Stem cells for myocardial repair

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Summary
There is a growing interest in the clinical application for stem cell as a novel therapy for treatment of acute myocardial infarction and chronic myocardial ischaemia. The initial premise is the transplanted exogenous stem cells can engraft and integrate with host myocardium for cardiac regeneration. However, recent experimental studies suggest that multiple mechanisms, including remodelling of extracellular matrix, enhancement of neovascularisation and recruitment of endogenous stem cells are more likely to contribute to the beneficial effects of stem cell therapy that direct trans-differentiation of stem cells into functional myocardium. Among different potential cell sources, bone marrow-derived cells and skeletal myoblasts have been tested in pilot clinical studies. Phase VII randomised controlled clinical trials suggest that intracoronary or intramyocardial injection of bone marrow-derived cells may be safe and feasible strategies for treatment of acute myocardial infarction as well as chronic myocardial ischaemia. In addition, these studies show a modest, but significant improvement in left ventricular ejection fraction and clinical status of patients after cell transplantation. Nevertheless, most of these studies included a relatively small sample size (<200) and short duration of follow-up (<6 months), and the clinical efficacy of stem cell therapy need to be confirmed by future clinical trials. Furthermore, the optimal timing, cell types and mode of delivery need to be addressed, and strategies to improve cell survival and engraftment should also be developed to overcome the potential hurdles related to cell-based therapy.

Keywords
Stem cells, cardiology, ischaemic heart disease

Introduction
Coronary artery disease (CAD) remains the major cause of morbidity and mortality worldwide (1). Despite recent advances in reperfusion therapy for acute myocardial infarction (MI) and pharmacotherapy for post-MI left ventricular (LV) remodelling, the incidence and mortality of post-MI heart failure are increasing (2). There is still major unmet need for treatment of post-MI heart failure due to progressive LV remodelling after initial insult to the myocardium. In patients with end-stage heart failure, the only available curative therapy is heart transplant which is limited by shortage of organs. As a result, there is tremendous interest in developing novel therapies for post-MI LV remodelling and dysfunction. In the last few years, stem cell therapies have been investigated as potential treatment for patients with acute MI and chronic myocardial ischaemia.

Mechanisms of action
The initial premise of cell-based therapy relies on the belief that exogenous cells or mobilised endogenous cells can transdifferentiate into mature cardiomyocytes (CMs) and integrates both electrically and mechanically with host CMs to improvement of cardiac function (3). Clinical evidence demonstrated that new endogenous or exogenous cells can incorporate into host myocardium via trans-differentiation and/or cell fusion (4). However, emerging data have demonstrated that cellular mediated paracrine effects are likely the major mechanism for the improvement in LV function. Our recent experimental studies (5) and others (6) have shown that the transplanted cells can secrete pro-angiogenic cytokines to enhance neovascularisation. The paracrine factors secreted from the transplanted cells exert anti-apoptotic effects, alter the restoration of extracellular matrix and recruit endogenous stem cells (7). Furthermore, there is very limited direct trans-differentiation and long-term survival of transplanted cells (8, 9). Indeed, direct injection of cellular extract can provide a similar beneficial effects on cardiac function as intact cell therapy (10).

Different types of cell sources
Different types of stem or progenitor cells have been investigated for cardiac repair including skeletal myoblast, bone marrow (BM)-
Skeletal myoblasts

Skeletal myoblasts are skeletal muscle precursor cells which can be isolated from skeletal muscle biopsies and expanded *ex vivo* to the quantities sufficient for autologous transplantation (11). Experimental studies have demonstrated that transplanted skeletal myoblasts engraft and differentiate into myotubes after transplantation, and improve cardiac function in animal model of MI (12). However, the major concern of skeletal myoblast is the lack of connexin-43 expression after *in vitro* differentiation and results in the failure of electrical integration with the host myocardium to cause proarrhythmias (13). Indeed, ventricular tachyarrhythmias were observed after skeletal myoblast transplantation in the early clinical trials and remains a potential safety issue (11).

