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NANODEVICE-BASED GENOME EDITING TO ENHANCE CRISPR/CAS9 STABILITY AND EFFICIENCY

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Abstract. *Since a decade ago, the importance of CRISPR and the CRISPR-associated system (Cas) in the field of genome modification has increased. The limited intracellular delivery effectiveness of this method makes it difficult to transport Cas payloads and sgRNA despite its adaptability. Nanomaterials including liposomes, polymers, gold nanoparticles, and inorganic nanoparticles have been used successfully for gene transfer. Here, we briefly cover the many CRISPR/Cas delivery systems and their related difficulties, then we go through the different nanotechnological ways for CRISPR/Cas delivery, and look at the numerous issues that CRISPR-based plant genome editing encounters. It also covered the challenges of delivering CRISPR/Cas9 utilising nanotechnology and the regions that must be targeted to benefit from this editing approach.*

Keywords: *crispr/cas, nanotechnology, crispr/cas9, delivery.*

РЕДАКТИРОВАНИЕ ГЕНОМА НА ОСНОВЕ НАНОУСТРОЙСТВ ДЛЯ ПОВЫШЕНИЯ СТАБИЛЬНОСТИ И ЭФФЕКТИВНОСТИ CRISPR/CAS9

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Аннотация: *С тех пор, как десять лет назад важность CRISPR и CRISPR-ассоциированной системы (Cas) в области модификации генома возросла. Ограниченная эффективность внутриклеточной доставки этого метода затрудняет транспортировку полезных веществ Cas и sgRNA, несмотря на его адаптивность.*

Наноматериалы, включая липосомы, полимеры, наночастицы золота и неорганические наночастицы, успешно использовались для переноса генов. Здесь мы кратко рассмотрим множество систем доставки CRISPR / Cas и связанные с ними трудности, затем рассмотрим различные нанотехнологические способы доставки CRISPR / Cas и рассмотрим многочисленные проблемы, с которыми сталкивается редактирование генома растений на основе CRISPR. В нем также рассматривались проблемы, связанные с внедрением CRISPR/Cas9 с использованием нанотехнологий, и регионы, на которые необходимо ориентироваться, чтобы извлечь выгоду из этого подхода к редактированию

Ключевые слова: *crispr/cas, нанотехнологии, crispr/cas9, доставка*

CRISPR/CAS9 ТУРУКТУУЛУГУН ЖАНА НАТЫЙЖАЛУУЛУГУН ЖОГОРУЛАТУУ ҮЧҮН NANODEVICE НЕГИЗДЕЛГЕН ГЕНОМ ТҮЗӨТҮҮ

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Аннотация. *Он жыл мурун, ГЕНОМДУ өзгөртүү жаатында CRISPR жана CRISPR менен байланышкан тутумдун (Cas) мааниси жогорулаган. Бул ыкманын клетка ичиндеги жеткирүү эффективдүүлүгү анын ыңгайлуулугуна карабастан, кассалык жүктөрдү жана sgRNA ташууну кыйындатат. Наноматериалдар, анын ичинде липосомалар, полимерлер, алтын нанобөлүкчөлөр жана органикалык эмес нанобөлүкчөлөр генди өткөрүп берүү үчүн ийгиликтүү колдонулган. Бул жерде, биз кыскача көп CRISPR/Cas жеткирүү системаларын жана алардын байланышкан кыйынчылыктарды каттыйт, анда биз CRISPR/Cas жеткирүү үчүн ар кандай нанотехнологиялык жолдор менен барып, CRISPR негизделген өсүмдүк геном түзөтүү көптөгөн маселелерди жаратат. Ошондой эле, CRISPR/Cas9 Нанотехнологияны жана ушул түзөтүү ыкмасынан пайда алуу үчүн максаттуу болушу керек болгон аймактарды колдонуу кыйынчылыктарын каттыган.*

