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Techniques to induce cardiac regeneration post myocardial infarction

Hirani S[1], Dunn C[2]

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Abstract
Myocardial infarction (MI) causes the death of cardiomyocytes (CMs), the primary cardiac muscle cells responsible for controlling the beating of the heart and enabling the heart to pump blood. Due to the extremely low turnover rate of CMs, after myocardial infarction the heart never fully repairs itself. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), a DNA detection and editing system found in prokaryotes, and the use of induced pluripotent stem cells (iPSCs) have featured prominently in cardiac research in recent years. The potential of CRISPR and Cardiac Stem Cells (CSCs) to induce cardiac regeneration harnesses several mechanisms affecting the heart: reprogramming cardiac fibroblasts into CMs, transcription factor activated dedifferentiation of senescent CMs into replication competent cells and inducing cardiac progenitor cells to proliferate into CMs. These techniques have shown promising preliminary results in animal models. Various clinical trials are underway, primarily utilizing allogenic, off-the-shelf transplanted iPSCs along with the manipulation of endogenous genes and transcription factors utilizing CRISPR to enable recapitulation of heart functionality and CM survivability post MI.

Keywords
Myocardial infarction, Cardiomyocyte, CRISPR, Stem cells, Cardiac regeneration, Induced pluripotent stem cells, Cardiac progenitor cells, Fibroblast, DNA

[1]Corresponding Author: Serena Hirani, Oakton High School, 2900 Sutton Rd, Vienna, VA 22181, USA. serenahirani@gmail.com
[2]Mentor, Connor Dunn, Polygence, 333 Ravenswood Ave Building B, Menlo Park, CA 94025, USA.

Introduction

Background

Acute Myocardial Infarction (MI), commonly known as a heart attack, may be a potentially fatal condition caused by a significant decrease of blood flow to the heart. It is usually caused by a blood clot or atherosclerosis, leading to a buildup of plaque in the coronary arteries (1). This sudden inability of the heart to function causes necrosis, or cell death, in large areas of the heart muscle tissue and a buildup of inflexible scar tissue which does not have the ability to contract and pump blood (2). Risk factors for MI include but are not limited to: obesity, high cholesterol levels, hypertension, lack of exercise, drug and tobacco use, family history, and stress (3). While these factors may raise an individual's risk of MI, it is impossible to predict a sudden cardiac event. MI is a leading cause of death in both the United States and worldwide. When the event is not fatal, the long-term prognosis is still very poor, with an increased risk of death for individuals for at least 7 years post-MI (4, 5). Additionally, MI can lead to other serious complications such as heart failure, sudden cardiac arrest, or arrhythmias, all potentially fatal, with a poor prognosis (5).

The endogenous regenerative capacity of mammalian cardiac cells, specifically CMs, is low. Immediately after birth, mammals have a high cardiac cell turnover rate, but soon thereafter, most cells enter the G_0 post-mitotic phase of the cell cycle, effectively terminating regeneration. At the age of 25, CM turnover rate drops to around 1%, and by age 75, it is as low as 0.45% (6). A promising area of research regarding increasing cell turnover rate is the study of amphibian and zebrafish cardiac regeneration. It has been shown that zebrafish can regenerate their hearts even after 20% damage, greatly exceeding human regenerative capacity (6). While not the main subject of this review, it is relevant to note that it is unknown why adult mammalian cardiac cells have such a low regenerative capacity. One theory as to why mammals have limited cardiac cell turnover rate is that the CM centrosome loses its integrity soon after birth. Centrosomes play a major role in mitosis and the cell cycle, allowing cells to regenerate and duplicate, a feature not present in human CMs. Zebrafish and amphibians, along with very young mammals, have intact cardiac centrosomes, allowing CMs to regenerate (7).

