

## Pluripotent Stem Cells and Repair of Myocardial Infarction

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### Abstract

Pluripotent stem cells mainly refer to Embryonic Stem (ES) cells and induced Pluripotent Stem (iPS) cells. These two pluripotent stem cell types show significant similarities in their global histone modifications, gene expression patterns, and differentiation potentials. ES and iPS cells hold great promise in the field of regenerative medicine because they can give rise to all three germ layers, including cardiac lineages. Transplantation of ES cells and iPS cell-derived cardiomyocytes (ES- or iPS-CMs) has emerged as a promising treatment for ischemic heart disease. Stem cell grafts may be implanted in areas of myocardial infarction to restore cardiac function by regenerating cardiomyocytes and inducing neovascularization. The objective of this review is to briefly present the current research in the field of repairing infarcted myocardium using ES cells and iPS cells.

### Introduction

Heart failure is a leading cause of morbidity and mortality worldwide [1]. Although percutaneous coronary interventions can effectively treat myocardium infarction (MI), the management of ventricular remodeling and chronic ischemic cardiomyopathy after an MI still remains as a challenge. Stem cell therapy is a promising new approach to restore cardiac function and prevent ventricular remodeling after an acute MI. However, the therapeutic effects of stem cells in heart disease have been limited. Furthermore, it is not clear whether adult stem cells, such as skeletal myoblasts [2], bone marrow mononuclear cells, mesenchymal stromal cells, and mesenchymal stem cells (MSCs) (from bone marrow, peripheral blood, and adipose tissue), can differentiate into cardiac muscle cells and fibers after transplanted into MI heart. The current thought is that the primary mechanisms by which adult stem cells can improve heart function after an MI involve paracrine effects, such as the release of cytokines, chemokines, and growth factors, which inhibit apoptosis and fibrosis formation, enhance contractility, and activate endogenous regenerative mechanisms through endogenous circulating or cardiac resident stem cells [3,4].

ES and iPS cells are similar in their capacity to differentiate into cardiac lineages and, therefore, improve heart function after MI. Hence, these two cell types hold great promise in cardiac regenerative medicine.

### ES Cells and Repair of Myocardial Infarction

The most well-known type of pluripotent stem cell is the ES cell, which was first isolated in 1981 from mouse blastocysts by two independent groups [5,6], and again in 1998 from human blastocysts by Thomson's group [7]. ES cells possess the ability of self-renewal, indefinite proliferation and differentiation into all the cell types found in the body. Human ES (hES) cells have indisputable cardiomyogenic abilities and have been extensively investigated for the repair of heart failure by implantation into the heart. The three most frequently used methods to differentiate ES cells into functional cardiomyocytes are [1] co-culture with mouse visceral endoderm-like stromal cells [2,8],

spontaneous embryoid body (EB) differentiation in suspension and monolayer differentiation [3,9,10]. In addition, cytokines and small molecules can enhance cardiac differentiation [11-13].

Several groups have postulated the possibility of regenerating the myocardium by transplanting undifferentiated ES cells, ES-derived cardiac progenitor cells, such as Flk1 positive cells, islet1 positive cells, or ES-CMs. However, the tumorigenic potential of transplanted ES cells was proved to be a hindrance. Studies on transplantation of hES-CMs in mice, rats, and guinea-pigs [10,14,15] have shown that hES-CMs are safe and survive in the heart and improve heart function in areas of MI. One current challenge is to derive phenotypically stable cardiac cell populations from human ES cells in numbers sufficient for repair of MI. Recent evidence has shown that more than one billion hES-CMs cells can be produced and cryopreserved with good viability [16]. These cryopreserved hES-CMs have been transplanted via intramyocardial delivery into a non-human primate monkey heart model of myocardial ischemia, and they generated extensive remuscularization in the infarcted heart after reperfusion. The grafts were perfused by host vasculature, and electromechanical junctions formed between graft and host cardiomyocytes within 2 weeks of engraftment. Importantly, there was electromechanical coupling as indicated by the synchronization of the grafts' regular calcium transients to the host's electrocardiogram. Moreover, grafting of hESC-CMs attenuated remodeling process of MI heart [14,16,17].

Although hES cells have shown the greatest cardiac differentiation potential, their clinical use has been hampered by their tumorigenic potential, their immunogenic properties, and the ethical issues related to their embryonic origin. For these reasons, the discovery of iPS cells, which closely resemble ES cells but can be easily derived from adult cells, has provided an exciting alternative for bypassing these ethical and immunogenic concerns [18-20].

