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Review article

The significance of bioengineered nanoplatforms against SARS-CoV-2: From detection to genome editing

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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 polyprotein 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 by the ether has revealed the lipid envelope of the virus [1]. From a historical point of view, discovery and isolation of the virus in humans was performed in 1960s, however, it could not be cultivated by the standard methods [9]. In 1965, development of novel cultivating techniques facilitated inoculation of the isolated virus into the volunteers [10] followed by identification of a variety of corona viruses in animals [11]. Other types of coronaviruses in humans have been identified from 2003 to 2019 [11,12]. Coronaviruses usually target the epithelial cells and transmit between the hosts *via* various routes [1]. They can induce infections of the respiratory tracts in humans including the bronchitis and pneumonia and other symptoms such as those in the gastrointestinal tract that could be mild to fatal [13]. 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.

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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 preventing chronic diseases such as cancer, neurological disorders, or infections of the respiratory tract induced by the coronavirus [22,23]. Because of low immunogenicity of peptides, peptide vaccines are usually applied along with the immune-stimulants such as adjuvants [24]. In recent years, computational techniques have been applied for designing multi-epitope peptide vaccines capable of triggering remarkable immune responses [25]. However, the results should be validated during the well-designed experiments *in vitro* and *in vivo*. Recently, peptide-presenting NPs have been suggested as promising candidates for overcoming some limitations associated with the peptide vaccines [26].

Hyper-variable viruses have remained as one of the major healthcare concerns. For developing new prophylactic plans based on the epitopes and specific targeting the conserved regions of the hyper-variable viruses, determining the immunogenic domains of viral proteins which are able to elicit protective immune responses, is of great importance [27]. Identifying the conserved domains of proteins shared in various viruses and capable of eliciting protective immune responses can provide novel perspectives in developing epitope-based vaccines. Discovery of the protective monoclonal antibodies (mAbs) capable of targeting the protein motifs which are widely shared, enables characterizing the peptides capable of mimicking the epitopes and evoke protective immune responses [28]. Evaluating post-immunization and post-infection sera and characterizing and cloning mAbs have been suggested as an appropriate approach for identifying the protective epitopes [29]. Protective mAbs against the protected regions could play an essential role in the immunogen design and therapy [30]. Noteworthy, performing experimental procedures for identifying protective epitopes of mAbs and T-cell activating peptides are usually burdened due to some limitations including the technical ones. This has led to the development of various epitope-related predictive evolvable algorithms which can be used for addressing the observational research [31]. Identifying the peptides capable of eliciting efficient T-cell responses against the hyper-variable viruses can result in the development of novel vaccine formulations which evoke B- and T-cell protective responses (Fig. 1). This might be of great value for identifying the hyper-variable genomic hotspots in the coronavirus.

Besides application for analyzing the empirical data and improving the efficiency of identification, *in silico* strategies can be used for predicting the immunogens for being included in new vaccine approaches based on epitopes [32]. Data obtained from the modular immune *in vitro* construct system and clinical studies have indicated that activation of all components of the immune system is required for inducing appropriate and long-lasting immune response [33]. In general, vaccination efficiency depends on various factors such as the vaccine strain, nature of disease, proper schedule of vaccination, appropriate modeling approaches for predicting the immunization process performance, continuous monitoring particularly after presenting a novel vaccine, maintenance of high rates of immunization, or idiosyncratic reactions [34]. Regarding the development of effective and safe vaccines, various challenging issues should be addressed such as several stages of the life cycles of pathogens, diversity of pathogenic and physiologic oligomers and isoforms, host-related or vaccine attenuation failures, lack of appropriate response due to the various factors including the genetic ones, complexity of the immunopathology, epitope nature, identifying the pathological epitopes which could evoke specific antibody response, inappropriate or various degrees of immune responses or difficulties in prediction of this type of responses, and high rates of mutations in RNA viruses that may protect them against the immune responses [26,35,36]. Identifying about 149 mutation sites across SARS-CoV-2 genome has created more problems in development of an effective vaccine [37]. Recently, increasing efforts have been attracted towards designing the vaccines against COVID-19 using a variety of methods such as altering the nucleotide sequence of the viral genes and modification of mRNA structures or adding a stabilization domain to the synthetic proteins of virus for improved protein recognition by the immune system of host [38,39]. Noteworthy, vaccine safety and efficiency should be assessed using various virus strains in the animal models. In general, designing process of mRNA vaccine include antigen selection, sequence optimization, modified nucleotide screening, evaluating the immune response, performing appropriate safety tests, and providing optimized delivery system [39,40]. Strategies for the development of mRNA vaccine against SARS-CoV-2 include; i) using mRNA for expressing receptor binding domain (RBD) and S protein, and ii) application of mRNA for expressing virus-like particles (VPLs) [39]. In humans, the safety of platforms based on mRNA for vaccine delivery has been previously reported [41], however, there is no well-established data regarding those against COVID-19. DNA vaccines which usually include antigen encoding plasmid DNA molecules, are currently under development against SARS-CoV-2 [42]. High pathogenicity of the virus may negatively affect the successful passing of the clinical research phases. Virus-like particles (VLPs) capable of mimicking the conformation and organization of native viruses can be applied for evaluating the mechanisms of viral infections, efficiency of vaccines, therapeutic agents, or delivery of drugs [43,44]. They can be applied for determining SARS-CoV vaccine-induced antibodies and enable assessment of vaccine efficiency in the absence of appropriate animal models [45].

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, MF59 through the creation of an 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

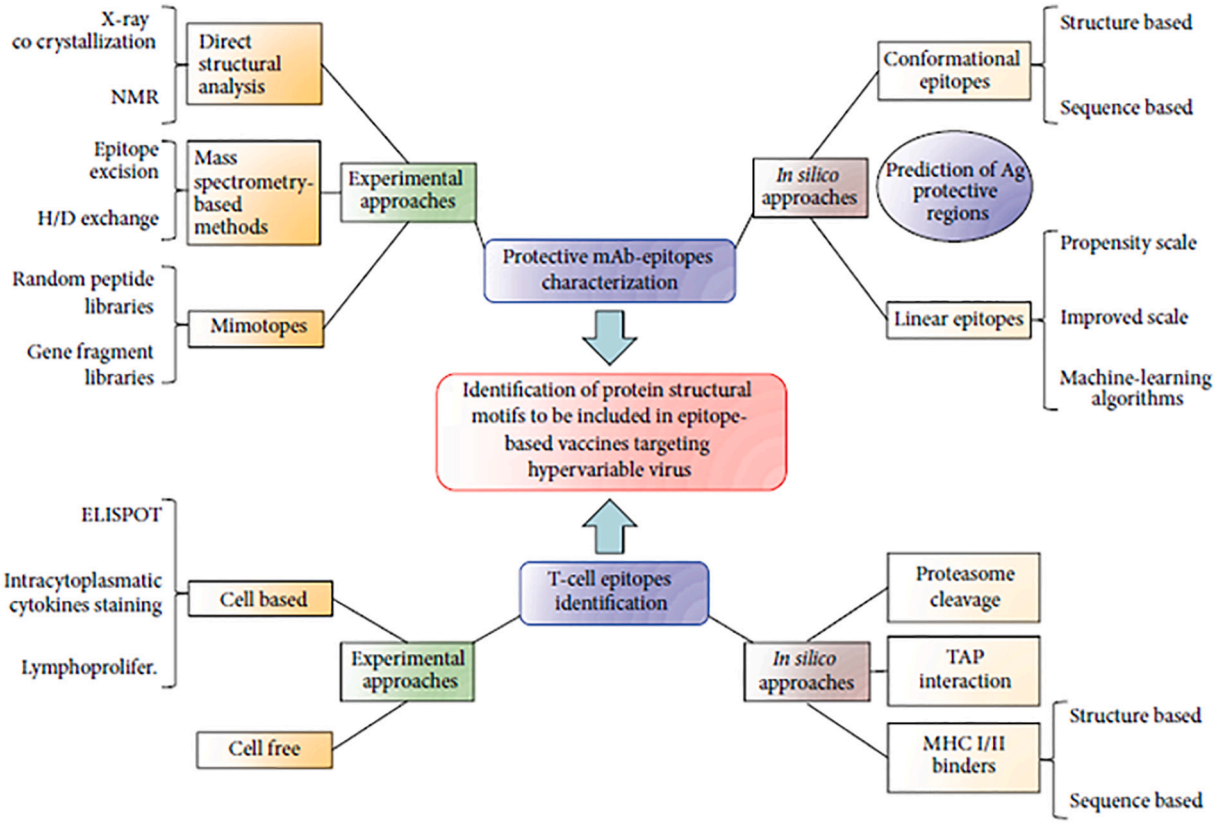

Fig. 1. Identification of the protein structural motifs for being included in the epitope-based vaccines for targeting the hypervariable viruses. Synergistic application of the methods including the *in silico* and experimental approaches has been illustrated.
Adapted from Ref. [27]

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)

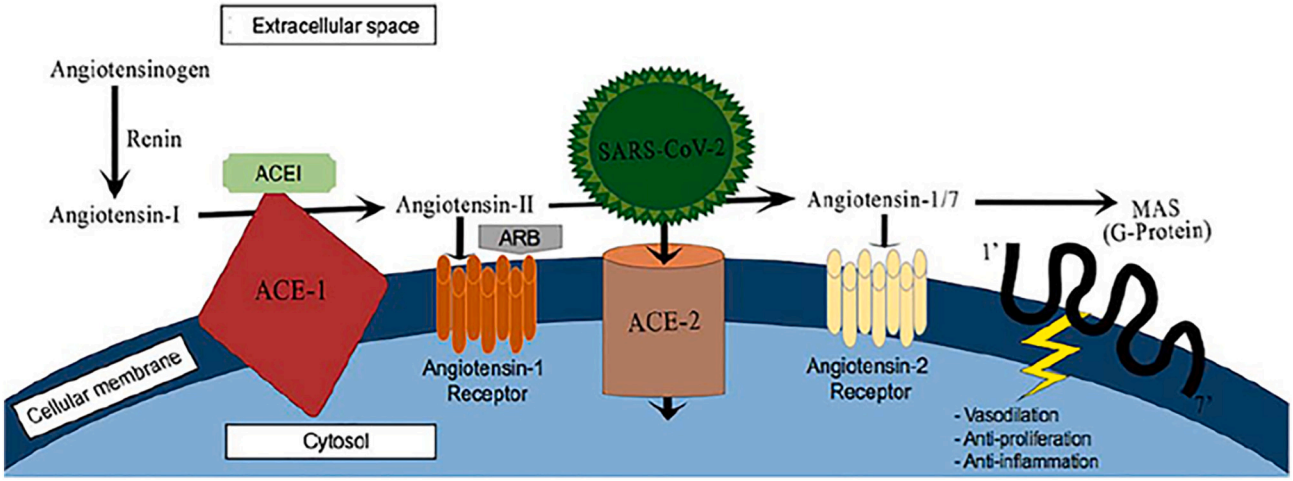

Fig. 2. Mechanisms of action of ACEI, ARB, and SARS-COV2 infection through ACE-2 receptors. ACEI: angiotensin-converting enzyme inhibitors, ARB: angiotensin receptor blocker, ACE-2: angiotensin-converting enzyme 2.
Adapted from Ref. [71].

polymerases can inhibit the replication of coronavirus and viral macrodomain has been suggested as an appropriate target for antivirus 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

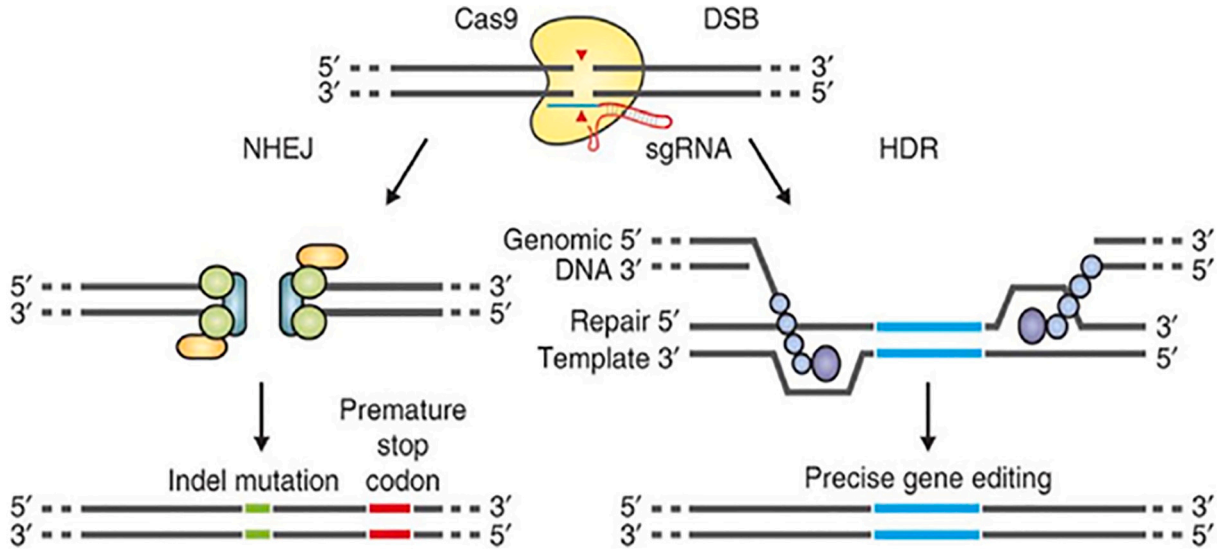

Fig. 3. DSB creation at specific sites is of key significance for genome editing. CRISPR-Cas9-induced DSB repair mechanism including the HDR and NHEJ pathways has been illustrated. (DSB: double strand breaks).
Adapted from Ref. [111].

