

# Advancing Therapeutics: CRISPR-Cas9 and Emerging Drug Delivery Systems

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**Abstract:** The field of genome editing in health, agriculture, and biotechnology has seen a revolutionary surge in productivity brought about by the CRISPR-Cas9 technology. This post looks into the genesis and distinct features of the system, with particular emphasis on the indispensable tools of the trade: the Cas9 enzyme and the guide RNA. It notes the extraordinary precision with which the system yields exact genome edits, conditions that CRISPR creates for the development of novel medical therapies, and the momentous rise of church-state issues in bioengineering that promise to impact the agricultural sector. The second focus area is how well the CRISPR-Cas9 system can be delivered using nanoparticles, including lipid-based and polymeric nanoparticles, and viral vectors. These are very effective delivery methods, but they aren't perfect and have a scope of improvements that lie ahead. Should the CRISPR-Cas9 system find success in research domains, it's likely that current delivery methods, including nanoparticles, will be a part of that success. There's at least one expert opinion that foresees the current research scenario in the field as already placing our understanding of CRISPR-Cas9 on the cutting edge of genome editing breakthroughs.

**Keywords:** Nanoparticles; CRISPR-Cas9; Genome editing; Drug delivery systems; Precision medicine; Viral vectors; Gene therapy

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## 1. Introduction

Attention has turned to those that are rapid and precise, with the emphasis currently resting with CRISPR-Cas9 because of its speed, simplicity and relatively low cost.

This system is better than earlier techniques like zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) and has utility in a broad array of issues, comprising scientific research, medical treatment, gene editing, and agribusiness. (Gaj et al., 2013).

CRISPR-Cas9 consists of two essential components: Cas9 or molecular scissor, guide RNA which clears Cas9 where on the DNA it has to search. Circulating from bacterial immune systems, the CRISPR-Cas9 platform was used to carry specific gene modifications in varying organisms including human beings. (Jinek et al., 2012).

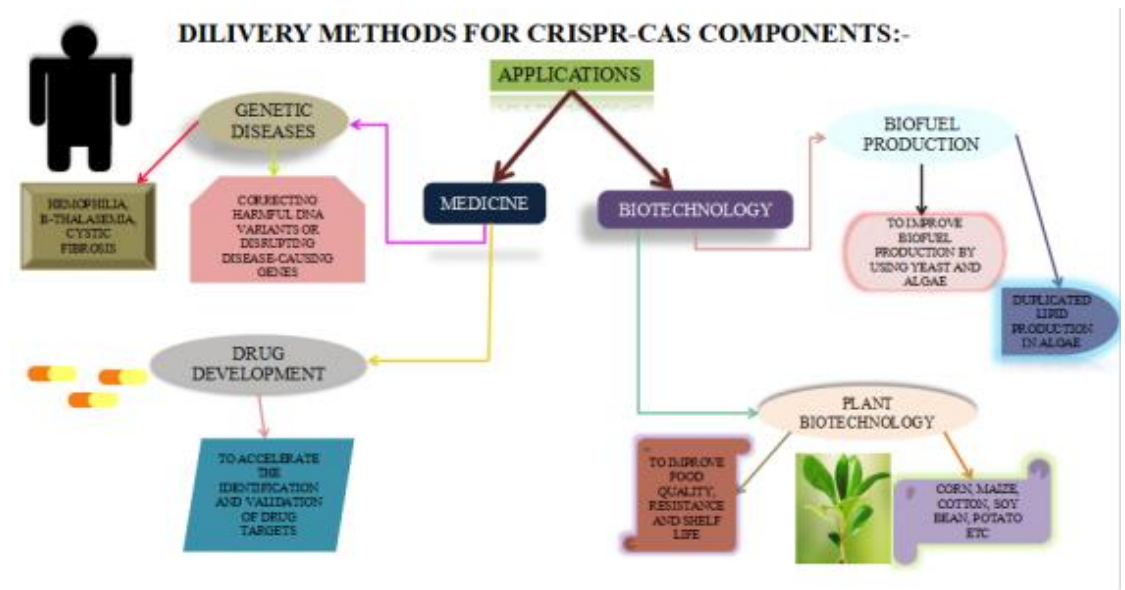
Cas 9 and gRNA unite and locate the gene sequence in the DNA chain after which Cas9 separates the sequence into two individual strands. Such cellular repair mechanisms deliver these changes through either non homologous end joining (NHEJ) or homology directed repair (HDR). It is exuberantly versatile for research, therapy, and agriculture because of its authentication for precise genetic modifications based on its-programmable design. It does this with a unparalleled accuracy to that of other genome editing tools, with lower off target effects and improved outcomes. (Cong et al., 2013).