### iPS Cells and Repair of Myocardial Infarction

In 2006, Takahashi and Yamanaka [8] were the first to reprogram somatic cells into ES-like pluripotent stem cells by introducing 4 key pluripotent factors: Oct-3/4, Sox2, c-Myc, and Klf4. The

reprogrammed cells were called iPS cells because they resembled ES cells in morphology and developmental potential. For this groundbreaking contribution, Yamanaka et al. were rewarded the Nobel Prize in 2012. Similar reprogramming was soon accomplished in various mouse and human tissues by transducing a defined set of viral-transcription factors [21-23]. However, viral-transcription factors integrate into the host genome and may lead to tumorigenesis. To avoid this serious complication, non-viral integration methodologies or virus-free transfection were developed [24-28]. Recently, iPS cells have been successfully generated by the induction of only small-molecules [29]. These non-viral integration methodologies can be very useful for generating iPS cells for safe clinical applications.

iPS cells, similar to ES cells, show unlimited self-renewal and demonstrate pluripotency by giving rise to lineages of all three germ layers. Further, iPS cells can be induced to differentiate into functional cardiomyocytes, which are very similar to those generated from hES cells. Human iPS (hiPS) cells can differentiate into cardiac cells with nodal-, atrial-, and ventricular-like phenotypes that are responsive to  $\beta$ -adrenergic stimulation [9,30], and display gene expression patterns and electrophysiological properties similar to those of ES-CMs [30-32]. When iPS cells were transplanted by intramyocardial delivery into the infarcted hearts of immunocompetent mouse models, they successfully differentiated into cardiomyocytes, smooth muscle cells, and endothelial cells, and this resulted in significantly improved cardiac function [33].

However, owing to their embryonic characteristics and viral reprogramming factors, although transplanted undifferentiated iPS cells contributed to the cardiac lineages in heart tissue, these engrafts had the potential for tumorigenesis [33]. To bypass this serious limitation, one strategy is that cardiac cells were derived from iPS cells *in vitro* before implantation. These transplanted hiPS-CMs remained within the infarcted heart and decreased cardiac remodeling after ischemic damage. Importantly, no tumor formation has been reported. This occurred despite the immature state of the cardiomyocytes generated using current protocols [34]. One limitation of MI modeling is that it requires high yields of phenotypically mature hiPS-CMS. Unfortunately, the efficiency of the process of cardiac differentiation from iPS cells is still low for cardiac regenerative medicine applications. To increase the efficiency of hiPS-derived cardiac differentiation, strategies of enhancing hES-CMs formation are being investigated [13,35,36]. Alternative strategy is using non-viral integration methodologies or virus-free transfection or small molecules to generate iPS cells, which will be conducive to avoid tumorigenesis through viral vector genomic DNA integration [24-29].

iPS-derived cardiac progenitors have been demonstrated to be another promising source for MI therapy. Cardiac progenitors have an ability of proliferation and differentiation into cardiomyocytes, smooth muscle cells and endothelial cells [37]. For example, iPS cell-derived NK2 homeobox 5 (Nkx2-5) positive cardiac progenitors or iPS cell-derived fetal liver kinase-1 (Flk-1)+ progenitor cells showed multipotency and capable of differentiating into endothelial cells, smooth muscle cells and cardiomyocytes. These cardiac progenitor derived cardiomyocytes are capable of forming electrically and mechanically coupled large-scale cell cultures with mature electrophysiological properties *in vitro* [37,38]. *In vivo*, studies showed that iPS cell-derived Flk-1 positive progenitor improved cardiac function in a mouse model of acute MI [40]. Another type of iPS cell-derived cardiac progenitor, LIM-homeobox transcription factor islet-1

(Isl1) positive cells were demonstrated to survive and to differentiate into cardiac lineage after transplanted into MI hearts [41].

Long term survival and remain (Long term survival and maintaining) of engrafts in MI heart have been the challenge. Recently, three dimension (3D) tissue engineered have been (Recently, three dimensional (3D) tissue has been engineered and) applied for constructing tissue patch by seeding purified iPS-CMs for repairing the post-MI heart. 3D tissue patch seeded hiPS-CMs were beating and showed a cardiac muscle-like structure with anastomosing vessels with the host's jugular arteries and veins after they were ectopically transplanted to the neck portion of other rats [42]. Heart function of MI mouse was significantly improved by injection into the infarct of this PEG-fibrinogen (PF) scaffold seeded with iPS cells engineered to secrete matrix metalloproteinase [9]. Therefore, survival and integration of allografts in the ischemic heart can be significantly improved with the use of bioengineered therapeutic cells [43]. Transplanting patched cardiac tissue may become a new treatment option for heart failure.

In summary, there exist significant similarities between iPS cells and ES cells, such as similar pluripotency, indistinguishable global histone modification and gene expression patterns [19,44], and advanced histocompatibility match. ES cells applications in cell therapy have been promising, but ethical concerns have precluded the cells' use in clinical settings because they are derived from embryos. iPS cells provided an exciting alternative to ES cells because iPS cells closely resemble ES cells, can be easily derived from cells from adult patients, and bypass these ethical and immunogenic concerns.

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