

# "Stem Cell Research in the Cardiac Field: Where Are We Now?"

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**C**ardiovascular diseases are the leading cause of morbidity and mortality in developed countries. In 2006, heart disease accounted for 34.2% of all deaths or 1 in every 2.9 deaths in the United States (US). The human heart does not have an abundance of cells that naturally repair or replace damaged heart muscle. Accordingly, the human body cannot, without medical assistance, repopulate regions of scar tissue within the heart with functioning muscle. Stem cell therapy for the heart or cellular cardiomyoplasty is fast emerging as a new therapeutic option for these patients.

## Introduction

Myocardial infarction (MI), commonly known as a heart attack, occurs when a blockage in a coronary artery severely restricts or completely stops blood flow to a portion of the heart. When blood supply is greatly reduced or blocked for more than a short period of time, heart muscle cells die. If the healthy heart muscle cells do not replace the dead cells within approximately two months, the injured area of the heart becomes unable to function properly.

Congestive heart failure, or CHF, is a debilitating condition that occurs as the heart becomes progressively less able to pump an adequate supply of blood throughout the body resulting in fluid accumulation in the lungs, kidneys and other body tissues. Persons suffering from NYHA Class II or worse heart failure experience high rates of mortality, frequent hospitalization and poor quality of life. CHF has many causes, generally beginning in patients with a life-long history of high blood pressure or after a patient has suffered a major heart attack or some other heart-damaging event. CHF itself may lead to other complicating factors such as pulmonary hypertension, edema, pulmonary edema, liver dysfunction and kidney failure. Although medical therapy for CHF is improving, it remains a major debilitating condition. According to the American Heart Association Heart Disease Statistics – 2007 Update, the estimated, total direct and indirect costs of heart failure in the US in 2006 were approximately \$33.2 billion.

Heart disease has been the leading cause of death from 1950 through 2003 within the United States according to the US Department of Health and Human Services. In addition, heart failure is the single most frequent reason for hospitalization in the elderly according to a 2007 study entitled "Long-Term Costs and Resource Use in Elderly Participants with Congestive Heart Failure" (Liao, L.,

et al). The American College of Cardiology/ American Heart Association 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult, or the ACC/AHA Guidelines, provides recommendations for the treatment of chronic heart failure in adults with normal or low LVEF. The treatment escalates and becomes more invasive as the heart failure worsens. Current treatment options for severe, chronic heart damage include, but are not limited to, heart transplantation and other surgical procedures, bi-ventricular pacers, drug therapies, ICDs, and ventricular assist devices. Therapies utilizing drugs, ICDs and bi-ventricular pacers are currently by far the most commonly prescribed treatments for patients suffering from NYHA Class II or NYHA Class III heart failure. Since the therapies generally each address a particular feature of heart disease or a specific subgroup of heart failure patients, the therapies are often complementary and used in combination.

The heart failure treatment industry generally has a history of adopting therapies that have proven to be safe and effective complements to existing therapies and using them in combination with existing therapies. Although a number of the therapies described above have proven to improve the cardiac function of a damaged heart, no currently available heart failure treatment has demonstrated an ability to generate new muscle tissue within the scarred regions of a heart. Stem cell therapy offers new hope to patients who have otherwise limited choices.

## Cardiovascular Disease as a Target for Stem Cell Therapeutics

As mentioned, ischemic heart disease is a major health problem in the world. Although there have been many advances in myocardial reperfusion strategies and novel pharmacological approaches, therapies for treating acute and chronic myocardial ischemic damage remain limited. Experimental animal studies and clinical trials suggest that the transfer of stem and progenitor cells into the myocardium has a favorable impact on tissue perfusion and contractile performance. Neovascularization and myocyte formation have also been shown. Differentiation of administered stem cells, cell fusion and release of paracrine signals by injected stem cells are potential mechanisms. There has been an attempt to treat almost all cardiovascular disease conditions with stem cells including acute myocardial infarction, heart failure, angina and chronic ischemia in the heart.

