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# Stem Cell Therapy: The Methods in the Madness

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## Abstract

The diagnosis and treatment of heart failure remains meagre even with therapeutic progression in recent times. The possibility of cardiac transplantation is of great risk and restricted by a dearth of donors. Earlier heart was considered to be a terminally differentiated organ inept to regenerate. However, several preclinical and clinical studies have demonstrated that heart could revive to normal function following cell therapy. Over years, investigators have looked at the potential and usage of several kinds of stem cells, which could radically improve the understanding of the regenerative capacity of the heart. Stem cell-related cardiomyocytes regeneration strategies have shown that cell based therapies can mend cardiac function and the repercussions of this are triggering great anticipation. We discuss here current knowledge and status of regenerating adult mammalian heart by stem cell therapy. We also consider the various stem cell and progenitor-cell types that might regenerate the myocardium and review the major clinical trials of them.

**Keywords:** Cardiac stem cell therapy; Pluripotent Stem cells; Cardiomyocytes; Cardiovascular disease

**Abbreviations:** MI: Myocardial Infarction; HSCs: Haematopoietic Stem Cells; iPSCs: Induced Pluripotent Stem Cells; CSC: Cardiac Stem Cells; CPCs: Cardiac Progenitor Cells; ESC: Embryonic Stem Cell; ESC-CM: Embryonic Stem Cell Derived Cardiomyocytes; TNF: Tumor Necrosis Factor; BMCs: Bone Marrow–Derived Stem Cells; PBSCs: Peripheral Blood–Derived Stem and Progenitor Cells; MHC: Major Histo-Compatibility Complex; VEGF: Vascular Endothelial Growth Factor; GvHD: Graft Versus Host Disease; LVEF: Ejection Fraction of Left Ventricle; SPECT: Single Photon Emission Tomography; SP: Side Population; CSps: Cardiosphere; CDCs: Cardiosphere Derived Cells.

## Introduction

Cardiovascular diseases are the major cause of death worldwide with ischemic heart diseases topping the list with about 7.4 million deaths in 2012 alone (WHO). While elder people are more susceptible to heart failure; men are more prone to myocardial infarction [1]. Myocardial Infarction (MI) represents a condition in which there is no enough blood supply to heart muscles leading to heart failure. Whereas, there may be multiple causes for cardiac failure like hypertension, congenital cardiac disease, blockage in coronary artery, cardiomyopathy (chronic disease of heart muscle), defects in heart valves, but the end result is death of myocytes. Moreover, the condition in these diseases is further worsened due to the inability of the heart to regenerate at the same rate as the cells are lost during MI and thereby rendering the heart relatively less functional or more often leading to heart failure. Current clinical practice for heart failure is surgery to bypass or open (stent) blocked arteries to improve blood supply to (re-vascularize) the heart. However, as the stent is not native to the body, it spurs an immune response. Furthermore, one of the shortcomings of vascular stents is the potential for restenosis via the growth of a thick smooth muscle tissue inside the lumen, the so-called neointima. Patients with end-stage congestive heart failure are deliberated for cardiac transplantation, but the demand for this therapeutic methodology significantly overshadows the disposal of donor hearts.

Mammalian myocardium do harbour mechanisms for endogenous regeneration, but there are several hindrance like inflammation, fibrosis etc. which arrest the cardiac repair (Table 1). Beside there are several reports which suggests that the potential of cardiomyogenesis declines with age [2]. Moreover non-functional fibrous tissue replaces the dead myocardium. Cardiomyocytes have been reported to essentially undergo programmed cell death (apoptosis) [3] and remodelling prior to heart attack. Following remodelling there is reduction in Left Ventricular (LV) function [4]. Healing of the heart will require addition of new cardiomyocytes which could take over the function of lost cardiomyocytes. However, though it was earlier considered that the adult cardiomyocytes are terminally differentiated cells i.e. they do not regenerate [5], but now it is well established that cardiac cells present in heart differentiates into various cell types [6]. Heart harbour many stem cell types. The adult rat heart contains a population of undifferentiated cells which express surface antigens typically found in hematopoietic stem cells (HSCs): c-kit, MDR1, and Sca-1 [7]. Adult cardiac cell with Sca-1 marker differentiates into beating cardiomyocytes [8]. But that is not enough to treat heart attack.