However, there are a number of concerns associated with the application of the CRISPR-Cas9, including off-target effects and problems with the correct delivery of the machineries. Preparations are underway in order to regulate the technology among others enhancement of Cas9 and design of more effective gRNAs. Deliverance of the CRISPR composites comprising of Cas9, tracrRNA and guide RNA into the cell is currently being depicted using methods like viral vectors and nanoparticles. (Wang et al., 2016). Further, the innovative area of such usage concerns is ethical about it, especially in the human germline variations of the technique.

Thus, as a practically universal genome ‘scalpel’ allowing a user to cut, replace, deactivate or modify DNA in essentially any way desired, CRISPR-Cas9 is capable of controlling almost any gene activity in a cell. Thus, while having a number of disadvantages, if its flaws are provided and elementary ethical standards are met, it can benefit technological growth in science in a broad range of fields, providing novel approaches to solve significant biological and global challenges. (Doudna & Sternberg, 2017).

## 2. The Role and Significance of CRISPR-Cas9 in Genome Editing

CRISPR-Cas9 technology represents a revolutionary milestone in genome editing, driven by the coordinated action of its two main components: either cytosine deaminase or Cas9 endonuclease and the guide RNA (gRNA); an adenoviral vector with targeted gene editing potential gRNA is as small as 20 nucleotides, which binds directly to the target DNA sequence at the PAM site. The Cas9 system is known as molecular scissors and it cuts the DNA at three positions back from the PAM sequence and makes DSB. These double-strand breaks (DSBs) are subjected to NHEJ, which leads to insensitive insertion or deletion (indels) while HDR enables goal-directed genetic engineering (Barrangou & Marraffini, 2014). Originally a tool for bacterial immunity, CRISPR-Cas9 has become suiting a myriad of needs from altering crop characteristics to progress in clinical genetics. Furthermore, it is more flexible, easier to operate and accurate than previous methods such as ZFN and TALENs. (Cong et al., 2013; Mali et al., 2013).

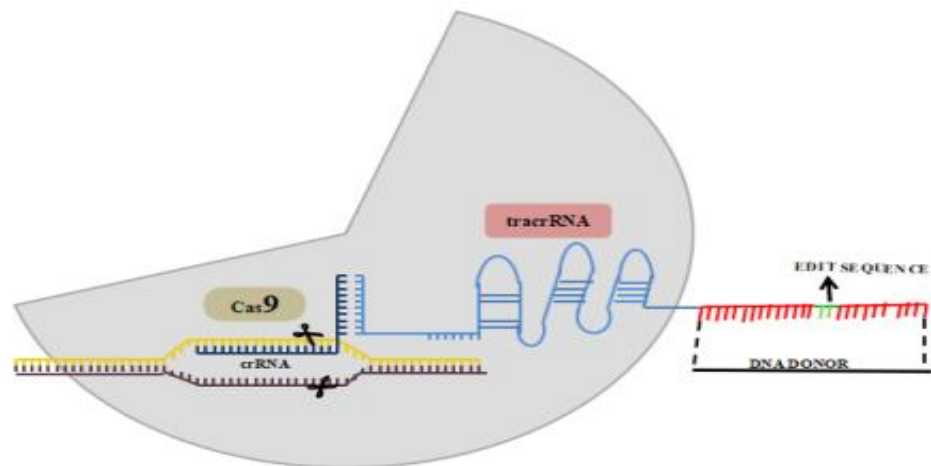


**Fig.** Delivery components ensure efficient transport of CRISPR–Cas elements—such as Cas proteins and guide RNA—into target cells for precise genome editing

