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USE OF STEM CELLS IN REGENERATIVE CARDIOVASCULAR MEDICINE

(Review Article)

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Abstract

Cardiovascular disease (CVD) is a major cause of morbidity and mortality worldwide, with its lifetime risk exceeding over 60%. Though various medications and procedures have managed to play a role in reducing mortality, none have shown to be permanent. The idea of stem cells is to generate an original solution that provides normal physiological responses. When applied to cardiology, it holds tremendous promise for rapid myocardial regeneration. The selection of the most appropriate type of cell is essential for its efficient application. If done successfully, it will negate temporary solutions such as a stent, defibrillators, and medications. This article discusses all the studies that applied stem cells in cardiac pathologies and reveals the benefits as well as outcomes. It helps us understand the limitations one may come across while experimenting in this field and introduces issues that will need further research.

Introduction

Cardiovascular disease (CVD) remains the leading cause of death worldwide, killing 17 million people each year. It is estimated by the World Health Organization (WHO) that this number will reach 24 million by 2020 [1]. CVD contains multifactorial pathologies that are both genetic and environmental. Lifestyle changes, pharmacological or surgical intervention are current strategies against CVD. The effect of drug treatment differs for each individual and surgery is not viable in all patients. New strategies or approaches have to be considered to better understand the pathogenesis of CVD and broaden the diagnostic and therapeutic plan, especially in the case of heart failure (HF).

Stem cells are one of the human body's key cells that can develop into more than 200 cell types. Stem cells are undifferentiated cells that are found in the embryonic, fetal, and adult stages of life and give rise to differentiated cells that create tissue and organ structures. Stem cells construct the foundation for the entire body's tissue and organ system. It mediates various roles in host disease development, growth, and tissue repair processes. There are four types of stem cells, i.e., unipotent, multipotent, pluripotent, and totipotent, depending on the trans-differentiation ability. Self-renewal, clonality, and potency are the key characteristics of stem cells. Between different stem cells, these properties can differ. Blastocyst-derived embryonic stem cells (ESCs) have a greater capacity for self-renewal and potency, whereas adult tissue stem cells have minimal self-renewal because they do not proliferate freely and can only differentiate into tissue-specific cells. Stem cells can be classified as embryonic stem cells (ESCs), tissue-specific progenitor stem cells (TSPSCs), mesenchymal stem cells (MSCs), umbilical cord stem cells (UCSCs), bone marrow stem cells (BMSCs), and iPSCs on the level of regenerative applications. Stem cell transplantation for induction of tissue regeneration of malignant cells may be autologous, allogeneic, and syngeneic. Tissue typing of human leukocyte antigens (HLA) for tissue and organ transplantation as well as the use of immunosuppressants is advised to prevent the effects of host-versus-graft rejection [2,3,4].

In patients with advanced HF after MI, stem cell transplantation has been reported to enhance cardiac function as a new treatment strategy. Stem cell transplantation, as shown in many basic research and clinical trials, can boost tissue perfusion, contribute to angiogenesis, and retain or regenerate myocardial tissue. For myocardial infarction (MI), stem cell-derived sheet engineering provides desirable advantages in comparison to direct stem cell transplantation and scaffold tissue engineering. Induced pluripotent stem cells can form vascularized networks that would allow the manufacturing of thick human cardiac tissue and have proven to be successful in MI therapy compared to other sheets.

History

In the mid-nineteenth century, the evolution of stem cells led to the invention that other cells could be developed by certain cell types. In the following years, it was discovered that the bone marrow

contained hematopoietic SC and stromal cells. In the late 1950s, Dr. Thomas conducted the first successful transplant. Identical twins were taken in this case to avoid the concern of graft vs host disease. The first successful nontwin allogeneic transplantation was not conducted until 1968. In 1973, a young boy with a genetic immunodeficiency condition received multiple marrow transplants from a donor recognized as a match in Denmark. That was considered the first successful unrelated donor transplant. In 1979, at the Hutchinson Hospital, the first successful unrelated donor transplant for a patient with leukemia took place [5]. Bone marrow transplantation has since expanded dramatically in the 1990s.

