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Clinical Trials in the Therapeutic Application of Embryonic and Adult Stem Cells for Cardiovascular Disease

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ABSTRACT

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality worldwide, representing a group of disorders characterized by loss of cardiac function as a result of irreversible damage to cardiomyocytes which results in scar tissue formation. Stem cell therapy are a viable option to improve cardiac function and to promote the repair and regeneration of the myocardium. Several preclinical and clinical trials have shown that transplantation of functional and healthy SCs can promote myocardial regeneration and repair. Here, we focus on the therapeutic applications of embryonic stem cells and adult stem cells to human CVD.

Keywords: Cardiovascular disease, Embryonic stem cells, Adult stem cells, Cardiac stem cells, Clinical trials, Therapy

Abbreviations: NYHA: New York Heart Association; LVEF: Left Ventricular Ejection Fraction; AMI: Acute Myocardial Infarction

INTRODUCTION

Cardiovascular disease (CVD) is currently the leading cause of death worldwide as it results in an irreversible damage to the cardiomyocytes and consequently a decline in the overall function of the heart. The discovery of stem cells with their potential to differentiate to any cell type appears to be the clue to the much anticipated cardioregeneration. Stem cell differentiation is the process whereby a cell change from one cell type to another, usually to a more specialized type. Stem cells (SCs) have the ability to divide for indefinite periods in culture and can differentiate into specialized cells-including daughter stem cells [1]. The ability of SCs to undergo such differentiation is called potency [2]. Plasticity is the ability to convert cells from one lineage to another thereby creating tissues other than that from which they were isolated [1]. The more cell types in the body that a SC can differentiate into, the higher its potency.

Differentiation capacities of pluripotent versus adult stem cells

Pluripotent SCs can differentiate into any of the three embryonic germ layers (endoderm, mesoderm, and ectoderm), and their derivatives, and thus any cell within the mature organism. However, they cannot form extraembryonic tissues [2]. Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are examples of pluripotent cells. Multipotent SCs can differentiate into multiple cell types of the parent organ only, they are more specialized than pluripotent ESCs and iPSCs and are thus limited in their potency potential [3]. Multipotent SCs are often referred to as progenitors, precursors, or somatic stem cells. Adult SCs which can be derived from various adult tissues or organs, for examples bone marrow derived hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), skeletal myoblasts (SMs), cardiac stem cells (CSCs), are good examples of multipotent stem cells (Table 1).

Table 1 Examples of adult stem cells

Isolates	Source	Brief Remarks	References
Hematopoietic stem cells	Bone marrow	They are the most characterized and have been employed in clinical settings for their therapeutic roles in hematopoietic malignancies.	[4]
Germline stem cells	Basal layer of seminiferous tubules	*C. elegans* and Drosophila, are the most studied. They are easily identified and have greater tendency for easy genetic manipulations.	[5]

Mammary stem cells	Mammary glands	Studies has revealed that the anatomy and homeostasis of the ductal tree are preserved by bipotent and unipotent mammary stem cells.	[6]
Epidermal stem cells	Basal layer of the epidermis	Highly efficient in self-renewal for extended period of time and possess ability to differentiate to any lineage within their tissue of origin	[7]
Neural stem cells	Subventricular zone of the lateral ventricle and the subgranular zone of the hippocampus	Possess ability to self-renew and differentiate to neurons, oligodendrocytes and astrocytes. Setbacks in assessing neural stem cells include, insufficient samples, poor therapeutic specificity, and efficacy, with chances for immune rejection	[8]
Intestinal stem cells	Base of the crypt of the intestinal epithelium	Consist of crypt plasticity leading to the conversion of progenitor cells to intestinal stem cells. However, there is insufficient data on the intestinal stem cells of humans.	[9]
Hair follicle stem cells	Bulge region of the epithelial stem cells in the hair follicle	They differentiate to the seven concentric layers of a mature hair follicle. They are within the early placode epithelium before noticeable of the bulge	[10]
muscle satellite cells	Basal lamina of myofibers	They are underneath the basal lamina and exterior to the myofiber plasma membrane. They are identified by immunofluorescence stained by marker such as transcription factors and membrane proteins.	[11]

Embryonic stem cells, derived from the inner cell mass of the pre-implantation blastocyst stage embryo, are pluripotent SCs [12]. They have the ability to differentiate into any one of the somatic cell types of the endoderm, mesoderm or ectoderm and are very plastic in culture [13]. In contrast, adult stem cells (also called progenitor, precursors or somatic stem cells) are less plastic in culture, multipotent and lineage specific, possessing the ability to differentiate only into specific cell types of the tissue of origin in which they reside [3]. In comparison with ESCs, adult SCs are limited in their differentiation capabilities and are less plastic. For example, adult neural stem cell progenitors will only become cells of the brain, namely neurons, astrocytes, and oligodendrocytes [13]. However, extensive plasticity of adult stem cells has now been described, allowing the cells to differentiate across tissue lineage boundaries to other cell types of other lineages, a process referred to as trans-differentiation [12]. Unlike adult SCs, ESCs are prone to form teratomas, an encapsulated tumour with tissue components of the three embryonic germ layers.