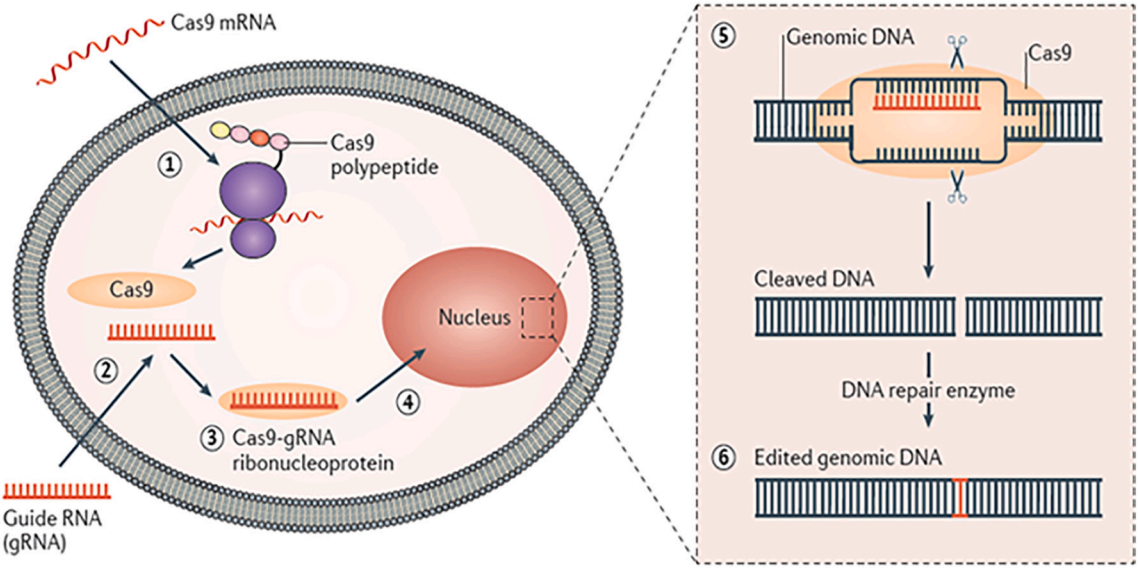

Fig. 4. The emergence of CRISPR technology has enabled gene knockout upon mRNA delivery which encode gene-editing nucleases. The mechanism of CRISPR- and mRNA-mediated gene editing has been illustrated. Transfection of target cell with mRNA which encodes Cas9 nuclease facilitates translation by the ribosomal complex (step 1). Transfection of guide RNA into the target cell (step 2) is followed by the formation of Cas9-gRNAc complex and a ribonucleoprotein with high affinity for DNA sequence (step 3), ribonucleoprotein complex translocation to the nucleus (step 4) and binding to the DNA sequence which is complementary to gRNA, double-stranded DNA cut (step 5) which can be repaired by the end joining leading to the gene knockout or replacement by the newly-inserted DNA piece in the repair template (step 6).
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 C_2C_2 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 occurence 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 (specially 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 *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, nanoemulsions, 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]. Silver NPs (Ag-NPs) by interacting with cell surface receptors inhibit virus entry into the host cells and prevent its reproduction [148]. They interact with the genome of double-stranded RNA viruses and inhibit their replication [149]. Ag-NPs undergo color alterations because of the localized surface plasmon resonance effect [150]. Ag-NPs modified by mercaptoethane sulfonate or tannic acid have been shown to prevent infections induced by type 1 or 2 of the herpes simplex virus *via* inhibiting the penetration, attachment, and spread of virus [151,152]. Gold NPs (AuNPs) because of their distinctive electric, catalytic, and photonic characteristics and capability of interactions with a variety of biomolecules such as RNA aptamers, single-stranded DNA, and antibodies can be applied in the viral

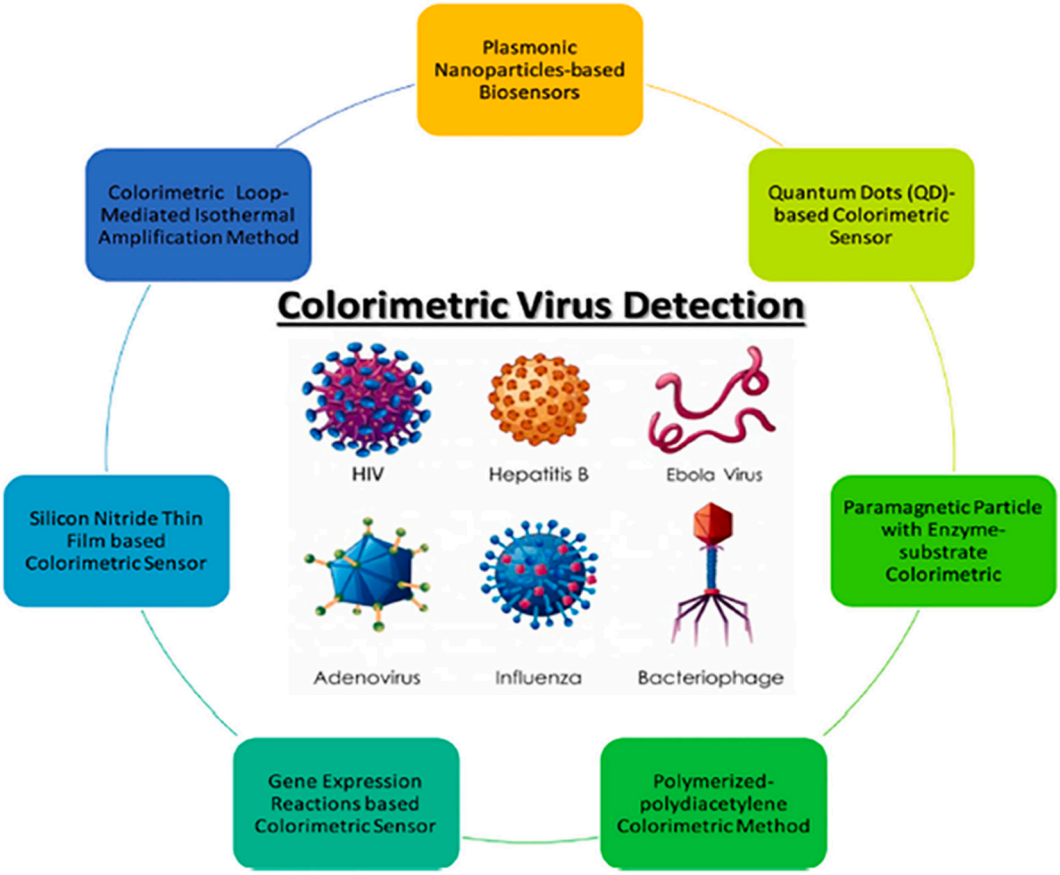

Fig. 5. Schematic illustration of the nanotechnology-based technologies for colorimetric viral detection.
Adapted from Ref. [154].

detection settings [153]. 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 scanometric 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 nanoreserviors 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

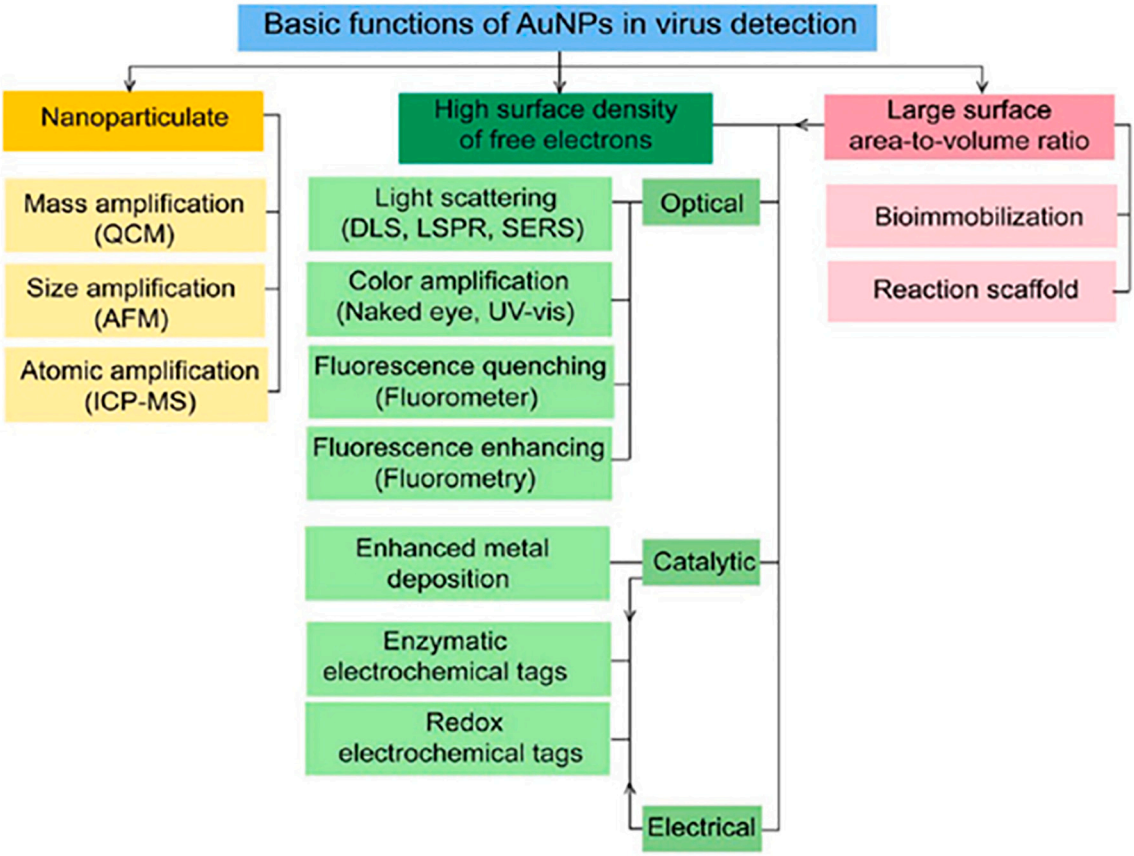

Fig. 6. Schematic illustration of the properties and functions of gold nanoparticles in virus detection. These nanoparticles with high sensitivity and specificity are capable of transducing various signal types including the electrical, optical, and catalytic signals. They could also be applied as the 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].