Recent clinical trials have shown that cell sheet technology has enhanced the ejection fraction, restored the dysfunctional cardiac wall, increased vascular genesis, and reduced fibrosis in models of heart disease. Cell sheets would be considered a potential treatment despite issues like lack of nutrition or increased transplant time window.

Methodology

There is a range of stem cells used in cardiovascular therapy the same way, there are multiple pathways to deliver this tissue to the pathological sites.

Some of these cell types include:

A. Human embryonic stem cell-derived cardiomyocytes (hESC-CMs)

Before embryonic stem cells can be differentiated into cardiomyocytes, they need to undergo expansion with the help of fibroblasts.

The expansion phase of undifferentiated hESC is performed on mouse embryonic fibroblasts (MEFs) in a medium consisting of DMEM/F12 supplemented with 20% KnockOut serum replacement (Invitrogen), L-glutamine, non-essential amino acids, beta-mercaptoethanol, and BFGF. Cells are then subjected to collagenase IV and trypsin, as well as the ROCK inhibitor Y-27632 to enhance cell survival. Human ESCs were depleted of MEFs before cryopreservation.

The embryonal carcinoma cells and embryonic stem cells found in mice were dissociated into single drops that then aggregated and formed spheroids with 2 layers (inner ectoderm like layer and outer endodermal layer). These spheroids were then termed embryoid bodies [6].

Cardiac differentiation was induced using an embryoid body for which they were re-suspended into low-attachment plates in StemPro-34 medium (Invitrogen) supplemented with L-glutamine, ascorbic acid, transferrin, and monothioglycerol. For the further direction of cell differentiation toward the cardiovascular lineage, the basal media was supplemented with bone morphogenetic protein 4 (BMP4, R&D) for 24 hours followed by BMP4, 6 ng/ml Activin A (R+D), and BFGF for 3 days. On day 4 of differentiation, the embryoid bodies are to be dissociated into single cells using trypsin and seeded onto Matrigel-coated plates at a density of 105 cells/cm2 in StemPro. The medium needs to be changed every 3–4 days thereafter until day 14 of differentiation [7].

At days 14 of differentiation for hESC-CMs, cultures are heat shocked with a 30-min exposure to 43°C media, followed by a return at 37°C in fresh media supplemented with cyclosporine A. One day later, cultures underwent a 1-hr pretreatment with Y-27632 (Rho-associated kinase inhibitor; 10 μM) and then were harvested with 0.25% trypsin/0.5 mM EDTA (Invitrogen). After being washed with DMEM/F12 supplemented with DNase (Invitrogen, 100 U/ml), 10 × 106 cells were suspended into a syringe that consisted of growth factor-reduced Matrigel in basal StemPro-34 medium (50% vol/vol), supplemented with L-glutamine, ascorbic acid, transferrin, monothioglycerol, cyclosporine A (200 nM, Wako), and Y-27632 (10 μM) [7].

B. Human induced-pluripotent stem cell-derived cardiomyocytes

iPSC colonies can be differentiated into functional cardiomyocytes using a variety of methods, which are very similar to those traditionally employed to produce cardiomyocytes from hESCs as they are both very similar in characteristics and differentiation potential. Currently, the most common method of generating cardiomyocytes from iPSCs is the embryoid body (EB) differentiation system which coaxes the iPSCs to differentiate into the cardiac lineage [8].

C. Adipose tissue-derived mesenchymal stem cell

Human adipose tissue has been developed as a novel source for multipotent stem cells and is considered more suitable in regenerative therapy. Their primary benefit is that they can be harvested

quickly and repeatedly using a minimally invasive process. ADSCs can be differentiated from tri-germ lineages into different cell types, comprising, for example, osteocytes, adipocytes, neural cells, endothelial vascular cells, cardiomyocytes, beta-pancreatic cells, and hepatocytes.

Intriguingly, immunosuppressive properties and low immunogenicity characterize ADSCs. Collagenase digestion, accompanied by centrifugal density gradient separation, is the most commonly used technique to separate ADSCs from fat tissue. ADSCs exhibit a spindle-shaped morphology in vitro and lack the intracellular droplets of lipids. Isolated ADSCs are usually expanded with a basal medium containing 10 percent fetal bovine serum.

