

Stem Cell Therapy for Cardiac Repair

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Heart regeneration via stem cell therapy could improve the functional outcome for millions of patients. A goal of cardiac stem cell research is to foster the engraftment of new, beating cardiac cells into the ischemic region of the heart after a myocardial infarction. The key elements of cell therapy for myocardial repair reviewed here are the source of cells and the mechanisms by which these cells improve cardiac function. Injection of stem cells into the heart of animals ignited the field by showing some functional cardiac improvement. Unfortunately, few injected cells are retained in the heart or become a new, beating myocardium, and clinical trials have shown moderate improvement of human heart function. The causes of the minimal functional improvement are still unknown, but blood vessel formation (angiogenesis) or secretion of growth factors or cytokines are likely candidates. Cells appropriate for human therapy might be mesenchymal stem and progenitor cells from bone marrow or the heart itself. A more controversial cell source, embryonic stem cells, have a nearly unlimited self-renewal potential and can differentiate into beating cardiac myocytes. However, all of these cell sources and the mechanisms of improvement need further research, with the differentiation of stem cells into functional cardiac cells a difficult but most beneficial hurdle to leap.

KEY WORDS: mesenchymal stem cells, regenerative medicine, resident cardiac stem cells, stem cell therapy

There are currently 5 million patients with heart failure in the United States¹ for whom recovery of cardiac function could prove beneficial. A goal of cardiac stem cell research is to foster the engraftment of new, beating cardiac myocytes into the ischemic region of the heart after a myocardial infarction. Differentiation of cells into cardiac myocytes that are both mechanically and electrically coupled to the healthy cardiac myocytes is one way to achieve this goal. However, recent clinical trials indicate that other mechanisms probably predominate, including stem cell differentiation into cells of vascular lineage, resulting in better blood supply to the ischemic region. Another biologically useful action is the release of cytokines and growth factors from the injected cells leading to reduced cell death or enhanced recovery of host ventricular muscle cells. Growth factors may also help stem cells migrate into the damaged

heart or activate a local population of stem cells residing within the heart.

The cell sources and mechanisms of cardiac improvement are 2 key elements of stem cell therapy reviewed here. Although the initial excitement surrounding cell-based therapy focused on stem cell differentiation into cardiac myocytes, clinical trials have shown that most of the injected cells are not retained in the heart.² Thus, injection of stem cells has not yet significantly improved heart function.

Sources of Cells

The optimal source of cells for regenerating damaged myocardium is a topic of intense research. Initial animal experiments using skeletal muscle precursor cells showed a general improvement of heart function. Specifically, skeletal myoblasts that were transplanted into the infarcted myocardium of rabbits remained viable and improved myocardial performance.³ However, later clinical trials showed that these cells were not electrically coupled to the native heart cells and, in some cases, caused ventricular arrhythmias. Indeed, one would not expect electrical coupling of these cells since they lack the ability to make gap junctions. More recently, stem cells isolated from bone marrow, embryonic stem cells, and resident cardiac stem cells have been used. High-quality reviews on stem cell therapies for the heart can be consulted for detailed information.^{4–6}

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Bone Marrow Cells—Mesenchymal Stem Cells and Endothelial Progenitor Cells

The most prevalent source of cells used in clinical studies has been from bone marrow. Bone marrow cells as well as subpopulations from within the bone marrow including multipotent mesenchymal stem cells and endothelial progenitor cells have been studied. These subpopulations are sorted according to their expression of membrane receptor proteins so-called *cell-surface markers*. Whole bone marrow cell populations can be incubated with antibodies directed against cell-surface markers and attached to either magnetic beads or fluorescent tags. Magnetic columns or flow cytometry is then used to isolate the cells of interest from the whole population. In this fashion, mesenchymal stem and endothelial progenitor cells can be isolated from the bone marrow. However, a principal problem is that the cell-surface markers that determine a true cardiac stem cell have not yet been defined. As a result, there are a vast number of possible combinations of the various markers and cell subpopulations to be studied. It is not surprising that the outcomes of therapy have been variable since the subpopulations yielded from laboratory to laboratory have not been standardized. Currently, stem cells from bone marrow are usually identified first by the absence of surface markers of more committed hematopoietic lineages (termed *lineage negative*). Then they are further sorted by surface markers for mesenchymal stem cells (including but not limited to c-kit and sca-1) and endothelial progenitor cells (including but not limited to c-kit, vascular endothelial growth factor receptor 2, AC133, CXCR4, and CD34).⁷ Mesenchymal stem cells have also been separated from whole bone marrow by their ability to adhere to plastic substrates, justifying another name given to them—plastic adherent cells.

