Heart Fail Rev (2010) 15:447–456
DOI 10.1007/s10741-010-9160-z

# Stem cells for heart failure in the aging heart

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Published online: 12 March 2010


**Abstract** Despite a wide range of therapeutic interventions, the prognosis for most patients with heart failure remains poor. The identification of stem cells with the ability to generate cardiomyocytes and vascular cells and promote local repair and survival pathways has highlighted the ability of the heart to undergo regeneration and potentially provides a new therapeutic strategy for treatment of the failing heart. In recent years, however, clinical trials aimed at exploiting the beneficial effects of stem and progenitor cells to treat patients with cardiovascular disease have resulted in mild improvements at best, suggesting that these cells and/or the conditions in which they find themselves are not conducive to cardiac repair. Heart failure is most prevalent among older individuals, and a growing body of evidence suggests that with increasing age, cardiac stem and progenitor cells undergo senescent changes that impair their regenerative capacities. Moreover, environmental alterations over time appear to impact the capacity of these cells to improve cardiac function. Understanding these senescent changes may lead to the development of new and improved approaches to exploit the potential of stem cells to repair the aging heart. In this review, age-associated alterations in cardiac stem cell function are discussed, as well as strategies that are being investigated to promote cardiac regeneration in the patient with heart failure.

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**Keywords** Stem cells · Aging · Heart failure · Regeneration

## Introduction

Heart failure can be defined as the inability of the heart to pump blood sufficiently to meet the demands of the body. It can result from a number of etiologies, including hypertension, myocardial infarction and neurohormonal disorders [1]. In response to these pathologies, the heart initially undergoes physiological hypertrophy whereby myocytes grow in size to increase ventricular wall thickness in order to adapt to the increased wall stress. This adaptation becomes increasingly ineffective however and leads to pathological (or decompensated) hypertrophy and cardiac dysfunction associated with fibrosis and cardiomyocyte death. In fact, it has been estimated that up to one-third of cardiomyocytes may be lost in the patient with heart failure [2, 3].

While many tissues such as those of the liver, blood, skin and skeletal muscle are able to undergo some degree of cell turnover to replace damaged cells, the heart has a very limited regenerative capacity. Kajstura et al. estimated that the rate of cardiomyocyte proliferation in the healthy human heart is approximately 14 myocytes per million. In patients with end-stage ischemic heart disease, the rate rises 10-fold to 140 myocytes per million [4], yet this degree of turnover appears to be insufficient to compensate for the massive loss of cardiomyocytes experienced in chronic heart failure.

Despite limited cardiomyocyte proliferation in the adult heart, other cell types in the body appear to be capable of cardiogenic differentiation and could therefore be employed to replace damaged cardiomyocytes and

blood vessels after cardiac injury. These cell types and their potential application to the treatment of heart failure are described herein. The challenges associated with the impairment of stem cell function in the aging heart will also be discussed.

## Sources of cells for cardiovascular replacement

### Bone marrow-derived cells

The bone marrow is a rich source of stem cells that are able to generate not only blood cells but also differentiate into other cell types, including liver cells, neurons, and skeletal muscle [5]. In 2001, Orlic et al. [6] demonstrated that c-kit$^{pos}$lin$^{neg}$ bone marrow cells could be transplanted into the hearts of rodents that had undergone myocardial infarction where they are able to differentiate into both cardiomyocytes and vascular cells. Studies by other groups using similar approaches appear to support these findings and suggest that different stem cell populations, including hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) can generate cardiomyocytes in the ischemic heart [7, 8].

There has been some controversy as to whether these stem cell populations genuinely give rise to new cardiac cells: findings from a number of labs suggest that this apparent regeneration may in fact be due to fusion of donor cells with existing cardiomyocytes [9]. On the other hand, studies have also shown that bone marrow–derived cells have the capacity to differentiate into cardiomyocytes in vitro in the absence of mature cardiomyocytes, thus eliminating the possibility of cell fusion [10]. In reality it may be that there is a mixture of de novo differentiation as well as cell fusion in the injured heart.

