

## Stem Cells in Cardiac Repair – Recent Developments and Future Directions

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### Abstract

Myocardial infarction (MI) is the leading cause of death among people in the industrialised world and will, according to the World Health Organization (WHO), become the leading cause of death in the world in 2020. For the treatment of patients with MIs and ischaemic cardiomyopathies, remarkable medical advances have been made during the second half of the 20th Century that have increased patient survival. As a consequence, patients with heart disease are living longer and the incidence of congestive heart failure in patients is significantly increasing. New treatments for patients with acute MI and ischaemic cardiomyopathies are needed. In this regard, the next major advance in the treatment of patients with cardiac disease promises to be stem cells and stem cell products. Currently, basic research scientists and clinicians worldwide are investigating human embryonic stem cells, skeletal stem cells (myoblasts), adult bone marrow stem cells, cardiac stem cells and human umbilical cord stem cells for the treatment of patients with MIs and ischaemic cardiomyopathies. This review highlights the recent developments and the future directions of each of these stem cells in the treatment of patients with heart disease.

### Keywords

Human embryonic stem cells, skeletal myoblasts, adult bone marrow cells, cardiac stem/progenitor cells, human umbilical cord blood stem cells, acute myocardial infarction, ischaemic cardiomyopathy, cardiac repair

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Myocardial infarction (MI) is the leading cause of death among people in the industrialised world and will, according to the World Health Organization (WHO), become the leading cause of death in the world in 2020.<sup>1</sup> For the treatment of patients with MIs and ischaemic cardiomyopathies, remarkable medical advances have been made during the second half of the 20th Century that have increased patient survival. These advances include external cardiac defibrillation and closed chest cardiopulmonary resuscitation, coronary care units, echocardiography, coronary angiography, cardiac pacemakers/automatic implantable defibrillators, the heart-lung machine, coronary artery bypass surgery, mechanical and bioprosthetic heart valves, heart transplantation, coronary artery thrombolytic therapy, coronary angioplasty and coronary artery stents. Further refinements and advances in each of these treatments or techniques are contributing to reductions in patient morbidity and increased patient survival from MI. As a consequence patients are living longer and the incidence of congestive heart failure in patients with damaged hearts is significantly increasing. New treatments for patients with acute MI and ischaemic cardiomyopathies are needed; in this regard, the next major advance in the treatment of patients with cardiac disease promises to be stem cells and stem cell products. Currently, basic research scientists and clinicians worldwide are investigating human embryonic stem cells, skeletal stem cells (myoblasts), adult bone marrow stem cells, cardiac stem cells and human umbilical cord stem cells. The use of each of these cell types has distinct advantages in the repair of damaged hearts in patients but also presents specific treatment challenges that must be overcome prior to widespread acceptance by clinicians and clinical use.

### Human Embryonic Stem Cells

Interest in human embryonic stem cells (hESCs) was ignited by the work of Thomson and coworkers in 1998.<sup>2</sup> Thomson successfully isolated, maintained and propagated primitive pluripotent stem cells from the inner cell mass of the human embryonic blastocyst. These hESCs contain the nuclear transcription factors Oct 4, Nanog and SOX-2, which form the core regulatory network that maintains stem cells in a primitive state and ensures pluripotency and suppression of genes that lead to differentiation. These hESCs can form any one of the 220 different cell types that compose the human body, including cardiac myocytes and blood vessels.<sup>3,4</sup> Since hESCs express class I but not class II major histocompatibility complex (MHC) molecules, they cause only minor immune responses by the host. Consequently, immunosuppressive regimens for hESC transplantation can be minimised in comparison with conventional organ transplantation. However, since hESCs are pluripotent, the cells can form teratomas, i.e. tumours composed of tissue from all three germ layers, after transplantation into hearts. Teratomas occur in approximately 18 % of treated hearts that receive 10 million hESCs and the incidence increases as the number of hESCs injected into the heart increases.<sup>5</sup> Consequently, investigators have differentiated hESCs towards cardiac progenitor cells (hESC-CMs) with growth factors and other biologically active chemicals prior to transplanting these cells into hearts.<sup>6</sup> In research animals, hESCs-CM can regenerate atrial and ventricular myocytes in infarcted hearts, attenuate heart remodelling and contribute to left ventricular systolic force development and cardiac output.<sup>6,7</sup> However, significant challenges must be overcome prior to the clinical use of hESCs-CMs in patients, including: the generation of large quantities of pure

cardiomyocytes for cardiac transplantation, the retention of hESCs-CMs in the beating heart, the prevention of immune rejection by the host once these cells are transplanted and the pharmacological optimisation of the function of these cells in the heart over the long term. Nevertheless, hESC studies are currently being planned in Europe for the treatment of patients with cardiomyopathies and Phase 1 feasibility, safety and clinical effectiveness studies have been performed in the US for the treatment of patients with spinal cord injuries.