Current treatments and interventions for MI are limited, and mostly focus on preventing further MI events, rather than repairing the heart (8). There may be some differences in treatment for a STEMI (complete blockage) and NSTEMI (partial blockage), but many treatments remain essentially similar. During a cardiac event, anticoagulants are administered to the patients to dissolve any clots that may be creating blockage. Operations such as a balloon angioplasty (a balloon is guided to the point of blockage and inflated to pack the plaque against artery walls and allow blood flow to continue) or a cardiac stent placement (a small piece of metal mesh is placed in the artery to improve blood flow) may be necessary. Additionally, individuals who have suffered MI are placed on a strict regime of medications for the rest of their life, often with significant side effects (3, 8).

CRISPR knock in for increased CM regeneration, division and turnover and knock out for decreased CM apoptosis

CRISPR-Cas is a natural viral defense mechanism found in the immune systems of many archaea and bacteria (9, 10). CRISPR recognizes specific viral sequences and is used in conjunction with guide RNA (gRNA) to direct the Cas protein to a specific point on the strand of the DNA of interest and cut out the DNA sequence specified by the gRNA, thus rendering it inactive and unable to harm the host organism (9).

There are numerous types and subtypes of Cas proteins, classified by their structure, phylogenetic traits, and ability to effectively protect organisms. Each Cas protein has a specific structure, and not all are completely effective in cleaving viral DNA due to their distinct structures (10, 11). Cas9 is a protein that is often used in research because of its developed structure, which includes all the parts necessary to effectively cleave DNA. The CRISPR-Cas9 protein can produce CRISPR RNA (crRNA), an important piece in creating gRNA, and successfully cleave the DNA of interest. Other Cas proteins are often dependent on other compounds to perform their tasks (10).

In many microorganisms, CRISPR-Cas9 is an integral part of their immune system. When a foreign viral DNA sequence is detected, the viral genome is processed into shorter segments of 26-72 bp called spacers. These spacers are inserted into the bacterial genome sequence (the CRISPR sequence) and are thus stored in the memory of the organism (9). In the event of infection by the same virus, CRISPR "remembers" the genome sequence and immediately begins the process of fighting it. CRISPR repeat sequences and spacers are first transcribed into RNA and broken into smaller sections of crRNA. crRNA is joined with a very short DNA sequence complementary to the target strand to form gRNA, which guides the Cas9 protein to the correct spot on the viral sequence and cleaves the DNA at this point, rendering it ineffective and unable to replicate again (9).

In 2012, two teams of scientists reached the conclusion that bacterial CRISPR systems could be adapted to target a specific site by changing the crRNA sequence. Both studies, one conducted by Virginijus Siksnys and his team and the other by Jennifer Doudna and her team, were published at approximately the same time (12, 13). A year later, in 2013, CRISPR was successfully adapted for the first time for use in eukaryotic cells, by Feng Zhang and his team (14). Since then, CRISPR has been used in numerous studies to 'knock out' or 'knock in' certain genes and inhibit or activate transcription respectively. It is used in studies of monogenic disorders such as sickle cell anemia and cystic fibrosis. Additionally, CRISPR has been used to disable the HIV-1 provirus by excising the HIV genome from infected patients (15).

In a recent study, knockout of a specific gene, *thioredoxin-interacting protein* (*TXNIP*), significantly increased cardiac function post-MI (16). Jiao and his team tested the importance of *TXNIP* in the reduction of cardiomyocyte apoptosis in wild type mice. The CRISPR-Cas9 system was used to knock out the gene in the *TXNIP*-KO group and to ensure the gene was present in the *TXNIP*-KI group. MI was induced in the mice and they were observed over the course of a month for changes in specific protein expression and cardiomyocyte apoptosis (cell death). It was

found that mice in the *TXNIP*-KO group had improved cardiac recovery post-MI compared to those in the *TXNIP*-KI group. The TXNIP-KO group exhibited a greater reduction in both cardiomyocyte apoptosis and myocardial fibrosis (tissue scarring), as well as a greater increase in angiogenesis (blood vessel formation) (16).

