Therapeutic Use of Stem Cells in the Management of Coronary Artery Disease and Heart Failure; Current Trends, Progress, and Challenges.

ABSTRACT.

In recent years, stem cell therapy has been considered a novel therapy for human subjects with coronary artery disease and heart failure. While stem cells of different types have been successfully isolated in laboratory settings and transplanted into animal models, doubts still exist on the successful outcome of stem cell therapy for cardiomyocyte regeneration in human subjects who develop coronary artery disease and heart failure. Given the limited progress on stem cell therapy for this indication in human subjects, we aim to understand normal cardiomyocyte embryogenesis and apply it as a guide in identifying an ideal stem choice, critical growth, and transcription factors for cardiomyocyte regeneration as well as in the implementation of stem cell therapy for in human subjects with coronary artery disease and heart failure. Ethical limitations, safety, and long-term complications are critical in identifying the ideal stem cell choice. The route of infusion of the ideal stem cell, dose, and timing of administration must also be standardized for a favorable clinical outcome. All stem cell sources evaluated for this clinical indication in animal and human studies were associated with some level of structural remodeling and improved cardiac performance following infusion. In the search for and identification of the ideal stem cell type, ethical choices with limited complications of restenosis, arrhythmias, ischemic vasculopathy, graft rejection, and malignant transformation would therefore rank high for the successful implementation of the therapeutic use of stem cells in the management of coronary artery disease and heart failure in human subjects. Original research and review articles from March 1995 – March 2022 were searched online. Search engines included Google Scholar, PubMed, Web of Science, and National Institute of Health. The keywords for the literature search included the use of stem cells for treatment, stem cell therapy, coronary artery disease, coronary heart disease, ischemic heart disease, and heart failure. Manual searches were also conducted for articles related to the pool generated from the online search. Statistical data were obtained from American Heart Association, Center for Disease Control (CDC), World Health Organization (WHO), and clinicaltrials.gov. From the articles generated, relevant sections were reviewed to understand the progress made with the therapeutic use of stem cells for managing coronary artery disease and heart failure in human subjects. The sources of stem cells, transcription factors, isolation methods, routes, and timing of infusion of different stem cell types evaluated for this clinical indication in preclinical and clinical studies were reviewed and outlined. The clinical outcomes of using different types of stem cells from both studies were also reviewed and compared, as well as ethical limitations, study limitations, and resultant adverse effects.

Key words : Heart Failure, Coronary Artery Disease, Stem Cells

INTRODUCTION.

Cardiovascular diseases comprise disorders affecting the structure and function of the heart and vessels. They remain the leading cause of morbidity and mortality in advanced countries, accounting for an estimated 17.9 million or 32 percent of deaths globally and over 868 thousand

deaths in the United States each year.[1] According to WHO, it is projected that the human population over 65 years of age will increase significantly from 2010 to 2030, with an increase in cardiovascular diseases by 40.5%, translating to about 27 million people with hypertension, 8 million with coronary artery disease(CAD), and 3 million with heart failure.[2] Cardiovascular diseases include congenital heart diseases, valvular heart diseases, cardiac rhythm disturbances, cardiomyopathies, pericardial diseases, hypertension, cerebrovascular disease (CVD), peripheral artery disease (PAD), coronary artery disease (CAD) and heart failure.

Coronary artery disease accounts for most of the disease burden of cardiovascular diseases. According to the American Heart Association (AHA), about 20.1 million, or 7.2 percent of adults over the age of 20, suffer from coronary artery disease in the United States.[3] Its prevalence is found to be higher in men (8.3%) than women (6.2%), with over 365 thousand deaths recorded among both sexes in 2018 alone.[4] In the spectrum of CAD, disease conditions include stable angina, Prinzmetal angina, unstable angina, and myocardial infarction. Unlike the others, episodes of myocardial infarction are followed by loss of viable cardiomyocytes and repair by fibrosis, scar tissue formation, reduced contractile function, and heart failure as clinical outcomes.[5]

Despite remarkable advances in treating coronary artery disease and heart failure, available pharmacological and interventional treatment options only improve patient outcomes, limit scar tissue formation, and prevent adverse cardiac remodeling but do not address the loss of viable cardiomyocytes.[6] About 50% of patients who develop heart failure die within five years, while others end up with terminal heart failure with a heart transplant as the treatment option for the loss of viable cardiomyocytes. According to reports from the International Society of Heart and Lung Transplantation (ISHLT), even though there is a continuous increase in the number of heart transplants performed globally every year, the number of candidates on the waiting list far exceeds available donor organs.[7] Out of about 50 thousand candidates on the waiting list, only about 5 thousand transplants are carried out yearly.[8] About 300 patients die each year while waiting for a heart transplant, and about 280 become too sick to receive one.[9] For those patients who meet the criteria for transplant and eventually obtain a donor's heart, concerns about long-term allograft survival still abounds. They remain at risk of rejection, infection, coronary allograft vasculopathy, and malignancy. The 3-year survival rate among patients with heart transplants presently stands at about 75%, with approximately 4% annual death rate after that.[7, 8]

With the discovery of the regenerative potential of cardiomyocytes, stem cells are emerging as a novel and promising therapeutic approach for the definitive treatment of coronary artery disease and heart failure.[2] Previously thought to be terminally differentiated with none to minimal regenerative capability, the 2001 study by Orlic et al. challenged this view about cardiomyocytes with the isolation and transplantation of c-kit hematopoietic stem cells from the bone marrow of green fluorescent protein (GFP) mice to the heart of wild-type female mice.[10] Preclinical and clinical studies have subsequently shown some benefits with the therapeutic use of stem cells for coronary artery disease and heart failure. With its successful implementation, a considerable reduction in the disease burden of cardiovascular diseases and heart failure is anticipated globally.

Review of Cardiomyocyte Embryogenesis.

At the beginning of human embryogenesis, conception is followed by repeated cell divisions of the developing zygote, known as cleavage during the first week of development. These cell divisions result in the formation of the 16-cell stage morula and the 32-cell stage blastocyst. The cells of the developing zygote segregate into the embryoblast, or inner cell mass, and the trophoblast, or outer cell mass, layers at the morula stage. At about the beginning of the second week of development, the trophoblastic cells differentiate into extra-embryonic tissues for implantation of the blastocyst, and the embryoblast layer differentiates into the bilaminar germ disc comprising the hypoblast and the epiblast. While the hypoblast cells develop into the yolk sac, the epiblast cells undergo delamination, epithelial-to-mesenchymal transition (EMT), and invagination down the primitive streak [11, 12] [5]. These epiblast cells invade the hypoblast in 2 waves of cell migration to form the endoderm and the mesoderm, with the remnant cells becoming the ectoderm. These processes are completed by the end of the second week of human embryogenesis and describe gastrulation, in which the three canonical germ layers of the embryo are formed.

Following gastrulation, the heart is known to be the first organ formed from the mesoderm starting at the end of the second week of development [13] [14]. The migration of mesodermal cells to the anterior axis of the developing embryo forms the cardiac crescent from which the paired heart fields arise.[15] [5] While the progenitor cells of the first heart field (FHF) arise from the anterolateral aspect of the crescent, the progenitor cells of the second heart field (SHF) emerge from its medial aspect. In the development of the heart, the cells of the FHF form the primary heart tube, which acts as a scaffold for the elongation of the developing heart tube by cells of the SHF [5]. In the adult heart, the left ventricle is formed from the FHF, and the right ventricle and outflow tracts are derived from the SHF, with both fields contributing to the formation of the atria and inflow vessels.[5]

In the development of cardiomyocytes, migration and proliferation of progenitor cells occur, with differentiation into adult cardiomyocytes and integration with other cell types in the developing heart.[16] Among these, studies have shown proliferation to induce growth in postnatal cardiomyocytes to be the primary process contributing to cardiac embryogenesis [17]. Cardiomyocytes typically develop through a regulated activation and repression of signaling pathways by transcription factors, receptors, ligands, and microRNAs. This complex process is also known to involve the addition of mesenchymal stem cells with high proliferative potential.

