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The Role of CRISPR-Cas9 in Gene Editing

Mohammed J. Kadhim
Universitas Granada,Spanyol

*Korespondensi Penulis: mohamedaoda6@gmail.com**

***Abstract**. The CRISPR-Cas9 technique is a contemporary technology that has transformed the process of genetic modification, allowing scientists to target genomes in living organisms with precision and simplicity. Numerous practical applications of significant importance, including the introduction of new genes into living cells, are facilitated by the CRISPR-Cas9 system's advanced level of gene targeting, precision, and simplicity of use. An enigmatic process involving repeated DNA sequences in E. coli initiated the emergence of this CRISPR-Cas9 mechanism. After observing this phenomenon in bacteria, scientists initiated an investigation into its function. It was noted that E. coli employs it as a self-defence mechanism against bacteriophage attacks and plasmids. CRISPR-Cas9, a novel method for genetic modification, was created as a result of this investigation. This system has been re-engineered by scientists to be more broadly applicable, allowing for the modification of the genetic code of virtually any organism, surpassing the scope of self-defence. The CRISPR-Cas9 system-based gene editing mechanisms have the potential to produce substantial results that were previously difficult to achieve. Nevertheless, the question of genetic modification through the application of this technology prompts debates and demands for the resolution of the ethical, social, and legal issues that are associated with its application. The potential applications of the CRISPR-Cas9 system are seemingly boundless.*

***Keywords**: CRISPER Cas9, Gene Editing, Human germline, Ethics.*

Abstrak. Teknik CRISPR-Cas9 adalah teknologi kontemporer yang telah mengubah proses modifikasi genetik, memungkinkan para ilmuwan menargetkan genom pada organisme hidup dengan presisi dan sederhana. Berbagai aplikasi praktis yang sangat penting, termasuk pengenalan gen baru ke dalam sel hidup, difasilitasi oleh penargetan gen tingkat lanjut, presisi, dan kemudahan penggunaan sistem CRISPR-Cas9. Proses misterius yang melibatkan rangkaian DNA berulang pada E. coli mengawali munculnya mekanisme CRISPR-Cas9 ini. Setelah mengamati fenomena ini pada bakteri, para ilmuwan memulai penyelidikan terhadap fungsinya. Telah dicatat bahwa E. coli menggunakannya sebagai mekanisme pertahanan diri terhadap serangan bakteriofag dan plasmid. CRISPR-Cas9, sebuah metode baru untuk modifikasi genetik, diciptakan sebagai hasil dari penyelidikan ini. Sistem ini telah direkayasa ulang oleh para ilmuwan agar dapat diterapkan secara lebih luas, memungkinkan modifikasi kode genetik hampir semua organisme, melampaui cakupan pertahanan diri. Mekanisme pengeditan gen berbasis sistem CRISPR-Cas9 berpotensi menghasilkan hasil substansial yang sebelumnya sulit dicapai. Namun demikian, pertanyaan tentang modifikasi genetik melalui penerapan teknologi ini memicu perdebatan dan tuntutan penyelesaian masalah etika, sosial, dan hukum yang terkait dengan penerapannya. Potensi penerapan sistem CRISPR-Cas9 tampaknya tidak terbatas.

Kata Kunci: CRISPER Cas9, Gene Editing, Human germline, Etika.

1. INTRODUCTION

CRISPR is a genetic system or a collection of DNA sequences that are inherently present in the genomes of prokaryotic cells, including Escherichia coli bacteria. CRISPR sequences are DNA remnants that are derived from bacteriophages that infiltrate prokaryotic bacterial cells. In essence, the Cas9 enzyme fragments the phage's DNA or genetic material into smaller parts when it attacks a bacterial cell. This enzyme cleaves a specific chain of nucleotides into smaller fragments, thereby targeting the phage DNA. RNA directs the Cas9 enzyme. It targets a particular segment of the phage's DNA by binding to a guide RNA

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molecule (gRNA) that is transcribed from the CRISPR genetic system. The CRISPR Cas9 system is transmitted to human cells by the co-transfection of plasmids encoding the Cas9 nucleases and the requisite RNA elements, which commonly is sgRNA (single guide RNA). This has been done already. Using microarrays, the oligonucleotide synthesis of a set of single guide RNA has been accomplished owing to the ease of use of the system. Such packages can be designed to contain several single guide RNAs for every gene in the organism (Guan et al., 2016; Irfan et al., 2024).

