Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
The significance of bioengineered nanoplatforms against SARS-CoV-2: From detection to genome editing

Parichehr Hassanzadeh

Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 13169-43551, Iran

ARTICLE INFO

Keywords:
Coronavirus
COVID-19
Nanoplatforms
Virus detection
Genome editing

ABSTRACT

COVID-19 outbreak can impose serious negative impacts on the infrastructures of societies including the healthcare systems. Despite the increasing research efforts, false positive or negative results that may be associated with serologic or even RT-PCR tests, inappropriate or variable immune response, and high rates of mutations in coronavirus may negatively affect virus detection process and effectiveness of the vaccines or drugs in development. Nanotechnology-based research attempts via developing state-of-the-art techniques such as nanomechatronics ones and advanced materials including the sensors for detecting the pathogen loads at very low concentrations or site-specific delivery of therapeutics, and real-time protections against the pandemic outbreaks by nanorobots can provide outstanding biomedical breakthroughs. Considering the unique characteristics of pathogens particularly the newly-emerged ones and avoiding the exaggerated optimism or simplistic views on the prophylactic and therapeutic approaches including the one-size-fits-all ones or presenting multiple medications that may be associated with synergistic toxicities rather than enhanced efficiencies might pave the way towards the development of more appropriate treatment strategies with reduced safety concerns. This paper highlights the significance of nanoplatforms against the viral disorders and their capabilities of genome editing that may facilitate taking more appropriate measures against SARS-CoV-2.

1. Introduction

Coronaviruses include the related viruses group capable of causing diseases in the birds and mammals [1]. In comparison to other RNA viruses, coronavirus has the largest size of genome and spike peplomers projecting from its surface [2]. Regarding the genetic recombination and content, high-degree plasticity has been detected in the genomes of coronaviruses (CoVs) [3,4]. Large genomes of CoVs could be associated with increased mutation probabilities and facilitated viral attachment or entry into the cells by using multiple receptors [5,6]. Attachment of the spike glycoprotein to the host-cell receptor is followed by the cleavage of protease, activation of the spike protein, virus entry into the host cell via endocytosis or viral envelop fusion with cell membrane, un-coating the virus and RNA genome entrance into the cytoplasm, translation of the reading frame of viral genome by the host ribosome and forming a polypeptide that can be cleaved by its own proteases into various non-structural proteins which are implicated in transcription and replication of RNA [7,8]. Inactivation of the ether has revealed the lipid envelope of the virus [1].

In December 2019, pneumonia outbreak was detected as a novel coronavirus strain which imposed a pneumonia pandemic, COVID-19 [14]. Unfortunately, the virus may coexist with humans for long periods of time and the symptoms may occur via activation of the patients’ immune responses [15,16]. In this respect, strengthening hosts’ immune systems or controlling the hyperactive immune responses or inflammation can provide more efficient protective effects.
2. A quick look at the proposed strategies against the COVID-19 and the challenging issues

2.1. Antibody tests and real-time RT-PCR

Recently, FDA has warned about the limitations of antibody tests for COVID-19 screening that may be due to the slow body response to the virus, inability to confirm that people are no longer virus carriers, showing false negative or positive results, technical difficulties in production of proteins (as antigens) in the laboratories and determining which proteins are the most appropriate ones for antibody production [17]. Despite the easy screening and evaluation of the immunity, rapid antibody testing cannot be a suitable substitute for COVID-19 diagnosis by real-time RT-PCR which has been shown as one of the most precise methods for evaluation and detection of the presence of genetic materials of RNA viruses including the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [18]. High specificity and sensitivity, providing reliable diagnosis at the early stage of infection and high-confidence detecting the low-copy targets, being faster than other viral isolation techniques, and reduced risks of error or contamination are the major advantages of real-time RT-PCR [19]. However, the technique is associated with various limitations such as short detection window, difficult scalability, and high costs [20]. Inappropriate collecting methods or irregular viral loads can increase the rates of false negative results including those related to the infections of COVID-19 [21]. Therefore, RT-PCR may not be applied as a unique test for guiding the healthcare plans.

2.2. Vaccines

Development of vaccines has been a significant breakthrough in biomedicine leading to the reduced mortality rates. Besides profound influence on preventing or managing the infectious diseases, vaccines including the peptide-based ones have attracted growing interests for influence on preventing or managing the infectious diseases, vaccines which proteins are the most appropriate ones for antibody production cannot be a suitable substitute for COVID-19 diagnosis that may be due to the slow body response to the virus, inability to confirm that people are no longer virus carriers, showing false negative or positive results, technical difficulties in production of proteins (as antigens) in the laboratories and determining which proteins are the most appropriate ones for antibody production [17]. Despite the easy screening and evaluation of the immunity, rapid antibody testing cannot be a suitable substitute for COVID-19 diagnosis by real-time RT-PCR which has been shown as one of the most precise methods for evaluation and detection of the presence of genetic materials of RNA viruses including the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [18]. High specificity and sensitivity, providing reliable diagnosis at the early stage of infection and high-confidence detecting the low-copy targets, being faster than other viral isolation techniques, and reduced risks of error or contamination are the major advantages of real-time RT-PCR [19]. However, the technique is associated with various limitations such as short detection window, difficult scalability, and high costs [20]. Inappropriate collecting methods or irregular viral loads can increase the rates of false negative results including those related to the infections of COVID-19 [21]. Therefore, RT-PCR may not be applied as a unique test for guiding the healthcare plans.

For accelerating development of vaccine against SARS-CoV-2, adjuvants should be applied for increasing the immune response. In this sense, aluminum adjuvant via phagocytosis facilitation, slowing antigen diffusion from the site of injection, and stimulation of T helper type 2 immune response, MPLs can match the creation of immune environment at the site of injection and recruiting the immune cells for inducing antigen-specific immune response, or adjuvant system series (AS01-AS04) could be suitable [46–48]. In general, optimal adjuvant is selected based on the vaccine design process. Combination of various adjuvant types can be used for increasing the immune response efficiency [49]. As aforementioned, safety concerns have remained to be addressed relating the development of drugs or vaccines including those against COVID-19 [50]. Besides the biological activities, membrane fusion, and receptor
binding capabilities, S protein has been shown as a promising antigen candidate for development of vaccine. However, it can induce severe damages in the liver and increased infection [51, 52]. This necessitates performing more rigorous research on both function and structure of S protein.

2.3. Drugs

Some reports have suggested in vitro and in vivo efficiency of hydroxychloroquine and chloroquine (FDA-approved drugs against the malaria, rheumatoid arthritis, and lupus) against SARS-CoV-2 [53, 54]. Besides disappointing or not-well-established findings, these drugs may be associated with toxic effects on heart, kidney, liver, or neurons [55] that may negatively affect their efficiency. The experimental antiviral agent, remdesivir, has been represented for preventing the replication of this rapidly spreading single-stranded RNA virus. The drug has shown therapeutic potentials against SARS-CoV-2 in vitro and MERS-CoV in non-human primates [56]. Initial findings of a few studies indicate the relatively rapid recovery and improved rates of survival in some COVID-19 patients receiving remdesivir [57, 58]. Observational data should be confirmed by well-designed, multicenter, and peer-reviewed clinical trials for demonstrating the suitability and efficiency of the drug which has shown various side effects including the hepatotoxicity. Furthermore, Chinese researchers have not found encouraging data during a double-blind, randomized, and placebo-controlled study [59]. Regarding ritonavir-lopinavir, preliminary report indicate disappointing results [60]. Favipiravir as an inhibitor of RNA polymerase has shown efficiency against the viral infections such as influenza [61]. Few clinical trials in China have represented favipiravir as an anti COVID-19 therapeutic agent due to the rapid clearance of virus and increased rate of improvement in patient’s chest imaging [62]. This necessitates further confirmations by more vigorous and controlled trials.

Besides the viral target proteins, inhibitors which target the interaction of virus with host factors could be promising candidates (host-directed therapeutics) against the viral infections [63, 64]. In general, drugs capable of direct targeting the virus appear to provide more therapeutic efficiency. SNG001, an experimental medication against the chronic obstructive pulmonary disorder is currently under evaluation in a phase-II clinical trial for its potential effectiveness against the COVID-19 [38]. Recently, enhanced morbidity and mortality in the hypertensive patients affected by COVID-19 has been reported [65]. Regarding the inhibitors of angiotensin converting enzyme (ACE) or antagonists of angiotensin receptor, there are no well-established studies demonstrating the potential harms or benefits of these drugs in COVID-19 patients [66, 67]. Despite the implication of ACE-2 in the viral entry (Fig. 2) and COVID-19 pathogenesis [68], it could not be inhibited by ACE inhibitors which are usually prescribed in the clinic [69].

