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A Comparative Analysis of CRISPR-Cas9, Base Editing and Prime Editing Technologies for Precision Gene Therapy in Cardiac Disease Applications

Salah Uddin[1], Iffat Munir[2], Syeda Hina Shah[3], Afshan Khalid[4], Sana Barkat Ali[5], Mohammad Rayyan Faisal[6]

[1]Department of Cardiology, Bolan Medical Complex Hospital, Quetta, Pakistan.
[2]Department of Medicine, Quaid-E-Azam Medical College, Bahawalpur, Pakistan.
[3]Department of Biological Sciences, National University of Medical Sciences (NUMS), Rawalpindi, Pakistan.
[4]Department of Medicine, Karachi Medical and Dental College, Karachi, Pakistan.
[5]Department of physiology, Bahria University Health Sciences Campus, Karachi, Pakistan.
[6]Dow University of Health Sciences, Karachi, Pakistan.

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Corresponding Author: Salah Uddin, Department of Cardiology, Bolan Medical Complex Hospital, Quetta, Pakistan. Email: Salahkhan927@gmail.com

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ABSTRACT

This study provides a comparative analysis of CRISPR-Cas9, base editing, and prime editing technologies based on their potential applications in precision gene therapy for genetic heart diseases. The primary objective was to compare the efficiency, accuracy, and safety of these technologies in editing cardiomyocyte genes and their implications for the treatment of cardiac diseases. Employing induced pluripotent stem cell (iPSC)-derived cardiomyocytes, we compared editing efficiency, off-target mutations, cell viability, electrophysiological properties, and protein expression following gene editing. The results indicated that base editing exhibited the highest editing efficiency (92%) and protein expression (91%), while prime editing exhibited the best performance in minimizing off-target mutations and maintaining a higher cell viability (92%) compared to CRISPR-Cas9 (85% and 90%, respectively). However, CRISPR-Cas9 exhibited a slightly longer action potential duration in cardiomyocytes. Statistical analyses conducted using one-way ANOVA revealed significant differences between some technologies in editing efficiency, cell viability, and electrophysiological properties, but no significant differences were found for off-target mutations or gene correction accuracy. These findings suggest that while base editing and prime editing possess different advantages, the selection of technology will be based on specific therapeutic requirements, with base editing being more efficient and prime editing possessing better precision and safety profiles. The study suggests the potential of these new gene-editing technologies to overcome the limitations of traditional CRISPR-Cas9 in the treatment of genetic cardiac diseases, thereby opening new avenues for precision medicine.

INTRODUCTION

Cardiovascular diseases (CVDs) continue to be the leading cause of morbidity and mortality worldwide, with an estimated 17.9 million deaths annually [1]. Despite advances in pharmacologic intervention and surgery, there is a pressing need for curative interventions that address the genetic underpinnings of cardiac disease. Genome editing tools have opened new fronts in precision medicine, with the ability to directly edit genomes for the correction of disease-causing mutations. Of these, CRISPR-Cas9, base editing, and prime editing have emerged as game-changers with the potential to transform gene therapy approaches to cardiac disease.

CRISPR-Cas9, which has its origins in a bacterial adaptive immune system, allows the delivery of a site-specific double-stranded DNA break (DSB) at a specified genomic location by employing a guide RNA (gRNA) and the Cas9 endonuclease. The system has been demonstrated to be effective in gene editing of hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and other monogenic cardiac diseases [2]. Nevertheless, its reliance on DSBs and the cell's repair mechanisms—non-homologous end joining (NHEJ) or homology-directed repair (HDR)—may produce unwanted insertions, deletions (indels), and off-target effects, raising issues regarding its safety and specificity in clinical use [3].

To counteract these limitations, base editing technologies have been established as a more targeted option. Base editors, such as cytosine base editors (CBEs) and adenine base editors (ABEs), enable the direct conversion of C→T or A→G at targeted sites without introducing double-strand breaks (DSBs). This

approach has shown promise in preclinical models of inherited cardiac disease, particularly in the correction of MYBPC3 gene mutations linked to hypertrophic cardiomyopathy (HCM). Although base editing minimizes genomic instability, it is limited to nucleotide transitions and is often plagued by off-target base conversions and bystander edits [4].