The intracoronary administration of bone marrow cells have been shown to improve recovery of left ventricular contractile function in patients with acute myocardial infarction such as in the REPAIR-AMI clinical trial. It is thought that the bone marrow cells

(and other cell types such as adipose tissue derived stem cells) contribute to functional regeneration of the infarcted myocardium and enhance neovascularization of ischemic myocardium.

For treating heart failure, catheter-delivered muscle stem cells have shown promise. The CAuSMIC clinical trial, showed evidence that the patients that received intracardiac muscle stem cells showed less cardiac remodeling (an increase in the size of the heart that signifies worsening of function) than the control patients. The treated patients also demonstrated marked improvement in heart failure symptoms after both six and twelve months.

## Stem Cells for Cardiac Repair

Over the past decade, several indications using a variety of different types of stem cells have emerged including embryonic stem cells<sup>1, 2, 3, 4</sup> cardiac myocytes<sup>5, 6</sup> bone marrow derived stem cells<sup>7, 8</sup> adipose derived stem cells<sup>9, 10</sup> peripheral blood stem cells and skeletal myoblasts<sup>11, 12</sup> for use in acute MI, chronic ischemia, and chronic heart failure. In addition to cellular therapy for the existing heart, some groups are attempting to create a bioartificial heart using cardiac or endothelial cells.<sup>13, 14</sup>

Cardiomyocytes derived from human embryonic stem cells offer the potential to repair the infarcted heart. Laflamme, et. al. demonstrated that human embryonic stem cells (hESCs) can graft in the infarcted rat heart and preserve regional and global contractile function.<sup>15</sup> Despite the many advances in the pre-clinical laboratories, embryonic stem cells have yet to enter into the clinical setting for cardiac indications.<sup>16, 17, 18, 19</sup> There are many hurdles to overcome with regards to hESCs such as transplantation of human embryonic stem cells into mice has led to the formation of tumors.<sup>20</sup> In addition, their use is potentially limited due to cell regulations, ethical considerations, and genetic manipulation. Geron Corporation has reported that additional large animal studies are required prior to advancing this platform to the clinic for use in congestive heart failure or acute myocardial infarction.

The recent discovery of cardiac stem cells obtained directly from adult cardiac tissue could lead to new treatments for heart failure patients.<sup>21</sup> It is believed that these cells are inherently programmed to reconstitute cardiac tissue. In addition these cells which are present in cardiac tissue are believed to play a role in the clinical benefit observed in other stem cell trials. The proteins secreted after stem cell therapy may promote regeneration in a paracrine effect which is related to the inherent cardiac stem cells.<sup>22</sup> These cells have only recently been introduced into the field and there are still several obstacles to overcome prior to advancing this platform to the clinic. Cardiac stem cells have been difficult to isolate and to expand *ex vivo* into meaningful numbers without losing differentiation potential and several groups are exploring ways to improve this cell population.<sup>23</sup>

Bone marrow derived stem cells have been utilized in a variety of indications within the heart. These cells have primarily been utilized to assist in the angiogenesis process and to assist in the revascularization of hibernating tissue. Typical clinical applications have been in acute MI and myocardial ischemia. Pompili, et. al. (Arteriocyte, Inc.) demonstrated long term clinical and perfusion improvements in the absence of adverse events when injecting bone marrow derived stem cells into patients with myocardial ischemia.<sup>24</sup>

Osiris therapeutics is currently enrolling patients in a phase II study utilizing culture expanded allogeneic mesenchymal stem cells derived from bone marrow for the treatment of patients who have just experienced their first acute myocardial infarction. Osiris' phase I double blind, placebo controlled study of 53 patients demonstrated safety in the acute MI setting. Patients receiving the treatment had fewer adverse events, required less hospitalizations, had reduced incidence of arrhythmias, and had a significant and durable improvement in cardiac function. The cells are believed to interact with the immune cells in the body, reducing inflammation and assisting in tissue repair.<sup>25</sup> Angioblast systems recently announced positive three month interim efficacy results from the first 20 patients enrolled in a phase 2 heart failure trial utilizing bone marrow derived mesenchymal cells. Randomized placebo control patients demonstrated an 11% mean decrease in ejection fraction while patients receiving the low dose showed a 37% mean increase in ejection fraction.<sup>26</sup> Bone marrow derived stem cells work best in an acute setting or in hibernating tissue mainly due to the fundamental characteristics of mesenchymal stem cells. Bone marrow derived mesenchymal stem cells are medium-dependent and tend to acquire the characteristics of the cells they are in contact with. For this reason, these cells are not appropriate for the scar tissue of a chronic myocardial infarct (i.e., into the fibrotic area, because they would tend toward differentiating into more fibrosis).<sup>27</sup>