Furthermore, application of ACE inhibitors or antagonists of the angiotensin receptor may enhance ACE-2 expression and susceptibility of patients to the viral propagation and entry into the host cells [70]. These drugs have not influenced mortality or morbidity in 112 patients affected by both cardiovascular disease and COVID-19 [71]. Altogether, there is no appropriate scientific evidence or clinical trial data suggesting discontinuation of these medications in COVID-19 patients with co-existing cardiovascular disorders. Evaluating the accuracy of the contradictory hypotheses necessitates designing additional research projects.

EK1 and HR2-derived peptides as the inhibitors of entry/fusion appear to be effective against SARS-CoV-2 infections [72]. Furthermore, clustered regularly- interspaced short palindromic repeats (CRISPR)-Cas13d system has been suggested for targeting and cleaving the genome of SARS-CoV-2 [73]. Intracellular poly(ADP-ribose)
polymers can inhibit the replication of coronavirus and viral macromdomain has been suggested as an appropriate target for antiviral treatment [74]. Further evaluations are required for supporting these reports.

Based on the receptor-mediated endocytosis of coronavirus, targeting of endocytosis has been suggested as a promising approach against SARS-CoV-2 [75]. Over the last decade, huge amounts of costs and time for drug-related research and design has provoked development of more advanced strategies and techniques. Artificial intelligence (AI) technologies have offered immense opportunities to analyze large-scale multivariate data, solve complicated problems, make precise decisions, identify novel compounds including the biomarkers and predicting their interactions, bioactivities, targets, and treatment outcomes, provide novel insights into the disease pathomechanisms, and rapidly design more effective therapeutics and carriers [76]. Platforms powered by AI can be used for patient matching with relevant trials in the clinical settings leading to the improved cost-effectiveness and error reduction. A number of drugs targeting AP-2-associated protein kinase-1, as the host kinase capable of regulating clathrin-dependent endocytosis have been detected and baricitinib has been suggested as an appropriate drug against COVID-19 due to its inhibitory effect on the kinase activity [77,78]. Inhibitors of p21-activated protein kinase-1, an enzyme which is involved in the viral entry and replication and can prevent micropinocytosis, have been suggested as therapeutic agents against COVID-19 [79,80]. Noteworthy, inhibitors like caffeic acid, ketorolac, triptolide, or propolis are associated with low solubility and problems in cell penetrability [81]. In this respect, newer inhibitors such as minnelide and frondoside-A with improved solubility and potency have been developed [82,83] that needs further evaluations.

The beneficial effects of corticosteroids for suppressing the hyperinflammatory response in some COVID-19 patients has remained controversial. There is no well-established clinical evidence for supporting corticosteroid therapy against the lung injury associated with COVID-19 [84]. Recently, a retrospective study has shown the beneficial effects of low dose corticosteroids in the critically-ill COVID-19 patients [85]. Because of its high potency, dexamethasone may be considered for suppressing the hyperinflammatory or immunologic reactions in patients with severe conditions, however, after appropriate infection control. Furthermore, dexamethasone may worsen the present infection or reactivate the previously managed one and intensify the negative impacts of COVID-19 on various body organs including the heart that may result in serious adverse effects. In this respect, further evaluations are of critical importance to determine the appropriateness of corticosteroids including dexamethasone against COVID-19 even in severe conditions.

Preventing activation of Fc receptors may also be helpful for reducing the inflammatory response induced by SARS-CoV-2 [86]. Furthermore, blocking interleukin-6 (IL-6) receptors or granulocyte-macrophage colony stimulating factor may reduce SARS-CoV-2-induced immunopathology [87].

2.4. Convalescent plasma (CP) therapy

In the case of an infection for which there is no particular treatment approach, CP therapy (providing specific antibodies with human origin) has been suggested as a promising strategy [88]. Regarding COVID-19, CP can be obtained from a recovered patient with humoral immunity against the disease [89]. However, evaluation of the safety and efficiency of CP therapy has remained challenging that may be due to imprecise mechanism of action or inappropriate clinical trials [88].

2.5. Monoclonal antibodies (mAbs)

mAbs can be applied for direct attacking and neutralizing the virus, preventing the infection of host cells, or blocking the spike proteins for preventing virus attachment to the host cells [90]. In patients recovered from the infections of COVID-19, reproducing or engineering of the functional copies may provide antibodies for mimicking or increasing the immune system attack against the SARS-CoV-2 [91]. Tocilizumab as a mAb against the receptor of IL-6 which has received FDA approval for treating patients with giant cell arteritis and idiopathic or rheumatoid arthritis [92], has been recently applied as the immunosuppressive drug in COVID-19 patients in critical conditions in Italy and China and promising results have been reported [93]. Meanwhile, performing various clinical trials in different countries is required for obtaining more conclusive data.

2.6. Tissue engineering (TE)

TE techniques which can be applied for repairing or replacing the damaged tissues and organs, improving the efficiency of traditional treatment strategies against a variety of disorders, screening of drugs, or immunomodulation [94,95], may also be useful against the viral outbreaks via designing the viral models, vaccine platforms, and systems for delivery of therapeutics [96]. Tissue-engineered lung models can be used for evaluating the developmental process of tissue and its
physiological or pathological conditions including the infections induced by viruses [97]. However, TE may be associated with a variety of limitations that necessitates application of the modeling approaches and newer technologies including 3D printing for obtaining biocompatible supporting materials [98].

2.7. Drug repurposing

Drug repurposing with present antivirals is also an encouraging approach for treatment of the viral infections [99]. Noteworthy, the safety and efficiency of the aforementioned investigative, preventive, and treatment strategies including the traditional medicines or their combination with conventional drugs against SARS-CoV-2 have not been fully approved by health authorities [100,101]. Indeed, there is no therapeutic agent with absolute safety. Development of the efficient preventative or treatment strategies for limiting infections necessitates consideration of the unique properties of pathogens including the viruses, selection of the appropriate sample size, sharing of datasets extracted from high-quality evaluations, and identifying the limitations of studies that might result in the increased efficiency and reduced safety concerns.

3. The significance of editing the viral genomes

Genome editing is a genetic engineering type which targets the genetic material insertion towards the specific sites. It may occur as a natural process without needing to the genetic engineering [102]. Genome editing by the engineered enzymes; nucleases (transcription activator-like effector, zinc finger, or mega-nucleases), and clustered regularly-interspaced short palindromic repeats system have been selected as the breakthrough and methods of the year by the prestigious journals, Nature methods and Science [103,104]. These nucleases induce double strand breaks (DSB) in the genes in a site-specific manner. Creation of DSB at specific sites is of critical importance in editing of genes (Fig. 3). The breaks can be repaired via the homologous or non-homologous pathways leading to the targeted-mutations [102].

In general, editing of the genome depends on the mechanics of DSB repair through which various enzymes join DNA ends or a template (homologous sequence) is applied to regenerate the missing sequence of DNA at break point. In this context, a vector is created with appropriate genetic components within the sequence which is homologous to DSB sequence leading to the desired alteration being inserted at DSB site [105]. Efficient editing the genomes of plants and animals has been performed since 2012 [106]. Using the engineered nucleases for removing or adding the genomic components (i.e., editing), may be beneficial in various fields of biomedicine such as evaluating the functions of genes, development of the complex systems, gene mutations in a targeted manner, chromosome rearrangements, gene labeling, or gene therapy in which the defective genes are replaced by normal alleles at appropriate locations [106]. The emergence of CRISPR technology has provided the possibility of gene knockout upon mRNA delivery which encode gene-editing nucleases (Fig. 4), [107].

In the viral diseases, CRISPR can be applied for targeting host or virus for disrupting the genes which encode the viral receptor proteins [108]. This system may also facilitate disease eradication and enable taking genes from the human cells or replacing genes capable of activating cancer or other undesired defects. Because of its efficiency and precision, CRISPR can be applied for establishment of the large-scale productions [108].

