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STEM CELL THERAPY IN CARDIOVASCULAR DISEASE

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ABSTRACT

Cardiovascular disease is the leading cause of death worldwide. Of the almost 17 million people who die each year from cardiovascular causes, over 11 million die as a result of cardiac disease and 5.5 million deaths are related to stroke. Myocardial infarction carries a short-term mortality rate of about 7% (with aggressive therapy), and congestive heart failure an even more distressing 20% one-year mortality. Stem cell therapy is an exciting new frontier in the battle against cardiovascular disease that has sparked intense research as well as criticism. In the pursuit of this endeavor, it has become apparent that we need to better understand the processes that lead to both damage and repair if we are to realise the true potential of stem cell therapy. The current evidence suggests that stem cell therapy has great promise for attenuating remodeling and transforming inert scar into biochemically functional myocardium. However, the past decade has shown that translating the potential of stem cell therapy into actual practice is not easy, and many hurdles would need to be overcome before this therapy attains its full potential. Finally, the discovery of various cardiac stem cell populations has renewed interest in the innate regenerative capacity of the human heart.

KEYWORDS: Stem cells, cardiovascular disease, myocardial infarction, heart, therapy.

INTRODUCTION

Cardiovascular disease (CVD), which includes hypertension, coronary heart disease (CHD), stroke, and congestive heart failure (CHF), has ranked as the number one cause of death in the United States every year since 1900 except 1918, when the nation struggled with an

influenza epidemic.[1] In 2002, CVD claimed roughly as many lives as cancer, chronic lower respiratory diseases, accidents, diabetes mellitus, influenza, and pneumonia combined. According to data from the 1999-2002, National Health and Nutrition Examination Survey (NHANES), CVD caused approximately 1.4 million deaths (38.0 percent of all deaths) in the U.S. in 2002. Nearly 2600 Americans die of CVD each day, roughly one death every 34 seconds. Moreover, within a year of diagnosis, one in five patients with CHF will die. CVD also creates a growing economic burden; the total health care cost of CVD in 2005 was estimated at $393.5 billion dollars.

Cardiovascular disease is the leading cause of death worldwide. Of the almost 17 million people who die each year from cardiovascular causes, over 11 million die as a result of cardiac disease and 5.5 million deaths are related to stroke. Myocardial infarction carries a short term mortality rate of about 7% (with aggressive therapy), and congestive heart failure an even more distressing 20% one-year mortality.[2] Despite significant strides in therapy, thanks to newer treatment modalities and risk-reduction strategies, the global burden remains substantial.[3,4,5.6] This continued health problem has prompted research into new therapeutic approaches. Stem cell therapy is a relatively new frontier in the battle against cardiovascular disease that has sparked intense research and criticism. With the discovery of various stem cell populations possessing cardiogenic potential, and the subsequent ability to isolate and expand these cells, the notion of a restorative therapy has begun to take shape. Although much knowledge has been gained through more than a decade of research, numerous barriers to true cardiac regeneration remain. In the pursuit of this endeavour, it has become apparent that we need to better understand the processes that lead to both damage and repair if we are to realise the true potential of stem cell therapy. The discovery of the proliferative capacity and plasticity of various stem cell populations has sparked much interest and debate regarding their use as a potential therapy.

HEART FAILURE (HF)

The majority of CVD is composed of cardiac diseases which can be broadly divided into either ischemic (e.g., coronary artery disease and myocardial infarction) or non-ischemic heart disease (e.g., valvular heart disease and hereditary cardiomyopathy). Regardless of the underlying cause, however, HF is the final common stage of many diseases associated with the heart.[7] Based on recent statistics, more than 900,000 people are living with HF in the UK, which represents about 5% of medical hospitalizations.[8] Approximately 5.8 million

people are affected with HF in the USA and over 23 million worldwide.[9] Under medical treatment, 20-30% of HF patients die in the first year of diagnosis and 45-60% after 5 years, respectively.[10,11] Thus, HF has become a major public health issue in terms of high mortality rate and enormous healthcare expenditure.[12]

