Review Article

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Insights in Stem Cells

2016
Vol. 2 No. 2:8

# Cell-Based Therapy of Ischemic Heart Failure

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**Citation:** Lisboa S, Gaballa M. Cell-Based Therapy of Ischemic Heart Failure. Insights Stem Cells. 2016, 2:2.

## Abstract

Ischemic heart failure (IHF), heart failure secondary to myocardial infarction, is the number one killer in the US. Despite all the major advances in cardiac interventions, its relevance is on the rise. There is an urgent need for an effective alternative therapy. Stem cells have the potential to ameliorate many chronic illnesses such as IHF. Like any new therapy, several unanswered questions need to be addressed. The first question to be addressed is which type of stem cell should be used? There are several types of cells that have previously been investigated including embryonic stem cells, inducible pluripotent cells, and adult cells. Among the most commonly used adult cell type are fat cells, cord blood, bone marrow, cardiac cells, and myoblasts. Many cell-based strategies have been utilized, for example, one strategy has been directed differentiation of embryonic and inducible pluripotent stem cells have provided an unlimited number of cardiomyocytes for cell replacement of the lost cardiac cells in IHF. A second strategy has been exogenous stem cells may be generated and used as progenitors to regenerate the myocardium. A third strategy has been adult cardiac progenitor cells may be created by partial de-differentiation of tissue cardiac cells and delivered to regenerate the injured myocardium. The second question to be addressed is what are the mechanisms of action of these cells? This question may be complicated by the difficulty in identifying the cells' active ingredients, effective dose, treatment duration, and specific targeted pathology. A common mechanism of action of all those strategies appears to be the paracrine factors such as growth factor or microRNA secreted by those exogenous cells. This hypothesis is supported by the findings that most cell-based therapies lead to an improvement in cardiac function with minimal structural contribution of exogenous cells' to the damaged myocardium. This review covers the recent advancements in cell therapy such as iPSCs technology and the generation of modified adult cardiac progenitor cells. Using cells derived from bone marrow, cord blood, fat cells and others is covered in several other review articles. The third question to be addressed is what is an efficient delivery route to the targeted organ? Common cell delivery techniques are discussed in this review and more attention is given to the retrograde coronary vein delivery technique and the cardiac patch technology. Finally, the next possible phase of personalizing cell therapy is discussed, in which, gene and cell therapies are combined. With the recent advances of gene editing combined with more modern and simpler technologies, such as CRISPER/CAS9, genetically editing tissue-derived cells prior to implantation into injured hearts is quickly becoming a reality. There are many excellent review articles that have discussed additional cell delivery techniques such as systemic infusion, direct intramyocardial injection, and intracoronary artery infusion, but this review focuses on the progress in cell-based therapy for the last three years (2014-2016).

**Keywords:** Cardiomyocytes; Stem cells; Cell-based therapy

**Received:** April 02, 2016; **Accepted:** August 22, 2016; **Published:** August 26, 2016

## Introduction

Heart failure is the number one killer of men and women in the United States. Despite spending more than $35 billion annually on the treatment of heart disease in the US, the number of patients progressing to heart failure is increasing. Currently, there is no strategy to reverse or halt the progression of heart failure. Over the past few years, stem cell transplantation has risen as a new therapeutic strategy for treating IHF. A large number of preclinical as well as clinical studies were performed using adult bone marrow-derived cells with mixed results. The majority of the studies showed modest improvement in cardiac function in IHF. Among the studies tested adult cell types, cardiac c-Kit+ progenitor cells proved to be potential candidates as therapeutic agents; however, the low cardiogenic potential of transplanted adult cells and c-Kit+ cells may present a major obstacle. Therefore, a new strategy is needed to improve cardiac function in heart failure patients.

Embryonic stem cells (ESCs) are a logical candidate for cardiac regeneration due to their ability to differentiate to cardiac cells. These cells have the highest potential to differentiate to any type of cell depending on their cellular microenvironment. However, their tumoro-genesis property and allogenicity hampered their adoption in clinical application. Inducible pluripotent stem cells (iPSCs) are developed to overcome the allogenicity issue. These cells are produced by inducing a patient's own cells, such as a skin fibroblast, to de-differentiate to embryonic-like stem cells.

