Stem Cell Transplantation in Myocardial Infarction

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Congestive heart failure, which is most commonly caused by myocardial infarction, is the most frequent cause of hospitalization in the United States in patients over the age of 65. Although current pharmacotherapy can inhibit neurohormonal activation, this falls short of preventing left ventricular remodeling and the development of congestive heart failure. Stem cells are undifferentiated pluripotent cells that have the potential to proliferate and differentiate into cardiomyocytes. Cellular cardiomyoplasty, which is the replacement or regeneration of cardiomyocytes through cell transplantation, is a potential therapeutic approach to prevent left ventricular remodeling after myocardial infarction. The majority of the data on stem cell transplantation comes from preclinical animal studies. Although the results are interesting and perhaps safe, early phase I clinical studies are small and very preliminary. Data from large, randomized controlled trials are needed to clarify the short- and long-term effects of cellular cardiomyoplasty.
[Rev Cardiovasc Med. *2004;5(2):82-98*]

Key words: Stem cells • Myocardial infarction • Coronary angioplasty • Myocardial regeneration

Congestive heart failure, which is most commonly caused by myocardial infarction, affects approximately 5 million patients in the United States and is the most frequent cause of hospitalization in the United States in patients over the age of 65.[1] The physician's goal during acute myocardial infarction is to establish reperfusion and salvage as much myocardium as possible. If reperfusion is not achieved expeditiously, left ventricular systolic dysfunction,

ventricular remodeling, and ultimately congestive heart failure may ensue as a result of cardiomyocyte loss, elevated cardiac filling pressures leading to increases in diastolic and systolic stress, and the release of a milieu of deleterious neurohormones and mediators.[2,3] Despite pharmacologic and mechanical revascularization techniques, there is no effective therapy that replaces scarred myocardium with viable, functioning myocardium. Mortality rates remain high, with 50% of congestive heart failure patients dying within 5 years of diagnosis.[4] The development of newer and more effective treatment options for patients with congestive heart failure is imperative, given the increasing number of cases and their economic impact on health care.[5,6] Neurohormonal inhibition of the sympathetic nervous system and renin-angiotensin-aldosterone system, as well as mechanical left ventricular assist devices, are limited in regenerating lost cardiomyocytes during myocardial infarction.[7]

The dogma that the heart is a terminally differentiated postmitotic organ incapable of self-renewal has recently been challenged. After myocardial infarction, left ventricular remodeling is in part determined by neovascularization and increased apoptosis, especially in the border zone of the infarct.[8] Although cells derived from resident cardiomyocytes or circulating stem cells have regenerative capacity after myocardial infarction,[9] their ability to minimize the deleterious effects of ventricular remodeling and recovery of cardiac function is limited. The surviving cardiomyocytes bordering the infarct zone become hypertrophied following myocardial infarction as part of an adaptive mechanism to compensate for the loss of myocardium.[10,11] Late reperfusion of vascular beds in the area of infarct in human and animal models resulted in beneficial effects on ventricular remodeling and survival.[12,13] However, after myocardial infarction, normal angiogenesis is usually insufficient to meet the greater demands for oxygen and nutrients and to prevent apoptosis of hypertrophied cardiomyocytes and ventricular remodeling.[14] Therefore, increasing perfusion to infarcted myocardium to enhance oxygen and nutrient delivery through the formation of new blood vessels has the potential to improve cardiac function. Congestive heart failure after myocardial infarction may ensue and contribute to significant morbidity and mortality when normal reparative mechanisms are overwhelmed despite optimal medical therapy. Therefore, the next logical step would be to repopulate the injured cells that have undergone necrosis with stem cells, in order to regenerate cardiomyocytes and reverse ventricular remodeling.

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We give an overview of the pathophysiologic background on cellular cardiomyoplasty, which is the replacement or regeneration of cardiomyocytes through cell transplantation. We discuss the potential donor cells and the mode of stem cell delivery. We also give an overview of the clinical trials, the associated controversies, and the future of stem cell therapy.

Table 1
Mediators of Stem Cell Mobilization, Migration, and Attachment

Mediator	Action
Granulocyte colony-stimulating factor	Mobilizes stem cells from bone marrow into circulation
Stem cell factor	Mobilizes stem cells from bone marrow into circulation
Vascular endothelial growth factor	Stimulates nascent blood vessel formation; homing signal crucial to the recruitment of circulating progenitor cells to assist the repair mechanisms in myocardial infarction
Stromal cell–derived factor-1	Homing signal crucial to the recruitment of circulating progenitor cells to assist the repair mechanisms in myocardial infarction

Cell-Based Myocardial Regeneration

Stem cells are precursor cells capable of proliferation, self-renewal, and differentiation into specialized tissues and organs, including cardiomyocytes.[15,16] The genetic and cellular

mechanisms that initiate transdifferentiation of stem cells are poorly understood.

The repopulation of cardiomyocytes to regenerate new myocardium can mitigate the remodeling process. This can be accomplished with one of the following methods. Necrotic myocardium can be replaced by transplanting cells that differentiate into cardiomyocytes or promote neovascularization. Another method involves cytokines such as granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF), which increase bone marrow stem cell mobilization, homing, and engraftment to infarcted myocardium (Table 1).[17] The endogenous repair process after myocardial necrosis can also be enhanced with specific growth factors, such as insulin-like and hepatocyte growth factors, that stimulate cardiomyocyte replication and attract cardiac resident stem cells.[18,19]