STEM CELLS

Stem cells are a population of immature tissue precursor cells capable of self-renewal and provision of *de novo* and/or replacement cells for many tissues. Embryonic stem cells can be obtained from the inner cell mass of the embryonal blastocyst. Although it was recently shown that human embryonic stem cells can differentiate into cardiomyocytes,[13] because of the immunogenicity and rejection, as well as ethical considerations, these cells may be restricted to experimental in vitro studies and their therapeutical potential remains to be determined. Also, these cells may act as an unanticipated arrhythmogenic source after intramyocardial transplantation.[14] In contrast, adult human stem cells (hematopoietic, mesenchymal) are found in mature tissues, e.g., the bone marrow. Plasticity of adult stem cells can probably generate lineages of cells different from their original organ of origin. Thus, these cells can be used for organ regeneration and for cellular repair in various species, as well as in humans. Over the past decade, several different stem cell types have been studied in an effort to find the best source for cardiac regeneration. Each stem cell population has its own advantages and complications (Table 1).

Table 1: Characteristics of stem cell populations used for cardiac repair

Stem cell	Derived from	Advantages	Disadvantages	Clinical trials
Embryonic stem cells	Inner cell mass of the pre-implantation blastocyst	Theoretically unlimited self-renewal capacity; pluripotent	Ethical considerations; teratoma formation; graft-versus-host disease	None
Bone marrow mononuclear cells, hematopoietic stem cells, circulating progenitor cells	Bone marrow, peripheral blood (also umbilical cord, placenta)	Easy to isolate; proven safe and feasible to implant	Controversy whether true cardiovascular differentiation takes place	Large-scale trials: modest and transient benefits; significant reduction in subsequent cardiovascular events[15-22]
Mesenchymal stem cells	Bone marrow (adherent cells), adipose tissue	Easy to isolate and expand in culture; less immunogenic than other lines; multipotent	Large heterogeneity; heterotopic differentiation (e.g. ossification)	Safety and feasibility, and small-scale studies[23]

Endothelial progenitor cells	Bone marrow, peripheral blood	Mobilized from bone marrow or present in peripheral blood; important in neovasculogenesis	Heterogeneity; small populations; reduced in individuals with cardiovascular comorbidities	Safety and feasibility, and small-scale studies[24-29]
Skeletal myoblasts	Mature muscle (between the sarcolemma and basement membrane)	Extensive scalability; resistance to ischaemia; multipotent; no teratoma formation	Potential for arrhythmias; lack of cardiomyocytes differentiation (dysynchronous beating)	Large-scale clinical trials: no benefits[30]
Cardiac stem cells	Special niches in the myocardium (in deep tissue at the atria and apex)	Resident cells; robust cardiovascular differentiation potential; reduced tumour formation.	Stem cell pool appear to undergo senescence; scalability largely unknown	None

Stem cell therapy for the heart accounts for a third of publications in the regenerative medicine field.[31] Mummery *et al.* have recently reviewed the use of both adult and embryonic stem cells, such as bone marrow-derived stem cells, which include hematopoietic stem cells (HSCs), and mesenchymal stem cells (MSCs), endogenous cardiac progenitor cells (CPCs), human embryonic stem cells (hESCs), induced pluripotent stem cells, and hESC derived cardiomyocytes.[31] The use of bone marrow-derived stem cells such as HSCs and MSCs to repair cardiac tissues was predicated on the hypothesis that these cells could differentiate into cardiomyocytes and supporting cell types. Although the presence of CPCs in fetal hearts is well established, the presence of CPCs in postnatal or adult heart remains controversial, and the possibility that the so-called CPCs from postnatal hearts are bone marrow cells has remained unresolved. Transplanted cardiomyocytes isolated from *in vitro* differentiation of hESCs and induced pluripotent stem cells could engraft in the heart to form a synctium with each other, but not with the recipient heart. This failure to couple with the recipient cardiomyocytes could cause arrhythmia, a potentially fatal condition.

