

## Targeted Gene Delivery Using CRISPR/Cas9 Precision Editing at the Cellular Level

Ziegler Zhang\*

Department of Pharmaceutical Analysis and Nuclear Pharmacy, University Bratislava, Slovakia

### Abstract

The advent of CRISPR/Cas9 technology has revolutionized the field of genetic engineering, enabling precision gene editing at the cellular level. This article explores the application of CRISPR/Cas9 in targeted gene delivery, focusing on its use in precise, efficient genetic modifications in both somatic cells and germline cells. CRISPR/Cas9 offers a transformative approach to correcting genetic defects, treating genetic diseases, and enhancing the understanding of gene function. The article covers the underlying mechanism of CRISPR/Cas9, its advantages over previous gene-editing methods, current therapeutic applications, challenges in gene delivery, and potential risks. Moreover, we examine future developments and the ethical considerations surrounding the use of CRISPR/Cas9 in therapeutic settings. The goal of this review is to present an overview of the progress and potential of CRISPR/Cas9 in targeted gene delivery and its broader implications for personalized medicine.

**Keywords:** CRISPR/Cas9; Gene delivery; Gene editing; Precision medicine; Genetic diseases; Targeted therapy; Genetic modification; CRISPR therapeutics; Cellular gene editing; Genetic engineering

### Introduction

Gene therapy has long been heralded as a potential solution for treating genetic disorders by directly correcting the underlying genetic mutations. Traditional gene therapy methods involved the delivery of therapeutic genes using viral vectors, with the goal of replacing defective genes or introducing new genetic material into a patient's cells. While these approaches showed promise, they often faced significant limitations, including off-target effects, immune reactions, and challenges in delivering genes to the right cells in the body [1].

In 2012, the discovery of the CRISPR/Cas9 system revolutionized the field of gene editing. CRISPR, which stands for "Clustered Regularly Interspaced Short Palindromic Repeats," is a bacterial immune defense mechanism that has been repurposed for precision gene editing. The Cas9 protein, which works alongside CRISPR RNA, enables the targeted cutting of DNA at specific locations in the genome, allowing for precise modifications to the DNA sequence [2].

CRISPR/Cas9 has gained significant attention for its potential in gene therapy because of its simplicity, efficiency, and ability to make specific changes to the genome at a fraction of the cost and time compared to previous technologies. With the ability to directly edit the DNA in living organisms, CRISPR/Cas9 has opened new doors in both basic research and therapeutic applications. In particular, the ability to deliver CRISPR/Cas9 components (Cas9 protein and guide RNA) to the targeted cells in the body in a controlled manner is key to achieving precision genetic modification. This article delves into the mechanism of CRISPR/Cas9, its use in targeted gene delivery, the challenges and advancements in the field, and its potential to revolutionize medicine [3].

### Description

CRISPR/Cas9 functions as a molecular scissors that can cut DNA at precise locations within the genome. The system is composed of two main components. CRISPR RNA (crRNA) This RNA sequence guides the Cas9 protein to the specific location of the genome that needs to be edited. The crRNA sequence is complementary to the target DNA sequence, ensuring that the cut is made at the correct site [4].

**Cas9 Protein** This is an endonuclease that unwinds the DNA and makes a double-strand break at the targeted location. Once the DNA is cut, the cell's natural repair mechanisms—non-homologous end joining (NHEJ) or homology-directed repair (HDR)—are activated. These repair processes can be harnessed to insert, delete, or correct specific genes. The simplicity and versatility of the CRISPR/Cas9 system allow for a wide range of genetic modifications, from knocking out genes to inserting new genes or correcting mutations in existing ones. The targeted delivery of the CRISPR/Cas9 components into the desired cells is a critical step in ensuring that the gene editing is localized and precise [5].