Ачкыч сөздөр: *crispr/cas, нанотехнология, crispr/cas9, жеткирүү.*

Introduction. The discovery of DNA double helix structure marks the beginning of new era of life science. Among the scientist it remains always fascinating to make changes in the genetic material of different organism. The increasing curiosity among researchers for alternation or manipulation of genetic material had enabled them to discovery of the different innovative genome editing techniques. The era of genome editing have seen lots of

development with the advent of techniques like zinc finger nucleases (ZFNs), transcription activator-like effector nucleases and clustered regularly interspaced short palindromic repeats (CRISPR)- Cas associate nucleases. In this decade the CRISPR have gained tremendous importance as the most potential genome editing technique as it is RNA-programmable technology which allows precise and efficient engineering or corrections of mutations, alternation of gene expression and marking of DNA in a wide spectrum of cell types and organism in the three domains of life. Recently the Noble prize for Chemistry in 2020 was awarded to Jennifer Doudna and Emmanuelle Charpentier for CRISPR/Cas9. CRISPR-Cas9 is making revolution in the field of life-science while working in different laboratories across the world. The recent advances in the CRISPR technologies like CRISPRi, CRISPRa, CRISPR multiplexing and base editing is becoming boon in the research field. In field of life-science CRISPR have potential application to understand gene function to understand biology in health and disease, rewire biological pathways and to create better models for human diseases. In the field of medicine and health CRISPR have potential to cure for genetic disorders like sickle cell anemia, muscular dystrophy, cystic fibrosis., etc. CRISPR can be potentially used in oncogenic studies for engineering of immune cells to target cancer cells. CRISPR possess antimicrobial and antiviral applications such as developing sequence-specific alternatives to broad spectrum antibiotics and also have potential of engineering resistance to HIV and other pathogens in human cells. CRISPR have potential of drug development and can work as gene drivers for control of disease vector. In the field of agriculture CRISPR is used in crop engineering to create cultivars with resistance to drought, climate change and various biotic and abiotic stresses. CRISPR can be used potentially in the dairy industry for vaccination of probiotics like *S. thermophiles* against phages. CRISPR possess potential of engineering of industrial microorganism for biofuel and biomaterials production (Charpentier, 2017).

Despite of the tremendous potential application and versatility of CRISPR-Cas system there are certain challenges while delivering CRISPR-Cas9 at the target site. The present conventional methods of biomolecule delivery have critical drawbacks like low efficiency of gene transmission, narrow species range of application and tissue damage. Also the molecular size of Cas9 is larger to enter in the target cell. CRISPR- Cas9 needs to work on the nuclear genome and thus the components of CRISPR-Cas system need to enter into the nucleus. Thus the delivery of CRISPR-Cas systems remains a

challenging issue to the date, thus to address this issue the present review focus on the role of nanotechnology approaches to enhance efficiency and stability of CRISPR-Cas9.

Different delivery approaches of CRISPR/Cas9 and its current challenges The efficient delivery of CRISPR/Cas9 system at the target site seems challenging. The molecular weight of CRISPR/Cas9 is approximately 160kDa (Jinek *et al*, 2014) and it forms an RNP complex after that the long phosphate backbone of the sgRNA impairs a net negative charge to the complex (Sun *et al*, 2015). Due to the large molecular weight and net negative charge it makes tedious for entering of Cas9 RNP in the cellular membrane. It is very crucial that once inside the cell after the entry of Cas9 protein and sgRNA they must survive the degradation procedure in the cell and needs to enter into the nucleus and needs to operate on the nuclear genome to enable efficient gene editing (Yip, 2020). So, choice of an appropriate delivery strategy for the CRISPR/Cas9 system remains always of crucial importance for the achievement of efficient and precise gene editing.

Here discussed the different delivery approaches used for CRISPR/Cas9 delivery to target site and the challenges faced during CRISPR/Cas9 delivery. For the delivery of CRISPR/Cas9 wide variety of possible delivery methods are being use currently. Delivery of CRISPR/ Cas9 can be classified into major two types: cargo and delivery vehicle. In the case of CRISPR/Cas9 cargoes, three common approaches that were reported consist of: 1) DNA plasmid encoding both the Cas9 protein and the guide RNA, 2) mRNA for Cas9 translation alongside a separate guide RNA, and 3) Cas9 protein along with guide RNA (ribonucleoprotein complex). It often depends on the delivery vehicle which of these three cargoes can be packaged, and is the system is usable *in vitro* and/or *in vivo* (Lino *et al*, 2 018).