Adipose tissue consists of adipocytes and a mononuclear cell fraction that contains a mesenchymal stem cell population. These cells are very similar in nature to bone marrow derived stem cells and in some cases have advantages over bone marrow derived stem cells.<sup>28, 29, 30</sup> In one study by Zhang, et. al, adipose derived stem cells (ADSCs) exhibited a higher percentage of differentiating into cardiomyocytes when compared to bone marrow mesenchymal stem cells (MSCs). These results along with an advantage in tissue content, homology, growth and differentiation rate indicate that ADSC may be better suited for cellular cardiomyoplasty than MSCs.<sup>31</sup> *In vitro* differentiation, including morphological changes, spontaneously beating foci and the expression of cardiac-specific markers, has been demonstrated by multiple laboratories. Early *in vivo* work with a murine model of myocardial cryoinjury demonstrated positive engraftment of ADSCs at the site of injury. Two functional animal studies using *in vivo* infarction models were recently presented at the meeting of the International Fat Applied Technology Society (IFATS): The first, a 60-min left coronary artery occlusion model, found improvements in cardiac function at 12 weeks in treated rats. Analysis showed improved ejection fraction and contractility, and a reduction in left ventricular remodeling and compensatory hypertrophy. The second model consisted of a 30-min left anterior descending artery occlusion followed by direct myocardial injection of cells. They found a significant improvement of cardiac function, as measured by ejection fraction, cardiac output and stroke volume, at 1 month.<sup>32</sup> In another pre-clinical study at the Jordan University of Science and Technology involving the injection of human ADSCs into the myocardium of infarcted rats, there was as much as a 90 percent reduction in scar size versus the control group.<sup>33</sup> In addition, bone marrow collection is extremely painful and often yields a low volume of blood. By contrast, large volumes of adipose tissue can be

easily obtained under local anesthesia with little patient discomfort.<sup>34</sup>

Cytori Therapeutics has recently completed enrollment in a study to investigate adipose derived stem cells in chronic heart disease. The phase I PRECISE trial was carried out at several centers in Europe with enrollment of 27 patients. The independent data safety and monitoring board had not identified any safety concerns and six month results are expected in the first half of 2010.<sup>35</sup> Cytori is also currently recruiting for a phase I study at two centers in Europe to establish the safety and feasibility of Adipose derived stem cells in patients with acute myocardial infarction (MI).<sup>36</sup> Bioheart Inc. has also begun studies utilizing adipose derived stem cells for cardiovascular diseases. Bioheart has recently received CE mark on the TGI 1200 system for automated processing of adipose tissue to obtain a mixture of adipose derived stem cells. The TGI 1200 system is currently being marketed in all countries recognizing the CE mark for using in Acute MI, chronic ischemia, and critical limb ischemia.<sup>37</sup>

There has been some research in the use of peripheral blood and collection of stem cells for use in cardiovascular diseases. Baxter has recently completed a phase II trial of 150 patients to assess the safety and efficacy of CD34+ cells in patients with chronic myocardial ischemia. Patients receive granulocyte colony stimulating factor to mobilize the hematopoietic (blood-forming) CD34+ cells from their bone marrow to their bloodstream. Then, a cell separation system collects a mononuclear cell preparation rich in CD34+ stem cells from the patient's bloodstream and separates the cells using magnetics for use in the heart.<sup>38</sup> Theravita utilizes stem cells culture expanded from peripheral blood to treat end stage patients suffering from coronary artery disease, cardiomyopathy or congestive heart failure.