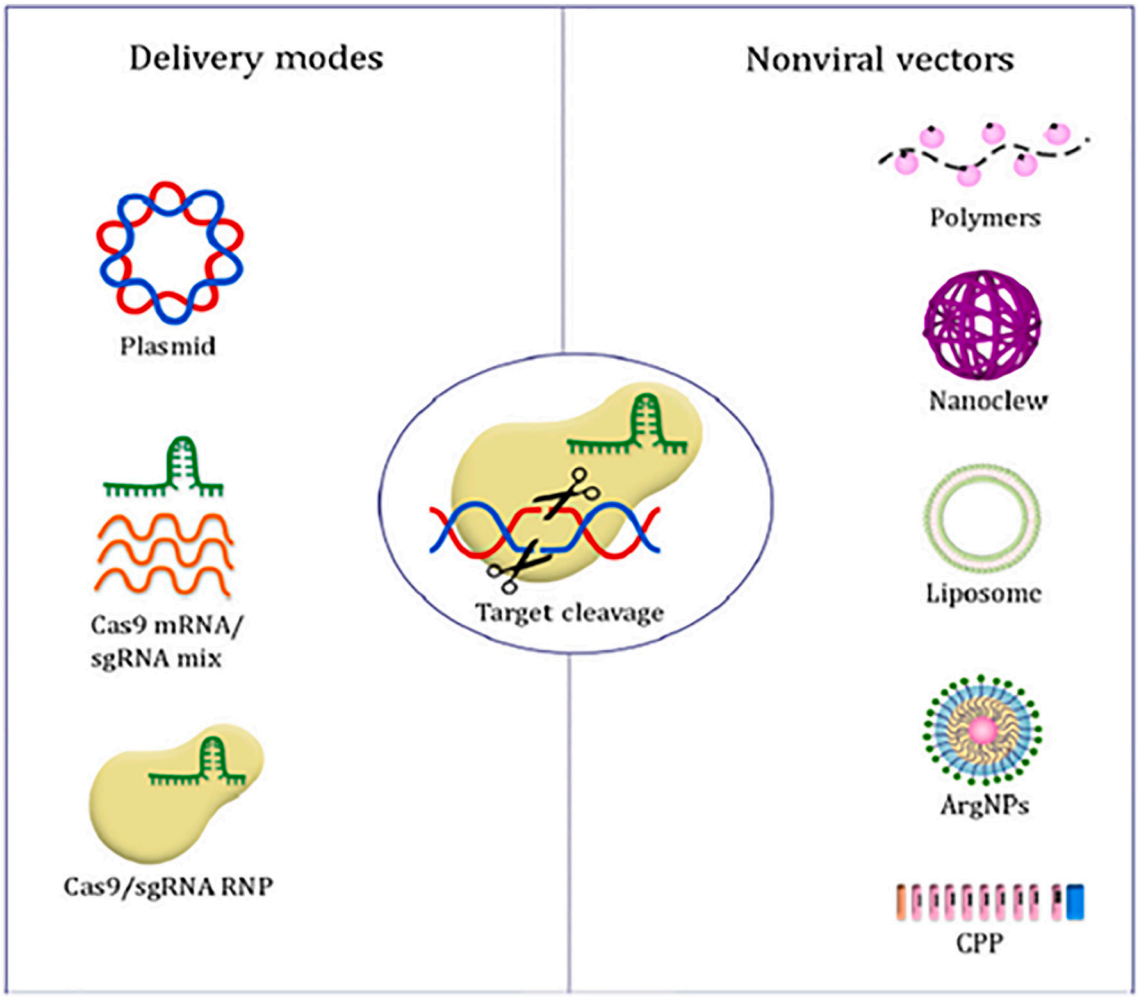

Fig. 7. Schematic representation of CRISPR-Cas9 system-based gene editing using the non-viral vectors. Engineering the non-viral nanoparticles including the liposomes and polymeric, inorganic, or lipid nanoparticles provides the possibility to overcome many of the limitations associated with the physical or viral approaches for delivery of CRISPR-Cas9 and attenuating off-target effects.
Adapted from Ref. [258].

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

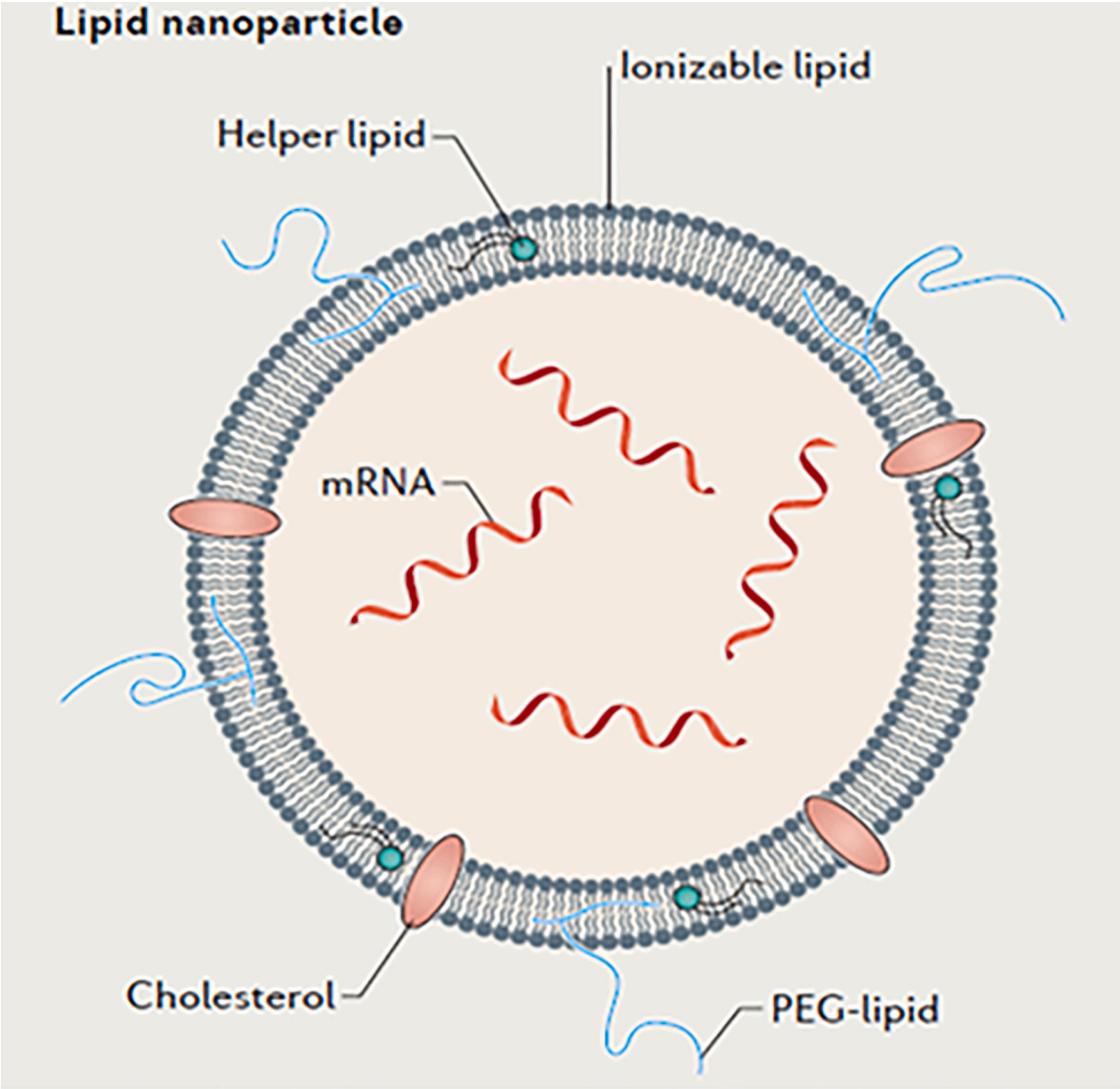

Fig. 8. A synthetic lipid nanoparticle for mRNA delivery. Lipid nanoparticle usually contains the helper lipid, cholesterol, ionizable lipid, and polyethylene glycol-lipid. mRNA is entrapped into the nanoparticle.
Adapted from Ref. [107].

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 nanoplatforms 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

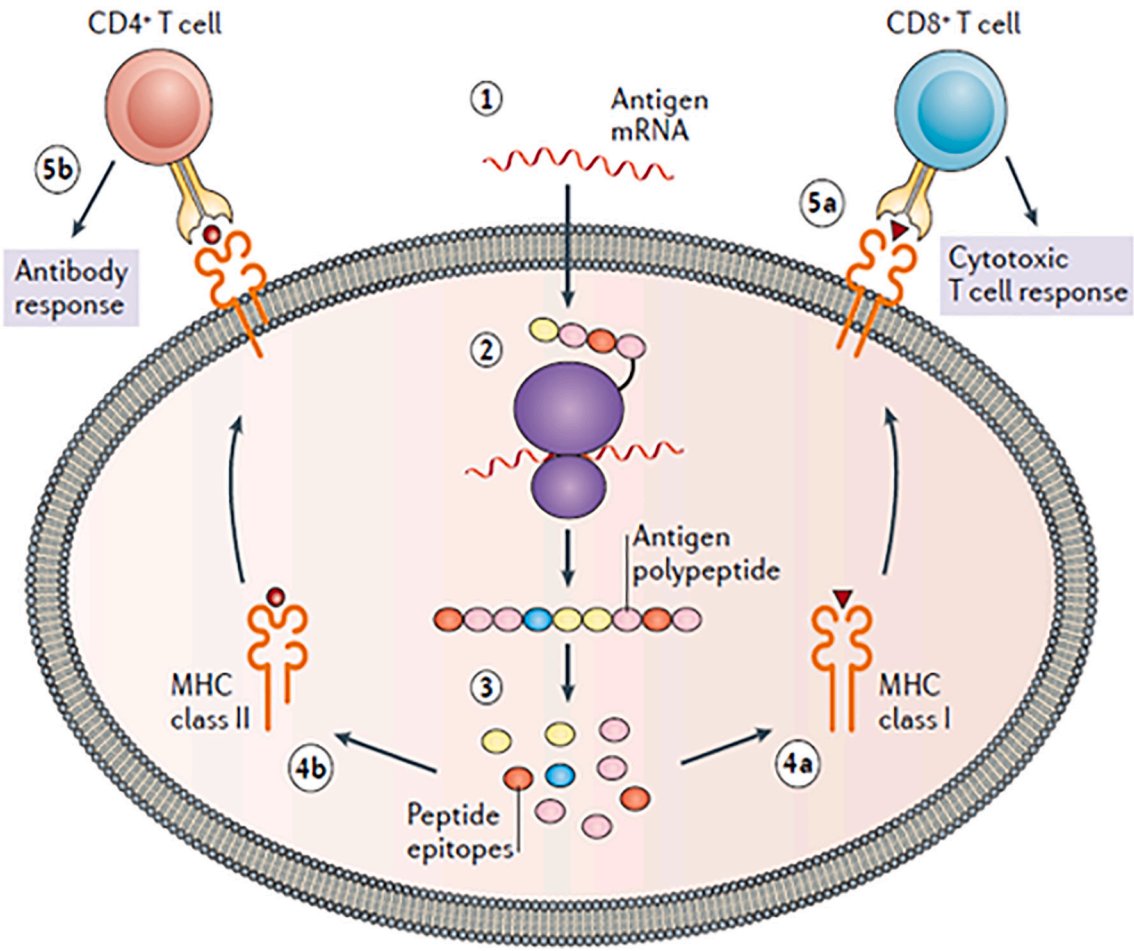

Fig. 9. mRNA-mediated vaccination mechanism. Antigen-encoding mRNA transfection into the antigen-presenting cell cytosol (step 1) is followed by mRNA translation into the antigenic peptide (step 2) which could be processed into the smaller epitopes (step 3) capable of binding to the major histocompatibility complex (MHC) class I or II (steps 4a and 4b). After trafficking of MHCs to the cell surface, their antigenic epitopes are presented to $CD8^+$ T cells (step 5a, cytotoxic T cell response) and $CD4^+$ T cells (step 5b). This might provide cellular immunity or antibody response.
Adapted from Ref. [211].

