Advanced International Journal for Research (AIJFR)
E-ISSN: 3048-7641 ● Website: www.aijfr.com ● Email: editor@aijfr.com

Nanoparticle-Mediated CRISPR-Cas9 Delivery: Current Strategies, Pharmacological Challenges and Therapeutic Applications in Genetic Disorders

Shouvik Mondal [1], Kriti Kumari [2]

[1]Assistant Professor, Department of Pharmaceutics, Netaji Subhas University, Jamshedpur, 831012
[2]Research Scholar, Department of Pharmaceutics, Netaji Subhas University, Jamshedpur, 831012

ABSTRACT

The CRISPR-Cas9 genome-editing system has emerged as a promising technology for the precise correction of disease-causing mutations and has shown significant potential in treating various genetic disorders. However, the successful clinical application of CRISPR-Cas9 largely depends on the development of safe and efficient delivery systems. Among the available approaches, nanoparticle-mediated delivery has gained considerable attention for its low immunogenicity, high biocompatibility, improved targeting, and capacity to deliver diverse CRISPR cargoes. This review summarises the current strategies of nanoparticle-mediated CRISPR-Cas9 delivery, including lipid, polymeric, hybrid, inorganic, and biomimetic nanoparticles. It also discusses major pharmacological challenges such as off-target effects, biodistribution, endosomal escape, immunogenicity, toxicity, and regulatory considerations. Furthermore, recent therapeutic applications in genetic disorders, including sickle cell disease, β-thalassemia, Duchenne muscular dystrophy, cystic fibrosis, hereditary transthyretin amyloidosis, and cancer, are highlighted. Finally, future perspectives on advanced nanocarrier design and clinical translation are discussed. Nanoparticle-mediated CRISPR-Cas9 delivery holds great promise for advancing precision medicine and developing effective therapies for genetic disorders.

Keywords: CRISPR-Cas9; Nanoparticles; Genome editing; Genetic disorders; Gene therapy; Drug delivery.

1. INTRODUCTION

Genetic disorders result from inherited or acquired alterations in the genome that disrupt normal cellular functions and contribute to a wide range of debilitating and life-threatening diseases. Conventional therapeutic strategies, including pharmacological treatment, enzyme replacement therapy, and supportive care, often alleviate symptoms without correcting the underlying genetic abnormalities. The emergence of genome-editing technologies has revolutionised molecular medicine by enabling precise modification of disease-causing genes. Among these technologies, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated protein 9 (CRISPR-Cas9) system has become the most

versatile and efficient gene-editing platform because of its simplicity, programmability, high editing efficiency, and cost-effectiveness. Since its development, CRISPR-Cas9 has demonstrated remarkable potential for the treatment of numerous inherited and acquired diseases, including sickle cell disease, β-thalassemia, cystic fibrosis, Duchenne muscular dystrophy, retinal disorders, and various cancers. [1–4]

The CRISPR-Cas9 system consists of a Cas9 endonuclease guided by a single-guide RNA (sgRNA), which directs the nuclease to a complementary target DNA sequence adjacent to a protospacer adjacent motif (PAM). Cas9 induces a site-specific double-stranded DNA break that is subsequently repaired through endogenous cellular mechanisms, primarily non-homologous end joining (NHEJ) or homology-directed repair (HDR), enabling targeted gene disruption, correction, or insertion. Despite its exceptional therapeutic potential, the clinical success of CRISPR-Cas9 is critically dependent on the development of safe, efficient, and tissue-specific delivery systems capable of transporting genome-editing components into target cells while minimising off-target effects and immune responses. [4–7]

Efficient delivery remains one of the greatest barriers to the clinical translation of CRISPR-Cas9-based therapies. Viral vectors such as adeno-associated viruses (AAVs), adenoviruses, and lentiviruses have demonstrated high transfection efficiency; however, their clinical application is limited by immunogenicity, insertional mutagenesis, restricted cargo capacity, manufacturing complexity, and potential long-term safety concerns. Consequently, nanoparticle-based non-viral delivery systems have emerged as promising alternatives due to their superior biocompatibility, design flexibility, lower immunogenicity, and ability to deliver diverse CRISPR cargoes, including plasmid DNA, messenger RNA (mRNA), single-guide RNA, and ribonucleoprotein (RNP) complexes. [5–10]

Recent advances in nanotechnology have enabled the development of lipid nanoparticles, polymeric nanoparticles, lipid-polymer hybrid nanoparticles, gold nanoparticles, silica nanoparticles, magnetic nanoparticles, extracellular vesicles, and other biomimetic nanocarriers for CRISPR-Cas9 delivery. These nanocarriers protect gene-editing components from enzymatic degradation, enhance cellular uptake, facilitate endosomal escape, improve intracellular trafficking, and allow controlled release at target sites. Furthermore, surface modification with targeting ligands, antibodies, peptides, and polymers has significantly improved tissue specificity while reducing systemic toxicity, thereby enhancing the therapeutic efficacy of genome editing. [6–11]

