REVIEW ARTICLE

Human Induced Pluripotent Stem Cell-Based Cardiac Tissue Engineering for Myocardial Infarction

Edwina Rugaiah Monayo[1,2], Jeanne Adiwinata Pawitan[3,4,5]

ABSTRACT

Objective: **To outline progress in induced pluripotent stem cells (iPSCs) in generating iPSC-derived cardiovascular cell lineages, and cardiac tissue engineering.**

Materials and methods: **A data search for original articles was conducted on PubMed, using keywords: "induced pluripotent stem cells" OR "iPSCs" AND "myocardial infarction" OR "heart failure" AND "cardiomyocyte" on December 18, 2021. Search results were filtered and selected by titles and abstract to get relevant articles, which contained data on iPSC-related tissue engineering for heart tissue repair after myocardial infarction. Data were grouped and synthesize as text.**

Results and Discussion: **we got 35 articles that provide information on cell based therapy, iPSCs, advancement in creating efficient techniques for generating iPSC-derived cardiovascular cell lineages, and recent cardiac tissue engineering advances in myocardial infarction animal models. iPSCs can be derived from skin fibroblasts by expressing four transcription factors that were found to be critical for cellular reprogramming into inner cell mass derived ESC-like cells, namely Oct3/4, c-Myc, Sox2 and Klf4. Cardiomyocytes can be produced from iPSC using special medium and supplements. Tissue engineering can be made possible by 3D printing technique and showed promising results upon transplantation.**

Conclusion: **Tissue engineering using patient-derived human iPSC-cardiomyocytes holds great potential in medicine because it might avoid the problem of possible immune rejection.**

KEY WORDS

pluripotent stem cell, cardiomyocytes, iPSC, myocardial infarction

INTRODUCTION

Cardiovascular disease, which includes everything from congenital abnormalities through adult myocardial infarction, is the leading cause of death worldwide[1]. A common consequence of myocardial infarction (MI) is heart failure (HF). Although pharmacological and surgical therapies have improved the prognosis following an MI, there is currently no effective way to restore the lost tissue, making heart failure the greatest cause of mortality and morbidity globally[2].

Despite major improvements, the most of invasive procedures are just preservation, i.e., an attempt to keep heart tissue intact and functional for as long as feasible without structural repair. Heart failure, on the other hand, is almost always unavoidable due to the progressive nature of cardiovascular disease (CVD). Many individuals with end-stage heart failure will eventually require a heart transplant due to the etiology and severity of their condition. There is a strong need for alternate therapeutic approaches because there are few therapy alternatives and the number of hearts available for donation is limited[3].

The underlying problem of cardiac tissue loss is not addressed by current medicines for heart failure. To reduce mortality and the need for heart transplants, new medicines are required. The use of stem cell-based therapies in the treatment of cardiac disease are consistent with the hypothesis that the myocardium's natural self-renewal mechanisms are there, but may not be sufficient to restore the infarcted cardiac muscle. Following initial reports of cell therapy in the treatment of heart disease, investigations continue to be carried out rapidly. In addition, several studies in animals and clinical trials in humans have been carried out to maintain the capacity of multiple stem cell populations to promote cardiac function and reduce infarct size[4].

Regenerative medicine has the capacity to heal myocardial tissue

Received on December 20, 2022 and accepted on December 26, 2022

1) Doctoral Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia
Jakarta, Indonesia
2) Faculty of Medicine, Universitas Negeri Gorontalo
Gorontalo, Indonesia
3) Department of Histology, Faculty of Medicine, Universitas Indonesia
Jakarta, Indonesia
4) Stem Cell Medical Technology Integrated Service Unit
Dr. Cipto Mangunkusumo General Hospital
Faculty of Medicine, Universitas Indonesia
Jakarta, Indonesia
5) Stem Cell and Tissue Engineering Research Center
Indonesia Medical Education and Research Institute
Faculty of Medicine, Universitas Indonesia
Jakarta, Indonesia
Correspondence to: Jeanne Adiwinata Pawitan
(e-mail: jeanneadiwip@gmail.com)

ORCID ID:
Edwina Rugaiah Monayo: 0009-0004-8310-2463
Jeanne Adiwinata Pawitan: 0000-0002-6551-5238

