International Journal of Creative and Open Research in Engineering and Management
ISSN: **3108-1754 (Online)**
Volume 02 Issue 06 June-2026 | Impact Factor: 3.5

A theragnostic approach to block the pathogenicity of coronavirus during crises of' antimicrobial resistance

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Running head: CRISPR against coronavirus

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How to Cite this Article:

Khalid, Z. (2026). A theragnostic approach to block the pathogenicity of coronavirus during crises of' antimicrobial resistance. International Journal of Creative and Open Research in Engineering and Management, 02(6). https://doi.org/10.55041/ijcope.v2i6.316

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Abstract

The COVID-19 pandemic has emphasized the need for advanced therapeutics that are rapid, sensitive, and multiplexed to treat the virus and its variants. One promising technique that has emerged for combating viruses is CRISPR, a genome editing tool that can enable precise and rapid theragnostic with just the knowledge of a microbe's genome sequence. In this overview, we discuss the different types of CRISPR tools, their mechanisms, and their applicability in detecting viral variants. However, the practical applications of CRISPR/Cas systems are currently limited by the lack of efficient delivery equipment. Although adeno-associated viruses (AAV) and nanoparticles are potential delivery systems, they require extensive optimization for in vivo gene editing. Despite the challenges and limitations, the use of CRISPR as a theragnostic tool shows promise for blocking the pathogenicity of coronaviruses.

Key words: CoV – CRISPR/Cas - Theragnostic – Nanoparticles

Introduction

The term "coronaviruses" (CoVs) relates to a class of viruses that have spikes like crowns on their outer surfaces [1]. The respiration, neurons, gut, and liver are affected by CoVs in living-organisms [2]. On December 8, 2019, Wuhan was the site of the first coronavirus outbreak, which later expanded from there to worldwide. WHO has given the term COVID-19 for infection ended up by disastrous Coronavirus (WHO). Later, coronavirus was given termed as "Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2)" by ICTV because of its resemblance with the SARS-CoV. Over 215 nations worldwide have reported more than 757,264,511 confirmed cases of COVID-19 and roughly 6,850,594 fatalities (WHO). There are numerous hypotheses regarding the arrival of SARS-CoV-2 virus. The SARS-CoV-2 Omicron variants have several mutated versions; K417 N, T478 K, E484 A, and N501 Y, which are linked to variations in virulence and transmissibility [3]. Recognizing viral disease zoonosis may help in preventing the recurrence of similar

events, especially in context with the emergence of SARS-CoV-2. Three main factors, including changes in human behavior, changes in the environment, and changes in the variety of microbes, can be blamed for the occurrence or recurrence pathogens and their transmission to the susceptible hosts [4]. SARS-CoV-2 are extremely contagious among people as having +ssRNA genome. Coronavirus is a member of the Corona-viridae family's Beta-coronavirus genus [5]. The MERS-CoV and SARS-CoV coronaviruses are among the other viruses in the same genus with their devastating effects on humans. SARS-CoV-2 genome has 29.9 kb size [6] that codes for 16 non-structural proteins, including RNA-Dependent RNA Polymerase, besides other structural proteins including spike protein, an envelope protein, membrane protein, and nucleoprotein [7]. The polybasic cleavage site that is coded by spike protein's genome increases the viral infectivity and mode of transmission, making it unique. [7, 8, 9, 10]. Since SARS-CoV-2 symptoms resemble those of the flu, a precise diagnosis of the infection is crucial to control mortality. SARS-CoV-2 detection currently uses the real-time RT-PCR techniques that have been approved by the WHO but due to quick mutation in virus might eventually made the available diagnostic methods useless [11]. Nonsynonymous mutations help viruses to evolve and adapt better that are produced by nearly eighty percent of these recurrent substitutions. [12]. Since there are no effective medications or vaccines for COVID-19, only supportive management methods are employed to treat illness worldwide [12, 13]. The global havoc caused by SARS-CoV-2 needs better diagnostic and therapeutic management. CRISPR/Cas technology may be a potent, ground-breaking, and comprehensive method for limiting viral replication in host cells and controlling viral transmission by targeting viral RNA for its degradation. In this review we have concentrated on the mechanism of CRISPR/Cas, and it's possible implementations that help to develop the precise, focused, and sensitive COVID-19 diagnostics as well as a possibly secure, safe, and efficient CRISPR based anti-viral in the interest of preserving the lives of billions and billions of people many of whom are susceptible to infect by COVID 19.

CRISPR/Cas system

Clustered Regularly Interspaced Palindromic Repeat Sequences (CRISPR) is one of the trendiest biological tools related to gene-editing technology. More than thousands of publications about CRISPR technology have been published after 2013, indicating its exponential progress. CRISPR, technology was acknowledged during research on the *E. coli*iap gene as a distinct region in the 3′ end structural domain. Five highly homologous segments of the CRISPR, each 29 nucleotides long and separated by 32 nucleotides, made up the sequence [14].

