimal cardiac graft size and limit adverse homing [28]. In addition, it is currently not known whether ex vivo expansion of bone marrow stem cells to increase cell dose may hamper their cardiac homing ability, as is seen in bone marrow homing [29, 30].

*Myocardial Injections*

Orlic et al. [22] have been able to repair infarction in mice by injecting bone marrow stem cells into the border zone of the infarct. The cells injected there reported to migrate from the site of injection to the infarcted myocardium and to reconstitute up to 68% of the infarct area. However, these areas represent very small infarct areas compared to infarctions in mammals such as humans possessing larger hearts. In this regard, stem cells injected into the border zone are less likely to repair infarction to the same extent in humans. This 'size' issue, among other factors as well, clearly stresses the need for caution when interpreting or extrapolating results from animal studies (mostly on mice) to humans [31].

Direct delivery of stem cells using a intracoronary, transendocardial or transepicardial approach could provide an alternative strategy to enhance cell engraftment and optimal cell spreading into the infarct territory. However, the attempts to deliver stem cells directly into the infarct area might bear a conceptual flaw, because the inoculation of undifferentiated stem cells into the infarct territory could move these cells away from host cardiomyogenic signals, which are needed for optimal transdifferentiation [32, 33]. In addition, some reports suggest that the cardiac regeneration process in (some) vertebrates and in cytokine-treated mice (see below) proceeds from the border zone to the inside of the injured region, which suggests that cell-to-cell contact with the peri-infarct host cells may also be important for the cardiac differentiation of undifferentiated stem cells [34–38].

Thus when direct injections into the infarct area are considered, bone marrow stem cells may require preprogramming in vitro to become committed to differentiate into cardiomyocytes.

In fact, most experimental studies that used direct intramyocardial injections have been performed with myogenically committed cells, such as skeletal myoblasts, 5-azacytidine-treated mesenchymal stem cells or fetal cardiomyocytes, which are less dependent on local signals for cardiac differentiation [39–41]. However, intramyocardial injections may injure the myocardium or induce arrhythmias, and intramyocardial injections of myoblasts produce islet-like formations which limits electromechanical coupling with the host myocardium [27, 42].

*Intracoronary Delivery*

Although the safety and technical feasibility of intracoronary cell delivery has been demonstrated in patients with myocardial infarction, its ability to disseminate stem cells directly and globally in the infarct area remains uncertain, because several animal studies suggest that intravascularly delivered stem cells home in the peri-infarct zone in contrast to those primarily inoculated into the infarct area [10, 18, 33, 42, 43]. In other words, stem cells are believed to migrate out of the vasculature in the peri-infarct zone and than move to the infarcted area, in the same manner as inflammatory cells home to the site of myocardial infarction [33]. This indirect pathway could limit an effective local accumulation. However, in most of these studies, stem cell engraftment was assessed in animals with a permanently occluded infarct-related artery or before native revascularization of the infarct territory could have occurred, which could have limited direct cell delivery to the infarct area [10, 18, 33]. On the other hand, spatially and/or temporally restricted homing signals could also explain why border zone rather than infarct area engraftment might occur with intravascular delivery. Nevertheless, the intracoronary delivery of stem cells could offer a higher cell concentration than the intravenous delivery route and seems to be less hazardous than direct intramyocardial injections at the early infarct stage.

*Cytokine Therapy*

Cytokines and chemokines such as stem cell factor (SCF), SDF-1 and its receptor CXCR4 are essential for cardiogenesis during embryonic development in rodents and are implicated in the recruitment of bone marrow stem cells [19, 44]. The cytokine granulocyte colony-stimulating factor (G-CSF or filgrastim) is also widely used in humans to mobilize stem cells and progenitor cells from the bone marrow to the blood stream [45]. Using this cytokine and SCF in mice, Orlic et al. [38] demonstrated a spectacular myocardial regeneration of the infarct area with a definite hemodynamic and survival benefit as compared to the controls. Although the investigators propose that the cell responsible for cardiac regeneration after this cytokine challenge might be a mobilized hematopoietic bone marrow stem cell, it still remains speculative whether the regenerating cells in fact have a bone marrow origin. It also remains largely uncertain how granulocyte monocyte-colony stimulating factor (GM-CSF) improves collateral flow in patients with coronary artery disease [46].