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 a 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 knockdown 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 recently 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 composing 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*-formulation of poly(β-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 inducing 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 the liquid phase which can be easily identified (within 5 min) by unveild 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-existed 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 mutation 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 siRNAs to the lungs. In the Rhesus macaque affected 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]. Advancements 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, cuting 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

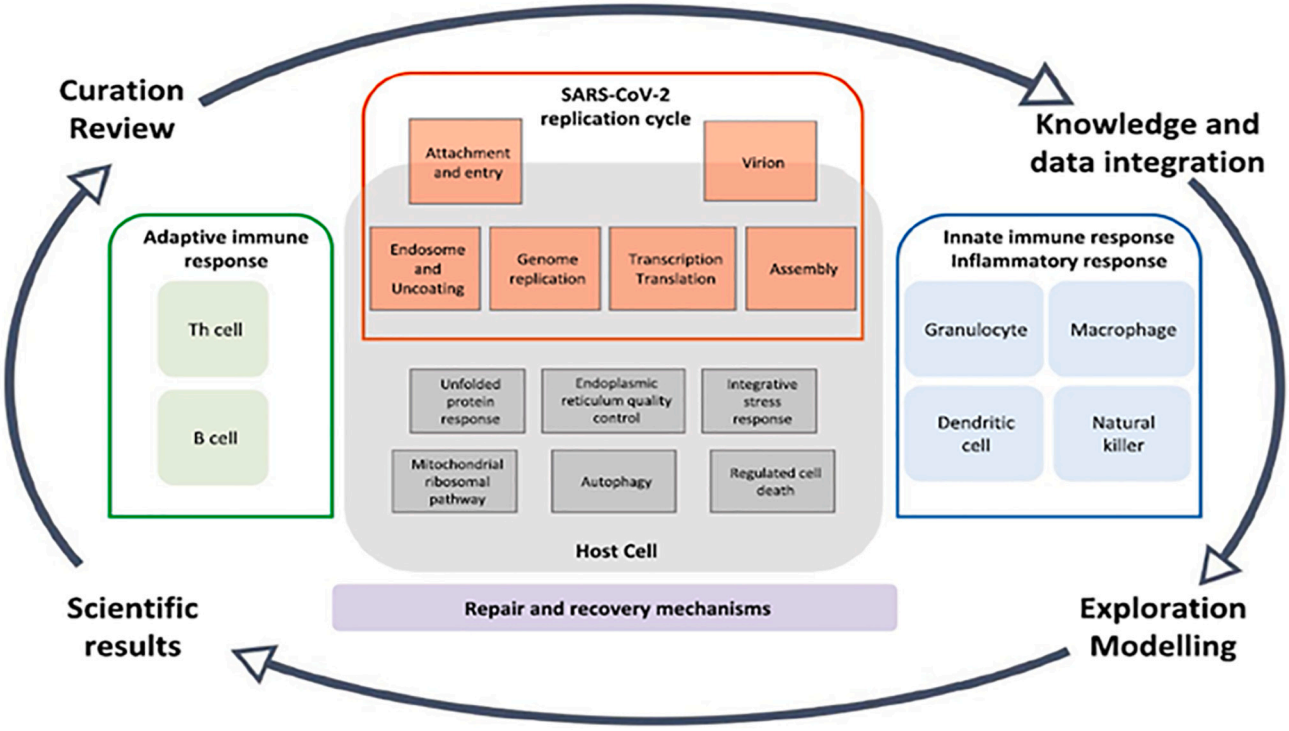

Fig. 10. An overview of the map project of COVID-19. The replication cycle and interactions of SARS-CoV-2, immune system reaction, and the mechanisms of repair have been highlighted in the map.
Adapted from Ref. [234].

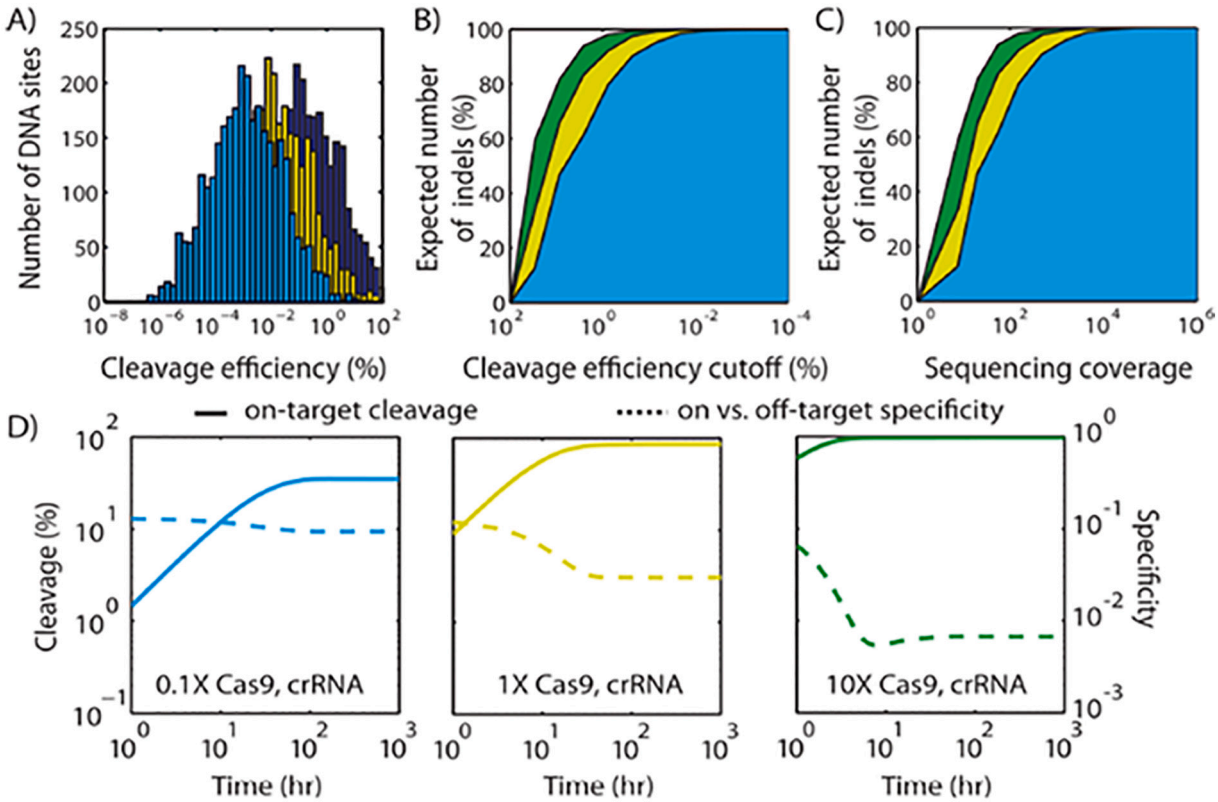

Fig. 11. Prediction of the genome editing in human using a biophysical model. A: Distributions calculated by the model demonstrate the numbers of DNA sites in the human genome which are cleavable with different efficiencies after application of LTR-B crRNA with baseline (in yellow), 10-fold lower or 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].

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 nanomamchines 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 enables 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 α-*N*-acetylgalactosaminidase (α-NAGA) protein is followed by the immunosuppression and virus spread throughout the body [254]. α-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 α-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 α-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

order to guide enzymes including Cas9 to the desired DNA target [257]. Using nanobots including the micropropellers 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 nanomedicine 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 optimisms or propaganda regarding the currently available or newly-developed therapeutics may be associated with catastrophic outcomes.

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Declaration of competing interest

Author declares no conflicts of interest.

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References

[1] J.D. Almeida, D.M. Berry, C.H. Cunningham, D. Hamre, M.S. Hofstad, L. Mallucci, K. McIntosh, D.A. Tyrrell, Virology: coronaviruses, Nature 220 (5168) (1968) 650.
[2] P.C. Woo, Y. Huang, S.K. Lau, K.Y. Yuen, Coronavirus genomics and bioinformatics analysis, Viruses 2 (8) (2010) 1804–1820.
[3] T.T.-Y. Lam, et al., Identification of 2019-nCoV related coronaviruses in Malayan pangolins in southern China, bioRxiv (2020), https://doi.org/10.1101/2020.02.13.945485.
[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. Pöhlmann, Proteolytic activation 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.
[9] J.S. Kahn, K. McIntosh, History and recent advances in coronavirus discovery, Pediatr. Infect. Dis. J. 24 (11 Suppl) (2005), https://doi.org/10.1097/01.inf.0000188166.17324.60. S223-7, discussion S226.
[10] E.J. Kendall, M.L. Bynoe, D.A. Tyrrell, Virus isolations from common colds occurring in a residential school, Br. Med. J. 2 (5297) (1962) 82–86.
[11] S. Su, G. Wong, W. Shi, J. Liu, A.C. Lai, J. Zhou, et al., Epidemiology, genetic recombination, and pathogenesis of coronaviruses, Trends Microbiol. 24 (6) (2016) 490–502.
[12] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, et al., A novel coronavirus from patients with pneumonia in China, 2019, N. Engl. J. Med. 382 (8) (2020) 727–733.
[13] V.M. Corman, D. Muth, D. Niemeyer, C. Drosten, Hosts and sources of endemic human coronaviruses, Adv. Virus Res. 100 (2018) 163–188.
[14] D.S. Hui, E. I Azhar, T.A. Madani, F. Ntoumi, R. Kock, O. Dar, et al., The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health—the latest 2019 novel coronavirus outbreak in Wuhan, China, Int. J. Infect. Dis. 91 (2020) 264–266.
[15] R.A. Neher, R. Dyrdak, V. Druelle, E.B. Hodcroft, J. Albert, Potential Impact of Seasonal Forcing on a SARS-CoV-2 Pandemic. MedRxiv, 2020.
[16] Matthew Zirui Tay, Chek Meng Poh, Laurent Rénia, Paul A. MacAry, Lisa F.P. Ng, The trinity of COVID-19: immunity, inflammation and intervention, Nat. Rev. Immunol. 20 (2020) 363–374.
[17] Stephen M. Hahn, Coronavirus (COVID-19) Update: Serological Tests, Food and Drug Administration, April 07, 2020.
[18] Linda J. Carter, Linda V. Garner, Jeffrey W. Smoot, Yingzhu Li, Qiongqiong Zhou, et al., Assay techniques and test development for COVID-19 diagnosis, ACS Cent Sci 6 (5) (2020) 591–605. May 27.
[19] M.J. Espy, J.R. Uhl, L.M. Sloan, S.P. Buckwalter, M.F. Jones, E.A. Vetter, J.D. C. Yao, et al., Real-time PCR in clinical microbiology: applications for routine laboratory testing, Clin. Microbiol. Rev. 19 (1) (2006) 165–256. Jan.
[20] Stephen A. Bustin, Tania Nolan, Pitfalls of quantitative real-time reverse-transcription polymerase chain reaction, J. Biomol. Tech. 15 (3) (2004) 155–166. Sep.
[21] Alireza Tahamtan, Abdollah Ardebili, Real-time RT-PCR in COVID-19 detection: issues affecting the results, Expert. Rev. Mol. Diagn. (2020) 1–2, https://doi.org/10.1080/14737159.2020.1757437.
[22] K.A. Chianese-Bullock, W.P. Irvin Jr., G.R. Petroni, C. Murphy, M. Smolkin, W. C. Olson, E. Coleman, et al., A multipeptide vaccine is safe and elicits T-cell responses in participants with advanced stage ovarian cancer, J. Immunother. 31 (2008) 420–430.
[23] J.F. Koellhoffer, C.D. Higgins, J.R. Lai, Protein engineering strategies for the development of viral vaccines and immunotherapeutics, FEBS Lett. 588 (2014) 298–307.
[24] Mariusz Skwarczynski, Istvan Toth, Peptide-based synthetic vaccines, Chem. Sci. 7 (2) (2016) 842–854. Feb 1.
[25] Arafat Rahman Oany, Abdullah-Al Emran, Tahmina Pervin Jyoti, Design of an epitope-based peptide vaccine against spike protein of human coronavirus: an in silico approach, Drug Design, Development and Therapy (8) (2014) 1139–1149.
[26] Ryan J. Malonis, Jonathan R. Lai, Olivia Vergnolle, Peptide-based vaccines: current progress and future challenges, Chem. Rev. 120 (6) (2020) 3210–3229.
[27] Matteo Castelli, Francesca Cappelletti, Roberta Antonia Diotti, Giuseppe Sautto, Elena Criscuolo, Matteo Dal Peraro, et al., Peptide-based vaccinology: experimental and computational approaches to target hypervariable viruses through the fine characterization of protective epitopes recognized by monoclonal antibodies and the identification of T-cell-activating peptides, Clin. Dev. Immunol. 2013 (2013) 1–12, https://doi.org/10.1155/2013/521231, 521231.
[28] T. Ben-Yedidia, R. Arnon, Epitope-based vaccine against influenza, Expert Review of Vaccines 6 (6) (2007) 939–948.
[29] T.H. Lee, B.-H. Song, S.-I. Yun, et al., A cross-protective mAb recognizes a novel epitope within the flavivirus NS1 protein, J. Gen. Virol. 93 (1) (2012) 20–26.
[30] N. Mancini, L. Solforosi, N. Clementi, D. De Marco, M. Clementi, R. Burioni, A potential role for monoclonal antibodies in prophylactic and therapeutic treatment of influenza, Antivir. Res. 92 (1) (2011) 15–26.
[31] J.M. Gershoni, A. Roitburd-Berman, D.D. Siman-Tov, N.T. Freund, Y. Weiss, Epitope mapping: the first step in developing epitope-based vaccines, BioDrugs 21 (3) (2007) 145–156.
[32] N. Seyed, F. Zahedifard, S. Safaiyan, et al., In silico analysis of six known leishmania major antigens and in vitro evaluation of specific epitopes eliciting HLA-A2 restricted CD8 T cell response, PLoS Negl. Trop. Dis. 5 (9) (2011), e1295.
[33] Nabil Ahmed, Stephen Gottschalk, How to design effective vaccines: lessons from an old success story, Expert Rev Vaccines 8 (5) (2009) 543–546. May.
[34] E. Miller, P.C.L. Beverley, D.M. Salisbury, Vaccine programmes and policies, Br. Med. Bull. 62 (1) (2002) 201–211.
[35] Ursula Wiedermann, Erika Garner-Spitzer, Angelika Wagner, Primary vaccine failure to routine vaccines: why and what to do? Human Vaccines & Immunotherapeutics 12 (1) (2016) 239–243.
[36] J.C. Frei, A.S. Wirchnianski, J. Govero, O. Vergnolle, K.A. Dowd, T.C. Pierson, M. Kielian, et al., Engineered dengue virus domain III proteins elicit cross-neutralizing antibody responses in mice, J. Virol. 92 (e01023–18) (2018).
[37] X. Tang, C. Wu, X. Li, Y. Song, X. Yao, X. Wu, Y. Duan, H. Zhang, Y. Wang, Z. Qian, On the origin and continuing evolution of SARS-CoV-2, Natl. Sci. Rev. (2020), nwaa036, https://doi.org/10.1093/nsr/nwaa036.
[38] Mohammed Uddin, Farah Mustafa, Tahir A. Rizvi, Tom Loney, Hanan Al Suwaidi, Ahmed H. Hassan Al-Marzouqi, et al., SARS-CoV-2/COVID-19: viral genomics,