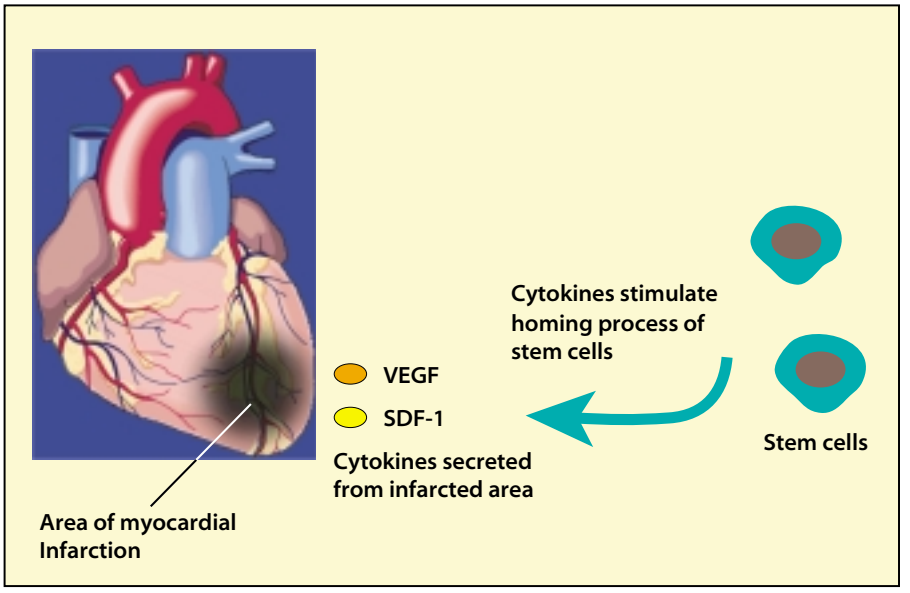

Figure 1. *The "homing process" involves stem cells migrating and attaching to the sites of injury or ischemia, which provides an environment that is favorable to growth and differentiation because of increased vascular permeability, cytokine release, and adhesion protein expression. The expression of vascular endothelial growth factor (VEGF) and stromal cell–derived factor-1 (SDF-1) is highly up-regulated in hypoxic tissue, supporting the hypothesis that these mediators may represent homing signals crucial to the recruitment of circulating progenitor cells to assist the endogenous repair mechanisms in the infarcted tissue.* www.medreviews.com

Mechanisms of Stem Cell Migration

One emerging concept is that some form of injury or inflammation is a prerequisite for the success of circulating-cell participation in differentiated tissue structure and function.[20] Once reperfusion is achieved in acute myocardial infarction, an intense inflammatory cascade is unleashed. The architecture of the left ventricle rearranges, leading to ventricular remodeling. The "homing process" involves stem cell migration to the sites of injury or ischemia, which provides an environment that is favorable to growth and function (Figure 1).[21] This microenvironment is a stimulus for homing and differentiation of stem cells of the appropriate lineage. It increases vascular permeability and expression of adhesion proteins like integrin, along with homing receptors that facilitate their attachment, which is mediated by cell-to-cell contact and chemoattractant release from local tissue injury.[22,23]

The migratory capacity of transplanted progenitor cells might be dependent on natural growth factors such as vascular endothelial growth factor (VEGF) and stromal cell–derived factor-1 (SDF-1).[24] The expression of VEGF and SDF-1 is highly up-regulated in hypoxic tissue, supporting the hypothesis that these factors may represent homing signals crucial to the recruitment of circulating progenitor cells to assist the endogenous repair mechanisms in the infarcted tissue.[25-28]

Transplanted stem cells must engraft and proliferate efficiently in a short period of time after myocardial infarction to derive a maximal clinical benefit.[29] With a smooth transition process, newly formed cardiomyocytes are required to be connected intercellularly through electrical coupling with other cardiomyocytes and the formation of connexin, an integral membrane protein constituent of gap junctions.[30] Paramount to the survival of the stem cells is simultaneous neovascularization to keep up with the metabolic requirements of the newly transplanted cells to perform contractile work.

Donor Cells

A wide variety of different donor cells that might replace necrotic myocardium have been used in both animal and clinical studies for stem cell therapy, each with their own advantages and disadvantages. Techniques have been developed to harvest, isolate, and expand potential donor cells (Table 2).

Fetal Cardiomyocytes

Fetal cardiomyocytes transplanted into murine models engrafted in host myocardium and fostered electrical pathways through the formation of

Table 2
Potential Donor Cells

Donor Cell	Advantages	Disadvantages
Fetal cardiomyocytes	Cardiomyocyte phenotype	Immunosuppression required Ethical debate Short survival Limited supply
Skeletal myoblasts	Lack of immunogenicity Autologous transplantation Fatigue-resistant, slow-twitch fibers Resistant to ischemia High potential for division in culture Easily accessible	Arrhythmogenic Lack gap junctions Require ex vivo expansion
Endothelial progenitor cells	Lack of immunogenicity Autologous transplantation	Require ex vivo expansion due to limited supply
Embryonic stem cells	Pluripotent Highly expandable	Ethical debate Lack of availability Tumor potential
Adult mesenchymal stem cells	Lack of immunogenicity Autologous transplantation Pluripotent Cryopreservable for future use	Unclear functional and electrophysiologic properties Difficult to isolate and purify Require ex vivo expansion

gap junctions.[31] Fetal cardiomyocytes transplanted into infarcted myocardium limited scar expansion, formed new cardiac tissue, and prevented postinfarction heart failure.[32-35] A superior growth potential to improve cardiac function was seen with fetal cardiomyocytes compared with adult or pediatric cardiomyocytes.[36] Transplantation of human fetal cardiomyocytes induced the formation of nascent blood vessels in the host myocardium, providing increased blood flow and a route to remove cellular debris after myocardial infarction.[37]

There are several disadvantages to the use of allogenic human fetal cardiomyocyte transplantation. Intense ethical and political debates concerning their use still exist. Furthermore, the limited quantity of cells available for myocardial regeneration and the need for expansion gives them a disadvantage when compared with other types of cells. Transplanted allogenic fetal cardiomyocytes only survived for a short duration because they were slowly eliminated due to immunorejection.[38] Thus, the future role of fetal cardiomyocytes in clinical trials seems to be limited, especially with the emergence of autologous cell transplantation.

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Skeletal Myoblasts

Skeletal myoblasts are precursor cells for new skeletal myocytes that reside within the muscle tissue. Skeletal myoblasts that were implanted into myocardium underwent myotube formation, withdrew from the cell cycle, and remained viable.[39,40] Skeletal myoblasts can be harvested from routine muscle biopsy and then transplanted into the myocardium, obviating the need for immunosuppression. However, skeletal muscle biopsy may not yield adequate numbers of cells for transplantation and therefore may require expansion.[40]

Skeletal myoblasts possess a high potential for replication in culture.[41] Because patients with coronary artery disease often have areas of poor perfusion, their strong resistance to ischemia allows skeletal myoblasts to survive and engraft in host myocardium.[42]