Despite our still evolving understanding of stem cell transplantation in treating cardiac disease, stem cell transplantation has already being tested in clinical trials. In a recent review of more than 20 clinical trials that primarily used adult stem cells, such as bone marrow stem cells, mobilized peripheral blood stem cells and skeletal myoblasts to treat heart disease,[32] the trends favored such transplantations to treat cardiac disease when measured using clinical end points of death, recurrence of AMI or hospitalization for heart failure. Several stem and progenitor cells showed a potential to improve cardiac regeneration (Figure 1).

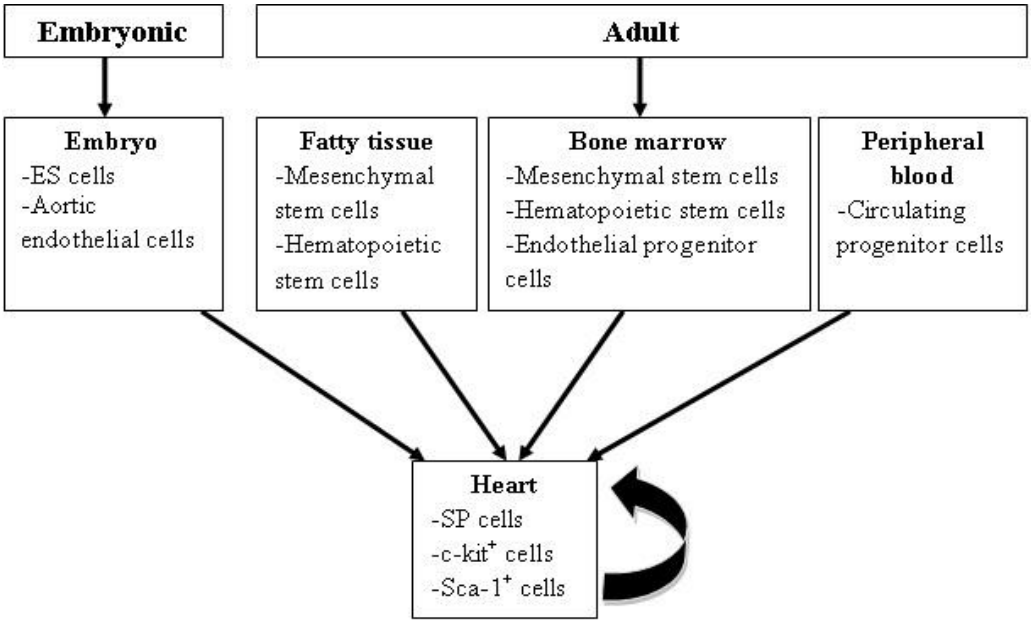

Figure 1: Sources of progenitor cells for cardiac regeneration

It has been proposed that stem cells release angiogenic ligands, protect cardiomyocytes from apoptotic cell death, induce proliferation of endogenous cardiomyocytes, and may recruit resident cardiac stem cells (Figure 2).[33-37] Regardless of the mechanisms, there appears to be general agreement that stem cell therapy has the potential to improve perfusion and contractile performance of the injured heart.[38,33-35,37,39]