Despite an enormous number of pre-clinical studies showing beneficial effects of cell-based therapy for heart failure, a large number of clinical trials conducted to date showed a modest effect on cardiac function in heart failure after cell therapy. Clinically, a new wave of studies, based on our current understanding of stem cell biology, need to be developed. There are three major issues to resolve in order to develop the next wave of cell-based therapy for heart failure. First, what is the best cell type to achieve the most beneficial therapeutic outcome after transplantation into an injured heart? There is an argument that may favor the use of cardiac-committed cells, such as partially reprogrammed adult cardiac-derived cells (modified cardiac progenitor cells) and inducible pluripotent stem cell-derived cardiac progeny. However, the optimal stage that these cells should be transplanted remains an unanswered critical question. Transplantation of fully differentiated ESC-derived cardiomyocytes has been shown to induce re-muscularization of an infarcted heart [1]. Early progenitors have a higher resistance to the hypoxic milieu in the injured myocardium tissue and possess a greater plasticity in response to local queues. The question still remains; at what stage of differentiation iPSC or ESC-derived progenitors should be used? The second issue is the obscurity of the mechanisms of action. The majority of published work supports the conclusion that the effects of therapeutic cells on the heart are mediated primarily by secreted factors, i.e. the therapeutic cells acting as a source of those biological factors. This may imply that the focus of future cell-based therapy should be on cell retention to allow cells to deliver sufficient amounts of factors. An option that may overcome this issue is to use therapeutic cells seeded in a 3-D extracellular scaffold. This may provide a better survival rate for cells. The third issue relates to cell manufacturing. This process is tedious and requires massive resources, and the need to overcome many regulatory hurdles [2].

## IPSCs

Advanced IHF is represented by a decrease in cardiomyocyte number. Supplementation of functional cardiomyocytes into the heart would be an appropriate therapeutic strategy. IPSCs' technology allows for large scale *in vitro* production of patient-specific functional cardiomyocytes. A number of studies have shown that transplantation of iPSC-derived cardiomyocytes (iPSC-CMs) into the injured heart improves cardiac function. However, the resultant cardiomyocytes-derived from human induced pluripotent stem cells (hiPSC-CMs) are similar to human fetal cardiomyocytes (CMs) and different than adult CMs, based on gene expression, electrophysiology, and morphology [3-5]. Specifically hiPSC-CMs are smaller, less electrically excitable, have a lower sensitivity to adrenergic stimulation, and have impaired excitation-contraction coupling relative to adult CMs [6-9]. It is unclear how those differences in hiPSC-CMs compared to adult CMs affect cardiac function after transplantation in injured hearts. Mature CMs are presumed to provide a functional advantage upon implantation; it is possible that less-mature CMs might be better equipped to survive in the infarcted myocardium's hostile environment.

Another limitation of the iPSCs' technology is that generating patient-specific iPSC-derived cardiomyocytes (iPSC-CMs) requires 2-3 months from the biopsy to producing CMs. Residual undifferentiated iPSCs may present a potential risk of teratoma formation [10]. To address this limitation, direct reprogramming strategies are currently being explored. For example, somatic cells can be directly converted to CMs in mice [11]. Recently, direct reprogramming of human fibroblasts was also achieved [11]. Despite these advances, the protocols of induced CMs (iCMs) are less efficient compared with the iPSC-CM protocols (5% vs. 90%) [12]. Additionally, iCMs are not fully characterized. Some studies suggest that iCMs are similar to ESC-CMs [13], whereas, others suggest they are less mature than hiPSC-CMs [14]. Once refined, trans-differentiation (by directly reprogramming fibroblasts in the scar tissue into functional myocardium *in vivo*) could revolutionize regenerative medicine by eliminating the risk of teratomas [15]. Clinical application of these new advances in cell-based therapy, including its feasibility, safety, and therapeutic efficacy need to be further investigated at the pre-clinical stage. In their recent review, Miyagawa et al., summarized current topics related to safety and efficacy of iPSC-CMs transplantation therapy for cardiac disease and discussed the prospects for this treatment in clinical studies [16].

## Modified Cardiac Progenitor Cells

Ischemic heart disease is more common in elderly patients. Cardiac c-Kit+ cells have been utilized in early stage clinical trials for chronic heart failure (CHF). However, these cells have modest cardiogenic potential that may limit their efficacy [17,18].

Modified autologous adult progenitor cells to promote the repair of post-infarct cardiac tissue may prove to be an effective therapy option [19]. This cellular therapy involves the isolation of adult progenitor cells from the patient, *in vitro* manipulation of these cells, and the subsequent transplantation of the cells back into the patient's own heart. An obstacle affecting this process is that progenitor cells recovered from an elderly patient's cardiac muscle tissue tend to be in senescence. As a result of this, hostile *in vitro* manipulations can cause the aged cells to undergo *in vivo* apoptosis following transplantation into the patient. Inhibition or reversal of senescence in adult progenitor cells may be a strategy that lower stem cell mortality and coerce aged stem cells into adopting more resilient phenotypes similar to that of their younger counterparts. In an excellent review written by Khatiwala et al. in 2016, the authors discussed a selection of the most efficient and most recent strategies used experimentally to enhance the effectiveness of the current stem cell therapies for ischemic heart diseases [20].