Besides stem cells, the bone marrow can also give rise to endothelial progenitor cells (EPCs), which can be mobilized in response to injury and can home to damaged tissues [11, 12]. Circulating levels of EPCs are decreased in patients with chronic heart failure and coronary artery disease. Moreover, the number of EPCs in the systemic circulation is inversely correlated with the risk of cardiovascular disease [13, 14], suggesting that these cells may play an important role in cardiovascular repair mechanisms. It has been shown that these cells can incorporate into newly forming vessels and may also secrete factors such as VEGF, FGF, GM-CSF and SDF-1 to promote local blood vessel growth [15, 16]. Importantly, different EPC populations have been tested in experimental models of myocardial infarction, chronic heart failure and cardiomyopathy and have been shown to limit the extent of myocardial injury [16, 17].

### Resident cardiac stem cells

In 2003, Beltrami et al. [18] identified a c-kit$^{pos}$ population of cells present in the myocardium that could be clonally expanded and could differentiate into cardiomyocytes, endothelial and smooth muscle cells in vitro. These resident cardiac stem cells represent a very small population (estimated at approximately one cardiac stem cell per 10,000 cardiomyocytes; [19]) but appear to have a large capacity for self-renewal and can be cultured and expanded for more than 1 year while maintaining their stem cell phenotype [18]. In a rat myocardial infarction model, these cells differentiate into cardiomyocytes and vascular cells and improve cardiac function. Similarly, Sca-1$^{pos}$, Isl-1$^{pos}$ and SSEA-1$^{pos}$ cells have been identified in cardiac tissues that can differentiate into cardiomyocytes both in vitro and in vivo [20, 21]. Additionally, Messina et al. [22] have shown that cardiac stem cells present in human myocardial biopsies can be cultured to generate "cardiospheres", which can differentiate both in vitro and in vivo into cardiomyocytes and vascular cells that contribute to the preservation of cardiac function after myocardial infarction. Thus, one could envisage that cardiac biopsies could provide a source of autologous cardiomyocytes for therapeutic cardiac regeneration.

### Other sources of stem cells

Besides the bone marrow, MSCs from adipose tissue can also differentiate into cardiomyocytes and vascular cells and ameliorate the effects of cardiac damage [23]. The ability of skeletal muscle progenitors to replace damaged cardiomyocytes has also been explored. Skeletal myoblasts are present in small numbers surrounding skeletal muscle fibers in the adult. These satellite cells can proliferate and engraft in areas of muscle damage and contribute to preservation of function [24]. Given the contractile nature of these cells, their ability to replace cardiac muscle was hypothesized to be of benefit in models of cardiac injury. Indeed, experimental studies in both small and large animals have demonstrated that skeletal myoblast transplantation results in reduced ventricular remodeling. However, these cells do not differentiate into cardiomyocytes and importantly, do not become electrically coupled to the surrounding myocardium, resulting in arrhythmogenesis. Despite promising early-phase clinical studies suggesting that myoblast transplantation may improve cardiac function [24], the recent phase II multicenter MAGIC trial of patients with left ventricular dysfunction showed that myoblast transplantation did not improve ejection fraction and was associated with an increased number of arrhythmic events [25]. Thus, these progenitors do not appear to be a viable cell source for safe cell-based cardiac repair.

Embryonic stem cells can be driven to differentiate into both cardiomyocytes and vascular cell types [26]. While this may provide a useful source of cells for drug discovery and screening, use of embryonic stem cells would involve allogeneic transplantation and therefore issues of rejection currently preclude their use as a practical cell source for cell transplantation. Umbilical cord blood is a rich source of HSCs, MSCs and EPCs that are reported to have greater proliferative potential than comparable cells from adult tissues and can generate cardiomyocytes as well as many other cell types [27]. One possible advantage of umbilical cord blood–derived stem cells is that due to their immunological immaturity, they may be amenable to allogeneic transplantation. Further assessment is however required to establish the immunogenicity of these cells and their potential use as a source of cells for cardiovascular regeneration and repair.

It has recently been shown that mature fully differentiated cells can be reprogrammed via the transfection of retroviruses expressing four transcription factors (nanog, sox 2, c-myc and klf-4) to become stem cells capable of generating cell types of all three germ layers [28]. This discovery has paved the way for a completely new avenue of stem cell–based therapy and allows for the possibility of producing new cells from a patient's own somatic cells, thus ruling out issues of rejection from allogeneic cell transplantations. There is evidence that iPS cells can generate cardiomyocytes and vascular cell types [29], though the ability of these cells to contribute to cardiovascular repair has yet to be tested. While the application of these cells to patients with heart failure may be many years away, the generation of these cells provides a novel source of patient-specific cardiomyocytes that will aid in our understanding of the mechanisms underlying cardiac dysfunction and will provide a valuable supply of human cardiomyocytes to aid in drug discovery and elucidation of mechanisms of disease.