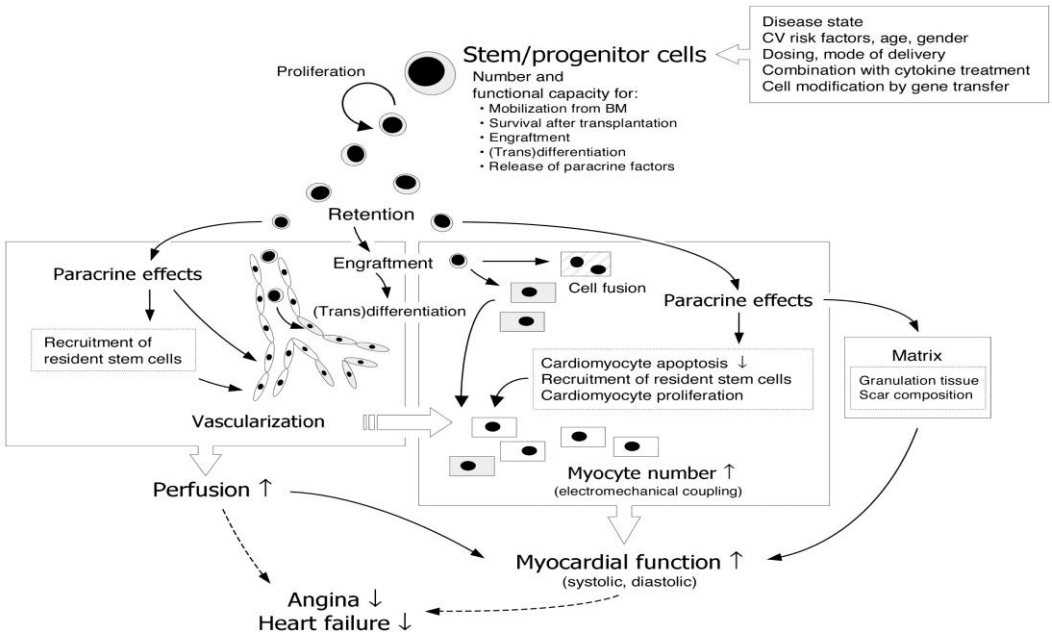

Figure 2: Therapeutic stem cell transplantation for myocardial regeneration. Stem and progenitor cell numbers and functional capacity are influenced by a patient's age, gender, cardiovascular risk factors, and underlying disease state.

POTENTIAL DONOR CELLS

Conceptually, a variety of stem and progenitor cell populations could be used for cardiac repair. Each cell type has its own profile of advantages, limitations, and practicability issues in specific clinical settings. Studies comparing the regenerative capacity of distinct cell populations are scarce. Many investigators have therefore chosen a pragmatic approach by using unfractionated bone marrow cells (BMCs),[40-51] which contain different stem and progenitor cell populations, including HSCs, endothelial progenitor cells (EPCs), and mesenchymal stem cells (MSCs). Ease of harvest and lack of extensive requirement for ex vivo manipulation are additional advantages of using unselected BMCs.

Endothelial Progenitor Cells

The endothelium is a layer of specialized cells that lines the interior surface of all blood vessels (including the heart). This layer provides an interface between circulating blood and the vessel wall. Endothelial progenitor cells (EPCs) are bone marrow-derived stem cells that are recruited into the peripheral blood in response to tissue ischemia.[52] EPCs are precursor cells that express some cell-surface markers characteristic of mature endothelium and some of hematopoietic cells.[53,54-56] EPCs home in on ischemic areas, where they differentiate into new blood vessels; following a heart attack, intravenously injected EPCs home to the damaged region within 48 hours.[57]

$CD133^+$ Cells

The cell surface antigen CD133 is expressed on early HSCs and EPCs, both of which collaborate to promote vascularization of ischemic tissues.[58] $CD133^-$ cells can integrate into sites of neovascularization and differentiate into mature endothelial cells. Because CD133 expression is lost on myelomonocytic cells, this marker provides an effective means to distinguish “true” $CD133^+$ EPCs from EPCs of myelomonocytic origin.[59]

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are precursors of non-hematopoietic tissues (e.g., muscle, bone, tendons, ligaments, adipose tissue, and fibroblasts) that are obtained relatively easily from autologous bone marrow. They remain multipotent following expansion *in vitro*, exhibit relatively low immunogenicity, and can be frozen easily. While these properties make the cells amenable to preparation and delivery protocols, scientists can also culture them under special conditions to differentiate them into cells that resemble cardiac myocytes. This property enables their application to cardiac regeneration. MSCs represent a rare population

of $CD34^-$ and $CD133^-$ cells present in bone marrow stroma (10-fold less abundant than HSCs) and other mesenchymal tissues.[60] MSCs can readily differentiate into osteocytes, chondrocytes, and adipocytes. One advantage of using these cells in human studies is their low immunogenicity; allogeneic MSCs injected into infarcted myocardium in a pig model regenerated myocardium and reduced infarct size without evidence of rejection.[61]