In the past couple of years, numerous clinical trials have advocated the usage of stem cells as an impending therapeutic modality to address this unmet clinical requirement. Different stem cell types such as ESCs, iPSCs, HSCs, MSCs etc have been used in both animal studies and patients to stimulate the restoration of damaged myocardium. Pluripotent stem cells harbour the potential to generate functional cardiomyocytes. Stem cells hold the ability to form new differentiated cell types including cardiomyocytes. It has been already shown that

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|---|---|
| Resident cardiac stem cells | • Cells are insufficient in number<br>• Stem-cell potential declines with age<br>• Sparse proliferation and differentiation<br>• Signs of inflammation, fibrosis and scanty blood vessel regeneration |
| Bone-marrow stem-cell | • Inadequate mobilization<br>• Inefficient homing<br>• Inadequate multipotency<br>• Signs of inflammation, fibrosis and little angiogenesis |
| Cardiomyocytes | • Inadequate mobilization and Inefficient homing<br>• Inadequate multipotency<br>• Signs of inflammation, fibrosis and insufficient angiogenesis |

**Table 1**: Potential hurdles to endogenous cardiac repair.

| **Attributes** | **ESCs*** [26] | **iPSCs*** [40] | **EpiSCs*** [108] | **MSCs*** [48] | **HSCs*** [3] | **CSs* and CDCs*** [117] | **CSCs*** [111] | **Skeletal myoblast** [47] |
|---|---|---|---|---|---|---|---|---|
| Source | Blastocyst of embryo | Reprogramming of somatic cell | Epiblast layer of mammalian embryo | Bone marrow, Umbilical cord. | Bone marrow And circulating blood | Cardiac ex-plant culture | Found in 'stem cell niches' of adult mammalian heart. | Skeletal muscle fibers |
| Potency | Pluripotent | Pluripotent | Pluripotent | Pluripotent | Multipotent | Multipotent | Multipotent | Multipotent |
| Immunogenicity | Yes | No | Yes | Low | Low | Yes | Yes | Yes |
| Teratoma formation | Yes | Yes | Yes | Yes | No | No | No | No |
| *In vitro* proliferation | Yes. | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Chimera formation | Yes | Yes | No | No | No | No | No | No |
| Patient specific | No | Yes | No | Yes | Yes | Yes | Yes | Yes |
| Ethical issues | Yes | No | Yes | No | No | No | No | No |

*ESC= Embryonic Stem Cell; *iPSCs = induced Pluripotent Stem Cell; *EpiSCs= Epiblast Stem Cells; *MSCs= Mesenchymal Stem Cells; *HSCs= Hematopoietic Stem Cells; CSs= Cardiospheres; CDCs= Cardiosphere-Derived Cells; CSCs= Cardiac Stem Cells.

**Table 2:** Comparison of ESC, iPSCs, EpiSCs, MSCs, HSCs, CSP, CSCs and skeletal myoblasts.

several types of stem cells such as HSCs as well as bone marrow- derived mesenchymal stem cells could regenerate cardiomyocytes [9]. Further these stem cells can regenerate to various vascular cells. Makino et al. have reported that mouse bone marrow-derived mesenchymal stem cells (CMG cells) differentiate into cardiomyocytes after 5'-azacytizine treatment [10]. Some vertebrates, such as newts [11,12], zebrafish [12], and neonatal mice [13] regenerates myocardium following experimental injury [14]. Induced Pluripotent Stem cells (iPSCs), Bone marrow derived stem cells, endogenous Cardiac stem cell (CSCs), embryonic stem cell and Cardiosphere are supposed to have considerable potential in myocardial regeneration. Different stem cell types vary in their cardiomyocytes regeneration potential (Table 2). The main cardiac cell types can now be generated in clinics, and gradually we are learning the rules for building myocardium and keeping it alive after transplantation but it's not that easy. Adult stem cells hold great promise incardiac regeneration [15]. The major challenge facing the field of adult CPCs (Cardiac Progenitor Cells) is to develop protocols with higher yields of definitive cardiomyocytes. Researchers studying pluripotent stem cells need to identify the optimal stage of differentiation and demonstrate that these cells can be used without tumorigenesis. Several discrepancies have been reported in autologous bone marrow stem cell trials. There are several disagreements regarding individual trials. As per meta-analysis data, trials with fewer discrepancies were tend to have smaller effect on ejection fraction of stem cell therapy and trials that had no discrepancy had no significant stem cell therapy effect [16]. The treatment of the patients suffering from MI or congenital heart failure with human stem cell based therapies has seized the imagination of scientists. In last one decade stem cell research, has made significant progress from bed to bench side which is exhibited by the existing preclinical data's. However, there are still hues and cry in society arguing that trials are still premature because mechanistic comprehensions are inadequately addressed.