Physical delivery, viral vectors and non-viral vectors are the three general categories in which the vehicles used to deliver the gene editing system cargoes are classified. Each delivery method has its own advantages and as well as demerits when it comes to its clinical application (Zuris *et al*, 2014). Electroporation and microinjection and are the most commonly used physical delivery methods. Electroporation utilizes short, intense electric field that creates pores of small size on the cell membrane. Such damage on cell membrane causes the inflow of large particles into the cell. Electroporation method has limitation as this *in vivo* method can't be widely adopted. The small range between the electrodes which are usually around 1 cm hinders the

transfer of target to large area of tissues (Sokotowska and Zabielska 2019).

Conduction of electro-transfer in clinical case is problematic as there is fear of organ damage due to high voltage (Durieux *et al*, 2004). Electroporation-mediated gene editing is expensive as the extensive optimization of Cas9-to-sgRNA ratios and specific electroporation conditions for each cell type are required. The strong electrical current created by electroporation results in high percentage of cell deaths, suggest that this method is not suitable for stress-sensitive cell types or tissue types (Yip, 2020). Microinjection consists of injecting Cas9 and sgRNAs directly within the cells with the use of microscope and needle. Microinjection is used widely in embryonic gene editing and for producing transgenic animals. Despite of high transduction efficiency and low cytotoxicity the major pitfall of microinjection delivery method is its labour intensiveness and is time consuming which hinders its application in small number of species (Duan *et al*, 2021). Viral delivery vectors consist of specifically designed adeno-associated virus (AAV), full sized adenovirus and lentivirus vehicles (Lino *et al*, 2018). Virus- mediated gene delivery is one of the most widely used method for gene delivery which consist of integration of CRISPR/Cas9-encoding sequences within the viral genome and once there is integration between the viral genome and CRISPR/Cas9 sequence this complex is released into the infected cells which is our target cell. During this process there is possibility of integrating the viral vectors into the host cells which might include adverse effects such as mutations, carcinogenesis and an immune response (Yip, 2020).

The virus mediated delivery of CRISPR/Cas9 has limitation as the load capacity of virus is minimal (Chew *et a/.*, 2016). Different non-viral vector systems have been developed and successfully used for safe delivery of CRISPR/Cas9 to the target cell. Some of the noteworthy non-viral delivery methods consist of polymeric materials, liposomes, cell-penetrating peptides (CPPs) and cationic nano carriers. Non-viral vectors are capable to accommodate components of a large size for delivery (Fig. 1). Non-viral vectors are having reduced or non-hazardous nature and are easy to generate which make many scientists to select this type of system for delivery of nucleases (Chandrasekaran *etal*, 2018).

