

# Stem Cells for Regeneration of Cardiac Tissues: Current Findings and Future Applications

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As the world population ages, the drive to find new treatments for accompanying health issues increases. The number one killer of people in the United States is heart disease. Heart disease manifests itself in many different clinical complaints. Many people suffer from congestive heart disease, coronary artery disease, and other idiopathic cardiomyopathies. There are many treatments for these conditions, often requiring surgery. These medical interventions are quite effective, often restoring normal heart function. The progression of heart disease has an accompanying progression of medical therapies. The last resort for medical intervention often is organ transplantation. The first successful transplant was performed at Stanford University in 1967. Since then, there have been many advances in the field of transplantation, whereby increasing the success rate. Transplantation is not an effective therapy for all patients. The waiting period for a donor heart ranges, on average, from 48 to 375 days depending on the health of the organ recipient (<http://www.OPTN.org/data>). Even with the many available medical and surgical treatments, after a myocardial infarction the prognosis for recovery is poor. Stem cell therapy is attempting to address this short coming as a novel means to regenerate viable myocardium.

Transplanting of stem cells directly into the myocardium or inducing migration of peripheral stem cells, such as hematopoietic stem cells (HSC), can serve as a therapeutic adjunct to cardiac disease (1–5). Embryonic stem cells (ESCs) have the potential to differentiate into all tissues types; however, ethical and governmental controversy has limited their clinical use. This review will present the basic concepts of stem cells and describe the stem cell research that is being reported related to cardiac tissue regeneration.

## OVERVIEW OF ESCs

The fertilized egg is said to be totipotent, meaning it has the potential to differentiate into any cell of the body. Approximately 4 days after fertilization, the totipotent cells specialize to form the blastocyst. Inside an early embryo are stem cells that have the ability to divide or self replicate for indefinite periods in culture and give rise to all of the somatic cells the body. The specialization of these cells is the result of the path they take as they differentiate from one of the three germ layers of the blastocyst. ESCs used in research are taken from day 5 blastocysts and grown up in cell culture. To derive these ESC cultures, most of the cells belonging to the trophectoderm are removed, and only the inner cell mass, about 30–34 cells, are used (6). The inner cell mass consists of pluripotent cells. Pluripotent stem cells give rise to multipotent stem cells, also known as adult stem cells (ASCs). These cells are able to differentiate into a limited number of cell lines, fewer than their totipotent precursor embryonic stem cells.

## ASCs

ASCs have two characteristics; first they have the ability to self-replicate over the lifetime of the organism. Also, they give rise to mature cells with specialized morphology and function. Even though rare in numbers, the primary function of ASC is to maintain specific cellular populations (6). Several tissue types have been found to contain adult stem cells, including bone marrow, peripheral blood, brain, spinal cord, dental pulp, blood vessels, skeletal muscle, epithelia of the skin and digestive system, cornea, retina, liver, and pancreas (6). ASCs have the ability to transdifferentiate from one tissue type to another. This is termed plasticity and can be seen when hematopoietic stem cells differentiate into neurons (3,7). Previously it was thought that ASC lineages were committed to become a certain class of progeny, but recently it has been dem-

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onstrated that, through relocation of ASC *in vivo*, reprogramming occurs (8). In the future, it will be important to determine what these new niches provide for the relocated ASC to induce transdifferentiation. We and others are determining whether the tissue-specific homing signals are secreted by damaged tissue to enhance ASC recruitment and induce differentiation. This concept has been supported by various other experiments, suggesting that injury to a target organ is sensed by distant ASC, which then migrate to the site of damage where they undergo tissue specific differentiation (3,9,10).

### **HEMATOPOIETIC STEM CELLS (HSCs)**

Of all the adult stem cells, HSC have been the most studied and characterized. HSCs are currently the only stem cell used for bone marrow reconstitution therapy. For HSCs to support replacement of injured cells at a distant tissue location, they first must be stimulated to migrate to the site of injury and then differentiate to replace the injured tissue. It is known that HSCs can renew themselves and differentiate into a variety of specialized cells of at least 8 to 10 distinct lineages of mature cells (11), mobilize into circulating blood, and undergo apoptosis (6). For HSCs to translocate to injured tissues chemokine-directed stimulation is necessary. Many potential mechanisms of HSC differentiation have been proposed. HSC may be stimulated extrinsically through growth factors and stromal interactions or stimulated intrinsically to support differentiation. More recently both intrinsic and extrinsic factors have been shown to play a role in HSC differentiation, where the extrinsic signals support survival and development of committed cells and intrinsic mechanisms account for the actual lineage commitment (11). Through the application of chemokines to facilitate migration and the subsequent addition of growth factors to stimulate differentiation, it is possible for HSCs to be used to repair diseased organs such as cardiac tissues.

### **THE ROLE OF STEM CELLS TO REPAIR DISEASED HEART TISSUE**

The formation of a noncontractile scar within cardiac tissue after a myocardial infarction (MI) offers much support to the notion that cardiac myocytes are not capable of regenerative cellular divisions. This basic and long-lived concept is being challenged by new developments in stem cell research. If nondifferentiated cells can be directed to migrate to the damaged area of the heart, where they would undergo terminal differentiation, the impact of the MI could be lessened. Without the intervention of auxil-

iary cells, the heart has limited options for recovery after ischemic insult. Intrinsic attempts to regain normal heart function after damage manifests itself in hypertrophy of the remaining myocytes. Often these, now abnormally large cells, are unable to maintain sufficient cardiac output (12). It is hypothesized that these negative responses can be truncated through the use of stem cell therapy. The stem cells have been shown to assist in contractile heart function in addition to playing a role in the initiation of angiogenesis (13).