Let us consider the various stem-cell and progenitor-cell types that might regenerate the myocardium and review the major clinical trials of such therapy.

## Pluripotent Stem Cells

The stem cells that demonstrate pluripotency are embryonic stem (ES) cells, embryonic carcinoma cells, embryonic germ cells, and induced pluripotent stem (iPS) cells. Amongst pluripotent stem cells, iPS cells and ES cells are most likely to be a novel impending source of cells for the treatment of various degenerative diseases including cardiovascular diseases owing to their self-renewal potency. Pluripotency and cardiomyocytes are identified by various markers expressed as proteins in cell.

### Embryonic stem cells (ESCs)

Embryonic stem (ES) cells are pluripotent in nature i.e. they can differentiate into any cell type present in the adult human. They unambiguously fulfil all the requirements of stem cells: clonality, self-renewal and multipotency [17]. ES cell can give rise to entire myocardium. ESCs are highly proliferating cells and have a potential to give rise to the cardiac cells. This gives ESCs an edge over other type of cells [18]. Human embryonic stem cells (hESCs) differentiate into myocytes with similar properties to cardiomyocytes [19]. The ESCs application in cardiac tissue regeneration started way back in 2002. Min JY et al injected ESCs into MI (myocardial infarction) rat model [20] for the first time and discovered that ESCs survives and regenerate cardiomyocytes and significantly improves heart function [21]. Many strategies exist to produce enough ESC derived cardiomyocytes (ESC-CMs) for cell replacement therapy [22-25]. But still they are not employed in treatment because of obstacles of immunological rejection. Moreover, they also cannot be employed due to ethical problem. ESCs are harvested from inner cell mass of human blastocysts by killing embryos [26]. ES cells show signs of teratomas when injected in vivo [27,28]. As information regarding pathways involved in ES cell

differentiation and heart embryonic development increases day by day, ES cell differentiation may become more controllable to reduce teratoma.

Methods to limit teratoma formation include genetic selection of differentiated ES cells [29] or differentiation of ES cells *in vitro* into cardiomyocytes or endothelial cells before injection [30,23]. Tumour-necrosis factor (TNF) promotes the differentiation of ES cells into cardiomyocytes [31]. An inherent difficulty in controlling the growth and differentiation of ES cells and other pluripotent stem cells is that the timing with which specific signalling pathways are activated is very crucial. For an instance, studies on mouse and zebra fish embryos disclosed that the role of the Wnt–beta-catenin pathway in cardiac development varies depending on the developmental stage [32]. HESCs (human embryonic stem cells) differentiate into cardiomyocytes in the presence of BMP4 and activin A [23]. However, number of pathways has been revealed to govern this primary step of cardiac differentiation: Nodal/Activin, BMPs, canonical Wnt signaling as well as Notch signalling [33].

