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 induced 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.

1 Introduction

A myocardial infarction is a medical condition where blood flow to the heart is cut off, that is 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 eventually 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). The main issue 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 (MPT) 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). As good as they may seem, these don't indirectly restore cardiac tissue or regenerate dead cardiomyocytes, and there are still so many limitations regarding these therapies. These therapies have a very hard time regenerating cardiomyocytes and replacing the scar tissue. First, these drugs that were mentioned, such as statins, beta blockers, and ACE inhibitors, work on the heart's environment and not the specific cells themselves. 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 make new cells appear if the old ones are damaged or dead. 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's already been formed. The heart is a very sensitive and complex muscle, and so most treatments focus on ensuring that no further damage takes place, rather than undoing the damage that has been done. Majority 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. 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). 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 pluripotent stem cells–derived cardiomyocytes (PSC-CMs) can engraft within the infarcted myocardium. Right after 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). Gene editing 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).

Despite the progress in genome editing approaches, studies remain fragmented. 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, more so in vivo.

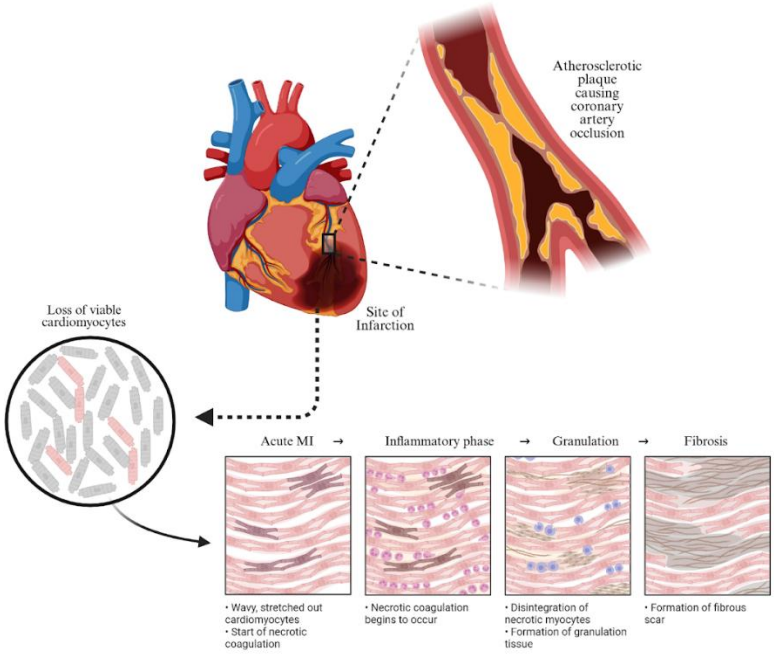

Figure 1. Pathophysiological progression following myocardial infarction. Information compiled from (Scalise et al., 2021). Created with BioRender. A Myocardial Infarction leads to coronary artery occlusion and reduced blood flow, which results in an ischemic injury to cardiomyocytes within the infarct 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.

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. The search focused on peer-reviewed articles from reputable journals, published between 2016 and 2026. Some sources were papers from more than 10 years back, as there were no recent studies done on it. 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

Myocardial infarction results in the irreversible loss of functioning and working heart cells, cardiomyocytes, which creates the need for regenerative strategies to help restore these contractile tissues (Tzahor & Poss, 2017). A myocardial infarction can have multiple causes, some include atherosclerosis, plaque rupture, coronary artery spasm, thrombosis, oxygen supply and demand imbalance, or even sometimes microvascular dysfunction. The most common cause of a heart attack is when fatty deposits build up in the coronary arteries, and this can prevent blood from reaching major arteries and result in a blood block. Plaques can rupture, exposing the inner contents to the blood. Coronary Artery Spasm is a sudden constriction of the coronary artery, automatically reducing blood flow and cardiac output. This can be caused by factors such as smoking, stress, and drugs like cocaine, or even endothelial dysfunction. Coronary Artery Spasm can be classified into temporary or prolonged ischemia, and when prolonged, it is classified as a MI (Matta et al., 2020). The heart needs oxygen to pump effectively. Certain prior medical conditions, like severe anemia, hypotension, tachycardia, or respiratory failure, can reduce organ delivery. And so if the demand for blood and oxygen exceeds the supply, then the cells begin to die, even without a blocked artery. Lastly, the microvascular dysfunction is when smaller coronary vessels fail to deliver enough blood, instead of a main artery getting blocked. Cardiomyocytes don't divide frequently, so the heart struggles to replace a large number of damaged cells post-injury. Taking tissue from other organs to transplant into the heart is not a standard procedure due to incompatibility. These tissues don't function like heart cells and can not contract in the coordinated way

the heart does. Heart transplants are limited by donor availability, and there is always a risk of rejection. Those with donor hearts require lifelong immunosuppressive therapy. This solution is also not scalable for millions of individuals who suffer from heart disease. However, certain approaches can be made to ensure that the heart remains as stable and fully functional as possible. Among these approaches, pluripotent stem cells–derived cardiomyocytes (PSC-CMs) offer a renewable source of human cardiac cells with high potential for myocardial repair.

Stem cells include two major types (human embryonic stem cells and induced pluripotent stem cells). One of the most common types of stem cells is human embryonic stem cells (hESCs) (Vazin & Freed, 2010). These are derived from early-stage embryos (blastocysts). Isolation of the inner cell mass (ICM) is critical, as it helps form these pluripotent hESCs. The hESCs are then grown in the lab using feeder layers and feeder-free systems. Feeder layers can provide support by secreting certain factors that maintain pluripotency and prevent differentiation. Feeder-free systems, on the other hand, use extracellular matrices to replicate environments without the need for animal cells. This makes the clinical studies much easier and accessible. To ensure that the cells can differentiate into other types of cells, markers such as OCT4, NANOG, and SOX2 are used to check for cell pluripotency (Swain et al., 2020). These are transcription factors, with OCT4 being the most important as it contributes to self-renewal and guides differentiation into specific lineages. SOX2/SOX3 helps to keep stem cells from prematurely going into mesendoderm, balancing OCT4's effects. hESCs are self-renewing and can differentiate into almost any kind of cell. However, hESC derivation is ethically controversial in some parts of the globe due to the limited supply of human embryo donors (Peng et al., 2020). This is one of the main reasons people turn to iPSCs. Induced pluripotent stem cells (iPSCs) are another type of stem cell that is lab-created based on somatic cells, usually reprogrammed to become any cell (Shown in Figure 2) (Cerneckis 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). Similar to hESCs, their pluripotency is verified through gene and protein markers, testing their ability to differentiate into a range of other somatic cells. Compared to hESCs, iPSCs reduce the likelihood of immune rejection significantly. Regarding the genetic aspect, there can be higher risks of genetic mutations when reprogramming occurs. Comparatively, ESC-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).

The general process of differentiation starts with the untouched PSCs turning into cardiac progenitors, later becoming cardiomyocytes. Figure 2 thoroughly depicts the starting Wnt pathway activation, detailing how the process allows for mesoderm formation. Later, Wnt inhibition occurs to promote 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. Growing the PSCs in 3D clusters and organoids can better mimic natural tissue and improve structural and functional maturation. Certain proteins, such as α-actinin, cTnT, and NKX2.5, are expressed when PSCs differentiate the way they are supposed to (Aykul et al., 2020). During differentiation, PSC-CMs can be evaluated using electrophysiological recordings, calcium imaging, and observation of spontaneous contractions to confirm well-developed cardiomyocyte-like properties (López-Muneta et al., 2022).

Direct intramyocardial injection is the preferred delivery method because of significantly higher chances of successful implementation in clinical trials. IV transfusions (using a catheter) and intracoronary routes aid in delivering the stem cells directly to the bloodstream or to the infarct site (Lian et al., 2012; Vekstein et al., 2022). When the PSC-CMs are directly delivered to the damaged heart tissue, most of the cells stay where they are initially injected. For intravenous delivery, the cells enter the systemic circulation, getting trapped in the lungs, spleen, or liver. Many don't end up reaching the heart and die right there. On the contrary, in intracoronary delivery, Cells enter the coronary arteries, but many get carried away with the blood flow. Additionally, other methods of delivery 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). 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. Their functional assessment depends on factors, such as

metabolic, electrophysiological, and morphological maturation (Eliwa et al., 2025).

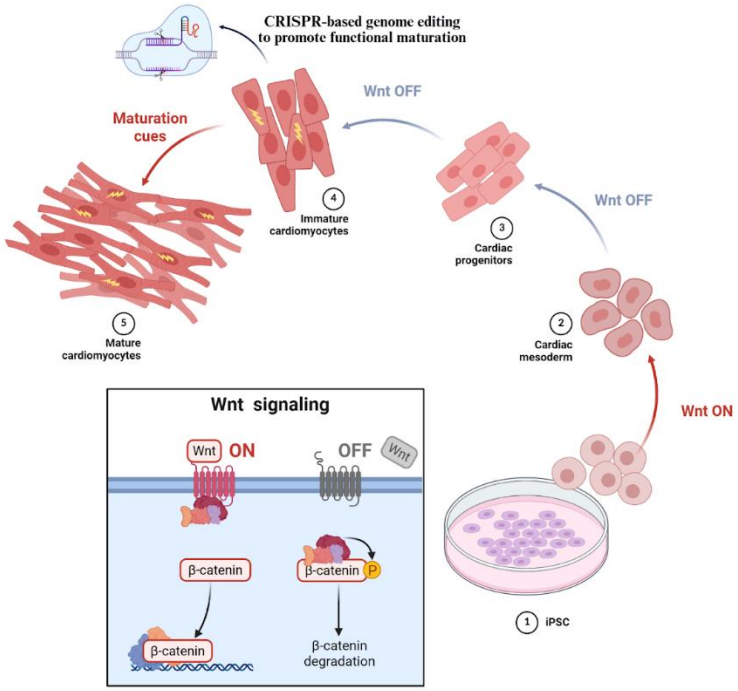

Figure 2. Differentiation of pluripotent stem cells into cardiomyocytes through stage-specific signaling, information compiled from Magro-Lopez et al., 2024. Created with BioRender. Early Wnt activation induces mesoderm formation, then it is followed by Wnt inhibition to promote cardiac progenitor specification. 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.