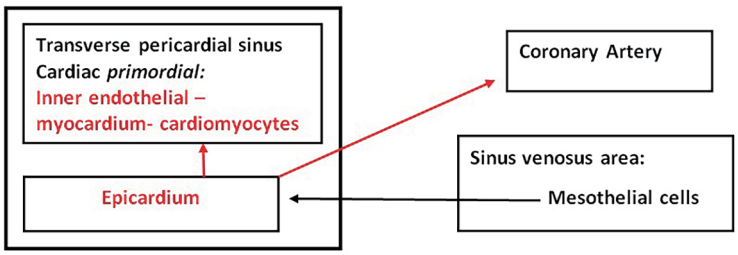

Figure 1: Myocardial cell source during cardiac development

that has been injured. Several studies have shown that stem cell transplantation into the heart can directly replace tissue lost after an MI, whilst stimulating innate repair pathways that can activate existing progenitor cells and commence regeneration. Many cells have been hypothesized as having regeneration potential, including those coming from blood, bone marrow, adipose tissue, and heart tissue. Despite the fact that each of these cell types can differentiate into new contractile cardiomyocytes, their differentiation efficiency is poor. True cardiomyogenic potential exists in embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) as more effective cell types for heart regeneration[5], and needs to be explored.

The goal of this mini review was to outline some of the researches using induced pluripotent stem cells (iPSCs), which represented advancement in creating efficient techniques for generating iPSC-derived cardiovascular cell lineages. We also described recent tissue engineering advances in myocardial infarction animal models that utilized cardiovascular cells from differentiated iPSCs, to take us one step closer to our ultimate goal of cell-based heart repair. Therefore, we addressed cell based therapy, iPSCs, iPSC derived cardiomyocytes, and cardiac tissue engineering.

MATERIALS AND METHODS

To write this narrative review, we conducted a data search on PubMed, for original articles using keywords: "induced pluripotent stem cells" OR "iPSCs" AND "myocardial infarction" OR "heart failure" AND "cardiomyocyte" on December 18, 2021. Inclusion criteria were original articles that were related to iPSCs for heart tissue repair after myocardial infarction, and the publication period of the last five years. The exclusion criteria were non-English articles. Data collection procedure: search results were filtered and selected by titles and abstract to get relevant articles, which contained data on iPSC-related tissue engineering for heart tissue repair after myocardial infarction. Data were grouped and synthesize as text and a Figure. We got 35 articles that were used to write this review article.

RESULT AND DISCUSSIONS

Cell Based Therapy

Cell therapy offers a promising paradigm for cardiac tissue regeneration. Due to the limited proliferative and self-healing capacity of cardiomyocytes in adults, cell therapy has become a popular option for heart repair and regeneration by restoring cardiomyocytes and damaged myocardial tissue[6].

Mammalian cardiomyocytes leave the cell cycle and lose their ability to multiply shortly after birth, which explains why cardiac muscle tissue lost to myocardial infarction or other kinds of heart disease cannot be properly regenerated via endogenous myocardial healing processes. Researchers have tried to promote cardiac repair by sending cardiomyocytes and some other cell types to injured areas, such as through stem cell (SC) therapy.

Self-replicating cells that really can generate, sustain, and substitute differentiated cells are known as stem cells, which are suitable to be used as regenerative cells. Regenerative cells that are generated from bone marrow include endothelial progenitor cells, hematopoietic progenitor cells and mesenchymal stromal/stem cells. In addition, peripheral blood and other fetal tissues could also be used to collect endothelial progenitor cells. A favorable effect of stem cells on post-MI ventricular function has been documented in several clinical trials; however, the specific mechanism of this benefit has yet to be determined. Paracrine effects on surrounding tissues, neovascularization and viable myocardium regeneration are some possibilities for the mechanism[7].

Embryonic SCs (ESCs) and adult or somatic SCs are the two basic types of SCs according to their source. Induced pluripotent cells (iPSCs), which are genetically reprogrammed "embryonic-like" cells (via pluripotent transcription factors), have been studied in recent years[8].

The risk of transplant rejection, tumorigenesis potential, genetic instability, low induction effectiveness (iPSCs), and ethical concerns are limitations for direct therapeutic use of pluripotent SCs, either ESCs or iPSCs. ESCs and iPSCs, which can differentiate into any form of cell in an organism, one of which is mesodermal-derived cardiomyocytes might be promising in heart regenerative medicine when differentiated into desired cell types[9,10]. For the purpose of cardiac regeneration potential, differentiation of iPSCs into limited types of multipotent cells, for example mesenchymal SCs and cardiac SCs, or adult unipotent SCs have been extensively studied. In addition, many different types of adult SCs have been used in recent clinical trials, including mesenchymal SCs (MSCs), multipotent bone marrow-derived stem cells (BM-SCs), which include BM-MSC, hematopoietic SCs (HSCs) and endothelial SCs, as well as skeletal myoblasts, cardiac SCs (CSCs), epicardial cells, human amniotic fluid-derived stem cells (hAF-SCs), and cardiomyocyte (CM)[8].

