Clinical Applications of Stem Cell Therapy for Regenerating The Heart

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ABSTRACT
An immediate reperfusion therapy after acute myocardial infarction (AMI) is a prerequisite to prevent further cardiac damage and minimize ventricular remodelling. Although a rigorous and sophisticated set of therapeutic procedure has been applied in the disease management, mortality rate has yet unchanged during the last twenty years. This fact necessitates an alternative or adjuvant therapy that is critically safe and capable of repairing the injured vascular as well as regenerating the infarcted myocardium without omitting the ethical considerations. Stem cell therapy could be the answer. It has gained major basic and clinical research interest, ever since its discovered potential to repair the injured vascular in 1997. Multiple cell types across lineages have been shown to be able to transdifferentiate into mature functioning cardiomyocytes either in vitro through similar phenotypical and genotypical characteristics or in vivo by regenerating the infarcted myocardium and improve contractile function. Although the exact repairing mechanisms are still in a major debate, numerous clinical trials have demonstrated favorable effects toward the use of autologous stem cells in AMI patients with considerably low side effects. Despite the relatively novel discovery, stem cell therapy offers a promising prospect to confer a better protection, prevent later complications, and perhaps reduce the mortality among patients with ischemic heart disease. This ultimate outcome would likely be achieved through a stringent and coordinated of either basic and clinical research.

Key words: stem cells, repair mechanisms, myocardial infarction, cardiomyocyte regeneration.

INTRODUCTION
Although significant progresses have been made in the treatment of coronary artery disease, there are still limitations possessed. As in the case of acute myocardial infarction (AMI), if not treated well, may cause necrosis of approximately one billion cardiomyocytes. This massive cell death is exacerbated by replacement of fibrous tissue in the necrotic area. The aftermath of these events are cavitary dilation and negative remodeling of left ventricle which further compromise cardiac contractile function that eventually ended by cardiac decompensation and terminal failure. Current therapies would allow for immediate reperfusion to the infarcted region, yet human heart has a limited ability to regenerate its own contractile cells. Although there is evidence for spontaneous regeneration after injury, the process are insignificant to be seen clinically.

Obviously, a spectrum of therapeutic strategies which are able to restore blood supply to the ischemic area as well as regenerating the infarcted heart is required. Stem cell therapy could become one of the answers. Stem cells are a cluster of cells that is characterized by their clonogenicity, self-renewal, and ability to differentiate into multiple cell lineages. There is convincing evidence that supports the ability of various types of stem cells in regenerating damaged myocardium after infarction. According to their origin, stem cells can be classified into two categories: embryonic stem cells (ESCs) and adult stem cells. ESCs are derived from the inner cell mass of an embryo during blastocyst phase. This type of cell retains its pluripotency, therefore, can give rise to endodermal, mesodermal, and ectodermal layers. On
the other hand, adult stem cells have their origin from postnatal somatic tissues, thus limiting their differentiation capacity to only multiple cell lineages (multipotent). However, there are considerable evidence which elucidated their capacity to transdifferentiate into distinct lineage (so called plasticity). This phenomenon also happens in the heart, in which, for instance, hematopoetic stem cells (HSCs) have been shown to transdifferentiate into functional cardiomyocyte (discussed later).

Due to the remarkable capacity of stem cells in regenerating the heart, we decided to review this topic in a concise, but comprehensive and deliberate manner. Several points will be elaborated, including current classification of stem cell type mostly used for regenerating the heart based on phenotype characterization, functional capacities, and their special characteristics, accompanied by the potential repair mechanism at a molecular extent, supported by practical evidence from clinical trials, and ultimately discussion related to current obstacles and direction of future research.

**Classification of Stem Cell Types Commonly Used for Heart Regeneration**

**Bone Marrow-derived or Circulating Blood-derived Progenitor Cells**

Cells isolated from bone marrow (BMCs) were shown to effectively improve cardiac function as reported by Orlic et al., in which intramyocardial injection of BMCs into the contracting wall bordering the infarcted heart of mice model resulted in 68 percent replacement with newly formed myocardium. Another study reported an alternative way of repair by BMCs, that is enhancing the neovascularization process thereby preventing myocytes apoptosis, reduces negative remodeling, and improves contractile function. Both mechanisms are likely to be true since unfractonated BMCs contain heterogenous stem cell populations, including: hematopoetic stem cells (HSCs), mesenchymal stem cells (MSCs), and endothelial progenitor cells (EPCs). Circulating blood-derived progenitor cells (CPCs) are basically similar to BMCs. The typical multipotent stem cells found in BMCs also exist in CPCs with EPCs predominate (more than 90% shows endothelial characteristics). This similarity had inspired scientists to compare BMCs versus CPCs efficacies, thence if none has proven to be superior to one another (as it does), CPCs are favored because of its aspiration feasibility.