epidemiology, vaccines, and therapeutic interventions, Viruses 12 (2020) 1–18, https://doi.org/10.3390/v12050526.
[39] Jinyong 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/vaccines8020153.
[40] Z. Jahanafrooz, B. Baradaran, J. Mosafer, M. Hashemzaei, T. Rezaei, A. Mokhtarzadeh, M.R. Hamblin, Comparison of DNA and mRNA vaccines against cancer, Drug Discov. Today 25 (2020) 552–560.
[41] Cuiling 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] V. Gonzalez-Nicolini, C.D. Sanchez-Bustamante, S. Hartenbach, M. Fussenegger, Adenoviral vector platform for transduction of constitutive and regulated tricistronic or triple-transcript transgene expression in mammalian cells and microtissues, J. Gene Med. 8 (2006) 1208–1222.
[43] N. Noranate, N. Takeda, P. Chetanachan, P. Sittisaman, A. A-nuegoonpipat, S. Anantapreecha, Characterization of chikungunya virus-like particles, PLoS One 9 (2014), e108169.
[44] H. Dong, H.C. Guo, S.Q. Sun, Virus-like particles in picornavirus vaccine development, Appl. Microbiol. Biotechnol. 98 (2014) 4321–4329.
[45] N.J. Temperton, P.K. Chan, G. Simmons, M.C. Zambon, R.S. Tedder, Y. Takeuchi, R.A. Weiss, Longitudinally profiling neutralizing antibody response to SARS coronavirus with pseudotypes, Emerg. Infect. Dis. 11 (2005) 411–416.
[46] H. Hogenesch, Mechanism of immunopotentiation and safety of aluminum adjuvants, Front. Immunol. 3 (2012) 406.
[47] T.F. Tsai, Fluad(R)-MF59(R)-adjuvanted influenza vaccine in older adults, Infect. Chemother. 45 (2013) 159–174.
[48] A.M. Didierlaurent, B. Laupeze, A. Di Pasquale, N. Hergli, C. Collignon, N. Garcon, Adjuvant system AS01: helping to overcome the challenges of modern vaccines, Expert Rev. Vaccines 16 (2017) 55–63.
[49] N. Petrovsky, Comparative safety of vaccine adjuvants: a summary of current evidence and future needs, Drug Saf. 38 (2015) 1059–1074.
[50] S. Jiang, Don't rush to deploy COVID-19 vaccines and drugs without sufficient safety guarantees, Nature 579 (2020) 321.
[51] L. Du, Y. He, Y. Zhou, S. Liu, B.J. Zheng, S. Jiang, The spike protein of SARS-CoV–a target for vaccine and therapeutic development, Nat. Rev. Microbiol. 7 (2009) 226–236.
[52] L. Liu, Q. Wei, Q. Lin, J. Fang, H. Wang, H. Kwok, H. Tang, K. Nishiura, J. Peng, Z. Tan, et al., Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection, JCI Insight (2019) 4.
[53] Awadhesh Kumar Singh, Akriti Singh, Altamash Shaikh, Ritu Singh, Anoop Misra, Chloroquine and hydroxychloroquine in the treatment of COVID-19 with or without diabetes: A systematic search and a narrative review with a special reference to India and other developing countries, Diabetes Metab Syndr 14 (3) (2020) 241–246.
[54] Mathieu E. Rebeaud, Florian Zores, SARS-CoV-2 and the use of chloroquine as an antiviral treatment, Front. Med. (2020), https://doi.org/10.3389/fmed.2020.00184.
[55] J. David, Browning. Pharmacology of Chloroquine and Hydroxychloroquine, Hydroxychloroquine and Chloroquine Retinopathy (2014 Apr 4) 35–63.
[56] Y. Wang, D. Zhang, G. Du, et al., Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial, Lancet (2020), https://doi.org/10.1016/S0140-6736(20)31022-9.
[57] J. Grein, N. Ohmagari, D. Shin, et al., Compassionate use of remdesivir for patients with severe COVID-19, N. Engl. J. Med. (2020), https://doi.org/10.1056/NEJMoa2007016.
[58] Calvin J. Gordon, Egor P. Tchesnokov, Emma Woolner, Jason K. Perry, Joy Y. Feng, Danielle P. Porter, Matthias Gotte, Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency, J. Biol. Chem. (2020), https://doi.org/10.1074/jbc.RA120.013679.
[59] Y. Wang, D. Zhang, G. Du, et al., Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial, Lancet (2020), https://doi.org/10.1016/S0140-6736(20)31022-9.
[60] B. Cao, Y. Wang, D. Wen, et al., A trial of lopinavir–ritonavir in adults hospitalized with severe COVID-19, N. Engl. J. Med. (2020), https://doi.org/10.1056/NEJMoa2001282.
[61] D.F. Smee, E.B. Tarbet, Y. Furuta, J.D. Morrey, D.L. Barnard, Synergistic combinations of favipiravir and oseltamivir against wild-type pandemic and oseltamivir-resistant influenza A virus infections in mice, Future Virol 8 (2013) 1085–1094.
[62] Q. Cai, M. Yang, D. Liu, J. Chen, D. Shu, J. Xia, X. Liao, Y. Gu, Q. Cai, Y. Yang, et al., Experimental treatment with favipiravir for COVID-19: an open-label control study, Engineering (2020), https://doi.org/10.1016/j.eng.2020.03.007 in press.
[63] I. Mejdrová, D. Chalupská, P. Plačková, et al., Rational design of novel highly potent and selective Phosphatidylinositol 4-Kinase IIIβ (PI4KB) inhibitors as broad-spectrum antiviral agents and tools for chemical biology, J. Med. Chem. 60 (1) (2017) 100–118.
[64] J.C. Saiz, N.J. Oya, A.B. Blázquez, E. Escribano-Romero, M.A. Martín- Acebes, Host-directed antivirals: a realistic alternative to fight Zika virus, Viruses 10 (9) (2018), E453.
[65] J. Liu, X. Zheng, Q. Tong, W. Li, B. Wang, K. Sutter, M. Trilling, M. Lu, U. Dittmer, D. Yang, Overlapping and discrete aspects of the pathology and pathogenesis of the emerging human pathogenic coronaviruses SARSCoV, MERS-CoV, and 2019-nCoV, J. Med. Virol. 92 (5) (2020) 491–494.
[66] G.M. Kuster, O. Pfister, T. Burkard, Q. Zhou, R. Twerenbold, P. Haaf, et al., SARS-CoV2: should inhibitors of the renin-angiotensin system be withdrawn in patients with COVID-19? Eur. Heart J. 41 (19) (2020) 1801–1803.
[67] P. Zhang, L. Zhu, J. Cai, et al., Association of inpatient use of angiotensin converting enzyme inhibitors and angiotensin II receptor blockers with mortality among patients with hypertension hospitalized with COVID-19, Circ. Res. 126 (2020) 1671–1681.
[68] 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.
[69] G.I. Rice, D.A. Thomas, P.J. Grant, A.J. Turner, N.M. Hooper, Evaluation of angiotensin-converting enzyme (ACE), its homologue ACE2 and neprilysin in angiotensin peptide metabolism, Biochem. J. 383 (2004) 45–51.
[70] Ankit B. Patel, Ashish Verma, COVID-19 and angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: what is the evidence? JAMA (2020) E1–E2, https://doi.org/10.1001/jama.2020.4812.
[71] Juan Simon Rico-Mesa, Averi White, Allen S. Anderson, Outcomes in patients with COVID-19 infection taking ACEI/ARB, Curr. Cardiol. Rep. 22 (2020), 31, https://doi.org/10.1007/s11886-020-01291-4.
[72] S. Xia, Y. Zhu, M. Liu, Q. Lan, W. Xu, Y. Wu, et al., Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein, Cell Mol Immunol (2020), https://doi.org/10.1038/s41423-020-0374-2.
[73] T.M. Nguyen, Y. Zhang, P.P. Pandolfi, Virus against virus: A potential treatment for 2019-nCov (SARS-CoV-2) and other RNA viruses, Cell Res. 30 (2020) 189–190.
[74] Matthew E. Grunewald, Yating Chen, Chad KunyI, Takashi Maejima, Robert Lease, Dana Ferraris, et al., The coronavirus macrodomain is required to prevent PARP-mediated inhibition of virus replication and enhancement of IFN expression, PLoS Pathog. (5) (2019) 1–24, https://doi.org/10.1371/journal.ppat.1007756.
[75] H. Wang, P. Yang, K. Liu, F. Guo, Y. Zhang, G. Zhang, C. Jiang, SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway, Cell Res. 18 (2008) 290–301.
[76] Parichehr Hassanzadeh, Fatemeh Atyabi, Rassoul Dinarvand, The significance of artificial intelligence in drug delivery system design, Adv. Drug Deliv. Rev. 151–152 (2019) 169–190.
[77] G. Neveu, A. Ziv-Av, R. Barouch-Bentov, E. Berkerman, J. Mulholland, S. Einav, AP-2-associated protein kinase 1 and cyclin G-associated kinase regulate hepatitis C virus entry and are potential drug targets, J. Virol. 89 (2015) 4387–4404.
[78] P. Richardson, I. Griffin, C. Tucker, D. Smith, O. Oechsle, A. Phelan, J. Stebbing, Baricitinib as potential treatment for 2019-nCoV acute respiratory disease, Lancet 395 (2020) e30–e31.
[79] P.N. Pascua, J.H. Lee, M.S. Song, S.J. Park, Y.H. Baek, B.H. Ann, E.Y. Shin, E. G. Kim, Y.K. Choi, Role of the p21-activated kinases (PAKs) in influenza A virus replication, Biochem. Biophys. Res. Commun. 414 (2011) 569–574.
[80] Y. Zhou, G. Simmons, Development of novel entry inhibitors targeting emerging viruses, Expert Rev. Anti-Infect. Ther. 10 (2012) 1129–1138.
[81] B.C.Q. Nguyen, H. Takahashi, Y. Uto, M.D. Shahinozzaman, S. Tawata, H. Maruta, 1,2,3-Triazolyl ester of ketorolac: A "click chemistry"-based highly potent PAK1-blocking cancer-killer, Eur. J. Med. Chem. 126 (2017) 270–276.
[82] S. Patil, L.G. Lis, R.J. Schumacher, B.J. Norris, M.L. Morgan, R.A. Cuellar, et al., Phosphonooxymethyl prodrug of triptolide: synthesis, physicochemical characterization, and efficacy in human colon adenocarcinoma and ovarian cancer xenografts, J. Med. Chem. 58 (2015) 9334–9344.
[83] B.C.Q. Nguyen, K. Yoshimura, S. Kumazawa, S. Tawata, H. Frondoside Maruta, A from sea cucumber and nymphaeols from Okinawa propolis: natural anti-cancer agents that selectively inhibit PAK1 in vitro, Drug Discov. Ther. 11 (2017) 110–114.
[84] C.D. Russell, J.E. Millar, J.K. Baillie, Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury, Lancet 395 (2020) 473–475.
[85] W. Zhou, Y. Liu, D. Tian, J. Cheng, M. Hu, M. Fang, Y. Gao, Potential benefits of precise corticosteroids therapy for severe 2019-nCoV pneumonia, Signal Transduct Target Ther 5 (2020) 18.
[86] Y. Fu, Y. Cheng, Y. Wu, Understanding SARS-CoV-2-mediated inflammatory responses: from mechanisms to potential therapeutic tools, Virol. Sin. (2020), https://doi.org/10.1007/s12250-020-00207-4.
[87] Y.F.B. Zhou, X. Zheng, D. Wang, C. Zhao, Y. Qi, R. Sun, et al., Aberrant pathogenic GM-CSF+ T cells and inflammatory CD14+CD16+ monocytes in severe pulmonary syndrome patients of a new coronavirus, BioRxiv (2020), https://doi.org/10.1101/2020.02.12.945576.
[88] L. Chen, J. Xiong, L. Bao, Y. Shi, Convalescent plasma as a potential therapy for COVID-19, Lancet Infect. Dis. (2020), https://doi.org/10.1016/S1473-3099(20)30141-9.
[89] O. Garraud, F. Heshmati, B. Pozzetto, F. Lefrere, R. Girot, A. Saillol, S. Laperche, Plasma therapy against infectious pathogens, as of yesterday, today and tomorrow, Transfus. Clin. Biol. 23 (2016) 39–44.
[90] Junghyun Gooa, Yuji Jeonga, Young-Shin Parka, Eunji Yang, Dae-Im Jung, Semi Rho, Characterization of novel monoclonal antibodies against MERS-coronavirus spike protein, Virus Res. 197863 (278) (2020) 1–10.
[91] R. Wölfel, V.M. Corman, W. Guggemos, et al., Virological assessment of hospitalized patients with COVID-2019, Nature (2020).
[92] Yoshiyuki Ohsug, The immunobiology of humanized anti-IL6 receptor antibody: from basic research to breakthrough medicine, J Translational Autoimmunity 3 (2020), 100030.
[93] Miguel A. González-Gay, José Mayo, Santos Castañeda, José M. Cifrián, José Hernández-Rodríguez, Tocilizumab: from the rheumatology practice to the

