# 6. MENDING A BROKEN HEART: STEM CELLS AND CARDIAC REPAIR

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## HEART FAILURE: THE DISEASE AND ITS CAUSES

Cardiovascular disease (CVD), which includes hypertension, coronary heart disease (CHD), stroke, and congestive heart failure (CHF), has ranked as the number one cause of death in the United States every year since 1900 except 1918, when the nation struggled with an influenza epidemic.[1] In 2002, CVD claimed roughly as many lives as cancer, chronic lower respiratory diseases, accidents, diabetes mellitus, influenza, and pneumonia combined. According to data from the 1999-2002 National Health and Nutrition Examination Survey (NHANES), CVD caused approximately 1.4 million deaths (38.0 percent of all deaths) in the U.S. in 2002. Nearly 2600 Americans die of CVD each day, roughly one death every 34 seconds. Moreover, within a year of diagnosis, one in five patients with CHF will die. CVD also creates a growing economic burden; the total health care cost of CVD in 2005 was estimated at $393.5 billion dollars.

Given the aging of the U.S. population and the relatively dramatic recent increases in the prevalence of cardiovascular risk factors such as obesity and type 2 diabetes,[2,3] CVD will continue to be a significant health concern well into the 21st century. However, improvements in the acute treatment of heart attacks and an increasing arsenal of drugs have facilitated survival. In the U.S. alone, an estimated 7.1 million people have survived a heart attack, while 4.9 million live with CHF.[1] These trends suggest an unmet need for therapies to regenerate or repair damaged cardiac tissue.

Ischemic heart failure occurs when cardiac tissue is deprived of oxygen. When the ischemic insult is severe enough to cause the loss of critical amounts of cardiac muscle cells (cardiomyocytes), this loss initiates a cascade of detrimental events, including formation of a non-contractile scar, ventricular wall thinning, (see Figure 6.1), an overload of blood flow and pressure, ventricular remodeling (the overstretching of viable cardiac cells to sustain cardiac output), heart failure, and eventual death.[4] Restoring damaged heart muscle tissue, through repair or regeneration, therefore represents a fundamental mechanistic strategy to treat heart failure. However, endogenous repair mechanisms, including the proliferation of

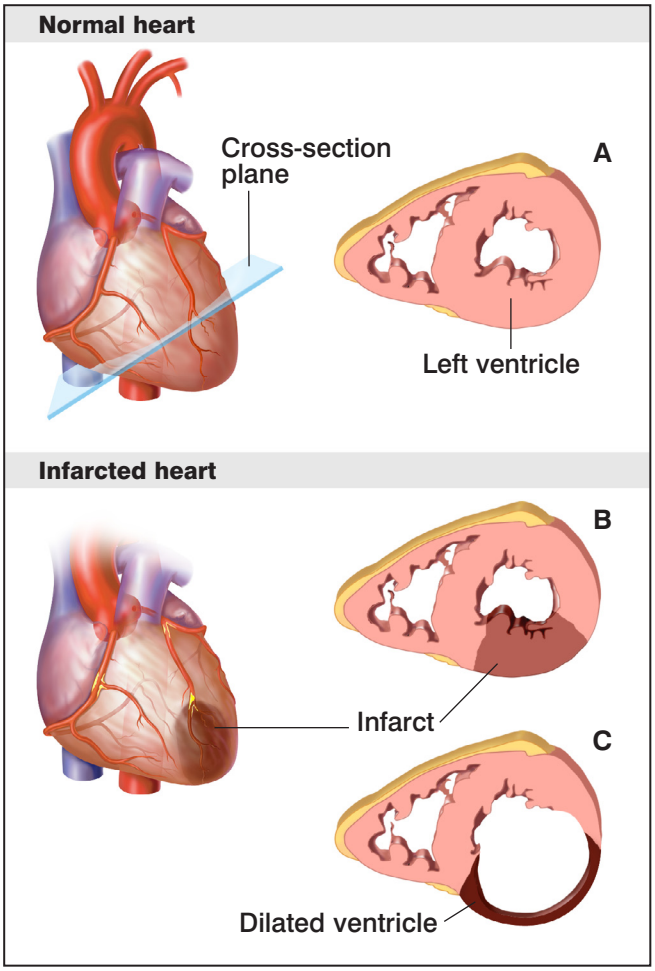




**Figure 6.1. Normal vs. Infarcted Heart.** The left ventricle has a thick muscular wall, shown in cross-section in A. After a myocardial infarction (heart attack), heart muscle cells in the left ventricle are deprived of oxygen and die (B), eventually causing the ventricular wall to become thinner (C).