In a rat model, the implantation of skeletal myoblasts resulted in the formation of viable grafts that decreased ventricular remodeling and increased global cardiac function and exercise capacity after myocardial infarction.[43] Left ventricular systolic function improved after autologous skeletal myoblasts were harvested, expanded in culture, and transplanted within infarcted myocardium through colonization of the fibrotic area by the skeletal myoblasts in a sheep model.[44] The improvement of myocardial contractility correlated with the quantity of the implanted myoblasts.[45] Skeletal myoblasts can be delivered to the heart by either direct intramyocardial or intra-arterial injection.[46,47]

However, future application of skeletal myoblasts may be limited because of their association with ventricular tachyarrhythmias. Several patients who underwent autologous skeletal myoblast transfer experienced ventricular tachyarrhythmias within weeks of transplantation.[48,49] In addition, skeletal muscle cells have fundamental biologic differences when compared with adult cardiomyocytes. Skeletal myoblasts are incapable of forming gap junctions, leading to failure of successful electromechanical coupling and rendering them unable to contract synchronously with the host myocardium after transplantation.[50]

Because adult stem cells are usually unavailable in adequate quantities from a patient, especially with intravenous harvesting, methods to isolate, purify, and expand endothelial progenitor cells ex vivo are required.

Endothelial Progenitor Cells

Neovascularization can salvage hibernating myocardium, inhibit the apoptosis of hypertrophied cardiomyocytes in the vicinity of the infarct that eventually leads to dilatation of the ventricle, and improve cardiac function after myocardial infarction. Endothelial progenitor cells circulate in peripheral blood and contribute to neovascularization. There is increased mobilization of endothelial progenitor cells from bone marrow into peripheral circulation in patients with acute myocardial infarction.[51-53] Myocardial VEGF expression is enhanced in myocardial infarction and is thought to be the main contributor to increased mobilization of endothelial progenitor cells in myocardial infarction.[27] Enhancing this naturally occurring phenomenon in myocardial infarction patients by delivering concentrated doses of these cells into the infarct-related artery may have beneficial clinical effects. Autologous harvesting from bone marrow makes endothelial progenitor cells an attractive source of donor cells. In a rat model, the intravenous injection of ex vivo–expanded endothelial progenitor cells homed to foci of myocardial neovascularization, improved vascularity and ventricular function, and decreased left ventricular scarring after myocardial infarction.[54] In a murine infarct model, human bone marrow–derived endothelial progenitor cells that were mobilized with G-CSF were intravenously injected into circulation.[55] The endothelial progenitor cells migrated to the infarct area, transdifferentiated into endothelial cells, increased capillary formation in the surrounding tissue, and decreased the number of apoptotic cardiomyocytes in the peri-infarct region. There was also a reduction in ventricular remodeling and improved ventricular function. In addition to their role in neovascularization, endothelial progenitor cells can also transdifferentiate into cardiomyocytes and contribute to myocardial regeneration.[56,57]

Because adult stem cells are usually unavailable in adequate quantities from a patient, especially with intravenous harvesting, methods to isolate, purify, and expand endothelial progenitor cells ex vivo are required. However, excessive manipulation and expansion of total bone marrow mononuclear cells may induce differentiation into nonendothelial lineage cells and affect their homing capacity.[58] A method to negate this effect is to expand and enrich the ex vivo–cultured endothelial progenitor cells in a prespecified culture system.[54] Patients with coronary artery disease treated with statins had higher numbers of circulating endothelial progenitor cells.[59] Statins also modulated the adhesiveness of the cells and accelerated endothelial repair at sites of arterial injury.[60] When autologous bone marrow cells that secrete the angiogenic factors VEGF and macrophage chemoattractant protein-1 (MCP-1) were implanted with transendocardial injections into infarcted myocardium, there was endothelial cell proliferation in ischemic porcine myocardium.[61] This led to increased collateralization at rest and during pharmacologic stress and increased segmental myocardial wall thickening during stress.

Embryonic Stem Cells

Embryonic stem cells, the most primitive of the stem cells that develop as the inner cell mass at day 5 of fertilization in the blastocyst, are undifferentiated cells that possess enormous developmental potential in their early stages.[62] They are the most versatile of all stem cells and have the ability to undergo an undetermined number of cell doublings and differentiate into specific cell types, including cardiomyocytes.[63,64] Embryonic stem cells

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injected directly into the myocardium successfully engrafted into the host, reduced the size of the infarct, and improved ventricular function and isometric contractility after myocardial infarction in a rat model 6 weeks after cell transplantation.[65] It is also believed that improvement in myocardial contractility is a function of embryonic stem cells serving as platforms for the release of cardioprotective factors such as VEGF.[65] The addition of VEGF to embryonic stem cells further enhanced the functional improvement of postinfarcted hearts.[66]

Currently, embryonic stem cells are not approved for human use. Despite the apparent benefits of using embryonic stem cells, their mainstream use in myocardial regeneration appears to be limited. It appears that recipients of animal embryonic stem cells will require immunosuppression to avoid rejection of the cellular transplant due to tissue incompatibility.[29] In addition, human embryonic stem cells are not as efficient as animal embryonic stem cells in conversion into cardiomyocytes. This must be taken into consideration when evaluating studies in mice to develop strategies for myocardial regeneration.[29] There is also the potential for tumorigenicity, including teratomas. The ethical, moral, and political issues surrounding their application, as well as the severe restrictions on human embryonic stem cell research in the United States and the limited supply, have prompted the search for an alternative source of stem cells.

Adult Mesenchymal Stem Cells

A population of rare progenitor cells known as mesenchymal stem cells (also known as marrow stromal cells) is found in bone marrow. Human adult mesenchymal stem cells have the capacity to colonize different tissue, replicate, allow for autologous transplantation, and appear to have multilineage differentiation capacity in vitro, with the ability to differentiate into specialized tissues, including cardiomyocytes, endothelial cells, and smooth muscle cells.[67-71] Autologous harvesting and transplantation of mesenchymal stem cells eliminate the concern over immunorejection and makes them a potential candidate for stem cell therapy after myocardial infarction. Due to the limited number of stem cells that can be harvested from bone marrow, measures to stimulate and increase their mobilization, including the administration of G-CSF, and expansion in culture may be necessary.

Autologous harvesting and transplantation of mesenchymal stem cells eliminate the concern over immunorejection and makes them a potential candidate for stem cell therapy after myocardial infarction.

