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Role of Stem Cells in Cardiac Diseases

Review Article

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Abstract

Ischemic heart disease is the leading cause of global mortality, and cardiovascular diseases represent a major challenge for researchers because of the overall health complications associated with them. Numerous studies have shown that it is exceedingly difficult to induce cardiomyocytes to divide, resulting in a flood of interest in attempting to treat cardiac ischemia through the delivery of new cardiomyocytes. To do so, researchers have started investigating the potential of using induced pluripotent stem cells (iPS) to derive functional cardiomyocytes.

The diverse cells that compose the heart and the subdivision of cardiomyocytes make it difficult to generate specific cells with specific genetic and functional signatures. Hence, it is essential to define the cardiac progenitor cells' identity and subsequently design the best strategy to differentiate them into the appropriate mature functional cells.

Recent studies in animal models and some clinical trials have shown the beneficial effects of these iPS cells in decreasing morbidity and improving heart function. Yet, many hurdles still need to be overcome before generalizing the conclusions reached so far. These are related to the nature of the manipulated cells, their delivery into the host, and their interaction with the host cells. This review touches upon the current knowledge of Cardiac Progenitor Cells (CPC), the role of iPS cells in understanding cardiac disease, as well as the clinical trials and animal models involving stem cells and cardiac disease.

Keywords: iPS Cells; Cardiac Disease; Myocardial Infarction; Animal Models.

Cardiac Progenitor Cells

The heart is the first organ to become fully functional during development in all organisms. Despite numerous studies in model organisms in the last two decades, the identity of the cardiac progenitor cell (CPC) remains unclear. However, studies have confirmed that most cardiac cells originate from the mesoderm. Work in recent years has shed light on a few genetic markers that are believed to be candidates for expression on the illusive cardiac progenitor cell, such as NKX 2.5, ISL-1, FLK-1, MESP1 and others [1-4].

In mouse embryonic development, cardiac progenitor cells (CPC) are believed to make their debut in a 24-hour window between days E6.5 and E7.5. Reports have indicated that the precursors for the heart forming cells must express MESP1. MESP1 expressing cells exist at day 6.5 in the primitive streak [5]. These cells, however, can give rise to non-cardiac lineages and thus MESP1 could be a marker of a progenitor cell that is upstream of the CPC [6].

As cells migrate away from the primitive streak and into the Anterior Lateral Plate Mesoderm, MESP1 expression drops and cardi-

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ac specific markers like NKX2.5 and ISL-1 begin to appear. These cells are now committed to making cardiac cells, mainly the future beating cardiomyocytes [7]. This ensues by day E7.5, and somewhere in between E6.5 and E7.5, a cardiac progenitor is born.

Recent work in human embryonic stem cells (hES) has identified a group of cells that are KDR^+ and are capable of producing all three cell-types needed to make the heart (cardiomyocytes, endodermal cells, and skeletal muscle cells). These cells represent the second wave of $FLK1^+$ cells during embryonic development, the first giving rise to cells with a hemangioblast fate [8]. How close the second wave of $FLK1^+$ cells is to the actual CPC is still not entirely clear, but equally exciting to the identification of these $FLK1^+$ cells was the use of hES cells to do so. This serves as a proof of concept that stem cells can be used as an adequate platform to model human embryonic cardiac development and to interrogate the CPC riddle further.

The fact that we have not yet characterized the CPC has not discouraged the use of cardiac stem cells in the clinic. On the contrary, the lack of a defined CPC has led to a hodge-podge of clinical trials with different "cardiac stem cells" and conflicting results. The identification of a definitive CPC will solve a very intriguing scientific question, and will most certainly have tremendous ramifications on the applications of cardiac stem cells in the clinic.