A strategy to augment cardiac-derived cells' cardiogenic potential, as previously reported by our lab, is by epigenetically modifying the cells using small molecules such as Class I Histone Deacetylases (HDACs) inhibition. We found that a selective inhibition of Class I HDACs (mocetinostat) *in vitro* induces increased expression of cardiac markers in c-Kit+ cells. Compared to untreated cells, transplantation of mocetinostat-treated c-Kit+ cells resulted in higher retardation of LV remodeling in CHF [19,21].

Another strategy to improve the cardiac potential of adult cardiac-derived cells is pretreatment with small molecules against TGF-β. We showed that intervention with TGF-β signaling by inhibiting TGF-β receptor type I or Smad 2/3 using small-molecule inhibitors improved c-Kit+ cell yield, attenuated epithelial to mesenchymal transition markers, stimulated the pluripotency marker Nanog, and improved efficiency of c-Kit+ cell differentiation toward cardiomyocyte-like cells *in vitro*. Our findings suggest that TGF-β inhibition positively modulates c-Kit+ cell phenotype and function *in vitro,* and this strategy may be considered in optimizing cardiac progenitor function and cell expansion protocols for clinical application [21].

A previous study from our lab, suggests that Notch-mediated reversible EMT process is a mechanism that regulates explant-derived c-Kit+ and c-Kit- cells. Specifically, Notch stimulation augmented, while Notch inhibition suppressed, mesenchymal transition in both c-Kit+ and c-Kit- cells. In c-Kit+ cells, Notch stimulation reduced, while Notch inhibition up-regulated expression of pluripotency marker such as Nanog and Sox2. Notch induction was associated with degradation of β-catenin in c-Kit- cells. In contrast, Notch inhibition resulted in β-catenin accumulation, acquisition of epitheloid morphology, and up-regulation of Wnt target genes in c-Kit- cells [22].

During cardiac aging, DNA damage and environmental stressors contribute to telomeric shortening and human cardiac progenitor cells acquire a senescent phenotype that leads to decreased stem cell function. Reversion of this phenotype through genetic modification may advance regenerative therapy. For example, Sussman et al. examined the protective effects of Pim1 to target alteration is cardiac progenitor cells induced by aging [23].

## Cell Delivery

Identifying the most efficient type of cell for ischemic heart failure may be insignificant if an efficient delivery route for that particular cell is absent. Therefore, the most efficient, up to date, cell delivery techniques are discussed in this review. Emphasis is placed on the retrograde coronary vein delivery technique and cardiac patch technology. Thus, delivery of therapeutic material by a retrograde venous infusion has been reported as a safe alternative to intracoronary and intramyocardial routes in preclinical large animal models [24,25]. Compared to standard antegrade coronary perfusion, venous interventions require no stop in forward blood flow; therapeutic product is retained in the myocardium allowing for longer exposure and uptake of delivered material. Unlike coronary arteries, veins are mostly disease-free, which makes this delivery route clinically attractive. Lastly, retrograde infusion provides a widespread delivery across the myocardium with an increased drug concentration in LV [26,27]. Therefore, we developed and applied a simplified retrograde coronary vein (RCV) infusion approach for a small animal model of heart failure. Using RCV delivery approach, we examined the efficacy of cardiac c-Kit+ cells in improving cardiac function in CHF rats. Our study showed that RCV infusion approach is an efficient technique for targeted cell delivery to the infarcted myocardium. Cardiac c-Kit+ cells, delivered using RCV infusion ameliorated progression of heart failure, improved cardiac function and retarded myocardial remodeling in heart failure rats [28].

## Tissue Engineering

For cardiac cell replacement therapy to be successful, a large number of cells are needed. The requirement of having a high number of cells and the long-term survival of those cells in the injured heart tissue makes it challenging for cellular therapy with cell injection. It is well established that the majority of injected cells die in the injured myocardium [29,30]. Cardiac patch technology may overcome some of the limitations of cellular injection therapy: 1) Large numbers of cells can be seeded into the patch; 2) cellular retention and survival is higher in the cardiac patch; 3) targeted delivery of the patch to the myocardium; 4) the size and shape of the patch can be controlled *in vitro*; and 5) the patch can be designed to provide a controlled release of bioactive molecules [31,32]. In addition, a higher degree of maturation could be achieved using a cardiac patch. A serious disadvantage of current cardiac patch therapy is the requirement for open chest surgery. This disadvantage excludes a large number of patients who would benefit from such therapy. However, new minimally invasive delivery systems may be developed in the near future. Also, it seems likely that a thick, vascularized cardiac patch containing adult-like CMs is needed. Also pertinent, is the requirement for standardized protocols for patch generation and maturation before translation to clinics. The beneficial outcomes of cardiac patch therapy have already been demonstrated in animal models [32].