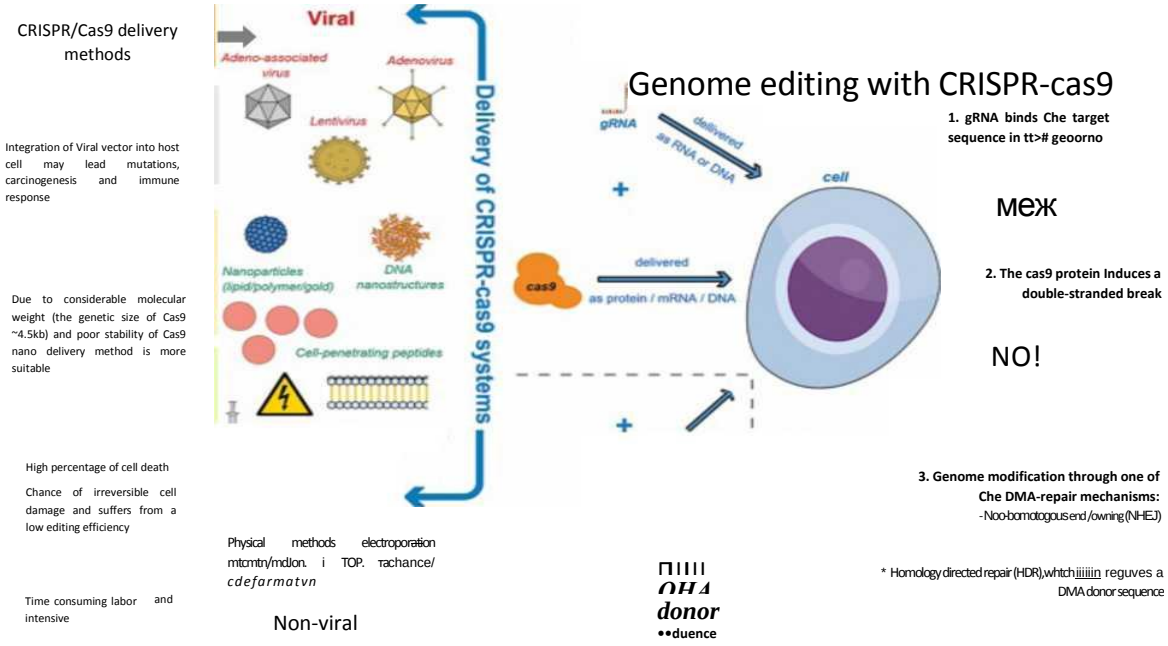


Fig 1 : Different delivery methods of CRISPR/Cas9

Nanoparticles boon to CRISPR delivery. As discuss earlier genome editing proteins faces challenges in efficient delivery at target site due to proteolytic instability and poor membrane permeability. Thus it seems demanding to develop a novel platform that can efficiently assemble protein into nanoparticles for intracellular delivery while maintaining biological activity of the protein (Fu *etal*, 2014).

There are different types of CRISPR nano-delivery approaches. Lipid based nanoparticles, polymer based nanoparticles, DNA nanostructures and gold nanoparticles are some well-known approaches of nano-delivery (Duan *et a!.*,2021).

Here we discussed the various CRISPR/Cas9 nano-delivery approaches in details (Fig. 2).

Liposome mediated CRISPR nano delivery. Liposomes are closed bilayer structure which is formed by hydrated phospholipids and have ability to encapsulate bioactive hydrophilic, amphipathic and lipophilic molecules into inner water phase or within lipid leaflets (Nisini *et al,* 2018). Many time liposomes have served as model systems for cellular membrane to discover of protein functionality regarding osmotic and pH stability (Alghuthaymi *et al,* 2021). Lipid nanoparticle is the classic delivery system used for nucleic acid transfer. In this method the nucleic acid which bears negative charge forms a complex with lipid having positive charges by electrostatic interaction and host-guest interaction, once the complex is formed is uptake by cellular endocytosis. Lipid nano-carriers have potential to transverse the membrane by the process of endocytosis and can protect the loaded

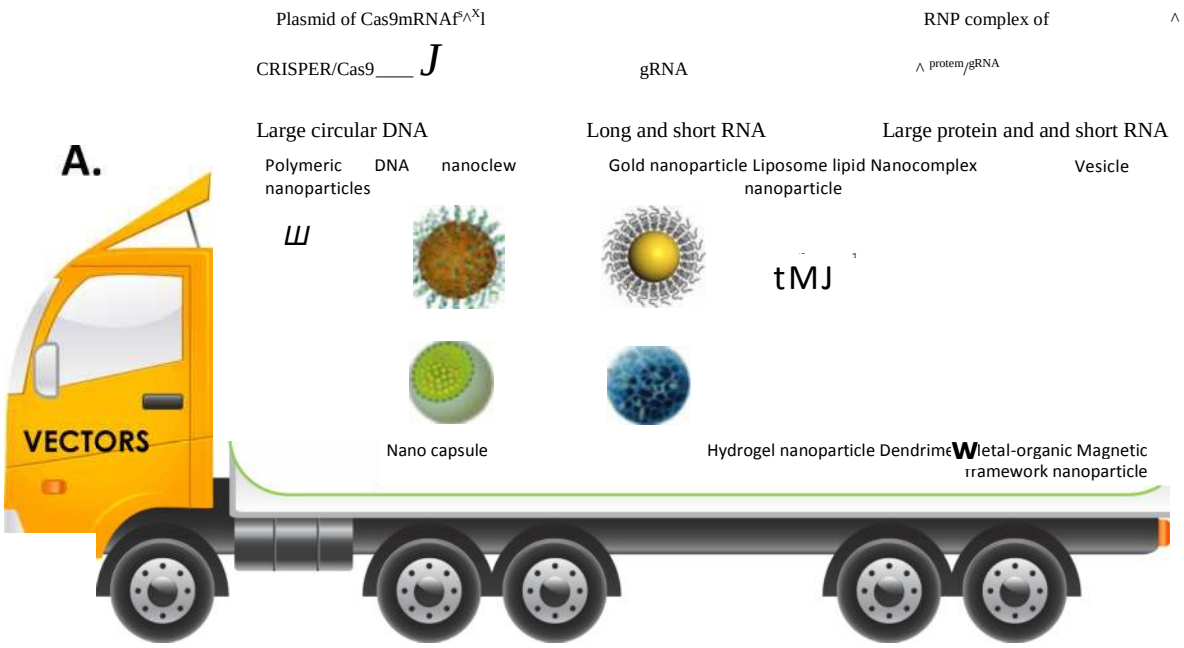
cargoes from nuclease degradation and immune response (Duan *et al,* 2021).