During decades, mice have been used as a disease model host. Using CRISPR system enables bridging the gaps between the animal disease models and clinical trials via creation of the transgenic models in large animals and non-human primates [109,110], targeting and eliminating the endogenous retroviruses leading to the reduced risk of disease transmission and immune barriers [108]. CRISPR-Cas9 as the most versatile technique for engineering or editing of the genomes and gene therapy, has enabled direct introducing of sgRNA and Cas9 protein into the zygotes for obtaining intended modifications of genes, bypassing cell targeting stage in the generation of transgenic lines, and significant reduction of generation time [111]. The most widely applied CRISPR-Cas9 machinery has been derived from the Streptococcus pyogenes and Cas9 endonuclease from F. novicida is capable of RNA targeting [112]. CRISPR-Cas9 system of F. novicida has been applied for targeting single-stranded RNA (+ssRNA) virus genome (hepatitis C virus, HCV) [113]. sgRNA and Cas9 targeted to 5′ and 3′ un-translated regions of HCV genome led to the significant reduction (50–60%) of the expression of viral protein [113]. These findings might be helpful for development of resistance to RNA viruses using the technology of gene editing.

Introducing CRISPR-Cas9 into the cells has been shown to increase the efficiency of viral mutations in site-specific and accelerated manner [114]. This system precisely control the mutation site and is a systematic strategy for constructing recombinant viruses with large genomes and

Fig. 3. DSB creation at specific sites is of key significance for genome editing. CRISPR-Cas9-induced DSB repair mechanism including the HDR and NHEJ pathways has been illustrated. (DSB: double strand breaks).
Adapted from Ref. [111].
significant inhibition of the replication of viruses (wild-type ones) with high-cleavage efficiency [115,116]. CRISPR-Cas9 has efficiently and rapidly edited Vaccinia virus genome and showed capability of simultaneous engineering of 2 viral genes that might significantly expand biomedical applications of the Vaccinia virus as a promising vector for a variety of vaccines against the infectious diseases, delivery of genes, and anticancer immunotherapies [117,118]. In usual, CRISPR-Cas system targets DNA in hosts or viruses but the system is also capable of RNA targeting and editing [114,119]. RNA-targeting CRISPR-Cas effector C2C2 system via cleavage of the viral RNA and preventing the replication of virus can be considered as a promising strategy against RNA viruses [120].

Acquiring a better understanding about the host response to SARS-CoV-2 might be of therapeutic significance. DNA or RNA editing by the endogenous deaminases can restrict the replication of particular viruses [121]. Following SARS-CoV-2 virus entry into the human body, two deaminase enzymes have shown the capability of editing the RNA of virus and affecting its replication [121,122] that might be of great significance against COVID-19. APOBECs and ADARs are deaminase families which are expressed in mammals and change nucleotide building blocks of RNA in the virus via amino group removal [123]. They are implicated in gene editing of the coronavirus that may influence the fate of virus and affected patient [122]. After single-nucleotide variant analysis in RNA sequencing data sets from the extracted fluid from the COVID-19 patient’s lung, low levels of the mutation occurrence were observed and nucleotide alterations were identified that may be due to the editing of RNA [122]. By comparing the genomes of MERS-CoV, SARS-CoV, and SARS-CoV-2, researchers have suggested that deaminase enzymes are involved, at least in part, in the mutations of these strains of coronavirus [122]. In this context, deaminase enzymes may be considered as potential targets for development of novel therapeutics against RNA viruses.

As previously reported, CRISPR-Cas9 machinery plays an important role in eukaryotic antiviral defense [124]. Following application of CRISPR-Cas machinery for producing DNA virus-resistant plants [125], CRISPR-Cas9 system has been reprogrammed for conferring immunity against RNA viruses in Arabidopsis and Nicotiana benthamiana plants leading to the reduced accumulation of the viral RNA and symptoms of infection [126]. Transgenic plants demonstrated inheritable resistance and a remarkable reduction of viral accumulation was observed in the progenies indicating the importance of CRISPR-Cas9 technology for producing plants with stable resistance to RNA viruses [127]. Such findings could be applicable to other species for direct targeting RNA viruses in the eukaryotes and preventing further infections.

Nucleases which can be programmable enable accelerated engineering of the genomes in cells or organisms [128], however, their targeted delivery may be quite challenging. In this respect, Cas9-sgRNA ribonucleoproteins-loaded murine leukemia VLPs have been applied for inducing efficient editing of genomes in a variety of primary cells and cell lines [129].

Application of targeted-RNA recombination has been shown as a promising approach for manipulating the genome of coronavirus (especially in 3′ part) [130]. The method consists of two steps for effective selection of the recombinant viruses (based on the host cell switching) including: i) preparing the interspecies chimeric coronavirus through which the ectodomain of spike glycoprotein can be replaced by that of the coronavirus distinct species tropism, ii) application of the chimeric virus as recipient for recombining with donor RNA which carries original spike gene. Afterwards, recombinant virus is isolated according to its natural cellular tropism. Further created mutations in donor RNA could be incorporated within the recombinant virus for generating the mutant virus [130,131].

Accurate and efficient editing of the large genome of a virus necessitates application of the straightforward and efficient technology (instead of the multiple-step and time-consuming ones) for selecting attenuated strains of vaccine and constructing mutant viruses or vectors for gene therapy [112]. This can be done via introducing RNA-guided nucleases into the host cells during the viral replication leading to the inhibition of replication [132]. Engineered replication defective viruses may also be applied for gene editing. In this sense, viral vectors as delivery devices for nucleases and DNA templates have been considered as useful tools in the settings of genome editing [133]. Lentiviral vectors can permanently induce genetic modifications in the target cells because of the integrase dependent mechanisms [134]. This type of mechanisms
are of critical significance for inducing stable genetic deficiencies in target cells [135]. Using adenoviral vectors for inducing Apc mutations or chromosomal inversion or rearrangement represent these vectors as the donor DNA template source for genome editing in a homology-directed manner and promising platforms which are able to introduce designer nucleases \textit{in vivo} [136]. However, activation of the adaptive and innate immune responses against the viral particles or presence of the designer nucleases for long periods of time in target tissues are challenging issues [137] which should be addressed. Ideally, editing of genomes should be performed in accurate and rapid manner for limiting the potential toxicities and off target effects because of the expression of effectors in a sustained fashion [138]. In order to minimize the limitations of the genome editing, enhancement of the specificity and safety of the engineered nucleases and improved detection capability of off target effects, obtaining a deeper knowledge about the genome repair machinery, high throughput sequencing, identifying the secondary targets and capturing the broken ends appear necessary [139, 140]. Altogether, application of an arrangement of tools but not one-fits-all ones appears more useful for genome delivery or editing.

4. Nanoplatforms for viral detection and genome editing

Imminent breakthroughs in nanotechnology have revolutionized theranostic strategies. Functional system engineering at molecular scales, designing nanovectors including the nanoparticles (NPs) capable of loading a variety of imaging or therapeutic agents for early detecting the transforming cells or diseases, targeted therapy, and treatment outcome monitoring [141, 142] might have huge impacts on biomedicine. Nanosensors have shown great potentials for detection of biosignatures of various disorders or too low concentrations of viruses and bacteria. Application of the colorimetric nanobiosensors for point-of-care viral detection is an appropriate strategy against the pandemic outbreaks [143], (Fig. 5). This might also be a promising approach against the COVID-19 pandemic.

Hybrid nano-biosystems containing virus-derived biomolecules conjugated to the NP surface which can serve as specific probes for virus detection are more advantageous over the conventional methods in terms of specificity, stability, sensitivity, and performance [144]. Nanocarriers due to their suitable physicochemical characteristics such as small size of particles, large surface area, and tunable charge of the particle surface which facilitate particle penetration across the cell membrane, improved solubility, stability, and bioavailability of the entrapped drugs, targeted delivery, and reducing drug resistance can be suitable reservoirs for antiviral agents [145]. A variety of liposomal formulations, polymeric NPs, nanosuspensions, dendrimers, solid lipid NPs, and niosomes have been presented against the viral infections [146]. Furthermore, graphene oxide and silver nanocomposites has shown promising antiviral activities [147]. Nanocarriers due to their suitable physicochemical characteristics such as small size of particles, large surface area, and tunable charge of the particle surface which facilitate particle penetration across the cell membrane, improved solubility, stability, and bioavailability of the entrapped drugs, targeted delivery, and reducing drug resistance can be suitable reservoirs for antiviral agents [145].

![Colorimetric Virus Detection](image)

Fig. 5. Schematic illustration of the nanotechnology-based technologies for colorimetric viral detection. Adapted from Ref. [154].
For virus detecting, Au-NPs are capable of performing various functions such as amplification of color, fluorescence enhancing or quenching, and light scattering. Bioconjugates of Au-NPs can be applied in the electrochemical, colorimetric, fluorometric, and scannometric systems for detecting human viral groups [154], (Fig. 6).

