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Genome Editing of Stem Cell-Derived Cardiomyocytes for Post-Infarct Repair

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Abstract

A myocardial infarction (MI), commonly known as a heart attack, can cause permanent loss of cardiomyocytes in the affected area, significantly limiting the heart's ability to regenerate damaged tissues and restore function. While the injured area may not be able to fully recover, recent advancements in stem cell therapies involve using pluripotent stem cells (iPSC) and human embryonic stem cell (hESC)-derived cardiomyocytes. Other therapies that are being used, such as drug delivery and bioengineered scaffolds, have many issues and complications, which is why stem cell-based therapies are preferred. The efficacy of these stem cell-derived cardiomyocytes, in a post-infarct environment, is dependent on certain edits in gene expression. Studies in this field focus on genome editing technologies such as CRISPR-Cas9 to modify certain genes involved in calcium-handling pathways, metabolic regulations, and electrophysiological development in these stem cells. Function maturation can be analyzed through gene expression profiles and contractility, in vitro and in vivo. In this review, we synthesize how different genome edits of various signaling proteins aim to improve the functional maturation of both iPSC and hESC-derived cardiomyocytes following a myocardial injury. Specifically, understanding how targeted genome edits enhance therapeutic potential. Synthesizing current approaches and finding connections between evaluations can help in the development of more effective stem-cell-based interventions.

Keywords: genome editing, CRISPR-Cas9, stem cell-derived cardiomyocytes, cardiac regeneration, induced pluripotent stem cells (iPSCs), cardiomyocyte maturation, myocardial infarction, regenerative medicine, cardiovascular disease

1. Introduction

A myocardial infarction is a medical condition in which blood flow to the heart is cut off, most commonly caused by plaque buildup in the coronary arteries (Młynarska et al., 2024). This obstruction prevents oxygen and nutrient delivery to cardiac tissue (Jiang et al., 2024). This ultimately results in the immediate death of surrounding cardiomyocytes and heart tissue (Salari et al., 2023). MI remains a leading cause of heart failure worldwide despite advances in reperfusion strategies and pharmacological therapies (Cho & Cho, 2021). Even though clinical interventions have improved short-term survival after certain ischemic diseases, myocardial infarction continues to have a significant global health burden. This is due to the long-term consequences of irreversible cardiac damage (Laforgia et al., 2022). Patients may develop arrhythmias, progressive heart failure, and strokes. Changes to the heart's structure (Left Ventricular Remodeling) are possible, as the heart may change in shape, size, and thickness over time, increasing pressure on the myocardium walls, leading to heart failure. As shown in Figure 1, myocardial infarction initiates many processes that prevent tissue repair. These include severe inflammation, hypertrophy, extracellular matrix (ECM) breakdown, fibroblast activation, which causes fibrosis/scarring, and angiogenesis (Antar et al., 2023). A critical barrier is that the adult human heart has negligible regenerative capacity, making cardiomyocyte loss post-MI largely irreversible (Carotenuto et al., 2021). Cardiomyocytes within the infarct zone (IZ) and nearby areas suffer significant damage due to both necrosis and apoptosis (Ma et al., 2025; Thankam & Agrawal, 2020).

In addition to physical and structural changes of these cardiomyocytes, a myocardial infarction causes major metabolic shifts within the affected cells (H. Liu et al., 2025; Zuurbier et al., 2020). Under standard physiological conditions, cardiomyocytes use efficient aerobic metabolism to meet the regular high energy demand (S. Liu et al., 2025). However, after an ischemic injury, these cells make a sudden transition to anaerobic glycolysis pathways. This shift causes a reduction in ATP production and inefficient cellular function. Alongside these metabolic alterations, disruptions in intracellular signaling cause eventual calcium overload (Buja, 2024). Due to the dysregulation of calcium-handling mechanisms, calcium concentration builds up within cardiomyocytes. This promotes mitochondrial permeability transition onset and triggers pathways that lead to cell death. Altogether, these biochemical and mechanical disturbances create a highly unfavorable and toxic environment. These hostile conditions create a post-infarct microenvironment that is resistant to cardiomyocyte survival and maturation (Bertero et al., 2024). As a result, strategies that try to fully repair the heart are insufficient, which is why there is a need for alternative therapeutic approaches (Akbaba et al., 2025).

Current therapies that are being used include drug therapies, bioengineered tissues, and certain medications (M. S. Ahmed et al., 2024). Therapies that involve a combination of drugs (e.g., Paromomycin/Neomycin) can inhibit certain proteins that disrupt cell growth, potentially inducing regeneration (J. Huang et al., 2023). Experimental drugs like ENPP1 inhibitors are created to prevent inflammation and scarring, which may lead to direct repair. Using hydrogels, which are essentially biocompatible materials that can deliver therapeutic agents (Musuc et al., 2024). Similarly, scaffolds can also deliver cells, growth factors, or other electrical signals directly to the injured area to reinforce functional repair. Medications, including statins, beta blockers, and angiotensin-converting enzyme inhibitors (ACE-I), may lower the chances of a second heart attack or heart failure in the near future (T. Huang et al., 2024). Despite the clinical utility of these interventions, they do not directly restore cardiac tissue or regenerate dead cardiomyocytes, and there are still a significant number of limitations regarding these therapies. These therapies have a very hard time regenerating cardiomyocytes and replacing the scar tissue. First, the drugs mentioned, such as statins, beta blockers, and ACE inhibitors, act on the heart's environment rather than on specific

cells themselves (Eliwa et al., 2025). These drugs work to reduce stress, inflammation, and other harmful signals, but they do not trigger the formation of new cardiomyocytes. The adult human heart has a limited ability to actually create new muscles, and so even the best of drugs cannot induce de novo cardiomyocyte proliferation. Scar tissue is structurally very stiff and non-contractile, so the heart lacks the ability to pump fully and effectively. Due to this, bioengineered tissues and hydrogels may help support the heart tissue, but they can rarely replace the scar tissue that has already been formed (Razavi et al., 2024). The myocardium is a highly specialized tissue with regenerative plasticity; thus, most treatments focus on ensuring that no further damage takes place, rather than restoring myocardial integrity. The majority of therapeutics given to an individual after a myocardial infarction focus on preventing a second heart attack, lowering stress on the heart, and improving circulation. Very rarely do any of these treatment options actually consider undoing the damage directly. The need for something more complex is crucial to ensure maximum regeneration.