A popular model for testing the efficacy of stem cell therapy is to induce a myocardial infarct in a rodent and determine whether there is improved function after treatment. Preliminary studies indicated that lineage negative bone marrow cells injected into the border zone of an infarct regenerate the myocardium in mice. Regeneration of heart tissue was observed in 40% of the treated mice, and new cells were found in 68% of the infarcted portion of the ventricle.⁸ This new heart tissue consisted of proliferating myofibroblasts and vascular structures as determined convincingly from tissue sections. The inability of other groups to obtain similar results has led to many questions about this early report.⁹ However, these initial studies have led to many of the clinical studies to date.

Both whole, unselected bone marrow cells and presorted mesenchymal stem cells from a patient's

own bone marrow (ie, autologous cells) have been used in human clinical trials. Mesenchymal stem cells from bone marrow retain their ability to differentiate into bone, cartilage, adipose tissue, and skeletal muscle.^{10,11} Autologous cells have improved cardiac performance in both animals and human beings with recent randomized human studies supporting the safety and feasibility of autologous bone marrow cell therapy. Two studies showed moderate but statistically significant improvement of left ventricular function at 3 and 4 months after intracoronary administration of these cells.^{12,13} These findings were not confirmed in a third study.¹⁴ The reasons for the different results are not known. It is clear that there are problems with intracoronary injection of cells because very few of the cells injected into the heart become cardiac myocytes. A major emphasis of current research is to define the subpopulation of those very rare cells in the bone marrow that may have the capacity to make contractile heart cells. To date, however, this has not been successful.

Endothelial progenitor cells are a second subpopulation within the bone marrow that can promote vasculogenesis leading to vessel regrowth, thereby improving oxygenation to tissue after damage.⁷ Preclinical trials indicate that endothelial progenitor cells contribute to 1% to 25% of vessel formation after ischemic injury for several diseases.⁷ In addition, endothelial progenitors may secrete growth factors and paracrine signals that prevent cardiac myocyte cell death.¹⁵ These cells are capable of vascular differentiation and secretion of growth factors promoting angiogenesis and increasing blood supply to the damaged heart. However, differentiation of stem cells into functional cardiac myocytes is a main goal of stem cell therapy, and the following cell types may be most promising to accomplish this.

Embryonic Stem Cells

Although ethical and political considerations have blunted research on human embryonic stem cells, they are an attractive source for cell-based therapy and have been used worldwide. Characterized as pluripotent, embryonic stem cells have nearly unlimited self-renewal potential and can differentiate into beating cardiac myocytes as demonstrated by electrical and mechanical coupling to the host myocardium (see the first article in this series, Curtis M, Russell B. Cardiac tissue engineering. *J Cardiovasc Nurs*. 2009;24(2): 87–92). However, one of their biggest limitations is that cancerous teratomas may form due to the ability of embryonic stem cells to divide and differentiate into almost any cell type. Thus, clinical trials with this cell source are lagging behind those for other kinds of stem cells. However, differentiation of embryonic

stem cells before implantation may both prevent oncogenesis and yield a pure cardiac myocyte population. Field and colleagues¹⁶ have used genetic selection to isolate cells that were 99.6% positive for heart muscle myosin. Embryonic stem cells were transfected with a fusion gene including the cardiac specific α -cardiac myosin heavy chain promoter, differentiated *in vitro*, and then selected. Implantation of these cells resulted in stable grafts in animals. Although there have yet to be clinical trials using embryonic stem cells, electromechanical coupling has been demonstrated between enriched cardiac cells derived from human embryonic stem cells that were transplanted into rat hearts, leading to increased myocardial wall thickness.¹⁷

