Current perspective of stem cell therapies for cardiac regeneration

Coronary artery disease (CAD) remains a major cause of morbidity and mortality worldwide. Despite recent advances in the prevention and treatment of coronary artery disease, it remains the major cause of morbidity and mortality worldwide [1]. Clinical studies confirm that new endogenous or exogenous stem cells can incorporate into host myocardium via transdifferentiation and/or cell fusion [9]. However, emerging evidence suggests that cellular mediated paracrine effects are likely to be the major mechanism that contributes to the improvement in LV function [10], as there is lack of significant CM transdifferentiation [11,12] and long-term survival of transplanted cells [13]. Experimental studies have shown that the transplanted cells can secrete proangiogenic cytokines.

Potential mechanisms for stem cell therapies

The concept of cardiac regeneration relies on the belief that exogenous cells or mobilized endogenous cells can transdifferentiate into mature CMs after transplanting into the micro-environment of the damaged myocardium. Furthermore, they should also integrate both electrically and mechanically with host CMs, thereby contributing to improvement of cardiac function [6]. Experimental studies show that several types of stem cells can improve LV function in animal models of MI or myocardial ischemia [7,8]. The potential mechanisms of stem cell therapy for cardiac regeneration are summarized in Figure 1.

Clinical studies confirm that new endogenous or exogenous stem cells can incorporate into host myocardium via transdifferentiation and/or cell fusion [9]. However, emerging evidence suggests that cellular mediated paracrine effects are likely to be the major mechanism that contributes to the improvement in LV function [10], as there is lack of significant CM transdifferentiation [11,12] and long-term survival of transplanted cells [13]. Experimental studies have shown that the transplanted cells can secrete proangiogenic cytokines.
to enhance neovascularization [14]. Furthermore, the paracrine factors secreted by the transplanted cells exert anti-apoptotic effects, alter the restoration of extracellular matrix and recruit endogenous stem cells [10,14]. Indeed, recent studies demonstrate that direct injection of bone marrow (BM) cell extract can provide comparable benefits as intact cell therapy in limiting LV remodeling and improve the LV function after MI [15].

**Cell types for cardiac regeneration**

A variety of stem or progenitor cells, including skeletal myoblast, BM-derived cells, placental/cord blood-derived cells, adipose tissue-derived cells, resident cardiac progenitor cells, embryonic stem cells (ESCs) and, recently, induced pluripotent stem (iPS) cells, have been investigated for cardiac regeneration (Figure 2). Table 1 summarizes the advantages and disadvantages of various cell types used for cardiac regeneration.

**Skeletal myoblasts**

Skeletal myoblasts, also known as muscle 'satellite cells', are skeletal muscle precursor cells found between the basal lamina and sarcolemma of skeletal muscle. They can be isolated from skeletal muscle biopsies and expanded *ex vivo* to the quantities sufficient for autologous transplantation [16]. Therefore, skeletal myoblasts appear to be an attractive candidate for myocardial regeneration. Preclinical experiments have demonstrated that transplanted skeletal myoblasts successfully engraft, differentiate into myotubules and improve post-MI cardiac function in animal models of MI [17–23]. These initial encouraging results paved the way for the first Phase I human trial in 2000 [24] followed by a number of pilot clinical trials [25–29]. However, the enthusiasm for using skeletal myoblasts for cardiac regeneration has faltered with concerns regarding an increased incidence of ventricular tachyarrhythmia after transplantation. The lack of connexin-43 expression in skeletal myoblasts after transplantation leads to the failure of electrical integration with the host myocardium and may contribute to an increased risk of proarrhythmias [30,31].

**BM-derived cells**

Bone marrow-derived cells are a heterogeneous cellular population consisting of approximately 98% differentiated cells, including hematopoietic cells, vascular cells, adipocytes, osteoblasts and osteoclasts, and approximately 2% stem or progenitor cells. The undifferentiated stem or progenitor cells in the BM are a mixed population of hematopoietic stem cells, endothelial progenitor cells (EPCs) and mesenchymal stem cells (MSCs). BM-derived stem or progenitor cells have attracted attention owing to
their potential to transdifferentiate into various cell types in the body, including neurons, hepatocytes, skeletal muscle and CM. Furthermore, they can be readily harvested and expanded \textit{ex vivo} for autologous transplantation without any immune rejection. Prior experimental studies suggest that transplantation of BM-derived cells into the border zone of acute MI results in \textit{in vivo} myogenesis and neoangiogenesis by the transplanted cell, which subsequently contributes to an improvement in cardiac function \cite{32}. However, recent studies using genetic rather than immunofluorescence techniques have demonstrated that transdifferentiation of BM cells into CMs is only a rare event \cite{11,12,33}. Therefore, paracrine effects of BM cells have been postulated as the major mechanism for the improvement of cardiac function after transplantation \cite{34}.

