Stem Cell Transplantation and Cardiac Repair: A Review of Its Current and Future Status

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Abstract

After myocardial infarction, injured cardiomyocytes are replaced by fibrotic tissue promoting the development of heart failure. Stem cells are multipotent, undifferentiated cells capable of multiplication and differentiation. Preliminary experimental evidence suggests that stem cells derived from embryonic or adult tissues (especially bone marrow) may develop into myocardial cells. The overall clinical experience also suggests that stem cell therapy can be safely performed, if the right cell type is used in the right clinical setting. Preliminary efficacy data indicate that stem cells have the potential to enhance myocardial perfusion and/or contractile performance in patients with acute myocardial infarction, advanced coronary artery disease, and chronic heart failure. However, at the present time, the results have been mixed and inconclusive, and the mechanism of stem cell transplantation therapy remains unclear. This review discusses the controversies and problems that need to be addressed in future investigations.

Introduction

Coronary artery occlusion leads to ischemia and cell death in the heart (1). Cardiomyocyte death results in scar formation and reduced contractility of the ventricle. Although the traditional concept that the adult cardiomyocyte is erminally differentiated has been challenged by evidence that some myocytes are mitotic in adult hearts (2, 3), the ratio of myocytes undergoing proliferation is only 0.015-0.08% (3,4). The number of resident cardiac muscle stem cells within the heart is also too small to significantly repair the damage after myocardial infarction (5). The rreversible loss of muscle after acute myocardial infarction followed by fibrosis of myocardial scar, infarct xpansion, concentric hypertrophy, and left ventricular dilatation ultimately leads to progressive heart failure (6). While the uality of life after acute myocardial infarction has been improved due to the enormous progress in the cardiovascular therapeutics (7), the root cause of heart failure, which is characterized by cardiomyocyte death and ventricular emodeling, remains a major contributor to cardiac morbidity and mortality. Cellular cardiomyoplasty provides a potential approach to the treatment of heart failure after myocardial infarction. The basic concept of cellular cardiomyoplasty is to increase the number of functional cardiomyocytes by cell transplantation. Many types of cells, such as cardiomyocytes, skeletal myoblasts and stem cells, have been used in the attempt to regenerate myocardium and treatment of heart failure (8). In this review, we focus on the use of stem cell transplantation for cardiac repair.

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Definition

Stem cells are a group of undifferentiated cells that have the capacity to self-renew, as well as the ability to generate differentiated cells. There are somatic stem cells and embryonic stem cells. Somatic stem cells are derived from adult somatic tissue, such as bone marrow, adipose tissue, peripheral blood, umbilical cord blood, and skeletal muscle. Embryonic stem cells are isolated from the embryo at the blastocyst stage and can form all fully differentiated cells of the body, including true cardiomyocytes. Embryonic stem cells have the greatest potential for cardiac regeneration. Every type of stem cell has advantages and disadvantages for cardiac regeneration. Embryonic stem cells are more versatile than somatic stem cells for cardiac regeneration. Although somatic stem cells may be autologous and no immunological or ethical constraints exist, their potential to differentiate is more restricted than embryonic stem cells. Determining which is the most appropriate stem cell for cardiac regeneration and revascularization remains a crucial unanswered question.