Skeletal myoblasts have been studied in over 2000 animals, 350 patients and was one of the first stem cells to enter into the clinic for cardiovascular diseases. A variety of groups have studied this platform with a variety of trial designs. The human body cannot, without medical assistance, repopulate regions of scar tissue within the heart with functioning muscle. Unlike other cell therapy types, skeletal myoblasts are designed to improve cardiac function by populating regions of scar tissue. Myoblasts, which are obtained from a biopsy of the thigh, are precursors to muscle cells that have the capacity to fuse with other myoblasts or with damaged muscle fibers to regenerate skeletal muscle. When injected into scar tissue within the heart wall, myoblasts have been shown to be capable of engrafting in the damaged tissue and differentiating into mature skeletal muscle cells. In a number of clinical and animal studies, the engrafted skeletal muscle cells have been shown to express various proteins that are important components of contractile function. The use of myoblasts obtained from a patient's own body helps to avoid certain challenges currently faced by other cell-based clinical therapies intended to be used for the treatment of chronic heart damage including tissue rejection and instances of the cells differentiating into cells other than muscle.

A recent study (Menesche, et. al) reported the final results of the MAGIC trial (sponsored by Genzyme Corporation) consisting of a Phase II, randomized, double blind, placebo controlled multicenter clinical trial in various countries in Europe to assess the safety and efficacy of skeletal myoblast implantation injected during CABG

surgery into the scarred region of the heart. Although the study failed to find any significant differences in Wall Motion or LVEF as measured by echocardiography, a significant decrease, or improvement, was documented (by 12-13% from baseline preoperative values) of LV Volume in patients receiving the high dose of cells whereas there were no significant changes in the placebo group. Reduction in LV volume generally is reflective of positive ventricular remodeling and improvement in the heart's ability to circulate oxygenated blood through the arteries. The team also reported a higher number of arrhythmic events early on in the myoblast treated patients, but noted that after 6 months, the rates of major cardiac adverse events and of ventricular arrhythmias did not differ between the treated and placebo patients.<sup>39</sup>

In another study (Dib, et. al) sponsored by Mytogen, (a wholly-owned subsidiary of Advanced Cell Technology, Inc.) myoblasts were tested for safety and feasibility in class II, III, and IV heart failure patients. When comparing the 12 treated subjects to the 11 control, there was no difference between groups in arrhythmias and no deaths. The treated subjects showed improvements in NYHA, Minnesota Living with Heart Failure Questionnaire, ventricular viability, and evidence of reverse ventricular remodeling. Based on this data, the FDA has allowed the company to proceed with a Phase II clinical trial.<sup>40</sup>

Bioheart has completed several trials for class II, III, and IV heart failure patients, including a phase I MYOHEART trial in the US and a Phase II SEISMIC trial in Europe. In these studies it was reported that 83 to 94% of the MyoCell treated heart failure patients improved, or did not worsen, in the Six Minute Walk, Quality of Life and/or heart failure class while only 6 to 17% worsened. In the SEISMIC Trial control group, 69% of the patients that received drugs only, worsened in exercise capacity and/or quality of life. MyoCell treated patients in both studies demonstrated substantial improvement in six-minute walk exercise testing and quality of life, which has led to the FDA approval of a Phase II/III randomized, double-blind, placebo-controlled clinical trial in the U.S.<sup>41</sup>

In addition, Bioheart announced that it plans to conduct clinical trials for its second-generation MyoCell SDF-1 myogenic cell composition (a combined cell and gene therapy) for treating advanced heart failure. It has been shown that the stem cell homing molecules Stromal-cell Derived Factor -1 (SDF-1) is transiently expressed following MI, and that re-establishment of this homing factors in myocardial tissue months after MI is sufficient to induce stem cell homing, vasculogenesis and recovery of myocardial function. SDF-1, is a family of CXC chemokines, and its receptor is CXCR4. The SDF-1/CXCR4 pathway is critical during embryogenesis for hematopoiesis, vascular development, and cardiac development. It has been reported that 1) cells expressing markers of hematopoietic stem cells or EPCs express CXCR4; 2) vascular endothelial growth factor (VEGF) induces CXCR4 in endothelial cells; and 3) SDF-1 functions as a chemoattractant for EPCs in vitro. SDF-1 also induces the expression of VEGF and induces angiogenesis in vivo.<sup>42</sup> SDF-1 and its receptor CXCR4 are crucial for bone marrow retention of hematopoietic stem cells and are involved in cardiogenesis and recruitment of endothelial progenitor cells to sites of ischemic tissue.<sup>43</sup> Others (Penn et. al.) have demonstrated