It is found that liposomes have distinct advantages in Cas9/sg RNA RNP delivery because the proteins and nucleic acid are protected from degradation in blood circulation due to lipid bilayers. And another one advantages of liposome is it have ability to enhance the endosomal escape by fusion with endosomal membrane (Deng *et al,* 2019). Cationic lipid-based vectors are found useful for efficient delivery of Cas9 and gRNA. Cationic lipids such as 1, 2- dioleoyl-3-trimethylammoniumpropane (DOTAP), N-[1 -(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), and 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE) are seems very useful in nanoparticle mediated CRISPR delivery (Li *et al,* 2015). Wanga *et al,* (2016) combined bioreducible lipid nanoparticles and Cre recombinase or anionic Cas9: single-guide (sg) RNA complexes and delivered these into cultured human cells and come to opinion that the delivery of bioreducible lipids into cultured human cells enables gene recombination and genome editing with more efficiency. Wei *et al,* (2020) reported a generalizable methodology allowing the use of engineered modified lipid nanoparticles for efficient delivery of RNPs (Ribonucleoprotein complexes) into cells and edit tissues including brain, muscle, liver and lungs. They make experiment of mice and the delivered RNPs had restored dystrophin expression in DMD mice and significantly decrease serum PCSK9 level in C57BL/6 mice. Zuris *et al,* (2014) reported that there is potential ability of intracellular delivery of protein when negatively supercharged proteins and cationic lipid nucleic acid are fused together. They found that the potent delivery of nM concentrations of *Cre* recombinase, TALE and Cas9-based transcription activators, and Cas9: sgRNA nuclease complexes in human cultured cell is possible by this approach. Also they used this approach for efficient delivery of *Cre* recombinase and Cas9: sgRNA complexes into the mouse inner ear *in vivo,* and achieved 90% Cre- mediated recombination and 20% Cas9- mediated genomic editing in hair cells. Polymeric nanoparticles mediated CRISPR/Cas9 delivery

There are evidences of cationic polymer nanoparticles been used frequently to deliver different nucleic acid, including mRNA and plasmid DNA. Polyethyleneimine and chitosan were the most widely used carriers for delivery of CRISPR/Cas 9. As like lipid nanoparticle, polymer nanoparticle also have ability to transverse the membrane through endocytosis and thus can prevent the loaded cargoes from immune response and nuclease degradation (Duan *et al,* 2021).