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. Studies in the past have shown that a limitation of PSC-derived cardiomyocytes is that they are very similar to fetal and neonatal cardiomyocytes, development-wise, rather than fully mature adult ventricular myocytes (Aigha & Raynaud, 2016). The immaturity is reflected through multiple levels of cellular organization, including gene expression, morphology, and functional behavior (Sun & Nunes, 2017). Adult ventricular cardiomyocytes are functionally and structurally specialized to help support a variety of needs that the heart requires. They help synchronize electrical conduction, promote efficient calcium cycling, and support sustained force generation (Giacomelli et al., 2017; X. Li

et al., 2019). Thus, cardiomyocytes containing these fetal-like properties create a significant mismatch when the cells are introduced to a damaged, hostile environment. They cannot meet the high demands that the heart requires, including mechanical, electrical, and metabolic performance. Structural, electrophysiological, calcium-handling, and metabolic deficiencies collectively limit the therapeutic effectiveness of PSC-CMs.

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-derived cardiomyocytes 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 needed to function. As shown in Figure 3, 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). Additionally, they lack a well-developed 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. PSC-CMs have a shorter sarcomere length and poor Z-line alignment, which disrupts efficient force transmission during contraction (Figure 3) (Shameem et al., 2025). This structural immaturity is accompanied by altered expression of contractile protein isoforms. An imbalance between MYH6 and MYH7 reflects an early developmental phenotype (Anfinson et al., 2022).

Beyond structural abnormalities, iPSC- and hESC-derived cardiomyocytes show profound immaturity in calcium-handling mechanisms that are crucial for coordinated contraction. PSC-CMs have an underdeveloped sarcoplasmic reticulum and reduced SERCA2a activity, which limits efficient intracellular calcium cycling (Gu et al., 2021; Kho, 2023). In adult cells, the SERCA2a pump quickly transports around 70% of calcium back into the SR for the next heartbeat. In immature PSC-CMs, SERCA is weak, so the cell must use the NCX exchanger to push all of the calcium completely out of the cell. This method is slower and less efficient. 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). For example, 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). Prolonged calcium handling also increases susceptibility to calcium overload, which can compromise contractile stability and cellular viability.

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, there is a significantly low expression of the KCNJ2 gene, which is responsible for the Kir2.1 channel that works on controlling the inward rectifier potassium channel (Kadota et al., 2020). In these pluripotent stem cells, the upstroke velocity range is ≈50 V/s, whereas in adult CMs it is stable at ≈250 V/s. This approximate 200 V/s upstroke velocity difference significantly impacts the availability of these sodium channels. 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).

PSC-derived cardiomyocytes 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). 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. These limitations highlight that successful cardiac regeneration requires not only cell survival but also advanced functional maturation.

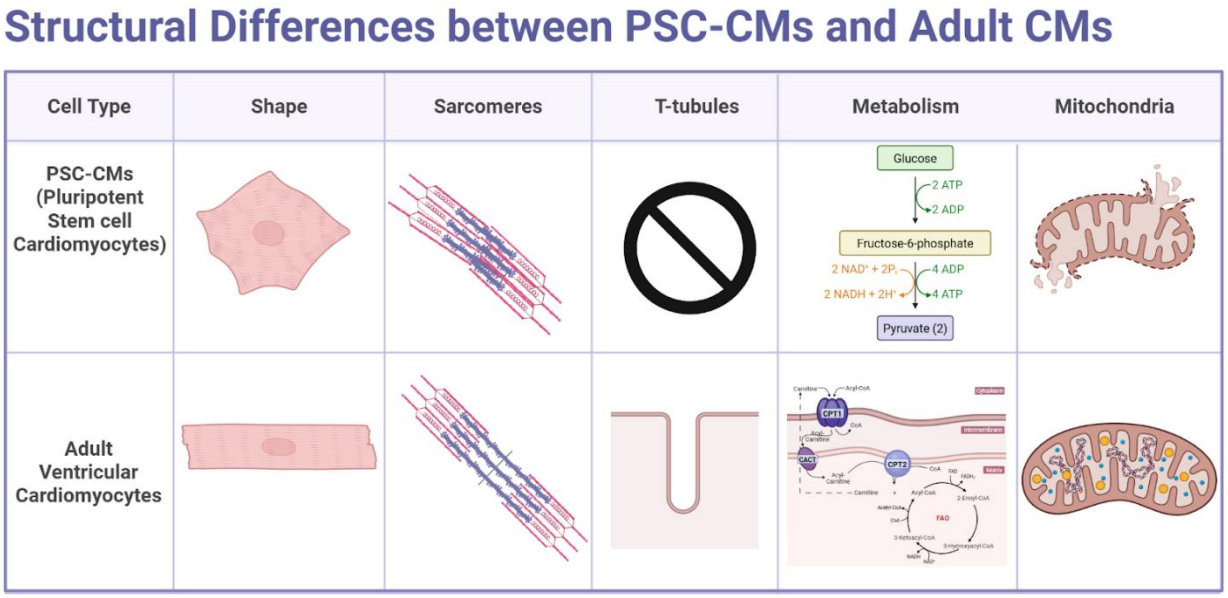

Figure 3. Structural Differences between PSC-CMs and Adult CMs. Information compiled from Moriwaki et al., 2025. Created with BioRender. Comparative structure analysis between immature stem cell-derived cardiomyocytes and fully developed adult heart cells, including factors like cell shape, sarcomere structure, metabolism, etc.

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 back 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. 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).

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.

Table 1. Genes targeted by CRISPR-based editing in human PSC-derived cardiomyocytes, editing strategies, functional outcomes, and relevance to cell maturation.

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

Table 2 - Target Genes using CRISPR-Cas9. Information compiled from Zhou et al., 2021. Categorization of various target genes regarding electrophysiology, calcium, structure, survival, and metabolic maturation alongside the functional goal.

3.4 CRISPR-based genome editing

CRISPR-Cas systems are the main technology for genome editing of stem cells (Caudal et al., 2024). With a combination of edits at certain parts of the DNA and RNA strands, we can try to improve the functionality of these stem cells. There are a few main CRISPR-Cas systems that include Cas9 nuclease, base editors, and prime editors (Valenti et al., 2019). To begin, Cas9 nuclease works to the DNA at a specific site, guided by an sgRNA (Park et al., 2022). Base editors change single nucleotides without cutting both the DNA strands. This lowers the risk of spontaneous and unwanted mutations. Lastly, prime editors include inserting or correcting small sequences precisely. This editing system is very useful for more complex, intricate edits. Different CRISPR tools have different precision, efficiency, and risk profiles, which is very important for PSC-CM applications where safety is crucial (Doudna & Charpentier, 2014). 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.

Based on different functional targets, there are different editing strategies that should be implemented. Regarding electrophysiology, targeting ion channels (e.g., KCNJ2 and SCN5A) can reduce automaticity or stabilize resting potential (Kantor et al., 2026). 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. For structural and contractile maturation, 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. 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 during later on in the differentiation, when the mitochondria of

the cardiomyocytes are still developing (Q. Zhou et al., 2021). 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. 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. 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). 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. Calcium handling is evaluated through analysis of sarcoplasmic reticulum calcium release and reuptake dynamics, transient amplitude, and beat rate regularity (Han & Entcheva, 2023). Structural maturation is examined by measuring sarcomere length, alignment, and contractile force generation. Metabolic function is assessed through mitochondrial activity, oxidative capacity, and ATP production, reflecting the energetic readiness of the cells. 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).

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. 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, we may be able 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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Declarations

Generative AI or other LLMs were not used to create or generate any parts of this paper. AI was used to clean up punctuation mistakes, minor grammar issues and small improvements.

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Biography

[Redacted for blind review]

EDITOR COMMENTS AND RECOMMENDATION:

The reviewers commend the author for tackling an ambitious research question and for their proficiency in presenting complex literature in a logical and digestible manner. Both reviewers suggest modifications to the text, specifically more formal, precise language, as well as additional elaboration or clarity in the sections detailed below. Given the reviewer feedback, the editor's recommendation is to accept the manuscript following major revisions.

REVIEWER 1:

Overall comments:

Originality & Significance

This paper does a good job highlighting an emerging and complex research area- using engineered stem cells to rebuild tissue after a myocardial infarction. It is a highly original topic and one I find quite significant. To improve the overall novelty, the author could strengthen the analysis section, by directly analyzing the literature instead of just summarizing. Section 3.4 is strong, but could be improved by making the author's individual ideas more clear.

Clarity & Structure

A major area of improvement in terms of clarity is connecting the very detailed biology information you provide to your overall research goal. You currently have many dense, long, paragraphs, and there are times where I am not sure why you are telling me the information that you are. I would break some of your paragraphs up and try to connect your background information as much as possible to the overall goal of your research, so the reader can follow along logically.

Use of Evidence & Research Methods

The methods section right now is a little vague. I think adding more specific details, like search terms, or inclusion/exclusion criteria would strongly improve the paper's rigor

Engagement with Literature

I think it is clear that the author has a mastery of many complex biological concepts. A major area to strengthen the paper, however, is to go beyond just repeating information and engage with the literature directly. This could be challenging experimental designs reported in papers, directly comparing the benefits or limitations of approaches, analyzing specific data or numerical quantifications, or outlining future experiments. The groundwork for this is already there, I think it will just take a little extra effort to put the paper over the edge

Grammar & Language

Generally, good with some room for improvement. I would review your paper's grammar to make sure you do not use things like contractions or very informal language.