Delivering CRISPR/Cas9 components into cells is an essential step for achieving effective gene editing. Several strategies have been developed for targeted gene delivery, each with its own advantages and challenges. These include viral vectors, lipid nanoparticles (LNPs), electroporation, and microinjection. Viral vectors, such as lentiviruses, adenoviruses, and adeno-associated viruses (AAVs), are commonly used for gene delivery due to their ability to efficiently introduce foreign genetic material into cells. In the case of CRISPR/Cas9, viral vectors can be engineered to carry both the Cas9 protein and the guide RNA (gRNA). However, the use of viral vectors poses several challenges, including immune responses and the risk of insertional mutagenesis [6].

Lipid nanoparticles are emerging as a promising non-viral method for CRISPR/Cas9 delivery. LNPs encapsulate the Cas9 protein and gRNA, protecting them from degradation and facilitating their entry into cells. LNPs are particularly effective for in vivo gene editing,

**\*Corresponding author:** Ziegler Zhang, Department of Pharmaceutical Analysis and Nuclear Pharmacy, University Bratislava, Slovakia, E-mail: zhangzieg16@yahoo.com

**Received:** 02-Dec-2024, Manuscript No: jabt-25-157730, **Editor Assigned:** 06-Dec-2024, pre QC No: jabt-25-157730 (PQ), **Reviewed:** 20-Dec-2024, QC No: jabt-25-157730, **Revised:** 25-Dec-2024, Manuscript No: jabt-25-157730 (R), **Published:** 30-Dec-2024, DOI: 10.4172/2155-9872.1000707

**Citation:** Ziegler Z (2024) Targeted Gene Delivery Using CRISPR/Cas9 Precision Editing at the Cellular Level. J Anal Bioanal Tech 15: 707.

**Copyright:** © 2024 Ziegler Z. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

including applications in tissues such as the liver and lung. LNP-based delivery systems have been used in several clinical trials, particularly for mRNA vaccines, and are now being adapted for gene editing. Electroporation uses an electric field to increase the permeability of the cell membrane, allowing the CRISPR/Cas9 components to enter the cell. This method is effective for delivering CRISPR/Cas9 to primary cells, stem cells, and embryos but may cause cell damage due to the electric shock [7].

Microinjection involves directly injecting the CRISPR/Cas9 components into the cytoplasm or nucleus of cells. While this method is highly efficient for specific applications, it is labor-intensive and not suitable for high-throughput or large-scale gene editing. The ability to precisely edit genes using CRISPR/Cas9 has broad applications, particularly in the treatment of genetic diseases. Some key areas of application include. Monogenic diseases, such as cystic fibrosis, sickle cell anemia, and Duchenne muscular dystrophy, are caused by mutations in a single gene. CRISPR/Cas9 offers a potential cure for these diseases by directly correcting the mutation at the DNA level. For example, researchers have used CRISPR/Cas9 to correct the mutation in the beta-globin gene in sickle cell anemia patients, showing promise for long-term treatment [8].

CRISPR/Cas9 has also shown potential in cancer therapy, particularly in engineering immune cells for adoptive cell therapy (ACT). By editing immune cells such as T-cells or natural killer (NK) cells, researchers aim to enhance their ability to target and destroy cancer cells. Additionally, CRISPR/Cas9 can be used to knock out genes in cancer cells that promote tumor growth or resistance to treatment. Targeted gene delivery using CRISPR/Cas9 has shown significant potential for treating inherited eye diseases such as Leber congenital amaurosis (LCA), a rare genetic disorder that causes blindness. In preclinical studies, CRISPR/Cas9 has been used to repair the defective gene in the retinal cells, restoring vision in animal models [9].

Researchers are investigating the use of CRISPR/Cas9 to repair or replace damaged genes in muscle and nerve cells, offering potential treatments for diseases such as muscular dystrophy and spinal muscular atrophy (SMA). The ability to correct genetic mutations at the cellular level could result in effective therapies that prevent or reverse the progression of these diseases [10].

