# Stem Cell Therapy for Acute Myocardial Infarction and Heart Failure- Past, Present and Future

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## Abstract

Heart failure (HF) after acute myocardiac infraction (AMI) is becoming a major problem in Taiwan. Because of the increase of the aged population in Taiwan, many of whom survive AMI, the country spends much money on the health care of these patients suffering from ischemic heart failure. In recent decades, in addition to conventional therapies with β-blockers and angiotensin-converting enzyme inhibitors (ACEIs), percutaneous transluminal coronary angioplasty (PTCA) and stenting, or surgical therapies with left ventricular assist device (LVAD), coronary artery bypass grafting (CABG) and ultimately cardiac transplantation, stem cell therapy has emerged to be a promising therapeutic option as the biologic characteristics of various stem cells have been more clearly elucidated and much progress has been made in the field of regenerative medicine. In this article, we provide internists a brief review of the literature regarding the background of stem cell therapy, the proposed mechanisms, and the cell types that have been studied for the potential use of cellular therapy, both in animals and humans, as well as problems and future prospects for stem cell therapy. Several clinical trials of cornerstone significance are also reviewed. ( J Intern Med Taiwan 2009; 20: 1-18 )

Key Words： Stem cells, Acute myocardial infarction, Heart failure, Pluripotent, Transdifferentiation, Paracrine effect

## Introduction

Myocardial infarction（MI）is the leading cause of congestive heart failure（CHF）and death in most developed countries[1]. Many patients with acute MI have been saved by recent treatment advances, but many of these same patients then suffer from ischemic heart failure. Heart transplantation provides hope for those patients with end-stage heart failure who have failed to respond to conventional therapies. Yet, a shortage of donors and the potential of post-operative complications, such as transplant vasculopathy and allograft rejection, limit the feasibility of heart transplantation[2]. Because of the increasing age of the Taiwanese population, enormous health care expenditures are consumed by aged patients with

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chronic ischemic cardiomyopathy; as such, finding a means to treat ischemic heart failure would not only benefit the patient but would also portend economic benefits. With research advances in stem cell biology and regenerative medicine, including tissue engineering and cell therapy, it is hoped that in the near future stem cell transplantation will be a promising treatment modality for patients with ischemic heart failure.

Pathophysilogically, post-MI heart failure is characterized by irreversible loss of cardiomyocytes which leads to progressive functional deterioration. Cardiomyoplasty, a procedure in which proliferating and functional cardiomyocytes are supplied via cell-based therapies to the injured myocardium, which then improves vascular supply and augments the myocardium's contractile function, is highly appealing as a new therapy. It is hoped that stem cell transplantation, by providing a potential source of new cells with its multipotent characteristics, will hold promise for patients with ischemic heart failure. The present review is aimed to increase clinicians' understanding of stem cells and their therapeutic potential for cardiovascular disease, with particular focus on MI and post-MI heart failure. We describe first the biological properties of stem cells and the background of stem cell therapy. Next, we describe the cell types used in stem cell therapy research as well as proposed therapeutic mechanisms, followed by a review of recent progress in animal and human clinical trials. Finally we outline continuing challenges and prospects for the future.

Table 1: Stem cell potency and cell source

| Potency | Cell source |
|---|---|
| Totipotent | ■ Fertilized oocytes. |
| | ■ Blastomeres of embryos at stage 2 through 8. |
| Pluripotent | ■ Embryonic stem cells. |
| | ■ Embryonic germ cells. |
| | ■ Embryonic carcinoma cells. |
| Multipotent | ■ Hematopoietic stem cells. |
| | ■ Mesenchymal stem cells. |
| Unipotent | ■ Skeletal myoblasts. |

## Biological properties of stem cells

Stem cells, regardless of their source, have three common defining characteristics[3]: 1.Self-renewing: the ability to divide indefinitely. 2. Potent: the ability to differentiate into one or more cell types. 3. Clonogenic: the ability to generate an exact duplicate.

According to their potential for differentiation, stem cells are classified into four groups: totipotent, pluripotent, multipotent and unipotent (Table 1). Totipotent stem cells can differentiate into cells of all three germ layers and trophoblasts and ultimately to a living organism. Pluripotent stem cells can generate cells of all three germ layers but not trophoblasts. Three types of pluripotent stem cells can be derived from mammalian embryos: embryonic stem (ES) cells, embryonic germ (EG) cells and embryonic carcinoma (EC) cells. Multipotent stem cells can generate cells of different lineage within one germ layer. Hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) are the best known multipotent stem cells[4]. Unipotent stem cells are tissue-specific progenitors that, after a few divisions, can differentiate into specific tissue types, and then become structurally and functionally integrated part of the specific tissue.

