Heart failure, a common consequence of ischemic heart disease, is a major cause of morbidity and mortality in the world. Pharmacological treatment with β-blockers and inhibitors of the renin–angiotensin–aldosterone system has improved the clinical outcomes in patients with heart failure. Likewise, mechanical unloading with left ventricular assist devices and resynchronization therapy have led to partial reversal of cardiac structural and molecular remodeling and symptomatic improvement. Despite these remarkable advances, however, mortality and morbidity of patients with heart failure, with or without reduced ejection fraction remains high. Moreover, heart transplantation, while an effective option, is available only for a selected number of patients and is not without considerable negative consequences. Furthermore, gene therapy still remains in early investigational stages and not yet ready for clinical applications. The high residual mortality and morbidity of patients with heart failure might be inherent to the shortcomings of the current therapeutic approaches, as none directly targets the underlying causal problem in heart failure, that is, loss of or intrinsically dysfunctional myocytes. Consequently, novel therapeutic approaches are necessary to further improve the clinical outcomes in patients with heart failure.

The heart is considered, by and large, a terminally differentiated organ with a limited intrinsic regenerative capacity that alone is insufficient to compensate for the pathological loss of cardiac myocytes during the postnatal period. The discovery of cardiac progenitor cells (CPCs) in the heart more than a decade ago along with the recent data showing that the existing myocytes undergo a gradual turnover have raised the potentials for regenerative cardiac repair. Likewise, the discovery of mesenchymal stem cells (MSCs), which was thought to have the potential to differentiate to cardiac myocytes, but yet to proven, or enhance differentiation of the endogenous cardiac stem cells has offered a cell transplantation approach for regenerative cardiac repair. Furthermore, advances in generation and characterization of cardiac myocytes from induced pluripotent stem cells using the Yamanaka factors or a combination thereof, have expanded the therapeutic options for cardiac repair. In addition, direct reprogramming of the resident fibroblasts to myocytes, whether using a defined set of transcription factors or microRNAs, has further advanced the field of regenerative cardiac repair. Finally, combination of different cell types has been used to gain additive and synergistic effects. Recent advances have offered considerable insight into molecular biology, self-renewal, and differentiation cardiac stem cells, as well as phenotypic characteristics that are expected to offer clinical applications.

The potential use of stem cells in repairing injured myocardium and improving heart failure has raised considerable excitement in patients, physicians, and researchers alike. The field, however, is in infancy and faces considerable challenges in attaining its goal of repairing the damaged myocardium and restoring cardiac function in ischemic heart disease (Table 1). Even the identity of the resident CPCs remains unsettled. Resident cells expressing the c-kit antigen but not markers of the hematopoietic or mast cells are considered bona fide CPCs sufficient and necessary to repair the damaged myocardium. And yet, genetic fate mapping experiments have shown minimal contribution of the c-kit+ cells to cardiac myogenesis. Human embryonic stem cells have been shown to differentiate to beating cardiomyocytes, SA nodal-like cells and mesodermal...
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Nonstandard Abbreviations and Acronyms

CPC cardiac progenitor cell
MSC mesenchymal stem cell

cells. However, their clinical use is overshadowed by the occurrence of serious cardiac arrhythmias in the transplanted animals, likely because of poor electromechanical coupling of the injected cells with the host cells. Moreover, the use of induced pluripotent stem cell–derived cardiac myocytes for cardiac repair is hampered by their immature phenotype and the presence of epigenetic and genetic changes. Resident and bone marrow cells and cortical bone–derived cells are considered as potential sources for differentiation to cardiac myocytes but lacking compelling evidence for cardiac myogenesis and perhaps exerting their salutary biological effects through paracrine mechanisms. Equally exciting and challenging are the discovery and characterization of other CPCs that could give raise to various cardiac cell types, including smooth muscle cells, endothelial cells, and fibroblasts. An important problem to overcome is the multiple comorbidities and their comedications of ischemic heart disease patients with heart failure that may affect cytoprotective signaling triggered by the different stem cell, as well as survival and differentiation properties of such stem cells in the injured tissue. Clearly, the rapid face of discoveries is dazzling and a complete coverage of the recent developments in cardiovascular stem cells would be beyond the scope of this article. We regret that many valuable works were not covered in part or at all included in the present overview on Recent Developments.

