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Nano-Engineered CRISPR Systems: Redefining Precision Gene Editing and Targeted Therapeutics

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ABSTRACT

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)–Cas systems have revolutionized genome editing by providing a precise, efficient, and programmable platform for genetic modification. Despite their immense therapeutic potential, the clinical translation of CRISPR technology is significantly limited by challenges associated with safe and efficient delivery of gene-editing components. Nanotechnology has emerged as a promising solution to overcome these barriers by enabling targeted, controlled, and non-viral delivery of CRISPR machinery. This review highlights the fundamental principles of CRISPR–Cas systems and comprehensively discusses various delivery strategies, including viral and non-viral approaches. Special emphasis is placed on nanotechnology-based delivery systems such as lipid nanoparticles, polymeric nanoparticles, inorganic nanocarriers, and exosome-mediated transport. These systems enhance gene stability, improve cellular uptake, reduce immunogenicity, and enable tissue-specific targeting. Furthermore, advanced strategies such as ligand-mediated targeting, stimuli-responsive release, and CRISPR ribonucleoprotein delivery are explored to improve editing precision and minimize off-target effects. The review also summarizes recent advancements, including DNA nanotechnology, base editing, and prime editing integrated with nanocarriers, which further expand the scope of gene therapy. Additionally, applications of CRISPR nanotechnology in cancer therapy, genetic disorders, infectious diseases, and diagnostics are discussed. Overall, the integration of CRISPR technology with nanotechnology offers a powerful and versatile platform for next-generation gene therapy, paving the way for safer and more effective clinical applications in the future.

Keywords : CRISPR–Cas9, Gene Editing, Nanotechnology, Nanocarriers, Targeted Drug Delivery, Lipid Nanoparticles, Polymeric Nanoparticles.

1. INTRODUCTION:

The fascinating tale of Clustered Regularly Interspaced Palindromic Repeats (CRISPR) began with discoveries made by a Japanese research team in 1987[1]. In order to withstand exposure to alien nucleic acids like viral genomes and plasmids, bacteria and archaea have developed a variety of defense mechanisms. These resistance mechanisms include restriction modification systems, phage abortive infection systems, stopping phage DNA injection, and preventing phage adsorption[2]. The adaptive microbial immune system, which is based on CRISPR (clustered regularly interspaced short palindromic repeats) and Cas (CRISPR associated) genes, has recently been discovered, expanding this defensive repertoire[3]. According to Barrangou et al. (2007), CRISPR and the related Cas genes provide an adaptive immunity that offers protection against bacteriophage infection. Short sequences from viruses and other mobile genetic elements are incorporated into the host's CRISPR locus by the CRISPR/Cas system, a highly adaptable and heritable resistance mechanism that directs the destruction of invasive nucleic acids by transcription and processing into small RNAs[4]. By storing memory in the form of viral DNA in the bacterial host chromosomes, the CRISPR–Cas system, an adaptive immune system in prokaryotes, guards against phage invasion. Direct repeats are repeating nucleotide sequences that encircle the viral DNA in the system. Sequences that encode proteins known as Cas proteins encircle the near end of these direct repeats. In order to direct reprogrammed endonucleases to the target gene, this mechanism was intentionally altered[4]. The five direct repeats of the CRISPR-Cas system, which include 24 nucleotide repetitive sequences, were first seen in 1987 when the gene that converts the alkaline phosphatase isozyme in Escherichia coli was discovered. The second instance was found in Haloferax mediterranei, which has 30–34 nucleotide sequences with direct repetitions separated by spacers, which are 35 bp length sequences. Short, inverted repetitions that resembled those in the previous sequence were also present in the direct repeats[5]. The repeating DNA sequences found in the genome close to the "DNA repair system" between 1993 and 2005 were identified by Ruud Jansen and associates in 2002 as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs)[6]. The relationship between the roles of CRISPR

and Cas genes as a system was computationally examined by Eugene V. Koonin and his associates in 2006. They found that this system resembled a prokaryotic RNA interference immune system[7]. According to a research, Streptococcus thermophilus transfers the CRISPR–Cas9 system to Escherichia coli, giving it protection against plasmid and phage infection [8]. Cas9 was created by David Bikard and colleagues as a transcriptional repressor that inhibits RNA polymerase (RNAP) from binding to promoter regions [9].

