Cardiac Progenitor Cells and Bone Marrow-Derived Very Small Embryonic-Like Stem Cells for Cardiac Repair After Myocardial Infarction

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Heart failure after myocardial infarction (MI) continues to be the most prevalent cause of morbidity and mortality worldwide. Although pharmacological agents and interventional strategies have contributed greatly to therapy, new and superior treatment modalities are urgently needed given the overall disease burden. Stem cell-based therapy is potentially a promising strategy to lead to cardiac repair after MI. An array of cell types has been explored in this respect, including skeletal myoblasts, bone marrow (BM)-derived stem cells, embryonic stem cells, and more recently, cardiac progenitor cells (CPCs). Recently studies have obtained evidence that transplantation of CPCs or BM-derived very small embryonic-like stem cells can improve cardiac function and alleviate cardiac remodeling, supporting the potential therapeutic utility of these cells for cardiac repair. This report summarizes the current data from those studies and discusses the potential implication of these cells in developing clinically-relevant stem cell-based therapeutic strategies for cardiac regeneration. (Circ J 2010; 74: 390–404)

Key Words: Cardiac function; Cells; Ischemic heart disease; Myocardial infarction; Reperfusion

Myocardial infarction (MI) results in loss of cardiomyocytes, scar formation, ventricular remodeling, and eventually heart failure. Pharmacologic, catheter-based, and surgical interventions have led to improved survival of patients with coronary artery disease, although they fail to regenerate dead myocardium. Consequently, reduced mortality is accompanied by increased morbidity because of ischemic heart failure. In recent years, stem cell-based therapy has emerged as a potential new strategy for cardiac repair.1 The ultimate goals of cell-based therapy are cardiomyocyte regeneration and coronary neovascularization, leading to clinical improvement without severe adverse effects. The optimal source of cells for repairing damaged myocardium is a topic of intense research. Important features of stem cells for cardiac regeneration include self renewal, clonogenicity, and the ability to differentiate into cardiomyocytes, endothelial cells and vascular smooth muscle cells.

Studies in animal models of MI have demonstrated that various subsets of adult primitive cells can regenerate functional cardiomyocytes, with improvement in cardiac structure and function. Small clinical trials of adult bone marrow (BM)-derived stem cell therapy in patients with MI and ischemic cardiomyopathy have recapitulated these beneficial effects in humans, with infarct size reduction and improved cardiac function. BM cells, as well as subpopulations from within the BM, including hematopoietic stem cells (HSCs, primarily a blood-forming cell), multipotent mesenchymal stem cells (MSCs, potential cardiomyogenic and angiogenic cell) and endothelial progenitor cells (EPCs, primarily an angiogenic cell), have been the most prevalent source of cells used in experimental and clinical studies. These subpopulations are sorted according to their expression of membrane receptor proteins, the so-called cell-surface markers. Entire BM cell populations can be incubated with antibodies directed against cell-surface markers and attached to either magnetic beads or fluorescent tags. Magnetic column or flow cytometry is then used to isolate the cells of interest from the entire population. In this fashion, particular characteristic HSCs, MSCs and EPCs can be isolated from the BM. MSCs have also been separated from the entire unfractioned population of BM by their ability to adhere to plastic substrates and by the absence of hematopoietic markers.2 However, a principal problem is that the cell-surface markers that determine a true cardiac stem cell have not yet been defined. As a result, there is a vast number of possible combinations of the various markers and cell subpopulations to be studied. Not surprisingly, the outcomes of therapy have been variable because the subpopulations yielded from laboratory to laboratory have not been standardized.

Over the past 10 years, researchers have applied various BM-derived stem/progenitor cells for cardiac reparative therapy in animal studies, such as lineage negative (lin−) e-kit positive (c-kitpos) BM stem cells,3–5 BM-derived MSCs,6,7 and EPCs.8,9 Despite these studies1–9 showing the transdifferentiation of BM-derived stem/progenitor cells into functional...
cardiomyocytes and vascular cells, other studies have suggested that the transplanted BM stem cells do not readily acquire a cardiac phenotype in the injured heart. Thus, promoting adequate transdifferentiation of the transplanted BM-derived stem/progenitor cells into functional cardiomyocytes and vascular cells remains a great challenge for researchers to use these cells in cardiac reparative therapy.

