Potential of different types of stem cells for cardiomyocyte regeneration

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ABSTRACT
Cardiac disorders are a major cause of death in the world today, causing a greater number of deaths as compared to even communicable diseases like AIDS, tuberculosis, diarrhea and malaria. Cardiac disease may occur due to either atherosclerosis or ischemia, both of which lead to decreased blood supply to the myocardium that would bring about necrosis of cardiac tissue, finally resulting in a decrease in the ejection fraction of the heart. Stem cells with their ability to divide and differentiate into many kinds of cells, help in regenerating the injured tissue in the heart that has lost its ability to relax and contract, thus resulting in an improvement in the overall functioning of the heart. Embryonic stem cells, haematopoietic stem cells, mesenchymal stem cells, induced pluripotent stem cells and resident cardiac stem cells are some of the types of cells capable of cardiac regeneration. This article describes the properties of these stem cells, their advantages, shortcomings and factors that affect their efficacy in cardiac regeneration.

KEYWORDS
Myocardial infarction; Stem cells; Differentiation; Regeneration.

INTRODUCTION
Heart attacks and congestive heart failure continue to be among the world’s major health challenges despite much advancement in cardiovascular medicine[1]. The destruction of cardiomyocytes can be a result of hypertension, decrease in the blood supply to the heart muscle caused by coronary artery disease, or a myocardial infarction leading to the sudden closing of the coronary artery causing anoxia. Despite improvements in surgical procedures, drug therapy and organ transplantation, a greater part of the patients with congestive heart failure die within few years of initial diagnosis. Scientists are now exploring ways to save lives by introducing new cells to replace dead or injured cells so that the weakened heart muscle can regain its pumping action. One important type of cell that can be regenerated is the cardiomyocyte, that contracts to eject the blood out of the ventricle. Two other cell types that are important for appropriate functioning of the heart are the vascular endothelial cell, which forms the inner lining of blood vessels and the non striated muscle cell, which forms the wall of blood vessels. Researchers have discovered that under highly specific growth conditions,
stem cells can develop into new cardiomyocytes and vascular endothelial cells in vitro[2]. This ability to provide tissue for the damaged heart may be exploited for human benefit. This approach has enormous advantages over heart transplant, particularly in the light of scarcity of donor hearts available to meet current transplantation needs. Until now, the lack of a suitable human cardiac cell source has been the major hindrance in regenerating the human myocardium, either by cardiac tissue engineering or by cell-based transplantation. Cardiomyocytes grow to be terminally differentiated soon after birth and lose their ability to divide and proliferate[3].

The potential of the following types of stem cells for cardiomyocyte regeneration are examined:

**Embryonic stem cells**

Embryonic stem cells (ESCs) are procured from the inner cell mass of 5-6 day blastocysts. They have unlimited expansion ability and unrestricted plasticity, offering a reliable source of cells that are restricted to the lineage of cells in need of repair and regeneration[4]. They are regarded as a single source of diverse cell types (endothelial, fibroblasts, cardiac muscle cells) required for cardiovascular regeneration[5]. ESCs are seen as an appropriate source for generation of unlimited quantities of cardiomyocytes[6]. In addition, they are capable of differentiating into other cell types like endothelial cells, thus showing the capability of forming all the required cardiac tissue components apart from cardiomyocytes[5].

ESCs have the ability to produce factors like VEGF, IGF, TNF α, ß, IL-1. Collectively, these paracrine factors help in survival, formation of new blood vessels, differentiation, remodeling and contraction[6]. When human ESCs were cultured in vitro in suspensions, embryoid bodies were formed. These embryoid bodies can differentiate along all the three germ layers. 8.1% of these embryoid bodies were seen to have spontaneously contracting areas. They also stained positively when stained with anti-cardiac myosin heavy chain. RT-PCR showed the expression of numerous cardiac-specific genes and transcription factors[8]. hESC-derived cardiomyocytes were seen to show properties of premature cardiac cells. ESCs are seen to possess the property of transdifferentiation, whereby they can differentiate into particular cell types by reprogramming[9]. This would allow the use of cells from autologous sources thus preventing the risk of rejection of exogenous cells due to a mismatch.