The word "CRISPR" was first adopted by Jansen et al. in 2002 to address the unique family of interspaced repeat sequences [15]. The in-depth study of the sequences revealed the spacer sequences to be different from the palindromic repeats. The interlining elements in between CRISPR arrays were found to be associated with the phage genome or extrachromosomal genome [15]. Most archaea and many eubacteria have CRISPR/Cas systems, which provide amazingly diverse adaptive immunity to defend against foreign invaders like bacteriophages and mobile genetic elements [16]. These are highly conserved sequences in which the size of CRISPR repeats varies between 23-47 bp and the size of spacers varies between 21-72 bp [16, 17]. The genome of bacteria may contain one or more than one CRISPR locus. These loci have different and highly irregular spacer sequences even amongst closely associated species [17]. These sequences are used to recognize the invading viral/plasmid genome. By adding new spacers after the infection by phage or archaea, new matching viral or plasmid genomes are recognized and destroyed. This technology enables the recognition and distinction between native genome from non-native genome [17]. CRISPR actively requires the presence of 6-20 CRISPR associated Cas genes present adjacent to the spacer sequence that codes for Cas proteins. This system is now used by scientists for biotechnological applications such as genome editing. The mechanism (Fig. 1) by which the CRISPR/Cas system degrades the invader’s genome occurs through three steps i.e., adaptation or acquisition, biogenesis or expression of CRISPR RNA (crRNA) and interference or targeting. The first step is the adaptation or acquisition of a spacer sequence. By acknowledging the protospacer adjacent motif (PAM), Cas proteins of the CRISPR system identify and differentiate between native and non-native genome. PAMs are 3-6 base pairs of nucleotide motifs which Cas proteins concede [17]. Just several base pairs

of nucleotides near the PAM motif are incorporated into the pathogen's CRISPR loci upon recognition of the non-native genome. It leads to the development of remembrance of non-native genome within microorganisms. Premature crRNA is produced as a consequence of the trans-activating CRISPR RNA (tracrRNA) joined with the integration of non-native sequence (pre-crRNA). Pre-crRNA is transformed into mature-crRNA after processing, which directs Cas endonuclease to introduce double-stranded breaks into the non-native genome [17]. Depending upon the specific function of crRNAs these are also known as guideRNAs. The double strand break would be vulnerable to the non-native genome that is adjacent to the PAM motif and complementary to the fully mature crRNA. Interference is the final step in which crRNAs acknowledge and make a complementary base pairing specific to non-native genome [17]. This causes the destruction of the invading genome-crRNA complex by cleavage. The Cas nuclease in Cas9 cuts the non-native genome at a specific region towards the start of proto- spacer adjacent motif (PAM). PAM is a brief, precise 3-4 nucleotide sequence that comes right after the target sequence and crucial for Cas nuclease to cleave it [16]. Any genetic change in PAM causes a difference in complementarity between the spacer and non-native genome, which is prohibited and contributes to infection susceptibility [17].

CRISPR/Cas systems were categorized into 2 different classes and 6 types on the basis of differences between the Cas genes and their proteins [18]. Class 2 systems only have a single Cas effector, whereas class 1 systems have multisubunit effector complexes made up of multiple Cas effectors, some of which are in charge of pre-crRNA processing, some of which are in charge of crRNA binding, and some of which are in charge of nuclease cleaving. Types I, III, and IV are present in Class 1 systems, and Types II, V, and VI are present in Class 2 systems. Several promising CRISPR/Cas variants, primarily in the class 1 system, have been created. These include a number of dead Cas effectors and other mutants represented by Cas9 (H840A) and Cas9 (D10A) [19]. The six most extensively studied types of CRISPR/Cas system are type I, type II, type III, type IV, type V and type VI. These are categorized on the basis of specific Cas proteins present within the system. All of these subtypes have Cas1 and Cas2 proteins that are crucial to the spacer [20].

Type I system

Except for transposon-encoded type I variants, type I CRISPR/Cas systems have a Cas3 protein as a distinguishing feature with varying numbers of Cas5, Cas6, and Cas7 [20]. This type is further broken down into six subtypes, I-A through I-G, each of which has one unique Cas8 homolog [20]. An N-terminal HD phosphohydrolase for DNA cleavage and a C-terminal DExH helicase domain for unwinding are the two most important domains of Cas3 [20].

Non-native genome is incorporated into the bacterial genome's CRISPR loci during CRISPR acquisition phase. Afterward, during crRNA biogenesis, tracrRNA helps translate CRISPR loci into the primary transcript, which is then processed into crRNA. A crRNA and the Cas9 endonuclease work together to cleave foreign DNA close to the PAM region when interference occurs. To perform DNA interference, all type I CRISPR/Cas systems for subtype I-E join together to form the crRNA guide complex or CRISPR associated complex for anti-viral defense (CASCADE). Identification of the PAM sequence by Cas8 triggers the interference mechanism [20]. When non-native genome binds to the CASCADE, a significant conformational change occurs that causes Cas3 to be recruited to the Cas8 docking site [20]. After that Cas3 nuclease is likely to decide whether to destroy the target genome [20].

Type II system

The Cas9 gene, which codes for a multi-domain protein involved in adaptation, biogenesis, and interference, is the distinctive for the type II system [21]. The pre-cRNA is processed by type II systems using tracrRNA and cellular RNase III. Two nuclease domains, the RuvC-like nuclease and the HNH nuclease domain, are enclosed within the massive Cas9, required to cleave the non-native genome [21]. There are currently three different types of type II systems: IIA, IIB, and IIC. Csn2, a particular gene thought to be involved in spacer integration, is present in type IIA. Instead of expressing the csn2 gene, type IIB encodes a fourth gene

belonging to the Cas4 family [21]. Cas4 plays a crucial function in identifying the 3′ nucleotide motif and 5′ PAM of protospacers [21]. The only three protein-coding genes in Type II-C are Cas1, Cas2, and Cas9. As the powerful genome manipulating tool, the type II CRISPR/Cas system is currently being developed, revolutionizing the biotechnology industry. The target cell needs to express Cas9 endonuclease and single guide RNA for genome editing (sgRNA). The presence of PAM sequence with both elements will cause a double strand break (DSB) at the genome's target sequence. A DNA repair process that employs a non-homologous end joining (NHEJ) or homologous recombination pathway can join DSB [21]. Gene addition, deletion, knockout, knock-in, and many other modifications can be made using this.