Apart from the studies using these typical BM-derived stem/progenitor cells for cardiac reparative therapy, a pool of resident cardiac progenitor cells (CPCs) has been discovered in the adult heart itself. More recently, a novel population of very small embryonic-like stem cells (VSELs) has also been identified in the BM. Transplantation of these CPCs and VSELs has been shown to induce effective cardiac repair after MI. This review will discuss the recent data from our studies dealing with CPCs and VSELs for cardiac reparative therapy.

**Myocardial Repair With CPCs**

**Discovery of Resident CPCs**

Traditionally, the heart has been viewed as a static organ incapable of repairing any form of damage. According to this paradigm, the number of myocytes is established at birth, and this population of terminally differentiated myocytes is irreplaceable throughout the life of the organ and the organism. In agreement with this notion of the heart, myocytes are permanently lost, and the heart has no reserve mechanism to compensate for cell death and the wear and tear resulting from the physiological demands of daily life. However, demonstration that small amplifying myocytes can divide after infarction or pressure overload, and that a responsive stem cell pool resides in the adult myocardium, has opened an innovative view of the heart as a dynamic organ capable of self-renewing. In 2003, Beltrami et al reported a population of resident CPCs in the adult rat heart that were identified by their expression of the surface antigen and stem cell marker (c-kit$^{\text{pos}}$) and by the absence of surface markers of more committed hematopoietic lineages (lin$^{\text{neg}}$). These lin$^{\text{neg}}$/c-kit$^{\text{pos}}$ CPCs are multipotent undifferentiated and give rise to myocytes, smooth muscle, and endothelial cells. Although rare overall, histological analysis revealed the CPCs reside in small clusters within cardiac niches. CPC clusters and cardiac niches exist in both the atria and ventricles. These cells

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**CPCs and VSELs for Cardiac Repair**

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can be isolated, cultured, and cloned, and expanded in vitro for therapeutic implication in vivo. The existence of CPCs that amplify and commit to the myocyte lineage in response to increased workload strongly supports the notion of potential cardiac regeneration. Unlike other adult stem cells, CPCs represent a logical source to exploit in cardiac regenerative therapy, because they are likely to be intrinsically programmed to generate cardiac tissue and to increase cardiac tissue viability. Given that CPCs have these unique characteristics, the identification of resident CPCs has created great excitement and sparked intense hope for myocardial regeneration with cells that are from the heart itself and are thereby inherently programmed to reconstitute cardiac tissue.

In Vivo Evidence of Cardiac Repair With CPCs
Myocardial repair requires the formation of myocytes and coronary vessels, and it cannot be accomplished by a cell already committed to the myocyte lineage. In the presence of an infarct, the generation of myocytes alone cannot restore contractile performance in the akinetic region; myocytes will not grow or survive in the absence of vessel formation. Resistance arterioles are critical for blood supply, and oxygen delivery is controlled by the capillary network. Similarly, the creation of vessels alone would not restore the dead myocardium or reinstitute contractile activity in the infarcted portion of the ventricular wall; vessels do not produce force.

Observation that CPCs injected locally in the infarcted myocardium of animals repair the necrotic tissue and improve ventricular function has formed the basis of a new paradigm in which multipotent CPCs are implicated in the normal turnover of myocytes, endothelial cells, smooth muscle cells, and fibroblasts.

However, the field of regenerative cardiology is in its infancy, and great caution has to be exercised in the implementation of this form of cellular therapy in humans before we have obtained the basic information concerning the ability of CPCs to migrate, divide, and differentiate. Unfortunately, there is not a good animal model that can be used to collect the information that is of tremendous importance in the application of CPC treatment to the patient population. In an attempt to develop strategies relevant to the future treatment of patients, new hypotheses have to be raised and various in vivo animal models have to be tested to move the field in a direction that defines CPC therapy clinically on an individual basis. This would maximize the efficacy of CPC administration in ischemic heart disease.