Recently, studies are going on improving CRISPR/Cas9 delivery efficiency *in vivo,* thus to achieve various gene editing targets. Zhang *et al,* (2019) synthesized cationic polymer polyethyleneimine- в-cyclodextrin (PC) and used it for efficient delivery of plasmid encoding Cas9 and sgRNA. In this study the Cationic polyethyleneimine- 0- cyclodextrin easily formed nanocomplexes with negatively charged Cas9/sgRNA plasmid and resulted in efficient cellular uptake and transfection in HeLa cells. Nanoclew-mediated CRISPR delivery DNA nanoclew is a unique fascinating technology for delivery of CRISPR technology. The very first report of DNA nanoclew was given by Sun *et a/.,* (2015). DNA nanoclew is nothing but a sphere-like structure of DNA which can be compared with a ball of yarn. The synthesis of this nanoclew is by rolling circle amplification in which palindromic sequences aids in the self-assembly of structure. The nanoclew can be loaded with a payload which can be specifically triggered for release by applying certain biological conditions. Sun *et al,* (2015) reported DNA nanoclew as safe and efficient delivery system for delivery of Cas9 protein and single-guide RNA. They used this bio-inspired vehicles and loaded them efficiently with Cas9/single-guide RNA complexes and after that delivered the complexes to the nuclei of human cells for target specific gene editing along with maintaining cell viability. Gold Nanoparticles Recently gold nanoparticles (AuNPs) are used for efficient delivery of CRISPR/Cas9 RNP. Cross linking of AuNPs with sulfhydryl (-SH) substances through Au-S bonds and manipulating their surface charge and hydrophobicity is easy (Levy *et al,* 2010). With the use of surface modified AuNPs cationic peptide, pCas9 can be adsorbed due to electrostatic interactions. Wang *et al,* (2018) used AuNPs carrying pCas9 protein and modified TAT peptide. The editing machinery is released by intravenous administration and the Cas9 is further released by a thermal effect triggered by a laser directed to the AuNPs. Here the cationic TAT peptide has role of guiding pCas9/sgPLK-1 (Polo like Kinase 1) to nucleus and destroy the PLK-1 gene this causes inhibition in the tumour growth. Lee *et al,* (2017) used CRISPR-Gold technology for delivery of Cas9 ribonucleoprotein and donor DNA *in vivo.* Here to deliver Cas9 ribonucleoprotein and donor DNA they used gold nanoparticles conjugated to DNA and complexed with cationic endosomal disruptive polymers as delivery vehicle. The results demonstrated that CRISPR-Gold have potential to repair the mutant dystrophin gene and reduced muscle fibrosis in X-linked muscular dystrophy (MDX) in mice. CRISPR-Gold approach for delivery of Cas9 protein is safer than viral delivery of CRISPR. This technology has proved its potential in minimising off-target DNA damage, precisely editing brain cells,

curing genetic diseases. CRISPR-gold technology can also be used to cure polygenic diseases like Huntington's disease by using dual sgRNA.



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IMM

O Delivery into targeted cell
O Transcription O Translation
O Form into Cas/gRNA RNP
0 Genome editing in nucleus

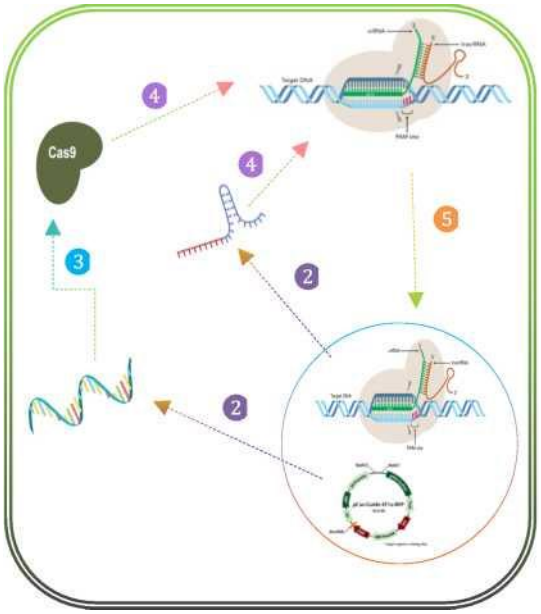

Fig. 2: Delivery of CRISPR-Cas for genome editing. A) Rational designs of delivery construct; B) Flow of genome editing by three forms of CRISPR/Cas