Type III system

All types of III CRISPR/Cas systems have Cas10 gene, which produces the Cas10 protein. Four conserved domains, including two Palm domains and one HD domain, are present in this protein. [17]. HD domain is the N-terminal cyclase-like domain with the Zn-metal ion, requires the RRM fold as the palm domain but lacks any enzymatic activity. It is believed that the palm domain's catalytic residues when get active degrade target RNA [17]. Type III CRISPR/Cas systems use crRNAs made from CRISPR arrays linked to type I or type II systems rather than expressing the Cas1 and Cas2 genes on their own [17]. There are currently 2 subtypes of type III systems, type IIIA and type IIIB, which can be distinguished by the addition of the csm2 and cmr5 genes, respectively. While type IIIB relies solely on other CRISPR/Cas systems found within that genome, type IIIA targets DNA and has Cas1, Cas2, and Cas6 while type IIIB lacks these genes. RNA target of the type IIIB system as well [21].

Type IV system

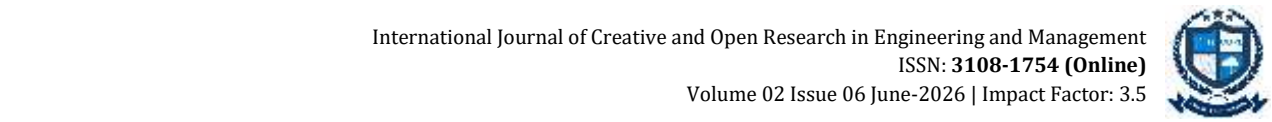
These CRISPR/Cas systems are found within many genomes of bacteria and plasmids. Type IV subtype lacks the Cas1 and Cas2 genes, just like subtype III A, and is frequently not connected to CRISPR arrays [20]. Furthermore, this is only CRISPR/Cas system with no detectable CRISPR cassette. The type IV system has an effector complex that includes 2 genes for RAMP proteins from the Cas5 (csf3) and Cas7 (csf2) [20]. One possibility for a characteristic gene for this type is csf1. Type IV systems can be divided into two different subtypes, the first of which has the DinG family helicase csf4 [20], while the second lacks DinG but typically has a gene for a small alpha helical protein, most likely a small subunit [20]. Similar to type III systems, type IV CRISPR-Cas system is potentially versatile equipment, could use crRNA from various CRISPR arrays [20].

Type V system

Subtypes V-A and V-B of the type V CRISPR-Cas system are also referred to as Cpf1(type V-A) or C2c1 (type V-B) [65]. A small and effective enzyme called Cas12 is the characteristic gene of this type, which makes staggered cuts in dsDNA. Cas12 enhances genome editing ability by processing its own guide RNAs [65]. Recently, it was found that Cas12a can indiscriminately cut up single-stranded DNA once activated by a target DNA molecule matching its spacer sequence [65]. Because of this characteristic, Cas12a is an effective tool for identifying trace amounts of target DNA in a mixture.

Type VI system

There are four subtypes of the Type VI CRISPR-Cas system: VI-A, VI-B, VI-C, and VI-D. also referred to as CasRx or C2c2. Because it targets RNA rather than DNA, Cas13 stands out in the CRISPR community [65]. It unleashes a nonspecific RNase activity and demolishes all nearby RNA, regardless of their sequence, once it is activated by the ssRNA sequence bearing complementarity to its crRNA spacer [65]. This characteristic has been used in vitro as an accurate diagnostic tool. In mammalian cells, these systems can also be used for effective, multiplexable, and targeted RNA knockdown or RNA sequence editing [65]. With no change to the genome's sequence, Cas13 is now a potentially important therapeutic for influencing gene expression.



In order to improve diagnostics and therapeutics against viruses, Cas proteins from the above mentioned CRISPR/Cas types and subtypes might be utilized.