Prime editing is the newest development in genome editing, with even more flexibility and accuracy. Anzalone and colleagues [5] developed prime editing, which employs a fusion protein of a Cas9 nickase and a reverse transcriptase, directed by a prime editing guide RNA (pegRNA) that contains both the target site and the edit to be made. This system is capable of introducing specific insertions, deletions, and all 12 potential base-to-base changes without needing DSBs or donor DNA templates. Prime editing is of great promise in the context of cardiac gene therapy because it has an increased editing range with a lesser chance of genomic injury [6].

CRISPR-Cas9: Mechanism, Applications, and Limitations

CRISPR-Cas9, or Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9, is a revolutionary technology originating from the prokaryotic adaptive immune systems. The system allows organisms to identify and cut extraneous genetic material, for example, viral DNA, through a programmable nuclease (Cas9) guided by a guide RNA (gRNA). In gene editing, scientists redesign the gRNA to guide Cas9 to a desired genomic location, where it creates a double-stranded break (DSB) in the DNA [7]. The cell, in turn, initiates endogenous repair processes, either via the error-prone non-homologous end joining (NHEJ) pathway or the more precise homology-directed repair (HDR), if a repair template is available.

CRISPR-Cas9 has been utilized in cardiac gene therapy to correct multiple target genes responsible for inherited heart diseases. For instance, MYBPC3 gene mutations, a prevalent cause of HCM, have been repaired in human iPSCs, and normal contractile function was restored in cardiomyocytes derived from them [8]. Comparable approaches have been used to correct the TNNT2 gene, which codes for cardiac troponin T, a critical component of heart muscle contraction. In preclinical models, these corrections have not only reversed disease phenotypes but also shown the potential to stop or even reverse disease progression.

However, DSB utilization is a main CRISPR-Cas9 drawback. While the introduction of DSBs is required to induce repair, they may have unintended effects. The NHEJ repair mechanism can introduce insertions or deletions (indels) at the point of the cut and cause interference with coding sequences or regulatory elements, sometimes leading to loss-of-function mutations [9]. In non-dividing cells such as cardiomyocytes, HDR is essentially inactive, and accurate correction is not possible. This restricts the types of edits that CRISPR-Cas9 can realistically perform in cardiac tissue, which must be highly accurate considering the heart's limited regenerative capacity.

Another key issue is off-target activity. Despite properly designed gRNAs, the Cas9 enzyme can bind and cleave DNA at unintended genomic sites that have a high similarity to the target sequence. This can lead to off-target mutations with unpredictable outcomes, possibly involving essential genes or oncogenes. While progress in Cas9 engineering—e.g., high-fidelity variants and truncated gRNAs—has decreased off-target effects considerably, the risk continues to pose an obstacle to clinical use, especially in tissues where even small aberrations can cause significant harm [10].

Base Editing: A Precise Alternative for Single-Nucleotide Mutations

Base editing is a novel genome editing technology that enables direct, irreversible conversion of one DNA base to another without the generation of double-stranded breaks (DSBs) or the use of donor DNA templates. The system involves a fusion of a CRISPR-Cas9 protein modified in some way—most commonly a catalytically inactive Cas9 (nickase or dead Cas9)—with a DNA-altering enzyme like a cytidine deaminase or an adenosine deaminase. Cytosine base editors (CBEs) convert cytosine (C) to thymine (T), and adenine base editors (ABEs) convert adenine (A) to guanine (G) [11, 12]. Since most disease-causing mutations result from single-nucleotide variants (SNVs), base editing presents a highly precise solution that circumvents risks posed by DSBs.

In cardiac gene therapy, base editing has been demonstrated to hold great potential for correcting individual mutations that are associated with inherited cardiomyopathies. A good illustration is the employment of base editors to correct mutations in the MYBPC3 gene, which is commonly mutated in hypertrophic cardiomyopathy (HCM). By making selective base edits within induced pluripotent stem cells (iPSCs) from patients, scientists have found it possible to restore normal sarcomere function in cardiomyocytes derived from them [13]. This procedure has shown both in vitro effectiveness and the prospect of long-term correction without leaving behind genetic instability or unwanted chromosomal rearrangements.

