

## Cardiovascular Applications of Stem Cell Therapy

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### Abstract

Despite remarkable progress in medical and surgical therapy for heart disease, congestive heart failure (CHF) and coronary artery disease (CAD) are leading causes of morbidity and mortality in the United States. Although implantation of a left ventricular assist device has recently emerged as a promising therapy for CHF, no other therapy holds as much promise for the treatment of patients suffering from cardiovascular disease other than cardiac regeneration. In this regard, there are substantial pre-clinical and clinical studies that have elucidated the safety and efficacy of cardiac stem cell-based therapy using a variety of cell lines to promote regeneration of the heart. In spite of promising results in both animal and human studies, the exact fate of these administered stem cells within the human heart is poorly understood as is the mechanism by which they promote myocardial recovery and regeneration. These limitations of our current knowledge base can be considered a critical issue limiting widespread application of stem cell-based therapy.

**Keywords:** Stem cells; Congestive heart failure; Coronary artery disease; Cardiac regeneration

### Introduction

One of the most common causes of death worldwide is acute myocardial infarction (AMI). The end-stage sequela of adverse remodeling after AMI is congestive heart failure (CHF). In spite of valid therapeutic advances in the last half century, CHF continues to be the leading cause of hospitalization for people aged 65 years and older [1]. According to the American Heart Association, CHF is estimated to affect 4.8 million Americans each year with an estimated total cost of \$ 39.2 billion in 2010 [2]. CHF is responsible for a significant decrease in physical and mental health, the end result of which is a clearly noticeable decreased quality of life [3,4].

AMI is associated with a loss of cardiomyocytes and this process was traditionally believed to be permanent as the heart was traditionally considered to be a terminally differentiated organ [5]. In the last decade, this paradigm has been challenged as different types of stem cells have been used for the purpose of cardiac regeneration including skeletal myoblasts, bone marrow derived mononuclear cells, mesenchymal stem cells and embryonic stem cells [6]. Furthermore, the discovery and demonstration of a resident cardiac stem cell population that is capable of proliferating and differentiating into cardiomyocytes has further challenged the traditional belief that heart is incapable of regenerating itself [7].

### Stem cell technology

Recent developments in stem cell biology have elucidated a significant differentiation plasticity of both embryonic and adult stem cells in human tissue. These findings have ignited the hopes of achieving cell-based replacement therapy for cardiovascular disease. Stem cells with potential applications in the treatment of cardiovascular disease can be obtained from a variety of sources including the blood, bone marrow, somatic cells, and within the heart itself.

The clinical trials that have been performed in patients with AMI, chronic ischemic heart failure and dilated cardiomyopathy have proven the safety and efficacy of stem cell therapy *in vivo* with varying degrees of success [6,8-16]. This review will provide an overview of these studies

and we will also discuss possible mechanisms behind successful cardiac stem cell therapy.

### Embryonic stem cells

Embryonic stem cells (ESCs) are pluripotent stem cells derived from the blastocyst stage. These cells are able to differentiate into cells of all three embryonic germ layers [17]. Differentiation of both mouse and human ESCs into cardiomyocytes has already been described in several studies [18,19]. Furthermore, there has been a demonstrated functional improvement of infarcted myocardium of rodents after transplantation with ESCs [20-25]. These administrated ESCs have the capacity to become engrafted, propagate action potentials, and improve left ventricular (LV) function through positive LV remodeling in animal models [24,26,29].

Although animal models of ESC therapy have produced exciting results, human applications of ESCs have proceeded cautiously. Factors that have limited further research on ESCs in the clinical arena include concerns about: the possibility of immune rejection of allografted cells, low yield of cardiomyocytes, and risk of teratoma formation [30,31].