During the period of cardiac development, epicardium may serve as a source of cells for the myocardium and coronary arteries, which produce multi-lineage progeny and secrete paracrine trophic signals. This epicardial cell population passes through an epithelial-to-mesenchymal transition, invades the myocardium to create coronary smooth muscle cells, cardiac fibroblasts and endothelial cells. Epicardial cells, when activated, can help in neovascularization and cardiac repair. As a result, mature epicardium can aid in myocardial regeneration (Figure 1)[11].

Despite the best efforts that have been made with adult SCs, none of the cells can live up to expectation period as a reliable treatment for cardiovascular disease. Even the most strong adult stem cells are insufficient for cardiac tissue regeneration and/or functional compensation for the loss of cardiac contractile parts, such as in infarction, cardiomyopathy or congenital heart disease. In this situation, obtaining a functional CMs necessitates the use of a different form of SC, namely, pluripotent SCs (PSCs) either ESCs or iPSCs[3].

hAF-SCs are pluripotent and immune-privileged[11]. However, they were unable be converted in vitro into functional CMs by Wnt pathway modification approach that was aimed at cardiac differentiation[12]. Fang *et al.*[13] transformed hAF-SCs to hAF-SC-iPSCs to facilitate cardiac differentiation. Their results revealed that hAF-SC-iPSC-CMs display cardiac-specific markers, have CM-like electrical characteristics, their immunogenicity was low, and spontaneously were able to contract. Grafted hAF-SC-iPSC-CMs were able to remain in infarcted mouse heart regions and recover post-MI cardiac function using less potent immunosuppressive medication. As a result, hAF-SC-iPSC-CMs could be qualified as candidates for cell therapy.

Biagi *et al.*[14] study showed that early-stage hiPSC-CMs could be used to regenerate myocardium segments in infarcted rats as an alternative therapy. Their findings showed that hiPSC-CMs on the 11th-15th days of differentiation, which were given 7 days following induction of MI, improved overall cardiac function by improving segmental contraction while retaining some proliferation capacity. Furthermore, interacting proteins were expressed in these human grafts (high levels of Pan-cadherin, low levels of Cav and low levels of Cx43). They discovered host circulation in all of the animals with human grafts, but no substantial immune rejection reaction. This proof-of-concept paper adds to the

effort to develop a high-quality regenerative cell-based therapy to treat individuals with degenerative cardiac disease, such as myocardial infarction.

Induced Pluripotent Stem Cells

iPSCs with all their cardiac derivatives are gaining traction as novel treatments for post-myocardial infarction repair. Transplants that are given to avoid the immune response are given using autologous iPSCs and their derivative grafts. Imaging examination revealed evidence of transplanted cells more than 2 years after surgery in the first clinical research for age-related macular regeneration, with no evident signals of rejection[15]. So it would be safe to claim that autologous iPSCs offer the opportunity to use PSCs for rejection-free cell therapy[16].

In search for a safe and effective cardiovascular regeneration therapy, iPSCs have a wide range of cell-based applications. Human pluripotent stem cells (hPSCs) have the ability to multiply indefinitely in their undifferentiated form and are pluripotent, which means they can develop into practically any somatic cell type when given specific progressive stimuli, making them a virtually limitless source of functional cardiovascular cells for cardiac regeneration[1].

Takahashi and Yamanaka[9] discovered a very first technique of reprogramming somatic cells in 2006. The idea was sparked by prior understanding of key regulatory genes that played a role in cellular identity. In their Nobel Prize-winning experiment, they were able to generate induced pluripotent stem cells (iPSCs) from somatic cells like skin fibroblasts by expressing four transcription factors that were found to be critical for cellular reprogramming into inner cell mass derived ESC-like cells, namely Oct3/4, c-Myc, Sox2 and Klf4.

Since then, scientists have been racing to enhance the effectiveness of iPSC reprogramming by tinkering with transcription factor combinations and selecting other transcription factors like Lin28 and Nanog[17]. It is also feasible to generate live, tumor-free complete organisms from iPSCs. Human iPSCs (hiPSCs) were created less than a year after their first generation in mice, by the same team of pioneering scientists and many others[3].

HiPSCs might have a lot of potential in cardiovascular regeneration following ischemia injury. The creation and optimization of methods to differentiate hiPSCs into epicardial cells, vascular smooth muscle cells (VSMCs), endothelial cells (ECs), epicardial cells, and CMs have received a lot of attention recently[1].

Endothelial cells are required for vasculogenesis and angiogenesis, due to their function to coat the inside of blood vessels. ECs make up around a quarter of the human heart and play an essential role in cardiac function regulation and response to pathophysiological stress[18]. Several scientists have developed a novel 2D procedure that does not require the use of a feeder to differentiate hiPSCs into ECs and generate functional hiPSC-EC populations. In animal models of cardiovascular illness, hiPSC-derived ECs have shown a remarkable capacity to stimulate neovascularization, thus potentially delivering considerable and long-term therapeutic advantages[13,19].

