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## Stem Cell Science:

Overviews of Selected Disease Areas

## Heart Disease

## HARVARD STEM CELL INSTITUTE

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**The Harvard Stem Cell Institute (HSCI)** is a scientific collaborative established in 2004 to fulfill the promise of stem cell biology as the basis for the cure and treatment of a wide range of chronic diseases and medical conditions. HSCI's unique effort unites experts across the disciplines, schools and departments of Harvard University and all its affiliated research hospitals.

HSCI also sponsors public education programs concerning the scientific, legal and ethical implications of stem cell research, conducts a summer research program for college students, and helps educate high school teachers about stem cell science. HSCI depends upon the vision and generosity of private individuals, foundation and corporate donors to carry on its work, due to current U.S. restrictions on federal funding of embryonic stem cell research.

## Introduction

The following scientific overview focuses on the use of stem cells – both in research and potential therapeutic applications – to address one of the most challenging, life-changing diseases and conditions of our time. This overview along with its companion pieces has two educational objectives: to make clear the opportunities and promise inherent in the basic science and to provide you with a picture of the research avenues that must be pursued to reach clinical applications. The overviews point to the areas where fundamental questions exist, where therapies need improvement, and where funding for research is urgently required.

Immense hope and scientific effort at institutions like the Harvard Stem Cell Institute have been invested in stem cells. Researchers seek the fullest understanding of how cells' fates are determined. They want to know how embryonic stem (ESC) cells recognize and respond to the signals that move them to become the full range of mature cells in an animal. They want to know how adult stem cells, whose fates are more restricted, contribute to the repair and regeneration of organs and tissues. Studying both types of cells in parallel will provide us information pertinent to developing stem cell therapies. With each experiment and clinical trial, researchers and clinicians move closer to these goals. This set of papers summarizes the current state of stem cell science in several key disease areas.

Stem cell research adds its own unique challenges to those faced by any early stage science. In fact, there are many cells along with their behavior that are still being discovered. In some cases the benefit, whether detailed fundamental knowledge, development of new drugs, or transplantable cells, is likely to be far in the future; in other cases, progress will come amazingly quickly. In stem cell research, as in few other contemporary scientific enterprises, success depends on supportive collaboration among diverse constituents: scientists and clinicians; local, state, and federal governments; universities and industry; and patients and their advocates. As a cross-institutional collaborative research and educational organization, HSCI is committed to meeting this challenge. We hope these disease overviews will help you better understand the research areas that matter to you.

I would welcome your thoughts about these overviews, your questions, and concerns. Contact me at [brock\\_reeve@harvard.edu](mailto:brock_reeve@harvard.edu).

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# Heart Disease

## Introduction

**H**ear disease has been the leading cause of death in the United States since 1900 with the exception of the flu pandemic in 1918. According to the American Heart Association, nearly 80 million (one in three) adult Americans are affected by cardiovascular disease today. Heart disease together with stroke will cost an estimated \$448.5 billion in direct health care and related costs (2).

As long ago as the ancient Egyptians, the heart has been viewed as a sacred entity, encompassing the soul and wisdom of an individual, acting as a focal point and linking all parts of the body. The injury and suffering brought on by World War II spurred rapid advances in the discovery and improvement of certain techniques such as antibiotics, anesthesia, and blood transfusions, which allowed physicians surgical access to the heart. Until 1952, heart surgery was performed without visualization and with very low rates of success. With the discovery that cooling the body could slow organ function, as well as with the invention of the heart-lung machine, open-heart surgery became feasible. Thus, surgeons could bypass the heart and maintain the circulation of blood and oxygen to the body. The first successful heart transplant was done in 1967 by Dr. Christiaan Barnard, though for a number of years the majority of heart transplant patients succumbed to organ rejection or infection. Later, Dr. Norman Shumway investigated the difficult problems of immune rejection and infection. Shumway is known for his pioneering use of cyclosporin, a drug that suppresses organ rejection without completely neutralizing the recipient's immune response.