Highly sensitive and simple Au-NPs-based assays have represented Au-NPs as promising nanoplatforms for detecting of various virus types [155]. Meanwhile, special attention should be paid to the application of advanced and rigorous methods for synthesis of the stable and uniform Au-NPs with several surface modification possibilities, appropriate conjugation of the specific targeting biomolecule to the NP surface, application of the negative and positive controls and reference tests, and in-field assessment of the novel Au-NPs-based techniques for confirming their specificity and sensitivity. In recent years, a highly selective colorimetric analysis has been performed for identifying the viral lysine using the molecular-driven Au nanorods [156].

Carbon nanotubes (CNTs) with enormous potentials in a variety of scientific domains such as nanomedicine, can be applied as nanoreservoirs for targeted delivery and controlled release of a variety of therapeutics. CNTs with superior biocompatibility, solubility, mechanical characteristics, and thermo-electrical conductivity have attracted a growing interest in theranostic fields such as high-resolution imaging and bio-sensing [157–162]. Besides the viral detection, CNTs can be applied as nanocarriers of antiviral agents and improve their efficiency. For instance, chemical linkage of ribavirin or isoprinosine on the surface of single-walled CNTs for carrying the drug across the cell membranes has led to the improved drug efficiency [163,164]. Besides Au-NPs and mesoporous silica NPs, CNTs can be used as CRISPR-Cas9 carriers for delivery and editing of genomes. CNTs-DNA capable of coding Cas9-protein and guide RNA is a suitable nanosystem for editing specific genomes [165]. Quantum dots capable of emitting the photons with specific wavelengths, can provide highly-sensitive and robust fluorescence perspective for point-of-care viral detection [166,167]. These semiconductor particles may be modified and along with various nanostructures can be applied for virus detecting [166]. As aforementioned, safe and efficient delivery of the elements of gene editing towards the specific region and providing targeted correction of genes devoid of off-target adverse effects may be quite challenging. In this context, various strategies have been proposed for improving the delivery efficiency and facilitating clinical translation of the technology of genome editing [168]. Engineering the non-viral NPs including the liposomes and polymeric, inorganic, or lipid NPs enables overcoming many of the limitations associated with the physical or viral approaches for delivery of CRISPR-Cas9 and attenuating off-target effects [168], (Fig. 7).

Lipid NPs include a wide range of materials capable of delivery of a variety of therapeutic agents including the nucleic acid-based ones. Systematic screening of various lipid NPs formulations is a promising approach for identifying the potent ones for tissue delivery of RNA-based therapeutics [169]. Deep-sequencing of the nucleic acid-based barcoded NPs in tissues after i.v. injection has facilitated identification of a lipid-NP formulation which is able to deliver functional sgRNA and siRNA to the endothelial cells [170]. Unchangeable shape and kinetic stability have made lipid NPs advantageous over the colloidal systems based on the lipids such as niosomes or liposomes. Lipid NPs improve the capacity of loading and stability of the entrapped active molecules and prevent their expulsion during the storage period [171–173]. They been assessed for delivery of the messenger RNA (mRNA) delivery for...

---

**Fig. 6.** Schematic illustration of the properties and functions of gold nanoparticles in virus detection. These nanoparticles with high sensitivity and specificity are capable of transducing various signal types including the electrical, optical, and catalytic signals. They could also be applied as the scaffolds for bio-immobilization. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) Adapted from Ref. [153].
preventing or treating a variety of diseases [107], (Fig. 8).

Based on the mRNA activity in the cytosol, it can prevent the risk of mutagenesis in the genome [174]. Despite the potentials of mRNA for preventing or treating a wide range of disorders and development of advanced in vitro transcription technology for controlling the immunogenicity and stability of mRNA, extensive applications of mRNA-based drugs necessitates designing the effective and safe vehicles for encapsulating and delivering mRNA payloads [175]. Lipid NPs are one of the most appropriate nanoscale platforms for delivery of a wide variety of mRNAs in vitro or in vivo [176]. These NPs have provided an effective transfection for the entrapped mRNA in mice tissues [177]. Lipid NP systems by encapsulating mRNA can be used for targeting the liver and using it as bioreactor for producing the therapeutic proteins including the hormones and monoclonal antibodies after intravenous injection [178, 179]. Following the intramuscular or intradermal injection of a single low dose, mRNA-lipid NP systems have provided highly-efficient vaccination (Fig. 9) against the Zika and influenza viruses [179].

mRNA-lipid NPs which code for the programmable nucleases enable highly-efficient and persistent genome editing of several gene targets in vivo [180]. Noteworthy, storing lipid-NPs for long periods of time is a challenging issue for successful clinical translation. In this respect, various conditions have been investigated for long-term storage of mRNA-encapsulated lipid-NPs and stability of NPs was evaluated using various concentrations of the cryoprotectants; mannitol, trehalose, or sucrose. It has been found that addition of trehalose or sucrose (5%, w/v) to the lipid NPs provides a suitable efficiency of mRNA delivery for about 3 months [181]. Microfluidic mixing process is a common technique for mRNA encapsulation in lipid-NP systems [182]. Microfluidic mixing method can be applied for development of appropriate lipid-NP systems capable of encapsulating the negatively-charged macromolecules such as gold NPs, mRNA, and plasmids. Lipid-NPs composed of the cationic lipid, PEG, cholesterol, distearoylphosphatidylcholine (DSPC), and siRNA could be appropriately constructed using the techniques of microfluidic mixing.

Molecular simulations have revealed the nano-structured core of the lipid-NPs with aqueous compartments holding siRNA. Besides the imaging applications, lipid-NP-siRNA systems could be used as promising therapeutics [182]. In recent years, microfluidic techniques have been applied for improving the reproducibility and quality of lipid NPs leading to more efficient tissue delivery of siRNA [183, 184]. Formation of lipid-NPs based on the fusion-dependent procedure provides a deeper knowledge about the formation mechanism and structures of the lipid-NPs-nucleic acid complex that might facilitate development of more potent lipid-NP-nucleic acid polymers for biomedical applications including the gene therapy [185].

As aforementioned, traditional therapeutics and vaccines against the viral disorders may be associated with a variety of side effects including the toxicity, resistance, and high costs. Over the last decade, increasing
interest has been attracted towards the mechanism and application of RNAi as a tool for manipulation or controlling the expression of genes and inhibiting viral replication [186,187]. RNAi is recognized as one the most efficient and specific methods for silencing the genes including the target transcripts of mRNA [188]. RNAi induced by the small-interfering RNA (siRNA) is capable of inhibiting the viral antigen expression and providing novel antiviral treatment approaches [189]. Because of the capability of gene silencing, activity in the cellular processes, and low cost, siRNA has been considered as an attractive medicinal modality. Simultaneous treatment with various shRNAs or siRNAs has been suggested against the viral infections and mutations [190].

Noteworthy, natural RNA may be associated with various challenging issues such as non-efficient delivery to the target tissues, short half-life because of the susceptibility to the nucleases, rapid clearance, immune response stimulation, and off target effects. Furthermore, specific targets are required for combating against the infections induced by viruses and avoiding the mutant escapes [191]. In general, clinical translation necessitates enhanced stability and reduced toxicity of RNAs, targeted delivery, and preventing mutant escapes. Modification of RNAs may result in the reduced immunogenicity and viral escape and enhanced stability, however, the modified RNAs may still demonstrate poor uptake into the cells because of their negative charge, hydrophilicity, and rapid clearance [192]. Failure of the synthetic siRNAs in crossing the biomembranes via the passive diffusion that may be due to their polyanionic nature and high molecular weights, has provoked the need to develop the technologies for transmembrane delivery of drugs [193]. In this respect, various delivery systems including the lipid NPs as one of the most advanced nanoplastforms for delivery of therapeutic agents including the biomolecules, have been designed for providing efficient uptake into the cells and accumulation at the target site or synergistic effects between the traditional drugs and RNAs for preventing the resistance [194]. Besides the ease of manufacturing and safety, lipid NPs can be functionalized for achieving proper tropism that might be of great significance when RNA interference is not organ-specific [195]. In recent years, the technology of lipid NPs has facilitated clinical translation of nanodrugs such as those for efficient delivery of siRNA against a variety of disorders such as amyloid polyneuropathy that prevented production of pathological proteins. Development of Onpattro, a lipid NP-based siRNA drug, has paved the way for clinical translation of the lipid NP nanomedicines which contain nucleic acids for expressing or silencing the target genes [196,197]. Increasing research efforts have been attracted towards the maximizing siRNA-lipid
NPs potency for gene silencing [198]. Cationic lipid NPs are promising nanosystems for delivery of siRNA against the RNA virus, HCV. Intravenous treatment has reduced core protein of the virus in transgenic mice [199]. Increasing clinical trials have also been carried out for evaluating siRNA-loaded NPs including the lipid-based ones against the infections induced by a variety of viruses such as HBV, HIV, and HCV [200]. siRNAs entrapped into the lipid NPs have successfully targeted Ebola virus outbreak strain and provided remarkable protection and fully recovery for the rhesus monkeys when the therapy was begun three days after the exposure [201]. In this sense, lipid NPs-delivered siRNAs have been suggested for counteracting this type of lethal viral disease in humans.