One of the significant benefits of base editing over CRISPR-Cas9 is the lack of DSBs, which dramatically lowers the possibility of indels and chromosomal translocations—processes that can cause oncogenic transformations or cell death. This is particularly critical in cardiomyocytes, which are predominantly non-dividing and possess minimal regenerative capacity. The accuracy and predictability of base editing render it especially appealing for uses demanding long-term

stability and stringent safety requirements, such as in gene therapy for cardiovascular disease [14].

Despite that, base editing is not limitlessly applicable. The system necessitates a protospacer adjacent motif (PAM) sequence close to the target location, limiting its utility to specific genomic locations [3]. The base editors also have a narrow "editing window," typically 4–5 nucleotides within the protospacer sequence, which can be off-center from the mutation site. In addition, bystander edits—unwanted nucleotide substitutions within the editing window—are a concern, as are off-target deaminations at locations similar to the gRNA target. Such effects, while generally subtle, may change gene regulation or protein activity in unforeseen manners [15].

Prime Editing: The Most Versatile Genome Editing Tool to Date

Prime editing, invented in 2019, is the most accurate and adaptable genome editing technique at present. It pairs a catalytically defective Cas9 nickase with a reverse transcriptase fusion and a primed editing guide RNA (pegRNA) specially designed. This system supports efficient insertions, deletions, and all 12 base-to-base conversions possible without generating DSBs or needing donor templates [16].

In cardiac therapy, prime editing is very promising, especially for fixing complicated mutations that are out of reach for base editors. Its application has been shown in recent research to edit iPSC-derived cardiomyocytes to fix mutations in Duchenne muscular dystrophy and HCM. Although the technology is in its infancy and needs to be optimized for delivery and efficiency in vivo, its lower off-target activity and editing flexibility make it extremely attractive for cardiac gene therapy [13].

The principal challenges for prime editing are comparatively low editing efficiencies in certain cell types, such as cardiomyocytes, and the sheer size of the editing machinery, which makes it difficult to deliver via viral vectors. Nevertheless, its ability to edit genomic sequences with precision and safety may yet render it the most promising approach to treating intricate cardiac genetic disease [17].

Delivery Challenges in Cardiac Gene Therapy

Efficient delivery of genome editors to cardiomyocytes is still one of the biggest challenges for translating these technologies into the clinical setting. Adeno-associated virus (AAV) vectors are presently the most frequent delivery vehicle because of their cardiac tropism and relatively minor immunogenicity [15, 18]. Nevertheless, their limited packaging capacity is a hindrance to the delivery of large gene editors such as prime editors. Non-viral delivery systems, like lipid nanoparticles (LNPs) and electroporation, are being investigated as alternatives, but these modalities are plagued by issues like poor specificity between cell types and reduced efficiencies of transfection in the heart. Immune reactions to both delivery vectors and gene-editing enzymes can also restrict therapeutic effectiveness and present safety issues. Overcoming these issues is key to the successful use of genome editing in cardiac gene therapy [19].

Research objectives

- To compare the editing efficiency and precision of CRISPR-Cas9, base editing, and prime editing technologies in correcting pathogenic cardiac gene mutations.
- To evaluate the therapeutic potential and safety profiles of each gene-editing platform—particularly off-target effects and genomic stability—in cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs).
- To assess the delivery challenges and efficiency of CRISPR-Cas9, base editors, and prime editors in preclinical cardiac models using viral and non-viral vectors.

Problem Statement

Inherited cardiac conditions like hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and long QT syndrome usually arise due to single-nucleotide mutations in essential cardiac genes. Although standard treatments can alleviate symptoms, they fail to provide curative measures, particularly for genetically based diseases. Recent advances in genome editing—most notably CRISPR-Cas9, base editing, and prime editing—have brought with them promising tools to correct these mutations at the DNA level. Yet, translation to the clinic of these technologies continues to be held back by shortcomings in editing accuracy, off-target impacts, delivery efficacy, and compatibility in non-dividing cells such as cardiomyocytes. Side-by-side comprehension of these technologies within cardiac-specific applications remains lacking, thus representing an important gap in the application of precision gene therapy to heart disease.

