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Review

Nanobased Platforms for Diagnosis and Treatment of COVID-19: From Benchtop to Bedside

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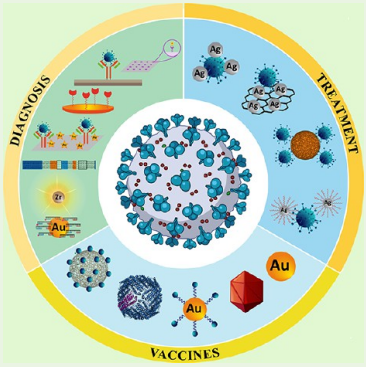
ABSTRACT: Human respiratory viral infections are the leading cause of morbidity and mortality around the world. Among the various respiratory viruses, coronaviruses (e.g., SARS-CoV-2) have created the greatest challenge and most frightening health threat worldwide. Human coronaviruses typically infect the upper respiratory tract, causing illnesses that range from common cold-like symptoms to severe acute respiratory infections. Several promising vaccine formulations have become available since the beginning of 2021. Nevertheless, achievement of herd immunity is still far from being realized. Social distancing remains the only effective measure against SARS-CoV-2 infection. Nanobiotechnology enables the design of nanobiosensors. These nanomedical diagnostic devices have opened new vistas for early detection of viral infections. The present review outlines recent research on the effectiveness of nanoplatforms as diagnostic and antiviral tools against coronaviruses. The biological properties of coronavirus and infected host organs are discussed. The challenges and limitations encountered in combating SARS-CoV-2 are highlighted. Potential nanodevices such as nanosensors, nanobased vaccines, and smart nanomedicines are subsequently presented for combating current and future mutated versions of coronaviruses.



KEYWORDS: *COVID-19, coronavirus, nanobiosensor, nanobased vaccine, SARS-CoV-2*

1. INTRODUCTION

Coronavirus pandemics have emerged rapidly in the 21st century, with catastrophic consequences.[1,2] The first severe acute respiratory syndrome coronavirus (SARS-CoV) pandemic, SARS-CoV-1, occurred in southern China in late 2002 and infected more than 8000 people with ∼10% mortality globally.[3] This was followed by the emergence of the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 that infected about 2494 people with a mortality rate of 34.4%.[4,5] In late 2019, the new SARS-CoV-2 pandemic, often referred to as coronavirus disease 2019 (COVID-19), emerged in Wuhan, China, and spread quickly to all countries around the world.[6] As of February 26, 2021, the World Health Organization (WHO) reported a total of 112 million confirmed cases with 2.5 million deaths.[7] Over the last 12 months or so, considerable efforts have been made to rapidly develop COVID-19 vaccines to protect and mitigate the effects of this deadly disease on the human population. However, with more than 2.5 million deaths to date, there is an urgent need for fast and reliable diagnostic and therapeutic approaches against SARS-CoV-2 infections. Many aspects of the currently available vaccine formulations remain to be clarified, including the safety of administration on the pediatric population and their effectiveness against emerging viral strains.

Nanotechnology has opened up new horizons in many different aspects of medical science, such as targeted gene delivery, targeted drug delivery, biosensor platforms, imaging, and diagnosis.[8,9] Nanomaterials have been developed to combat viral, bacterial, and fungal infections[10] because of their unique physicochemical characteristics, such as high surface area, nanoscale dimensions, and readily achievable surface modifications. These properties enable scientists to improve drug pharmacokinetics, control drug release, enhance drug solubility, facilitate cellular membrane passage, and enhance the bioavailability of pharmaceutics against a series of viruses such as human immunodeficiency virus, herpes simplex virus, and hepatitis B virus.[11,12] Nanomaterials are promising tools for the diagnosis and treatment of COVID-19.

The present review systematically outlines the recent advances reported in the literature on the use of nanoparticles as effective diagnostic and antiviral treatment tools against recently mutated coronaviruses. In addition, an overview of the

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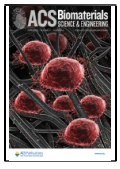

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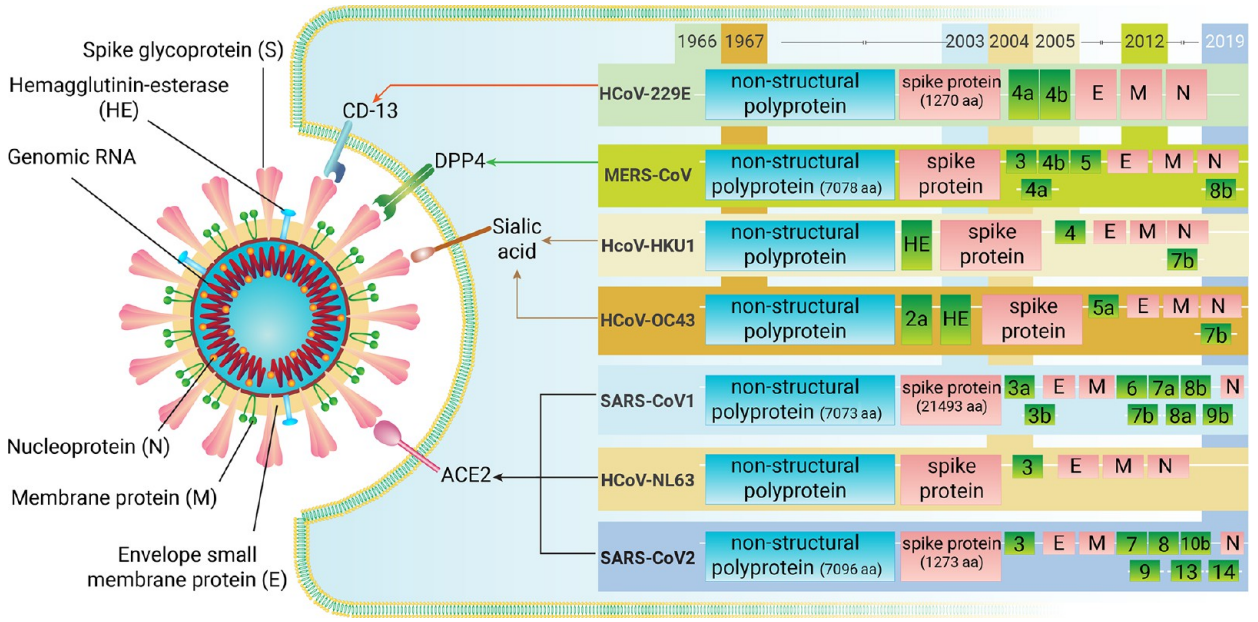

Figure 1. Biological properties of different types of human coronaviruses (HCoVs) that emerged over the years. In general, arrangements of the envelope (E), membrane (M), and nucleocapsid (N) proteins are different among the CoVs. The size of nonstructural proteins (NSPs) is varied in different CoVs strains. For example, 30 119 bp (7078 aa) in MERS-CoV, 29 844 bp (7096 aa) in SARS-CoV-2, and 29 751 bp (7073 aa) in SARS-CoV-1. The specific receptors used by CoVs are also different: 9-O-acetylated sialic acid is utilized by HCoV-OC43 and HCoV-HKU1, human aminopeptidase N (CD13) by HCoV-229E, dipeptidyl peptidase 4 (DPP4) by MERS-CoV, and angiotensin-converting enzyme 2 (ACE2) by HCoV-NL63, SARS-CoV1, and SARS-CoV2. Abbreviations: human coronaviruses, HCoVs; human aminopeptidase N, CD13; dipeptidyl peptidase 4, DPP4; angiotensin-converting enzyme 2, ACE2; nonstructural proteins, NSPs.

biological properties of all human coronaviruses is provided, with evaluation of their differences and site-specific infection of the human body. The challenges and limitations encountered by this technology are discussed. Nanotechnology offers multiple roles in combating coronavirus infections, such as nanosensors, nanobased vaccines, and smart medicine.