ADSCs display a stem cell-specific combination of surface markers, such as CD90, CD105, CD73, CD44, and CD166, and lack the expression of hematopoietic markers CD45 and CD344, close to MSCs extracted from the bone marrow [9].

D. Bone marrow-derived mesenchymal stem cells and mononuclear cells

Despite having many sources for mesenchymal cells in the body, the most common mesenchymal cells are derived from bone marrow. They not only show positive outcomes for proliferation but also carry immunosuppressive properties [10].

To isolate MSC from BM, it is fractioned by density and is then isolated in a medium that contains fetal bovine serum. They are given two days to adhere and any cells that are unable to do so are removed. This allows the remaining successful cells to grow for a few weeks. Initially, there will be a heterogeneous adherent cell layer including fibroblast-like and small round-shaped cells, while they appear uniformly spindle-shaped after several passages in culture. Cells that manage to form sheets will undergo a reaction with trypsin to expand further. Later on, a panel of monoclonal antibodies will be used against their expressed antigen to study their phenotype [11].

Unfortunately, research has shown that if these cells are given intravenously, they will be entrapped in other organs like the lungs or spleen. At the same time, if given intracoronary, it will need a long ischemic period to ensure that cells are evenly distributed which has shown to cause myocardial necrosis as a complication [10]. This can contribute as one of the reasons its use is limited.

E. Skeletal myoblasts

Skeletal myoblasts can be found between the basal lamina and sarcolemma. Damage to muscle or any degeneration induced by diseases can act as a trigger for its proliferation. Features like the high proliferative potential observed in vitro under appropriate culture conditions, maintenance of undifferentiated status, and resistance to ischemic stress makes these myoblasts favorable to be used for repair in cardiac insults [12].

A very significant and successful clinical study published in march 2003 where skeletal myoblasts were implanted in humans was done and resulted in areas of myoblast engraftment demonstrating healthy graft morphology even though the cells were located in some cases in a large area containing a mature scar. Furthermore, there was significant angiogenesis in the graft side of a patient. However, it was done in conjunction with coronary revascularization making it difficult to accredit the functional benefits to skeletal myoblast implantation alone, and hence, cell survival becomes difficult to assess.

Skeletal myoblasts are obtained from a biopsy and separated from the connective tissue. It is then digested with enzymes on multiple occasions with trypsin, EDTA, and collagenase at 37 Celsius to release satellite cells. They are then allowed to grow in a serum containing fetal bovine serum, recombinant human epidermal growth factor, and dexamethasone. Cell densities would have to be maintained during the process to avoid any possibility of myotube formation. This results in <75% of the culture surface being occupied by cells [4]. It is then washed and preserved in tuberculin syringes along with cryopreservation. This is done to maintain the integrity of the sample until it reaches the clinic where it is warmed.

Applications of Stem Cells in Cardiovascular Medicine

1. General Applications

a. Paracrine signaling: This function allows stem cells to influence the surrounding cardiac tissue by activating various signaling pathways, without functional cell-cell contact to the host tissue. Transplanted stem cells release biologically active molecules such as VEGF, TGF-B, EGF, that promote processes of regeneration like activating tissue intrinsic progenitor cells, recruitment of cells needed in tissue repair, reducing cardiac myocyte apoptosis and neovascularization [10].

2. Bone Marrow Mononuclear Cells

a. Ischemic Cardiomyopathy

In the setting of ischemic cardiomyopathy stem cells have been studied in patients by employing injections directly into the myocardium. These studies were often non-randomized and their efficacy was denoted in a nonrandomized study of 21 patients whose areas of viable but dysfunctional myocardium were injected with bone marrow-derived stem cells. At the end of 2 months, there was a significant decrease in the region of reversible ischemia (from 15 to 6% of total myocardium) and a 6% increase in ejection fraction in the treated patients but not in controls. During follow up at 4 months, significant mechanical improvement in the injected segments was confirmed with electromechanical mapping.