Bone marrow (BM)-derived cells

BM-derived cells are heterogeneous cellular population consist of ~98% differentiated cells (haematopoietic cells, vascular cells, adipocytes, osteoblasts and osteoclasts) and ~2% stem or progenitor cells (haematopoietic stem cells, endothelial progenitor cells [EPC] and mesenchymal stem cells [MSC]). BM-derived stem or progenitor cells have been shown to be able to trans-differentiate into various cell types of the body including neurons, hepatocytes, skeletal muscles and CMs. Similar to skeletal myoblast, BM cells can be readily harvested and expanded *ex vivo* for autologous transplantation without any immune rejection. Experimental studies suggested that transplantation of BM-derived cells into the acute or chronic ischaemic myocardium resulted in *in vivo* myogenesis and neo-angiogenesis, and improvement in cardiac function (5, 16). As discussed above, the paracrine effects of BM-derived EPC and MSC have been postulated as the major mechanism for the improvement of cardiac function after transplantation (6, 7). The encouraging results from pre-clinical studies with different types of BM-derived cells (including mononuclear cells, MSC and EPC) resulted in a series of pilot clinical trials investigating the potential therapeutic use of BM-derived cells in CAD patients. Currently, there is very limited data on the direct comparison of the safety and efficacy between these different types of BM-derived cell for therapeutic use.

Embryonic stem cells (ESC)

As compared with adult stem cells, ESC can be cultured indefinitely (self-renew) in an undifferentiated state without karyotypic alteration. Furthermore, functional CMs with cardiac-specific structural and functional properties can be consistently differentiated from ESCs with various methods (23). Therefore, ESCs have been considered as potential unlimited *ex vivo* cell source for cardiac repair. Experimental studies have demonstrated that transplantation of undifferentiated ESCs or their cardiac derivatives into infarcted myocardium can consistently improved LV function post-MI (24). Nevertheless, the current efficiency of *in vitro* cardiac differentiation from ESC remains low (typically less than 1%), and a heterogeneous population of CMs (pacemakers, atrial, and ventricular cells) with immature phenotypes are obtained. Together with the potential risk of immune rejection and tumour formation, these issues limited the clinical application of ESC for cardiac regeneration (25).

Induced pluripotent stem cells (iPSC)

Recent breakthrough in the generation of human iPSC has attached much attention due to its potential implication on tissue re-
generation (26). These iPSCs generated from the reprogramming process using a combination of transcription factors (Oct3/4, Sox2, Klf4 and c-Myc), exhibits essential features of ESCs including morphology, surface markers, gene expression profiles and telomerase activity. Experimentally, functional CMs could be differentiated from iPSCs and showed typical features resembling of those from ESC-derived CMs (27). One of the potential advantages of iPSCs over ESC is patient-specific stem cells can be obtained for autologous transplantation to avoid the ethical and immunological issues related to ESC. However, iPSCs still share some of the issues associated with ESC, including the low yield as well as the immature and heterogenous phenotypes with cardiac differentiation. Furthermore, the use of viral vectors to create iPSCs may lead to uncontrolled cellular proliferation, oncogenesis or abnormal development (28).

Mode of cell delivery

Optimal stem cell therapy depends on successful delivery, engraftment and survival of the transplanted cell. 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 (29). In addition to direct cell delivery, mobilisation of endogenous stem cell can be achieved by administration of cytokines or growth factors. Table 1 summarises the potential application as well as advantage and disadvantage of different methods of cell delivery.

In general, stem cells can be delivered by intravenous or intracoronary routes after coronary revascularisation in the setting of acute MI. The intravenous infusion is easy available, but have a low efficacy for cellular engraftment (<1%) due to the cell trapping in the other organs. Selective intracoronary cell injection using the over-the-wire infusion balloon catheter allows direct delivery of cell to the infarcted region. However, both intravenous and intracoronary routes are not applicable for patients with an occluded artery and not suitable for delivery of stem cells of larger size or limited migration ability, such as skeletal myoblasts due to risk of microembolisation.