2. HUMAN CORONAVIRUSES: AN OVERVIEW ON BIOLOGICAL PROPERTIES

To date, seven known coronaviruses (HCoVs) have been identified that infect humans. They belong to the family Coronaviridae and include SARS-CoV-1,[13,14] HCoV-229E,[15] HCoV-NL63,[15] HCoV-OC4),[16] HCoV-HKU1,[17] MERS-CoV,[18] and SARS-CoV-2.[19] The HCoV-229E and HCoV-NL63 are identified as *Alphacoronaviruses*, whereas HCoV-HKU1, HCoV-OC43, SARS-CoV1, SARS-CoV2, and MERS-CoV are classified as *Betacoronaviruses*.[20] As a coronavirus that infects humans, SARS-CoV-2 is genetically similar to SARS-CoV-1 (~79%) and MERS-CoV (~50%).[21]

Coronaviruses are enveloped, positively sensed, single-stranded RNA with spherical capsids (120−160 nm) that collectively resemble a crown with a solar shape.[22,23] The CoV genome is about 26.4−31.7 kb, which is the largest among RNA viruses with guanine and cytosine contents varying from 32 to 43%.[24] Genomic RNA acts as a mRNA (mRNA) which plays a key role in the replication of the viral genome and production of new infectious virus particles.[25] The 5′ untranslated region (5′ UTR) and 3′ untranslated region (3′ UTR) are the regions of mRNA involved in many regulatory aspects of gene expression with a major role in RNA−RNA interactions for binding with viral and cellular proteins.[26] A typical CoV comprises at least six-open reading frames (ORFs). Two-thirds of the genome consists of ORF1a and ORF1b, which produce two polypeptides, pp1a and pp1ab. These polypeptides are processed by viral proteases (e.g., 3-C-like protease (3CLpro), main protease (Mpro) and papain-like protease (PLpro)) for cleaving the 16 nonstructural proteins (NSPs) that are involved in genome transcription and replication.[27] The sizes of the NSPs vary in different CoV strains.[21]

Coronaviruses have four canonical structural proteins including spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N). Besides, there are several nonstructural proteins that are encoded by ORFs 10 and 11 on one-third of the genome near the 3′ end.[28] The S protein is a large glycosylated transmembrane protein (1160−1400 aa) that plays an essential role in the recognition of cellular receptors for infection of a susceptible cell. The size of this protein differs among the coronaviruses: 21493 aa, 1270 aa, and 1273 aa for SARS-CoV-1, MERS-CoV, and SARS-CoV-2, respectively.[21] The E protein is a small envelope protein (74−109 aa) responsible for the assembly of virions and curving of the viral envelope.[29] The M protein is an integral glycoprotein (250 aa), which has three transmembrane regions and interacts with other structural proteins to maintain the virion structure.[30] The N protein is a heavily phosphorylated nucleocapsid protein (500 aa), which has a key role in encapsulating the viral genome into helical nucleocapsid within the viral particles.[31] The arrangement of N, E, and M proteins among coronaviruses is different, as shown in Figure 1.[32,33] The *Betacoronavirus* genus has an additional structural protein, hemagglutinin-esterase (HE, 430 aa) and a transmembrane protein that forms homodimers.[33] The HE protein has acetyl-esterase function that is not necessary for in vitro viral replication. However, the HE protein may affect early viral infection in vivo by binding reversibly to O-acetylated sialic acids. The 3a/b and 4a/b proteins are other mature proteins

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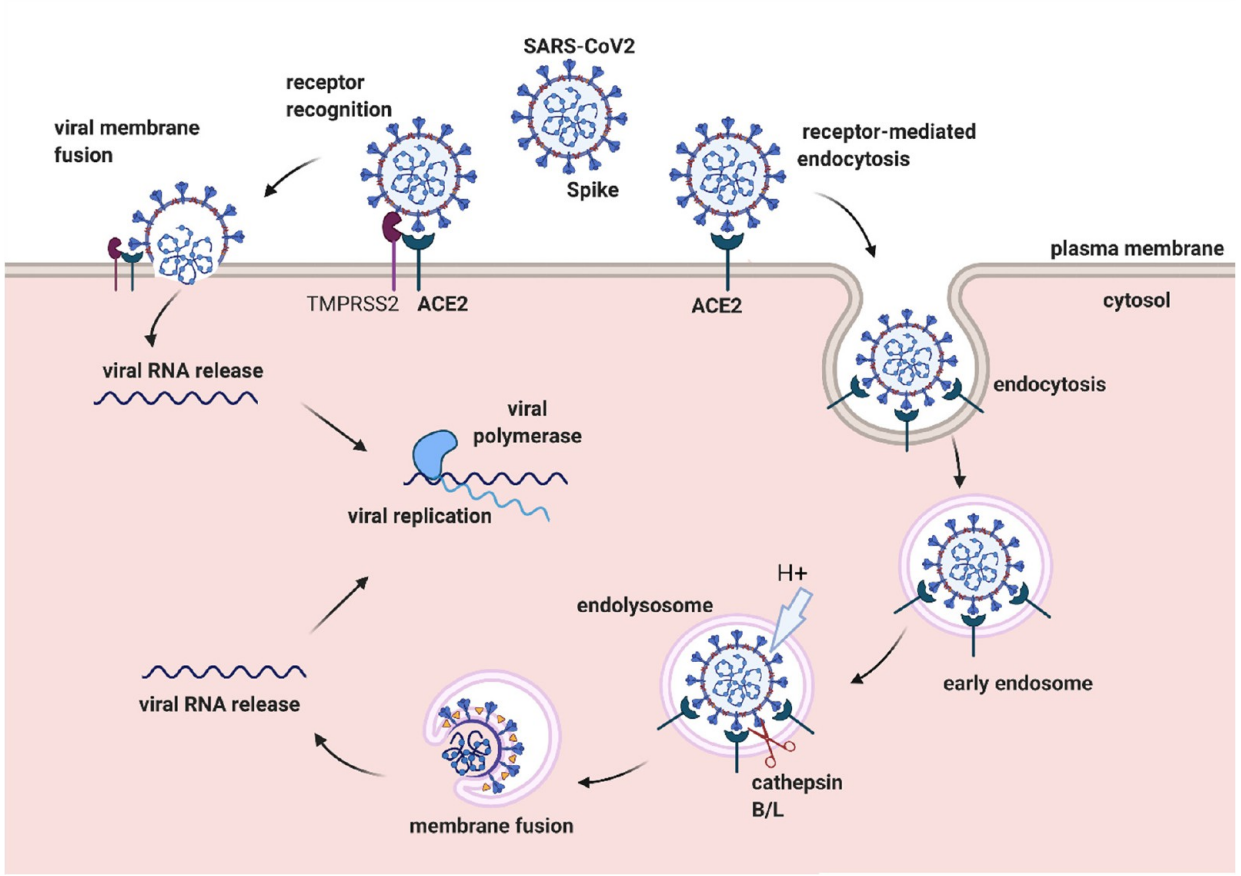

Figure 2. Schematic of the mechanism of entry of SARS-CoV-2 into a host cell. Binding of the SARS-CoV-2 to the cell surface is facilitated by host cellular proteins. The recognition and binding of virions occur via interaction between virion-associated spike protein and the host's ACE2 receptor. Activation of the spike protein is mediated by the cell surface serine protease TMPRSS2, which mediates the fusion of the viral membrane with the cell plasma membrane and the release of the viral RNA into the cytoplasm of the host cells. In the absence of the cell surface proteases, after the engagement of the ACE2 receptor, entry of the SARS-CoV-2 occurs via clathrin-mediated endocytosis. During endosome maturation, the low pH activates endosomal cysteine proteases cathepsin B/L, which prime the S protein, allowing membrane fusion and release of the viral RNA from the late endosomes/lysosomes. Abbreviations: angiotensin-converting enzyme 2, ACE2.

responsible for various important functions in virus replication and genome maintenance.[27]

The receptors utilized by human CoVs typically include 9-O-acetylated sialic acid by HCoV-OC43 and HCoV-HKU1,[34] human aminopeptidase N (CD13) by HCoV-229E,[35,36] dipeptidyl peptidase 4 (DPP4) by MERS-CoV,[37] and angiotensin-converting enzyme 2 (ACE2) by HCoV-NL63, SARS-CoV1, and SARS-CoV2.[35,38] In addition, protease can help CoVs enter cells. For example, transmembrane protease serine 2 (TMPRSS2) and airway trypsin-like protease TMPRSS11D activate the S protein in HCoV-229E, SARS-CoV-1 and SARS-CoV-2 infections,[39] while cathepsin L is activated in SARS-CoV and MERS-CoV.[40] After the virus enters a susceptible cell, the genome is transcribed and translated. Replication and transcription of the coronavirus genome occur with continuation/discontinuation of RNA synthesis that is mediated by a huge replicase complex.[41] The replicase complex is about 20 kb and contains up to 16 viral subunits along with a number of host cellular proteins.[42] After the cellular and molecular processes, the protein is assembled on the cell membrane. Genomic RNA that buds off the internal cell membranes is converted to the mature particle forms.[43]

3. MECHANISM OF ENTRY OF CORONAVIRUSES INTO CELLS

Blocking of entry of coronaviruses into the host cell is one of the basic approaches in preventing viral infections. Because the pathogenesis of coronaviruses has not been fully understood, the precise molecular mechanism by which the virus enters a cell is unknown.[44] Two routes are used by CoVs for entering human cells. These routes are categorized as direct delivery of the viral genome into the cytosol through fusion with the host cell membrane and through endocytosis (Figure 2).[45]

Coronaviruses enter the host cell through the interaction of their structural spike protein with cell surface receptors. The S1 subunit of the viral spike protein binds with its receptor through the receptor binding domain (RBD), after which fusion with the viral cell membrane commences through the spike S2 subunit.[46] The ACE-2 receptor is the major receptor for entry of the SARS-CoV-1 and SARS-CoV-2 into a human host.[47] Moreover, neuropilin-1 (NRP1) and CD147 were recently identified as host cofactors that enhancing the entry of SARS-CoV-2 via endocytosis.[48,49] Proteolytic cleavage at the S1/S2 and S2′ sites by host cell proteases is required for the conformational changes of the S protein and for the viral fusion with the cell membranes. The cell surface serine protease transmembrane serine protease 2 (TMPRSS2) and endosomal