This may be achieved through pluripotent stem cells–derived cardiomyocytes (PSC-CMs). These stem cells provide a strong alternative since these special cells can self-renew and differentiate into other types of cells in the body. If differentiated correctly, with the help of genome editing, they can prove to be very effective in regenerating damaged tissues. Induced pluripotent stem cells (iPSCs) are generated through the reprogramming of somatic cells into a pluripotent state, while human embryonic stem cells (hESCs) are derived from early-stage embryos (Taninokuchi Tomassoni et al., 2024). Donor somatic cells are crucial, as they are introduced to programming factors such as Yamanaka factors (OCT4, SOX2, KLF4, c-MYC) (Kirkeby et al., 2025; Aguirre et al., 2023). However, hESC derivation is ethically controversial globally due to the limited supply of human embryo donors (Peng et al., 2020). hESC-derived cells display more mature characteristics, whereas iPSCs offer greater accessibility for patient-specific disease modeling. Consequently, iPSCs are more favorable for clinical and ethical usage, despite their comparatively reduced maturation (Attwood & Edel, 2019). Stem cell-derived cardiomyocytes can be introduced through injection (surgeries and catheters), scaffolds/bioengineered patches, or through the bloodstream (intracoronary, intravenous) (Silver et al., 2021). Direct injection achieves higher engraftment efficiency than vascular delivery (M. Guo et al., 2025). Preclinical transplantation studies show that PSC-CMs can engraft within the infarcted myocardium. Following engraftment, PSC-CMs have been shown to electrically couple with host cardiomyocytes and contribute to partial restoration of contractile function (Hong et al., 2023). These findings suggest that PSC-CMs can integrate into damaged cardiac tissue and aid in cardiac contraction. Certain factors limit the maturation and differentiation of these stem cells, which can include calcium handling pathways, electrochemical signals, and the fetal characteristics of these stem cell-derived cardiomyocytes (Macarrón Palacios et al., 2024).

Genome editing has emerged as a strategy to overcome these challenges. Genome editing technologies, particularly CRISPR-based tools, allow for precise and durable modulation of genes (Pandey et al., 2025; Caudal et al., 2024). Researchers rely on three systems: Cas9 nuclease, base editors, and prime editors (Valenti et al., 2019; Park et al., 2022). To precisely insert or correct genes, this system relies on Homology-Directed Repair (HDR). However, because mature cardiomyocytes quickly become post-mitotic and stop dividing, this repair pathway shuts down, and Cas9 efficiency drops drastically. To solve this limitation, base editors change single nucleotides without cutting both DNA strands. (Doudna & Charpentier, 2014). Gene editing, using CRISPR, can directly alter cell-intrinsic regulatory networks and produce stable phenotypic changes that remain mostly intact after transplantation (Butterfield et al., 2025). Targeted genome edits have been explored in pathways regulating calcium handling. Additional genome editing methods focus on electrophysiological stability through changes in ion channel expression, isoform switching, and action potential shaping (Karakasis et al., 2025). Genome editing strategies

have also targeted metabolic maturation pathways, including mitochondrial biogenesis, oxidative phosphorylation, and substrate utilization (Pacesa et al., 2024).

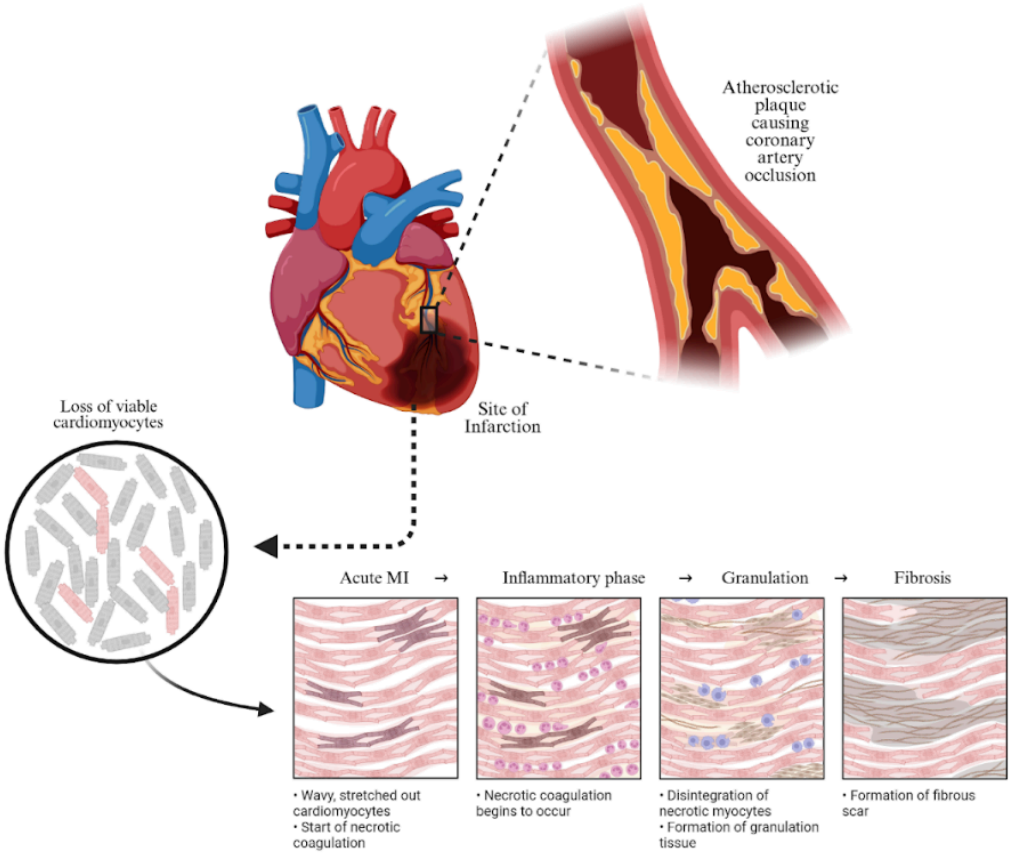

Figure 1: Pathophysiological progression following myocardial infarction. Information compiled from (Scalise et al., 2021). Created with BioRender.

Note: A Myocardial Infarction leads to coronary artery occlusion and reduced blood flow, which results in an ischemic injury to cardiomyocytes within the infarct zone. Acute cardiomyocyte necrosis triggers an inflammatory response characterized by immune cell infiltration. Over time, this process results in the formation of a fibrotic scar, causing irreversible loss of contractile tissue and impaired heart function.

Despite the progress in genome editing approaches, studies remain fragmented. The lack of standard protocols for stem cell differentiation and editing means that results are difficult to replicate across laboratories. While CRISPR was discovered in 2012, its application in human regenerative cardiology is still in its infancy (Liu & Olson, 2022). Previous studies focused on singular isolated gene targets rather than integrated maturation phenotypes (Seeger & Hoffmann, 2025). iPSC- and hESC-derived cardiomyocytes are often evaluated separately; thus, a direct comparison between these cell sources is

needed. Additionally, functional outcomes vary widely across in vitro, in vivo, and computational models, which makes it difficult to establish consistent conclusions (J. Wu et al., 2024). Oftentimes, studies have been done on cardiomyocytes, without fully implementing them back into the damaged heart through methods such as intravenous or intracoronary infusion. Therefore, the objective of this review is to synthesize current evidence on genome editing strategies that are designed to enhance the functional maturation of iPSC and hESC-derived cardiomyocytes in a post-myocardial infarction environment, with an emphasis on in vivo applications.