In general, BM-derived cells can be broadly divided into two main groups according to the expression of the surface antigen CD34. CD34$^+$ stem/progenitor cells are precursors of blood cells and endothelial cells in BM. Among

![Figure 2. Different types of stem/progenitor cells for cardiac regeneration.](image)

**Table 1. Comparison of the advantages and disadvantages of different types of cells for engraftment.**

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal myoblasts</td>
<td>Easy to obtain by isolation from skeletal muscle biopsies</td>
<td>A lack of connexin-43 expression after \textit{in vitro} differentiation results in failure of electrical integration with the host myocardium</td>
<td>[27,101]</td>
</tr>
<tr>
<td>BM-derived cells</td>
<td>Using \textit{ex vivo} expansion it is possible to produce sufficient quantities for autologous transplantation</td>
<td>Transdifferentiation into CMs may only be a rare event</td>
<td>[10–12,14,33]</td>
</tr>
<tr>
<td>ESCs</td>
<td>Able to undergo self-renewal in undifferentiated state without karyotypic alteration</td>
<td>Efficiency of spontaneous \textit{in vitro} cardiac differentiation is very low (&lt;1%) Potential risk of immune rejection and teratoma formation Unethical with sacrifice of early embryo</td>
<td>[25,70,102]</td>
</tr>
<tr>
<td>iPS cells</td>
<td>Patient specific and feasible for autologous transplantation</td>
<td>Low yield of cardiac differentiation Requires the use of viral vectors, which may lead to oncogenesis and genome abnormality</td>
<td>[28,70]</td>
</tr>
</tbody>
</table>

BM: Bone marrow; CM: Cardiomyocyte; EPC: Endothelial progenitor cell; ESC: Embryonic stem cell; iPS: Induced pluripotent stem; MSC: Mesenchymal stem cell.
them, EPCs are characterized by their coexpression of CD133 and/or kinase insert domain receptor [35]. Experimental studies suggest that BM-derived EPCs can be mobilized into systemic circulation, and contribute to postnatal neovascularization [36,37] and endothelial repair [38]. Therefore, the use of EPCs as selected by CD34+ or CD133+ may have the greatest potential for enhancement of neovascularization.

By contrast, CD34- cells are less well defined. They consist of MSC and multipotent adult progenitor cells. BM-derived MSCs are precursors of stromal cells in BM, which possess the capacity of multilineage differentiation, including CMs [39,40]. Despite the fact that a panel of markers including CD29, CD90, CD44 and CD73 (SH3 or SH4) has been described, there are no specific markers for their selection. Experimental studies have also shown that MSC transplantation increased myocardial perfusion, reduced apoptosis and infarct size, and improved LV function [41]. Compared with other BM-derived stem cells, MSCs appear to have immunoprivilege and may have the potential to be used for allogeneic transplantation [42]. Although the transplanted MSCs in host myocardium have the potential to transdifferentiate into CMs, in vitro differentiation of MSCs into functional CMs after transplantation into patients with MI is rarely observed. Multilineage differentiation capacity has also been described in a subset of multipotent adult progenitor cells, but specific markers for their identification are lacking [43,44]. The potential of differentiation of MSCs is rather limited and selective, and clinical use of this cell type remains to be discussed.

The encouraging results from these preclinical studies using different types of BM-derived cells have prompted a series of clinical trials investigating the potential therapeutic use of BM-derived cells in CAD patients. Indeed, the majority of recently completed or ongoing clinical trials in CAD patients are based on the use of BM-derived cells.

Adipose tissue-derived cells
Adipose tissue represents another abundant and readily available source of adult stem cells for cardiac regeneration. In addition to adipocytes, adipose tissue also contains MSCs and EPCs. Similar to BM-derived MSCs, adipose tissue-derived MSCs have also been shown to differentiate into CM [45] and endothelial lineages [46]. Experimental studies also demonstrate that adipose tissue-derived MSCs can engraft and acquire cardiac phenotypes in post-MI myocardium and improve cardiac function [47,48]. However, their therapeutic potential as compared with other sources of MSCs remains unclear.