Vascular smooth muscle cells, which have contractile and various factor-releasing properties, are another important component of blood arteries. They are present in the heart, have the ability to proliferate, and are easily obtained and cultured. Preliminary research suggests that smooth muscle cells implanted into the heart can help restore myocardial wall tension and flexibility, minimize dilatation, maintain cardiac function, and improve MI in animal models. However, the utilization of high-therapeutic-value hiPSC-derived VSMCs is a very new area of research that is still in its early stage[1].

Cardiomyocytes are the heart's most researched cell type, and they serve a critical function in increasing the blood flow throughout our circulatory system through regulated contraction and relaxation. Several researches have revealed promising results for the efficient production of hiPSC-CMs. The importance of the Nodal/Activin/TGF (Transforming growth factor), BMP (bone morphogenic protein), and Wnt signal transduction pathways in embryonic cardiac specification has been revealed in various publications, and has inspired several directed differentiation approaches to create hiPSC-CMs by imitating these developmental signaling cues[1,20,21].

Due to its particular characteristics, hiPSC has recently been proved to be a potent tool for simulating disease and treating a variety of human ailments, including degenerative neurological disease, cardiovascular disease and diabetes. Regardless of the tissue of origin, hiPSCs have the ability to self-renew and differentiate into all somatic cells found in the body. As a result, hiPSC is being thoroughly investigated in order to construct functional networks for the therapy of myocardial injury[22].

iPSC Derived Cardiomyocytes

Transplanted cells, such as cardiomyocytes that were produced from iPSCs, were able to integrate into host tissues, improve heart function, and minimize harmful remodeling processes in animal models of MI. Patient-specific iPSC-derived cells were thought to have considerable advantages over ESCs, such as less ethical concerns and immunological responses. Various studies, however, have reported genomic instability in iPSC lines as a result of pre-existing abnormalities in adult stem cells or mutations emerging during reprogramming and culture processes. The unexpected discovery that transplanted iPSC-derived cells are immunogenic raises yet another serious worry concerning iPSC safety. To resolve this concern, various new methods were developed that use a non-integrated gene delivery technique or insert proteins, modified messenger ribonucleic acids (mRNAs), and microRNAs (miRs) to reduce the danger of mutagenesis during the reprogramming process. Chemically induced iPSCs have recently been created using small molecule combinations. Despite these tremendous advancements, clinical translation of human iPSC-derived cells for the treatment of cardiovascular disease has yet to occur[6].

hPSC-derived cardiomyocytes (hPSC-CMs) have previously been proven to effectively repair damaged cardiac tissue and improve cardiac function[23]. One of the most significant obstacles is the possibility of post-transplant immunological rejection of allogeneic hPSC-CM. Currently patients must take strong immunosuppressive drugs for the rest of their lives to prevent the transplanted cells from being rejected. Kidney failure, life-threatening infections, and various malignancies are all possible side effects of this medicine. Using autologous iPSCs, however, this issue can be eliminated[13].

Guan *et al*.[24] performed cell preparation and differentiation to provide iPSC-CMs. Oct3/4-Sox2, Klf4 and cMyc were used to obtain human iPSC line from a healthy 32-year-old male. Human iPSCs that had not been differentiated were grown to 90 percent confluence and then differentiated into functional cardiomyocytes. During days 0 and 1, iPSCs were cultured in a medium (RPMI 1640 with B27 supplement minus insulin) supplemented with 6 μM CHIR-9902, which was a specific inhibitor of glycogen synthase kinase 3β that promotes the Wnt signaling pathway. The medium was changed to a medium without CHIR99021 on the second day. Then on the 3rd day and the next day, cells were cultures in a medium that was supplemented with 5 μM Wnt antagonist (IWR-1). After that, the medium was changed each day until day 8. The cells normally begin to contract spontaneously around 8 to 10 days of differentiation. On days 16-20 of development, purified iPSC-CMs were detached and 1 x 10^7 cells per cryogenic tube were cryopreserved. Before transplantation, cryopreserved cardiomyocytes were thawed in a 37°C temperature (for roughly two minutes) and coated in a 75 μl of 5 percent albumin solution.

Several studies used small compounds to modulate Wnt signaling and differentiated human iPSCs, which were reprogrammed from a healthy donor's peripheral blood mononuclear cells, into cardiomyocytes (hiPSC-CM)[25,26]. Cardiomyocyte preparation, generation, and cultures was done on basement membrane matrix-coated 6-well plates. Cardiomyocytes were isolated by adding 0.05 percent Trypsin/EDTA 2 mL in each well for 5 minutes at room temperature, followed by neutralization with 0.0125 percent trypsin inhibitor, and the isolated cardiomyocytes were gathered and transferred into a 50 mL conical tube on day 19 after differentiation. Cardiomyocytes were pelleted and suspended in 5 mL RPMI/B-27 cell culture medium[26].