RNA vaccines can also be packaged within the lipid NPs as vectors [202]. This type of vaccines are currently under development for fighting against the coronavirus pandemic. mRNA vaccines could be suitable substitutes to the traditional vaccine technology that may be due to their high potency and short cycles of production [203]. mRNA vaccine entrapped in the lipid NPs can generate vigorous immune responses [204]. It is possible to improve the tolerability of mRNA vaccine without influencing its potency [205].

Combination of the lipid NP-based delivery system and sgRNA has provided significantly increased activity in vivo in both rat and mouse models. It has been shown that lipid NP-based system is capable of delivery the components of CRISPR-Cas9 for obtaining the clinically-relevant genome editing levels in vivo and significant reduction of transthyretin. Lipid NP-based delivery by achieving >97% knockdown of the target protein after single dose and providing cumulative level of editing after multiple doses has represented lipid NP system as an effective platform for editing the genomes. The biodegradable and transient lipid NPs-based system containing poly(amidoamine), chitosan, or cell-penetrating peptides for CRISPR-Cas9 delivery has been well-tolerated and the levels of editing was preserved for about one year [206,207] indicating the ability of lipid NPs for providing knocking down and genome editing with high durability. Noteworthy, durability is an important factor which determines the efficiency of any gene editing process [208]. In general, engineering of lipid NPs for regulation of genes in specific cells or tissues is of great importance as the technology could provide the possibility of gene therapy in an organ-specific fashion [205]. Using the selective organ targeting (SORT) approach which is compatible with various techniques of genome editing and may be helpful for designing protein-replacement or gene correcting therapeutic agents in targeting organs, lipid NPs carrying the therapeutic agents based on the nucleic acids have been bioengineered for inducing lung-, liver-, or spleen-specific regulation of genes [209]. Cell- or organ-targeted SORT lipid NPs could be engineered for selective editing of the hepatocytes, endothelial cells, epithelial cells, T cells, or B cells [209]. Administration of tissue-specific mRNA-encapsulated lipid NPs has provided sustained production of the therapeutic proteins with minimal safety concerns [205]. In a study conducted by Cheng et al., i.v. treatment with SORT lipid-NPs led to the increased delivery of mRNA.
and editing of genes in an organ-specific manner [209]. Besides the lipid NPs, SORT method can be applicable to other forms of NP systems and enables rational designing of carriers for various loads such as proteins or a wide variety of drugs, high-degree editing in the specific organs or cells, and more efficient treatment of various diseases [209]. Synthetic NP vectors consisting of the nucleic acids poly(bamino esters) have been shown promising for delivery of nucleic acids against a variety of diseases [210]. For expanding this advantage for systemic mRNA delivery, hybrid lipid-polymer NPs have been developed to deliver mRNA into the lungs [211]. Co-formation of poly(b-amino esters) with polyethylene glycol-lipid has led to the development of mRNA formulations with enhanced stability and potency and capable of mRNA delivery to the lungs in mice following intravenous injection [211] indicating the effectiveness of degradable lipid-polymer NPs for systemic mRNA administration.

In general, the molar ratios and components of lipid NPs which usually consist of the cholesterol, phospholipids, polyethylene glycol lipids, and cationic lipids, have been optimized for ensuring efficient delivery of the nucleic acids to the tissue and providing potent silencing of genes at clinically-relevant doses [212]. Increasing the molar components of lipid NPs with additional molecules for tuning NP internal charge can facilitate the delivery of therapeutics in an organ-specific fashion and affect the cellular fate of NPs [213]. Addition of the cationic lipid has been shown necessary for increased efficiency of SORT-lipid NPs [209]. Using SORT-lipid NPs has also enabled CRISPR-Cas system-based genome editing in a tissue-specific fashion [209]. Generating SORT-lipid NPs holding Cas9 mRNA and guide RNA facilitated editing of genes in the extra-hepatic tissues. Using various formulations of SORT-lipid NPs in mice has enabled selective induction of target genome editing in a tissue-specific manner [209]. However, the precise mechanisms by which the lipid NPs can be directed towards the specific organs by SORT moderation have remained to be addressed. Identifying the underlying mechanisms would enable fine tuning of tissue- or organ-specificity of lipid NPs. Furthermore, the probable immunogenicity should be rigorously evaluated prior to the implementation of SORT technique for rational designing of lipid NPs for therapeutic modification of genes in various organs [207,209].

Using graphene oxide- or arginine-modified nanostructures or those consisting of Cas9-peptide for CRISPR-Cas9 delivery has provided suitable editing efficiency. NPs containing graphene oxide-PEG-polyethyleneimine are capable of protecting the cargo, sgRNA-Cas9, against the enzymatic hydrolysis [213,214]. Development of a customizable nanocapsule for delivery of the single-guide RNA (sgRNA) and Cas9 has enabled controlled stoichiometry of the components of CRISPR and efficient generation of targeted genome edits, and limited the potential safety concerns. This nanoplatform has efficiently delivered CRISPR-ribonucleoprotein complex for somatic genome editing in vitro and in vivo [214,215].

As aforementioned, coronaviruses including SARS-CoV and MERS-CoV are pathogens capable of causing widespread pneumonia outbreaks with high mortality and morbidity rates. Highly contagious nature and clinical importance of these viruses necessitate application of advanced detection techniques. Using Au-NPs for detecting coronaviruses is based on the development of specific and rapid detection of molecules via colorimetric and electrochemical assays [153]. These assays particularly the colorimetric one put an end to the requirement of skilled personnel or complex instrumentation and provides negative or positive results only in few minutes which can be easily identified (within 5 min) by unaided eye. Using the technique enables detecting target SARS virus nucleic acids with the sensitivity limit of about 100 fM [153] indicating the appropriateness of technique for early virus diagnosis that might be of great significance for these highly contagious viruses.

siRNA ability for prophylaxis or therapy of the coronavirus-related infections has been previously evaluated [216]. siRNA pre-existing in the host cells is capable of inhibiting the replication of SARS-CoV and further infections because of disrupting virus RNA and inactivating the replication machinery of the virus [217]. siRNA duplexes have been assessed for anti-SARS-CoV effects in the primate cells and active duplexes and showed prolonged inhibitory effects on the replication of SARS-CoV and further infection. Combination of the active sequences provided increased antiviral potency and reduced viral escape because of the reduction in siRNA target. In this sense, integrating siRNA duplexes has been shown as a promising approach for development of the antiviral therapeutics [217]. Application of siRNA duplexes for treating patients infected by CoV necessitates efficient delivery of this type of siRNAs to the lungs. In the Rhesus macaque infected by SARS coronavirus, siRNA has reduced viral load and replication and protected lungs against the diffuse alveolar damage. It has been represented as a safe biological agent with enormous potentials in targeted therapy or prophylactic antiviral regimens [218]. Further clinical evaluations are required for supporting such findings. M protein of SARS-CoV is critically involved in the viral integration and infections [219]. Wang et al. have created two siRNAs which targeted well-conserved and unexploited regions in M (membrane) gene. Both siRNAs specifically and effectively inhibited the expression of SARS-CoV membrane gene at new targeting sites [220].

Liver is one of the most vulnerable organs to the virus attacks. Patients affected by the liver diseases may be more vulnerable to the negative outcomes of COVID-19 [221]. Based on the reports, dendrimer-based lipid NPs have effectively delivered miRNAs/siRNAs for normalizing the functions of liver [222], mRNA-encapsulated lipid NPs can be applied for production of the therapeutic proteins and gene-editing complexes for correcting disease-induced mutations in the hepatocytes [223]. Single treatment with a lipid NP-based delivery system has led to an efficient editing of transthyretin gene in mouse liver and a significant and durable reduction of protein contents in the serum [223]. Nanoblades which can be used for homology-directed repairs, are capable of ribonucleoprotein cargo delivery in a rapid and transient fashion, mediating knock-in in various cell lines and genome-editing in a dose-dependent manner without significant influence on target cell proteome, programming with Cas9 proteins for mediating transient targeted gene activation or up-regulation [129]. Viral-derived Cas9-sgRNA ribonucleoproteins-loaded nanoblades have been capable of editing the genomes in mouse liver [129].