## 3. Overcoming Delivery Challenges: The Role of Nanomaterials in Enhancing CRISPR-Cas9 Efficiency

One of the biggest obstacles of applying CRISPR-Cas9 system as a genome editor is the efficient delivery of its components to the target cells. Some of these challenges includes; cellular uptake and the independency to exit the endosomal compartment. To overcome these challenges nanomaterials namely lipid based and polymeric nanoparticles have been developed to improve delivery of CRISPR-Cas9 components. The utilization of nanoparticles present specific benefits because of their high surface area, ease of surface modification and their ability to perform multiple functions that would enhance the delivery process. Among the types of nanocarriers, lipid-based nanoparticles (LNPs) demonstrate all the abilities to protect and enclose all components of CRISPR-Cas9 and deliver them into target cells or tissues. These nanoparticles are non-toxic, can readily incorporate into cell membranes and can efficiently transfect the genetic material into the cytoplasm. (Gao et al., 2016; Zhang et al., 2020).

Moreover, targeted engineered nanoparticles utilize the functional features inclining to accommodation size, surface charge, and functionalization. These attributes lead to enhancement of stability of the delivery system, its specificity to tissues of interest, and overall biocompatibility thereby minimizing off target effects. (Wang et al., 2016). Likewise, polymeric nanoparticles where CRISPR-Cas9 components are encapsulated, guarantees their appropriate release and uptake by target cells, leading to improved efficiency of gene editing. (Sun et al., 2020). Incorporation of these nanomaterials in CRISPR Cas 9 delivery has shifted gene therapy practices from earlier drawbacks like low uptake and target specificity. That said future iterations of these nanoparticles will probably be refined to work even better in CRISPR-Cas9 based therapies as future research continues to play out.



**Fig 2.** TracrRNA pairs with crRNA to guide the Cas9 nuclease to the target DNA sequence, enabling precise site-specific cleavage

### 3.1 Principles of the CRISPR-Cas9 System

The CRISPR-Cas9 system is a revolutionary tool in genome editing, relying on two core components: Cas9 and the guide RNA or gRNA for short here refers to the Cas protein and RNA molecule. Cas9 is a nuclease protein downloaded from bacterial immune system which controls the DNA at certain sites as molecular scissors. The nuclease domains help in the cleavage of the DNA strands, and recognition domain with gRNA and the target DNA. The gRNA acts as a guide and makes Cas9 protein to have focus on the intended locus on the genome. This guide contains a scaffold part, and a sequence which can be helpful in adaptation to the target DNA.

Specifically, when present in cells, the Cas9-gRNA complex recognizes and cleaves the desired target DNA strand. After this double-strand break (DSB) the further destiny of the genetic modification by the cell repair mechanisms non-homologous end joining (NHEJ) or homology-directed repair (HDR). Altogether, NHEJ often deploys insertions or

deleterious damage while HDR can be a highly selective process for correction allowing the CRISPR-Cas9 system to mediate both gene knockout and gene editing.

### 3.2 Comparative Advantages and Limitations of CRISPR-Cas9

CRISPR-Cas9 system has some outstanding benefits over the preceding methodologies of gene editing including Zinc Finger Nucleases and Transcription Activator-Like Effector Nucleases. It appears also to be stronger than the other method in terms of versatility, thus making it easier to adapt for use on different species and for targeting different genes.

Also, CRISPR-Cas9 is cheaper and much easier to practice, not mentioning the versatility it opens for the scientific and business worlds. (Jinek et al., 2012). Nonetheless, like all the other systems, the CRISPR-Cas9 is not without its pullbacks. A threat is genomic safety, which include the off-target effects where the Cas9 protein cleaves targeted or nontarget regions in the genome with convenient repercussions. To address this issue, design of the gRNA must be refined, and experimental conditions need to be enhanced.