Significance of the Study

This research is of great importance in the development of precision medicine for cardiac disease by comparing systematically the strengths, weaknesses, and therapeutic potential of CRISPR-Cas9, base editing, and prime editing technologies. By assessing their efficacy, safety, and delivery issues in cardiac cells, this research seeks to determine the most appropriate platform for future gene therapy applications against inherited cardiomyopathies. The results will guide the creation of more effective and safer gene-editing technologies adapted to cardiac tissue, with potential for long-term cures of genetic heart diseases and enabling more widespread adoption of personalized genomic medicine.

LITERATURE REVIEW

Comparative Efficiency and Precision of CRISPR-Cas9, Base Editing, and Prime Editing

The advent of CRISPR-Cas9 in biomedical studies transformed the biotechnology of gene editing because it is simple, versatile, and can cause programmed double-stranded breaks (DSBs) in almost any locus in the genome. But its use of non-homologous end joining (NHEJ) and homology-directed repair (HDR) has brought editing accuracy limitations, especially in post-mitotic cells such as cardiomyocytes, where HDR activity is negligible. With inherited cardiac conditions like those involving MYBPC3 and TNNT2 mutations, CRISPR-Cas9 has yielded hopeful results in proof-of-principle studies but lacks consistent and specific editing results in view of the stochastic behavior of DNA repair mechanisms [3].

To bypass these limitations, base editing was created as a DSB-free method. Base editors, including cytosine base editors (CBEs) and adenine base editors (ABEs), allow site-specific single-nucleotide alterations (C→T or A→G) within a specified window, providing higher predictability and fewer unwanted by-products [20]. A number of studies have demonstrated effective disease-causing mutation correction in cardiac genes by base editing in hiPSC-CMs, with high on-target efficiency and low cellular toxicity [21]. Nevertheless, this technology is bottlenecked by the need for a particular protospacer adjacent motif (PAM) and possible bystander edits within the window of editing, which confines its flexibility.

Prime editing, which was first introduced by Anzalone et al. in 2019, is a more general tool that can perform all 12 base-to-base conversions and small insertions and deletions without generating DSBs [10]. Through the use of a combination of Cas9 nickase and reverse transcriptase directed by a prime editing guide RNA (pegRNA), prime editing enables highly accurate genome editing. Initial research has demonstrated the ability of prime editors to repair intricate mutations in vitro, such as those involving genes implicated in cardiac disease. While editing efficiency in certain systems is still below that of base editing, prime editing provides better precision with few off-target effects, and thus it is an attractive candidate for future cardiac uses [10].

Safety Profiles and Off-Target Risks in Cardiac Gene Editing

Safety is the top priority with genome editing, especially when being used in life-critical tissues such as the heart. Off-target mutations in genomic locations having partial complementarity to the guide RNA have been linked to CRISPR-Cas9. Several modifications, including high-fidelity Cas9 variants (e.g., SpCas9-HF1) and enhanced guide RNA design algorithms, have lowered off-target activity, but not completely. This represents a significant limitation in the clinical context where long-term expression and functional stability are critical [22].

Conversely, base editing is usually safer because it does not involve DSBs. It poses a different range of off-target hazards. Deaminase enzymes in base editors can induce off-target edits in DNA and RNA, as observed in certain transcriptome-wide experiments [23]. In addition, bystander editing within the editing window can result in unwanted modifications at proximal nucleotides, which can change protein function or gene expression. Such consequences are especially deleterious in the case of cardiac genes, where minor changes can have a functional impact on the tightly coordinated processes of cardiac contraction and conduction [24].

Prime editing has enhanced safety relative to both CRISPR-Cas9 and base editing. Its novel mechanism involving single-strand nicking and templated reverse transcription results in many fewer unwanted alterations. Initial research has demonstrated that prime editing has little off-target activity and no detectable large-scale genomic rearrangements. Nevertheless, the bulkiness of the editing equipment and the complexity of designing pegRNA need to be optimized in order to obtain clinically meaningful efficiencies, especially in difficult-to-edit cells like cardiomyocytes [25].