In contrast to cardiomyocytes in lower animals like the zebrafish and salamander, which retain high proliferative potential into adulthood [18], several studies have described the adult mammalian heart as a postmitotic organ due to the observed decline in potential towards birth with differentiation into adult cardiomyocytes, synthesis of contractile proteins and expression of sarcomeres, radiocarbon dating studies in human subjects have however shown evidence of postnatal cardiomyocyte regeneration.[5] [17] [18] These studies have revealed cardiomyocytes younger than the age of human subjects as well as the active turnover of cardiomyocytes with a half-life of 4 - 8 years in individuals and clusters of cells positive for stem cell markers such as c-kit but negative for contractile proteins in heart tissue have also been identified.[17]

Adult cardiomyocytes may thus have a potential for regeneration via the differentiation of stem cells into adult cardiomyocytes, the effect of paracrine factors produced by stem cells, or crosstalk between both processes [17]. Sources of stem cells for cardiomyocyte proliferation and regeneration may be from resident cardiac stem cells, non-resident stem cells such as bone-marrow-derived stem cells, hematopoietic stem cells, multipotent neural crest cells, or from the de-differentiation of existing cardiomyocytes [17]. Transcription factors, growth factors, and signaling pathways are now known to play crucial roles in both cardiomyocyte embryogenesis and postnatal cardiomyocyte proliferation and differentiation in support of the regeneration potential [17]. The notion that the heart is a postmitotic organ thus appears to be inaccurate. Detectable cardiomyocyte proliferation has been shown to occur in normal hearts and induced heart failure in animal studies [17].

Adult cardiomyocyte progenitors share similar genetic markers with fetal cardiomyocyte progenitors, which possess the potential to differentiate into multiple cardiac cell types, and the parallels between developmental pathways and adult cardiomyocyte regeneration suggest that the same regulatory networks may be common to cardiac embryogenesis and adult cardiomyocyte regeneration.[19, 20] Having a detailed understanding of the migration pattern of cardiomyocyte progenitor cells and the roles of transcription factors in signaling pathways involved in proliferation and differentiation into cardiomyocytes during cardiac embryogenesis would provide insights into adult cardiomyocyte regeneration and aid in developing effective strategies for stem cell therapy in coronary artery disease and heart failure.

Role of signaling molecules in cardiomyocyte development and differentiation

Early cardiomyocyte development and differentiation express various cardiac-restricted transcription factors. Nkx2-5, expressed early in cardiac embryogenesis, plays a crucial role in the initiation and normal growth of the embryonic myocardium.[21] It also plays a role in gene expression and terminal differentiation of ventricular cardiomyocytes. Studies of mice lacking expression of Nkx2-5 have shown poor myocardium development beyond the early stages of cardiac looping.[11]

Myocardin is a cardiac-specific transcription cofactor that also plays a role in the early differentiation of cardiomyocytes. Studies have shown that it is responsible for in-vitro activation of early cardiac gene expression via the serum response factor (SRF) binding site. The in-vivo function is not fully understood but has been observed to be involved in the expression of Nkx2-5. GATA family, the zinc finger transcription factor, is usually expressed by the anterior endoderm and mesoderm.[11] It is responsible for cardiac fusion via the movement of the pool of progenitor cells to coalesce and form the linear heart tube. With the reduced function of GATA4/5, normal endoderm differentiation and the ventral migration of cardiac progenitor cells stops, leading to cardia bifida.[21] Mesp1 is activated by the T box transcription factor Eomes expressed by the earliest known cardiac precursors as a marker of cardiac mesoderm. It has a prominent lineage, but it contributes majorly to the development of the heart.[22] Mesp1 helps migrate progenitor cells through the node and primitive streak at gastrulation. Although the mesoderm is restricted to a cardiac fate by GATA, in the absence of Mesp1, progenitor cell migration stops, leading to complete or partial cardiac bifida. Mesp2 also contributes to the migration and differentiation of mesoderm from the primitive streak. When deficient, the cardiac and other mesodermal derivatives fail to develop.[21]

Irx4 is a homeodomain factor expressed at the outer curvature of the looping heart tube and is involved in chamber specialization via ballooning of the chambers. It is restricted to the development of the ventricular myocardium and negative regulation of the atrial chamber identity.[22] Irx4 is regulated by Nkx2.5 or dHand, the absence of which reduces its expression and leads to diminished ventricular differentiation.

Ventricular differentiation is regulated by Nkx2.5, dHand, Mef2c, and RXR but Irx4 is only affected when Nkx2.5 and dHand are absent. Low Irx4 causes decreased eHand expression in the developing heart and reduced expression of ANF in the ventricles after birth. Irx4-deficient mice develop cardiomyopathy due to impaired cardiac function.[21]

Pathogenesis of Coronary Artery Disease.

Anatomically, the heart comprises four chambers: right and left atria and right and left ventricles. Deoxygenated blood from the venous system usually drains into the right atrium and fills the right ventricle through the tricuspid valve. The right ventricle pumps the blood through the pulmonic valve into the pulmonary artery for gas exchange and oxygenation in the pulmonary circulation. The oxygenated blood then returns to the left atrium via the pulmonary veins and fills the left ventricle through the mitral valve. The oxygenated blood is pumped by the left ventricle through the aortic valve into the aorta and flows into the arterial system of different organs in the body.

The coronary circulation, which supplies the heart, thus arises from the aorta. Distal to the aortic valve, dilatations of the aortic root, known as the anterior aortic sinus and left posterior aortic sinus, give rise to the right and left coronary arteries, respectively. The left coronary artery (LCA) branches into the left anterior descending (LAD), which supplies the anterior interventricular septum and anterior wall of the left ventricle, and the left circumflex (LCX) arteries which supply the lateral and posterior walls of the left ventricle. In coronary artery disease, the LAD and its branches are the most affected due to increased turbulence of blood flow.

The branches of the right coronary artery (RCA) include the sinoatrial nodal artery, right marginal artery (RMA), and posterior descending artery (PDA). The right wall of the right ventricle is supplied by the RMA, while the PDA supplies the posterior interventricular septum and posterior wall of the right ventricle. In about 70% of individuals, a right dominant coronary circulation is seen with the atrioventricular nodal branch (AV nodal branch) arising from the PDA. In comparison, in about 10% of the population, the AV nodal branch arises from the LCX, forming a left dominant coronary circulation. In the remaining 20%, it arises from both PDA and LCX to form a co-dominant coronary circulation.

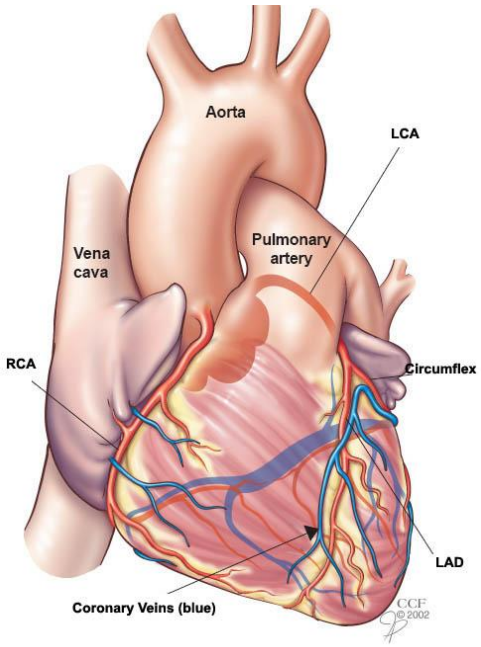

Figure 1. Representation of coronary arteries
Image from: https://my.clevelandclinic.org/health/diseases/16898-coronary-artery-disease

Histologically, the walls of arteries comprise three layers: tunica intima, tunica media, and tunica adventitia. One of the most discussed hypotheses in the multifaceted pathophysiology of coronary artery disease is the interplay of atherosclerosis which is a clinical condition with modifiable (age, sex, and gender) and non-modifiable risk factors (hyperlipidemia, hypertension, diabetes mellitus, thyroid disease), which begins with deposition of fibro-fatty atheromatous plaques in the tunica intima layer of blood vessels and results in chronic inflammation of large blood vessels.