5. The roles of AI techniques, modeling approaches, and nanorobots

Application of AI techniques for accelerating the process of nanofabrication and solving the associated problems, developing smarter and hybrid technologies, producing the nanoarchitectures with enhanced power of computation, and evaluating the impact of nanostructures on the biosystems [224] enable overcoming the potential limitations of the nanotech-based approaches. Computational modeling is an essential step for creating functional systems for delivery of nanotherapeutics and their clinical translation. Acquiring an improved understanding about the underlying mechanisms of the bio-events and bio-nano interactions and predicting the effects of formulation factors on distribution or delivery of the entrapped agent and dose-response are of critical importance for designing the nanotherapeutics with increased efficiency of targeting and reduced safety concerns. Patient-specific models capable of providing special opportunities can be applied for personalized theranostics [225–227]. In recent years, advancements in the structural and molecular biology and computational genomics have provided a deeper knowledge about the structures of protein targets in both virus and host that might result in the development of more efficient antiviral agents [228]. Computer simulations via application of the structural information and mutational analyses facilitate rational design of new antiviral agents including the nucleotide inhibitors [229]. Advance-ments in translational bioinformatics, structural biology, pharmacogenomics, in silico techniques, and virtual designing of ligands enable overcoming the newly-emerged and highly-mutating viruses and
development of vaccines and a variety of highly-specific antiviral drugs including those capable of inhibiting the proteins of virus [230,231]. Computationally-designed gRNAs targeting Vaccinia virus genes have facilitated evaluating the functions of genes [118]. Inverse computational fluid dynamics (CFD) modeling enables identification of the contaminants and their spread [232]. CFD models can be applied for investigating the coronavirus spread [233]. Using computational technique, a project has been recently designed (Fig. 10) to construct COVID-19 disease map for providing a comprehensive knowledge about the mechanisms of interactions between SARS-CoV-2 virus and host and development of models with high-quality capable of linking to the data repositories [234].

COVID-19 map can serve as the platform for visual assessment and computational analysis of the molecular procedures implicated in the entry and replication of virus and its interactions with host, recovery of the host cells, the mechanisms of repair, and immune responses [234]. Obtaining the improved knowledge of disease which is associated with complex pathomechanisms and multi-organ and multi-cellular nature of the infection, and facilitating the design process of efficient theranostics are other beneficial aspects of the presented map [234]. Various pathways are included in the map such as the replication cycle of virus and mechanisms of transcription, the effect of SARS-CoV-2 on the pulmonary blood pressure, heme catabolism, apoptosis, interferon 2 signaling, and SARS-CoV-2 proteins. COVID-19 diagram collections and metabolic model of the alveolar macrophages in a patient affected by the virus are also incorporated in the map [234,235]. Combination of the illustrative representations of the mechanisms of COVID-19 with underlying models in the map provides a suitable plan for the virologists, immunologists, and clinicians for collaboration with the computational biologists and data analyzers in order to build rigorous models and accurate interpretation of data [236,237]. It would also be possible to better understand host susceptibility characteristics including the age and gender, progression of disease, mechanisms of defense, and treatment response. Application of the map with other disease maps enables assessment of the comorbidities [234].

As aforementioned, the ability of precise genome modification and regulating particular genes might be of great significance in biomedicine. In order to better characterize the variables which control off-target or on-target activity of the Type II CRISPR-Cas9 system, a biophysical model of genome regulation and editing based on Cas9 has been developed for predicting the mechanism of controlling Cas9-based binding and cleavage dynamics [238]. Combining the kinetics and thermodynamics for modeling the formation, site selection, and diffusion of Cas9-crRNA and determining kinetic factors has led to the identification of DNA supercoiling as new mechanism which control binding of Cas9. Furthermore, the model enabled prediction of the binding frequency of Cas9-off-target across the human genomes (Fig. 11) and revealed the reason for high off-target activity [238] that might facilitate attenuation of this type of activity.

This quantitative, dynamical, and mechanistic CRISPR/Cas9 model accounts for the formation and expression kinetics of the active complex of Cas9:crRNA. It has been revealed that concentration of Cas9:crRNA controls off-target activity and low level of the expression of crRNA is suitable for formation of sufficient active dCas9:crRNA [238].

Over the last decade, remarkable advancements in nanotechnology has facilitated designing the intelligent nanomechanical platforms which are powered by the bio-molecular motors or various sources of chemical energy [239]. These significantly durable nanomachines enable monitoring the internal chemistry and activities of organs, and accessing the diseased regions [240]. Development of the biologically-inspired nanorobots with powerful engines capable of processing of information, sensing, actuation, signaling, entering the cells, cancer cell destroying, cutting out the defective genes, and combating a variety of diseases is an eminent breakthrough in biomedicine. Nano swimmers which are able to maneuver through the physiological fluids for targeted-therapy, bacteria nanobots, 3-D DNA nanomachines, and remote-controlled nanorockets capable of targeted delivery of therapeutics [241,242] can revolutionize the current theranostic

![Curation Review](image1)

![Knowledge and data integration](image2)

![Scientific results](image3)

![Exploration Modelling](image4)

Fig. 10. An overview of the map project of COVID-19. The replication cycle and interactions of SARS-CoV-2, immune system reaction, and the mechanisms of repair have been highlighted in the map.
Adapted from Ref. [234].
interventions. Besides predicting and preventing the hazards of chemicals, nanorobots can cross over the body for cell repair, assisting a malfunctioning organ, repairing tissues, evaluating brain activity, replacing heart pacemakers, targeted gene or drug delivery, improvement of the efficiency of currently available therapeutics, or monitoring the patient. Nanobots along with the wireless transmitters provide the possibility of modifying the treatment protocol [240, 243]. Besides detecting or destroying toxic agents, stimuli-responsive nanorobots can be used against a variety of disorders including the viral infections [244]. MRI-guided nanomachines provide the possibilities of precise magnetic particle localizations or simultaneous tracking and actuating NPs [245]. These nanoplatforms can be programmed for performing the biological functions at cellular level such as attacking cancer cells or viruses after systemic administration [240]. Folate substances may be attached onto the nanorobot body which are powered by the flagella motors [246]. This approach could be taken for drug delivery against the human viral infections [247]. A Biomolecule or drug is released from a nanorobot at distinct points via manipulation of the physiological parameters. Targeted release may be triggered by pH or temperature alterations [248]. Considering the unpredicted alterations of the physiological parameters, developing the externally-triggered nanomachines provides more appropriate controlled drug delivery [249].

Nucleic acid-based nanorobots capable of biosensing such as sensing the flow rate, delivery of the cellular-compatible message and biological activators, evaluating the inter- or intra-molecular forces, manipulating the molecules or NPs for fabricating more advanced nanoplatforms, controlling the chemical reactions, and triggering apoptosis [250, 251] might be the major components of the modern theranostic settings. These smart machines with new generation of nanomotors can move through the fluid environments and execute specific missions [252]. Advanced simulation methods enable acquiring a deeper knowledge about the mechanisms of nanorobot interactions within living organism [253]. Regarding the virus pandemics, application of the programmed nanorobots enables detecting various levels of specific proteins in the bloodstream [248] that might facilitate characterizing of a specific virus. Cell invasion by the influenza virus and secretion of $\alpha$-N-acetyl-galactosaminidase (NAGA) protein is followed by the immunosuppression and virus spread throughout the body [254]. $\alpha$-NAGA overexpression in bloodstream triggers the prognostic behavior of nanorobot and electromagnetic signals could be transmitted to a mobile phone and satellites followed by identification of the contaminated person position. Programmable nanorobot can sense and detect $\alpha$-NAGA concentration in bloodstream [254].