Nevertheless, these findings suggest that the use of growth factors such as G-CSF might be eminently pragmatic and could offer a noninvasive regenerative strategy in patients with myocardial infarction. Clinical trials in humans are currently under way to test whether the administration of G-CSF is safe and could benefit patients with coronary artery disease (www.clinicaltrials.gov). However, caution is warranted because the induction of granulocytosis in patients with myocardial infarction during a course of G-CSF might contribute to the development of myocardial reperfusion injury or plaque destabilization at the remote, nonculprit coronary lesions [45, 47, 48]. Additionally, the use of this cytokine in humans might be ineffective when initiated at the time of acute ischemia and infarction due to competition for migration and receptor occupancy of the injured heart by other cells such as neutrophils [21]. Furthermore, the angiogenic effects of GM-CSF may also involve the vasa vasorum of atherosclerotic plaques which might worsen atherosclerotic disease. Documentation of a possible atherogenic effect of GM-CSF, however, has been controversial [46]. Finally, although the short-term clinical toxicity in normal individuals seems acceptable, the long-term effects of even a brief course of G-CSF in normal humans are presently unknown [45].

## Stem Cell Plasticity: How Do Stem Cells Become Cardiomyocytes?

*Real Transdifferentiation, Cell Fusion, Heterogeneity of Stem Cell Populations?*

The making of Dolly the sheep and the multipotency of adult stem cells have ultimately demonstrated the 'genomic reprogrammability' (plasticity) of cells in response to altered cytoplasmatic or environmental factors [49–51]. However, recent reports have challenged 'stem cell plasticity' by demonstrating that stem cells are able to alter their identity not by reprogramming to fit in their new environment, but rather by fusion with preexisting host cells. For instance, stem cells have been thought to give rise to new hepatocytes in vivo, but genetic analysis of these newly formed hepatocytes has revealed fusion of stem cells with preexisting hepatocytes rather than their transdifferentiation [52, 53]. This issue has stressed the need for a better analysis of the reconstituted infarct to distinguish between true differentiation or fusion of stem cells with residual cardiomyocytes within the (peri-) infarct area [54]. However, the reports of cell fusion do not contest all evidence on the existence of adult stem cell plasticity and there is also indirect evidence that argues against cell fusion as a major mechanism implicated in 'cardiac regeneration' [12, 55–57]. Moreover, some authors hypothesize that fusion might even prevent resident cells from dying and the fused cells could also be intermediates (followed by reduction divisions) in the regeneration process [58, 59]. Finally, as will be discussed below, stem cells can also improve cardiac function, apart from bona fide cardiomyogenesis [60]. Nevertheless, caution is needed because the formation of fused, hybrid cells with extra chromosomes in patients treated with stem cells could spur cancer [62].

Another unresolved issue concerns the remarkable heterogeneous developmental potential of undifferentiated stem cells which replace all different cell types within the heart (blood vessel cells, cardiomyocytes). This has not yet been demonstrated at the single-cell level, because the bone marrow populations used in most experimental studies might have been impure enough to contain different stem cell types (e.g. mixture of mesenchymal stem cells, endothelial progenitors, a putative myocyte progenitor) each having a restricted potential rather than the reprograming of a single stem cell type towards the different cell lines in the heart [63]. Even the current stem cell sorting systems or purification protocols based on cell surface markers such as CD34, Sca-1 or c-kit may yield a heterogeneous selection of stem cells [63].