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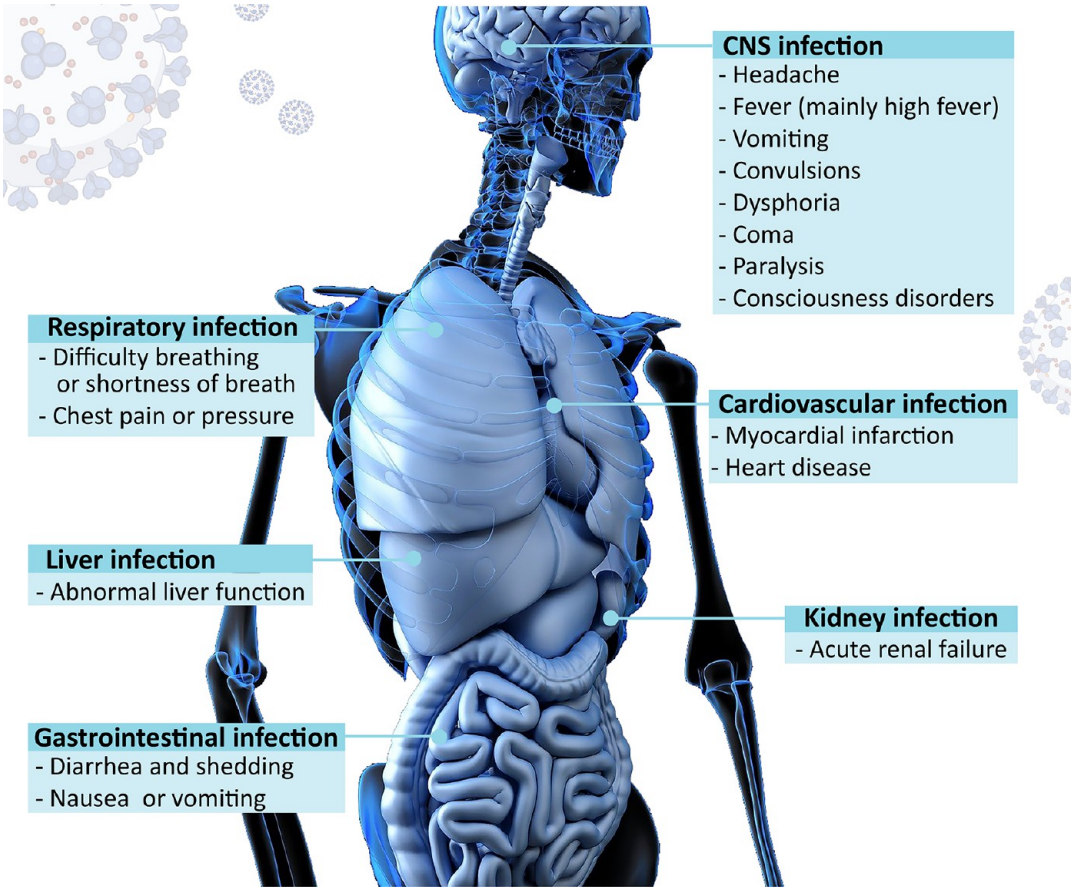

Figure 3. Host organs involved in coronavirus infection and the corresponding symptoms.

cysteine proteases cathepsin B and L (CatB/L) are responsible for the activation of the spike proteins.[50] After binding to the ACE2 receptor, host proteases on the cell surface mediate virus fusion at the level of the plasma membrane or with the endosomal membrane, with subsequent release of the viral genome into the host's cytosol.[51] Release of endocytosed virions into the cytosol is usually dependent on the pH of the endosomes, whereas direct entrance of the virions into the cytosol is pH-independent.

Recently, it has been proposed that Sars-CoV-2 employs clathrin-mediated endocytosis as the mechanism for cell entry.[52] However, similar to the SARS-CoV and other CoVs, SARS-CoV-2 may utilize multiple pathways to gain access into the host cell cytosol.[53−57] To date, 11 clinically approved generic drugs have been identified as potential candidates for blocking the routes of entry of SARS-CoV-2, including direct fusion with the cell membrane.

4. INFECTION OF HOST ORGANS BY CORONAVIRUS

Although human COVs generally cause upper respiratory tract infections with relatively mild symptoms, SARS-CoV-1, MERS-CoV and the recent SARS-CoV-2 have caused severe epidemics of acute respiratory syndromes. Because viruses are cleared by the immune system, viral infections typically remain in the respiratory tract with minimal local clinical consequences.[58] However, in some cases, viruses can evade the immune system and spread to other tissues, including the respiratory system, central nervous system, cardiovascular system, gastrointestinal system, liver, and kidney, where they induce other types of pathologies (Figure 3).[59,60]

4.1. Respiratory System Infection. The most common complication of coronaviruses is respiratory system infection. The clinical manifestations include fever, dry cough, dyspnea, and fatigue. Pulmonary manifestations of the recent COVID-19 pandemic have varied from asymptomatic infection to respiratory failure and death.[61] The main receptor for the entry of SARS-CoV-1 and SARS-CoV-2, the ACE2 receptor, is heterogeneously distributed in the upper and lower respiratory tract. It is expressed at high levels in the sinonasal cavity and pulmonary alveoli, as well as on the apical side of type II alveolar epithelial cells in the lung parenchyma. This partially explains the preference of lung cells as a target for replication of these viruses.[62] SARS-CoV-2 infection causes strong alveolar injury and acute interstitial pneumonia. The latter is characterized by macrophage infiltration, hyaline membrane formation, and alveolar wall edema and thickening.[63] There are also pulmonary vascular abnormalities with pulmonary vessel hyaline thrombosis, hemorrhage, neutrophils, and lymphocyte infiltration. These symptoms are collectively described as diffuse pulmonary intravascular coagulopathy.[64]

Among the factors that determine a poor prognosis of the COVID-19 disease, there is the huge inflammatory over-reaction due to the excessive increase in circulating proinflammatory cytokines. The latter include interleukin (IL-1), IL-6, IL-12, interferons, and tumor necrosis factor (TNF)-α. This "cytokine storm" ultimately leads to an acute respiratory distress syndrome (ARDS), which is characterized by endothelial cell dysfunction, damage of the vascular barrier, capillary leakage, and diffuse alveolar damage.[65]

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Other factors involved are enormous oxidative/nitrosative stress following the entrance of the virus, with the occurrence of apoptotic cell death and necrosis.[66] A severe form of ARDS with low oxygen saturation levels and respiratory failure is the leading cause of mortality for SARS-CoV-2.

The molecular mechanism involved in the pathogenesis of SARS-CoV-2 is characterized by cytokine dysregulation. Accordingly, cytokine blockers such as tocilizumab, sarilumab, and siltuximab monoclonal antibodies[67] or corticosteroids such as dexamethasone[68] are considered promising therapeutic candidates for counteracting lung hyper-inflammation. These medications generally improve clinical outcomes.

4.2. Central Nervous System Infection. Detection of CoVs RNA in human brain samples indicates that these viruses are neuroinvasive and neurotropic, with the capability of causing CNS diseases.[69] It has been demonstrated that HCoV-OC43 RNA has the potential to cause persistent infection in human CNS cells for at least one year in a murine model of acute viral encephalitis.[70] In murine CNS, neurons were the main target of viral infection; the neurons were degenerated via programmed cell death.[71] The S glycoprotein of the virus plays a major role in the neurodegenerative mechanism.[72] Infections involving HCoV-229E, HCoV-OC43, SARS-COV-1, and SARS-COV-2 have been identified in various human neurological diseases, such as Parkinson's disease, multiple sclerosis, and acute disseminated encephalomyelitis.[73−75] To date, there have been no reports on the presence of HCoV-HKU1, HCoV-NL63, or MERS-CoV in the central nervous system of humans. However, several studies have shown that neurological symptoms are associated with HCoV-HKU1, HCoV-NL63, and MERS-CoV.[76,77] SARS-CoV-2 was detected in capillary endothelial cells in the frontal lobe tissues obtained from the post-mortem examination.[78] According to that report, viral infections that cause neurodegenerative diseases can impair the function of the blood brain barrier and illicit a systemic inflammatory response.[79] The systemic inflammation triggered by coronavirus infection may cause neuroinflammatory reactions that increase susceptibility of the infected individual to neurological disorders.[58] Infection of the central nervous system may expedite the progression of neurodegenerative diseases in at-risk individuals.