**Hematopoetic Stem Cells**

HSCs are known to express c-Kit and CD133 surface antigen early in their formation which later permanently positive for CD33 and CD34. HSCs have been reported to transdifferentiate into cardiomyocytes by Jackson et al. They had eliminated BMCs population in mice model through irradiation and induced myocardial infarction then subsequently administer the isolated HSCs from transgenic donor expressing β-galactosidase (LacZ). As a result, there appeared to be LacZ-positive cells in the infarcted myocardium and endothelial cells (0.02% and 3.3% respectively). These findings suggest trans-differentiation of HSCs into cardiomyocytes and endothelial cells. The fact is strengthened by a report of extensive transdifferentiation of c-Kit+ BMCs into functional cardiomyocytes, replacing the old infarcted one. This further will be assured by lineage tracing and intravital imaging protocols to exclude cell fusion as the principal mechanism.

However, several studies did not find the evidence of HSCs transdifferentiation into cardiomyocytes as described through lineage tracing, intravital imaging, and functional characteristics. Other researchers have concluded that transdifferentiation is merely an overlooked phenomenon and HSCs incorporation into the infarcted myocardium could be explained by cell fusion (discussed later). Apart from these discrepancies, HSCs autologous transplantation have been proven to have cardioprotective effects in the event of coronary artery disease, both in vitro and in vivo.

**Mesenchymal Stem Cells**

MSCs are also identified in unselected BMCs. These group of cells express multiple surface markers, including CD29, CD44, CD71, CD90, CD106, CD120a, and CD124 but negative for CD34 and CD133. MSCs concentration is 10-fold lower when compared to HSCs. The usual MSCs capacity to give rise mesodermal layers is seen potential to differentiate into mature functioning cardiomyocytes. Indeed, exposure of murine MSCs to a certain DNA methylation inhibitor could promote MSCs differentiation into beating cardiomyocytes with typical phenotype identities, comprising positive RT-PCR for atrial natriuretic peptide (ANP), myosin light chain-2a (MLC2a) and -2v (MLC2v), GATA4, and Nkx2.5 which are all expressed in human cardiomyocytes. Other studies also confirmed the differentiation event of MSCs into cardiomyocyte-like cells after injected into healthy as well as infarcted myocardium.

The
most obvious clinical improvements enhanced regional wall motion and remodeling prevention in the vicinity of noninfarcted myocardium. However, the extent of differentiation activity is considered very low and inefficient. Shiota et al. have extracted MSCs via certain culturing processes and transplant it into murine infarct model. The result was good enough as marked by improved functionality despite a very low degree of donor cells incorporation (only 7 MSC-derived cardiomyocytes per heart). The fact was confirmed by Shim et al. in which they found a very low degree of cardiogenesis derived from human MSCs. The ability of MSCs to improve cardiac function clinically without adequate evidence of remuscularization have brought an idea of indirect pathway through paracrine mechanism. Furthermore, MSCs have been reported to have a low immunogenicity along with its ability to mediate immunomodulatory actions, thus allowing for a safer allogenic transplantation to be performed.  

**Endothelial Progenitor Cells**

EPCs were firstly isolated from human peripheral blood by Asahara et al in 1997. Ever since, EPCs were applied in various studies and exhibited pro-neovasculogenic function and capable of increasing perfusion into ischemic tissue. There are two types of EPCs. The former one which is discovered by Asahara also called “early EPCs” or endothelial colony-forming units (CFU-ECs). CFU-ECs were reported to be hematopoietic-derived progeny, therefore displaying characteristics of myeloid lineage rather than true EPCs. It expresses several surface antigens, e.g. CD31, CD34, CD45, vWF, VEGFR2 (KDR), and showed positive uptake of diL-AcLDL. Despite having characteristics of EPCs (expressing vWF, KDR, and able to uptake AcLDL), CFU-ECs were unable to form tube-like formation in Matrigel and only able to do so during co-culture with HUVEC. In Matrigel, CFU-ECs can only elongate its cytoplasm and form spindle-shape morphology, while upon HUVEC exposure, CFU-ECs only intervened around it. Moreover, CFU-ECs are capable of ingesting bacteria, similar to macrophages. This is perhaps because of same lineage shared by these cells as shown by CD14+ (monocyte/macrophage cell surface antigen).  

ECFCs appeared 2 to 4 weeks in the same culture with CFU-ECs and exhibited smooth cytoplasmic architectural with firm attachment in the plate. During growth period, it also forms cobblestone appearance – the characteristic morphology of endothelial cells. ECFCs express several phenotype markers, including CD31, CD105, CD144, CD146 but neither express CD14 nor CD45. ECFCs exhibited strong expression of endothelial genes, like VE-cadherin, Flt-1, KDR, eNOS, and vWF. Upon fluorescence intensity measurement, ECFCs were observed brighter than CFU-ECs, suggesting richer nitric oxide production which is the characteristic of endothelial cells. Ultimately, ECFCs forms tube-like formation, either in Matrigel or in HUVEC coculture. They were incorporated into it, instead of intervened.