The first in vivo study of CPCs for cardiac repair was reported by Beltrami et al in a rat model of MI. In that study, female Fischer 344 rats were subjected to the left coronary artery permanent ligation (non-reperfused MI) and 5 h later, BrdU-labeled lin<sup>−</sup> /c-kit<sup>+</sup> CPCs prepared from syngeneic male rats were injected with 2×10<sup>5</sup> cells in 2 opposite regions bordering the infarct. BrdU-labeled regenerating myocardium was found in infarcts both at 10 and 20 days. The regenerating band was thin and incompletely covered the infarcted area at 10 days, but was thicker and present throughout the infarct at 20 days. This regeneration had significantly reduced infarct size from 53% to 40% in the 10-day group, and from 70% to 48% in the 20-day group. The regenerated myocardium was constituted by BrdU-positive small myocytes, capillaries, and arterioles that appeared to mature over time. The myocytes expressed cardiac myosin heavy chain, α-sarcomeric actin, α-cardiac actinin, N-cadherin, and connexin 43. Cardiac regeneration increased wall thickness and ejection fraction, and improved LV end-diastolic pressure, developed pressure and dP/dt at 20 days. That study demon-

![Figure 2](image_url)
strated for the first time that injection of lin^neg/c-kit^pos CPCs into the border region of infarcted myocardium regenerates cardiac tissue and positively affects cardiac performance.

In a follow-up study, Rota et al confirmed that intramyocardial injection of CPCs led to the replacement of approximately 42% of the scar with newly formed myocardium, attenuated ventricular dilation and prevented the chronic decline in function of the infarcted heart. Subsequently, Bearzi et al reported that injection of human CPCs into the border zone of the infarcted myocardium of immunodeficient mice and immunosuppressed rats generated a chimeric heart that contained human myocardium composed of myocytes, coronary resistance arterioles, and capillaries. The regenerated human myocardium was structurally and functionally integrated with the rodent myocardium and contributed to the performance of the infarcted heart. Those in vivo studies have clearly shown that CPCs are effective in myogenesis when administered intramyocardially. However, delivery of CPCs through direct intramyocardial injection is an approach that would be impractical for widespread use in patients.

Because intracoronary delivery via a percutaneous procedure is a more clinically-relevant approach and the most widely used route for clinical studies of cell-based therapy in patients, we sought to validate the protective properties of the CPCs after delivered intracoronarily. With regard to the intracoronary approach, other adult stem cells, such as MSCs and cardiosphere-derived cells (CDCs), have been shown to produce microvascular occlusion after intracoronary delivery and raised concern over the use of this approach to deliver stem cells into the diseased myocardium. This is not surprising, as the diameter of MSCs and CDCs is >20 μm, exceeding the typical capillary luminal diameter of 7–10 μm. In contrast, CPCs are <10 μm, which is not larger than the capillary luminal diameter, providing an unique advantage over the other adult stem cells for intracoronary delivery. Furthermore, the study was performed in the setting of a permanent coronary occlusion, which differs importantly from the contemporary clinical situation in which the occluded artery is usually recanalized. Thus, we performed studies to examine CPC-mediated cardiac repair in more clinically relevant settings.

