

Human Pluripotent Stem Cell-derived Cardiomyocytes Treatment for Coronary Heart Disease

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Abstract. In the past few decades, stem cell therapy made remarkable progress in the medical field, demonstrating significant potential in the treatment of various diseases. Particularly, for cardiovascular disorders such as coronary heart disease (CHD), research has indicated that stem cell transplantation holds promise as an effective clinical therapeutic approach. Human pluripotent stem cell-derived cardiomyocytes, as a crucial cardiac cell type, have garnered widespread interest in cardiovascular disease research and the exploration of cardiac cell-based therapies. These cells can be cultured in vitro and are envisioned as an innovative avenue for treating heart ailments through transplantation. By culturing a substantial number of PSC-CMs ex vivo, potentially in conjunction with other cardiovascular cell types or progenitors, and subsequently transplanting them to the damaged region of the heart, myocardial regeneration and restoration of cardiac function can be achieved. Nevertheless, despite the significant potential of PSC-CMs, further in-depth research and validation are essential before considering their clinical application, as several critical challenges need to be addressed. This paper primarily focuses on the latest advancements and limitations concerning the use of PSC-CMs in the treatment of CHD, unveiling opportunities and hurdles in cardiac regenerative medicine.

Keywords: Pluripotent stem cell, Coronary Heart Disease (CHD), cardiomyocyte, cell therapy, human pluripotent stem cell-derived cardiomyocytes.

1. Introduction

Cardiovascular diseases (CVDs) have a significant impact on public health, with coronary heart disease (CHD) being one of the most well-known and extensively studied conditions. Obesity plays a crucial role in CVDs, contributing to 7%-44% of cases. Moreover, CVDs are responsible for more than two-thirds of the mortality associated with obesity, as indicated by the Body Mass Index (BMI) [1]. In the context of individuals' daily lives, numerous factors are potentially linked to the development of CHD, including hypertension, diabetes, smoking, and obesity. As researchers delve into potential treatments, the therapy of stem cells emerges as an advantageous choice for managing CHD. A distinctive characteristic of stem cells is self-renewal and cell-type differentiation. In the context of CHD, therapy for stem cells shows potential for repairing damaged cardiomyocytes and restoring heart function [2]. The following review explores the current condition of stem cell therapy for CHD currently and its potential to enhance patient outcomes.

2. Pathogenesis

CHD is primarily attributed to atherosclerosis, a pathological process characterized by the accumulation of diverse cellular components, lipids, and debris within the inner layer of blood vessels (vascular intima), resulting in plaque development caused by atherosclerosis. Traditionally, atherosclerosis has been linked to the deposition of cholesterol [3].

CHD etiology is influenced by both hereditary and environmental factors. Environmental factors encompass individual behaviors and lifestyle habits, such as dietary patterns and physical activity, which can significantly influence the initiation and progression of CHD [4]. On the other hand, genetic factors can impact lipid metabolism and blood pressure regulation, potentially fostering the development of atherosclerosis. Atherosclerosis represents a chronic inflammatory vascular condition

influenced by a complex interplay of conventional and nonconventional risk factors. Atherosclerosis's pathophysiology has been linked to several signaling pathways connected to inflammatory responses.

3. Stem Cells Classification

3.1. Categorization of Stem Cells

3.1.1 Totipotent stem cells

This kind of stem cell can differentiate between all the types of possible cells. Zygote is one of the examples which formed after egg fertilization [4].

3.1.2 Pluripotent stem cells

This type of cell could form all the cells from the germ layer, excluding extraembryonic structures [2]. Several instances of induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) could be produced from implanted embryos and preimplantation embryos, respectively. The remarkable full pluripotency of induced pluripotent stem cells enables them to grow into any type of cell [5]. Shinya Yamanaka pointed out that factors that are critical in the maintenance of stem cell totipotency are also capable of performing a function in stem cell induction.

3.1.3 Multipotent stem cells

This type of stem cells (MSCs), such as hematopoietic (adult) stem cells, can develop into different blood components for different functions, including phagocytosis and blood clotting. Despite having fewer cell types available to it, it can specialize in distinct cells from particular cell lineages.