### Induced pluripotent stem cells (iPS cells)

Within the nine years of their discovery, induced pluripotent stem cell (iPSCs) has gained much attention and hold great expectations towards myocardium regeneration. IPSCs have high capacity for cardiac regeneration as they have ability to differentiate into most of the body cell lineages. Besides that iPSCs have infinite growth too [26,34,35]. IPSCs are somatic cells generated by ectopic expression of OCT3/4, Sox2, KLF, c-Myc (Yamanaka's factors) [21]. This invaluable work of direct reprogramming was completed by Takahashi and Yamanaka using retrovirus mediated transduction into mouse fibroblasts [10]. IPSCs are similar to embryonic stem cell in pluripotency [36]. Therefore, iPSCs have potential to differentiate into cardiomyocytes in the cell culture [37-39]. Human iPS cells were established in 2007 [34,40]. The reprogramming factors can be transduced with adenoviruses, sendai viruses, plasmid vectors, and removable transposon systems [41-46]. Dinender K. Singla et al first successfully generated iPS cell line from cardiac ventricular cell type H92c using four stemness factor: Oct3/4, Sox2, Klf4, and c-Myc [15]. Thus, they laid the foundation to clinical use of iPS cell in myocardium repair. Advantages of iPS cell include that these are patient-specific pluripotent cell. For generation of iPSCs, autologous tissue is preferred over allogeneic tissue for the issue of immune-rejection. However, iPS technology cannot be translated directly from bench to bedside due to safety issues and fortunately porcine has evolved as a good model for testing iPSCs potential to regenerate myocardial tissues. Porcine cardiovascular system is more closely related to human in comparison to mice [47]. Pig pluripotent stem cells are known to improve left ventricular function and perfusion [48]. Moreover, differentiation of porcine iPSCs into rod photoreceptors and their successful integration into retina has also been reported [49]. However, development of optimal protocol and culture conditions specific for porcine iPSCs culture is prerequisite for further progress in this field.

iPSCs have tremendous proliferative capacity for myocardium regeneration [50]. However, there are certain disadvantages which are associated with iPS cell technology such as tumour formation. This has been shown in two separate studies in mice and rats where intramyocardial transplantation of iPS cell indicated tumorigenicity [51,52]. c-Myc is an oncogene. Therefore, strategies have been designed to eliminate c-Myc from reprogramming [53,54]. Various integrating delivery systems (like linear DNA vectors or viral vectors) for induction of pluripotency may lead to the tumor formation or insertional mutagenesis due to undesired transgene reactivation [55]. Moreover, integrated provirus may cause mutation into the gene in which it gets integrated, thereby leading to disruption of function. It takes much longer time to derive and characterize iPSCs from patient in comparison to cardiac or progenitor cell [56]. The reprogramming efficiency is very low. These cells might be immunogenic and they might have genome instability [57]. IPSCs tend to lose their pluripotency with time after few passages. iPS cell may cause immune response even in closely related recipient [58]. Despite all this, still there is a need for clinical grade iPS cell lines. Unless these hurdles are overcome, true clinical realization of iPS cells is not possible.

## Adult Stem Cells (Somatic Stem Cells)

Different adult stem cells can be seen as the right candidates for cell therapy. The findings of numerous stem cell populations possessing cardiogenic potential and the development of methods to isolate and expand these cells have shaped the concept of cell based recuperative therapy [59,60]. These cells can be characterized according to their tissue of origin. A variety of different stem cell types have been used for the clinical application. Diverse type of adult cardiac stem cells and progenitor cells, including human myoblasts, mesenchymal, $CD34^+$ and $CD133^+$ autologous human bone marrow–derived stem cells (BMCs), peripheral blood–derived stem and progenitor cells (PBSCs) have been used for the therapy for end-stage heart failure.

### Mesenchymal stem cell (MSCs)

Clinical application of embryonic stem cells (ESCs) and induced Pluripotent stem cells (iPS) have been limited by the fact that even one undifferentiated stem cell has the potential to give rise to a tumor and this aspect of ES cells and iPS cell compromise their application in clinical purposes. Recently, lot of attention from various labs has developed in MSCs. MSCs are safer as compared to iPS and ES cells as they don't give rise to tumor. Moreover, they are free from ethical concerns. Besides, the medical significance of MSCs till now appears predominantly derived from their non-stem/progenitor cell properties. Explicitly, MSCs yield extracellular vesicles, together with exosomes, and a multitude of cytokines and growth factors that subdue immune responses by deterring B- and T-cell propagation, monocyte maturation and by stimulating generation of regulatory T-cells and M2 macrophages [61]. Hence, even though few claim that on the basis of in vivo differentiation potential or ability to support haematopoiesis, MSCs shall be defined [62]. Others argue for a wider definition that places less prominence on the 'stem' properties of the cell and more on the trophic and immunomodulatory properties that render them potentially valuable in treating several diseases [63]. Because of this, MSCs could play a key role in the area of regenerative medicine. MSCs are being explored to replenish damaged tissue resulting from cardiovascular disease and myocardial infarction [64]. Globally, for the evaluation of the potential of MSCs based cell therapies there are 344 registered clinical trials in various phases [65]. MSC represents a stem cell population present in adult body tissues.