## The Problem of Heart Failure

Heart failure is characterized by reduced cardiac output as a function of impaired heart pumping ability that is unable to support the body. Risk factors associated with heart disease may be uncontrollable (family history, diabetes, age and post-menopausal changes) or controllable (smoking, excess body weight, hypertension, high cholesterol, stress and physical inactivity). Efforts aimed at the treatment of heart disease include prevention, minimizing invasive techniques and looking toward cellular repair and replacement strategies. The latter two are very promising due to the potential of stem cells.

According to the Mayo Clinic the current average one-year survival rate for heart transplant patients is nearly 90 percent; with a five-year average survival rate of 72 percent. However, as with most organ replacement therapies, there is a severe shortage of donors. Additionally, people afflicted with cardiovascular disease often do not need a whole new heart, but rather just need portions of their diseased heart repaired. Unfortunately, heart muscle cells do not regenerate sufficiently to completely repair damage, leading to a cumulative loss of these cells throughout our lives. The little “regeneration” that does occur is the remodeling and shifting of existing healthy cells to compensate for damaged ones. The healthy cells physically swell and work harder, potentially leading to other types of heart damage. In addition, scarring and remodeling causes surrounding healthy cells to become electrically asynchronous, making it harder to promote a regular heartbeat.

Methods for repairing injured heart muscle and valves aim to alleviate, improve, or prevent certain physiological states. Available drugs can lower cholesterol and blood pressure, prevent clotting, provide relief from chest pain, and improve or strengthen the heart’s ability to contract. Current medical procedures aim to circumvent the damaged area, open up a blocked vessel, and replace defective valves or the whole heart itself. Today’s therapies, however, are aimed at mitigating the progression of heart disease and injury to heart muscle cells that is cumulative and irreversible.

## Stem Cell Research and Heart Disease

Is there a way to replace damaged heart muscle cells with healthy functioning ones? Researchers are enthusiastically pursuing cardiac stem cells as part of the answer to this question. They are working to identify the adult cardiac cell type that holds the two basic properties of a stem cell: self-renewal (the ability to make more of itself) and differentiation (the ability to give rise to all types of cardiac cells).

The heart consists of three major types of cells; cardiomyocytes (heart muscle cells; see Figure 1), smooth muscle cells, and endothelial cells. In 2007, both Ken Chien of the Harvard Stem Cell Institute and Massachusetts General Hospital and Stuart Orkin's group of the Harvard Stem Cell Institute and the Dana Farber Cancer Institute published findings further defining the cardiac stem cell. These research groups independently identified multipotent progenitors to the distinct cardiac cell types. These studies are significant because they help to define the lineage of cardiac cells. This information has the important capacity to inform directed differentiation protocols in which embryonic stem cells could be turned into a variety of cardiac cell types. These cells could then represent a source for tissue engineering approaches to cardiac repair, such as creating grafts of healthy cells in the laboratory that could be transplanted into a patient at an injury site. Also, knowing which genes are important for cardiac cell differentiation could help in the development of approaches to stimulate a patient's endogenous cardiac stem cell population to make more cardiac cells. This approach would circumvent the need for risky transplants using foreign tissue. Finally, exploiting new advances in nuclear reprogramming in which fibroblast cells can be reprogrammed to be pluripotent stem cells could represent a useful cell source.

Another critical use of stem cells is as a platform for early-stage drug toxicity testing. Due to cardiotoxicity issues, many new drugs are either pulled from the market or their clinical trials are halted. This has resulted in injury to patients and millions of dollars lost by drug companies running these trials. If protocols were in place to reliably produce cardiac cells from stem cells, this would create a theoretically limitless supply of cells that could be used in large-scale screens to test drugs for cardiotoxicity. This enables scientists to identify potentially dangerous drugs at a very early stage of their development by causing adverse effects on cells in the laboratory. Screening large numbers of cells in the laboratory, instead of in a patient, is a cost-effective strategy and a potentially life-saving one.