For patients with chronic myocardial ischaemia not amenable to coronary revascularisation, direct intramyocardial injection via either surgical epicardial or transcatheter endocardial approaches due to low level of expression of homing signal for stem cells. Epicardial injection allows direct visualisation of the targeted regions but is only applicable to patients require coronary artery bypass grafting. On the other hand, catheter-based endocardial injection can be performed as stand-alone procedure, but requires the use of special designed injection catheter with or without the use of three dimensional 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 artery and with the use of certain larger cell types.

In patients with post-MI heart failure due to large area of infarcted and non-viable myocardium, direct injection of stem cells

<table>
<thead>
<tr>
<th>Strategies</th>
<th>Potential clinical applications</th>
<th>Clinical experience</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>Acute MI</td>
<td>+/-</td>
<td>Easy available and avoid the risk of any invasive procedure</td>
<td>Low efficacy of cell delivery</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No applicable to patients with an occluded artery</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Risk of systemic administration unclear</td>
</tr>
<tr>
<td>Intracoronary</td>
<td>Acute MI</td>
<td>++++</td>
<td>Possible wide use in catheterisation laboratory</td>
<td>Efficacy of cell delivery to the myocardium</td>
</tr>
<tr>
<td></td>
<td>Chronic MI</td>
<td>+</td>
<td>Limited risk of systemic administration</td>
<td>uncertain</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clinical trials ongoing</td>
<td>No applicable to patients with a occluded artery</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No applicable for stem cell with large cells</td>
</tr>
<tr>
<td>Catheter-based direct intramyocardial injection</td>
<td>Chronic myocardial ischaemia Acute MI</td>
<td>++++ +</td>
<td>Avoid the risk of open heart surgery</td>
<td>Need for specialised catheters and imaging technology to guide the procedure</td>
</tr>
<tr>
<td>Open heart direct epicardial injection</td>
<td>Chronic myocardial ischaemia Chronic MI</td>
<td>++ ++</td>
<td>Applicable to patients who need open heart surgery</td>
<td>Risk of mortality and morbidity of open heart surgery</td>
</tr>
<tr>
<td>Direct epicardial patch</td>
<td>Chronic MI</td>
<td>-</td>
<td>Applicable to patients who need open heart surgery</td>
<td>Need tissue engineering to create cellular patch</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Avoid uneven distribution of cell in the myocardium</td>
<td>Risk of mortality and morbidity of open heart surgery</td>
</tr>
</tbody>
</table>

Table 1: Advantages and disadvantage of potential clinical strategies for stem cell delivery.
into myocardium scar will result in low graft survival and differ-
entiation due to the lack of blood supply as well as paracrine sup-
ports from adjacent host CMs. Therefore, the use of bioengineer-
ing approaches, such as cardiac patches and injectable delivery ma-
tices are needed to improve cell retention, survival and differenti-
ation (30).

Clinical trials

Acute myocardial infarction (MI)

In patients with acute MI, several major randomised controlled clinical trials have been reported with the use of intracoronary ad-
ministration of autologous BM cells in patients who had under-
gone successful percutaneous coronary intervention of infarcted
related artery. As shown in Table 2, these studies yielded mixed
results on the effect of intracoronary administration of BM cells
(31–39). The conflicting results of these randomised controlled
trials may be due to the small patients sample size (<200), their dif-
fences in the study population, the dosage, preparation and types
of cells, timing of cell transfer, and the methodology of functional
assessment. Nevertheless, no major adverse effect was observed in
all these clinical trials. Several meta-analyses have also been re-
ported on the safety and efficacy of intracoronary BM cell therapy
during acute MI (40–43). Consistently, all these analysis demon-
strated a modest (~3–4%) but significant improvement in LV eje-
tion fraction and a small reduction in infarct scar size and LV di-
mension after intracoronary stem cell therapy as compared with
controls. This improvement in LV ejection fraction was similar to
those (~2.5%) observed in the REPAIR-AMI (Remodelling in
Acute Myocardial Infarction) trial which is the largest randomised,
controlled clinical study reported (36). Indeed, these magnitude of
benefits on LV function observed in stem cell therapy are compat-
able to those other established therapies for acute MI, such as reper-
fusion therapies, angiotensin-converting enzyme inhibitors, an-
giotensin receptor blockers and beta-blockers (44). The clinical ef-
cacy of intracoronary BM cells therapy as well as the optimal tim-
ing and dosage of cell administration will be addressed in several
larger ongoing trials, such as SWISS-AMI and BOOST-2 trials.