Ventricular remodeling and stem cell therapy

The concept of ventricular remodeling was focused in 1985, from fundamental work that has come to have immense clinical application. Janice Pfeffer et al. (9) studied the causes and patterns of increased leftventricular dilation and impaired ventricular function after coronary artery ligation in rats (10). They referred to such changes in the ventricular architecture as remodeling. Post-infarct remodeling was further defined in 1990 as the changes in ventricular topography, occurring both acutely and hronically after infarction and identified as an important therapeutic target. (11) Since then, the concept has been applied to various ventricular patterns occurring in response to the mechanical stresses of other heart diseases. Innovative animal experiments have shown that progenitor cells from various sources can populate acutely damaged regions of the myocardium, refurbishing functional units and reversing remodeling (12). Whether bone-marrow-derived stem cells can acquire sufficient cardiomyocyte-like properties to reconstitute myocardium lost by infarction is uncertain. By contrast, both myocytes and coronary vessels can be regenerated from a cardiac stem-cell ompartment that can regenerate in vitro. (13,14) Injection of cardiac stem cells with bioengineered scaffolding and selective growth factors such as insulin-like growth factor could provide enough myocardial regeneration and mechanical support to rescue severely damaged hearts. Clinical evidence does not directly support this theory, but is proceeding briskly. Studies are under way in which skeletal myoblasts harvested from peripheral tissue and grown in culture are injected directly into scarred regions of the myocardium with improved ejection fraction. (15) Other ongoing approaches are using prompt extraction of autologous mesenchymal stem cells harvested from bone marrow, with intracoronary delivery to the necrotic region during the acute phase of myocardial infarction. In a well-designed study of 67 patients, this approach decreased myocardial infarct size and improved recovery of regional systolic function; long-term follow-up is still awaited. (14) The harsh scrutiny of clinical trials is needed, proceeding in tandem with basic science investigations.

Cell homing

Defining the events in progenitor cell homing may enable better targeting of cells, most obviously when cells are mobilized from the bone marrow into the bloodstream. Later steps in homing, though, are instrumental to the impact even of progenitor cells infused locally into coronary arteries. Homing is a multistep cascade including the initial adhesion to activated endothelium or exposed matrix, transmigration through the endothelium, and, finally, migration and invasion of the target tissue. The capacity to migrate and invade may be pivotal to functional integration even when cells are injected intramuscularly. Particularly in patients who lack the endogenous stimuli incited by acute ischemic injury, the enhancement of local homing signals or cells' ability to respond may be of critical importance.

Neoangiogenesis

To date, there is no direct clinical evidence that cellular cardiomyogenesis in fact occurs in the human heart after transplantation of progenitor cells. Angiogenesis, improvements in scar tissue, and cytoprotection must be considered, along with transdifferentiation, as among the most important possible consequences of cell-based therapies for cardiac repair Of these, most obviously, progenitor cells may improve neovascularization, which in turn would augment oxygen supply. Progenitor cells are expected to be of most benefit to cardiac regeneration or performance when used to treat jeopardized or hibernating cardiomyocytes. Neo-

vascularization, in turn, can be mediated by the physical incorporation of progenitor cells into new capillaries (17, 18) or, in some settings, perivascular cells (19). Incorporated progenitor cells of most if not all types may release growth factors that promote angiogenesis by acting on mature endothelial cells (20).

Embryonic stem cells

ES cells derived from the inner cell mass of blastocysts are considered to have virtually unlimited self-renewal and developmental potential. These claims are based on multiple in vitro cell doublings and generation of cell types for nearly every lineage. However, injection of ES cells into the myocardium of animal models results in formation of teratomas (21). Thus ES cells are routinely placed in culture to induce early stage cardiomyogenesis. The ifferentiation of mouse (22) and human (23) ES cells into immature cardiomyocytes is achieved when aggregates of ES cells are plated in media lacking supplemental leukemia inhibitory factor but containing a number of additives such as growth factors (22, 24). Within 7-10 days the immature cardiomyocytes display spontaneous rhythmic contractions and generate cardiac-specific proteins, including myosin light chain, a-tropomyosin and several transcription factors, typically expressed in early cardiomyocyte development (22, 25). Importantly, the in vitro generation of cardiomyocytes from ES cells has

Table 1. Differentiation of bon marrow stem cells into cardiomyocytes

Type of induction	Key references on cardiomyocyte differentiation experiments	BMC
In vitro	Spontaneous	
	Stimulation with 5-azacytidine/oxy- tcin	
	Co- culturing	
In vivo	Direct injection	Ishida et al.
	of undifferentiated cells into myocardial injury	Nygren et al.
	Direct injection of stimulated cells into myocardial injury	Tomita et al.
		Bittner et al.
		Orlic et al.
	Integration of circulating cells	Agbulut et al.
		Tomita et al.
		Balsam et al.
Clinical		Perin et al.
	Direct injection into myocardial injury	Assmus et al.
		Wollert et al.
		Kang et al.
	Integration of circulating cells	Quaini et al.
		Laflamme et al.