## Challenges in Developing Cardiac Therapies from Stem Cells

Cell therapy approaches for heart disease must take into account many processes that are prerequisites for successful cardiac regeneration. For example, cells must travel to the area of damage and integrate into the correct location within the heart. These new cells must also couple electronically with themselves as well as surrounding cells and ensure that scarring does not occur. Additionally, a source of cells is needed to generate a network of blood vessels for the newly transplanted cardiomyocytes. Additional cells, other than cardiac progenitor cells in the heart, may enhance cardiac repair and regeneration (see Table 1). Researchers are attempting to identify those progenitor cells that will travel from the bloodstream to the heart and mature into the correct heart cell types needed to repair the damaged area. Additional cell types are being explored for their repair potential such as endothelial precursor cells. Endothelial cells are of particular interest to researchers because of their role in promoting the growth of new blood vessels.

Numerous clinical trials attempting to repair heart damage using stem cells have demonstrated improvements in cardiac output, or the ejection fraction, which is the volume of blood pumped out of the left ventricle. This result, though valuable, is not sufficient to demonstrate the efficacy and safety of a trial. Clinical trials need to be *double blind* (meaning that both the researchers and subjects do not know who belongs to the experimental group and who belongs to the control group), *randomized with a control* (meaning that the treatment is assigned to subjects at random with a placebo treatment included), result in *outcome data of clinical events* (in particular, any adverse effects), and patients need to be tracked over a *long-term time frame*.

Given the great need for effective cardiac therapies, researchers are testing different types of cells for their potential utility. This may be premature for at least a couple of reasons. One, our understanding of adult progenitor cells remains limited. Two, most clinical trials focus on introducing non-cardiac stem or progenitor cells into the heart or blood stream and are based on the hypothesis that these introduced adult stem cells have the ability to transdifferentiate — turn into another type of adult cell, in this case a cardiac muscle cell.

**Table 1****Candidate Stem Cells for Therapy** (adapted from Guan and Hasenfuss, 2007)**Types of stem cells suggested for cardiac cellular therapies***Embryonic stem cells*

- Embryonic stem cells (ESC)
- Embryonic germ cells

*Adult stem cells*

- Cardiac origin
- Hematopoietic stem cells (HSC): blood stem cells
- Mesenchymal stem cells (MSC): able to differentiate into bone, cartilage, skeletal muscle, and fat
- Endothelial precursor cells (EPC): blood vessel formation
- Multipotent adult progenitor cells (MAPC): a rare type of stem cell that has yet to be reproduced or found *in vivo*
- Side population cells (SPC): cell type isolated from various types of tissues that has ability for engraftment and long-term reconstitution
- Spermatogonial stem cells
- Skeletal myoblast (muscle) cells
- Skeletal-based precursors of cardiomyocytes
- Cord blood stem cells

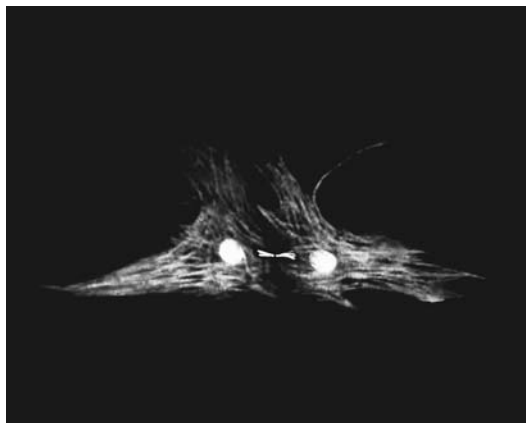
Transdifferentiation is the process by which one cell type turns into another cell type, for example, a blood cell into a heart muscle cell. Although several groups report transdifferentiation of blood cells, skeletal muscle cells, and a few other types of cells into cardiomyocytes, there is to date a lack of robust evidence for any type of adult stem cell transdifferentiating into cardiomyocytes. Criteria for successful transdifferentiation include: (1) multiple cell types must be derived from a *single stem cell*; (2) researchers must be able to generate *functional* mature cells of the different types of cells; (3) transdifferentiation must occur in the *absence* of cell fusion (see below); and (4) researchers must demonstrate the *robust contribution* to all derived cell types; which is essential especially for therapeutic application.