3.1.4 Oligopotent stem cells

Only a few distinct types of cells can be formed from this type of stem cell. Lymphoid and myeloid are two examples [4]. Instead of red blood cells, a myeloid stem cell can become white blood cells.

3.1.5 Unipotent stem cells

This type of cell is distinguished only by its limited differentiation skills and the ability to divide again. Examples are muscle stem cells and dermatophytes [4].

3.2. Categorization of Stem Cells Based on Source

3.2.1 Embryonic stem cells

ESCs could continually regenerate themselves. They continue to proliferate through cell division and have the property to maintain their stem cell status and function [5]. They originate from the developmental phase of the embryo, at which point the embryo has not been implanted in the uterus. The embryos, which are hollow small balls of cells called blastocysts, are around four or five days old.

3.2.2 Adult stem cells

A particular kind of stem cell observed in developed tissues and organs has been identified as adult stem cells. They have some self-renewal and multidirectional differentiation potential, but their differentiation is more limited compared to ESCs. Adult stem cells could become various categories of cells related to each tissue from whence they originated. Hematopoietic stem cells in the bone marrow, for instance, can develop into red, white, and platelets, whereas skin stem cells in the skin can develop into several skin cell types. Adult stem cells can be mobilized in response to tissue injury, inflammation, or disease and differentiate into specific cell types needed by the damaged tissue to promote tissue repair and regeneration.

4. Mechanism of Therapy for Stem Cell

The mechanism used to repair parts of the heart that are being damaged is not yet fully comprehended and may involve several direct or indirect pathways [6]. By obtaining a more in-depth understanding of the processes involved in cell transplantation, we can more precisely evaluate the causes and outcomes of regenerative therapy.

4.1. Cell Replacement

Cell replacement is a crucial mechanism driving the potential of stem cell therapy. A groundbreaking study demonstrated that autologous bone marrow cells (BMC) transplanted into injured mouse hearts could differentiate into cardiomyocytes *in vivo*, forming the foundation for stem cell transplantation and cardiac regeneration research. Additionally, transplanting MSC into ischemic regions of experimental animal hearts significantly increased the pool of immature cardiomyocytes [7]. Once engrafted, these stem cells migrated to the site of cardiac injury and, influenced by the surrounding microenvironment, transformed into cardiomyocytes expressing specific cardiac markers, representing a direct and potent mechanism for stem cell differentiation in tissue repair. However, it is important to acknowledge that there is emerging evidence of an elevated risk of tumorigenicity following direct stem cell transplantation. Therefore, gaining a comprehensive understanding of the complexities underlying cell replacement is vital in accurately assessing the rationale and effectiveness of regenerative therapies involving stem cells.

4.2. Paracrine Mechanism

Stephen et al. proposed the paracrine mechanism as the primary mode of action for the therapeutic benefits of transplanted cardiomyocytes, rather than simply cell replacement. Following transplantation, the cardiomyocytes secrete a significant quantity of extracellular vesicles known as exosomes [8]. These exosomes contain functional substances, including RNA and specific proteins, that participate in cell signaling processes. The exosomes play a crucial role in substance transportation, facilitating vascular regeneration, alleviating local ischemic symptoms, and restoring myocardial function. Furthermore, the exosomes released by the transplanted cardiomyocytes not only inhibit apoptosis in the damaged myocardium and stimulate neovascularization but also ameliorate the expression of local inflammatory factors, thereby mitigating inflammation-induced damage [9].

4.3. Mitochondrial Transfer Mechanism

Cardiac hypertrophy in response to heart failure induces a compensatory enlargement of myocardial cells, leading to a relative insufficiency of mitochondria within these cells, thereby contributing to the progression of heart failure. Additionally, ischemic injuries exacerbate mitochondrial damage in the myocardium, impairing energy production and compromising cardiac contractility. Remarkably, Lin et al. have demonstrated that transplanted cardiac mitochondria can be transferred to damaged recipient cells, facilitating the restoration of cellular bioenergetics.

Clinical investigations involving the direct transplantation of autologous cell-derived mitochondria into patients suffering from myocardial ischemic injuries have yielded promising results, with approximately 80% of cases demonstrating substantial improvements in cardiac function. This highlights the pivotal role of mitochondrial transfer in repairing impaired myocardial cells and restoring their energy-generating capacity.

