

# Cell therapy for cardiac repair

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Heart failure is a leading cause of morbidity and mortality worldwide. The current strategies for treatment are limited and new therapeutic approaches are needed. This review describes research performed in animal models of cardiac disease and clinical trials and discusses the mechanisms involved in possible beneficial effects of cell therapy. Cell therapy is a promising strategy to treat heart failure, as it aims to replenish the failing myocardium with contractile elements. However, cell therapy with adult progenitor cells induces a small improvement in heart function without significant cardiomyogenesis. Paracrine mechanisms are likely to be important. The most effective cell type for therapy remains unclear. Induced pluripotent stem cells have the greatest potential but more information on the properties of this cell type is needed. The integration of cells in the host myocardium and the routes of delivery remain controversial. The differentiation of cardiac cells from pluri- and multipotent cells and the understanding of their properties are growing points in cell therapy. More research is needed to correctly assess the physiological properties of differentiating cells, to dissect the role of the host environment in the integration and differentiation and to define the stage of differentiation required for cell transplantation.

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## Introduction

The understanding that a common mechanism of development of heart failure is the loss of ventricular cardiomyocytes leads to the notion of supplementing those losses by delivering cells directly into the diseased ventricle as a mode of treatment. During the last 15 years, there have been numerous studies performed in the field of cardiac cell therapy involving a wide range of animal models and also in large clinical trials. Some of the reported data have stimulated much interest, and the field continues to be an active area of ongoing research; there has also been considerable controversy over some of the key points and some important questions remain unanswered. In this review, the major recent developments, particularly about the cell types tested in clinical trials, will be summarized, and the outstanding issues discussed.

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## Animal studies of cardiac cell therapy

The majority of the animal studies of cardiac cell therapy have employed the strategy of directly injecting cells labelled using genetic or fluorescent markers into normal or diseased hearts. Such a study model enabled the phenotypic fate of the transplanted cells to be followed while monitoring the function of the diseased recipient ventricle.

### *Foetal and neonatal cardiomyocytes*

Cardiomyocytes would constitute the ideal donor cell for transplantation into diseased hearts, as they already possess the necessary structural and physiological attributes to integrate with the recipient myocardium. Following the demonstration by Soonpaa *et al.*<sup>1</sup> that stable grafts could be formed following murine foetal cardiomyocyte transplantation into syngeneic hosts, researchers injected cardiomyocytes from foetal and neonatal sources into rat hearts which had previously undergone myocardial infarction.<sup>2</sup> Implanted myocytes formed stable grafts within the myocardium and improved ventricular performance. As expected for differentiated cardiomyocytes, the implanted cells retained their contractile phenotype and even expressed gap junction connexins necessary for intercellular electrical communication. Synchronous  $\text{Ca}^{2+}$  transients in the transplanted cells and recipient cells could be visualized directly, showing that delivery of committed cardiomyocytes can form nascent myocardium within the recipient heart with appropriate electrical integration.<sup>3</sup>

The studies utilizing committed cardiomyocytes are only considered proof-of-principle studies since there is no readily accessible source of foetal or neonatal cardiomyocytes for treating heart failure patients. The other major potential obstacle that needs to be overcome with these cell types is the likely immune rejection. For these reasons, the majority of animal studies of cardiac cell transplantation have utilized types of cells which are available from autologous sources, as discussed below.

### *Skeletal myoblasts*

Myoblasts derived from skeletal muscle satellite cells were among the first to be utilized in cardiac cell transplantation experiments.<sup>4</sup> The advantages of skeletal myoblasts include their availability from autologous sources (which bypasses the immunological and ethical considerations associated with some of the other cell types that become

prohibitive when applied to humans), ability to proliferate and be expanded *ex vivo* and superior resistance to ischaemia compared with cardiomyocytes. Skeletal myoblasts are also well-known to be committed to a contractile tissue phenotype.<sup>5</sup>

Initially, it was hoped that these cells might transdifferentiate into cardiomyocytes following injection, or at least be genetically manipulated into acquiring more of the features of cardiomyocytes. However, it has become apparent that skeletal myoblasts remain committed to forming mature skeletal myotubes in the heart. Furthermore, mature myotubes as well as skeletal myoblasts that remain unfused appear to remain mechanically and electrically isolated from the recipient myocardium.<sup>6</sup> Another important consideration is the limited survival of the skeletal myoblasts following injection. Labelling the injected skeletal myoblasts with <sup>14</sup>C-thymidine and then monitoring <sup>14</sup>C radioactivity revealed that only ~7.4% survived at 72 h.<sup>7</sup> Clearly, the small numbers of surviving cells cannot contribute a substantial contractile force to the myocardium. However, despite these disappointing findings from characterization of the injected cells, there appears to be a modest improvement in ventricular performance.<sup>4</sup> This important paradox remains unexplained. A variety of interpretations have been put forward, some of which are discussed later.

### Bone marrow-derived cells

In 2001, Orlic *et al.*<sup>8</sup> reported that substantial cardiac regeneration might be induced in mice that had undergone myocardial infarction by injecting the *Lin*<sup>-</sup>, *c-kit*<sup>+</sup> subset of bone marrow cells. Their suggestion that locally delivered bone marrow-derived cells could generate new myocardium through transdifferentiation sparked much interest among clinicians as well as scientists since methods for obtaining and sorting large numbers of autologous bone marrow cells were already well-established, and because there was a good degree of clinical familiarity of transplanting such cells into patients suffering from haematopoietic disorders.