4.3. Gastrointestinal Infection. The relation between respiratory infection and the gastrointestinal tract has not been completely understood. Patients with respiratory infections typically have intestinal dysfunction. This is indicative of the crosstalk between the gastrointestinal tract and the lung.[59,80] A recent case study identified SARS-CoV-2 RNA in a stool specimen, where the virus utilized ACE2 receptor for the entry into the cells.[81] Indeed, ACE2 expression correlates with neutral amino acid transporter B0AT1 (SLC6A19) expression in the gastrointestinal tract, which increases the susceptibility of an individual to CoV infection.[82]

4.4. Cardiovascular Infection. Myocardial damage caused by CoV infection increases the complexity of patient treatment. Recent studies reported that MERS-CoV and SARS-CoV-2 can cause severe myocarditis and heart dysfunction.[83,84] The mechanism of severe myocardial damage caused by CoV infection may be related to the ACE2 cell surface receptor. Indeed, ACE2 is extensively expressed both in the lung and in the cardiovascular tract. Hence, ACE2-related signaling pathways may have a key role in heart dysfunction.[85] Other suggested mechanisms of myocardial injury include a cytokine storm that is triggered by an imbalance between type 1 and type 2 T-helper cells, and respiratory dysfunction and hypoxemia caused by SARS-CoV-2 that result in damage of the myocardial cells.[86]

4.5. Liver Infection. Post-mortem examination of patients infected by SARS-CoV identified the presence of a large number of virus particles in the lungs, liver vascular endothelium, and parenchymal cells.[87] In addition, SARS-CoV-1 RNA was demonstrated in hepatocytes by reverse transcription-polymerase chain reaction (RT-PCR).[88] Because the ACE2 receptor is abundantly expressed in the endothelial cells of liver, it has been proposed that SARS-CoV-1 utilizes this receptor for cell entry.[89] Both liver cells and bile duct epithelial cells express ACE2 receptors.[90] However, bile duct cells express more ACE2 receptors than liver cells. Because bile duct epithelial cells play an important role in liver regeneration and immune response,[91] it has been suggested that liver damage that occurs in CoV patients is attributed to the damage of bile duct cells and not the virus infection.

Liver enzymes and bilirubin levels increased in patients with MERS-CoV infection, whereas albumin levels decreased.[92−96] A more recent study reported that liver dysfunction in patients with severe SARS-CoV-2 infection was significantly more extensive than that patients with mild SARS-CoV-2 infection only.[81] In those patients with severe SARS-CoV-2 infection, the levels of liver enzymes such as alanine aminotransferase, aspartate transaminase, and gamma-glutamyl transferase are considerably high.[97]

Patients infected with coronavirus who have other liver comorbidities such as hepatitis B virus (HBV) or hepatitis C virus (HCV) infections are more susceptible to liver damage and the manifestation of acute hepatitis. This may be attributed to the promoted replication of the hepatitis virus during CoV infection.[98] The antibiotics, antiviral medication, and other drugs used for the treatment of CoV infection probably cause liver dysfunction.[99,100]

4.6. Kidney Infection. Studies have shown that CoVs (SARS and MERS-CoV) can attack the kidney and cause acute kidney injury.[49,101] It is well shown that ACE2 receptors are not only expressed in the lung, heart, liver, and brain but are also present in the kidney.[102,103] Thus, the virus can utilize this receptor for entry into the kidney. Patients suffering from SARS-CoV-2 infection have been reported to have a higher frequency of renal and kidney abnormalities.[104]

5. DIAGNOSIS

The innate immune system provides excellent defense against viruses, otherwise primary prevention is the only alternative option. For this reason, diagnosis remains the most effective approach to control virus infection.[105] There is growing interest in virus detection through the use of molecular-based techniques. These approaches have been classified into the amplification or nonamplification molecular-based techniques.[106]

Molecular-based techniques are more rapid and sensitive than serological techniques, either as a simple method for the manual detection of viruses or as a part of highly developed techniques.[107] Fully automated detection systems are generally preferred in medicine. Biosafety issues and time concerns associated with the clinical usage and study of viruses are eliminated with the use of such systems. Despite all the purported benefits associated with the newly developed molecular techniques, there are still potential restrictions regarding their accuracy, sensitivity, specificity, and even

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reproducibility. These restrictions are mainly caused by the genetic inconsistency of viruses.[108,109] In addition, these assays are expensive and time-consuming, requiring specific laboratory instruments as well as expert human resources.[110] Nanomaterials with unique properties, including optical, electronic, mechanical, and magnetic characteristics, are considered attractive substrates for biomedical imaging and clinical diagnosis.[111,112] Table 1 compares the rants and raves of common virus detection methods. A wide range of nanomaterials has been proposed for virus detection. These nanomaterials include metal, silica and polymeric nanoparticles, quantum dots (QDs), and carbon nanotubes (Table 2).[113]

According to the WHO, the current trend of CoV diagnostics is focused on the development of nucleic acid- or protein-based detection methodology for point-of-care testing (POCT).[120,121] Nanobiohybrid platforms, containing at least one component derived from virus (e.g., nucleic acid, antibody, antigen, or structural peptide) are conjugated to various NPs.[122] These systems rely on functioning of NPs as well as the activity of the conjugated biomolecules and/or compact multivalent probes for signal transduction.[123] These specific NP-based probes are used in a variety of optical, electrical, and electrochemical assays for single and multiple virus detections.[124]

A quantum dot-conjugated RNA oligonucleotide system has been designed for highly sensitive imaging. The system was installed on a biochip for the recognition of SARS-CoV-1 nucleocapsid (N) protein.[125] More recently, RT-PCR was combined with lateral flow immunoassay for rapid detection of MERS-CoV.[126] Nucleic acid testing can also be combined with the lateral flow assay. For example, a multiplex colorimetric paper-based analytical device was developed using AgNPs as a colorimetric substrate to detect the DNA associated with MERS-CoV infection.[127] Another system was developed using self-assembled nanostructure that consisted of AuNPs and quantum dots.[128] This platform was used as an immunosensor for the detection of Avian coronavirus (IBV) infected birds. Nanonested PCR was employed with AgNPs to distinguish between the variant and the classical strains of porcine epidemic diarrhea corona virus.[129] In another study, a method was developed for detection of IBV using magnetoplasmonic NPs and zirconium-quantum dots conjugated with IBV antibodies.[130] Notably, there was no reaction between the magnetoplasmonic NPs and Zr-quantum dots until the targeted virus was added.[131] Compared with conventional analysis, this immunosensor possesses remarkable advantages, including higher sensitivity, faster analysis and accuracy comparable to enzyme-linked immunosorbent assay. In 2019, a range of signal amplifying techniques were introduced, including thermal imaging and assembly of multiple AuNPs, for improving the lateral flow readout signals for the detection of MERS-CoV (Figure 4).[132]

An immunochromatographic strip (ICS) was introduced for the detection of IBV in infected chickens based on the use of IBV-specific monoclonal antibodies against S glycoprotein and N proteins.[133] Monoclonal antibody−colloidal gold conjugates were utilized as tracers during the preparation of ICS. The assembled ICS was identified as a specific test for IBV antigens, compared to RT-PCR.[133] Considering that RT-PCR is an expensive technique, the AuNP-ICS method appears to have the potential for rapid detection of different IBV strains in chickens.

Table 1. Advantages and Cons of Common Virus Detection Methods

	technique	detection base	advantages	disadvantages	ref
basic detection	cell culture	infection test	broad spectrum; low-cost	difficulty in maintaining cell cultures; lengthy test	114
	electron microscopy	viral particle	broad spectrum; low-cost	require the presence of ~106 virus particles/mL for detection; similarity of morphologies	115
serological detection	immunoblotting assay, neutralization assay, immunochromatographic test and complement-fixation test, enzyme-immunoassay/chemiluminescent immunoassay, radioimmunoassay, immunoprecipitation assay, hemagglutination-inhibition	viral protein	easy; low-cost	poor sensitivity; necessity for fresh reagents	116,117
molecular detection	polymerase chain reaction, reverse transcription polymerase chain reaction, loop-mediated isothermal amplification	viral nucleic acid	high sensitivity; easy to set up	extremely liable to contamination; not easy to quantitate results; high-skill operator required	118
nanobased detection	nanobiosensors	viral protein/nucleic acid	extremely high selectivity and sensitivity; high stability; fast response; portable system	pH and temperature influence the selectivity and sensitivity of biosensor	119