Resident Cardiac Stem Cells

Contrary to the longstanding thought that the heart is a terminally differentiated organ incapable of self-regeneration or repair, recent data suggest that the heart contains stem cells.¹⁸ The idea that heart growth can only be achieved through hypertrophy was challenged with data indicating that niches within the heart contain cells that divide and can replace damaged cardiac myocytes. These resident cardiac stem cells express the cell-surface marker, c-kit, and possess fundamental properties of stem cells in that they are self-renewing and capable of differentiation into multiple cell types.¹⁹ This subset of cells has been shown to migrate to damaged regions of the heart and generate new cardiac myocytes in rats, hence improving cardiac function.²⁰

Mechanism of Functional Cardiac Improvement

Differentiation of Stem Cells

Currently, there is no consensus among investigators with regard to the capability of bone marrow cells to differentiate into functional cardiac myocytes. The results of some studies suggest that stem cells from bone marrow can differentiate into cardiac myocytes,⁸ whereas the results of others do not.⁹ Although there is no evidence from human clinical trials that bone marrow cells injected into the heart differentiate into cardiac myocytes, there is evidence in animals that this occurs occasionally.

In vitro coculture studies of stem cells with primary cells and *in vivo* injection of stem cells in animals have illustrated the ability of stem cells to differentiate into cardiac cells. Primary cardiac myocytes harvested from neonatal rats induce cardiac myogenic differentiation of endothelial progenitor cells^{21,22} and mesenchymal stem cells.^{23,24} In addition, mesenchymal stem cells injected into pig²⁵ or sheep²⁶ hearts days after myocardial infarction express muscle-specific proteins, result

in long-term engraftment, reduce scar formation, and improve cardiac function.²⁵ The engrafted mesenchymal stem cells express proteins found in cardiac myocytes and in vascular endothelium and smooth muscle.²⁶ Although expression of cardiac proteins in these cells is a good first indication of cardiac differentiation, there is little evidence that these cells differentiate into beating heart cells *in vivo*. It is likely that injection of stem cells into the mechanically active heart may not be the best approach for cardiac regeneration.

The activation of a population of stem cells that reside within the heart may be a more promising possibility for heart regeneration. Results of some studies demonstrate “proof of principle” that a population of resident cardiac stem cells found within the heart can differentiate toward the cardiac lineage. Current research is directed toward understanding how this occurs and how to increase the number of these highly desirable functional cells. Importantly, the number and percentage of dividing cardiac stem cells were shown to be greater in acutely infarcted hearts and hearts with end-stage cardiomyopathy as compared with normal cardiac tissue.²⁷ In addition, cardiac stem cells have an increased commitment to the cardiac myocyte, smooth muscle, and endothelial cell lineages in the infarcted and end-stage hearts versus normal hearts.²⁷ Resident cardiac stem cells could be the best cell source for heart muscle regeneration if the proportion that becomes beating heart cells could be enhanced.

None of the clinical studies mentioned above^{12–14} provide evidence that intracardiac injection of cells isolated from bone marrow differentiates into cardiac muscle cells. However, differentiation of stem cells into vascular cells is commonly observed and represents another mechanism by which heart function can be improved after myocardial infarction. It is likely that clinical studies showing functional improvements associated with the direct delivery of bone marrow cells to the heart are mainly due to the secretion of factors that increase angiogenesis.^{28,29} A recent article suggests that cardiac stem cells engrafted within host rat myocardium divided and differentiated into endothelial cells, smooth muscle cells, and few cardiac myocytes.³⁰ Most of the cells formed coronary arteries, arterioles, and capillaries leading to increased myocardial blood flow, thus improving function.³⁰ In this particular study, before injection, the cardiac stem cells were activated with insulin-like growth factor 1 and hepatocyte growth factor. These and several other factors are known to influence stem cell function in the heart.