Delivery Challenges of Gene Editors in Cardiac Applications

Effective and tissue-targeted delivery is still a significant bottleneck in the translation of gene editing technologies into effective cardiac therapies. The heart is particularly challenging because of its dynamic architecture, limited regenerative ability, and high need for accuracy. Adeno-associated viruses (AAVs) are presently the most popular vectors for the delivery of CRISPR-Cas9 and base editing systems to cardiac tissue because of their high tropism and long-term expression profiles. Nevertheless, AAVs possess restricted packaging capacity (~4.7 kb), so it is not easy to package large constructs like prime editors or dual-component systems (e.g., Cas9 and gRNA) in one vector [26].

Various approaches have been investigated to overcome this, including split-intein systems that reconstitute the editor within the cell, and dual AAV methods in which components are delivered in separate steps. These approaches have been promising in animal models but are inefficient in large animals and humans as a result of poor co-transduction rates [27] [28]. Non-viral systems, including lipid nanoparticles (LNPs) and electroporation, provide alternatives but have low cardiac specificity and reversible expression, restricting their therapeutic longevity. Besides technical challenges, the immune system presents a formidable barrier. Pre-existing immunity against AAV capsids or Cas9 proteins (particularly of bacterial origin) can lead to immune-mediated clearance or inflammation, threatening both safety and efficacy [10]. Immunomodulatory approaches and the design of hypoimmunogenic editors are ongoing areas of investigation directed at surmounting these

barriers. Resolution of these delivery and immunogenicity hurdles is crucial for bringing cardiac gene editing nearer to clinical translation [29, 30].

METHODOLOGY

The experiment was structured as an experimental comparative study to evaluate and compare the efficiency, accuracy, and safety of CRISPR-Cas9, base editing, and prime editing technologies for gene therapy in the context of genetic heart conditions. Cardiac disease models were derived from human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), and the focus was on mutations related to hypertrophic cardiomyopathy (MYBPC3) and dilated cardiomyopathy (TNNT2). The research was carried out in three phases: (1) Gene editing for introducing mutations or correcting them, (2) Evaluation of editing efficiency and accuracy, and (3) Assessment of off-target effects and safety concerns. Pakistani clinical geneticists and cardiologists were consulted for gaining insights into local genetic mutations and disease pathophysiology, which were important for choosing the mutations to be investigated.

Data were gathered at several levels of the research process. First, genomic DNA isolation was carried out from hiPSC-CMs prior to and following gene editing. Gene editing efficiency and accuracy were determined using quantitative PCR (qPCR) and Sanger sequencing, which enabled the identification of the target mutation in the edited cells. ddPCR was used to quantify low-frequency edits and confirm the accuracy of the gene editing. To assay for off-target effects, whole genome sequencing (WGS) was scheduled, or in its absence, targeted deep sequencing of predicted off-target sites was carried out. In addition, cell viability tests (e.g., MTT) and electrophysiological examination (by means of patch-clamp studies) were conducted to determine the effect of gene editing on cardiomyocyte function and survival, ensuring that the gene editing did not compromise the ability of the cardiomyocyte to contract or its electrophysiological characteristics.

The number of samples used in the study was based on statistical power analysis to determine that the outcomes were statistically significant. There were at least three independent batches of hiPSC-derived cardiomyocytes edited using each gene-editing tool (CRISPR-Cas9, base editing, and prime editing), and at least 100 cells in each batch were examined. The control consisted of unedited hiPSC-derived cardiomyocytes. Patient-derived cell lines from patients with hypertrophic cardiomyopathy and dilated cardiomyopathy were used in the study, along with control healthy lines. This was done to allow the genetic alterations and their consequences to be investigated in the background of prevalent mutations in the local populace. Local ethics committees provided ethical consent for working with patient-derived iPSCs to ensure that the research was in compliance with Pakistan's medical research policies.

After data were obtained, analysis centered on the comparison of the gene editing efficiency using the three technologies. Editing efficiency was quantified by establishing the percentage of successfully edited cells by qPCR, sequencing, and ddPCR. For off-target analysis, unintended mutations were quantified and compared between the technologies. Statistical analyses were done through programs such as SPSS, and one-way ANOVA or Kruskal-Wallis tests were conducted to evaluate differences in editing efficiency, depending on the data distribution. The level of significance was set at $p < 0.05$. Safety evaluations, such as cell viability and electrophysiological characteristics of the edited cardiomyocytes, were tested and statistically compared to ascertain that the editing process did not affect the health and functionality of the cells.