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Table 2. Summary of Representative Engineered Nanomaterials Employed As Biosensors for Virus Detection[a]

	nanoparticles (NPs)	characteristics	target viruses	biosensor type	ref
inorganic nanoparticles	silver (AgNPs)	fluorescent properties of AgNPs introduce high sensitivity to optical-based biosensors	HBV HIV CoVs KSHV WNV influenza	optical/ electrochemical	141−144
	gold (AuNPs)	AuNPs have been used extensively for highly sensitive detection of viral diseases due to their unique optical and electrical properties	HTNV RVFV DENV HEV KSHV IAV HPV HIV CoVs	optical/ electrochemical	145−148
	magnetic (MNPS)	controllable by an external magnet; MNPs are extensively utilized in reusable biosensor platforms	IAV HBV CoVs	piezoelectric/ electrochemical	149,150
	zinc oxide (ZnO)	with piezoelectric properties, ZnO plays a main role in special sensors known as mechano-chemicals	HIV	piezoelectric/ electrochemical	151
	copper NPs	small size and high surface-to-volume ratio of copper NPs enable them to interact closely with viruses for easy detection	HBV IAV	electrochemical	152,153
	aluminum (AlNPs)	nanoporous morphology of AlNPs is the most prominent and attractive feature for designing biosensors; porous structure enhances the surface-to-volume ratio that results in an increased number of target molecules inside pores	DENV Ebola	electrochemical	154,155
	quantum dots (QDs)	QDs are nanosize particles with unique optical and electrical properties and are powerful tools for providing rapid and sensitive virus detection to facilitate early treatment and monitoring of viral disease	HIV HBV EBV CoVs	optical/ electrochemical	156−158
	silica NPs	many biomolecules, such as antigen-antibodies, peptides and DNA, can be attached to the surface of silica NPs, making this platform important for bioanalytical studies	HBV HPV	optical	159,160
organic nanoparticles	carbon nanotubes (CNTs)	CNT-based biosensors possess high selectivity and sensitivity due to their high surface area; this platform is also useful because of their ease of functionalization	HBV HPV influenza	electrochemical/ FET	161−163
	graphene oxide (GO)	size controllability of GO nanosheets and changes in their oxidation level are unique features for this biosensor platform to detect specific viruses	HBV HIV HIV-1 rotavirus	optical/ electrochemical/ potentiometric	164−168

[a]Abbreviations: hepatitis B virus, HBV; human immunodeficiency virus, HIV; human papilloma virus, HPV; dengue virus, DENV; Hantaan virus, HTNV; Rift Valley fever virus, RVFV; hepatitis E virus, HEV; Kaposi's sarcoma-associated herpesvirus, KSHV; influenza A virus, IAV; field effect transistor, FET.

Lateral flow detection of SARS-CoV-2 antigen has been used to improve COVID-19 diagnosis as a point-of-care approach.[134] In the lateral flow assays, a paper strip is coated with AuNP−antibody conjugates in the first line and with capture antibodies in the second. A urine or blood sample is placed on the strip, while the proteins of interest are placed on the membrane.[135] The viral antigens bind to the coated AuNPs in the first line as the sample runs through the membrane by capillary action. When the antigen/AuNP−antibody complex flows through the strip, it is immobilized by the capture antibodies in the second line and a colored line appears. The color of the complex (blue) is different from the color of the NPs (red) because of plasmon effect. Although this kind of assay shows 100% specificity for IgM and IgG, the clinical sensitivity and accuracy are different (57 and 69% for IgM, and 81 and 86% for IgG, respectively). Detecting both IgM and IgG yields a clinical sensitivity of 82%.[135]

An energy transfer system has recently been developed using recombinant spike protein receptor binding domain (RBD) conjugated to fluorescent quantum dots, AuNPs, and cells with green fluorescent protein tagged ACE2 receptors (ACE2-GFP) for facile monitoring of viral spike protein−ACE2 interaction (Figure 5).[136] In that study, fluorescence of the quantum dots was quenched upon their binding with AuNPs in the vicinity. Fluorescence was recovered by neutralizing SARS-CoV2 antibodies that compete with ACE2−AuNPs or blocking the binding of quantum dot−spike protein RBD to the ACE2−AuNPs. The in vitro bioimaging results demonstrated the potential ability of quantum dot−RBD internalization via dyamin/clathrin-dependent receptor-mediated endocytosis, with high affinity to the ACE2 extracellular domain. This platform is a promising biosensor for facile, rapid, and high-throughput cell-based screening of SARS-CoV-2 infection.

Another research group created an advanced field-effect transistor (FET) biosensor platform based on graphene sheets with a specific antibody against SARS-CoV-2 spike protein (Figure 6).[137] This biosensing platform could recognize surrounding alteration on their surface and provide ultra-sensitive sensing and low-noise detection. In addition, it could distinguish the SARS-CoV-2 antigen from the MERS-CoV

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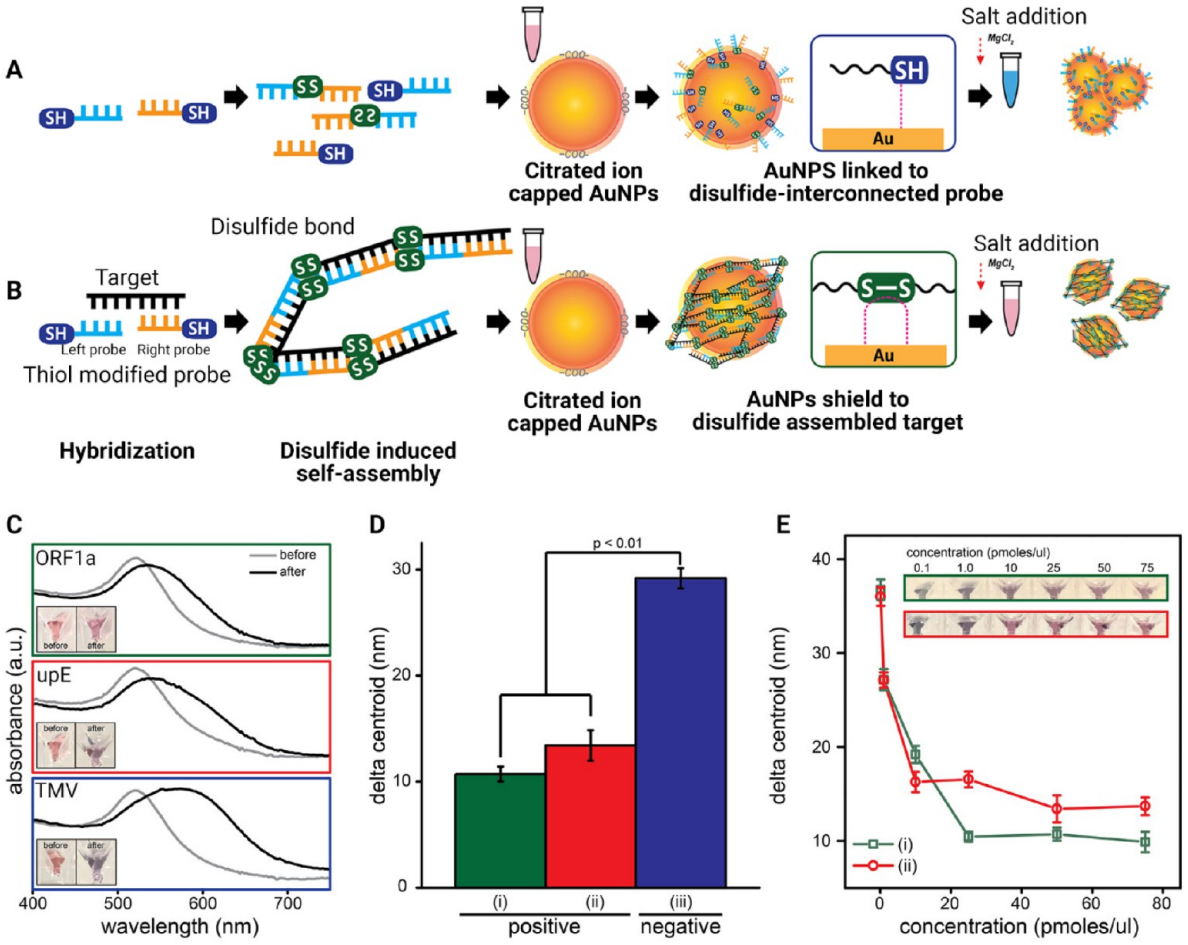

Figure 4. Colorimetric detection of DNA using gold nanoparticles (AuNPs): (A) Salt-induced AuNP aggregation in the absence of targets. (B) In the presence of targets, the disulfide coupling bonds induce self-assembly and prevent aggregation of the AuNPs. This results in color change that is visible to the naked eye. (C) Ultraviolet−visible light spectra of the AuNPs solution before and after adding salt in the presence or absence of disulfide-induced self-assembled targets (positive samples (open reading frames (ORF) 1a and upstream of E protein (upE)) and negative samples (tobacco mosaic virus (TMV)). (D) Average delta centroid of positive controls and the negative control at 0.1 M $MgCl_2$. (E) Limit-of-detection graph of the positive control according to the target concentration. Abbreviations: gold nanoparticles, AuNPs; open reading frames, ORF; upstream of E protein, upE; tobacco mosaic virus, TMV. Reproduced with permission from ref 132. Copyright 2019 American Chemical Society.

antigen. It is a potential device for rapid and highly sensitive detection of CoVs from clinical samples.

Metal NPs (e.g., Au, Zr and Ag NPs, as well as MoS_2 nanosheets) and quantum dots were employed for the detection of a range of coronaviruses.[138] Conjugating nanomaterials with colorimetric, electrochemiluminescence, immunosensing, photoluminescence, and chiroimmunosensing have also been considered as potential substrates for the detection of coronaviruses. Electrochemical devices appear to be a good alternative for the detection of new strains of coronaviruses because of their superior ability to combine with nanomaterials.[138] Using nanomaterials in this aspect decreases the time of analysis and increases sensitivity. This strategy opens new vistas in designing better systems with higher performance in the future.