### Induced pluripotent stem cells

Induced pluripotent stem (iPS) cells have been introduced as an alternative to ESCs. These cells arise from somatic cells that have been reprogrammed to an embryonic-like state [32-35]. The reprogramming occurs by insertion of a defined set of transcription factors that leads to

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the expression of embryonic stem cell factors including: OCT4, SOX2, c-Myc, KLF4, NANOG and LIN28 [33,34]. iPS cells might be an ideal source for cell-based therapy because they can directly be derived from the patient requiring therapy, without concerns for immune-mediated rejection [35]. These cells are also capable of forming all three cell types that exist within the heart: endothelial cells, smooth muscle cells, and functional cardiomyocytes [36-39].

Although iPS cells have a robust potential to replace ESCs and revolutionize cardiovascular stem cell-based therapies, concerns regarding their wide spread use include: poor cardiomyocyte differentiation, potential for tumorigenesis and some suggestion that the resultant cardiomyocytes maybe inefficient [39-41]. Human iPS lines exhibit broad epigenetic diversity which may limit their cardiac differentiation potential [39,42]. With regards to tumorigenesis, there is laboratory evidence that the epigenetic memory of the somatic cell of origin can influence the differentiation potential of iPS cells into tumor cells [43]. Another limitation of iPS cells is concern that the generation of iPS cells using retroviruses or lentiviruses, has the potential for insertional mutagenesis that can lead to malignant transformation following cardiac differentiation [44].

### Bone marrow cells

A variety of cells within the bone marrow have demonstrated the plasticity to form cardiac myocytes, collectively they are known as bone marrow cells (BMCs). Orlic et al. showed that BMCs transplanted into the infarcted myocardium of rodents can differentiate into cardiomyocytes and improve cardiac function [45]. Two other groups went on to demonstrate the safety of intramyocardial injections of BMCs in patients with severe ischemic cardiomyopathy and AMI [10,46]. Both studies showed an improvement in left ventricular function and myocardial perfusion. The effect of BMC therapy on left ventricular function after acute myocardial infarction (AMI) was tested in the BOOST trial (bone marrow transfer to enhance ST-elevation infarct regeneration) [9]. Sixty patients, who presented with ST-segment elevation MI and underwent successful primary percutaneous coronary intervention (PCI) with stent placement, were then randomized to receive intracoronary infusion of autologous BMCs versus standard care alone. Patients in the BMC group showed significant improvements in left ventricular function compared with the control group six months after stem cell transplantation [9]. Although, this reported change in left ventricular ejection fraction (LVEF) was no longer apparent at 18 months follow-up [47]. The paradox at 18 month follow-up has been explained by two subsequent studies. Ballsam et al. studied the ability of injected c-kit-enriched BMCs to regenerate myocardium in a mice infarct model. They demonstrated that transplanted BMCs express the hematopoietic marker CD45 and myeloid marker Gr-1 and develop into hematopoietic cells within the myocardium rather than cardiomyocytes [48,49]. Murry et al. also demonstrated that transplanted bone marrow derived hematopoietic stem cells failed to transdifferentiate into cardiomyocytes in the hearts of mice. These studies indicate that BMCs do not readily acquire a cardiac phenotype, and raise a cautionary note for clinical studies of infarct repair [49].

### Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are a group of clonogenic cells that exist in multiple locations within the human body including:

bone marrow stroma, peripheral blood, umbilical cord, adipose tissue, fetal liver, lung, synovial membrane and dental pulp [50-56]. Due to their ease of isolation, wide differentiation potential, and lack of immunogenicity; MSCs have been utilized in clinical medicine for a variety of application [57-61].

Human bone marrow-derived mesenchymal stem cells (BMSCs), or multipotent mesenchymal stromal cells, are a group of clonogenic cells present among the bone marrow stroma that are capable of multi-lineage differentiation [62]. BMSCs can be used to generate cardiomyocytes for use in a range of cardiovascular diseases [63,64]. Makino and colleagues have successfully induced BMSCs to differentiate into cardiomyocytes *in vitro* with 5-azacytidine [65]. Amado et al. demonstrated improved myocardial thickness, enhanced contractility and reduced scar formation in the infarct zone of a post-MI animal model [66]. In a swine model of chronic ischemic cardiomyopathy, Hare and his group reported the capacity of allogeneic BMSCs to engraft and differentiate into cardiomyocytes, smooth muscle cells and endothelium [67]. They injected male BMSCs into female swine and identified these cells within the myocardium by co-localization of the Y-chromosome by fluorescence *in situ* hybridization. They showed that cardiomyocyte differentiation was present in approximately 14% of Y-positive BMSCs as identified by co-staining for the cardiac structural proteins α-sarcomeric actinin, tropomyosin, GATA-4 and Nkx2.5. In addition, differentiated myocytes exhibited the capacity for coupling with host myocytes via connexin-43 and had the ability to promote angiogenesis as well [67].