Unfortunately, the results of Orlic *et al.* could not be reproduced by others.<sup>9,10</sup> Instead, the subsequent studies concluded that bone marrow-derived cells adopt mature haematopoietic fates. The studies identified only very small numbers of cardiomyocytes from the recipient myocardium that also expressed the genetic markers of cells that were injected. These cells, as well as occurring only with extremely low frequency, invariably contained genetic material from both injected and recipient cells, suggesting that they were the results of cell fusion rather than transdifferentiation. Further evidence that bone

marrow-derived cells fuse with recipient cardiomyocytes came from experiments utilizing *Cre/lox* recombination. Bone marrow cells harvested from mice expressing *Cre* recombinase in addition to eGFP were injected into the hearts of R26R reporter mice.<sup>11</sup> The R26R reporter cardiomyocytes each carried a copy of the *LacZ* reporter gene containing a loxP-flanked stop cassette. The karyotypes of cardiomyocytes expressing  $\beta$ -galactosidase were either tetraploid or hexaploid, indicating that they were the results of cell fusion. On the other hand, eGFP<sup>+</sup>/ $\beta$ -galactosidase<sup>-</sup> cells had appearances of small mononuclear cells.

A subset of bone marrow-derived cells, termed mesenchymal stem cells (MSCs), has attracted special interest. MSCs compose the stromal compartment of bone marrow and are not haematopoietic. In culture, they adhere to polystyrene surfaces and proliferate indefinitely. They express CD29 and CD90 and are negative for the surface markers found on other bone marrow-derived cells. MSCs have been shown to be able to differentiate into a variety of cell types *in vitro*, including adipocytes, chondrocytes,<sup>12</sup> osteoblasts and skeletal myoblasts.<sup>13</sup> In 1999, Makino *et al.*<sup>14</sup> demonstrated that exposure of MSCs to the DNA methylating agent 5-azacytidine can yield proliferating cells similar to those of foetal cardiomyocytes. Their described cells beat spontaneously, fused into tube-like structures that exhibited sarcomeres, stained positive for myocardial proteins and also had measurable action potentials.

Despite these special properties of MSCs, the results of their injection into hearts appear to be no different from that of other bone marrow-derived cells. The overall survival of bone marrow-derived cells after injection is low, and those few that do survive do not form mature cardiomyocytes which integrate with their native neighbours.<sup>15</sup> A number of research groups separately demonstrated cells which exhibited the markers of donor cells along with some of the proteins which are only expressed in cardiomyocytes,<sup>16</sup> but none specifically addressed the possibility of cell fusion. It is interesting, however, that all groups measured improvement in whole heart function.

### *Embryonic and induced pluripotent stem cells*

Embryonic stem (ES) cells are the ideal cell type for cardiac repair because of their pluripotency: they can form any cell in the heart with consequent extensive regenerative potential. There are, however, several problems with ES cells for cardiac repair, including the immunological incompatibility with the host myocardium and the tendency to form teratomas.<sup>17</sup> To date, few studies have used ES cells to treat heart disease in animal models. Using pro-survival factors, it was

shown that human ES cells transplanted in injured rat myocardium improved myocardial function.<sup>18</sup> However, despite the newly formed cardiomyocytes, the functional improvement was only temporary,<sup>19</sup> suggesting that other factors such as integration with existing myocardium remain important areas to address.

Takahashi and Yamanaka<sup>20</sup> first described a method to reprogramme fibroblasts to a pluripotent, embryonic-like, state. Several studies have shown that manipulating the expression of transcription factors transforms somatic cells into induced pluripotent stem (iPS) cells which are indistinguishable from ES cells.<sup>21</sup> This discovery resolves the ethical and immunological issues related to the use of ES cells and offers considerable promise for cardiac repair. Differentiation *in vitro* of iPS cells into functional cardiomyocytes is possible, and their use for treatment of injured myocardium is currently under investigation.

### *Endogenous (resident) cardiac stem cells*

The field of endogenous cardiac stem cells continues to be an area of active research, but also remains controversial. Of the side population, cells able to extrude the Hoechst 33528 dye the *c-kit*<sup>+</sup>/*Lin*<sup>-</sup> subset have been reported as self-renewing and able to give rise to blood vessels as well as cardiomyocytes.<sup>22</sup> Other research groups identified different markers of cardiac stem cells, such as the transcription factor Isl-1.<sup>23</sup>

Limited animal experimental data have been published regarding the results of endogenous cardiac stem cell transplantation.<sup>24,25</sup> Given that the ideal type of cell for cardiac transplantation would be self-renewing, survive engraftment, differentiate into cardiac cells and be non-arrhythmogenic, a true endogenous cardiac stem cell that can be readily harvested would be an excellent candidate. However, consensus on detailed characterization of the various types of proposed endogenous cardiac stem cells and robust methods of tracking their survival and phenotypic fate after transplantation are not yet established. Phase 1 clinical trials of the use of these cells for cardiac repair have recently begun and their results are awaited with interest.