Clinical Trials In Human Patients

The ClinicalTrials.gov lists >1000 clinical trials including >600 studies in the United States alone that tests effects of various stem cells in human patients (http://www.clinicaltrials.gov/). The list includes 71 including 38 active clinical trials in patients with heart failure using various stem cells, such as adipose-derived, mesenchymal, human embryonic, autologous CD133+ and CD34+ stem cells among the others. Autologous skeletal myoblasts were probably the first cell type used to regenerate functional myocardium. It was tested initially in a rabbit model of myocardial cryoinjury, which showed incorporation of the injected myoblasts and improved myocardial performance. Subsequent observational studies were followed by randomized clinical trials in human patients with heart failure injected with autologous skeletal myoblasts. The results in small size studies were somewhat promising. However, despite the encouraging results in small and observational studies, the overall results of larger clinical studies have not been impressive but rather null. Given the risk of cardiac arrhythmias associated with injection of myoblasts and the availability of other cell types, autologous skeletal myoblasts are not considered the prime cell type for heart failure therapy.

To date, the initial results of clinical trials with cardiac stem cells have been mostly promising, although they remain inconclusive in terms of long-term effects and often contradictory. The findings of REPAIR-AMI trial showed that intracoronary delivery of bone marrow cells in patients with acute myocardial infarction improved cardiac function, which were preserved on average 18-month and 5-year follow-up periods. The differences in the outcome might reflect differences in the study population characteristics and subtleties of the experimental design including preparation and characterization of the bone marrow–derived cells. Likewise, the mechanisms responsible for the beneficial effects of exogenously applied stem cells remain unclear, as data to show fate, function, and differentiation of the injected cells to cardiac myocytes, as well as production and secretion of paracrine factors are lacking. Two recent clinical trials SCIPIO and CADUCEUS, which used 2 different sets of CPCs, reported improved cardiac function. In both trials, the underlying mechanism(s) responsible for improved clinical outcomes remains to be determined but is speculated to be secondary to expression and secretion of paracrine factors rather than direct differentiation of the injected progenitor cells to cardiac myocytes. Paracrine factors released from the injected CPCs might direct many restorative processes, including myocardial protection, neovascularization, and cardiac remodeling. Consequently, there is considerable interest in identification and characterization of secretome of the CPCs for therapeutic gains. 

Table 1. Some of the Challenges Facing Clinical Use of Myocardial Regeneration

| 1. Does postnatal heart contain bona fide stem cells that could regenerate cardiac myocytes? |
| 2. What are the characteristics and markers of bona fide cardiomyogenic stem cells? |
| 3. How to provoke controlled proliferation and differentiation of bona fide cardiomyogenic stem cells to mature cardiac myocytes? |
| 4. What are the determinants—transcription factors, noncoding RNA and others—of differentiation of bona fide cardiomyogenic stem cells to mature myocytes? |
| 5. How to reduce or eliminate aging of the bona fide cardiac stem cells and enhance their survival under pathological conditions? |
| 6. How to enhance differentiation of other resident progenitor cells to mature cardiac myocytes? |
| 7. How to enhance recruitment and retention of the circulating progenitor cells to the heart and enhance their differentiation to mature myocytes? |
| 8. Which type of progenitor cells to inject or implant in the myocardium to obtain most efficient differentiation to mature cardiac myocytes? |
| 9. How to enhance engraftment of injected/implanted progenitor cells in the myocardium? |
| 10. How to reduce or eliminate antigenicity of the progenitor cells into the myocardium, and reduce or eliminate rejection? |
| 11. How to enhance survival of the injected/implanted cells in the heart? |
| 12. How to enhance cell–cell communications and electromechanical coupling among the transplanted cells as well as among the transplanted and the host cells? |
| 13. How to generate induced pluripotent stem cell–derived cardiomyocytes with molecular and phenotypic characteristics closer to mature cardiac myocytes? |
| 14. Is the recovery of myocardial function because of myocardiogenesis or secondary to expression and secretion of paracrine factors? And if the latter, what are these paracrine factors and how to garner their effects to enhance cardiomyogenesis? |
example, fibroblast growth factor 9, which is secreted from bone marrow cells, has been shown to promote myocardial vascularization and myocytes hypertrophy, preserves cardiac function, and reduce mortality in an experimental model of myocardial infarction. Fibroblast growth factor signaling is also implicated in suppression of autophagy and prevention of premature differentiation of CPCs. It is anticipated that several paracrine factors contribute to improvement in cardiovascular function in clinical trials of cardiac stem cells. Overall, the results of the clinical trials performed to date have been less than spectacular, despite the plausible rationale, which raises the necessity of novel approaches. Table 2 provides a partial summary of the published clinical trials of patients with heart failure using various types of progenitor cells.