## Background of stem cell therapy

Adult tissues such as bone marrow, peripheral blood, fat, skeletal muscle, skin, liver, even brain and heart had been reported to harbor stem or progenitor cells with the capability to trans-differentiate[5]. Human cell therapy with bone marrow-derived HSCs in order to reconstitute the hematopoietic system of a myeloablated host has been employed for more than 3 decades[6]. On the

other hand, cell therapy for cardiac diseases has been delayed until this decade; recent researches showing myocardial regeneration by intrinsic cardiac stem cells (CSCs) and bone marrow-derived stem cells (BMSCs) revised the concept of the heart as an organ of terminal differentiation[7]. As early as 1994, a research team reported that myocyte nuclear and possible cellular hyperplasia contribute to ventricular remodeling in the hypertrophied senescent heart in humans[8]. By studying pathologic anatomy, a group of researchers demonstrated that the adult human myocardium maintained some degree of cellular proliferating ability: 0.08% of the myocytes found in infarcted heart[9] and 0.015% of those found in failing heart[10,11,12] were demonstrated to be on mitosis. These findings of cycling myocytes undergoing mitosis in both physiologic and pathologic conditions, combined with the finding of chimerism of the transplanted heart in cases of sex-mismatched cardiac transplantation[13], raised the possibility of a population of stem cells or cardiac progenitor cells (CPCs) , either resident in the mammalian heart or recruited from the circulation, from which new myocytes and vascular cells could be derived[14]. In 2002, the first identification of a stem-cell like cell population in the adult heart was reported by Hierlihy et.al.[15]. Based on immunohistochemical analysis, these cells were found to be capable of differentiating into cardiomyocytes in vitro. In 2003, Beltrami and colleagues reported that the heart contains a c-kit+ cell population which was clonogenic and able to undergo self renewal and differentiation into cardiac cell lineages in vitro. When injected into the infarcted border of a syngenic rat, these cells can regenerate more than 50% of the contractile myocytes and vascular cells[16]. In the years following, many studies identified populations of intrinsic cardiac stem cells in the adult heart, based on analysis of cell markers such as c-kit, Sca-1, Abcg2 and isl1+[17-20]. In animal models, these CSCs, when injected intravascularly, were shown to home to the injured myocardium, differentiate into cardiomyocytes, and improve cardiac function. Although the origin of these cells remains debatable, the concept that the heart contains stem cells which play important roles for maintenance of myocardial homeostasis, or repair of the damaged myocardium, opens the door for further studies of stem cell therapy for cardiovascular diseases. The findings from animal studies that BMSCs can be mobilized to home to the injured myocardium and participate in myocardial regeneration after MI have encouraged much research in the field of cell-based therapy. The recruitment of BMSCs by dystrophic cardiac muscle was first reported in 1999 in the study of sex-mismatched bone marrow transplantation in female dystrophic mdx mice[21]. In the following years, several studies had demonstrated that BMSCs had the ability to home to areas of myocardial injury as early as 3 days after MI[22] and to participate in cardiac repair[23]. Side population cells from bone marrow were reported to undergo cardiomyogenic differentiation and angiogenesis after MI[24], and regenerate as much as 68% infarcted myocardium[25] when they were injected into the border zone of an acute infarct. In summary, much current research suggests that injured myocardium may served as a stimulant, recruiting BMSCs or CSCs, to home to the injured site and participate in myocardial repair and regeneration. Based on these research findings, stem cell therapy has emerged to be a novel and promising therapeutic modality for cardiovascular disease, especially for patients with MI and post-MI heart failure.

## Cell types

In the past 2 decades, several cell types have been used in animal studies of cellular therapy (Table 2). Among them, only skeletal myoblasts and bone marrow-derived stem cells have been used in

Table 2: Comparison of stem cell types

| | Donor supply | Allogenicity | Oncogenicity | Ethical concern | Clinical trials |
|---|---|---|---|---|---|
| Embryonic stem cells | ＋ | ＋ | ＋ | ＋ | No |
| Fetal cardiomyocytes | ＋ | ＋ | － | ＋ | No |
| Skeletal myoblasts | － | － | － | － | Yes |
| Bone marrow-derived stem cells (BMSCs) | － | － | － | － | Yes |
| Resident cardiac stem cells | ＋－ | － | Unknown | － | No |

－:Negligible.

＋－: Mild concern.

＋: Major concern.

human clinical trials. The cell types used in animal studies of cellular therapy include the following:

1.Embryonic stem cells (ESCs) : Embryonic stem cells are pluripotent cells that can give rise to cell types of all three germ layers. They were first isolated by in vitro culture of cells derived from the inner cell mass of blastocysts in mouse embryo in 1981[26]. Embryonic stem cells can readily differentiate into all cell types in the body including cardiomyocytes. Several different ESC lines have been manipulated to produce cardiomyocytes and stable intracardiac grafts when transplanted to mice with experimental myocardial infarction[27]. These cardiomyocytes respond well to $\beta$-adrenergic stimulation in vitro[28]. Cardiomyocytes can also be derived from a human ESC line[29] which was first isolated from human embryo at the blastocyst stage in 1998[30]. When human ESC-derived cardiomyocytes were tested in vivo in a porcine model with conduction block, they were observed to integrate electromechanically into the myocardium and reveal pacemaker activity[31]. This finding raised the possibility of biological pacemaker which could be derived from human ESCs. Several researchers have reported that the transplantation of ESC-derived cardiomyocytes in both rodent[32] and sheep[33] models of myocardial infarction improved ventricular function by creating new myocardial tissue.