## A new understanding of the mechanisms of action of "stem cells"

Stem cells are defined by two main characteristics: their ability to undergo self-renewal and their capacity to differentiate into cell types of multiple lineages. However, a number of lines of evidence suggest that the main mechanism by which many cells traditionally described as stem cells contribute to improved cardiac function may not in fact be through direct differentiation and replacement of injured tissues. Cell tracing studies to evaluate the level of engraftment of various stem and progenitor cell populations, including MSCs and EPCs, have shown that even after myocardial infarction very few cells home to or are retained at the site of injury and differentiate into cardiomyocytes or vascular cells. Indeed, many of these studies have demonstrated that the vast majority of labeled bone marrow or stem cells home to other organs, most notably the lungs, liver, spleen and kidney [30, 31].

Rather than engraftment and differentiation, there is mounting evidence that many cell types considered to be stem or progenitor cells may act primarily through paracrine signaling mechanisms in damaged tissues, eliciting pleiotropic effects that promote local regeneration, repair and/or preservation of function. A variety of different stem and progenitor cell populations have been studied for their ability to secrete cytokines and growth factors and it is now evident that these cells, including HSCs, MSCs, multipotent adult progenitor cells (MAPCs) and EPCs, produce dozens of signaling factors that can act on the local environment. These factors include pro-angiogenic and mitogenic factors such as VEGF, FGF, PDGF and angiopoietin-1, homing factors such as SDF1, cytoprotective factors including IGF-1 and thymosin-$\beta$4, inflammatory signals, such as MCP-1 and matrix-degrading enzymes including MMPs and plasminogen activator [32]. Thus, transplanted stem cells may act through a host of secreted factors to not only upregulate local angiogenic and regenerative mechanisms but may also promote cell survival, inhibit fibrosis and modulate inflammatory signals.

Perhaps most intriguingly in the context of heart failure, it has recently been hypothesized that these paracrine signals may also influence cardiac contraction. For example, Takahashi et al. [33] have shown that culture of adult rat ventricular cardiomyocytes in conditioned media from bone marrow cells preserves cardiac contractility. Furthermore, cardiomyocytes from failing rat hearts cultured in the presence of skeletal myoblasts or bone marrow mononuclear cells exhibit greater cardiac contractility and calcium handling than cardiomyocytes cultured alone. Importantly, this increased contractility does not require cell–cell contact, suggesting that soluble factors released from the skeletal myoblasts and bone marrow cells can regulate this process [34]. These studies may provide some explanation as to why stem and progenitor cells are able to confer functional benefit in chronic heart failure models where significant damage and scarring has already occurred and there is little opportunity for regeneration or prevention of fibrosis. Based on this accumulating evidence that paracrine signaling may be their main mechanism of action, these "stem" cells should probably be renamed (for example, "pro-regenerative" cells) to distinguish them from true adult stem cells, which are able to directly replace damaged cells in injured tissues. In the case of MSCs, the use of "mesenchymal stromal cells", as is already used by some investigators, may be more appropriate than "mesenchymal stem cells".

Besides the wide range of paracrine effects elicited by stem cells described earlier, MSCs appear to have immunomodulatory properties that may allow their application for allogeneic cell transplantation. These MSCs are able to suppress T lymphocyte activation and proliferation in vitro, increase regulatory T cell activity and suppress the production of inflammatory cytokines, including TNF$\alpha$ and IL-12 [35]. Moreover, MSCs appear to be somewhat immunoprivileged, since they lack major histocompatibility complex class II surface proteins and do not express costimulatory proteins for T-cell induction. Again, this process appears to be mediated in part by secretion of factors from the MSCs, including prostaglandin $E_2$, HGF, and TGF$\beta$ [23, 35]. In animal models, these cells can be transplanted into allogeneic hosts and have shown improvements in cardiac function post-myocardial infarction without eliciting immune responses [36]. Based on these promising findings, MSCs may represent a possible source of stem cells for allogeneic transplantation.