Detailed comments:

Abstract:

- Good abstract

Introduction:

- Minor comment but if define an abbreviation like MPT, you should use it at least two or three times in the paper, otherwise you don't need to make an abbreviation
- Again minor comment, but sometimes you use the author's first initial for the citation- it should always be just their last name for this citation style
- At times, it would be helpful to check your grammar for a more formal tone
    - For example, on line 88-89, use "do not" instead of a contraction.
- For some points, I think you need more citations.
    - For example, the block of text after "These therapies have a very hard time regenerating cardiomyocytes and replacing the scar tissue" on line 90 has no citations until the end of the paragraph. You present a lot of new information here so I think citations are warranted
- Your paragraphs are very dense, it might be helpful to break them up into some smaller paragraphs that have clear topic sentences to help the reader relate the information to your research question – it's helpful to orient the reader why you are telling them information when they're not as familiar with a topic as you are
    - For example, the sentence on line 124 that starts with "Genome editing … " should be it's own paragraph or line 88 when you are introducing the limitations of the existing drugs
- You tell me on line 108 that iPSCs are alternative treatment options, but it'd be helpful to set up earlier in the paragraph a tiny bit of background on these options and why logically they might be a good alternative. Some of this information comes later but it might be confusing to the reader if they are not familiar with the benefit of stem cells
- I think you should give a little bit of detail on CRISPR - not all the readers of this journal might be familiar with this concept
- Your figure 1 is very clear and helpful
- You state "studies remain fragmented". Instead of leaving it at this, you should engage with the literature to explain why. Is it a lack of standardized protocols? Is it because the field is too new? Providing a cited reason for this fragmentation would be a very strong addition to the paper

Methods:

- What search terms did you use?
- How did you define “reputable journal” ?
- How many articles were considered or excluded?
- Did you determine an article’s relevance using the abstract or full text
- You say “Some sources were papers from more than 10 years back, as there were no recent studies done on it.” - Does that mean you included that paper and your 2016-2026 range is incorrect or were these excluded. This is generally pretty confusing
- Overall, I think the methods section needs more detail to convince me that your review was “systematic.” Systematic reviews have very strict inclusion/exclusion criteria and generally aim to capture every paper within the topic during a time period

Analysis:

- In general, your analysis section in 3.1 and 3.2 is not really “analysis.” You do a good job summarizing complex literature. However, you are providing background information that would be more appropriate in an introduction section. Instead, you want to engage directly with the literature– what were the major conclusions, how did they do their experiments, were there any limitations? This is the type of analysis we would typically expect in this section. As a result, I’d consider massively restructuring your paper and moving some of the summary information to the introduction or creating a background section. Maybe even reminding your reader of your research question at this point could be helpful
    - For example, you begin 3.1 by telling me about cardiomyocytes, which you did already in the introduction and background on MIs.
    - Similarly, the background about iPSCs I wrote that you seemed missing in the introduction is here
- On line 234, you assume the reader knows what a Wnt pathway is, which is probably unlikely for a general audience
    - You want to tell them why this is relevant to your topic
- I think the synthesis starting at line 246 is better, I think again it could be really taken to the next level by directly interrogating the papers you cite more rigorously
    - For example, you say “Direct intramyocardial injection is the preferred delivery method because of significantly higher chances of successful implementation in clinical trials.” - maybe you could cite the data that supports that claim. Do multiple studies find this to be true? You don’t want to just repeat what other people say, you want to convince the reader by directly analyzing the sources

- In section 3.2, you present a lot of detailed biology. It might be helpful to orient the reader to why this is relevant to your stated goal of “targeted genome edits enhance therapeutic potential” – right now it’s a little bit easy to get lost and not understand why you are telling me so much information about iPSC. I’m sure, as the expert, it all makes perfect sense to you- but someone who is new to the field (and that’s usually the main reader of review papers!) might be a little confused at this point
    - For example, what is upstroke velocity? Is it important to understand for the purposes of your paper? I’d carefully consider all the information in 3.1 and 3.2 and try to relate it to your overall goal more directly.
    - You could also more explicitly compare/contrast, rather than just stating the need for comparison– you can create that comparison yourself using the literature
- Minor comment but genes are usually italicized (like KCNJ2), I’d do a quick check of this as you mention many genes
- Again, make sure major claims have citations
    - For example, “Another drawback to these stem cell-derived cardiomyocytes is the lack of a stable structure they possess.” could use a citation
- Table 1 and 2 are nice but I think might be better later in the paper
- 3.4 is a strong section overall. Something that I think would improve it is delineating what is your idea versus what the literature has already found/tried. How are your ideas building off previous successful or failed experiments? At this point, it’s not clear where you are adding intellectual contribution, although it is clear there is a lot of work here. This would also help it be more of an “analysis”
    - (I would still move some of the CRISPR background to the introduction)
- I think maybe even a table or diagram showing that would be needed to do to make CRISPR a viable treatment in the context of MI would be a really helpful demonstration of the novelty of this paper
- You could also be more specific about the value of different gene editing approaches
    - For example, you could more explicitly explain why base or prime editing is particularly valuable for cardiomyocytes
- One potential idea for this section is to group genes more into pathways rather than listing genes. While this section is organized it can kind of read like a catalog
    - For example, you could group the literature by the functional problem the researchers are trying to solve. For example, instead of a section on KCNJ2, have a section on "Overcoming Arrhythmogenic Risks” – it might make the clinical application more clear to the reader
- A minor technical detail – The paper suggests using CRISPR-Cas9 nuclease for edits throughout the differentiation and maturation process, but standard CRISPR-Cas9 relies

on Homology-Directed Repair (HDR) to insert or correct genes. HDR is almost exclusively active in dividing cells. Once cardiomyocytes begin to mature and stop dividing (post-mitotic), CRISPR-Cas9 efficiency for gene correction or insertion drops significantly. This is exactly why base Editors and prime editors (which you mention) are the "scientifically accurate" choice for late-stage maturation edits

Discussion:

- I like the discussion section and in general you bring up many good points
- A way to strengthen your paper is by providing some outlook based on your expertise
    - What are future directions of the field ? What sort of experiments or data would you want to see that address these limitations? This section is short in your conclusion and I think would be more appropriate here
- Another way to make your paper more mature is to engage with the literature and if there are any disagreements to your findings. Are there any papers that you found where results were contradictory? (e.g., Does one paper say a certain edit improves contraction while another says it has no effect?) Finding these "academic disagreements" is the hallmark of high-level literature engagement. This could go in the discussion or in the analysis section where appropriate

Conclusion:

- The conclusion section is overall good but missing citations

REVIEWER 2:

This manuscript presents an ambitious and well-researched review that integrates stem cell biology, cardiac physiology, and genome editing in the context of myocardial infarction. The topic selection is particularly strong and demonstrates a great level of intellectual curiosity and scope. The organization is logical and effective, moving from the clinical problem of myocardial infarction to current therapeutic limitations, then to stem cell-based approaches and the role of genome editing. In particular, the section discussing the immaturity of pluripotent stem cell-derived cardiomyocytes is especially well-developed. The use of qualitative comparisons and specific gene examples further strengthens the scientific depth of the work.

However, the manuscript would benefit from improving clarity and conciseness. Certain sections, particularly those describing the causes and pathology of myocardial infarction, are overly detailed and somewhat repetitive, which detracts from the central focus on genome editing and cardiomyocyte maturation. Streamlining these areas would improve overall readability and keep the emphasis on the main argument.

From a writing standpoint, the manuscript would benefit from a more consistently formal scientific tone and tighter phrasing. Some sentences are overly wordy or informal and could be revised for precision and clarity. In addition, the central thesis—that genome editing enables coordinated, multi-pathway maturation of stem cell-derived cardiomyocytes—could be more explicitly stated and reinforced throughout the paper.

Overall, this is a strong and promising manuscript with a solid conceptual foundation and impressive depth of knowledge. Even without revisions focused on conciseness and clarity, this is still a compelling scientific review that I would recommend for acceptance.

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

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[Affiliation Redacted for Blind Review]

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.

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. 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). 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 pluripotent stem cells–derived cardiomyocytes (PSC-CMs) can engraft within the infarcted myocardium. Right after 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). CRISPR-Cas9 is a precise genome-editing technology adapted from bacterial immune systems to cut and modify specific DNA sequences. It implements a guide RNA (sgRNA) to direct the Cas9 enzyme to a precise location. The cell then repairs this break, which allows for genes to be knocked out or inserted. 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).

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.

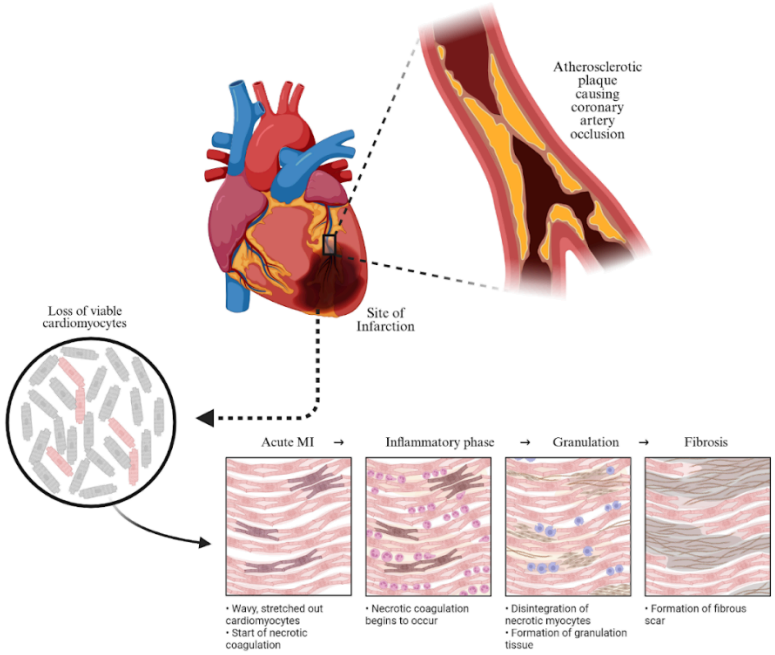

Figure 1. Pathophysiological progression following myocardial infarction. Information compiled from (Scalise et al., 2021). Created with BioRender. A Myocardial Infarction leads to coronary artery occlusion and reduced blood flow, which results in an ischemic injury to cardiomyocytes within the infarct 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.