Figure 1. Classification of CRISPR-Cas systems

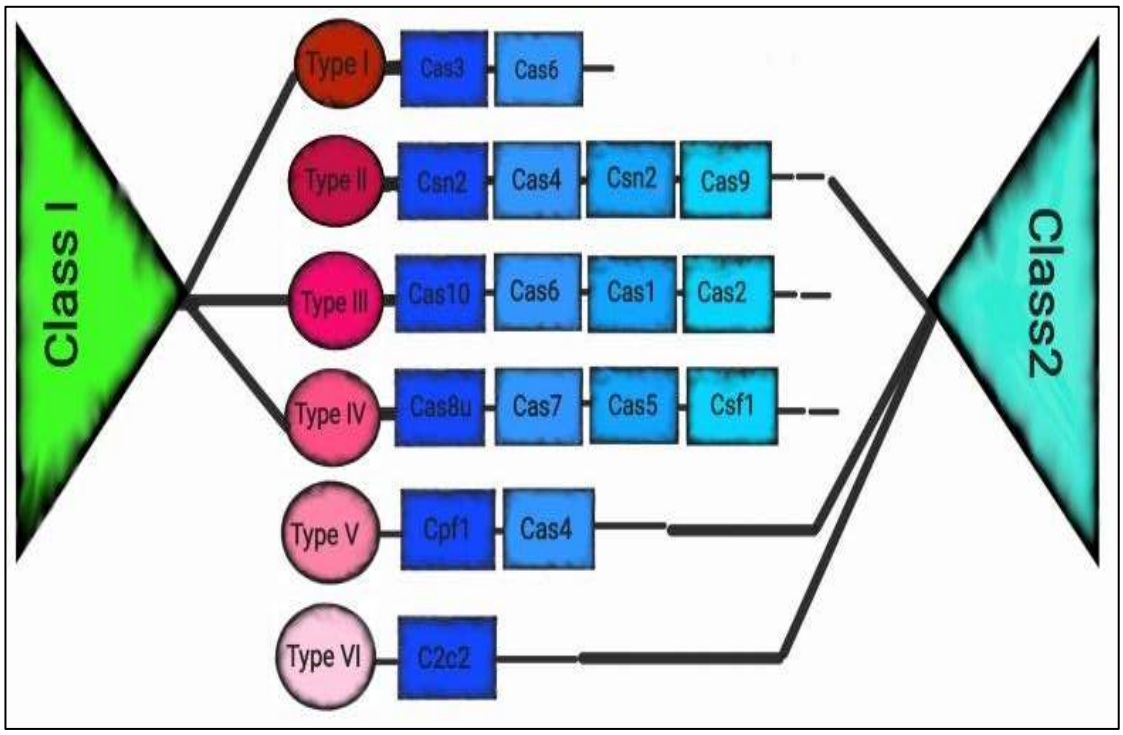


Table 1. Comparing the features of the CRISPR/Cas system

CRISPR system		Characteristic gene	Organism	Target molecule	crRNA	References
Types	Class					
I	1	Cas3	*Escherichia coli*	DNA	Single crRNA	[68, 70]
II	2	Cas9	*Francisellanovicida*	DNA	tracrRNA:crRNA	[68, 70]
III	1	Cas10	*Staphylococci* sp.	DNA and RNA	Single crRNA	[66, 69, 70]
IV	1	csf1	*Pseudomonas aeruginosa*	-	-	[67]
V	2	Cas12	*Francisellanovicida*, *Acidaminococcus* sp., *Lachnospiraceae*sp., *Prevotella*sp.	DNA (dsDNA or ssDNA)	Single crRNA/tracrRNA:crRNA	[68, 70]

VI	2	Cas13	*Leptotrichiashahii*,	RNA (ssRNA)	Single crRNA	[68, 70]

CRISPR as diagnostic

CRISPR/Cas systems have been shown to be used as diagnostic techniques for detecting nucleic acids that may frequently replace real-time PCR [32, 33]. Diagnostic tools based on CRISPR/Cas have more sensitivity and specificity than conventional PCR, but traditional techniques are used because of low estimation costs. The application of CRISPR/Cas to molecular diagnostics may alter the profile of global diagnostic and healthcare systems [34, 35]. CRISPR/Cas system regardless of target nucleic acid is utilized to create the pathogen-detection tool known as the CRISPR/Cas9-mediated lateral flow nucleic acid assay (CASLFA) [18, 24].

After the COVID-19 flu, epidemic broke out globally, there's an urgent necessity for instant and

user-friendly screening techniques. Although CRISPR-based tools have shown considerable detectability within 30–60 minutes, still to be recognized by US Food and Drug Administration

approval (FDA). Using Cas12a or Cas13 nuclease, newly created CRISPR-based diagnostic technique has been operating under the "collateral cleavage activity" theory. Following the targeted cleavage of CRISPR-RNA (crRNA), the Cas12a/Cas13 nuclease, a component of the CRISPR tool, is activated. When activated, it non-specifically cleaves all nearby ssDNA/RNA molecules, a process known as collateral cleavage or trans-cleavage. A novel nucleic acid-based diagnostic tool has been produced using this property to create fluorophores ssDNA/RNA reporter probes that can be visualized as bands in a paper strip [36, 37]. The reporter probes could be cleaved by the Cas protein as a result of the crRNA's targeting of the viral RNA, producing a positive band on the paper strip. The recently created Specific High-sensitivity Enzymatic Reporter Unlocking (SHERLOCK) technology uses the activity of the crRNA/Cas13a protein complex to precisely identify RNA molecules and cleave collateral RNA in addition to target RNAs. This technique recognizes specific RNA molecules by using fluorescent dye-tagged non-targeted reporter RNA [38]. The S and ORF1ab genes serve as two signatures of COVID-19 that are recognized by two crRNAs, allowing the SHERLOCK system based on CRISPR-Cas13 to diagnose SARS-CoV-2 [37]. The DNA endonuclease targeted CRISPR trans-reporter (DETECTR) method, similar to the SHERLOCK system, combines reverse transcription with isothermal recombinase polymerase amplification to amplify the pathogenic DNA. The Cas12a nuclease is activated when the Cas12a/crRNA complex recognizes a target, and it then exhibits indiscriminate cleavage of reporter ssDNA with a fluorescence tag. Crude DNA isolated from

clinical samples and human cell culture has been successfully used by DETECTR to distinguish between human papillomavirus 16 (HPV16) and human papillomavirus 18 (HPV18)

within an hour [39]. For the diagnosis of COVID-19, two specific crRNAs targeted to the E and N genes with detection ranges of 70–300 copies per microliter of sample input were chosen. reverse transcription and loop-mediated isothermal amplification (LAMP) were used in the isothermal amplification stage to quickly identify COVID-19 in just 30 minutes [40].