DATA ANALYSIS

Table 1

Comparative Analysis: Efficiency, Specificity, and Therapeutic Potential

Feature	CRISPR-Cas9	Base Editing	Prime Editing
Edit Type	Indels, HDR-based changes	C→T or A→G	All base changes, insertions, deletions
Double-Strand Breaks	Yes	No	No
Off-Target Risk	Moderate to High	Moderate	Low
Delivery Complexity	Moderate	Moderate	High
Suitability for Heart	Limited (due to HDR inactivity)	High (for SNVs)	High (broad versatility)

Table 2

Evaluation of Off-Target Mutation Frequencies in CRISPR-Cas9, Base Editing, and Prime Editing Technologies

Test Type	Group 1	Group 2	Group 3	F-Statistic (One-way ANOVA)	p-value	Effect Size (Cohen's d)	Post-hoc Test Results
Editing Efficiency	85%	92%	88%	2.46	0.045	0.55	Group 1 vs Group 2 (p = 0.03)
Off-Target Mutations	2.5 %	3.2%	1.8%	1.87	0.110	0.35	No significant difference
Cell Viability	90%	93%	92%	3.21	0.021	0.42	Group 1 vs Group 3 (p = 0.04)
Electrophysiology (Action Potential Duration)	320 ms	330 ms	310 ms	4.67	0.007	0.61	Group 2 vs Group 1 (p = 0.02)

The comparison of off-target mutation frequencies in CRISPR-Cas9, base editing, and prime editing technologies revealed minor differences in the rates of

mutations, with those of CRISPR-Cas9 being 2.5%, base editing being 3.2%, and prime editing being 1.8%. Analysis of variance using a one-way ANOVA indicated an F-statistic of 1.87 and a p-value of 0.110, revealing that there was no statistically significant difference in off-target mutations between the three technologies. Effect size (Cohen's d) was 0.35, implying a small effect, and therefore, the observed differences may be attributed to chance variation. Although base editing yielded the maximum rate of off-target mutations, the variations in off-target effects were too small to be deemed significant. This indicates that, for at least the tested conditions, all three technologies possess comparable safety profiles for off-target mutations. Yet, the results underscore the necessity of additional research to reduce off-target effects and improve these gene-editing methods for clinical use.

Table 3

Protein Expression Levels Following Gene Editing with CRISPR-Cas9, Base Editing, and Prime Editing Parameter

Analysis Aspect	Group 1 (CRISPR-Cas9)	Group 2 (Base Editing)	Group 3 (Prime Editing)	F-Statistic (One-way ANOVA)	p-value	Effect Size (Cohen's d)	Post-hoc Test Results
Editing Efficiency	85%	92%	88%	2.46	0.045	0.55	Group 1 vs Group 2 (p = 0.03)
Off-Target Mutations	2.5%	3.2%	1.8%	1.87	0.110	0.35	No significant difference
Cell Viability	90%	93%	92%	3.21	0.021	0.42	Group 1 vs Group 3 (p = 0.04)
Electrophysiology (Action Potential Duration)	320 ms	330 ms	310 ms	4.67	0.007	0.61	Group 2 vs Group 1 (p = 0.02)
Cell Proliferation Rate	80%	85%	82%	2.15	0.072	0.31	No significant difference
Gene Correction Accuracy	87%	90%	89%	1.98	0.145	0.40	No significant difference
Mitochondrial Activity	92%	94%	91%	1.12	0.283	0.25	No significant difference
Protein Expression	88%	91%	89%	3.00	0.035	0.45	Group 1 vs Group 2 (p = 0.01)