Mesenchymal stem cells implanted into swine are capable of engraftment in host myocardium and differentiation into myogenic lineage, as demonstrated by the expression of muscle-specific proteins, attenuation of contractile dysfunction and pathologic thinning, and improvement of inotropic function after myocardial infarction.[72-74] When postinfarcted pigs underwent intramyocardial transplantation with human mesenchymal stem cells, there was an improvement in global cardiac function and perfusion within the infarcted myocardium.[75,76] Cotransplantation of human mesenchymal stem cells with human fetal cardiomyocytes resulted in further improvement in cardiac function and rest perfusion.[75] The improvement in blood flow suggests that human mesenchymal stem cells may provide a source for neovascularization and release VEGF to induce new capillary formation.

In addition to cardiomyocyte generation, scar area reduction, and neovascularization, mesenchymal stem cell transplantation is thought to improve myocardial function by increasing the density of sympathetic nerves. There was overexpression of cardiac tenascin, a gene involved in nerve regeneration,[77-79] cardiac remodeling,[80] vascular remodeling,[81-83] and neointimal proliferation,[84] in a swine model of myocardial infarction when mesenchymal stem cells were injected into infarcted myocardium.[85] Mesenchymal cells also increased cardiac nerve sprouting in both atria and ventricles, and increased the magnitude of atrial sympathetic hyperinnervation. However, heterogeneous sympathetic nerve sprouting affects automaticity, triggered activity, refractoriness, and conduction velocity of myocardial cells and, therefore, may represent a substrate for lethal ventricular arrhythmia.[86-90]

The DNA demethylating agent 5-azacytidine has been used to induce the differentiation of stem cells into cardiomyocytes.[69] Adult mesenchymal stem cells isolated from fatty tissue differentiated into cardiomyocytes after exposure to 5-azacytidine in a rabbit model.[91] It has also been shown that mesenchymal stem cells isolated from whole bone marrow and treated with 5-azacytidine differentiated into cardiac-like cells in ventricular scar tissue and induced angiogenesis and improved myocardial function after autotransplantation.[50] Therefore, in vitro culture of mesenchymal stem

cells with 5-azacytidine prior to transplantation into myocardial scar tissue may facilitate successful engraftment.

Mode of Delivery of Stem Cells

Intramyocardial Injection

Recent studies have implemented three different modes of delivery of stem cells (Table 3). Stem cells were harvested from bone marrow and injected directly into the contracting myocardial wall bordering the infarct.[92] Transplantation of bone marrow stem cells led to the regeneration of a significant amount of contracting myocardium, reduction in the infarct area, and improvement in cardiac hemodynamics. Because direct myocardial injections are invasive, this mode of delivery may be the preferred route when a surgical procedure is planned. Direct myocardial injections may also be the preferred mode of delivery in patients with chronic congestive heart failure. The homing process that is up-regulated in myocardial infarction due to increased levels of VEGF may be less intense and less conducive to cell engraftment in the chronic condition. It also appears that skeletal muscles are better suited to delivery by direct myocardial injections because of the potential for embolization when a large quantity of cells is implanted.[22] It is likely that a smaller quantity of cells is needed to achieve the desired effect with direct intramyocardial injection compared with intracoronary or intravenous administration. Direct myocardial injection of cells is a simple process and allows for direct visualization and surveillance of the target zones.[22] However, the surgical approach of stem cell therapy is associated with well-known operative risks of an open-heart operation.[22]

Table 3
Mode of Delivery of Stem Cells

Route of Injection	Comments
Intramyocardial	Smaller number of cells to achieve engraftment compared with intracoronary or intravenous administration
	Simple and can be performed by direct inspection of the potential target zones
	May lead to "islands" of cells in the infarcted myocardium, providing a substrate for electrical instability and ventricular tachyarrhythmias
Intracoronary	Delivers the maximum concentration of cells to the site of infarct and peri-infarct tissue during the first passage
	Allows the stem cells to home in and engraft into the areas bordering the infarct zone in a homogenous manner
Intravenous	Simplest and least invasive method
	Homing of stem cells to noncardiac organs may limit the number of cells reaching the infarct region

Direct myocardial injection of cells is a simple process and allows for direct visualization and surveillance of the target zones. However, the surgical approach of stem cell therapy is associated with well-known operative risks of an open-heart operation.

Stem cells can also be implanted percutaneously with catheter-based myocardial injections guided by left ventricular electromechanical mapping with the NOGA system (Biosense Webster, Diamond Bar, CA).[93] The NOGA system includes a mapping catheter that implements a low-intensity, active magnetic field energy and sensors allowing for real-time, three-dimensional mapping. This allows for positioning of the catheter and measurement of the electrical and motion capabilities of the heart. Cells may then be directly injected with high precision into nonviable areas of the myocardium with an injection-needle catheter, thus offering an advantage over the more invasive surgical approach and its associated risks.[94,95]

Intracoronary Injection

A nonsurgical, safer mode of delivery of stem cells after myocardial infarction can be accomplished through selective intracoronary injection to the infarct-related artery with an over-the-wire balloon catheter.[96] Intracoronary delivery of stem cells appears to be superior to direct intramyocardial and intravenous administration in clinical practice because it allows all the cells to flow through the infarct and peri-infarct tissue during the first passage. Intracoronary administration into the infarct-relat-

ed artery allows the stem cells to home in and engraft to the border zone of the infarct homogenously. This may explain the lack of tachyarrhythmias associated with the intracoronary infusion of autologous endothelial progenitor cells and bone marrow cells. This is different from skeletal myoblasts injected into scarred tissue, which creates an island of arrhythmogenicity.[97] Still-unresolved issues of intracoronary delivery of stem cells include the optimal number of cells and the optimal duration of the infusion, as they may adversely affect coronary perfusion and induce myonecrosis.

Intravenous Injection

Intravenous injection is the simplest and least invasive method of administration of stem cells and obviates the need for cardiac surgery or cardiac catheterization.[55] However, there needs to be a greater degree of homing of the stem cells to reach the heart. In this mode of delivery, the optimal dose of stem cells is of particular importance. However, because of the long circulation time, cells could be lost by extraction toward noncardiac organs and fail to home to the area of infarct.