cardiomyocytes under conditions of severe blood vessel stress or vessel formation and tissue generation via the migration of bone-marrow-derived stem cells to the site of damage, are in themselves insufficient to restore lost heart muscle tissue (myocardium) or cardiac function.[5] Current pharmacologic interventions for heart disease, including beta-blockers, diuretics, and angiotensin-converting enzyme (ACE) inhibitors, and surgical treatment options, such as changing the shape of the left ventricle and implanting assistive devices such as pacemakers or defibrillators, do not restore function to damaged tissue. Moreover, while implantation of mechanical ventricular assist devices can provide long-term improvement in heart function, complications such as infection and blood clots remain problematic.[6] Although heart transplantation offers a viable option to replace damaged myocardium in selected individuals, organ availability and transplant rejection complications limit the widespread practical use of this approach.

The difficulty in regenerating damaged myocardial tissue has led researchers to explore the application of embryonic and adult-derived stem cells for cardiac repair. A number of stem cell types, including embryonic stem (ES) cells, cardiac stem cells that naturally reside within the heart, myoblasts (muscle stem cells), adult bone marrow-derived cells, mesenchymal cells (bone marrow-derived cells that give rise to tissues such as muscle, bone, tendons, ligaments, and adipose tissue), endothelial progenitor cells (cells that give rise to the endothelium, the interior lining of blood vessels), and umbilical cord blood cells, have been investigated to varying extents as possible sources for regenerating damaged myocardium. All have been tested in mouse or rat models, and some have been tested in large animal models such as pigs. Preliminary clinical data for many of these cell types have also been gathered in selected patient populations.

However, clinical trials to date using stem cells to repair damaged cardiac tissue vary in terms of the condition being treated, the method of cell delivery, and the primary outcome measured by the study, thus hampering direct comparisons between trials.[7] Some patients who have received stem cells for myocardial repair have reduced cardiac blood flow (myocardial ischemia), while others have more pronounced congestive heart failure and still others are recovering from heart attacks. In some cases, the patient's underlying condition influences the way that the stem cells are delivered to his/her heart (see the section, "Methods of Cell Delivery" for details). Even among patients undergoing comparable procedures, the clinical study design can affect the reporting of results. Some studies have focused on safety issues and adverse effects of the transplantation procedures; others have assessed improvements in ventricular function or the delivery of arterial blood. Furthermore, no published trial has directly compared two or more stem cell types, and the transplanted cells may be autologous (i.e., derived from the person on whom they are used) or allogeneic (i.e., originating from another person) in origin. Finally, most of these trials use unlabeled cells, making it difficult for investigators to follow the cells' course through the body after transplantation (see the section "Considerations for Using These Stem Cells in the Clinical Setting" at the end of this article for more details).

Despite the relative infancy of this field, initial results from the application of stem cells to restore cardiac function have been promising. This article will review the research supporting each of the aforementioned cell types as potential source materials for myocardial regeneration and will conclude with a discussion of general issues that relate to their clinical application.

## MECHANISMS OF ACTION

In 2001, Menasche, *et.al.* described the successful implantation of autologous skeletal myoblasts (cells that divide to repair and/or increase the size of voluntary muscles) into the post-infarction scar of a patient with severe ischemic heart failure who was undergoing coronary artery bypass surgery.[8] Following the procedure, the researchers used imaging techniques to observe the heart's muscular wall and to assess its ability to beat. When they examined patients 5 months after treatment, they concluded that treated hearts pumped blood more efficiently and seemed to demonstrate improved tissue health. This case study suggested that stem cells may represent a viable resource for treating ischemic heart failure, spawning several dozen clinical studies of stem cell therapy for cardiac repair (see Boyle, *et.al.*[7] for a complete list) and inspiring the development of Phase I and Phase II clinical trials. These trials have revealed the complexity of using stem cells for cardiac repair, and considerations for using stem cells in the clinical setting are discussed in a subsequent section of this report.

The mechanism by which stem cells promote cardiac repair remains controversial, and it is likely that the cells regenerate myocardium through several pathways. Initially, scientists believed that transplanted cells differentiated into cardiac cells, blood vessels, or other cells damaged by CVD.[9-11] However, this model has been recently supplanted by the idea that transplanted stem cells release growth factors and other molecules that promote blood vessel formation (angiogenesis) or stimulate "resident" cardiac stem cells to repair damage.[12-14] Additional mechanisms for stem-cell mediated heart repair, including strengthening of the post-infarct scar[15] and the fusion of donor cells with host cardiomyocytes,[16] have also been proposed.