Other Nanoparticles Yue *et al,* (2017) used graphene oxide (GO) polyethylene glycol (PEG)- polyethylenimine (PEI) nanocarrier for delivering the high-molecular weight complex of SgRNA/ Cas 9 for endocytosis, endosomal escape, nuclear entry and gene editing. The result found that the GO nanocarrier can be utilised successfully for efficient gene editing in human AGS cells with an efficiency of ~ 39%. The results also proved that this nanocarrier had ability to protect sgRNA from enzymatic degradation, conferring extremely high stability to sgRNA/Cas 9complexes. Inorganic Nanoparticles like gold have been used for the delivery of CRISPR component this proves the potential of inorganic nanoparticles in CRISPR delivery. Other inorganic nanoparticles like carbon nanotubes (CNTs), bare mesoporous silica nanoparticles (MSNPs) and dense silica nanoparticles (SiNPs) have been used for delivery of many genes but the use of these inorganic nanoparticles for CRISPR/Cas9 delivery is not reported yet. Nanotechnology to tackle CRISPR challenges in plants Considering the challenges in plant for CRISPR, nanotechnology can be a way to address it. The challenges like delivery, Tissue culture and regeneration, species dependence, low HDR efficiency can be better resolved with help of nanotechnology. Overlook Table 1 which summarises the CRISPR challenges in plants and their solutions through nanotechnology.

Delivery. The critical challenge to CRISPR/Cas9 in plant is its delivery. The common abiotic transfection techniques like heat shock, electroporation lipid and polymer mediated delivery which are widely used for microbes and animals are typically ineffective in intact plant. In genetic engineering field nanotechnology have contributed in efficient delivery into diverse plant species and tissues. In addition to targeted delivery of nanotechnology contributes in controlled cargo release and cargo protection from degradation. The delivery of DNA and proteins into plant cell with use of nanotechnology has been successful, but still the use of nanomaterial for delivery of CRISPR/Cas9 in plants in not yet reported due to the distinct physiochemical characters of CRISPR reagents and the requirement of high delivery efficiencies to enable genome editing in plants (Demirer *et al,* 2021).

Tissue culture Plant tissue culture is core in transformation experiment. In recent years nanoparticles are used successfully in tissue culture techniques to eliminate microbial contamination and have synergetic effect on callus induction, organogenesis, somatic embryogenesis and genetic transformation.

Species dependence. The inability of transformation tools to be effective for wide range of plant species is one of the major hurdles for widespread application of plant CRISPR editing. Some reason for plant species dependence is the

inefficiency to deliver cargoes to all species, obstacles of *in vivo* sgRNA validation and the prerequisite of PAM site of nuclease with unsuitable genomic composition in some species. Nanoparticles have been in aspect of delivery in wide range of plant species including *Arabidopsis,* tobacco, maize, wheat, spinach, arugula, watercress and cotton (Ortigosa *et al,* 2014, Demirer *et al,* 2019, Kwak *et al,* 2019, Santana *et al,* 2020). These studies interpret that the gateway of nanoparticles into plant cell is mechanical phenomenon and so it not largely disturbed by plant genetics or signalling pathways. It is now crucial to work on the aspect of delivering Cas9 component through nanotechnological approach in diverse species.

Low HDR efficiency. Potential benefit of nanotechnology is it can increase the HDR efficiency in plants via multiple approaches. One of promising way to increase HDR efficiency is using the nanomaterial that can efficiently deliver the double or single stranded (template) DNA to nucleus of plant cell. Coming through the recent studies in animal, negatively charged nanoparticles can be utilized to increase the HDR efficiency in plants (Nguyen *et al,* 2020). In this study the Cas9-sgRNA complex is stabilized by nanoparticles along with carrying donor template interacting with Cas RNPs to free the template to the nucleus. It was found that in this approach HDR efficiency was enhanced approximately two-four folds in human T cells and could give substantial increase in plants. The challenging aspect in this regarding plant is the designing of nanoparticles in such a way that it should appropriately allow the stable carrying of RNP and donor DNA.