To summarize, a large number of animal model studies involving various types of cells have been reported, describing only limited success in generating substantial numbers of nascent cardiomyocytes and their functional integration in the myocardium. Although injected foetal cardiomyocytes can provide additional myocardium and ES cells and CSCs have larger potentials for cardiomyogenesis, the formation of new cardiomyocytes by transdifferentiation of skeletal myoblasts or bone marrow-derived cells has not been convincingly demonstrated.

However, several studies have surprisingly reported moderate improvements in whole heart function in post-infarct hearts following transplantation of skeletal myoblasts or bone marrow-derived cells. This paradox remains unaccounted for and will be specifically addressed in the remainder of this review.

## Clinical trials of cardiac cell therapy

### *Skeletal myoblasts*

After ~10 years of preclinical testing resulting in more than 40 studies, skeletal myoblasts were the first to be tested in clinical trials.<sup>26</sup> Because autologous skeletal myoblasts need to be expanded *ex vivo* over several weeks, these trials were performed in patients with chronic heart failure, rather than acute myocardial infarction. Autologous skeletal myoblasts were prepared from muscle biopsy samples and expanded using foetal bovine serum. Six of the trials that have been published are summarized in Table 1. As can be seen, most of these studies entailed coronary artery bypass graft surgery, although in the POZNAN trial myoblasts were delivered via the trans-coronary route.<sup>27</sup>

These trials have demonstrated that hundreds of millions of skeletal myoblasts can be grown from muscle biopsies and subsequently injected into the heart without early procedural complications. Long-term engraftment of skeletal myoblasts, featuring clusters of myotubes aligned parallel to host cardiomyocytes, has been visualized by microscopy of explanted hearts up to 18 months after transplantation.<sup>28,29</sup> However,

**Table 1** Clinical trials of skeletal myoblast transplantation in chronic ischaemic disease.

Study	<i>n</i>	Control	Cell dose	CABG	Outcome
Menasche <i>et al.</i> <sup>30</sup>	97	Placebo injection	$4-8 \times 10^8$	Yes	No change in EF at 6 months. EDV and ESV decreased at 6 months
Hagege <i>et al.</i> <sup>58</sup>	10	None	$8.7 \times 10^8$	Yes (remote)	Improved symptoms. Increased EF by 6.7% at 4 years. VT arrhythmias in three patients
Gavira <i>et al.</i> <sup>31</sup>	12	None	$2.2 \times 10^8$	Yes	Increased EF by 20% at 1 year. Improved viability of injected segments
Dib <i>et al.</i> <sup>28</sup>	30	None	$5 \times 10^7$	Yes	Increased EF by 8% at 2 years. VT arrhythmias in three patients. Skeletal myotubes were visualized in hearts, which were explanted later
Siminiak <i>et al.</i> <sup>59</sup>	10	None	$1.1 \times 10^7$	Yes	Increased EF by 6.8% at 1 year
Siminiak <i>et al.</i> <sup>27</sup>	10	None	$5.2 \times 10^7$	No	Increased EF by 3–8% at 6 months. Improved NYHA

EF, ejection fraction; EDV, end-diastolic volume; ESV, end-systolic volume; VT, ventricular tachycardia.

myotube grafts were only very small compared with the ventricles, and given the large numbers of cells injected, this implies that the vast majority of cells were lost, either to inefficient seeding or high rates of cell death.

No meaningful conclusion can be drawn regarding the efficacy in augmentation of function in the injected areas from these early trials. The large randomized controlled trial by Menasche *et al.*<sup>30</sup> measured decreased ventricular luminal dimensions 6 months after skeletal myoblast transplantation but found no change in ejection fraction. On the other hand, other studies measured clear improvements in ejection fraction.<sup>28,31</sup> The main limitation of these trials is that their interpretation has been made difficult by concomitant coronary artery surgery, which sometimes included the region receiving myoblast injections. Furthermore, there are differences among studies that make direct comparisons difficult, including differences in cell culture processes (which may influence myoblast viability and differentiation) and the end points used to judge efficacy (including tool of cardiac function assessment), and the variable baseline function of the engrafted regions.

Patient safety has been a concern, especially following the detection of ventricular tachycardia in 4 out of 10 patients in the early study carried out by Menasche *et al.*<sup>26</sup> As a precaution, and also to assess the incidence and timing of graft-related arrhythmias, this group implanted internal cardiac defibrillators in all subsequent patients. In the later study, arrhythmias were detected in 12–17% of patients who received skeletal myoblast transplantation compared with 6% in control patients ( $P = ns$ ).

### Bone marrow cells

Whereas 10 years of preparation preceded the clinical trials of skeletal myoblast transplantation, the early clinical trials of bone marrow-derived cell transplantation were reported within 6 months of the publication by Orlic *et al.* Subsequently, there have been many randomized clinical trials published.