Radio frequency identification device and complementary metal oxide semiconductor transponder system are applied in the architecture of nanorobot for positioning in vivo [256]. Furthermore, embedment of nanobiosensors in the structure of nanorobot facilitates $\alpha$-NAGA monitoring. Detecting the overexpression of protein indicates time of viral contamination [255]. Making only a single mistake in the genome editing may result in the catastrophic outcomes. Sometimes, CRISPR-Cas9 may target wrong genes [257]. For obtaining a better knowledge about the error types which may be made by CRISPR, a genome-sequencing chip has been developed for evaluating the interactions between the genomes and CRISPR, performing machine learning approaches to find true binding sites, simulating binding affinities, designing short RNA for being matched with particular DNA sequence in

![Fig. 11. Prediction of the genome editing in human using a biophysical model. A: Distributions calculated by the model demonstrate the numbers of DNA sites in the human genome which are cleavable with different efficiencies after application of LTR-B crRNA with baseline (in yellow), 10-fold lower or higher (in blue and green, respectively) concentrations of crRNA and Cas9, B: Number of off-target mutations, C: Required sequencing coverage for identifying the number of off-target mutations with high certainty (99%), D: Modification dynamics of human genome calculated by the model. On-target cleavage and on- and off-target cleavage ratio have been demonstrated by the solid and dashed lines, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) Adapted from Ref. [238].]
order to guide enzymes including Cas9 to the desired DNA target [257]. Using nanobots including the micropumps coated with nickel, the potential challenges associated with CRISPR delivery may be overcome [258] indicating the significance of these nanoplatforms in genome editing.

6. Concluding remarks

Effective management or treatment of COVID-19 necessitates performing well-designed basic and clinical investigations. Focusing on the intracellular trafficking machinery and inter-patient variables might provide smarter insights into the disease pathomechanism and treatment response. Nanotechnology with capabilities of covering a wide range of highly-advanced devices and biomaterials for early diseases diagnosis and targeted delivery of theranostics including the proteins or genes has provided outstanding breakthroughs in biomedicine. Development of the scalable NPs with improved tissue penetration and proof-of-concept nanorobots capable of early detection of pathogens and destroying them, editing the genomes, and smart delivery of therapeutics could be of great significance against the disease. Modeling approaches with conclusive influences on nanomaterials play an important role in designing nanomaterials with optimal properties and activities. High-performance multi-scale modeling and simulation methods with ever-increasing predictability and power provide a deeper knowledge about the bio-interactions and disease pathomechanism that might result in the development of more efficient therapeutics with improved outcomes.

Even after development of the preventive or therapeutic agents including the membrane-modifiers and inhibitors of the virus entry or membrane anchoring, they should undergo long-term safety, efficiency, and immunogenicity assessments. In this respect, exaggerated optimistic or propaganda regarding the currently available or newly-developed therapeutics may be associated with catastrophic outcomes.

Role of the funding source

This work did not receive any specific grant from the funding agencies in public, commercial, or not-for-profit sectors.

Declaration of competing interest

Author declares no conflicts of interest.

Acknowledgements

Not applicable.

References

[1] J.D. Almeida, D.M. Berry, C.H. Canningham, D. Hamre, M.S. Hofstad, L. Mallucc, K. McIntob, D.A. Tyrell, Virology: coronaviruses, Nature 220 (5168) (1968) 650.
[2] P.C. Woo, Y. Huang, S.K. Lau, K.Y. Yuen, Coronavirus genomics and bioinformatics analysis, Virus 2 (8) (2010) 1804–1820.
[3] T.-Y. Lam, et al., Identification of 2019-nCoV related coronaviruses in Malayan pangolins in southern China, bioRxiv (2020), https://doi.org/10.1101/2020.03.13.945485.
[4] N. Wang, et al., Structural definition of a neutralization-sensitive epitope on the MERS-CoV S1-NTD, Cell Rep. 28 (2019) 3395–3405.
[5] C. Sun, et al., SARS-CoV-2 and SARS-CoV spike-RBD structure and receptor binding comparison and potential implications on neutralizing antibody and vaccine development, bioRxiv (2020), https://doi.org/10.1101/2020.02.16.951723.
[6] F. Li, et al., Structure of SARS coronavirus spike receptor-binding domain complexed with receptor, Science 309 (2005) 1864–1868.
[7] G. Simmons, P. Zmora, S. Gierer, A. Heurich, S. Pohlmam, Protodeactive of the SARS-coronavirus spike protein: cutting enzymes at the cutting edge of antiviral research, Antivir. Res. 100 (3) (2013) 605-614.
[8] S. Zhang, et al., Structural definition of a unique neutralization epitope on the receptor-binding domain of MERS-CoV spike glycoprotein, Cell Rep. 24 (2018) 441-452.
epidemiology, vaccines, and therapeutic interventions. Viruses 12 (2020) 1–18, https://doi.org/10.3390/v12010005.

[39] Jingyong Zhang, Hao Zeng, Jiang Gu, Haibo Li, Lixin Zheng, Quanming Zou, Progress and prospects on vaccine development against SARS-CoV-2, Vaccines 8 (2020) 1–12, https://doi.org/10.3390/vaccines80100153.

[40] Z. Jahangirooroz, A. Baradaran, J. Mousafir, H. Hashemi, T. Rezaei, et al., Comparison of DNA and RNA vaccines against cancer, Drug Discov. Today 25 (2020) 552–560, https://doi.org/10.1016/j.drudis.2020.03.006.

[41] Cailin Zhang, Giulietta Maruggi, Hu Shan, Junwei Li, Advances in mRNA vaccines for infectious diseases, Front. Immunol. 10 (2019) 594, https://doi.org/10.3389/fimmu.2019.00594.

[42] J.C. Saiz, N.J. Oya, A.B. Bl, I. Mejdrov, D.F. Smee, E.B. Tarbet, Y. Furuta, J.D. Morrey, D.L. Barnard, Synergistic J. Grein, N. Ohmagari, D. Shin, et al., Compassionate use of remdesivir for patients with COVID-19? Eur. Respir. Rev. 29 (2020) 102714, https://doi.org/10.1183/1971-6702.0102714.

[43] P. Zhang, L. Zhu, J. Cai, et al., Association of inpatient use of antiviral converting enzyme inhibitors and antiviral II receptor blockers with mortality among patients with hypertension hospitalized with COVID-19, Circ. Res. 126 (2020) 1120–1122, https://doi.org/10.1161/CIRCRESAHA.120.319291.

[44] P. Zhou, X.-L. Yang, X.-G. Wang, et al., A pneumonia outbreak associated with a new coronavirus of probable bat origin, Nature 579 (7798) (2020) 270–273, https://doi.org/10.1038/d41586-020-00976-2.

[45] G.I. Rice, D.A. Thomas, P.J. Grant, A.J. Turner, N.M. Hooper, Evaluation of angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers: what is the evidence? JAMA 324 (2020) 1094–1104, https://doi.org/10.1001/jama.2020.9993.

[46] Jinyong Zhang, Hao Zeng, et al., Virus-like particles as potential therapeutic tools against SARS-CoV-2, Expert Rev. Anti-Infect. Ther. 18 (2020) 1–10, https://doi.org/10.1080/14787210.2020.1729850.

[47] D. Yang, Overlapping and discrete aspects of the pathology and pathogenesis of MERS-CoV, J. Viral. 274 (2021) 119289, https://doi.org/10.1016/j.jvi.2021.119289.

[48] Parichehr Hassanzadeh, Fatemeh Atyabi, Rassoul Dinarvand, The significance of broad-spectrum antiviral agents and tools for chemical biology, J. Med. Chem. 60 (2017) 1074–1118, https://doi.org/10.1021/acs.jmedchem.6b01560.

[49] E. Vlahdelidou, C.N. Sakellaris, I. Zolotas, S. Pappas, M. Vlahakis, Comparative safety of vaccine adjuvants: a summary of current epidemiology, vaccines, and therapeutic interventions, Viruses 12 (2020) 1, https://doi.org/10.3390/v12010001.

[50] N. Garcon, Adjuvant system AS01: helping to overcome the challenges of modern vaccines for infectious diseases, Front. Immunol. 10 (2019) 594, https://doi.org/10.3389/fimmu.2019.00594.

[51] N.J. Temperton, P.K. Chan, G. Simmons, M.C. Zambon, R.S. Tedder, Y. Takeuchi, et al., Overlapping and discrete aspects of the pathology and pathogenesis of MERS-CoV, J. Viral. 274 (2021) 119289, https://doi.org/10.1016/j.jvi.2021.119289.
Parichehr Hassanzadeh, Fatemeh Atayi, Elham Arabi, Rassoul Dinivar, Application of carbon nanotubes as the carriers of the cannabinoid, 2-arachidonoylethanolamide (2-AG): towards a novel treatment strategy in colitis, Life Sci. 193 (2018) 64–76.