The Hippo Pathway is a kinase signaling cascade that controls tissue growth by limiting cell proliferation and division (17). It has been studied extensively in relation to cardiomyocytes, as these specific cells are unable to regenerate at an efficient rate. Liu *et al.* examined the potential of using gene therapy to knock down, or reduce the expression of, the *Sav* gene, a gene that plays a major upregulatory role in the Hippo Pathway (18). In this study, pig hearts were used as a model due to their similarity to human hearts. Adeno-associated virus 9 (AAV9) was used to deliver *Sav* short hairpin RNA to the cardiomyocytes. AAV carrying green fluorescent protein (GFP) was used as a control. Three months post-injection, the pig hearts were examined for changes. Pig hearts treated with AAV9-*Sav*-shRNA showed a 14.3% increase in ejection fraction (a measurement of left ventricle function) compared to the pig hearts treated with AAV9-GFP. AAV9-Sav-shRNA treated hearts also showed signs of reduced myocardial fibrosis and increased cardiomyocyte division (18).

Differentiation of, and dedifferentiation into, Stem cells

Stem cells are the original basic cells of the body, found in all multicellular organisms, and are defined by their ability to self-renew and differentiate into cells with specific functions (19) They all start out unspecialized. Stem cells exist in all forms of development, and are classified based on their potency, which changes with the stages of development and other factors (19).

Human embryonic stem cells (ESCs) are formed during the blastocyst phase of embryonic development, about 4-5 days after the formation of the blastocyst (20, 21). These cells are totipotent, meaning they have the potential to differentiate into any human body cell and into an entire organism. The inner cell mass (ICM) of the blastocyst contains stem cells that are pluripotent - able to differentiate into all human body cells, but not the placenta, and therefore not an entire organism (21). These pluripotent cells are most often used in scientific and medical research and are usually taken from extra embryos from in vitro fertilization (IVF) (20). As these cells continue to differentiate and specialize, they become multipotent stem cells, which can differentiate into a specific lineage, or small group, of related cells. For example, neural stem cells (NSCs) can form neurons, astrocytes, and oligodendrocytes; hematopoietic stem cells (HSCs) form different types of blood cells; and mesenchymal stem cells (MSCs) form mesenchymal tissue, including bone, cartilage, muscle, connective tissue, etc. (22). Lastly, unipotent stem cells can only form one specific kind of cell, however, they still have the ability to self-renew (21). This self-renewal is what allows for stem cells to repair damage in the body and create new cells, and is the basis for a large amount of research on medical treatments using stem cells.

In 2006, Yamanaka and Takahashi found that with the addition of certain transcription factors

- the Yamanaka factors - somatic cells could be turned back into induced cardiac progenitor cells (iPSCs) as shown in Figure 1 (23). The Yamanaka factors are Oct4, Sox2, Klf4, and cMyc, 4 transcription factors that play a major role in the replication of DNA (24). The introduction of this technology has opened doors to a multitude of treatments and research models.

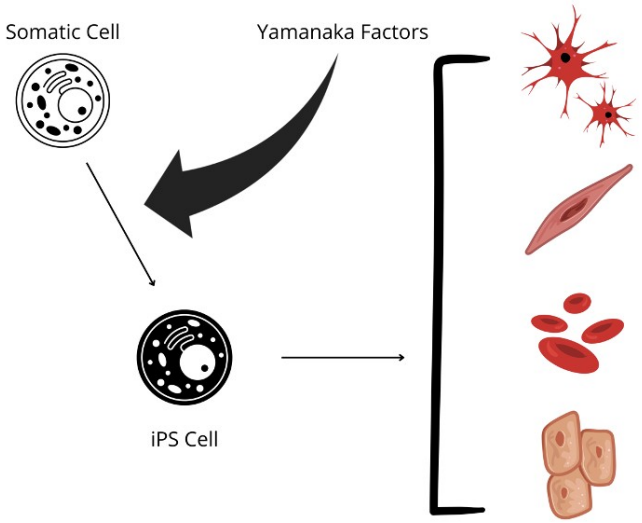

Figure 1: Somatic cells are induced into pluripotent stem cells with the ability to renew and differentiate into a variety of different body cells including but not limited to: neurons, skin cells, muscle cells, and blood cells. Adapted from Cheung et al., 2012.

