IJPT
1735-2657/07/62-217-227
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

IJPT 6:217-227, 2007

RIDR

REVIEW ARTICLE

Are Stem Cells the Next Therapeutic Tool for Heart Repair?

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Received January 24, 2007; Revised June 6, 2007; Accepted July 9, 2007

This paper is available online at *http://ijpt.iums.ac.ir*

ABSTRACT

Cardiovascular disease remains the leading cause of morbidity and mortality in the United States and Europe. In recent years, the understanding that regenerative processes exist at the level of the myocardium, has placed stem cell research at center stage in cardiology. A stem cell is a cell that has the ability to divide (self replicate) for indefinite periods often throughout the life of the organism. Myocardial regeneration with stem-cell transplantation is a possible treatment option to reverse the deleterious hemodynamic and neurohormonal effects that occur after myocardial infarction and can lead to congestive heart failure. Embryonic stem (ES) cells, bone marrow (BM) stem cells, myoblasts, fetal cardiomyocytes, endothelial progenitors, resident cardiac progenitor cells, and tissue-derived stem cells are potential sources of stem cells. They regenerate heart by differentiation in the endothelial and cardiac lineages, neovascularization as well as influence on the local environment by the release of paracrine factors. Early phase I clinical studies indicate that stem-cell transplantation is feasible and may have beneficial effects on ventricular remodeling after myocardial infarction. The ongoing rigorously- designed trials will contribute greatly to this emerging and exciting new therapeutic approach for diseases of the cardiovascular system.

Keywords: *Myocardial infarction, Stem cell, Cardiomyocytes and Differentiation*

Stem cells therapy is one of the most fascinating areas of biology today and stem cells are the trend setters of regenerative or reparative medicine. Stem Cells are unspecialized cells, can divide and renew themselves for long periods of time and become specific specialized cell types of the body i.e. stem cell is an undifferentiated cell in the body with undetermined function capable of forming various tissues under definite signals received from the body [1]. By definition, stem cells are cells that are clonogenic (capable of producing exact duplicates), self-renewing (capable of dividing indefinitely), and potent (capable of differentiating into multiple cell lineages) [2].

According to their potential for differentiation, several generations of stem cells can be distinguished viz totipotent, pluripotent, multipotent, or unipotent [3]. Stem cells in the very early stages of embryonic development are often referred to as totipotent, or omnipotent. Totipotent cells can give rise to any type of cell: cells of the trophoblast and cells of the 3 germ layers (endoderm, mesoderm, and ectoderm), all of which are necessary for complete embryonic development thus developing a living organism [4]. Stem cells that can give rise to cells of all 3 embryonic germ layers but not to trophoblasts are considered pluripotent [5]. Stem cells that give rise to cells of different lineages within a single germ layer are considered multipotent. After a limited number of divisions, unipotent stem cells begin to differentiate and give rise to cells of specific tissue types. These tissue-specific cells then impart function and structure to tissues and organs by becoming their integral units [6].

In 1998, James Thomson at the University of Wisconsin-Madison isolated cells from the blastocyst, and developed the first human embryonic stem cell lines [7]. Stem cell can be obtained from several sources like spare embryos, special purpose embryos, cloned embryos, aborted fetuses, umbilical cords, adult tissue or organs and cadavers.

Coronary arteries are the blood vessels that supply blood and oxygen to the heart muscle. Fatty deposits build up in blood vessel walls and narrow the passageway for the movement of blood. Coronary artery disease results from a chronic inflammatory disease of the vascular wall and leads to vessel occlusion and organ damage [8, 9]. Coronary artery disease can be treated pharmacologically (anticoagulants, fibrinolytics and antiplatelets) [10] or surgically (Percutaneous Transluminal

Coronary Angioplasty (PTCA) and Coronary Artery Bypass Surgery (CABG)).Gene therapy for therapeutic angiogenesis and to prevent restenosis is done with the genes of Growth Factor Protein, Low-density lipoprotein receptor, Hypoxia Response Element (HRE), COX-I, tissue factor pathway inhibitor [11].

Limitations of current therapies have led to research aimed at regenerating and repairing ischemically damaged myocardium through stem-cell therapy. How can stem cells play a part in repairing the heart? One important type of cell that can be developed is the cardiomyocyte (myogenesis), the heart muscle cell that contracts to eject the blood out of the heart's main pumping chamber (the ventricle). Besides, vascular endothelial cell, which forms the inner lining of new blood vessels, and the smooth muscle cell, which forms the wall of blood vessels [12, 13].