Microfluidic devices incorporated as organ-on-a-chip are considered another point-of-care system.[139] These devices consist of a palm-sized chip fixed with the reaction chambers and micrometer-sized channels. The chip is made of various materials, including polymers, glass, or papers. The device mixes and separates liquid samples by capillary, vacuum, or electrokinetic forces.[139] Microfluidic devices have benefits such as portability, miniaturization and the use of small sample volume for rapid detection. For example, a smartphone-based point-of-care microfluidic platform has been developed. The system was fabricated with ZnO nanorods and polydimethylsiloxane (PDMS) to detect antibodies against specific infections such as human immunodeficiency virus (HIV) infection through colorimetric detection.[140] This platform showed 100% clinical sensitivity and 87% specificity for HIV detection in 96 patients in Rwanda. Microfluidics may be modified further for the detection of coronavirus RNA or protein.

6. THERAPY

6.1. Nanomaterials to Combat Coronaviruses. Nanomaterials have been introduced as antiviral agents or drug delivery platforms for combating CoV infections.[169] In 2014, a research group patented a mixture of silver colloid, titanium dioxide (TiO_2) NPs and a dispersion stabilizer with antibacterial, antifungal, and antiviral behavior.[170] The platform offers antiviral activity against CoVs such as porcine epidemic diarrhea virus (PEDV) and swine transmissible gastroenteritis virus (TGEV). When the platform concentration is diluted by 1000-fold, virus growth is inhibited at a rate of 99.9 and 93.0% for PEDV and TGEV, respectively. This

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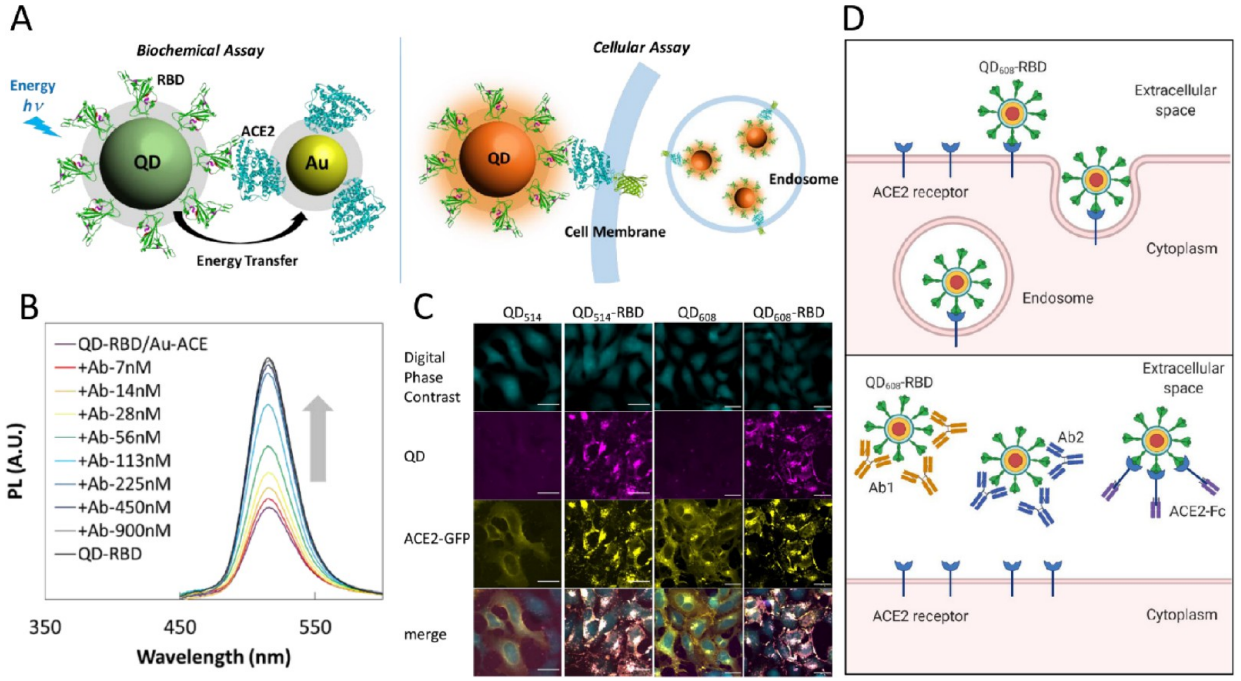

Figure 5. (A) Schematic of the biochemical assay using energy transfer from quantum dot-conjugated Spike Protein-RBD domain (QD-RBD) to AuNP-ACE2 (top left) and the cellular assay using QD-RBD interaction with ACE2 (with or without green fluorescent protein (GFP) modification at the end of the C-terminal) on the cell membrane (top right). (B) Evaluation of the efficacy of neutralizing antibody Ab1 that is specific for SARS-CoV-2, showing the fluorescence recovery of QD_{514}-RBD in the presence of neutralizing antibody Ab1. (C) In vitro live imaging shows that QD-RBD domain induces the translocation of ACE2 and is internalized into cells, (D) Schematic of the QD-RBD internalization via receptor-mediated endocytosis and inhibition using antibodies Ab1, Ab2, and ACE2-Fc. Abbreviations: angiotensin-converting enzyme 2, ACE2; gold nanoparticles, AuNPs; quantum dot, QD; green fluorescent protein, GFP. Reproduced with permission from ref 136. Copyright 2020 American Chemical Society.

activity was reliant on the platform concentration, which means that the usage dose has to be in tune with the virus in which the platform is designed to inhibit.[170]

In the same year, the induced immune responses of four silver nanoconjugates on TGEV-infected swine testicle cells were investigated.[171] These nanomaterials included AgNPs, two Ag nanowires with mean lengths of 60 and 400 nm, and silver colloids. Silver NPs and the two types of Ag nanowires protected the testicle cells against TGEV infection and reduced the number of apoptotic cells. In contrast, the silver colloids were not capable of inhibiting cellular entry by TGEV.[171] Graphene oxide−silver (GO−Ag) nanoconjugates that possess antiviral activities against nonenveloped and enveloped viruses were developed by other researchers.[172] Different dilutions of GO−Ag solution were incubated with diluted solutions of feline coronavirus. The supernatant was analyzed using a virus inhibition assay after removing the GO-Ag pellets. The GO-AgNPs were able to detect nonenveloped and enveloped viruses by binding of the AgNPs to the negatively charged sulfur groups of the viral proteins, whereas pristine GO inhibited only enveloped viruses at noncytotoxic concentrations.[172]

A diphyllin-based therapeutic device was developed for the treatment of feline infectious peritonitis (FIP) caused by feline coronavirus.[173] Diphyllin is a vacuolar ATPase required for endosomal acidification inhibition in Felis catus whole fetus-4 cells. The inhibitory behavior of diphyllin against FIP was enhanced by generating a diphyllin nanocarrier with poly(ethylene glycol)-*block*-poly(lactide-coglycolide). Diphyllin NPs demonstrated antiviral activity; even a high dosage of the NPs was tolerated by mice.[173] Although this system was not a candidate for preparing vaccines, the study verified the efficacy of nanoformulations against coronaviruses.

Another nanoplatform was developed using *N*-(2-hydroxypropyl)-3-trimethyl chitosan (H-HTCC) to produce nano/microspheres (NS/MS) for adsorbing coronaviruses.[174] The copy number of viral RNA decreased when H-HTCC-NS/MS was added to the viral suspensions. The result is indicative of a good correlation between virus concentration and the amount of added biomaterial.[174] In another novel therapeutic approach, Ag_2S nanoclusters were fabricated for restraining the proliferation of PEDV in treated Vero cells (Figure 7).[175] The Ag_2S nanoclusters were capable of inhibiting the synthesis of negative-strand RNA and preventing viral budding. The Ag_2S nanoclusters regulated the expression of interferon-stimulating genes as well as the production of pro-inflammation cytokines. This resulted in the protection against PEDV infection.[175]

Although a lot of studies illustrated the antiviral activities of nanomaterials against coronaviruses, further investigation is needed to develop antiviral nanomedications against SARS-CoV, MERS-CoV, and SARS-CoV-2.