## The limitations of endogenous stem cells in the aging heart

Cardiac stem and progenitor cells clearly have a remarkable capacity for expansion and appear to elicit a range of protective responses that can limit the extent of cardiac injury in experimental models of myocardial infarction and heart failure. Yet, cardiovascular disease is the number one cause of death in the US and affects approximately 36% of the population [37], suggesting that the endogenous functions of stem cells are insufficient to counteract the deleterious effects of heart disease in the vast majority of individuals. A number of reasons for this discrepancy can be postulated. Perhaps most importantly, patients who suffer from myocardial infarction and heart failure are generally older. Indeed, approximately 38% of individuals aged 40–59 have some form of cardiovascular disease, while this almost doubles to approximately 73% in the 60–79 age range [37]. Moreover, older patients are more likely to suffer from co-morbidities including hypertension and diabetes. Mounting evidence suggests that stem cell function becomes impaired with increasing age and disease, and additionally, the microenvironmental regulation of stem cells is also altered under these conditions. Some of these age- and disease-associated impairments are described in the following paragraphs.

Aging may have a negative influence on the mobilization of stem and progenitor cells from the bone marrow. Analysis of HSCs and EPCs in peripheral blood suggest that circulating levels of these cells may decrease with age [38, 39], although other studies dispute this [40]. What is apparent, however, is that regardless of age, stem and progenitor cell numbers are significantly decreased in patients with cardiovascular diseases, including coronary artery disease and diabetes, compared to healthy age-matched individuals [13]. Besides having lower baseline levels of circulating stem and progenitor cells, the ability to mobilize more EPCs in response to various stimuli also appears to be impaired with increased age and disease. For example, while exercise can increase circulating EPC levels in young individuals, this mobilization is blunted in older subjects [39]. Similarly, the increase in circulating EPCs observed after coronary artery bypass graft surgery in younger patients is significantly reduced in patients over the age of 69 [38].

Coupled with the decline in circulating stem and progenitor cell number is the increase in functional impairment of these progenitor cell populations with age and/or cardiovascular disease. In an experimental model of myocarditis-induced dilated cardiomyopathy, bone marrow–derived EPC numbers are decreased compared to controls, while the number of spleen-derived EPCs increased. Despite these opposing changes in levels, EPCs from both sources have a decreased ability to adhere to fibronectin, mature endothelial cells or cardiomyocytes, suggesting that they may have impaired migratory capacity in vivo [17]. Similarly, EPCs in human and rodent studies have a reduced capacity to migrate toward angiogenic stimuli in vitro [40] and human EPCs from older individuals have impaired proliferative and survival capacity compared to those from young subjects [40]. The cardiac differentiation capacity of bone marrow cells from old versus young mice is also impaired [10], suggesting that aging stem cells have a more limited capacity for repair via cardiac cell regeneration.

While stem cells are often thought as having an endless capacity for self-renewal and proliferation, it has become clear that like somatic cells, stem cells are prone to the intrinsic changes associated with aging and senescence that lead to impaired function. Anversa et al. have demonstrated that there is a decrease in resident c-kit$^{pos}$ cardiac stem cells in histological samples from aged patients with heart failure and those stem cells that are present are more likely to express the senescent marker p16$^{INK4a}$ and exhibit shortened telomeres compared to stem cells from healthy human hearts [19, 41]. These senescent changes are likely due to both intrinsic alterations with age, such as decreased telomere activity, as well as environmental insults such as oxidative stress, which can lead to DNA damage.

The environmental influence on stem cell function has been exemplified in heterochronic parabiosis experiments in which the circulatory system of a young mouse is directly connected to that of an old mouse. These studies demonstrate that exposure of aging stem cells to systemic factors derived from young mice increases their

proliferative capacity and their ability to contribute to muscle regeneration after injury [42]. Thus, circulating factors play an important role in maintenance of stem cell function but appear to be decreased with age. Indeed, the growth factors VEGF, FGF, PDGF and angiopoietin have all been shown to be downregulated in aging animals. These factors have pleiotropic effects on stem and progenitor cells, including cell proliferation, differentiation, migration and survival; therefore, decreases in the heart with increased age have a significant impact on angiogenic and cardioprotective pathways [43–45]. Experimental studies have shown that restoration of these factors in the aging animal can limit the extent of myocardial injury after infarction, suggesting that such approaches may have benefit in a clinical setting [43, 44].