These were first isolated from bone marrow [66,67]. They constitute 0.001–0.01% of bone marrow cells [68,69]. Almost all tissues (adipose tissue, muscle, liver, lung, umbilical cord, bone marrow) harbour MSCs and can be successfully proliferated in in vitro conditions [70]. However, it has been reported that there could be a surge of five major histocompatibility complex (MHC II) during in vitro culturing of MSCs. This indicates that one should use freshly isolated MSCs [71]. MSCs play important role in regulating haematopoietic environment [72]. MSC ablation disrupts haematopoiesis [73]. MSCs proliferate promptly

in vitro and owes potential to give rise to tissues of mesenchymal origin such as chondrocytes, adipocytes and osteocytes [74,75]. Human MSCs have multiple differential potential [76]. Human MSCs are different from murine MSCs in marker expression and behaviour [18]. MSCs differentiate into myogenic phenotype [77] and cardiomyocytes [78]. However, MSCs regenerative capacity decreases with ageing. Therefore, they cannot be used in therapy for old patients. MSCs can be found in small numbers in peripheral blood [67]. MSCs express cell surface markers CD73, CD105 [66], CD29, CD44, CD90 [79,80] and lack CD34 and CD45 [81,82]. Presence or absence of these markers and adherence to plastic in standard culture conditions are the minimal criteria to define MSCs [53]. MSC are observed to differentiate into cardiac like cells in the presence of 5-aza cytidine [46,83]. Besides, cardiomyocytes can also be obtained from MSCs by supplementing culture medium with ascorbic acid, dexamethasone and insulin [84]. MSCs trans-differentiate into cardiomyocytes phenotype when cultured along with ventricular myocytes [85]. Human MSCs are known to differentiate into cardiomyocytes when transplanted into adult murine heart [86]. In pigs, allogeneic MSCs safely engraft and express protein normally restricted to cardiomyocytes, vascular endothelium and smooth muscles [77]. Autologous MSC transplantation induces VEGF (vascular endothelial growth factor) and neovascularization in ischemic myocardium [87].

MSCs direct transplantation has shown improvement in vasculature in myocardial infarction model in canine [88], murine [89], porcine [90], and rat [91,92]. MSCs can differentiate and incorporate into vasculature as either smooth muscle cells or endothelium in differential amounts [93]. MSCs are better candidates for myocardial regeneration as they have the ability to evade and suppress host immune response to certain extent in comparison to EPCs, CSCs, and embryonic stem cells [94]. Human MSCs have the advantages of easy isolation [95], rapid proliferation [96], genetic stability and ease with tissue engineering, tolerant to immunological rejection [97] and potential to repair tissue damage. MSCs tend to generate tumor when cultured ex -vivo prior to transplantation [98]. As soon as the immunomodulatory prospective of MSCs was recognized in vitro and in initial preclinical models, MSCs were promptly carried to the clinic. Clinical administration of allo-MSCs was first achieved by Le Blanc et al. in 2004 [99]. A nine year-old boy suffering from treatment-resistant, grade IV GvHD (Graft versus host disease (GVHD) is an immune-mediated disease resulting from a complex interaction between donor and recipient adaptive immunity) was administered MSCs. Haplo identical MSCs were collected form his mother. After 170 days of bone marrow transplantation there was a rapid recovery after each MSC infusion and the patient survived beyond 1 year. However, the 24 patients suffering with acute grade IV GvHD who were not administered MSC therapy died an average of 2 months after bone marrow transplantation. This revolutionary case study showed initial sight of MSCs' therapeutic prospective [95].