However, it is essential to acknowledge that the cardiomyocytes derived from stem cells currently employed in transplantation trials still manifest a relatively immature phenotype compared to fully developed adult human cardiomyocytes [2, 9]. This disparity in maturation might potentially restrict their optimal integration with the host myocardial tissue and, in turn, could contribute to the incidence of post-procedural ventricular arrhythmias. As such, further investigations are warranted to elucidate

and optimize the mechanisms underlying mitochondrial transfer and enhance the efficacy and safety of regenerative therapies for heart disorders.

5. Advancements in Cardiac Therapy

In France, during coronary artery bypass surgery, fibrin patches containing cardiovascular progenitor cells made from human ESCs were implanted into the epicardial surface. [10]. Over the course of four days, the cardiovascular progenitor cells were exposed to BMP-2 and the tyrosine kinase inhibitor SU-5402, which specifically targets the fibroblast growth factor receptor. SSEA-1 immunomagnetic sorting was used to isolate progenitor cells expressing positive markers for pluripotency loss. Each patch received five to ten million progenitor cells, which were then sutured onto the epicardium and placed over the infarcted area. Six people with persistent ischemic heart disease participated in this investigation. The patients additionally took immunosuppressive regimens [9]. Except for one patient who passed away immediately after surgery, all patients had uncomplicated postoperative recovery. No tumor development was found, and none of the five patients who survived had any ventricular arrhythmias. The particular contributions of cell treatment vs bypass grafting of the coronary arteries to the therapeutic advantages are yet unknown, however, reports suggest symptomatic improvement in all patients. Additionally, the survival status of the introduced cells after completion of immunosuppressive treatment remains unclear. Further investigation will be necessary to clarify the therapeutic benefits and engraftment effectiveness of this cardiovascular progenitor cell treatment. Nevertheless, it is very promising that this strategy offers a viable route for using stem cells to treat coronary heart disease. [11].

The Field team demonstrated that cardiomyocytes, when injected into the hearts of adult mice, have been able to endure and ingratiate themselves into the host myocardium. Subsequently, the issue of human cell sourcing has been addressed through the development of hESC and iPSC technologies, along with the establishment of efficient protocols for generating cardiomyocytes and other cardiac cell types in nearly unlimited quantities [12].

IPSCs are considered one of the most promising sources of cell replacement therapy due to their capacity to proliferate and develop into various cell groups indefinitely. In an animal experiment, we explored the differentiation potential of hiPSC-CPCs in vitro, and further observed the effect of its transplantation in vivo on acute myocardial infarction in rats. The findings demonstrated that cardiac precursor cells produced from induced pluripotent stem cells had an elevated level of differentiation efficiency in vitro, could survive and develop into cardiomyocytes after transplantation in vivo, could prevent ventricular remodeling, and could enhance heart function. Even if the various mechanisms that might regulate how stem cells differentiate into blood arteries or myocardium remain to be investigated, this study lays a theoretical foundation for the future repair of myocardial infarction using CPC derived from ESCs or iPSCs [13].

Cardiomyocytes produced from PSC-CMs have been successfully transplanted into different animals. The transplanted cardiomyocytes have demonstrated viability, integration, and functional benefits within the injured heart. This is achieved by establishing gap junction connections between the transplanted parts and the host tissue, facilitating remuscularization and electrical coupling with the host tissue. This integration enables the transplanted cardiomyocytes to synchronize their contractions with the surrounding tissue, contributing to the overall contractile function of the injured heart. The successful outcomes in these animal models show promising potential for the therapeutic application of PSC-CMs in treating heart-related conditions in humans. However, further research and clinical studies are required to thoroughly evaluate the safety and efficacy of this strategy before considering its widespread clinical application [2].

6. Human Pluripotent Stem Cell-derived Cardiomyocyte Production and Limitations

6.1. Production of PSC-CMs

Cardiomyocytes could be separated from PSCs through numerous protocols which include two distinct approaches: adherent and suspension culture. In both approaches, the administration of growth factors and Mirco molecules at accurate timing and optimal dosages plays a critical role in emulating cardiac development. Typically, cell beating initiates around 10 days after differentiation. Despite significant progress in enhancing differentiation efficiency in the past decade, the elimination of undifferentiated cells remains pivotal for clinical applications. Numerous surface markers are available for cardiomyocyte purification, requiring antibody labeling. Additionally, regulated glucose or glutamine deprivation with lactate supplementation can be used to metabolically purify cardiomyocytes [11].