Figure 3. Administration of cardiac progenitor cells improves postinfarction ventricular remodeling in rats after myocardial infarction: multiple anatomical parameters obtained in hearts arrested in diastole. *P<0.05 between untreated and treated rats. Data are mean±SEM. LV, left ventricular. (Reprinted from Dawn et al.)
in which MI was created after ischemia and reperfusion and CPCs were delivered via the intracoronary route. In an acute MI setting, 35 rats were subjected to a 90-min coronary occlusion and received EGFP-labeled clonogenic CPCs intracoronarily at 4 h after reperfusion. CPCs were delivered to the coronary arteries at a dose of $1 \times 10^6$ cells/rat via a catheter positioned in the aortic root during two 20-s occlusions of the ascending aorta. At 35 days after CPC delivery, immunofluorescent staining and confocal microscopic images of the treated hearts showed that the transplanted EGFP$^{\text{pos}}$ cells were mainly engrafted in the infarcted region, although scarce numbers of EGFP$^{\text{pos}}$ cells were found in the surviving noninfarcted LV myocardium (Figure 1). EGFP$^{\text{pos}}$ CPC-derived new cells were characterized by the expression of $\alpha$-sarcomeric actin, GATA-4, MEF2C, connexin 43 and N-cadherin (Figure 1), suggesting that the transplanted CPCs differentiated toward cardiac lineages and induced myocardial regeneration. Echocardiographic analysis showed that

![Figure 4](Image)

**Figure 4.** Morphometric analysis in rats after myocardial infarction. (A) Representative Masson's trichrome-stained myocardial sections from a vehicle-treated and a CPC-treated rat. Scar tissue and viable myocardium are blue and red, respectively. (B) Quantitative analysis of LV morphometric parameters. Data are means±SEM. CPC, cardiac progenitor cell; LV, left ventricular. (Reprinted from Tang et al.44)
CPCs prevented further decline of fractional shortening, LV ejection fraction, LV anterior wall thickness and attenuated the increase in LV dimension, area and volume (Figure 2), suggesting an improvement in LV systolic performance. Pathologic analysis showed that treated hearts had a smaller LV chamber diameter and volume, and a higher wall thickness-to-chamber radius ratio and LV mass-to-chamber volume ratio (Figure 3). In addition, CPC treatment decreased the infarct size by 29%. That study demonstrates that CPCs are effective in limiting infarct size, attenuating LV remodeling, and ameliorating LV function after acute MI when delivered intracoronarily.

Clinically, however, this approach would be problematic because administration of autologous CPCs to patients with acute MI would necessitate an endomyocardial biopsy followed by isolation and expansion of c-kitPOS CPCs from the tissue fragment, all of which would require weeks, so CPC transplantation would have to be postponed until after the acute phase of the infarct. By the time a sufficient number of autologous CPCs would be ready for therapeutic use, the...
inflammatory response to acute injury would have abated and a scar would have formed. At this chronic stage, the local myocardial environment is very different from that of an acute MI because the expression of the cytokines and adhesion molecules that are upregulated during the acute phase and play an important role in the chemotaxis, homing, and differentiation of stem cells is absent or markedly diminished.

For example, CPCs are known to express CXCR-4, the receptor for stromal-derived factor-1 (SDF-1), a cytokine that plays a pivotal role in orchestrating the chemotaxis, homing, and differentiation of various progenitor/stem cells. The expression of SDF-1 in the myocardium increases markedly after ischemia/reperfusion, peaking at 1 day, but resolving by 7 days. The migration, survival, and differentiation of CPCs are also modulated by hepatocyte growth factor (HGF) and insulin-like growth factor-1 (IGF-1), both of which are upregulated shortly after ischemia/reperfusion and then subsequently decline. In view of the marked downregulation of SDF-1, HGF, IGF-1, and other signals in the setting of a mature scar, it is unclear whether transplanting CPCs at this stage would still be effective.