It's possible to add new genetic material to the target CRISPR-Cas9 site or fix pathogenic mutations with the help of CRISPR-Cas9. CRISPR-Cas9 can also be used to provide additional components like Dna that after the editing step regulates the expression of the gene. Because of its accuracy and ease of use, the CRISPR-Cas9 system can be utilized in various fields. These include general biological research, and more specialized areas such as journalist genetics, genetic therapy and medicine. Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs) are also methods that are used but are not as common owing to their complexity as compared to CRISPR/Cas9. These systems (ZFNs and TALENs) were developed by scientists after many years of work, and it was these systems that made it possible to hurriedly discover CRISPR-Cas9. Many researchers have reported successful results in treatment of different rare genetic diseases and even cancer immunotherapy employing CRISPR-Cas9 technology. Considering the possibilities, it is logical to assume that CRISPR-Cas9 will be able to target many therapeutic areas with (Irfan et al., 2024; T. Zhang, 2014).

Applications Of Crispr-Cas9 In Gene Editing

The precision and accuracy of CRISPR-Cas9 has changed the world of gene editing. The treatment of genetic disorders like cystic fibrosis and sickle cell anemia may become possible through direct replacement of defective DNA. Also, it accelerates the process of drug development by making it possible for researchers to create accurate models of the diseases. In agriculture, CRISPR-Cas9 can be used to enhance sustainability, productivity, nutritional quality, and disease resistance of crops. In addition, it is critical to non-biomedical research because it allows scientists to study gene expression and control in a way that is radically more straightforward.(Cong et al., 2013; Fu et al., 2013; Gallo et al., 2017; Liang et al., 2015; Yang et al., 2013; X.-H. Zhang et al., 2015).

CRISPR-Cas9 System In Medicine

The CRISPR-Cas9 technology is one of the most powerful tools within gene therapy, medical research, and medicine. It has a primary use in medicine which is the study of functions of genes and mechanisms of diseases. Scientists can disrupt specific genes in human cells or other model systems to understand better how one can develop more complex medical approaches to genetic diseases. In addition, the CRISPR-Cas9 system allows the elimination of mutatons that are responsible for the diseases, directly in a patient's cells which enables therapeutic interventions. In addition, CRISPR-Cas9 is able to correct mutations that cause genetic blood disorders like beta-thalassemia in human patient-derived stem cells (Fu et al., 2013; Gallo et al., 2017; Yang et al., 2013).

Further, the understanding of CRISPR-Cas9 tools has emerged as case of great importance with respect to cancer research and treatment. This method efficiently hits and tears apart genes associated with cancer development while making modifications that boost responsiveness of cancer cells to treatment. For example, one study showed how a gene that leads to resistance to chemotherapy was knocked out, and how this positively affected cancer treatment outcomes (Ma et al., 2014).

The potential of CRISPR-Cas9 includes elsewhere gene removal; it can also be charity for diagnostic purposes. By detecting specific DNA arrangements accompanying with certain diseases, this technology enables rapid and accurate diagnosis of genetic mutations and viral infections. It has also become possible to explain cases of hypoglycaemia thanks to CRISPR-Cas9 technology by introducing a point mutation in the MEN1 gene within stem cells. Despite the significant role of CRISPR-Cas9 in the field medicine, there remain experiments that essential to be addressed and considered. For instance, off-target effects and ethical issues (which will be discussed later) remain subjects of debate and controversy.

With that being said, we cannot ignore the potential of CRISPR-Cas9 in medicine. The tool can be used to develop specific gene therapies, facilitate prompt and proper diagnosis, and increase knowledge of gene functions and means of infection. If accomplished, these changes would fundamentally change the scope of medicine and greatly improve the ability to manage complex medical issues, especially those of genetic origins (Cong et al., 2013; Hsu et al., 2013; Ma et al., 2014).

