RESEARCH PAPER

Nanotechnology Based CRISPR Delivery Platforms in Therapeutic Genome Engineering

Akshay R. Yadav[1], Monali M. Upare[2,4]*, Sanjeevani R. Desai[3], Rahul A. Sachdeo[4], Sarang S. Mahamuni[5], Nutan V. Shah[3,4]

[1,2,5]*Department of Pharmaceutical Chemistry, Krishna Charitable Trust's Krishna College of Pharmacy, Karad, Maharashtra, India-415539*

[3]*Department of Pharmaceutics, Krishna Charitable Trust's Krishna College of Pharmacy, Karad, Maharashtra, India-415539*

[4]*Research Scholar, Krishna Vishwa Vidyapeeth (Deemed to be University), Krishna Institute of Pharmacy, Karad, Maharashtra, India-415539*

ABSTRACT

The CRISPR-Cas system has revolutionized gene editing, but its therapeutic application faces delivery challenges, especially within living systems. Nanotechnology has emerged as a transformative tool to enhance CRISPR delivery by improving cellular uptake, stability, and precision targeting. Various nanocarriers-lipid-based, polymeric, inorganic, and biomimetic are being designed to overcome the limitations of viral vectors. These systems facilitate intracellular trafficking, endosomal escape, and nuclear transport of CRISPR elements. Functional customization and stimuli-responsive properties further refine their performance. This review provides a comprehensive overview of platforms and their potential in treating cancers, inherited disorders, and infectious diseases. Emphasis is placed on integrating nanomedicine with precision editing for patient-specific therapies. Advancements in smart nanocarriers may shape the future of minimally invasive, next-generation gene therapies...

Keywords: CRISPR-Cas, Nanotechnology, Gene Editing, Nanocarriers, Targeted Delivery, Genome Engineering

How to cite this article: Yadav AR, Upare MM*, Desai SR, Sachdeo RA, Mahamuni SS, Shah NV, Nanotechnology Based CRISPR Delivery Platforms in Therapeutic Genome Engineering .Int J Drug Deliv Technol. 2026;16(2s): 177-184; DOI: 10.25258/ijddt.16. 177-184

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

A fundamental component of molecular biology for a long time, gene editing allows scientists to simulate illnesses, comprehend gene function, and investigate potential treatments. Initially, scientists used labor-intensive and imprecise techniques like random mutagenesis, which allowed them to change genes but gave them little control over the location of the changes.[1] Although targeted gene alteration was eventually made possible by homologous recombination, its effectiveness was still poor, especially in mammalian cells.[2]

Even though they were more accurate than previous techniques, their design was still somewhat complicated and expensive. RNA-guided nucleases were eventually discovered as a result of additional innovation spurred by the need for easier, more versatile gene editing tools. [3,4] This method promised programmability, meaning that the editing enzyme could be directed to a new target by simply changing the guide sequence. It opened the door to a new age of potent and widely available genome editing tools.[5]

Need for Advanced Delivery Methods

The precision of the CRISPR machinery itself is simply one factor that affects genome editing efficacy; another is the machinery's ability to be carried to the appropriate cellular and subcellular location with safety and dependability. The effectiveness of editing, activity duration, off-target dangers, and, eventually, therapeutic result are all influenced by delivery.[6]

Table 1: Three main forms of CRISPR components typically delivered[7,8]

Delivery Format	Description	Key Advantages
Plasmid DNA	Circular DNA carrying the genetic code for Cas protein and guide RNA. Enters the nucleus for expression.	Stable and easy to produce Long-lasting expression - Suitable for research applications
mRNA + Synthetic sgRNA	Delivers messenger RNA for Cas protein along with separately	Rapid onset of action No risk of DNA integration

**Author for Correspondence: monaliupare1518@gmail.com*

	synthesized guide RNA. Acts in cytoplasm.	Safer for therapeutic use
Ribonucleoprotein (RNP)	Pre-assembled complex of Cas protein bound to guide RNA. Delivered in active form.	Immediate activity post-delivery Short duration limits off-target effects High precision