However, there are a few drawbacks of using ESCs as a source of stem cells for cardiac regeneration. Embryoid bodies contain a large number of cell types, making cardiogenesis extremely inefficient. Also, undifferentiated hESC may lead to the spontaneous formation of large, benign masses of haphazardly differentiated tissues called teratomas[10-12]. Ethical issues are a major hurdle in the use of hESC for research and therapy[13]. This is because production of a hESC line involves the destruction of an embryo. Stem cells derived from the embryo are allogenic and carry the risk of rejection when transplanted into the host[14].

It was seen that when ESCs were transplanted into mice after Doxorubicin induced cardiomyopathy, it led to significant decrease in various adverse pathological mechanisms as well as enhancement in cardiac regeneration[15]. In another study, when mice ESCs growing on collagen I/II scaffolds was modified with adhesion peptides RGD (arginine-glycine-aspartic acid), the embryoid bodies were observed to differentiate efficiently into beating cardiomyocytes on collagen scaffolds[16]. The use of microRNAs as modulators in ESCs, was seen to provide enhanced cardiomyocyte differentiation, resulting in enhanced cardiac repair, regeneration and function in mice[17]. SSEA-4, Tra 1-60, Tra-181 and Oct-4 are the prominent ESC markers and on differentiation to cardiac cells, they express cardiac troponin I[18].

**Haematopoietic stem cells**

Haematopoietic stem cells (HSCs) are located in the bone marrow[13]. There are many sub-populations of cells in the bone marrow, and these need to be sorted based on various markers[14]. Significant cardiac improvements were seen in patients administered with CD34+ bone marrow cells[16]. These are multipotent cells with a promising potential to treat an array of conditions.

HSCs are taken from the patient to whom they must be administered for treatment. Thus, they are considered to be an autologous source of stem cells and can easily cross the immunological barrier, posing no risk of rejection in the host. Unlike hESC, which is fraught by ethical issues, HSCs have no such complications as they
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do not involve the destruction of an embryo. HSCs are derived easily from the hip bone of the patient and pose no problems of lack of donors[13]. HSCs exhibit plasticity and can give rise to all types of blood cells, cardiac tissue and blood vessels[19]. This is credited to their capacity for multilineage differentiation and self-renewal[20]. Nevertheless, since HSCs are derived from the patient’s own body, there is a time delay between isolation of cells, purification, and consequent selection of adequate number of cardiac progenitors ex-vivo and finally introduction of these cells into the patient.

Cell therapy is seen to be highly effective only when a well defined population of cells is administered[13]. Knowledge of the mechanisms by which stem cells undergo extravasation, followed by homing at the site of cardiac injury is extremely important to optimize therapeutic use of stem cells[19]. Very few of the injected cells are seen to develop into cardiomyocytes[21].

In a placebo controlled phase II study conducted to evaluate the outcome administration of granulocyte colony stimulating factor (G-CSF) on transplanted HSCs, it was seen that early treatment with G-CSF resulted in increased perfusion to the infracted area, which may be due to enhanced neovascularization[24]. In a rabbit model, it was noted that in the initial stages of myocardial ischemia, the increase in cytokines TNFá and VEGF was accompanied by mobilization and consequent homing of HSCs into the infracted myocardium[25]. The various markers that prove differentiation into cardiomyocytes include cardiac á/â myosin heavy chain, sarcomeric myosin heavy chain and cardiac troponinI[26].

Mesenchymal stem cells

Among the various kinds of stem cells used for cardiac function restoration, numerous pre-clinical trials have been performed on mesenchymal stem cells (MSCs), in order to evaluate their effectiveness in regenerating the heart after a myocardial infarction[27]. They exhibit multipotency and are precursors to muscle, bone, tendon, ligaments, adipose tissue and fibroblasts[28,29]. They can be isolated, expanded in the lab to a sufficient quantity and then used for regeneration into cardiomyocytes, smooth muscles and endothelial cells due to their capacity of multi-lineage differentiation[30,31]. Due to their capacity for quick division in culture, MSCs from one donor may be collected, cultured in-