Nonetheless, several problems must be overcome before initiating the clinical use of ESC for cellular therapy. First, the allogenic origin of ESC raises concern about allograft rejection and the need for immunosuppressive agents. Second, the inherent characteristic of ESC is the ability to form teratoma before they differentiate. Although the teratogenicity is lost with differentiation into more specified cell types, such as cardiomyocytes[34], the formation of teratoma raises concerns about ESCs' malignant potential. Third, ethical considerations prevent the large scale clinical use of human ESCs, because an embryo must be sacrificed. Recent progress, which is encouraging, has demonstrated that by *in vitro* reprogramming of fibroblasts, a pluripotent ESC-like cell line can be produced[35]. This reprogramming provides the opportunity to obtain ESC from a human's own somatic cells, which eliminates the need to destroy an embryo and the use of immunosuppressive agents.

2.Fetal cardiomyocytes: Fetal cardiomyocytes are derived from fetal heart and are grown by tissue culture. They are not true stem cells because they are already differentiated[3]. These cells can proliferate in utero and have the potential to mature into cardiomyocytes. Due to this biological feature, it was hope that fetal cardiomyocytes would help augment cardiac function in the jeopardized myocardium. In animal studies, fetal cardiomyocytes have been transplanted by direct epicardial injection into a rodent myocardium with experimental MI.

Research has shown the possibility of successful engraftment of transplanted cells to host myocardium, with the presence of intercalated discs and intercellular connections[36], resulting in increased ejection fraction and decreased dyskinesis[37-38]. The above-mentioned engrafted fetal cardiomyocytes can survive for at least 6 months after transplantation. But like ESCs, the use of fetal cardiomyocytes has been hindered by their strong immunogenicity and the ethical considerations inherent in their use.

3.Skeletal myoblasts: Skeletal myoblasts, also called satellite cells, are cells that normally reside under the basement membrane of skeletal muscle[3]. They are actually early-committed muscle cells instead of true stem cells[39]. Physiologically, they can respond to injury by proliferating and then fusing with other cells[40] to repair damaged muscle tissue. Skeletal myoblasts were the first cells to be studied in animal experiments and clinical trials because of their autologous origin, high proliferative ability and resistance to ischemia. In animal studies of heart failure, transplanted skeletal myoblasts have been demonstrated to differentiate into muscle cells and engraft to the host myocardium with resulting functional improvement[41-44]. A recent study of pacing-induced canine heart failure model showed attenuation of myocardial remodeling with significant reduction of myocardial fibrosis and apoptosis, as well as improvement of left ventricular ejection fraction[45]. However, after further analysis, it was found that the engrafted cells did not transdifferentiate into cardiomyocytes[46] and couple electromechanically with the host myocardium[47]. The electromechanical instability raises the risk of ventricular arrhythmia which had been reported both in vitro and in vivo in the past few years[48-50]. The incidence of arrhythmia is closely related to the following two factors. Concerning the numbers of engrafted myoblasts, it has been demonstrated that reentry was not induced in cocultures containing few myoblasts (1% to 5 %) , but when the number of myoblasts exceeded 20%, inducible reentry occurred consistently[48]. According to research in an animal model comparing the effect of myoblasts and BMSCs, there is a positive correlation between the numbers of transplanted cells in the pacing site and the dispersion of activation time[51]. The second determinant of arrhythmogenesis is the location of the cell injection. Investigators have also found that myoblast transplantation in the infarct border zone resulted in more arrhythmia compared with central scar injection[52]. This arrhythmia difference by zone may be explained by the fact that the conduction velocity, which is already decreased in the border zone, is decreased further by the transplanted cells. Other limitations in skeletal myoblast transplantation include prolonged culture time and early decay of cell survival with a low rate of cell engraftment over time.

4.Bone marrow-derived stem cells: Bone marrow-derived stem cells, also called bone marrow progenitor cell (BMPCs) , are the cell type most widely used in cellular therapy because of their easy accessibility, autologous origin and the fact that they contain multiple subpopulations of cells with the ability to transdifferentiate into both myocardial and vascular cells. The cell population of the bone marrow can be established and characterized by specific cell surface markers such as CD[34], lineage marker, c-kit and stem cell antigen-1 (Sca-1)[53]. To date, hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs) have been the subpopulation cells most extensively used in the animal models and clinical trials of cell therapy.