However, during transplantation into hindlimb ischemia model, both cell types improve perfusion into ischemic area close to normal state. Another study had transplanted CFU-ECs and ECFCs independently to mice model before extracted and stained for human anti-CD31. In mice treated with ECFCs, there existed human CD31+ cells around the capillaries which determined ECFCs ability to participate in blood vessel formation. In contrast, CFU-ECs failed to do this. The fact that CFU-ECs and ECFCs are not clonally related have dramatic implications in which these two cells exhibited different phenotype and characteristics. Even though CFU-ECs cannot form endothelial cells, their contribution to neovascularization is remarkable due to paracrine effects exerted. CFU-ECs exhibited higher VEGF and IL-8 concentration, cytokines which orchestrate majorly angiogenic processes. It is perhaps this mechanism that explains similar efficacies in clinical settings between the use of CFU-ECs or ECFCs for reperfusion therapy.

EPCs were also reported to transdifferentiate into cardiomyocytes. Badorff et al. extracted CD34 CD133 cells from healthy humans and co-culture it with rat cardiomyocytes. After 6 days, EPCs expressed cardiomyocyte proteins, including troponin I, ANP, after 6 days, EPCs expressed cardiomyocyte proteins, including troponin I, ANP, multiple sarcomeric actinin, and MEF-2. Further analysis of calcium transients exhibited periodic oscillations, similar in amplitude and duration with adjacent rat cardiomyocytes. Transdifferentiation pathway is assured by several studies, thus excluding cell fusion process. Yet, it occurred with very low efficiency (nearly <1% EPCs express α-sarcomeric actinin).
Another study did not confirm EPCs transdifferentiation into cardiomyocytes. According to their report, EPCs transdifferentiation was simply artifacts resulting from autofluorescence. Moreover, the EPCs definition that is used and applied for extraction in studies confirming EPCs transdifferentiation points to CFU-ECs of hematopoetic lineage which actually does not have capacities to be called “true EPCs”.41-43

**Skeletal Myoblasts**

Skeletal myoblasts are usually known as satellite cells. They are classified as progenitor cells which normally reside in basal lamina of muscle fibers. Myoblasts can be clonally expanded and differentiate into myotubes. When transplanted into infarcted myocardium, myotubes form will not be integrated but clustering in a specific foci.56 Myoblast transplantation in infarct model exhibited improvement in myocardial contractility either atrial or ventricular.

**Resident Cardiac Stem Cells**

The ability of the heart to repair itself after injury has suggested the existence of certain stem cell types which reside in the myocardium. Indeed, further investigations have revealed multiple resident cardiac stem cells (CSCs) in the human heart. Side population cells (SP) cells were firstly isolated by Hierlihy et al.57 Cells with SP phenotype comprise about 1% among mice cardiomyocytes. It has been shown that SP cells exhibited α-actinin after cocultured with mature cardiac myocytes. There are SP cells which express Sca-1 but CD31-. After coculture with rat ventricular myocytes, they were positive for cardiac-specific genes (Nkx2.5) but troponin I and connexin 43. Several studies have confirmed this finding with similar cardiomyocyte repopulation.61,62 However, cell fusion cannot be excluded in the later studies. Other type of CSCs termed cardiospheres was discovered. Cardiospheres resulted from mild enzymatic digestion of human cardiomyocytes that appear as rounded and phase bright cells which clustered in suspension. Cardiospheres exhibited stem cells characteristics (self-renewing and clonogenic) and expressed endothelial and stem cell markers (KDR, CD31 and CD34, c-Kit, Sca-1, respectively). These cells were positive for cardiac phenotypes like troponin I, ANP, and MHC. However, it did not contract spontaneously unless cocultured with rat cardiomyocytes. Cardiospheres were reported to differentiate into mature cardiomyocytes in infarcted mice model.

Due to small proportion of CSCs it is relatively difficult to expand these cells and apply it directly into clinical context. However, CSCs are undoubtedly more efficient and natural for cardiogenesis when compared to another cells derived from bone marrow directly or skeletal myoblasts that is non-heart origin.

**Pluripotent Stem Cells**

As explained before, embryonic stem cells (ESCs) are theoretically capable of differentiating into all cell types involved in 3 germ layers in vitro and in vivo. Indeed, ESCs were able to differentiate into cardiomyocytes (hESC-CMs) and exhibited firm cardiac phenotypes when assessed transcriptionally, immunocytochemically, ultrastructurally, and functionally. It displayed cardiac-specific transcription factors (Nkx2.5, GATA4, MEF2c), sarcomeric proteins (cardiac troponin I, T, MHCs, and actins), other cardiac-specific proteins (ANP, MLC2V, MLC2A), as well as spontaneous beating activity. However, the work of ESCs are hampered by ethical concerns besides higher risk of forming teratoma due to improper growth of undifferentiated cells upon intracardiac transplantation.