## METHODS OF CELL DELIVERY

Regardless of which mechanism(s) will ultimately prove to be the most significant in stem-cell mediated cardiac repair, cells must be successfully delivered to the site of injury to maximize the restored function. In preliminary clinical studies, researchers have used several approaches to deliver stem cells. Common approaches include intravenous injection and direct infusion into the coronary arteries. These methods can be used in patients whose blood flow has been restored to their hearts after a heart attack, provided that they do not have additional cardiac dysfunction that results in total occlusion or poor arterial flow.[12, 17] Of these two methods, intracoronary infusion offers the advantage of directed local delivery, thereby increasing the number of cells that reach the target tissue relative to the number that will home to the heart once they have been placed in the circulation. However, these strategies may be of limited benefit to those who have poor circulation, and stem cells are often injected directly into the ventricular wall of these patients. This endomyocardial injection may be carried out either via a catheter or during open-heart surgery.[18]

To determine the ideal site to inject stem cells, doctors use mapping or direct visualization to identify the locations of scars and viable cardiac tissue. Despite improvements in delivery efficiency, however, the success of these methods remains limited by the death of the transplanted cells; as many as 90% of transplanted cells die shortly after implantation as a result of physical stress, myocardial inflammation, and myocardial hypoxia.[4] Timing of delivery may slow the rate of deterioration of tissue function, although this issue remains a hurdle for therapeutic approaches.

## TYPES OF STEM CELLS INVESTIGATED TO REGENERATE DAMAGED MYOCARDIAL TISSUE

Embryonic and adult stem cells have been investigated to regenerate damaged myocardial tissue in animal models and in a limited number of clinical studies. A brief review of work to date and specific considerations for the application of various cell types will be discussed in the following sections.

### *Embryonic Stem (ES) Cells*

Because ES cells are pluripotent, they can potentially give rise to the variety of cell types that are instrumental in regenerating damaged myocardium, including cardiomyocytes, endothelial cells, and smooth muscle cells. To this end, mouse and human ES cells have been shown to differentiate spontaneously to form endothelial and smooth muscle cells *in vitro*[19] and *in vivo,*[20,21] and human ES cells differentiate into myocytes with the structural and functional properties of cardiomyocytes.[22-24] Moreover, ES cells that were transplanted into ischemically-injured myocardium in rats differentiated into normal myocardial cells that remained viable for up to four months,[25] suggesting that these cells may be candidates for regenerative therapy in humans.

However, several key hurdles must be overcome before human ES cells can be used for clinical applications. Foremost, ethical issues related to embryo access currently limit the avenues of investigation. In addition, human ES cells must go through rigorous testing and purification procedures before the cells can be used as sources to regenerate tissue. First, researchers must verify that their putative ES cells are pluripotent. To prove that they have established a human ES cell line, researchers inject the cells into immunocompromised mice; i.e., mice that have a dysfunctional immune system. Because the injected cells cannot be destroyed by the mouse's immune system, they survive and proliferate. Under these conditions, pluripotent cells will form a teratoma, a multi-layered, benign tumor that contains cells derived from all three embryonic germ layers. Teratoma formation indicates that the stem cells have the capacity to give rise to all cell types in the body.

The pluripotency of ES cells can complicate their clinical application. While undifferentiated ES cells may possibly serve as sources of specific cell populations used in myocardial repair, it is essential that tight quality control be maintained with respect to the differentiated cells. Any differentiated cells that would be used to regenerate heart tissue must be purified before transplantation can be considered. If injected regenerative cells are accidentally contaminated with undifferentiated ES cells, a tumor could possibly form as a result of the cell transplant.[4] However, purification methodologies continue to improve; one recent report describes a method to identify and select cardiomyocytes during human ES cell differentiation that may make these cells a viable option in the future.[26]

This concern illustrates the scientific challenges that accompany the use of all human stem cells, whether derived from embryonic or adult tissues. Predictable control of cell proliferation and differentiation requires additional basic research on the molecular and genetic signals that regulate cell division and specialization. Furthermore, long-term cell stability must be well understood before human ES-derived cells can be used in regenerative medicine. The propensity for genetic mutation in the human ES cells must be determined, and the survival of differentiated, ES-derived cells following transplantation must be assessed. Furthermore, once cells have been transplanted, undesirable interactions between the host tissue and the injected cells must be minimized. Cells or tissues derived from ES cells that are currently available for use in humans are not tissue-matched to patients and thus would require immunosuppression to limit immune rejection.[18]

## Skeletal Myoblasts

While skeletal myoblasts (SMs) are committed progenitors of skeletal muscle cells, their autologous origin, high proliferative potential, commitment to a myogenic lineage, and resistance to ischemia promoted their use as the first stem cell type to be explored extensively for cardiac application. Studies in rats and humans have demonstrated that these cells can repopulate scar tissue and improve left ventricular function following transplantation.[27] However, SM-derived cardiomyocytes do not function in complete concert with native myocardium. The expression of two key proteins involved in electromechanical cell integration, N-cadherin and connexin 43, are downregulated *in vivo,*[28] and the engrafted cells develop a contractile activity phenotype that appears to be unaffected by neighboring cardiomyocytes.[29]