(1) Hematopoietic stem cells: Hematopoietic stem cells can be isolated from bone marrow by selective sorting of a particular set of surface receptors (Linage-, c-kit+, Sca-1+, CD34lo, CD 38hi )[54,55].They can give rise to cell lines of the lymphoid and hematopoietic systems and have been used clinically

in bone marrow transplantation for more than 3 decades. Definite transdifferentiaton of HSCs into cardiomyocytes has not been proven. Nonetheless, several studies in animal model of acute myocardial ischemia have demonstrated that transplanted HSCs can differentiate into cardiomyocytes and coronary vessels, and regenerate up to 68% of the infarcted myocardium[24,25,56] . However, some investigators, in repeating the experiments in a similar animal model, found donor cells in the heart expressing hematopoietic markers instead of cardiac specific markers[57-59].Overall, transplantation of HSCs have demonstrated the ability to regenerate myocardium and improve cardiac function, although the underlying mechanisms remain controversial.

(2)Mesenchymal stem cells (MSCs): Mesenchymal stem cells, also called bone marrow stromal cells, are multipotent cells which reside in the stroma of bone marrow. They normally can differentiate into osteocytes, chondrocytes and adipocytes. Under certain conditions, such as coculture with cardiomyocytes[60] or exposure to 5-azacytidine[61], a cysteine analog capable of altering the expression of genes that may regulate differentiation, MSCs have been shown to acquire a cardiomyocyte phenotype and differentiate into cardiomyocyctes in vitro. They also can be induced to differentiate into endothelial cells when cultured with vascular endothelial growth factors[62,63]. When transplanted into rodent, porcine or canine models of MI, MSCs have been shown to home to the injured myocardium[64] and enhance vascular density[62,63], resulting in a reduction of infarction size and improved ventricular function and cardiac regeneration[65,66]. MSCs can be obtained easily from autologous bone marrow, expanded in vitro with preserved potency, and stored for future use by cryopreservation. The beneficial effect of MSCs seems to be augmented by cotransplantation with other stem cells, as shown in a swine model[67].When transduced with a virus encoding for Akt, an anti-apoptotic gene, MSCs have been shown to prevent the pathological remodeling of the LV after MI, regenerate myocardium up to 80 % and normalize the cardiac function completely[68], all which indicate the great potential of MSCs in cell therapy.

(3)Endothelial progenitor cells (EPCs): Endothelial progenitor cells are functional precursors of endothelial cells. They can be identified by the expression of cells surface markers such as CD34 +, AC 133+, c-kit and vascular endothelial growth factor receptor 2 (VEGF2)[69]. EPCs can be isolated from either the bone marrow (BM) or the peripheral blood and expanded in vitro. As early as 1997, EPCs derived from the BM were observed to home into ischemic foci and contribute to the local angiogenesis[70]. This finding triggered the notion of therapeutic angiogenesis as a strategy for cell therapy. In 2000, research on the transplantation of ex vivo expanded EPCs in a rodent ischemic model reported significant therapeutic benefit through the combination effect of EPCs-contributed neovascularization and indirect stimulation of host vessel formation[71]. In the following years, several animal studies demonstrated the incorporation of EPCs into new vessels, with enhanced angiogenesis of the ischemic tissue and reduction of apoptosis in the treated animals[72,73]. HSCs, MSCs, and EPCs are easily obtained from BM or peripheral blood, of autologous origin and most importantly quite safe, as shown in animal experiments. No major complications such as arrhythmia, emboli, or inflammation have been noted. These encouraging results have led to a number of clinical trials which will be discussed in the following section.

5.Resident cardiac stem cells (CSCs): Intrinsic CSCs were first found by Hierlihy et al. in 2002. Since then, cells with stem cell characteristics have been found to reside in the heart[15-18], both in normal and pathological conditions such as aortic stenosis with cardiac hypertrophy[74]. Several cell markers such as c-kit, Sca-1, Abcg2, isl1+ and MDR1 have been

used to identify these cell populations from myocardium[16-18,20,75] .The origin of these subsets of CSCs is considered to come from the cardioblasts during embryogenesis[76]. There is, however, the possibility of an extracardiac source of circulating stem cells replenishing the pool of CSCs, as indicated by the analysis of posttransplant organs of sex-mismatched heart transplants. Cardiac stem cells isolated from rats have been shown to regenerate infarcted myocardium and improve cardiac function by intravascular administration[19], reconstituting more than 50% of the myocardium, and forming both new vessels and cardiomyocytes[16]. In a canine model with experimental MI, injection of growth factors was demonstrated to recruit resident cardiac stem cells to regenerate infarcted myocardium resulting in improvement of cardiac wall motion and self repair[77].Stem cells with c-kit+ isolated from surgical specimen of human heart have been demonstrated to be clonogenic, giving rise to myocytes, endothelial cells, and vascular smooth muscle cells in differentiation medium[78]. In preliminary experiment, local injection of these human-derived cells into the infarcted myocardium of immunodeficient mice was shown to regenerate myocytes and coronary vessels of human origin. The main problem with CSCs is that their numbers decrease rapidly with age. In patients over 60 years old with MI or heart failure, the numbers and functional capacity of resident CSCs remain doubtful[79]. Additional research examining the definitive site of resident (CSCs) will help obtain these stem cells, using targeted endomyocardial biopsy, and then expand their numbers in vitro so as to be sufficient for cellular therapy.