Recent cellular technology has allowed the discovery of several alternatives in generating pluripotent cells. Among these are spermatogonial stem cells (SGSC), parthenogenetic stem cell (PSC), and induced pluripotent stem cells (iPS). SGSC are derived from human testis and responsible for maintaining spermatogenesis. Recently, scientists have discovered that SGSC can transform into ESC-like cells under transplanted in vivo to a mouse infarct model, these cells migrated to the injured myocardium and differentiated into cardiomyocytes while expressing α-actin, cardiac troponin I, and connexin 43. Several studies also confirmed this finding with similar cardiomyocyte repopulation. However, cell fusion cannot be excluded in the later studies. Other type of CSCs termed cardiospheres was discovered. Cardiospheres resulted from mild enzymatic digestion of human cardiomyocytes that appear as rounded and phase bright cells which clustered in suspension. Cardiospheres exhibited stem cells characteristics (self-renewing and clonogenic) and expressed endothelial and stem cell markers (KDR, CD31 and CD34, c-Kit, Sca-1, respectively). These cells were positive for cardiac phenotypes like troponin I, ANP, and MHC. However, it did not contract spontaneously unless cocultured with rat cardiomyocytes. Cardiospheres were reported to differentiate into mature cardiomyocytes in infarcted mice model.

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Another type of CSCs was reported by Oh et al. It expressed Sca-1 antigen but c-Kit+. It was also positive for cardiac-specific genes (Nkx2.5) but exhibited no spontaneous beating activity. When
proper culture, thus able to give rise into multiple cell types, including cardiomyocytes.\textsuperscript{74,75} The same outcome may also be achieved through parthenogenesis of PSC that is available only in pre-menopausal women.\textsuperscript{76}

Alternatively, EPC-like cells can be generated by inducing typical mature cells with certain transcription factors (so called iPS). The four transcription factors were transduced into commonly differentiated human cells (e.g. skin fibroblasts) by viral vectors and allowed these cells to be reprogrammed across 3 germ layers, exactly similar to ESCs.\textsuperscript{77-79} Through this technology, ethical concerns due to embryonic manipulation and allogenic transplantation can be avoided besides reducing the risk of immunogenic rejection.\textsuperscript{8} Furthermore, iPS cells have been shown to be able to differentiate into cardiomyocytes.\textsuperscript{77,80} The cells positively expressed cardiac phenotypes, including Nkx2.5, GATA4, MEF2c, ANP, MLC2v, and MLC2a transcription factors, positive immunostain for sarcomeric MHC, and troponin C, and exhibited calcium transients upon field stimulation.\textsuperscript{80} iPS once had been worried for its safety aspects since it used lentiviral vectors to deliver the genetic materials needed for reprogramming the cells. However, the use of polycistronic retroviral vectors and non-viral vectors has been established very well.\textsuperscript{81-84}

**WORKING HYPOTHESES OF MYOCARDIAL REPAIR BY STEM CELLS AT THE MOLECULAR LEVEL**

The precise mechanism of stem cell therapy in improving heart function after injury is unknown yet. However, several theories have elaborated the plausible pathway in which stem cells work. Resident cardiac stem cells (CSCs) are known to proliferate soon after infarction and replace the dead myocardium with newly functional cardiomyocytes.\textsuperscript{54-56} However, the CSCs capacity to differentiate in adult hearts are limited.\textsuperscript{2} Yet this direct lineage differentiation is favorable to be developed scientifically either through genetic modifications\textsuperscript{85-87} or factor stimulations.\textsuperscript{88,89} Another evidence have shown that certain CD45\textsuperscript{+} CSCs were indirectly mobilized from bone marrow, soon after myocardial infarction to replace the resident CSCs in the heart.\textsuperscript{59}

A second pathway that potentially be involved during heart repair is stem cells mobilization from bone marrow. BMCs contain multiple progenitor cells, i.e. HSCs, MSCs, EPCs, and others. These cells were thought to migrate into injured heart and undergo transdifferentiation to become novel cardiomyocytes.\textsuperscript{11,12} Transdifferentiation means the multipotent stem cells will differentiate across the conventional lineage, tissue, and germ layer.\textsuperscript{7} This process has deviated the former view of hierarchical dogma, in which certain multipotent-type of cells can only give rise to similar differentiated cells.\textsuperscript{90,91}