2. Methods

To ensure a proper analysis of current advancements in regenerative cardiology, a systematic literature search was conducted using databases such as PubMed and Google Scholar. Certain keywords used to find the sources in the databases include CRISPR-Cas9, genome editing for PSC-CMs, myocardial infarction, and stem cell-derived cardiomyocytes. The search focused on peer-reviewed articles from reputable journals such as Nature Communications, Nature Reviews, and Cell. There was no particular inclusion/exclusion criterion except for checking for relevancy using keywords and date of publication. Typically, sources were chosen if they were published between 2016 and 2026. However, select sources from more than 10 years back were included because there were no recent studies done on those specific topics, or because they were necessary foundational sources, such as the seminal work from Jennifer Doudna regarding the fundamentals of CRISPR. Sources were evaluated based on their relevance to CRISPR-Cas9 applications, stem cell-derived cardiomyocytes, and post-infarct functional maturation. Afterwards, Zotero was used for citations to ensure bibliographical accuracy. Finally, BioRender was used to create all the figures in this paper.

3. Analysis

3.1. The Generation of PSC-CMs

In the past 10 years, the progress made on using genome editing on stem cells to improve the functionality of the myocardium following an injury has been limited, with few definitive claims. A primary limitation in evaluating this process is that the standard differentiation protocols frequently result in structurally immature cells.

Pluripotent stem cells can be generated from embryos (hESCs) or reprogrammed somatic cells (iPSCs). While they can differentiate, each form has its drawbacks and limitations regarding practical considerations. Ultimately, their functional assessment depends on multiple factors, such as metabolic, electrophysiological, and morphological maturation (Eliwa et al., 2025). The general process of differentiation begins with untouched PSCs differentiating into cardiac progenitors, which later become cardiomyocytes. In the evaluated literature, researchers look closely at Wnt signaling pathways, which are protein networks that regulate key cellular processes. Such processes include cell fate, polarity, and organ formation. After binding to certain receptors, Wnt proteins initiate signaling that guides stem cell self-renewal and tissue regeneration. Figure 2 thoroughly depicts the starting Wnt pathway activation, detailing how the process allows for mesoderm formation. Later, Wnt inhibition promotes cardiac specification (Nicholson et al., 2022). BMP and Activin A refine mesoderm differentiation, while FGF works on the expansion and survival of developing cardiac progenitors (Magro-Lopez et al., 2024). Chemicals can mimic these pathways to produce cardiomyocytes without expensive proteins.

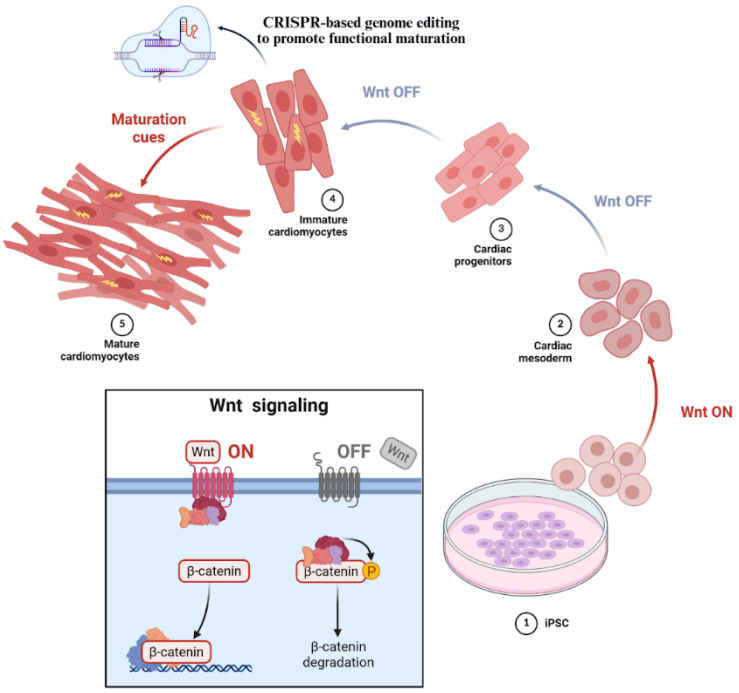

Figure 2: Differentiation of pluripotent stem cells into cardiomyocytes through stage-specific signaling, information compiled from Magro-Lopez et al., 2024. Created with BioRender.

Note: Early Wnt activation induces mesoderm formation, then it is followed by Wnt inhibition to promote cardiac progenitor specification. Cardiac progenitors further differentiate into immature cardiomyocytes exhibiting a fetal-like phenotype. Post-differentiation maturation cues, including metabolic, mechanical, and electrophysiological signals, drive progression toward an adult-like cardiomyocyte state. Genome editing strategies, such as CRISPR-Cas9–mediated modifications, can be applied at the immature cardiomyocyte stage to enhance functional maturation.

From an experimental standpoint, researchers have attempted to optimize these protocols by substituting recombinant proteins with small-molecule modulators. However, a recurring conclusion in the literature is that while chemical manipulation improves financial scalability, it often fails to produce fully mature functional properties. Comparative trials done (Aykul et al., 2020) demonstrate that small-molecule substitution often compromises long-term phenotypic fidelity. Consequently, evaluating these differentiation protocols requires rigorous functional validation; researchers commonly deploy a combination of electrophysiological recordings, high-resolution calcium imaging, and quantitative observation of spontaneous contractions to verify whether the resulting PSC-CMs possess true cardiomyocyte-like properties (López-Muneta et al., 2022).

Nonetheless, a major methodological limitation identified across these studies is that the standard 2D cultures fail to replicate the complex cardiac extracellular matrix; consequently, recent research has pivoted toward 3D clusters and organoids to better mimic natural tissue and force structural maturation.

Beyond differentiation efficiency, the optimal route for clinical translation is still heavily debated. When examining the experimental outcomes of these delivery routes, quantitative cell-tracking data demonstrate a severe physiological trade-off: while intravenous delivery results in catastrophic cell loss due to pulmonary and hepatic entrapment, direct intramyocardial injection yields significantly higher local retention rates, making it the mathematically superior route for clinical trials despite its invasiveness (Lian et al., 2012; Vekstein et al., 2022).

When examining the experimental outcomes of these delivery routes, cell-tracking data demonstrates a severe physiological trade-off: while intravenous delivery results in high cell loss due to pulmonary and hepatic entrapment, direct intramyocardial injection yields significantly higher local retention rates, making it the preferred route for clinical trials despite its invasiveness.