Cardiac Disease Modeling via Stem Cells

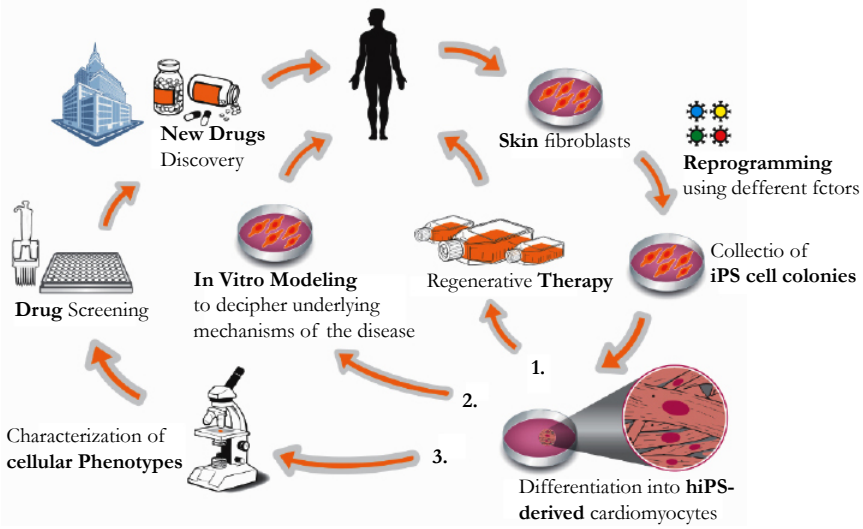
Stem cells are an excellent tool to model cardiac diseases. Examples of cardiac diseases that have been studied through stem cell modeling include LEOPARD syndrome, biological pacing [9, 10] and long QT (LQT) syndrome [11, 12]. LEOPARD syndrome is an autosomal dominant developmental disorder, where the main disease phenotype is hypertrophic cardiomyopathy. In a study by Carvajal-Vergara et al., iPS cells were generated from a patient with a mutation in the PTPN11 gene, which encodes the SHP2 phosphatase. LEOPARD syndrome hiPS cells-derived cardiomyocytes are spontaneously hypertrophied *in vitro* and present a higher level of sarcomeric organization compared with hES cells or wild-type hiPS cells-derived cardiomyocytes. Therefore these characteristics are associated to a potential hypertrophic situation in patients [13]. LQT syndrome is a heritable disease correlated to prolongation of the QT interval on an electrocardiogram and could lead to a ventricular tachyarrhythmia which might cause an unexpected cardiac death [14]. Bellin et al. was able to generate iPS cells from two patients affected with LQT syndrome type 1, who have mutations in the KCNQ1 gene encoding the repolarizing potassium channel mediating the delayed rectifier I (Ks) current [15]. Noteworthy, cardiomyocytes derived from LQT syndrome type 1 patients exhibited extended action potentials compared with cells from control subjects and also had an increased sensitivity to catecholamine-induced tachyarrhythmia. This phenotype, which represents one of the most principal clinical features of these syndromes, was attenuated by beta-blockade. Thus, LQTs 1 patient-specific iPS cell-derived cardiomyocytes entirely replicated the disease phenotypes [16].

In these instances though, stem cells are being used more as a model of disease than as a treatment. Indeed, effective pacing devices may make the use of stem cells to treat arrhythmias merely an intellectual exercise, but it will certainly shed light on disease pathophysiology. Similarly, using stem cells to model cardiac abnormalities such as the long QT syndrome will provide valuable insight about disease pathophysiology as well as a great platform to test drugs safely (Figure 1).

Stem cell use in cardiac Clinical Trials

The appeal of using stem cells in cardiac disease is understandable. The heart has traditionally been viewed as a post-mitotic organ incapable of regenerating damaged tissue. Simultaneously, cardiac disease is the most frequent cause of death in the US, accounting for a 25%, 24.6% and 24.4% of all deaths in 2008, 2009, and 2010 respectively [17-19].

Figure 1. Schematic diagram of the potentials of human induced pluripotent stem (hiPS) cells in cardiovascular diseases. Skin fibroblasts are obtained from diseased patients, reprogrammed into iPS cells, and further differentiated into specific hiPS-derived cardiomyocytes. These differentiated cells can be utilized in 1. Gene correction and regenerative medicine, 2. *In-vitro* models to decipher underlying mechanisms and pathophysiology of some cardiac disorders, and 3. Drug screening for new drugs discovery.



Subsequently, the use of stem cell therapies as part of cardiac disease treatment is gaining interest. A quick search on the NIH clinical trials database for the key words "stem cells" and "Heart" will yield upwards of 550 worldwide registered trials in various stages of progress with more than half of these trials taking place in the USA (www.clinicaltrials.gov). That being said, the vast majority of these trials are focused on treating myocardial ischemia and heart failure.