A recent review also pointed out the problems in correctly identifying cardiomyocytes. The authors identify potential microscopic artifacts that may account for misleading results in their own studies that may translate to many other studies. The most common method for identifying new cardiomyocytes is immunohistochemistry (IHC): tagging cardiomyocytes with a fluorescent flag, exciting the flag with a laser and counting the number of flags with the presumption that it is equal to the number of cardiomyocytes. A particular type of white blood cell, associated with heart attacks, can be easily mistaken for a new cardiomyocyte if not examined properly. Additionally, there is a significant amount of autofluorescence, a natural, and unfortunately common, complication with IHC. The authors demonstrate that an area previously damaged by a heart attack in a mouse may glow fluorescent green, suggesting that the mouse has generated new cardiomyocytes even though the mouse never even received the green flag (see Figure 1).

Many reports of cardiomyocyte regeneration may also be inflated due to cell fusion events. A cell fusion event may appear indistinguishable from that of transdifferentiation, since the merging of an introduced stem cell with an existing cardiomyocyte may result in the stem cell acquiring the characteristics of the existing cardiomyocyte. In addition, there is a lack of scientific evidence that introduced adult stem cells remain in the heart after transplantation, much less integrate and function properly. Clinical studies have indicated that less than 3 percent of infused bone marrow cells actually remain in the heart. In addition some of the positive clinical benefits measured may in fact be attributed to factors secreted by the bone marrow cells, which promote repair by stimulating endogenous cell populations.

In short, cardiac repair represents some unusual challenges for researchers. The range of approaches being explored, coupled with researcher's ever-expanding toolbox, is cause for cautious optimism as we move closer toward feasible approaches to cardiac repair.

**FIGURE 1.**  
Neonatal rat cardiomyocytes as seen through a microscope, showing a cell that has divided into two. Researchers hope someday to use stem cells as a source for cardiomyocytes to repair damaged hearts. Figure reprinted with permission from CSHL Press. Engel, F.B. et al. *Genes Dev.* 2005; 19: 1175-1187.



REFERENCES:

American Heart Association. "Cardiovascular disease kills one in three; burden of disease varies across the country," <http://www.americanheart.org/presenter.jhtml?identifier=4475>

Guan K, Hasenfuss G. "Do stem cells in the heart truly differentiate into cardiomyocytes?" *J Mol Cell Cardiol.* 2007, 43, 377-87.

Mayo Foundation for Medical Education and Research, "Heart transplant: A treatment for end-stage heart failure," <http://www.revolutionhealth.com/conditions/heart/heart-failure/surgery/heart-transplant/end-stage-failure>.

Moretti, A, Caron, L, Nakano, A., Lam, J.T., Bernshausen, A., Chen, Y., Qyang, Y., Bu, L., Sasaki, M., Martin-Puig, S., Sun, Y., Evans, S., Laugwitz, K.-L., and Chien, K.R. Multipotent Embryonic Isl1+ Progenitor Cells Lead to Cardiac, Smooth Muscle, and Endothelial Cell Diversification." *Cell* 2006 127, 1151-1165.

Rosenzweig A. "Cardiac cell therapy—mixed results from mixed cells." *N Engl J Med.* 2006, 355, 1274-7.

Wu, S.M., Fujiwara, Y., Cibulsky, S.M., Clapham, D.E., Lien, C., Schultheiss, T.M., and Orkin, S.H. "Developmental Origin of a Bipotential Myocardial and Smooth Muscle Cell Precursor in the Mammalian Heart." *Cell* 2006, 127, 1137-1150.

Zhang S., Wang D., Estrov Z., Raj S., Willerson, J.T., Yeh, E.T. "Both cell fusion and transdifferentiation account for the transformation of human peripheral blood CD34-positive cells into cardiomyocytes in vivo." *Circulation* 2006, 25, 3803-7.

Reinecke H, Minami E, Poppa V, Murry CE. (2004). "Evidence for fusion between cardiac and skeletal muscle cells." *Circ Res.* 2004, 2, 56-60.

Laflamme, M., and Murray, C.E. "Regenerating the heart." *Nature Biotechnology* 2005, 23, 845-856.

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