Figure 1 : Representative human ESC-derived cardiomyocytes, differentiated using the monolayer protocol (top timeline in a), immunostained for á-actinin (red) and CX43 (green). Nuclei are shown in blue. Representative human iPSC-derived cardiomyocytes, differentiated using the monolayer protocol (top timeline in a), immunostained for á-actinin (green) and the transcription factor NKX2.5 (red). Intracellular [Ca$^{2+}$] ([$Ca^{2+}$]) transients in a human ESC-derived cardiomyocyte before (black) or after (red) the application of diltiazem, an L-type Ca$^{2+}$-channel blocker. The absence of [Ca$^{2+}$] transients after diltiazem treatment indicates that extracellular Ca$^{2+}$ is required to initiate intracellular Ca$^{2+}$ release, just as in adult cardiomyocytes. $F/F_0$ denotes the change in fluorescence intensity. Human ESC-derived cardiomyocytes show the characteristic action-potential properties of either working chamber (top) or nodal (bottom) cardiomyocytes, indicating early subtype specification[37].

vitro and then used for treatment of numerous patients\[29\]. MSCs do not express HLA class II normally, and modify T-cell responses.

The regenerative capacity of MSCs decreases with age, thus their effectiveness comes down drastically when used as an autologous source in old patients\[29\]. Besides, cardiac cell therapy for older patients will require MSCs from young donors for greater renewal capacity and this involves allogenic transplantation\[32,33\]. Although MSCs do not normally express HLA Class II, recent research has suggested that MSCs may switch immune states in vivo to express HLA Class II and induce an immunogenic response\[33,34\].

In a recent study, aged MSCs in mice were initially exposed to glucose depletion to enhance age affected function. This resulted in enhanced expression of paracrine factors like IGF-1, FGF-2, VEGF and SDF-1α in hearts transplanted with preconditioned aged MSCs. Therefore it was concluded that pre-conditioning of aged MSCs with glucose depletion can restore the ability of aged cells to repair senescent infarcted myocardium by improving proliferation and delaying senescence\[35\]. In another study it was investigated whether trimetazidine (TMZ) could improve survival of MSCs in conditions of hypoxia. It was also tested whether TMZ could influence the rates of survival, differentiation, and subsequent activities of transplanted MSCs in rat hearts that had been subjected to acute myocardial infarction (AMI). It was concluded that implantation of MSCs combined with TMZ treatment resulted in up-regulation of anti-apoptotic proteins and was superior to administration of MSCs alone, due to superior MSCs viability and cardiac function recovery\[36\]. A study conducted to evaluate the effect Tongxinluo (TXL) treatment around transplanted MSCs in swine hearts with acute myocardial infarction (AMI), concluded that TXL treatment resulted in a significant survival and differentiation potential of implanted MSC by inhibition of apoptosis and oxidative stress accompanied by significant benefits in cardiac function\[38\].

MSC differentiated into cardiomyocytes express markers like Nkx2.5, human atrial natriuretic peptide, myosin light chain-2α and GATA-4\[39\].

**Induced pluripotent stem cells**

Somatic cells can be reprogrammed into the pluripotent state by the viral transduction of transcription factors like Oct 3/4, Sox2, Klf4, and c-Myc\[40\] or by fusion of somatic cells with pluripotent cells\[41\]. These cells can display pluripotency both in vitro and in vivo, differentiating into cells derived from all three germ layers\[40\]. Thus, generation of induced pluripotent stem cells (iPSCs) is considered a ground breaking step towards generation of patient-specific pluripotent stem cells for various applications\[42\].

iPSCs have the capacity to differentiate into atrial, nodal and ventricular cells, with properties similar to the native cardiomyocytes. They are also seen to differentiate into smooth muscles and endothelial cells, supplying the infarcted area with sufficient blood, leading to an overall improvement in cardiac functioning\[43\]. iPSCs are endowed with all the advantages of ESCs, without involving any ethical issues. These can therefore be used as an alternative to ESCs\[44\].

On the contrary, iPSCs give rise to numerous types of cardiomyocytes. Such a mixed population of cells raises concerns of pro-arrhythmia effects. Moreover, the ability of iPSCs to mature after transplantation is being investigated. Presence of undifferentiated cells may result in the formation of tumors consequent to transplantation\[44\]. In addition, use of viral vectors for transduction of transcription factors may pose potential problems in the host. These are now being replaced by adenoviruses and plasmid mediated transfections\[45,46\].