A review of some of the major clinical trials involving stem cells and heart disease is summarized in Table-1 and reveals some interesting findings. Paramount among those is the identification that the practice of stem cell injection into cardiac tissue is a safe one. One of the few findings that were reproduced in different

Table 1. Stem cell clinical trials in cardiac ischemia and heart failure.

Trial	Patients	Type of Cell	Source of cells	Size of study	Method of administration	Endpoint	Outcome
Focus-CCTRN [21]	Chronic ischemic heart failure receiving maximal medical therapy	Bone marrow mononuclear cells	Bone marrow	· 61 treated with BMC · 31 control	Transechocardial injection of BMC into LV endocardial regions	· Efficacy at 6 months measured by LVESV, maximal oxygen consumption	· The treatment was not efficacious. No significant changes were detected between patients treated with BMC or control
CADUCEUS [22]	Ischemic heart disease patients 2-4 weeks post MI	Cardishphere derived autologous stem cell	Endomyocardial Biopsy	· 17 treated with CDC · 8 Control	Catheter infusion of cells into infarct related artery	· Safety · Efficacy	· Safety: 1 incidence of Q-wave MI in 1 patient was regarded as related or likely related to the study · Efficacy at 6 mnths: Reduction in scar size - increase viable heart mass - increase regional contractility - increase systolic wall thickening. No change in End Diastolic Volume - No change in End Systolic Volume - No difference in LVEF with control
SCIPIO [23]	Ischemic heart disease patients 113 days post CABG	c-kit (+) lineage (-) cardiac stem cells	Atrial biopsy	· 16 treated with CSC · 7 control	Catheter infusion of cells into infarct related artery	· Safety · Efficacy	· Safety: no association with adverse effects for up to 1 year · Efficacy: Reduction in scar size - increase in LVEF - Decrease in NYHA score - increase in MLHFQ score
POSEIDON [24]	Patients with chronic LV dysfunction due to ischemic injury	Mesanchymal stem cell	Iliac crest bone marrow aspirate	· 31 patients treated with different dose of MSC from allogenic and autologous sources	Transendocardial injection into infarcted myocardium	· Safety: serious and non serious adverse events · Efficacy: NYHA class - O2 consumption - walk test - MLFHQ	· Safety: 6.7% presence of SAE at 30 days. 33% and 53.3% presence of SAE in allogenic and autologous group at 1 year respectively. · Efficacy: autologous MSC associated with 6 min walk test and MLHFQ - Allogenic MSC reduced LVED volume - Allogenic and autologous MSC reduced mean EED and spherecity index
SWISS-AMI [25]	Acute ST elevation myocardial infarction with SPCI	Bone marrow mononuclear cells	iliac crest bone marrow aspirate	· 65 early BMNC treatment · 63 late BMNC treatment · 67 control	Intracoronary infusion into previously infarcted vessel within 5-7 days (early) or 3-4 weeks (late)	· Change in LVEF at 4 months	· No difference between control group, early group, or late group in 4 month LVEF
TIME [26]	Acute Myocardial infarction with successful reperfusion	Bone marrow mononuclear cells	Bone marrow aspirate	· 79 treatment · 41 placebo	Intracoronary infusion into previously infarcted vessel at 3 days vs 7 days	· Efficacy at 6 months measured by LVEF and LV wall motion Safety: Major adverse cardiac events	· Safety: No major adverse effect in the treatment group · Efficacy: The treatment had no significant effect on LVEF or wall motion between groups treated at 3 days, 7 days, or control groups
Late TIME [27]	Acute Myocardial infarction with successful reperfusion	Bone marrow mononuclear cells	Iliac crest bone marrow aspirate	· 58 treatment · 29 placebo	Intracoronary infusion of BMC at 2-3 weeks post MI	· Efficacy at 6 months measured by LVEF and LV wall motion	· The treatment had no significant effect on LVEF or wall motion between groups treated at 2-3 weeks or control groups
C-CURE [28]	Chronic heart failure of ischemic origin	Cardiopeitc stem cell	Iliac crest bone marrow aspirate	· 20 treatment · 13 placebo	Endoventricular injection of cells	· Safety and feasibility · Efficacy of treatment	· Safety: No evidence of cardiac or systemic toxicity identified by study Feasibility: In 75% of patients who underwent biopsy, adequate stem cells were produced and injected back into the heart · Efficacy: Improved LVEF, decreased LVES volume, improved 6 min walk distance, improved quality of life scores
MAGIC [29]	Patients with ischemic left ventricular dysfunction requiring CABG surgery	Skeletal muscle myoblast	Muscle biopsy from the thigh	· 67 treated with skeletal muscle myoblast at 2 different doses · 30 control	Multiple site injection of cells into a kinetic myocardium	· Safety: Presence Major cardiac events · Efficacy: Global or LV function change	· Safety: No major adverse cardiac events resulted from treatment · Efficacy: No difference in LV function between patients who received skeletal muscle myoblast or placebo