Possible therapeutic applications of ESCs and adult SCs to CVDs

Embryonic stem cells

ESCs are able to differentiate into cardiomyocytes when exposed to cardio-instructive cues like activin A and bone morphogenetic protein. The resulting ESC-derived cardiomyocytes can form contracting areas and link electromechanically with the host cells following transplantation [14,15]. However, ethical/political issues surrounding their use, the risks of teratoma formation and immunogenicity, have severely limited their clinical application in humans [13,16]. At present, only one clinical case has been reported. The ESCORT trial (in France) involve the transplantation of ESC-derived cardiomyocytes implanted in a fibrin scaffold and delivered surgically into the infarcted area of a patient with severe heart failure [17]. Three months after the procedure, patient experienced symptomatic improvement (move from NYHA Class III to NYHA Class I), new-onset contractility on echocardiogram and improved left ventricular ejection fraction (LVEF) from 26% to 36% [9]. Despite the limitations, ESCs still remain a vital laboratory tool for understanding differentiation and pluripotency in the cardiogenic process [16].

Skeletal myoblasts

SMs are the first SCs to be explored for cardiac application [18]. Though SMs do not differentiate into cardiomyocytes *in vivo*, their phenotypic similarity to cardiac muscle, ease of isolation/expansion *in vitro*, the relative resistant to ischemia and the ability to derive autologous cells drives their application as a SCs source for cardiovascular disease (CVD) [19]. Preclinical studies show much promise for ischemic heart regeneration [20]. Some clinical studies done (like POZNAN trial and others) revealed some improvement in ventricular function (increased LVEF) and NYHA class levels [21,22]. The SEISMIC trial reveals minimal change in NYHA classification with no improvement in global LVEF [23]. The SMs differentiated into myotubes (rather than cardiomyocytes) with no gap junctions which causes conduction block in the heart and a significant risk for ventricular arrhythmias [21,22]. As such, clinical studies involving SMs for CVD has diminish in recent years.

Bone marrow-derived stem cells

The bone marrow (BM) contains several types of SCs including, MSCs, HSCs, endothelial progenitor cells (EPCs) and mononuclear cells (BMMNCs) [13]. They can differentiate into cardiomyocytes and vascular endothelial cells, are easily harvested and can offer autologous cells for transplantation. These cells have been extensively tested for CVD in several preclinical and clinical studies. Martin-Rendon, et al. reviewed thirteen randomized controlled clinical trials (a total of 811 patients) involving intracoronary delivery of autologous bone marrow stem cells (BMSCs) to treat AMI [24]. The LVEF improved by 3% ($p<0.01$), the left ventricular end-systolic volume (LVESV) was significantly reduced by 4.74 ml ($p<0.01$) and the myocardial lesion improved by 3.51% ($p<0.01$) [24], thus indicating the potential role of BMSCs for CVD.

Mononuclear cells

The year 2002 witnessed the first clinical trial of BMMNCs in patients with acute myocardial infarction (AMI) [25]. The study revealed improvement in global LVEF with enhancement of myocardial perfusion. Other recent trials (like SWISS-AMI [26], BOOST [27], and TOPCARE-AMI [28,29] trials) showed similar results. However, the LateTIME double blind trial revealed no significant improvement in regional and global LVEF after 2-3 weeks [30,31]. Overall, BMMNCs studies have shown promise in the treatment of patient with AMI, though recent neutral results from other studies casts doubt on the potential therapeutic application of this new tool.

Mesenchymal stem cells

MSCs are also located in the adipose tissue, umbilical cord blood and placenta. They can differentiate into muscle, bone, cartilage, and adipose tissues. They can be relatively easily harvested from autologous bone marrow. They have low immunogenicity. Most importantly, they can differentiate into cardiomyocytes and endothelial cells *in vivo* when transplanted for AMI or non-injury in experimental models [32,33]. Their therapeutic action is postulated to be by the secretion of soluble paracrine factors [34]. Several clinical trials have been reported. The BOOST trial involves intracoronary transplantation of autologous BM-derived MSCs in AMI patients receiving percutaneous coronary intervention [35]. Global LVEF was increased by 6.7% compared to 0.7% in the control group [35]. Hare, et al. conducted a randomized, double-blind controlled phase 1 clinical trial in 2009 to assess the safety and efficacy of intravenously delivered BM-derived MSCs [36]. There were improvements in LVEF (as assessed by cardiac MRI) and global symptoms [36]. The REPAIR-AMI trial shows similar outcomes [37].