For a cardiac patch to prove effective, it needs to be thick, but transplanting tissue sections >400 μm thick have a poor survival rate due to diffusion limitations [33]. One strategy to produce thicker tissues involves stacking layers of cardiac tissue. Previously, we demonstrated that transplantation of scaffold-free cardiac cell sheets improved cardiac function of injured heart tissue [34]. A similar scaffold-free approach was developed where cultured CM monolayers are released as intact cell sheets from a temperature-sensitive culture surface [35].

Finally, an optimum location of a cardiac patch implantation is still unclear. Typically, a cardiac patch is implanted onto the epicardial side of the heart [32,36-38]. A limitation of epicardial placement is that epicardial cells are not CMs; thus, they physically separate the patch and the host myocardium and interfere with the electrical coupling. It is well established that during development, epicardial-derived cells support myocardial growth by providing progenitor cells and mitogens [39,40,]. After injury, epicardial cells migrate into the myocardium to give rise to both new blood vessels and CMs [39,40]. A future strategy of cell therapy may incorporate the role of the epicardium in myocardial repair by introducing bioactive peptides into the cardiac patch to mobilize epicardial cells, which may eliminate the graft-host tissue barrier. An example of these molecules is thymosin β4, which was shown to promote migration of epicardial cells into the myocardium and induce both vascularization and CM survival. Another example is Follistatin-like 1, which was shown to promote integration, limit fibrosis, and improve cardiac function, CM proliferation and vascularization when delivered via an acellular epicardial patch [15].

In addition to hurdles of cell-based therapy stated above, there are numerous unanswered questions pertaining to the following: cellular handling and preparation, regenerative ability of the damaged heart tissue, inflammatory status, injection timing, endogenous cardiogenic and angiogenic potential, specific targeted pathology, and clinical endpoints.

Since the discovery of angiotensin converting enzyme inhibitors and angiotensin receptor blockers more than a decade ago, the progress in developing heart failure, novel therapies have stagnated despite the large number of randomized clinical trials in heart failure patients. There is a recent incremental improvement in outcomes that was shown with combination therapy, blockade of angiotensin receptors and neprilysin inhibition [41]. This stagnation in drug development for heart failure may be explained by the lack of drugs that address the major cause of heart failure, mainly the loss of cardiomyocytes, progression in myocardial fibrosis, and the inflammatory status of the injured heart tissue. Cellular therapy may directly correct some of those specified abnormalities. In order for this to be accomplished, a clear understanding of stem cell-based mechanisms of action needs to be elucidated before cell adaptation of standard operating procedures to specific types of heart failure patients (for example, ischemic versus non-ischemic cardiomyopathy, hypertrophic, dilated cardiomyopathy, etc.) and the stage of the disease.

Elucidating the mechanisms of action is of paramount importance in designing an efficient cell-based therapy. The secretion of paracrine biofactors from exogenous cells appears to be a common mechanism of cell improvement of cardiac function, which led some investigators to speculate that “cardiac therapy may be achieved by development of cell-free strategies” [2]. This conclusion is unlikely, simply due to the sheer number of paracrine factors, such as growth factors, miRNAs, etc., that cells secrete in a given microenvironment. Even if one could create a cocktail of those factors, figuring out the appropriate doses, spatial, and temporal profiles of those factors may be too complicated knowing that some of these factors may work synergistically, while others may be inhibitory. Recent reports demonstrated that those factors are clustered in extracellular membrane vesicles, typically secreted by cells and readily detectable in body fluids and cell culture conditioned media. This observation may eliminate the need of separating individual factors in a cell-free manner. These factors could be delivered collectively as extracellular vesicles. These vesicles offer advantages over the delivery of specific factors that only target a single signaling pathway.

## Conclusion

The stem cell therapy field is progressing, but more basic studies are needed to answer the questions raised in this review. At this time, it appears that modified autologous cardiac-derived cells is a good candidate for cell therapy of IHF, and the retrograde perfusion via the coronary vein is an efficient cell delivery approach. This conclusion is based on the reviews of many contributions in the field and our own published data. This data also provided the scientific basis to start a Phase-1 clinical trial at our institution in the impending future. Despite the many challenges that cell-based therapy has faced during the last few decades, personalized cell therapy for IHF where gene and cell therapies are combined is not far off. With the recent advances of gene editing technologies, such as CRISPER/CAS9, genetically editing patient-derived progenitor cells prior to transplantation into damaged heart tissue is now possible. In such a scenario, one could identify a genetic abnormality in a patient’s cells, correct that abnormality using gene editing and deliver those modified cells back to the same patient. Thus, stem cell therapy will soon realize its potential as a potent treatment for IHF.

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