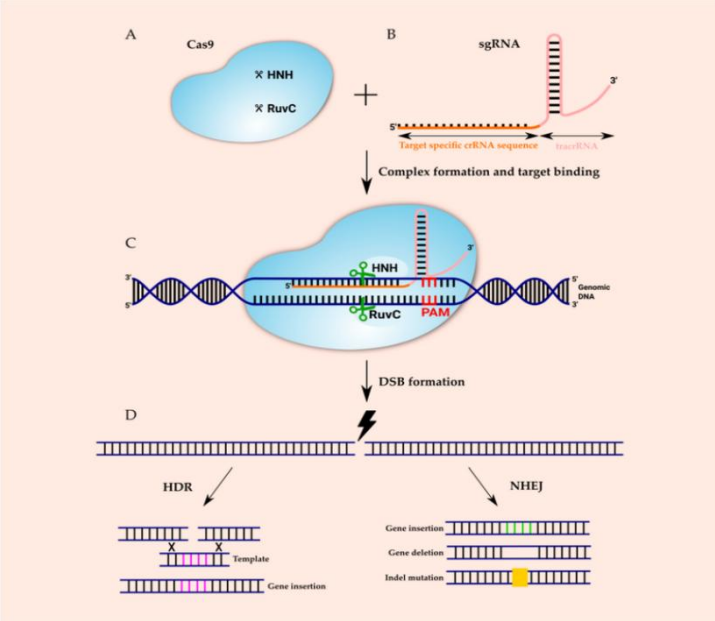

Fig 1. CRISPR–Cas 9 system molecular mechanism

2. CRISPR-CAS SYSTEM

There are three ways to distribute the CRISPR-Cas system. The first option involves inserting the Cas9 protein's sgRNA and mRNA into the cytoplasm, thereafter the mRNA is transcribed. Nevertheless, the mRNA molecule's low stability leads to its quick disintegration and, thus, a short gene editing lifetime. The third type, the Cas9–sgRNA RNP complex, is a safe and efficient substitute that is constrained by molecular weight and extensive active Cas9 production[9,10]. The strategies can be classified into two main categories: viral and non-viral delivery systems[11].

2.1 Non-viral strategies:

The two primary subcategories of non-viral solutions are physical approaches and nanoparticles. These methods, in contrast to viral delivery systems, have been shown to be effective and do not restrict the size of the payload. However, cell survival and tissue damage have proven to be significant challenges, especially for physical delivery modalities [12].

A. Microinjections

Microinjections consist of various needles of micron size which act as carrier for biomolecules. DNA plasmids, mRNA, or cas9 protiens with sgRNA are delivered by the microinjections with the advantage of minimal off-target side effects [13].

Positive outcomes have been obtained from recent studies employing microinjections to deliver CRISPR-Cas in many living species. Numerous successful experiments with arthropods, mice, and even human embryos have been conducted; nevertheless, the efficacy of microinjections in numerous different live animals has not been completely investigated [14].

B. Electroporation

Another common physical technique is electroporation, which involves temporarily disrupting the phospholipid bilayer of the plasmatic membrane with controlled electric pulses to increase cell membrane permeability and enable intracellular transport and, consequently, the introduction of nucleic acids into particular cells[15].

For a number of reasons, this approach differs from other widely used delivery methods like microinjections and is appropriate for all kinds of CRISPR-Cas cargo. First off, this technique may be applied both in vitro and in vivo because the administration does not require a microscope. Additionally, there have been reports of increased embryonic survival rates[16].

C. Hydrodynamic Delivery

Hydrodynamic delivery involves quickly injecting a fluid—typically a saline solution—into the animal's tail vein in an amount equal to 8–10% of its body weight. This liquid is loaded with gene editing cargo into the animal's bloodstream; the rapid flow of saline solution causes the liver to expand, raising hydrodynamic pressure and momentarily rupturing the endothelium and cell membranes. This allows the CRISPR-Cas cargo to enter the cells, especially hepatocytes and the kidney, lung, muscle, and heart cells [17].

2.2 Viral Delivery Strategies

Viral systems are popular substitutes, but they have some drawbacks. For instance, viruses need HEK 293T cells to produce viral-like particles with Cas9 and sgRNA, which then infiltrate the target cells and are either investigated in vitro or subsequently implanted into the intended organism [18].

A. Adenoviral Vectors

Double stranded DNA viruses which have the ability to affect the dividing and non-dividing cells are called adenoviruses. After inoculation, certain characteristics of these viruses enable the efficient transfer of genetic material; the viral genome does not integrate with the host genome, reducing adverse consequences such as insertional mutagenesis. AdVs are among the top options for viral delivery systems because of their low immunogenicity and efficient transduction [19].