Skeletal Myoblasts

Skeletal myoblasts, or satellite cells, are progenitor cells that normally lie in a quiescent state under the basal membrane of mature muscular fibers. Myoblasts can be isolated from skeletal muscle biopsies and expanded *in vitro*. Myoblasts differentiate into myotubes and retain skeletal muscle properties when transplanted into an infarct scar.[62-65] Although myotubes do not couple with resident cardiomyocytes electromechanically, myoblasts transplantation has been shown to augment systolic and diastolic performance in animal models of myocardial infarction.[66]

Resident Cardiac Stem Cells

The modest functional effects of transplanted progenitor cells from bone marrow and skeletal muscle in human studies stimulated further research into the natural regenerative mechanisms of the cardiac tissue. The heart has traditionally been viewed as a post mitotic organ because mature cardiomyocytes withdraw from the cell cycle and cease to proliferate. The presence of resident cardiac stem cell (CSC) population(s) capable of differentiating into cardiomyocyte or vascular lineages suggests that these cells could be used for cardiac tissue repair.[67-71] Interestingly, contradictory data began to accumulate as cardiomyocyte proliferation and cycling were found under certain pathological conditions – namely ischaemia and hypertension[72,73,74] This idea was further advanced with the discovery of male cardiomyocytes and endothelial cells in donor female cardiac tissue transplanted into a male recipient.[75,76] These findings raise the possibility that Y-chromosome positive, male cells migrated either from the recipient atrial stump or the bone marrow into the cardiac tissue and differentiated into functional cardiomyocytes. Moreover, estimates of the death rate levels of adult cardiomyocytes also led to the consideration of a pool of cardiac progenitor cells.[77] This evidence prompted a search to locate such resident cardiac cells. CSCs hold great promise for clinical applications, although it is conceivable that the bone marrow may contain a stem cell population with similar properties.[78]

Embryonic Stem Cells

Embryonic stem (ES) cells are totipotent stem cells derived from the inner cell mass of blastocysts. Under specific culture conditions, ES cells differentiate into multicellular embryoid bodies containing differentiated cells from all three germ layers including cardiomyocytes. Human ES cell–derived cardiomyocytes display structural and functional properties of early-stage cardiomyocytes that couple electrically with host cardiomyocytes when transplanted into normal myocardium.[79,80] ES can potentially give rise to the variety of cell types that are instrumental in regenerating damaged myocardium, including cardiomyocytes, endothelial cells, and smooth muscle cells. ES cells that were transplanted into ischemically-injured myocardium in rats differentiated into normal myocardial cells that remained viable for up to four months,[81] suggesting that these cells may be candidates for regenerative therapy in humans.

ROUTE OF CELL ADMINISTRATION

The appropriate route of cell administration to the damaged organ is an essential prerequisite for the success of organ repair. High cell concentrations within the area of interest and prevention of homing of transplanted cells into other organs are desirable. Therefore, targeted and regional administration and transplantation of cells should be preferred;

- In regional heart muscle disease, as in myocardial infarction, selective cell delivery by intracoronary catheterization techniques leads to an effective accumulation and concentration of cells within the infarcted zone. This can be realized in humans with bone marrow-derived cells.[82] With intracoronary administration, all cells must pass the infarct and peri-infarct tissue during the immediate first passage. Accordingly, with the intracoronary procedure, the infarct tissue can be enriched with the maximum available number of cells at all times. Further developments of catheterization systems for various clinical studies are needed.