Based on these pre-clinical studies, early stage clinical trials with intravenous, intracoronary and intramyocardial delivery of MSCs have been conducted [68-70]. In a randomized evaluation of intravenous allogeneic MSCs versus placebo, Hare et al. showed that this form of cell therapy was safe in post-MI patients with an improvement in ejection fraction by echocardiography of 6% at 3 months for patients receiving intravenous MSCs [68]. Intramyocardial injection of MSCs was also recently studied in 8 patients with a chronic ischemic cardiomyopathy. This study demonstrated an overall improvement in regional contractility which strongly correlated with a reduction in both the end-diastolic and end-systolic volumes [69].

### Skeletal myoblasts

Cell-based cardiac repair has been studied with the transplantation of autologously derived skeletal myoblasts [71-76]. Several clinical studies have been performed based on the fact that these cells can readily be harvested from the patient, proliferated *in vitro* and then be reintroduced to the patient without a need for immunosuppression as they are autologously derived [71,77-79].

A variety of preclinical and clinical studies have demonstrated that engrafted skeletal myoblasts transdifferentiate into cardiomyocytes and result in functional improvement [71,75,78-81]. There are however, conflicting views regarding the utility of skeletal myoblasts in treating cardiomyopathy patients. These include their commitment to their skeletal muscle lineage and perhaps more importantly their failure to electromechanically couple with cardiomyocytes which can lead to malignant ventricular arrhythmias [76-82]. Indeed, the ventricular arrhythmias arising from the lack of electromechanical integration represents a major problem facing skeletal myoblast transplantation [83-85].

## Endothelial progenitor cells

Endothelial progenitor cells (EPCs) are either bone marrow derived or found circulating in the peripheral blood [86]. They express AC133 and other endothelial cell surface markers [87-89]. Endothelial cells have a pivotal role in the efficient development and function of cardiovascular tissue [90]. In addition, their role in the development of mature endothelium is key as the endothelium mediates angiogenesis, inflammation, vasculogenesis and thrombosis [91].

Given the EPCs ability to self-renew and their potential application in myocardial repair after AMI, EPCs have received significant research attention [92,93]. For instance, when EPCs are injected into animal models of ischemia, they rapidly incorporate into new sites of vascularization [92,94]. Their role in neoangiogenesis results in decreased apoptosis of hypertrophied myocytes, long-term salvage and survival of viable myocardium and a reduction in collagen deposition that leads to a sustained improvement in cardiac function [95].

## Cardiac stem cells

Over the past few decades stem cells have been characterized and isolated from many adult tissues. Despite this, the search for cardiac stem cells (CSCs) has been considered unprofitable until recently. This is because of a traditionally accepted paradigm in cardiac biology that considered the human heart as a post-mitotic and terminally differentiated organ with no intrinsic regenerative capacity [96-98]. This belief, however, has been challenged recently with evidence of cardiac cell division after AMI and through the identification of stem cell-like cells within the heart. These cells appear to have the potential to regenerate contractile cardiomyocytes and restore the normal coronary vascular bed [5,99].

The differentiation potential of stem cell-like cells isolated from the heart has been studied both *in vitro* and *in vivo* [7]. These studies have demonstrated that these undifferentiated stem cell-like cells express stem cell markers, such as c-kit or Sca1 [100]. These cells which originate from the endomyocardium have the ability to proliferate and differentiate into cardiomyocytes, smooth muscle cells and endothelial cells [7,101-104].