With the recognition that two essential factors—recent tissue damage and a sufficient number of circulating stem cells—are required for successful colonization and transdifferentiation of bone marrow stem cells into a variety of tissue, a different approach to cellular cardiomyoplasty was studied. In a noninvasive approach for myocardial regeneration, bone marrow stem cells were mobilized with G-CSF and SCF injections.[17] G-CSF, a cytokine that has been used extensively in clinical stem cell transplantation for lineage-specific stem cell renewal, mobilizes stem cells in the bone marrow by disrupting the homing mechanisms of stem cells through the proteolytic cleavage of vascular cell adhesion molecule-1 (VCAM-1).[98,99] The stem cells mobilized with G-CSF and SCF decreased mortality, infarct size, cavity dilatation, and diastolic stress in a murine infarct model.[17] In addition, there were improvements in ejection fraction and hemodynamics due to the formation of new cardiomyocytes with arterioles and capillaries. Connexin was also identified in the newly formed cardiomyocytes derived from bone marrow cells, suggesting that gap junctions formed between cardiomyocytes.

Clinical Studies

Preclinical studies in animal models suggest that cardiac transfer of stem cells enhances myocardial regeneration and neovascularization after myocardial infarction. Based on these studies and the recognition of the plasticity of adult stem cells, it has been proposed that stem cells may be used for myocardial regeneration in patients with myocardial infarction. Currently, however, the clinical data on stem cell transplantation is in its infancy and is very limited. Preliminary clinical data on stem cell transplantation have mainly come from small nonrandomized, and a few randomized, controlled trials (Table 4).

Based on clinical studies and the recognition of the plasticity of adult stem cells, it has been proposed that stem cells may be used for myocardial regeneration in patients with myocardial infarction. Currently, however, the clinical data on stem cell transplantation is in its infancy and is very limited.

In a study by Hamano and associates, when autologous mononuclear bone marrow cells were injected into the infarcted myocardium during coronary artery bypass grafting, scintigraphy demonstrated an improvement in myocardial perfusion in the area of the myocardium treated with cells in 3 of the 5 patients and no change in 2 patients after 1 year.[100] New collateral vessels were formed at the site of injection. One disadvantage to this approach is that the cells were delivered by injection during bypass surgery, making it unfeasible in most patients with myocardial infarction.

In a nonsurgical approach reported by Strauer and colleagues, the safety and efficacy of intracoronary transplantation of autologous mononuclear bone marrow cells was reported in 10 patients with preserved left ventricular ejection fraction 5-9 days after myocardial infarction.[96] Bone marrow cells were harvested and cultivated for autologous transplantation. Subsequently, 2-3 mL of the cell suspension was delivered by high-pressure infusions at the site of the former infarct occlusion, with the balloon inflated at low pressures for 2-3 minutes to allow the cells to migrate into the tissue and prevent reflux of cell suspension into the aorta. With balloon catheter delivery of autologous bone marrow cells to the infarcted region, there was a reduction in infarct size after 3 months, as determined by myocardial perfusion defect, and an increase in stroke volume. The treated patients also demonstrated improved wall motion and left ventricular end-systolic volume, cardiac function, and metabolism of the infarcted myocardium compared with 10 patients in the

Table 4
Clinical Trials of Cellular Cardiomyoplasty

Study	Patients, N	Procedure	Follow-up Period	Donor cell	Complications
Hamano et al[100]	5	Myocardial injection during CABG	1 y	Bone marrow cells	None
Strauer et al[96]	10	Intracoronary infusion during PTCA	3 mo	Bone marrow cells	None
Assmus et al[97]	20	Intracoronary infusion during PTCA	4 mo	Progenitor cells	None
Menasché et al[48]	10	Myocardial injection during CABG	10.9 mo	Skeletal myoblasts	1 death
Stamm et al[102]	6	Myocardial injection during CABG	3-9 mo	Bone marrow cells	2 patients experienced SVT
Pagani et al[49]	5	Myocardial injection during LVAD	68-191 d	Skeletal myoblasts	4 patients experienced arrhythmias; 1 LVAD death
Tse et al[93]	8	Myocardial injection during catheterization	3 mo	Bone marrow cells	None
Perin et al[103]	14	Myocardial injection during catheterization	4 mo	Bone marrow cells	1 death; no arrhythmias
Wollert et al[104]	30	Intracoronary infusion during PTCA	6 mo	Bone marrow cells	None
Brehm et al[105]	20	Intracoronary infusion during PTCA	3 mo	Bone marrow cells	None
Smits et al[106]	5	Myocardial injection during catheterization	6 mo	Skeletal myoblasts	1 patient experienced VT

CABG, coronary artery bypass grafting; LVAD, left ventricular assist device; PTCA, percutaneous transluminal coronary angioplasty; SVT, supraventricular tachycardia; VT, ventricular tachycardia.

control group. Given that patients were revascularized percutaneously at an average of 12 hours after the onset of symptoms, it is believed that the improvement in left ventricular function was due to coronary angiogenesis and myocardial regeneration from transplanted bone marrow cells and not entirely from the revascularization. The authors, throughout the diagnostic or therapeutic procedure and at 3 months follow-up, reported no adverse events.

The Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) pilot trial compared the effect of direct intracoronary infusion of autologous circulating progenitor cells and bone marrow cells in 20 patients who underwent primary angioplasty for acute myocardial infarction.[97] The intracoronary infusion of cells via an over-the-wire balloon catheter advanced into the previously deployed stent increased coronary perfusion indices of the infarcted regions, as measured by stress echocardiography. There was also improved global and regional contractility and attenuation of postinfarction left ventricular remodeling. In order to more reliably relate the functional characteristics of the transplanted progenitor cells to quantitative measures of outcomes at 4-month follow-up, serial contrast-enhanced magnetic resonance imaging (MRI) demonstrated the migratory capacity of the progenitor cells to their physiologic chemoattractants, was the major determinant of infarct remodeling, and had a beneficial effect on the postinfarction remodeling process and regeneration enhancement in patients with acute myocardial infarction.[24] No adverse outcomes were reported, including any evidence of myonecrosis and inflammation accompanying the intracoronary injection,