Despite these advances, several pharmacological challenges remain before nanoparticle-mediated CRISPR-Cas9 delivery can achieve widespread clinical application. Important concerns include biodistribution, pharmacokinetics, pharmacodynamics, biodegradability, biocompatibility, immune activation, off-target genome editing, intracellular stability, large-scale manufacturing, regulatory compliance, and long-term biosafety. Addressing these limitations is essential for improving therapeutic precision and ensuring safe clinical translation. [10–14]

Encouraging preclinical studies and early clinical trials have demonstrated the potential of nanoparticle-mediated CRISPR-Cas9 therapy in treating hereditary transthyretin amyloidosis, sickle cell disease, β-thalassemia, muscular dystrophy, metabolic disorders, retinal diseases, and several forms of cancer. These advances highlight the growing importance of integrating nanotechnology with genome editing to develop targeted, personalised, and minimally invasive therapies for genetic disorders. [12–15]

This review comprehensively discusses the current strategies employed in nanoparticle-mediated CRISPR-Cas9 delivery, examines the major pharmacological challenges that limit clinical translation, and highlights recent therapeutic applications in genetic disorders. Additionally, future perspectives on advanced nanocarrier design, targeted delivery approaches, and emerging clinical developments are presented to provide insights into the future of precision genome-editing therapeutics.

Table 1: Comparison of nanoparticle delivery systems [10, 16, 20, 21, 22]

Strategy	Advantages	Limitations	Applications
Lipid Nanoparticles (LNPs)	High efficiency, clinically advanced	Liver accumulation	Gene therapy
Polymeric Nanoparticles	Controlled release, stable	Cytotoxicity	Cancer, genetic disorders
Lipid–Polymer Hybrid NPs	High stability, improved delivery	Complex formulation	Targeted gene editing
Inorganic Nanoparticles	Easy functionalization	Potential toxicity	Targeted delivery
Extracellular Vesicles (EVs)	Biocompatible, low immunogenicity	Difficult production	Precision medicine
Targeted Nanoparticle Delivery	High specificity, reduced off-target effects	Complex design	Personalised gene therapy

2. CURRENT STRATEGIES FOR NANOPARTICLE-MEDIATED CRISPR-CAS9 DELIVERY

Efficient delivery of CRISPR-Cas9 components is a critical determinant of successful genome editing. An ideal delivery system should protect the gene-editing machinery from enzymatic degradation, facilitate cellular uptake, promote endosomal escape, and achieve targeted intracellular release while minimising toxicity and immune responses. Nanoparticle-based delivery systems have emerged as promising non-viral alternatives because of their high loading capacity, tunable physicochemical properties, and improved safety profile compared with viral vectors. Depending on their composition and mechanism of action, these nanocarriers can deliver CRISPR-Cas9 as plasmid DNA, messenger RNA (mRNA), single-guide RNA (sgRNA), or ribonucleoprotein (RNP) complexes. [16-20]

A. Lipid Nanoparticles (LNPs)

Lipid nanoparticles are the most clinically advanced non-viral delivery systems for CRISPR-Cas9. They consist of ionizable lipids, phospholipids, cholesterol, and polyethene glycol (PEG)-lipids, which collectively improve stability, encapsulation efficiency, and intracellular delivery. LNPs efficiently deliver Cas9 mRNA and sgRNA to target tissues, particularly the liver, and have demonstrated promising therapeutic outcomes in hereditary transthyretin amyloidosis and other genetic diseases. Their biodegradability, scalability, and reduced immunogenicity make them attractive candidates for clinical translation. [16, 20, 22]

B. Polymeric Nanoparticles

Polymeric nanoparticles prepared from biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA), polyethyleneimine (PEI), chitosan, and poly(β-amino esters) are widely investigated for

CRISPR delivery. These systems provide structural stability, controlled release, and protection of nucleic acids against degradation. Surface modifications further enhance cellular uptake and reduce cytotoxicity, making polymeric nanoparticles suitable for targeted gene-editing applications. [17-19]

C. Lipid–Polymer Hybrid Nanoparticles

Lipid–polymer hybrid nanoparticles combine the structural strength of polymeric cores with the biocompatibility of lipid shells. This hybrid design improves encapsulation efficiency, prolongs circulation time, enhances endosomal escape, and increases gene-editing efficiency. Such nanoparticles have shown considerable potential for targeted delivery in cancer therapy and inherited genetic disorders. [18, 19, 22]