In addition to cardiomyocyte production, endothelial cell preparation from human umbilical vein endothelial cells (HUVEC) and fibroblast preparation from human cardiac fibroblast cell line (HCF) (adult ventricular type) were also carried out. After the three types of cells were prepared, a co-culture of the three types of cells, namely hiPSC-CM, HUVECs, and HCFs, was performed in RPMI/B-27 cell media to produce a stock solution of mixed cells in a 50 mL conical tube, which should be frozen. The hiPSC-CM (70): HCFs (15): HUVECs (15) ratio in a concentration of 165,000 cells per mL was made. Then using a multi-channel pipette, approximately 200 μL of the mixture, which contained 33,000 cells, was put into 96-well U-bottom plates with ultra-low attachment, and the 96-well plates were incubated for 3 days at 37°C with 5% CO2 and 95% humidity, before transplantation[26].

In 2020, Fang *et al*.[13] developed hAFSC-iPSCs. hAFSC-iPSCs and hESCs, which served as positive controls for cardiac differentiation using RUES2 cells, were grown in 1% glutamine, 15% fetal bovine serum (FBS), and 1% penicillin/streptomycin containing Minimum Essential Media (MEM) at 370C in a 5% CO2 environment. Human basic fibroblast growth factor (bFGF, 4 ng/mL) was added to the medium and was used to expand hAFSC-iPSCs and hESCs. They cultured

hAFSC-iPSCs as well as hESCs to convert them directly into cardiomyocytes using a serum-free, monolayer technique using serial administration of bone morphogenetic protein4 (BMP4) and activin A.

In 2020, Samura *et al*.[20] carried out the process of hiPS-cell line culture and differentiation to cardiomyocytes. The hiPS-cell line was cultivated and maintained in 4 ng/mL human bFGF containing primate embryonic stem (ES) cell media. Further, a bioreactor system was used to generate cardiac differentiation[27]. In short, using a dissociation solution, hiPS-cell lines were isolated and moved to ultralow attachment culture plates in mTeSR1 medium, which was a special medium for maintaining hiPSCs, and supplemented with Y-27632 (Rho kinase inhibitor). Following embryoid body formation, the culture medium was replaced with a differentiation medium that contained StemPro34, 2 mmol/L l-glutamine, 1-thioglycerol and 50 mg/mL ascorbic acid, as well as various human recombinant proteins such as BMP4, bFGF, activin A, vascular endothelial growth factor, and IWR-1[20,27].

Biagi *et al*.[14] employed fresh early-stage hiPSC-CM in their work (differentiation on days 11-15). Using an episomal reprogramming technique, healthy donor erythroblasts were used to make hiPSCs. Another study by Cruvinel *et al*.[28] used a monolayer-based approach to distinguish cardiomyocytes. Cells that had been singled out were plated (2.5 x 10^5 cells/cm^2) in 5 μM Y-27632 containing Essential 8™ (E8) medium. The E8 media was replaced every day until the cells reached 100 percent confluence. After that, the medium was changed with RB-medium (RPMI supplemented with B27 without insulin) and 4 μM CHIR99021 [Glycogen synthase kinase (GSK-3) inhibitor that is a potent pharmacological activators of the Wnt signaling pathway]. After a 24-hour period, the medium was changed to 10 ng/mL BMP4 containing RB-medium. On the second day, the medium was replaced with a fresh RB-medium with the addition of 2.5 μM KY2111, which induces stem cell differentiation, and XAV939 (a small-molecule inhibitor of tankyrase 1/2 that also serves as a Wnt pathway inhibitor). Starting on day-4, fresh RPMI that was supplemented with 500 μg/mL Dulbecco's Phosphate-Buffered Saline (DPBS), 213 μg/mL ascorbic acid, 35% bovine serum albumin (BSA) and 2 μg/mL plasmocin was used and changed every 48 hours. Cells were cultured for 30 days under these particular conditions before being submitted to investigations as single cells[14,28].

iPSC-CM holds the possibility of regenerative therapies. However, recent studies in animal heart models found that implantation of hiPSC-CM via needle injection, engineered cardiac tissue (ECT) and cell sheet resulted in a minimal engraftment and limited therapy effectiveness in severe cardiac failure[29]. The keys to hiPS-CM lifetime are preserved blood supply to the transplanted tissue, decreased immunogenicity post transplant, and a favorable environment that improves viability of cardiomyocytes[30]. Implantation of a cell sheet paired with blood vessel induction due to omentum usage both in the recipient heart and the transplanted sheet, has been reported to boost hiPS-CM survival due to blood supply in earlier studies[31]. Laminin, particularly laminin-221, is a significant component of the extracellular matrix (ECM) that has the strongest affinity for cardiomyocytes. Laminin-221 was found to improve the metabolic and mechanical activities of hiPS-CMs in vitro[20].