It based on high-fidelity Cas9 variants and gRNA design to enhance specificity, and accuracy of the system, eliminating off-target effects which are still a problem in clinical application. (Hsu et al., 2013; Kleinstiver et al., 2016).

Overcoming these challenges, CRISPR-Cas9 method can be further optimized and fine tuned and indeed extend the number of its applications across numerous biological disciplines including agriculture or gene treatment.

### 3.3 Nano-Particle Based Delivery Systems

Lipid based and polymeric nanoparticles are on the cutting edge in the development of CRISPR-Cas9 delivery system. Cationic lipids of lipid-based nanoparticles condense and protect the CRISPR-Cas9 elements, enabling their uptake by cells. This makes them suitable for delivering their payloads selectively; furthermore, based on experience with other viral vectors, the immunogenicity is relatively low. (Liu et al., 2020). In contrast, biodegradable organopolymers give safeguard to the CRISPR-Cas9 particles while giving a go steady release proforma-an element which is critical for discerning nucleic dumps. Some of these aspects like size, charge and surface chemistry of the nanoparticles remarkably have an impact on its ability and efficiency of gene delivery. (Kumar et al., 2019). When combined, lipid-based and polymeric nanoparticles address fundamental issues in CRISPR-Cas9 delivery and enhancing the editing process.

### 3.4 Viral Vectors as Nanocarriers

Adeno-associated viruses (AAVs) have become some of the most suitable viral delivery vectors for CRISPR-Cas9 components in vivo. Such non-pathogenic viruses are preferred due to their capacity of entering target cells as well as transferring such essential elements as Cas9 and gRNA. According to the characteristics of AAVs, the long-term transgene expression is more suitable for therapeutic applications. (Mingozzi & High, 2013). However, immunogenicity of AAV vectors and pre-existing immunity in some individuals are shown to lower the therapeutic efficacies of these vectors. Further studies should be oriented to explain the ways of AAVs interacting with the host immune system to improve gene therapy. (Britt, 2017).

Lentiviral vectors also make another really good choice because of bringing in large numbers of genomes, integrating them into stable locations, and offering optimum gene expression for the longer run. These vectors are especially advantageous when it comes to transfecting rather big DNA pieces or several genes at a time that is why they will suit both ample range of applications (Zhu et al., 2015). However, the possibility of mutagenesis created by lentiviral integration has to be further discussed to weigh the benefits against the hazards. Comparing AAVs and lentiviral vectors, both systems point to the

fact that the choice of the delivery strategy is critically important for the overall efficiency and safety of the CRISPR-Cas9 applications.

### 3.5 Enhancing CRISPR-Cas9 Efficiency with Nanomaterials

Nanomaterials are known to be vital in enhancing CRISPR-Cas9 by regulating the properties of the nanomaterials. They improve the efficiency and specificity of Cas9 entry into the cell, its endosomal release and the ability to edit the genome of target cells. (Zhang et al., 2018). Dressing up CRISPR-Cas9 components with nanomaterials like gold nanoparticles or quantum dots strengthen the structure of the complex, increases the enzymatic activities, and increases the specificity of DNA binding. (Rai et al., 2016).

Genome editing has now become one of the most targeted areas in nanotechnology. For example, enhancement in the DNA cleavage efficiency of gold nanoparticles has been achieved by conjugating them with single stranded DNA. (Zhu et al., 2019).

Nanosheets of graphene oxide (GO) have a vast surface area that affords great compatibility with particles that have charges similar to those of GO and allows accurate targeting and delivery of CRISPR components.

GO nanosheets enhance the target specificity of the editing process and when conjugated with cell-specific targeting vectors (Zhao et al., 2016). Likewise, mesoporous silica nanoparticles (MSNs) with the opportunity for the regulation of pore size and surface functionalization has the capability of carrying CRISPR-Cas9 components to target cells. (Liu et al., 2020). Single-walled carbon nanotubes (SWCNTs) also include intrinsically cellular permeability that facilitates the encapsulation of both Cas9/gRNA complexes because of high aspect ratio and capacity to cross cell membranes (Zhang et al., 2017).