Table 2: Widely explored nanocarriers[9,10]

Nanocarrier Type	Description	Key Attributes
Lipid-Based Nanoparticles (LNPs)	Soft vesicles made from lipid molecules, commonly used for delivering RNA-based therapies	Proven success in mRNA vaccine platforms Efficient in encapsulating and protecting Cas9 mRNA and guide RNA
Polymeric Nanoparticles	Engineered from synthetic or natural polymers, designed to carry and release genetic material in a controlled manner.	Customizable surface Tunable degradation rates Suitable for sustained release
Inorganic Nanoparticles	Composed of metals or minerals like gold, silica, or iron oxide, known for their stability and imaging potential.	Useful in diagnostics and delivery Easy to functionalize Physically robust
Biomimetic Systems	Nanocarriers coated with cell-derived membranes or constructed to mimic biological vesicles like exosomes.	Improved biocompatibility Reduced immune detection Effective in targeted delivery

Mechanism of Action (Cas9, gRNA, PAM Sequence)

Primarily made up of the Cas9 protein and a guide RNA (gRNA) that guides Cas9 to a particular DNA target, CRISPR/Cas is a potent genome-editing tool. To provide precise binding, the gRNA combines the activities of tracrRNA and crRNA. To start cleavage, Cas9 needs a short DNA sequence known as PAM (also referred to as "NGG") close to the target site. Cas9 causes a double-strand break after binding. The cell fixes this break using either Homology-Directed Repair, which permits precise editing, or Non-Homologous End Joining, which may damage the gene.[11,12]

Current Therapeutic and Research Applications

Since its implementation, CRISPR/Cas has revolutionized fundamental research and is progressively advancing in the clinical field. It is frequently employed in lab settings for high-throughput genomic screening, gene function research, and knockout model creation. Its ease of use and adaptability have sped up synthetic biology and functional genomics initiatives in a variety of organisms. CRISPR has the potential to be used in therapeutic development to treat genetic illnesses such Duchenne muscular dystrophy, β-thalassemia, and sickle cell disease.[13]

Delivery Challenges in Clinical Translation

Delivering CRISPR components safely and effectively is still one of the largest challenges in clinical translation, despite its therapeutic potential. The delivery mechanism affects the length of gene-editing activity, immunogenicity, and off-target consequences in addition to efficiency. Although viral vectors are quite effective, they have drawbacks such immunological reactions, limited cargo capacity, and the possibility of genetic integration. Non-viral methods, especially those based on nanoparticles, are becoming more popular due to their adjustable characteristics and decreased immunogenicity. Effective cell-specific targeting, endosomal escape, and nuclear localization of the CRISPR machinery are still difficult to achieve, though.[14]

Nanocarriers

The ability to safely and accurately transfer genetic material to the targeted cells is crucial to the development of successful gene treatments. [21] These nanoscale carriers are perfect for delivering gene editing because they can encapsulate or bind nucleic acids, shield them from enzyme degradation, and make it easier for them to pass across cellular membranes.[15]

Controlled delivery and reduced off-target effects are made possible by the precise customization of nanocarriers in terms of size, surface chemistry, and payload release mechanisms. This adaptability makes it possible to distribute several CRISPR formats, such as ribonucleoprotein complexes (RNPs), mRNA, or plasmid DNA, in a way that minimizes damage and immune activation while optimizing efficiency.[16]

Targeting Potential and Biocompatibility

The ability of nanocarriers to target is one of its main advantages. Nanoparticles can be targeted to particular cell types or tissues by functionalizing their surfaces with ligands, antibodies, or aptamers.[17] This improves therapeutic precision and lowers systemic exposure. This characteristic is especially crucial in diseases like cancer or neurological disorders that need for cell-selective editing.[18]

Types of Nanocarriers for CRISPR Delivery

Lipid-Based Nanoparticles (LNPs):