Placental or cord blood-derived cells
Placental or cord blood are also a rich source of stem cells, including hematopoietic stem cells, MSCs and unrestricted somatic stem cells. In general, they have a higher proliferative potential compared with similar cell types that are derived from adult BM. Recently, a nanofiber-based ex vivo human umbilical cord blood stem cell expansion technology made it feasible to obtain an appropriate number of therapeutic stem cells. Additionally, proangiogenic growth factor overexpression in progenitor cells can potentially improve autologous or allogeneic stem cell therapy for ischemic diseases [49]. Another study suggested that human umbilical cord blood cells specifically migrate to infarcted rather than normal myocardium, where engraftment and neoangiogenesis occur, and beneficially influence remodeling processes [50].

Furthermore, these cells can be easily obtained and cryopreserved after birth for future autologous transplantation. However, there are very limited data on the use of placental or cord blood-derived cells for cardiac regeneration [51].

Resident cardiac stem cells
The presence of resident cardiac stem cells in the adult mammalian heart has been increasingly recognized [3]. Several investigators confirmed the existence of resident cardiac stem cells, which are self-renewing, clonogenic, and can differentiate into CMs, smooth muscle and endothelial cells [52]. These cells can be identified by the expression of c-kit and sca-1, as well as their phenotype and ability to differentiate in cell culture [53]. Resident cardiac stem cells have been successfully isolated from surgical and endomyocardial biopsy in a clinical setting, and expanded ex vivo for autologous transplantation. In experimental models of MI, transplantation or mobilization of resident cardiac stem cells can engraft and acquire cardiac and vascular phenotypes in infarcted myocardium, and improve cardiac function [54]. Recently, a method has been presented for the isolation of adult human stem cells from endomyocardial biopsy specimens. Cardiosphere-derived cells are cardiogenic in vitro, and have promoted cardiac regeneration in a murine model [55]. However, by eliminating the presence of myogenic tissue...
in the cardiac biopsy, Andersen et al. argued that cardiospheres cultured from neonatal and adult rats/mice are indeed without cardiomyogenic potential [56], suggesting a murine model may be unsuitable as a source of cardiac stem cells.

**Embryonic stem cells**

Compared with adult stem cells, ESCs possess two unique properties. First, ESCs can be cultured indefinitely (i.e., they self-renew) in an undifferentiated state without karyotypic alteration. Second, ESCs maintain the potential to differentiate into all cell types of the body (pluripotency) [57,58]. In fact, genuine CMs with cardiac-specific structural and functional properties can be consistently differentiated from ESCs using various methods [59]. Furthermore, various subtypes of CMs including pacemaker, atrial and ventricular CMs have been identified [60]. As a result, ESCs have been regarded as a potentially unlimited ex vivo cell source for cell-based therapies. Numerous preclinical studies involving transplantation of undifferentiated ESCs or their cardiac derivatives into infarcted myocardium have been reported [61]. Consistently, transplantation of undifferentiated ESCs or ESC-derived CMs improved LV function post-MI (Table 2).

However, there are many obstacles to the clinical use of ESCs for cardiac regeneration. First, the efficiency of in vitro cardiac differentiation from ESCs remains low (typically <1%), thus making it very difficult to achieve the number of CMs needed for therapeutic application. Second, cardiac differentiation from ESCs results in a heterogeneous population of CMs (pacemakers, atrial and ventricular cells), which could give rise to arrhythmia caused by a mismatch between the transplanted cells and the host CMs [62]. Third, CMs derived from ESCs are in fact structurally and functionally immature relative to their adult counterparts. The immature electrophysiological properties of ESC-derived CMs may contribute to pro-arrhythmia [63]. Fourth, the potential risks of tumor formation and immunogenicity of ESCs and ESC-derived cells also need to be addressed [64,65]. Finally, the most important disadvantages of ESC usage are the controversial problems associated with ESCs, including the low yield, as well as the immature and heterogeneous phenotypes with cardiac differentiation. Furthermore, the use of viral vectors to create iPS cells may lead to insertional mutagenesis causing uncontrolled cellular proliferation, oncogenesis or abnormal development [70]. Expression of oncogenic factors should be carefully monitored in the iPS cells to avoid teratoma formation upon iPS cell therapy. However, such problems can be solved by non-viral administration of the four pluripotency-inducible factors, which include the use of non-integrating genetic elements [71,72], excision of genetic elements after iPS formation [73-76] or recombinant transduction-tagged proteins [77].