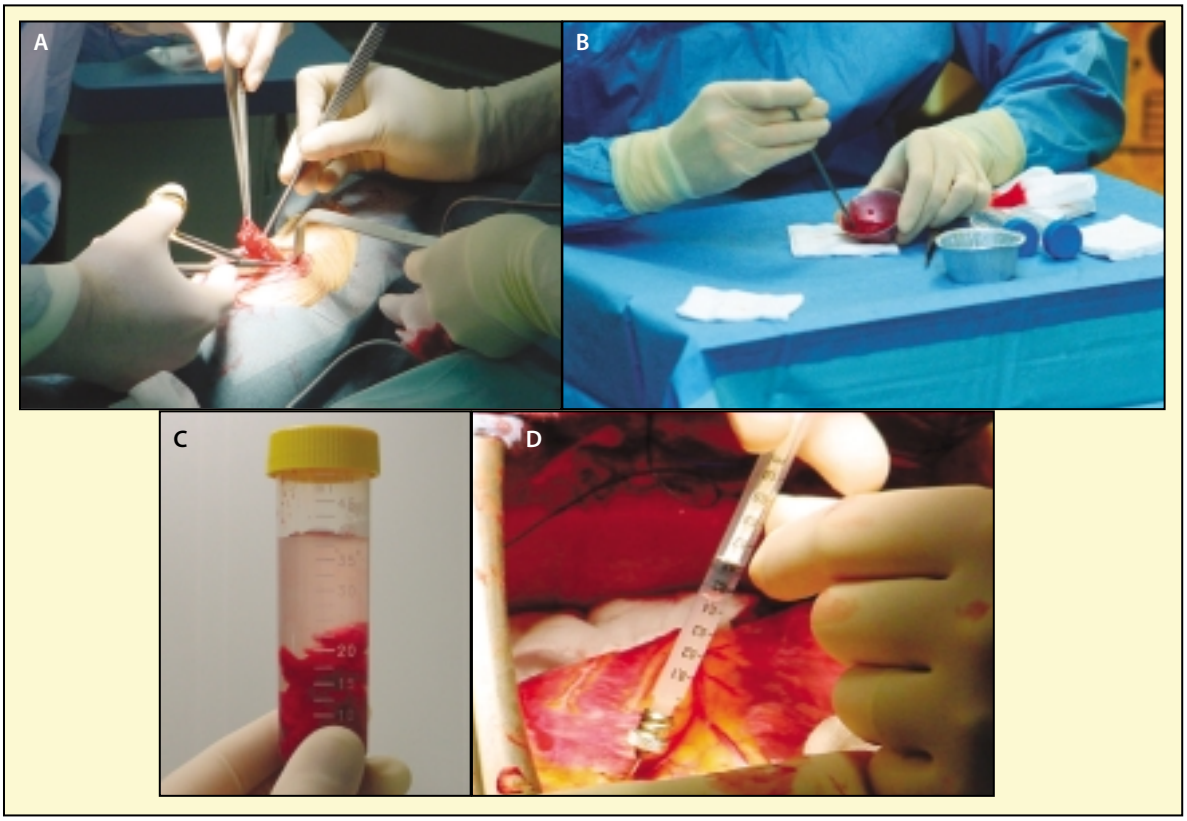

Figure 2. (A) *Skeletal myoblasts were harvested by obtaining a 10-15 g piece of vastus lateralis from the patient's thigh,* **(B)** *minced, and* **(C)** *stored in a preservation medium.* **(D)** *The cell suspension was then injected within and near the peri-infarct zone with a customized 27-gauge needle under cardioplegic arrest. Injections were made tangentially to reduce the risk of intracavitary cell delivery. Images provided by A. Hagége.*

suggesting that infusion is safe and does not lead to occlusion of some small vessels.

Approximately 700 million to 1200 million skeletal myoblasts were directly injected into the postinfarction myocardial scar during coronary artery bypass grafting in 10 patients with ischemic heart failure (Figure 2).[48] At 11-month follow-up, the 10 patients who received the injections of the skeletal myoblasts showed an improvement in New York Heart Association functional class and left ventricular ejection fraction. Survival of transplanted cells was inferred by improvements in deoxyglucose metabolism on positron emission tomography. There was one patient death secondary to a stroke.[101] However, on histologic examination, the formation of skeletal myotubes was clearly evident. Sustained ventricular tachycardia was present in 4 patients and required the implantation of an automatic implantable cardioverter-defibrillator. Given that this study was not controlled, whether the improvements in functional class and left ventricular ejection fraction were the result of the coronary artery bypass grafting or the injected myoblasts is not readily apparent.

In another study involving cell transplantation during coronary artery bypass grafting, autologous bone marrow cells were injected in the infarct border zone in 6 patients with myocardial infarction.[102] At 3–9 month follow-up, there was an improvement in infarct tissue perfusion based on single-photon emission computed tomography (SPECT) myocardial perfusion scintigraphy in 5 patients. These results suggest that AC133+ stem cells may play a role in angiogenesis in the infarcted myocardium. All patients were alive at follow-up, but 2 patients had supraventricular tachycardia.

Five patients with ischemic cardiomyopathy with refractory heart failure, awaiting cardiac transplantation, received injections of autologous skeletal myoblasts in the area of scarred myocardium concomitant with left ventricular assist device implantation.[49] Histologic examination revealed skeletal myoblast survival and differentiation into mature myofibers (Figure 3). Because of the presence of the left ventricular assist device, assessment of functional improvement in the regional myocardial segments with cardiac MRI was not possible. Skeletal myoblasts did not elicit an immune response. Similar to a previous clinical study, ventricular tachyarrhythmias were encountered in 3 patients.[48]

Using the transendothelial

approach, autologous bone marrow mononuclear cells harvested from the iliac crest were implanted with the use of the percutaneous catheter-based transplantation technique in 8 patients with severe ischemic heart disease and normal mean ejection fraction.[93] Left ventricular electromechanical mapping with the NOGA system was constructed to assist the direct injection of bone marrow cells into the myocardium, with a mean of 16 injections at 11 targeted ischemic regions. At 3-month follow-up, patients reported less anginal episodes and fewer consumed nitroglycerin tablets and demonstrated improved myocardial perfusion and segmental contractility on cardiac MRI. There were no acute procedural complications or long-term sequelae, including ventricular tachyarrhythmias, myonecrosis, or the development of cardiac tumor.