The stem cell niche also plays an important role in the generation, mobilization and function of stem and progenitor cells. Of all the bone marrow–derived stem and progenitor cell populations, HSCs and their interaction with their microenvironment are probably the most well studied. Primitive HSCs reside in the endosteal niche in close proximity to stromal cells, which are thought to maintain the cells in a quiescent state. Movement of these HSCs to the vascular niche (i.e. the region of sinusoid endothelial cells) promotes HSC proliferation, differentiation and trans-endothelial migration. With age, the interaction of HSCs with bone marrow stromal cells appears to be impaired, suggesting that changes in cell adhesion molecules such as cadherins and integrins occur. Indeed, V-CAM and $\alpha$4- and $\alpha$5-integrin expression by HSCs is downregulated in older animals [46].

Dynamic changes in the composition of the extracellular matrix (ECM) are observed in the heart post-myocardial infarction. These changes are associated with conversion of cardiac fibroblasts to myofibroblasts, at least a portion of which are thought to be derived from HSCs [47]. Myofibroblasts secrete ECM proteins, including collagen and fibronectin, as well as growth factors and cytokines such as TNF$\alpha$ and IL-1$\beta$ that play important roles in the early inflammatory response to cardiac injury (reviewed in [47]). The secretion of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) by myofibroblasts also contributes to the regulation of ECM and subsequent fibrosis post-injury. In the early phase, these extracellular changes are protective, promoting homing of inflammatory cells, wound healing and may contribute to preservation of force generation. Continued ECM deposition by myofibroblasts, however, leads to scar formation and systolic dysfunction. With normal aging, both human and rodent hearts exhibit changes in levels of ECM proteins, concomitant with alterations in the activity of MMPs and TIMPs: while collagen content generally increases, MMP levels decrease [48–50]. In experimental models, aging mice show blunted responses to myocardial infarction, including reduced influx of macrophages and neutrophils and decreased collagen deposition coupled with reduced numbers of myofibroblasts. As a result, these older mice exhibit adverse remodeling and worsened cardiac function compared to young mice [51]. Thus, changes in the interaction of stem cells with their environment not only alter their generation and mobilization but may contribute to the age-associated changes in cardiac remodeling in response to injury.

## Cell transplantation for patients with heart failure

Clearly, the changes with age and disease that affect stem cell function are multifactorial and may play an important role in the progression of cardiac dysfunction to heart failure. Identifying mechanisms to inhibit or reverse these changes may help to prevent or at the least slow the progression of cardiovascular disease. Indeed, based on supportive experimental evidence, a number of strategies have made the transition to clinical trials.

The main focus of clinical trials for cardiovascular disease to date has been on the transplantation of autologous, unfractionated bone marrow cells to the ischemic injury site via intramyocardial or intracoronary delivery. This strategy limits safety concerns, since patients receive their own cells, and to date no significant safety issues have arisen in these trials. Based on the experimental evidence that stem cell transplantation may play a role in the early stages after injury, most trials have focused on cell transplantation within the first week following myocardial infarction. Despite promising and wide-ranging experimental evidence that bone marrow transplantation may limit myocardial damage, these studies have shown modest success. Meta-analyses demonstrate that the transplantation of autologous, unfractionated bone marrow after acute myocardial infarction combined with standard care results in an average increase in ejection fraction of 3–4% at 6-month follow-up, compared with standard treatment alone [52]. This discrepancy between the experimental and clinical data may reflect the fact that animal studies largely focus on the treatment of young and essentially healthy animals that undergo experimental myocardial injury, while the clinical trials have generally involved the treatment of older patients. Despite the fact that these changes seem modest, a recent comparison of the functional improvements seen in stem cell trials with trials examining existing therapies (including coronary angioplasty, angiotensin-converting enzyme inhibitors and beta-blockers) suggests that the improvements seen using stem cells are comparable to those observed with the use of current therapeutic strategies [53]. Thus, the stem cell clinical trial

data may not be as discouraging as previously thought and certainly suggest that stem cell–based therapies may be worth pursuing, at least for acute myocardial infarction.