I. POTENTIAL SOURCES OF CARDIAC STEM

Recent experimental and clinical observations have suggested that cell transplantation could be of therapeutic value for the treatment of heart disease. This approach was based on the idea that transplanted donor cardiomyocytes would integrate with the host myocardium and thereby directly contribute to cardiac function [14].

Stem-cell transplantation (also called cell cardiomyoplasty) generates contractile cells that integrate both functionally and structurally into the surrounding viable muscle. This integration occurs via the formation of cell-to-cell contacts such as cardiac-specific intercalated discs consisting of desmosomes and gap junctions. The desmosomes give myocardial tissue the necessary physical strength; the gap junctions, which consist of the cardiac muscle-specific protein connexin 43, enable communication between cardiomyocytes that results in coordinated and synchronous contraction of myocardial tissue [15]. The contractility of engrafted cells can occur naturally (for example, in the case of fetal cardiomyocytes or skeletal myoblasts) or be induced by transdifferentiation (for example, in the case of adult stem cells). These structural characteristics indicate that the developing new myocytes are coupled electrically and mechanically [16]. Endothelial cells and fibroblasts have also been used to restore function to nonviable myocardium, but the results have been substantially inferior to those achieved with contractile cells [4, 5, 17].

The microenvironment plays a fundamental role in the transdifferentiation of stem cells. Human mesenchymal stem cells (from adult bone marrow), when engrafted into murine hearts, seem to differentiate into cardiomyocytes that are indistinguishable from the host's cardiomyocytes [18] and can form functional gap junctions with one another, with cell lines expressing cardiac connexins, and with adult cardiac myocytes [19]. Although its mechanism is incompletely understood, the "homing" of stem cells to the injured myocardium is essential, as it concentrates the implanted cells in an environment favorable to their growth and function. Ischemia or hypoxia may increase vascular permeability, enhance the release of chemoattractive factors, and promote the expression of adhesion proteins, which may facilitate the homing process [20].

Embryonic stem (ES) cells, bone marrow (BM) stem cells, fetal cardiomyocytes, myoblasts, endothelial progenitors, resident cardiac progenitor cells, and tissue-derived stem cells are potential sources to improve neovascularization and left ventricular (LV) function after myocardial infarction [21, 22, 23].

(A) EMBRYONIC STEM CELLS (ESS)

Three types of pluripotent stem cell lines have been established from mammalian embryos: Embryonic Stem (ES), Embryonic Germ (EG) and Embryonic Carcinoma (EC) cells [24]. Embryonic stem (ES) cells are derived from the inner cell mass of the blastocyst-stage embryo, late in the first week after fertilization [25, 26, 7]. Embryonic stem cells must be obtained when an embryo is in early development, that is, when the fertilized egg has divided to form about 1000 cells [27].

Embryonic stem cells are pluripotent, weakly immunogenic and they can readily differentiate into nearly any cell in the body, for example, mouse ES cells can be directed *in vitro* to yield vascular structures [5, 28, 29]. ES cells can be cultured for extended periods and genetically manipulated without loss of their pluripotential capacity (as assessed by their ability to participate in all aspects of the development of the embryo proper when reintroduced *in vivo*) [30, 31, 32]. ES cells 14of a disorganized array of differentiated or partially differentiated cell types that are derived from the three primary germ layers of the embryo-the endoderm, mesoderm, and ectoderm [33] including cells of the hematopoietic, endothelial, cardiac and neuronal tissues [32].

In *vitro* differentiation of ES cells into cardiomyocytes occurs in embryoid bodies. This differentiation can be divided into 3 stages: early, intermediate, and terminal. In the early stage, pacemaker-like cells are produced, and in the intermediate stage, atrial and ventricular cells and cells of the heart conduction system develop. During the terminal stage, well-organized bundles of myofibrils can be observed with clearly distinguishable A, I, and Z bands, and intercalated discs that contain desmosomes and gap junctions. Cardiomyocytes derived from terminally differentiated ES cells respond to β-adrenergic stimulation in vitro [4]. These unique pluripotent cell lines can be propagated in the undifferentiated state in culture and coaxed to differentiate into cell derivatives of all three germ layers, including cardiomyocytes [34]. Human ES (hES) cells differentiate into spontaneously beating cells with a cardiomyocyte phenotype. The morphology and ultrastructure of these cells are organized with sarcomeric structures, formation of intercalated disks, desmosomes, and gap junctions, characteristic of cardiomyocytes [4, 35, 36] and the presence of a functional syncitium with action potential propagation [36, 37] and even displaying atrial and ventricular subtypes. Electrical recordings from these cells, changes in calcium-ion movement within the cells, and contractile responsiveness to catecholamine hormone stimulation by the cells were similar to the

recordings, changes, and responsiveness seen in early cardiomyocytes observed during mammalian development [36, 38]. Furthermore, hES cells can be genetically engineered to gain an improved resistance to ischemia [4, 5]. However, hES cells have drawbacks like potential immune rejection, difficult-to-obtain pure cell cultures, and limited proliferative capacity.