**Rejuvenation of Cardiac Stem Cells**

Stem cells are not exempt from senescence. As a result, resident cardiac stem/progenitor cells in older humans are expected to have a decreased reparative capacity in response to myocardial injury. Consequently, there is considerable interest in rejuvenating the endogenous CPCs. Several molecules are implicated in rejuvenation of CPCs, including Pim-1 kinase, NOTCH1 signaling, and telomerase, just to name a few. Pim-1 kinase has been shown to impart antisenescence and anti-apoptotic effects in CPCs, as well as in MSCs. Genetic modification of aged human CPCs with Pim-1 kinase results in remarkable rejuvenation of the CPCs associated with enhanced proliferation, increased telomere lengths, and decreased susceptibility to replicative senescence. Likewise, activation of NOTCH1 signaling pathway is implicated in rejuvenation of myogenic responses to satellite muscle cells. Activation of telomere–telomerase axis is known to contribute to cell survival and proliferation, and to prevent cellular senescence. Madonna et al recently identified a subpopulation of adipose tissue–derived MSCs that expresses high levels of myocardin (MYOCD), a nuclear transcription cofactor for myogenic genes, and telomerase reverse transcriptase, the catalytic subunit of telomerase. Adipose tissue-mesenchymal stem cells

### Table 2. A Partial Summary of Controlled Clinical Trials of Stem Cell Delivery in Ischemic Heart Disease

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Study Design</th>
<th>Route of Administration</th>
<th>Sample Size</th>
<th>Number of Cells</th>
<th>Follow Up</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal myoblasts</td>
<td>Nonrandomized</td>
<td>Transendocardial</td>
<td>Treated: 6; Controls: 6</td>
<td>210±150×10^6</td>
<td>12 mo</td>
<td>Improved LVEF and walking distance</td>
</tr>
<tr>
<td>Skeletal myoblasts</td>
<td>Nonrandomized</td>
<td>Intramyocardial</td>
<td>Treated: 12; Controls: 14</td>
<td>5×10^6</td>
<td>12 mo</td>
<td>Improved myocardial viability, reperfusion, and function</td>
</tr>
<tr>
<td>Skeletal myoblasts</td>
<td>Nonrandomized</td>
<td>Transendocardial</td>
<td>Treated: 14; Controls: 28</td>
<td>3±50×10^6</td>
<td>4 y</td>
<td>No benefits, increased risk of arrhythmias</td>
</tr>
<tr>
<td>Skeletal myoblasts</td>
<td>Randomized</td>
<td>Intramyocardial</td>
<td>Treated: 97; Controls: 30</td>
<td>400–800×10^6</td>
<td>6 mo</td>
<td>Improved cardiac function</td>
</tr>
<tr>
<td>Skeletal myoblasts</td>
<td>Double-blind</td>
<td>Transendocardial</td>
<td>Treated: 12; Controls: 11</td>
<td>30–600×10^6</td>
<td>12 mo</td>
<td>Improved myocardial viability and function</td>
</tr>
<tr>
<td>BM-MNC</td>