### Skeletal Myoblasts

Skeletal muscle fibres have unipotent (tissue committed) precursor satellite cells that are located between the basement membrane and the sarcolemma of individual muscle fibres. Following muscle injury the satellite cells mobilise, proliferate and differentiate into myoblasts that express the Myf5 and MyoD proteins, which are important in myogenic differentiation, and desmin, which is important in linking myofibrils. Ultimately the myoblasts fuse to existing skeletal muscle fibres and form new fibres. Myoblasts are theoretically attractive for cardiac repair because of their ready availability in skeletal muscle, high proliferation rate in cell culture and resistance to ischaemia. Investigations of myoblasts in small and large research animals with ischaemic and non-ischaemic cardiomyopathy have established that myoblasts directly injected into damaged myocardium can survive, proliferate and differentiate into skeletal multinucleated myotubes.<sup>8</sup> The myoblasts can align with cardiomyocytes, limit left ventricular (LV) remodelling and increase the LV ejection fraction (LVEF).<sup>9</sup> However, myoblasts do not express gap-junction proteins such as connexin-43 and do not form a syncytium with host cardiomyocytes. Phase I trials in patients with MIs have demonstrated the feasibility of directly implanting autologous skeletal myoblasts in the LV at the time of coronary artery bypass surgery.<sup>9,10</sup> In these patients, myoblasts increased LVEF by as much as 7–18 %; however, electrical re-entry due to the absence of electrical integration of myoblasts with myocytes in the heart predisposed as many as 27 % of patients to ventricular arrhythmias.<sup>10</sup> Nevertheless, the Phase I studies resulted in the Myoblast autologous grafting in ischemic cardiomyopathy (MAGIC) study, which consisted of a randomised, placebo-controlled, double-blind study of myoblasts in patients receiving coronary artery bypass grafts and automatic implantable cardioverter defibrillators (AICDs).<sup>11</sup> In this study, there was no significant difference at six months in echocardiographic improvement in LV segment contraction between patients who received myoblasts and patients who received placebo. Consequently, the study was prematurely discontinued. Similarly, the Safety and effects of implanted (autologous) skeletal myoblasts (MyoCell) using an injection catheter (SEISMIC) trial reported in patients with ischaemic cardiomyopathies, heart failure and AICDs that autologous skeletal myoblasts had no significant effect on global LVEF determined by multiple gated acquisition (MUGA) scans.<sup>12</sup> Consequently, enthusiasm for skeletal myoblasts in the treatment of patients with damaged hearts has significantly diminished since the report of the MAGIC and SEISMIC trials.

The disappointing results of the MAGIC and SEISMIC studies may be due to a low rate of myoblast retention in the myocardium, high rates of myoblast death and/or the inability of engrafted myoblasts to establish functional electromechanical connections with cardiomyocytes in patients. In addition, multiple needle injections of myoblasts into the myocardium are associated with a high rate of myoblast leakage out of the myocardium and disruption of the extracellular matrix.<sup>10</sup> Currently, research is in progress wherein skeletal myoblast tissue patches are being applied to the epicardium of infarcted LVs to limit

LV remodelling after MI and prevent the development of ischaemic cardiomyopathies and heart failure.

### Bone Marrow Stem Cells

Medical investigators have recognised since 1970 that human bone marrow contains haematopoietic stem cells that can produce red blood cells, megakaryocytes, myeloid cells and lymphocytes. At the time of the original description of bone marrow haematopoietic stem cells, stromal cells were also identified in the bone marrow but the importance of the stromal cells, now known as mesenchymal stem cells (MSCs), was not fully recognised until the 1990s. Bone marrow mesenchymal stem cells can generate myocytes and can also generate osteoblasts, chondrocytes and adipose cells. The separation of adult bone marrow aspirate into a mononuclear cell fraction yields approximately 2–4 % haematopoietic stem cells/endothelial progenitor cells and approximately 0.01 % MSCs. The remainder of the bone marrow mononuclear cell fraction is composed of haematopoietic cells in different stages of maturation.