(B) BONE MARROW-DERIVED STEM CELLS (BMSCS)

Bone marrow is composed of various types of cells of specific phenotypes and function. The concept of progenitor-cell transfer for enhancing cardiac repair has raised new therapeutic prospects [39]. Bone marrow cells can be transplanted either as total, unfractionated bone marrow or as a well-defined subpopulation of BMSCs. Bone marrow is a potential source of multipotent stem cells for cell cardiomyoplasty, particularly because of its easy accessibility, autologous origin, and ability to transdifferentiate into both cardiomyocytes and coronary vessels [40, 41, 42].

Total unfractionated bone marrow is usually aspirated from the iliac crest and immediately injected into damaged myocardium. Total bone marrow contains very few multipotent cells capable of transdifferentiating into cardiomyocytes, per unit of volume. Another, slightly more complicated method is to inject a well-defined subpopulation of multipotent BMSCs into damaged myocardium. [43]

(i) Hematopoietic Stem Cell (HSC)

HSCs are constantly being generated in the bone marrow where they differentiate into mature types of blood cells. Indeed, the primary role of HSCs is to replace blood cells, only an estimated 1 in 10,000 to 15,000 cells in the bone marrow is a hematopoietic (blood forming) stem cell (HSCs) [44, 45]. The mobilization of HSCs from bone marrow can apparently be enhanced by cytokine factors, as shown by studies in a rat model of MI [43, 46, 47]. Treatment with cytokine stem-cell factor (SCF) which binds to the c-kit tyrosine kinase receptor, and granulocyte colony-stimulating factor (G-CSF) led to a 250-fold increase in circulating HSCs. It was reported that, in the presence of an acute myocardial infarct, cytokine-mediated translocation of BMC resulted in a significant degree of tissue regeneration 27 days later. Ejection fraction progressively increased and hemodynamics significantly improved as a consequence of the formation of 15×10^6 new myocytes with arterioles and capillaries connected with the circulation of the unaffected ventricle [47]. The phenotypes of engrafted cells develop cardiomyocytes, endothelial cells, and smooth muscle cells.

(ii) Mesenchymal Stem Cells (MSCs)

Mesenchymal stem cells (MSCs) also known as bone marrow stromal cells, are multipotent. MSCs are self-renewing clonal precursors of nonhematopoietic tissues derived from mesodermal germ layer [48]. They are relatively easy to obtain from autologous bone marrow, to expand in vitro without sacrificing multipotency, and to cryopreserve for future use. Normally, MSCs can differentiate into osteocytes, chondrocytes, and adipocytes. Under certain conditions, however, they can differentiate into other cell types. When cultured with vascular endothelial growth factor (VEGF), MSCs can differentiate into endothelial cells [19]. When treated with the DNA-demethylating agent 5-azacytidine (5-aza), they can differentiate into cardiomyogenic (CMG) cells, which can subsequently form myotubes connected by intercalated discs that beat spontaneously and synchronously [49]. The cells showed a fibroblast-like morphology, but the morphology changed after 5-azacytidine treatment in ~30% of the cells; they connected with adjoining cells after one week, formed myotube-like structures, began spontaneously beating after two weeks, and beat synchronously after three weeks. They expressed atrial natriuretic peptide and brain natriuretic peptide and were stained with anti-myosin, anti-desmin, and anti-actinin antibodies [50]. These cells had several types of action potentials, such as sinus node-like and ventricular cell-like action potentials. All cells had long action potential duration or plateau, a relatively shallow resting membrane potential, and a pacemaker-like late diastolic slow depolarization [51]. They also expressed cardiac-specific transcription factors in a pattern that is seen in the early embryonic heart and display functional adrenergic and muscarinic receptors on their surfaces [19].

The exact mechanisms by which MSCs may improve cardiac function remain controversial. One candidate mechanism is de novo angiogenesis, as shown recently in rats whose ischemic hearts were injected with 5-aza-treated MSCs [52, 53, 54]. Another possible mechanism is cell fusion, which would theoretically allow damaged myocardial and endothelial cells to preserve their structural and functional integrity by commandeering additional cytoplasmic material and new genetic material from the fused cells [55].