CRISPR/Cas components is lipid-based nanoparticles (LNPs). An ionizable or cationic lipid core, helper lipids (such DSPC or DOPE), cholesterol for membrane stability, and a polyethylene glycol (PEG)-lipid coating that lengthens circulation time and inhibits aggregation make up

the structural makeup of LNPs. They are appropriate for intracellular delivery because they can encapsulate mRNA or ribonucleoproteins (RNPs) and aid in endosomal escape. The success of mRNA-based COVID-19 vaccines shows that LNPs are the only non-viral nanocarriers currently licensed by the FDA for therapeutic nucleic acid delivery. NTLA-2001, created by Intellia Therapeutics, is a well-known case study in the gene editing field. It treats transthyretin (ATTR) amyloidosis by delivering CRISPR-Cas9 in vivo via LNPs.[19]

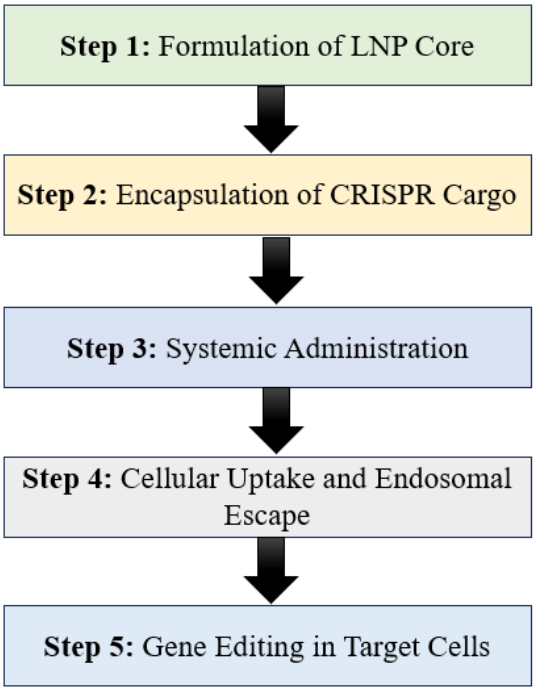

Figure 1: LNP-Based Delivery of CRISPR/Cas Components

Clinical trials have demonstrated the formulation's encouraging safety and effectiveness, which represents a significant breakthrough in systemic CRISPR delivery.[20]

Polymeric Nanoparticles

Because polymeric nanoparticles can form stable compounds with nucleic acids and have structural tunability, they offer yet another flexible platform for CRISPR delivery. Although cationic polymers, such polyethyleneimine (PEI), help with membrane contact and gene condensation, their high charge density frequently results in cytotoxicity. By exploiting the acidic environment inside cellular compartments, novel pH-sensitive polymers allow endosomal escape, releasing their payload only after absorption. Such polymers are designed to boost editing efficiency and minimize premature release. However, polymeric systems still face significant obstacles in the form of immune activation and possible cytotoxicity, which call for surface modifications such PEGylation or conjugation with targeted ligands to improve biocompatibility and lower systemic toxicity.[21]

Inorganic Nanoparticles

Magnetic nanoparticles (MNPs), silica, and gold (AuNPs) are examples of inorganic nanocarriers that have special benefits for CRISPR delivery, especially in real-time imaging and diagnostic tracking. Thiol-modified oligonucleotides or peptides can be readily added to gold nanoparticles to functionalize them, allowing for cell-specific delivery and excellent loading efficiency. Applications like as photothermal activation or imaging are also made possible by their optical characteristics.[22]

The porous nature of silica nanoparticles allows for regulated release and high payload capacity, while MNPs can be directed by external magnetic fields to achieve specific localization. Biocompatible coatings like PEG, peptides, or antibodies are frequently used to functionalize inorganic nanoparticles in order to enhance cellular absorption and decrease immune clearance.Their application is mainly restricted to preclinical and exploratory studies due to their long-term biocompatibility and clearance pathways, despite their advantages in stability and imaging.[23]

Biomimetic and Hybrid Systems

Biomimetic nanocarriers, which imitate natural biological systems, offer a state-of-the-art method of delivering CRISPR. Exosome-mimetic vesicles, which are made from synthetic analogs or cell membranes, enable cargo delivery with excellent immunological evasion and biocompatibility. Through surface proteins, these vesicles spontaneously engage with recipient cells, facilitating effective uptake.[24]