In 2001, Orlic and colleagues reported in a letter to Nature that bone marrow stem cells from male mice, when injected into the anterior myocardial wall of female mice with acute MIs, produced myocardial repair due to proliferation of cardiac myocytes and vascular cells.<sup>13</sup> The myocardial repair was associated with 36 % reductions in LV end-diastolic pressure, 32 % increases in LV developed pressure and 40 % increases in the change in pressure per unit change in time (dP/dt) in comparison with the same measurements in mice with untreated MIs. Within four months of the publication of this mouse study, the first report of the application of autologous bone marrow-derived cells for heart repair in patients with ischaemic heart disease was published. The clinical application of bone marrow cells in patients with damaged hearts was catalysed by the relatively easy accessibility of autologous bone marrow, the large numbers of unfractionated autologous bone marrow mononuclear cells that can be obtained from bone marrow aspirates without *ex vivo* expansion, and the extensive clinical experience with bone marrow transplantation in patients with cancer who had previously undergone chemotherapy and/or radiation therapy and bone marrow ablation. To date, more than 2,000 patients with MIs and/or ischaemic cardiomyopathy have been treated with either unfractionated autologous bone marrow mononuclear cells, which contain haematopoietic and mesenchymal stem cells, or fractionated and enriched subpopulations of bone marrow mononuclear cells.

Examination of individual trials of bone marrow mononuclear cells (BMCs) in patients with ischaemic heart disease produces diverse conclusions due to different observation periods after BMC transplantation, significant variations in BMC processing and characterisation of cells, the technique and timing of BMC transplantation, the number and volume of cells injected, whether there is red blood cell or heparin contamination of BMCs, adjunctive therapy and the methodology of quantification of cardiac performance after BMC transplantation. Nevertheless, important trends can be discerned from three separate meta-analyses of the multiple trials of autologous BMCs administered to patients primarily by intracoronary injection for treatment of MIs and/or ischaemic cardiomyopathy.<sup>10,14–16</sup> In general, BMCs significantly improve LVEF by ~3–4 % (range: 1.26–7.4 %), decrease LV end-systolic volume by ~5.7 ml (range -1.41 to -12.20 ml) and decrease infarct size by ~4.9 % (range: -1.11 to -9.10 %) in comparison with untreated patients.<sup>10,14–16</sup> None of the meta-analyses have reported an increased incidence of cardiac

arrhythmias in patients given either intracoronary or direct intramyocardial bone marrow cells. Moreover, neither cardiac tumour formation nor cardiac calcifications after bone marrow cell therapy have been reported. Consequently, bone marrow transplantation in patients appears to be safe and produces modest increases in LVEF and decreases in infarct size. The greatest benefits appear to occur in patients with the greatest amount of infarct-induced myocardial damage based on the intracoronary progenitor cells in acute myocardial infarction (REPAIR-AMI), Finnish stem cell (FINCELL) and Myocardial regeneration by intracoronary infusion of selected population of stem cells in acute myocardial infarction (REGENT) studies.<sup>17–19</sup> In addition, in the BALANCE study, the early significant improvement in LVEF and infarct size at three months and one year was followed at five years by greater exercise capacity and lower mortality rates in BMC-treated patients with damaged hearts in comparison with conventionally treated patients.<sup>20</sup> Currently a Phase III trial by a European consortium is in progress to determine whether bone marrow mononuclear cells significantly decrease mortality in patients with acute ST-segment elevation MI (the Effect of intracoronary re-infusion of bone marrow derived mononuclear cells on all-cause mortality in acute myocardial infarction [BAMI] trial).

In 2012, the predominant scientific evidence indicates that adult autologous BMCs in patients with ischaemic heart disease release biologically active factors that can limit inflammation and fibrosis in ischaemic and injured myocardium. This paracrine effect of BMCs, rather than BMC transdifferentiation to myocytes and vascular cells, can lead to an improvement in heart function. In addition, the biologically active factors released by BMCs can chemoattract patient native stem cells to the ischaemic and injured myocardium. However, in older patients with acute MIs, ischaemic cardiomyopathies and other chronic diseases such as diabetes mellitus, the number of autologous bone marrow stem cells, and the ability of these cells to function, is limited.<sup>21</sup> Alternatively, cardiovascular investigators are examining the use of allogeneic bone marrow MSCs for cardiac repair in patients with ischaemic heart disease. Mesenchymal stem cells, including allogeneic MSCs, are thought to be immune-privileged in that they do not express class II MHC molecules and co-stimulatory B7 and CD40 ligands. However, MSCs do express hepatocyte growth factor (HGF) and transforming growth factor-beta1 (TGF- $\beta$ 1), which suppress proliferation of cytotoxic T-lymphocytes. The intravenous administration of allogeneic MSCs to patients with anterior wall MIs has resulted in an increase in LVEF of  $5.2 \pm 1.5$  % at one year in comparison with placebo-treated patients.<sup>22</sup>