At the Texas Heart Institute, allogeneic MSC injections were performed in a canine model of chronic ischemia induced by ameroid constriction. The MSCs differentiated into smooth muscle cells and endothelial cells, which resulted in increased vascularity and improved cardiac function both at rest and under stress [55]. One group found that MSCs genetically engineered to over-express the serine threonine kinase Akt, which transmits a powerful survival signal, became 17 times more resistant than normal MSCs to the hypoxic environment of the ischemic myocardium. Moreover, when transplanted into areas surrounding ischemic myocardium, these genetically altered cells inhibited myocardial remodeling and collagen deposition and almost completely normalized systolic and diastolic function. These cells could not, however, induce new blood vessel growth. Thus, mesenchymal stem cells genetically enhanced with Akt1 can repair infarcted myocardium, prevent remodeling and nearly normalize cardiac performance [56]. Clearly, MSC therapy can restore some function to the ischemic heart. In addition, this ability is apparently enhanced when other stem cells are used as well, as recently shown in an ischemic swine

model in which human MSCs and fetal cardiomyocytes were transplanted together [57].

(iii) Endothelial Progenitor Cells (EPCs)

During embryonic development, just after gastrulation, a kind of cell called the hemangioblast, which is derived from mesoderm, is presumed to be the precursor of both the hematopoietic and endothelial cell lineages [58]. The occurrence of a first major cardiovascular event (acute myocardial infarction, hospitalization, revascularization, or death from cardiovascular causes) was associated with reduced endothelial progenitor-cell levels [59]. EPCs derived from bone marrow circulate in the peripheral blood and have been implicated in neoangiogenesis after tissue ischemia has occurred [60, 61, 62]. EPCs are capable of proliferating and differentiating into endothelial cells and are therefore ideal candidates for vascular regeneration [63]. Intracoronary injection of EPCs cells may improve left ventricular function after acute myocardial infarction [64].

(C) FETAL AND NEONATAL CARDIOMYOCYTES

Fetal and neonatal cardiomyocytes are already differentiated and do not, in practical terms, divide. Nevertheless, they can be used for cellular cardiomyoplasty [65]. These cells are obtained from fetal and neonatal hearts, respectively, and then grown in tissue cultures. These cells have also been shown to connect with host cardiomyocytes through intercalated discs containing desmosomes and gap junctions-a sign of their structural and functional integration into the host myocardium. In animal models, the cells were successfully engrafted through epicardial injection into ischemic myocardium [66, 67].

(D) SKELETAL MYOBLASTS (SMS)

Adult skeletal muscle, unlike myocardium, has retained an efficient regenerating mechanism. Skeletal myoblasts have autologous origin, high proliferative potential, commitment to a myogenic lineage, and resistance to ischemia. The last property is an especially important one for cells intended for implantation in the hypoxic environment of a postinfarction scar [68, 69]. Also, cellular and molecular approaches to strengthening the injured or weakened heart, focusing on strategies to replace dysfunctional, necrotic, or apoptotic cardiomyocytes with new cells of mesodermal origin [70]. Histologically, skeletal myoblasts (SMs) are committed progenitors of skeletal muscle cells. Each mature skeletal myofiber bears a few myogenic cells known as satellite cells (SCs). These myogenic cells remain quiescent and in undifferentiated state under the basal lamina, until recruited for repair or regeneration of damaged muscle tissue [71]. SCs obtained from adult skeletal muscle, when implanted into injured myocardium, will multiply and may be influenced by the cardiac environment to undergo ``milieu-dependent'' differentiation, thus repairing the damaged myocardium [72]. Studies in rats and human beings confirmed that implanted SMs could repopulate scar tissue, resulting in ventricular wall thickening, elevated ejection fraction, and improved contractility [73].

(E) RESIDENT CARDIAC STEM CELLS

Several investigators have recently identified a population of stem cells within the myocardium that are capable of differentiating into cardiac myocytes, which are similar to BMSCs [74]. The recognition that the adult heart possesses a stem cell compartment that can regenerate myocytes and coronary vessels has raised the unique possibility to rebuild dead myocardium after infarction, to repopulate the hypertrophic decompensated heart with new better functioning myocytes and vascular structures, and, perhaps, to reverse ventricular dilation and wall thinning [75]. It has recently been reported that these cells can be harvested from cardiac biopsies. Injecting these cells in the setting of myocardial infarction can promote cardiomyocyte formation with associated improvements in systolic function [76]. Cardiac progenitor cells isolated from adult myocardium have a phenotype (Sca-1+, lin−, CD45−, CD31+, CD38+) [77]. Myocardial regeneration necessitates the utilization of a more primitive multipotent cell such as the c-*kit*-positive cardiac stem cell [78]. At present, these cells are limited in number and require *ex vivo* separation and expansion over several weeks.