B. Adeno-Associated Viruses

As AdVs, these vectors are DNA double-stranded viruses that can infect both dividing and non-dividing cells; as one of the most popular options, they possess an adequate safety profile with mild immunogenicity and off-target side effects. These viruses do not insert their genome into the host chromosomes. Instead, the viral genetic material binds into specific loci of mitochondrial DNA known as integration sites, and this mechanism maintains the safety profile of AAVs since it does not contribute to tumorigenesis [20].

Limitation of this system is cloning capacity which led to limited payload, potential cytotoxicity and isolated mutations [21].

C. Lentivirus

Lentivirus is a single-stranded RNA virus with strong cloning and cargo capabilities that can infect both dividing and non-dividing cells. Its ability to be pseudotyped with other viral proteins sets it apart from the aforementioned vectors. It also has a low level of immunogenicity, good packaging and transduction potential, and minimal effects on the cell cycle. Lentiviruses, in contrast to AdVs, incorporate their genome into the host's genetic material, which may result in insertional mutagenesis and other unintended consequences[22].

To lessen the possible negative consequences, researchers have suggested substitutes such integrating defective lentiviruses (IDLVs). This method increases the safety of LV vectors by introducing a single point mutation to an integrase, which hinders lentivirus integration. On the negative side, IDLVs exhibit reduced transgenic expression. Additionally, employing a self-inactivating Cas9 protein to lessen Cas9 off-target negative consequences is a workable substitute to lower the mutational danger [23].

3. Targeted Delivery Strategies in CRISPR Nanotechnology

Targeted delivery is one of the most critical aspects of CRISPR-Cas9 gene editing when combined with nanotechnology. Efficient targeting ensures that gene-editing components reach the desired cells or tissues, minimizing off-target effects and toxicity. Below is a structured explanation suitable for your article or notes [24,25,26].

3.1. Ligand-Mediated Targeting[27]

This strategy uses specific ligands attached to nanoparticles that bind to receptors on target cells.

- Ligands include:
- Antibodies
- Peptides (e.g., RGD peptides)
- Aptamers
- Example:
- Folate receptors on cancer cells can be targeted using folic acid-conjugated nanoparticles.

Advantage: High specificity toward diseased cells

Application: Cancer gene therapy

3.2. Receptor-Mediated Endocytosis[27]

Nanoparticles are designed to enter cells via receptor binding and internalization.

- Nanocarriers bind to overexpressed receptors
- Enter cells through endocytosis pathways
- Common in tumor-targeting systems

Key Benefit: Enhanced cellular uptake of CRISPR components

3.3. Stimuli-Responsive Delivery Systems[27,28]

These systems release CRISPR components in response to specific stimuli:

Types of stimuli:

- **pH-sensitive** (tumor microenvironment is acidic)
- **Redox-sensitive** (high glutathione levels inside cells)
- **Enzyme-responsive**
- **Temperature/light-responsive**

Advantage: Controlled and site-specific release

Example: pH-sensitive lipid nanoparticles releasing Cas9 in tumor cells

3.4. Cell-Penetrating Peptides (CPPs)[27,28]

CPPs facilitate transport of CRISPR components across cell membranes.

- Examples:
- TAT peptide
- Penetratin

Advantages:

- Improved intracellular delivery
- Useful for hard-to-transfect cells

3.5. Lipid-Based Nanoparticles (LNPs)[27,28]

Widely used non-viral delivery system for CRISPR.

- Encapsulate:
- Cas9 mRNA
- sgRNA
- Can be modified with targeting ligands

Example:

- Liver targeting using ionizable lipids

Advantages:

- Biocompatible
- Clinically advanced (used in mRNA vaccines)

3.6. Polymer-Based Nanocarriers[27,28]

Polymers like PLGA, chitosan, and PEI are used for CRISPR delivery.

- Can be engineered for:
- Controlled release
- Surface functionalization

Advantages:

- Stability
- Tunable properties

3.7. Exosome-Mediated Delivery[27]

Natural vesicles used as delivery vehicles.

- Derived from cells
- Can carry CRISPR/Cas9 components

Advantages:

- Low immunogenicity

- High biocompatibility
- Natural targeting ability

3.8. Magnetic Nanoparticle Targeting[27,28]

Magnetic fields guide nanoparticles to target sites.