Within one year of this breakthrough, Osiris therapeutics initiated enrolling patients for the first comprehensive clinical trials of allo-MSCs in the therapy of acute GvHD and acute myocardial infarction [100]. Ever since, usage of MSCs in clinical trials rose and numerous MSC cell preparations from academic and corporate institutions are being explored in nearly 350 clinical trials (>80% of which are phase 1 or 2). Clinical trials investigating the safety and efficiency of MSCs have used both allogeneic (190) and autologous (150) cells. However, as far as clinical state of MSCs stem cell therapy is concerned, the results of the clinical trials were somewhat mixed i.e. some of the initial studies exhibited that MSCs affects immune function in human [94] and mouse [101] in in vitro culture and some of the most chronic and elusive inflammatory conditions in rodent models shown sign of optimism for the prospect of treating them for e.g., MSC therapy in animal model of MI showed increased cardiac output [102]. Moreover, these findings were corroborated by the recently completed phase 1 trial, in patients within 10 days of acute MI, using a single infusion of allogeneic MSCs [103].

Intra coronary delivery of MSC under BOOST Trial showed substantial improvement in LVEF over control in initial stages but after 18 months this difference was not significant [104]. So, long term tracking of intravenously injected MSCs is required. Cardiovascular cell therapy research network in the united states conducted FOCUS-CCTRN (First Mononuclear Cells Injected In the UNITED States conducted by CCTRN) trial in which patients suffering from chronic heart failure were administered with autologous bone marrow first mononuclear cells showed no significant improvement in Left ventricular(LV) ejection fraction nor improvement of LV end systolic volume, maximal oxygen consumption and reversibility on single photon emission tomography (SPECT). However, trans endocardially injected autologous bone marrow derived cells into the heart were found to be feasible and safe. Moreover, there were indications of higher cell counts of CD34 and CD133 which was linked with LVEF (Ejection Fraction of Left Ventricle) elevation [105]. In clinical trials both auto and allo MSCs are being used.

In POSEIDON trial, the safety and efficiency of auto- and allo-MSCs were equated after subjected into the remodelled cardiac injury of patients with chronic cardiac ischemia [78]. Unfortunately, after thirty days, clinical improvement was partial in both groups. Though, both cell sources were found to be safe, evaluations of auto and allo MSCs are required in impending studies.

## Hematopoietic Stem Cells (HSCs)

Beside Mesenchymal stem cells, bone marrow harbour another set of stem cells called Hematopoietic Stem cells. Some researchers referred highly enriched HSC population isolated from bone marrow as 'side population (SP) cells' [106]. HSCs are multipotent in nature and gives rise to all blood types. Hematopoietic stem cells are also reported to transdifferentiate into various cell types such as skeletal muscle [27,107], hepatocytes [56], epithelial cells [52], neurons [108,109] endothelial cells [106] and cardiomyocytes [106,96]. HSCs are known to adopt mature haematopoietic fates in ischemic myocardium [110]. In an experiment, Jackson *et al.* identified the source of stem cell population arising after ischemic myocardium damage. They transplanted side population cells into lethally irradiated mice and then blocked coronary artery to create ischemic condition for 60 minutes. They found that engrafted 'side population cells' migrated to ischemic cardiac muscle and differentiated into cardiomyocytes and endothelial cells [106].

However, HSCs are not the good candidate for myocardial regeneration. Though Orlic *et al.* reported that adult bone marrow population rich in haematopoitic stem cells can transform themselves into myocardium and vasculature cell types within 9 days of their direct injection into ischemic heart; on contrary Charles *et al.* reports that HSCs do not transdifferentiate into cardiomyocytes in myocardial infarcts. They reported that HSCs do not readily acquire cardiac phenotype [111]. Similar report from Fukuda and Fujita in an attempt to determine the origin of bone marrow (BM) derived cardiomyocytes in mice, suggested mesenchymal stem cells to be better option in comparison to HSCs. MSCs can mobilize and differentiate into cardiomyocytes after myocardial infarction. Their result proved the origin of the BM-derived cardiomyocytes to be MSCs [71].

### Cardiosphere (CSps) and cardiosphere derived cells (CDCs)

Adult mammalian heart contains endogenous cardiac committed cells [17,112-115]. When cardiac surgical biopsy specimens are grown in culture they yielded Cardiosphere (CSps) [59]. CSps (self assembling spherical clusters) comprises a niche like environment when cultured in semi suspension on poly-D-lysine. Cardiac committed cells proliferate on the periphery in a gradient fashion while undifferentiated cells proliferate in core. Fibronectin can be used to retrieve the cell numbers required for cell therapy as CSp-derived cells (CDCs) can be cultured as monolayer on fibronectin. [116]. Cardiosphere are plated to create CDCs. Cardiosphere (CSps) and Cardiosphere derived cell (CDCs) can also be obtained from stromal cells and cardiac atrial appendage stem cells [117].