Similar findings were observed in the IACT study where intracoronary infusions of cells were given. This trial had a sample size of 18 patients with a history of MI and followed a non-blinded observational study method. The controls consisted of a group that did not receive therapy (cellular). Results noted during follow up at 3 months after intracoronary injection of BMMCs were: Reduction in size of infarct by 30%, increased ejection fraction (by 15%), and increased movement velocity of infarct wall by 57%. However, there were no noticeable changes in the control group that did not receive therapy. This concludes that if stem cell transplantation was applied in ischemic cardiomyopathy, the results were favorable in regards to bone marrow cells [13].

b. Acute Myocardial Infarction

There have been many studies done in the past to check the application of bone marrow infusions in patients with MI. TOPCARE-AMI was a trial conducted by infusing bone marrow-derived mononuclear cells into AMI patients. This research was followed up for 5 years and the end result concluded proving long term safety of intracoronary delivery of BMMNCs, as well as improvement of left ventricular ejection fraction.

Another meta-analysis study of 2626 patients also showed significant results. The infarct size and left ventricular chamber enlargement underwent significant reduction and these findings were consistent in long term follow up. Furthermore, it was deduced that BMMNC therapy reduced the incidence of death, recurrent MI, and stent thrombosis in patients with ischemic heart disease [6].

c. Chronic ischemic cardiomyopathy and heart failure

FOCUS-CCTRN was a phase 2 trial conducted in patients suffering from chronic ischemic cardiomyopathy. This trial studied the 6-month efficacy of trans-endocardial delivery of BMMNCs on myocardial function and perfusion. The results showed significant improvement in stroke volume and LVEF, which correlated with higher bone marrow CD34+ and CD133+ progenitor cell counts. These conclusions were used to derive the notion that certain bone marrow-derived cell populations may provide a greater regenerative benefit and thereby determine clinical efficacy.

Based on this the ACT34-CMI (Adult Autologous CD34+ Stem Cells) investigators conducted a double-blind, randomized, phase II clinical trial to evaluate the safety and efficacy of intra-myocardial injections of autologous CD34+ cells in patients with refractory chronic myocardial ischemia. At 6 months and 12 months, there was good exercise tolerance and reduction in angina episodes compared to the control group. This supported the idea that bone marrow cells played a greater significance in patients with refractory angina [6].

3. Mesenchymal Stem Cells

a. Immunomodulation

MSCs known for their immunomodulatory properties can influence inflammatory processes after AMI and in HF. Studies have shown that MSCs can regress the proliferation and cause apoptosis of T cells. They can stimulate the Treg cell generation and promote a phase that resolves inflammation after MI. This allows for significant wound healing. However, the function of this is highly dependent on pro-inflammatory cytokines such as interferon (IFN)-γ and tumor necrosis factor (TNF-α).

Recently, Luger et al. showed that intravenous application of human BM-derived MSCs reduced the number of NK cells and neutrophils by 25 - 50% in hearts 7 days after MI which was which would avoid adverse remodeling, especially in mice with large infarcts [10].

b. Neovascularization

To replenish the damaged tissue with needed oxygen and nutrients, the process of new blood vessel formation is important. MSCs from different sources are capable of releasing pro-angiogenic factors contributing to the formation of new blood vessels. Additionally, it also showed that the release of cytokines increased capillary density. The notion was heavily backed up by a study by Timmers et al who injected conditioned human MSCs into MI pigs. Post 3 weeks, the animal showed increased capillaries in the area [10].

4. Skeletal Myoblasts

a. Resistance to Ischemia.

- Skeletal myoblasts are resistant to ischemic stress and differentiate into the myogenic lineage. Many clinical trials have been conducted using skeletal myoblasts and the results indicate the reduction of left ventricular modeling and interstitial fibrosis along with improved systolic and diastolic benefits.

b. Use in HF patients.