Unlike skeletal myoblasts, hundreds of millions of autologous bone marrow-derived cells can be obtained quickly without the need for *ex vivo* expansion. This enabled most of the clinical studies to be aimed towards treatment of patients who suffered recent acute myocardial infarction (summarized in Table 2), although others have performed bone marrow-derived cell transplantation in patients with chronic heart failure (Table 3). Another difference between the trials using bone marrow-derived cells compared with skeletal myoblasts is that most of those injecting bone marrow-derived cells have done so using

**Table 2** Randomized controlled trials of bone marrow cell transplantation following myocardial infarction.

Study	<i>n</i>	Follow up	Cell dose	Assessment method	Outcome
Chen <i>et al.</i> <sup>38</sup>	69	6 months	$6 \times 10^{10}$	Echo	EF increased 18%
Ge <i>et al.</i> <sup>34</sup>	20	6 months	$4 \times 10^7$	SPECT/Echo	EF increased 6.7%
Janssens <i>et al.</i> <sup>35</sup>	67	4 months	$1.7 \times 10^8$	MRI	No effect
Kang <i>et al.</i> <sup>39</sup>	56	6 months	$1.4 \times 10^8$	MRI	EF increased 5.2%
Lunde <i>et al.</i> (ASTAMI) <sup>60</sup>	100	6 months	$8.7 \times 10^7$	SPECT/Echo/ MRI	No effect
Meyer <i>et al.</i> (BOOST) <sup>61</sup>	60	18 months	$2.5 \times 10^9$	MRI	EF increased 6.7% at 6 months. No effect at 18 months*
Schachinger <i>et al.</i> (REPAIR-AMI) <sup>36</sup>	204	4 months	$2.4 \times 10^8$	LV angiography	EF increased 2.5%
Meluzin <i>et al.</i> <sup>62</sup>	66	3 months	$10^8$	SPECT/Echo	EF 3%. No effect when $10^7$ cells injected
Li <i>et al.</i> <sup>63</sup>	70	6 months	$7.3 \times 10^7$	Echo	EF increased 5.5%
Penicka <i>et al.</i> <sup>40</sup>	24	4 months	$2.6 \times 10^9$	SPECT/Echo	No effect <sup>†</sup>

All studies used the intracoronary injection route. All studies demonstrated satisfactory patient matching. Trial terminated early because of adverse events and no significant benefit. Echo, echocardiography; SPECT, single-photon-emission computed tomography; MRI, magnetic resonance imaging. \*EF at 18 months was higher than at baseline, but showed no statistically significant difference compared to control. <sup>†</sup>Patients with severe heart failure recruited.

**Table 3** Randomized controlled trials of bone marrow cell transplantation in chronic heart disease.

Study	<i>n</i>	Follow up	Cell dose	Function assessment	Outcome
Erbs <i>et al.</i> <sup>64</sup>	26	3 months	$6.9 \times 10^6$	MRI	Increased EF by 7.2%
Assmus <i>et al.</i> (TOPCARE-CHD) <sup>65</sup>	75	3 months	$2.1 \times 10^8$	LV angiography	Increased EF by 2.9%
Hendrikx <i>et al.</i> <sup>32</sup>	20	4 months	$6 \times 10^7$ *	MRI	No effect
Kang <i>et al.</i> (MAGIC Cell-3-DES) <sup>39</sup>	40	6 months	$1.4 \times 10^8$	MRI	No effect

All studies demonstrated satisfactory patient matching. SPECT, single-photon-emission computed tomography; MRI, magnetic resonance imaging. \*In this trial, cells were injected intramuscularly during CABG.

percutaneous approaches. The commonest method has been intracoronary perfusion under high pressures during coronary revascularization. Some studies employed direct intramuscular cell injection during coronary artery bypass surgery.<sup>32,33</sup> Other trials have employed percutaneous endocardial injection, which enables electromechanical mapping to help identify viable myocardium for appropriate delivery.

As can be seen in Tables 2 and 3, the results for both acute myocardial infarction and chronic disease have been mixed. Although most of



these studies were at least partially blinded during follow-up, only three have carried out blinded placebo treatment in their recruited controls, in the form of saline injection.<sup>34–36</sup> The inconsistency of results, together with the modest improvements measured, has prompted a variety of interpretations. Some have commented that non-standardization of protocols, including of cell preparation, timing of injection, baseline patient characteristics and method of evaluation of cardiac function might account for the variation on the results seen.<sup>37</sup> For example, Chen *et al.*<sup>38</sup> used purified MSCs, whereas most others injected unfractionated bone marrow mononuclear cells. Several investigators mobilized bone marrow-derived cells by administering systemic granulocyte colony-stimulating factor prior to cell harvest,<sup>39</sup> which may have had additional effects. Subgroup analysis of the data obtained by Schächinger *et al.* suggested that bone marrow-derived cell transplantation is more beneficial when performed more than 5 days after the onset of myocardial infarction. Finally, Penicka *et al.*<sup>40</sup> commented that the negative finding from their trial, which was terminated early, may be related to the fact that they recruited only patients with ejection fraction <40%.

With increasing skepticism over the collective results, several systematic meta-analyses of bone marrow-derived cell transplantation trials have been published.<sup>41–43</sup> These have concluded that modest yet statistically significant benefits exist in terms of left ventricular dimensions, ejection fraction and infarct size. However, no new insight into the underlying physiological mechanisms of action was provided. The meta-analysis of 18 studies by Abdel-Latif *et al.* identified no correlation between the number of cells injected and benefit to the patient, whereas the Cochrane Review suggested that injection of more than 10<sup>8</sup> bone marrow cells was optimal. The relationship between changes in LVEF and patient clinical outcomes remains poorly characterized. In some cases, patient outcomes improve substantially following medical or interventional therapies that yield small increases in LVEF.<sup>44</sup> Nonetheless, many researchers in the field, including the authors of these meta-analyses, have expressed doubt over the clinical relevance of such small benefits.<sup>42,45</sup>

With regard to future directions, opinions vary. Some have suggested that larger randomized controlled trials, perhaps even with repeated treatment, are warranted for further subgroup analysis.<sup>46</sup> On the other hand, others have suggested a moratorium on new clinical trials which might be exposing patients to risk until additional insight is gained from further animal studies.<sup>45</sup> The suggested key points for future research include optimum cell type, timing and method of delivery, and perhaps most importantly, the underlying physiological mechanisms of action.