Other formulation techniques presented lipid nanoparticles as capable of carrying both Cas9 and gRNA simultaneously further improving intracellular delivery and gene edit efficiency. This development emphasizes the compatibility of CRISPR-Cas9 technique and nanomaterials with a concentration on optimizing their performance as well as the issues solved in gene editing. The integration of lipid nanoparticles with CRISPR-Cas9 is the potential strategy to tackle the challenges that are associated with cellular uptake and targeted delivery and enhance the efficiency and specificity of gene editing (Sun et al., 2019).

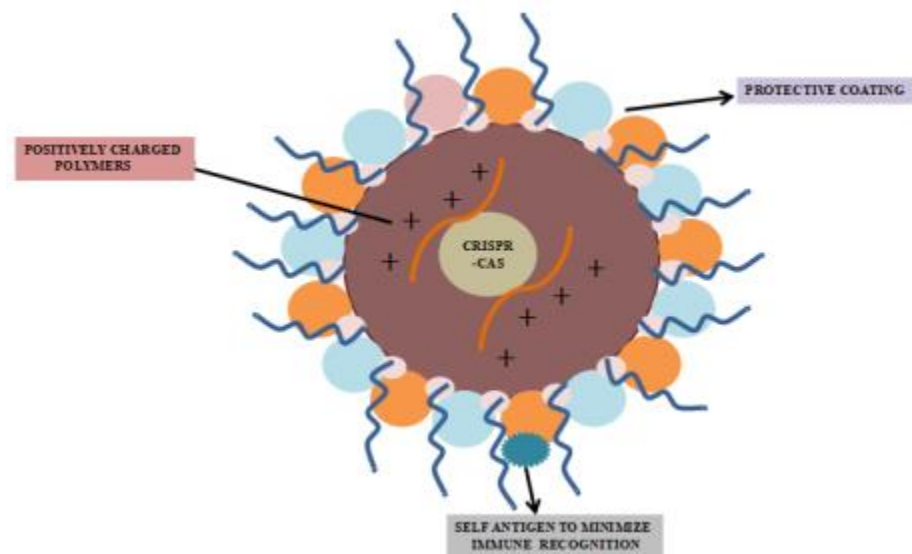
### 4. CRISPR-Cas9 in Precision Medicine

CRISPR-Cas9 system is one of the most advanced personal genomic technologies that has benefitted eradicating disease genes. Integrating CRISPR-Cas9 technology with next-generation sequencing (NGS), cancer linked genes can be knocked out / edited and the compounds can selectively target cancer cells. For the diagnosis and treatment of cancer, the personalised treatment based on people's genetic characteristics is more frequent, which NGS detects and locates for treatment strategies targeting on the abnormal function of cancer cells (Wang et al., 2018).

Saturation editing a promising approach involves modifying, each nucleotide within a gene to distinguish polymorphic mutations from pathogenic ones and become even more specific in genetic therapies. Aside from cancer treatments, CRISPR-Cas9 has the promise treating inherited blindness, which already achieved important landmarks in precision medicine (Davis et al., 2020). The use of viral enveloped vectors inclusive of AAVs and lentiviral vectors and integration of nanotechnology is a model and unique method in gene manipulation. They tackle issues such as; Side effects and therapeutic efficacy and hence improve the delivery as well as the targeting ability of the CRISPR-Cas9 systems (Zhou et al., 2019). As research and development of gene therapy continues, the specialty sub-specialty of personalized medicine is expected to see drastic improvement. CRISPR-Cas9 is expected to develop further to enhance specificity and efficiency in its therapeutic function.

#### 4.1 Surface Modifications for Enhanced Nanoparticle-mediated Delivery of CRISPR-Cas9

Incorporation of cell-penetrating peptides also remains important to increasing the stability and bioavailability of nanoparticles, which are central to the delivery of Cas9-gRNA complexes. Such changes are useful to guarantee that nanoparticles can easily enter target cells and cause the release of the necessary genetic substances for editing (Saha et al., 2018). Yet there are limitations; some nanomaterials can be cytotoxic, and provoke immune reactions. Solving such issues is very sensible for the stability and effectiveness of the nanoparticle-associated delivery systems. The understanding of such limitations will inform subsequent studies focused on improving the safety and efficacy of the nanocarriers to boost the performance of the CRISPR-Cas9 gene editing platforms.