To address this issue, we performed another study to determine whether administration of CPCs is effective in regenerating cardiac tissue and alleviating postinfarction LV remodeling and dysfunction when these cells are infused intracoronarily in the setting of an old MI (scar) produced by temporary coronary occlusion followed by reperfusion. Rats were subjected to a 120-min coronary occlusion followed by reperfusion. At 1 month after coronary occlusion/reperfusion, at a time when the acute inflammatory response has resolved and the formation of the scar is complete, rats received vehicle or EGFP-labeled c-kitpos CPCs intracoronarily at a dose of 1×10^6 cells/rat via a catheter positioned in the aortic root; 35 days later, morphometric analysis revealed that the amount of viable myocardium within the risk region was 25% greater (P<0.01) and the thickness of the anterior (infarcted) wall 24% greater (P<0.05) in CPC-treated than in vehicle-treated rats (Figure 4). Cardiac function assessed by both echocardiography (Figure 5A) and hemodynamics (Figure 5B) showed significant improvement at 35 days in the CPC-treated rats compared with the vehicle group. Correlation analysis revealed that the hemodynamic parameters were significantly related to the amount of viable myocardium in the risk region (Figure 6), suggesting that the functional improvement noted in CPC-treated rats was accounted for, in part, by the greater proportion of viable tissue in the risk region.
region. In addition, CPC treatment reduced the myocardial collagen content in both the infarcted and noninfarcted regions (Figure 7), suggesting less fibrosis in the CPC-treated myocardium. Taken together, these results demonstrate that intracoronary administration of CPCs in the setting of a myocardial scar produces beneficial structural and functional effects within a relatively short timeframe (35 days). To delineate the possible role of cardiac regeneration from transplanted EGFP\textsuperscript{pos} CPCs in these beneficial effects, we performed a comprehensive pathologic analysis of 28–40 sections per heart to quantitate the newly formed EGFP\textsuperscript{pos} myocardial cells. Although previous studies of CPCs in models of acute MI\textsuperscript{13,27,28,35} have shown robust engraftment and differentiation of the transplanted cells, surprisingly, we were unable to find any EGFP\textsuperscript{pos} cells in 10 of 17 CPC-treated hearts. In the remaining 7 hearts, EGFP\textsuperscript{pos} cells accounted for only \(\sim2.6\%\) of the risk region and \(\sim1.1\%\) of the noninfarcted region, values that were insufficient to account for the functional improvement. Importantly, in the CPC-treated group, the increase in viable tissue within the infarcted region and the improvement in systolic LV performance were observed not only in the subset of 7 hearts that had EGFP\textsuperscript{pos} cells expressing cardiomyocyte, endothelial, and vascular smooth muscle cell markers, but also in the subset of 10 hearts in which EGFP\textsuperscript{pos} cells were not found. Taken together, these findings suggest that although transplanted CPCs gave rise to new cells expressing markers specific for cardiac myocytes, endothelial cells, and vascular smooth muscle cells, direct
Figure 8. Effect of cardiac progenitor cell (CPC) transplantation on c-kit<sup>pos</sup> cells in rats after myocardial infarction. (A) Representative confocal microscopic images from a vehicle-treated and a CPC-treated rat showing c-kit<sup>pos</sup> cells (red) and BrdU<sup>pos</sup> cells (green) in the risk region. Yellow arrows indicate c-kit<sup>pos</sup> cells that are BrdU positive; white arrows show c-kit<sup>neg</sup> cells that are BrdU positive. (B) Quantitative analysis of c-kit<sup>pos</sup> cells and double positive (c-kit<sup>pos</sup>/BrdU<sup>pos</sup>) cells in normal, vehicle-treated, and CPC-treated hearts (the last group is subdivided into EGFP<sup>pos</sup> and EGFP<sup>neg</sup> subgroups). (C) Quantitative analysis of c-kit<sup>pos</sup> cells in the risk and noninfarcted regions in normal, vehicle-treated, and CPC-treated hearts [as in (B), the last group is subdivided into EGFP<sup>pos</sup> and EGFP<sup>neg</sup> subgroups]. (D) Quantitative analysis of double positive (c-kit<sup>pos</sup>/BrdU<sup>pos</sup>) cells expressed as a percent of all c-kit<sup>pos</sup> cells. (E) Correlation between the number of c-kit<sup>pos</sup> cells and the percent of viable myocardium in the risk region (r=0.59, P<0.01). Data are means±SEM. (Reprinted from Tang et al.)
Figure 9. Cardiac commitment of endogenous cardiac progenitor cells (CPCs) after transplantation of exogenous CPCs in rats after myocardial infarction. The commitment of endogenous CPCs (c-kitpos/EGFPneg cells) to the cardiac lineage was assessed by quantitating endogenous CPCs that expressed Nkx2.5 and MHC in vehicle- and CPC-treated hearts. New myocytes formed in the last 2 weeks of life were identified by measuring α-sarcomeric actinpos and BrdUpos cells. (A) Representative confocal microscopic image of a c-kitpos/EGFPneg/Nkx2.5pos/MHCpos cell obtained in serial sections of a CPC-treated heart. (B) Quantitative analysis of c-kitpos/EGFPneg/Nkx2.5pos/MHCpos cells in the risk and noninfarcted regions of vehicle- and CPC-treated hearts (the CPC-treated group is subdivided into 2 subgroups: with EGFPpos cells [EGFPpos] and without EGFPpos cells [EGFPneg]). The number of c-kitpos/EGFPneg/Nkx2.5pos/MHCpos cells is normalized to both number of cells (10^4 nuclei, Upper panels) and area (mm^2, Lower panels). Because cell density in the CPC-treated, EGFPpos hearts was higher (because of clusters of EGFPpos cells), the magnitude of the increase in endogenous CPCs in CPC-treated EGFPpos hearts was greater when the number of cells was normalized to area (mm^2) than to the number of nuclei. (C) Representative confocal microscopic image of α-sarcomeric actinpos/BrdUpos cells in a CPC-treated heart. (D) Quantitative analysis of the number of α-sarcomeric actinpos/BrdUpos cells in the risk and noninfarcted regions of vehicle- and CPC-treated hearts (subdivided into EGFPneg and EGFPpos subgroups). Data are means±SEM. (Reprinted from Tang et al. 44)
formation of new parenchyma from the transplanted CPCs was not the major reason for the benefits observed, suggesting that paracrine mechanisms are likely to play an important role. Indeed, we found that infusion of exogenous CPCs promoted proliferation of endogenous cells, including endogenous c-kit+ CPCs, in both the risk and noninfarcted regions (Figure 8), and the increase in the number of endogenous CPCs was also associated with an increase in the number of endogenous CPCs that exhibited apparent cardiogenic commitment in both regions (Figure 9). These findings suggest that exogenous CPCs promote proliferation of endogenous CPCs, resulting in formation of new cardiac cells, and support the concept that one such paracrine mechanism is activation of endogenous CPCs.