- Iron oxide nanoparticles used
- External magnet controls localization

Application: Localized tumor targeting

3.9. Viral-Mimicking Nanoparticles[28]

Synthetic nanoparticles designed to mimic viral efficiency.

- Combine:
- High transfection efficiency
- Reduced toxicity

Goal: Replace viral vectors safely

Targeted delivery strategies in CRISPR nanotechnology are essential to improve precision, safety, and therapeutic efficacy. Combining multiple strategies—such as ligand targeting with stimuli-responsive release—offers promising advancements for future gene therapy applications[29].

4. STRATEGIES FOR CRISPR LOADING INTO NANOPARTICLES

CRISPR/Cas gene-editing components are increasingly being delivered into target cells via nanoparticle-based delivery methods. For effective cellular absorption, successful genome editing, and protection of the editing machinery, CRISPR systems must be loaded into nanoparticles efficiently. Depending on the kind of CRISPR cargo—plasmid DNA, mRNA, or ribonucleoprotein (RNP) complexes—a number of approaches have been devised [30].

4.1. Encapsulation of CRISPR Components

Encapsulation is one of the most widely used strategies for loading CRISPR components into nanoparticles. In this method, CRISPR materials such as Cas9 mRNA, guide RNA (gRNA), or plasmid DNA encoding CRISPR systems are enclosed within the nanoparticle matrix during the formulation process. Encapsulation protects the genetic material from enzymatic degradation and premature clearance in the bloodstream. Materials such as lipid nanoparticles, polymeric nanoparticles (PLGA, chitosan), and lipid–polymer hybrid nanoparticles are commonly used. This strategy ensures stable delivery and controlled release of CRISPR components once the nanoparticle reaches the target cells [31].

4.2. Electrostatic Complexation

Because CRISPR nucleic acids are negatively charged, they can bind electrostatically with positively charged polymers or lipids to create complexes. Stable nanoparticle complexes are created when cationic substances like polyethyleneimine (PEI), chitosan, or cationic lipids attach to RNA molecules or CRISPR plasmids. Strong nucleic acid binding and great loading efficiency are made possible by this quite straightforward and effective method. Additionally, electrostatic complexation increases the efficacy of gene editing by facilitating cellular absorption through endocytosis [32].

4.3. Surface Conjugation

Using chemical linkers or affinity interactions, CRISPR components are affixed to the surface of nanoparticles in surface conjugation techniques. Functional compounds like amine, thiol, or carboxyl groups can be added on the surface of nanoparticles to enable CRISPR molecules to attach covalently. This method allows CRISPR complexes to be presented and oriented in a controlled manner, perhaps improving contact with target cells. Additionally, surface functionalization improves tissue-specific distribution by enabling the inclusion of targeted ligands like peptides, aptamers, or antibodies [33,34].

4.4. Loading of CRISPR Ribonucleoprotein (RNP) Complexes

Loading preassembled Cas9 protein and guide RNA complexes (RNPs) into nanoparticles is another sophisticated tactic. There are many benefits of delivering CRISPR in RNP form:

- Rapid gene editing activity after delivery
- Reduced risk of off-target effects
- Transient presence of CRISPR components in cells

Nanoparticles such as gold nanoparticles, lipid nanoparticles, and polymeric nanocarriers can effectively encapsulate or bind RNP complexes and facilitate their transport into cells.

4.5. Lipid Nanoparticle Self-Assembly[35].

During formulation, lipid nanoparticles can self-assemble with CRISPR nucleic acids. Under certain pH levels, ionizable lipids bind with DNA or CRISPR RNA to form stable complexes. Cas9 mRNA and guide RNA are frequently delivered via this method, especially in in vivo gene editing applications. Effective encapsulation, endosomal escape, and enhanced intracellular delivery are all made possible by lipid nanoparticles.

4.6. Stimuli-Responsive Loading Systems

Advanced nanoparticle systems are designed to release CRISPR components in response to specific stimuli, such as:

- pH changes
- Enzymes
- Temperature
- Redox conditions

These stimuli-responsive nanoparticles ensure that CRISPR cargo is released only at the target site, thereby improving editing efficiency and minimizing off-target effects [36].

5. ROLE OF NANOTECHNOLOGY IN GENE DELIVERY

In order to treat or prevent illnesses, gene therapy entails introducing genetic material into cells, such as DNA, RNA, or siRNA. Although they are highly effective, traditional viral vectors (adenovirus, lentivirus, and retrovirus) have a number of drawbacks, including limited gene carrying capacity, immunogenicity, and insertional mutagenesis. Because they offer safer, more regulated, and targeted gene delivery, nanotechnology-based delivery technologies have become attractive substitutes. Nanotechnology protects and transports genetic material to certain cells or tissues using nanoscale carriers (1–100 nm) [37].