**Fig 3.** Surface functionalization of nanoparticles—through ligands, polymers, or charge tuning, enhances cellular uptake, targeting specificity, and stability of CRISPR-Cas9 cargo during delivery

### 5. Key Applications of CRISPR-Cas9

#### 5.1 Human Genetic Diseases

CRISPR-Cas9 has offered a fresh hope for individuals with genetic diseases since it has been found to correct mutations that bring on diseases. Genetic disorder like Sickle-Cell Anemia, Cystic Fibrosis, and Retinitis Pigmentosa can all be cured by CRISPR-Cas9 gene editing. This technology permits alteration of specific genes, a concept that is the basis of the concept of personalized medicine. However, therapeutic interventions are supposed to be prescribed and administered being individualized to meet specific therapeutic goals with the least possible adverse effects including off-target effects as well as immune responses (Doudna & Charpentier, 2014).

#### 5.2 Agriculture

CRISPR-Cas9 has opened a new frontier in agriculture food production in that it offered increased crop yield, nutrition quality and disease resistance. The cells have been modified using CRISPR-Cas9 to make ungenearing changes in chromosomes to possess features such as wraps against pathogens among other ways as highlighted greatly enhance the ease of constructing stronger produce capable to tackle deformities. tow examples include rice, wheat, and tomatoes, and demonstrate how the CRISPR technology could change the global food supplies. They include gene fixes to crops where improvement in resistance to diseases has been realized showing the efficiency of the technology in the production of high yielding and resistant crops.

Nonetheless, the application of CRISPR-Cas9 to agriculture brings a number of acknowledged regulatory and ethical issues. The application of GMOs and gene-edited plants is not universally regulated and thus requires systematic reconsideration of the existing laws. However, these and other ethical questions such as the effects of gene editing on the environment and the biased application of the science among different people and nations have to be resolved before the said technology can go mainstream. It is clear that CRISPR-Cas9 is both a very promising tool in the fields of genetic diseases and agriculture, but precaution must be taken in such developments (NASEM, 2016).

Thus, the CRISPR-Cas9 crui- sle has a huge potential for the further development of medicine and agriculture. Nevertheless, some problems including efficient delivery methods, avoidance of side effects, social legislation restrictions, and ethical questions should be solved to reveal the main advantages of this technology. Such challenges can however be addressed through continuous research and uptake of research collaborations among the institutions and or researchers in ensuring safe use of CRISPR-Cas9.

## **6. Challenges and Considerations in CRISPR-Cas9 Genome Editing**

### **6.1 Off-target Effects and Specificity**

Of all the limitations of the CRISPR-Cas9 system however it's arguably one of the most challenging setbacks where off-target effects are concerned. These are effects due to unwanted changes in the genetic context of target homologous sequences. Such an inaccuracy can cause a shift in genetic information, it may result in advantageous or completely disastrous consequences. To this end, the researchers are working on raising the specificity of the functional CRISPR-Cas9 system through improving the enzyme Cas9 and using specific RNA sequences for targeting the gene of interest while avoiding non-target genes. Furthermore, the computational techniques and analytical tools are also being employed to predict the possible off-target site and safe target sequence in the organism. These are important for enhancing the accuracy and efficiency of CRISPR-Cas9 mainly in gene regulation or gene therapies (Hsu et al., 2013). That is why overcoming off-target effects, increasing the selective effect of CRISPR-Cas9, designing strategies to increase specificity is crucial for enhancing both safety and efficiency of the genome editing process at the molecular level. The future is credited in these areas to bandwidth expand the possibility for CRISPR-Cas9 both for treatment and agriculture.