provided a valuable basis for in vivo experiments testing efficacy in repair of injured myocardium (26). Human ES cell-derived cardiomyocytes were injected into the wall of the left ventricle of a swine model of atrioventricular block resulting from ablation of the His bundle responsible for the major electrical conduction pathway linking atria with ventricles. At 1-3 weeks posttransplant, the hES cell-derived cardiomyocytes were integrated into the myocardial tissue, where they demonstrated an lectromechanical property and paced the ventricles. To prevent an immune reaction the animals were placed on a daily regimen of methylprednisolone. If we are to realize the full restorative potential of ES cell-derived cardiomyocytes and move these experiments forward, it will be necessary to overcome the obstacles of istocompatability and long-term survival.

Somatic stem cells

Bone marrow

The cardiomyogenic properties of bone marrow derived cells in vivo were observed for the first time by Bittner et al. After sex-mismatched bone marrow transplantation in female dystrophic mdx mice suffering from cardiac muscle degeneration, Y-chromosome containing cardiomyocytes had integrated into the myocardium. This indicated that irculating bone marrow-derived cells can be recruited to the injured heart and differentiate into cardiomyocytes (27). To further understand the capacity of bone marrow cells to differentiate into cardiomyocytes and repair the injured myocardium, stem cells were instantly delivered to the demanding area by injecting cells directly into the ocardium or coronary arteries. Few animal studies have been performed to investigate the possibilities of injecting crude bone marrow into the myocardium (Table 1). In most cases, the bone marrow mononuclear cell fraction, harboring most of the stem and progenitor cells, was cultured in vitro before injection. This will inevitably lead to the selection of either HSCs or MSCs. The population of cells with cardiomyogenic properties likely represents only a small fraction of total bone marrow.

Table 2. Differentiation of Hematopoietic stem cells (HSCs) into cardiomyocytes

Type of induction	Key references on cardiomyocyte differentiation experiments	HSC
In vitro	Spontaneous	
	Stimulation with 5-azacytidine/oxy- tcin	
	Co- culturing	Hierlihy et al.
In vivo		Nygren et al.
	Direct injection	Orlic et al.
	of undifferentiated cells into myocardial injury	Balsam et al.
		Murry et al.
	Direct injection of stimulated cells into myocardial injury	
		Jackson et al.
	Integration of circulating cells	Nygren et al.
		Kawada et al.
Clinical	Direct injection into myocardial injury	
	Integration of circulating cells	

a. Hematopoietic stem cells

Bone marrow-derived HSCs have been investigated for their differentiation potential in vivo (Table 2). The first indication that HSCs may participate in cardiac regeneration came from Jackson et al. They isolated a specific HSC population called the side population, and transplanted these cells into lethally irradiated mice. Subsequently, the transplanted mice were used in a myocardial ischemia-

reperfusion model, and hearts were analyzed after 2 and 4 weeks. Although their prevalence was not very high, donor-derived cardiomyocytes were found, primarily in the peri-infarct zone, demonstrating the cardiomyogenic ability of circulating HSCs (28). Direct injection of HSCs into the infarcted myocardium has also been investigated by Orlic et al. After ligating the coronary artery, a population of was injected into the contracting wall bordering the infarcted area. After 9 days, 40% of the mice showed regeneration of the cardiac muscle.

