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CRISPR-BASED NANOTHERAPEUTICS: A NEXT-GENERATION APPROACH FOR TARGETED GENE DELIVERY

Shreya Singh[1]

[1] Institute of Pharmacy, Amity University Uttar Pradesh (Lucknow Campus), AB-2 Building, 2nd–5th Floor, Near Malhaur Railway Station, Gomti Nagar Extension, Lucknow – 226010, Uttar Pradesh, India.

Corresponding Author: Shreya Singh

Abstract:

The advent of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas systems has revolutionized the field of gene therapy, offering unparalleled precision, programmability, and efficiency for genome editing. However, translating CRISPR-based therapeutics into clinical applications remains constrained by major challenges in the safe and targeted delivery of the CRISPR components—Cas nucleases and guide RNAs—into specific cells and tissues. Recent advances in nanotechnology have provided innovative solutions to these limitations, leading to the emergence of CRISPR-based nanotherapeutics. Nanocarriers such as lipid nanoparticles, polymeric systems, inorganic nanoparticles, and biomimetic exosomes enable protection of CRISPR cargos from enzymatic degradation, facilitate endosomal escape, and improve tissue-specific targeting while minimizing off-target effects and immunogenicity. This review comprehensively discusses the principles and mechanisms of CRISPR/Cas systems, various nanoplatforms engineered for their delivery, and design strategies for optimizing safety and efficacy. Special emphasis is given to the applications of CRISPR–nanocarrier combinations in cancer therapy, genetic and infectious diseases, neurological disorders, and regenerative medicine. Furthermore, recent preclinical progress, ongoing clinical trials, regulatory considerations, and future perspectives are highlighted. The convergence of genome editing and nanotechnology represents a transformative step toward next-generation, personalized, and precision gene therapies with potential to redefine the future of molecular medicine.

Keywords: CRISPR/Cas9, Nanoparticle, Gene editing, Targeted delivery, Personalized medicine, Genome engineering