## Putative mechanisms mediating the benefits of cell therapy

Medical therapies were not always fully understood before gaining clinical acceptance. However, elucidation of their underlying cellular mechanisms provides the most promising path towards their optimization while maintaining safety. It is difficult to predict the best cell type, dose, and delivery method for cardiac cell therapy in the absence of knowledge of their physiological mechanisms of action. To obtain such information by trial and error would take much time and resources and expose patients to substantial risk.

The clinical trials and the preclinical experiments using animal models outlined above were originally initiated with the aims of true cardiac regeneration. It was hypothesized that supplementation of the diseased heart with the correct type of cells would rectify the deficiency in numbers.<sup>8</sup> However, the animal studies have shown that substantial primary remuscularization does not happen. Foetal cardiomyocytes form very small numbers of functionally integrated myocardial tissue following injection, but even this does not happen with bone marrow cells or skeletal myoblasts. Despite this, many studies have measured improvements in failing hearts. Even at the level of individual cardiomyocytes substantial functional changes can be demonstrated.<sup>47</sup> The numerous clinical trials have suggested between them that a modest functional benefit might exist, but the lack of human cardiac tissue for histological analysis has meant that, apart from some exceptions,<sup>28</sup> clinical studies have not been able to follow the phenotypic fate of the cells injected into human hearts. The demonstration that the functional characteristics of a diseased myocardium can be altered by cell therapy without any remuscularization has resulted in a search for alternative mechanisms of action. Some of the proposed mechanisms, which are not mutually exclusive, are discussed below. The extent of contribution of each of these processes and how they might be optimally manipulated for patient benefit should be the main consideration for future research in cardiac cell therapy.

### *Effects on the native cardiomyocytes*

Cardiomyocytes are the dominant cell type in the normal heart with respect to volume and are the sole generators of contractile force. Studies of isolated single cardiomyocytes from failing human myocardium obtained in the setting of transplantation have demonstrated derangements in excitation-contraction coupling,<sup>48</sup> resulting in depressed contractility.<sup>49</sup> In our lab, we studied the chronic effects of skeletal myoblast or bone marrow cell transplantation on the

functional characteristics of recipient cardiomyocytes in a post-infarct rat heart.<sup>47</sup> Injection of either type of cell attenuated hypertrophy and normalized the impaired contractile performance. Overall,  $\text{Ca}^{2+}$  handling dynamics were improved by either cell type. However, there were differences between the two types of injected cell with respect to the individual components of excitation-contraction coupling that were affected, suggesting that cardiomyocytes might be influenced in more ways than one.

### *Modulation of the extracellular matrix*

In body tissues, extracellular matrix (ECM) formation and digestion and maintenance of optimal tissue structure are related to the fine balance between the activities of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinase (TIMP). Pathological ventricular remodelling involves maladaptive changes in the ECM, which leads to further myocardial deterioration. A net increase in ECM leads to interstitial fibrosis, and although this can be useful in limiting ventricular enlargement, it also decreases tissue compliance and adversely affects systolic and diastolic performance.

Murtuza *et al.*<sup>50</sup> first showed that transplantation of skeletal myoblasts results in decreased levels of collagen in the ECM, correlating with attenuated up-regulation of MMP. The injection of MSCs into infarcted rat hearts attenuates the increases in cardiac expression of collagen and TIMP-1 without affecting the levels of MMP-1, resulting in favourable effects on ventricular dimensions and performance.<sup>51</sup> Thus, there is interesting albeit limited data to support the hypothesis that cell transplantation may modulate the ECM to augment mechanical performance of the myocardium.

### *Neoangiogenesis*

This hypothesis relates to both acute myocardial infarction and chronic ischaemia and states that injected cells contribute towards the increased formation of collateral blood vessels. In the case of acute myocardial infarction, there is ongoing loss of cells at the border region, and it is proposed that cell transplantation attenuates the ongoing cell loss by increasing perfusion to these tenuously supplied areas.<sup>52</sup> Direct evidence for this is provided by the study utilizing radioactive microspheres to demonstrate an increase in regional myocardial blood flow after transplantation of neonatal cardiomyocytes.<sup>53</sup> The same study revealed higher capillary densities in histological ventricular sections.

In the case of bone marrow-derived cell transplantation, it has been suggested that the endothelial progenitor cells (EPCs), which are generally attributed with a role in new vessel formation, might play a central role in myocardial neoangiogenesis. Following intravenous injections of human EPC preparations into athymic rats which had undergone myocardial infarction, they can be found incorporated in newly formed coronary vessels.<sup>54</sup> Injection of other types of human bone marrow cells does not show this effect. Injection of EPCs also reduces the extent of myocardial infarction, resulting in relatively preserved LV function. These observations should be interpreted with caution. For example, it is plausible that mature endothelial cells within the EPC preparations incorporated in the coronary vessels. Nonetheless, EPCs and their transplantation into hearts continue to be an area of interest.