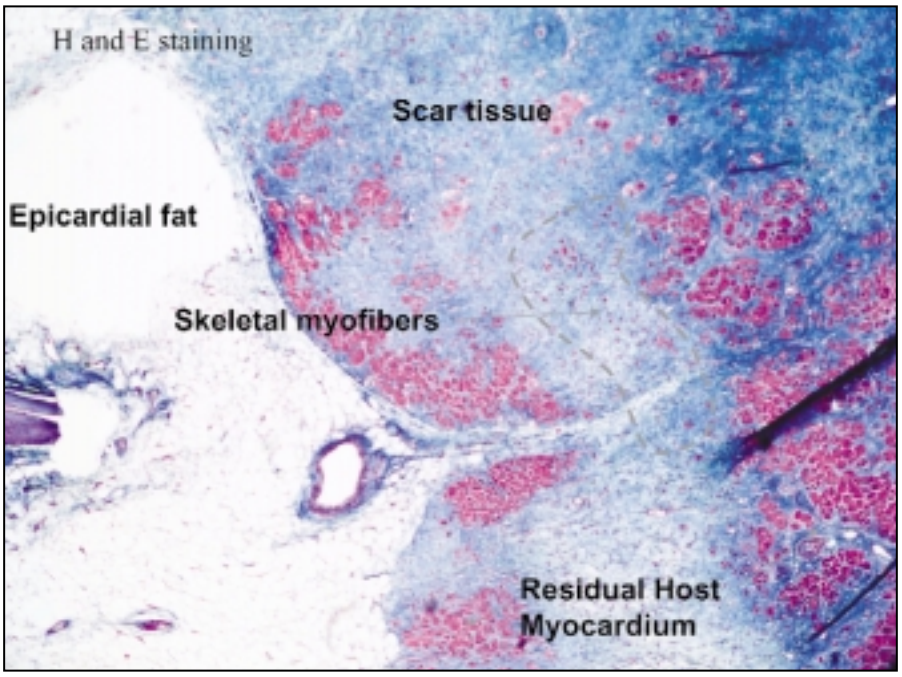

Figure 3. *This histologic analysis is from a patient with end-stage ischemic cardiomyopathy who underwent autologous transplantation into ischemic-damaged myocardium during left ventricular assist device implantation. Hematoxylin-eosin staining demonstrates that autologous myoblasts survived and formed viable grafts in heavily scarred myocardial tissue. Image provided by F. Pagani.* www.medreviews.com

In another clinical trial using the transendothelial approach,[103] autologous bone marrow mononuclear cells were implanted into hibernating myocardium via percutaneous catheter-based endocardial injections guided by left ventricular electromechanical mapping with the NOGA system in 14 patients with severe coronary artery disease and an ejection fraction < 40% with no option for revascularization. Injections took place 4 hours after the bone marrow cells were harvested from the iliac crest.

The early clinical investigations suggest that autologous bone marrow cell transplantation into the infarct-related artery is feasible and safe. However, because of the nonrandomized design of the studies, the effects of intracoronary bone marrow cell transfer on ventricular remodeling after myocardial infarction have remained uncertain.

At 2-month follow-up, treated patients experienced an improvement in New York Heart Association class and Canadian Cardiovascular Society Angina score. There was also a significant reduction in total reversible stress defect and improvement in global left ventricular function in treated patients, as determined by SPECT. At 4-month follow-up, there was an increase in left ventricular ejection fraction from 20% to 29% and a reduction in end-systolic volume post-treatment. There was also a significant mechanical improvement of the injected segments on electromechanical mapping. Neovascularization was the presumed mechanism of the improvement in myocardial contractility as perfusion was increased in the hibernating myocardium. The one death in the treatment group was thought to be from sudden cardiac death. No major periprocedural complications or significant tachyarrhythmias were reported.

The early clinical investigations suggest that autologous bone marrow cell transplantation into the infarct-related artery is feasible and safe. However, because of the nonrandomized design of the studies, the effects of intracoronary bone marrow cell transfer on ventricular remodeling after myocardial infarction have remained uncertain. In the Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration (BOOST) trial, one of the first randomized controlled trials, 60 patients with acute myocardial infarction and successful primary percutaneous coronary intervention were randomized to receive either autologous bone marrow cells delivered to the

infarct-related artery via an over-the-wire balloon catheter or optimal medical therapy.[104] Patients who received cell therapy had significant improvement of left ventricular function at 6-month follow-up with cardiac MRI. The procedure was safe, with no deaths or myonecrosis in patients who received autologous bone marrow cells. There was also no evidence of enhanced arrhythmogenicity as assessed by electrophysiologic studies associated with the transplantation of the autologous bone marrow cells.

In another clinical trial, 20 patients were treated with intracoronary administration of autologous bone marrow cells or standard therapy after myocardial infarction.[105] At 3-month follow-up, treated patients had smaller infarct sizes and increased ejection fraction, glucose uptake on positron emission tomography, and myocardial perfusion. These results suggest the intracoronary delivery of autologous bone marrow cells improves cardiac function, perfusion, and metabolism secondary to neovascularization and myogenesis within the infarcted region.

Five patients with ischemic heart failure after an anterior wall myocardial infarction received percutaneous catheter-based intramyocardial injections of autologous skeletal muscle myoblasts guided by left ventricular electromechanical mapping with the NOGA system.[106] At 6-month follow-up, the 5 patients who received the injections of the skeletal myoblasts showed an improvement in left ventricular ejection fraction, with increased wall thickening at the target areas on regional wall analysis by cardiac MRI. One patient had asymptomatic runs of nonsustained ventricular tachycardia and received an implantable cardioverter-defibrillator.

Assessment of Stem Cell Transplantation Success With Noninvasive Imaging

Previously, verification of the status of the transplanted stem cells had only been possible with histologic analysis. Despite providing optimal imaging for transcatheter therapy, X-ray fluoroscopy is limited in permitting precise percutaneous endomyocardial drug delivery. Refinement of noninvasive imaging modalities is needed to provide solid scientific validation of cardiac regeneration from a functional and structural perspective.[23]

It appears that advances in cardiac MRI have propelled it to the forefront in the noninvasive assessment of transplanted stem cells. Cardiac MRI has been used to characterize the engraftment of mesenchymal stem cells labeled with MRI-visible contrast agents injected intramyocardially after myocardial infarction in a swine model.[107] Cardiac MRI characterizes the size and the location of each intramyocardial injection and the extent of mesenchymal stem cell retention. Stem cells can also be labeled with iron particles to noninvasively monitor their distribution in the body after transplantation.[108] Cardiac MRI also allows for precise targeted catheter-based implantation of myogenic precursor cells into the infarcted myocardium and the detection of cells loaded with iron oxide in the infarcted myocardium.[109] Real-time cardiac MRI provides precise three-dimensional size and localization of targeted percutaneous endomyocardial drug delivery within the ventricular wall.[110] It is believed that application of cardiac MRI may one day be used to help deliver stem cells in the failing myocardium for therapeutic purposes.