(F) OTHER STEM CELLS

These include peripheral blood CD34+ cells and fibroblasts. Adult peripheral blood CD34+ cells are of interest as potential cardiac stem cells, because they can be obtained from autologous sources without the need for painful bone marrow aspiration and known to be able to transdifferentiate [79]. Fibroblasts of autologous origin have the potential to inhibit host matrix degradation, to improve diastolic function by replacing scar tissue with more elastic muscle-like tissue, and to enhance regional hypertrophy and neovascularization. In addition, they may prevent infarct expansion and ventricular remodeling by increasing scar thickness, which reduces wall stress [77].

II. REGENERATION MECHANISM

Cardiac regeneration by stem cells occurs by several mechanisms. These include (i) differentiation in the endothelial and cardiac lineages, (ii) neovascularization as well as (iii) influence on the local environment by the release of paracrine factors. (Fig 1)

(i) Differentiation and/or fusion

Cells might differentiate into the cardiac lineage and thereby contribute to cardiac regeneration. Injection of various bone marrow-derived stem cells after myocardial infarction leads to an engraftment of these cells in the border zone of the infarcts [40, 80]. Badorff et al. demonstrates that EPCs isolated from the peripheral blood of healthy adult volunteers and patients with CAD

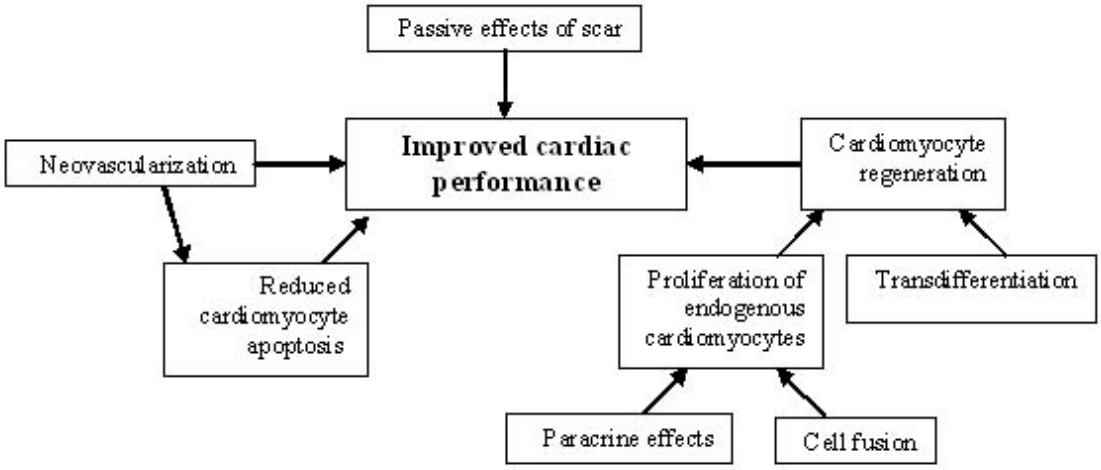

Fig 1. Different mechanism of regeneration and cardiac repair. Adopted from Boyle et al. [34]

are capable of transdifferentiating into cardiac myocytes when co-cultured with rat cardiomyocytes [81].

Originally, cell fusion referred to the joining of two fully differentiated cells [82]. The concept of cell fusion has evolved and, currently, indicates the coalescence of a stem cell and a differentiated cell [83, 84]. Fusion of a resident primitive cell or BMC with a preexisting myocyte that subsequently re-enters the cell cycle and leads to the formation of a myocyte progeny has been proposed as an alternative mechanism of growth in the adult heart [77, 85, 86]. Also the expression of cardiac marker proteins was not the result of a differentiation process, but rather reflected cell-cell fusion. Such events were reported initially for ES cells, and now have been confirmed with HSCs [80].

(ii) Improvement of neovascularization

After myocardial infarction, the newly formed capillary network in the infarcted myocardium cannot adequately keep up with the tissue growth needed for contractile compensation and cannot meet the higher demands of the surviving hypertrophied cardiomyocytes, leading to further expansion of the infarct and fibrosis of the myocardium [87]. Thus, neovascularization represents a potentially important process by which increasing perfusion to infarcted myocardium may reduce ventricular dilatation and improve cardiac function through the rescue of hibernating myocardium and decreased apoptosis of hypertrophied cardiomyocytes [88].