However, transdifferentiation has been questioned by several studies and considered as an overlooked phenomena.\textsuperscript{82,93} The existence of a certain cell type with a specific stain in the responsible myocardium can be a product of two kinds of artifacts, autofluorescence due to label transfer to neighboring cells\textsuperscript{82-95} and/or cell fusion.\textsuperscript{22,55,96-98} The first artifact can happen if someone use cell lineage markers that can easily be exchanged between cultured cells or between donor and recipient cells following transplantation. This is common for chemical labels such as bromodeoxyuridine (BrdUrd), fluorescent tracker dyes, and fluorescent-labeled particles.\textsuperscript{93-95} This kind of artifact can be minimized by using indelible cell lineage markers (i.e. genetic markers), although transgene products can undergo exchange under certain circumstances.\textsuperscript{99,100}

Cell fusion is a process in which the donor and recipient cells merge together following in vivo transplantation.\textsuperscript{101,102} The fused cells contain genetic information from both of them. The cells may appear as binucleated, a mononucleated cell with tetraploid synkaryon, or a cell that is normal karyotypically if reductive division occurs.\textsuperscript{7,103} Oh et al\textsuperscript{15} had reported that cell fusion accounted for as much as 50\% of the differentiated cells that replace the damaged myocardium as shown by transgenic models. However, it does not exclude the possibility that these cells may have been differentiated before fused with the existing myocardium. A study by Kajstura et al\textsuperscript{104} exhibited an immature profile of the newly formed cardiomyocytes from bone marrow transplantation. The cells often loosely attached with the existing myocytes. This finding indicates that bone marrow cells transplanted formerly did not undergo cell fusion, but instead had transdifferentiated into novel cardiomyocytes and subsequently replaced the old infarcted myocardium. A continuing debate will likely exist until this process can be explained with other strategies.

Besides direct repair through differentiation and/or transdifferentiation, improved myocardial function can also be explained by paracrine mechanism.\textsuperscript{32,33,105}
Various sources of cellular origin and plausible mechanisms of cardiac repair by stem cells therapy. Cardiomyocytes in the infarcted region can be regenerated through differentiation of resident cardiac stem cells (rCSCs) which are occupied in the heart itself or by transdifferentiation of multiple cell types (e.g. bone marrow-derived progenitor cells, pluripotent stem cells, etc) after recruitment and mobilization to the injured site. However, this mechanism has been a major dispute since cell fusion phenomena and paracrine effects cannot be excluded and also exert favorable effects towards repair process. Abbreviation: BMCs., bone marrow-derived or circulating blood-derived progenitor cells; EPCs., endothelial progenitor cells; ESCs., embryonic stem cells; G-CSF., granulocyte-colony stimulating factor; HSCs., hematopoietic stem cells; iPS., induced pluripotent stem cells; MMP., matrix metalloproteinase; MSCs., mesenchymal stem cells; PSCs., parthenogenetic stem cells; rCSCs., resident cardiac stem cells; SGSCs., spermatogonial stem cells; STAT3., signal transducers and activators of transcription protein 3.
Paracrine signaling performed by several factors or cytokines can mobilize stem cells from their niches and directly home into the injured area. Among them, stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF) can stimulate myogenesis and angiogenesis in the infarcted region. G-CSF is also able to accelerate infarct healing through facilitating macrophage infiltration into the necrotic tissue as well as activating matrix metalloproteinase (MMP). It can suppress myocyte apoptosis by activating STAT3, a transcription factor that is responsible for inhibiting apoptosis. VEGF also plays an important role in angiogenesis. Systemic VEGF administration has led to increased EPCs proliferation, mobilization from bone marrow, and migration to the injured area thereby promoting neovascularization and improved perfusion. Stromal cell-derived factor (SDF-1) is also essential in mobilizing stem cells from bone marrow to vascular zone as well as promoting their proliferation and angiogenic capacity.

Given these evidences therefore, it is unsurprising if transplantation of unselected BMCs can improve myocardial function dramatically. The process thus involving myocardium contractility improvement through direct differentiation and/or transdifferentiation of stem cells to generate newly functional cardiomyocytes as well as improve neovascularization through a complex paracrine signaling and EPCs differentiation into mature endothelial cells.

CLINICAL TRIAL EVIDENCE

To date, numerous human clinical trials have been conducted in order to assess the efficacy and safety aspects of stem cell therapy for the heart. However, a major portion of the study used BMC (either fractionated or not) and/or CPC with several studies applied skeletal myoblasts. To our knowledge, there is no clinical trial which assesses similar parameters by using resident CSC yet. This happened apparently because of difficulties in cell expansion, limited numbers of CSC in the heart, and existing uncertainties regarding CSC phenotypes with the optimum quality for treating patients with coronary artery disease. Whereas BMC or CPC (especially unfractionated) is preferred because they can readily be aspirated and administered with minimal manipulation.