Taken together, our studies using rats with acute or chronic MI have demonstrated that intracoronary administration of exogenous CPCs produces beneficial structural and functional effects and activates endogenous CPCs. Although exogenous CPCs can differentiate into new cardiac cells, this mechanism is not sufficient to explain the benefits, suggesting paracrine

**Figure 10.** Echocardiographic assessment of left ventricular (LV) function in mice after myocardial infarction. Representative two-dimensional (A,C,E) and M-mode (B,D,F) images from vehicle-treated (A,B), CD45+ cell-treated (C,D), and very small embryonic-like stem cell (VSEL)-treated (E,F) mice 35 days (d) after coronary occlusion/reperfusion. The infarct wall is delineated by arrowheads (A,C,E). Compared with the vehicle-treated and CD45+ cell-treated hearts, the VSEL-treated heart had a smaller LV cavity, thicker infarct wall, and improved motion of the infarct wall. (G–J) Transplantation of VSEL improved echocardiographic measurements of LV systolic function 35 d after myocardial infarction. Data are means±SEM. n=11–14 mice per group. *P<0.05 vs group II at 35 d; #P<0.05 vs group I at 35 d; §P<0.05 vs values at 96 h in respective groups. BSL, baseline. (Reprinted from Dawn et al.)

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effects, among which our data identify activation of endogenous CPCs.