The novel strategy for the treatment of vascular insufficiency was termed therapeutic angiogenesis [89]. It has been shown by various groups that bone marrow contains HSCs can differentiate to endothelial cells and increase the formation of new capillaries in tissues that are ischemic [90, 91, 92, 93, 94, 95]. Also, *ex vivo* expanded hEPCs may have utility as a "supply-side" strategy for therapeutic neovascularization [96, 97]. Bone marrow from adult humans contains endothelial precursors with phenotypic and functional characteristics of embryonic hemangioblasts, and that these can be used to directly induce new blood vessel formation in the infarct-bed (vasculogenesis) and proliferation of preexisting vasculature (angiogenesis) after experimental myocardial infarction [93]. Various cultivation methods were used to isolate these endothelial precursor cells from the peripheral blood. The isolated cells showed expression of endothelial marker proteins including VE-cadherin, von Willebrand factor, KDR and endothelial nitric oxide synthase (eNOS) [98]. Recruitment of smooth muscle cells provides these vessels with essential viscoelastic and vasomotor properties and enables accommodating the changing needs in tissue perfusion. This later stage is called arteriogenesis and has a major role in collateral growth [99]. Endothelial progenitor cells showed incorporation into sites of physiological and pathological neovascularization *in vivo* after either systemic injection or using direct intramyocardial transplantation [100]. In contrast to differentiated endothelial cells, transplantation of progenitor cells successfully enhanced vascular development by in situ differentiation and proliferation within ischemic organs [1].

(iii) Paracrine effects

Bone marrow-derived stem and progenitor cells home to sites of ischemia. This may allow the local release of factors acting in a paracrine manner on the surrounding ischemic tissue. Bone marrow-derived mononuclear cells (BMCs) release angiogenic growth factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and angiopoietins, thereby enhancing the local angiogenic response.[101,102,103].

III. ADMINISTRATION ROUTE

The mode of stem cell delivery is to achieve the ideal concentration of cells needed to repair the injured myocardial region with the lowest risk to patients [104].

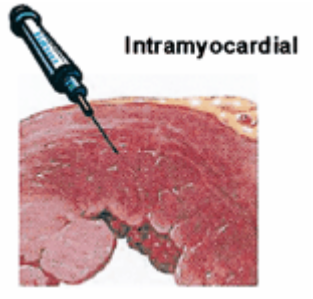

Fig 2. Diagrammatic representation of intramyocardial injection
Adopted from Strauer et al. [106]

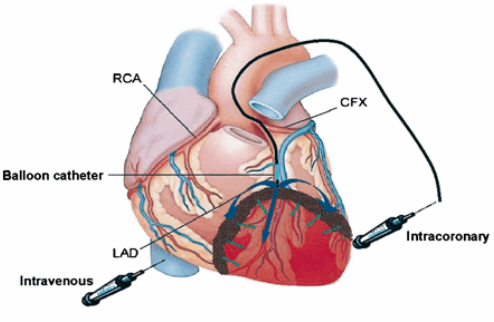

Fig 3. Diagrammatic representation of Intracoronary and intravenous injection Adopted from Strauer et al.[106]
RCA = Right Coronary Artery;
LAD = Left Anterior Descending coronary artery;
CFX =Circumflex Artery.

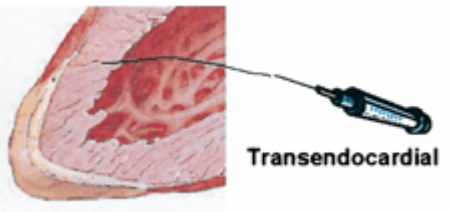

Fig 4. Diagrammatic representation of transendocardial injection.
Adopted from Strauer et al. [106]

i. Intramyocardial Injection

Orlic and colleagues [46] isolated bone marrow stem cells and directly injected them in the margin bordering the infarct of the left ventricle of mice. These cells migrated into the region bordering the infarction and differentiated into cardiomyocytes and endothelial cells, generating de novo myocardium, improving cardiac function, and leading to neovascularization. Direct intramyocardial injection may require fewer cells to achieve engraftment compared with intracoronary or intravenous administration. A different approach is to implant stem cells through percutaneous catheter-based myocardial injections guided by electromechanical mapping [105]. (Fig 2)