5.1. Improved Gene Stability and Protection from Nucleases

Nucleases found in blood and bodily fluids may easily break down genetic molecules including DNA, mRNA, and siRNA. By encasing or complexing these delicate molecules within nanoparticles, nanotechnology-based carriers provide protection. Nucleic acids are shielded from enzymatic breakdown during circulation in the body by nanocarriers such liposomes, polymeric

nanoparticles, and lipid nanoparticles. This protection greatly lengthens the genetic material's circulation duration and stability, improving its capacity to reach the target area. Furthermore, until the genetic payload reaches the intended location of action, the nanoscale structure aids in preserving its biological activity and structural integrity[38] .

5.2. Targeted Delivery to Specific Tissues

The potential for tailored distribution is one of the biggest benefits of nanotechnology in gene delivery. Surface ligands, antibodies, peptides, or aptamers that identify certain receptors on target cells can be added to nanoparticles. With little contact to healthy tissues, this tailored technique guarantees that the therapeutic gene is mostly delivered to the sick tissue, such as tumor cells, inflammatory tissues, or certain organs. Targeted administration lowers systemic toxicity and increases therapeutic effectiveness. For instance, through receptor-mediated endocytosis, nanoparticles made with tumor-targeting ligands can aggregate specifically in cancer cells [39].

5.3 Reduced Immunogenicity Compared to Viral Vectors

Conventional viral vectors employed in gene therapy frequently cause immunological reactions, which can result in toxicity, inflammation, or the vector's quick removal from the body. Non-viral solutions based on nanotechnology often have higher safety profiles and less immunogenicity. Lipids, biodegradable polymers (PLGA), and natural polymers (chitosan), which are frequently employed in the production of nanoparticles, are generally biocompatible and less likely to cause severe immunological responses. Because of their decreased immunogenicity, nanoparticles may be administered repeatedly, which is frequently required for long-term gene therapy therapies or chronic illnesses [40].

5.4 Ability to Deliver Large Genetic Payload

The size of the therapeutic gene that may be delivered is restricted by the limited gene carrying capacity of many viral vectors. Larger genetic resources, such as several genes, big plasmid DNA, or combinations of other nucleic acids, can be accommodated by nanoparticles. This feature makes it possible to deliver complicated therapeutic structures, such huge plasmids, many therapeutic genes at once, or gene-editing systems (like CRISPR-associated components). Because of this, nanocarriers provide more versatility when creating cutting-edge gene therapy techniques [41].

5.5. Controlled and Sustained Release

Genetic material may be released over a long period of time in a regulated and maintained manner using nanoparticles. Researchers can control the pace at which the genetic material is released by altering the nanocarrier's composition, shape, and rate of disintegration. For instance, in physiological settings, polymeric nanoparticles composed of biodegradable polymers like PLGA progressively break down, releasing the contained nucleic acids in a regulated way. This prolonged release lengthens the duration of gene expression, lessens the need for frequent doses, and improves the overall therapeutic result [42].

5.6. Possibility of Co-Delivery of Drugs and Genes

Therapeutic genes and traditional medications can be delivered simultaneously within a single carrier system thanks to nanotechnology. In conditions like cancer, where combining gene therapy with chemotherapy might improve therapeutic efficacy, this co-delivery technique is very helpful.For example, siRNA or plasmid DNA that targets drug resistance genes in tumor cells can be encapsulated in nanoparticles together with a chemotherapeutic medication. This integrated strategy can minimize negative effects by increasing therapeutic efficacy, overcoming medication resistance, and lowering necessary drug dosages [43].

By increasing stability, simplifying targeted transport, lowering immunogenicity, permitting the delivery of huge genetic payloads, offering controlled release, and enabling combination drug–gene therapy, nanotechnology greatly improves gene delivery. Nanotechnology-based systems are promising substitutes for traditional viral gene delivery vectors because of these benefits [44].

6. RECENT ADVANCEMENT IN CRISPR NANOTECHNOLOGY

Because of its extreme accuracy and programmability, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology has revolutionized genome editing. Nevertheless, a crucial constraint is the effective and secure delivery of CRISPR components (Cas proteins, sgRNA, and mRNA). By enhancing the stability, targeting, and delivery efficiency of CRISPR systems, nanotechnology has become a crucial enabling platform [45].