Chronic myocardial ischaemia/ infarction

In contrast to acute MI, there are only a few small randomised con-
trolled clinical trials on stem cell therapy for treatment of refrac-

Table 2: Randomised controlled trials in acute myocardial infarction.

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of patients</th>
<th>Cell types &amp; numbers (x10⁶)</th>
<th>Time to therapy (days)</th>
<th>Follow-up duration (months)</th>
<th>Primary endpoint</th>
<th>LVEF (%)</th>
<th>Infarct size</th>
<th>Side effects</th>
</tr>
</thead>
</table>
| BOOST (31, 32) | 30 vs. 30 controls | 2460 BMC with gelatine-polysucci-
-nate density gradient (9.5 CD34⁺) | 4.8 | 18 | LVEF (MRI), safety | 6 months: +6.7% vs. +0.7% (↑ 6%) | Nil |
| Jannsens et al. (33) | 33 vs. 34 controls | 304 BMC with Ficoll (2.8 CD34⁺) | 1 | 4 | LVEF (MRI) | +3.4% vs. +2.2% (NS) | Nil |
| ASTAMI (34) | 50 vs. 50 controls | 68 BMC with Lymphoprep Ficoll (0.7 CD34⁺) | 6 | 6 | LVEF, infarct size (MRI/ SPECT/Echo) | +1.2% vs. +4.3% (NS) | Nil |
| MAGIC CELL-3-DES (35) | 25 vs. 25 controls | 1500 G-CSF mobiliz-
ed PBC (7x10⁶ CD34⁺) | 4 | 6 | LVEF (MRI) | +5.5% vs. 0% (↑ 5.5%) | Nil |
| REPAIR-AMI (36) | 95 vs. 92 controls | 236 BMC with Ficoll (3.6 CD34⁺) | 4.4 | 12 | LVEF (LV angiography) | 5.5% vs. +3.0% (↑ 2.5%) | Nil |
| Meluzin et al. (37) | 44 (high & low) vs. 22 controls | High: 100 BMC Low:10 BMC | 7 | 3 | LVEF (SPECT) | High: +5%; Low: +3%; Control: +2% (↑ 2.0%) | Nil |
| REGENT (38) | 117 (selected & unselected) vs. 20 controls | Unselected: 178 BMC Selected: 1.9 CD34/CXCR4⁺ | 7 | 6 | LVEF (MRI) | Unselected: +3% Selected: +3% Control: 0% (NS) | Nil |
| FINCELL (39) | 40 vs. 40 controls | 402 BMC | 2–6 | 12 | LVEF (ECHOC) | +7.1% vs. +1.2% | Nil |

BMC, bone marrow cells; G-CSF, granulocyte colony stimulating factor; PBC, peripheral blood cells; LVEF, left ventricular ejection fraction; MRI, magnetic resonance imaging; NA, not available; NS, non-significant.
Stem cells in cardiovascular biology and medicine

For treatment of refractory angina, clinical trials are mainly focused on the use of intramyocardial injections of BM cells as guided by catheter-based three-dimensional electromechanical mapping (45) into chronic ischaemic myocardium which are not amenable to conventional coronary revascularisation (46–47). Table 3).