Cardiac Tissue Engineering

A number of recent studies have found that cardiac tissue engineering technologies can give better therapeutic outcomes. Cell-based therapies were recently studied and showed promise in minimizing myocardial damage and limiting cardiac remodeling after MI[6]. Clinical trials, on the other hand, have thus far failed to show a meaningful improvement, because only a tiny percentage of implanted cells survived in the infarcted myocardium. A lot of evidence implies that the therapeutic advantages of cell transplantation are primarily due to paracrine effect rather than engrafted cells. A recent research has found that tissue engineering technique can boost stem cell engraftment, resulting in greater therapeutic results[32]. Engineered cardiac tissues, which are derived from hiPSC, can be utilized for heart disease modelling and drug substances in vitro screening, as well as developing functional cardiac tissue for myocardial infarction therapy[22,33].

Cardiac stem cell therapy, which involves injecting pluripotent stem cells into the myocardium, has been created as a new treatment option for heart failure. Several studies have shown that employing various cell sources, cardiac function in injured hearts can be improved, as well as tissue regeneration. However, many fundamental challenges with this technology remain unsolved, including low cell retention rates, insufficient nutrition delivery in tissues, and lack of effective integration with host tissues[34].

Tissue engineering technologies were developed as an alternative to cell-based therapies due to their limitations. The creation of stable heart tissue using 3D printing with a scaffold for transplantation therapy is a common strategy for cardiac tissue engineering. Nevertheless, there are significant disadvantages to this scaffold-based strategy, including immunogenicity of the scaffold, mechanical limitations, and quick disintegration.

Ong *et al*.[35] have announced a new biomaterial-free 3D bioprinting approach for developing functioning cardiac patches for heart cell preservation and tissue transplantation. In addition, in vivo regeneration capability of the heart tissue was investigated by Yeung *et al*.[26]. The cardiac patches, which were transplanted in a mouse model, were manufactured by a 3D bio-printing technology of cellular spheroids that were made from a co-culture of human IPSC derived cardiomyocytes, fibroblasts, and endothelial cells. In a mouse model, they did an in vivo investigation in which the unique biomaterial-free heart tissue engineered patch was implanted and showed long-term retention as well as increased functional and vascularity. The biomaterial-free technique utilized in this study decreased the danger of infection induced by the scaffold material and reduced the possibility of inflammatory reaction[26].

Yokoyama *et al*.[21] used a fibronectin (FN) combined with gelatin (G) covering to prepare cardiac tissues from 3D-hiPSC-CMs as 3D-hiPSC-CM tissues (3D-hiPSC-CTs). Fibronectin was chosen for this construction because of its ability to promote the synthesis of several proteins found in cardiomyocytes via α6β1receptor, as well as for cardiomyocyte ischemia resistance and also the provision of a scaffold for cell attachment, which might potentially boost cell survival following transplantation. Fibronectin coated 3D-hiPSC-CT could be a possible heart transplantation alternatives, with the capability of improving survival and efficacy of treatment in situations of myocardial infarction. Therefore, they predicted that FN-G coated 3D-hiPSC-CTs would ameliorate ischemic heart function as a result of basement membrane matrix remodeling and cardiac protein remodeling[21].

Samura *et al*.[20] created a 3-dimensional engineered cardiac tissue (3D-ECT) with hiPS-CMs, laminin-221 and fibrin gel. To create a 3D-ECT, polypropylene sheets and a 6-well plate, which was gelatin-coated, were prepared. hiPS-CMs (5 x 10^6 cells) in 100 μL of cardiac medium, which contained DMEM that was supplemented with 10% FBS, combined with 10 μL fibrinogen and 10 μL laminin-221 or 10 μL PBS without Ca and Mg, were used. hiPS-CMs, fibrinogen, and laminin-221 or PBS were mixed and put onto a gelatin gel, followed by a 5 μL thrombin solution, which was added to the mixture and subsequently coated with a polypropylene sheet (10 mm x 10 mm). Laminin-221 increased the metabolic and mechanical capabilities of hiPS-CMs in vitro, and 3D-ECT showed beneficial effect in treatment of a mouse model with ischemic cardiomyopathy, implying that incorporating laminin-221 into hiPS cell-derived cardiac constructs could improve therapeutic outcomes[20].

Tissue engineering technologies have been developed as an alternative to stem cell-based therapies to overcome these limitations. In mouse models, a variety of techniques for heart tissue engineering have been used to demonstrate long-term retention and increased function and vascularity[26].