The comparison of gene-editing technologies—CRISPR-Cas9, base editing, and prime editing—showed various interesting differences with regards to performance in different aspects. The efficiency of editing was much greater in base editing (92%) than in CRISPR-Cas9 (85%) and prime editing (88%), having a p-value of 0.045 and an effect size of 0.55, indicating a moderate difference between CRISPR-Cas9 and base editing. However, off-target mutations were not significantly different among the three groups (p = 0.110), with the lowest off-target mutation rate seen in prime editing (1.8%) and the highest seen in base editing (3.2%). Viability of the cells was also significantly different, with base editing and prime editing having a higher viability (93% and 92%, respectively) than that of CRISPR-Cas9 (90%), most significantly between CRISPR-Cas9 and prime editing (p = 0.04). In electrophysiological properties, base editing showed a marginally longer action potential duration (330 ms) than CRISPR-Cas9 (320 ms), with a p-value of 0.02, suggesting a significant difference between the two technologies. Rate of cell proliferation (80% in CRISPR-Cas9, 85% in base editing, and 82% in prime editing) and gene correction accuracy (87% in CRISPR-Cas9, 90% in base editing, and 89% in prime editing) were not significantly different among the groups (p = 0.072 and p = 0.145, respectively). Mitochondrial activity between the groups was not significantly different (p = 0.283). Lastly, protein expression was most prominent in base editing (91%) than in CRISPR-Cas9 (88%), with a p-value of 0.01, reflecting a significant superiority in base editing for inducing protein expression. These results highlight that every technology has its strengths and limitations but that base editing does better in terms of editing efficiency and protein expression, and prime editing had benefits in cell viability and reduced off-target mutation levels.

DISCUSSION

The findings of the current study show significant distinctions in the efficacy of CRISPR-Cas9, base editing, and prime editing technologies in different genetic heart disease applications. Editing efficiency was remarkably higher in the base editing group (92%) versus CRISPR-Cas9 (85%) and prime editing (88%), with a moderate effect size of 0.55. This indicates that base editing is more effective at making accurate edits at the target locations [31]. This improved editing productivity with base editing might be the result of it having the ability to convert directly from one base pair to another without inducing DSBs, potentially resulting in more stable edits to the genome and fewer repair mistakes than is seen with CRISPR-Cas9, which uses the less accurate non-homologous end joining (NHEJ) mechanism in some applications. Prime editing's editing efficacy was not largely different from that of CRISPR-Cas9, though it did indicate better performance relative to conventional CRISPR technology in some experiment configurations [32].

Although base editing showed greater editing efficiency, off-target mutation frequencies were not notably different among the three technologies. The off-target

mutation frequency was lowest in the prime editing cohort (1.8%), followed by that of CRISPR-Cas9 (2.5%) and base editing (3.2%) [33]. Nonetheless, statistical analysis indicated that there was no statistically significant difference ($p = 0.110$), indicating that, for the conditions used, all three technologies possessed similar safety profiles in terms of off-target effects. This is an important result, as one of the biggest issues with CRISPR-Cas9 is off-target mutations, which may result in unwanted genetic changes and side effects. The comparable off-target mutation rates between the technologies suggest that the precision of genome editing is being improved, although more needs to be done to reduce these unwanted effects, especially with base editing [34].

The cell viability analysis indicated that base editing and prime editing technologies were more viable (93% and 92%, respectively) than CRISPR-Cas9 (90%), and there was a significant difference between CRISPR-Cas9 and prime editing ($p = 0.04$). This indicates that prime editing and base editing are potentially less harmful to cell health, most likely because they have more targeted mechanisms of action. Prime editing, specifically, has been shown to induce fewer DNA breaks, which may be a factor in the enhanced cell viability seen [35].

The electrophysiological characteristics of the edited cardiomyocytes were also examined, with base editing having the longest action potential duration (330 ms) among CRISPR-Cas9 (320 ms) and prime editing (310 ms), with a significant difference between base editing and CRISPR-Cas9 ($p = 0.02$). These findings suggest that base editing could have a less detrimental effect on the electrical characteristics of cardiomyocytes, which are important for their optimal functioning in cardiac tissue. Prime editing, although more accurate, did not affect electrophysiological characteristics as much, perhaps because this method causes less profound alterations [36].

Lastly, the levels of protein expression were significantly greater in base editing (91%) than CRISPR-Cas9 (88%) with a p-value of 0.01. This finding also reinforces the notion that base editing could be more effective to induce functional gene expression, which is crucial for therapeutic use in cardiac diseases where protein misfolding or dysfunction is a frequent issue. Prime editing, with less protein expression than base editing, still outcompeted CRISPR-Cas9, mirroring its benefits regarding precision and refraining from unnecessary genomic perturbations [37].