### **6.2 Safety Concerns with Viral Vector Delivery**

Delivery of CRISPR-Cas9 precursor components into target cells is commonly accomplished using viral vectors because of their effectiveness. However, they present several safety concerns, such as immune reactions, and possible insertional mutagenesis.

Despite their potential to deliver genetic information into the cells targeting the therapy of genetic diseases, viral vectors stimulate immune responses that may be toxic to the patient, inserts into the host genome can disrupt essential genes or regulatory sequences, and viral genomes themselves present considerable risks (Doudna & Charpentier, 2014). To manage such risks, it is possible to propose profound mechanisms for the testing and estimation of safety. It is noteworthy that in order to get rid of potential risks, the researchers need to make a complete dedication to risk reduction and use viral vector-based CRISPR-Cas9 systems responsibly and following the principles of GSPs (Hsu et al., 2014).

### **6.3 Ethical and Regulatory Considerations**

As with any novel technology, the use of CRISPR-Cas9 system has its ethical and legal concerns. Informed by this issue, questions concerning the limits of genetic modification and intervention on the workings of the embarked natural environment arise (Gantz & Bier, 2015). Research on germline editing is currently considered especially ethical because of the heritable changes it brings to future generations which could have unpredictable

consequences (Lanphier et al., 2015). That is why the issues connected with germline editing are ethical and require further examination and regulation. Moreover, the use of CRISPR-Cas9 differs from one country to another, which makes the regulation more a challenge. To avoid cases of misuse of this advancement and make sure that the technology is only and only being used appropriately then there is need to create sound policies that should be adopted at the state level (Lander et al., 2019).

#### **6.4 Delivery Mechanisms and Cellular Uptake Optimization**

This precision of targeting genetic editing is highly dependent with the ability to transport and deliver the CRISPR-Cas9 components into the target cell. There is still a predominance of viral vectors and the further development of other methods, such as nanoparticles and electroporation, due to their lower immunogenicity and higher target specificity (Zhang et al., 2020). Changing the affinity of some of the CRISPR components can also affect the integration of these components into the cellular machinery and therefore enhance their editing capacity. Nevertheless, there are various drawbacks, which have not been optimized, such as different cell-type delivery and off-target issue so, the improvement of CRISPR-Cas9 technology's safety and efficiency (Ran et al., 2013).

Many opportunities have been seen using the versatility of the CRISPR-Cas9 platforms across different fields, but main issues remain on off-target concern, a delivery system, and regulations on its usage.

These concerns have to be addressed to enhance efficiency of CRISPR-Cas9 for genetic, therapeutic and agricultural purposes as well as adhering to the proper ethical standard. The technology has already advanced, progress related to the specificity and efficiency of gene editing has been made, Cas9 high-fidelity variants has been created and guide RNA designs that minimized off target effects (Slaymaker et al., 2016).

It has been revealed further that lipid based and polymeric nanoparticles based delivery systems have the potential of enhancing the efficiency of CRISPR-Cas9. These nanoparticles can protect Cas9 and the guide RNA, thus improving the uptake by the cell and effective gene editing (Zhou et al., 2018). There are also several other features that are relevant to in vivo application of viral vectors: adeno-associated virus (AAV) and lentiviral vectors have longer transgene expression and broader application potentialities (Mingozzi & High, 2017).

### **7. Real-World Impact**

CRISPR-Cas9 has applicability in most of the health care sectors, where it can be used to cure diseases whose root cause is genetic. Nevertheless, this brings close to health care facility the issues of safety, ethicality as well as affordability of the advancements in clinical care (Schmidt et al., 2020). For instance, application of CRISPR-Cas9 in gene therapy process means that one has to ensure that off-target actions are substantially low and delivery systems are both effective and non-cytotoxic. From the Taiwanese example, one can infer about the necessity of developing the set of profound regulations that would cover the usage of the genome editing technologies in clinical practice (Chen et al., 2020).

#### **7.1 Areas Requiring Enhancement**

High specificities of Cas9 variants and guide RNA designs have been successfully enhanced, but the problem of decreasing off-target side-effects remains complex (Kleinstiver et al., 2016).