<td>Double-blind</td>
<td>Transendocardial</td>
<td>Treated: 14; Controls: 7</td>
<td>25.6±6.3×10^6</td>
<td>4 mo</td>
<td>Improved myocardial function and perfusion</td>
</tr>
<tr>
<td>BM-MNC</td>
<td>Open-label randomized</td>
<td>Intracoronary</td>
<td>Treated: 52; Controls: 23</td>
<td>205±110×10^6</td>
<td>3 mo</td>
<td>Improved myocardial function</td>
</tr>
<tr>
<td>BM-MNC</td>
<td>Randomized</td>
<td>Intramyocardial</td>
<td>Treated: 10; Controls: 10</td>
<td>60±31×10^6</td>
<td>4 mo</td>
<td>Improved regional but not global cardiac function</td>
</tr>
<tr>
<td>BM-MNC</td>
<td>Randomized</td>
<td>Intracoronary</td>
<td>Treated: 14; Controls: 14</td>
<td>20–32×10^6</td>
<td>3 mo</td>
<td>Improved myocardial viability and function</td>
</tr>
<tr>
<td>BM-MNC</td>
<td>Randomized</td>
<td>Intracoronary</td>
<td>Treated: 24; Controls: 23</td>
<td>12×10^6</td>
<td>6 mo</td>
<td>Improved diastolic function</td>
</tr>
<tr>
<td>BM-MNC</td>
<td>Randomized</td>
<td>Intramyocardial</td>
<td>Treated: 42; Controls: 23</td>
<td>84–56×10^6</td>
<td>6 mo</td>
<td>No effects on infract size or cardiac function</td>
</tr>
<tr>
<td>BM-MNC</td>
<td>Randomized</td>
<td>Transendocardial</td>
<td>Treated: 20; Controls: 10</td>
<td>30×10^6</td>
<td>6 mo</td>
<td>Symptomatic improvement</td>
</tr>
<tr>
<td>BM-PC</td>
<td>Randomized</td>
<td>Intramyocardial</td>
<td>Treated: 10; Controls: 10</td>
<td>22×10^6</td>
<td>6 mo</td>
<td>Improved cardiac function</td>
</tr>
<tr>
<td>BM-PC</td>
<td>Nonrandomized</td>
<td>Intramyocardial</td>
<td>Treated: 20; Controls: 20</td>
<td>5.8×10^6</td>
<td>6 mo</td>
<td>Improved cardiac function</td>
</tr>
<tr>
<td>BM-PC</td>
<td>Randomized</td>
<td>Intracoronary</td>
<td>Treated: 28; Controls: 27</td>
<td>123×10^6</td>
<td>12 mo</td>
<td>Improved cardiac function, exercise tolerance, and reduced mortality</td>
</tr>
<tr>
<td>CD34+</td>
<td>Randomized</td>
<td>Intracoronary</td>
<td>Treated: 55; Controls: 55</td>
<td>113±26×10^6</td>
<td>5 y</td>
<td>Improved cardiac function, exercise tolerance, and survival</td>
</tr>
<tr>
<td>CSCs</td>
<td>Randomized open-label</td>
<td>Intracoronary</td>
<td>Treated: 16; Controls: 7</td>
<td>1×10^6</td>
<td>12 mo</td>
<td>Improved cardiac function and reduced infract size</td>
</tr>
<tr>
<td>CSCs</td>
<td>Randomized open-label</td>
<td>Intracoronary</td>
<td>Treated: 17; Controls: 8</td>
<td>12.5–25×10^6</td>
<td>12 mo</td>
<td>Increased viable myocardium and reduced infract size</td>
</tr>
</tbody>
</table>

BM-MNC indicates bone marrow–derived mononuclear cells; BM-PC, bone marrow progenitor cells; CSCs, cardiac stem cells; and LVEF, left ventricular ejection fraction.
Novel Delivery Systems for Stem Cell Therapy