### *Paracrine effects on the recipient myocardium*

It has been demonstrated that injection of MSCs into animal models of ischaemic hearts and hindlimbs might also increase collateral perfusion, albeit without direct integration into the new vessels,<sup>52</sup> which suggests that neoangiogenesis may be augmented via indirect as well as direct mechanisms. Furthermore, growth media conditioned by exposure to MSCs enhanced the proliferation of cultured endothelial and smooth muscle cells in a dose-dependent manner. Analysis of these growth media revealed increased levels of various cytokines, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), placental growth factor and monocyte chemoattractant protein-1. Inhibition of VEGF and bFGF using antibodies only partly attenuated these effects, implying that multiple cytokines had exerted their influence in combination. These experiments show that MSCs can increase the collateral circulation through paracrine mechanisms.

Similar studies supporting the presence of a paracrine effect were independently performed using unfractionated bone marrow mononuclear cells,<sup>55</sup> and also MSCs overexpressing AKT.<sup>56</sup> The growth media that were conditioned by exposure to these bone marrow cells contained higher concentrations of various cytokines and protected isolated cardiomyocytes from hypoxic insults. When directly injected into rat hearts undergoing acute myocardial infarction, the conditioned media decreased the sizes of infarcts and increased blood vessel densities, with corresponding improvements in cardiac function.

In addition to the protective effects during acute infarction, paracrine secretions also influence the contractile characteristics of chronically failing myocardium. Cardiomyocytes isolated from post-infarct remodelled hearts have impaired contractile and  $\text{Ca}^{2+}$  handling characteristics.

However, experiments performed in our laboratory show that co-culture for 48 h in the presence of either bone marrow cells or skeletal myoblasts improves in  $\text{Ca}^{2+}$  handling and contractility.<sup>57</sup>

Following the demonstrations of beneficial effects of cell-free conditioned media, the paracrine theory of cell transplantation has increasingly become the subject of discussion among scientists and also those performing clinical trials. However, although commonly invoked, the paracrine theory remains undefined. At its minimum, it postulates that locally secreted substances from the injected cells benefit their surrounding myocardium, but the cellular targets and the precise physiological effects resulting in the potential benefit have not been described. Furthermore, there is only a vague consensus on the primary disease process that is targeted by cell transplantation. Most of the published studies mentioned above that have led to the paracrine theory have focused on protection of cardiomyocytes from death during an acute myocardial infarction, but the majority of reports in the field have put their main emphases on the function of the whole heart in the long term. Therefore, studies in cardiac cell transplantation attempting to elucidate their underlying mechanisms need to clarify their goal as an initial step—limitation of cardiomyocyte death, or attenuation of the ensuing maladaptation of the surviving cardiomyocytes.

Large-scale clinical trials continue to be performed world-wide, although cell therapy is far from being an established treatment for heart failure. The volume of animal model studies published by numerous research groups demonstrates that cell transplantation is capable of delivering a clear functional benefit. Thus, it appears that cell transplantation holds considerable promise for the treatment of heart failure, and further research is warranted. However, clinical trials have shown mixed results and meta-analyses have demonstrated only small benefits. Subsequently, many researchers in the field of cardiac cell transplantation are increasingly promoting a 'return from bedside to bench'. In this regard, it appears that current research should aim to identify the similarities between the animal and human studies and address the disparities between animal and human studies as a priority. Further characterization of the pathophysiology of heart failure and elucidation of the physiological mechanisms responsible for the beneficial effects mediated by transplanted cells under specific disease states would hold the best promise towards optimizing the future results. Finally, more research is needed to assess the value of promising new cell types, such as CPCs or iPS cells, in their ability to differentiate in cardiac tissue and to dissect the role of the host environment in the integration and differentiation of the transplanted cells.