- The transendocardial and transpericardial route of application has been used in large animal experiments[83] and was also recently tested in patients.[84] The main potential advantage of the surgical procedure is injection under visualization, which allows anatomic identification of the target area and even distribution of the injections. The safety and feasibility of catheter-based transendocardial injection was demonstrated in large animal studies,[85] and initial clinical experience in 19 patients using intramyocardial gene transfer showed similar safety profiles.[86] Current clinical experience is limited to one injection system, using

electromechanical mapping to generate 3-dimensional left ventricular reconstruction before the injection. Intraventricular catheter manipulation, however, can injure the myocardium, inducing ventricular premature beats and short runs of ventricular tachycardia. In certain cases, this precludes injection to the more arrhythmogenic zones, and it may extend the duration of the procedure and should always be carefully monitored. Each injection catheter is tested for cell biocompatibility to assure no mechanical or functional damage to cells being propelled under pressure through the narrow injection needle. Future developments with steerable transendocardial injection and delivery systems with mapping of the injured zone are needed. Transendocardial injection of autologous bone marrow cells has also been performed as part of several pilot and phase I studies. Safety and feasibility data are still pending and efficacy parameters need large randomized clinical trials.

- The intravenous route of administration is easiest. The main disadvantage, however, is that approximately only 3% of normal cardiac output will flow per minute through the left ventricle, and it is also limited because of transpulmonary first-pass attenuation effect on the cells. Therefore, this administration technique will require many circulation passages to enable infused cells to come into contact with the infarct-related artery. During that time, homing of infused cells to other organs will considerably reduce the number of cells that will populate the infarcted area.

- Some major cell types, such as skeletal myoblasts, have the disadvantage of emboligenic potency when delivered systemically. Therefore, intramyocardial injection during open-heart surgery has been tested. This procedure has also been used in humans.[87] However, the therapeutic effect is limited because of severe arrhythmogenic complications. Another approach implanted autologous bone marrow cells during open-heart surgery and could show improvement in myocardial perfusion in 3 of 5 treated patients.[88]

DETECTION OF TRANSPLANTED STEM CELLS

An important clinical problem will be the identification and localization of transplanted autologous stem cells within the injured area of the heart. The transplanted cell or cell population is a single unit in a complex biological network of other cells. Therefore, for both localization and fate mapping of stem cells within the target organ, specific cell markers are desirable. Thus, analysis of stem cell behavior will presume.

1. *In situ* labeling of a single cell or a transplanted cell population

2. Transplantation of already in vitro labeled cells or cell populations.

For labeling in animal experiments, retroviral transduction with a marker gene or labeling with thymidine or bromodeoxyuridine (BrdU) has been used. For clinical detection of stem cells, magnetic labeling and in vivo tracking of bone marrow cells by the use of magnetodendrimers or radioactive detection methods may be useful. Myocardial biopsies in humans hardly will be justifiable under these circumstances. Thus, localization and fate mapping of stem cells in the region of myocardial injury will represent an important task for experimental and clinical stem cell research in the future, as well as for the assessment of time course of proliferation in the recipient new cell homes and for the evaluation of proper cell function after full transdifferentiation. Preliminary results through the detection of the reporter gene *LacZ*, by identification of β-galactosidase–positive cells in tissue section and chromosome analysis by fluorescence in situ hybridization (FISH) techniques are encouraging.[89]

HOW TO INCREASE STEM CELL THERAPEUTIC EFFICACY

Attempts at increasing efficacy of stem cells for cardiac indications have taken several avenues of investigation: increasing trafficking efficacy; enhancing plasticity of administered cells; and increasing growth factor production. Endowment of these features as been performed by gene transfection or modification of culture conditions such as exposure to cytokines or hypoxia. Another interesting approach is addition of chemotactic agents to the area of tissue injury to enhance trafficking.[90]

CLINICAL EVIDENCE FOLLOWING STEM CELL THERAPY

1. Acute Myocardial Infarction

Modern reperfusion strategies and advances in pharmacological management have resulted in an increasing proportion of AMI survivors at heightened risk of developing adverse LV remodeling and heart failure.