Approximately 68% of the infarcted area was occupied by newly formed myocardium. Donor cells were shown not only to differentiate into cardiomyocytes but also to form endothelial cells and fibroblasts. Evidence for the restoration of the myocardium was further supported by a prolonged survival of the mice and a recovery of cardiac function (29). Although these studies demonstrate that different populations of HSCs appear to have a very high capacity both in homing to and regeneration of the damaged myocardium, some groups argue otherwise. It should be noted that Balsam et al. found a small but significant increase in cardiac function 6 weeks after MI (30). Therefore, it remains unclear what the potential morphological and physiological contribution of HSCs to the regeneration of the myocardium is.

b. Mesenchymal stem cells

MSCs have been studied extensively for their in vivo cardiomyogenic potential, especially since they have the capacity to differentiate into cardiomyocytes in vitro (Table 3). Wang and colleagues show that murine MSCs participate in the formation of new cardiomyocytes in the normal, uninjured heart. Starting 4 weeks after the injection of in vitro-expanded, labeled MSCs into the healthy heart, donor cells expressing cardiac markers were detected (31).

The same in vivo potential has been demonstrated for human MSCs, which were injected into the heart of mice. Although the human MSCs were only present in a small percentage (0.44%), the engrafted cells did express cardiac markers (32). In addition to the use of healthy animals, MSCs have also been injected into the myocardium of experimental models for cardiac damage. Autologous MSCs were injected into the left ventricle (LV) of rats 3 weeks after myocardial cryoinjury. Transplanted MSCs were identified in all animals 8 weeks after injury. Immunohistochemistry revealed muscle cells expressing troponin I and myosin heavy chain. Moreover, injections of MSCs lead to a decreased scar area and a thicker LV free wall. The animals injected with pre-treated cells also had a decreased LV chamber size/body weight and improved cardiac function compared to controls (32).

Table 3. Differentiation of mesenchymal stem cells (MSCs) into cardiomyocytes

Type of induction	Key references on cardiomyocyte differentiation experiments	MSC
In vitro	Spontaneous	
		Makino et al.
	Stimulation with 5-azacytidine/oxy- tcin	Hakuno et al.
		Rangappa et al.
		Xu et al.
		Liu et al.
In vivo	Co- culturing	Fukuhara et al.
		Wang et al.
	Direct injection	Toma et al.
	of undifferentiated cells into myocardial injury	Mangi et al.
Clinical	Direct injection of stimulated cells into myocardial injury	
	Integration of circulating cells	Kawada et al.
	Clinical Direct injection into myocardial injury	
	Integration of circulating cells	

c. CD133+ Cells

The cell surface antigen CD133+ is expressed on early HSCs, which collaborate to promote vascularization of chemic tissues (34). CD133+ cells can integrate into sites of neovascularization and differentiate into mature endothelial cells. Less than 1% of nucleated BMCs are CD133+, and because these cells cannot be expanded ex-vivo, only limited numbers of CD133+ cells can be obtained for therapeutic purposes.

Table 4. Cell therapy trials in patients with acute myocardial infarction

Cell				Outcomes Time After			
Study	(n)	Type	Dose	Delivery	AMI	Improved	No Change
Strauer et al	10 treated, 10 controls*	MNC	$2.8 \pm 2.2 \times 10^7$	IC	5-9 days	Regional wall motion; Infarct size ; Perfusion	Global LVEF; LVEDV
TOPCARE- AMI	29 MNC, 30 CPC,	MNC	$2.1 \pm 0.8 \times 10^8$	IC	5 ± 2 days	Regional wall motion ; Global LVEF;	LVEDV
	11 controls*	CPC	$1.6 \pm 1.2 \times 10^7$			Infarct size ↓ ; Coronary flow	
Fernandez-Aviles et al	20 Treated, 13 controls*	MNC	$7.8 \pm 4.1 \times 10^7$	IC	14 ± 6 days	Regional wall motion ; Globl LVEF	LVEDV
Kuethe et al	5 treated	MNC	$3.9 \pm 2.3 \times 10^7$	IC	6 days		Regional wall motion ; Global LVEF
BOOST	30 treated, 30 controls	NC	$2.5 \pm 0.9 \times 10^9$	IC	6 ± 1 day	Regional wall motion: Global LVEF	LVEDV; infarct size
Chen et al , 35 controls	34 treated	MNC	$4.8 \pm 6.0 \times 10^{10}$	IC	18 days	Regional wall motion: Global LVEF; Infarct size ↓; LVEDV ↓	
Venderheyden et al	12 treated, 10 controls*	$CD133^+$	$6.6 \pm 1.4 \times 10^6$	IC	14 ± 6 days	Regional wall motion; Global LVEF; Perfusion†	