Parichehr Hassanzadeh, Elham Arabi, Fatemeh Atayi, Rassoul Dinivar, Carbon nanotubes provide longer lasting gastroprotective effects than anandamide in an in vitro model of gastric injury. Life Sci. 193 (2018) 2235–2368.

Shu, J., Li, A.-G., Huang, J.-Q., Huang, Y.-Q., Huang, G.-X. Wang. Anti-betanodavirus activity of inosiprinosine and improved efficacy using carbon nanotubes based drug delivery system, Aquaculture 512 (2019), 734377.

B. Zhu, G.-L. Liu, F. Ling, G.-X. Wang. Carbon nanotube-based nanocarrier loaded with ribavirin against grass carp reovirus, Antivir. Res. 118 (2015) 29–38.

G.S. Demirel, et al. High aspect ratio nanomaterials enable delivery of functional genetic material without DNA integration in mature plants, Nat. Nanotechnol. 14 (2019) 456–464.

Y. Li, J. Jing, K. Ding, J. Gao, Z. Peng, Y. Li, L. Shen, M. Gao, Detection of Epstein-Barr virus infection in cancer by using highly specific nanoprobe based on dsDNA capped CdTe quantum dots, RSC Adv. (4) 2014 (2014) 22545–22550.

W. Zhao, W.-P. Zhang, Z.-L. Zhang, R.-L. He, Y. Lin, M. Xie, H.-Z. Wang, D.-W. Pang. Robust and highly sensitive point-of-care virus detection based on immunomagnetic separation, Anal. Chem. 84 (2012) 2358–2365.

Min Qiu, Zachary Glass, Quaiding Xu. Nonviral nanoparticles for CRISPR-gRNA-based genome editing: an in vivo study of potential dual delivery systems, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) E9494–E9592.

E.T. Wang, et al. Barcoded nanoparticles for high throughput in vivo discovery of targeted therapeutics, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 2060–2065.

Parichehr Hassanzadeh, Fatemeh Atayi, Rassoul Dinivar, Ahmad Reza Dehpour, Mortezaz Azhadzardeh, Mskitn Dinivar, Application of nanostructured lipid carriers: the prolonged protective effects for sesamol in in vitro and in vivo models of ischemic stroke via activation of PI3K signalling pathway, DARU Journal of Pharmaceutical Sciences 25 (2017), 25, https://doi.org/10.1186/s40401-017-0191-z.

Parichehr Hassanzadeh, Elham Arabi, Fatemeh Atayi, Rassoul Dinivar, Fusion-dependent formation of lipid nanoparticles containing CRISPR/Cas9-polyamine-DNA polyplexes, J. Biol. Chem. 278 (2003) 44826–44831.

P.Y. Lu, Y.F. Xie, M.C. Woodie, siRNA-mediated antitumorism for drug target validation and drug discovery, Curr. Opin. Mol. Ther. 5 (2003) 225–234.

M.S. Weinberg, P. Arbuthnot, Progress in the use of siRNA as a therapy for chronic hepatitis B virus infection, Vaccine Medicine 24 (4) (2010) 28–34.

K.A. Whitehead, R. Langer, D.G. Anderson, Knocking down barriers: advances in siRNA delivery, Nat. Rev. Drug Discov. 6 (2007) 129–138.

Y.C. Tam, S. Chen, P.R. Curlis, Advances in lipid nanoparticles for siRNA delivery, Pharmaceutics 5 (2013) 498–507.

R. Cortesi, M. Campioni, L. Ravani, M. Dechrebs, M. Pinotti, E. Esposito, Cationic lipid nanoparticles as carriers for nucleic acids, New Biotechnol. 31 (1) (2014) 44–54.

H. Wood, FDA approves pantoprazole to treat barrett esophagitis, Biomol. Ther. 16 (2007) 255–260.

J.A. Kulkarni, et al., Fusion-dependent formation of lipid nanoparticles containing CRISPR/Cas9-polyamine-DNA polyplexes, J. Biol. Chem. 278 (2003) 44826–44831.
P. Hassanzadeh

[235] A. Renz, L. Widerspick, A. Drager, FBA Reveals Guanylate Kinase as a Potential Target for Antiviral Therapies against SARS-CoV-2 (Version 3), Preprint at, 2020, https://doi.org/10.5281/ZENODO.3752641.

[236] M. Ostaszewski, et al., Community-driven roadmap for integrated disease maps, Brief. Bioinform. 20 (2019) 659–670.

[237] J.M. Villevecce, P. Koi, B.H. Habermann, Tools for visualization and analysis of molecular networks, pathways, and –omics data, Adv. Appl. Bioinforma. Chem. AABC 8 (2015) 11–22.

[238] Iman Farasat, Howard M. Salis, A biophysical model of CRISPR-Cas9 activity for rational design of genome editing and gene regulation, PLoS Comput. Biol. (1) (2016) 1–33, https://doi.org/10.1371/journal.pcbi.1004724.

[239] A. Cavalcanti, B. Shirinzadeh, R.A. Freitas Jr., L.C. Kretly, Medical nanorobot architecture based on nano bioelectronics, Recent Pat Nanotechnol 1 (2007) 1–10.

[240] B.S. Yamaan Saadeh, Dinesh Vyan, Nanorobotic applications in medicine: current proposals and designs, Am J Robot Surg 1 (1) (2014) 4–11.

[241] D. Murphy, B. Challacombe, T. Nedar, G. Elhage, K. Althoefer, L. Seneviratne, et al., Equipment and technology in robotics, Arch. Esp. Urol. 60 (2007) 349–354.

[242] Parichehr Hassanzadeh, Fatemeh Ataybi, Rassoul Dinarvand, Creation of nanoparticles via physical-chemical stimuli of polydopamine/gold composites, Sci. Rep. 6 (2016) 22650.

[243] S. Martinez, J.B. Mathieu, O. Felfoul, A. Chau, A. Elbouboussou, S. Tamaz, et al., Computer-assisted protocol for endothelial cell targeting using a clinical MRI system for controlling unheated microdevices and future nanorobots, Comput Aided Surg 13 (2008) 340–352.

[244] B. Melab, B. Campuzano, M.J. Lozano, A. Cassia, N. Lim, et al., Micromachine-enabled capture and isolation of cancer cells in complex media, Angew Chem Int Ed Engl 50 (2011) 4161–4164.

[245] R.R. Patel, Nanorobotics in nanomedicine, Asian J Pharm Sci Res 3 (2013) 15–22.

[246] S. Martinez, O. Felfoul, M. Mohammadi, J.B. Mathieu, Interventional procedure based on nanorobots propelled and steered by flagellated magnetic bacteria for direct targeting of tumors in the human body, Conf Proc IEEE Eng Med Biol Soc 2008 (2008) 2497–2500.

[247] M.B. Sonamna, Nanobotics: the future of medical treatments, Int J Sci Tech Res 4 (2015) 276–278.

[248] E. Torelli, M. Marini, S. Palombo, L. Piantanida, C. Polano, A. Scarpellini, et al., A DNA origami nanorobot controlled by nucleic acid hybridization, Small 10 (2014) 2978–2986.

[249] S.M. Douglas, L. Bachelet, G.M. Church, A logic-gated nanorobot for targeted transport of molecular payloads, Science 335 (2012) 831–834.

[250] B. Belkam, M. Sitti, Bacterial flagella-based propulsion and on/off motion control of microscale objects, Appl. Phys. Lett. 90 (2007) 1–3.

[251] A. Dube, G. Sharma, M. Mavroidis, M.S. Tomassone, K. Nikitezuk, M. Yarmuch, Computational studies of viral protein-nan actuators, J Comp Theor Nanosci 1 (2004) 18–28.

[252] J.J. Skehel, D.C. Wiley, Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin, Annu. Rev. Biochem. 69 (2000) 531–564.

[253] A. Dubey, G. Sharma, M.S. Tomassone, K. Nikitezuk, M. Yarmuch, Computational studies of viral protein-nan actuators, J Comp Theor Nanosci 1 (2004) 18–28.

[254] J.J. Skehel, D.C. Wiley, Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin, Annu. Rev. Biochem. 69 (2000) 531–564.

[255] A. Dubey, G. Sharma, M. Mavroidis, M.S. Tomassone, K. Nikitezuk, M. Yarmuch, Computational studies of viral protein-nan actuators, J Comp Theor Nanosci 1 (2004) 18–28.

[256] J.J. Skehel, D.C. Wiley, Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin, Annu. Rev. Biochem. 69 (2000) 531–564.