To date, the safety and feasibility of transplanting SM cells have been explored in a series of small studies enrolling a collective total of nearly 100 patients. Most of these procedures were carried out during open-heart surgery, although a couple of studies have investigated direct myocardial injection and transcoronary administration. Sustained ventricular tachycardia, a life-threatening arrhythmia and unexpected side-effect, occurred in early implantation studies, possibly resulting from the lack of electrical coupling between SM-derived cardiomyocytes and native tissue.[30,31] Changes in pre-implantation protocols have minimized the occurrence of arrhythmias in conjunction with the use of SM cells, and Phase II studies of skeletal myoblast therapy are presently underway.

## Human Adult Bone-Marrow Derived Cells

In 2001, Jackson, *et.al.* demonstrated that cardiomyocytes and endothelial cells could be regenerated in a mouse heart attack model through the introduction of adult mouse bone marrow-derived stem cells.[9] That same year, Orlic and colleagues showed that direct injection of mouse bone marrow-derived cells into the damaged ventricular wall following an induced heart attack led to the formation of new cardiomyocytes, vascular endothelium, and smooth muscle cells.[11] Nine days after transplanting the stem cells, the newly-formed myocardium occupied nearly 70 percent of the damaged portion of the ventricle, and survival rates were greater in mice that received these cells than in those that did not. While several subsequent studies have questioned whether these cells actually differentiate into cardiomyocytes,[32,33] the evidence to support their ability to prevent remodeling has been demonstrated in many laboratories.[7]

Based on these findings, researchers have investigated the potential of human adult bone marrow as a source of stem cells for cardiac repair. Adult bone marrow contains several stem cell populations, including hematopoietic stem cells (which differentiate into all of the cellular components of blood), endothelial progenitor cells, and mesenchymal stem cells; successful application of these cells usually necessitates isolating a particular cell type on the basis of its' unique cell-surface receptors. In the past three years, the transplantation of bone marrow mononuclear cells (BMMNCs), a mixed population of blood and cells that includes stem and progenitor cells,

has been explored in more patients and clinical studies of cardiac repair than any other type of stem cell.7

The results from clinical studies of BMMNC transplantation have been promising but mixed. However, it should be noted that these studies have been conducted under a variety of conditions, thereby hampering direct comparison. The cells have been delivered via open-heart surgery and endomyocardial and intracoronary catheterization. Several studies, including the Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration (BOOST) and the Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) trials, have shown that intracoronary infusion of BMMNCs following a heart attack significantly improves the left ventricular (LV) ejection fraction, or the volume of blood pumped out of the left ventricle with each heartbeat.[34-36] However, other studies have indicated either no improvement in LV ejection fraction upon treatment[37] or an increased LV ejection fraction in the control group.[38] An early study that used endomyocardial injection to enhance targeted delivery indicated a significant improvement in overall LV function.[39] Discrepancies such as these may reflect differences in cell preparation protocols or baseline patient statistics. As larger trials are developed, these issues can be explored more systematically.

## Mesenchymal (Bone Marrow Stromal) Cells

Mesenchymal stem cells (MSCs) are precursors of non-hematopoietic tissues (e.g., muscle, bone, tendons, ligaments, adipose tissue, and fibroblasts) that are obtained relatively easily from autologous bone marrow. They remain multipotent following expansion *in vitro,* exhibit relatively low immunogenicity, and can be frozen easily. While these properties make the cells amenable to preparation and delivery protocols, scientists can also culture them under special conditions to differentiate them into cells that resemble cardiac myocytes. This property enables their application to cardiac regeneration. MSCs differentiate into endothelial cells when cultured with vascular endothelial growth factor[40] and cardiomyogenic (CMG) cells when treated with the DNA-demethylating agent, 5-azacytidine.41 More important, however, is the observation that MSCs can differentiate into cardiomyocytes and endothelial cells *in vivo* when transplanted to the heart following myocardial infarct (MI) or non-injury in pig, mouse, or rat models.[42-45] Additionally, the ability of MSCs to restore functionality may be enhanced by the simultaneous transplantation of other stem cell types.[43]

Several animal model studies have shown that treatment with MSCs significantly increases myocardial function and capillary formation.[5,41] One advantage of using these cells in human studies is their low immunogenicity; allogeneic MSCs injected into infarcted myocardium in a pig model regenerated myocardium and reduced infarct size without evidence of rejection.[46] A randomized clinical trial implanting MSCs after MI has demonstrated significant improvement in global and regional LV function,[47] and clinical trials are currently underway to investigate the application of allogeneic and autologous MSCs for acute MI and myocardial ischemia, respectively.