D. Inorganic Nanoparticles

Inorganic nanocarriers, including gold nanoparticles, silica nanoparticles, magnetic nanoparticles, and calcium phosphate nanoparticles, possess unique optical, magnetic, and physicochemical properties that facilitate efficient CRISPR-Cas9 delivery. Gold nanoparticles are particularly attractive because of their ease of surface functionalization and low toxicity, whereas magnetic nanoparticles enable magnetically guided targeted delivery. [17,20]

E. Extracellular Vesicles and Biomimetic Nanocarriers

Extracellular vesicles (EVs), including exosomes, have gained attention as natural delivery vehicles due to their excellent biocompatibility, low immunogenicity, and intrinsic ability to transport nucleic acids between cells. Biomimetic nanocarriers, such as cell membrane-coated nanoparticles, further enhance immune evasion and tissue-specific targeting, improving the therapeutic potential of CRISPR-Cas9. [21, 23]

F. Targeted Nanoparticle Delivery

To improve editing specificity and minimise off-target effects, nanoparticles are frequently functionalized with targeting ligands such as antibodies, peptides, aptamers, carbohydrates, and small molecules. These ligands recognise specific cell surface receptors and promote receptor-mediated endocytosis, enabling selective delivery to diseased tissues while reducing systemic toxicity. Stimuli-responsive nanoparticles that release CRISPR components in response to pH, enzymes, redox conditions, or external stimuli are also being developed to enhance therapeutic precision. [22-25]

Overall, advances in nanoparticle engineering have significantly improved the safety, efficiency, and specificity of CRISPR-Cas9 delivery. Continued optimisation of nanocarrier design is expected to accelerate the clinical translation of genome-editing therapies for a wide range of genetic disorders. [16, 20, 22, 25]

3. PHARMACOLOGICAL CHALLENGES IN NANOPARTICLE-MEDIATED CRISPR-CAS9 DELIVERY

Despite significant advances in nanoparticle-mediated CRISPR-Cas9 delivery, several pharmacological challenges continue to limit its widespread clinical application. The therapeutic success of genome editing depends not only on the efficiency of gene modification but also on the safety, stability, biodistribution, and pharmacokinetic behaviour of the delivery system. Addressing these challenges is

essential for the successful translation of CRISPR-Cas9 therapeutics from laboratory research to clinical practice. [26-29]

Table 2: Pharmacological challenges and possible solutions in nanoparticle - mediated CRISPR-Cas9 delivery [10, 16, 20, 29]

Challenge	Impact	Possible Solution
Biodistribution	Poor tissue targeting	Ligand-functionalized nanoparticles
Endosomal trapping	Low editing efficiency	pH-responsive nanoparticles
Off-target editing	Unwanted mutations	High-fidelity Cas9, optimized sgRNA
Immunogenicity	Reduced efficacy	Biocompatible nanocarriers
Biocompatibility & biodegradability	Potential toxicity	Biodegradable materials
Manufacturing and Regulatory Challenges	Clinical translation issues	Standardised GMP production
Ethical and Clinical Considerations	Challenges in clinical adoption	Regulatory oversight and long-term monitoring

A. Bio distribution and Pharmacokinetics

After systemic administration, nanoparticles encounter multiple biological barriers that influence their circulation time, tissue distribution, and accumulation at target sites. Nanoparticle size, surface charge, composition, and surface modification significantly affect pharmacokinetic properties. Rapid clearance by the mononuclear phagocyte system (MPS), particularly in the liver and spleen, may reduce delivery efficiency and therapeutic outcomes. Strategies such as PEGylation and ligand-mediated targeting have been employed to prolong circulation time and enhance tissue-specific delivery. [26, 27, 29]

B. Cellular Uptake and Endosomal Escape

Efficient intracellular delivery remains a major obstacle for CRISPR-Cas9 therapeutics. Following endocytosis, many nanoparticles become trapped within endosomes, where degradation of CRISPR components may occur before reaching the nucleus. Several nanocarrier designs incorporate ionizable lipids, pH-responsive polymers, and membrane-disruptive peptides to facilitate endosomal escape and improve genome-editing efficiency. [27, 29, 30]

C. Off-Target Genome Editing

Off-target cleavage is one of the most important safety concerns associated with CRISPR-Cas9 technology. Unintended DNA modifications may lead to mutations, chromosomal rearrangements, or disruption of essential genes. Improving guide RNA design, engineering high-fidelity Cas9 variants, optimising nanoparticle delivery, and controlling intracellular exposure time are effective strategies for reducing off-target effects and improving editing precision. [29-33]