BMC: Bone Marrow Mononuclear Cells. LV: Left Ventricle. LVESV: Left Ventricular End Systolic Volume. MI: Myocardial Infarction. CDC: Cardioshpere Derived Cells. LVEF: Left Ventricular Ejection Fraction. CABG: Coronary Artery Bypass Graft. CSC: Cardiac Stem Cell. NYHA: New York Heart Association. MLHFQ: Minnesota Living With Heart Failure Questionnaire. MSC: Mesenchymal Stem Cells. EED: Early Enhancement Defect. BMNC: Blood Mono Nuclear Cells. SPCI: Successful Percutaneous Intervention. BMC: Bone Marrow Mono Nuclear Cells.

trials was the safety of stem cell treatments. Indeed, there seems to be minimal to no adverse outcomes from administration of stem cells into the heart. Some of the concerns had previously included the differentiation of the stem cells into cancerous cells within the heart, as well as the interruption of the highly regulated cardiac conduction system and the subsequent development of arrhythmias, among others – all of which are apparently not a major concern when using stem cells to treat cardiac disease.

Another important take away from the various trials is the feasibility of such a treatment. The idea of culturing stem cells from the body (and in some instances creating iPS cells), expanding them, and injecting them back into the heart may have seemed like stuff of science fiction a few decades ago. The abundance of clinical trials doing just that, and with efficiency, is proof of the feasibility of such procedures.

It's not all-good news though. Some of the limitations of the trials include a short follow up period that ranged from 4 months to 1 year, conflicting outcomes, and a lack of a clear understanding what is happening at the molecular and biological level. As far as follow up time goes, a longer follow up period of up to 5 years will surely be more informative in terms of efficacy and safety, and future trials will likely tackle this point. The more important limitation, however, is the contradictory results reported by the trials. The lack of standardization between the trials with regards to the type of stem cells used for treatment, patient population, timing, dose, end points, and many other variables resulted in clinical trials that are not easily comparable to one another and often yielding contradictory results. What is not contradic-

Table 2. iPS cell use in animal models of cardiac disease.