## Resident Cardiac Stem Cells

Recent evidence suggests that the heart contains a small population of endogenous stem cells that most likely facilitate minor repair and turnover-mediated cell replacement.[7] These cells have been isolated and characterized in mouse, rat, and human tissues.[48,49] The cells can be harvested in limited quantity from human endomyocardial biopsy specimens[50] and can be injected into the site of infarction to promote cardiomyocyte formation and improvements in systolic function.[49] Separation and expansion *ex vivo* over a period of weeks are necessary to obtain sufficient quantities of these cells for experimental purposes. However, their potential as a convenient resource for autologous stem cell therapy has led the National Heart, Lung, and Blood Institute to fund forthcoming clinical trials that will explore the use of cardiac stem cells for myocardial regeneration.

## Endothelial Progenitor Cells

The endothelium is a layer of specialized cells that lines the interior surface of all blood vessels (including the heart). This layer provides an interface between circulating blood and the vessel wall. Endothelial progenitor cells (EPCs) are bone marrow-derived stem cells that are recruited into the peripheral blood in response to tissue ischemia.[4] EPCs are precursor cells that express some cell-surface markers characteristic of mature endothelium and some of hematopoietic cells.[19,51-53] EPCs home in on ischemic areas, where they differentiate into new blood vessels; following a heart attack, intravenously injected EPCs home

to the damaged region within 48 hours.[12] The new vascularization induced by these cells prevents cardiomyocyte apoptosis (programmed cell death) and LV remodeling, thereby preserving ventricular function.[13] However, no change has been observed in non-infarcted regions upon EPC administration. Clinical trials are currently underway to assess EPC therapy for growing new blood vessels and regenerating myocardium.

### *Other Cells: Umbilical Cord Blood Stem Cells, Fibroblasts, and Peripheral Blood CD34⁺ Cells*

Several other cell populations, including umbilical cord blood (UCB) stem cells, fibroblasts (cells that synthesize the extracellular matrix of connective tissues), and peripheral blood $CD34^+$ cells, have potential therapeutic uses for regenerating cardiac tissue. Although these cell types have not been investigated in clinical trials of heart disease, preliminary studies in animal models indicate several potential applications in humans.

Umbilical cord blood contains enriched populations of hematopoietic stem cells and mesenchymal precursor cells relative to the quantities present in adult blood or bone marrow.[54,55] When injected intravenously into the tail vein in a mouse model of MI, human mononuclear UCB cells formed new blood vessels in the infarcted heart.[56] A human DNA assay was used to determine the migration pattern of the cells after injection; although they homed only to injured areas within the heart, they were also detected in the marrow, spleen, and liver. When injected directly into the infarcted area in a rat model of MI, human mononuclear UCB cells improved ventricular function.[57] Staining for CD34 and other markers found on the cell surface of hematopoietic stem cells indicated that some of the cells survived in the myocardium. Results similar to these have been observed following the injection of human unrestricted somatic stem cells from UCB into a pig MI model.[58]

Adult peripheral blood $CD34^+$ cells offer the advantage of being obtained relatively easily from autologous sources.[59] Although some studies using a mouse model of MI claim that these cells can transdifferentiate into cardiomyocytes, endothelial cells, and smooth muscle cells at the site of tissue injury,[60] this conclusion is highly contested. Recent studies that involve the direct injection of blood-borne or bone marrow-derived hematopoietic stem cells into the infarcted region of a mouse model of MI found no evidence of myocardial regeneration following injection of either cell type.[33] Instead, these hematopoietic stem cells followed traditional differentiation patterns into blood cells within the microenvironment of the injured heart. Whether these cells will ultimately find application in myocardial regeneration remains to be determined.

Autologous fibroblasts offer a different strategy to combat myocardial damage by replacing scar tissue with a more elastic, muscle-like tissue and inhibiting host matrix degradation.[4] The cells may be manipulated to express muscle-specific transcription factors that promote their differentiation into myotubes such as those derived from skeletal myoblasts.[61] One month after these cells were implanted into the post-infarction scar in a rat model of MI, they occupied a large portion of the scar but were not functionally integrated.[61] Although the effects on ventricular function were not evaluated in this study, authors noted that modified autologous fibroblasts may ultimately prove useful in elderly patients who have a limited population of autologous skeletal myoblasts or bone marrow stem cells.