Hematopoietic stem cells and endothelial progenitor cells

HSCs can differentiate into myeloid and lymphoid cell lineages. EPCs present in the peripheral blood can differentiate into vascular endothelial cells which can help to repair ischemia through neovascularisation. Phenotypic markers for HSCs and EPCs includes CD34 AND CD133. Several clinical trials done (in AMI/ischemic cardiomyopathy patients) using CD34+ or CD133+ cell types show much improvements in LVEF, myocardial perfusion, contractile function, LVESV levels and oxygen consumption [38-41]. EPCs have been shown to contribute to new vessels formation in myocardial ischemic experimental models [42,43]. We await the results of the clinical trials assessing the ability of EPCs to form new vessels in human myocardium.

Adipose-derived MSCs

They are phenotypically similar to BM-MSCs. They can differentiate into cardiomyocyte *in vitro*, they have low immunogenicity and they are relatively easily harvested by liposuction. Animal model studies with adipose-derived MSCs have shown great promise [44-46]. The Precise Trial and the MyStromalCell Trial are ongoing clinical trials assessing the benefits of this cells in human CVD. Preliminary results for the Precise Trial show improvements in LV mass and motion score index after 18 months [47,48].

Cardiac stem cells

The heart used to be known as a post-mitotic organ with no regenerative capacity. However, we now know that a small population of resident stem cells (known as cardiac stem cells, CSCs) exists in niches in the adult heart that are capable of cardiac cellular turnover throughout life replacing the dying cells [49,29]. These group of adult stem cells in the heart discovered by Beltrami and colleagues were shown to express markers of stem cells (ckit, Mdr1, and Sca1) [49]. The CSCs have then been extensively characterized into seven types: ckit cells [50], Sca1+ [51], IsI1+

cells [52], side population cells (Abcg2/Mdr1+) [53], cardiac mesangioblasts [54], epicardial progenitors [55] and cardiosphere-derived cells (ckit+/flk1+/Sca1+) [56]. They have the ability to give rise to myocytes, smooth muscles and endothelial vascular cells. They can be harvested during heart surgery or endomyocardial biopsy, then expanded in culture. Experimental and clinical trials with CSCs are very promising [50]. Two clinical trials stand out. The SCIPIO trial [42], a phase 1 clinical trial were autologous ckit+ CSCs was transplanted via intracoronary injections in patients with ischemic cardiomyopathy. There was 12% increase in LVEF and decrease in infarct size [57]. In the CADUCEUS trial (another phase 1 clinical trial), autologous CDCs were transplanted by intracoronary injection in myocardial infarction patients. Six months' post-injection, MRI reveals reduction in scar mass, increases in viable heart tissue and contractility. No adverse effect was reported 1 year after these 2 trials [58].

Induced pluripotent stem cells

Induced pluripotent stem cells utilizes cells from the individual and can thus avert immune rejection and repeated immunosuppressive therapy and its accompanying complications [59]. The iPSCs have the potential to differentiate into the three obligate cardiac cell types such as the cardiomyocytes, smooth muscles and endothelial cells [60-63]. Although the discovery of iPSCs has been a major breakthrough in the field of regenerative medicine, a lot of research however is still required for its clinical application as it is faced with drawbacks of high cost and time delay in production, inconsistent result, mutation post reprogramming, and retained epigenetic memory of the source tissue [64,65].

CONCLUSION

SCs therapy hold great promise for heart disease and may revolutionize the treatment of CVD. Much progress has already been made in a relatively short time and the results have been encouraging and generally safe. At present, no single SCs type has proven itself to meet sufficient standard for universal application of these cells clinically. Each SCs seems to have their own advantages and disadvantages. Large-scale clinical trials are needed if we must standardize and optimize the use of these cells and newer protocols must be established to circumvent their limitations. The ongoing TransACT 1 and 2 trials (using BM-derived CD133+ cells in MI patients) in the Bristol Heart Institute Hospital UK, are fascinating. SCs may soon become a powerful tool to be use by clinicians to mend a broken heart. Lastly, the most appropriate mode of delivery, time of delivery, retention and survival of cells still require further research.

Conflict of Interest

The authors declare that there is no conflict of interest.

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