CDCs differentiate into all the three types of cardiac lineages as seen in animal cardiomyopathy model [118-120]. It has been established that both human and porcine CDCs differentiate into electrically functional myocytes [121], CADUCEUS clinical trial further supports that treatment in ischemic patients with Cardiosphere Derived Cells (CDCs) decrease scarring after myocardial infarction and regenerate viable myocardium [102,122-124] but there was negligible effect on Left ventricular ejection fraction [125]. CADUCEUS stands for Cardiosphere-Derived autologous Stem Cells to reverse ventricular dysfunction. Raj Makkar and colleagues report that Cardiosphere-derived cells (CDCs) reduce scarring after myocardial infarction. Since there were no improvements in patient's ejection fraction, it would be essential to address the following two points. First, CDCs contain sub populations, including mesenchymal cells. It would be interesting to know which fractions are responsible for the clinical effects, and whether the mechanisms of action are direct (differentiation into cardiomyocytes or vascular endothelial cells), indirect (cell cell contact or paracrine effects) or both [122].

Even though cardiac tissues contain cardiac fibroblasts, the use of cardiospheres minimizes fibroblast contamination. To test the therapeutic potential of CS/PCs three clinical trial have been conducted namely; SCIPIO (ClinicalTrials.gov Identifier NCT00474461), CADUCEUS (Cardiosphere-Derived autologous Stem Cells to Reverse ventricular dysfunction; ClinicalTrials.gov Identifier NCT00893360) and ALCADIA (Autologous Human Cardiac-Derived Stem Cell to Treat Ischemic cardiomyopathy; ClinicalTrials.gov Identifier NCT00981006).

### Skeletal myoblasts

Skeletal myoblasts were the first cells to be used to regenerate myocardium [108,126]. In study of myocardial infarction in animals and humans, injection of skeletal myoblast had shown successful engraftment of skeletal muscle fibers, graft survival and functional advantages [127]. Taylor et al reported improved myocardial performance when they transplanted skeletal myoblast into cryoinfarcted myocardium of some rabbits. They found Islands of different sizes comprising elongated, striated cells that retained characteristics of both skeletal and cardiac cells in the cryoinfarct [128]. For example, intramyocardial injection of autologous cultured skeletal muscle-derived stem cells into patient with old myocardial infarction is safe and shows increased global and regional left ventricular function [78,129,130]. Skeletal myoblast are not so promising. They do not beat in synchrony with the surrounding myocardium [129]. They tend not to integrate electrically with heart cells. Myoblasts are resistant to ischemia. Human trials of myoblasts in heart failure are going on. But it is seems unlikely that skeletal myoblasts will be able to truly regenerate myocardium.

### Cardiac stem cells

Few stem cells are present in heart too. These are called Cardiac Stem Cells. These can express the cell-surface markers c-kit or Sca1 [112]. A definitive marker for CSCs has not yet been identified. Adult Cardiac Sca-1+ cells are transformed into functional cardiomyocytes in vitro by treatment with oxytocin [7]. Cardiac Stem cells are concentrated in specific areas of cell for example atria or pericardium [131]. No clinical data using CSCs are available yet. It is hard to proliferate CSCs in vitro. CSCs reside in clusters consistent with the existence of cardiac niches. The factors that attract these cells out of their putative niches to an injury site remains undefined. The question also remains whether CSCs stably reside in the heart or are derived from other tissues such as the bone marrow, as has been suggested for Kit+ cells. Cardiac stem cells also express MSC markers such as such as CD90 and CD105 and ESC markers Rex1, Nanog and Sox2 [123]. Cardiac stem cells (CSCs) can generate all the components of the myocardium. CSCs can be expected to be more effective than HSCs in rebuilding dead ventricular tissue. This is because HSCs have to reprogram themselves to produce progeny differentiating into cardiac cell lineages. Such an intermediate phase is avoided by the direct activation and migration of CSCs to the site of injury.