demonstrated that cell-based delivery of the gene led to an improved treatment effect over direct adenoviral injection of the gene, and suggested that adenoviral vectors that encode secreted factors could potentially offer greater efficacy in combination with myoblast transplantation.<sup>44</sup> In animal studies, myoblasts combined with SDF-1 provided a 54 percent improvement of heart function compared to 27 percent for the original MyoCell composition while the placebo control treated animals declined by 10 percent.<sup>45</sup>

### Rationale for Stem Cell Based Approaches to Treat Heart Diseases

Transplanting stem cells into the diseased or damaged heart may result in several potential benefits. Both myogenesis and angiogenesis are goals of using cellular therapy for heart diseases. The ultimate goal is to fully replace damaged heart tissue with new healthy tissue that is capable of improving cardiac function. Another animal study (Prosper et. al.), indicates that the use of myoblasts in the heart post-infarct may result in an increase in vasculogenesis and changes in matrix remodeling with decreased fibrosis.

Stem cells may serve as protein factories, releasing beneficial proteins such as SDF-1 which is able to recruit stem cells with angiogenic potential from the bone marrow. Others suggest that the cells release angiogenic growth factors such as VEGF which can further improve blood supply in the damaged heart.

### Current Hurdles and the Future of Stem Cells

In addition to the various types of cells which can be utilized in the heart in a variety of indications, there are countless other variables and potential hurdles to consider. For example, the method and timing of delivery may drastically affect the outcome in the patients. There are pros and cons associated with the different routes of

delivery from intravenous to direct muscle injection and from catheter based to open heart surgery.<sup>46</sup>

The mechanism by which implantation of cell therapy may improve heart function is not completely clear. Proposed mechanisms for cell therapy improving cardiac function include but are not limited to the cells inducing reverse remodeling, participating in active contraction, secreting beneficial proteins and growth factors, growing new healthy tissue within damaged areas of the heart, decreasing fibrosis, and inducing vasculogenesis. Successful cell therapy depends on a number of factors including delivery to the target tissue, cell survival and engraftment, and integration into the micro-environment. All these steps constitute a potential goal for cell manipulation aiming to improve the overall outcome of such therapy. A better understanding of the mechanisms by which cells improve cardiac function will be important to direct further research.

### Conclusion

The future of cell therapy within the cardiac field also faces financial and regulatory hurdles. The regulatory pathway for biologics is not well established and there are several unknowns with regards to the requirements for commercialization. The inconsistencies associated with the regulatory pathway have to do in part to the lack of understanding of the mechanisms of action of the biologic products. This makes for a complicated, lengthy, and expensive timeline when attempting to bring a biologic product to market. At a time of financial instability, obtaining the funds to take a stem cell product through the complicated pathway can be challenging. The statistics associated with heart failure are staggering and heart failure is a human tragedy and epidemic. Stem cell treatments are already offering new options to patients and the age of regenerative medicine is only just beginning.



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*Bioheart, Inc. is committed to delivering intelligent devices and biologics that help monitor, diagnose and treat heart failure and cardiovascular diseases. Its goals are to improve a patient's quality of life and reduce health care costs and hospitalizations. Specific to biotechnology, Bioheart is focused on the discovery, development and, subject to regulatory approval, commercialization of autologous cell therapies for the treatment of chronic and acute heart damage. Its lead product candidate, MyoCell®, is an innovative clinical muscle-derived stem cell therapy designed to populate regions of scar tissue within a patient's heart with new living cells for the purpose of improving cardiac function in chronic heart failure patients. The Company's pipeline includes multiple product candidates for the treatment of heart damage, including Bioheart Acute Cell Therapy, an autologous, adipose tissue-derived stem cell treatment for acute heart damage, and MyoCell® SDF-1, a therapy utilizing autologous cells that are genetically modified to express additional potentially therapeutic growth proteins. For more information on Bioheart, visit [www.bioheartinc.com](http://www.bioheartinc.com)*



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