## CONSIDERATIONS FOR USING THESE STEM CELLS IN THE CLINICAL SETTING

As these examples indicate, many types of stem cells have been applied to regenerate damaged myocardium. In select applications, stem cells have demonstrated sufficient promise to warrant further exploration in large-scale, controlled clinical trials. However, the current breadth of application of these cells has made it difficult to compare and contextualize the results generated by the various trials. Most studies published to date have enrolled fewer than 25 patients, and the studies vary in terms of cell types and preparations used, methods of delivery, patient populations, and trial outcomes. However, the mixed results that have been observed in these studies do not necessarily argue against using stem cells for cardiac repair. Rather, preliminary results illuminate the many gaps in understanding of the mechanisms by which these cells regenerate myocardial tissue and argue for improved characterization of cell preparations and delivery methods to support clinical applications.

Future clinical trials that use stem cells for myocardial repair must address two concerns that accompany the delivery of these cells: 1) safety and 2) tracking the cells to their ultimate destination(s). Although stem cells appear to be relatively safe in the majority of recipients to date, an increased frequency of non-

sustained ventricular tachycardia, an arrhythmia, has been reported in conjunction with the use of skeletal myoblasts.[30,62-64] While this proarrhythmic effect occurs relatively early after cell delivery and does not appear to be permanent, its presence highlights the need for careful safety monitoring when these cells are used. Additionally, animal models have demonstrated that stem cells rapidly diffuse from the heart to other organs (e.g., lungs, kidneys, liver, spleen) within a few hours of transplantation,[65,66] an effect observed regardless of whether the cells are injected locally into the myocardium. This migration may or may not cause side-effects in patients; however, it remains a concern related to the delivery of stem cells in humans. (*Note:* Techniques to label stem cells for tracking purposes and to assess their safety are discussed in more detail in other articles in this publication).

In addition to safety and tracking, several logistical issues must also be addressed before stem cells can be used routinely in the clinic. While cell tracking methodologies allow researchers to determine migration patterns, the stem cells must target their desired destination(s) and be retained there for a sufficient amount of time to achieve benefit. To facilitate targeting and enable clinical use, stem cells must be delivered easily and efficiently to their sites of application. Finally, the ease by which the cells can be obtained and the cost of cell preparation will also influence their transition to the clinic.

## CONCLUSIONS

The evidence to date suggests that stem cells hold promise as a therapy to regenerate damaged myocardium. Given the worldwide prevalence of cardiac dysfunction and the limited availability of tissue for cardiac transplantation, stem cells could ultimately fulfill a large-scale unmet clinical need and improve the quality of life for millions of people with CVD. However, the use of these cells in this setting is currently in its infancy — much remains to be learned about the mechanisms by which stem cells repair and regenerate myocardium, the optimal cell types and modes of their delivery, and the safety issues that will accompany their use. As the results of large-scale clinical trials become available, researchers will begin to identify ways to standardize and optimize the use of these cells, thereby providing clinicians with powerful tools to mend a broken heart.

## REFERENCES


1. American Heart Association. *Heart Disease and Stroke Statistics–2005.* Dallas: American Heart Association; 2005.
2. Mokdad AH, Ford ES, Bowman BA, et al. Prevalence of obesity, diabetes, and obesity-related health-risk factors, 2001. *JAMA.* 2003;289:76-79.
3. Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. *JAMA.* 2002;288:1723-1727.
4. Rosenstrauch D, Poglajen G, Zidar N, Gregoric ID. Stem cell therapy for ischemic heart failure. *Tex Heart Ist J.* 2005;32:339-347.
5. Mangi AA, Noiseux N, Kong D, et al. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med.* 2003;9:1195-1201.
6. Lietz K, Long JW, Kfoury AG, et al. Outcomes of left ventricular assist device implantation as destination therapy in the post-REMATCH era: implications for patient selection. *Circulation.* 2007;116:497-505.
7. Boyle AJ, Schulman SP, Hare JM. Is stem cell therapy ready for patients? Stem cell therapy for cardiac repair. *Circulation.* 2006;114:339-352.
8. Menasche P, Hagege AA, Scorsin M, et al. Myoblast transplantation for heart failure. *Lancet.* 2001;357:279-280.
9. Jackson KA, Majka SM, Wang H, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest.* 2001;107:1395-1402.
10. Condorelli G, Borello U, De Angelis L, et al. Cardiomyocytes induce endothelial cells to transdifferentiate into cardiac muscle: implications for myocardium regeneration. *Proc Natl Acad Sci USA.* 2001;98:10733-10738.
11. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. *Nature.* 2001; 410:701-705.
12. Kocher AA, Schuster MD, Szaboles MJ, et al. Neovascularization of ischemic myocardium by human bone-marrow derived angioblasts prevents cardiomyocyte apoptosis, reduces remodelling and improves cardiac function. *Nat Med.* 2001;7:430-436.
13. Schuster MD, Kocher AA, Seki T, et al. Myocardial neovascularization by bone marrow angioblasts results in cardiomyocyte regeneration. Am J Physiol Heart Circ Physiol. 2004;287:H525-H532.
14. Gnecchi M, He H, Liang OD, et al. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. *Nat Med.* 2005;11:367-368.
15. Fujii T, Yau TM, Weisel RD, et al. Cell transplantation to prevent heart failure: a comparison of cell types. *Ann Thorac Surg.* 2003;76:2062-2070.