PSC-CMs can contract, produce force, and spread excitement, but when their shape, function, and transcriptional studies are taken into consideration, fetal CMs are the ones they most closely resemble. In vitro, miniaturization of cardiac illnesses, comprising channelopathy, cardiomyopathy, and cardiometabolic disorders, has been accomplished by scientists utilizing patient-induced PSCs. It ought to be emphasized that despite the fetal phenotype being beneficial for modeling disease and testing for drugs, human PSC-CMs still lag behind the mature adult phenotype. Various techniques are proposed to promote the maturation of PSC-CMs in culture, comprising chemical treatment, mechanical and electrical stimulation, and prolonged cultivation. The most cutting-edge approach combines these stimuli with a multidimensional culture of tissues. Nevertheless, as of now, there is no evidence indicating that the in vitro phenotype of human PSC-CMs exactly matches that of adults. [2, 11].

Following transplantation, in terms of the size of cells, cell-cell junctions, sarcomere construction, and mitochondrial structures, PSC-CMs experience morphological maturity. To mature, they depend mainly on cues via the host tissue. Interestingly, the maturation process of transplanted PSC-CMs is time-dependent and gradual. Notably, even after 3 months following transplantation into rat hearts, human PSC-CMs were unable to acquire complete maturity. In contrast, after transplantation, rat primary CMs or mouse iPSC-CMs showed full maturation. The entire growth of human CMs in rodent hearts may be hindered by the stark electrophysiological differences between human and rodent CMs. Alternatively, the three-month period could not be long enough to achieve complete development. However, after 3 months, indications of maturity appeared when human PSC-CMs were transplanted into non-human monkey hearts, showing the beneficial impact of the cardiac environment [11].

6.2. Limitations and Solutions

Upon transplantation of PSC-CMs into non-human primates, the occurrence of ventricular tachycardia was noted, signifying a potential cardiac health hazard, albeit with some studies postulating its temporary nature. Gaining a comprehensive comprehension of this therapeutic modality assumes paramount importance in mitigating the risk of arrhythmias [9].

In mature hearts, the regulation of heart rate is primarily governed by specialized cells known as pacemaker cells, responsible for generating rhythmic electrical signals that trigger contractions in other cardiac cells. To unravel the ion channels involved in the maturation process of cardiac cells, RNA sequencing was employed to identify the expression patterns at different developmental stages. Subsequently, CRISPR-based genome editing was utilized to precisely target and knockout specific ion channel genes, as well as activate others, allowing for the identification of key players contributing to arrhythmogenic currents [7].

Through meticulous efforts, the researchers successfully engineered a cell line of stem cells with three depolarization genes knocked out and one repolarization gene activated. Remarkably, the resulting cardiomyocytes exhibited electrical quiescence reminiscent of adult myocardium but

displayed contractile responses upon electrical stimulation, mimicking natural pacing [7]. Upon transplantation into the heart, these cardiomyocytes matured into functional adult cells, seamlessly integrating with the existing myocardium, and achieving synchronization with the heart's natural rhythm, all while mitigating the risk of dangerous arrhythmias.

This pioneering discovery signifies a remarkable stride in the realm of cardiac regeneration. Although further evaluation of engineered cells is warranted, the researchers are confident that this innovative approach holds significant promise in effectively addressing the challenges associated with regenerating human hearts.

7. Conclusion

This comprehensive review elucidates the therapeutic modalities and developmental advancements of PSC-CMs within the context of cardiac regeneration. PSCs offer a prolific source of cardiac cells that can be harnessed for potential transplantation into non-human primate hearts to replace ischemic regions. Numerous studies in cardiovascular diseases have revealed the enormous significance of stem cell-based treatments for the medical management of coronary artery disease. With the continuous progress and maturation of regenerative medicine, PSC-CMs are poised to unveil expansive horizons in the domain of cardiovascular applications.

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