Disease model	Animal Used	Stem cell used	Endpoint	Result
MI [30]	Rat	MSC / BCL-2 engineered	· Revascularization · Cell survival · Functional improvement in LV at 6 weeks	· Decreased apoptosis · Increased survival · Smaller infarct size · Improvement in LV function
MI [31]	Rat	MSC	· Infarct size · Functional improvement in LV at 6 weeks	· No impairment in LV function · Abnormal colonies detected
MI [20]	Mouse	ESC	· Fate of ES cells injected into heart · Effect of ES cell injection on cardiac size and function	· ES cells became cardiomyocytes · Reduced cardiac modeling · Improved cardiac function
MI [32]	Mouse	iPS	· Effect of iPS cell injection on cardiac function	· Improved cardiac function · Decreased remodeling
MI [33]	Mouse	BM vs. UCB	· Cardiac function and histological assessment	· Scar size was same in BM, UCB and Control · Decreased apoptosis in BM and UCB · Only BM improved contractility
MI [34]	Mouse	hUCB	· Effect of cells on cardiac repair following MI	· hUCB migrated to infarcted region and aided in remodeling
MI [35]	Rat	BM-MNC	· Safety and therapeutic effectiveness	· Increased neovascularization · Increased EF
HF [36]	Sheep	UCMNC	· Cardiac function and histological assessment	· Enhanced RV diastolic function · Increased angiogenesis
HF [37]	Rabbit	BMC	· Contractility and capillary density	· Increased contractility in treated hearts · Increased vascularization in treated hearts
MI [38]	Rabbit	BM-MSC	· Assess cardiac function	· Markedly improved cardiac function · Decrease area of fibrosis · Increase number of capillaries
MI [39]	Dog	CSC	· Assess effect of resident CSC on infarcted heart	· Marked recovery in contractile function Vascularization of scarred tissue
HF [40]	Dog	MSC	· Contractility and histopathological change	· Improved EF in treated dogs after 60 days · Increased vascularization
MI [41]	Sheep	MPC	· Dose dependent effect on LV post MI	· Low dose increased LVEDV and LVESV · Lower doses increased vascular density at border zone · All doses increased EF
MI [35]	Pig	BM-MNC	· Therapeutic effectiveness of BM-MNC injection	· Improved cardiac function · Increased capillary density at scar site · Increased regional blood flow
MI [42]	Pig	EPC	· Post MI effect of EPC and EPC conditioned media injection	· Increased ventricular function and cardiomyocyte size at 2 months in both groups
MI [43]	Pig	MSC	· Effect of MSC on cardiac nerve density	· Increased cardiac nerve sprouting

MI: Myocardial Infarction. MSC: Mesenchymal Stem Cell. LV: Left Ventricle. ESC: Embryonic Stem Cell. iPS: induced Pluripotent Stem Cell. BM: Bone Marrow. UCB: Umbilical Cord Blood. hUCB: Human Umbilical Cord Blood. BM-MNC: Bone Marrow Mono Nuclear Cell. EF: Ejection Fraction. HF: Heart Failure. UC-MNC: Umbilical Cord Mono Nuclear Cells. RV: Right Ventricle. BMC: Bone Marrow derived Stem Cells. CPC: Cardiac Progenitor Cells. MPC: Mesenchymal Precursor Cells. EPC: Endothelial Progenitor Cells. ADSC: Adipocyte Derived Stem Cell

tory, however, is that these conflicting results cast a shadow of doubt on any favorable results that the trials have reported. And more importantly, they emphasize the necessity of conducting a standardized trial with clear endpoints and parameters and a large patient population to yield powerful results that will provide an unequivocal answer to the question of efficacy of stem cell treatment in cardiac disease.

Animal models that studied the efficacy of stem cell treatment in cardiac disease have reported that the stem cells injected into the heart are mostly not differentiating into cardiomyocytes *in vivo*. Rather, improvement in cardiac function were shown to be due to a paracrine effect exerted by these stem cells as well as an increase in the neovascularization of scared tissues, though exactly how and what is happening is still unclear. Human trials have so far not addressed this issue clearly. Thereby, understanding the molecular effects of stem cells in cardiac disease will help design better clinical trials in the future by elucidating the best type of cells to use, as well as the timing and location of stem cell injection.

Animal models

Mice, rodents, sheep, rabbits, dogs, and pigs have been used to interrogate the efficacy of using stem cells in the treating cardiac disease. Though the endpoints varied wildly, the results were consistent in different animal models across the board, as summarized in Table-2. Unlike human trials, animal models show a clear benefit in using stem cells to treat chronic heart failure and acute myocardial infarctions. This result has been repeated in almost all the species used to model the disease. Neovascularization is the most commonly identified culprit in recovery, and it was detected in most of the trials. While most trials claimed that the enhanced cardiac function was also partly due to a paracrine effect, a few trials identified the generation of new cardiomyocytes in the heart. One such exception is the Singla et al. study [20], where the injection of embryonic stem cells into a mouse heart after an MI resulted in all three cardiac cell types of the heart. While this result is quite encouraging, it faces a major obstacle in human application – namely the use of human embryonic stem cells in research.

Conclusion

Stem cell use especially iPS cells in cardiac disease holds great promise for the future. And while many trials have produced encouraging results, there needs to be a well-conducted study that can tackle the shortcomings of present trials and follow a standardized protocol that will lend greater credibility to the results that such a trial will produce.

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