All of the aforementioned CRISPR/Cas system-based nucleic acid detection techniques demand numerous manual steps and independent nucleic acid amplification, which exacerbates the detection procedures and increases the danger of carryover contamination. All-In-One Dual CRISPR/Cas12a (AIOD-CRISPR) assay method was developed, which makes it possible to more quickly, extremely precisely, and visually diagnose viral nucleic acids [41]. This assay streamlines the identification process and lowers the risk of carryover contamination by incubating all the reagents needed to detect viral nucleic acids in a single pot at 37°C. SARS-CoV-2 and HIV genomic RNA have been swiftly and accurately detected using the AIOD-CRISPR assay method [37, 41]. For the assay, a paper probe based on lateral flow is not required. Instead, the tubes can be

visually detected by taking pictures of them under a blue LED light source. The application of this technique could result in the creation of COVID-19 point-of-care molecular diagnostic tools for the next generation. Researchers in India have created the FnCas9 Editor Linked Uniform Detection Assay (FELUDA), a point-of-care low-cost test, using a Cas9 from *Francisellanovicida* that is highly sensitive to nucleotide mismatches [42].

CRISPR as therapeutic

Viruses are tiny, contagious, acellular microbes that can infect humans, animals, and plants. They are made up of DNAs or RNAs that carry specific genes and an encasing protein shell [22]. Currently, the SARS-CoV-2 infestation poses a constant risk to both population's and world's peace and healthcare. SARS-CoV-2 permits its frequent distribution from individual-to-material and material-to-individual, emphasizing the need for specific and cost-effective treatments. CRISPR/Cas-based nucleic acid therapeutic is the most recent and cutting-edge tool for SARS-CoV-2 and consequently it has been reported widely. Recently, researchers at the Broad Institute of the Massachusetts Institute of Technology and Harvard University in the United States illustrated that the Cas13 enzyme used in cell culture to prevent the replication of three ssRNA viruses, including the lymphocytic choriomeningitis virus, influenza A virus, and vesicular stomatitis virus. The scientists identified highly conserved potential Cas13 crRNA target sites in the viral RNA and used cell culture models to compile the collection of anti-viral crRNAs that could be multiplexed in a combinatorial manner. In mammalian cells, the crRNA-directed Cas13 enzyme showed that viral RNA could be effectively reduced. Similar to other nucleic acid-based treatments like shRNAs, the CRISPR/Cas13 system was employed to limit viral proliferation. Also, the team demonstrated how multiplexing, such as a CRISPR array or numerous crRNAs, could significantly enhance the efficacy of Cas13-based therapies. The CRISPR array could be processed by Cas13 to produce unique crRNAs that could target various viral RNAs. Cas9-based CRISPR anti-viral have been reported to cause target site mutations [23, 24], but Cas13 treatment did not result in any crRNA target-site mutations [25]. These reports show the Cas13 enzyme's potential as a powerful anti-viral to treat SARS-CoV-2 infection.

There are currently no immediate treatments available to treat outbreaking corona viral flu, and it will likely take months for a vaccine to be developed. CRISPR/Cas13 is only recognized as an efficient system that uses sequence-specific crRNAs to prevent bacteriophage infection in host bacterial cells [26, 27]. A similar strategy could be used in the creation of a COVID-19 treatment that specifically targets +ssRNA genome of SARS-CoV-2. It has recently been suggested to make use of the CRISPR/Cas13d mechanism to precisely deteriorate SARS-CoV-2 genetic material via constructing crRNAs that are directed to virus' ORF1ab (replicase-transcriptase) and S (spike) genes [3]. Due to its extremely conserved sequence of amino acids and structural properties, the SARS-CoV-2 virus's RdRp gene may also serve as an appropriate goal for CRISPR-Cas13-mediated virus RNA deterioration [28]. It is easier to target the various virus variations that are constantly changing due to the rapid development of crRNAs since Cas13d protein has the particular trait that its cleavage isn't dependent on PAM-like sequences [69]. The research team suggested that patients with COVID-19 are acquiring Cas13d effector through recombinant adeno-associated virus (AAV). The Cas13d effector's diminutive size is anticipated to make it easier for the AAV to transport CRISPR cassettes with more than 2 different crRNAs, resulting in effective virus eradication and a decreased risk of the development of resistance. Additionally, the accessibility of an AAV serotype that is specific to lung tissue can aid in the targeted delivery of CRISPR.