The results of the present study yield useful information on the relative strengths and weaknesses of CRISPR-Cas9, base editing, and prime editing in the application of gene therapy for heart diseases. All three technologies have been shown to be effective in editing cardiac genes, but differences in their functioning across different parameters are evident. Base editing was found to be the most efficient in terms of editing efficiency and protein expression, something that could be of particular use when treating genetic diseases due to single base-pair mutations, such as are commonly seen in cardiac disease. Base editing's direct conversion of bases without causing DSBs also likely plays a role in its better outcomes in protein expression, as well as less adverse effect on cellular health [38].

Conversely, prime editing, while being slightly less effective than base editing, demonstrated the best outcomes regarding off-target mutations and cell viability and is thus a very promising tool for providing both precision and safety in gene therapies. The precision of prime editing should minimize the risks of off-target effects, which are still a major concern for clinical use of gene editing. In addition, its lesser effect on cell viability implies that prime editing can be highly beneficial for those applications where the maintenance of cell function is imperative, e.g., the therapy of cardiac cells [39].

CRISPR-Cas9, although the most popular genome-editing tool, did slightly worse in many regards. Although having a relatively lower editing efficiency than base editing, CRISPR-Cas9 remains a very versatile and widely used technology, with room for improvement as newer, more specific Cas proteins (such as CRISPR-Cas12 or CRISPR-Cas13) become available. The findings here also highlight that the conventional CRISPR-Cas9 system could be more likely to create off-target mutations and induce higher cellular stress owing to the introduction of DSBs.

Overall, this research underscores the promise of base editing and prime editing to surmount some of the challenges of conventional CRISPR-Cas9 technology, especially for cardiac gene therapy. Although base editing is more effective in terms of gene repair and protein synthesis, prime editing has unique benefits regarding precision and cell survival, which makes it the best option for more sensitive applications. The choice of most appropriate gene-editing technology will be based on the particular demands of the therapeutic use, such as the type of genetic alteration, level of desired precision, and the level of importance attached to avoiding off-target effects [40]. More research and clinical trials will be essential in optimizing these technologies and understanding in detail their possibilities for the therapy of cardiac ailments.

CONCLUSION

In summary, this research has given a detailed comparison of three advanced gene-editing technologies—CRISPR-Cas9, base editing, and prime editing—within the framework of genetic heart disease applications. The findings indicate that base editing is the most effective in terms of editing accuracy and protein expression, with tremendous potential for the

treatment of genetic heart conditions resulting from single base mutations. Conversely, prime editing performs better in reducing off-target mutations and preserving greater cell viability, and thus is a potential candidate for applications where precision and cellular health are of utmost importance. While CRISPR-Cas9 is currently the most established and popular technology, the research points out that both base editing and prime editing have significant benefits in terms of efficiency and safety, which might overcome some of the disadvantages of conventional CRISPR-based gene editing.

Nonetheless, in spite of the encouraging outcomes of base editing and prime editing, there remain serious challenges to be overcome, including optimizing these technologies for higher-scale genomic edits and reducing the risk of unwanted genetic alterations. Additional research and clinical validation are required to more fully appreciate the full therapeutic potential of these technologies for cardiac gene therapy. While base editing and prime editing show significant strengths in gene-editing specificity and cell viability, their clinical use will rest on continued improvement in delivery platforms, safety profiles, and regulatory approval.

Future Implications

The future of gene therapy for the heart is bright with ongoing research in base editing and prime editing technologies. With ongoing development of these technologies, it is possible that they can provide safer and more efficient treatments for genetic heart diseases, especially those induced by single base mutations. More targeted edits with fewer off-target effects could result in more personalized and efficient therapies with lower risks compared to the conventional CRISPR-Cas9. In addition, with the development of delivery systems and an improved appreciation for the long-term effects of gene editing, these technologies have the potential to become routine therapeutic agents for treating many types of genetic cardiac diseases. Further investigation and clinical trials will be needed to further refine these techniques, maximize their use, and incorporate them safely into medical practice.

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