Although encouraging results have been reported in cardiac cell therapy, only a few of the transplanted cells survive in the myocardium and integrate into the host myocardium. Transplanted cells quickly disappear from the site of injection because they are removed by the blood flow and degraded by specific enzymes located in the extracellular microenvironment. However, despite a quick disappearance from the myocardium, CPCs impart considerable improvement on cardiac function, implying a paracrine mechanism. Several approaches have been suggested to overcome these hurdles. Conventional strategies such as overexpression of prosurvival genes, such as Akt, β adrenergic stimulation, cotransplanting with others, such as the endothelial cells, modification of the extracellular matrix and immune system are used to enhance survival and retention of CPCs in the heart. Recently, there has been increasing focus on development of novel biomaterials that are coated with stem cells are functionalized with growth, mitotic and chemotactic factors, cytokines, and other biologically active materials. These new biomaterials are biocompatible and biodegradable polymers made of poly (ε-caprolactone) or poly(lactic-co-glycolic acid) that allow prolonged and controlled delivery of growth factors in situ and better cell retention in the transplanted area.

The combination of the stem cells, biomaterials and growth factors may enhance the efficacy of cell therapy by mobilizing endogenous stem/progenitor cells in vivo, promoting cell proliferation and differentiation, and augmenting cell engraftment and survival in the injured myocardium (Figure). Likewise, transplantation of ATMSCs coated on fibrin polymers and CPCs with immobilized insulin growth factor type 1 on peptide nanofibers has been shown to be beneficial. The use of cardiac-specific decellularized matrices might also serve as platforms for injectable biomaterials to deliver stem cells in a more sustainable and effective manner. The so-called environmentally responsive systems are designed to match the release of the functional molecular with a patient’s physiological need at the appropriate time or the correct site. They are constituted of sensitive hydrogels that can control the release of drugs by changing the gel structure according to environmental stimulation, such as temperature, pH, or ion concentration. Poly N-isopropylacrylamide hydrogel is a typical example of temperature-sensitive hydrogels, which shows sol-to-gel transformation at a critical solution temperature of ≈35°C. This polymer releases the drug when it transforms from gel-to-sol and is of particular interest in those clinical situations, such as tissue ischemia, characterized by low temperature in the tissue. The interest in pH-sensitive polymers is in their capability of releasing a drug when the environmental pH decrease and hence, promoting proliferation and differentiation of CPCs on such conditions.

Stem Cell Therapy Without the Cells

The improved cardiac function observed in preclinical studies using traditional stem cell transplantation is in discord with the data showing poor long-term stem cell engraftment. Systemically administered c-kit+ cells, bone marrow cells, adipose tissue–derived cells and blood-derived endothelial progenitor cells exhibit low homing efficiency, and limited capacity for transdifferentiation into cardiomyocytes post transplantation. Thus, the prevailing assumption is that the injected stem cells do not contribute directly to replenishing cardiomyocyte populations in the heart. This notion has shifted the focus on paracrine effects derived from the stem cell secretome, such as growth factors, microRNA, antioxidants, proteasomes, and exosomes, as the underpinning mechanisms responsible for improved cardiac function after stem cell transplantation. Consequently, there is a considerable interest in identification and characterization of the paracrine factors, which might offer the opportunity to achieve the effects of stem cell transplantation without truly injecting them, and hence, the so called stem cell therapy without the cells. Current secretome-based approaches have shown some promise in preclinical models. For example, exosomes have been implicated in mediating some of the proangiogenic paracrine effects of CD34+ stem cells and cardioprotection by remote conditioning.

The Road Ahead: Toward Clinical Application

There are currently several clinical studies that are investigating clinical uses of various stem cells in myocardial repair and regeneration. The ongoing multicenter trials, such as ADVANCE (NCT 2010-022153-42), BAMI-01 (NCT 2012-001495-11), or 2011-01-01REPEAT (NCT 2011-000595-33), are expected to provide more compelling evidence for the clinical use of stem cells and offer insight into the mechanisms of their effects (reviewed in the study by Sanganalmath and Bolli). Currently, the paracrine mechanisms are considered...
the key events responsible for neoangiogenesis and cardioprotection imparted by the transplanted stem cells in the ischemic myocardium. New insights in the nature of the secretome and their mechanisms of effects might further enhance the clinical use of cardiac regeneration. Likewise, alternative approaches to enhance differentiation of the endogenous CPCs and direct reprogramming of the resident noncardiac cells to cardiac cells would be expected to offer further opportunities to treat human patients with ischemic heart disease and consequent heart failure.

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None.

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