SARS-CoV-2 would be prevented by Anti-viral CRISPR prophylaxis in huMAN cells (PAC-MAN) which is a COVID-19 therapy based on CRISPR/Cas13 technology. Human's lungs are shown to be an efficient target for SARS-CoV-2 and influenza A virus (IAV) genomic sequence degradation [29]. The PAC-MAN strategy might be a viable anti-viral approach that control the new viral strains and might be quickly put into effect in a global epidemic situation. To get the best outcome, it is essential to tackle the significant challenge of delivering PAC-MAN in animal studies to the target organ. Such CRISPR-based anti-virals may be delivered

using viral vectors such as AAV, adenovirus, and lentiviral vectors [30]. Non-viral vectors are less prevalent than viral vectors, but they nevertheless offer significant benefits, including simplicity in scaling up, absence of immunogenicity, and delivery of the CRISPR/Cas system as RNPs, which minimizes off-target effects. Gold, lipopolysaccharide and polysaccharide, cell-penetrating proteins, and lipid-based nanoparticles are other examples of additional non-viral vectors [31]. Because it specifically targets highly conserved areas in the genome of virus, CRISPR/Cas13-based approach works against viral strains that replicate rapidly.

Delivery system

A major difficulty in the formulation of CRISPR based theragnostic is the requirement of an effective delivery system. Although CRISPR/Cas is a potent technique for editing genome, it has few delivery systems limiting its therapeutic potential [31]. There are two types of delivery systems, physical and chemical. Bacteriophages and plasmid fall into the category of physical delivery system also known as viral delivery systems. Nanoparticles are included in chemical delivery systems [33]. A far more popular method for delivering CRISPR is through viral vectors, and many scientists also chose the AAV as a delivery tool [43]. However, many such recombinant viruses have limitations to carry transgenes up to a certain size. It is challenging to develop a recombinant AAV that carries genes which encode Cas proteins also with crRNA/gRNA. But most Cas proteins have got large molecular weights and to tackle this problem, just recently, a few light weighted Cas proteins, including Cas14, cjCas9, ScCas9, and others, were discovered. These proteins are also able to be forwarded by rAAV and utilized for their desired uses [44, 45, 46, 47]. Although viruses are advantageous for carrying foreign genes with the limitations of toxicity, immunogenicity and short-term Cas expression inside the host. Non-viral vectors based on lipids and inorganic particles, on the other hand, are free of these restrictions and may be investigated as a substitute for delivering CRISPR/Cas systems for therapeutic purposes [31]. To overcome the drawback associated with the viral delivery system, nanoparticles are being explored [48]. Presently, nanoparticles provide an ideal delivery platform for CRISPR/Cas systems, including cations, Lipid Nanoparticles (LNPs), DNA nanoparticles, lipid complexes, gold nanoparticles, and zeolite imidazole frameworks [48, 49]. Traditionally, lipid nanoparticles are used to deliver nucleic acids. The delivery efficiency of LNPs is relatively low and hence requires some modifications [49]. The Lipid nanoparticle is the first non-viral delivery system found to be capable of co-delivering Cas9 mRNA and sgRNA in-vitro and in-vivo [50]. When administered locally, LNPs become unstable. In the current state of technology, stable lipid nanoparticle delivery systems with reduced liposomal toxicity are still difficult to develop [50]. This is an auspicious technology which demands more investigation utilizing in vivo models to establish the therapeutic efficacy that can overcome challenges posed by infectious diseases.

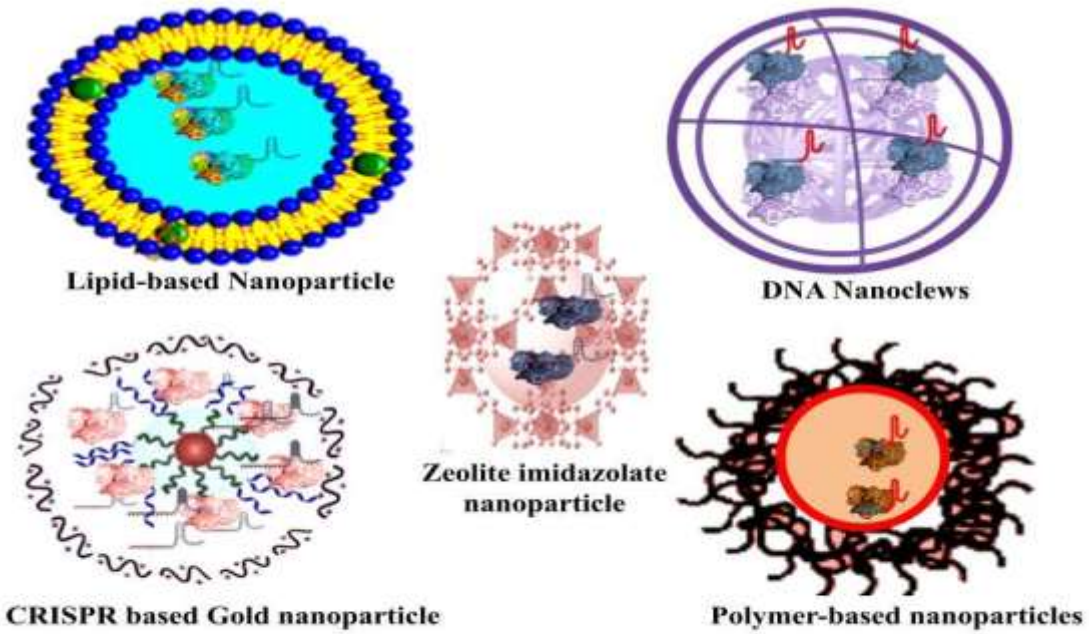


Figure 2. Various kinds of nanoparticles that are frequently used in medical applications and have tremendous opportunities to deliver CRISPR/Cas systems.