## Mechanisms of beneficial effects of stem cell therapy

Several mechanisms have been proposed to explain the beneficial effects of stem cell therapy, including transdifferentiation, cell fusion, paracrine effect, angiogenesis, and dedifferentiation.

Transdifferentiation: Transdifferentiation refers to the change of cell phenotype from one lineage to another. In 1998, the first proposed evidence of transdifferentiation was the observation that bone marrow-derived progenitors contribute to muscle regeneration[80]. In vitro demonstration of direct transformation of bone marrow derived-stromal cells into functional cardiomyocytes was reported later by Makino et al. in 1999[61]. In a study of HSC transplantation into infarcted mice, the formation of new myocytes, endothelial and smooth muscle cells was reported, newly formed myocardium was shown to occupy 68% of the ventricle 9 days after transplantation[25]. In 2003, a group of researchers found, on the basis of chromosomal markers, that CD34+ cells isolated from the peripheral blood were able to transdifferentiate directly into cardiac cell types in vivo[81]. Subsequent studies also demonstrated that when bone marrow-derived stem cells were transplanted into ischemic heart, they expressed the cardiac-specific markers troponin[82] and myosin[56], indicating transformation into functional cardiomyocytes. On the other hand, some contradictory results also have been presented[57-59]. When investigators repeated the experiment in animal models, no direct evidence of transdifferentiation was observed. The role of transdifferentiation as a beneficial effect mechanism in stem cell therapy remains controversial.

Cell fusion: Cell fusion describes a fusion of the transplanted and host cells with a concomitant transfer of the donor's cell content and genetic material to the host cell[4]. Cell fusion has been proposed as another mechanism responsible for stem cell plasticity and tissue repair in an in vivo model in which bone marrow-derived cells were demonstrated to fuse with cardiac myocyte[59]. Several studies have found evidence that cell fusion is the major source of bone marrow-derived

hepatocytes[83-85]. Theoretically, cell fusion allows damaged myocardium and endothelial cells to preserve their structural and functional integrity by adapting additional cytoplasmic and genetic material from the fused cells. Yet, considering cell fusion's low frequency in co-cultured stem cells (estimated at 1:10000 or 1:100000 cells)[83], and transplant models (less than 1% of cardiac myocytes)[86], cell fusion dose not appear to account for the full contribution of tissue generation in stem cell therapy.

Paracrine effect and angiogenesis: Paracrine effect refers to the fact that transplanted cells may release some kind of cytokines and growth factors that can stimulate proliferation and differentiation of host tissue. Several studies suggest that the myocardium may have an intrinsic potential to repair its minor tissue injuries[9,11,13,87]. When tissue injuries outweigh the self-repairing capacity of the heart, overt heart failure ensues. Myocardial repair is a very complex process that involves certain cytokines acting as a mediator in each step[88]. The damaged tissue secretes stem cell factor (SCF), stromal-derived factor-1 (SDF-1), granulocyte-colony stimulating factor(G-CSF), vascular endothelial growth factor(VEGF), monocyte chemoattractant protein-1 (MCP-1) as well as other substances, which may mobilize the stem cells to the injured sites[89-92]. In a genetically engineered mouse model, Fazel et al. documented that cardiac repair with bone marrow-derived progenitors was associated with the induction of angiogenic cytokines and enhanced angiogenesis[93]. Under experimental ischemic conditions, cell death of cultured cardiomyocytes and endothelial cells was demonstrated to be prevented by factors secreted by bone marrow stromal cells[94]. Considering that the magnitude of functional improvement obtained in transplanted heart is disproportionate to the amount of transdifferentiation observed, paracrine effect and angiogenesis seems to be a more relevant and well accepted beneficial mechanism in stem cell therapy when compared to above mentioned mechanisms.

Dedifferentiation: Dedifferentiation refers to a process in which well-differentiated tissue-specific cells regress to more primitive and multipotent cells and regain their plasticity in order to redifferentiate to cells of other tissue types. In the animal kingdom, zebrafish have been found to fully regenerate the heart after a 20% left ventricular resection[95]. Urodele amphibians repair amputated limbs, tails, eyes and the heart structure through a process that seems to involve the dedifferentiation of mature cell near the edge of the wound[4]. At present, dedifferentiation of cells in adult mammals has yet to be clearly documented. Some investigators have demonstrated that cell cycle activity can be induced in terminally differentiated adult cardiomyocytes in vivo by genetic modifications[96]. Enhanced cardiomyocyte cycle activity is accompanied by favorable post-infarction ventricular remodeling and cardiac function improvement[97,98]. The long-held view that tissue specific stem cells are predetermined to be able to give rise only to a particular cell type has been challenged by recent study[99]. Nonetheless, additional evidence is needed to support dedifferentiation as a mechanism of stem cell plasticity and to define its contribution to cellular therapy.