D. Immunogenicity and Toxicity

Both CRISPR-Cas9 components and nanoparticle carriers can induce immune responses. Cas9 proteins derived from bacterial species may trigger adaptive and innate immune reactions, while certain nanoparticle materials may activate inflammatory pathways or complement systems. Therefore, the

development of biocompatible, biodegradable, and low-immunogenic nanocarriers is critical for ensuring patient safety during repeated administration. [26, 28, 30]

E. Biocompatibility and Biodegradability

Nanoparticles intended for clinical use must exhibit excellent biocompatibility and controlled biodegradation without producing toxic metabolites. Lipid nanoparticles and biodegradable polymeric systems generally demonstrate favourable safety profiles; however, inorganic nanoparticles may accumulate in tissues and require careful toxicity evaluation. Long-term biocompatibility studies remain essential before clinical approval. [26, 27, 31]

F. Manufacturing and Regulatory Challenges

Table 3: Regulatory considerations for clinical translation of nanoparticle - mediated CRISPR-Cas9 therapeutics. [20, 26, 29, 31, 45]

Agency	Requirement
FDA (Food and Drug Administration)	Safety and efficacy
EMA (European Medicines Agency)	Toxicology assessment
ICH (International Council for Harmonisation)	Quality guidelines
GMP (Good Manufacturing Practice)	Manufacturing standards
GLP (Good Laboratory Practice)	Preclinical studies
GCP (Good Clinical Practice)	Clinical trials

Large-scale manufacturing of nanoparticle-based CRISPR delivery systems requires reproducible synthesis, high encapsulation efficiency, product stability, and strict quality control. Regulatory agencies require comprehensive evaluation of pharmacokinetics, toxicology, immunogenicity, efficacy, and long-term safety before clinical approval. The absence of standardised manufacturing protocols and regulatory guidelines continues to slow the clinical translation of many nanoparticle formulations. [26, 29, 31]

G. Ethical and Clinical Considerations

Clinical implementation of CRISPR-Cas9 also raises ethical concerns regarding unintended genetic modifications, germline editing, patient consent, equitable access to treatment, and long-term monitoring of treated individuals. International regulatory frameworks emphasise responsible research practices and rigorous clinical evaluation to ensure the safe and ethical application of genome-editing technologies. [32-35]

Overall, overcoming these pharmacological challenges will require interdisciplinary collaboration among pharmaceutical scientists, nanotechnologists, molecular biologists, clinicians, and regulatory authorities. Continued innovation in nanoparticle engineering and genome-editing technology is expected to improve the safety, specificity, and clinical success of CRISPR-Cas9-based therapies. [26-35]

4. THERAPEUTIC APPLICATIONS OF NANOPARTICLE-MEDIATED CRISPR-CAS9 DELIVERY IN GENETIC DISORDERS

Nanoparticle-mediated CRISPR-Cas9 delivery has demonstrated significant therapeutic potential in the treatment of a wide range of inherited and acquired genetic disorders. By enabling efficient, targeted,

and non-viral delivery of genome-editing components, nanoparticle-based systems overcome many limitations associated with viral vectors, including immunogenicity, limited cargo capacity, and insertional mutagenesis. Recent preclinical studies and early-phase clinical trials have highlighted the potential of these nanocarriers in correcting disease-causing mutations and improving therapeutic outcomes. [36-40]

A. Sickle Cell Disease and β-Thalassemia

Sickle cell disease (SCD) and β-thalassemia are among the most extensively studied genetic disorders for CRISPR-Cas9 therapy. Nanoparticle-mediated delivery enables efficient editing of hematopoietic stem cells by targeting the BCL11A gene or correcting mutations in the HBB gene, thereby restoring fetal haemoglobin production and reducing disease severity. These approaches have shown promising results in both preclinical and clinical studies. [41, 45]

B. Hereditary Transthyretin Amyloidosis

Hereditary transthyretin (ATTR) amyloidosis is the first disease successfully treated using in vivo CRISPR-Cas9 genome editing delivered through lipid nanoparticles. Systemic administration of lipid nanoparticles carrying Cas9 mRNA and guide RNA targeting the TTR gene resulted in a substantial reduction in circulating transthyretin protein levels, demonstrating the feasibility of nanoparticle-mediated gene editing in humans. [39, 45]

C. Duchenne Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is caused by mutations in the DMD gene, leading to progressive muscle degeneration. CRISPR-Cas9 delivered via nanoparticles has shown the ability to restore dystrophin expression by correcting disease-causing mutations in animal models. Polymeric and lipid-based nanoparticles have demonstrated encouraging therapeutic efficacy with reduced systemic toxicity. [36, 37]