### c-kit+ cardiac progenitor cells

New cardiomyocytes are formed from adult cardiac stem cell throughout the life. Main pool of cells identified responsible for this are c-kit positive cells. c-kit is receptor tyrosine kinase. It is expressed on surface of different cell types. It binds to Stem Cell Factor (SCF). c-kit is also called CD117. c-kit+ cells are from bone marrow [45]. These are multipotent stem cells. These differentiate into main cell types of heart: myocytes, smooth and endothelial vascular and connective tissue cells [112,132,79]. It is known that population of c-kit+ cell increase in infarcted myocardium [131]. c-kit+ cell mobilize from bone marrow to infarcted myocardium through peripheral blood. The process is called Homing. Homing can be controlled. Ge Zhang *et al* found that controlled release of Stromal Cell–Derived Factor-1alpha in situ increases c-kit+ cell homing to the infarcted heart [132].

However, recently it was shown in SCIPIO trial that myocytes obtained from c-kit+ cells were functionally insignificant but there was marked increase in cardiac endothelial cells. This suggests that c-kit+ cells can better cause neovascularization rather regeneration of myocytes [3].

## Recent Advances

A breakthrough came from Inagawa et al 2012 when they demonstrated that a combination of 3 cardiac transcription factors Gata4, Mef2c, and Tbx5 (GMT) reprograms fibroblasts directly into functional cardiomyocytes in vitro [133]. This has opened a new approach to cell therapy for direct myocyte regeneration in heart. This emerging strategy will definitely be superior to others in terms of immunogenicity. Another important advancement was made with the transplantation of iPS cells into a Japanese women eye suffering from visual impairment. She was the first person to receive tissue derived by IPS technology. A team of researchers lead by Yasuo Kurimoto at the Institute for Biomedical Research and Innovation, next to the RIKEN Center for Developmental Biology (CDB), grew a sheet of retinal tissue from a woman's skin cells, then implanted it into her eye. This has opened the door for the use of iPS cells in the Stem cell therapy of heart [92].

## Future Prospects and Concluding Remarks

It's evident that stem cells hold the potential to be used for therapy in various diseases. However, still it's a long way to go in terms of clinical application. Various researchers across the world are trying to use human stem cells derived cardiomyocytes for therapy but most

of the trials are falling short of expectations. At this point in time, an absolute necessity is the explicit, proficient and selective differentiation of stem cells into cardiomyocytes to implement stem cell therapy as a treatment. Various, stem cell types can regenerate myocardial cells but the risk of tumorigenesis is associated with them.

Even a single undifferentiated stem cell can give rise to a tumor. So, it is imperative to fine tune a protocol before we can consider stem cell therapy transition from bench to bed side. MSCs are safer for therapy as compared to other PS cells as they don't give rise to tumor and they are immune privileged. Moreover, they are free from ethical concerns. However, the injected stem cells do not sustain in in vivo conditions. Furthermore, mesenchymal stem cells (MSCs) are prone to lose their pluripotency with age. Hence, the other viable option is to generate engineered heart tissue from human stem cell derived cardiomyocytes for regeneration of decease heart. So far different methods have been developed to produce 3D cardiac tissue constructs however cardiac tissue engineering is a very new field and lots of hurdle exists in any speculation regarding the success of this technique would be farfetched right now.

Endogenous or resident cardiac cells could also play key role provided we learn their controlled proliferation. As they are present in heart themselves there is no need to graft cells. However, they are available in insufficient number in heart and show inadequate proliferation and differentiation. After years of anticipation scientists do not agree to whether cell infusions invigorate the human heart. For example available clinical data suggest that autologous bone marrow derived cells can repair cardiac function after a heart attack. The trial suggests that the cells will decrease the death rate by 20% to 25%. The numerous studies of the similar therapy, in patients with the same ailment that have unsuccessful to make a difference. Moreover, most of the studies are done in mouse and mouse is not a typical representative of mammals. In mouse studies, most of the times we witness significant effects or improvements. But as the study shifts on large animal for phase III trial we observe moderate to no improvement. The rationale behind this is that mice used for lab studies are often not healthy and young. As a result they don't echo the actuality of patients. However, from the standpoint of stem cell biology, discovery of stem cells therapeutic potential within animal models has optimistically triggered the field and we hope to make significant advances ahead.

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