## Clinical trials (Tables 3, 4)

1.Bone marrow stem cells (BMSCs): The pioneering and promising results of BMSCs in animal studies led to the first non-randomized clinical trial in 2002, which was comprised of 10 patients with intracoronary injection of BMSCs (a mononuclear faction of bone marrow aspirate) 7 days after acute myocardial infarction(AMI)[100]. Decreased infarction region, and left ventricular end systolic volume (LVESV) with increased stroke volume index (SVI) were demonstra-

ted after 3 months follow-up. In the same year, the TOPCARE-AMI (Transplantation of Progenitor Cells And Regeneration Enhancement in Acute Myocardial Infarction) trial compared BMSCs and circulating progenitor cells via intracoronary transplantation[101].The trial showed no significant differences between these two groups. Similar increases in left ventricular ejection fraction (LVEF) and overall improvement in LVESV were reported after 1 year follow-up[102]. In 2004, the first randomized controlled trial, BOOST (Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration) trial was conducted in 60 patients with AMI after coronary angioplasty. Thirty patients who received intracoronary injection of BMPCs 5-7 days post-AMI showed significant improvement of LVEF at 6 months follow-up, compared with another 30 patients receiving only optimal medical treatment[103]. The LVEF improvement was not sustained at 18 months follow-up, as evaluated by MRI[104], and there was no statistically significant difference in the incidence of arrhythmia or post-angioplasty coronary restenosis. In the same year, in order to overcome the limitation of a relatively small number of cells available for study, the MAGIC trial tried to use granulocyte-colony stimulating factor (G-CSF) to mobilize CD34+ stem cells from bone marrow, and then infusing the mobilized peripheral blood stem cells intracoronarily in patients with coronary stenting[105]. Although an improvement of systolic function was observed in patients receiving cellular therapy at 6 months follow-up, an increased rate of in-stent restenosis was also noted. An increased risk of MI or death, another adverse outcome of G-CSF used in patients with stable ischemic heart disease, was also reported in 2005[106]. In 2006, a double-blind randomized placebo-controlled trial with stem cells in acute ST-elevation myocardial infarction (STEMMI trial) also demonstrated a lack of beneficial effects in patients treated with G-CSF mobilized marrow progenitor cells[107]. In the same In year, four larger randomized, placebo-controlled, double blind trials were reported. Patient numbers

Table 3: Clinical Trials of Bone Marrow-derived Cells in Acute Myocardial Infarction and Chronic Heart Failure

| Authors (year) | Patient number | Administration route | Follow-up | Results | Reference |
|---|---|---|---|---|---|
| Strauer et al. (2002) | 10 | Intracoronary | 3 months | Decreased infarct size and LVESV. Increase SVI. | 100 |
| Schächinger V et al. (2004) | 59 (30BMC +29CPC) | Intracoronary | 12 months | Increased LVEF. Decreased infarct size and LVESV. | 102 |
| Wollert et al. (2004) | 30 | Intracoronary | 6 months | Improved LVEF | 103 |
| Kang et al. (2004) | 10/10 | Intracoronary (G-CSF/PBSC) | 6 months | Increased LVEF in PBSC patients | 105 |
| Janssens et al. (2006) | 32 | Intracoronary | 4 months | Decreased infarct size. No difference in myocardial flow. | 108 |
| Lunde et al. (2006) | 50 | Intracoronary | 6 months | No difference in LVEDVand infarct size. | 109 |
| Schächinger et al. (2006) | 204 | Intracoronary | 12 months | Decreased combined death, MI recurrence and revascularization. | 110 |
| Assmus et al.(2006) | 24 (CPC), 23 (BMC) | Intracoronary | 6 months | Significantly Increased LVEF | 111 |

BMC, bone marrow cell; CPC, circulating progenitor cells; G-CSF, granulocyte-colony stimulating factor; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVEDV, left ventricular end-diastolic volume; PBSC, peripheral blood stem cell; SVI, stroke volume index.
(Modified from Curr Opin Pharmacol. 2006)

Table 4: Clinical Trials of Skeletal Myoblasts in Chronic Ischemic Heart Failure

| Authors (year) | Patient number | Administration route | Follow-up | Results | Reference |
|---|---|---|---|---|---|
| Menasché et al. (2003) | 10 | Transepicardial | 10.9 months (average) | Improved NYHA, LVEF, and regional thickening | 114 |
| Smits et al. (2003) | 5 | Transendocardial | 6 months | Improved LVEF, and regional thickening | 120 |
| Siminiak et al. (2004) | 10 | Transepicardial | 12 months | Improved LVEF | 117 |
| Dib et al. (2005) | 30 | Transepicardial | 9~27 months | Improved LVEF, reduced LV systolic and diastolic volume | 118 |
| Hagège et al.(2006) | 9 | Transepicardial | 18-58 months | Increased LVEF, low re-hospitalization incidence | 115 |
| Gavira et al. (2006) | 12 | Transepicardial | 12 months | Increased LVEF, decreased WMSI, no arrhythmia. | 119 |
| Biagini et al. (2006) | 10 | Transendocardial | 12 months | Increased LVEF during low dose dobutamine infusion. | 122 |

LVAD, left ventricular assist device; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; WMSI, wall motion score index.
(Modified from Curr Opin Pharmacol. 2006)

ranged from 32 to 204 per study. Three trials used intracoronary infusion of BM-derived mononuclear cells 4 to 8 days after AMI[108-110], whereas one trial used BM-derived progenitor cells 3 months after MI[111]. Decreased infarction size with augmented regional systolic function and global ejection fraction were demonstrated in most trials. One trial reported significant decrease of combined death, MI recurrence and revascularization incidence[110]. Recently, non-expanded peripheral blood-derived mononuclear cells have been shown to promote improvement of cardiac function in patients with AMI via intracoronary injection[112]. Although these trials have not shown substantial benefits in all patients, the feasibility, efficacy and most importantly the safety profile have been documented. Because of these advancements, there is an encouraging atmosphere for developing large-scale multicenter collaborative studies in the future.