Cell membrane-coated nanoparticles, in which artificial cores are covered in membranes from cancer cells, platelets, or red blood cells, represent another intriguing tactic. Tissue-specific targeting and immunological stealth are conferred by this coating.Lastly, in order to capitalize on the advantages of each system, hybrid nanoassemblies blend several components, including lipids, polymers, and inorganic cores. These multipurpose systems provide unmatched adaptability in precision gene therapy by enabling the co-delivery of medicinal compounds, imaging agents, or gene editors.[25]

Design Parameters for Effective CRISPR Delivery

The effectiveness and accuracy of CRISPR-Cas systems' distribution into target cells are critical factors in determining their therapeutic potential. Systems based on nanocarriers offer flexible instruments for this aim, but their efficacy is controlled by a number of important biological and physicochemical design factors. To create safe and effective CRISPR-based treatments, it is essential to comprehend and optimize these factors.[26,27]

Size, Charge, and Surface Chemistry

When it comes to biodistribution, cellular absorption, and excretion from the body, nanocarrier size is crucial. While larger particles may have trouble entering cells or permeating tissues, too-small particles may be quickly removed. Surface charge is just as important. Since cationic particles interact more easily with the negatively charged cell membrane, slightly positive or almost neutral charges are frequently ideal for cellular absorption. However, cytotoxicity and non-specific interactions with serum proteins can result from highly cationic surfaces, as those of unaltered polyethyleneimine (PEI). Thus, to balance

charge, improve biocompatibility, and lessen immune recognition, surface modifications like PEGylation or ligand attachment are used.[28]

Endosomal Escape Mechanisms

To release CRISPR components into the cytoplasm or nucleus after being ingested by cells, nanocarriers must avoid the endosomal pathway. Failure to do so may result in loss of therapeutic action and lysosomal breakdown. Numerous approaches have been devised to tackle this difficulty. The proton sponge effect, which is frequently observed in cationic polymers such as PEI, promotes osmotic swelling and buffers the endosomal environment, ultimately resulting in endosome rupture. As an alternative, release may be facilitated by lipids or membrane-disruptive peptides that cause the endosomal membrane to become unstable in an acidic environment.[29]

Targeted vs. Systemic Delivery

Effective gene editing sometimes requires delivery to specific cells or tissues. Targeted administration is made possible by surface ligands that recognize and bind to cell-specific receptors. This approach increases local concentration at the site of sickness while reducing systemic exposure and negative effects. For example, ligands that target the folate or transferrin receptors have been used to direct nanoparticles to cancer cells. On the other hand, disorders involving extensive tissue involvement are better suited for systemic administration. Systemic dosing, however, raises the possibility of immune clearance and off-target effects. For safe and efficient systemic circulation, it is therefore essential to customize nanoparticle characteristics including size, charge, and stealth coatings.[30]

Stimuli-Responsive Release (pH, Redox, Enzyme)

Nanocarriers are increasingly being made to release their cargo in response to pathogenic or intracellular cues in order to increase precision. By limiting unintentional gene editing, these stimuli-responsive devices make sure that CRISPR components are only released under particular circumstances. Redox-responsive mechanisms cleave disulfide bonds to allow intracellular release by taking advantage of the high glutathione content inside cells. Enzyme-sensitive carriers provide tissue-selective release in response to overexpressed enzymes (such as matrix metalloproteinases) in disease areas.These smart designs improve therapeutic efficiency, reduce toxicity, and increase the likelihood ofsuccessful gene editing at the intended location.[31]

Therapeutic Applications of NanoCRISPR Platforms

CRISPR delivery made possible by nanotechnology, or nanoCRISPR, has produced innovative new treatment options for a number of diseases. They can be used to treat inherited, infectious, neurological, and malignant disorders.