To overcome these barriers, recent investigations have shifted focus from liquid cell suspensions towards more biomaterial-based delivery systems. Consequently, current bioengineering research has pivoted to other methods of delivery, which may include using scaffolds, cardiac patches, and hydrogels, even if they may be less potent (Kobayashi et al., 2023). Cardiac engineering can also be done by directly injecting the stem cells into artificial tissue before being used on the heart (Z. Liu et al., 2024).

3.2. Immaturity of stem cell-derived cardiomyocytes

The functional quality of PSC-derived cardiomyocytes is evaluated by comparing their structural, electrophysiological, calcium-handling, and metabolic properties to mature adult ventricular cardiomyocytes. To achieve the ultimate goal of using targeted genome edits to enhance therapeutic potential, it is first critical to analyze the exact baseline deficiencies that these edits must correct. A rigorous cross-study synthesis confirms a major translational bottleneck: baseline unedited PSC-CMs remain developmentally arrested at a fetal or neonatal stage (Aigha & Raynaud, 2016). This creates a profound functional mismatch when evaluating these cells against adult parameters. This immaturity is reflected through multiple levels of cellular organization, including gene expression, morphology, and functional behavior (Sun & Nunes, 2017).

In contrast, adult ventricular cardiomyocytes are functionally and structurally specialized to support the heart's diverse needs. They help synchronize electrical conduction, promote efficient calcium cycling, and support sustained force generation (Giacomelli et al., 2017; X. Li et al., 2019). Consequently, when fetal-like PSC-CMs are introduced to a damaged, hostile environment post-myocardial infarction (MI), a severe functional mismatch occurs. They simply cannot meet the heart's rigid mechanical, electrical, and metabolic demands, making the targeted correction of these baseline deficiencies the primary hurdle for regenerative medicine.

3.2.1. Structural and Morphological Deficiencies

One of the most noticeable manifestations of PSC-CM immaturity is their underdeveloped cellular morphology in comparison to adult ventricular heart cells. As depicted in Figure 3, PSC-CMs are typically smaller and rounder compared to a more elongated and larger developed cardiomyocyte. The length of these cells aids in supporting efficient contraction that is required for the minimum cardiac output.

Furthermore, morphological tissue-profiling studies by Shameem et al. (2025) confirm that the internal structure of these

cells shows significant maturity gaps:

1. Sarcomeric Alignment: PSC-CMs display poorly organized sarcomeres with reduced alignment, in contrast to the highly ordered sarcomeric framework that is observed in mature cardiomyocytes (Mohammadzadeh & Lejeune, 2025). PSC-CMs have a poor Z-line alignment, which disrupts efficient force transmission during contraction (Figure 3) (Shameem et al., 2025).

2. T-Tubule Network: A T-tubule network is a crucial structural feature that is essential for rapid and uniform excitation–contraction coupling that occurs in adult cardiomyocytes (Setterberg et al., 2021). These deficiencies limit the ability of PSC-CMs to generate synchronized and forceful contractions.

3. Protein Isoforms: This structural immaturity is accompanied by an imbalance between MYH6 and MYH7, which reflects an early developmental phenotype rather than an adult one (Anfinson et al., 2022). Consequently, this structural phenotype identifies the transcriptional regulation of the MYH6/MYH7 loci as a high-priority target for genome editing to force structural elongation.

3.2.2. Calcium-Handling Mechanisms

Beyond structural abnormalities, iPSC- and hESC-derived cardiomyocytes show profound immaturity in calcium-handling mechanisms, which prevents coordinated contraction. PSC-CMs have an underdeveloped sarcoplasmic reticulum and reduced SERCA2a pump activity (Gu et al., 2021; Kho, 2023). While adult cells rely on SERCA2a to quickly transport around 70% of calcium back into the SR for the next heartbeat, immature PSC-CMs rely on a slower, less efficient NCX exchanger to push all of the calcium completely out of the cell.

Consequently, these cells rely heavily on sarcolemmal calcium influx rather than rapid calcium release and reuptake from internal stores, which results in prolonged calcium transients and slower relaxation kinetics (C. Bompotis et al., 2016). Optical mapping of calcium-binding dyes by Koivumäki et al. (2018) quantified this deficit, demonstrating that the time values regarding the duration of these transients are around 100-200+ ms. Their decay times are approximately 400–800+ ms. This contrasts with adult values of 40–60 ms and 100–300 ms, respectively. (Koivumäki et al., 2018). This prolonged handling increases susceptibility to intracellular calcium overload, compromising contractile stability and cell viability under stress. To bridge this physiological gap, therapeutic genetic strategies must focus on upregulating SERCA2a expression or modulating its key inhibitor, phospholamban (PLN), to optimize internal calcium recycling.

3.2.3. Electrophysiological Immaturity and Arrhythmogenic Risk

Electrophysiological immaturity further distinguishes PSC-derived cardiomyocytes from adult ventricular cardiomyocytes and poses significant challenges for cardiac integration (R. E. Ahmed et al., 2020). PSC-CMs typically exhibit a more depolarized resting membrane potential and prolonged action potential duration compared to mature cardiomyocytes (Y. Guo & Pu, 2020). In PSC-CMs, KCNJ2 expression is significantly reduced, which encodes the Kir2.1 subunit of the inward rectifier potassium channel (Kadota et al., 2020).

In PSC-CMs, the upstroke velocity range (the speed at which the cell depolarizes during an action potential) is ≈50 V/s, whereas in adult CMs it is stable at ≈250 V/s. This approximate 200 V/s difference in upstroke velocity impairs electrical

signal propagation, making it impossible for transplanted cells to sync smoothly with the host tissue. The persistence of pacemaker channel expression contributes to spontaneous automaticity, a feature that is largely absent in adult ventricular myocytes. In addition, immature gap junction organization can result in weak electrical coupling between transplanted PSC-CMs and the host myocardium (Sottas et al., 2018). These distinct electrophysiological deficits provide a clear roadmap for genome editing: knocking in or overexpressing KCNJ2 while silencing pacemaker genes represents a mandatory step toward achieving electrical safety.

3.2.4. Metabolic Profile and Environmental Adaptation

Finally, PSC-CMs exhibit a metabolic profile that reflects early developmental stages rather than adult cardiac metabolism. PSC-CMs predominantly rely on glycolysis for energy production, whereas adult cardiomyocytes depend largely on fatty acid oxidation and mitochondrial oxidative phosphorylation (Garbern & Lee, 2021). This metabolic immaturity is accompanied by low mitochondrial density and poorly developed cristae organization, limiting ATP-generating capacity (Leveille et al., 2017).