The progression of atherosclerotic plaques to the level of critical stenosis (70%) can present with cardiac ischemia, plaque rupture, vasomotor instability and spasm, thrombus formation and propagation, and subsequent cholesterol and thrombo-embolism of coronary microcirculation. Severe plaque accumulation can result in thrombus formation with partial (subtotal) occlusion of the vessel (seen in unstable angina and acute subendocardial myocardial infarction) or complete occlusion (seen in acute transmural myocardial infarction). In some cases, thrombi can break off from the site of formation and propagate as emboli to cause occlusion in a different microvascular location.

In coronary artery disease, arterial occlusion and decreased cardiac muscle perfusion occur. It is a heterogeneous group of clinical syndromes ranging from angina pectoris (chest pain) to myocardial infarction. The occlusion of the artery can be due to constriction and dysfunction of the blood vessels, blood clots, and atherosclerosis. Patients with coronary artery disease are usually asymptomatic, or there is an occlusion blocking more than 70% of the blood vessels (critical stenosis). Patients present differently based on the etiology of coronary artery disease; a patient with stable angina might present with chest pain on exertion.[23] When the occlusion to blood vessels lasts for more than 20- 40 minutes, coagulative necrosis of the myocardium results from loss of oxygen supply to the heart muscle. A possible consequence of myocardial infarction

is cardiac remodeling with dilation and hypertrophy of the myocardium, leading to systolic and diastolic dysfunction that progresses to heart failure.[24]

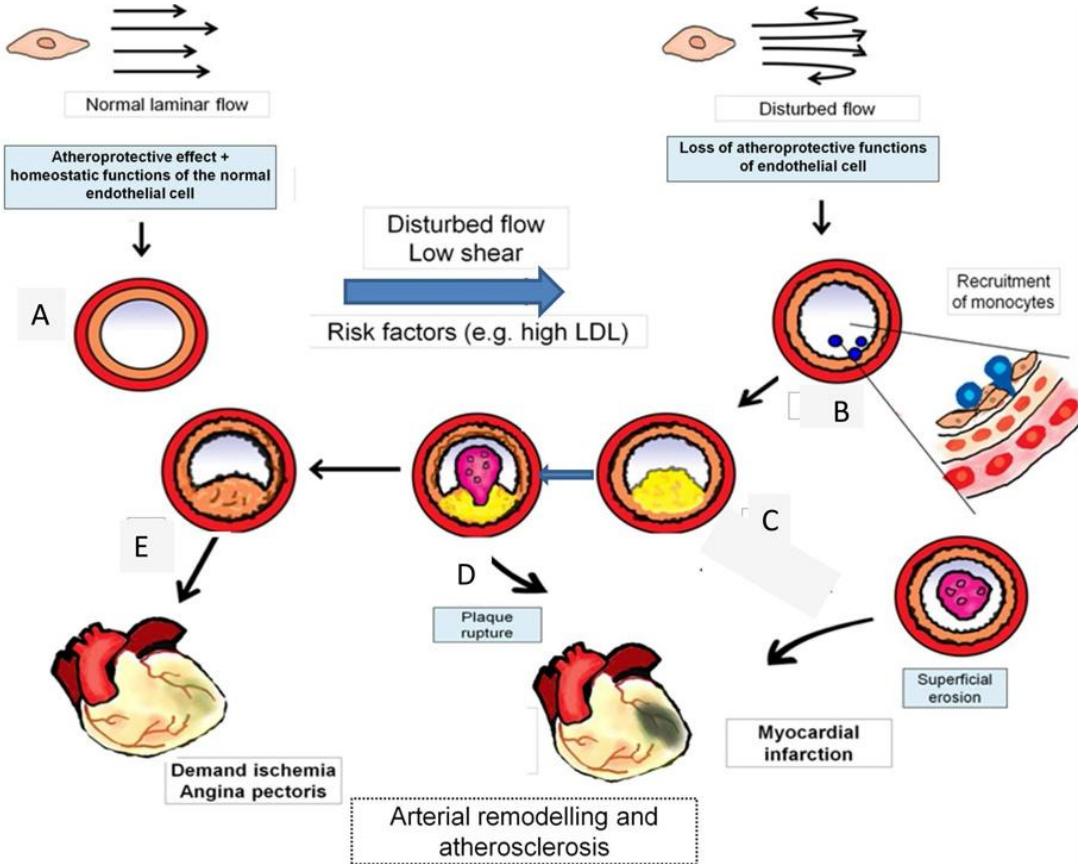

Figure 2. Coronary arterial remodeling as a result of atherosclerosis.
Image from: https://europepmc.org/article/pmc/4330973

Arterial occlusion is one of the elements in the complex pathophysiology of coronary artery disease. Dysfunction of the coronary microcirculation interferes with normal blood flow regulatory mechanisms, like the ion channels. This leads to hypoxic injury, loss of contractile force, impaired cardiomyocyte relaxation, tissue necrosis, and fibrosis and serves as a connection between coronary artery disease and heart failure (HF). Regardless of the risk factors and etiology, a possible contributor to HF is the increase in systemic pro-inflammatory factors like interleukin 6, tumor necrosis factor-alpha, pentraxin-3, reactive oxygen species (ROS), increase in the activity of vascular cell adhesion molecule 1(VCAM-1) and E-selectin.[25]

There is a general dysfunction of the primary compensatory mechanism to increased cardiac demand via coronary blood flow reserve (CFR) via the endothelium, myogenic, nervous, and metabolic mechanisms. This will interfere with various ion channels such as Ca^{++}-dependent signal through the L-type Ca^{++} channels, sodium ion channels, voltage-gated potassium channels, vasoactive substances (nitric oxide) release, and sympathetic and parasympathetic mechanisms in the heart.[25, 26]

Atherosclerotic plaque changes do not necessarily lead to myocardial ischemia, symptomatic coronary artery disease, and subsequent heart failure. The primary pathophysiological mechanism is most likely myocardial microvascular systolic dysfunction. The coronary microvascular dysfunction will foster plaques development through the increase of shear stress and the prolonged exposure of the coronary vessel wall to low-density lipoproteins (LDL), generation of reactive oxygen species (ROS), production of inflammatory mediators and advanced glycation end-products (AGEs), promoting cardiomyocyte hypertrophy, fibrosis and microvascular rarefaction which are the main histological alterations seen in HF.[27]

Ion channel dysfunction plays the most central role in the pathophysiology of CAD and HF. An abnormal intracellular Na+ concentration, downregulation of K+ channels, and Ca++ cycling defects are major determinants of heart failure. Calcium channels regulate vasodilators such EDHF, NO, and prostacyclin. ATP-sensitive potassium channels in the heart regulate contractility, relaxation, and coronary vascular tone by regulating intracellular calcium concentration.[28] The by-products like hydroxyl radicals (OH) generated from the mitochondria in the reperfusion phase determine the risk of loss of contractile force.[25]

In heart failure, many channels play a contributing role. These include the voltage-gated potassium channels involved in regulating metabolism via the oxidation-reduction process and the Transient Receptor Potential (TRP) channels in the cardiomyocytes involved in regulating the calcium current (influx) through the sarcolemma. TRP channels function in cellular proliferation, differentiation, migration, contraction, and relaxation. Studies have shown that the TRP channels are involved in hypertrophy, remodeling, intravascular pressure elevation, atherosclerotic plaque progression, ischemia, post-ischemic angiogenesis, and heart failure.[25]

STEM CELL THERAPY.

Stem cells possess the ability to differentiate into different cell types and thus serve as a reservoir system for tissue repair and regeneration. Stem cells have a capacity for asymmetric cell division and self-renewal to maintain the stem cell pool and terminal differentiation into adult functional cells. Embryonic stem cells are one of the two major classes of stem cells. They are undifferentiated cells harvested from the inner cell mass of the blastocyst of a developing embryo making their use controversial. In contrast, adult stem cells are partially differentiated cells located in tissue beds or niches.[29, 30]

Following a myocardial infarction episode, stem cell therapy has been observed to mediate endothelial, vascular smooth muscle cell, and cardiomyocyte regeneration by a paracrine mechanism of secretion of cytokines, transcription factors, and growth factors such as transforming growth factor-β (TGF-β), stromal-derived factor-1 (SDF-1) and vascular endothelial growth factor (VEGF). These factors promote neovascularization, differentiation of progenitor cells into adult cardiomyocytes, decrease the apoptosis of surviving cardiomyocytes, and induction of tissue repair.[29, 31]

In preclinical and clinical studies, different stem cell types have been evaluated for cardiomyocyte regeneration. They fall into two broad classes: some stem cells are endogenous to the cardiovascular system, while others are exogenous to the cardiovascular system. The specific types under each category are explained below.