A better identification assay and clonal analysis with fate mapping of single-cell colonies cells could resolve this issue and will enable us to compare the effectiveness of different stem cell types and therefore give insight in the best suited donor cell or direct us to a combination of different stem cells to rebuild the injured heart [39, 63].

*Cardiomyogenic Signals for Transdifferentiation*

Once implanted in the infarcted heart, local environmental factors interact with the engrafted stem cells, reprograming their genetic repertoire with resulting transdifferentiation into cardiomyocytes. So there is cardiac differentiation in response to organ- or tissue-specific cues, called 'milieu-dependent differentiation'. Most mechanisms that mediate stem cell differentiation into cardiomyocytes in vivo are as yet undetermined, but electromechanical stimulation and paracrine factors from cardiac and other host cells are likely to be involved [32, 33, 64, 65]. Also contact with surrounding cells and extracellular matrix may play a key role in stem cell grafting and differentiation [37]. All these signaling pathways initiate the cardiac gene program, driving the stem cells to differentiation into cardiomyocytes with the expression of cardiac-specific proteins, such as cardiac troponin or α-myosin heavy chain (α-MHC) [22, 64]. The likelihood or efficacy of cardiac differentiation probably depends on the stem cell type used and the local quantitative and qualitative cues encountered at the time of transplantation.

These local conditions or cues that dictate cardiac differentiation in vivo, however, may not be sufficiently strong to efficiently mediate lineage switch in the infarct territory, as has been suggested by several studies [32, 33, 66]. Moreover, it is currently not known whether the ex vivo expansion of stem cells could hamper their response to cardiomyogenic signals.

These findings could force us to enhance the differentiation signals in vivo or to use precommitted cells with cardiogenic lineage instead of uncommitted or undifferentiated stem cells [32, 66, 67]. The in vitro commitment of undifferentiated stem cells to a cardiomyogenic cell line has been performed by coculturing stem cells with cardiomyocytes and END-2 cells or using myogenic agents such as 5-azacytidine and many other chemicals [68, 69]. However, it is currently not known whether these cardiogenic precommitment strategies are safe or could adversely affect the cardiac homing ability of stem cells, their dividing capacity or alternative differentiation into non-cardiomyocytes, such as endothelial cells which make up an important part of the cell population in normal cardiac tissue. Whether these more differentiated cells will be more effective than less differentiated or undifferentiated cells should be investigated and an optimal differentiation point determined.

Most attention has been focused on the renewal of parenchymal cells (cardiomyocytes), but the restoration of the extracellular matrix and vascular supply is also an important issue because both tissue components are essential for the structural and functional support of the newly formed cardiomyocytes. For instance, neovascularization of the infarct territory may be an important key to successful cardiomyoplasty by any delivery method, because a suboptimal angiogenic milieu may limit the engraftment, spreading, growth and differentiation of stem cells in the infarct area. Although the normal cardiac repair process involves neoangiogenesis, it is not clear whether this would be sufficient for the above-mentioned stem cell kinetics [70]. In addition, one should also take into account the unfavorable dynamic changes in microvessel density in infarct zones, as described in dogs, which might also have a significant impact on the survival of the newly formed cardiomyocytes [71]. Finally, from a chronobiological point of view, we do not know whether we should await for this pathological neovascularization to have occurred before delivering stem cells, because the process of neoangiogenesis is likely to proceed in close temporal relationship with cardiomyocyte renewal, as suggested from species with intrinsic cardiac regenerative capacity and animals treated with stem cells [22, 35, 38].

Although stem cells by themselves can contribute to neoangiogenesis and vasculogenesis of the infarct territory, additional genetic or cellular pro-angiogenic interventions to improve infarct revascularization may be required [72–74]. As endothelial progenitors have been demonstrated to colonize and contribute to angiogenic sites in animal models, they could hold promise for improved infarct revascularization and hence, cardiac regeneration [75].