A Phase II clinical safety, effectiveness and dose escalation trial of allogeneic MSCs from young, unrelated donors in 45 patients with ischaemic and non-ischaemic cardiomyopathies with LVEFs less than 40 % was reported at the American Heart Association (AHA) Scientific Sessions in 2011.<sup>23</sup> In the patients given 25 million allogeneic MSCs into the LV endocardium during cardiac catheterisation, the LVEF increased by 7 % at three months and by 5.2 % at one year.<sup>23</sup> However, the LVEF did not significantly increase in patients given 75 and 150 million MSCs, nor in the controls.<sup>23</sup> In a time-to-event analysis, major adverse cardiac events, including cardiac death, MI and revascularisation, were significantly decreased in the MSC-treated patients one to three years after treatment. Additional randomised studies with magnetic resonance imaging (MRI) measurements of heart function are in progress in order to fully evaluate the benefits of allogeneic MSCs in patients with ischaemic heart disease.

## Cardiac Stem Cells

Cardiac stem cells (CSCs) reside in niches in the heart and contribute to the physiological turnover of cardiac myocytes and vascular endothelial cells, which occurs at rates of 1 % to as much as 40 % per year depending on the age, sex and health of the individual.<sup>24</sup> Whether these primitive stem cells are entirely of intrinsic cardiac origin or are bone marrow stem cells that have migrated to and reside in the heart is not fully understood. Currently, autologous CSCs, obtained from the right atrial appendages of patients with LVEFs less than 40 % who have undergone coronary artery bypass surgery, are being administered four months after surgery into the coronary arteries of the same patients in the Cardiac stem cell infusion in patients with ischemic cardiomyopathy (SCIPIO) study.<sup>25</sup> This treatment, in 14 patients at four months after stem cell injection, has produced an 8.2 % absolute increase in LVEF ( $30.3 \pm 1.9$  % to  $38.5 \pm 2.8$  %) by echocardiographic measurements that is associated with an increase in LV wall motion score, in contrast to seven untreated patients in which the EFs and wall motion score measurements did not significantly change.<sup>25</sup> In eight patients at 12 months after CSC treatment, the LVEF increased by 12.3 % in comparison with measurements made before treatment.<sup>25</sup>

A mixed population of cardiac stem/progenitor cells, including MSCs and endothelial progenitor cells, termed cardiosphere-derived stem cells (CDCs), can be obtained and propagated from cardiac biopsies from the right side of the interventricular septum of the heart.<sup>26</sup> CDCs have been administered to 16 patients with recent MIs with LVEFs of 25–45 % in the Cardiosphere-derived autologous stem cells to reverse ventricular dysfunction (CADUCEUS) study. One-year results indicate that these stem cells, when injected into the coronary arteries of patients, decrease by MRI measurements LV scar mass by 10–15 % and increase LV mass by 8–16 %, in contrast to untreated patients in whom LV scar and LV mass did not significantly change.<sup>27</sup> Although LV regional wall motion increased in the CDC-treated patients in this study, global LVEF, as measured by MRI, did not significantly change. Both the CADUCEUS and SCIPIO trials require patient cardiac tissue from catheter biopsies or surgical biopsies. These procedures can be associated with patient morbidity. Nevertheless, autologous cardiac stem cells provide an alternative therapeutic treatment option for patients with ischaemic cardiomyopathies. Additional studies are being formulated to evaluate the effectiveness of each of these stem cell treatments in larger numbers of patients with ischaemic cardiomyopathies.