There have been a handful of clinical trials examining the effects of autologous bone marrow transplantation for the treatment of patients with chronic heart failure. Some studies have shown dramatic effects. For example, Silva et al. [54] performed transendocardial delivery of autologous bone marrow mononuclear cells in patients with severe ischemic heart failure who were heart transplant candidates. While no change in ejection fraction or end-systolic and -diastolic function was observed at 2- and 6-month follow-up, exercise capacity was so improved that 4 of the 5 subjects were no longer eligible for transplantation. This was a very small study that did not incorporate a control group; nevertheless, the results are encouraging. A number of other small-scale studies have shown improvements in ejection fraction, exercise capacity and/or NYHA heart failure class, but there are as many trials that have shown no significant benefit [55–57]. Thus, while there remains some promise for the use of autologous bone marrow cell transplantation for chronic heart failure, more large-scale studies are required to fully evaluate its benefits. Importantly, very little is known about the mechanisms by which stem cells affect or modify cardiac function in the patient with chronic heart failure. In the acute setting (post-MI), stem cells appear to act on multiple pathways to promote cell survival, angiogenesis, regulation of inflammation, etc. However, in chronic heart failure where the potential for new blood vessel formation or cell survival in fibrotic regions is limited, the mechanisms of action remain to be determined. Ideally, stem cells might elicit anti-hypertrophic effects, prevent further fibrosis and promote enhanced contractility and new myocyte formation. To date, however, there is little evidence to suggest that these changes actually occur. Thus, a greater understanding of the mechanisms that underlie the potential therapeutic effects of bone marrow cells in the setting of chronic heart failure will help to define the most successful strategies to harness the potential of these cells.

Despite the somewhat limited improvements observed in early stem cell trials for heart disease, much can be learned from these studies. Notably, the approaches used so far appear to be safe, with no adverse effects or evidence of aberrant differentiation. Refinement of the use of stem cell–based therapies should focus on a number of areas. Firstly, the selection of patients may be important to the success of these approaches. Results of the REPAIR-AMI trial, for example, suggest that the patients who benefit most from stem cell therapy may be those with the largest cardiac functional deficit. In this study, the improvement in ejection fraction among all patients in the group receiving bone marrow transplantation was less than 3%. However, when patients were stratified according to their baseline ejection fractions at the start of the study, it was found that those subjects who had ejection fractions below the median (<48.9%) experienced greater benefit when evaluated 6 months later (i.e. an average 6.6% increase in ejection fraction; [58]).

While most clinical trials to date (particularly those in later phases) have focused on the transplantation of unfractionated autologous bone marrow, the selection of specific cell populations may improve success in patients with cardiovascular disease. A number of specific cell populations have already been investigated, including $CD133^{pos}$ and $CD34^{pos}$ bone marrow cells [59, 60]. Although these approaches appear to be safe, long-term follow-up is needed to confirm this, and large, randomized studies are required to fully evaluate their efficacy.

As previously mentioned, one limitation of autologous stem cell therapies is the possible decline in number of stem cells that are mobilized upon cardiac injury. Stem cell mobilization may be increased by a number of pharmacological agents. Granulocyte-colony stimulating factor (G-CSF) has been employed in rodent models of heart disease and shown to be cardioprotective [61]. In the clinic, however, G-CSF treatment has shown limited efficacy. A number of trials have examined the effects of G-CSF treatment on top of standard care in patients who had recently experienced a myocardial infarction. While patients in the FIRSTLINE-AMI trial displayed significant improvement in functional parameters 4 months post-MI, no improvement was found in the cardiac function of patients treated with G-CSF in a number of other trials [62]. These data point to the fact that while increasing the pool of circulating stem cells may be an important strategy for improving cardiac repair mechanisms post-injury, this may need to be coupled with approaches to improve stem cell homing and/or function so that the newly mobilized cells reach the appropriate destination and act in a beneficial manner.

Delivery of stem cells directly to the site of injury (for example by intramyocardial or intracoronary injection) versus intravenous infusion can significantly increase the number of cells that remain in the myocardium. Despite this, however, the numbers of cells that are retained still remain relatively low, i.e. generally in the 1–5% range [30, 31]. Alternative strategies have therefore been explored in animal models. SDF-1, for example, is upregulated in the border zone and infarct region in the first few days after myocardial infarction and has been found to play an important role in recruitment of CXCR4+ stem cells to the injury site in rodent models [63]. Exogenous delivery of SDF-1 enhances stem cell recruitment and leads to improvement in heart function [63]. Anversa et al. [64] have demonstrated that the growth factors HGF and IGF-1

are also able to promote the homing of local resident cardiac stem cells (positive for the c-met receptor and IGFR1) to ischemic myocardium where they appear to differentiate into cardiomyocytes and vascular cells. These factors have not been tested in the clinic however, so the therapeutic benefits of these factors in patients with heart disease remain unknown. Transfection of VEGF or FGF to increase stem cell homing and angiogenesis has shown promise in animal models of heart disease, but there has been limited success in the clinic [65, 66]. These findings point to the fact that targeting just one or two factors for upregulation in stem cells from cardiovascular disease patients may have limited benefits.