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## References

- 1 Soonpaa MH, Koh GY, Klug MG *et al.* Formation of nascent intercalated disks between grafted fetal cardiomyocytes and host myocardium. *Science* 1994;264:98–101.
- 2 Li RK, Jia ZQ, Weisel RD *et al.* Cardiomyocyte transplantation improves heart function. *Ann Thorac Surg* 1996;62:654–60.
- 3 Rubart M, Pasumarthi KB, Nakajima H *et al.* Physiological coupling of donor and host cardiomyocytes after cellular transplantation. *Circ Res* 2003;92:1217–24.
- 4 Taylor DA, Atkins BZ, Hungspreugs P *et al.* Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat Med* 1998;4:929–33.
- 5 Collins CA, Olsen I, Zammit PS *et al.* Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. *Cell* 2005;122:289–301.
- 6 Rubart M, Soonpaa MH, Nakajima H *et al.* Spontaneous and evoked intracellular calcium transients in donor-derived myocytes following intracardiac myoblast transplantation. *J Clin Invest* 2004;114:775–83.
- 7 Suzuki K, Murtuza B, Beauchamp JR *et al.* Dynamics and mediators of acute graft attrition after myoblast transplantation to the heart. *FASEB J* 2004;18:1153–5.
- 8 Orlic D, Kajstura J, Chimenti S *et al.* Bone marrow cells regenerate infarcted myocardium. *Nature* 2001;410:701–5.
- 9 Balsam LB, Wagers AJ, Christensen JL *et al.* Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature* 2004;428:668–73.
- 10 Murry CE, Soonpaa MH, Reinecke H *et al.* Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature* 2004;428:664–8.
- 11 Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM *et al.* Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature* 2003;425:968–73.
- 12 Friedenstein AJ, Chailakhyan RK, Gerasimov UV. Bone marrow osteogenic stem cells: in vitro cultivation and transplantation in diffusion chambers. *Cell Tissue Kinet* 1987;20:263–72.
- 13 Ferrari G, Cusella-De AG, Coletta M *et al.* Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 1998;279:1528–30.
- 14 Makino S, Fukuda K, Miyoshi S *et al.* Cardiomyocytes can be generated from marrow stromal cells in vitro. *J Clin Invest* 1999;103:697–705.
- 15 Dai W, Hale SL, Martin BJ *et al.* Allogeneic mesenchymal stem cell transplantation in postinfarcted rat myocardium: short- and long-term effects. *Circulation* 2005;112:214–23.
- 16 Mangi AA, Noiseux N, Kong D *et al.* Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med* 2003;9:1195–201.
- 17 Nussbaum J, Minami E, Laflamme MA *et al.* Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response. *FASEB J* 2007;21:1345–57.
- 18 Laflamme MA, Chen KY, Naumova AV *et al.* Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol* 2007;25:1015–24.
- 19 van Laake LW, Passier R, Doevendans PA *et al.* Human embryonic stem cell-derived cardiomyocytes and cardiac repair in rodents. *Circ Res* 2008;102:1008–10.
- 20 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663–76.
- 21 Park IH, Zhao R, West JA *et al.* Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 2008;451:141–6.
- 22 Beltrami AP, Barlucchi L, Torella D *et al.* Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 2003;114:763–76.

- 23 Moretti A, Caron L, Nakano A *et al*. Multipotent embryonic isl1+ progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. *Cell* 2006;**127**:1151–65.
- 24 Dawn B, Stein AB, Urbanek K *et al*. Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function. *Proc Natl Acad Sci USA* 2005;**102**:3766–71.
- 25 Gonzalez A, Rota M, Nuszynska D *et al*. Activation of cardiac progenitor cells reverses the failing heart senescent phenotype and prolongs lifespan. *Circ Res* 2008;**102**:597–606.
- 26 Menasche P, Hagege AA, Scorsin M *et al*. Myoblast transplantation for heart failure. *Lancet* 2001;**357**:279–80.
- 27 Siminiak T, Fiszer D, Jerzykowska O *et al*. Percutaneous trans-coronary-venous transplantation of autologous skeletal myoblasts in the treatment of post-infarction myocardial contractility impairment: the POZNAN trial. *Eur Heart J* 2005;**26**:1188–95.
- 28 Dib N, Michler RE, Pagani FD *et al*. Safety and feasibility of autologous myoblast transplantation in patients with ischemic cardiomyopathy: four-year follow-up. *Circulation* 2005;**112**:1748–55.
- 29 Hagege AA, Carrion C, Menasche P *et al*. Viability and differentiation of autologous skeletal myoblast grafts in ischaemic cardiomyopathy. *Lancet* 2003;**361**:491–2.
- 30 Menasche P, Alfieri O, Janssens S *et al*. The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. *Circulation* 2008;**117**:1189–200.
- 31 Gavira JJ, Herreros J, Perez A *et al*. Autologous skeletal myoblast transplantation in patients with nonacute myocardial infarction: 1-year follow-up. *J Thorac Cardiovasc Surg* 2006;**131**:799–804.
- 32 Hendrikx M, Hensen K, Clijsters C *et al*. Recovery of regional but not global contractile function by the direct intramyocardial autologous bone marrow transplantation: results from a randomized controlled clinical trial. *Circulation* 2006;**114**(Suppl 1):I101–7.
- 33 Stamm C, Kleine HD, Choi YH *et al*. Intramyocardial delivery of CD133+ bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: safety and efficacy studies. *J Thorac Cardiovasc Surg* 2007;**133**:717–25.
- 34 Ge J, Li Y, Qian J *et al*. Efficacy of emergent transcatheter transplantation of stem cells for treatment of acute myocardial infarction (TCT-STAMI). *Heart* 2006;**92**:1764–7.
- 35 Janssens S, Dubois C, Bogaert J *et al*. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. *Lancet* 2006;**367**:113–21.
- 36 Schachinger V, Erbs S, Elsasser A *et al*. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med* 2006;**355**:1210–21.
- 37 Rosenzweig A. Cardiac cell therapy—mixed results from mixed cells. *N Engl J Med* 2006;**355**:1274–7.
- 38 Chen SL, Fang WW, Ye F *et al*. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol* 2004;**94**:92–5.
- 39 Kang HJ, Lee HY, Na SH *et al*. Differential effect of intracoronary infusion of mobilized peripheral blood stem cells by granulocyte colony-stimulating factor on left ventricular function and remodeling in patients with acute myocardial infarction versus old myocardial infarction: the MAGIC Cell-3-DES randomized, controlled trial. *Circulation* 2006;**114**(Suppl 1):I145–51.
- 40 Penicka M, Horak J, Kobylka P *et al*. Intracoronary injection of autologous bone marrow-derived mononuclear cells in patients with large anterior acute myocardial infarction: a prematurely terminated randomized study. *J Am Coll Cardiol* 2007;**49**:2373–4.
- 41 Abdel-Latif A, Bolli R, Tleyjeh IM *et al*. Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. *Arch Intern Med* 2007;**167**:989–97.
- 42 Lipinski MJ, Biondi-Zoccai GG, Abbate A *et al*. Impact of intracoronary cell therapy on left ventricular function in the setting of acute myocardial infarction: a collaborative systematic review and meta-analysis of controlled clinical trials. *J Am Coll Cardiol* 2007;**50**:1761–7.
- 43 Martin-Rendon E, Brunskill SJ, Hyde CJ *et al*. Autologous bone marrow stem cells to treat acute myocardial infarction: a systematic review. *Eur Heart J* 2008;**29**:1807–18.
- 44 Reffelmann T, Konemann S, Kloner RA. Promise of blood- and bone marrow-derived stem cell transplantation for functional cardiac repair: putting it in perspective with existing therapy. *J Am Coll Cardiol* 2009;**53**:305–8.