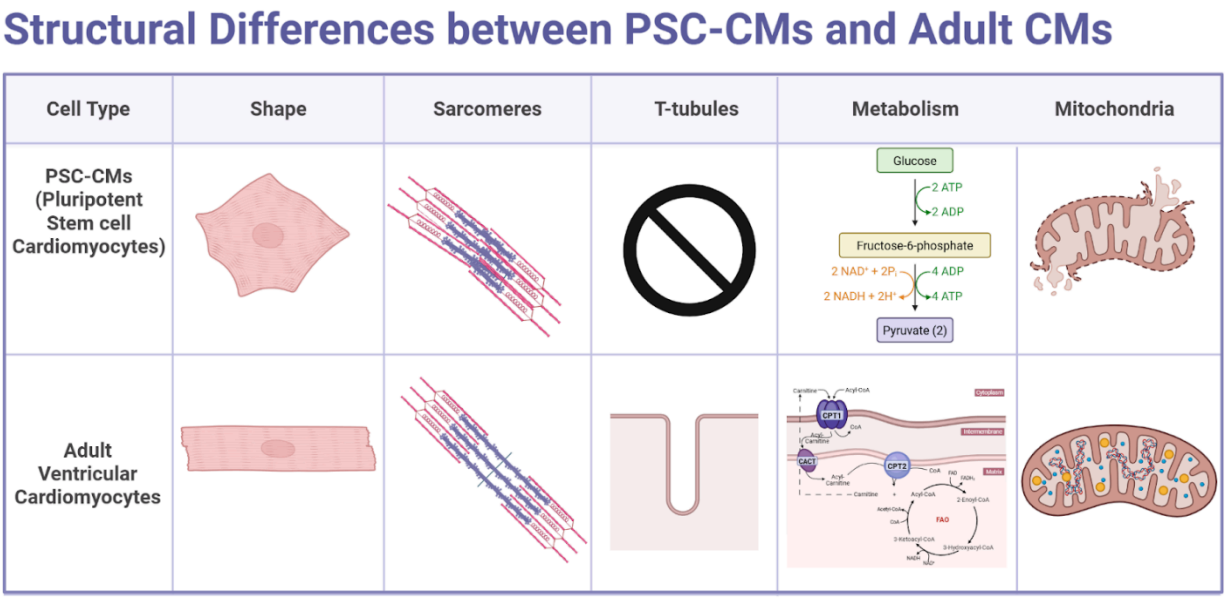

Figure 3: Structural Differences between PSC-CMs and Adult CMs. Information compiled from Moriwaki et al., 2025. Created with BioRender.

Note: Comparative structure analysis between immature stem cell-derived cardiomyocytes and fully developed adult heart cells, including factors like cell shape, sarcomere structure, metabolism, etc.

As a result, PSC-CMs demonstrate reduced oxidative capacity and limited ability to meet the high energetic demands of sustained cardiac contraction. This metabolic profile also impairs adaptation to ischemic stress, a critical limitation in the infarcted myocardium (Vučković et al., 2022). Following myocardial infarction, transplanted PSC-derived cardiomyocytes

encounter a hostile environment characterized by hypoxia, inflammation, and mechanical stress (Ibrahim et al., 2025). Many transplanted cells exhibit poor survival, inadequate electrical coupling, and insufficient mechanical contribution to the host myocardium. Ultimately, these multi-systemic limitations highlight that successful cardiac regeneration requires moving beyond mere cell survival; genome editing strategies must comprehensively reprogram metabolic, electrophysiological, and structural pathways to force advanced functional maturation before clinical translation is viable.

3.3. Key Genome Editing Targets

The combined structural, calcium-handling, electrophysiological, and metabolic immaturity of PSC-CMs limits their ability to survive and function under the hostile environments of an infarcted heart (Feric & Radisic, 2016). Recent studies have evaluated different gene targets and editing strategies to enhance the functional integration of PSC-CMs (Neofytou et al., 2020). Other than genome editing, there are various techniques available to improve maturation, such as 3D tissue engineering, nanotechnology/nanobodies, and electrical pacing (Oikonomopoulos et al., 2018). Options such as pharmacological modulation, protein stabilization, or pathway-level interventions can also be used in a lab or clinical setting (C. A. Wu et al., 2023). However, if you remove the cell from the scaffold or stop the electrical pulse, the cell often regresses to its immature form or loses its specialized function over time. This is why genome editing can be considered beneficial since it directly alters the biological blueprint of the stem cell itself. Sometimes using other methods leads to toxicity and unintended side effects. Genome editing allows for surgical precision, so the risk, while still present, is lower than that of other conventional approaches (Akbarian & Chen, 2022). Because these limitations are molecular and gene-regulated, editing specific genes can shift PSC-CMs to adult-like behavior. Such methods involve CRISPR-Cas9 and other advanced genome editing techniques such as base editing or prime editing (Uddin et al., 2020).

Regarding their electrophysiological makeup, PSC-CMs have depolarized resting potentials, meaning they are more excited and unstable compared to adult heart cells (H. Zhou et al., 2021). Mature cardiomyocytes have negative resting potentials that enable them to respond quickly and predictably to a range of signals. Adult cells have specific spike patterns, which trigger a heartbeat (Goversen et al., 2018). PSC-CMs have longer and irregular spikes, which can cause uncoordinated contractions and arrhythmias. Genes that are crucial to target include the *KCNJ2* gene (Table 1). This gene is primarily responsible for making a protein named Kir2.1, which helps set and adjust the resting potential. If you increase *KCNJ2* expression in PSC-CMs, the cells' resting potential becomes more negative (like adult cells). PSC-CMs behave electrically more like mature heart cells, reducing arrhythmia risk (Karbassi et al., 2020). Other genes that work to help with the electrical signals of the stem cells include *HCN4*, *CACNA1H*, *and SLC8A1*, which are involved in automaticity. These aid in the tendency of the cells to beat on their own.

Editing these genes with CRISPR-Cas9 cuts can reduce spontaneous beatings, helping the transplanted cells sync with the heart. Pluripotent stem cell-derived cardiomyocytes are immature in their ability to handle calcium, which is necessary for proper heart contraction post-MI (Marchiano et al., 2023). In functioning adult heart cells, calcium travels in and out of the cell and the sarcoplasmic reticulum (SR) quite efficiently. This enables strong and coordinated contractions. However, stem cells have slow and irregular calcium transients, leading to weakly timed contractions. Genes that work to address include the *RYR2*. This is the ryanodine receptor that works to release calcium from the SR (Ernst et al., 2023). Mutations in this gene can cause irregular calcium waves and can potentially trigger arrhythmias. *SERCA2a* pumps calcium back into the SR after there is a contraction. If it's underactive, relaxation is slow, and calcium builds up inside the cell. Additionally, *PLN* (phospholamban) works to regulate *SERCA2a* activity. *CASQ2* helps to store calcium inside the SR, aiding with buffer levels (Table 1) (Steinberg et al., 2023).