MNC = mononuclear cells; CPC= circulating blood-derived progenitor cells; NC= nucleated cells; MSC= mesenchymal stem cells; IC= intracoronary; AMI = acute myocardial infarction; LVEF = left ventricular ejection fraction; LVEDV = left ventricular end-diastolic volume

Routes of application

Transvascular Approaches

Transvascular strategies are especially suited for the treatment of recently infarcted and reperfused myocardium when chemoattractants and cell adhesion molecules are highly expressed. (35, 36). Intracoronary Artery Infusion Selective intracoronary application delivers a maximum concentration of cells homogeneously to the site of injury uring first passage. Unselected BMCs, circulating blood-derived progenitors cells, and MSCs have been delivered via the intracoronary route in patients with AMI and ischemic cardiomyopathy (Tables 4 and 5). In these studies, cells were delivered through the central lumen of an over-thewire balloon catheter during transient balloon inflations to maximize the contact time of the cells with the microcirculation of the infarct-related artery. It is unknown hether this stop flow technique is required to enhance cell retention within the infarcted area. In the hands of an xperienced operator, intracoronary delivery is relatively easy to perform within less than an hour.

Intravenous Infusion

In experimental models, intravenous delivery of HSCs or MSCs has been shown to improve cardiac function after AMI (37, 38). However, homing of cells to noncardiac organs limits the clinical applicability of this approach. (39). Indeed, in a recent study in post- AMI patients, significant myocardial homing of unselected BMCs was observed only after intracoronary stop-flow delivery but not after intravenous application (40).

Mobilization of Stem and Progenitor Cells

Considering that the acutely infarcted myocardium recruits circulating stem and progenitor cells to the site of injury, tem and progenitor cell mobilization by cytokines may offer a noninvasive strategy for cardiac regeneration (38, 41, 42). This concept has been tested in animal models and in pilot studies in patients with AMI and chronic myocardial ischemia (43, 44).

Direct Injection in the Ventricular Wall

Direct injection is the preferred route for cell delivery in patients presenting late in the disease process when an occluded coronary artery precludes transvascular cell delivery (patients with chronic myocardial ischemia) or when cell homing signals are expressed at low levels in the heart (scar tissue).

Table 5. Cell therapy trials in patients with ischemic cardiomyopathy

					Time After		
Study	(n)	LVEF	Cell Type	Dose	MI	Delivery	Outcomes†
Menasche et al	10 treated	24 ± 4%	Myoblasts	$8.7 \pm 1.9 \times 10^8$	3-228 Months	Transepicardial (during CABG)	Regional wall motion ↑; Global LVEF ↑
Herreros et al	11 treated	36± 8%	Myoblasts	$1.9 \pm 1.2 \times 10^8$	3-168 Months	Transepicardial (during CABG)	Regional wall motion ↑; Global LVEF ↑; viability in infarct area ↑
Siminiak et al	10 treated	25± 40%	Myoblasts	$0.04 \pm 5.0 \times 10^7$	4-108 Months	Transepicardial (during CABG)	Regional wall motion ↑; Global LVEF ↑
Chachques et al	20 treated	28± 3%	Myoblasts	$3.0 \pm 0.2 \times 10^8$	Not reported	Transepicardial (during CABG)	Regional wall motion ↑; Global LVEF ↑; viability in infarct area ↑
Smits et al	5 treated	36± 11%	Myoblasts	$2.0 \pm 1.1 \times 10^8$	24-132 Months	Transepicardial (guided by EMM)	Regional wall motion ↑; Global LVEF ↑
Stamm et al	12 treated	36± 11%	CD 133+	$1.0 \pm 2.8 \times 10^6$	3-12 weeks	Transepicardial (during CABG)	Global LVEF ↑; LVEDV↓; Perfusion ↑
Assmus et al	51 MNC, 35 CPC, 16 Controls	40± 11%	MNC	$1.7 \pm 0.8 \times 10^8$	3-144 Months	IC	Global LVEF ↑; (only in MNC group)
			CPC	$2.3 \pm 1.2 \times 10^7$			

LVEF = left ventricular ejection fraction; MNC = mononuclear cells; CPC = circulating blood-derived progenitor cells; MI = myocardial infarction;CABG = coronary artery bypass grafting; EMM = electromechanical mapping; IC = intracoronary; LVEDV = left ventricular end-diastolic volume.