Some of these cardiac stem cell-based tissue engineering techniques showed promise as a regenerative therapy. Therefore, there is high expectation to turn this technology into a flexible method for treating end-stage heart failure caused by a MI.

CONCLUSION

To overcome the limitations of stem cell-based therapies, tissue engineering approaches have been developed as alternative treatments. Tissue engineering using patient-derived Human iPSC-CMs holds great potential in medicine because it might avoid the problem of possible immune rejection.

ACKNOWLEDGEMENT

This work was supported by a research grant from Ministry of Education and Culture and Ministry of Research and Technology of the Republic of Indonesia, Hibah PRN-BOPTN 2021, contract no. PKS-186/UN2.INV/HKP.05/2021.

REFERENCES

1. Jiang Y, Lian XL. Heart Regeneration With Human Pluripotent Stem Cells: Prospects And Challenges. Bioactive Materials. 2020; 5(1): 74-81.
2. Roger VL, Go AS, Lloyd-Jones DM, *et al*. Heart Disease And Stroke Statistics--2012 Update: A Report From The American Heart Association. Circulation. 2012; 125(1): e2-e220.
3. Samak M, Hinkel R. Stem Cells in Cardiovascular Medicine: Historical Overview and Future Prospects. Cells. 2019; 8(12): 1530.
4. Rikhtegar R, Pezeshkian M, Dolati S, *et al*. Stem Cells As Therapy For Heart Disease: iPSCs, ESCs, CSCs, and Skeletal Myoblasts. Biomed Pharmacother. 2019; 109: 304-13.
5. Chow A, Stuckey DJ, Kidher E, *et al*. Human Induced Pluripotent Stem Cell-Derived Cardiomyocyte Encapsulating Bioactive Hydrogels Improve Rat Heart Function Post Myocardial Infarction. Stem Cell Reports. 2017; 9(5): 1415-22.
6. M ler P, Lemcke H, David R. Stem Cell Therapy in Heart Diseases - Cell Types, Mechanisms and Improvement Strategies. Cell Physiol Biochem. 2018; 48(6): 2607-55.
7. Minicucci MF, Azevedo PS, Polegato BF, Paiva SA, Zornoff LA. Heart Failure After Myocardial Infarction: Clinical Implications And Treatment. Clin Cardiol. 2011; 34(7): 410-4.
8. Wernly B, Mirna M, Rezar R, *et al*. Regenerative Cardiovascular Therapies: Stem Cells and Beyond. Int J Mol Sci. 2019; 20(6): 1420.
9. Takahashi K, Yamanaka S. Induction Of Pluripotent Stem Cells From Mouse Embryonic And Adult Fibroblast Cultures By Defined Factors. Cell. 2006; 126(4): 663-76.
10. Machiraju P, Greenway SC. Current Methods For The Maturation Of Induced Pluripotent Stem Cell-Derived Cardiomyocytes. World J Stem Cells. 2019; 11(1): 33.
11. Liu YW, Roan JN, Wang SP, *et al*. Xenografted Human Amniotic Fluid-Derived Stem Cell As A Cell Source In Therapeutic Angiogenesis. Int J Cardiol. 2013; 168(1): 66-75.
12. Liu YW, Fang YH, Su CT, Hwang SM, Liu PY, Wu SN. The Biochemical And Electrophysiological Profiles Of Amniotic Fluid-Derived Stem Cells Following Wnt Signaling Modulation Cardiac Differentiation. Cell Death Discov. 2019; 5: 59.
13. Fang YH, Wang SPH, Gao ZH, *et al*. Efficient Cardiac Differentiation of Human Amniotic Fluid-Derived Stem Cells into Induced Pluripotent Stem Cells and Their Potential Immune Privilege. Int J Mol Sci. 2020; 21(7): 2359.
14. Biagi D, Fantozzi ET, Campos-Oliveira JC, *et al*. In Situ Maturated Early-Stage Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes Improve Cardiac Function by Enhancing Segmental Contraction in Infarcted Rats. J Pers Med. 2021; 11(5): 374.
15. Mandai M, Watanabe A, Kurimoto Y, *et al*. Autologous Induced Stem-Cell-Derived Retinal Cells for Macular Degeneration. N Engl J Med. 2017; 376(11): 1038-46.
16. Yamanaka S. Pluripotent Stem Cell-Based Cell Therapy-Promise and Challenges. Cell Stem Cell. 2020; 27(4): 523-31.