- The first known use of skeletal myoblasts was in a single patient with severe ischemic HF as reported by Menasche et al. The process involved implanting autologous SMs into post-infarction scar during coronary artery bypass graft (CABG) to remote myocardial areas. On a follow up conducted 5 months later, the Echo and PET scan showed that the contraction and viability of grafter scar were healthy. These benefits were also evident symptomatically in the patient.
- Another Phase I study was conducted recruiting 12 patients and using the trans-epicardial approach to deliver autologous SM. By noticing an increase in LVEF and improved viability in PET, it can be concluded that the use of skeletal myocytes would benefit HF patients via the increase in functional cell mass [12].

5. Human Induced Pluripotent Stem Cells

a. Model a disease

- Human-induced pluripotent stem cells have the ability to model diseases given their ability to differentiate into any cell type within the body. Studies have shown that patients with inherited arrhythmias, such as long QT syndrome can be studied by producing its induced pluripotent cells. These iPSCs can capture the disease phenotype and hence provide a platform for research on the pathology and investigation of different compounds as a means to discover a novel treatment. Modeling the long QT syndrome- An extensive study was done by Moretti et all to show that human-induced pluripotent cells can reiterate the exact phenotype of the disease. They found and targeted an AD inheritance of a 596 G-A missense mutation in the KCNQ1 gene which has been known to be affiliated with QT syndrome. 4 patients, 2 with LSQT1, and 2 as the control group were compared for this study. When the iPSC-CMS cells obtained from these patients were assessed via electrophysiological parameters, a prolonged AP duration and slowed repolarization velocity was observed compared to the control group. These findings are consistent characteristics of long QT syndrome and further prove that iPSC-CMs can model diseases [8]. iPSCs are patient-specific, hence they can bypass the obstacle of tissue rejection often seen in transplant procedures. This gives induced pluripotent stem cells a major advantage over other cell types [8].
- A study was done by Nelson et al where iPSCs were delivered into the myocardium of infarcted hearts in mice. This procedure was followed by ligating the LAD artery. Evidence showed that the graft provided promising results in contractility and wall thickness while also regenerating the surrounding tissues [8].

6. Human Embryonic Stem Cells

a. Gives rise to numerous differentiated cells. hESCs were found to have various advantages over other types: immortality, ability to indefinitely proliferate in culture while maintaining the undifferentiated phenotype, and the capacity to form derivatives of all three germ layers [14].

b. Drug testing

A major part of drug development is testing new products for clinical use which requires affirmation that the product does not have any significant toxicities that can result in cardiac dysfunction or arrhythmias.

Detection of potential cardiac toxicities can help pharmaceuticals save millions of dollars and promote the use of these funds towards beneficial clinical trials. To validate this notion there is a high demand for human model cells, whether healthy or damaged. Here hESCs come into play as recent studies have demonstrated that hESC-CMs allow a great opportunity for electrophysiological drug screening since the cells are tolerant to drugs that are cardiac or not [8].

7. Adipose Tissue Cells

a. When ADSCs were used for studies in animal models they showed improvement in cardiac function and repair. Some of the improvements included successful differentiation into cardiac tissue. There were also paracrine effects such as angiogenesis, recruitment of local cells, reduction in fibrosis, and less induced apoptosis. This helped the infarcted tissues to revascularize and prevent cell death [15].

b. Recent studies have shown that NRGI- Neuregulin-1, an endothelial-derived factor, can synergize with the ADSCs in cardiac repair. These suggest that the ADSC-NRG1 combination could be used for future clinical studies, perhaps with NRG1 expressed from the ADSCs directly, or from co-administered microparticles.

c. A great application of ADSCs was done in the APOLLO trial on patients with ST-elevation. This Phase I clinical trial was based in Spain with a sample size of 9 analyzable patients and was carried out for 6 months after the infusion of adipocytes cells via the coronary artery. Results showed an increase in EF, better perfusion, and reduction in the infarct by half [15].