fight against COVID-19, a virus infection with multiple faces, Expert. Opin. Biol. Ther. 1 (2020) 1–8, https://doi.org/10.1080/14712598.2020.1770222.
[94] A. Khademhosseini, R. Langer, A decade of progress in tissue engineering, Nat. Protoc. 11 (2016) 1775.
[95] P. Hassanzadeh, Tissue engineering and growth factors: updated evidence, Biomed Rev 23 (2012) 19–35.
[96] Alexander M. Tatara, Role of tissue engineering in COVID-19 and future viral outbreaks 26 (2020) 468–474.
[97] A.J. Miller, J.R. Spence, In vitro models to study human lung development, disease and homeostasis, Physiology (Bethesda) 32 (2017) 246.
[98] Parichehr Hassanzadeh, Fatemeh Atyabi, Rassoul Dinarvand, Tissue engineering: still facing a long way ahead, J. Control. Release 279 (2018) 181–197.
[99] O. Méndez-Lucio, J. Tran, J.L. Medina-Franco, N. Meurice, M. Muller, Toward drug repurposing in epigenetics: olsalazine as a hypomethylating compound active in a cellular context, ChemMedChem 9 (2014) 560–565.
[100] H. Lu, Drug treatment options for the 2019-new coronavirus (2019-nCoV), Biosci Trends (2020), https://doi.org/10.5582/bst.2020.01020.
[101] Z. Wang, X. Chen, Y. Lu, F. Chen, W. Zhang, Clinical characteristics and therapeutic procedure for four cases with 2019 novel coronavirus pneumonia receiving combined Chinese and Western medicine treatment, Biosci Trends (2020), https://doi.org/10.5582/bst.2020.01030.
[102] K.M. Esvelt, H.H. Wang, Genome-scale engineering for systems and synthetic biology, Mol. Syst. Biol. 9 (1) (2013) 641, https://doi.org/10.1038/msb.2012.66.
[103] Method of the Year 2011, Nat. Methods 9 (1) (2012) 1, https://doi.org/10.1038/nmeth.1852.
[104] Science News Staff, Breakthrough of the Year: CRISPR Makes the Cut, 2015.
[105] M. Jasin, Genetic manipulation of genomes with rare-cutting endonucleases, Trends Genet. 12 (6) (1996) 224–228.
[106] M.A. McMahon, M. Rahdar, M. Porteus, Gene editing: not just for translation anymore, Nat. Methods 9 (1) (2011) 28–31.
[107] Khalid A. Hajj, Kathryn A. Whitehead, Tools for translation: non-viral materials for therapeutic mRNA delivery, Nat Rev 2 (2017) 1–17, https://doi.org/10.1038/natrevmats.2017.56 (17056).
[108] R. Barrangou, J.A. Doudna, Applications of CRISPR technologies in research and beyond, Nat. Biotechnol. 34 (9) (2016) 933–941.
[109] J. Moon, M. Kim, Applications of CRISPR/Cas9 for gene editing in hereditary movement disorders, JMovement Disorders 9 (3) (2016) 136–143.
[110] P.D. Hsu, E.S. Lander, F. Zhang, Development and applications of CRISPR-Cas9 for genome engineering, Cell 157 (6) (June 2014) 1262–1278.
[111] J.A. Doudna, E. Charpentier, The new frontier of genome engineering with CRISPR-Cas9, Science 346 (2014).
[112] L. Cong, F.A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, et al., Multiplex genome engineering using CRISPR/Cas systems, Science 339 (2013) 819–823.
[113] A.A. Price, T.R. Sampson, H.K. Ratner, A. Grakoui, D.S. Weiss, Cas9-mediated targeting of viral RNA in eukaryotic cells, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 6164–6169.
[114] P. Horvath, R. Barrangou, CRISPR/Cas, the immune system of bacteria and archaea, Science 327 (2010) 167–170.
[115] P.D. Hsu, D.A. Scott, J.A. Weinstein, F.A. Ran, S. Konermann, et al., DNA targeting specificity of RNA-guided Cas9 nucleases, Nat. Biotechnol. 31 (2013) 827–832.
[116] J.E. DiCarlo, J.E. Norville, P. Mali, X. Rios, J. Aach, et al., Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems, Nucleic Acids Res. 41 (2013) 4336–4343.
[117] B. Moss, Reflections on the early development of poxvirus vectors, Vaccine 31 (2013) 4220–4222.
[118] Ming Yuan, Wensheng Zhang, Jun Wang, Chadwan Al Yaghchi, Jahangir Ahmed, Louisa Chard, et al., Efficiently editing the Vaccinia virus genome by using the CRISPR-Cas9 system, J. Virol. 89 (2015) 5176–5179.
[119] David B.T. Cox, Jonathan S. Gootenberg, Omar O. Abudayyeh, Brian Franklin, Max J. Kellner, Julia Joung, Feng Zhang, RNA editing with CRISPR-Cas13, Science 358 (6366) (2017) 1019–1027. Nov 24.
[120] Omar O. Abudayyeh, Jonathan S. Gootenberg, Silvana Konermann, Julia Joung, Ian M. Slaymaker, David B.T. Cox, et al., C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector, Science 353 (2016) 5573–5578.
[121] A. Phuphuakrat, R. Kraiwong, C. Boonarkart, D. Lauhakirti, T.-H. Lee, P. Auewarakul, Double-stranded RNA adenosine deaminases enhance expression of human immunodeficiency virus type 1 proteins, J. Virol. 82 (2008) 10864–10872.
[122] S. Di Giorgio, et al., Evidence for host-dependent RNA editing in the transcriptome of SARS-CoV-2, Sci. Adv. (2020), https://doi.org/10.1126/sciadv.abb5813.
[123] Jeffrey Chen, Thomas MacCarthy, The preferred nucleotide contexts of the AID/APOBEC cytidine deaminases have differential effects when mutating retrotransposon and virus sequences compared to host genes, PLoS Comput. Biol. 13 (3) (2017), https://doi.org/10.1371/journal.pcbi.1005471. Mar. (e1005471).
[124] A. Scheben, F. Wolter, J. Batley, H. Puchta, D. Edwards, Towards CRISPR/Cas crops – bringing together genomics and genome editing, New Phytol. 216 (2017) 682–698.
[125] T.R. Sampson, S.D. Saroj, A.C. Llewellyn, Y.-L. Tzeng, D.S. Weiss, A CRISPR/Cas system mediates bacterial innate immune evasion and virulence, Nature 497 (2013) 254–257.
[126] O.O. Abudayyeh, J.S. Gootenberg, P. Essletzbichler, S. Han, J. Joung, J.J. Belanto, et al., RNA targeting with CRISPR–Cas13, Nature 550 (2017) 280–284.
[127] Tong Zhang, Qiufeng Zheng, Xin Yi, Hong An, Yaling Zhao, Siqi Ma, Guohui Zhou, Establishing RNA virus resistance in plants by harnessing CRISPR immune system, Plant Biotechnol. J. 16 (8) (2018) 1415–1423, https://doi.org/10.1111/pbi.12881.
[128] M. Jinek, et al., A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity, Sci. N. Y. NY 337 (2012) 816–821.
[129] Philippe E. Mangeot, Valérie Risson, Floriane Fusil, Aline Marnef, Emilie Laurent, Juliana Blin, et al., Genome editing in primary cells and in vivo using viral-derived Nanoblades loaded with Cas9-sgRNA ribonucleoproteins, Nat. Commun. 10 (2019) 1–15, https://doi.org/10.1038/s41467-018-07845-z.
[130] P.S. Masters, P.J. Rottier, Coronavirus reverse genetics by targeted RNA recombination, Curr. Top. Microbiol. Immunol. 287 (2005) 133–159.
[131] Cornelis A.M. de Haan, Bert Jan Haijema, Paul S. Masters, Peter J.M. Rottier, Manipulation of the coronavirus genome using targeted RNA recombination with interspecies chimeric coronaviruses, Methods Mol. Biol. 454 (2008) 229–236.
[132] Yanwei Bi, Le Sun, Dandan Gao, Chen Ding, Zhihua Li, Yadong Li, et al., High-efficiency targeted editing of large viral genomes by RNA-guided nucleases, PLoS Pathog. 10 (2014) 1–11 (e1004090).
[133] Xiaoyu Chen, Manuel A.F.V. Gonçalves, Engineered viruses as genome editing devices, Mol. Ther. 24 (2016) 447–457.
[134] J. Mátrai, M.K. Chuah, T. VandenDriessche, Recent advances in lentiviral vector development and applications, Mol. Ther. 18 (2010) 477–490.
[135] L. Naldini, Ex vivo gene transfer and correction for cell-based therapies, Nat Rev Genet 12 (2011) 301–315.
[136] K. Suzuki, C. Yu, J. Qu, M. Li, X. Yao, T. Yuan, et al., Targeted gene correction minimally impacts whole-genome mutational load in human-disease-specific induced pluripotent stem cell clones, Cell Stem Cell 15 (2014) 31–36.
[137] V. Louis Jeune, J.A. Joergensen, R.J. Hajjar, T. Weber, Pre-existing anti-adeno-associated virus antibodies as a challenge in AAV gene therapy, Hum Gene Ther Methods 24 (2013) 59–67.
[138] E.K. Brinkman, T. Chen, M. Amendola, B. van Steensel, Easy quantitative assessment of genome editing by sequence trace decomposition, Nucleic Acids Res. 42 (2014) e168.
[139] D. Carroll, Progress and prospects: zinc-finger nucleases as gene therapy agents, Gene Ther. 15 (22) (2008) 1463–1468.
[140] J.A. Zuris, D.B. Thompson, Y. Shu, J.P. Guilinger, Efficient delivery of genome-editing proteins in vitro and in vivo, Nature 33 (2015) 73–80.
[141] T. Kubik, K. Bogunia-Kubik, M. Sugisaka, Nanotechnology on duty in medical applications, Curr. Pharm. Biotechnol. 6 (2005) 17–33.
[142] P. Hassanzadeh, Nanopharmaceuticals: innovative theranostics for the neurological disorders, Biomed Rev 25 (2014) 25–34.
[143] Parichehr Hassanzadeh, New perspectives in biosensor technology, Gastroenterology and Hepatology From Bed to Bench 3 (3) (2010) 105–107.
[144] X. Wang, L.-H. Liu, O. Ramstroem, M. Yan, Engineering nanomaterial surfaces for biomedical applications, Exp. Biol. Med. 234 (2009) 1128–1139.
[145] L. Singh, H.G. Kruger, G.E. Maguire, T. Govender, R. Parboosing, The role of nanotechnology in the treatment of viral infections, Ther Adv in Infect Dis 4 (4) (2017) 105–131.
[146] Zohreh Fasili, Freshteh Mehri, Hossein Ali Ebrahimi, Zhaleh Jamali, Elham Mohammad Khanlou, Farzad Kahrizi, et al., Applying nanoparticles in the treatment of viral infections and toxicological considerations, Pharmaceutical and Biomedical Research 5 (2019) 1–20.
[147] Y.-N. Chen, Y.-H. Hsueh, C.-T. Hsieh, D.-Y. Tzou, P.-L. Chang, Antiviral activity of graphene–silver nanocomposites against non-enveloped and enveloped viruses, Int. J. Environ. Res. Public Health 13 (2016) 430.
[148] J.L. Elechiguerra, J.L. Burt, J.R. Morones, A. Camacho-Bragado, X. Gao, H. H. Lara, M.J. Yacaman, Interaction of silver nanoparticles with HIV-1, J. Nanobiotechnology 3 (2005) 6.
[149] R.G. Kerry, S. Malik, Y.T. Redda, S. Sahoo, J.K. Patra, S. Majhi, Nano-based approach to combat emerging viral (NIPAH virus) infection, Nanomed. Nanotechnol. Biol. Med. 18 (2019) 196–220.
[150] M. Mancuso, L. Jiang, E. Cesarman, D. Erickson, Multiplexed colorimetric detection of Kaposi's sarcoma associated herpesvirus and Bartonella DNA using gold and silver nanoparticles, Nanoscale 5 (2013) 1678–1686.
[151] D. Baram-Pinto, S. Shukla, N. Perkas, A. Gedanken, R. Sarid, Inhibition of herpes simplex virus type 1 infection by silver nanoparticles capped with mercaptoethane sulfonate, Bioconjug. Chem. 20 (2009) 1497–1502.
[152] P. Orlowski, E. Tomaszewska, M. Gniadek, P. Baska, J. Nowakowska, J. Sokolowska, Z. Nowak, M. Donten, G. Celichowski, J. Grobelny, et al., Tannic acid modified silver nanoparticles show antiviral activity in herpes simplex virus type 2 infection, PLoS One 9 (2014), e104113.
[153] Mohamed Shehata Draz, Hadi Shafiee, Applications of gold nanoparticles in virus detection, Theranostics 8 (2018) 1985–2017.
[154] V.X. Ting Zhao, T.I. Wong, X. Ting Zheng, Y. Nee Tan, X. Zhou, Colorimetric biosensors for point-of-care virus detections, Materials Science for Energy Technologies 3 (2020) 237–249.
[155] S.M. Shawky, D. Bald, H.M.E. Azzazy, Direct detection of unamplified hepatitis C virus RNA using unmodified gold nanoparticles, Clin. Biochem. 43 (2010) 1163–1168.
[156] Wang, P. Zhang, C.M. Li, Y.F. Li, C.Z. Huang, A highly selective and colorimetric assay of lysine by molecular-driven gold nanorods assembly, Biosens. Bioelectron. 34 (2012) 197–201.
[157] Parichehr Hassanzadeh, Elham Arbabi, Fatemeh Rostami, Fatemeh Atyabi, Rassoul Dinarvand, Carbon nanotubes prolong the regulatory action of nerve growth factor on the endocannabinoid signaling, Physiol Pharmacol 19 (2015) 167–176.