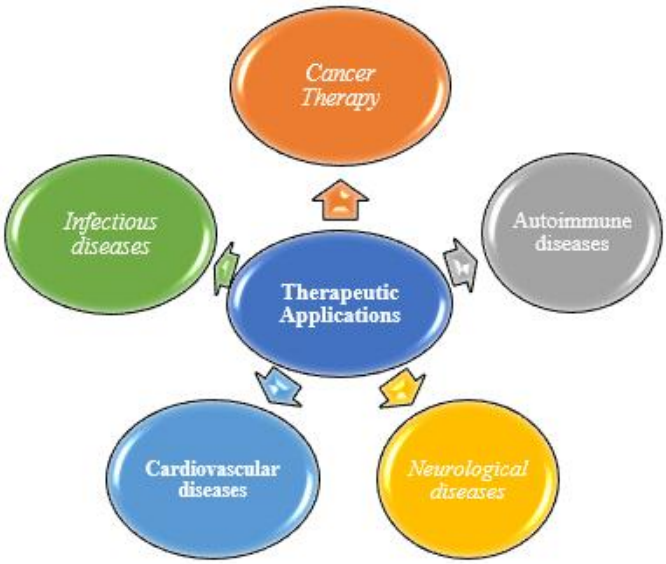

Figure 2: Therapeutic Applications of NanoCRISPR

Genetic Disorders

This technique provides a therapeutic solution without necessitating long-term viral integration. CRISPR components can be effectively delivered by nanocarriers that can pass cell membranes, particularly in muscle tissues, to restore partial or complete dystrophin function, giving affected persons hope for long-term therapeutic benefit.

Cancer Therapy

One of the main applications of nanoCRISPR technology is the therapy of cancer. One well researched method for preventing tumor growth is gene deletion of oncogenes or survival pathways. For instance, preclinical cancer models have been used to evaluate CRISPR-mediated disruption of the EGFR or KRAS gene utilizing nanoparticle systems. Immune regulation, particularly by altering immune checkpoint regulators, is another exciting avenue.

Infectious Diseases

CRISPR technologies also offer a new approach to combat viral infections by selectively targeting viral genomes. There is promise for a viable treatment because CRISPR-Cas9 has been utilized to eliminate HIV proviral DNA that has been integrated into host cells. Similarly, CRISPR can target HPV to break oncogenic viral genes like E6 and E7, which cause cervical and other cancers. During the COVID-19 pandemic, CRISPR systems that target RNA, like as Cas13, were studied for their potential to brak down the genome of SARS-CoV-2. Rapid antiviral effects were shown by nanoparticles that could carry these systems into respiratory cells. These investigations demonstrate how NanoCRISPR can be used to treat both acute and chronic viral infections.[32]

Neurological Diseases

Because of the blood–brain barrier (BBB), diseases like Huntington's and Alzheimer's present delivery issues. Gene editors can be delivered to neurons by nanoCRISPR systems that are designed with BBB-penetrating characteristics, such as surface charge tuning or ligand modification.In animal models of Huntington's disease,

CRISPR has been used to silence the mutant HTT gene. Similarly, via gene editing upstream regulatory regions, nanoCRISPR techniques for Alzheimer's disease seek to decrease amyloid-β or tau formation, providing a route to disease change as opposed to symptom management.[33]

Clinical Trials and Market Outlook

Recent years have witnessed a boom in the clinical development of NanoCRISPR technologies, which is a reflection of both growing investment in precision gene editing techniques and scientific maturation.

Ongoing and Completed NanoCRISPR Trials

CRISPR is now being delivered by nanotechnology in a number of clinical trials, mostly for the treatment of rare and hereditary diseases. Most of these investigations use lipid nanoparticles to deliver CRISPR-Cas9 components in vivo due of their shown safety and efficacy.[34]

Table 3: In vivo, human applications of NanoCRISPR systems[35,36]

Trial Code	Disease Target	Delivery System	CRISPR Format	Status	Sponsor
NCT04601051	Transthyretin Amyloidosis (ATTR)	Lipid Nanoparticles (LNPs)	mRNA + sgRNA	Phase I Completed	Intellia Therapeutics
NCT05442346	Hereditary Angioedema (HAE)	LNPs	mRNA + sgRNA	Ongoing	Intellia / Regeneron
NCT05309789	Sickle Cell Disease	LNPs	Cas9 RNPs	Early Phase	Beam Therapeutics

Companies and Platforms in Development

Numerous biotechnology firms are developing exclusive NanoCRISPR delivery systems, frequently aimed for both in vivo and ex vivo uses. These businesses concentrate on creating delivery systems that are suited for immune evasion, disease specificity, and improved editing accuracy.