D. Cystic Fibrosis

Cystic fibrosis results from mutations in the CFTR gene, causing impaired chloride ion transport and progressive pulmonary disease. Nanoparticle-based CRISPR delivery systems have been explored to correct CFTR mutations in airway epithelial cells, providing a promising strategy for long-term treatment while minimising immune responses associated with viral vectors. [38, 45]

E. Inherited Retinal Disorders

Inherited retinal diseases such as Leber congenital amaurosis and retinitis pigmentosa have become attractive targets for CRISPR-Cas9 therapy due to the accessibility of ocular tissues. Nanoparticle-mediated delivery enables localised genome editing with reduced systemic exposure and has demonstrated restoration of retinal function in preclinical studies. [42, 45]

F. Cancer Gene Therapy

Nanoparticle-mediated CRISPR-Cas9 systems are also being investigated for cancer treatment by targeting oncogenes, tumour suppressor genes, and immune checkpoint pathways. Delivery of CRISPR components to tumour tissues can inhibit tumour growth, enhance antitumor immunity, and improve the efficacy of immunotherapies while reducing off-target toxicity. [40-45]

G. Other Genetic Disorders

Beyond these diseases, nanoparticle-mediated CRISPR-Cas9 delivery is being explored for Huntington's disease, haemophilia, metabolic disorders, lysosomal storage diseases, spinal muscular atrophy, and several rare monogenic disorders. Continuous improvements in nanocarrier engineering and genome-editing technologies are expected to expand the therapeutic applications of CRISPR-Cas9 in precision medicine. [36, 43, 45]

Overall, nanoparticle-based CRISPR-Cas9 delivery represents a transformative approach for treating genetic disorders by combining efficient genome editing with targeted, safe, and minimally invasive delivery. Ongoing clinical trials and technological innovations are likely to accelerate its translation into routine clinical practice. [36-45]

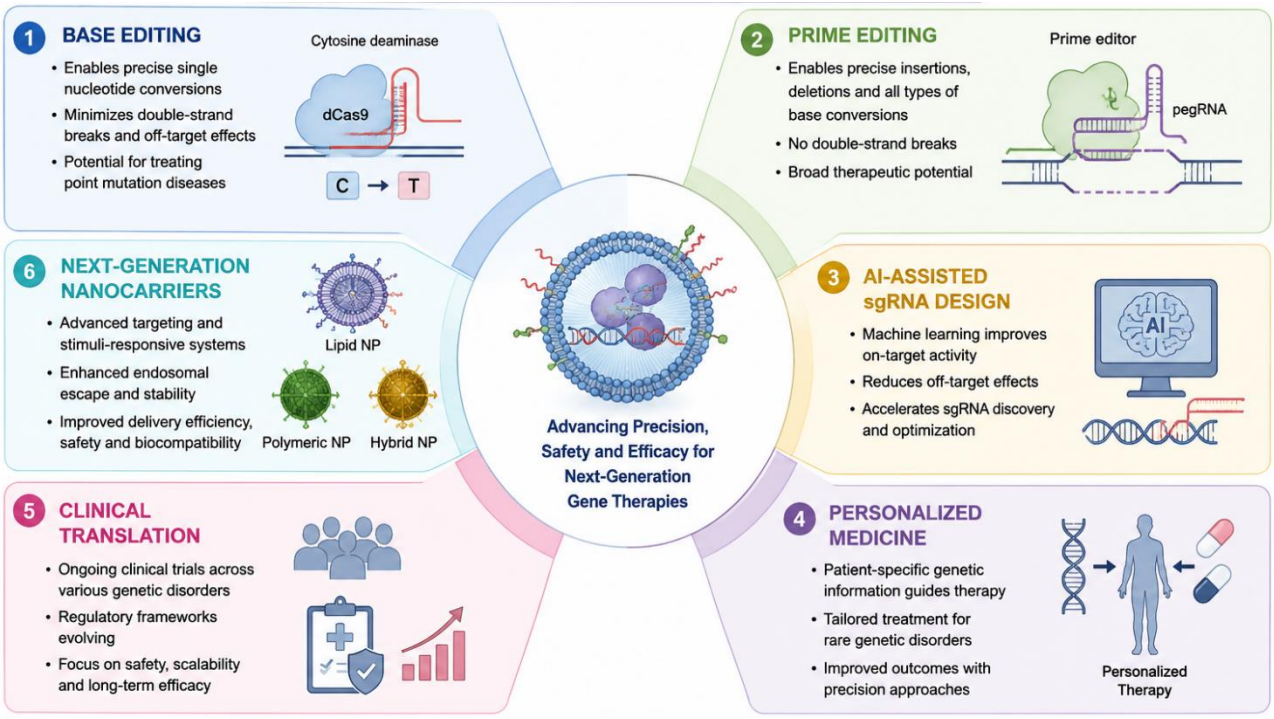
5. FUTURE PERSPECTIVES

Nanoparticle-mediated CRISPR-Cas9 delivery is rapidly advancing toward clinical translation, with continuous improvements in nanocarrier design, genome-editing precision, and targeted drug delivery. Future research is expected to focus on developing next-generation nanoparticles with enhanced biocompatibility, biodegradability, prolonged circulation time, and efficient tissue-specific targeting. Surface engineering using antibodies, peptides, aptamers, and cell membrane coatings may further improve selective delivery while minimising off-target effects and systemic toxicity. [36-39]