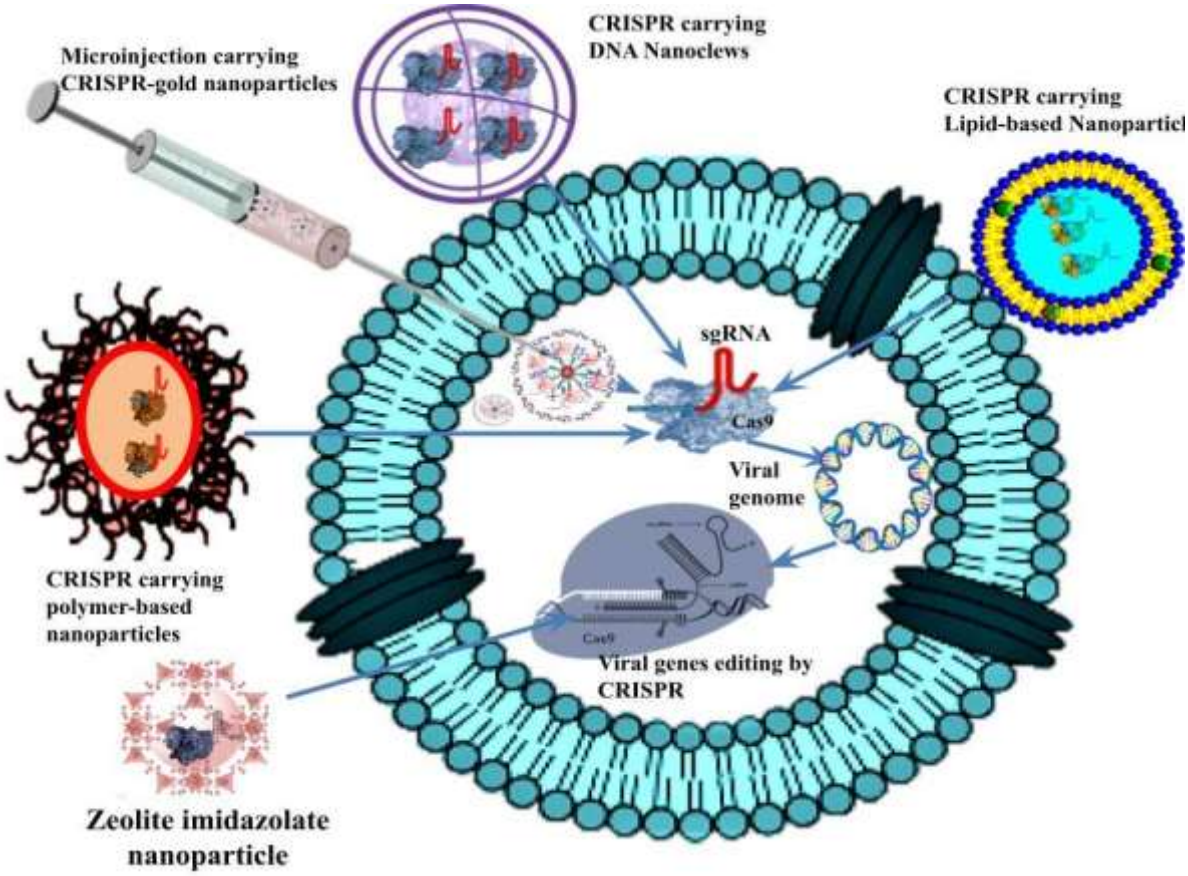


Figure 3. Delivery of various CRISPR-based nanoparticles to the target viral genome in vivo.

Limitations

1. Lack of delivery system

Animals can typically be systemically exposed to both modified and unmodified vectors. Exogenous CRISPR genes are protected from internal deterioration by modified vectors, but non-modified vectors may do so. The CRISPR/Cas system may alter normal tissues when it enters non-target cells, which could have unexpected effects. To lessen the entry of genes into unintended cells, a better delivery system, coated with biofilm or polypeptide to improve the onsite direct of foreign genes is required [51, 52, 53]. In response to alterations in the abrasive conditions in the target organ's microenvironment, gene delivery should be designed as environmentally friendly nanostructures. The nanostructures' core enters the cell through endocytosis after being first exposed by the breakdown of their outer shell in a particular microenvironment. An effective way to target infectious tissue on-site without affecting non-infectious tissues is needed that can be induced by various factors to release its genes [54].

2. Off-Target Site Of action

A specific area of the target site must be targeted by PAM sequences in order for many Cas proteins, including Cas9 and Cas12a. The PAM sequences are very specific to the CRISPR/Cas system type, and the use of this technology for therapeutic purposes is severely constrained in the absence of a specific PAM sequence close to the target region. These CRISPR tools need to be able to change to fit the targets they select in order to be more effective. Research has been done to create PAM variants of Cas9 and Cas12a that could recognize more than one PAM in order to accomplish this. Other essential Cas proteins, like Cas13 variants, also require designing to enhance their efficacy and selectivity [55, 56].

3. RNA Destabilization and Mosaicism's Occurrence

Due to RNase's widespread presence, RNA is prone to damage. It might significantly affect how well the CRISPR-based diagnostic system works. The occurrence of mosaicism, which occurs when transduced cells are used before the editing or cleavage of target nucleic acids, is another restriction on the use of the CRISPR system for therapeutic applications [60].