2 Background

Stem cells include two major types (human embryonic stem cells and induced pluripotent stem cells). One of the most common types of stem cells is human embryonic stem cells (hESCs) (Vazin & Freed, 2010). These are derived from early-stage embryos (blastocysts). Isolation of the inner cell mass (ICM) is critical, as it helps form these pluripotent hESCs. The hESCs are then grown in the lab using feeder layers and feeder-free systems. Feeder layers can provide support by secreting certain factors that maintain pluripotency and prevent differentiation. Feeder-free systems, on the other hand, use extracellular matrices to replicate environments without the need for animal cells. This makes the clinical studies much easier and accessible. To ensure that the cells can differentiate into other types of cells, markers such as OCT4, NANOG, and SOX2 are used to check for cell pluripotency (Swain et al., 2020). These are transcription factors, with OCT4 being the most important as it contributes to self-renewal and guides differentiation into specific lineages. SOX2/SOX3 helps to keep stem cells from prematurely going into mesendoderm, balancing OCT4's effects. hESCs are self-renewing and can differentiate into almost any kind of cell. However, hESC derivation is ethically controversial in some parts of the globe due to the limited supply of human embryo donors (Peng et al., 2020). This is one of the main reasons people turn to iPSCs. Induced pluripotent stem cells (iPSCs) are another type of stem cell that is lab-created based on somatic cells, usually reprogrammed to become any cell (Shown in Figure 2) (Cerneckis 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). Similar to hESCs, their pluripotency is verified through gene and protein markers, testing their ability to differentiate into a range of other somatic cells. Compared to hESCs, iPSCs reduce the likelihood of immune rejection significantly. Regarding the genetic aspect, there can be higher risks of genetic mutations when reprogramming occurs. Comparatively, ESC-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).

CRISPR-Cas systems are the main technology for genome editing of stem cells (Caudal et al., 2024). Researchers rely on three systems: Cas9 nuclease, base editors, and prime editors (Valenti et al., 2019). Traditional Cas9 nucleases use a single-guide RNA (sgRNA) to generate double-stranded breaks (DSBs) at designated genomic sites (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, lowering the risk of spontaneous, unwanted mutations. Similarly, prime editors precisely insert or correct small sequences, making them incredibly useful for more complex, intricate edits in non-dividing cells. This editing system is very useful for more complex, intricate edits. Different CRISPR tools have different precision, efficiency, and risk profiles, which is very important for PSC-CM applications where safety is crucial (Doudna & Charpentier, 2014).

3 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. The search focused on peer-reviewed articles from reputable journals, published between 2016 and 2026. Some sources were papers from more than 10 years back, as there were no recent studies done on it. 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.

4 Analysis

4.1 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.

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).

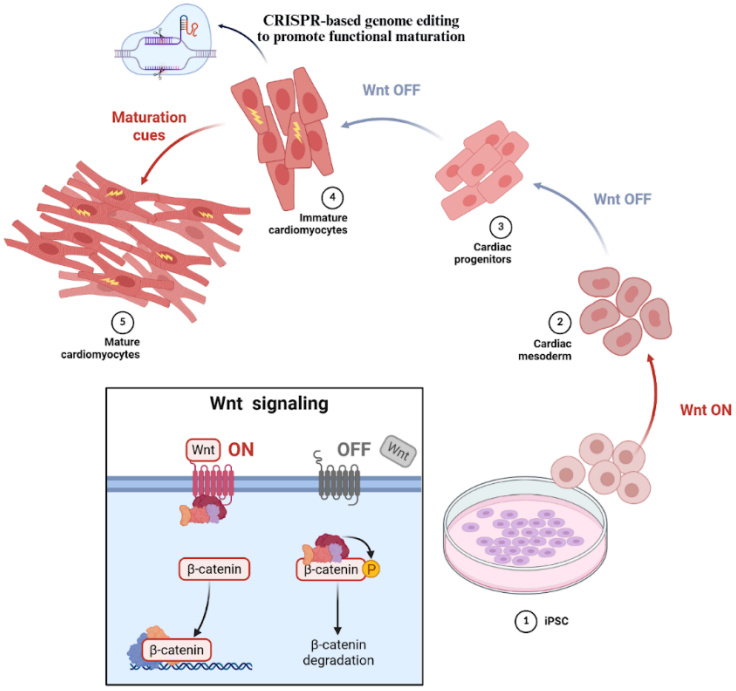

Figure 2. Differentiation of pluripotent stem cells into cardiomyocytes through stage-specific signaling, information compiled from Magro-Lopez et al., 2024. Created with BioRender. Early Wnt activation induces mesoderm formation, then it is followed by Wnt

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.

4.2 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.

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.

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.

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.

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).

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.

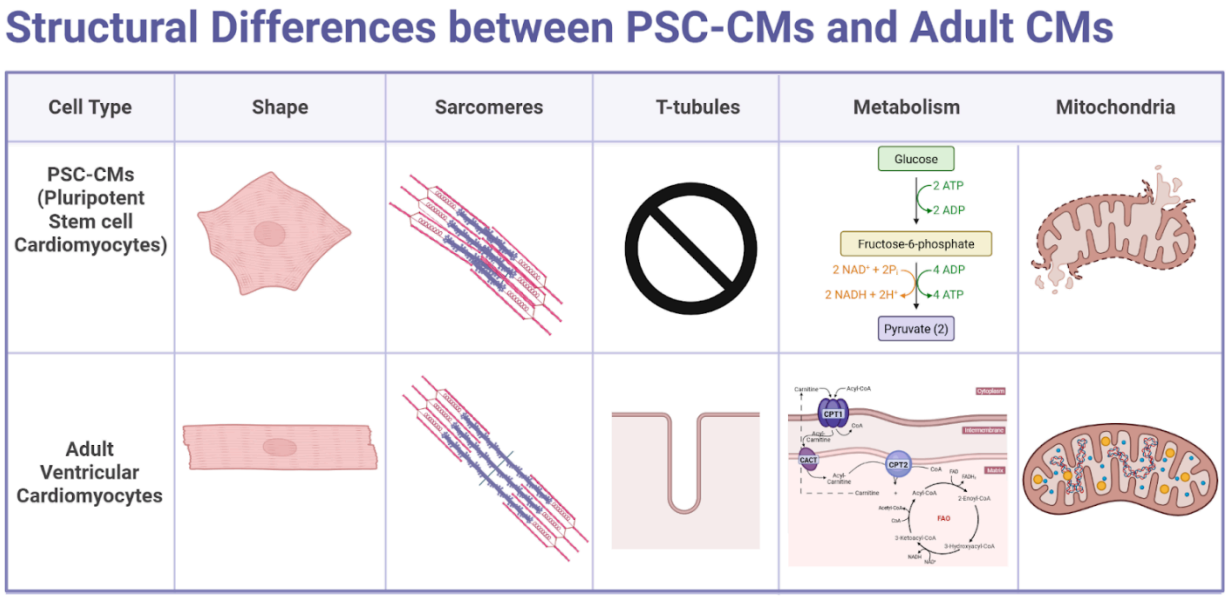

Figure 3. Structural Differences between PSC-CMs and Adult CMs. Information compiled from Moriwaki et al., 2025. Created with BioRender.

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

4.3 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).

4.4 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. 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.

4.4.1 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.

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.

4.4.2 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.

4.4.3 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.

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

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

4.4.4 Screening and Functional Validation

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).

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.

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

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

Table 3 - Target Genes using CRISPR-Cas9. Information compiled from Zhou et al., 2021. Categorization of various target genes regarding electrophysiology, calcium, structure, survival, and metabolic maturation alongside the functional goal.

5 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. 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).

6 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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Declarations

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

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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.

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Biography

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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.

1 Introduction

A myocardial infarction is a medical condition ~~where~~ in which blood flow to the heart is cut off, ~~that is~~ 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 ~~eventually~~ 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). ~~The main issue~~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 ~~(MPT)~~ 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). ~~As good as they may seem~~Despite the clinical utility of these interventions, ~~these~~ they do not ~~indirectly~~ directly restore cardiac tissue or regenerate dead cardiomyocytes, and there ~~are~~are still ~~so~~ a significant number of~~many~~ limitations regarding these therapies. These therapies have a very hard time regenerating cardiomyocytes and replacing the scar tissue. First, ~~these~~ the drugs ~~that were~~ mentioned, such as statins, beta blockers, and ACE inhibitors, ~~work~~ act on the heart's environment ~~and not the~~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.~~make new cells appear if the old ones are damaged or dead.~~ 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~~'s~~ already been formed (Razavi et al., 2024). The myocardium is a highly specialized tissue with regenerative plasticity; thus, ~~heart is a very sensitive and complex muscle, and so~~ most treatments focus on ensuring that no further damage takes place, rather than ~~than undoing the damage that has been done~~restoring myocardial integrity. ~~Majority~~ 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. These stem cells provide a strong alternative since these special cells can self-renew and differentiate

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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). 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 pluripotent stem cells–derived cardiomyocytes (PSC-CMs) can engraft within the infarcted myocardium. Right after 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). CRISPR-Cas9 is a precise genome-editing technology adapted from bacterial immune systems to cut and modify specific DNA sequences. It implements a guide RNA (sgRNA) to direct the Cas9 enzyme to a precise location. The cell then repairs this break, which allows for genes to be knocked out or inserted. 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).

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

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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, ~~more so in vivo.~~with an emphasis on in vivo applications.