2. Experimental Background

In one of the earliest studies, HSCs were injected into the infarct border zone after coronary artery ligation in mice. Several days later, the infarcted area was replaced by newly formed myocardium with HSC-derived myocytes and vascular structures.[38] Transdifferentiation to cardiomyocytes and vascular structures has also been reported after transfer of $CD34^+$ cells into mice with AMI.[91]

3. Clinical Trial Experience

Inspired by the exciting experimental data, several trials were initiated to test whether cell therapy is safe and feasible in patients after AMI. Some have decried the clinical trials as being premature without a more complete understanding of the underlying mechanisms,[92] whereas others have pointed out that the clinical trials are justified by the potential benefits of cell therapy.[93] The clinical trials may be categorized into studies using unselected BMCs or selected cell populations.

- ***Unselected Bone Marrow Cells***

The combined experience from more than 100 patients suggests that intracoronary delivery of unselected BMCs (all nucleated cells or mononuclear cell fraction only) is safe in the short- and mid-term (several months).[40,41,43-46] No bleeding complications were noted after bone marrow harvest. Intracoronary BMC infusions did not appear to inflict additional ischemic damage to the myocardium or to promote a systemic inflammatory reaction, because no further increases in serum troponin or CRP levels were observed. No increased rates of in-stent restenosis were observed after transfer of unselected BMCs.[43,44,46] The effects of BMC transfer were observed on top of the benefits associated with established interventional and medical strategies to promote functional recovery after AMI.[46]

- ***Selected Bone Marrow Cell Population***

The Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE AMI) trial compared unselected mononuclear BMCs with circulating blood-derived progenitor cells (mostly EPCs). Both cell types appeared to have similar safety and efficacy profiles.[41,43]

- ***Stem and Progenitor Cell Mobilization***

Stem cell mobilization with stem cell factor (SCF) and/or granulocyte colony-stimulating factor (G-CSF) has been proposed to stimulate myogenesis and angiogenesis in the infarcted area and to improve cardiac function after AMI in mice.[94,95] By contrast, treatment with SCF and G-CSF enhances vascularization of the infarcted area but does not improve cardiac function in baboons after AMI.[96] Perhaps, reperfusion of the infarct-related artery before cytokine therapy would have permitted better access of mobilized cells to the infarct center in this large animal model.[96] Of note, G-CSF may accelerate infarct healing by enhancing macrophage infiltration and matrix metalloproteinase activation[97] and suppress

cardiomyocyte apoptosis by activating the cytoprotective STAT3 transcription factor,[98] suggesting that stem cell–independent mechanisms may contribute to the effects of G-CSF after AMI.

CONCLUSIONS AND FUTURE PERSPECTIVES

Given the worldwide prevalence of cardiac dysfunction and the limited availability of tissue for cardiac transplantation, stem cells could ultimately fulfill a large-scale unmet clinical need and improve the quality of life for millions of people with CVD. However, many unresolved questions about experimental and clinical cardiology are still open for future research (Table 2).

Table 2: Key issues to be addressed following stem cell therapy for CVD.

S. No.	Issues
1.	The long-term fate of transplanted stem cells in the recipient tissue.
2.	The ability of transplanted stem cells to find their optimum myocardial "niche."
3.	The potency of stem cells to transdifferentiate into heart muscle cells.
4.	The optimal angiogenic milieu needed for transplanted cells in hypoperfused tissue.
5.	The capability of the recipient tissue to enable an enhanced environment to offer optimum, milieu-dependent differentiation of engrafted cells.
6.	Specific detection of engrafted cells or cell populations by labeling techniques.
7.	The optimal time course of availability and application for stem cell replacement therapy in cardiovascular disease.
8.	The arrhythmogenic potential of implanted cells.
9.	The specific characterization of the progenitor cells that should be measured to predict therapeutic effect of transplanted cells.
10.	Development of safe and reproducible catheter-based delivery systems for depositing stem cells to recipient heart muscle.

Additional research is needed to explore the therapeutic merits of cell transplantation techniques while accepting the likelihood that possible adverse side effects may occur. With regard to the clinical practicability, ethical problems, and hazards of immunogenity, actual and future research will focus preferably on adult stem cells, whereas research on embryonic stem cells may emerge presumably into comparable clinical relevance in the years to come.

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