**Mode of cell delivery**

Successful cell therapy depends on engraftment and survival of the transplanted cell. Furthermore, systemic administration of stem cells such as intravenous administration relies on nonorgan-specific cell delivery and may increase the risk of adverse systemic side effects. The homing and engraftment of stem or progenitor cells after administration relies on the method of cell delivery, the characteristics of transplanted cells and the host environment [34]. One of the practical approaches to select the mode of delivery is based on the physiologic or disease status of the host myocardium (Figure 3).

In the setting of acute MI, the upregulation of local homing signals, such as stem cell factor, stromal cell-derived factor-1 and VEGF, enhances extravasation, migration and retention of transplanted cells in the infarcted myocardium [78]. Therefore, stem cells can be delivered by intravenous or intracoronary
Table 2. Summary of previous studies of embryonic stem cell transplantation into the myocardium.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Cell type</th>
<th>Animal model</th>
<th>Time to injection</th>
<th>Follow-up</th>
<th>Findings</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klug et al. (1996)</td>
<td>mESC-D3-derived CMs</td>
<td>Adult dystrophic mice with uninjured myocardium</td>
<td>NA</td>
<td>7 weeks</td>
<td>Grafted cells formed intracardiac graft in the mouse myocardium</td>
<td>[103]</td>
</tr>
<tr>
<td>Min et al. (2002)</td>
<td>mESC-D3-derived CMs</td>
<td>Rat myocardial infarct model with left anterior descending artery ligation</td>
<td>30 min</td>
<td>6 weeks</td>
<td>Improved left ventricular systolic function; grafted cells transformed into CMs</td>
<td>[104]</td>
</tr>
<tr>
<td>Behfar et al. (2002)</td>
<td>mESC-CGR8 (undifferentiated)</td>
<td>Rat myocardial infarct model with left coronary artery ligation</td>
<td>4 weeks</td>
<td>5 weeks</td>
<td>Grafted cells transformed into CMs; improved left ventricular systolic function</td>
<td>[105]</td>
</tr>
<tr>
<td>Yang et al. (2002)</td>
<td>mESC-D3-derived CMs</td>
<td>Mouse myocardial infarct model with left coronary artery ligation</td>
<td>15 weeks</td>
<td>6 weeks</td>
<td>Reduced infarct size and reduced postinfarct remodeling; better improvement in VEGF with substantial angiogenesis</td>
<td>[106]</td>
</tr>
<tr>
<td>Min et al. (2003)</td>
<td>mESC-D3-derived CMs</td>
<td>Rat myocardial infarct model with left anterior descending artery ligation</td>
<td>20 min</td>
<td>32 weeks</td>
<td>Improved survival rate; reduced infarct size; improved left ventricular systolic function; grafted cells transformed into CMs; increased capillary densities</td>
<td>[107]</td>
</tr>
<tr>
<td>Hodgson et al. (2004)</td>
<td>mESC-CGR8-derived CMs</td>
<td>Rat myocardial infarct model with left anterior descending artery ligation</td>
<td>8 weeks</td>
<td>12 weeks</td>
<td>Improved left ventricular ejection fraction and reduced postinfarct remodeling; grafted cells transformed into CMs</td>
<td>[108]</td>
</tr>
<tr>
<td>Kehat et al. (2004)</td>
<td>hESC-H9.2-derived CMs</td>
<td>Swine complete atrioventricular block model</td>