[158] Parichehr Hassanzadeh, Fatemeh Atyabi ElhamArbabi, Rassoul Dinarvand, Application of carbon nanotubes as the carriers of the cannabinoid, 2-arachidonoylglycerol: towards a novel treatment strategy in colitis, Life Sci. 179 (2017) 66–72.
[159] Parichehr Hassanzadeh, Elham Arbabi, Fatemeh Atyabi, Rassoul Dinarvand, Nerve growth factor-carbon nanotube complex exerts prolonged protective effects in an in vitro model of ischemic stroke, Life Sci. 179 (2017) 15–22.
[160] Parichehr Hassanzadeh, Fatemeh Atyabi, Rassoul Dinarvand, Application of carbon nanotubes for controlled release of growth factors or endocannabinoids: a breakthrough in biomedicine, Biomedical Reviews 27 (2017) 41, https://doi.org/10.14748/bmr.v27.2105.
[161] Parichehr Hassanzadeh, Elham Arbabi, Fatemeh Atyabi, Rassoul Dinarvand, Carbon nanotubes provide longer lasting gastroprotective effects for anandamide in stressinduced gastric ulcer in rat, Physiol Pharmacol 22 (2018) 38–47.
[162] A. Mohammadi, A.B. Moghaddam, R. Dinarvand, S. Rezaei-Zarchi, Direct electron transfer of polyphenol oxidase on carbon nanotube surfaces: application in biosensing, Int. J. Electrochem. Sci. 4 (2009) 895–905.
[163] S. Zhu, J. Li, A.-G. Huang, J.-Q. Huang, Y.-Q. Huang, G.-X. Wang, Anti-betanodavirus activity of isoprinosine and improved efficacy using carbon nanotubes based drug delivery system, Aquaculture 512 (2019), 734377.
[164] 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.
[165] G.S. Demirer, et al., High aspect ratio nanomaterials enable delivery of functional genetic material without DNA integration in mature plants, Nat. Nanotechnol. 14 (2019) 456–464.
[166] Y. Li, L. 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 dBSA capped CdTe quantum dots, RSC Adv. 4 (2014) 22545–22550.
[167] 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 fluorescence approach for point-of-care virus detection based on immunomagnetic separation, Anal. Chem. 84 (2012) 2358–2365.
[168] Min Qiu, Zachary Glass, Qiaobing Xu, Nonviral nanoparticles for CRISPR-based genome editing: is it just a simple adaption of what have been developed for nucleic acid delivery? Biomacromolecules 20 (2019) 3333–3339.
[169] Cory D. Sago, Melissa P. Lokugamage, Kalina Paunovska, Daryll A. Vanover, Christopher M. Monaco, et al., High-throughput in vivo screen of functional mRNA delivery identifies nanoparticles for endothelial cell gene editing, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) E9944–E9952.
[170] E.T. Wang, et al., Barcoded nanoparticles for high throughput in vivo discovery of targeted therapeutics, Proc. Natl. Acad. Sci. 114 (2017) 2060–2065.
[171] Parichehr Hassanzadeh, Fatemeh Atyabi, Rassoul Dinarvand, Ahmad-Reza Dehpour, Morteza Azhdarzadeh, Meshkat Dinarvand, 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/s40199-017-0191-z.
[172] Parichehr Hassanzadeh, Elham Arbabi, Fatemeh Atyabi, Rassoul Dinarvand, Ferulic acid-loaded nanostructured lipid carriers: a promising nanoformulation against the ischemic neural injuries, Life Sci. 193 (2018) 64–76.
[173] Parichehr Hassanzadeh, Elham Arbabi, Fatemeh Rostami, Fatemeh Atyabi, Rassoul Dinarvand, Aerosol delivery of ferulic acid-loaded nanostructured lipid carriers: a promising treatment approach against the respiratory disorders, Physiol Pharmacol 21 (2017) 331–342.
[174] Y. Granot-Matok, E. Kon, N. Dammes, G. Mechtinger, D. Peer, Therapeutic mRNA delivery to leukocytes, J. Control. Release 305 (2019) 165–175.
[175] C.D. Sago, et al., High-throughput in vivo screen of functional mRNA delivery identifies nanoparticles for endothelial cell gene editing, Proc. Natl. Acad. Sci. Unit. States Am 115 (2018) E9944–E9952.
[176] W. Zhao, et al., Lipid polymer hybrid nanomaterials for mRNA delivery, Cell. Mol. Bioeng 11 (2018) 397–406.
[177] N. Pardi, et al., Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes, J. Control. Release 217 (2015) 345–351.
[178] A. Thess, et al., Sequence-engineered mRNA without chemical nucleoside modifications enables an effective protein therapy in large animals, Mol. Ther. 23 (2015) 1456–1464.
[179] N. Pardi, et al., Administration of nucleoside-modified mRNA encoding broadly neutralizing antibody protects humanized mice from HIV-1 challenge, Nat. Commun. 8 (2017), 14630.
[180] A. Conway, et al., Non-viral delivery of zinc finger nuclease mrna enables highly efficient in vivo genome editing of multiple therapeutic gene targets, Mol. Ther. 27 (2019) 866–877.
[181] Pengxuan Zhaoa, Xucheng Houa, Jingyue Yana, Shi Dua, Yonger Xuea, Wenqing Lia, et al., Long-term storage of lipid-like nanoparticles for mRNA delivery, Bioactive Materials 5 (2020) 358–363.
[182] A.K.K. Leung, Y.Y.C. Tam, S. Chen, I.M. Hafez, P.R. Cullis, Microfluidic mixing: a general method for encapsulating macromolecules in lipid nanoparticle systems, J. Phys. Chem. B 119 (2015) 8698–8706.
[183] N.M. Belliveau, et al., Microfluidic synthesis of highly potent limit-size lipid nanoparticles for in vivo delivery of siRNA, Mol. Ther. Nucleic Acids 1 (2012), e37.
[184] D. Chen, et al., Rapid discovery of potent siRNA-containing lipid nanoparticles enabled by controlled microfluidic formulation, J. Am. Chem. Soc. 134 (2012) 6948–6951.
[185] J.A. Kulkarni, et al., Fusion-dependent formation of lipid nanoparticles containing macromolecular payloads, Nanoscale 11 (2019) 9023–9031.
[186] Q. Pan, H.W. Tilanus, H.L.A. Janssen, L.J.W. Van Der Laan, Prospects of RNAi and microRNA-based therapies for hepatitis C, Expert. Opin. Biol. Ther. 9 (6) (2009) 713–724.
[187] F.L. Tan, J.Q. Yin, RNAi, a new therapeutic strategy against viral infection, Cell Res. 14 (6) (2004) 460–466.
[188] A. De Fougerolles, H.P. Vornlocher, J. Maraganore, J. Lieberman, Nat. Rev. Drug Discov. 6 (2007) 443–453.
[189] Chang-Jer Wu, Yi-Lin Chan, Antiviral applications of RNAi for coronavirus, Expert Opin. Investig. Drugs 15 (2006) 89–97.
[190] P.Y. Lu, Y.F. Xie, M.C. Woodle, siRNA-mediated antitumorgenesis for drug target validation and therapeutics, Curr. Opin. Mol. Ther. 5 (2003) 225–234.
[191] Q. Du, H. Thonberg, J. Wang, C. Wahlestedt, Z. Liang, A systematic analysis of the silencing effects of an active siRNA at all single-nucleotide mismatched target sites, Nucleic Acids Res. 33 (2005) 1671–1677.
[192] M.S. Weinberg, P. Arbuthnot, Progress in the use of RNA interference as a therapy for chronic hepatitis B virus infection, Genome Medicine 2 (4) (2010) 28–34.
[193] K.A. Whitehead, R. Langer, D.G. Anderson, Knocking down barriers: advances in siRNA delivery, Nat. Rev. Drug Discov. 8 (2009) 129–138.
[194] Y.C. Tam, S. Chen, P.R. Cullis, Advances in lipid nanoparticles for siRNA delivery, Pharmaceutics 5 (2013) 498–507.
[195] R. Cortesi, M. Campioni, L. Ravani, M. Drechsler, M. Pinotti, E. Esposito, Cationic lipid nanosystems as carriers for nucleic acids, New Biotechnol. 31 (1) (2014) 44–54.
[196] H. Wood, FDA approves patisiran to treat hereditary transthyretin amyloidosis, Nat. Rev. Neurol. 14 (2018) 570.
[197] Akin Akinc, Martin A. Maier, Muthiah Manoharan, Kevin Fitzgerald, Muthusamy Jayaraman, Scott Barros, et al., The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs, Nat. Nanotechnol. 14 (2019) 1084–1087.
[198] Muthusamy Jayaraman, Steven M. Ansell, Barbara L. Mui, Ying K. Tam, Jianxin Chen, Xinyao Du, et al., Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo, Angew. Chem. Int. Ed. 51 (2012) 8529–8533.
[199] T. Watanabe, T. Umehara, F. Yasui, et al., Liver target delivery of small interfering RNA to the HCV gene by lactosylated cationic liposome, J. Hepatol. 47 (6) (2007) 744–750.
[200] Josune Torrecilla, Alicia Rodríguez-Gascón, María Ángeles Solinís, Ana del Pozo-Rodríguez, Lipid nanoparticles as carriers for RNAi against viral infections: current status and future perspectives, Biomed. Res. Int. 2014 (1–17) (2014) 161794, https://doi.org/10.1155/2014/161794.
[201] Emily P. Thi, Chad E. Mire, Amy C.H. Lee, Joan B. Geisbert, Joy Z. Zhou, Krystle N. Agans, et al., Lipid nanoparticle siRNA treatment of Ebola virus Makona infected nonhuman primates, Nature 521 (7552) (2015) 362–365. May 21.
[202] Andreas Reichmuth, Matthias Oberli, Ana Jeklenec, Robert S. Langer, mRNA vaccine delivery using lipid nanoparticles, Ther. Deliv. 7 (5) (2016) 319–334.
[203] R.P. Deering, S. Kommareddy, J.B. Ulmer, L.A. Brito, A.J. Geall, Nucleic acid vaccines: prospects for non-viral delivery of mRNA vaccines, Expert Opin. Drug Deliv. 11 (2014) 885–899.
[204] S. John, O. Yuzhakov, A. Woods, J. Deterling, K. Hassett, C.A. Shaw, G. Ciaramella, Multi-antigenic human cytomegalovirus mRNA vaccines that elicit potent humoral and cell-mediated immunity, Vaccine 36 (2018) 1689–1699.
[205] Kimberly J. Hassett, Kerry E. Benenato, Eric Jacquinet, Aisha Lee, Angela Woods, Olga Yuzhakov, et al., Optimization of lipid nanoparticles for intramuscular administration of mRNA vaccines, Molecular Therapy: Nucleic Acids 15 (1) (2019) 1–11.
[206] Jonathan D. Finn, Amy Rhoden Smith, Mihir C. Patel, Lucinda Shaw, Madeleine R. Youniss, Jane van Heteren, et al., A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing, Cell Rep. 22 (2018) 2227–2235.
[207] Brittany E. Givens, Youssef W. Naguib, Sean M. Geary, Eric J. Devor, Aliasger K. Salem, Nanoparticle-based delivery of CRISPR/Cas9 genome-editing therapeutics, AAPS J. 20 (108) (2018) 1–22, https://doi.org/10.1208/s12248-018-0267-9.
[208] F. Schmidt, D. Grimm, CRISPR genome engineering and viral gene delivery: a case of mutual attraction, Biotechnol. J. 10 (2015) 258–272.
[209] Qiang Cheng, Tuo Wei, Lukas Farbiak, Lindsay T. Johnson, Sean A. Dilliard, Daniel J. Siegwart, Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR–Cas gene editing, Nat. Nanotechnol. 1 (2020) 1–10, https://doi.org/10.1038/s41565-020-0669-6.
[210] Roy van der Meel, Einar Sulheim, Yang Shi, Fabian Kiessling, Willem J.M. Mulder, Twan Lammers, Smart cancer nanomedicine: strategic directions to improve translation and exploitation, Nat. Nanotechnol. 14 (11) (2019) 1007–1017. November.
[211] James C. Kaczmarek, Asha K. Patel, Kevin J. Kauffman, Owen S. Fenton, Matthew J. Webber, Michael W. Heartlein, et al., Polymer–lipid nanoparticles for systemic delivery of mRNA to the lungs, Angew. Chem. 128 (2016) 1–6.
[212] J.A. Kulkarni, D. Witzigmann, S. Chen, P.R. Cullis, R. van der Meel, Acc. Chem. Res. 52 (2019) 2435–2444.
[213] R. Mout, M. Ray, G. Yesilbag Tonga, Y.W. Lee, T. Tay, K. Sasaki, et al., Direct cytosolic delivery of CRISPR/Cas9-polyamine-DNA polyplexes, J. Biol. Chem. 278 (45) (2003) 44826–44831.
[214] H. Yue, X. Zhou, M. Cheng, D. Xing, Graphene oxide-mediated Cas9/sgRNA delivery for efficient genome editing, Nanoscale 10 (3) (2018) 1063–1071.

