

Cardiovascular Diseases: Recent Developments in Regenerative Medicine

Abstract

Advent of regenerative medicine has opened new therapeutic interventions for patients with cardiovascular diseases. The stem cell based cardiac repairing and regeneration along with improved vascularisation can improve diseased heart. Clinical studies over last decade have evaluated the cardiogenic potential of embryonic, induced pluripotent, cardiac, mesenchymal and bone marrow derived stem cells. The clinical studies have raised the hope of successful translation of regenerative cells from bench to bed side. Intrinsic factors guiding the stem cells to site of injury i.e. homing, transformation into cardiomyocytes, and angiogenesis have been assessed in recent years. However, complete understanding of mechanisms co-relating and regulating the process need to be explored. This review focuses on characteristics of stem cells under investigations, for clinical trials, analyzing safety, feasibility, efficacy and mechanism underlying cardio protective and cardio regenerative process. Further, the approaches involving scaffold based 3-dimensional cardiac generation will also be analysed.

Keywords: Mesenchymal stem cells; Cardiomyocytes; Cardiac regeneration; Embryonic stem cells; Pluripotent stem cells; ISSCR

Mini Review

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Abbreviations: CSCs: Cardiac Stem Cells; BMMNCs: Bone Marrow Mononuclear Cells; ESCs: Embryonic Stem Cells; iPSCs: Induced Pluripotent Stem Cells; MSCs: Mesenchymal Stem Cells; bFGF: Basic Fibroblast Growth Factor; IL: Interleukin; TNF: Tumor Necrosis Factor; ISSCR: International Society for Stem Cell Research

The heart failure is one of leading cause of morbidity and mortality all over the world. More than 5 million patients are suffering from chronic heart failure post myocardial infarction caused due to ischemic heart disease [1]. The disease develops over a period of time due to loss of cardiomyocytes. The condition is further aggravated due to complications related to obesity, hypertension, diabetes, smoking and alcohol consumption. The currently available treatments regime include application of β -blockers, targeting of rennin-angiotension-aldosterone system using ACE (angiotensin converting enzymes) inhibitors, ARBs (angiotensin II receptor blockers) and aldosterone antagonists [2]. In selected patients cardiac resynchronization therapy and implantable defibrillators are also recommended [3]. Though, the treatment improves condition of patients symptomatically, no remarkable change in mortality or morbidity is observed. Heart transplant is not feasible option due to unavailability of donors and possible immune rejection. New interventions, based on stem cell driven regeneration appear to be promising in current scenario. The cardiac regeneration can bring endogenous repair through formation of new cardiomyocytes and improved vascularisation.

The regenerative capacity of adult human myocardium was considered to be limited as myocardial cells are terminally differentiated. However, discovery of cardiac progenitor cells developed the possibility of heart tissue repair through

cardiomyocytes generation. The process involves replacement of damaged myocardial cells with new one, derived from pluripotent cells. The major source for pluripotent cells are human embryonic (hESCs) and induced pluripotent stem cells (iPSC). The hESCs are considered to be promising candidate for cardiac cells development as their cardiogenic potential is established and further they can differentiate into multilineage smooth muscle and endothelial cells, required in myocardial tissue. However, clinical use of hESCs and iPSCs still needs detail investigation to assess safety and efficacy. hESCs are derived from embryo which differs from parent in their genome. Similarly iPSCs also demonstrate genetic variability. The risk of immune rejection in such genetically variable cells is high. Both these cells type, if infused in undifferentiated state can lead to teratoma formation [4,5]. Another aspect which limits the application of hESCs is ethical issues associated with use of embryonic cells.

Bone Marrow Derived Stem Cells

Bone marrow is potential source of stem cells comprising of mixed population of cells mainly primary early stage committed cells, hematopoietic stem cells, endothelial progenitor cells and MSCs. Several studies have evaluated the functional myocardial activity by infusing bone marrow mononuclear cells (BMMNCs). In one major clinical trial, REPAIR, patients with acute myocardial infarction (AMI) were treated with bone marrow derived stem progenitor cells. 204 patients received intracoronary administration of autologous stem cells. After one year, significant reduction in occurrence of adverse events and improved vascular repair was observed. These beneficial effects were observed even after two years of follow up [6-9]. Similar, results were observed in different studies using administration of CD34 $^{+}$ cells in patients

of angina and myocardial ischemia demonstrated improvement in frequency of angina and exercise tolerance [10,11]. However, despite of moderate success in bone marrow derived stem cells, some of the trials demonstrated little or no improvement after cell transplant. In a set of randomized trials, under category FOCUS-CCTR and Late TIME trials, BMMNCs were infused to assess several parameters including myocardial perfusions, oxygen consumption and left ventricular function. The end result of trials concluded that cellular infusion did not improve oxygen consumption and left ventricular dysfunction [12-14].