In a study conducted to assess the effect of scar tissue composition on engraftment of iPSCs into infarcted myocardium, a tricell patch (Tri-P) was created with induced pluripotent stem cell-derived cardiomyocytes, endothelial cells and mouse embryonic fibroblasts and affixed over the entire infarcted area in adenylyl cyclase 6 (AC6) overexpressing mice, 7 days after myocardial infarction. Application of Tri-P in AC6 mice resulted in higher induced pluripotent stem cell engraftment accompanied by angiomyogenesis and improvement in LV function\[47\]. In another study conducted to determine if the acquired cardiogenicity was affected by nuclear reprogramming, mouse embryonic fibroblasts were reprogrammed with or without c-MYC. It was concluded that nuclear reprogramming independent of c-MYC enhances production of pluripotent stem cells with innate cardiogenic potential\[48\]. Another study used an excisable polycistronic lentiviral vector to demonstrate proficient derivation of iPSCs.
free of exogenous reprogramming transgenes. The presence of the transgenes in the induced pluripotent cells is a matter of concern and this excisable lentivirus vector helps to replace the integrated reprogramming factors.\(^\text{[49]}\)

The markers observed on differentiation of iPSCs into cardiomyocytes are Nkx2.5, âMHC, Mlc2v, and cTnT.\(^\text{[50]}\)

**Cardiac stem cells**

Unlike previously believed, the adult heart is seen to have a potential for cardiac regeneration. This is noticed primarily after a myocardial infarction or a pressure overload.\(^\text{[51]}\) This ability is attributed to the resident cardiac stem cells (CSCs) present in the heart. However, this capacity is not sufficient to repair the heart completely after a myocardial infarction.\(^\text{[52]}\) But if these cells are transplanted in a large number exogenously, they are seen to have positive effects in the functioning of the heart after a cardiac arrest.

CSCs are of two types: myogenic CSCs (mCSCs) which give rise to cardiomyocytes and vascular CSCs (vCSCs), which yield endothelial and smooth muscle cells.\(^\text{[53,54]}\) Thus, use of CSCs can give rise to all kinds of cells required to improve overall cardiac function.\(^\text{[55]}\) CSCs are obtained from the heart and are seen to successfully form only cardiac cells, without the formation of teratomas as is the case with ESCs.

However, CSCs are seen to undergo apoptosis due to decreased telomerase activity leading to telomerase shortening. This results in reduced life of the administered CSC leading to deterioration in cardiac functioning.\(^\text{[56]}\)

Adult feline hearts, treated with Isoproterenol to induce cardiac injuries were monitored for increase in cellular proliferation by incorporation of 5-Bromodeoxyuridine. During recovery, a significant increase in the number of cells that was positive for 5-Bromodeoxyuridine was observed.\(^\text{[57]}\) CSCs are selected based on expression of the markers c-kit, Sca-1 and MDR-1.\(^\text{[58]}\)

**CONCLUSION**

A comparative study of the different types of stem cells used in cardiac repair has revealed that each category has its pros and cons. ESCs have enormous potential, but they are limited by several ethical issues surrounding its use. This has spurred the discovery of alternate sources of stem cells. HSCs and MSCs are limited by lack of regenerative capacity and controversy over their commitment to cardiac lineage. iPSCs are restricted by the use of viral vectors for reprogramming and CSCs by their reduced telomerase activity.

Stem cell therapy does offer new promise, but it cannot succeed without a thorough understanding of the mechanisms involved, which is achievable only through extensive research, apart from validation through clinical trials. In short, stem cell research has immense potential to unravel novel avenues for regenerative medicine and help in decreasing mortality rates due to cardiovascular diseases.

**TABLE 1 : Outcomes of stem cell therapy for cardiac regeneration.**

<table>
<thead>
<tr>
<th>Type of cell</th>
<th>Outcomes</th>
<th>Reference</th>
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<tbody>
<tr>
<td>ESCs</td>
<td>Preserved LV dimensions and wall thickening.</td>
<td>58,59</td>
</tr>
<tr>
<td></td>
<td>Improved left ventricular systolic function and enhanced remodeling.</td>
<td>61,62</td>
</tr>
<tr>
<td>HSCs</td>
<td>Prevent deleterious remodeling and initiates speedy recovery.</td>
<td>30,31</td>
</tr>
<tr>
<td></td>
<td>Improvement in cardiac function and inhibition of cardiac remodeling.</td>
<td>40,61</td>
</tr>
<tr>
<td></td>
<td>Reconstitution of a functional ventricular wall, reduced infarct size and LV remodeling halted.</td>
<td>23,55</td>
</tr>
</tbody>
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