One such application is the potential to make use of iPSCs in drug models. The pharmaceutical industry often has a lack of suitable models to test potential treatments in early stages (25). Stem cell models offer a solution to this issue. While traditional models rely on cell samples from humans, iPSC lines have the ability to differentiate into all three germ layers in vitro, providing scientists with a more elaborate and efficient model to test potential therapies. Additionally, iPSC models have the capability to outcompete conventional mouse models due to the phenotypic differences between mice and humans (25). In 2014, Axiogenesis, a company specializing in in vitro cell and tissue models, announced the use of their Cor.4U® cardiomyocytes, derived from human iPSCs, for drug safety testing. The FDA made use of the Cor.4U® cells to test electrophysiological properties of the cells in response to treatment with specific compounds (26). In 2022, Axol Bioscience announced their own iPSC derived CMs used for drug testing. The FDA launched a group to test the ability of the cells to produce cardiotoxicity in vitro for research purposes (27). Stem cells are on the forefront of cardiac research not only for their potential for treatments, but because of their use in drug testing.

Discussion

CRISPR and gene transduction can be used to reprogram cardiac fibroblasts

While CMs are responsible for the function of contracting the heart to keep blood flowing, they only make up about 30-40% of the cell population in the heart. In contrast, fibroblasts, a major component of the extracellular matrix (ECM) and the cells that make up the main structure of the heart as well as the primary cells responsible for electrical signaling in the heart, make up the other 60-70% of cardiac cell numbers (28). Post-MI, damaged CMs are replaced with scar tissue made up of fibroblasts in order to preserve heart structure. However, this reduces cardiac function because fibroblasts are not as flexible and do not allow for effective heart contractions (29). Recently, CRISPR has been used to potentially reprogram fibroblasts into iCPCs with the activation of certain genes responsible for the formation and proliferation of cells. This is a promising area of study that can be implemented in post-MI cardiac care as well as in cardiac disease modeling.

Recently, it was shown that with the addition of cardiac transcription factors, fibroblasts can be reprogrammed into iCPCs (30). Dead Cas9 (dCas9), a deactivated form of Cas9 which binds to the target DNA but does not cleave it, preventing transcription of the target DNA, was used with the CRISPR activation (CRISPRa) - synergistic activation mediator (SAM) system to precisely activate certain cardiac genes and allow cardiac fibroblasts to be reprogrammed (30). CRISPRa-SAM was delivered into tail tip fibroblasts (TTFs) of mice using a modified lentivirus to target the promoter regions of 11 genes shown to be critical in the development and regulation of mammalian cardiac cells. Three genes, *Gata4*, *Nkx2.5*, and *Tbx5*, endogenous genes involved in processes affecting cell proliferation, were shown to be the most important for reprogramming fibroblasts into cardiac progenitor cells based on how cells reacted when specific genes were activated or deactivated in the target cells. This same study also tested whether fibroblasts derived from other tissues in the body, like lung tissue, could differentiate into iCPCs with the activation of these same endogenous genes. These cells, after being suspended in the growth medium for 14 days, showed no signs of pluripotency, but were able to form cells that expressed cardiac troponin T (cTnT), a prominent feature of CMs or CM-like cells (30).