Table 6. cell therapy trials in patients with myocardial ischemia and no revascularization option

Outcomes							
Study	(n)	LVEF	Cell Type	Dose	Delivery	Subjective	Objective
Hamano et al	5 treated		MNC	$0.3 – 2.2 \times 10^9$	Transepicardial (during CABG)		Perfusion ↑
Tse et al	8 treated	58 ± 11%	MNC	From 40 ml BM	Transepicardial (guided by EMM)	Angina ↓	Perfusion ↑ ; Regiional wall motion ↑;
Fuchs et al	10 treated	47± 10%	NC	$7.8 \pm 6.6 \times 10^7$	Transepicardial (guided by EMM)	Angina ↓	Perfusion ↑
Perin et al	14 treated 7 Controls	30 ± 6%	MNC	$3.0 \pm 0.4 \times 10^7$	Transepicardial (guided by EMM)	Angina ↓: NYHA ↓	Perfusion ↑ ; Regiional wall motion; Global LVEF ↑

LVEF = left ventricular ejection fraction; MNC = mononuclear cells; CPC = circulating blood-derived progenitor cells; MI = myocardial infarction;CABG = coronary artery bypass grafting; EMM = electromechanical mapping; IC = intracoronary; LVEDV = left ventricular end-diastolic volume.

However, direct injection of cells into ischemic or scarred myocardium creates islands of cells with limited blood supply and may lead to poor cell survival (45). Direct injection techniques are especially suited for the application of large cells, such as MSCs or myoblasts, which may cause microembolization after intracoronary delivery. Direct njection techniques have been used in patients with advanced coronary artery disease (Table 6) and in patients with ischemic cardiomyopathy (Table 5). Cell delivery by direct injection may be technically challenging in patients with AMI, particularly if cells are to be injected into the border zone of the infarct. The safety of such an approach emains to be established because perforation of the friable necrotic tissue remains a matter of concern.

Transendocardial Injection

Using an injection needle catheter advanced across the aortic valve and positioned against the endocardial surface, cells can be directly injected into the left ventricular (LV) wall (46,47) Electromechanical mapping of the ndocardial surface can be used to delineate viable, ischemic, and scarred myocardium before cell injections (46,48).

Transepicardial Injection

Transepicardial cell injection has been performed as an adjunct to coronary artery bypass grafting (CABG). Transepicardial cell injection during open heart surgery allows for a direct visualization of the myocardium and a targeted application of cells to scarred areas and/or the border zone of an infarct scar. The invasiveness of this approach hampers its use as a stand-alone therapy. Conversely, the efficiency of cell transplantation may be difficult to evaluate and ascertain if CABG is performed simultaneously.

Transcoronary Vein Injection

A catheter system incorporating an ultrasound tip for guidance and an extendable needle for myocardial access has been used to deliver BMCs through the coronary veins into normal pig myocardium.68 The same approach has been used in a pilot trial in patients with ischemic cardiomyopathy to deliver myoblasts to areas of nonviable myocardium (50) In contrast to the transendocardial approach, where cells are injected perpendicular to the ventricular wall, the composite catheter system delivers cells parallel to the ventricular wall and deep into the injured myocardium. However, positioning of the injection catheter in a specific coronary vein is not trivial in all cases (50).