*Time Window for Optimal Transdifferentiation*

The fate or main differentiation pathway of engrafted stem cells (cell death, cardiomyogenesis, neovascularization or scar formation) is likely to be determined by the local conditions at the time of implantation. In other words, stem cells such as mesenchymal stem cells have different fates, depending on the microenvironment they engraft [33]. However, when stem cells are introduced into the infarct area at the early stage of myocardial infarction, a high cell death rate and therapeutic failure is observed, which is due to inflammation and limited sur-

vival factors (e.g. oxygen) in the early course following infarction [24]. On the other hand, undifferentiated stem cells such as mesenchymal stem cells might transdifferentiate to fibroblasts in the fibrogenic microenvironment when encountered at the time of scar formation, to which they might even contribute and worsen the arrhythmic substrate [11, 33, 76, 77]. Given these possible time constraints for optimal transdifferentiation after infarction when undifferentiated stem cells are injected directly into the infarct area, cell transplantation seems to be most successful after the inflammatory reaction has resolved, but before scar expansion [40]. However, the exact 'time point' for stem cell transplantation after myocardial infarction is not known. Finally, the 'exact delivery time' not only relates to an optimal differentiation window, but it also relates to the accessibility and the acceptance of the infarct territory, which are determined by the homing and adhesion signals, and the changing vasculature in healing infarctions. But it is not known whether all these determinants overlap, because little is known about the tissue and molecular kinetics that mediate homing/adhesion and differentiation of stem cells. Therefore, the understanding of the evolution and maturation of the injured myocardium at the molecular level will also be essential if today's interest in rebuilding the infarcted heart is to prove successful, as has been formulated by Sun et al. [78].

## The Repaired Infarction: Structural and Functional Assessment

The in vivo cardiac developmental potential of adult stem cells, such as mesenchymal stem, cells has been demonstrated by labeling these cells genetically, which allows transplanted cells to be readily identified upon histological examination. These tracers have been colocalized with cardiac specific markers such as cardiac troponin or transcription factors such as Nkx 2.5 within the same cell to establish cell origin as well as cell identity [79]. So far, only histological and cytological analysis of tissue specimens has enabled identification of the integration and differentiation of implanted stem cells into the myocardium [79]. Other techniques such as magnetic or radioactive labeling of stem cells will enable us to identify and monitor noninvasively in vivo stem cell migration, homing and engraftment for future experimental studies [18, 80, 81]. Recent MRI studies in rats have demonstrated highly efficient detection of labeled hematopoietic and mesenchymal stem cells in vivo even at single-cell resolution [82]. Molecular imaging might broaden the horizon of noninvasive monitoring in the future by assessing the cardiac differentiation process of stem cells in the injured heart [83].

In order to assess a successful cardiomyoplasty, it is necessary to demonstrate that the transdifferentiated stem cells in the infarct area are phenotypically like the native, resident cells, but one should prove that these transdifferentiated cells are also functional, improving cardiac function. In most studies, however, besides newly formed 'mature' cardiomyocytes, the regenerated infarct area also consisted of 'immature myocytes' or 'muscle-like cells', and vascular structures with endothelial and smooth muscle cells, all derived from implanted bone marrow stem cells [38]. Additionally, a disconcertingly wide variability of cardiac stem cell engraftment and repaired infarct area (0–90% of the infarct zone) has been reported in the literature [22, 25]. This variability might in part be attributed to the divergent experimental settings, such as infarction model, animal size, number and source of stem cell(s) used, but also the delivery time and technique [24, 27, 40, 84]. Other reasons summarized in reference 79, such as the technique used to track the fate of the injected cells, may also account for the quantitative differences between the reports [79].

Although short-term structural and functional improvement has been obtained in most of these animal studies, the long-term viability and the chronic evolution of the reconstituted heart and its response to physiological and pathophysiological stimuli remains to be determined [85].