- 45 Nadal-Ginard B, Fuster V. Myocardial cell therapy at the crossroads. *Nat Clin Pract Cardiovasc Med* 2007;4:1.
- 46 Bartunek J, Dimmeler S, Drexler H *et al.* The consensus of the task force of the European Society of Cardiology concerning the clinical investigation of the use of autologous adult stem cells for repair of the heart. *Eur Heart J* 2006;27:1338–40.
- 47 Lee J, Stagg MA, Fukushima S *et al.* Adult progenitor cell transplantation influences contractile performance and calcium handling of recipient cardiomyocytes. *Am J Physiol Heart Circ Physiol* 2009;296:H927–36.
- 48 del Monte F, O’Gara P, Poole-Wilson PA *et al.* Cell geometry and contractile abnormalities of myocytes from failing human left ventricle. *Cardiovasc Res* 1995;30:281–90.
- 49 Davies CH, Davia K, Bennett JG *et al.* Reduced contraction and altered frequency response of isolated ventricular myocytes from patients with heart failure. *Circulation* 1995;92:2540–9.
- 50 Murtuza B, Suzuki K, Bou-Gharios G *et al.* Transplantation of skeletal myoblasts secreting an IL-1 inhibitor modulates adverse remodeling in infarcted murine myocardium. *Proc Natl Acad Sci USA* 2004;101:4216–21.
- 51 Xu X, Xu Z, Xu Y *et al.* Effects of mesenchymal stem cell transplantation on extracellular matrix after myocardial infarction in rats. *Coron Artery Dis* 2005;16:245–55.
- 52 Kinnaird T, Stabile E, Burnett MS *et al.* Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation* 2004;109:1543–9.
- 53 Reffelmann T, Dow JS, Dai W *et al.* Transplantation of neonatal cardiomyocytes after permanent coronary artery occlusion increases regional blood flow of infarcted myocardium. *J Mol Cell Cardiol* 2003;35:607–13.
- 54 Kocher AA, Schuster MD, Szabolcs MJ *et al.* Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 2001;7:430–6.
- 55 Takahashi M, Li TS, Suzuki R *et al.* Cytokines produced by bone marrow cells can contribute to functional improvement of the infarcted heart by protecting cardiomyocytes from ischemic injury. *Am J Physiol Heart Circ Physiol* 2006;291:H886–93.
- 56 Gneocchi M, He H, Noiseux N *et al.* Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. *FASEB J* 2006;20:661–9.
- 57 Lee J, Stagg MA, Siedlecka U *et al.* Identification of cell-specific soluble mediators and cellular targets during cell therapy for the treatment of heart failure. *Regen Med* 2008;3:953–62.
- 58 Hagege AA, Marolleau JP, Vilquin JT *et al.* Skeletal myoblast transplantation in ischemic heart failure: long-term follow-up of the first phase I cohort of patients. *Circulation* 2006;114(Suppl 1):I108–13.
- 59 Siminiak T, Kalawski R, Fiszler D *et al.* Autologous skeletal myoblast transplantation for the treatment of postinfarction myocardial injury: phase I clinical study with 12 months of follow-up. *Am Heart J* 2004;148:531–7.
- 60 Lunde K, Solheim S, Aakhus S *et al.* Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *N Engl J Med* 2006;355:1199–209.
- 61 Meyer GP, Wollert KC, Lotz J *et al.* Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months’ follow-up data from the randomized, controlled BOOST (BOne marrOW transfer to enhance ST-elevation infarct regeneration) trial. *Circulation* 2006;113:1287–94.
- 62 Meluzin J, Mayer J, Groch L *et al.* Autologous transplantation of mononuclear bone marrow cells in patients with acute myocardial infarction: the effect of the dose of transplanted cells on myocardial function. *Am Heart J* 2006;152:97.e5–15.
- 63 Li ZQ, Zhang M, Jing YZ *et al.* The clinical study of autologous peripheral blood stem cell transplantation by intracoronary infusion in patients with acute myocardial infarction (AMI). *Int J Cardiol* 2007;115:52–6.
- 64 Erbs S, Linke A, Adams V *et al.* Transplantation of blood-derived progenitor cells after recanalization of chronic coronary artery occlusion: first randomized and placebo-controlled study. *Circ Res* 2005;97:756–62.
- 65 Assmus B, Honold J, Schachinger V *et al.* Transcoronary transplantation of progenitor cells after myocardial infarction. *N Engl J Med* 2006;355:1222–32.