Direct reprogramming of fibroblasts into CMs, bypassing the iCPC phase, also holds some promise for efficient cardiac repair and disease modeling (31). A 2010 study found that with the in vivo introduction of a combination of transcription factors crucial to cell development, namely Gata4, Mef2c, and Tbx5, functional cardiac or mesodermal fibroblasts could be directly reprogrammed into CM-like cells that have a very similar structure to CMs and are able to perform CM functions such as contracting spontaneously. In the study, combinations of 14 different transcription factors were transduced into hearts of mice using retroviruses. When certain transcription factors from this pool were added or removed, the number of cardiomyocytes present increased or decreased (31). For example, the removal of five factors, Baf60c, Hand2, Hopx, Hrt2, and Pitx2c, increased the amount of CMs produced while the removal of Gata4 sufficiently decreased the amount of CMs

produced. This process of testing different combinations of transcription factors was performed until three crucial factors that increased differentiation were determined, Gata4, Mef2c, and Tbx5 (Figure 2). The study concluded that fibroblasts could be reprogrammed into CM-like cells in vivo, when exposed to the three main transcription factors (31).

Figure 2: Cardiac fibroblasts are reprogrammed into CM-like cells with the introduction of a combination of important transcription factors including Gata4, Mef2c, and Tbx5.

Stem Cells can be used to Induce Proliferation of Cardiomyocytes

With the relatively recent discovery of the Yamanaka factors, the field of cardiac research has gained an important tool in studying cardiac regeneration and creating disease models. The main issue with cardiac cells, specifically with CMs, post-MI, is they do not have the ability to self-renew, unlike stem cells (6, 19). The use of stem cells in cardiac regeneration has become a prominent area of research and presents the possibility of differentiating stem cells into CMs which can be transplanted into damaged hearts to promote regeneration.

The embryoid body (EB) method of differentiation has shown some promise in creating CMs. This method involves the formation of EBs, which are sphere-shaped structures similar to embryos, created using pluripotent stem cells (32, 33). They are similar to embryos in that they create all three different germ layers, but are different in that they are often inconsistent in their structure. However, they are similar enough to simulate CM differentiation (32). In a 2009 study, human iPSC lines were produced and made to form EBs, which then differentiated into cells of all three germ layers, including CMs and other cardiac cells. This was proven by a positive staining of cardiac troponin-1, sarcomeric α-actinin, and connexin-43, all hallmark indicators of CM cells (32).

A 2018 study investigated the potential for bone-marrow mesenchymal stem cells (BMSCs) to differentiate into cardiac cells with the introduction of specific microRNAs (miRNA) and cytokines (34). BMSCs are an ideal source of stem cells in research due to their availability and capacity to proliferate. Though often restricted due to its carcinogenicity, the chemical 5-Azacytidine (5-aza) has been used successfully in the past to induce differentiation of BMSCs into cardiomyocytes. Due to its limitations, however, 5-aza is not a method of differentiation that can be carried into clinical

research (34). *miR-1* has been shown by Zhao et al. to edit gene expression to induce BMSCs into both cardiomyocytes, and cardiac fibroblasts by lowering the expression of Dll-1, an inhibitor of cardiac gene expression (34, 35). Other miRNAs are used in slightly different ways to inhibit or promote expression of genes that play an important role in cardiomyocyte differentiation in BMSCs (34).

Stem cell factors play a major role in allowing senescent cardiomyocytes, or cardiomyocytes that have stopped dividing, to regain the ability to replicate (36). A 2022 study examined the effectiveness of two synthetic modified mRNAs in promoting cardiomyocyte regeneration, an SRF153 mutant (STEMIN) and the mutant YAP5SA (36). Post-MI mice models were injected with the two modified mRNAs and evaluated for reentry of cells into the S phase of the cell cycle, extent of myocardial fibrosis, and number of cardiomyocyte nuclei present. One month post-MI, it was found that mice treated with STEMIN and YAP5SA modified mRNA showed significant improvement in cardiac function. These hearts expressed a number of stem cell factors including NANOG and OCT4 that allowed the senescent cardiomyocytes to be dedifferentiated and able to replicate. Myocardial fibrosis was also greatly reduced and treated hearts had a 17-29-fold increase in cardiomyocyte nuclei (36).