Another question that deserves attention is the ability to deliver the components of CRISPR and, in particular, for in vivo use. Methods of packaging CRISPR-Cas9 efficiently with minimal toxicity and immunogenicity are critical to the growth of clinic applications (Lino et al., 2018). Similarly, germline editing has also drawn some ethical and regulatory issues that need some resolution via international cooperation (Lanphier et al., 2015).

## 7.2 Prospects for Future Research

The next steps involve enhancing the efficiency and specificity of the existing delivery methods avoiding off-target effects and exploring new fields of application for CRISPR-Cas9, especially within the context of precision medicine and agriculture. New trends in base and prime editing suggest ways for making targeted genetic alterations with relative safety and pose unprecedented ways for gene therapy in the future (Anzalone et al., 2019).

## 8. Future Trajectory

In the next five to ten years, CRISPR-Cas9 technology will largely extend its application to lots of researchers as a basic reagent, while its therapeutic application will expand further. But challenges that concern off-target effects, the Nanoparticle delivery systems, and ethical issues will still remain. These and other difficulties will have to be addressed both by interdisciplinary communication and by integrative approach in order to unleash potential that has been disrupted in the medical and agrarian fields by the new molecular technology of CRISPR-Cas9 (Charpentier & Doudna, 2013).

### 8.1 Precision Medicine

CRISPR-Cas9 is already the new generation plausibly one of the strands of personalized medicines that has improvised the handling of genetic diseases through genotypes at the nucleotide level. Nevertheless, it is imperative to note that practice of developing crisper based therapies faces limitations in accepting them as standard therapies, important issues of safety, ethics, and regulatory concern will have to be duly addressed.

Government collaboration with multiple economic stakeholders will be required to overcome these problems and make these therapies available for patients who need them (Korman et al., 2020).

## 9. Conclusion

Over past five years the advancement in the genome editing using CRISPR-Cas9 has been phenomenal and it has paved the way for an innovative discovery. The CRISPR-Cas9 system has revolutionalized the research of animals, medical sciences, human gene therapy, and plant biology with an emphasis on improving the crop's attributes. The generation of the genetic

knock-out mutants along the improvements in multiplex genome editing have shown diverse utility in plant sciences.

Studies focusing on the interactions between nanoparticles and CRISPR-Cas9 have received much attention because of the well-developed surface area and the ability to modify the nanoparticle properties. Such properties let nanoparticles transfer components of the CRISPR/Cas9 system to target cells. Lipid nanoparticles, polymer nanoparticles, and mesoporous silica needles have all also been identified as capable of successful encapsulation and effective delivery of CRISPR/Cas9 complexes by other carriers. This works not only in solving some of the delivery concerns but also improves the precision and effectiveness of genome editing.

As for future work, the future scientific work is directed towards refining the CRISPR/Cas9 system, reducing off-target effects, and designing appropriate nanocarriers for the targeted delivery system for cell/tissue specific uses. Each of these advancements offer tremendous potential for therapeutic application with genetic diseases, cancer, and infections. They will open the possibility of realizing individualized therapies and targeted therapies, which will provide great expectations for the future development of healthcare. Nevertheless, there are still serious difficulties when it comes to such accomplishments. Safety, feasibility and efficiency of the CRISPR/Cas9; rigorously controlling the negatives of the genome editing; clearing out ethical measures; and developing a

sound necessary regulatory standards are the key ways to unlock the full CRISPR/Cas 9 genome editing.

To this end, any future research agenda will require more integration between the scientists, the clinicians, the policymakers as well as the numerous regulatory bodies to engage with these complexities and ultimately create the much-needed new realizations in this revolutionary field. All in all, CRISPR/Cas9 technology that has evolved in the last five years would be considered by many as one of the most incredible molecular biology breakthroughs. These advances create more opportunities for scientific and agricultural analysis in addition to the therapeutic applications. The combination of nanomaterials with CRISPR/Cas9 is leading to further advancements of pioneering information and compelling treatments that may change the course of patient care and treatment in the future

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