Graphical Abstract

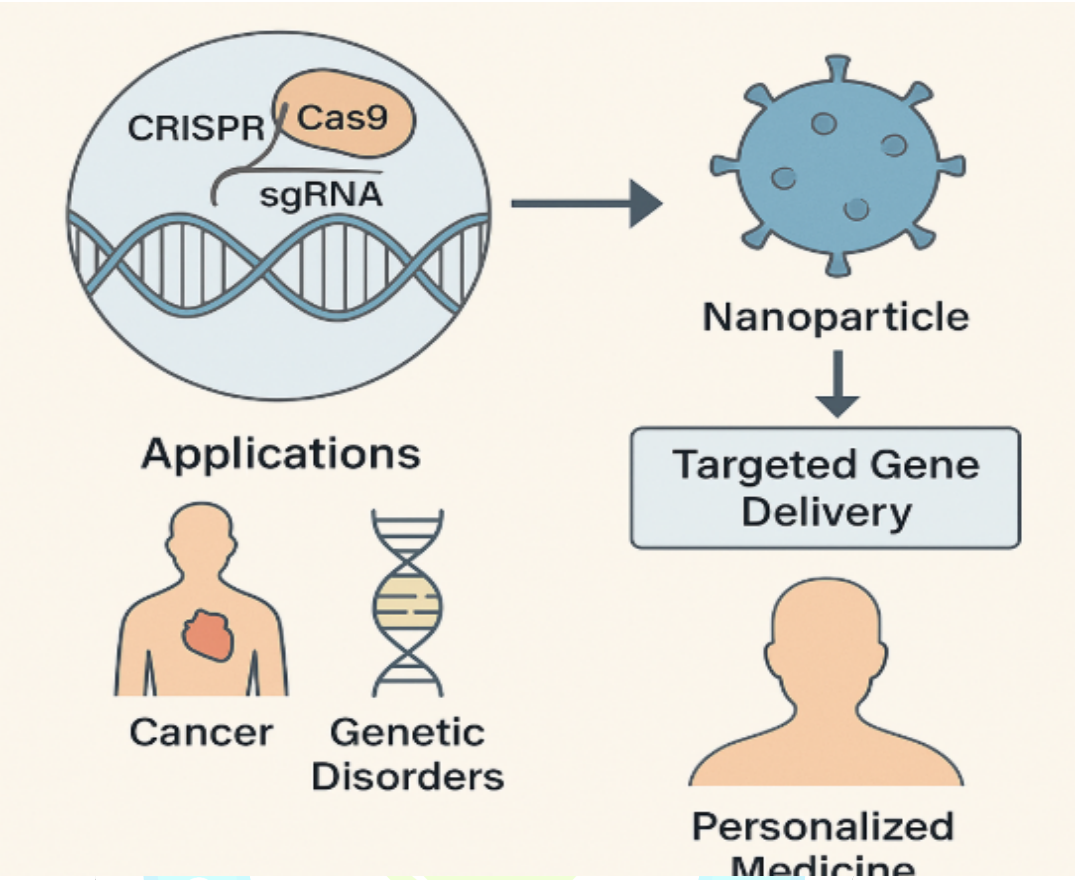

Fig 01 : Schematic representation of CRISPR-based nanotherapeutics for targeted gene delivery.

The CRISPR/Cas9 system, guided by a single guide RNA (sgRNA), is encapsulated within a nanoparticle carrier to enhance protection, stability, and cellular uptake. The nanocarrier facilitates targeted gene delivery to specific tissues, enabling precise genome editing for therapeutic applications such as cancer and genetic disorders. The integration of CRISPR technology with nanotechnology offers a promising route toward personalized and precision medicine.

1. Introduction

The advancement of genome-editing technologies has revolutionized therapeutic design by enabling direct modification of disease-causing genes rather than symptomatic management. Among these, the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system has emerged as a highly versatile and precise genome-editing platform. However, its clinical translation remains limited by challenges in **in vivo** delivery of the Cas9 protein and guide RNA (gRNA). Viral vectors, while effective, often raise concerns regarding immunogenicity, insertional mutagenesis, limited cargo capacity, and manufacturing complexity.

Nanotechnology has emerged as an alternative for the safe, targeted, and efficient delivery of CRISPR components. Nanoparticles (NPs) protect the editing machinery from enzymatic degradation, prolong circulation time, and enable tissue-specific accumulation through passive or active targeting mechanisms. The integration of nanotechnology with CRISPR/Cas systems has the potential to accelerate the development of next-generation therapeutics in cancer, genetic, and infectious diseases.[1]

2. Fundamentals of the CRISPR/Cas System

CRISPR/Cas systems are adaptive immune mechanisms naturally present in bacteria and archaea. The Class 2 Type II CRISPR/Cas9 system is the most commonly used in genome editing, relying on an engineered single-guide RNA (sgRNA) that directs the Cas9 nuclease to specific genomic loci adjacent to a protospacer adjacent motif (PAM). Cas9 introduces a double-strand break (DSB), repaired by cellular DNA repair pathways—non-homologous end joining (NHEJ) or homology-directed repair (HDR).[2]

Major limitations include off-target cleavage, inefficient HDR, and challenges in intracellular delivery. Non-viral delivery systems such as nanoparticles have been shown to improve the efficiency of CRISPR transport to the nucleus while minimizing toxicity and immunogenicity.[3]

3. Nanotechnology in Gene Delivery: An Overview

Nanocarriers (1–1000 nm) possess tunable physicochemical properties such as particle size, charge, and surface chemistry that allow for precise control over pharmacokinetics, biodistribution, and cell uptake.[4] These nanosystems can encapsulate plasmids, mRNA, or Cas9–sgRNA ribonucleoprotein (RNP) complexes, preventing degradation and facilitating endosomal escape.