6.2. Nanobased Gene Therapy of Coronaviruses. Ribonucleic acid interference (RNAi) mediated by small interfering RNA (siRNA) is an effective strategy to inhibit the replication of RNA viruses. Antiviral siRNA therapy offers several advantages compared to conventional antiviral drugs and vaccines. These advantages include rapid action with high specificity and efficacy at different viral stages, the use of a less amount of siRNA to reduce viral RNA, and high homology of siRNA with cognate viral RNA.[176] Therapy based on RNAi is a potentially promising approach to overcome SARS-CoV-2

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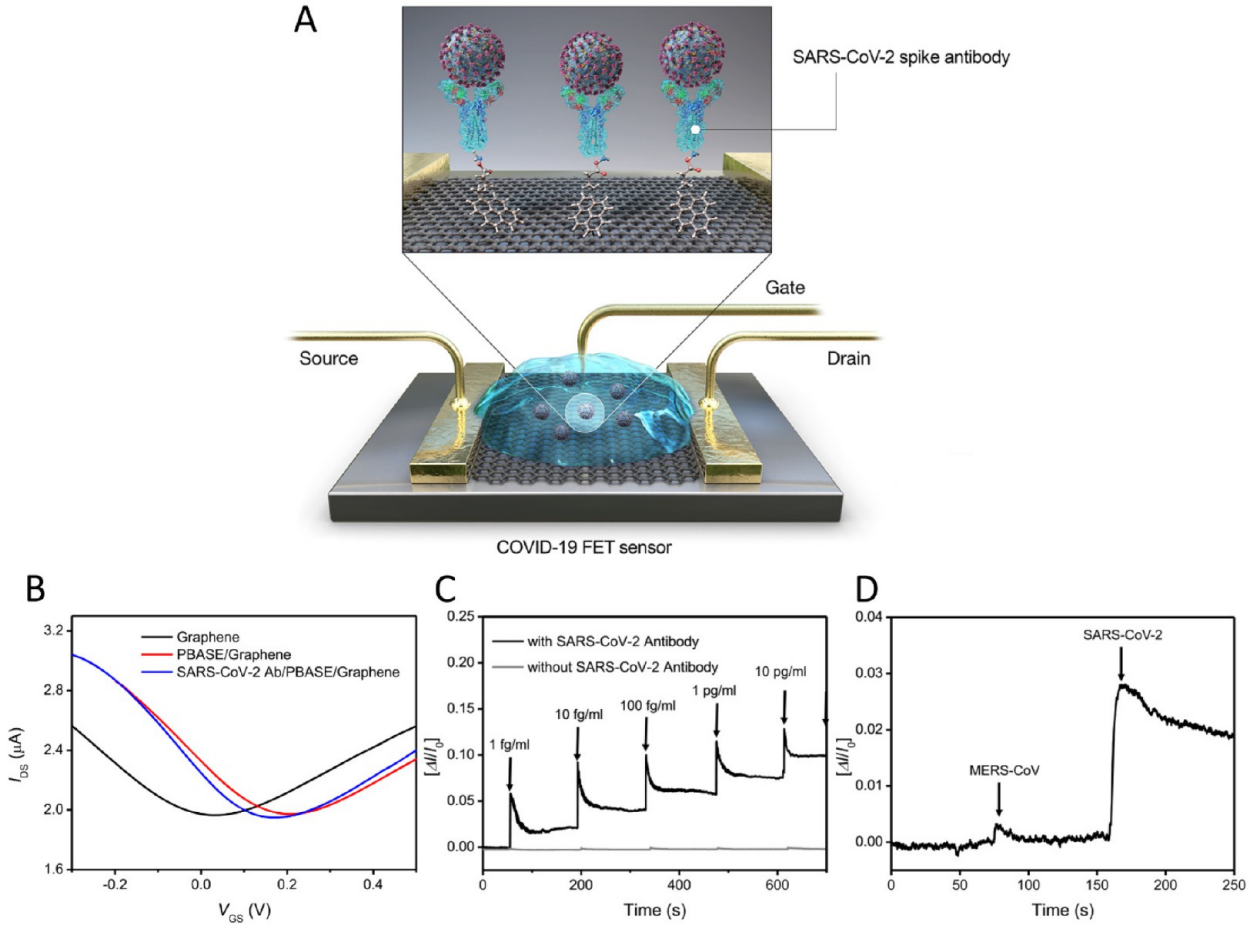

Figure 6. Operation procedure of the SARS-CoV-2 field effect transistor (FET) sensor. (A) Graphene is used as the sensing material. The SARS-CoV-2 spike antibody is conjugated onto the graphene sheet via 1-pyrenebutyric acid *N*-hydroxysuccinimide ester, which is an interfacing molecule and probe linker. (B) Transfer curves of the SARS-CoV-2 FET sensor in steps of the antibody conjugation (VDS = 0.01 V). (C) Real-time response of the FET-biosensor toward SARS-CoV-2 antigen protein in phosphate-buffered saline. (D) Elective response of the COVID-19 FET sensor toward target SARS-CoV-2 antigen protein and MERS-CoV protein. Abbreviations: field effect transistor, FET. Reproduced with permission from ref 137. Copyright 2020 American Chemical Society.

infection. In this regard, accurate characterization of the coronavirus genome enables rapid development of effective therapeutic anti SARS-CoV-2 RNAi activators.[177] Because the genomic sequences of SARS-CoV and SARS-CoV-2 have high homology (~79% at the nucleic acid level), the results derived from SARS-CoV may be extrapolated to SARS-CoV-2 (Figure 8A).[178]

Several recent studies have found that RNAi is effective against SARS-CoV.[179] A research group reported that the use of expression cassettes (plasmid-mediated siRNAs) that produced six antiviral RNAi activators could target specific sites of the viral genome. Pretransfection of Vero cells with the siRNA-expressing plasmids pSR02 and pSR03 prior to the infection of those cells with SARS-CoV resulted in blocking the replication of the ORF1b sequence of the virus genome.[180] Targeting the S sequence effectively inhibit viral infection and replication because the S gene is a good target in SARS-CoV.[181] RNAi activators that target both S and ORF1b regions of the viral genome have been investigated as the potential drug candidates.[182,183] Based on these valuable results derived from the use of RNAi against SARS-CoV, gene therapy via RNAi may revolutionize the treatment of COVID-19.[184] The therapeutic potential of RNAi in combating MERS-CoV has been investigated by using two siRNAs, Smad7-1 and Smad7-2, to knockdown MERS-CoV in both human lung and kidney cell lines. It was found that Smad7 effectively inhibited viral replication and infection in host cells.[101]

Although specific targeting of the viral genome sequence is the strength of antiviral siRNA therapy, targeted delivery of siRNA into a cell with inadequate endosomal escape is another potential approach.[185] Application of siRNA is typically hampered by rapid enzymatic degradation of the siRNA, fast clearance and inability of SiRNA in entering cells.[186] These challenges are mostly due to unstable negatively charged siRNA bases that stimulate unwarranted immune response, and random insertion of the siRNA into chromosomes that results in gene dysfunctions.[187] These restrictions may be overcome by using nontoxic, biocompatible nanocarriers prepared from polymers, lipids, hybrid (polymer/lipid) NPs, nanohydrogels, silica, dendrimers, iron oxide NPs and AuNPs.[188−190] Among these, lipids and polymers are considered promising platforms for siRNA delivery because of their highly biocompatible and biodegradable nature. For example, poly(lactic acid), polycaprolactone, poly(glycolic acid) and their copolymers have been approved by the United States Food and Drug Administration for targeted siRNA delivery in vivo.[187,191,192]

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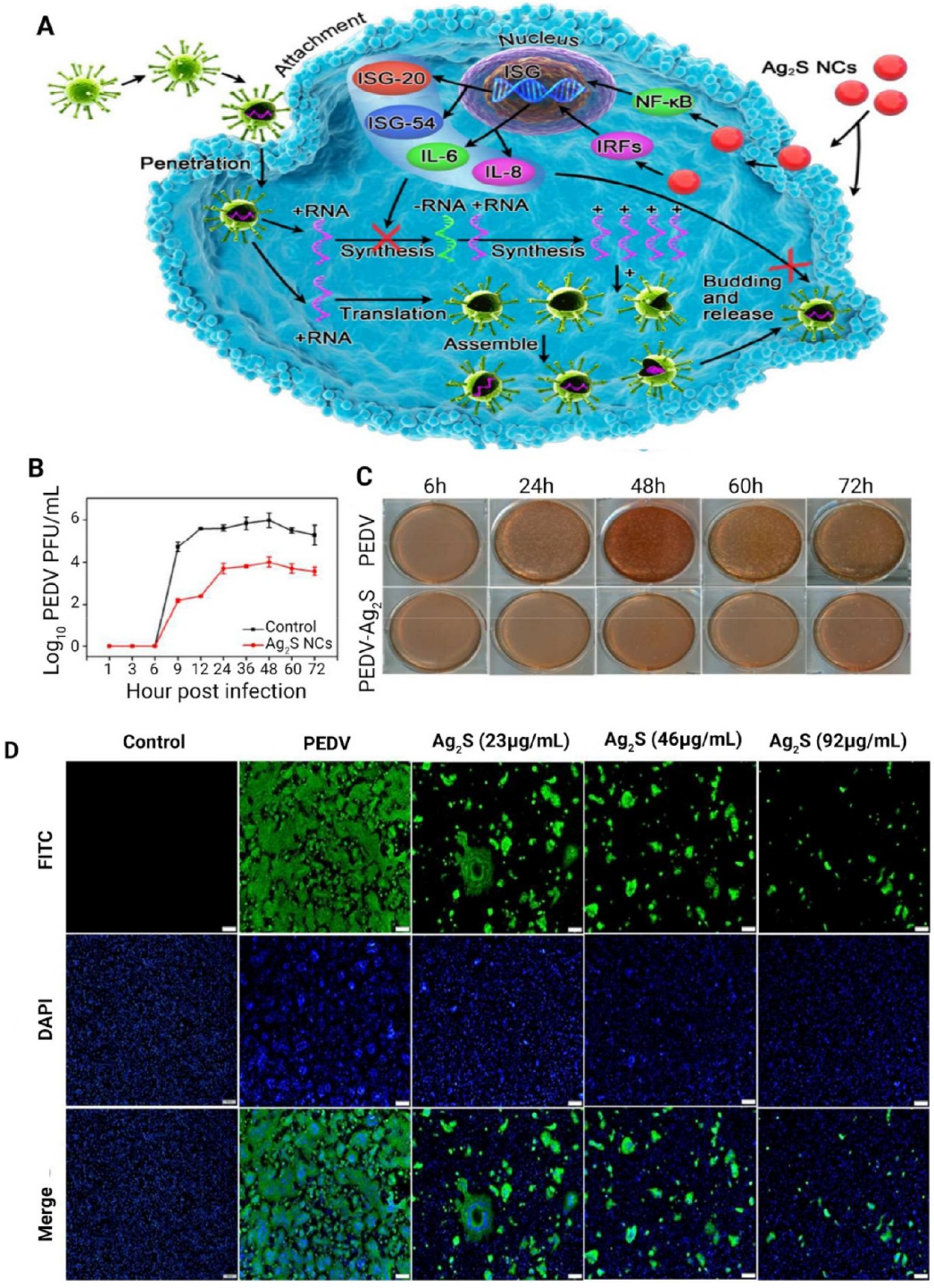