(ii) Intracoronary Infusion

A percutaneous transluminal coronary catheter can be used for intracoronary administration of bone marrow–derived stem cells after myocardial infarction [107] (Fig 3). Stem cells can be infused directly into the coronary arteries in a homogenous manner and have a greater likelihood of remaining in the injured myocardium as a result of the activation of adhesion molecules and chemokines [64]. The advantage of an intracoronary infusion is that the cells can be directed to a particular territory. This is advantageous over intravenous administration because it can deliver the maximum concentration of cells to the site of infarct and peri-infarct tissue during the first passage. Intracoronary administration into the infarct artery allows the stem cells to home in and incorporate into the areas bordering the infarct zone in a homogenous manner. This is in contrast to direct myocardial injection, which may lead to "islands" of cells in the infarcted myocardium, providing a substrate for electrical instability and ventricular tachyarrhythmias [108]. High-pressure injection of stem cells into the infarct region may facilitate transendothelial passage and migration into infarcted myocardium [64]. The quantity of cells and time of infusion should be carefully considered to avoid coronary flow impairment and myocardial cell necrosis. This technique may not be suitable for certain types of larger stem cells, such as skeletal myoblasts, which may be prone to embolization [109]. The timing of intracoronary stem cell therapy affects the therapeutic response in patients with reperfused myocardial infarction. The natural course of healing the infarction and the presence of putative homing signals within the damaged myocardium appear to favor cell engraftment during the transendothelial passage in the early days after reperfusion. However, the adverse inflammatory environment, with its high oxidative stress, might be deleterious if cells are administered too early after reperfusion [110].

(iii) Intravenous Injection

Intravenous administration of stem cells is an attractive and practical mode of delivery because it does not require cardiac surgery or catheterization (Fig 4). Microenvironmental factors, expression of matrix and adhesion molecules by injured tissue, homing receptors, and various factors relating to migration are believed to be involved in the homing process of stem cells [20]. Intravenously injected cells may become trapped in other organs (e.g. liver, spleen, lung, etc) so that only a small portion enters the coronary circulation and migrates into ischemic myocardium. But, BM-MSCs delivered by left ventricular (LV) cavity infusion migrate to and colonize the infarcted heart in significantly higher amounts than after intravenous infusion [111]. Homing signals are also present at other sites in the body, particularly in lymphoid tissues. Dosing will likely play an important role in the viability of this method [108]. One day after transplantation of neonatal

Table 1. Clinical Studies of Stem Cell Therapy for Cardiac Repair

Study	Treated(n)	Cell Type	Mode of Delivery	Associated procedure	Primary End Point
Strauer et al. [64]	10	BMMNCs	Intracoronary	PTCA	Safety
Pagani et al. [76]	5	Skeletal myoblasts	Open heart surgery	LVAD	Histology
Menasche et al. [99]	10	Skeletal myoblasts	Open heart surgery	CABG	Safety and feasibility
TOPCARE-AMI [108,117]	59	BMMNCs/ CPCs	Intracoronary	PTCA	Safety and feasibility
Tse et al. [105]	8	BMMNCs	Endomyocardial	Catheterization	Safety and feasibility
Perin et al. [118]	14	BMMNCs	Endomyocardial	Catheterization	Safety
BOOST [119]	30	BMMNCs	Intracoronary	PTCA	LVEF
IACT study [120]	18	BMMNCs	Intracoronary	PTCA	Not stated
Janssens et al. [121]	33	BMMNCs	Intracoronary	_	LVEF
Galinanes et al. [122]	14	Bone marrow	Open heart surgery	–	Safety
Fuchs et al. [123]	10	Bone marrow	Endomyocardial	PTCA	Safety and feasibility
MAGIC cell [124]	20	GCSF-mobilized PBMNCs	Intracoronary	_	Safety and feasibility
Ozbaran et al. [125]	6	GCSF-mobilized PBMNCs	Open heart surgery	–	Safety and feasibility
Erb et al. [126]	13	GCSF-mobilized CPCs	Intracoro119nary	PTCA	CFR and LVEF
Dib et al. [127]	30	Skeletal myoblasts	Open heart surgery	–	Safety and feasibility
POZNAN [128]	10	Skeletal myoblasts	Transcoronary-venous	PTCA	Safety and feasibility
Smits et al. [129]	5	Skeletal myoblasts	Endomyocardial	PTCA	Safety and feasibility
Herreros et al. [130]	12	Skeletal myoblasts	Open heart surgery	–	Safety and feasibility

cardiac myocytes into rat hearts, only 24% of the originally injected cells remained in the heart [112].

(iv) Transendocardial and Transpericardial injection

The transendocardial and transpericardial route of application has been used in large animal experiments [113] and was also recently tested in patients [114]. A transendocardial approach can be used in which a needle catheter is advanced across the aortic valve and positioned against the endocardial surface [105, 115] (Fig 5). Cells can be injected directly into the left ventricle. Electrophysiological mapping can be used to differentiate sites of viable, ischemic, or scarred myocardium.