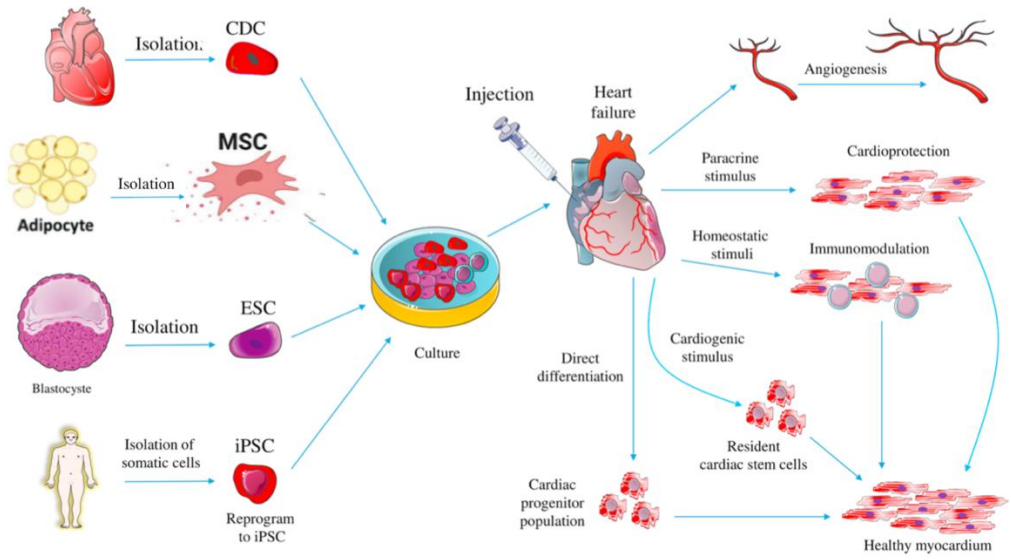

Figure 3. Schematic illustration of stem cell therapy for myocardial regeneration
Image adapted from https://europepmc.org/article/pmc/4330973

Endogenous Cardiac Stem Cells.

There have been several studies on using endogenous stem cells as a treatment for patients with coronary artery disease and heart failure. Some proposed treatment mechanisms include; transplantation, stimulation of cardiomyocyte proliferation, and unlocking the cell cycle arrest of cardiomyocytes in the adult heart.[3, 20] Endogenous cardiac stem cells are distinguished based on phenotype.[32] The two most commonly studied endogenous stem cells for therapy are C-kit cardiac stem cells (CSCs) and cardiosphere-derived cells (CDCs).

C-kit+ Cardiac stem cells (CSCs).

CSCs are resident cells found in the atrial and ventricular tissue of an adult heart. CSCs are commonly obtained through biopsy of the atrial appendages from patients undergoing surgical revascularization and isolated by surface-antigen enrichment.[32, 33] Through self-renewal and multipotency assays, isolated c-kit+ CSCs are multipotent and can expand in vitro at the clonal level. They have the potential to differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells.[3] c-kit CSCs have low to moderate cardiac transcription factors such as GATA binding proteins 4 and Cardiac specific homeobox NKx2.5. Isolation of C-kit+ CSCs is dependent on the expression of C-kit+ and the absence of CD45. Studies have found that there are subpopulations of C-kit+ progenitor cells with different functional capabilities.[34]

Experiments of animal models that involved the transplantation of human C-kit+ CSCs into infarcted myocardium of rats resulted in the regeneration of cardiac myocardium that was confirmed with an enhanced green fluorescent protein (EGFP).[29] The pretreatment of C-kit+ with Ephrin A was observed to cause greater retention of transplanted cells, improved regeneration capacity with a two-fold decrease in infarct size, and a significant increase in ejection fraction compared to treatment with only CSCs.[32] Ephrin A is an essential signaling

molecule for cell adhesion and movement. Clinical study of c-kit transplantation in patients with ischemic heart disease resulted in significant improvement of cardiac function with short-term safety.[3]

Some of the limitations of using c-kit is that much of the reported outcomes are mainly dependent on immunostaining, making false positives possible. Conversing studies have found a negative result that CSCs do not produce significant efficacy. A study on the transplantation of CSCs into infarcted adult mouse hearts showed a lack of cardiomyogenic differentiation.[3] Another study with human heart samples suggested that the observed c-Kit+ CSCs could have been mast cells. [3, 34, 35]

Cardiosphere-Derived Cells (CDCs).

CDCs are clonogenic cardiac progenitor cells with multilineage potentials both in vitro and in vivo. CDCs are a mixture of stromal, mesenchymal, and progenitor cells obtained via percutaneous endomyocardial biopsies and cultured to yield cardiosphere-derived cells comprising c-kit cells at the core and cells expressing extracellular markers at the periphery.[36] CDCs are administered via intracoronary injection in pre-clinical animal models and clinical studies with human patients.

These cardiac progenitor cells were first identified in 2003 through the expression of tyrosine kinase receptors and the absence of common hematopoietic lineage markers such as CD3, CD14, CD16, CD19, CD20, CD45, and CD56 in the mammalian heart.[37] Studies on the use of CDCs for the treatment of acute myocardial infarction (MI) were found to improve left ventricular systolic function (LVSF) and reduce infarct scar size in various preclinical trials of coronary artery disease.[37] Results from the pre-clinical trials are somewhat consistent with outcomes found in clinical studies showing decreased scar mass, increased viable mass, and improved regional function consistent with therapeutic regeneration compared to the control group.[31, 38]

Tissue regeneration was noted to persist long after transplantation of CDC cells; however, despite the improvement in scar size and regional function, there were no reported improvements in global cardiac function.[36] The use of autologous CDCs have been determined in preclinical models to be safe; however, a study has observed some serious adverse events such as acute myocardial infarction, chest pain, and coronary revascularization following CDCs cell infusion in patients with acute MI.[36, 37, 39] Concerns have also been raised on the original study involving CDCs and reported efficacies.[34]

Exogenous Cardiac Stem Cells.

Exogenous stem cells are cardiac progenitor cells not resident to the heart. They include mesenchymal stromal cells, embryonic stem cells (ESCs), induced pluripotent stem cells (IPSCs), bone marrow stem cells (BMCs), bone marrow-derived mononuclear cells (BM-MNCs), endothelial progenitor cells (EPCs), adipose tissue-derived stem cells (ADSCs) and hematopoietic stem cells (HSCs).

Mesenchymal stromal cells (MSCs).

MSCs are multipotent stem cells that can differentiate into cells of mesenchymal origin, like chondrocytes, adipocytes, myocytes, and osteoblasts.[16] They are easily accessible as they can

be derived from the bone marrow, fibroblast, adipose tissue, and umbilical cord.[40] MSCs are easily isolated, expanded, and require minimal genetic modification. Both allogeneic and autologous forms of MSCs have immunomodulatory properties mediated by the absence of MHC II, modulation of T-cell phenotype due to lack of B7 costimulatory molecule, and secretion of anti-inflammatory factors.[16]

Despite poor engraftment and poor survival rate of MSCs, the preclinical and clinical studies showed improved cardiac function, contractility, LVEF, and decreased remodeling, amongst other positive outcomes. These results were achieved by paracrine signaling, which helps recruit progenitor cells or activation of resident stem cells for angiogenesis and cardiac embryogenesis, enhances vascularization, stabilizes the extracellular matrix, reduces fibrosis and scar formation, and causes cell homing.[16, 41] Increased cell retention has been observed with intramyocardial injection of MSCs.[41]

Differentiation of MSCs to cardiomyocytes can be induced by 5-azacytidine (DNA methylation agent) and by a hypoxic environment of the infarcted tissue which stimulates expression and release of growth factors like vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and insulin-like growth factor (IGF). [16, 40]

Embryonic stem cells (ESCs).