Table 4: Emerging genome-editing technologies and their potential advantages for CRISPR-based therapeutics [24, 29, 31, 32, 33]

Technology	Major Advantage
Base Editing	No double-strand break
Prime Editing	High precision
CRISPRa	Gene activation
CRISPRi	Gene silencing
AI-assisted Design	Optimized sgRNA
Biomimetic Nanoparticles	Improved targeting

Figure 1: Future directions of CRISPR/Cas9 therapeutics highlighting base editing, prime editing, AI-assisted sgRNA design, personalised medicine, next-generation nanocarriers, and improved clinical translation. [20, 24, 29, 32, 33]



The emergence of novel CRISPR technologies, including base editing, prime editing, and CRISPR-associated nucleases with improved specificity, offers new opportunities for treating previously untreatable genetic disorders. Combining these advanced editing platforms with smart nanoparticles capable of stimuli-responsive drug release may significantly enhance therapeutic efficacy and safety. Artificial intelligence and machine learning are also expected to play an important role in optimising nanoparticle formulation, predicting biodistribution, and designing guide RNAs with reduced off-target activity. [40-43]

Despite encouraging progress, several challenges remain before widespread clinical implementation becomes possible. Large-scale manufacturing, reproducibility, regulatory approval, long-term safety evaluation, ethical considerations, and cost-effectiveness must be addressed through collaborative efforts involving researchers, clinicians, pharmaceutical industries, and regulatory agencies. Continued interdisciplinary research will accelerate the development of personalised genome-editing therapies for a broad spectrum of inherited diseases. [44, 45]

6. CONCLUSION

Nanoparticle-mediated CRISPR-Cas9 delivery has emerged as one of the most promising strategies for safe and efficient genome editing. Compared with viral vectors, nanoparticle-based systems offer improved biocompatibility, flexible cargo loading, lower immunogenicity, and greater potential for targeted delivery. Considerable progress has been achieved in the development of lipid nanoparticles, polymeric nanoparticles, hybrid nanocarriers, inorganic nanoparticles, and biomimetic delivery systems, resulting in enhanced gene-editing efficiency and improved therapeutic outcomes.

Although significant pharmacological challenges such as off-target effects, immune responses, biodistribution, intracellular delivery, and regulatory issues continue to limit clinical translation, ongoing advances in nanotechnology and genome engineering are steadily overcoming these barriers. Successful clinical studies in diseases such as hereditary transthyretin amyloidosis and promising results in sickle cell disease, β-thalassemia, Duchenne muscular dystrophy, cystic fibrosis, retinal disorders, and cancer demonstrate the tremendous therapeutic potential of nanoparticle-mediated CRISPR-Cas9 delivery.

Overall, the integration of nanotechnology with CRISPR-Cas9 genome editing represents a transformative approach for precision medicine. Continued innovation in nanoparticle engineering, targeted delivery systems, and gene-editing technologies is expected to expand clinical applications and provide safer, more effective, and personalised treatments for a wide range of genetic disorders in the future.