Outcome

A major problem seen in stem cell therapy was the common immunologic reaction known to any transplantation. Allogenic stem cells are known for positive outcomes in cardiac function, but the differentiation often results in immunologic rejection [16]. To provide this therapy, research was carried out to study the effects of interleukin 6 on cell differentiation. Interleukin 6 often is secreted as a normal physiological response to transforming growth factor-b. The research observed that the idea of cell differentiation caused a low level of interleukin-6 which increased leukocyte cell mediation damage ($P< 0.01$) [17]. It further proved the idea that the immunologic response may merely be due to cell differentiation rather than the cell phenotype. By defining that interleukin downregulation is one of the factors, a decrease in rejection can be obtained by restoring interleukin.

The second response of immunologic response is due to major histocompatibility classes. Sometimes, the stem cell recipients have performed antibodies against foreign HLA antigens. The use of mismatched allogeneic cells can lead to a reaction and cause graft failure. The mechanism of this reaction can simply be defined as MHC I protein binding to microglobulin beta 2 which is detected by T cells and destroyed [18]. It brought up the idea of whether the absence of B2M would avoid such a reaction. Research conducted showed that when cells were deficient in B2M, there was no mediated reaction by T cells. Moreover, the memory cells which are mediated by MHC II also aided in the rejection of graft in terms of vascularization. This embarked on an idea that if somehow major histocompatibility complexes I and II were knocked out, the cells would not be able to elicit a response. A study created a B2M/CIITA double-knockout mutation and injected the stem cells. After 8-10 days of differentiation, the cell function was confirmed by ELISA. The outcome of the result showed that there was no effect on the differentiation, but the T cell marker was significantly decreased in HLA knockout mutation in comparison to the control group [19]. Both the research brings out a major factor that if certain interleukin factors are restored, and some immune-mediated proteins are knocked out, the immunological rejection can be decreased to a great extent.

Though many factors can alter the results of stem cell transplantation, one of the main modulators is aging. There is evidence that transplant stem cells interact with neighboring cardiac myocytes and play a role in differentiation. Older individuals are often associated with cardiovascular and other diseases that would alter overall body function. Whether it is stressors like reactive oxygen species or a decline in DNA protein turnover, stem cell differentiation is highly compromised by these factors [20]. The ineffective stem cells can risk the stem cells to express senescence-associated factors. Senescence of a cell can be defined as irreversible cell cycle arrest in response to stressors. It leads to molecular and biological changes which overall promotes carcinogenesis. It also leads to the induction of cytokines and growth factors like

IL-6, IL-8, and TNF-a [21]. These cytokines are also secreted by atherosclerotic patients at a higher level. The solution is increasing the expression to SIRT3. Sirtuin 3 is a mitochondrial deacetylase that reduces oxidative stress and enhances differentiation [22]. This is usually low in age-related stem cells and increasing the overall expression, promises differentiation.

The incidence of arrhythmia also brings the efficacy of stem cells to great doubt. An experiment done by Dr. Mesache et al. observed the efficacy of skeletal myoblasts in ischemic cardiomyopathy patients. Out of 10 patients, 4 patients experienced episodes of sustained ventricular tachycardia and an internal defibrillator had to be inserted to prevent the risk of arrhythmia [23]. It is said that the lack of gap junction contributes to the onset of arrhythmia. The major adhesion protein known as N-cadherin and gap junction protein called connexin 43 is expressed normally in undifferentiated myoblasts. However, after differentiation, there is evidence that they are down-regulated [24]. Many investigations done such as by Suzuki et. al have managed to increase the overexpression of these proteins and reduce the risk of arrhythmia. However, the effectiveness of skeletal myoblasts as stem cells has been so positive, that risk of arrhythmia incidence outweighs the overall cardiac function [10]. The arrhythmia incidence, although significant, can be managed by medications and a defibrillator.

Conclusions

Progress in the field of regenerative medicine has been remarkable in the past few decades which gives us a promising future in improving the prognosis of cardiovascular insults. Although certain challenges like immunological reactions, expression of senescence factors, and arrhythmia induction persist, newer technologies continue to be developed. Studies managed to show that when different types of stem cells were used to differentiate into cardiac myocytes, it showed favorable results in terms of ejection fraction, wall thickness, contractility. Furthermore, it even introduced the idea of understanding disease by replicating its phenotype and opened doors to drug experimentation on a deeper level.

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