As well as improving the homing of cells using growth factors and cytokines, the use of structural support systems may also be a viable approach for directing the delivery of cells to injury sites and improving their retention in these regions. Such systems may consist solely of matrix- or scaffold-like hydrogels that are injected into the damaged tissues or may be a combination of matrix and stem cells (or stem cell–derived cardiomyocytes) that are engineered as patches or sheets that can enhance contraction of the ventricle. While beyond the scope of this review, such technologies have recently been described in the excellent review by Martinez and Kofidis [67].

As stem cells from patients with cardiovascular disease may be limited in number and regenerative function, stem cells from young, healthy individuals may provide a potent alternative for cardiovascular repair. Indeed, rodent studies have shown that transplantation of bone marrow cells from young animals to aging hosts can reverse the impairments in cardiovascular repair pathways [68]. As mentioned earlier, MSCs and MAPCs are cell populations that appear to have multifactorial actions that help to repair damaged tissues, as well as immunosuppressive properties that make them amenable to allogeneic transplantation. Thus, MSCs and MAPCs from young, healthy subjects might be used to treat older patients. Moreover, since these stem cells have a large ability for self-renewal, thousands of doses could be generated from a single batch of MSCs, thus allowing the development of standardized "off-the-shelf" therapies. Clinical trials are currently under way to test for safety and efficacy in patients with acute myocardial infarction and heart failure, and outcomes are eagerly anticipated as the potential of a "universal donor" cell-based therapy is an exciting prospect with immense potential to improve cardiovascular function in older patients.

## Conclusions

Clinical trials to date demonstrate that autologous cell-based therapies for cardiovascular repair are feasible and safe. However, the efficacy of such approaches has been limited. The design of future trials may need to include combined therapies that increase stem cell mobilization, homing and/or improve function (some of which are outlined in Table 1). Alternatively, given the multifactorial effects and putative immunoprivileged status of MSCs and MAPCs, the possibility of an off-the-shelf allogeneic

**Table 1** Potential stem cell-based strategies for treatment of heart failure in the elderly patient

| Approach | Methods |
|---|---|
| Increase SC number | Mobilization of endogenous SCs (e.g. by G-CSF, erythropoietin, etc.) |
| | Harvest endogenous SCs from bone marrow/circulation and expand ex vivo prior to transplantation |
| Increase SC homing and/or retention at site of injury | Harvest endogenous SCs from bone marrow/circulation and deliver directly to site of injury |
| | Local injection of homing factors (e.g. SDF-1, HGF) or plasmids expressing homing factors to promote SC recruitment to injury site |
| | Local transplantation/injection of scaffolds or patches to promote ingrowth and/or retention of SCs at site of injury |
| Improve function | Engineering of SCs to over-express growth factors (e.g. VEGF, HGF), survival factors (e.g. Akt), etc. to promote homing, migration, differentiation, proliferation, survival, etc. |
| | Delivery of allogeneic stem cells (e.g. MSCs) from young, healthy donors |
| Improve cardiac microenvironment | Delivery of growth factors to injury site to promote cell proliferation, differentiation, migration, etc. as well as homing |
| | Local transplantation of scaffolds or hydrogels to promote cell attachment, differentiation, patterning, etc. |
| Structural replacement of damaged cardiomyocytes | Transplantation of scaffolds or patches to promote homing and patterning of endogenous SCs |
| | Transplantation of scaffolds or patches seeded with SCs or SC-derived cardiomyocytes |

SC *stem cell*. Note that many of these approaches used in isolation may be insufficient to improve cardiac function. However, combined strategies may prove effective in the clinical setting

therapy for cardiovascular disease represents a potentially transformative approach to cardiac repair. Much of the focus of stem cell–based clinical trials has been on acute myocardial infarction, since our understanding of the mechanisms of heart failure is considerably more limited. Nevertheless, these approaches may have similar benefits in the context of chronic heart failure. A greater understanding of the pathophysiology of this syndrome as well as the senescent changes that occur in aging individuals is necessary to fully exploit the potential of these cells to deliver effective therapeutic strategies.

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