The isolation and characterization of the CSCs give us an opportunity to explain and explore the regenerative capacity of the heart and ignites the hope that in the near future we might be able to manipulate these human CSCs to hone to the regions of damaged myocardium and promote cardiac repairs that regenerate functional myocardium. Similar work has already been done in the rodent myocardium [105,106]. However, the rarity of CSCs coupled with their complex isolation procedure presents a significant challenge to the widespread clinical application of CSCs.

## Mechanisms of action

Almost all cell-based therapies have demonstrated an improvement of left ventricular function and ameliorated adverse cardiac remodeling, yet the mechanism behind the benefit of stem cell therapy is not fully understood [107]. Suggested mechanisms of stem cell benefit include: transdifferentiation into the various cellular constituents of the heart; fusion with other cardiac cells; incorporation and integration of implanted stem cells into native myocardial fibers; neovascularization; production of paracrine factors; and activation of endogenous repair mechanisms [92,93,101,102,107-113].

Transdifferentiation is the most common mechanism behind cardiac regeneration and this refers to the process by which stem cells adopt the characteristics of their surrounding tissues. Several animal and clinical studies have indicated that those stem cells transplanted into infarcted myocardium differentiate into new cardiomyocytes, endothelial cells and smooth muscle cells [45,49,64,65,114-116]. Transdifferentiation as a proposed mechanism for cardiac repair has been challenged by other groups and continues to remain a controversial issue [48,49,117]. Fusion of the administrated cells with host cells is another proposed mechanism of stem cell benefit. Cell fusion results when there is a transfer of cell contents, including genetic material from transplanted cells into host cells [118-120]. Several studies conducted in mice and pig models have demonstrated that BMCs engrafted into the myocardium, fuse with host cardiomyocytes [121,122]. Other animal studies proposed mechanisms by which stem cell therapy may have beneficial effects including the secretion of paracrine factors that have both anti-apoptotic and neoangiogenic properties [122-124].

Neovascularization leads to the formation of new blood vessels and improves perfusion to the infarcted myocardium; thus limiting apoptosis within these areas. Neovascularization as a result of BMSCs and BMCs is postulated to occur because these cells secrete angiogenic factors like vascular endothelial growth factor, fibroblast growth factor, hepatocyte growth factor and angioprotein-1 [94,125-128]. Also, BMSCs secrete some cytokines and growth factors including interleukin-1, interleukin-6, tumor necrosis factor- $\alpha$  and transforming growth factor- $\beta$  that play a pivotal role in the tissue response to injury [125].

Finally, the recent identification of CSCs and their potential for myocardial and vascular regeneration raises the possibility that these cells may be activated by other stem cells to promote endogenous myocardial repair [5,7,99,102]. This is supported by a recent study that shows that bone marrow derived c-kit (+) cells stimulate endogenous cardiomyocyte progenitors and promote cardiac repair [129]. Thus, stimulation of endogenous cardiogenic progenitor activity appears to be a critical mechanism of cardiac stem cell therapy [129].

## The future of cardiac stem cell therapy

The prospect of cell-based therapies for cardiovascular disease has led to excitement within the scientific community as cardiovascular disease continues to be the leading cause of death worldwide. There is a growing body of evidence from both the basic sciences as well as through translational studies that prove the ability of stem cells to improve left ventricular function. There are however, some critical questions regarding cardiac stem cell therapy that remain to be answered. These include: what is the best class of stem cells for cardiac cell therapy; what is the mechanism of benefit underlying cardiac stem cell therapies; what is the best vehicle for delivery of these cells into the myocardium; how can we activate resident CSCs and finally how can we promote engraftment and incorporation of transplanted stem cells within the heart? To help answer these questions and promote the future of cardiac stem cell-based therapies we need to continue to foster collaboration between basic scientists and clinical researchers. Such a partnership will help us understand how cardiac stem cell-based therapies can be optimized to guide future clinical studies. Finally, if we can promote an environment that facilitates stem cells to successfully repair and regenerate the heart, we can expect to see resultant

improvements in myocardial function, with clinical benefit to follow.

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