<td>Same session</td>
<td>1–3 weeks</td>
<td>Pacemaker activity from the grafted cells</td>
<td>[109]</td>
</tr>
<tr>
<td>Hime et al. (2004)</td>
<td>mESC (undifferentiated)</td>
<td>Mouse myocardial infarct model</td>
<td>Same session</td>
<td>12 h–36 days</td>
<td>Tracking with MRI</td>
<td>[110]</td>
</tr>
<tr>
<td>Kofidis et al. (2004)</td>
<td>mESC (undifferentiated)</td>
<td>Rat heterotopically transplanted heart with/without myocardial infarct</td>
<td>NA</td>
<td>2 weeks</td>
<td>Improved left ventricular fractional shortening and reduced postinfarct remodeling</td>
<td>[111]</td>
</tr>
<tr>
<td>Kofidis et al. (2004)</td>
<td>mESC (undifferentiated)</td>
<td>Mouse myocardial infarct model with left anterior descending artery ligation</td>
<td>Same session</td>
<td>2 weeks</td>
<td>Improved left ventricular fractional shortening and reduced postinfarct remodeling</td>
<td>[112]</td>
</tr>
<tr>
<td>Naito et al. (2004)</td>
<td>mESC-derived CMs</td>
<td>Rats with uninjured myocardium and cyclosporine</td>
<td>NA</td>
<td>1 week</td>
<td>Grafted cell survival</td>
<td>[113]</td>
</tr>
<tr>
<td>Laflamme et al. (2005)</td>
<td>hESC-H1- and H7-derived CMs</td>
<td>Athymic rats with uninjured myocardium</td>
<td>NA</td>
<td>4 weeks</td>
<td>Grafted cells formed stable expanding graft in the rat myocardium; graft fiber stands aligned in parallel with host myocardium</td>
<td>[114]</td>
</tr>
<tr>
<td>Ke et al. (2005)</td>
<td>mESC-D3 (undifferentiated)</td>
<td>Mouse myocardial infarct model with left coronary artery ligation</td>
<td>15 min</td>
<td>8 weeks</td>
<td>Grafted cells were harbored into host myocardium; increase in wall thickness and reduced infarct size in the patch-transplanted group</td>
<td>[115]</td>
</tr>
<tr>
<td>Menard et al. (2005)</td>
<td>mESC-CGR8-derived CMs</td>
<td>Sheep myocardial infarct model with left circumflex artery embolization</td>
<td>2 weeks</td>
<td>1 month</td>
<td>Grafted cells survived and differentiated into mature CMs in postinfarct myocardium in both immunosuppressed and immunocompetent sheep; improved left ventricular ejection fraction</td>
<td>[116]</td>
</tr>
<tr>
<td>Swijnenburg et al. (2005)</td>
<td>mESC-D3 (undifferentiated)</td>
<td>Mouse myocardial infarct model with coronary artery ligation</td>
<td>5 min</td>
<td>1–8 weeks</td>
<td>Teratoma formation at 2 weeks; vigorous inflammatory infiltrate at 4 and 8 weeks</td>
<td>[117]</td>
</tr>
<tr>
<td>Xue et al. (2005)</td>
<td>hESC-H1</td>
<td>Uninjured guinea pig myocardium</td>
<td>NA</td>
<td>NA</td>
<td>Electrical coupling between the transplanted hESC-derived pacemaker and the myocardium</td>
<td>[118]</td>
</tr>
<tr>
<td>Singla et al. (2006)</td>
<td>mESC-R1 and HM1 (undifferentiated)</td>
<td>Mouse myocardial infarct model with coronary artery ligation</td>
<td>3–5 h post-MI</td>
<td>2 weeks</td>
<td>Grafted cells survived in postinfarct myocardium, but no noninjured hearts; grafted cells transformed into CMs, vascular smooth muscle and endothelial cells demonstrated by colocalization immunolabeling; improved cardiac function and reduced postinfarct remodeling</td>
<td>[119]</td>
</tr>
</tbody>
</table>