Mesenchymal Stem Cells

Adult cells like mesenchymal stem cells (MSCs) are also capable of driving heart repair. MSCs are characterized by self renewal ability, low immunogenicity, no transplant rejection in host body and low tumorigenicity. They lack major histo-compatibility complex II (MHC II) and B7 co-stimulatory molecule expression, so can easily escape the immune system and overcome host rejection. Though, MSCs lack the ability to differentiate into cardiomyocytes, they contribute to neo vascularization and cardiomyocyte protection [15]. Several studies have investigated the ability of MSCs in improving cardiac functions. In PRECISE trial patients with ischemic cardiomyopathy were treated with adipose derived regenerative cells. Maximum oxygen consumption, ventricular function and exercise capacity was improved in treated group patients which suggested a reduction in inducible ischemia up to 18 months [16]. Some studies used allogenic source of MSCs as they are considered to be immunoprivileged and immunosuppressed [17,18]. A comparative study analysed two types of bone marrow transplants using allogenic and autologous source of cells. In this randomized phase 1/2 POSEIDON study, both types of cells demonstrated safety and potential to regenerate by reducing infarct size and ventricular remodelling [19]. In another study, Cardiopoietic stem Cell therapy in heart failure (C-CURE), bone marrow derived mesenchymal stem cells, treated with a particular cardiogenic cocktail, were used for infusion in patients with heart failure. The safety and feasibility was observed, with indications of benefit. Though some concerns were raised about the methodology of trial, the reply by investigators was submitted and published in same journal. Meanwhile, Cardio3biosciences, which conducted C-CURE trial, is working on phase III study of trial [20,21].

Cardiac Stem Cells

Another group of stem cells are categorized as cardiac stem cells (CSCs). They are heterogeneous group of cells residing in specific heart areas as atria or pericardium and are characterized by expression of c-kit surface marker [22,23]. Several trials used CSCs in their clinical study. Stem Cell Infusion in Patients with Ischemic Cardiomyopathy (SCIPIO) trial used c-kit⁺ CSCs in patients with heart failure due to IHD in phase 1 trial. The results were encouraging as it suggested intracoronary infusion of CSCs led to improved LV systolic function and reduced infarct size. An unprecedented increase in viable myocardium was reported due to therapeutic regeneration [24]. At almost same time, Makkar et. al. published a report demonstrating the use of cardiosphere derived cells (CDCs) in patients with left ventricular dysfunction

in a randomized phase 1 trial of cardiosphere derived cells for heart regeneration after myocardial infarction (CADUCEUS). The results demonstrated that infusion was safe and an increase in viable myocardial tissue was observed. The scar size reduction was claimed to be 3-5 times better than in case of bone marrow mononuclear cells used in other studies [25].

The clinical assessment of stem cell based intervention demonstrated feasibility and safety of transplant; however, the efficacy remained an issue. The variable efficacy of transplants can be understood on basis of mechanism governing mode of action of administered cells.

Mechanism Underlying Myocardial Activity Conferred by Stem Cell Transplants

Cardiogenic cells including ESCs and iPSCs stimulate heart repair by stimulating differentiation to cardiomyocytes. However, exact mechanism regulating the process is not completely understood. Paracrine signalling has been attributed main driving force behind cardiac repair. The mechanism can be categorized into cardio protection, angiogenesis, myogenesis and endogenous repair. The cardio protection is conferred by secretory anti-apoptotic factors. Once inside body, MSCs inhibit activation of transcription factor NF- κ B in B cells, which in turn attenuates secretion of pro-inflammatory factors TNF- α and IL-6, and promotes anti-inflammatory cytokine IL-10. TNF- α and IL-6 are toxic to cardiomyocyte activity and cause reduced contractile activity and induce apoptosis [26]. MSCs regulate immune response and induce IL-10 secretion through monocytes and macrophages which in turn inhibits NF- κ B nuclear factor. Along with cardioprotection, MSCs secrete angiogenic vascular endothelial growth factor (VEGF) and arteriogenic basic fibroblast growth factor (bFGF). VEGF increases capillary wall permeability, induces cell proliferation, migration and vascularisation. On other hand, bFGF promotes smooth muscle formation as part of angiogenesis [27,28].