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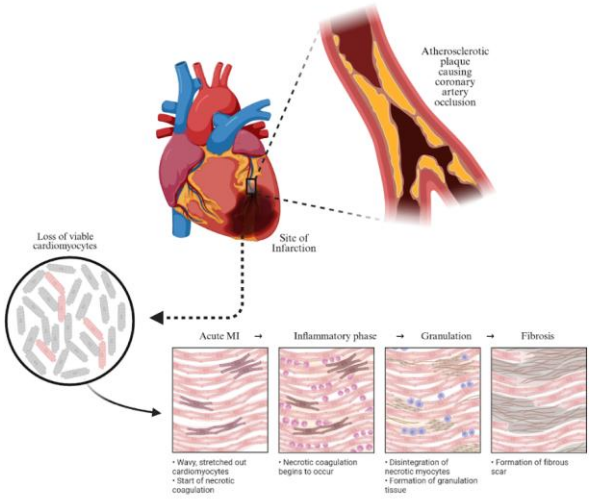

Figure 1. Pathophysiological progression following myocardial infarction. Information compiled from (Scalise et al., 2021). Created with BioRender. A Myocardial Infarction leads to coronary artery occlusion and reduced blood flow, which results in an ischemic injury to cardiomyocytes within the infarct 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.

2 Background

Stem cells include two major types (human embryonic stem cells and induced pluripotent stem cells). One of the most common types of stem cells is human embryonic stem cells (hESCs) (Vazin & Freed, 2010). These are derived from early-stage embryos (blastocysts). Isolation of the inner cell mass (ICM) is critical, as it helps form these pluripotent hESCs. The hESCs are then grown in the lab using feeder layers and feeder-free systems. Feeder layers can provide support by secreting certain factors that maintain pluripotency and prevent differentiation. Feeder-free systems, on the other hand, use extracellular matrices to replicate environments without the need for animal cells. This makes the clinical studies much easier and accessible. To ensure that the cells can differentiate into other types of cells, markers such as OCT4, NANOG, and SOX2 are used to check for cell pluripotency (Swain et al., 2020). These are transcription factors, with OCT4 being the most important as it contributes to self-renewal and guides differentiation into specific lineages. SOX2/SOX3 helps to keep stem cells from prematurely going into mesendoderm, balancing OCT4's effects. hESCs are self-renewing and can differentiate into almost any kind of cell. However, hESC derivation is ethically controversial in some parts of the globe due to the limited supply of human embryo donors (Peng et al., 2020). This is one of the main reasons people turn to iPSCs. Induced pluripotent stem cells (iPSCs) are another type of stem cell that is lab-created based on somatic cells, usually reprogrammed to become any cell (Shown in Figure 2) (Cerneckis 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). Similar to hESCs, their pluripotency is verified through gene and protein markers, testing their ability to differentiate into a range of other somatic cells. Compared to hESCs, iPSCs reduce the likelihood of immune rejection significantly. Regarding the genetic aspect, there can be higher risks of genetic mutations when reprogramming

occurs. Comparatively, ESC-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).

CRISPR-Cas systems are the main technology for genome editing of stem cells (Caudal et al., 2024). Researchers rely on three systems: Cas9 nuclease, base editors, and prime editors (Valenti et al., 2019). Traditional Cas9 nucleases use a single-guide RNA (sgRNA) to generate double-stranded breaks (DSBs) at designated genomic sites (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, lowering the risk of spontaneous, unwanted mutations. Similarly, prime editors precisely insert or correct small sequences, making them incredibly useful for more complex, intricate edits in non-dividing cells. This editing system is very useful for more complex, intricate edits. Different CRISPR tools have different precision, efficiency, and risk profiles, which is very important for PSC-CM applications where safety is crucial (Doudna & Charpentier, 2014).

32 Methods

To ensure a proper analysis of current advancements in regenerative cardiology, a systematic literature search was conducted usinng databases such as PubMed and Google Scholar. The search focused on peer-reviewed articles from reputable journals, published between 2016 and 2026. Some sources were papers from more than 10 years back, as there were no recent studies done on it. 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.

43 Analysis

43.1 The Generation of PSC-CMs

Myocardial infarction results in the irreversible loss of functioning and working heart cells, cardiomyocytes, which creates the need for regenerative strategies to help restore these contractile tissues (Tzahor & Poss, 2017). A myocardial infarction can have multiple causes, some include atherosclerosis, plaque rupture, coronary artery spasm, thrombosis, oxygen supply and demand imbalance, or even sometimes microvascular dysfunction. The most common cause of a heart attack is when fatty deposits build up in the coronary arteries, and this can prevent blood from reaching major arteries and result in a blood block. Plaques can rupture, exposing the inner contents to the blood. Coronary Artery Spasm is a sudden constriction of the coronary artery, automatically reducing blood flow and cardiac output. This can be caused by factors such as smoking, stress, and drugs like cocaine, or even endothelial dysfunction. Coronary Artery Spasm can be classified into temporary or prolonged ischemia, and when prolonged, it is classified as a MI (Matta et al., 2020). The heart needs oxygen to pump effectively. Certain prior medical conditions, like severe anemia, hypotension, tachycardia, or respiratory failure, can reduce organ delivery. And so if the demand for blood and oxygen exceeds the supply, then the cells begin to die, even without a blocked artery. Lastly, the microvascular dysfunction is when smaller coronary vessels fail to deliver enough blood, instead of a main artery getting blocked. Cardiomyocytes do not divide frequently, so the heart struggles to replace a large number of damaged cells post-injury. Taking tissue from other organs to transplant into the heart is not a standard procedure due to incompatibility. These tissues do not function like heart cells and can not contract in the coordinated way the heart does. Heart transplants are limited by donor availability, and there is always a risk of rejection. Those with donor hearts require lifelong immunosuppressive therapy. This solution is also not scalable for millions of individuals who suffer from heart disease. However, certain approaches can be made to ensure that the heart remains as stable and fully functional as possible. Among these approaches, pluripotent stem cells-derived cardiomyocytes (PSC-CMs) offer a renewable source of human cardiac cells with high potential for myocardial repair.

Stem cells include two major types (human embryonic stem cells and induced pluripotent stem cells). One of the most common types of stem cells is human embryonic stem cells (hESCs) (Vazin & Freed, 2010). These are derived from early-stage embryos (blastocysts). Isolation of the inner cell mass (ICM) is critical, as it helps form these pluripotent hESCs. The hESCs are then grown in the lab using feeder layers and feeder-free systems. Feeder layers can provide support by secreting certain factors that maintain pluripotency and prevent differentiation. Feeder-free systems, on the other hand, use extracellular matrices to replicate environments without the need for animal cells. This makes the clinical studies much easier and accessible. To ensure that the cells can differentiate into other types of cells, markers such as OCT4, NANOG, and SOX2 are used to check for cell pluripotency

~~(Swain et al., 2020). These are transcription factors, with OCT4 being the most important as it contributes to self-renewal and guides differentiation into specific lineages. SOX2/SOX3 helps to keep stem cells from prematurely going into mesendoderm, balancing OCT4's effects. hESCs are self-renewing and can differentiate into almost any kind of cell. However, hESC derivation is ethically controversial in some parts of the globe due to the limited supply of human embryo donors (Peng et al., 2020). This is one of the main reasons people turn to iPSCs. Induced pluripotent stem cells (iPSCs) are another type of stem cell that is lab-created based on somatic cells, usually reprogrammed to become any cell (Shown in Figure 2) (Cerneckis 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). Similar to hESCs, their pluripotency is verified through gene and protein markers, testing their ability to differentiate into a range of other somatic cells. Compared to hESCs, iPSCs reduce the likelihood of immune rejection significantly. Regarding the genetic aspect, there can be higher risks of genetic mutations when reprogramming occurs. Comparatively, ESC-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).~~

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 ~~starts with the untouched PSCs turning into cardiac progenitors, later becoming~~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 ~~occurs to promote~~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.

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 ~~Growing the PSCs in 3D clusters and organoids can better mimic natural tissue and improve structural and functional maturation. Certain proteins, such as α-actinin, cTnT, and NKX2.5, are expressed when PSCs differentiate the way they are supposed to~~ (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 ~~During differentiation, PSC-CMs can be evaluated using electrophysiological recordings, calcium imaging, and observation of spontaneous contractions to confirm well-developed 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 ~~Direct intramyocardial injection is the preferred delivery method because of significantly higher chances of successful implementation in clinical trials. IV transfusions (using a catheter) and intracoronary routes aid in delivering the stem cells directly to the bloodstream or to the infarct site~~ (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.

~~When the PSC-CMs are directly delivered to the damaged heart tissue, most of the cells stay where they are initially injected. For intravenous delivery, the cells enter the systemic circulation, getting trapped in the lungs, spleen, or liver. Many do not end up reaching the heart and die right there. On the contrary, in intracoronary delivery, Cells enter the coronary arteries, but many get carried away with the blood flow.~~ To overcome these barriers, recent investigations have shifted focus from liquid cell suspensions towards more biomaterial-based delivery systems. ~~Additionally~~Consequently, current bioengineering research has pivoted to other methods of ~~, 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). ~~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. Their functional assessment depends on multiple factors, such as metabolic, electrophysiological, and morphological maturation (Eliwa et al., 2025).~~

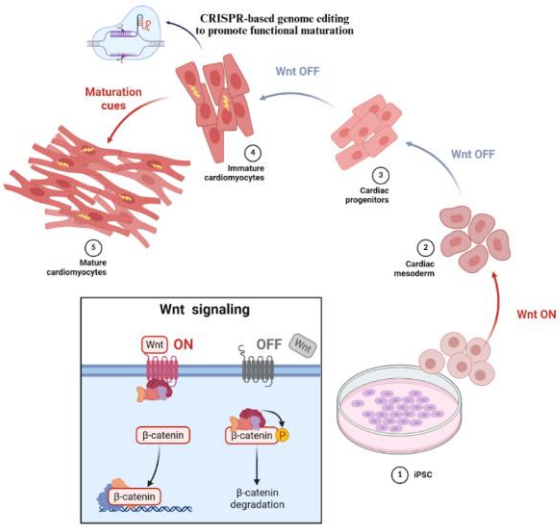

Figure 2. Differentiation of pluripotent stem cells into cardiomyocytes through stage-specific signaling, information compiled from Magro-Lopez et al., 2024. Created with BioRender. Early Wnt activation induces mesoderm formation, then it is followed by Wnt inhibition to promote cardiac progenitor specification. 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.