There are a variety of options for the origin of the stem cells used in the treatment of numerous disorders. Possibly the most widely publicized is the use of embryonic stem cells. The ESC has immense therapeutic potential because its completely undifferentiated nature. An ESC line, through the use of a specific growth factor milieu, can be driven to develop into numerous types of cell lines. Brustle et al. were able to propagate a sample of mouse ESC that was manipulated to become neuronal cells (14). This commitment to one cell lineage is dependent upon the microenvironment and growth factors that initiate the differentiation to the neuronal cell type. These ESCs become either oligodendrocytes or astrocytes (14). This ability to change cell type after the initial commitment further expands the possibility for stem cell modulation through the manipulation of the microenvironment in which the ESC are grown.

ESCs show great promise in the treatment of the damaged myocardium after MI. Through the use of mouse models, it has been shown that ESCs can differentiate into cardiomyocytes both *in vitro* and *in vivo*, forming intracardiac grafts (15). Because of these encouraging results, similar studies have been performed using human ESCs. *In vitro*, these cells have been shown to differentiate into human cardiomyocytes (16). Even though the results from the murine models were the driving factor to begin research on human ESC, there are notable differences in the way these two cell types behave in the differentiation process. Human ESCs do not need a feeder layer to go through the differentiation process if the cells are in the presence of leukemic inhibitory factor. In addition, fewer human ESCs than mouse ESCs can be stimulated to differentiate and begin spontaneous contraction (17).

Despite the myriad of techniques for delivering the stem cells to the heart near the site of injury, the method most frequently used is intramyocardial injection. The benefit of this method is that the stem cells do not have to migrate from a patch or graft to the site of injury, a process that requires a complex mixture of intercellular signals. Also, because ESCs lack many of the cell surface proteins that may cause an immune response, they are less likely to be viewed as allogenic (18). Intramyocardial injection comes with its own set of risks. It is possible for the injected cells to go directly into the left ventricle, where they may be

distributed throughout the systemic circulation, thus inhibiting the implantation in the left ventricular wall. When compared with a sham injection of cell free media, the animals that received an injection containing ESC had significantly improved cardiac function 6 weeks after cell transplantation (2). Another indication of the success of the implant of the ESC is the presence of gap junctions between the transplanted cells and host myocardium (2). These successes make use of ESC in the treatment for loss of cardiac function as a result of an MI a very likely alternative to current treatments.

In an attempt to circumvent the issues that surround the use of ESC, other stem cell populations are being investigated for possible efficacy in the regeneration of cardiomyocytes. Skeletal myoblasts have shown some promise in this field. For these techniques the skeletal muscle stem cells must be harvested and grown up in culture before injection into the heart to get enough cells to make a measurable change in cardiac function (19). Although the evidence for skeletal stem cells does not support the idea that the implanted cells become cardiomyocytes, it does suggest that these myoblasts change phenotype to support the cardiac workload after implantation (20). Although this is a step in aiding the remodeling process of the ischemically damaged cardiomyocytes, it does not represent an end point for the use of skeletal stem cells. In the future, it may be possible to transdifferentiate these stem cells into fully differentiated cardiac tissue, not simply take on the role of cardiomyocytes.

The use of bone marrow-derived HSCs is another option for a source of stem cells that is not shrouded in as much controversy as ESC. HSCs are injected in the same manner as ESC but are also applied to the site of the injured tissue using a patch that holds the HSC seeded in it. Directly attaching the patch to the damaged area minimizes the number of cells that will be lost in the systemic circulation. It has been shown that bone marrow-derived HSCs can differentiate into cardiac muscle (3,21). Other studies have shown that these stem cells not only migrate to the damaged area but that the stem cells also take part in the regeneration of damaged fibers (9). One of the most promising aspects of using HSC is that they have been shown to take part in angiogenesis, that is, the HSC migrate into the heart, where they form into new blood vessels (22). They can also change the growth of pre-existing collaterals (13). The revascularization process is of the utmost importance to the viability of the newly formed cardiomyocytes. Without adequate blood supply, these newly formed cells would suffer the same fate as the cells that were damaged by the MI, having no delivery blood to bring the necessary nutrients or remove waste products. By creating novel vessels, the stem cells are ensuring that once completely incorporated into the cardiac tissue, sufficient blood supply will be available.

The realm of treatment of damaged tissue resulting from a MI is changing dramatically. The use of stem cells, regardless of their origin, is a frontier that promises to continue to push the scope of scientific knowledge and its clinical application. The concept of inducing myocardial regeneration after MI to limit tissue damage is fairly new and only recently have these ideas begun to come to fruition. Research in the area of stem cells is gaining momentum at a startling rate partially as a result of the fact that stem cell therapy has been touted as the wonder drug of the 21st century. Although the advances in this field seem innumerable, many questions remain unanswered. It has been observed that stem cells migrate to the site of injury, but the inducers of this migration need to be defined (23). Our laboratory is actively investigating the role of specific cytokines, chemokines, and growth factors that could be used in combination with stem cells to facilitate significant engraftment and differentiation in the cardiac tissue. Clearly, stem cell therapy is well established in bone marrow transplantation, and it is apparent that in the near future this technique will be successfully applied to other organ systems.

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