In recent human trials, the increased viability of the infarct area and enhanced dobutamine-responsive contractility in some patients at follow-up has suggested the propagation of cardiac cells following stem cell engraftment, despite any histological proof [42, 43]. Other major limitations of these trials relate to the low number of patients included and the lack of randomized control groups. Also, results from long-term follow-up are still lacking and only surrogate endpoints could be evaluated [85]. Finally, most human stem cell studies have been done in patients that underwent simultaneous surgical or percutaneous revascularization, so that the effectiveness of stem cell transplantation may be confused with the revascularization procedure and the spontaneous recovery of stunned myocardium [86].

The suggested mechanisms by which stem cells improve cardiac function after myocardial infarction include an active contribution to contractile function and/or passive improvement of the heart mechanics [79]. Both mechanism and other, as yet undetermined, influences as

well seem to be mediated through direct and indirect cellular or noncellular processes of the injected stem cells [87, 88]. Noncellular effects, for instance, may include the secretion of cytokines or growth factors by bone marrow cells that may enhance the angiogenic repair process following myocardial infarction [88, 89].

According to Laplace's law, the introduction of cells that improves 'infarct scar thickening' may prevent left ventriculardilatation and attenuate left ventricular wall stress and remodeling after myocardial infarction, without contribution to myocardial contraction. These nonspecific cellular effects have been studied by introducing different cell types into the scar lesion, such as smooth muscle cells. By this passive mechanism, even noncontractile cells such as fibroblasts could be beneficial [79, 89].

Theoretically, the best functional improvement can be achieved when engrafted stem cells differentiate to cardiomyocytes, which provide for enhanced contractile performance. When coupled with host myocytes (as demonstrated by connexin-43 and cadherin staining), they may create an electromechanical continuum which ensures the orderly propagation of electrical signals and coordinated synchronous contractions, which rescue systolic function. However, thus far, an electromechanical function of transdifferentiated bone marrow cells in situ has never been demonstrated, but novel methods could provide insight in this issue [79, 90].

The improvement in cardiac performance and regional function may also result from neovascularization induced by stem cells directly or indirectly, increasing the perfusion of adjacent hibernating host cardiomyocytes [32, 91]. Moreover, this enhanced neovascularization leads to a reduction of apoptosis of the compensating myocytes in the peri-infarct region and prevents myocardial fibrosis with subsequent beneficial effects on postinfarction remodeling [72]. In this regard, the beneficial effects of transplanted stem cells are likely to be attributed to enhanced revascularization without directly contributing to systolic contraction, and this could be a major mechanism by which stem cells improve cardiac function [79]. In a recent pilot trial in humans, this enhanced neovascularization may have contributed to an improved left ventricular function. Enhanced neovascularization was suggested by almost complete normalization of the coronary blood flow reserve and an improved nuclear perfusion [43]. As already mentioned, it remains unclear whether stem cell fusion with host cardiac myocytes provide for a physiological repair mechanism. Theoretically, fused, hybrid cells could be intermediates (followed by reduction divisions) in the regeneration process or fusion of stem cells could even rescue the threatened residual (peri-) infarct cardiomyocytes. This issue clearly needs further investigation.

In most studies, the assays used to evaluate the improvement of cardiac function after cellular therapy have failed to distinguish the different underlying mechanisms of cardiac improvement. However, it may be of critical importance to understand these mechanisms when envisaging strategies aimed at enhancing cellular intervention [79].

## Adverse Reactions

Stem cells lost due to failure of cardiac engraftment can home to different organs such as the lungs, liver and many other organs [92]. The fate of these systemically engrafted mesenchymal stem cells differentiated to chondrocytes, adipocytes and bone marrow stromal cells has been assessed [77]. They also engraft in the brain where they adopt neural or astrocyte phenotypes [93].

It has also been hypothesized that mesenchymal stem cells may even be capable in participating in ongoing cellular turnover and replacement within an engrafted organ [94].