These cells originate from the inner cell mass of the blastocysts of a 3-5day old embryo. First isolated from mouse blastocysts in 1981, ESCs are totipotent cells with the ability to differentiate into cardiac, neuronal, or pancreatic cells derived from the three germ layers.[42] The first clinical study of the ESCs used the cellular scaffold method of stem cell delivery.

In animal models, the ESCs electromechanically coupled to the host cells at a spontaneous rate and they express cardiac restricted transcription factors such as GATA binding protein 4 (GATA-4), NK2 homeobox5 (NKX2–5), Myocyte-Specific Enhancer Factor 2C (MEF2C) and Irx4 which influences their ability to engraft and improve cardiac function.[40] Revascularization of the infarcted heart was observed in both rat and monkey models, but it was associated with the formation of arrhythmias.[29]

In human studies, pretreatment of ESCs with fibroblast growth factor receptor inhibitors (FGFRI) and Bone morphogenic protein-2 (BMP-2) induces the cells to express insulin gene enhancer protein (ISL-1), stage-specific embryonic antigen (SSEA), which was associated with improvement of LVEF by 10%, and no adverse effects were observed.[29] The retention rate of ESCs transplanted can be increased by prosurvival factors like Activin A, BMP4, and activation of Akt pathway or heat shock preconditioning.[41]

Although ESCs have great potential in the regeneration of cardiomyocytes, there have been limited clinical studies done due to the high ethical concern and lack of availability because of their origin. There are concerns raised on its immunogenicity as there has been increasing evidence suggesting that the ESCs express specific human leukocyte antigen subclasses, increasing the risk for graft rejections.[40] However, immunosuppressive therapy like

cyclosporine decreases this risk. In addition, other adverse effects that have been observed include tumorigenicity and teratoma formation. [43]

Induced pluripotent stem cells (iPSCs).

Initially, iPSCs were derived from already differentiated adult somatic stem cells reprogrammed into ESC-like cells by introducing transcription factors: Oct4, Sox2, Klf4, cMyc via retroviral mechanisms.[16] Although iPSCs have easily accessible source tissue, low risk of immune rejection, and low ethical concern, they share similar characteristics with ESCs: morphology, proliferation, differentiability, potency, expression of embryonic markers (SSEA-1), teratoma formation, and formation of the embryoid body in vitro.[40, 41]

In the murine model, intramyocardial injection of iPSCs showed improvement of LVEF and regional wall movements on echocardiography, but there was a report of teratoma in immunodeficient mice. Subsequent studies of undifferentiated iPSCs showed the presence of teratoma in both immunodeficient and immunocompetent mice. However, repeated experiments for differentiated iPSCs from embryoid body-beating aggregates showed no tumorigenicity.[44]

Currently, iPSCs have not been used for human clinical studies due to various safety concerns like genetic instability. In cloning, differentiation, and reprogramming of the cells, epigenetic memory causes mutations in the cell genome, such as chromosomal aberrations: duplications, translocations, deletions, and single nucleotide variants. These mutations play a role in the tumorigenic potential of iPSCs.[45]

The transcription factors initially used for reprogramming show oncogene potential, which can create teratoma.[41] The challenges associated with the use of iPSCs aside development of teratoma include poor electromechanical coupling of the stem cells to host cells, immunogenicity in the allogeneic iPSCs, a discrepancy in the method of selection, reprogramming, and several cell passages.[44]

Bone marrow cells (BMCs; multi-potent cells)

Bone marrow-derived stem cells derived from the tibia and femur of mice were sorted by fluorescence-activated sorting from transgenic mice expressing GFP to ensure the stem cells were lin-c-kit+. The lin-c-kit+ BMCs were injected into the walls surrounding the infarcted areas after coronary ligation, with newly formed cardiomyocytes occupying 68% of the infarcted area observed 9 days post-transplantation.[45] Some of the mice samples did not show the above result, which was attributed to the difficulty in injecting the stem cells and immunogenic reactions to the BMCs.

In mice with lin-c-kit positivity, which has a high capacity to differentiate, the left ventricular end-diastolic pressure (LVEDP) decreased by 38%. The repair mechanism involved proliferation, migration, and differentiation of the stem cells initiated by signals from the injured myocardium. The newly formed cardiomyocytes express cardiac protein like connexin 43, which suggests cellular coupling and functional competence of the newly formed myocardium.[46]

In clinical studies, BMCs are harvested from the posterior iliac crest. Patients are admitted within 5-7 days of symptoms onset of ST-elevation myocardial infarction (STEMI). Following baseline MRI, aspirated BMCs are then processed by 4% gelatine-polysuccinate density gradient sedimentations to reduce the volume of preparation. MRI was also used to assess the cardiac function after transferring BMCs into the infarcted hearts. Results observed that the global left ventricular ejection fraction (LVEF) at baseline (determined 3·5 days [SD 1·5] after PCI) was 51·3 (9·3%) in controls and 50·0 (10·0%) in the bone-marrow cell group (p=0·59). After 6 months, mean global LVEF had increased by 0·7 percentage points in the control group and 6·7 percentage points in the bone-marrow-cell group (p=0·0026).

Transfer of bone-marrow cells was also observed to enhance left-ventricular systolic function, primarily in myocardial segments adjacent to the infarcted area. There was no increase in the risk of adverse clinical events such as in-stent restenosis and proarrhythmic events.[47]

Bone marrow derived mononuclear cells (BM-MNCs).

BM-MNCs include a heterogenous population of monocytes, lymphocytes, hematopoietic, and endothelial stem cells. BM-MNCs have several advantages, including easy accessibility, minimal ex-vivo processing, and reduced immunogenicity. Regenerative potential for infarcted myocardium was first observed in 2001, after which many preclinical and clinical studies were conducted to assess this potential.

BM-MNCs were obtained from the posterior iliac crest via aspiration, and density gradient centrifugation was administered by intramyocardial injection or intracoronary injection to patients with MI.[40] Based on multiple trials carried out to assess the efficacy of these stem cells, the outcome has been inconsistent. The initial clinical trial, like the BOOST Trial, showed a 6.7% improvement in LVEF, and when re-evaluated at an 18months follow-up, there was no more improvement in LVEF, and at a 5-year follow-up.

The REPAIR AMI Trial was used to assess accurate cell delivery time to get results with significant benefits; it was concluded that cells delivered five or more days after PCI produced substantial improvement in LV function that lasted for two years. The BALANCE Study confirmed a similar outcome with a gain of LV function lasting for five years, amongst other benefits. These beneficial effects were ascribed to angiogenesis, trans-differentiation of stem cells into smooth muscle cells, endothelial cells, and cardiomyocytes, and secretion of growth factors. Subsequent studies showed no significant functional or structural benefits.[43] Contrary to the initial clinical trials, subsequent trials like the TIME, SWISS AMI, and LATE TIME AMI trials showed that BM-MNCs do not affect the improvement of LV function regardless of the time of cell delivery.

The discrepancy in the results of BM-MNCs could be due to the different methods of cell handling, isolation, storage, cell delivery, cell selection, and expansion techniques used in the various clinical trials.[16] Due to the heterogeneous population of BM-MNCs, there might be a particular subpopulation of a cell that results in the beneficial effects which are yet to be identified, and the various trials could have minimized or maximized the impact of that cell by the difference in their method of cell processing.

The variation in imaging techniques used for post-transplantation evaluations in the different studies contributed to this discrepancy in the results. There was no improvement in LVEF, infarct size, or LV volumes in studies using MRI to assess the functional benefits of the myocardium after implantation as opposed to studies that used echocardiography or left ventriculography for assessments.[48]

Wharton's Jelly-derived mesenchymal stem cell (WJ-MSCs).