Losordo et al. (46) demonstrated intramyocardial injection of granulocyte colony-stimulating factor mobilising CD34+ cells improved angina frequency and exercise capacity without any change in SPECT perfusion. In contrast, our recent studies (PROTECT-CAD) (47) and van Ramshorst et al. (48) have shown that intramyocardial injection of BM mononuclear cells was associated with significant improvement in exercise capacity and clinical status (47, 48). As determined by cardiac magnetic resonance imaging, patients received BM cell therapy also showed a modest but significant improvement in LVEF (3–5%) (47, 48). Although no overall improvement in SPECT perfusion was observed in the study by Losordo et al. (46) and PROTECT-CAD trial (47), van Ramshorst et al. (48) demonstrated a significant improvement in SPECT perfusion after BM cell injection. The reasons for the conflicting results on SPECT remain unclear, but may be related to the differences in the dosage of cell, study sample size and method of SPECT analysis. More importantly, no significant adverse event, including cardiac arrhythmias was observed after direct intramyocardial injection of BM cells (46–48). Nevertheless, patients with significant

Table 3: Randomised controlled trials in chronic myocardial ischaemia.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Cell types &amp; numbers (x10⁶)</th>
<th>Follow-up duration (mths)</th>
<th>Primary endpoints</th>
<th>Secondary endpoints</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Losordo et al. (46)</td>
<td>18 vs. 6 controls</td>
<td>0.05–0.5 per kg G-CSF mobilised PBC</td>
<td>6</td>
<td>Anginal frequency: −12.6 vs. −4.5 (↓8.1, NS) Safety: NS</td>
<td>SPECT perfusion score: −1.5 vs. −2.2 (NS) Exercise time: +0.5 vs. +0.3 mins (NS)</td>
</tr>
<tr>
<td>PROTECT-CAD (47)</td>
<td>19 vs. 9 controls</td>
<td>42 BMC with Ficoll (1.38 CD34+)</td>
<td>6</td>
<td>Exercise time: ↑53%</td>
<td>LVEF (MR0): +3.7% vs. −0.4% (↑5.4%) SPECT perfusion score: −0.5 vs. +2.4 (↑2.7, NS)</td>
</tr>
<tr>
<td>Van Ramshorst et al. (48)</td>
<td>25 vs. 25 controls</td>
<td>98 BMC with Ficoll</td>
<td>6</td>
<td>SPECT perfusion score: −3.4 vs. −1.1 (↓2.44)</td>
<td>LVEF (MR0): +3% vs. −1% (↑3%) Exercise capacity: +9W vs. +2W (↑7W)</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.

Table 4: Randomised controlled trials in congestive heart failure.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Cell types &amp; numbers (x10⁶)</th>
<th>Follow-up duration (months)</th>
<th>Primary endpoint</th>
<th>LVEF (%)</th>
<th>LV dimension</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOPCARE-CHD (49)</td>
<td>24 (PBC), 24 (BMC) vs. 23 (controls)</td>
<td>Intracoronary injection: 22 PBC or 205 BMC with Ficoll</td>
<td>3</td>
<td>LVEF (LV angiogram) PBC: −0.4%; BMC: +2.9%; Control: −1.2%</td>
<td>LV EDV: PBC: −3%; BMC: 0%; Control: −3% (NS) LV ESV: PBC: −2%; BMC: −2%; Control: −1% (NS)</td>
<td>Nil</td>
</tr>
<tr>
<td>MAGIC (50)</td>
<td>63 (high and low dose) vs. 34 controls</td>
<td>Epicardial injection: 400 skeletal myoblast (low dose); 800 skeletal myoblast (high dose)</td>
<td>6</td>
<td>LVEF and changes in number of akinetic segments (Echo) High dose: +5.2% Low dose: +3.4% Control: + 4.4% (NS)</td>
<td>LV EDV (ml/m): High: −12.6 Low: −3.9 Control: +5.9 (↓12.8) LV ESV (ml/m): High: −8.3 Low: −6.5 Control: −2.1 (↓8.1)</td>
<td>Non-significant higher number of arrhythmic events in myoblast group</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1; LV, left ventricular; ESV, end-systolic volume; EDV, end-diastolic volume.
LV dysfunction were excluded in these trials, therefore, the effects of intramyocardial BM cell transplantation in patients with ischaemic LV dysfunction need to address in future clinical trials.