CM: Cardiomyocyte; hESC: Human embryonic stem cell; mESC: Mouse embryonic stem cell; MI: Myocardial infarction; NA: Not applicable.
routes after coronary revascularization. The intravenous infusion is the simplest approach, but has a low efficacy for cellular engraftment (<1%) due to cell trapping in the other organs. Selective intracoronary cell injection via an over-the-wire infusion balloon catheter enables the maximum dose of cells to be delivered to the infarcted region. However, neither intravenous nor intracoronary routes are suitable for patients with an occluded artery or for delivery of stem cells of a larger size or with a limited migration ability, such as skeletal myoblasts, owing to the risk of microembolization.

For patients with chronic myocardial ischemia, the homing signals are expressed at low levels such that intravenous or intracoronary delivery will provide limited cell engraftment. In this setting, direct intramyocardial injection via either surgical epicardial or transcatheter endocardial approaches is needed for optimal cell delivery. Epicardial injection allows direct visualization of the targeted regions but is only applicable to patients requiring coronary artery bypass grafting. By contrast, catheter-based intramyocardial injection can be performed as a standalone procedure, but requires the use of specially designed injection catheters, with or without the use of 3D electromechanical mapping systems to guide the injection into the targeted regions. These techniques allow direct cell delivery into the targeted regions, even in patients with occluded arteries and when using certain larger cell types.

Finally, in patients with post-MI heart failure, owing to the large area of infarcted and nonviable myocardium, direct injection of stem cells into the myocardium scar will result in low graft survival and differentiation. This is caused by the lack of blood supply as well as paracrine supports from adjacent host CMs. Furthermore, transplanting viable cells into the scar may also increase the risk of pro-arrhythmias [62]. Therefore, the use of bioengineering approaches, such as cardiac patches and injectable delivery matrices, may be needed to improve cell retention, survival and differentiation [79]. However, the safety and efficacy of this approach has not been tested in clinical trials owing to the lack of optimal techniques to create viable 3D cellular patches.

**Clinical experiences**

The clinical use of stem cell therapy for treatment of cardiac diseases is still in its infancy. To date, only pilot cohort studies or small scale Phase I–II randomized trials have been reported for the treatment of acute MI and chronic myocardial ischemia with congestive heart failure and/or refractory angina. In this article, only data from randomized controlled trials or meta-analyses will be discussed.

**Acute MI**

In patients with acute MI, the majority of randomized controlled clinical trials have focused on the use of intracoronary administration
of autologous BM cells in patients who had undergone successful percutaneous coronary intervention at the infarct region. These studies yielded mixed results regarding the effect of intracoronary administration of BM cells owing to the relatively small patient sample size (<200), differences in the study population, the dosage, preparation and types of cells, timing of cell transfer, and the methodology of functional assessment. Nevertheless, none of these studies have shown major adverse effects after intracoronary BM cell delivery [60–68].

As a result, several meta-analyses, including randomized studies only, or both randomized and cohort studies, have been performed to determine the potential therapeutic benefits of intracoronary BM cell therapy during acute MI [80–82]. All these analyses demonstrated a modest (~3–4%) but significant improvement in LV ejection fraction, and a small reduction in infarct scar size and LV dimension after intracoronary stem cell therapy as compared with controls. This improvement in LV ejection fraction was similar to those (~2.5%) observed in the Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial, which is the largest randomized, controlled clinical study reported [83]. Interestingly, the benefit of stem cell therapy appears to be greater in those patients with more severe LV dysfunction after acute MI [83,84]. Indeed, the magnitude of benefits on LV function observed in stem cell therapy is comparable to those of other established therapies for acute MI, such as reperfusion therapies, angiotensin-convertase enzyme inhibitors, angiotensin receptor blockers and β-blockers [85]. Several larger ongoing studies, such as Swiss Multicenter Intracoronary Stem Cells Study in Acute Myocardial Infarction (SWISS-AMI) and the Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration (BOOST)-2 trials, will confirm the results of these meta-analyses, and should provide more information on the optimal timing and dosage of cell administration. Finally, the observed beneficial effects of stem cell therapy on surrogate markers, such as LV ejection fraction and dimension, will also need to be validated in subsequent clinical trials using clinical outcomes as study end points.

Chronic myocardial ischemia
In contrast to acute MI, there are only a few small randomized controlled clinical trials on stem cell therapy for the treatment of refractory angina caused by chronic myocardial ischemia. These trials mainly focused on the use of intramyocardial injections of BM cells into chronic ischemic myocardium that are not amenable to conventional coronary revascularization [76–78]. In these studies, cell transplantation was performed by 3D electro-mechanical mapping-guided catheter-based intramyocardial injection into the ischemic myocardium, as described previously [79,80]. Losordo et al. studied the use of three different doses of granulocyte colony-stimulating factor mobilizing CD34+ cells in patients with chronic myocardial ischemia [86]. Overall, they demonstrated a reduction in angina frequency and an increased exercise capacity without any change in SPECT perfusion [76]. The other two trials on the use of BM mononuclear cells showed significant but modest improvement in exercise capacity and LV ejection fraction [77,78]. Furthermore, a significant improvement in SPECT perfusion was also observed in the study by van Ramshorst et al. [87]. The reasons for the conflicting results on SPECT perfusion observed in these trials remain unclear, but are likely to be caused by sampling variation, dosage of cells used and the method of assessment of SPECT perfusion. Furthermore, no significant adverse events, such as cardiac arrhythmias, were observed after direct intramyocardial injection of BM cells [76–80]. Interestingly, assessment of cardiac function by cardiac MRI in two of the larger trials, which included patients with relatively normal LV ejection fraction, consistently showed a similar degree of improvement (3–5%) after BM cell transplantation [77,78]. The effects of BM cell transplantation in patients with ischemic LV dysfunction need to be addressed in future clinical trials.