Another drawback to these stem cell-derived cardiomyocytes is the lack of a stable structure they possess (Joshi et al., 2024). Some of which include poorly organized sarcomeres, in contrast to the highly organized structure that fully matured cells have. PSC-CM struggles with generating a strong force throughout the heart. Key genes that work with this aspect include *MYH6 / MYH7.* These are isoforms of myosin heavy chain proteins. Adult cells use more of the *MYH7* gene to ensure efficient contraction. To address the disorganized sarcomeres, *ACTN2*, alpha-actinin, helps to form Z-lines, anchoring the sarcomeres. Certain transcription factors (e.g., *TBX5, GATA4*) guide sarcomere assembly and organization (Lornage et al., 2019). PSC-CMs rely on glycolysis for energy, like fetal cells, but adult cardiomyocytes rely on fatty acid oxidation and ATP production (Abu Shelbayeh et al., 2023). This difference limits their ability to sustain prolonged contraction after transplantation. The main regulator of mitochondrial biogenesis and oxidative metabolism is the *PPARGC1a* (PGC-1α) gene (Idrees et al., 2025). The gene *TFAM* supports mitochondrial DNA function and replication.

Arguably, one of the most important factors in ensuring stem cells' ability to regenerate damaged tissue is their ability to survive in a toxic environment (Cipriano et al., 2025). After we inject the stem cells into the damaged heart tissue, the PSC-cardiomyocytes often die because of hypoxia. Due to the post-infarct environment, oxygen levels are significantly lower. There are also signs of inflammation and mechanical stress. Potential genes to target include the Anti-apoptotic regulators (including the BCL2 family). Their primary function is to help prevent programmed cell death (Vogler et al., 2025). After a myocardial infarction, stress response regulators can also be used to improve resistance to oxidative stress. Paracrine support factors (e.g., CRYAB) offer tissue support, including angiogenesis (Zhang et al., 2019). The need for a combination of editing strategies is crucial. A single gene edit usually isn't enough because PSC-CMs have multiple immaturities (electrical, structural, calcium, metabolic) (McCarty et al., 2020). Timing and combination of edits are critical. Regarding temporal edits, during the early progenitor stage, editing ion channels to stabilize the electrophysiology is most important.

However, later on, during the differentiation stage, it's best to edit the metabolic and structural genes when the networks are still developing. Combinatorial editing can help incorporate multiple genes. Numerous genes could be targeted, such as reducing spontaneous beatings, boosting calcium handling capability, and increasing IK1 for stable resting potential (Y. Li et al., 2021). Overall, there are 5 major gene target categories: electrophysiology, calcium, structure, metabolism, and survival. Stage-specific and combinatorial editing is crucial to ensure proper maturation and improved PSC-CM performance for cardiac regeneration. Next, understanding how CRISPR-based genome-editing strategies, including specific tools, delivery methods, and stage-dependent implementations, can maximize benefits and modify these targets (Azeez et al., 2024).

3.4. CRISPR-based genome editing

Using the CRISPR components in the PSCs efficiently and safely without causing unwanted damage or toxicity can pose a challenge (Nishiga et al., 2022). Viral deliveries (e.g., lentivirus, AAV) are highly efficient, but may increase risk for genetic mutations (Moldavskii et al., 2025). There are non-viral deliveries such as electroporation, lipid nanoparticles, and RNP complexes. These are much safer with a lower risk of insertional mutagenesis. Additionally, timing considerations should be factored in since different stages of PSC-CM development require different edits to maximize their potency. In the early progenitor stage, electrophysiology or calcium-handling edits are good, and for the late differentiation stage, structural/metabolic edits are needed (De Haan et al., 2021). The goal is to use certain edits while minimizing off-target gene mutations and cytotoxicity.

To achieve this safety goal, implementing high-fidelity Cas9 variants, base editors, or prime editors is particularly valuable

for cardiomyocytes. Because differentiated cardiomyocytes are post-mitotic cells, they lack robust Homology-Directed Repair (HDR) pathways, making traditional double-strand breaks dangerous (Doetschman & Georgieva, 2017). Our analysis highlights that base and prime editing are crucial here because they allow for precise single-nucleotide or structural corrections without creating double-strand breaks, drastically reducing indels, p53 toxicity, and apoptosis in donor cells.

Based on different functional targets, there are different editing strategies that should be implemented.

3.4.1. Overcoming Arrhythmogenic Risks (Electrophysiology)

Regarding electrophysiology, targeting ion channels (e.g., *KCNJ2 and* SCN5A) can reduce automaticity or stabilize resting potential (Kantor et al., 2026). However, it is important to take into account that while some studies show that forcing the *KCNJ2* expression that stops arrhythmias, counter-studies argue that overexpressing this gene can artificially shorten the Action Potential Duration (APD) too much. A recent study on the overexpression of the *KCNJ2* gene on iPSC-CMs via lentiviral transduction drew conclusions that excessive IK1 current density can excessively shorten the action potential duration, creating a substrate for arrhythmias (Zhou et al., 2023).

To build off these conflicting studies, we propose that instead of using strong, unregulated constitutive promoters to force *KCNJ2*, researchers should tether *KCNJ2* expression to highly tunable, cardiac-specific synthetic promoters. This represents our original strategy to optimize IK1 density to safe, adult-like thresholds without triggering the extreme APD shortening found in previous failed viral experiments.

3.4.2. Correcting Excitation-Contraction Decoupling (Calcium Handling)

To work with calcium handling, upregulating the *RYR2*, *SERCA2a*, *CASQ2*, or downregulating inhibitory factors like PLN are critical (J. Zhou et al., 2023). Edits can be done in later stages for proper calcium cycling networks. Additionally, knocking out or editing PLN to release SERCA2A is known to improve recycling and contraction; however, some literature reviews and research note that completely deleting the PLN can cause a long-term calcium overload in the mitochondria. This excessive calcium can lead to quickened apoptosis, especially when exposed to the stressful conditions of a real heart attack.

To resolve this issue, we propose avoiding a complete knockout of PLN. Instead, our strategy utilizes base or prime editing to introduce a localized missense mutation at the exact *PLN-SERCA2a* binding interface. This allows us to build upon past work by partially releasing *SERCA2a* inhibition to boost contraction, while avoiding the catastrophic mitochondrial calcium overload and cell death highlighted in previous literature reviews.

3.4.3. Resolving Structural, Metabolic, and Survival Deficiencies

Studies show that modifying the *MYH6/MYH7* ratios and enhancing *ACTN2* or transcription factor activity leads to improved structure of the stem cells (Htet et al., 2024). This is usually done after the cardiac commitment, post-formation of sarcomeres. We note that keeping this strict post-commitment timeline is necessary so that early cytoskeletal stiffness does not interfere with early cell expansion.