Table 4 summarises two randomised controlled clinical trials on the use autologous BM or skeletal myoblast in patients with congestive heart failure after MI. In TOPCARE-CHD study, patients with chronic MI (>3 months) were randomised to receive intracoronary transplantation of BM cells or circulating progenitor cells from peripheral blood (49). As compared with controls, patients received intracoronary BM cell transplantation but not circulating progenitor cells showed a modest but significant improvement in LVEF (2.9%) at three months, but no difference in LV dimension was observed. The use skeletal myoblast has been investigated in patients with ischaemic cardiomyopathy. The Myoblast Autologous Grafting in Ischaemic Cardiomyopathy (MAGIC) trial compared the safety and efficacy of low-dose (400 × 10⁶) or high-dose (800 × 10⁶) autologous skeletal myoblasts versus placebo in patients with ischaemic cardiomyopathy who underwent coronary artery bypass grafting (50). Due to the safety concern of proarrhythmia, all patients were treated with implantable cardioverter-defibrillators before transplantation. Although, there were no significant differences in regional or global LV function, and arrhythmia events between patients treated with skeletal myoblast versus controls, patients treated with higher dose of cells had a significant decrease in LV end-diastolic and end-systolic volume, suggesting the possibility of reverse LV remodelling after stem cell therapy (50).

Future perspectives

As discussed above, experimental data and pilot human clinical trials suggest that cell-based therapy is a safe and feasible option in patients with CAD. Despite much heterogeneity in different clinical trials, including cell types, dosage, study design, mode of delivery and methodology, there is a modest beneficial effect on the LVEF with the use of intracoronary or intramyocardial BM injection in patients with acute MI or chronic myocardial ischaemia, which beyond the magnitude of conventional therapy with a reasonable safety margin. In addition to define the optimal cell type, dosage and modes of delivery, further refinement of the therapeutic efficacy of cell-based therapy are needed.

First, the use of autologous BM- or MSC-derived cells is confounded by the depletion and/or functional impairment of those stem cells associated with aging, diabetes and severe CAD (51). Furthermore, the cell preparation and selection procedure can also affect the clinical efficacy of stem cell (52). The use of allogenic cell products with limited immunogenicity, such as MSC derived from different tissues or standardised non-cellular products such as conditioned medium or cell extract from different cell source (53) may overcome these problems. Furthermore, in vivo modification such as hypoxic preconditioning or over-expression of angiogenic growth factors before cellular transplantation may also enhance the efficacy of stem cell therapy (54, 55).

Second, transplantation of stem cells that can differentiate into functional CMs and integrate with host CMs is required for post-MI heart failure due to massive loss of CMs. Among different cell types, resident cardiac stem cell, ESC and iPSCs are potential candidates due to their better potential for proliferation and differentiation into functional CMs. Nevertheless, these cell types also have their own limitation as discussed above. Most importantly, generation of terminally differentiated and homogeneous functional CMs from these different types of stem cells are essential to avoid the issues of teratoma formation and proarrhythmias.

Third, in vitro cell imaging also confirmed the rate of sustained cell engraftment and integration after transplantation remains low (56). Therefore, strategies to enhance cell survival after transplantation is equally important to optimal the therapeutic benefits of stem cells therapy. The low rate of cell engraftment is attributed by the loss of transplanted cells during the cell delivery procedure and the mechanical contraction. Furthermore, multiple mechanisms including ischaemia, inflammatory reaction, apoptosis and loss of cell-cell interactions are likely contribute to the poor survival of transplanted cells. The potential solutions to improve cell engraftment and survival included engineering of transplanted cells to enhance cell homing and in vitro survival (54, 55, 57), and the use of cardiac patches and injectable delivery matrices (30).

References

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