Therapeutic Efficacies

The TOPCARE-AMI trial used unfractionated BMC versus CPC with 90% EPCs composition compared with placebo in post-AMI patients. Because autologous CPC required 3 day-culture period, the therapy was applied on average of 4 days post-AMI. Cells were infused via intracoronary route with stop-flow balloon catheter technique, i.e. low pressure inflation of balloon catheter to achieve complete blood flow occlusion in 3 minutes followed by progenitor cell infuision and subsequent reflow through deflation. This procedure is commonly performed in order to maximize cell migration and adhesion. At 4-month follow-up, a significant improvement in global left ventricular ejection fraction (LVEF) and regional wall motion in the infarct zone can be observed among patients receiving BMC and/or CPC versus control. Whereas differences between two groups can be omitted. Global LVEF and regional wall motion increased up to 9 and 1.2 percentage points in both treatment groups from baseline to follow-up, respectively. Of note, regional wall motion in the infarct border zone was found to improve most. Similar findings were observed in the BOOST trial. This randomized study involved 60 patients either received mononucleated BMC on average of 4.8 days after percutaneous coronary intervention (PCI) or standard therapy as the control group. The results were improvement in regional LV contractility (around infarct zone) and global LVEF increment as much as 6 percentage points compared to control during 6-month follow-up. However, this result cannot be sustained after 18 and 61 months later (LVEF differences decreased to 2.8 and 0.8 percentage points, respectively with statistical insignificance). Seemingly, patients with more extensive infarct and worse cardiac contractility (severely depressed LVEF) benefit most from stem cell therapy and sustained these favorable effects longer than any other groups.

The current biggest trial – REPAIR-AMI trial which involved 204 patients randomized to receive either BMC or placebo after PCI also concluded the same notion with the former studies, i.e. LVEF increment by 2.5 percentage points compared with placebo during 4-month follow-up. Further analysis of 54 patients who underwent serial MRI investigations revealed the sustained LVEF percentage point-difference of 2.8 between treated and control group at 12-month follow-up period. Another study with similar number of AMI patients – the HEBE trial – assigned randomly 200 subjects with either BMC or CPC transfusion versus control after PCI with stent placement. Cell transfusion was given intracoronarily and clinical outcomes were measured using MRI, both at baseline and 4-month follow-up. Final results of HEBE trial were presented at AHA.
meeting 2008 which showed no improvement on global or regional left ventricular systolic function. However, a post-hoc analysis concluded that patients with initial dilated left ventricle experienced no further dilation after treatment.

Different results were obtained from ASTAMI trial. In this study, 100 patients were randomly assigned to receive BMC or placebo after undergoing PCI 6 days before. Cardiac functions (LVEF, LV volumes, and infarct size) were measured by using single-photon emission CT (SPECT), echocardiography, and MRI. At 6- and 12-month follow-up period, no significant improvement was observed regarding LVEF, LV volumes and infarct size. Two factors might account for this difference, i.e. investigators in ASTAMI trial performed different cell isolation protocol (Lymphoprep, storage in NaCl with plasma) when compared to another study (REPAIR-AMI, Ficoll, storage in X-vivo 10 medium with serum). Moreover, ASTAMI trial infused fewer cells than those in the REPAIR-AMI trial (68 x 10^6 vs. 198 x 10^6 cells). The cell isolation protocol used in ASTAMI trial has been shown to have impaired function either in vitro (low migratory and colony forming abilities) or in vivo (unable to reperfuse blood flow in an ischemic model). However, Leuven AMI trial also found similar results, i.e. no LVEF improvement when assessed by MRI during 4-day and 4-month follow up, respectively. The study design and instruments were similar to others except that BMC was transplanted within 24 hours after PCI treatment. The timing factor is essential since BMC transfusion during 24 hours post-AMI was proven inferior to those administered in 4 to 7 days post-AMI in the context of LVEF improvement, decreased LV end-diastolic and end-systolic dimensions, and decreased incidence of restenosis.

The efficacy of BMC and/or CPC therapy was also evaluated for chronic heart failure. The increased prevalence of the disease is believed to be in part due to increase in ischemic heart disease incidence. The heart after infarction may form scar tissues, lose its vascularization and undergo negative remodeling. Indeed, TOPCARE-CHD trial tried to compare BMC and CPC efficacy in ischemic heart failure patients. This randomized controlled-crossover study involved 75 patients with minimally 3 months after AMI divided into three groups, i.e. BMC, CPC, or placebo. The treated groups were subsequently exchanged after 3 months’ follow-up. The cells were transfused into the coronary artery supplying the most dyskinetic left ventricular region. At 3-month follow-up, patients receiving BMC were shown to have LVEF improvement by 2.9 percentage points, superior to CPC (-1.4 percentage points) and control (-1.2 percentage points) groups. BMC group also achieved better regional contractility (0.26 percentage points) compared to other groups (CPC and control; -0.03 and -0.06, respectively – insignificant).