16. Nygren JM, Jovinge S, Breitbach M, et al. Bone marrow-derived hematopoietic cells generate cardiomyocytes at low frequency through cell fusion, but not transdifferentiation. *Nat Med.* 2004;10:494-501.

17. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation.* 2002;106:1913-1918.

18. Oettgen P. Cardiac stem cell therapy: need for optimization of efficacy and safety monitoring. *Circulation.* 2006;114:353-358.

19. Vittet D, Prandidni MH, Berthier R, et al. Embryonic stem cells differentiate in vitro to endothelial cells through successive maturation steps. *Blood.* 1996;88:3424-3431.

20. Marchetti S, Gimond C, Iljin K, et al. Endothelial cells genetically selected from differentiating mouse embryonic stem cells incorporate at sites of neovascularization in vivo. *J Cell Sci.* 2002;115:2075-2085.

21. Yamashita J, Itoh H, Hirashima M, et al. Flk1-positive cells derived from embryonic stem cells serve as vascular progenitors. *Nature.* 2000;408:92-96.

22. Kehat I, Kenyagin-Karsenti D, Snir M, et al. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest.* 2001;108:407-414.

23. Kehat I, Gepstein A, Spira A, Itskovitz-Eldor J, Gepstein L. High-resolution electrophysiological assessment of human embryonic stem cell-derived cardiomyocytes: a novel in vitro model for the study of conduction. *Circ Res.* 2002;91:659-661.

24. Westfall MV, Pasyk KA, Yule DI, Samuelson LC, Metzger JM. Ultrastructure and cell-cell coupling of cardiac myocytes differentiating in embryonic stem cell cultures. *Cell Motil Cytoskel.* 1998;36:43-54.

25. Min JY, Yang Y, Converso KL, et al. Transplantation of embryonic stem cells improves cardiac function in postinfarcted rats. *J Appl Physiol.* 2002;92:288-296.

26. Huber I, Itzhaki I, Caspi O, et al. Identification and selection of cardiomyocytes during human embryonic stem cell differentiation. *FASEB J.* 2007;21:2551-2563.

27. Dowell JD, Rubart M, Pasumarthi KB, Soonpaa MH, Field LJ. Myocyte and myogenic stem cell transplantation in the heart. *Cardiovasc Res.* 2003;58:336-350.

28. Reinecke H, MacDonald GH, Hauschka SD, Murry CE. Electromechanical coupling between skeletal and cardiac muscle. Implications for infarct repair. *J Cell Biol.* 2000;149:731-740.

29. Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, Charpak S. Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *Proc Natl Acad Sci USA.* 2003;100:7808-7811.

30. Menasche P, Hagege AA, Vilquin J-T, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *J Am Coll Cardiol.* 2003;41:1078-1083.

31. Siminiak T, Kalawski R, Fiszer D, et al. Autologous skeletal myoblast transplantation for the treatment of post-infarction myocardial injury: phase I clinical study with 12 months of follow-up. *Am Heart J.* 2004;148:531-537.

32. Murry CE, Soonpaa MH, Reinecke H, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature.* 2004;428:664-668.

33. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature.* 2004;428:668-673.

34. Assmus B, Schachinger V, Teupe C, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation.* 2002;106:3009-3017.

35. Schachinger V, Assmus B, Britten MB, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. *J Am Coll Cardiol.* 2004;44:1690.

36. Wollert KC, Meyer GP, Lotz J, et al. Intra-coronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet.* 2004;364:141-148.

37. Janssens S, Dubois C, Bogaert J, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised, controlled trial. *Lancet.* 2005;367:113-121.

38. Cleland JG, Freemantle N, Coletta AP, Clark AL. Clinical trials update from the American Heart Association: REPAIR-AMI, ASTAMI, JELIS, MEGA, REVIVE-II, SURVIVE, and PROACTIVE. *Eur J Heart Fail.* 2006;8:105-110.

39. Perin EC, Dohmann HFR, Borojevic R, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation.* 2003;107:2294-2302.

40. MacKenzie TC, Flake AW. Human mesenchymal stem cells: insights from a surrogate in vivo assay system. *Cells Tissues Organs.* 2002;171:90-95.