CRISPR-Cas9 System in Nanotechnology

A huge challenge is to further develop a way in which CRISPR-Cas9 would work together with technology or nanotechnology. These challenges come from a wider societal need. Because as scientists agree, the goal is to bypass human limitations and endeavours through the aid of genetics and CRISPR technology, while managing to create innovative technologies that can make lives easier. Nanotechnology is an emerging area of science that promises to change many fields of human activity including biology and medicine. One of the main goals is to develop nanoparticles that will transport CRISPR-Cas9 components such as guide RNA and Cas9 proteins into specific cells or tissues. This can be done with an acceptance that it would greatly improve the efficiency of genome editing and significantly cut down the unwanted effects (Abdallah et al., 2025; Cho et al., 2014; Hendel et al., 2015; Kim et al., 2015).

CASPER-Cas9 in Clinical Research

Clinical research that uses CRISPR-Cas9 helps us better understand how diseases work and how to create cures for them. For instance, CRISPR-Cas9 has been used to target the PCSK9 gene, which plays a role in regulating cholesterol levels. For example, a study showed that CRISPR-Cas9 might be used as a treatment to reduce cholesterol levels in people with familial hypercholesterolemia by targeting the PCSK9 gene, which plays a role in cholesterol regulation. A significant area of clinical research is the potential of CRISPR-Cas9 to cure hereditary disorders. One study demonstrated that it was possible to fix mutations that cause disease in the tissues or cells of a patient by employing the CRISPR-Cas9 system. In a different work, the CRISPR-Cas9 system was utilized to modify the CCR5 gene in hematopoietic stem cells in order to provide resistance to HIV infection. Researchers are highly interested in the potential of employing the CRISPR-Cas9 system to locate and destroy cancer cells in the human body accurately. In one study, scientists were able to determine whether the CRISPR-Cas9 system might selectively kill cancer cells by targeting the genes that contribute to the development of leukaemia. Also, very similarly, cellular therapies are designs by taking the patient's cells out of the body, doing the editing with CRISPR-Cas9, and putting the cells back into the body. For instance, a clinical trial conducted at the University of Pennsylvania modified the T cells of some patients suffering from advanced cancer with CRISPR-Cas9. The intent was for those modified T cells to target and kill the cancer cells. Regardless, CRISPR-Cas9 is quite promising and highly developed; it has reasonable risks and ethical questions that still need to be resolved on its way to extensive practical application. The risks are those associated with long-term safety, delivery systems, potential unintended effects, and ethical issues concerning saline germline edits. These risks shall be well examined (Begagić et al., 2024; Kim et al., 2015).

CRISPR-Cas9 Technology in Biotechnology

CRISPR-Cas9 technology is truly a core and powerful biotechnology tool for gene regulation and genome editing. The primary application of CRISPR-Cas9 in biotechnology is gene editing, which allows the making of precise interventions into the DNA sequences of living beings, including plants, animals, bacteria, and yeast. For instance, a yeast strain has been edited using CRISPR-Cas9 technology, which is an example of how it can be used to edit strains containing economically useful yeast metabolites.

In addition, CRISPR Cas9 has specific applications for the control of gene expression and functional genomics. Exact manipulation of gene expression permits the study of gene functions and the evaluation of biological system complexities. By using the CRISPR-Cas9 system to target gene regulatory elements, the functional interplay of specific genes can be understood.

CRISPR-Cas9 technology now means it is possible to establish experimental animals that serve as effective models for some human ailment. This represents an important advance in the field. The method introduces specific mutations associated with human genetic diseases, so that it is possible to develop a treatment that can cure such hereditary afflictions and also how they work better. For instance, a pig model of cardiovascular illness was developed utilizing CRISPR-Cas9 technology, which shows how important this technology is for understanding human diseases in animals. Like any other technology, CRISPR-Cas9 requires careful consideration of ethical issues when it is used in biotechnology. Research and talks are still happening to address concerns about the hazards and ethical difficulties that come with using CRISPR-Cas9 in different biotechnology applications (Wang et al., 2022; H. Zhang et al., 2021).