17. Masip M, Veiga A, Izpisúa Belmonte JC, Simón C. Reprogramming With Defined Factors: From Induced Pluripotency To Induced Transdifferentiation. Mol Hum Reprod. 2010; 16(11): 856-68.
18. Zhou P, Pu WT. Recounting Cardiac Cellular Composition. Circ Res. 2016;118(3):368-70.
19. Oh JE, Jung C, Yoon YS. Human Induced Pluripotent Stem Cell-Derived Vascular Cells: Recent Progress and Future Directions. J Cardiovasc Dev Dis. 2021; 8(11): 148.
20. Samura T, Miyagawa S, Kawamura T, et al. Laminin-221 Enhances Therapeutic Effects of Human-Induced Pluripotent Stem Cell-Derived 3-Dimensional Engineered Cardiac Tissue Transplantation in a Rat Ischemic Cardiomyopathy Model. J Am Heart Assoc. 2020; 9(16): e015841.
21. Yokoyama J, Miyagawa S, Akagi T, Akashi M, Sawa Y. Human Induced Pluripotent Stem Cell-Derived Three-Dimensional Cardiomyocyte Tissues Ameliorate The Rat Ischemic Myocardium By Remodeling The Extracellular Matrix And Cardiac Protein Phenotype. PLoS One. 2021; 16(3): e0245571.
22. Yu D, Wang X, Ye L. Cardiac Tissue Engineering for the Treatment of Myocardial Infarction. J Cardiovasc Dev Dis. 2021; 8(11): 153.
23. Liu YW, Chen B, Yang X, *et al*. Human Embryonic Stem Cell-Derived Cardiomyocytes Restore Function In Infarcted Hearts Of Non-Human Primates. Nat Biotechnol. 2018; 36(7): 597-605.
24. Guan X, Xu W, Zhang H, *et al*. Transplantation Of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes Improves Myocardial Function And Reverses Ventricular Remodeling In Infarcted Rat Hearts. Stem Cell Res Ther. 2020; 11(1): 73.
25. Ong CS, Fukunishi T, Zhang H, *et al*. Biomaterial-Free Three-Dimensional Bioprinting of Cardiac Tissue using Human Induced Pluripotent Stem Cell Derived Cardiomyocytes. Sci Rep. 2017; 7(1): 4566.
26. Yeung E, Fukunishi T, Bai Y, Bedja D, Pitaktong I, Mattson G, Jeyaram A, Lui C, Ong CS, Inoue T, Matsushita H, Abdollahi S, Jay SM, Hibino N. Cardiac Regeneration Using Human-Induced Pluripotent Stem Cell-Derived Biomaterial-Free 3D-Bioprinted Cardiac Patch In Vivo. J Tissue Eng Regen Med. 2019; 13(11): 2031-9.
27. Matsuura K, Wada M, Shimizu T, *et al*. Creation of human cardiac cell sheets using pluripotent stem cells. Biochem Biophys Res Commun. 2012; 425(2): 321-7.
28. Cruvinel E, Ogusuku I, Cerioni R, *et al*. Long-term single-cell passaging of human iPSC fully supports pluripotency and high-efficient trilineage differentiation capacity. SAGE Open Med. 2020; 8: 2050312120966456.
29. Li J, Minami I, Shiozaki M, *et al*. Human Pluripotent Stem Cell-Derived Cardiac Tissue-like Constructs for Repairing the Infarcted Myocardium. Stem Cell Reports. 2017; 9(5): 1546-59.
30. Miyagawa S, Fukushima S, Imanishi Y, *et al*. Building A New Treatment For Heart Failure-Transplantation of Induced Pluripotent Stem Cell-derived Cells into the Heart. Curr Gene Ther. 2016; 16(1): 5-13.
31. Kainuma S, Mitsuno M, Toda K, *et al*. Dilated Left Atrium As A Predictor Of Late Outcome After Pulmonary Vein Isolation Concomitant With Aortic Valve Replacement And/Or Coronary Artery Bypass Grafting. Eur J Cardiothorac Surg. 2015; 48(5): 765-77.
32. Kwon SG, Kwon YW, Lee TW, Park GT, Kim JH. Recent Advances In Stem Cell Therapeutics And Tissue Engineering Strategies. Biomater Res. 2018; 22: 36.
33. Fan C, Fast VG, Tang Y, *et al*. Cardiomyocytes From CCND2-Overexpressing Human Induced-Pluripotent Stem Cells Repopulate The Myocardial Scar In Mice: A 6-Month Study. J Mol Cell Cardiol. 2019; 137: 25-33.
34. Wang F, Guan J. Cellular Cardiomyoplasty And Cardiac Tissue Engineering For Myocardial Therapy. Adv Drug Deliv Rev. 2010; 62(7-8): 784-97.
35. Ong CS, Fukunishi T, Nashed A, *et al*. Creation of Cardiac Tissue Exhibiting Mechanical Integration of Spheroids Using 3D Bioprinting. J Vis Exp. 2017: 125: 55438.