Compared with viral vectors, nanoparticle-based systems exhibit lower immunogenicity,

scalable production, and the ability to co-deliver multiple therapeutic agents. However, physiological barriers (renal filtration, RES clearance, tissue penetration) and intracellular barriers (endosomal sequestration, nuclear import) must still be overcome.[5]

4. CRISPR-Based Nanotherapeutic Platforms

4.1 Lipid-Based Nanoparticles (LNPs)

LNPs composed of ionizable lipids, cholesterol, phospholipids, and PEG-lipids are widely used for nucleic acid delivery. They can encapsulate Cas9 mRNA or RNP complexes and facilitate endosomal release. Recent studies have demonstrated >90% gene knockdown in murine liver models following systemic administration of CRISPR-LNP systems.[6]

4.2 Polymeric Nanocarriers

Cationic polymers such as polyethyleneimine (PEI), chitosan, and poly(lactic-co-glycolic acid) (PLGA) are effective for CRISPR plasmid delivery due to their tunable charge and biodegradability. Functionalization with ligands and PEGylation enhances tissue targeting and serum stability.[7]

4.3 Inorganic Nanoparticles

Inorganic nanocarriers (gold, silica, and magnetic NPs) offer unique optical, magnetic, and photothermal properties that can be harnessed for combined imaging and therapy. Gold nanoparticles (AuNPs) have been used for **Cas9 RNP** delivery, demonstrating efficient gene disruption and photothermal activation.[8]

4.4 Exosome- and Cell Membrane-Coated Nanocarriers

Exosomes and cell-membrane-coated NPs mimic natural vesicles, reducing immunogenicity and promoting homing to target tissues. These biomimetic carriers are promising for the delivery of CRISPR systems across biological barriers.[9]

4.5 DNA and RNA Nanostructures

DNA and RNA origami nanostructures can be engineered to encapsulate Cas9/gRNA complexes, offering precise control over structure and stimuli-responsive release.[10]

5. Design Strategies for CRISPR–Nanocarrier Complexes

The design of CRISPR–NP complexes depends on the nature of the cargo (plasmid, mRNA, or RNP), particle size, charge, and targeting moieties. Active targeting ligands (antibodies, peptides, aptamers) can direct NPs to specific cell types. Endosomal escape remains a critical challenge, often addressed using pH-sensitive lipids or proton-sponge polymers.[11]

Surface PEGylation enhances circulation time, while biodegradable polymers (PLGA, PBAE)

improve safety. Additionally, nuclear localization sequences (NLS) facilitate efficient transport of Cas9 to the nucleus.[12]

6. Applications of CRISPR-Based Nanotherapeutics

6.1 Cancer Therapy

CRISPR–nanoparticles (NPs) enable silencing of oncogenes, reactivation of tumor suppressors, and modulation of immune checkpoints (e.g., PD-1/PD-L1). Lipid, polymeric, and biomimetic NPs enhance tumor targeting through passive and active mechanisms while minimizing systemic toxicity. Combination with chemotherapeutics or immunomodulators can produce synergistic anti-tumor effects, as shown in preclinical tumor models with reduced growth and improved survival.[13]

6.2 Genetic Disorders

Nanoparticle-mediated CRISPR delivery has shown promise in hereditary diseases such as β-thalassemia, Duchenne muscular dystrophy (DMD), and cystic fibrosis. NPs protect CRISPR cargos, improve cellular uptake, and allow tissue-specific targeting of stem or progenitor cells. Co-delivery with repair templates enhances precision editing, reduces off-target effects, and supports permanent gene correction in preclinical models.[14]

6.3 Infectious Diseases

CRISPR–NPs targeting viral DNA or RNA (e.g., HIV, HBV, SARS-CoV-2) enable efficient viral clearance with minimal host toxicity. Multiplexed targeting reduces viral escape, and NP-mediated delivery allows both transient and permanent inhibition of viral replication, demonstrating potential as therapeutic and prophylactic agents.[15]