43.2 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. ~~Studies in the past have shown that a limitation of PSC-derived cardiomyocytes is that they are very similar to fetal and neonatal cardiomyocytes, development-wise, rather than~~ A rigorous cross-study synthesis confirms a major translational bottleneck: baseline unedited PSC-CMs remain developmentally arrested at a fetal or neonatal stage ~~fully mature adult ventricular myocytes~~ (Aigha & Raynaud, 2016). This creates a profound functional mismatch when evaluating these cells against adult parameters. ~~The~~ This immaturity is reflected through multiple levels of cellular organization, including gene expression, morphology, and functional behavior (Sun & Nunes, 2017).

In contrast, a~~A~~dult ventricular cardiomyocytes are functionally and structurally specialized to ~~help support a variety of needs that the heart requires~~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.~~Thus, cardiomyocytes containing these fetal-like properties create a significant mismatch when the cells are introduced to a damaged, hostile environment. They cannot meet the high demands that the heart requires, including mechanical, electrical, and metabolic performance. Structural, electrophysiological, calcium-handling, and metabolic deficiencies collectively limit the therapeutic effectiveness of PSC-CMs.~~

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 ~~derived cardiomyocytes~~ 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~~, needed to function.~~

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: ~~As shown in Figure 3,~~ 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). ~~Additionally, they lack a well-developed tubule network.~~
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. ~~PSC-CMs have a shorter sarcomere length and poor Z-line alignment, which disrupts efficient force transmission during contraction (Figure 3) (Shameem et al., 2025).~~
3. Protein Isoforms: This structural immaturity is accompanied by ~~by altered expression of contractile protein isoforms. An~~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.

Calcium-Handling Mechanisms

Beyond structural abnormalities, iPSC- and hESC-derived cardiomyocytes show profound immaturity in calcium-handling mechanisms ~~that are crucial for coordinated contraction~~, which prevents coordinated contraction. PSC-CMs have an underdeveloped sarcoplasmic reticulum and reduced SERCA2a pump activity ~~, which limits efficient intracellular calcium cycling~~ (Gu et al., 2021; Kho, 2023). While~~In~~ adult cells rely on ~~, the~~ SERCA2a ~~pump~~ to quickly ~~transports~~ transport around 70% of calcium back into the SR for the next heartbeat. ~~. In~~ immature PSC-CMs~~, SERCA~~ rely on a slower, less efficient ~~is weak, so the cell must use the~~

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NCX exchanger to push all of the calcium completely out of the cell. This method is slower and less efficient.

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. For example, 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.Prolonged calcium handling also increases susceptibility to calcium overload, which can compromise contractile stability and cellular viability.

Electrophysiological Immaturity and Arrhythmogenic Risk

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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, there is a significantly low expression of the KCNJ2 gene, which is responsible for the Kir2.1 channel that works on controllingKCNJ2 expression is significantly reduced, which encodes the Kir2.1 subunit of the inward rectifier potassium channel (Kadota et al., 2020).

In these pluripotent stem cells,In PSC-CMs, the upstroke velocity range (the speed at which the cell depolarizes during an action potential) the upstroke velocity range is ≈50 V/s, whereas in adult CMs it is stable at ≈at ≈250 V/s. This approximate 200 V/s difference in upstroke velocity difference impairs electrical signal propagation, making it impossible for transplanted cells to sync smoothly with the host tissuesignificantly impacts the availability of these sodium channels. 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.

Metabolic Profile and Environmental Adaptation

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Finally, PSC-CMs ~~derived cardiomyocytes~~ 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).

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.~~These limitations highlight that successful cardiac regeneration requires not only cell survival but also advanced functional maturation.~~

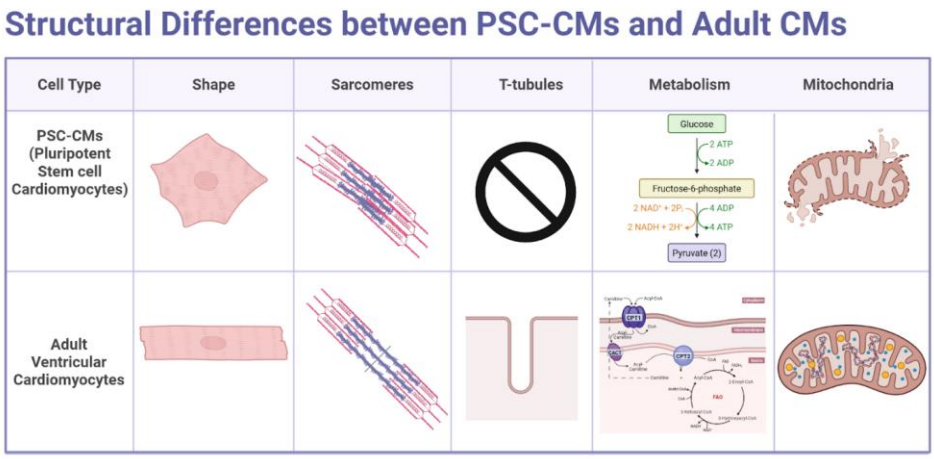

Figure 3. Structural Differences between PSC-CMs and Adult CMs. Information compiled from Moriwaki et al., 2025. Created with BioRender.

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

~~4~~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 ~~back~~ 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).

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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 *PPARGC1α* (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).

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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.

Table 1. Genes targeted by CRISPR-based editing in human PSC-derived cardiomyocytes, editing strategies, functional outcomes, and relevance to cell maturation.

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

~~Table 2 – Target Genes using CRISPR-Cas9. Information compiled from Zhou et al., 2021. Categorization of various target genes regarding electrophysiology, calcium, structure, survival, and metabolic maturation alongside the functional goal.~~

~~4~~3.4 CRISPR-based genome editing

~~CRISPR-Cas systems are the main technology for genome editing of stem cells (Caudal et al., 2024). With a combination of edits at certain parts of the DNA and RNA strands, we can try to improve the functionality of these stem cells. There are a few main CRISPR-Cas systems that include Cas9 nuclease, base editors, and prime editors (Valenti et al., 2019). To begin, Cas9 nuclease works to the DNA at a specific site, guided by an sgRNA (Park et al., 2022). Base editors change single nucleotides without cutting both the DNA strands. This lowers the risk of spontaneous and unwanted mutations. Lastly, prime editors include inserting or correcting small sequences precisely. This editing system is very useful for more complex, intricate edits. Different CRISPR tools have different precision, efficiency, and risk profiles, which is very important for PSC-CM applications where safety is crucial (Doudna & Charpentier, 2014).~~ 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. 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.

4.4.1 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.

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.

4.4.2 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.

4.4.3 Resolving Structural, Metabolic, and Survival Deficiencies

~~For structural and contractile maturation, s~~Studies show that modifying the *MYH6/MYH7* ratios and enhancing *ACTN2* ~~or~~ 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.

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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 ~~during~~ 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.

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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.

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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.

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

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

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4.4.4 Screening and Functional Validation

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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.

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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).

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

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Electrophysiology	KCNJ2, HCN4, CACNA1H	Stabilize RMP, reduce automaticity
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Metabolism	PPARGC1A, TFAM	Increase oxidative capacity
Survival	BCL2, CRYAB	Improve resistance to hypoxia

Table 3 - Target Genes using CRISPR-Cas9. Information compiled from Zhou et al., 2021. Categorization of various target genes regarding electrophysiology, calcium, structure, survival, and metabolic maturation alongside the functional goal.

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54 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. 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).

6~~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~~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, ~~we may be able~~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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Declarations

Generative AI or other Artificial Intelligence was not used to create or generate any parts of this paper. AI, including tools like ~~was~~Grammarly, was used to clean up punctuation mistakes, ~~minor~~ grammar issues, and small improvements in sentences.

Mentor Disclosure 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.

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Biography

[Redacted for blind review]

Dear Reviewer 1,

I am immensely grateful for the comprehensive, insightful, and highly rigorous evaluation of my manuscript. Every suggestion has been thoroughly integrated into this revised text. The manuscript's focus has been significantly shifted away from passive literature summaries toward critical analysis, exact data quantifications, structural reorganization, and clarity improvements.

Below is my detailed, point-by-point response to each comment, indicating exactly where edits were made in the revised document.

Part 1: Overall Comments (Originality, Clarity, & Methodology)

- **Comment 1 (Originality & Significance):** To improve the overall novelty, the author could strengthen the analysis section by directly analyzing the literature instead of just summarizing. Section 3.4 is strong, but could be improved by making the author's individual ideas clearer.
    - **Response:** I have restructured my presentation of literature data throughout the Analysis section (now titled Section 4). Rather than treating historical data as a simple list of findings, I now explicitly evaluate the literature through a framework of functional trade-offs and operational bottlenecks. Section 4.4 has been explicitly expanded to clearly delineate the author's precise technical perspectives on the timing, safety profiles, and molecular choices of genome-editing systems, including the added tables.
- **Comment 2 (Clarity & Structure):** *A major area of improvement in terms of clarity is connecting the very detailed biology information you provide to your overall research goal. You currently have many dense, long paragraphs... break some up and connect background information as much as possible to the overall goal.*
    - **Response:** Agreed. Dense biology paragraphs have been carefully subdivided, and explicit topic sentences have been introduced to systematically tie downstream cellular phenomena back to the overarching goal. I have broken down paragraphs to make it more readable.
- **Comment 3 (Use of Evidence & Research Methods):** The methods section right now is a little vague. I think adding more specific details, like search terms or inclusion/exclusion criteria, would strongly improve the paper's rigor.
    - **Response:** I have revised the Methods section (Section 3) to convert it from a general narrative into a rigorous, systematic review design.
- **Comment 4 (Engagement with Literature):** *Go beyond just repeating information and engage with the literature directly. This could be challenging experimental designs reported in papers, directly comparing the benefits or limitations of approaches, analyzing specific data or numerical quantifications, or outlining future experiments.*

    - **Response:** I have introduced rigorous cross-study data points and numerical quantifications directly into the text. For example, in Section 4.2, I contrast the electrophysiological parameters of unedited cell products with adult parameters using specific quantitative metrics—specifically highlighting the upstroke velocity mismatch that currently drives targeted genomic interventions.
- **Comment 5 (Grammar & Language):** *Review your paper's grammar to make sure you do not use things like contractions or very informal language.*
    - **Response:** A sweep of the entire manuscript was conducted. Every contraction (e.g., *"don't"*) has been completely removed and replaced with formal academic language (e.g., *"do not"*).