- Lipid Nanoparticles (LNPs) in CRISPR Delivery

One of the most sophisticated and popular non-viral delivery methods for CRISPR components is lipid nanoparticles. They make it easier for Cas9 mRNA and single guide RNA (sgRNA) to be effectively encapsulated and delivered into target cells. Recent advancements in ionizable lipid formulations have increased gene editing efficiency, decreased toxicity, and improved endosomal escape. As a major platform in CRISPR nanotechnology, LNPs are presently being investigated in clinical trials for in vivo gene editing applications due to their biocompatibility and scalability [45].

- Polymeric Nanoparticles and Their Advancements

CRISPR components are released in a regulated and prolonged manner by polymeric nanoparticles, such as those composed of polyethyleneimine (PEI) and poly(lactic-co-glycolic acid) (PLGA). The creation of stimuli-responsive polymers, which release their payload in reaction to environmental triggers like pH shifts or redox conditions, is one example of recent developments. Additionally, surface changes have been added to increase their efficacy as CRISPR delivery vehicles by lowering cytotoxicity and improving cellular absorption [46].

- Inorganic Nanoparticles for CRISPR Delivery

The great loading capacity and simplicity of functionalization of inorganic nanoparticles, such as mesoporous silica nanoparticles and gold nanoparticles, have drawn interest. CRISPR ribonucleoprotein complexes can be efficiently delivered by gold nanoparticles, which can also be designed for targeted delivery. Large surface areas and controlled release characteristics provided by mesoporous silica nanoparticles enhance the stability and effectiveness of gene editing. In preclinical research, these systems have demonstrated encouraging outcomes, especially in cancer treatment [47].

- Exosomes and Extracellular Vesicles

Extracellular vesicles and exosomes are a new, physiologically based method of delivering CRISPR. These naturally occurring nanocarriers have good biocompatibility, minimal immunogenicity, and built-in targeting capabilities. In order to enable effective and targeted genome editing, recent research has concentrated on designing exosomes to transport CRISPR/Cas9 ribonucleoprotein complexes. They are very promising for in vivo applications because of their capacity to overcome biological barriers [48].

- DNA Nanotechnology and CRISPR Systems

By creating programmable DNA nanostructures like DNA origami and nanocages, DNA nanotechnology has brought new approaches to CRISPR delivery. CRISPR components can be precisely arranged in space and released under regulated conditions because to these structures. The creation of DNA-based nanocarriers for multiplex gene editing and biosensing applications is one recent discovery that greatly improves the adaptability and accuracy of CRISPR systems [49].

- Advanced CRISPR Editing Technologies

To increase accuracy and lessen off-target effects, recent advancements in CRISPR technology, like as base editing and prime editing, have been combined with nanotechnology. These cutting-edge editing techniques increase safety by allowing gene repair without creating double-strand DNA breaks. In order to effectively distribute these advanced editing tools to target cells and increase their therapeutic potential, nanocarriers are essential [50].

- CRISPR Ribonucleoprotein (RNP) Delivery

An important development in genome editing is the delivery of CRISPR ribonucleoprotein complexes, which are made up of guide RNA and Cas protein. This method minimizes off-target effects and lowers long-term toxicity by enabling quick and temporary gene editing. By preventing RNPs from degrading and facilitating their effective entrance into cells, nanoparticles increase the effectiveness and safety of editing [51].

- Emerging Trends in CRISPR Nanotechnology.

The creation of intelligent and stimuli-responsive nanocarriers that release CRISPR components in response to certain biological triggers like pH, enzymes, or temperature is one of the emerging topics in CRISPR nanotechnology. To accomplish tissue-specific gene editing, targeted delivery methods utilizing ligand-functionalized nanoparticles are also being investigated. Furthermore,

extremely specialized guide RNAs are being designed using artificial intelligence, which lowers off-target effects and boosts overall effectiveness [52].

- Applications of CRISPR Nanotechnology

Applications for CRISPR nanotechnology are numerous and span many different industries. Targeted editing of oncogenes is made possible by nanoparticle-mediated CRISPR systems in cancer therapy, which enhances treatment results. These technologies help rectify disease-causing mutations in vivo in genetic illnesses. Targeting viral genomes in infectious disorders is another use of CRISPR-based nanotechnology. Additionally, the combination of CRISPR with nanotechnology has resulted in the creation of extremely sensitive biosensors for diagnostics as well as novel methods for crop enhancement in agriculture [53].

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