6.4 Neurological Disorders

Crossing the blood–brain barrier is challenging, but ligand-modified and biomimetic NPs have improved delivery of CRISPR components to neurons for neurodegenerative disorders. Precise editing can reduce pathogenic protein accumulation, restore cellular homeostasis, and slow disease progression, while imaging or stimuli-responsive platforms allow monitoring of delivery and efficacy.[16]

6.5 Regenerative Medicine

Nanocarrier-based CRISPR delivery enables gene correction in stem and progenitor cells for tissue repair. Edited cells can be used for tissue engineering or in vivo transplantation, and combination with growth factors or immunomodulators further enhances functional recovery.[17]

7. Preclinical and Clinical Progress

Preclinical studies demonstrate that LNP-mediated Cas9 mRNA and sgRNA delivery can achieve efficient editing in hepatocytes, with reduced off-target activity.[18] Although most clinical trials still rely on ex vivo viral delivery, in vivo NP-mediated CRISPR is advancing rapidly.[19] Regulatory agencies emphasize the need for safety, reproducibility, and ethical oversight before human application.

8. Challenges and Limitations

Key limitations include off-target editing, immunogenicity of nanomaterials, limited penetration in solid tissues, and challenges in large-scale manufacturing.[20] The long-term safety of nanoparticle formulations and genome edits also warrants comprehensive evaluation.[21]

9. Future Perspectives

The field of CRISPR-based nanotherapeutics is poised for a transformative leap, driven by synergistic innovations in artificial intelligence, nanotechnology, biomaterials engineering, and precision medicine. The forthcoming decade will witness a paradigm shift toward intelligent, programmable, and patient-specific delivery systems that will redefine how genome-editing tools are designed, optimized, and clinically deployed.[22]

9.1. AI-Guided Design and Predictive Modeling of Nanocarriers

Artificial intelligence (AI) and machine learning (ML) are revolutionizing drug delivery research by enabling *data-driven design* and *predictive optimization* of nanocarriers. Traditional trial-and-error formulation methods are gradually being replaced by algorithmic approaches that integrate physicochemical parameters, biological interactions, and pharmacokinetic behavior.

AI models can analyze extensive datasets encompassing nanoparticle size, surface charge, lipid composition, and polymer structure to predict in vivo biodistribution, cellular uptake, and gene-editing efficiency.[23]

Moreover, *reinforcement learning* and *genetic algorithms* can simulate iterative optimization cycles to identify ideal nanoparticle compositions that maximize CRISPR cargo stability while minimizing immunogenicity.[24]

For example, AI-based computational tools have been successfully applied to optimize lipid nanoparticle (LNP) formulations used in mRNA and Cas9 mRNA delivery systems, achieving higher encapsulation efficiencies and reduced off-target activity. The development of “digital twins” of biological systems — virtual replicas that simulate biological environments such as the liver, tumor microenvironment, or blood–brain barrier (BBB) — can allow virtual

screening of nanocarrier designs before experimental validation.[24] This integration of AI with experimental nanomedicine will accelerate translation timelines and significantly reduce formulation costs.

9.2. Development of Next-Generation CRISPR Systems (Cas12, Cas13, Cas14)

The evolution of CRISPR systems beyond Cas9 has unlocked new frontiers in precision gene editing. The next-generation nucleases — **Cas12**, **Cas13**, and **Cas14** — differ in size, specificity, and target type, enabling diversified therapeutic applications.