For metabolic maturation, it's best to activate *PGC-1α*, *TFAM*, or other metabolic regulators (Yao et al., 2016). This would result in the best outcomes if applied later on in the differentiation, when the mitochondria of the cardiomyocytes are still

developing (Q. Zhou et al., 2021). We hypothesize that editing during this specific development window allows the overexpression to work synergistically with endogenous mitochondrial maturation.

To ensure the stem cell's survival and engraftment, edits on the anti-apoptotic (BCL2) or stress-response genes have to be done (Ardehali et al., 2011). This can be applied either before transplantation or during late differentiation to prepare cells for harsh environments. We advocate for a targeted induction of BCL2 right before cell delivery to provide a temporary "molecular shield" against host ischemic pathways.

Single edits usually do not fix all PSC-CM deficiencies; cells need multiple edits across pathways. Using multiplex CRISPR addresses this issue, as it incorporates multiple sgRNAs to edit several genes at once. There are also potential combinatorial approaches, such as reducing automaticity, improving calcium cycling, and enhancing sarcomere organization. Combinatorial editing ensures that PSC-CMs are electrically, structurally, metabolically, and functionally mature (W. Li et al., 2025). Stage-specific edits reduce interference between pathways.

Table 1: Multiplex CRISPR Framework for Viable Myocardial Infarction (MI) Therapy.

Target Clinical Pathway	Associated Genes	Recommended Tool	Targeted Functional Outcome	Our Analytical Strategy / Solution to Risks
Arrhythmogenic Risk	KCNJ2, SCN5A	Prime Editor / CRISPRa	Suppress automaticity; stabilize resting potential (~−80 mV).	Use cardiac-specific synthetic loops
Calcium Decoupling	PLN, SERCA2a	Base Editor	Accelerate calcium transient reuptake and recycling.	Avoid total knockouts. Use precise base edits at the binding interface
Structural Immaturity	MYH6/7, ACTN2	Prime Editor	Optimize sarcomere length (~2.0 μm) and isotropic alignment.	Restrict editing to post-cardiac commitment
Energetic Failure	PGC-1\alpha, TFAM	Base Editor / CRISPRa	Shift metabolism from glycolysis to mature fatty acid β-oxidation.	Apply during late-differentiation window
Graft Mortality	BCL2	CRISPRa	Deliver robust resistance to host tissue ischemia, hypoxia, and ROS bursts.	Deploy directly pre-transplantation as an acute survival shield

Note: The following table demonstrates the integrated blueprint for combining these strategies into a single viable treatment for Myocardial Infarction (MI), clarifying our contribution relative to literature limitations.

3.4.4. Screening and Functional Validation

Table 2: Genes targeted by CRISPR-Based editing in human PSC-derived cardiomyocytes, editing strategies, functional outcomes, and relevance to cell maturation.

Gene Target	Editing Strategy (CRISPR/Overexpression)	Functional Effect on PSC-CM Maturation	Outcome
KCNJ2 (Kir2.1)	CRISPR-mediated **overexpression** of KCNJ2	Adult heart cells are electrically quiet when they are at rest. In contrast, cardiomyocytes are too electrically excitable and unstable. KCNJ2 creates a potassium channel (Kir2.1) Enhances inward rectifier potassium current (I_{k1}), leading to a more negative resting membrane potential and a more adult-like action potential. These electrical changes support improved calcium handling, metabolic maturation, and structural organization in cardiomyocytes.	3–5× increase in I_{k1} density and ~10–15 mV hyperpolarization of resting membrane potential.
SERCA2a (ATP2A2)	CRISPR activation or gene delivery to upregulate SERCA2a	Enhances calcium reuptake into the sarcoplasmic reticulum, supporting more mature excitation–contraction coupling and calcium handling in cardiomyocytes.	2–3× increase in SERCA2a expression and significantly faster Ca^{2+} decay kinetics (≈30–40% reduction in Ca^{2+} transient duration).
RYR2 (Ryanodine receptor 2)	CRISPR/Cas9 **mutagenesis/correction**	Modulates sarcoplasmic reticulum calcium release, leading to more adult-like calcium transients and improved excitation–contraction coupling.	≈20–30% increase in Ca^{2+} transient amplitude and reduced spontaneous Ca^{2+} leak events.
TNNT2 (Cardiac troponin T)	CRISPR mutagenesis using changed mRNA	Demonstrates the importance of sarcomere organization in maintaining cardiomyocyte structural integrity and functional maturation.	results in disrupted sarcomere assembly and >40% reduction in contractile force generation.

Note: Categorization of various target genes regarding electrophysiology, calcium, structure, survival, and metabolic maturation alongside the functional goal. Information compiled from Zhou et al., 2021.

Implementing high-fidelity Cas9 variants or base/prime editors to reduce unwanted mutations. We can thoroughly screen edit PSCs using whole-genome sequencing, karyotyping, and pluripotency markers. Avoiding prolonged culture periods post-edit can reduce genetic drift (Chen & Liu, 2023). Functional validation is essential to confirm that genome edits result in meaningful improvements rather than solely genetic alterations (Haake & Steenpass, 2025).

1. Electrophysiological properties are assessed by measuring resting membrane potential, action potential duration, and ion channel activity to determine whether edited PSC-CMs exhibit adult-like electrical behavior.

2. Calcium handling is evaluated through analysis of sarcoplasmic reticulum calcium release and reuptake dynamics, transient amplitude, and beat rate regularity (Han & Entcheva, 2023).

3. Structural maturation is examined by measuring sarcomere length, alignment, and contractile force generation.

4. Metabolic function is assessed through mitochondrial activity, oxidative capacity, and ATP production, reflecting the energetic readiness of the cells.

5. Finally, survival and engraftment potential are evaluated by testing resistance to hypoxia, oxidative stress, and inflammatory conditions (Streiff & Sachse, 2022). Together, these validation approaches ensure that CRISPR-based edits translate into functional improvements relevant to cardiac repair.

While many CRISPR-Cas tools can assist with delivery methods, stage-specific and combinatorial strategies, safety measures, and functional validation, there are still translational challenges and limitations that are encountered when injecting the cells into a functioning human heart (Soma et al., 2024).

Table 3: Target Genes using CRISPR-Cas9.

Category	Target Genes	Functional Goal
Electrophysiology	KCNJ2, HCN4, CACNA1H	Stabilize RMP, reduce automaticity
Calcium	RYR2, SERCA2a, PLN, CASQ2	Improve SR cycling
Structure	MYH7, ACTN2, TBX5, GATA4	Enhance sarcomere organization
Metabolism	PPARGC1A, TFAM	Increase oxidative capacity
Survival	BCL2, CRYAB	Improve resistance to hypoxia

Note: Categorization of various target genes regarding electrophysiology, calcium, structure, survival, and metabolic maturation alongside the functional goal. Information compiled from Zhou et al., 2021.