Congestive heart failure
There are only two randomized controlled clinical trials on the use of autologous BM or skeletal myoblast cells in patients with congestive heart failure after MI. In the Transplantation of Progenitor Cells in Patients With Persistent Left Ventricular Function After Myocardial Infarction (TOPCARE-CHD) study, patients who had chronic MI for more than 3 months were randomized to either receive an intracoronary transplantation of BM cells or circulating progenitor cells from peripheral blood [81]. Patients who received intracoronary BM cell transplantation but not circulating progenitor cells showed a modest but significant improvement in LV ejection fraction (2.9%) at
Review

Early pilot studies highlighted safety concerns regarding proarrhythmia in coronary artery bypass grafting in ischemic cardiomyopathy and indication for transplantation versus placebo in patients with ischemic cardiomyopathy. Therefore, the use of skeletal myoblasts has been investigated in patients with ischemic cardiomyopathy and indication for coronary artery bypass grafting [88]. Owing to the safety concern regarding proarrhythmia in the early pilot studies [89], all patients were treated with implantable cardioverter-defibrillators before transplantation. At 6 months, there were no significant differences in regional or global LV function as determined by echocardiogram and arrhythmia events among these three groups. However, patients receiving the highest dose of cells had a significant decrease in LV end-diastolic and end-systolic volume compared with controls, suggesting the possibility of reverse remodeling [82].

**Potential hurdles & future perspective**

Although stem cell therapies appear to be a promising therapeutic approach in patients with severe CAD, there are several major issues that need to be resolved. First, current evidence supports the notion that stem cell-mediated paracrine effects, including enhancement of neovascularization and extracellular matrix remodeling, prevention of apoptosis and recruitment of endogenous cardiac stem cells by multiple cytokines that could enhance homing signal from the injury site [90], rather than direct replacement of damaged myocardium, are the major mechanisms of action of stem cell therapies, especially with the use of adult stem cells. Nevertheless, the optimal cell types will also depend on the clinical setting. In patients with acute MI or chronic myocardial ischemia, the primary objectives are to salvage the ischemic myocardium by improving myocardial perfusion; and to preserve cardiac function by limiting LV remodeling. Therefore, the paracrine effects mediated by BM- or MSC-derived cells may be sufficient to achieve the therapeutic target. In addition, some authors suggested that MSC-derived cells may have immunomodulatory effects, which may subsequently modulate the inflammatory response to acute MI, thereby limiting the amount of initial tissue damage [91]. Recent studies have failed to demonstrate any differences in clinical efficacy of direct intracoronary administration of unselected mononuclear BM cells versus CD34+/CXCR4+ BM cells [84]. However, a similar improvement in LV ejection fraction was achieved with selected CD34+/CXCR4+ BM cells despite the fact that the total number of cells injected was approximately 100-times lower than unselected BM cells. Whether the use of a selected BM cell population at a higher dosage may further improve the efficacy of the stem cell therapy remains unclear.

Second, the clinical application of autologous BM-derived cells, including MSCs, is still limited by the depletion and/or functional impairment of those stem cells associated with aging, diabetes [92] and severe CAD [93]. Furthermore, the process of cell preparation and selection can also significantly impact on the clinical efficacy [94]. The potential solutions for these hurdles include the use of standardized allogenic cell products or extracts from healthy donors with limited immunogenicity, such as MSCs derived from different tissues or conditional medium with cytokines secreted from different cell sources during culturing [95]. In addition, genetic manipulation of autologous cells to enhance their functions, such as overexpression of endothelial nitric oxide synthase, may also overcome hereditary defects of patients’ stem cells [96].