**Myocardial Repair With VSELs**

More recently, a novel population of very rare (~0.01%) Sca-1\(^{+}\)/Lin\(^{neg}\)/CD45\(^{neg}\) cells has been identified from adult BM mononuclear cells\(^{15}\) and human cord blood.\(^{46}\) These cells are very small (~6 μm),\(^{47}\) and express (among other lineage markers) cardiac markers, including Nkx2.5/Csx, GATA-4, and MEF2C. Previous studies have shown that VSELs can differentiate in vitro into several lineages, including cardiac and vascular lineages,\(^{15,48}\) and are mobilized into the peripheral blood after acute MI.\(^{49}\) To test therapeutic potential of VSELs, we performed an in vivo study to examine whether BM-derived VSELs promote myocardial repair after a reperfused MI.\(^{50}\) Mice underwent a 30-min coronary occlusion followed by reperfusion and received an intramyocardial injection of \(1 \times 10^4\) Sca-1\(^{+}\)/Lin\(^{neg}\)/CD45\(^{neg}\) EGFP-labeled VSELs at 48 h after MI. A total of 5 injections were made in the peri-infarct region in a circular pattern at the border between infarcted and noninfarcted myocardium. To separate cell-specific from nonspecific actions, Sca-1\(^{+}\)/Lin\(^{neg}\)/CD45\(^{neg}\) VSELs were directly compared with Sca-1\(^{+}\)/Lin\(^{neg}\)/CD45\(^{pos}\) cells, which are highly enriched among HSCs and differ from VSELs only with respect to CD45 expression.

At 35 days after MI, VSEL-treated mice exhibited improved global and regional LV systolic function (Figure 10).

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**Figure 11.** Morphometric assessment of left ventricular (LV) remodeling in mice after myocardial infarction. Representative Masson’s trichrome-stained myocardial sections from vehicle-treated (A), CD45\(^+\) hematopoietic stem cell-treated (B), and very small embryonic-like stem cell (VSEL)-treated (C) hearts. Scar tissue and viable myocardium are blue and red, respectively. Note that the LV cavity is smaller and the infarct wall thicker in the VSEL-treated heart. (D–H) Morphometric measurements of LV structural parameters. Data are mean±SEM. n=11–14 mice per group. *P<0.05 vs group II. (Reprinted from Dawn et al.\(^{50}\))
cessation of LV remodeling (Figure 11) and attenuated myocyte hypertrophy in surviving tissue (Figure 12) compared with vehicle-treated controls. In contrast, transplantation of Sca-1<sup>pos</sup>/Lin<sup>neg</sup>/CD45<sup>pos</sup> cells failed to confer any functional or structural benefits. Scattered EGFP<sup>pos</sup> myocytes and capillaries were present in the infarct region in VSEL-treated mice, but their numbers were very small. These results indicate that transplantation of a relatively small number of CD45<sup>neg</sup> VSELs is sufficient to improve LV function and alleviate myocyte hypertrophy after MI, supporting their potential therapeutic utility for cardiac repair. The ability of VSELs to alleviate postinfarction LV remodeling warrants further investigation of their therapeutic utility and may have significant implications for the design of future studies of BMC-mediated cardiac repair both in animals and in humans.

**Conclusion**

Cell-based therapy for acute and chronic ischemic heart disease is an exciting new approach that is in the early stages of development. Development of this therapy has proceeded in an orderly and rigorous fashion, with appropriate parallel conduct of clinical trials and mechanistic research. As is the case with all therapies at an early stage, complete consensus in clinical trials does not exist. The totality of evidence supports the safety and provisional efficacy of cell-based therapies. Future challenges include optimizing the cell type and delivery method. The potential impact of cell-based therapy could revolutionize cardiovascular medicine. This review has provided proof of concept that administration of a relatively small number of CPCs or BM-derived VSELs is sufficient to attenuate deterioration of LV function and structure in animals after MI. The mechanism remains uncertain, but cannot consist solely of regeneration of transplanted cell-derived cardiomyocytes. The evidence that transplantation of exogenous CPCs promotes activation of endogenous CPCs supports the concept of possible paracrine effects. Because the small cell diameter of CPCs (<10 μm) and VSELs (<6 μm) is unlikely to cause microvascular occlusion, these cells appear to be suitable for intracoronary delivery, which is the most clinically relevant route for cell-based cardiac reparative therapy. It seems plausible that the next decade will witness a large number of innovative and exciting clinical trials utilizing CPC or VSEL-based approaches for reparative therapy of ischemic heart disease.

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