Myocardial infarction leads to accumulation of collagen resulting into fibrosis. Under fibrotic environment expression of many genes, growth factors and cytokine is downregulated resulting into inhibition of endogenous cardiac repair [29]. Under such environment, MSCs transplantation brings about modulation of matrix by metalloproteinase resulting into matrix degradation activity. MSCs control fibroblast activity by down regulating synthesis of type I and type III collagen synthesis and finally inhibit ventricular remodelling. Along with matrix regulation, MSCs cause endogenous cardiac repair through stimulation of, c-kit⁺ cardiac stem cells and cardiomyocyte cell division [30,31].

Researchers have proposed another way through which MSCs may impart cardiac repairing function. The MSCs along with other factors, secrete exosomes which are vesicles containing biological molecules primarily proteins, mRNA, microRNA and lipid [32]. Exosomes play vital role in cell-cell interaction and mediating bidirectional exchange of material. These exosomes induce angiogenesis in endothelial cells by direct transfer of mRNA. Timmers L et al. [33] demonstrated that media conditioned with hMSC reduce infarct size by 59% in animal model of myocardial ischemia [33].

The safety, feasibility and efficacy of stem transplant in cardiovascular diseases have been studied in numerous trials using wide range of stem cells. However, each approach is associated with various benefits and limitations. Though, ESCs and iPSCs are potentially capable of generating new cardiomyocytes, infusion of undifferentiated cell may lead to teratomas formation. Further, use of ESCs involves ethical concerns. Adult stem cells like CSCs and MSCs appear to be potentially more feasible for clinical transplants. CSCs directly or indirectly through MSCs can be induced for endogenous cardiac repair. Additionally, they are reported to not be associated with tumor formation, which is major advantage. MSCs exerts cardio-protective and regenerative effect through several mechanisms including secretion of growth factors VEGF, bFGF, metalloproteinases, exosomes and stimulation of endogenous c-kit⁺ CSCs. Though, MSCs themselves lack the ability to differentiate into cardiomyocytes, they provide beneficiary effect through paracrine signals.

Tissue Engineering

Along with cell based approaches for damaged cardiac repair, researchers have explored the concept of in-vitro 3 dimensional generation of complete heart. Emerging trends in tissue engineering indicate towards possible generation of cardiac structures. The methodology involves re-population of cardiomyocytes and endothelial cells around a natural (extra-cellular) or synthetic matrix based scaffold. Reprogrammed pluripotent iPSCs are primary source of cells, incorporated in engineered construct. The selection for scaffold depends on elasticity, biocompatibility and biodegradability of material. Further, the structure must be interconnected, appropriately porous and supportive for cell proliferation and differentiation of stem cells. The extracellular matrix protein (ECMP) based biomaterials are natural option for scaffolds developments in cardiac engineering and regeneration as they are bio-compatible. However, the decellularized matrices are superior to ECMP based as later one does not mimic the complexity and structure of native tissue. The decellularized matrixes are obtained through detergent treatment of intact cardiac tissue. The process retains intact vasculature and complex 3D arrangement of collagens, elastin and glycosaminoglycan [34]. The decellularized scaffold was successfully tested initially in rat, by recellularization of neonatal cardiomyocytes [35]. However, human organ generation using decellularized scaffolds is limited due to unavailability of intact hearts. Due to this animal based heart structures closely resembling to humans are under investigation. Synthetic polymers which have been investigated till now are based on poly (lactic acid) (PLA), poly(ethylene glycol) (PEG), poly(caprolactone) (PCL), poly(l-lactide-co-caprolactone) (PLCL), poly(glycerol sebacate) (PGS), and polyurethane (PU) [36,37].

Future Directions

Clinical trials conducted over last few years have generated substantial data on implications of regenerative medicine on cardiovascular disease. Most of data are based on results of early phase I and phase II studies. Contradictory results in several studies have raised concern over methods adopted during trial design. To deal with such issues and bring transparency in clinical trials procedures, International Society for Stem Cell Research (ISSCR) has issued revised guidelines to be followed. These

guidelines are aimed to encourage best practices in translational and clinical research. Under current scenario, in many cases, where conventional treatment fails to provide prolonged relief to cardiovascular patients, new interventions are required which can provide less invasive, sustained and cost effective benefits. Stem cell based repair or transplant of in-vitro developed cardiac structure in cardiovascular diseases open a wide array of opportunities to healthcare system where scientists, clinicians and developers can contribute keeping ethical values in practice.

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