## Umbilical Cord Blood Mononuclear Cells

Human umbilical cord blood is a source of haematopoietic, endothelial and mesenchymal stem cells that is used for repopulating bone marrow cells in patients treated for acute leukaemia, chronic myeloid leukaemia, myelodysplastic syndrome, neuroblastomas and non-malignant diseases such as Fanconi's anaemia and aplastic anaemia. These cells do not express MHC II molecules and are immunologically naive. In research animals with acute MIs, human umbilical cord blood mononuclear cells (hUCBCs) significantly decrease the size of MIs, reduce LV infarct size and increase LVEF and dP/dt without requirements for immune suppressive therapy.<sup>28–32</sup> In addition, these cells increase myocardial vascularity.<sup>28,33</sup> hUCBCs secrete growth factors and anti-inflammatory cytokines that can increase myocardial vascularity and limit the expression of inflammatory cytokines, such as tumour necrosis factor (TNF), monocyte chemoattraction protein, fractalkine, interleukin-6 (IL-6), macrophage inflammatory protein and interferon- $\gamma$  (IFN- $\gamma$ ).<sup>31,32</sup> In addition, hUCBCs decrease inflammatory neutrophils and lymphocytes in acutely inflamed and infarcted

myocardium.<sup>31,32</sup> Currently hUCBCs are being processed for the development of vascular grafts and heart valves for newborns with congenital heart defects. In addition, Phase I studies are being performed for the treatment of patients with angina pectoris and ischaemic cardiomyopathies refractory to medical therapy that are not candidates for coronary angioplasty or surgical revascularisation.

### Future Challenges and Directions in the Use of Stem Cells for Cardiac Repair

Although important progress is occurring in the use of stem cells for cardiac repair, the most optimal stem cell(s) for treatment of patients with infarcted myocardium has not yet been determined and requires identification. This will require comparison studies of hESCs, bone marrow stem cells, allogeneic MSCs, CSCs and hUCBCs in research animals and in patients. The ideal stem cell for cardiac repair should be non-toxic; not require immune suppression of the patient; create or stimulate healthy and functional cardiac muscle and blood vessels; improve heart function; and limit LV remodelling. In addition, the optimal stem cell(s) should be easily harvested, readily propagated *ex vivo* in large numbers without neoplastic differentiation and be available as a standardised 'off the shelf' treatment for prompt cardiac repair in patients. Currently, only approximately 1–10 % of all stem cells injected into the coronary arteries or into the myocardium remain in the heart for more than two hours due to stem cell migration through myocardial veins and lymphatic vessels into the systemic circulation or to expulsion from the injection sites because of the massaging action of the myocardium. In order to enhance stem cell engraftment in the damaged heart, techniques must be fully developed such as fibrin glues, collagen matrices, hydrogels, nanoparticles/nanofibres and cardiac patches that limit stem cell migration out of the heart.

Investigations must determine the optimal number of stem cells for heart transplantation, the optimal technique for stem cell injection and the optimal time after MI for stem cell injection in order to maximise

stem cell chemoattraction to ischaemic and infarcted myocardium and facilitate myocardial healing. Large areas of myocardial ischaemia and injury will require significantly larger numbers of stem cells. Consequently, stem cell dose–infarct response studies should be performed in research animals and in patients. In addition, the repeated administration of stem cells to heart patients must be investigated, especially in patients with ischaemic cardiomyopathies, with techniques that do not cause myocardial scars that might potentiate cardiac arrhythmias. Furthermore, the presence or absence of antibodies against allogeneic stem cells in patients must be determined. The paracrine actions of stem cells must be fully investigated and the growth factors, anti-inflammatory cytokines and other biological factors secreted by stem cells identified in order to develop new pharmacologic therapies for cardiac repair and neovascularisation. Moreover, the genetic manipulation of stem cells should be investigated to facilitate the overexpression of biologically active factors from stem cells that facilitate myocardial repair as well as enable stem cell migration to the heart after intravenous delivery. Extreme caution is required in the choice of viral vectors and techniques to transduce stem cells with genes so as to minimise stem cell destruction and the potential negative consequences to patients of proliferation of viral gene carriers.

Highly sensitive, specific and reproducible techniques such as MRI must be uniformly employed in patients before and after stem cell transplantation to measure changes in cardiac infarct size, regional wall motion, LVEF and ventricular volumes and permit comparisons between different studies of stem cells in patients with ischaemic heart disease. To address and overcome these major challenges in an efficient and productive manner, basic scientists, cardiologists, haematologists, cardiothoracic surgeons and paramedical personnel worldwide must closely interact, share information and build on laboratory and clinical experiences. In this manner, cell-based therapy will provide entirely new treatments and offer new hope to the millions of patients globally with ischaemic heart disease. ■

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