Part 2: Detailed Comments
Introduction:

- **Comment:** *If you define an abbreviation like MPT, you should use it at least two or three times in the paper; you don't need to make an abbreviation.*
    - **Response:** Checked and corrected. To maintain editorial cleanliness, the term has been contextualized fully, and standard abbreviations are strictly retained for recurring usage of that word throughout the text.
- **Comment:** *Sometimes you use the author's first initial for the citation - it should always be just their last name for this citation style.*
    - **Response:** As I used Zotero Software, it manually added the initial of the author to differentiate authors with the same last name. I have tried manually changing/deleting these initials, but it causes an error within the Zotero Software.
- **Comment:** *Onlinese 88-89, use "do not" instead of a contraction.*
    - **Response:** Corrected. All instances of contractions in this section and throughout the rest of the paper have been changed to the formal *"do not"*.
- **Comment:** *The block of text after "These therapies have a very hard time regenerating cardiomyocytes..." on line 90 has no citations until the end of the paragraph. You present a lot of new information here, so I think citations are warranted. **
    - **Response:** Citations have been added to support every standalone physiological claim within this paragraph, specifically drawing from *Eliwa et al. (2025)* and Razavi et al. (2024) to support the data.
- **Comment:** *Break up dense paragraphs into smaller paragraphs with clear topic sentences (e.g., line 124 starting with "Genome editing…", or line 88 introducing drug limitations).*
    - **Response:** These changes have been implemented exactly as requested. Paragraphs covering drug limitations and the introduction of genome editing have been cleanly separated and oriented with strong topic transitions.
- **Comment:** *On line 108, it'd be helpful to set up earlier in the paragraph a tiny bit of background on iPSCs and why, logically, they might be a good alternative.*

    - **Response:** I have integrated an analytical introduction to iPSCs earlier in the background section.
- **Comment:** *Give a little bit of detail on CRISPR - not all the readers of this journal might be familiar with this concept.*
    - **Response:** I have added an explanatory foundational section on the basic mechanics of CRISPR editing.
- **Comment:** *You state, "studies remain fragmented". Instead of leaving it at this, you should engage with the literature to explain why. Providing a cited reason for this fragmentation would be a very strong addition.*
    - **Response:** I have explicitly elaborated on this statement.

Methods:

- **Comment:** What search terms did you use? How did you define “reputable journal”? How many articles were considered/excluded? Did you use abstract or full text? Clarify the > 10-year-old paper range confusion.
    - **Response:** Section 3 has been carefully fleshed out to reflect rigorous systematic methodology. I have clarified that initial relevance screening was conducted using abstract filtering, followed by a meticulous full-text appraisal against my pre-specified inclusion parameters. I have also explicitly clarified that papers published outside my primary 2016–2026 window were strictly limited to foundational control references where modern data was entirely unavailable.

Analysis:

- **Comment:** *Sections 3.1 and 3.2 are not really “analysis.” You are providing background information that would be more appropriate in an introduction section... massively restructure your paper and move some summary info to the intro or background section.*
    - **Response:** I have completely restructured the organization of the manuscript. Baseline summary definitions regarding basic cardiomyocyte biology and iPSC development have been shifted entirely out of the analysis domain and integrated into a dedicated Section 2 (Background). This leaves Section 4 (Analysis) free to focus purely on engineering evaluations, comparative differentiation methodologies, and targeted molecular edits.
- **Comment:** *On line 234, you assume the reader knows what a Wnt pathway is... tell them why this is relevant to your topic.*
    - **Response:** I have added clear explanatory text stating that the Wnt pathway is a complex protein network that directly governs cell fate, tissue morphogenesis, and stage-specific differentiation. I explicitly map out its relevance by detailing how biphasic Wnt activation, followed by Wnt inhibition, acts as the key molecular switch to drive mesoderm formation and subsequent cardiac lineage specification.

- **Comment:** *In Section 3.2, orient the reader to why this detailed biology is relevant to your stated goal... what is upstroke velocity, and is it important? Carefully relate all info in 3.1 and 3.2 to your overall goal.*
    - **Response:** I have carefully contextualized the biological subsections. The text now explicitly states that upstroke velocity is a direct metric of action potential depolarization speed that dictates electrical propagation velocity.
- **Comment:** *Genes are usually italicized (like KCNJ2), I'd do a quick check of this.*
    - **Response:** Corrected. The genes mentioned in the text (e.g., *KCNJ2, MYH6, MYH7, ACTN2, TBX5, GATA4*) have been changed to standard italicized formatting.
- **Comment:** *Make sure major claims have citations. (e.g., "Another drawback to these stem cell-derived cardiomyocytes is the lack of a stable structure they possess")*
    - **Response:** Citations have been systematically added across all major statements. The specific morphological statement regarding structural layout and force transmission constraints is now fully cited using data from Joshi et al. (2024).
- **Comment:** *3.4 is a strong section overall. Delineate what is your idea versus what the literature has found/tried... How are your ideas building off previous experiments? Move CRISPR background to intro.*
    - **Response:** Implementations are complete. General CRISPR background details have been moved to Section 2. In Section 4.4, I now clearly present my original synthesis: framing CRISPR interventions not merely as a tool for single-gene knockouts, but as a coordinated multi-stage strategy.
- **Comment:** *Group genes more into pathways rather than listing genes... have a section on "Overcoming Arrhythmogenic Risks" rather than KCNJ2.*
    - **Response:** I have deleted the "catalog-style" gene lists. The analytical sections are now entirely driven by the clinical or functional problems being solved, grouping genes dynamically under broad physiological challenges such as structural/morphological maturation, electrophysiological integration, and metabolic substrate switches.
- **Comment:** *Technical detail: The paper suggests using CRISPR-Cas9 nuclease for edits throughout the differentiation process, but standard CRISPR-Cas9 relies on HDR... Once cardiomyocytes become post-mitotic, HDR drops significantly. This is exactly why base editors and prime editors are the choice for late-stage edits.*
    - **Response:** I thank the reviewer for this excellent technical correction. I have completely overhauled my description of editing systems in Section 2.

Discussion & Conclusion:

- **Comment:** *Strengthen your paper by providing some outlook based on your expertise. What are future directions of the field? Move future directions from conclusion to here.*
    - **Response:** I have expanded the Discussion section to incorporate a forward-looking perspective.
- **Comment:** *Engage with the literature and if there are any disagreements to your findings... Finding these "academic disagreements" is the hallmark of high-level literature engagement.*
    - **Response:** I have explicitly added an evaluation of active academic debates within the literature.
- **Comment:** *The conclusion section is overall good, but missing citations.*
    - **Response:** Completely agree. However, the only reason citations were not added is that I treated the conclusion as a section to deliver my own findings, where it is either my original thoughts or a summary of the topic (which has previous citations above).

Thank you again for helping me elevate the scientific depth and precision of this manuscript. I truly appreciate you taking the time to give me such thorough, crucial feedback.

Best regards,

[Author Name Redacted for Blind Review]

Dear Editor and Reviewer 2,

I am immensely grateful for the comprehensive, insightful, and highly rigorous evaluation of my manuscript. Every suggestion has been thoroughly integrated into this revised text. The manuscript's focus has been significantly shifted away from passive literature summaries toward critical analysis, exact data quantifications, structural reorganization, and clarity improvements.

Below is my detailed, point-by-point response to each comment, indicating exactly where edits were made in the revised document.

- **Comment 1 (Streamlining Myocardial Infarction Pathology):** *Certain sections, particularly those describing the causes and pathology of myocardial infarction, are overly detailed and somewhat repetitive, which detracts from the central focus on genome editing and cardiomyocyte maturation. Streamlining these areas would improve overall readability.*
    - **Response:** I completely agree with this assessment. I have edited Section 1 (Introduction) and Section 2 (Background) to trim away redundant clinical descriptions of coronary artery disease. This ensures that the pathological framework acts strictly as a brief setup for the core focus of the paper.
- **Comment 2 (Formal Scientific Tone and Phrasing):** *From a writing standpoint, the manuscript would benefit from a more consistently formal scientific tone and tighter phrasing. Some sentences are overly wordy or informal and could be revised for precision and clarity.*
    - **Response:** I have carefully polished the prose throughout the manuscript. Sentences have been shortened, repetitive clauses removed, and wordy passive descriptions have been rewritten into precise, active scientific phrasing.
- **Comment 3 (Reinforcing the Central Thesis):** *The central thesis—that genome editing enables coordinated, multi-pathway maturation of stem cell-derived cardiomyocytes—could be more explicitly stated and reinforced throughout the paper.*
    - **Response:** I appreciate this vital suggestion. I have revised my Introduction to state this core thesis explicitly. I have woven this central thesis into the transition sentences of every single major section.

Thank you again for helping me elevate the scientific depth and precision of this manuscript. I truly appreciate you taking the time to give me such thorough, crucial feedback.

Best regards,

[Author Name Redacted for Blind Review]

Overall comments:

Great job at engaging with the literature! I appreciate your attention to my comments and believe the paper is vastly improved. I recommend accept with a few minor edits I suggest below.

Originality & Significance

The paper is highly original and demonstrates a very mature understanding of the literature. In particular, the connection to CRISPR, along with considering modern prime editing, is very interesting.