Benefits And Future Prospects

The CRISPR-Cas9 technology has taken on substantial roles in innumerable arenas, including medicine, science, nanotechnology, and biotechnology. Current investigation and discoveries are paving the way for major advancements in the prospective future regarding CRISPR-Cas9 system [55, 56]. Future "tests" and research are focused on improving the precision and specificity of CRISPR-Cas9 editing. Efforts are being made to enhance the efficiency of homology-directed repair (HDR), as well as to reduce off-target effects and unintended genomic modifications, the current initiatives aim to minimize these off-target effects and increase HDR efficiency through several approaches, including the use of high-precision Cas9 variants and modulated guide RNA designs. These efforts and studies will enhance the accuracy and safety of CRISPR-Cas9 system across different applications in the future.

Investigators are struggling to extend the abilities of the CRISPR Cas9 system concerning to Gene editing further on than DNA modulation. This is done by improving RNA targeted CRISPR technique to perfectly edit RNA and do the precise manipulation to it. For example, Cas13 enzymes target programmable RNA and allow accurate modification to individual nucleotides. This will allow researchers to modify RNA molecules directly, which will create new opportunities for treating diseases at the RNA level. In one work, CRISPR/Cas13 was used to modify programmable RNA in human cells. CRISPR-Cas9 has great promise as a therapeutic tool for the treatment of genetic disorders and cancers. Future studies will focus on enhancing CRISPR-based therapies for genetic diseases and neoplasms. Furthermore, CRISPR-Cas9 holds promise as a tool for precision oncology, where specific mutations in cancer germs may be targeted. One study showed that researchers were able to treat a hereditary form of blindness in a clinical trial using CRISPR-Cas9 (Zaman et al., 2021).

The method of editing numerous genes and the capacity to target and change genomic locations simultaneously are two possible future applications of the CRISPR Cas9 system that are still being researched. The ability to target several genomic regions will allow for a more thorough investigation of genetic pathways and their complicated networks. Through our commitment to chemical innovation, this problem will sooner or later lead to a better understanding of diseases and higher uses for biotechnology as well as synthetic biology. The discussion about the hazards and ethic issue s of safe handling CRISPR/Cas 9 in the future and in all areas appli- cations is never finished. Despite the fact that CRISPR-Cas9 technology has a lot of potential and is promising, the future of its applications and research will be determined by ethical considerations.

Risks And Ethical Considerations

One of the biggest problems with CRISPR-Cas9 gene editing is that no one can predict its safety. Specifically, there are off-target effects and unwanted modifications. The goal of human gene editing is certainly to reduce human suffering from genetic disorders, cancer, and the like. Anything that is technically possible to reduce human suffering and the burden of disease can and should be used, as Steven Pinker argues. However, this can only be done with strict controls and constant oversight, especially when there is a possibility of altering the human germline.

In reality, gene manipulation technology is not restricted to the correction of genetic modifications that are linked to specific severe and incurable diseases; rather, it aims to reprogram the genome to incorporate characteristics that it does not naturally possess. For example, the Cas9 enzyme is primarily responsible for cleaving the DNA sequence. However, it can also target specific genomic regions to identify gene locations and induce regulatory effects that can activate or inhibit the expression of specific genes without affecting the genetic sequence.

This approach appears to be an innovative therapeutic strategy for certain neurological disorders; however, it raises concerns about the possibility of modifying gene expression for specific purposes, potentially leading to the regulation of individual behaviour or even that of entire population groups. While this method could fall under the definition of a medical intervention, it has generated considerable speculation about its potential use as a means to control gene expression towards that specific goal and thus be used to structure behaviour at the level of the individual and society. These technologies require a thorough understanding of methods for modifying genes as well as a detailed knowledge of the ethical discussions and health dangers of genetic changes (Di Cristina et al., 2025).