## Discussion

Despite the promising potential of CRISPR/Cas9, several challenges remain in achieving efficient and safe targeted gene delivery. One of the biggest concerns with CRISPR/Cas9 gene editing is the possibility of off-target effects, where the Cas9 protein cuts the DNA at unintended locations. These off-target edits can lead to unwanted mutations, potentially causing harm to the patient. Researchers are working to refine the accuracy of CRISPR/Cas9 by optimizing the guide RNA and improving the specificity of Cas9 variants to minimize off-target effects. The use of CRISPR/Cas9 components, particularly when delivered via viral vectors or nanoparticles, can trigger immune responses in the body. These responses may reduce the effectiveness of gene therapy and cause adverse side effects. Developing non-immunogenic delivery systems and improving the immunotolerance of CRISPR components are key areas of ongoing research.

Efficiently delivering CRISPR/Cas9 components to the right cells in the body remains a major challenge. Many tissues, particularly those in deep organs like the brain or heart, are difficult to target with current delivery methods. Advances in nanotechnology and tissue-specific

targeting are expected to improve the precision and efficiency of CRISPR delivery systems. The use of CRISPR/Cas9 in human germline editing (i.e., editing the DNA of embryos or reproductive cells) raises significant ethical concerns. Germline editing has the potential to permanently alter the human gene pool, with unknown long-term consequences. Ethical debates continue around whether germline editing should be allowed, and regulatory frameworks are still evolving to address these concerns.

The field of CRISPR/Cas9 is rapidly advancing, with numerous improvements in both the technology and its delivery methods. Some promising developments include Base Editing and Prime Editing. These are newer forms of CRISPR technology that allow for even more precise and accurate editing of genetic sequences, with minimal errors. These technologies have the potential to reduce off-target effects and improve the safety of CRISPR-based therapies.

**In Vivo Gene Editing** The development of more efficient in vivo delivery systems, such as optimized viral vectors and lipid nanoparticles, holds promise for enabling gene therapy without the need for ex vivo modification of cells. This could lead to simpler and more effective treatments for genetic diseases. Personalized Medicine As our understanding of the human genome expands, CRISPR/Cas9-based therapies may become an integral part of personalized medicine, allowing for the development of individualized treatments based on a person's genetic makeup.

## Conclusion

CRISPR/Cas9-based gene editing offers unprecedented precision and efficiency in genetic modification, with the potential to revolutionize the treatment of genetic diseases. Targeted gene delivery using CRISPR/Cas9 can correct mutations, repair damaged genes, and provide long-term therapeutic benefits. However, challenges related to off-target effects, immune responses, and efficient delivery to target cells need to be addressed before widespread clinical applications can be realized. Despite these hurdles, ongoing advancements in CRISPR technology and delivery systems, coupled with growing understanding of gene function, are paving the way for future breakthroughs in gene therapy. With continued research, CRISPR/Cas9 could become a cornerstone of precision medicine, offering hope for millions of patients suffering from genetic disorders.

## Acknowledgement

None

## Conflict of Interest

None

## References

1. Landers JP (2008) Handbook of capillary and microchip electrophoresis and associated microtechniques. CRC Press Boca Raton.
2. Eriksson L, Johansson E, Kettaneh-Wold N, Wikström C, Wold S (2008) Design of Experiments principles and applications, Umetrics Academy Umea Sweden.
3. Anselmo AC, Mitragotri S (2014) An overview of clinical and commercial impact of drug delivery systems. J Control Release 190: 1528.
4. Dawidczyk CM (2014) State-of-the-art in design rules for drug delivery platforms: Lessons learned from FDA-approved nanomedicines. J Control Release 187: 13344.
5. Amidon GL (1995) A theoretical basis for a biopharmaceutical drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res 12: 41320.

6. Yu LX (2002) Biopharmaceutics classification system: the scientific basis for biowaiver extensions. *Pharm Res* 19: 9215.
7. Yu LX (1996) Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption. *Adv Drug Deliv Rev* 19: 35976.
8. Shi Y (2009) Recent advances in intravenous delivery of poorly water-soluble compounds. *Expert Opin Drug Deliv* 6: 126182.
9. Shoghi E (2013) Solubility-pH profiles of some acidic, basic and amphoteric drugs. *Eur J Pharm Sci* 48: 291300.
10. Voelgyi G (2010) Study of pH-dependent solubility of organic bases Revisit of Henderson-Hasselbalch relationship. *Anal Chim Acta* 673: 406.