Clarity & Structure

I think in general, the clarity is improved greatly by reducing some of the analysis background information. However, I think the introduction of the "Background" section is a little confusing. How is this section different than the introduction? You already introduced a lot of the information in the "Background" section in the "Introduction." I think the clarity of the paper would be better by removing the background section and instead condensing this information and adding it, where appropriate, to the "Introduction." I know you probably worked really hard on gathering all this information, and it can be painful to delete information that you think is essential, but I think your mastery of the topic already is demonstrated in other parts of the paper :)

Use of Evidence & Research Methods

The level of analysis of the existing literature is much improved and appropriate for the topic. The analysis of CRISPR-Cas9 and its relevance is particularly really nice now.

I think my comments about the methods section being a little imprecise still hold. Your response to me suggests that maybe you did update this section, but on the draft I have, the methods section is the same so it'd be good to check and make sure you are addressing the following:

- For example, how do you define "reputable journal"? Also, you are missing details like what specific search terms you used– you want to be detailed enough so that someone could find a similar body of literature to yours if they wanted to replicate your results. It is also typical to include inclusion/exclusion criteria for the literature you analyzed. As an example, you might say that you excluded clinical trials. If you did not have a specific inclusion/exclusion criteria, it is fine to say that. Another detail that is a bit confusing is what "*Some sources were papers from more than 10 years back, as there were no recent studies done on it"* means? You said earlier only papers 2016-2026 were considered. Does that sentence mean that you actually also included earlier studies? I would revise to make this clearer- do not say 2016-2026 if the range was larger.

Engagement with Literature

There is a substantial improvement in the engagement with literature! Great work! As a final polish, I think there are sections missing some citations. Here are some examples that seem like they have missing:

- *“Because differentiated cardiomyocytes are post-mitotic cells,”*
- *“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.”*
- Table 2
- *“In the future, moving towards more Base Editing or Prime Editing… “*

Grammar & Language

The grammar looks improved to me!

I’m not sure what you mean by “*As I used Zotero Software, it manually added the initial of the author to differentiate authors with the same last name…..”* It’s fine to have authors with the same last name. It’s actually very standard in the field, a lot of people have the same name (even first name). I wouldn’t manually change how the author's name was represented in the article. You want to change the name not within the article but within the zotero database. You can do it there and then go to the Zotero tab in Google Docs and hit “refresh.” It should automatically update for you.

Overall comments:

Originality & Significance

This paper does a good job highlighting an emerging and complex research area- using engineered stem cells to rebuild tissue after a myocardial infarction. It is a highly original topic and one I find quite significant. To improve the overall novelty, the author could strengthen the analysis section, by directly analyzing the literature instead of just summarizing. Section 3.4 is strong, but could be improved by making the author's individual ideas more clear.

Clarity & Structure

A major area of improvement in terms of clarity is connecting the very detailed biology information you provide to your overall research goal. You currently have many dense, long, paragraphs, and there are times where I am not sure why you are telling me the information that you are. I would break some of your paragraphs up and try to connect your background information as much as possible to the overall goal of your research, so the reader can follow along logically.

Use of Evidence & Research Methods

The methods section right now is a little vague. I think adding more specific details, like search terms, or inclusion/exclusion criteria would strongly improve the paper's rigor

Engagement with Literature

I think it is clear that the author has a mastery of many complex biological concepts. A major area to strengthen the paper, however, is to go beyond just repeating information and engage with the literature directly. This could be challenging experimental designs reported in papers, directly comparing the benefits or limitations of approaches, analyzing specific data or numerical quantifications, or outlining future experiments. The groundwork for this is already there, I think it will just take a little extra effort to put the paper over the edge

Grammar & Language

Generally, good with some room for improvement. I would review your paper's grammar to make sure you do not use things like contractions or very informal language.

Decision: revise and resubmit

Detailed comments:

Abstract:

- Good abstract

Introduction:

- Minor comment but if define an abbreviation like MPT, you should use it at least two or three times in the paper, otherwise you don't need to make an abbreviation
- Again minor comment, but sometimes you use the author's first initial for the citation- it should always be just their last name for this citation style
- At times, it would be helpful to check your grammar for a more formal tone
    - For example, on line 88-89, use "do not" instead of a contraction.
- For some points, I think you need more citations.
    - For example, the block of text after "These therapies have a very hard time regenerating cardiomyocytes and replacing the scar tissue" on line 90 has no citations until the end of the paragraph. You present a lot of new information here so I think citations are warranted
- Your paragraphs are very dense, it might be helpful to break them up into some smaller paragraphs that have clear topic sentences to help the reader relate the information to your research question – it's helpful to orient the reader *why* you are telling them information when they're not as familiar with a topic as you are
    - For example, the sentence on line 124 that starts with "Genome editing … " should be it's own paragraph or line 88 when you are introducing the limitations of the existing drugs
- You tell me on line 108 that iPSCs are alternative treatment options, but it'd be helpful to set up earlier in the paragraph a tiny bit of background on these options and why logically they might be a good alternative. Some of this information comes later but it might be confusing to the reader if they are not familiar with the benefit of stem cells
- I think you should give a little bit of detail on CRISPR - not all the readers of this journal might be familiar with this concept
- Your figure 1 is very clear and helpful
- You state "studies remain fragmented". Instead of leaving it at this, you should engage with the literature to explain why. Is it a lack of standardized protocols? Is it because the field is too new? Providing a cited reason for this fragmentation would be a very strong addition to the paper

Methods:

- What search terms did you use?
- How did you define "reputable journal" ?
- How many articles were considered or excluded?
- Did you determine an article's relevance using the abstract or full text
- You say "Some sources were papers from more than 10 years back, as there were no recent studies done on it." - Does that mean you included that paper and your 2016-2026 range is incorrect or were these excluded. This is generally pretty confusing
- Overall, I think the methods section needs more detail to convince me that your review was "systematic." Systematic reviews have very strict inclusion/exclusion criteria and generally aim to capture every paper within the topic during a time period

Analysis:

- In general, your analysis section in 3.1 and 3.2 is not really “analysis.” You do a good job summarizing complex literature. However, you are providing background information that would be more appropriate in an introduction section. Instead, you want to engage directly with the literature– what were the major conclusions, how did they do their experiments, were there any limitations? This is the type of analysis we would typically expect in this section. As a result, I’d consider massively restructuring your paper and moving some of the summary information to the introduction or creating a background section. Maybe even reminding your reader of your research question at this point could be helpful
    - For example, you begin 3.1 by telling me about cardiomyocytes, which you did already in the introduction and background on MIs.
    - Similarly, the background about iPSCs I wrote that you seemed missing in the introduction is here
    -
- On line 234, you assume the reader knows what a Wnt pathway is, which is probably unlikely for a general audience
    - You want to tell them why this is relevant to your topic
- I think the synthesis starting at line 246 is better, I think again it could be really taken to the next level by directly interrogating the papers you cite more rigorously
    - For example, you say “Direct intramyocardial injection is the preferred delivery method because of significantly higher chances of successful implementation in clinical trials.” - maybe you could cite the data that supports that claim. Do multiple studies find this to be true? You don’t want to just repeat what other people say, you want to convince the reader by directly analyzing the sources
- In section 3.2, you present a lot of detailed biology. It might be helpful to orient the reader to why this is relevant to your stated goal of “targeted genome edits enhance therapeutic potential” – right now it’s a little bit easy to get lost and not understand why you are telling me so much information about iPSC. I’m sure, as the expert, it all makes perfect sense to you- but someone who is new to the field (and that’s usually the main reader of review papers!) might be a little confused at this point
    - For example, what is upstroke velocity? Is it important to understand for the purposes of your paper? I’d carefully consider all the information in 3.1 and 3.2 and try to relate it to your overall goal more directly.
    - You could also more explicitly compare/contrast, rather than just stating the need for comparison– you can create that comparison yourself using the literature
- Minor comment but genes are usually italicized (like *KCNJ2),* I’d do a quick check of this as you mention many genes
- Again, make sure major claims have citations
    - For example, “Another drawback to these stem cell-derived cardiomyocytes is the lack of a stable structure they possess.” could use a citation
- Table 1 and 2 are nice but I think might be better later in the paper
- 3.4 is a strong section overall. Something that I think would improve it is delineating what is your idea versus what the literature has already found/tried. How are your ideas

building off previous successful or failed experiments? At this point, it's not clear where you are adding intellectual contribution, although it is clear there is a lot of work here. This would also help it be more of an "analysis"
    - (I would still move some of the CRISPR background to the introduction)
- I think maybe even a table or diagram showing that would be needed to do to make CRISPR a viable treatment in the context of MI would be a really helpful demonstration of the novelty of this paper
- You could also be more specific about the value of different gene editing approaches
    - For example, you could more explicitly explain *why* base or prime editing is particularly valuable for cardiomyocytes
- One potential idea for this section is to group genes more into pathways rather than listing genes. While this section is organized it can kind of read like a catalog
    - For example, you could group the literature by the functional problem the researchers are trying to solve. For example, instead of a section on KCNJ2, have a section on "Overcoming Arrhythmogenic Risks" – it might make the clinical application more clear to the reader
- A minor technical detail – The paper suggests using CRISPR-Cas9 nuclease for edits throughout the differentiation and maturation process, but standard CRISPR-Cas9 relies on Homology-Directed Repair (HDR) to insert or correct genes. HDR is almost exclusively active in dividing cells. Once cardiomyocytes begin to mature and stop dividing (post-mitotic), CRISPR-Cas9 efficiency for gene *correction* or *insertion* drops significantly. This is exactly why base Editors and prime editors (which you mention) are the "scientifically accurate" choice for late-stage maturation edits

Discussion:

- I like the discussion section and in general you bring up many good points
- A way to strengthen your paper is by providing some outlook based on your expertise
    - What are future directions of the field ? What sort of experiments or data would you want to see that address these limitations? This section is short in your conclusion and I think would be more appropriate here
- Another way to make your paper more mature is to engage with the literature and if there are any disagreements to your findings. Are there any papers that you found where results were contradictory? (e.g., Does one paper say a certain edit improves contraction while another says it has no effect?) Finding these "academic disagreements" is the hallmark of high-level literature engagement. This could go in the discussion or in the analysis section where appropriate

Conclusion:

- The conclusion section is overall good but missing citations