Finally, stem cells such as endothelial progenitors and multipotent adult progenitor cells (MAPC) have been demonstrated to incorporate into the angiogenic vasculature of growing tumors [61, 95]. Using isotope labeling in rats, Gao et al. [92] demonstrated that culture-expanded mesenchymal stem cells lodge in the lungs after systemic infusion. The clinical implications of these lodged cells in the pulmonary system, however, have not yet been critically evaluated. On the other hand, there has been no significant acute toxicity when autologous or allogeneic mesenchymal stem cells are administered intravenously in humans [96].

The fates of stem cells in the different organs at long term are not known, but in contrast to (undifferentiated) embryonic stem cells, there are no reports that adult stem cells can form neoplasms after systemic engraftment (in small animals with relative short life spans!). However, when expanded in culture, adult stem cells can dedifferentiate and take on embryonic stem cell properties [97]. Expanding cells in culture could result in unintended alterations in the intrinsic properties or biological behavior, and cells that have been cultured for long periods may be dangerous because, theoretically, stem cells cultured in vitro may accumulate genetic mutations [97]. Finally, the

reports of stem cell fusion also implicates possible risks for tumour formation because hybrid, hyperploid cells may be unstable with ensuing uncontrolled growth [98].

Taking all this into account, one should be aware of the possible contribution of (undifferentiated) stem cells to the progress of pathological processes, vascularization of tumor tissues or even their cancerous transformation [67, 95, 98, 99, 100].

Currently, the realistic prospects for bone marrow stem cell therapy on a large scale for cardiac repair in humans appears to be remote and one of the reasons could be the electrophysiological properties of the engrafted stem cells. When cultured in vitro, cardiomyocytes derived from mouse embryonic stem cells reveal arrhythmogenic properties with action potential heterogeneity, protracted automaticity, reentry and frequent spontaneous and easily inducible triggered activity [101]. In addition, the ischemic milieu that surrounds the engrafted cells could exacerbate arrhythmogenesis [101]. In the early embryonic heart, each cell possesses an intrinsic pacemaker activity and as heart development progresses, the cells differentiate to atrial or ventricle myocytes and the overall rhythm is dictated by a small number of pacemaker cells [102]. This developmental heterogeneity has also been demonstrated in mesenchymal stem cell cultures with cardiomyogenic commitment [68]. It seems very likely that during the transdifferentiation process, the engrafted stem cells in the infarct area not only recapitulate the morphological characteristics but also the different electrophysiological stages of cardiac embryogenesis, creating a substrate for arrhythmogenesis in the regenerating heart. However, in contrast to skeletal myoblasts, none of the animal or human experimental studies using bone marrow stem cells has revealed enhanced malignant arrhythmias. Several reasons may account for these findings. The first and most obvious reason is that the possible arrhythmic complications remains to be determined in the long term. Second, stem-cell-derived cardiomyocytes may behave differently in vivo than in vitro, being less arrhythmogenic in vivo [101]. Finally, some studies suggest that the formation of new endothelial cells might be a more common event than the attempted cardiomyocyte renewal [10].

## Conclusion

Despite many breakthroughs in cardiovascular medicine, the complications of a myocardial infarction such as chronic congestive heart failure remain a serious worldwide problem.

Stem cells could provide a promising strategy to repair myocardial infarctions and prevent postinfarct congestive heart failure. However, before this regenerative strategy can be applied in in humans, many questions should be resolved first.

As with most reviews on stem cells in cardiology, this review also raises more questions than answers. Which and how many stem cells are needed to restore an infarct area? When should stem cells be delivered after myocardial infarction and what kind of delivery method is the most appropriate? We should also focus on the mechanisms that home stem cells to the heart and how stem cell differentiation proceeds in the injured heart. How could we restore a normal extracellular matrix and an optimal vascular supply in the infarct area? Which adverse reactions could be expected in the short term and in the long term?

It is clear that as long these questions remain unsolved, much scientific work needs to be done.

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