2.Skeletal myoblasts: Skeletal myoblasts were the first cell type to be used in the clinical trial of cell therapy. In 2001, Menasché et al. reported the first transplantation of autologous myoblasts in a patient with severe ischemic heart failure by epicardial injection at the time of coronary artery bypass grafting (CABG)[113]. In a following series of studies, patients with a low ejection fraction (＜35 %) , a history of MI, and an indication for CABG, were enrolled to test the feasibility of large-scale skeletal myoblast expansion and to document the safety profile of multiple cell injections on postinfarction scar[114]. The patients were followed up for an average duration of 52 months[115]. Symptomatic improvement with reduced incidence of heart failure and hospitalization was reported. Ejection fraction increased from a baseline value of 24.3%± 4% to 31 %± 4.1% and remained stable thereafter. As had been demonstrated earlier in animal studies, episodes of ventricular arrhythmia occurred in 5 patients, which prompted the implantation of an implantable cardioverter defibrillator (ICD) in each patient. One patient died 17.5 months after surgery; the autopsy showed clusters of myoblasts in his scar tissue[116]. Similar clinical trials were conducted by other three groups of researchers, except in these trials each patient received concomitant recanalization at the time of cell transplantation[117-119]. All of these similar studies demonstrated an improvement of cardiac function with increased ejection fraction and viability in

the myoblast-injected segments, as assessed by echocardiography and positron emission tomography (PET) . Only one of these three groups reported no occurrence of cardiac arrhythmia in twelve patients on one-year follow-up[119]. In addition to these surgical trials, catheter-based trials have been reported by three research teams using endovascular approach guided by either ultrasound or electromechanical mapping[120-122]. The procedural feasibility and efficacy of improving ejection fraction and regional wall motion were demonstrated. Yet, complications with ventricular arrhythmia and sudden death were also reported in these catheter-based trials. Additionally, transplantation of skeletal myoblasts is hampered by the prolonged culture time and low rate of myoblast survival over time. These limitations need to be overcome before skeletal myoblasts transplantation is deemed an acceptable treatment option for patients with ischemic heart failure.

## Conclusion and Future Direction

Hypotheses regarding using contractile cells to supporting failing hearts were first introduced in 1989[123]. Several cell types, as mentioned above, have been used in animal studies of cellular therapy for AMI and ischemic heart failure with promising results. Much progress has been made in these endeavors in the past two decades. Irrespective of the route by which the cells were administered, the feasibility, efficacy and particularly the safety concerns of stem cell therapy have been documented in several clinical trials using skeletal myoblasts and BMSCs. However, before stem cell therapy can be translated into a full clinical application, many challenges must be resolved, including:

1. The exact mechanism of stem cell therapy: Several potential mechanisms have been proposed, such as transdifferentiation, cell fusion, angiogenesis, paracrine effects or dedifferentiation, but not one of them solely can explain the effects of stem cell therapy in clinical situations. Further investigation of the genetic expression profiles of the stem cells will help to understand the pathways that influence cell survival, proliferation and differentiation. Identification of a common molecular pathway underlying different cell therapies is crucial to develop a novel strategy for stem cell therapy.
2. How to label and track the delivered cells exactly to determine the cell fate after homing and engraftment: In vivo imaging of the transplanted cells is necessary for monitoring the location, magnitude and duration of cell survival, providing a better understanding of the mechanisms of functional improvement after cell therapy. Much progress has been made in recent years in the technology of in vivo imaging, such as optical imaging with bioluminescence and fluorescence, single-photon emission computer tomography (SPECT), PET magnetic resonance imaging (MRI) and multimodality imaging with dual optical/MRI contrast agents[124] and nanoparticles generating simultaneous MRI, ultrasound and fluorescence contrast[125]. Most imaging modalities provide noninvasive morphological and functional data of the transplanted cells. More investigation is needed for further assessment of in vivo biological phenomenon and greater understanding of the molecular mechanisms of cell-based therapies.
3. Which cell type should be chosen for certain individuals or disease condition: Regarding the capability of transdifferentiation, embryonic stem cells hold the greatest potential for stem cell therapy. At the same time, previous studies have indicated that enhancement of vascular support with local angiogenesis and neovascularization plays a vital role for the beneficial effect of cell therapy. Cotransplantation with two types of cells with myogenic and angiogeneic potential may be

a practical treatment modality in the near future. Recently, c-kit+ cells derived from BM and heart tissue are attracting more attention. They were shown capable of giving rise to myocytes, endothelial cells and vascular smooth muscle cells in differentiation medium[78]. The numbers of c-kit+ cells increases markedly in AMI. In mutant mice, they were demonstrated to create a proangiogenic milieu to rescue the infarcted heart by enhanced angiogenesis[93]. More study is needed to further investigate the role of c-kit cells in the process of myocardial repair and regeneration.