Skeletal myoblast can be used as an alternative cellular source for chronic heart failure (HF) and being the first to undergo clinical trial in these patients. Several study groups have successfully isolated and expanded myoblast from skeletal biopsies. The first RCT that tested skeletal myoblast in HF patients – MAGIC trial involved 97 subjects that received transepicardial injections containing myoblast or placebo during CABG surgery. There was no significant difference between control and treated groups regarding regional or global LVEF improvement. However, LV end-diastolic and end-systolic volumes were significantly reduced in the treated group. Myoblast transplantation has been shown to have unique favorable effects. Its ability to tolerate hypoxia is theoretically suitable for HF patients in which loss of vascularization becomes one of the major issues. Moreover, myoblasts are able to release paracrine factors to induce resident CSC differentiation into mature functioning cardiomyocytes despite its inability to stimulate angiogenesis locally.

### Adverse Effects

To date, the most notable risk which might be taken into account is in-stent restenosis after BMC therapy. In TOPCARE-AMI study, one patient experienced in-stent thrombosis either in the target vessel or in an unrelated coronary artery after BMC infusion, which was subsequently followed by cardiogenic shock two days later. Although patient’s intrinsic factors play the major role towards in-stent thrombosis (it was discovered later that this patient acquired genetically severe anti-thrombin III deficiency), cell therapy might become the inducer as well. However, there is no increased risk of in-stent restenosis globally as has been reported by FINCELL study which had evaluated BMC recipients with intravascular ultrasonography 6 months after therapy (Table 1). This notion has also been confirmed by three meta-analyses. Intramyocardial calcification was reported after BMC injection in rats with acute AMI, yet there is no evidence found in human related to this case. So far, none of the trials has reported an
increased risk of arrhythmias post–BMC therapy. This fact has been investigated by BOOST trial\textsuperscript{119} through Holter monitoring method (principally used to identify the frequency premature ventricular beats and non-sustained ventricular tachycardia) and FINCELL trial\textsuperscript{142} through microvolt T-wave alternans, Holter monitoring, and signal-averaged electrocardiography evaluations. Ultimately, BMC therapy did not induce inflammatory proteins (troponin T, c-reactive protein) or leukocyte blood count elevation, indicating that this therapy itself did not inflict further ischemic damage.

In patients who received skeletal myoblasts therapy there is a serious concern related to a greater incidence of arrhythmias in this group. In the early nonrandomized trial, 4 out of 10 patients that had received myoblast injections during CABG surgery experienced sustained ventricular tachycardias from 11 to 22 days after the procedure.\textsuperscript{134} Another clinical studies that used skeletal myoblasts also report the same adverse effects.\textsuperscript{148,149} In one report involving 5 patients with end-stage HF underwent LV assist device implantation with concomitant myoblasts injections, 2 of these patients developed ventricular tachycardias immediately after surgery, despite the fact that one of them had a previous history of arrhythmias.\textsuperscript{136} These arrhythmias can be fatal as marked by two sudden deaths after transedocardial myoblasts therapies.\textsuperscript{137} Increased incidence of

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**Table 1. Several randomized-controlled trials assessing the effects of BMC therapy after AMI**

<table>
<thead>
<tr>
<th>Trial name (participant number)</th>
<th>Type of cell used (dose)</th>
<th>Onset of delivery</th>
<th>Type of reperfusion therapy</th>
<th>Route of delivery</th>
<th>Evaluation procedure [time]</th>
<th>Clinical outcome (treatment vs. control)</th>
<th>Safety profile</th>
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| TOPCARE-AMI (20)\textsuperscript{36} | Circulating blood-derived progenitor cells/CPCs CD34+ /CD45+ (7.35±7.31x10\textsuperscript{6}) | 4 days | PCI stent | i.c. with stop-flow method | LV angiography, stress echocardiograph, intracoronary Doppler for coronary flow reserve, FDG-PET | Global LVEF ↑ (baseline 51.6±9.6% to 60.1±8.6%), regional wall motion in the infarct zone ↑ (-1.5±0.2 to -0.5±0.7SD/obj), LVEFv (baseline 56.1±20 mL to 42.2±15.1 mL) | • The procedure did not inflict further cardiac damage (as shown by persisted CRP level, leukocyte blood count and troponin T↑).
• One patient in the treatment group suffered restenosis 3 days after procedure.
• No arrhythmia or heart failure detected among patients from both groups. |
| BOOST (60)\textsuperscript{108,121} | 128 ml bone marrow aspirate (CD34+) | 5.1±1.3 days | PCI stent | i.c. with stop-flow method | Cardiac MRI (contrast enhanced), Holter recordings [6, 18, and 61 months, respectively] | Global LVEF ↑ (6.7% at 6 months, 5.9% at 18 months, -2.5% at 61 months), systolic wall motion in the infarct zone ↑ | • The procedure did not inflict further cardiac damage (as shown by no troponin T increment 24h after procedure).
• No adverse events, restenosis, or arrhythmia were observed. |
| FINCELL (77)\textsuperscript{142} | 360 x 10\textsuperscript{6} and 2.6 x 10\textsuperscript{6} (mononuclear and CD34+ cells, respectively) | 2 to 6 days | Thrombolytic therapy with subsequent PCI DES-coated stent | i.c. with stop-flow method | Transthoracic LV echocardiograph, LV angiograph, IVUS, Holter monitoring, microvolt T-waves alternans, signal-averaged ECG [6 months] | Global LVEF ↑ (7.1±2.3 % vs. 1.2±11.5%) | • 3 patients experienced mild vasovagal reactions during bone marrow aspiration.
• 1 patient had failed blood re-flow after PCI stenting.
• No difference when compared with control group regarding adverse events and malignant arrhythmia. |
| REPAIR-AMI (187)\textsuperscript{122} | 236±174 x 10\textsuperscript{6} and 48±24 x 10\textsuperscript{6} (mononuclear and CD34+ cells, respectively) | 3 to 7 days | PCI stent | i.c. | LV angiography [4 months] | Global LVEF ↑ (5.5±7.3% vs. 3.0±6.5%), combined clinical end point of death, recurrent MI, or revascularization procedure at 1 year-period | • The incidence of arrhythmia or syncope were indifferent among both groups. |