4. Discussion

Certain genome edits using CRISPR-Cas9, regardless of what the target gene may be, carry risks of off-target mutations in the cell and can lead to instability. When using CRISPR-Cas9, the guide RNA can oftentimes bind to similar or identical sequences elsewhere in the genome. This risk increases when the target sequence is similar to other genomic regions and when the guide RNA is not as specific as it should be. In addition, these instabilities in prolonged in vitro PSC cultures have been associated with certain alterations that can lead to chromosomal abnormalities known as culture adaptation. Such abnormalities contribute to aneuploidy, CNVs (copy number variations), and certain point mutations in the DNA sequence. Studies have shown that risks regarding these alterations contribute to ongoing concerns regarding tumorigenicity (Naeem et al., 2020). If PSC-CMs are directly implemented into the infarct zone of the human heart without showing signs of proper maturation or completed differentiation, signs of teratoma formation can occur (Aussel et al., 2025). Transplanted PSC-derived cardiomyocytes can present arrhythmogenic risks due to the electrical mismatch between grafted cells and the host myocardium. These underdeveloped cells cannot match the electrical rhythm that the adult heart produces. On the contrary, strategies that target individual pathways may result in partial maturation, which can be insufficient to support the stable electrical behavior the heart requires to fully function (Yu et al., 2022). Effective cardiac repair requires coordinated maturation and electrophysiological integration to ensure synchronized excitation–contraction coupling. T-tubule development and ion channel localization must occur.

Genome-editing studies rely on in vitro models that do not fully replicate the complexity of the human heart. Differences in size, beating rate, and organization limit the ability to extrapolate findings from rodent models to the human myocardium. This limitation is especially relevant given that many in vitro studies are conducted in systems that do not accurately mimic post-myocardial infarction conditions (Jin et al., 2025). Model systems, therefore, have limited predictive power for clinical and long-term therapeutic outcomes, highlighting the need for improved translational models.

As CRISPR-Cas9 technology continues to evolve, future directions include deriving small parts within these stem cells, such as exosomes, and using them in combination with genome editing. In the future, moving towards more Base Editing or Prime Editing can be more precise as they allow for single-nucleotide changes without breaking the DNA strand, massively reducing the risk of cultural adaptations or teratomas (Matsoukas, 2020). Additionally, more in vivo experiments would significantly expand our knowledge and understanding of how accurate these genome edits are in organisms, rather than in controlled petri dishes in vitro. Using human cardiac organoids paired with high-throughput microelectrode arrays in vitro can be utilized before going into vivo. However, for potential work to be done in vivo, the field must move from rodents to porcine models as they share similar heart rates and anatomy of humans.

Overall, many translational challenges and limitations are associated with the implementation of stem cell-derived cardiomyocytes into the human heart, including issues related to gene editing, electrical behavior, abnormalities, and overall success rates (Khalil & McCain, 2021).

5. Conclusion

To conclude, myocardial infarction, as a result of CAD, remains one of the leading causes of heart failure around the world. The human heart cannot regenerate itself; thus, the damage can lead to irreversible death of cardiomyocytes in the vicinity of the infarction. While studies and clinical trials have been explored, implementing the use of drug therapies,

medications, and tissue engineering, very few offer promising solutions regarding full or even partial regeneration. Be that as it may, introducing stem cells (iPSCs and hESCs) can be a method for the restoration of cardiomyocytes.

Both human embryonic pluripotent stem cells (hESCs) and induced pluripotent stem cells (iPSCs) have the potency to differentiate and possibly contribute to cardiac regeneration. Nonetheless, they face certain issues regarding their maturation and how effective they will be if integrated into a damaged heart. However, using CRISPR-Cas9 to edit certain genes, it is feasible to overcome some of these functional limitations and induce maturation. The most potent genes to target, regarding the PSC-CMs electrophysiology, calcium handling capabilities, structural differences, and metabolic immaturity, involve adjusting the SERCA2a, KCNJ2, MYH6/MYH7, and PGC-1α, TFAM, respectively. Using a combinatorial edit and incorporating multiple sgRNAs would likely result in an improved outcome.

In the future, there is an arising need for improved maturation strategies, safe genome-editing protocols, and better preclinical models. If these challenges are overcome, the translation potential can be endless, including advanced treatment of cardiovascular disease and cardiac regeneration. Despite the challenges, the potency of genome edits using CRISPR-Cas9 can greatly affect the PSC-CMs in maturation, functionality, and survival in the infarct heart. With future studies regarding this topic to acknowledge and address ongoing hurdles, gene therapy and stem cell therapy hold unlimited possibilities in regenerative medicine.

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AI Tools Statement

Generative AI or other artificial intelligence was not used to create or generate any parts of this paper. AI, including tools like Grammarly or Google Gemini, was used to clean up punctuation mistakes, grammar issues, and make small improvements in sentences.

Author Biography

Rajanya Bishi is a rising junior at Farmington High School in Connecticut, driven by a deep fascination with regenerative cardiology. Her current research explores the intersections of stem cell therapies, genome-editing technologies, and cardiac tissue regeneration, reflecting her broader interest in translating advances in regenerative medicine into future cardiovascular therapies. Her research and academic writing have been recognized with a Top 10% national ranking in the Thermo Fisher Scientific Junior Innovators Challenge, the Young Scientist Award, the Connecticut Science and Engineering Fair (CSEF) Life Science Medallion, a placement in the John Locke Institute Global Essay Prize, and an Ohio Senatorial Citation.

Beyond research, Rajanya volunteers in the Cardiac Medical Rehabilitation Unit at the Hospital for Special Care and at UConn Health, experiences that have reinforced her commitment to patient-centered medicine and earned her the international Barbara James Service Award. She has also completed physician shadowing experiences in cardiology, pediatrics, and rheumatology.

Rajanya is a two-time NCWIT Aspirations in Computing Connecticut Affiliate Winner and a Connecticut Lieutenant Governor's Computing Challenge All-Stars honoree for developing technology-driven solutions with social impact. She serves as President of the CSEF Club and is an appointed member of the Future Problem Solving Program International Student Advisory Council, where she advises executive leadership on strategic initiatives. Outside of medicine and academics, Rajanya is an accomplished Bharatanatyam (classical Indian) dancer with over a decade of training, performing at regional and national cultural showcases.

Mentor Contribution Statement

This manuscript was completed under the mentorship of **Dr. Hamidreza Shaye** (Post-Doctoral at Stanford University) and **Dr. Lauren Tetz** (Post-Doctoral at Vanderbilt University). Their assistance was limited to technical and structural guidance. They provided instructions on how to use research software such as Zotero, for citation management, and

BioRender, for the creation of figures. Neither mentor contributed to writing this paper, nor did they generate any of the core arguments or analysis presented. All content and final writing are the original work of the author.