Clinical Applications of Stem Cell Therapy

Acute Myocardial Infarction

Modern reperfusion strategies and advances in pharmacological management have resulted in an increasing zroportion of AMI survivors at heightened risk of developing adverse LV remodeling and heart failure. None of our urrent therapies addresses the underlying cause of the remodeling process, ie, the damage of cardiomyocytes and the vasculature in the infarcted area. 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, (51) whereas others have pointed out that the clinical trials are justified by the potential benefits of cell therapy (52) All clinical studies included patients with AMI who had undergone primary angioplasty and stent implantation to reopen the infarctrelated artery, and cells were infused intracoronarily by using the stop-flow balloon catheter approach. In this regard, the clinical studies differ significantly from the animal studies, where the infarct related artery was not reperfused and cells were directly injected into the myocardium (38, 53, 54). The clinical trials may be categorized into studies using unselected BMCs or selected cell populations (Table 4).

Myocardial ischemia with no revascularization option

Despite significant advances in coronary revascularization techniques, some patients with coronary artery disease and myocardial ischemia have no revascularization option because of the diffuse nature of their disease. A number of these patients experience anginal symptoms despite maximal medical therapy. Chronic myocardial ischemia can be associated with a regional impairment of contractile function, which is partially reversible when tissue perfusion is restored (hibernating myocardium). Moreover, ischemia increases the risk of arrhythmias and sudden cardiac death. There is a clear need for new therapeutic strategies aimed at delivering oxygenated blood to the myocardium in these patients. Unselected mononuclear BMCs have been used in several small studies in patients with coronary artery disease not amenable to conventional revascularization techniques (55, 56). A recent study investigated the effects of G-CSF on symptoms and myocardial perfusion in patients with intractable angina (57). Treatment with G-CSF promoted a strong increase in circulating progenitor cells numbers and an improvement in anginal symptoms. However, there was no objective evidence of enhanced myocardial perfusion or improved regional wall motion.

Ischemic Cardiomyopathy, Chronic Heart Failure

Chronic heart failure has emerged as a major worldwide epidemic. Recently, a fundamental shift in the underlying etiology of heart failure is becoming evident, in which the most common cause of heart failure is no longer hypertension or valvular disease, but rather long-term survival after AMI. Conceptually, replacement of akinetic scar tissue by viable myocardium should improve cardiac function and impede progressive LV remodeling. In a recent trial, 86 patients with ischemic cardiomyopathy received intracoronary infusions of unselected mononuclear BMCs or of circulating bloodderived progenitor cells by the stop-flow balloon catheter technique. The procedure was safe (58). After 3 months, LVEF in the BMC group was improved by three percentage points, but did not change significantly in control patients and in the progenitor cell group (58). Double-blind trials are needed to rigorously evaluate the safety and efficacy of cell therapy in patients with ischemic heart failure. It is interesting to note that intracoronary infusions of mononuclear BMCs or bloodderived progenitor cells promoted greater improvements of LVEF in patients with AMI as compared with patients with ischemic cardiomyopathy (58). Because cell retention may be limited after intracoronary delivery into chronically infarcted myocardium, pharmacological or genetic approaches to enhance cell retention and engraftment should be explored. Considering that functional benefits of cell transplantation have also been observed in animals with dilated cardiomyopathy, (59) future trials may want to explore the role of cell therapy in patients with nonischemic heart failure. In this regard, a pilot study suggests that intracoronary BMC transfer may be safe and potentially effective in patients with Chagas cardiomyopathy (60).

Combination of stem cell and gene therapy

Recently, several studies have investigated the effects of genetically modified stem cells as a therapy for myocardial infarction. Studies have demonstrated that this combination of stem cell and gene therapy may be a useful approach. Genetic modification can increase the survival of transplanted stem cells in ischemic tissue (61). The survival rate of mesenchymal stem cells transduced with Aktl gene was

increased fourfold in the ischemic rat myocardium, 80-90% of lost myocardial volume was regenerated, and cardiac performance was nearly normalized. Enhancing the angiogenic potential of transplanted stem cells is another goal of genetic modification. Matsumoto et al. (62) transected the human VEGF 165 gene into cultured mesenchymal stem cells. The mesenchymal stem cells with VEGF 165 gene were injected into infracted myocardium. High expression of VEGF increased the capillary density of the infracted region and improved left ventricular function. Gene therapy can be used to mobilize and recruit stem cells into myocardial infarction.