Secreted Factors From Stem Cells

Once scientists can develop the right kind of premyocyte stem cells, they will still need to direct

Clinical Pearl

- Clinical trials designed to improve heart function by injecting stem cells are underway but only minor gains have been achieved thus far.
- The mechanism of these minor gains is most likely angiogenesis, not regeneration of new beating heart cells.
- Future research should focus on use of the optimal stem cell type and physical and chemical cues needed for their differentiation and maturation.

them to where they are needed for repair. This process of direction is called *homing* or the movement of cells toward the injured region where they adhere, engraft, and are not flushed away. The homing of stem cells from tissues such as bone marrow to the heart in response to various factors has been explored. Earlier animal studies showed that direct injection of 2 signaling proteins, stem cell factor and granulocyte-colony stimulating factor, facilitated the homing of bone marrow cells to the infarct border zone.³¹ Mortality was reduced, with an improvement seen in infarct size, ejection fraction, and hemodynamics. In another study, mice treated with granulocyte-colony stimulating factor had a higher number and better migration of resident cardiac stem cells into the infarct zone.³² Finally, vascular endothelial growth factor increased angiogenesis and homing of endothelial progenitors from the bone marrow.³³ Thus, patients with end-stage coronary artery disease were able to walk longer before reporting angina, a modest trend toward improved left ventricular function. These results suggest that it is very likely that the regenerative capability of bone marrow and cardiac stem cells requires a combination of growth factors.

Conclusion

Of the many challenges for successful cardiac regeneration with stem cell therapy, the cell source is the most important. The primitive stem characteristics and differentiation potential of embryonic stem cells make them the ideal candidate for treatment of heart disease. However, their clinical use is currently limited due to the potential to form cancer cells and the debate about ethical considerations. At this time, bone marrow cells have been most used in clinical trials and have shown potential to improve heart function. However, the exact mechanisms responsible for improved function are unclear. Reasons for different outcomes among studies may include the use of various subpopulations of mesenchymal stem and endothelial progenitor cells. For example, one subpopulation might release growth factors benefi-

cial to the undamaged cells of the heart, whereas another, albeit in low frequency, might differentiate toward the cardiac myocyte lineage. It is clear that soluble cues from the microenvironment of the heart play a key role in the differentiation and control of stem cell function. Physical cues imposed by the mechanically dynamic heart likely play a large role, and tissue engineering approaches (see the third article in this series, Biehl J, Russell B. Introduction to stem cell therapy. *J Cardiovasc Nurs.* 2009;24(2); 98–103) may hold the key to better differentiation of stem cells into cardiac myocytes, homing of stem cells to the damaged cardiac tissue and controlled division of the cells.

To date, the formation of vasculature has been proven a beneficial outcome of stem cell therapy. However, a main goal of cardiac differentiation of stem cells in vivo has not yet been realized. The body readily builds new vessels and uses its own adult progenitor stem cells to accomplish this; however, researchers have been unsuccessful in engrafting injected stem cells in the heart and in producing enough new contractile myocytes to improve heart function significantly. There is a high therapeutic potential for resident cardiac stem cells, and increasing the numbers of these cells may be the most practical way to enhance cardiac regeneration in people with heart disease.

Stem cell therapy is a very exciting field with great potential for heart regeneration. Early clinical trials have shown moderate improvement in heart function after treatment, and research focused on potentiating these improvements is important for future success of stem cell therapy. Although the field has progressed rapidly to clinical trials using various populations of stem cells, much more research is needed to characterize the potential of the various cell populations used for stem cell therapy and to understand the basic science regulating the differentiation, proliferation, and integration with the host heart.

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