- **Cas12** recognizes and cleaves double-stranded DNA with single RNA guidance, but unlike Cas9, it exhibits *collateral cleavage activity* on single-stranded DNA, making it useful for both therapeutic and diagnostic applications (e.g., SHERLOCK and DETECTR platforms).[25]
- **Cas13** targets RNA molecules directly, allowing transient modulation of gene expression without permanent DNA alterations, which is particularly valuable for treating viral infections (e.g., SARS-CoV-2, influenza) and transient genetic diseases.[26]
- **Cas14**, the smallest known nuclease, can target single-stranded DNA with ultra-high precision, offering promise for *in vivo* editing where delivery constraints exist due to size limitations.[27]

The combination of these compact CRISPR enzymes with nanocarriers such as LNPs, polymeric micelles, and exosome-mimetic vesicles may enable efficient co-delivery of multiple editing tools, thereby achieving *multiplexed genetic modulation*. Future designs are expected to incorporate hybrid systems, where Cas12/Cas13 are co-delivered with *AI-optimized lipid nanoparticles* to achieve cell-type-specific editing and minimal immunogenic response.

9.3. Integration with Organoid and Organ-on-Chip Models for Precision Screening

The translation of CRISPR-based nanotherapeutics from bench to bedside remains hindered by the limited predictability of conventional 2D cell cultures and animal models. Emerging organoid and organ-on-chip (OoC) technologies are addressing this challenge by providing physiologically relevant, human-derived microenvironments for therapeutic testing.[28]

Organoids — self-organized 3D cultures derived from stem cells — replicate the architecture and functionality of human tissues such as liver, brain, lung, and tumor microenvironments.[29] Meanwhile, organ-on-chip systems integrate microfluidic channels lined with living cells to mimic the dynamic mechanical and biochemical cues of human organs.[(8)]

When integrated with CRISPR-based nanotherapeutics, these platforms allow:

- Real-time visualization of gene-editing events at the cellular and tissue levels.
- Assessment of nanoparticle biodistribution, off-target editing, and immune interactions.
- Preclinical safety and efficacy screening under human-relevant conditions.

For instance, liver organoid models have been employed to evaluate CRISPR–LNP delivery efficiency and hepatotoxicity, offering an ethical and cost-effective alternative to animal testing.[30]

In the future, multi-organ-on-chip systems could simulate entire physiological networks — allowing simultaneous assessment of systemic distribution, metabolism, and gene-editing outcomes across interconnected organ systems.

9.4. Stimuli-Responsive and Biomimetic Nanocarriers

A promising direction lies in the development of stimuli-responsive nanocarriers that release CRISPR cargo in response to specific environmental triggers such as pH, temperature, redox state, or enzymatic activity.[31]

For example:

- **pH-sensitive liposomes** can release Cas9–sgRNA complexes in acidic tumor microenvironments.
- **Enzyme-sensitive polymeric nanoparticles** can degrade in the presence of tumor-associated proteases, ensuring site-specific release.
- **Redox-responsive carriers** can exploit intracellular glutathione gradients to trigger cytosolic release of CRISPR components.

Parallelly, biomimetic nanocarriers, such as cell membrane–coated nanoparticles, exosomes, and virus-like particles (VLPs), provide immune-evasive and tissue-homing capabilities.[32] These systems mimic the biological membranes of red blood cells, leukocytes, or cancer cells, enabling “self-camouflaged” delivery that reduces immune clearance and enhances targeting specificity.

Recent progress in *exosome engineering* — integrating Cas9 mRNA and sgRNA within naturally secreted vesicles — has shown remarkable success in achieving efficient, non-immunogenic genome editing *in vivo*.[33]

Combining stimuli-responsiveness with biomimicry will create hybrid nanocarriers that respond intelligently to biological signals, delivering CRISPR tools precisely when and where they are needed.

9.5. Toward Personalized and Precision Nanomedicine

The ultimate goal of these technological advances is to realize personalized CRISPR nanomedicine — tailored therapeutic strategies based on a patient's genomic profile, disease progression, and immune characteristics.[34]

Integration of *multi-omics data* (genomics, proteomics, transcriptomics) with *AI-optimized nanocarrier systems* can enable the customization of delivery vehicles for individual patients. Furthermore, combining CRISPR-based gene correction with AMP-loaded or immunomodulatory nanocarriers could create dual-action systems for infections, cancer, and inflammatory disorders — bridging the gap between *gene therapy* and *advanced drug delivery science*.