Third, structural regeneration of damaged myocardium is required for post-MI heart failure caused by massive loss of CMs. In this situation, transplantation of stem cells that can differentiate into functional CMs and integrate with host CMs is essential to achieve the therapeutic target. Unfortunately, the majority of adult stem cells rarely transdifferentiate into functional CMs and fail to integrate fully with host CMs. Among different cell types, resident cardiac stem cells, ESCs and iPS cells have better potential than other adult stem cells for proliferation and differentiation into functional CMs. Nevertheless, these cell types also have their own limitation as discussed previously. Most importantly, the generation of terminally differentiated and homogeneous functional CMs from these different types of stem cells may be needed to avoid the issues of teratoma formation and proarrhythmias.
Finally, the rate of sustained cell engraftment and integration after transplantation remains low. In preclinical studies, less than 10% of injected cells remain in the host myocardium after the first day and less than 1% of transplanted cells can still be detected after 4 weeks [97]. Indeed, a histological study also confirmed that the majority of stem cells did not engraft or survive after transplantation [13].

As a result, enhancement of cell engraftment, as well as survival, is mandatory for optimizing the therapeutic benefits of stem cell therapy for post-MI heart failure. The low rate of cell engraftment is caused by the loss of transplanted cells during the cell delivery procedure and mechanical contraction. The potential solutions to improve cell engraftment included tissue engineering of transplanted cells to enhance cell homing, such as overexpression of stromal cell-derived factor-1 [98], or nitric oxide synthase enhancers [99], and to improve cell delivery using cardiac patches and injectable delivery matrices [79]. On the other hand, multiple mechanisms, including ischemia, inflammatory reactions, apoptosis and loss of cell–cell interactions, are likely to contribute to the poor survival of transplanted cells. As a result, different therapeutic strategies to enhance angiogenesis via co-administration of EPCs or growth factors [92], and to prevent apoptosis by cellular preconditioning, such as hypoxia [93] or coexpression of Akt [100], have been investigated to improve cell survival. Furthermore, embedding cells into biomaterial as cardiac patches or injectable matrices can also improve cell survival by promoting cell-to-cell contact and act as barriers to reduce inflammatory reactions.

**Conclusion**

The lack of optimal therapeutic options for the treatment of organ failure has motivated the pursuit for an alternative biological therapeutic approach. In the last decade, preclinical experimental data, as well as pilot human clinical trials, suggest that damaged myocardium can be repaired by cell-based therapies using different types of stem cells. Despite much heterogeneity in clinical trials, including cell types, dosage, study design, mode of delivery and methodology, it has been demonstrated that there is a modest beneficial effect on the LV ejection fraction with the use of BM-derived cellular therapy in patients with acute MI or chronic myocardial ischemia, which goes beyond the magnitude of conventional therapy with a reasonable safety margin. Future challenges in the field include optimizing the cell type, dosage, modes of delivery and the choice of relevant functional assessment.

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**Executive summary**

**Background**

- Clinical applications of stem cell therapies have been investigated as a novel therapy for the treatment of acute myocardial infarction and chronic myocardial ischemia.

**Mechanisms of action**

- Current evidence suggests that cell-mediated paracrine effects, rather than direct cardiac transdifferentiation, after different types of stem cell transplantation, are more likely to contribute to the observed beneficial effects of stem cell therapies.

**Cell types**

- Among the different types of stem cells being investigated, bone marrow-derived cells are the most commonly tested in clinical trials.

**Clinical experiences**

- Phase I/II randomized controlled clinical trials suggest that intracoronary or intramyocardial injection of bone marrow-derived cells may be a safe and feasible strategy for the treatment of acute myocardial infarction as well as chronic myocardial ischemia. Initial results show that these cell-based therapies are associated with a modest but significant improvement in left ventricular ejection fraction and the clinical status of patients after cell transplantation. The clinical efficacy of stem cell therapies needs to be confirmed by future clinical trials.

**Future perspective**

- Future investigations should address the optimal timing, cell types and modes of delivery, and develop strategies to improve cell survival and engraftment in order to overcome the potential hurdles related to the current applications of cell-based therapies.

- The possible reason for improved cardiac function and survival of engrafted stem cells by paracrine mechanisms should not be ignored either. Identification of these cell-derived paracrine factors could lead to effective therapies without delivery of the cells themselves. Local intramyocardial delivery of cytokines or growth factors could be more reproducible with defined composition.
Current perspective of stem cell therapies for cardiac regeneration

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