Wharton's Jelly is a gelatinous mucous connective tissue surrounding the vein and two umbilical cord arteries. WJ-MSCs originate from embryonic epiblasts. The umbilical cord of female subjects is collected with consent during cesarean section. WJ-MSCs are then scraped from the sub-amnion to the perivascular region with a scalpel and isolated non-enzymatically. [49, 50]

WJ-MSCs possess properties between hESCs and adult stem cells. There are similarities between the gene expression of WJ-MSCs and hESCs. WJ-MSCs express the core markers characteristics of undifferentiated hESCs at a lower level and early cardiac transcription factors.[49] Reports have shown that stem cells with pluripotent differentiation potential can be isolated from WJ-MSC in humans. Such cells possess characteristics for MSCs, such as the ability to adhere to plastic, expression of specific surface markers, and differentiation into cells of mesenchymal origin, including cardiomyocytes. Functional analysis has also shown that WJ-MSCs signature genes are involved in immune, cytoskeletal, and chemokine regulation, cell adhesions, and signaling.[49, 51]

WJMSCs retain markers for both hESCs & MSCs and express low stemness markers and high levels of early cardiac transcription factors genes. WJMSCs act as an alternative to autologous stem cell therapies for MI. WJ-MSCs have a greater expansion capability and faster growth in vitro and do not impose ethical concerns as ESCs.[52]

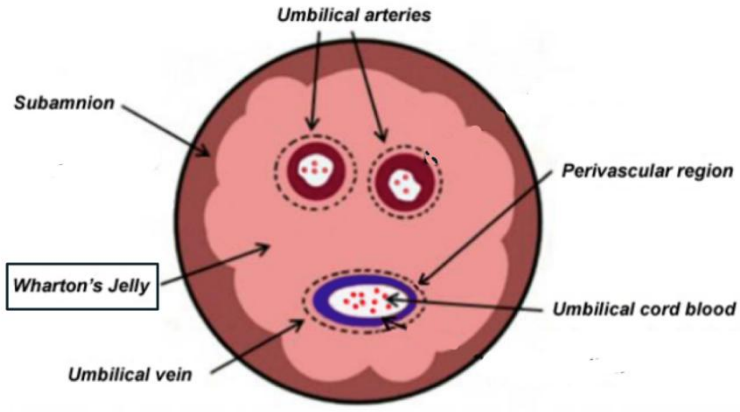

Figure 4. Depiction of Wharton's Jelly within the human umbilical cord

Image adapted from Kim et al. [51]

Adipose tissue-derived stem cells (ADSCs).

ADSCs are immune-competent cells, endothelial progenitor cells, and mesenchymal stem cells. The mechanism of ADSCs in MI is suspected to be through the paracrine release of anti-

apoptotic, immunomodulatory, and proangiogenic factors to stimulate cardiomyocyte regeneration and neo-angiogenesis in infarcted border zones.[53]

ADSCs are obtained by liposuction procedure of the periumbilical region, isolated using Cytori Celution device, and administered via intracoronary infusion or trans-endocardial injection.[53] Administration of ADSCs immediately after acute MI improved LV function, myocardial perfusion, and global LVEF in preclinical studies.[41] The preclinical intracoronary administration of ADSCs showed similar findings to the clinical studies that trend towards improved cardiac function accompanied by significant improvement in perfusion defect, about 50% reduction in myocardial scar formation, and LV mass.[16] Adipose-derived stem cells exhibited a higher percentage of differentiation into cardiomyocytes when compared to bone marrow (MSCs). Still, the most beneficial advantage of ADSCs is the easy accessibility and abundant cell source.[43, 54]

In preclinical animal models, ADSCs were reported to differentiate into cardiomyocytes and endothelial cells in vivo. Several clinical studies have used ADSCs for patients with acute MI. However, very few released their outcomes. While target lesion revascularization was observed as an adverse event, using ADSCs is considered safe with no unanticipated adverse effects.[53] ADSCs do offer advantages such as higher yield from higher stem cell density, less invasive and painful extraction method, and higher cardiomyocyte differentiation percentage compared to (MSC).[41]

Endothelial progenitor cells (EPCs)/Hematopoietic stem cells (HSCs).

HSCs can be isolated from bone marrow through selective sorting for a specific surface antigen (Lin-c kit+). The endothelial progenitor cells are a subpopulation of HSCs and express its surface marker; [40] ckit surface marker is thought to be responsible for HSCs regenerative potential for infarcted myocardium.[16] These stem cells are easily accessible, have standardized isolation methods, have a low risk of immunogenicity, and promote vascularization.[31]

In preclinical studies, HSCs/EPCs directly isolated from the peripheral blood or bone marrow expanded in vitro and administered into the coronary circulation showed improved LVEF, decreased fibrosis, and formation of functional and structural competent myocytes expressing expression contractile proteins like desmin, connexin43 & N-cadherin. Similar positive outcomes were observed in human studies.[40, 55] A subsequent study was done to compare the effectiveness of BM-MNCs and HSCs showed no significant differences between the two groups.[16]

TYPES, SOURCES, BIOMARKERS, ISOLATION & ROUTES OF ADMINISTRATION OF STEM CELLS.

Cell Type	Source	Biomarker, Surface markers or Transcription factor	Method of Isolation & Differentiation	Route of administration
Endogenous (CSCs) + Ephrin A1. [16, 32]	• Atrial appendages. [56]	• C-kit+, Sca-1.	• Enzymatic digestion, flow magnetic cell sorting using Sca-1 coupled magnetic beads. RT-PCR. Isolated by surface antigen enrichment. [34]	• Intracoronary infusion
Cardiac progenitor cells (CDCs). [32]	• Endomyocardial biopsy. [32]	• C-kit membrane marker: CD29, CD34+, CD90, CD105. • Transcription factor: C-kitISl-1, Nkx2.5, MEF2C, NANOG, OCT4 & GATA-4.	• Enzymatic digestion, plated culture	• Intracoronary injection
hiPSCs. [16, 44]	• Fibroblast, keratinocyte, human cord blood, peripheral blood lymphocyte. [45]	• Co transferring transcription factors (oct-4, Sox-2, Klf4, c-Myc)	• Plated on 0.1% gelatin-coated culture dishes • timed application of specific growth factor & reprogramming. [57]	• Cellular scaffold applied to an epicardial surface via sternotomy incisions or using small incision thoracoscopic techniques. [58]
hESCs. [29, 43]	• Inner cell mass of the blastocyst[40]	• SSEA-1 progenitor surface marker, cardiac gene Isl-1 & Mef2,	• Culture, flow cytometry & cell sorting	• Fibrin patch containing the hESCs is placed in the pocket between the epicardium and pericardium. [43]
BMCs (multi-potent)	• Iliac crest. [59]	• CD34+	• Preparation via 4% gelatine-polysuccinate density gradient sedimentation, flow cytometry. [47]	• Intramyocardial injection. • Cytokine migration • Intracoronary infusion via angioplasty balloon catheter
BM-MNCs. [59, 60]	• Posterior iliac crest. [43]	• CD34+/CD133+	• Aspiration, density gradient centrifugation. [40]	• Intracoronary injection via stop flow balloon catheter • Intramyocardial transplantation
MSCs. [40, 60-62]	• Muscle, skin, adipose tissue. [41]	• ALCAM/CD44 adhesion molecules •CD29, CD73, CD90, CD105	• No standard protocol. [16]	• Intracoronary injection, • Intravenous injection

WJ-MSCs. [49, 51]	• Embryonic or extraembryonic mesodermal tissue at day 13 of embryonic development. [16].	• CD29, CD73, CD90, CD105. • Transcription factors: Flk-1, Isi-1, Nkx2.5. oct-4, Sox-2, GATA-4, GATA-5, GATA-6, connexin-43	• Non-enzymatic method.	• Intracoronary infusion. [50]
ADSCs. [54]	• Subcutaneous adipose tissue. [41]	• SM- CD73, CD105, CD90	• Liposuction from subcutaneous Non-enzymatic and enzymatic dissociations called stromal vascular fraction.	• Trans-endocardial injection, Intracoronary infusion. [16, 53]
HSCs/EPCs. [16, 31, 41]	• Bone marrow, Peripheral blood,	• Lin- C-kit+ / CD133+, FLK-1, VEGFR	• Density gradient centrifugation or sedimentation. • Selective sorting via particular surface antigen. [40]	• Cytokine mobilization for EPC recruitment; G-CSF therapy, statins

Table 1: showing different stem cell sources, surface markers and/or transcription factors, mechanism of isolation and differentiation, and the route of stem cell transplantation.

ROUTES OF ADMINISTRATION OF STEM CELLS: ADVANTAGES AND DISADVANTAGES.