Stem Cells and CRISPR can Work Together to Repair the Heart

A significant limitation of stem cells is their limited survival after transplantation into the subject (37). This undermines the purpose of using stem cells, because they often fail in vivo despite their in vitro effectiveness. Pan et. al studied CRISPR gene regulation with a dCas9 system to improve the survivability of stem cells in ischemic hearts post-transplantation. In their study, specific genes such as *Akt* and *oxygenase-1* activated metabolic pathways that increased cell survival. CRISPR-dCas9 was used to regulate these genes in ischemic heart patients to improve stem cell survivability (37).

CRISPR is also widely used as a research tool. Recently, CRISPR knockout screen technology was used by Xu et.al. to determine essential genes involved in cardiac progenitor cell formation (38). CRISPR knockout screening is a process that involves creating a population of cells where each cell has a specific gene that has a loss of function. Using this method, it is possible to determine which genes are important for a certain process based on the ability of cells to perform that function in its absence (39). In the case of this cardiac progenitor cell study, CRISPR knockout screening was a used to knockout the entire human genome (one gene knocked out per cell) to determine that *ZIC2* is an essential gene in cardiac progenitor formation (38).

Limitations of Cardiac regeneration with Stem Cells and CRISPR

The relatively recent developments in research involving CRISPR and stem cells have significantly changed the landscape of regenerative medicine. While they will no doubt continue to be on the forefront of modern medicine, it is extremely important to consider some limitations involving the use of CRISPR and stem cells.

The predominant issue with CRISPR remains its propensity for causing off-target effects. As the CRISPR system is meant to cleave DNA

and knock out or replace genes, there is always a possibility of unintended genetic modification that affect different parts of the genome. CRISPR-Cas9, in some contexts, has an off-target effect frequency of greater than 50%, making it very inefficient to cause specific, targeted, changes (40). Importantly, there are some ways the frequency of off-target activity can be reduced. Altering the gRNA sequence to make it more probable to guide the Cas9 protein to the correct locus is one proposed solution. Another is to control the concentration of the Cas9-gRNA complex when it is delivered to increase its location specificity. It should be noted that even with these changes, there still remains a relatively high chance of off-target effects. While these effects are sometimes minimal and worth the risk, other times they can affect necessary physiological functions (40).

Certain aspects of the CRISPR-Cas system are still not fully understood, including the delivery mechanism. There are three categories of delivery that are most often used: physical delivery, viral vectors, and non-viral vectors (41). However, each has its drawbacks. Physical delivery includes microinjection, electroporation, and hydrodynamic delivery. Microinjection is the most commonly used out of the three due to its guaranteed delivery of CRISPR to the correct location. However, it is time consuming and costly. Electroporation, while not as accurate, is much easier and more universally used. Hydrodynamic delivery is usually not used due to its effect on surrounding tissue and high rate of physiological complications (41). Other methods of delivery all have their own limitations as well. Viral vectors, specifically adeno-associated virus (AAV) and lentivirus are most commonly used in the delivery of CRISPR-Cas9. While they are the most efficient, there is a risk of excessive activation of the immune system in response to inactive viral vectors (41). Non-viral vectors include systems such as lipid nanoparticles and cell-penetrating peptides (CPPs). While non-viral vectors are not used as often, they are a growing area of research. Each mechanism of delivery has its own merits, however, there is always a risk of off-target events and effects on surrounding tissue (41).

Use of stem cells have ethical limitations. A major concern for many scientists is the source of ESCs (42), since they are harvested from the blastocysts of newly formed embryos. Most often, ESCs are harvested from leftover embryos that were not needed in IVF treatments and were donated to science (43), which may alleviate this concern.