IV. CLINICAL TRIALS

The possibility that cardiac cell-repair therapy might become a clinical reality is a challenge worthy of the current state of technological and scientific expertise.Various preclinical animal studies show the potential to regenerate myocardium and improve perfusion to the infarct area to improve cardiac function but also suggest that stem cells may have proarrhythmic effects [116]. A phase I clinical study is performed out at Minneapolis Heart Institute Foundation for "Bone Marrow Stem Cell Infusion Following a Heart Attack". AMORCYTE MYOCARDIAL REPAIR STUDY is in progress at Emory University Texas Heart Institute. Early phase I clinical studies indicate that stem-cell transplantation is feasible and may have beneficial effects on ventricular remodeling after myocardial infarction. Future randomized clinical trials will establish the magnitude of the benefit and the effects on arrhythmias after stem-cell therapy [88]. (Table 1)

V. SAFETY

Safety is always a major concern in dealing with clinical trials involving stem cells. Implanted stem cells may differentiate into fibroblasts rather than myocytes. This may enhance scar formation, further depressing myocardial function and creating a substrate for life-threatening arrhythmias [116]. It has recently been suggested that skeletal myoblasts that have been genetically engineered to express gap junction protein connexin 43 exhibited decreased arrhythmogenicity [131]. There have been conflicting reports regarding the potential for increased restenosis after stem cell transplantation. In another study, a high rate of restenosis was observed after intracoronary delivery of peripheral blood stem cells mobilized with granulocyte colony-stimulating factor in the setting of myocardial infarction and stent placement [132]. There may also be life-threatening consequences if stem cells incompletely integrate into the myocardium and adversely affect electrical conduction and syncytial contraction of the heart [133]. Tumor formation associated with embryonic stem cells, such as teratomas, may also occur. Late-onset toxicity may occur from using whole populations of bone marrow mononuclear cells, which contain different organ-specific stem cells. These nonessential cells may incor-

porate into regenerating myocardium, resulting in the generation of noncardiac tissues [134].

CONCLUSION

Stem cells remain a highly promising therapeutic modality that could address the large, unmet clinical need of treating patients throughout the world with significant cardiac dysfunction that cannot be adequately treated with conventional therapeutic approaches or cardiac transplantation because of the limited availability of this resource.

The potential sources are Embryonic stem (ES) cells, bone marrow (BM) stem cells, myoblasts, fetal cardiomyocytes, endothelial progenitors, resident cardiac progenitor cells, and tissue-derived stem cells. Bone marrow derived stem cells are mostly used and will be very efficient therapy for heart repair.

Stem cells regenerate heart by differentiation in the endothelial and cardiac lineages, neovascularization as well as influence on the local environment by the release of paracrine factors. The basic mechanisms of stem-cell differentiation that lead to the formation of solid-organ tissue are still not completely understood. However, translational research, including clinical studies, is already being performed to develop potential treatment strategies. Manipulation in vitro of stem cells by providing optimal culture condition, which include various cytokines and growth factors to augment organ-specific engraftment, may be needed to facilitate in vivo incorporation.

Different routes of administration are intracoronary, intravenous, intramyocardial and transmyocardial. Further information is also necessary on the homing and organ-specific differentiation signals required for various stem cells, and this includes characterization of integrin and other adhesion molecule structure/function relationships on these cells. These studies will need extensive use of cellular controls and functional endpoints including imaging modalities that are clinically appropriate and can be validated with explanted tissue analysis.

Implanted stem cells may differentiate into fibroblasts rather than myocytes, which may enhance scar formation, further depressing myocardial function and creat life-threatening arrhythmias. Genetically engineered skeletal myoblasts express gap junction protein connexin 43 exhibited decreased arrhythmogenicity.

Many important fundamental questions about stem-cell transplantation still remain unanswered. The optimal donor cell and the optimal number of stem cells to be transplanted have not been determined. There is a threshold of the number of stem cells needed to generate adequate heart muscle to contribute to cardiac function. Adult stem cells are limited in supply in each patient and therefore are difficult to isolate and purify. The stem cells from the patient's body must be isolated and expanded in culture to obtain a sufficient amount for stem-cell transplantation.

Currently, the effectiveness of stem-cell transplantation alone is difficult to interpret because the clinical studies have been done in conjunction with percutaneous or surgical revascularization. Thus, larger double-blinded, controlled studies with therapeutic end points are imperative to clarify the role of stem-cell transplantation for myocardial regeneration.

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