9.6. Translational and Regulatory Outlook

While the technological trajectory is promising, the path to clinical translation demands rigorous validation of long-term safety, off-target effects, and ethical governance. Standardized frameworks for nanocarrier characterization, batch reproducibility, and immune compatibility will be crucial for regulatory approval.[35]

The establishment of AI-integrated quality-by-design (QbD) protocols and GMP-compliant nanomedicine production pipelines will further ensure scalability and reliability. In parallel, adaptive clinical trial designs leveraging real-time data analytics could accelerate approval for life-threatening genetic diseases.

Future advances will include AI-guided design of nanocarriers, development of next-generation CRISPR systems (Cas12, Cas13, Cas14), and integration with organoid and organ-on-chip models for precision screening.[22] Combining CRISPR with stimuli-responsive or biomimetic nanocarriers will further enhance safety and specificity, paving the way for personalized nanomedicine.[36]

10. Conclusion

CRISPR-based nanotherapeutics exemplify a transformative approach in genome editing by integrating the precision of CRISPR/Cas systems with the versatility of nanotechnology. Nanocarriers—ranging from lipid and polymeric nanoparticles to biomimetic vesicles—enhance the stability, cellular uptake, and tissue-specific delivery of CRISPR components, enabling effective interventions in cancer, genetic disorders, infectious diseases, neurological conditions, and regenerative medicine. Advances in AI-guided nanocarrier design, stimuli-responsive systems, and next-generation CRISPR nucleases (Cas12, Cas13, Cas14), alongside

organoid and organ-on-chip models, are accelerating preclinical optimization and translational potential. Despite challenges such as immunogenicity, off-target effects, and large-scale production, these innovations pave the way toward personalized, precision, and programmable therapeutics. Collectively, CRISPR-based nanomedicine holds the promise to redefine molecular medicine, offering patient-specific interventions with unprecedented accuracy, efficacy, and safety.

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12. Conflict of Interest

The authors declare that they have no conflict of interests.

13. References

1. Givens BE, Naguib YW, Geary SM, Devor EJ, Salem AK. Nanoparticle-based delivery of CRISPR/Cas9 genome editing therapeutics. *J Control Release.* 2019;271:109-127. doi:10.1016/j.jconrel.2017.12.015
2. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. *Science.* 2014;346(6213):1258096. doi:10.1126/science.1258096
3. Hejabi F, Abbaszadeh MS, Taji S, O'Neill A, Farjadian F, Doroudian M. Nanocarriers: A novel strategy for the delivery of CRISPR/Cas systems. *Front Chem.* 2022;10:957572. doi:10.3389/fchem.2022.957572
4. Patil SD, Rhodes DG, Burgess DJ. Complexation and membrane transport characteristics of plasmid DNA and cationic liposomes. *AAPS J.* 2005;7(1):E45-E55.
5. Kim H, Kim JS. A guide to genome engineering with programmable nucleases. *Nat Rev Genet.* 2014;15(5):321-334.
6. Wang HX, Li M, Lee CM, Chakraborty S, Kim HW, Bao G. CRISPR/Cas9-based gene editing for disease modeling and therapy: Challenges and opportunities for nonviral delivery. *Chem Rev.* 2017;117(15):9874-9906.
7. Wang M, Zuris JA, Meng FT, et al. Efficient delivery of genome-editing proteins using bioreducible lipid nanoparticles. *Proc Natl Acad Sci U S A.* 2016;113(11):2868-2873.
8. Baig MMFA, Chien WT, Chair SY. Nanotechnological approaches for targeted

delivery of CRISPR/Cas systems for genomic modifications. *Biomater Sci.* 2025;13:4597-4638. doi:10.1039/D5BM00711A