A limitation to the widespread use of iPSCs in cardiac repair is their extremely low efficiency (44). While the discovery of the Yamanaka factors represented a turning point in stem cell research, they are not perfect. There is still a very low percentage of cells that can gain pluripotency with the Yamanaka factors. In early studies of induced pluripotency, less than 1% of somatic cells gained pluripotency (44). While there has been some progress since then, it is still an inefficient process. Additionally, there is a possibility of spontaneous differentiation or proliferation of iPSCs, which can form tumors (44).

Another obstacle in the use of stem cells is that the inflammatory environment in the heart is not conducive to stem cell survival and retention. In fact, stem cells often undergo

significant attrition when transplanted into a human heart post-MI (45). Chiamvimonvat et.al., studied the potential of using soluble epoxide hydrolase (sEH) inhibitors to decrease inflammation in the host heart to allow stem cells the possibility of surviving longer (46). They found that TPPU, an sEH inhibitor, results in a significant improvement in the number of surviving stem cells. These investigators are continuing to research sEH inhibitors in relation to stem cells with a view to commencing clinical trials (46).

Future Directions

Until now, the techniques mentioned in this review have been mostly used in basic or translational research in animal models. By addressing some of the limitations mentioned earlier, scientists have been able to commence clinical trials of transplanted CMs or iPSCs.

In recent years, off-target effects with CRISPR have been studied and ideas involving the detection and prediction of off-target effects have been proposed, using systems such as genome sequencing and more targeted detection strategies (47). The delivery of CRISPR is also an issue that is being extensively researched to determine the most efficient system. In some studies, the side effects of viral vectors are being reduced. Additionally, non-viral vectors are being studied and may be the ideal system in the future (41).

The ESC controversy has led to the increased adoption of iPSCs since they bypass the embryonic stage and utilize body cells that are easier to harvest. However, these are not a perfect solution and they are still being studied to improve their efficiency.

There are ongoing pre-clinical and clinical trials that aim to bring these treatments to patients in the near future. A clinical trial with a completion date in 2023 is studying the use of human iPSC-derived cardiomyocytes to improve left ventricular systolic function (LVEF) in patients with ischemic cardiomyopathy, a condition in which the heart's ability to pump blood is reduced due to left ventriclular dysfunction (48). In this study, patients receive allogenic transplants of iPSC-derived cardiomyocytes and are assessed for a number of measures including improved LVEF, adverse effects, and abnormal vital signs (48). Another ongoing clinical trial is also studying the use of allogenic human iPSCs in treating heart failure (49). Patients are given a direct intramyocardial injection of iPSC-derived cardiomyocytes and examined at 1, 3, 6, and 12 months post-injection for changes in LVEF, a 6 minute walk test, adverse effects, etc (49). A number of other studies with similar objectives are currently being conducted with the use of stem cells.

Gene therapy is also a subject of clinical trials in recent years. A study completed in 2015 tested the safety and efficacy of adenovirus-delivered *VEGF-D* gene therapy in improving the myocardial perfusion (a test of cardiac blood flow) in patients with severe coronary artery disease (50). After a 1-year follow up, the researchers concluded that the treatment was safe, feasible, and showed a statistically significant increase in myocardial blood flow after in the treated group. Additionally, the quality of life, measured by a 15D questionnaire score, increased in the treated group from 0.787 ± 0.108 to 0.803 ± 0.101 at 3 months post-treatment (50).

Conclusion

The use of CRISPR-Cas systems and Stem cells have provided major breakthroughs in disease modeling and cardiac regenerative medicine in recent years. The use of allogenic, off-the-shelf, iPSCs is likely to become increasingly widespread in the near future, as is the use of CRISPR and small molecules to increase the former's efficiency. The manipulation of endogenous genes and transcription factors by CRISPR can allow greater differentiation, regeneration and turnover of iPSCs as well as decrease apoptosis and increase their resistance to the post MI inflammatory environment. These techniques are at the forefront of cardiac research, making it possible to accomplish a once-thought impossible task, viz. that of regenerating a damaged heart. It is only a matter of time before these techniques become safer, more effective and technically and ethically feasible for widespread use in post MI patients.

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