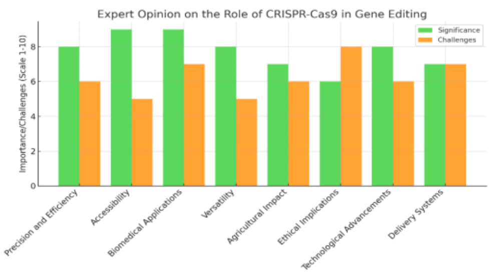
Advances in genome-editing technologies, such as CRISPR-Cas9, are increasing the potential and ease of use of these processes, and will inevitably create conflicts between individual goals and common ethics. The ethical and societal implications of these developments mirror those from other areas of human health, especially advances in tailored but expensive medical therapies. These issues outspread beyond national regulatory frameworks, highlighting their global nature and emphasizing the need for the constant and widespread dissemination of information regarding uncompleted developments and potential risks.

Expert Opinion

One of the most controversial and transformative breakthroughs in modern biology that has taken CRISPR-Cas9 by a storm is the invention of editing DNA. Because of the great level of precision, accuracy, and ease of use, it is now possible to alter the genome of an organism with great efficiency. According to leading experts, gene modification is not a highly specialized task anymore, and almost any lab around the world can conduct advanced technologies that were traditionally limited to highly sophisticated centres. Its ability to edit almost any part of the DNA has been one of the major advantages of this technique, and this is due to the fact that it is very easy to design the guide RNA. Because of its versatility, CRISPR-Cas9 can be employed to address many scientific and medical problems, including developing new treatments and making models of illnesses. Moreover, its capability to facilitate accurate modifications has opened doors for exploration into the roles of genes, complex genetic networks, and disease-causing mutation repairs. Nevertheless, the limitations of the technology are also a worrying aspect for the professionals. A significant area of concern for most still remains off-target effects, more so in therapeutic scenarios where unintentional variations may have dire significances. To counteract these threats, researchers are pointing towards validation as well as the development of new Cas types or other editing mechanisms and improved editing means. In addition, there are challenges related to delivery, with proper delivery of the CRISPR components to the appropriate tissues or cells being an important aspect of achieving clinical efficacy.

CRISPR-Cas9 technology offers tremendous possibilities, but professionals involved in ethics appear to recognize the necessity of an ethical analysis of what that entails. Germline editing does pose a deep concern for the implications of human enhancement and its effects on equity and on future generations. It appears there is agreement among scientists that the ethical and legal frameworks must be established, that they must be effective in curtailing abuse but also ensure the most effective harnessing of the benefits.

Experts generally agree that CRISPR-Cas9 has revolutionized the way that we understand and work with DNA, allowing for capabilities that eight to 10 years ago were unimaginable. Although potential barriers remain, the relentless advancement and refinement of the technology make it a cornerstone of modern biotechnology with tangible benefits across agriculture, medicine and beyond. Here is a bar chart showing how experts view CRISPR-Cas9 as a gene editing tool. Blue bars are used when you are emphasizing the importance of a particular aspect, while red bars are for highlighting the related issues or challenges. Let me know if you need any changes or more details.



Future Plans And Obstacles

- Off-target Effects: Improving CRISPR-Cas9 specificity to lessen unintentional edits.
- Delivery methods: The emergence of safe and effective ways to deliver CRISPR-Cas9 to targeted cells and tissues.
- Ethical concerns: Exploring the ethical implications of gene editing, particularly in relation to human embryos.

2. CONCLUSIONS

CRISPR-Cas9 gene-editing technology covered many essential elements of this method. In this paper we explored multiple applications of CRISPR-Cas9 in the commercial biotechnology, medical, Nano technological and clinical settings. Moreover, we explored predicted future applications and the projected impact of this technology in numerous industries, and it will certainly be extensive. In addition, the current discussions, ethical implications, and perils concerning this technology were also factored in. There are ongoing debates about the ethical, social, and legal implications of gene-editing humans and using humans as gene-editing technology utilizing CRISPR-Cas9. CRISPR-Cas9 is said here to be used in a humane, safe and sound manner. It is essential that we understand exactly what this technology means to ensure there is no ambiguity around the ethical and legal aspects of this technology, but also the benefits it can bring.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article

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