9. Yang Z, Shi J, Xie J, et al. Large-scale generation of functional exosomes for CRISPR delivery. *Nat Biomed Eng.* 2020;4(1):87-99.
10. Li J, Pei H, Zhu B, et al. Self-assembled DNA nanocapsules for CRISPR/Cas9 delivery. *Angew Chem Int Ed.* 2018;57(23):7483-7487.
11. Xu CF, Chen GJ, Luo YL, Zhang Y, Zhao G, Zhao LY. Rational designs of in vivo CRISPR-Cas delivery systems. *Adv Drug Deliv Rev.* 2021;168:3-29.
12. Liu C, Zhang L, Liu H, Cheng K. Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications. *J Control Release.* 2017;266:17-26.
13. Chen X, Gonçalves MA. Engineered viruses as genome editing devices. *Mol Ther.* 2016;24(3):447-457.
14. Yin H, Kauffman KJ, Anderson DG. Delivery technologies for genome editing. *Nat Rev Drug Discov.* 2017;16(6):387-399.
15. Nguyen DN, Roth TL, Li PJ, et al. Polymer-stabilized Cas9 nanoparticles and modified repair templates increase genome editing efficiency. *Nat Biotechnol.* 2020;38(1):44-49.
16. Lee B, Lee K, Panda S, Gonzales-Rojas R, Chong A, Bugay V. Nanoparticle delivery of CRISPR components for brain gene editing. *Sci Adv.* 2021;7(10):eabe7376.
17. Qiu M, Glass Z, Chen J, et al. Lipid nanoparticle-mediated codelivery of Cas9 mRNA and single-guide RNA achieves liver-specific genome editing in mice. *Nat Biotechnol.* 2021;39(8):997-1000.
18. Gillmore JD, Gane E, Taubel J, et al. CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. *N Engl J Med.* 2021;385(6):493-502.
19. Charlesworth CT, Deshpande PS, Dever DP, et al. Identification of pre-existing adaptive immunity to Cas9 proteins in humans. *Nat Med.* 2019;25(2):249-254.
20. Xu CF, Chen GJ, Luo YL. Rational designs of in vivo CRISPR delivery systems. *Adv Drug Deliv Rev.* 2021;168:3-29.
21. Haque AKMA, et al. Toxicity considerations in nanoparticle-mediated gene therapy. *Adv Drug Deliv Rev.* 2023;200:114048.
22. Xu X, Gao D, et al. AI-driven optimization of CRISPR delivery nanoplatforms. *Adv Sci.* 2024;11(5):2302879.
23. Agam K, Mishra N, Rajinikanth PS, Saraf SA, Kanaujia KA. Antimicrobial peptides

as antimicrobials for wound care management: A comprehensive review. *J Drug Deliv Sci Technol.* 2024;95:105570. doi:10.1016/j.jddst.2024.105570.