The intrinsic regenerative potentials for stem cells are essential to regenerate an infarcted heart. Certain variables, such as methods of administering these stem cells, are also necessary to achieve the most favorable outcome. The method of administration can influence the level of stem cell retention and survival rate of cells and could produce adverse effects in some patients. Different stem cell administration/delivery methods include intravascular infusion, intramuscular injection, cellular scaffolds (cell-sheet technology), and mobilization of stem cells.[63]

Intravascular infusion

This strategy of stem cell delivery includes intracoronary and intravenous infusion. Stem cells are infused into the coronary artery in the intracoronary method of cell delivery compared to the intravenous approach that involves cell delivery through the coronary sinus. The efficacy of intravascular cell infusion depends on the ability of the stem cells to reach their destination as large portions of stem cells are usually trapped in other organs like lungs, liver, and kidneys.[31, 41]

Some advantages of the intravascular infusion method include procedural practicality, simplicity in technique, and lack of need for specialized equipment.[63] A randomized clinical trial that was conducted to evaluate the efficacy and safety of stem cell therapy based on the route of stem cell administration among three groups of patients with MI following coronary artery stenting found improved cardiac function and exercise capacity among patients who received stem cells via intracoronary therapy compared to 2 other groups; a group of patients who had granulocyte-colony stimulating factor (G-CSF) as a method of mobilizing peripheral blood and a control group. Interestingly, there was also a higher rate of re-stenosis due to neointimal growth

observed in the patient group who received stem cells via intracoronary infusion.[64] However, it is predicted that the timing of stem cell mobilization and the use of drug-eluting stents may resolve this issue.[50, 64]

Though the intravascular method is less invasive, it is associated with a low survival rate and poor cell retention.[52] With large stem cells such as ESCs and iPSCs, administration via this method is associated with the occlusion of blood vessels and the extension of the ischemic zone.[58] The method is also inappropriate for MSCs as it could cause fibroblast formation in scarred myocardium and regeneration of myocytes in the unaffected area of the ventricle.[55] Other adverse events, such as uncontrolled differentiation of stem cells, microinfarction, and myocardial calcification, were observed in animal studies that involved intracoronary infusion.[64]

Intramuscular Injection

This is the most important method of stem cell delivery when there are low levels of cell homing signals, occluded coronary arteries, and large stem cells. Intramuscular injection involves direct stem cell delivery to the heart via epicardial or trans-endocardial injection.[63] Epicardial injection results in better stem cell retention but is a more invasive cell delivery route than trans-endocardial injection.

However, this route is complex and unfavorable because of adhesions from coronary artery bypass grafting (CABG) and the inability to access the posterior aspect of the heart easily.[16] With the trans-endocardial injection of stem cells, this method involves imaging to monitor the injection of cells into targeted areas and is minimally invasive. The overall safety of using the approach is still questionable, and perforation of the infarcted regions can occur.[63] Intramuscular injection method causes mechanical injury and biochemical stress to transplanted cells and is associated with poor stem cell survival rate due to insufficient blood supply. [62]

Cellular scaffolds

In this method, the stem cells are patched onto the epicardium of the heart to target the areas of interest. This is done by creating a pocket between the epicardium and pericardial flap. A fibrin patch containing the differentiated stem cells is slipped into the pocket, and the open borders of the pocket are sutured.[43]

Delivery of cells using cellular scaffold leads to increased cell retention and improvement of cardiac function by paracrine effects. Other advantages include the lack of mechanical or biochemical stress to the transplanted stem cells or host myocardium following transplantation and the best approach for cell delivery in emergencies.[41] The use of this approach for delivering ESCs resulted in decreased risk of ventricular arrhythmias, reduced cell damage, preservation of cardiac function, and improved patients' survival rate.[16] Poor cell survival due to insufficient blood supply is associated with using cellular scaffolds.[41]

Mobilization of stem cells

This is a non-invasive approach to cell delivery for cardiomyocyte regeneration. It uses cytokines like granulocyte-colony stimulating factors or injectable hydrogels to mobilize stem cells toward the injury sites.[41] Endogenous stem cells are mobilized by increasing the progenitor pool, enhancing differentiation and efficacy. HSCs are cells mobilized using G-CSF from the bone

marrow and peripheral blood for easy extraction from the patient's blood sample.[16] A disadvantage is that G-CSF is also associated with mobilizing immune cells leading to non-specific inflammation.[64]

OUTCOMES OF PRECLINICAL & CLINICAL STUDIES OF STEM CELL THERAPY FOR CORONARY ARTERY DISEASE & HEART FAILURE.

Cell Type	Pre-Clinical Studies Outcomes		Clinical Studies Outcomes	
	Structural	**Functional**	**Structural**	**Functional**
Endogenous (CSCs) [29, 43]	• 1.5% new cardiomyocytes in infarct & border zone (5% of entire heart cardiomyocyte compliment)	• Improved cardiac function after 4 weeks	• 3.3% decrease in infarct mass after 6 months • Increased LV viable mass by 12.2% & 15.7% decrease in scar size after 12 months & 24 months respectively. [32]	• Improved LVEF and maximum oxygen consumption after 6 months [32]
Endogenous (CDCs) [48]	• Ventricular remodeling, stimulated angiogenesis,	• Increased LVEF, superior paracrine effect in immunodeficient mice after 3 weeks [29]	• Decreased scar size, increased viable heart mass after six months [5, 62]	• Improved regional function of damaged myocardium is observed after 6 months & 1 year • Improved systolic wall thickening & contractility [3]
hiPSCs	• High integration capacity with recipient myocardium. alleviate adverse modeling processes • Improved cardiac function, decreased LV remodeling • Decreased apoptosis • Decreased infarct size. [29, 58]	• Improved LVEF 4 weeks after transplantation • Decrease ESV • Generate & improve cardiac contractility [44]	No known reports	No known reports

hESCs	• Formation of new myocytes. Increase cardiac mass • Weakening of LV remodeling in rat models • Electromechanical coupling to host cells at a spontaneous rate • Improved re-muscularization of infarcted heart in monkey model [29, 41]	• Improvement of LV systolic activity • Improved LVEF in rat models after two months	• High integration capacity with recipient myocardium	• Increased LVEF by 10% at 3 months follow up, improved contractility • Improved systolic motion of the cell-treated segments of the infarcted heart. • Results from a 6 min walk test showed increased distance covered(350-467m) [16, 29, 43]
BMCs (multi-potent)	• Formation of new myocytes & vascular structure, activation & growth of resident progenitor cells via paracrine effect [55]	• Colonized dead tissues and gave rise to contracting myocardium occupying 68% of an original infarct.	• Decreased MI size, improved maximal vascular conductance capacity [43]	• Improved LVEF, increased contractility at six months follow-up [59]
BM-MNCs[40]	• Improved contractility. Decreased morbidity & mortality [6, 16]	• Significant improvement of LVEF at three months post-transplant evaluation. [60]	• Myocardial regeneration, neovascularization, decreased infarct size after 1 year [31]	• Increased regional contractility, increased perfusion after 3 months • Slightly improved ejection fraction, decreased coronary plaques after 4 years [3]
MSCs	• Promotes regeneration of infarcted myocardium via paracrine effect. • Improvement In tissue metabolism. [55, 60]	• Reconstitution of dead myocardium correlated with the improvement of ventricular function & the reappearance of wall motion activity	• Reversed modeling, increased viability of infarct wall, increased regional contractility at 6 months follow up [40]	• Improved LVEF, decreased LVEDV after 6 months
WJ-MSCs	• Significant change in LV wall motion. [50]	• Improvement in Ejection fraction following MI. [52]	• Improved myocardial viability, improved infarct area perfusion, slightly reduced infarct size. [16]	• Increased LVEF, improved LVEDV & LVESV 18 months. [16, 41]
ADSCs	• Reduced infarct size. • Improved heart function by increasing angiogenesis and decreasing the degree of fibrosis in the infarcted tissue. [53, 63]	• Improvement in LVEF at 1 month post-transplant evaluation. [65]	• Decrease myocardial scar tissue (infarct size), improved LV mass and motion at 18 months follow up, improved perfusion. [16]	• Maximal improvement of oxygen consumption. [63]

HSCs/EPCs	• Newly formed myocardium replaced 38% of the infarcted area. (Promotes cardiac tissue regeneration) • Promotes neovascularization • decreased fibrosis reported after 1 month. [29, 55, 66]	• Myocytes became functionally competent and expressed contractile proteins, desmin, connexin 43, N-cadherin. • Improved ventricular hemodynamics	• Significant decrease in infarct size, regeneration of contracting cardiomyocyte after 6 months. [40]	• Improved LVEF after 6 months in patients with severe LV dysfunction if started early, decreased LV

Table 2: shows structural and functional outcomes reported for individual stem cells in preclinical (animal) studies and clinical (human) trials.