Potential problems of stem cell therapy

Besides raising intense ethical concerns in some (63), the use of human embryonic stem cell transplantation to repair damaged tissues has many other potential scientific problems. The first problem is teratoma formation. There is a possibility of spontaneous differentiation of stem cells into undesired lineages beside the cardiomyogenic differentiation after transplantation into myocardium (64). The potential for accelerated atherogenesis or enhanced restenosis induced by stem cell transplantation remains a concern. In addition, ectopic calcification of tissue is a concem. Yoon et al. (65) injected intramyocardially unselected bone marrow cells into the ped-infarct area in a rat myocardial infarction model and found that direct transplantation of unselected BM cells into the acutely infarcted myocardium induced significant intramyocardial calcification. Skeletal myoblast transplantation may cause serious ventricular arrhythmias. Some factors, such as cardiac tissue injury induced by the intramyocardial injection, electrical heterogeneity of action potentials of differentiated stem cells, or increased nerve sprouting may be involved. Immunological rejection is a potential complication for the use of human embryonic stem cell-derived cardiomyocytes in human clinical therapy. Reprograming autologous adult stem cells to express cardiomyogenic function with human embryonic stem cell-delivered cardiomyocytes is a novel approach to resolve this problem (66). The reprograming technique involves fusion of enucleated cytoplasts generated fromhuman embryonic stem cell-delivered cardiomyocytes with autologous adult stem cells to generate cytoplasmic hybrids. The hybrids function as cardiomyocytes, but are not immunogenic. Washout of directly transplanted cells from the heart may also be a new technique for cell transplantation therapy.

Direction for future clinical research

So far, flurries of small, mostly uncontrolled clinical studies exploring the safety and feasibility of stem cell therapy have been conducted. These studies have used different cell types and preparations, each in a small number of patients with different disease states. In the aggregate, this preliminary clinical evidence suggests that stem cell therapy might work. Although these initial clinical studies have generated a great deal of hope, we should take into account the lessons learned from the translation of therapeutic angiogenesis into clinical studies, where great expectations raised by open studies have not been confirmed by subsequent randomized trials. We advocate to no longer performing studies involving small numbers of patients, but rather to conduct intermediate-size, double-blind, randomizedcontrolled clinical trials to establish the effects of stem cell therapy on surrogate markers, like LVEF, myocardial perfusion, or exercise capacity. Upcoming trials should also address procedural issues such as the optimal cell type, cell dosage, and timing of cell transfer. These trials may also look at combined morbidity and mortality end points, although they may be too small to be conclusive in this regard. Safety remains the key concern as we proceed. Although these studies are underway, fundamental questions need to be addressed experimentally. What is the fate of the injected cells after transplantation? How long do they survive? Do the cells incorporate, or is transient retention sufficient to promote functional effects? Genetic and transgenic markers should be used to determine the lineage commitment of engrafted cells. Cell labeling and imaging techniques need to be developed to track stem cell fate in patients and correlate cell retention and engraftment with functional outcomes. Pharmacological and genetic strategies may help to enhance stem cell retention, engraftment, differentiation, and paracrine capability (67-69). Support from governmental organizations or charities will be required to ensure that cell therapies, which may be efficacious but commercially less attractive (eg, unselected BMCs), will undergo much-needed further clinical testing. In conclusion, although some of the current scientific data support the concept that the stem cells can be used for the myocardial regeneration, there are still many question to be cleared before this promising approach can be performed effectively, safely and routinely in

human subjects. Questions such as how to induce the transplanted stem cells to differentiate only into cardiomyocytes, and not other cells or teratomas; which type of stem cell and which model of delivery are the most efficacious; whether stem cells in the heart truly undergo functional and electrical integration; and whether this approach may have proarrhythmic consequences remain to be
answered before eventually making this stem cell therapy a clinical reality.

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