24. Kanaujia KA, Wagh S, Pandey G, Phatale V, Khairnar P, Kolipaka T, et al. Harnessing marine antimicrobial peptides for novel therapeutics: A deep dive into ocean-derived bioactives. *Int J Biol Macromol.* 2025;307(Pt 3):142158. doi:10.1016/j.ijbiomac.2025.142158.
25. Shukla AK, Yadav VK, Yadav V, Bharti V, Kanaujia KA. A better method for pharmaceutical quality control for impurity profile data. *[Journal Name]*. 2024;[Volume(Issue)]:[Page-range].
26. Yadav VK, Yadav V, Bharti V, Kanaujia KA. Matrix tablets: benefits, drawbacks and hopes for the future. *[Journal Name]*. [Year];[Volume(Issue)]:[Page-range].
27. Tiwari V, Sharma M, Yadav VK, Kanaujia KA, Yadav VP, Bharti V, … Yadav MK. Solubility enhancement and estimation of paracetamol and domperidone by hydrotropic agents. *Lancet.* 2024;373(9658):121.
28. Kanaujia KA, Shukla AK, Yadav VK, Yadav MK, Kumar S. Beyond borders: Tirzepatide unveiled – A comprehensive review of dual targeting therapies in diabetes and obesity management. *Biochem Cell Arch.* 2024;24(2).
29. Shukla AK, Tiwari A, Yadav VK, Yadav VP, Kanaujia KA, Mishra MK, Verma MSG. Preliminary phytochemical, isolation, characterization, antioxidant, and antimicrobial activity assessments of *Achyranthes aspera L. Biosci Biotechnol Res Asia.* 2025;22(1):401.
30. Shukla AK, Yadav VK, Verma M, Kanaujia KA, Jaiswal A, Gupta V. Expert Systems in Preformulation and Formulation Development with Special Reference to SeDeM System: An Innovative, Problem Solving, Intelligent and Optimization Algorithm Tool. *Curr Indian Sci.* 2024;2(1):e2210299X338978. doi:10.2174/012210299X338978241015155050.
31. Kanaujia KA, Maurya N, Arya DK. Exploring the medicinal potential of *Trillium govanianum* (a threatened plant): Current insights, challenges, and future prospects. *World J Pharma Pharmaceut Sci.* 2023;12:433-463.
32. Precisely Targeted Nanoparticles for CRISPR-Cas9 Delivery in Cancer and Genetic Disorders. *Nanomaterials.* 2023;15(7):540. doi:10.3390/nano15070540
33. Xu X, Gao D, et al. AI-driven optimization of CRISPR delivery nanoplatforms. *Adv*

Sci. 2024;11(5):2302879.

34. Haque AKMA, et al. Toxicity considerations in nanoparticle-mediated gene therapy. *Adv Drug Deliv Rev.* 2023;200:114048.
35. Liu C, Zhang L, Liu H, Cheng K. Delivery strategies of CRISPR-Cas9 gene-editing systems. *J Control Release.* 2017;266:17–26.
36. Chen JS, Ma E, Harrington LB, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science.* 2018;360(6387):436–439.
37. Abudayyeh OO, Gootenberg JS, Konermann S, et al. RNA targeting with CRISPR-Cas13. *Nature.* 2017;550(7675):280–284.
38. Harrington LB, et al. Programmed DNA destruction by miniature CRISPR–Cas14 enzymes. *Science.* 2018;362(6416):839–842.
39. Yin H, Kauffman KJ, Anderson DG. Delivery technologies for genome editing. *Nat Rev Drug Discov.* 2017;16(6):387–399.
40. Zhang YS, et al. Multisensor-integrated organs-on-chips for testing personalized therapies. *Sci Transl Med.* 2017;9(398):eaad7816.
41. Wörsdörfer P, et al. Generation of liver organoids from induced pluripotent stem cells for in vitro testing of gene therapies. *Stem Cell Reports.* 2023;18(1):55–72.
42. Xu CF, Chen GJ, Luo YL. Rational designs of in vivo CRISPR delivery systems. *Adv Drug Deliv Rev.* 2021;168:3–29.
43. Yang Z, Shi J, Xie J, et al. Large-scale generation of functional exosomes for CRISPR delivery. *Nat Biomed Eng.* 2020;4(1):87–99.
44. Qiu M, Glass Z, Chen J, et al. Lipid nanoparticle-mediated codelivery of Cas9 mRNA and single-guide RNA achieves liver-specific genome editing in mice. *Nat Biotechnol.* 2021;39(8):997–1000.
45. Gillmore JD, Gane E, Taubel J, et al. In vivo CRISPR–Cas9 gene editing for transthyretin amyloidosis. *N Engl J Med.* 2021;385(6):493–502.
46. Charlesworth CT, et al. Identification of pre-existing adaptive immunity to Cas9 proteins in humans. *Nat Med.* 2019;25(2):249–254.