4. Sensitivity of RNA Secondary Structure

The effectiveness of RNA-specific Cas proteins like Cas13 may be impacted by the secondary structure that RNA typically acquires inside of cells. In order to reduce the Cas13 proteins' sensitivity to RNA secondary structure, it might be necessary to understand the mechanisms that underlie this sensitivity [57].

5. Bio-compatibility

In order to lessen the likelihood of it having negative effects inside the host, it is necessary to develop appropriate vectors for host cells. The vector needs to be biocompatible and should have a significant ability to cross the cell due to the challenging process from entry to function [58]. When designing the delivery system, it is incorporating the immune response is crucial that will happen after the material is absorbed by the body. The body's immune responses are reportedly triggered by widely used Cas9 proteins made from *S. pyogenes* and *S. aureus*. To successfully evade immune reactions, a modified Cas9 lacking exons that initiate immune responses was delivered [59]. In contrast to viruses, lipids, and exosomes, which can successfully evade immune clearance in vivo, synthetic chemical nanoparticles require a protective surface coating. Examples include modified PEG, which helps to keep the polymer stable in the blood environment, or the presence of modified CD47 protein [61, 62, 63]. Exosomes from plants have a higher propensity to evade immune response. Because mammalian and plant pathogens differ significantly, it is also safer to deliver CRISPR/dCas9 systems via plant exosomes. The study of how plant exosomes can deliver genes, however, is still in its infancy [58, 64]. Plant exosomes' innate resistance to immunological clearance and low

infectiousness draw attention to their potential use in achieving the objective. Targeting particular genes with low antigenicity may stop the delivery tool from eluding an immune response.

Challenges and Future Perspective

Despite the progress that has been made in the field of infections, it is only when new, dangerous viruses, like SARS-CoV-2, appear in an unprepared population that humans become immobile. There is an immediate need to create theragnostic tools that are portable, quick, and practical so they can be used in settings where SARS-CoV-2 is more common, like airports and community health centers. Furthermore, because SARS-CoV-2 can cause both clinical and non-clinical symptoms in people, it can spread quickly throughout a region among infected but asymptomatic individuals. These individuals aren't actually tested, and as a result, they continue to infect others with the virus. The availability of a sensitive and portable diagnosis may aid in widespread testing of infected individuals in order to stop the spread of these viruses.

The CRISPR/Cas system enables the creation of novel, highly targeted anti-viral therapeutics and molecular diagnostics at the point-of-care without the requirement of high-end tools. CRISPR-based diagnosis could meet this requirement because it is sensitive and quicker than the current real-time PCR-based standard diagnosis method for the diagnosis of COVID-19. Despite the fact that the CRISPR-based therapies have not yet received approval, the US FDA has granted an emergency use authorization license for the CRISPR-based diagnostic. Although it is having accuracy and delicateness, there are still many challenges to be solved in terms of infrastructure requirements, the need for skilled labor, and cost. The development of diagnostics for some viruses, such as dengue, Zika, and others, has proven to be very successful when using the CRISPR/Cas system. The effectiveness, speed, and affordability of CRISPR/Cas-based diagnostics like DETECTR and AIOD-CRISPR assay method have been established. Although they have not yet been commercialized, paper-based diagnostics like SHERLOCK can be a very user-friendly tool for diagnosing virus infection at the point-of-care and in remote locations with few resources. These diagnostic tools make it possible to thoroughly screen the affected populations, assisting in regular surveillance and preventing the virus's spread within communities. The genomes of RNA viruses continue to accumulate advantageous mutations, which leads to the emergence of anti-viral resistance. Thus, formulation of medication to fight the viruses is incredibly challenging. But with the development of anti-viral therapeutic based on CRISPR that combats these viruses before the genomic mutation of RNA viruses might halt the emergence of anti-viral resistance.

Conclusion

SARS COV-2 is a serious issue that keeps coming up and necessitates quick action. It is pertinent to constantly investigate the opportunities to overcome it. CRISPR based theragnostic is a novel approach to be investigated for the same. Its specific targeting against the foreign antigen eliminates the possibility of nonspecific killing of the nontargeted tissues of host system. Moreover, any break in the chromosomal DNA by targeting through CRISPR is deadly to the infectious agents. Careful application of CRISPR based drug can be a potential therapy against chronic and re-emerging infections. There is a need for effective delivery systems such as phages and nanoparticles to deliver foreign genes efficiently. Adeno-associated viral vectors are prominently used to deliver foreign genes for SARS COV-2 variants in host cells with several limitations. Therefore, nanoparticles should be developed in conjugation with a variety of materials like synthetic polymer, protein, and polysaccharides to restrict the limitations. Bio-polymeric and degradable nanoparticles hold enormous applications as a vehicle for CRISPR/Cas delivery. CRISPR/Cas technique in the last ten years has gained enormous attention due to its multidimensional nature. While the mechanism behind CRISPR action is known, it still requires effective methodologies to overcome the hurdles associated with it. More research must be done to study the efficiency of CRISPR’s theragnostic. Alternative delivery systems and methods to enhance the existing efficacy of the delivery also must be investigated. There are numerous possibilities that CRISPR/Cas offers and need to be thoroughly studied such as deletion or manipulation of a targeted gene or delivery via CRISPR/Cas.

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