ADVERSE EFFECTS & LIMITATIONS PROFILE IN PRECLINICAL & CLINICAL STUDIES OF STEM CELL THERAPY FOR CORONARY ARTERY DISEASE & HEART FAILURE.

Cell Type	**Pre-clinical Studies Adverse Effects/Limitations**		**Clinical Studies Adverse Effects/Limitations**	
	Adverse effects	**Limitations**	**Adverse effects**	**Limitations**
Endogenous (CSCs)	• Fibrosis in the border zone of injection site	• Optimal cell or combination of cells for transplantation within a group is not well defined. [32]	• No important complication reported. [29]	• Limited clinical studies. and small sample sizes. • Most studies are in vitro & pre-clinical trials • Restricted cell quantity • Access via myocardial biopsy is invasive • reduction in available c-kit csc available for tissue biopsy with age • Inadequate cell characterization. [29, 32]
Endogenous (CDCs)	• Possibility of Microvascular occlusion • Thrombus formation. [32, 39]	• No known research limitation from reports	• Risk of MI • Ventricular tachycardia, ventricular fibrillation, and cardiac tumor formation were reported after 6 months. [36]	• Conflicting results on 12 months evaluation findings • No measurable functional improvement in end-systolic volume, end-diastolic volume, or LVEF in 12 months findings

hiPSCs[16, 22, 41, 44, 58]	• Potential to react with beta-adrenalin which can lead to increased spontaneous beating rate, decreased AP duration and arrhythmias • Risk of tumorigenicity especially in immunodeficient mice. [45, 67]	• Insufficient studies • Genomic instability. • Poor cell engraftment • Risk of tumorigenicity • Poorly defined infusion time, rate, & cell delivery method	• No known clinical study findings	• Therapeutic benefits remain largely unknown due to no available clinical study • Lack of standardized isolation method • High safety concerns due to genetic variation and long-term instability • High genetic variations may necessitate a larger number of participants requiring more time and cost.
hESCs	• Intravenously injected hESCs can engraft and colonize all organs with possible development of neoplastic lesions • Immune rejection of allogeneic hESCs • Likelihood to form teratoma at implantation site. [40]	• Sporadic reports on efficacy. • Reconstituted tissue did not possess the characteristics of functionally competent myocardium in other study	• Graft rejection due to immunogenicity. • Risk of tumorigenesis and malignant transformation, when administered via intramyocardial injection of undifferentiated ESCs • Risks of arrhythmias. [16, 29]	• High ethical & political concerns • Limited human studies.
BMCs (multi-potent)	•Difficulty injecting cell into LV of mouse due to thickness of LV wall (<1mm) • Heart beats of about 600 times/min observed	• Highly problematic method of administration	• Proarrhythmic effect • Risk of in-stent restenosis with use of granulocyte colony stimulating factor for mobilization of peripheral blood. [59, 64]	• No significance in LVEF when administered within 24 hours of MI. [3, 29]
BM-MNCs	• Low engraftment rate • Lack of differentiation. [55, 60]	• Contradictory findings between clinical and preclinical models	N/A	• poor cell survival • Benefits are transient. • No cell detected after 2 weeks

MSCs	• Intracoronary delivery resulted in fibroblast formation in scarred region • Regeneration of myocyte in unaffected portion of ventricular wall. [55]	• Contradictory findings between clinical and preclinical models • Characteristics of what constitute as MSCs poorly defined or conflicting • No definite consensus on properties of MSC.	• Thrombogenic and potential to form microvascular occlusion. •Potential to cause ischemic damage in infused myocardium. [39]	• Limited studies • Lack of definitive marker for MSCs • Lack of report from long-term follow-up • Autologous MSC cannot be isolated and expanded in less than 14 days thus reducing their usefulness for treatment of AMI. •Desired dosage difficult to obtain. [16, 50]
WJ-MSCs[50]	• Rats model demonstrated decline in cardiac function (ejection fraction) and other functional variables over time compared to human studies which shows improvement overtime. [52]	• Limited Studies	• Ectopic tissue formation •Increased levels of tumor-associated antigen. [5]	• Limited studies.
ADSCs	• No known adverse effect found in reports	• Poor cell retention and survival rate. [68]	• No unanticipated adverse effects reported after therapy. [53, 65]	• The procedure to extract ADSCs is invasive • Poor cell quality from donors due to age and comorbidities. [16] •Limited studies.
HSCs/EPCs	• No known adverse effect found in reports	• Limited studies	• Increased risk of restenosis and atherosclerotic disease progression, proangiogenic capacity • Potential to increase tumor vascularization • Mild to moderate bone pain & muscle discomfort. [40]	• Benefits vary with studies. • Not enough study on amount of application that increases adverse effect •Require careful monitoring)

Table 3: Showing comparison of adverse effects and limitations between pre-clinical (animal) studies and clinical (human) trials.

CONCLUSION

The response to cardiac injury in coronary artery disease typically involves loss of viable cardiomyocytes, inflammation, repair by fibrosis, scar tissue formation, and cardiac remodeling, which results in a decrease in cardiac contractility and systolic function. While current treatment guidelines address most of these clinical outcomes, stem cell therapy offers the added advantage of regeneration of viable cardiomyocytes. This potential therapeutic approach expectedly offers a better response profile to injury by decreasing fibrosis, scar tissue formation, and cardiac remodeling. Stem cell therapy is thus able to offer a general improvement in cardiac function, clinical outcomes, and prognosis while reducing progression to heart failure following episodes of coronary artery disease when compared to established treatment guidelines.

In this review, we focused on the therapeutic potential of stem cells in the management of coronary artery disease and heart failure. Normal cardiac embryogenesis was reviewed to highlight the significance of transcription and growth factors in signaling pathways involved in the migration, proliferation, and differentiation of resident stem cells from the paired heart fields into functional adult cardiomyocytes. Findings from preclinical and clinical studies employing different sources of stem cells were evaluated, and the clinical outcomes, adverse effect profile, as well as ethical and study limitations were compared to guide in identifying the ideal choice of stem cell. Among many sources, future direction points towards the use of human inducible pluripotent stem cells which avoids major ethical limitations especially associated with embryonic stem cells. However, more clinical studies are needed to standardize the method of administration of stem cells, dose, and timing to improve retention, proliferation, and differentiation potential, as well as for essential and safe transcription and growth factors needed for the recruitment of progenitor cells, activation of resident stem cells and optimum cardiomyocyte regeneration.

The stem cell choice with the least scar tissue formation, fibrosis, and cardiac remodeling would be ideal to restore cardiac contractility and systolic function following episodes of coronary artery disease. Pharmacotherapies would also have to be explored to address acute and long-term adverse effects of arrhythmias, thrombosis, microvascular occlusions, immunogenicity, rejection risk, restenosis, and tumorigenicity to improve prognosis. Stem cell therapy promises to be a viable and ideal therapeutic option in the nearest future and may be explored either in combination with established treatment guidelines for patients with coronary artery disease and heart failure or as a separate choice for therapy.

COMPETING INTERESTS

The authors have declared no competing interests exist.

Non-standardized abbreviations

CSCs: C-kit cardiac stem cells. CDCs: cardiosphere-derived cells. hiPSCs: human induced pluripotent stem cells. hESCs: human embryonic stem cells. BMCs: Bone Marrow derived stem cells. BM-MNCs: Bone marrow-derived mononuclear cells. MSCs: Mesenchymal stromal cells. WJ-MSCs: Wharton's Jelly-derived mesenchymal stem cell. ADSCs: Adipose tissue-derived stem cells. HP-BMCs: Hypoxia-preconditioned bone marrow cells. HSCs: hematopoietic stem cells. EPCs: Endothelial progenitor cells. AMI: acute myocardial infarction. MI: Myocardial infarction.

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