4. The optimal number of cells to be transplanted and the optimal timing of cell delivery to obtain the best possible effects: No consensus has been reached concerning the optimal number and timing of cell transplantation with each cell type. These considerations need to be addressed in the future large-scale randomized double-blind controlled trials.
5. The route of cell administration to obtain optimal therapeutic benefit: Stem cells ordinarily are transplanted by the following routes: intramyocardial, intracoronary, catheter-guided endoventricular injections or transvenous injection through coronary sinus. Each cell type may have its preferred route of administration. For example, skeletal myoblast transplantation is precluded by intracoronary injection because of the cell size. Wash-out effect is another problem for cell therapy[126]. There is evidence that a substantial number of the intramyocardially injected cells leak either into the pericardium or through the venous and lymphatic drainage. The use of a cell-seeded biocompatible sheet covering the targeted area has been shown to be more effective than injected myoblasts alone for the reduction of fibrosis and the enhancement of local angiogenesis, resulting in functional improvement[127,128]. Another approach is embedding the cells into a injectable biocompatible matrix or mixing the cells with growth factor-loaded nanopeptides. Both provide the injected cells with a tridimensional microenvironment in the myocardium, promoting cell survival and engraftment[129,130].
6. The potential risk of lethal arrhythmia in certain cell type transplantation: Skeletal myoblasts are well known for inducing lethal arrhythmia. The potential risk of arrhythmia prompted the routine implantation of an ICD in each patient in most clinical trials, including 97 patients enrolled in the MAGIC trial[131], which is one of the largest clinical trials for myoblast transplantation. Several studies have demonstrated that the careful selection of injection site[52] and the genetic modification of skeletal myoblasts to express gap junction protein before transplantation[48] may be helpful in reducing the incidence of lethal arrhythmia.
7. The necessity of immnosuppresive agents and the teratogenic risk, especially in embryonic stem cell transplantation: Immunorejection, teratogenesis and ethical concerns are major hurdles for the future development of stem cell therapy. The derivation of isogenic cells from recipient patients by either somatic cell nuclear transfer(SCNT) or somatic cell reprogramming is a promising solutions for the future. In animal experiments, in vitro reprogramming of fibroblasts into a pluripotent ESC-like state has been achieved[35,132]. The reprogramming would provides the opportunity to generate patient-specific pluripotent stem cells in the future, eliminating the concern of immunorejection and minimizing ethical problems.
8. The ethical and moral considerations regarding the use of stem cells, especially on embryonic stem cells and fetal cardiomyocytes: Stem cell research raises important ethical issues related to donors of gametes and embryos with possible destruction of an embryo. In addition to the

general ethical consideration on translating scientific research from laboratory to bedside, more stringing oversight is needed for stem cell research. A task force concerning the clinical investigation of the use of autologous adult stem cells for repair of the heart has been organized by the European Society of Cardiology[133] to oversee the situation of clinical trials and publish papers concerning the consensus on future studies.

In the near future, more well-designed large-scale multicenter randomized controlled studies are needed to resolve the above-mentioned problems in order for stem cell therapy to be accepted as a novel therapeutic modality for patients with AMI and heart failure. Although not all of the many challenges inherent to stem cell therapy for patients with AMI and heart failure can be resolved at one time, it is hoped that through vigorous investigation and subsequent information-sharing, stem cell therapy will become a success story for these AMI and post-MI heart failure patients.

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# 幹細胞治療在急性心肌梗塞以及心臟衰竭的應用—過去，現在，未來

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## 摘 要

急性心肌梗塞後的心臟衰竭已變成台灣人民健康的一大隱憂。隨著越來越多的病患從急性梗塞中存活，這些慢性缺血性心衰竭病患的照護，已造成政府極大的醫療負擔。在最近數十年裡，除了傳統藥物治療如乙型阻斷劑（β-blockers）、血管張力素轉換酶抑制劑(ACEIs)、冠狀動脈氣球擴張術及支架植入以及外科手術如左心室輔助器，冠狀動脈繞道手術或心臟移植，隨著幹細胞生物特性逐漸被了解以及近年來再生醫學蓬勃發展，幹細胞療法已成爲心肌梗塞和心臟衰竭的前瞻性療法。在本文我們將回顧文獻中有關幹細胞療法的背景、可能的機轉，各類幹細胞在人類及動物模式中使用的研究成果，以及幹細胞療法可能面臨的問題和將來的展望，期能增加臨床醫師對幹細胞在心血管疾病治療的瞭解。