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The fate and tissue distribution of human endothelial progenitor cells after injection into rats after myocardial infarction was elaborated after being radioactively labeled with [^{111}In]indium oxine.[111] ^{111}In-oxine is a radioactive tracer that has also been used commercially to monitor blood cells in the setting of heightened inflammatory states with scintigraphy for clinical applications.[112] Therefore, given the need to fully assess the detailed physiologic response to stem cell therapy, there still remains the need for newer techniques that current noninvasive imaging modalities are unable to accomplish in order to detect the newly introduced cells into the myocardium and to follow their cellular function, growth, and proliferation over time.

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Controversies in Stem Cell Transplantation

Despite the potential for stem cell therapy in patients with myocardial infarction, more questions arise even as our understanding of cellular cardiomyoplasty improves. What is the optimal donor cell and the optimal number of stem cells? When should the cells be transplanted? Which mode of delivery is optimal? Can we expand the cells in vitro to optimal levels prior to transplantation? Can we extend stem cell therapy to patients with chronic ischemic heart disease and nonischemic cardiomyopathy? Is stem cell therapy safe and effective in the long term?

Despite the promise that stem cell transplantation may hold, serious concerns about its safety have yet to be answered.

Each potential donor cell has its associated ethical, biologic, or technical limitations and advantages.[113] A major hurdle that must be overcome is the small number of stem cells that can be isolated from the adult patient in order to reach the minimum for successful transplantation. Recent advances in the propagation of the multipotent adult stem cell and the administration of various mediators and cytokines, such as G-CSF and SCF, to promote proliferation and mobilization of stem cells to the site of injury, may hold the key to unlocking this dilemma.[17,114] However, expansion of stem cells ex vivo in culture may be required and may alter their intrinsic characteristics, leaving them unable to precisely restore injured or diseased tissues.[20] In order to enhance the organ-specific engraftment process, fine manipulation of stem cells with a culture microenvironment that contains various cytokines and growth factors is needed.[115]

The optimal time for stem cell harvesting and transplantation is not clear. The fresh administration of stem cells after minimal manipulation appears to be the ideal approach. A longer period of time from harvesting to implantation might increase the risk of infection.[22] It appears that the inflammation seen within the first 48 hours after myocardial infarction interferes with the stem cells' regenerative capacity and adversely affects the engraftment process and survival of the stem cells.[116] Conversely, stem cells should be transplanted prior to excessive formation of scar tissue, which appears to occur at day 14 post–myocardial infarction.[117] Transplantation between 7 and 14 days post–myocardial infarction appears to be the optimal time for cell delivery.

Extension of stem cell therapy to patients with chronic heart failure may encounter several hurdles. Chronically failing hearts with no recent infarct may not respond to cells injected into the artery as they would in myocardial infarction. These patients also appear to have dysfunctional progenitor and stem cells.

A novel and more practical method of stem cell procurement for myocardial regeneration is from peripheral blood. Procurement of adult stem cells from peripheral blood transdifferentiates into cardiomyocytes, endothelial cells, and smooth muscle cells, with evidence of neovascularization and significant augmentation of transdifferentiation with local tissue injury.[118]

Safety

Despite the promise that stem cell transplantation holds, serious questions about its safety have yet to be answered. The differentiation of stem cells into fibroblasts, as opposed to myocytes, which may contribute to further scarring and incomplete integration of stem cells into the myocardium, may increase the risk of ventricular tachyarrhythmias.[119] Alteration of electrical conduction and normal syncytial contraction from poor integration of stem cells into the myocardium can have potentially life-threatening effects.[29] The potential of homing to noncardiac organs and differentiation into cardiomyocytes, especially during intravenous stem cell delivery, is also a potential complication, leading to unknown sequelae.

Although it might be related to the intrinsic properties of the infarcted myocardium with left ventricular systolic dysfunction, a major concern with stem cell transplantation is the potential for life-threatening ventricular tachyarrhythmias, especially with skeletal myoblasts. Although the exact mechanisms are unknown, there are several proposed hypotheses to explain ventricular tachyarrhythmias (Table 5).[120] One explanation is that the harvested skeletal myoblasts

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Table 5
Potential Mechanisms for Cardiac Arrhythmias in Stem Cell Transplantation

• Electrical heterogeneity of action potentials between the native and transplanted stem cells
• Intrinsic arrhythmic potential of transplanted cells
• Increased nerve sprouting induced by stem cell transplantation
• Local tissue injury induced by intramyocardial injection

are unable to completely communicate with the surrounding myocardium because of the lack of connexin (gap junctions). It therefore seems reasonable, as of now, to implant skeletal myoblasts in only those patients with an implantable cardioverter-defibrillator.

Summary

Despite the plethora of data on stem cell transplantation in animal infarct models, its role in sustaining clinical benefit in post–myocardial infarction patients is currently unknown. The exciting clinical potential of the use of stem cells to regenerate infarcted myocardium must be tempered by the reality of its unknown role in the treatment of myocardial infarction, especially given the initial excitement and promise of angiogenesis studies that failed to live up to their expectations and the disappointing results seen in gene therapy trials. There is ongoing debate over whether stem cell transplantation will play a major role in the routine treatment of myocardial infarction to prevent ischemic cardiomyopathy; perhaps it will serve more as an adjunct to existing therapies rather than a cure. Concomitant percutaneous or surgical revascularization makes the effectiveness of stem cell therapy difficult to assess. The role of clinical investigation to clarify the therapeutic clinical effect of stem cells cannot be overstated. ■

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Main Points

- Myocardial infarction is the leading cause of congestive heart failure and death in the industrialized world.
- Stem cells are undifferentiated pluripotent cells that possess the potential for self-renewal and differentiation into cardiomyocytes.
- Cellular cardiomyoplasty, which is the replacement or regeneration of cardiomyocytes through cell transplantation, offers a potential treatment option to reverse the deleterious hemodynamic and neurohormonal effects seen after myocardial infarction that can lead to congestive heart failure.
- Preclinical animal studies have shown the potential to regenerate myocardium and improve perfusion to the infarct area to improve cardiac function.
- Early phase I clinical studies suggest that stem cell transplantation is feasible and has the potential for beneficial effects on ventricular remodeling after myocardial infarction.
- Future randomized clinical trials will establish the magnitude of benefit from stem cell therapy.

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