REFERENCES

1. M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J. A. Doudna, and E. Charpentier, "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity," Science, vol. 337, no. 6096, pp. 816–821, 2012.
2. L. Cong, F. A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, et al., "Multiplex genome engineering using CRISPR/Cas systems," Science, vol. 339, no. 6121, pp. 819–823, 2013.
3. P. Mali, L. Yang, K. M. Esvelt, J. Aach, M. Guell, J. E. DiCarlo, et al., "RNA-guided human genome engineering via Cas9," Science, vol. 339, no. 6121, pp. 823–826, 2013.
4. J. A. Doudna and E. Charpentier, "The new frontier of genome engineering with CRISPR-Cas9," Science, vol. 346, no. 6213, Art. no. 1258096, 2014.
5. C. A. Lino, J. C. Harper, J. P. Carney, and J. A. Timlin, "Delivering CRISPR: A review of the challenges and approaches," Drug Deliv., vol. 25, no. 1, pp. 1234–1257, 2018.
6. D. C. Luther, Y. W. Lee, H. Nagaraj, and V. M. Rotello, "Delivery approaches for CRISPR/Cas9 therapeutics in vivo: Advances and challenges," Expert Opin. Drug Deliv., vol. 15, no. 9, pp. 905–913, 2018.
7. D. Wilbie, J. Walther, and E. Mastrobattista, "Delivery aspects of CRISPR/Cas for in vivo genome editing," Acc. Chem. Res., vol. 52, no. 6, pp. 1555–1564, 2019.
8. X. Xu, T. Wan, H. Xin, D. Li, H. Pan, J. Wu, and Y. Ping, "Delivery of CRISPR/Cas9 for therapeutic genome editing," J. Gene Med., vol. 21, no. 7, Art. no. e3107, 2019.
9. H. Yin, K. J. Kauffman, and D. G. Anderson, "Delivery technologies for genome editing," Nat. Rev. Drug Discov., vol. 16, no. 6, pp. 387–399, 2017.
10. C. F. Xu and J. Wang, "Delivery systems for CRISPR/Cas9 genome editing: Progress and challenges," Acta Pharm. Sin. B, vol. 11, no. 8, pp. 2151–2171, 2021.
11. L. Li, S. Hu, and X. Chen, "Non-viral delivery systems for CRISPR/Cas9-based genome editing: Challenges and opportunities," Biomaterials, vol. 171, pp. 207–218, 2018.
12. J. D. Gillmore, E. Gane, J. Taubel, J. Kao, M. Fontana, M. L. Maitland, et al., "CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis," N. Engl. J. Med., vol. 385, no. 6, pp. 493–502, 2021.

13. H. Li, Y. Yang, W. Hong, M. Huang, M. Wu, and X. Zhao, "Applications of genome editing technology in the targeted therapy of human diseases: Mechanisms, advances and prospects," Signal Transduct. Target. Ther., vol. 5, Art. no. 1, 2020.
14. P. Wang, L. Zhang, Y. Xie, N. Wang, R. Tang, W. Zheng, et al., "Genome editing for cancer therapy: Delivery of CRISPR/Cas9 by nanoparticles," Front. Bioeng. Biotechnol., vol. 9, Art. no. 673286, 2021.
15. T. Wei, Q. Cheng, Y. L. Min, E. N. Olson, and D. J. Siegwart, "Systemic nanoparticle delivery of CRISPR-Cas9 ribonucleoproteins for therapeutic genome editing," Nat. Nanotechnol., vol. 15, pp. 1012–1019, 2020.
16. B. E. Givens, Y. W. Naguib, S. M. Geary, E. J. Devor, and A. K. Salem, "Nanoparticle-Based Delivery of CRISPR/Cas9 Genome-Editing Therapeutics," AAPS J., vol. 20, no. 6, Art. no. 108, 2018.
17. B. Ashok, N. A. Peppas, and M. E. Wechsler, "Lipid- and polymer-based nanoparticle systems for the delivery of CRISPR/Cas9," J. Drug Deliv. Sci. Technol., vol. 65, Art. no. 102728, 2021.
18. Y. Gong, S. Tian, Y. Xuan, et al., "Lipid and polymer mediated CRISPR/Cas9 gene editing," J. Mater. Chem. B, vol. 8, no. 20, pp. 4369–4386, 2020.
19. X. Duan, K. Ouyang, X. Xu, et al., "Nanoparticle delivery of CRISPR/Cas9 for genome editing," Front. Genet., vol. 12, Art. no. 673286, 2021.
20. V. Madigan, F. Zhang, and J. E. Dahlman, "Drug delivery systems for CRISPR-based genome editors," Nat. Rev. Drug Discov., vol. 22, no. 11, pp. 875–894, 2023.
21. Q. Zheng, W. Wang, Y. Zhou, et al., "Synthetic nanoparticles for the delivery of CRISPR/Cas9 gene editing system: Classification and biomedical applications," Biomater. Sci., vol. 11, no. 16, pp. 5361–5389, 2023.
22. M. Kim, Y. Hwang, S. Lim, et al., "Advances in nanoparticles as non-viral vectors for efficient delivery of CRISPR/Cas9," Pharmaceutics, vol. 16, no. 9, Art. no. 1197, 2024.
23. H. Y. Lee, Y. Y. Choi, J. W. Kim, et al., "Nanocarriers for the delivery of the CRISPR/Cas9 system," Curr. Opin. Biotechnol., 2025.
24. A. Pickar-Oliver and C. A. Gersbach, "The next generation of CRISPR-Cas technologies and applications," Nat. Rev. Mol. Cell Biol., vol. 20, no. 8, pp. 490–507, 2019.
25. Y. Li, J. Zhao, X. Wang, et al., "Precisely targeted nanoparticles for CRISPR-Cas9 delivery in clinical applications," Adv. Drug Deliv. Rev., 2025.
26. Das S, Mondal S, inventors. Formulation and in vitro study of Ibuprofen loaded crosslinked sodium alginate and gellan gum microspheres. 2017.
27. Z. Glass, M. Lee, Y. Li, and Q. Xu, "Engineering the delivery system for CRISPR-based genome editing," Trends Biotechnol., vol. 36, no. 2, pp. 173–185, 2018.
28. B. H. Yip, "Recent advances in CRISPR/Cas9 delivery strategies," Biomolecules, vol. 10, no. 6, Art. no. 839, 2020.
29. P. Wang, L. Zhang, W. Zheng, et al., "Therapeutic genome editing using CRISPR/Cas9: Delivery strategies and biomedical applications," Signal Transduct. Target. Ther., vol. 7, Art. no. 1, 2022.
30. F. Dilnawaz and S. Acharya, "Nanoparticle-based CRISPR/Cas delivery: An emerging tactic for cancer therapy," Curr. Med. Chem., vol. 30, no. 31, pp. 3562–3581, 2023.