41. Davani S, Marandin A, Mersin N, et al. Mesenchymal progenitor cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a rat cellular cardiomyoplasty model. *Circulation.* 2003;108(suppl II):II-253-II-258.

42. Makino S, Fukuda K, Miyoshi S, et al. Cardiomyocytes can be generated from marrow stromal cells in vitro. *J Clin Invest.* 1999;103:697-705.

43. Min J-Y, Sullivan MF, Yang Y, et al. Significant improvement of heart function by cotransplantation of human mesenchymal stem cells and fetal cardiomyocytes in postinfarcted pigs. *Ann Thorac Surg.* 2002;74:1568-1575.

44. Shake JG, Gruber PJ, Baumgartner WA, et al. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. *Ann Thorac Surg.* 2002;73:1919-1926.

45. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation.* 2002;105:93-98.

46. Amado LC, Saliaris AP, Schuleri KH, et al. Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. *Proc Natl Acad Sci USA.* 2005;102:11474-11479.

47. Chen S-I, Fang W-W, Ye F, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cells in patients with acute myocardial infarction. *Am J Cardiol.* 2004;94:92-95.

48. Beltrami AP, Barlucchi L, Torella D, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell.* 2003;114:763-776.

49. Messina E, De Angelis L, Frati G, et al. Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circ Res.* 2004;95:911-921.

50. Smith RR, Barile L, Cho HC, et al. Unique phenotype of cardiospheres derived from human endomyocardial biopsies. *Circulation.* 2005;112(suppl II):II-334.

51. Eichmann A, Corbel C, Nataf V, Vaigot P, Breant C, Le Dourain NM. Ligand-dependent development of the endothelial and hematopoietic lineages from embryonic mesodermal cells expressing vascular endothelial growth factor receptor 2. *Proc Natl Acad Sci USA.* 1997; 94:5141-5146.

52. Sato TN, Quin Y, Kozak CA, Audus KL. Tie-1 and Tie-2 define another class of putative receptor tyrosine kinase genes expressed in early embryonic vascular system. *Proc Natl Acad Sci USA.* 1993;90:9355-9358.

53. Suri C, Jones PF, Patan S, et al. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell.* 1996;87:1171-1180.

54. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol.* 2000;109:235-242.

55. Mayani H, Lansdorp PM. Biology of human umbilical cord blood-derived hematopoietic stem/progenitor cells. *Stem Cells.* 1998;16:153-165.

56. Ma N, Stamm C, Kaminski A, et al. Human cord blood cells induce angiogenesis following myocardial infarction in NOD/scid-mice. *Cardiovasc Res.* 2005;66:45-54.

57. Hirata Y, Sata M, Motomura N, et al. Human umbilical cord blood cells improve cardiac function after myocardial infarction. *Biochem Biophys Res Commun.* 2005; 327:609-614.

58. Kim B-O, Tian H, Prasongsukarn K, et al. Cell transplantation improves ventricular function after a myocardial infarction: a preclinical study of human unrestricted somatic stem cells in a porcine model. *Circulation.* 2005; 112 (suppl I):I-96-I-104.

59. Korbling M, Katz RL, Khanna A, et al. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N Engl J Med.* 2002;346:738-746.

60. Yeh ET, Zhang S, Wu HD, Korbling M, Willerson JT, Estrov Z. Transdifferentiation of human peripheral blood $CD34^{+}$-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells in vivo. *Circulation.* 2003;108:2070-2073.

61. Etzion S, Barbash IM, Feinberg MS, et al. Cellular cardiomyoplasty of cardiac fibroblasts by adenoviral delivery of MyoD ex vivo: an unlimited source of cells for myocardial repair. *Circulation.* 2002;106(12 Suppl 1):I125-I130.

62. Smits PC, van Genus RJ, Poldermans D, et al. Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure: clinical experience with six-month follow-up. *J Am Coll Cardiol.* 2003;42:2063-2069.

63. Dib N, McCarthy P, Campbell A, et al. Feasibility and safety of autologous myoblast transplantation in patients with ischemic cardiomyopathy. *Cell Transplant.* 2005;14:11-19.

64. Pagani FD, DerSimonian H, Zawadzka A, et al. Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans: histological analysis of cell survival and differentiation. *J Am Coll Cardiol.* 2003;41:879-888.

65. Dow J, Simkhovich BZ, Kedes L, Kloner RA. Washout of transplanted cells from the heart: a potential new hurdle for cell transplantation therapy. *Cardiovasc Res.* 2005;67:301-307.

66. Muller-Ehmsen J, Whittaker P, Kloner RA, et al. Survival and development of neonatal rat cardiomyocytes transplanted into adult myocardium. *J Mol Cell Cardiol.* 2002;34:107-116.