Table 4: Companies and Platforms in Development[37,38]

Company	Delivery Platform	Lead Indication
Intellia Therapeutics	Lipid Nanoparticles (LNP)	ATTR, HAE
Editas Medicine	AAV and Non-Viral Nanoparticles	Retinal disorders, Sickle Cell
Beam Therapeutics	Base Editing + LNPs	Sickle Cell Disease, Liver Disease
CRISPR Therapeutics	Cas9 mRNA + Nanoformulation	β-thalassemia, Oncology
Precision BioSciences	ARCUS platform + Nanocarriers	Cancer, Genetic Liver Disorders

Challenges and Future Perspectives

NanoCRISPR technologies are a significant advancement in the field of gene therapy because of their unmatched precision and therapeutic potential. However, before preclinical findings can be widely used in clinical settings, a variety of scientific, technological, and sociological challenges need to be addressed. For the field to flourish sustainably, it will be crucial to comprehend and address these problems.[39]

Personalized Nanomedicine Integration

Modular platforms that enable quick switching of targeting ligands, payload types, and release mechanisms are necessary to achieve this degree of customisation. However, in terms of cost-effectiveness, production schedules, and regulatory approval, individualized nanomedicine also adds more complication. For future technologies to be clinically viable, scalability and personalization must be balanced.

AI in Nanocarrier Design

A viable way to speed up development is to integrate machine learning (ML) and artificial intelligence (AI) into this procedure. Designing safer and more effective systems is made possible by AI-driven models that forecast how modifications in nanoparticle characteristics affect biological activity. Without requiring significant experimental testing, these technologies can also be used to forecast in vivo performance, simulate interactions with biological membranes, and screen large libraries of compounds.AI has the ability to drastically cut costs and development durations by minimizing the trial-and-error stage. However, integrating AI techniques necessitates cooperation amongst fields, such as computational biology, materials science, and clinical pharmacology, as well as the use of high-quality datasets and standardized methodologies.[40,41]

Ethical and Societal Implications

Like any gene-editing technologies, NanoCRISPR presents social and ethical issues that need to be well thought out. Important ethical concerns are raised by topics including germline editing, consent in pediatric or fetal operations, and fair access to treatments. In order to overcome these issues, transparent governance, public involvement, and international regulatory cooperation will be crucial. Gaining the public's trust requires being transparent about the dangers, advantages, and protections. Preventing inequalities in healthcare outcomes will depend on making sure NanoCRISPR-based treatments are accessible and affordable worldwide. [42]

CONCLUSION

The success of nanocarrier-enabled in vivo genome editing is demonstrated by the advancements made thus far, especially in clinical-stage trials that target sickle cell disease and transthyretin amyloidosis. These developments are supported by the creation of a variety of nanocarriers, including lipid-based, polymeric, inorganic, and biomimetic ones, which provide increased intracellular

delivery, cellular targeting, decreased off-target effects, and design flexibility. The design of the delivery vehicle must navigate the biological constraints of the human body while striking a balance between biocompatibility and efficacy. Furthermore, regulatory pathways need to evolve in parallel with technological innovation, fostering transparent frameworks that encourage innovation while safeguarding public health. In summary, NanoCRISPR represents a dynamic convergence of gene editing and nanomedicine with the power to redefine therapeutic paradigms. While challenges persist, the momentum in research, clinical trials, and technological innovation signals a promising future one where precise, safe, and personalized genome editing may become a routine part of medical care.

ACKNOWLEDGMENT
We express our sincere thanks to KCT's Krishna College of Pharmacy Karad and Krishna Vishwa Vidyapeeth, Karad and for providing the necessary facilities to carry out the work.
SOURCE OF SUPPORT: Nil.
CONFLICT OF INTEREST: None
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