[215] Guojun Chen, Amr A. Abdeen, Yuyuan Wang, Pawan K. Shahi, Samantha Robertson, Ruosen Xie, et al., A biodegradable nanocapsule delivers a Cas9 ribonucleoprotein complex for in vivo genome editing, Nat. Nanotechnol. 14 (10) (2019) 974–980. October.
[216] Chang-Jer Wu, Yi-Lin Chan, Antiviral applications of RNAi for coronavirus, Expert Opin. Investig. Drugs 15 (2006) 89–97.
[217] B.-J. Zheng, Y. Guan, Q. Tang, et al., Prophylactic and therapeutic effects of small interfering RNA targeting SARS-coronavirus, Antivir. Ther. 9 (2004) 365–374.
[218] B.-j. Li, Q. Tang, D. Cheng, et al., Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque, Nat. Med. 11 (2005) 944.
[219] M. Frieman, R. Baric, Mechanisms of severe acute respiratory syndrome pathogenesis and innate immunomodulation, Microbiol. Mol. Biol. Rev. 72 (2008) 672–685.
[220] Ying Wang, Ying-Li Cao, Fan Yang, Yun Zhang, Shu-Hui Wang, Li Liu, Small interfering RNA effectively inhibits the expression of SARS coronavirus membrane gene at two novel targeting sites, Molecules 15 (2010) 7197–7207.
[221] Ulrich Spengler, Liver disease associated with non-hepatitis viruses, Encyclopedia of Gastroenterology (2020) 363–376.
[222] Qiang Cheng, Tuo Wei, Yuemeng Jia, Lukas Farbiak, Kejin Zhou, Shuyuan Zhang, et al., Dendrimer-based lipid nanoparticles deliver therapeutic FAH mRNA to normalize liver function and extend survival in a mouse model of Hepatorenal Tyrosinemia type I, Adv. Mater. (2018) 1–10 (1805308).
[223] Roy van der Meel, Nanotechnology for organ-tunable gene editing, Nat. Nanotechnol. 1 (2020) 1–2, https://doi.org/10.1038/s41565-020-0666-9.
[224] G.M. Sacha, P. Varona, Artificial intelligence in nanotechnology, Nanotechnology 24 (452002) (2013) 1–13, https://doi.org/10.1088/0957-4484/24/45/45200.
[225] P. Hassanzadeh, Computational modelling: moonlighting on the neuroscience and medicine, Biomed Rev 24 (2013) 25–31.
[226] Parichehr Hassanzadeh, Fatemeh Atyabi, Rassoul Dinarvand, Ignoring the modeling approaches: towards the shadowy paths in nanomedicine, J. Control. Release 280 (2018) 58–75.
[227] Parichehr Hassanzadeh, Fatemeh Atyabi, Rassoul Dinarvand, Application of modelling and nanotechnology-based approaches: the emergence of breakthroughs in theranostics of central nervous system disorders, Life Sci. 182 (2017) 93–103.
[228] L.G. Ferreira, R.N. Dos Santos, G. Oliva, A.D. Andricopulo, Molecular docking and structure-based drug design strategies, Molecules 20 (7) (2015) 13384–13421.
[229] J. Šebera, A. Dubankova, V. Sychrovsky, D. Ruzek, E. Boura, R. Nencka, The structural model of Zika virus RNA-dependent RNA polymerase in complex with RNA for rational design of novel nucleotide inhibitors, Sci. Rep. 8 (1) (2018), 11132.
[230] K. Shameer, K.W. Johnson, B. Readhead, Rapid therapeutic recommendations in the context of a global public health crisis using translational bioinformatics approaches: a proof-of-concept study using Nipah virus infection, bioRxiv (2018) 333021, https://doi.org/10.1101/333021.
[231] J.P. Martinez, F. Sasse, M. Brönstrup, J. Diez, A. Meyerhans, Antiviral drug discovery: broad-spectrum drugs from nature, Nat. Prod. Rep. 32 (1) (2015) 29–48.
[232] T.F. Zhang, Q. Chen, Identification of contaminant sources in enclosed environments by inverse CFD modeling, Indoor Air 17 (2007) 167–177.
[233] S. Mazumdar, S.B. Poussou, C.-H. Lin, S.S. Isukapalli, M.W. Plesniak, Q. Chen, Impact of scaling and body movement on contaminant transport in airliner cabins, Atmos. Environ. 45 (2011) 6019–6028.
[234] Marek Ostaszewski, Alexander Mazein, Marc E. Gillespie, Inna Kuperstein, Anna Niarakis, Henning Hermjakob, et al., COVID-19 disease map, building a computational repository of SARS-CoV-2 virus-host interaction mechanisms, Scientific Data 7 (2020) 136, https://doi.org/10.1038/s41597-020-0477-8.
[235] A. Renz, L. Widerspick, A. Dräger, 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. Villaveces, P. Koti, 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 Vyas, Nanorobotic applications in medicine: current proposals and designs, Am J Robot Surg 1 (1) (2014) 4–11.
[241] D. Murphy, B. Challacombe, T. Nedas, O. Elhage, K. Althoefer, L. Seneviratne, et al., Equipment and technology in robotics, Arch. Esp. Urol. 60 (2007) 349–354.
[242] Parichehr Hassanzadeh, Fatemeh Atyabi, Rassoul Dinarvand, Creation of nanorobots: both state-of-the science and state-of-the-art, Biomedical Reviews 27 (2016) 37–44.
[243] G.M. Patel, G.C. Patel, R.B. Patel, J.K. Patel, M. Patel, Nanorobot: A versatile tool in nanomedicine, J. Drug Target. 14 (2006) 63–67.
[244] Y.S. Lee, J.Y. Bae, H.Y. Koo, Y.B. Lee, W.S. Choi, A remote-controlled generation of gold@polydopamine (core@ shell) nanoparticles via physical-chemical stimuli of polydopamine/gold composites, Sci. Rep. 6 (2016) 22650.
[245] S. Martel, J.B. Mathieu, O. Felfoul, A. Chanu, E. Aboussouan, S. Tamaz, et al., A computer-assisted protocol for endovascular target interventions using a clinical MRI system for controlling untethered microdevices and future nanorobots, Comput Aided Surg 13 (2008) 340–352.
[246] S. Balasubramanian, D. Kagan, H.C.M. Jack, S. Campuzano, M.J. Lobo-Castañon, N. Lim, et al., Micromachine-enabled capture and isolation of cancer cells in complex media, Angew Chem Int Ed Engl 50 (2011) 4161–4164.
[247] R.R. Patel, Nanorobotics ideas in nanomedicine, Asian J Pharm Sci Res 3 (2013) 15–22.
[248] S. Martel, O. Felfoul, M. Mohammadi, J.B. Mathieu, Interventional procedure based on nanorobots propelled and steered by flagellated magnetotactic bacteria for direct targeting of tumors in the human body, Conf Proc IEEE Eng Med Biol Soc 2008 (2008) 2497–2500.
[249] M.B. Somanna, Nanobots: the future of medical treatments, Int J Sci Tech Res 4 (2015) 276–278.
[250] E. Torelli, M. Marini, S. Palmano, L. Piantanida, C. Polano, A. Scarpellini, et al., A DNA origami nanorobot controlled by nucleic acid hybridization, Small 10 (2014) 2918–2926.
[251] S.M. Douglas, I. Bachelet, G.M. Church, A logic-gated nanorobot for targeted transport of molecular payloads, Science 335 (2012) 831–834.
[252] B. Behkam, M. Sitti, Bacterial flagella-based propulsion and on/off motion control of microscale objects, Appl. Phys. Lett. 90 (2007) 1–3.
[253] A. Dubey, G. Sharma, C. Mavroidis, M.S. Tomassone, K. Nikitczuk, M. L. Yarmushc, Computational studies of viral protein nano-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) 531–569.
[255] S.P. Ahuja, J.R. Myers, A survey on wireless grid computing, J. Supercomput. 37 (1) (2006) 3–21.
[256] L. Ricciardi, I. Pitz, S.F. Al-Sarawi, V. Varadan, D. Abbott, Investigation into the future of RFID in biomedical applications, Proc. SPIE - Int. Soc. Opt. Eng. 5119 (2003) 199–209.
[257] Si Chen, Heng Sun, Kai Miao, Chu-Xia Deng, CRISPR-Cas9: from genome editing to cancer research, Int. J. Biol. Sci. 12 (12) (2016) 1427–1436.
[258] Huan Deng, Wei Huang, Zhiping Zhang, Nanotechnology based CRISPR/Cas9 system delivery for genome editing: Progress and prospect, Nanoresearch 1 (2019) 1–14, https://doi.org/10.1007/s12274-019-2465-x.