**Abbreviations:** DES., drug eluting stent; ECG., electrocardiogram; FDG-PET., F-18-fluorodeoxyglucose–positron emission tomography; i.c., intracoronary; IVUS., intravascular
arrhythmias was also confirmed in the MAGIC trial.\textsuperscript{138} The pathogenesis of myoblast-induced arrhythmias is unknown but perhaps due to the independent myoblasts contractions that are not synchronized with the corresponding myocardial contractility.\textsuperscript{150} The ability of myoblasts to generate its own action potentials may induce life-threatening extrasystoles via electrotonic interaction.\textsuperscript{140,151} This might happen considering the electromechanically nature of myoblasts that are not incorporated to the surrounding myocardium and accompanied by the formation of cell cluster (instead of dispersing) in a certain infarct region.\textsuperscript{36,152,153} A novel delivery technique that allows a more dispersed and even myoblasts distribution might become the solution besides benefit-to-risk assessment when applied to certain patient’s conditions.\textsuperscript{150}

Current Limitations and Future Directions

The heterogeneity of research results either basic or clinical studies emphasize the need of universal protocols that cover the most reliable scientific sources and subsequent integration into one package of standardized procedure. This is especially critical for cell isolation and expansion protocols (mainly for CSC) in which considerable variations of techniques and results were obtained, as well as a uniform convention of cellular phenotypes in order to define a precise nomenclature of each stem and progenitor cells thereby reducing the existed scientific bias. In the case of variable clinical results despite apparent similar cell isolation and expansion protocols in several trials necessitates an effort to evaluate cell quality and functionality in clinical settings. Indeed several studies have elucidated the impact of several factors (e.g. diseases, medications) to endogenous stem cells qualities and functions.\textsuperscript{153-160} Therefore, determining these components may lead to a better selection of cell types and help to estimate prognosis after therapy.

Current trials have not assessed the impact of cell therapy in a quite long period. Most of them only conducted follow-up study for as long as 6 month-period with exceptions to the BOOST,\textsuperscript{119-121} ASTAMI,\textsuperscript{126,127} and REPAIR-AMI\textsuperscript{122} trials (up to 5 years, both 12 months, respectively), suggesting the need to conduct a longer follow-up period. Furthermore, there are limited data regarding clinical outcomes other than global and regional ventricular functions. The overall efficacy of cell therapy on complications, functional performance, and mortality should be evaluated immediately considering a great deal of trials have been done to date. Ultimately, the second-generation trials should address in details regarding the timing of cell transfer, utilization of certain specified cell types, and dose of therapy. These investigations should be accompanied by a more effective and proper mode of cell delivery (e.g. improvements in needle model for intramyocardial injection which promote cell dispersion and limit immediate washout)\textsuperscript{161-164} as well as the use of a detailed imaging technique that facilitates a better characterization of target organs thereby improving cell delivery.\textsuperscript{164,165} In addition, proper imaging techniques are required to be regulated so that specific clinical results can be evaluated in a uniformed manner (e.g. the use of MRI instead of angiography or echocardiography for assessing LV dimensions and systolic function).\textsuperscript{1}

CONCLUSION

After more than a decade of discovery, stem cell therapy has progressed significantly in the area of understanding and direct applications to the troubled human hearts. Even though its mechanistic insights and precise functions of each cell types have not been elaborated completely, the therapeutic significance of stem cells has far exceeded the corresponding scientific knowledge. However, still plenty of information should be elucidated before stem cell therapy can exert its optimum effects in the treatment of coronary artery disease. In the future, stem cell technology can become an important adjuvant therapy in the treatment of various heart diseases. This ultimate outcome is likely to be achieved with a continuous coordination as well as persevered basic and clinical research.

REFERENCES

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