31. J. A. Doudna, "The promise and challenge of therapeutic genome editing," Nature, vol. 578, no. 7794, pp. 229–236, 2020.
32. A. C. Komor, Y. B. Kim, M. S. Packer, J. A. Zuris, and D. R. Liu, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage," Nature, vol. 533, no. 7603, pp. 420–424, 2016.
33. A. V. Anzalone, P. B. Randolph, J. R. Davis, A. A. Sousa, L. W. Koblan, J. M. Levy, et al., "Search-and-replace genome editing without double-strand breaks or donor DNA," Nature, vol. 576, no. 7785, pp. 149–157, 2019.
34. Samanta A, Mondal S, Roy D, Nayak AK. Design and release kinetics of liposomes containing abiraterone acetate for treatment of prostate cancer. Sensors International. 2021 Jan 1;2:100077.
35. H. Frangoul, D. Altshuler, M. D. Cappellini, Y. S. Chen, J. Domm, B. K. Eustace, et al., "CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia," N. Engl. J. Med., vol. 384, no. 3, pp. 252–260, 2021.
36. B. T. Staahl, M. Benekareddy, C. Coulon-Bainier, et al., "Efficient genome editing in the mouse brain by local delivery of engineered Cas9 ribonucleoprotein complexes," Nat. Biotechnol., vol. 35, no. 5, pp. 431–434, 2017.
37. K. Lee, M. Conboy, H. M. Park, F. Jiang, H. J. Kim, M. A. DeWitt, et al., "Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA in vivo induces homology-directed DNA repair," Nat. Biomed. Eng., vol. 1, Art. no. 889, 2017.
38. R. Mout, M. Ray, G. Yesilbag Tonga, Y. W. Lee, T. Tay, K. Sasaki, et al., "Direct cytosolic delivery of CRISPR/Cas9-ribonucleoprotein for efficient gene editing," ACS Nano, vol. 11, no. 3, pp. 2452–2458, 2017.
39. M. Wang, J. A. Zuris, F. Meng, H. Rees, S. Sun, P. Deng, et al., "Efficient delivery of genome-editing proteins using bioreducible lipid nanoparticles," Proc. Natl. Acad. Sci. U.S.A., vol. 113, no. 11, pp. 2868–2873, 2016.
40. B. Lee, K. Lee, S. Panda, R. Gonzales-Rojas, A. Chong, V. Bugay, et al., "Nanoparticle delivery of CRISPR into the brain rescues fragile X syndrome phenotypes in mice," Nat. Biomed. Eng., vol. 2, no. 7, pp. 497–507, 2018.
41. D. P. Dever, R. O. Bak, A. Reinisch, et al., "CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells," Nature, vol. 539, no. 7629, pp. 384–389, 2016.
42. W. H. Yeh, H. Chiang, H. A. Rees, A. S. B. Edge, and D. R. Liu, "In vivo base editing of post-mitotic sensory cells," Nat. Commun., vol. 9, Art. no. 2184, 2018.
43. J. A. Zuris, D. B. Thompson, Y. Shu, et al., "Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo," Nat. Biotechnol., vol. 33, no. 1, pp. 73–80, 2015.
44. D. S. D'Astolfo, R. J. Pagliero, A. Pras, et al., "Efficient intracellular delivery of native proteins," Cell, vol. 161, no. 3, pp. 674–690, 2015.
45. H. X. Wang, M. Li, C. M. Lee, S. Chakraborty, H. W. Kim, G. Bao, and K. W. Leong, "CRISPR/Cas9-based genome editing for disease modelling and therapy: Challenges and opportunities for nonviral delivery," Chem. Rev., vol. 117, no. 15, pp. 9874–9906, 2017.