Table 1. CRISPR challenges in plants and their solutions with through nanotechnology

Challenges	Insights on Nanotechnology solution	Reference
Delivery		
Large size, high local charge density, low stability of Cas9	Cas9RNP covalent attachment on nanoparticle surfaces with cleavable chemical linkers to release the RNP complex in target's proximity	Demirer *et al,* 2021
Donor DNA delivery	Nanoparticle can be used to deliver the Donor DNA by forming complex between the DNA and nanoparticles	Demirer *et al,* 2020
Tissue culture		
Inability to transform plant germline cells	High tensile strength nanomaterials can be used to transform large pollen surface apertures	Demirer *et al,* 2021
	Shoot apical meristem and flowers can be transformed with combination of nanoparticles and microinjection approach.	Hu *et al,* 2020
Inefficiency to deliver cargoes to all species	Gateway of nanoparticle into plant cells is a mechanical phenomenon and may not hinder by plant genetics and signalling	Demirer *et al,* 2021
Low HDR efficiency		
Deficit in simultaneous reach of Cas and sgRNA into plant nucleus	Negatively charged nanoparticles can be utilized to stabilize Cas-sgRNA complex along with modified donor template	Nguyen *et al,* 2020
Cas protein and sgRNA synthesis timescale variation	Delivery of Cas nuclease, sgRNA and donor template in time staggered	Demirer *et al,*
in the cell	manner with use of nanoparticles: sgRNA should be delivered at point where Cas reaches its maximum cellular expression, this can be achieved by sequential delivery, controlled cargo release or	2021

delayed sgRNA degradation in cells.

Challenges to Nanotechnology based CRISPR/Cas9 delivery Nanotechnology mediated CRIPSPR/Cas9 delivery using different nanoparticles including liposome, polymer, DNA nanoclew, gold have their own distinct benefits over the other delivery system for CRISPR/Cas9 The nanoparticles surround the large-sized DNA or protein and exhibit low immunogenicity to hosts. Nanoparticles are easily prepared than viral vectors (Wang *et al,* 2017). Blood consists of some nucleases and proteases and these can act on naked plasmid or protein by degrading them in system circulation. The plasmid or protein that is delivered in cells can be recognised by the host immune system as foreign entity and might be cleared. Encapsulating the elements of CRISPR/Cas9 system by nanoparticles could prevent them from degrading by the proteases and nucleases and thus improves the circulation time in host (Hendel *et al,* 2 015)

Despite of the potential of nanoparticles mediated CRISPR/Cas9 delivery there are several challenges for the nanoparticles mediated CRISPR/Cas9 delivery which need to be solved. Nanoparticles encapsulation prevent the CRIPSPR/Cas9 plasmid from degradation but the problem is once it enters the nucleus the endo-lysosome is quite critical for the optimization of nanoparticles. The CRISPR/Cas9 system have its origin from bacteria this can trigger host immune response to some extent. To reduce the immunogenicity of the CRISPR/Cas9 system the nanoparticles properties like particle size, surface charge and hydrophilicity should be stringently optimized (Aggarwal *et al,* 2009). Accumulation of nanoparticles have effect on editing efficiency and off-target effect so focus should be made on the size and surface modification when fabricating nanoparticles (Kobayashi *et al,* 2014). While designing nanoparticles some critical parameters such as phagocytic clearance and cytoplasmic mobility needs to consider (Li *et al,* 2018). Scale-up of nanoparticles is very important factor which should be considered. There is urgent need to focus on optimizing the characteristics, enhancing reproducibility and enlarging scale-up of nanoparticles.

Conclusion and prospects. CRISPR-Cas based editing is successful in plant and animals. However, there are still certain challenges while delivery of the CRISPR cargoes and transformation procedures. We have discussed how nanotechnology can address these challenges. Nanotechnology is boon for CRISPR/Cas9 delivery. This review summarises role of nanotechnology to enhance CRIPSR/Cas9 stability and efficiency. Despite of the potential of this technology we are still facing bottleneck when broadening the use of

nanotechnology in CRISPR based genome editing. There are many technological unknowns regarding use of nanomaterial in editing aspect such as the upper limit of DNA and protein size and amount that can be effectively delivered by nanomaterials. Also, what is the frequency of off-target editing through nanotechnological approach is still a question which need to be resolved. In future emphasis should be given on plant editing through use of nanotechnological approach as until date there is no example of plant genome editing through CRISPR based editing with the use of nanotechnology. Questions are still there regarding the use of nanotechnology in plant genome editing like whether the regulations of edited plants through nanotechnological approach will be different from traditional edited one, did the nanomaterial will still exist in plants after editing. These issues need to be focused and solution needed to found to harvest the benefits of use of nanotechnology in CRISPR-based genome editing.

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