Figure 7. (A) Schematic of the antiviral mechanism of Ag_2S nanoclusters against viruses, including four consecutive steps of attachment, penetration, replication and budding. Treatment with Ag_2S nanoclusters inhibits the synthesis of viral negative-strand RNA and prevents viral budding. The activation of interferon-stimulated genes and the up-regulation of pro-inflammatory cytokines play a key role in the inhibitory effect of Ag_2S nanoclusters. (B) Growth curves of porcine epidemic diarrhea virus (PEDV) with/without treatment with Ag_2S nanoclusters. (C) Plaque reduction assay after neutral red staining. Pictures were taken 2−3 days after infection. (D) Immunofluorescence assay of PEDV-infected cells with/without treatment with different concentrations of Ag_2S nanoclusters (bar: 100 μm). Abbreviations: porcine epidemic diarrhea virus, PEDV. Reproduced with permission from ref 175. Copyright 2018 American Chemical Society.

Lipid-based NPs, including solid-lipid NPs, nanostructured lipids, and liposomes, are also suitable for the preparation of siRNA delivery systems.[193] Nanocarriers preserve the encapsulated siRNA from degradation by serum nucleases, prolong their circulation and promote their access to destined sites.[194] Polycationic lipids or polymers maintain their low endosomal pH by increasing influx of protons and water. This causes the endosomes to rupture and release the loaded therapeutics into the cytosol.[195] Delivery of antiviral siRNA through commercially available cationic lipid structures such as

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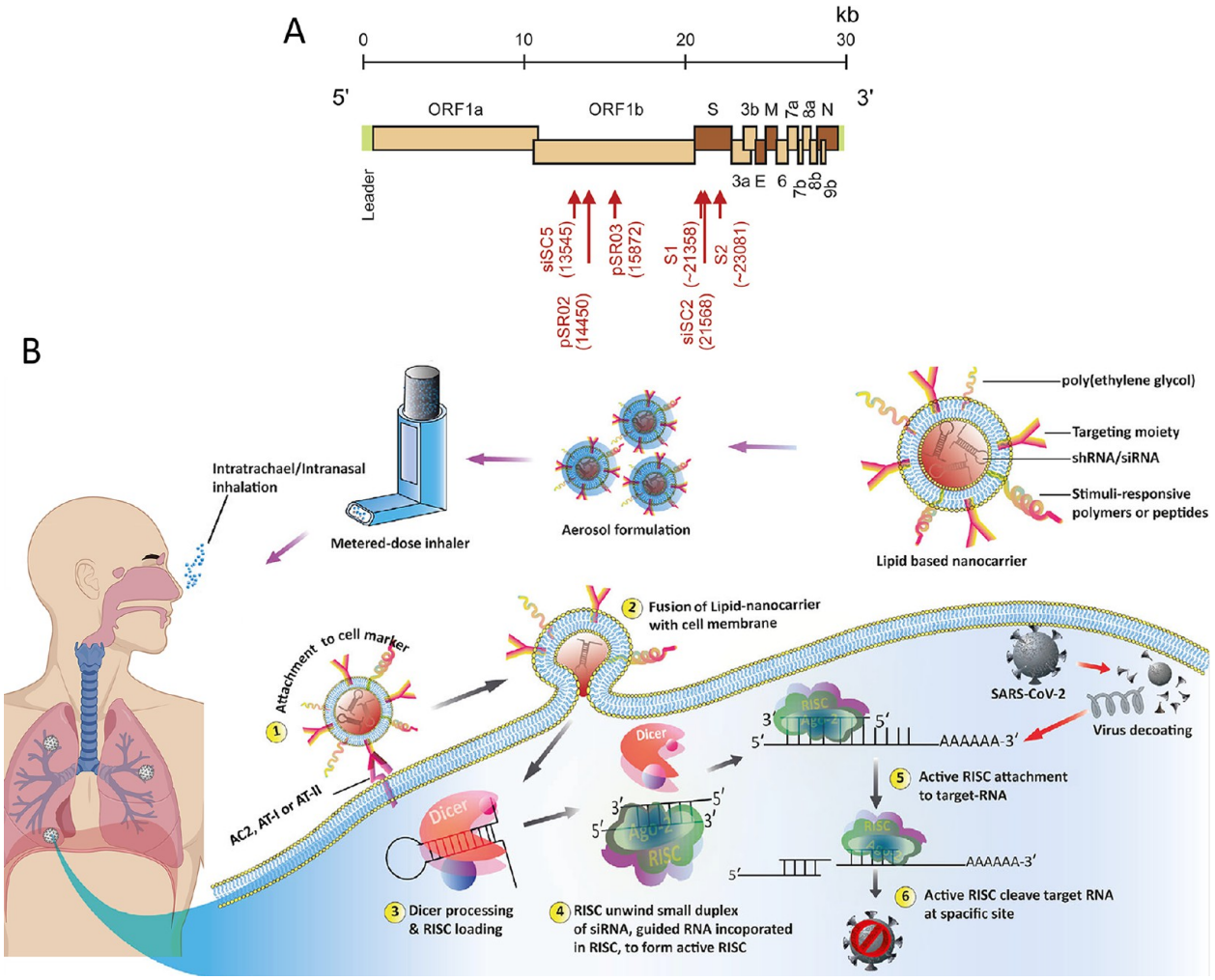

Figure 8. (A) Genome of SARS-CoV with targeted sites by RNAi activators. Reproduced with permission from ref 177. Copyright 2015 Elsevier, (B) Schematic of the proposed SARS-CoV-2 treatment through the use of multifunctional nanocarriers that deliver antiviral siRNA into the respiratory system to combat viral infection. Reproduced with permission from ref 200. Copyright 2020 Wiley.

oligofectamine, lipofectamine (Invitrogen), lipofectin, TransIT TKO (Mirus), and RNAifect (Qiagen) have demonstrated promising results.[195] Poly(lactic-*co*-glycolic acid) (PLGA), lipid, and polymer−lipid nanocarriers are suitable for loading of inhalable antiviral siRNA as well as for aerosol-based pulmonary delivery of antiviral siRNA.[196] Cholesterol-conjugated lipid nanoparticles (LNPs) have also been developed for the delivery of an mRNA vaccine against SARS-CoV-2.[197] Histidine-lysine copolymer and spermine-liposome conjugate-based nanocarriers have also been approved for siRNA delivery to target specific sequences in the SARS-CoV genome.[198] Coronavirus-infected mice that were treated with intranasally delivered nanoformulated antiviral siRNA showed very positive effects. Considering these successful achievements, the use of cationic-liposomal encapsulated antiviral-siRNA and their aerosol formulation appears to be a reasonable treatment for SARS-CoV-2 infection.[199] A lipid/polymer-based nanocarrier modified with functional molecules (i.e., antibodies or aptamers) was effective in delivering siRNA to target sites through intranasal or intratracheal administration via an inhaler (Figure 8B).[200] The use of antibodies against alveoli-specific surface markers type-I and II (AT-I and AT-II) is a good alternative for functionalization of nanocarriers and the subsequent delivery of therapeutic siRNA to lung cells and other organs that express these markers. The surface of nanocarriers may also be functionalized with polyethylene glycol and pH-sensitive histidine-lysine peptide for prolonged circulation and endosomal release of siRNA to the cytosol for inducing the RNA interference pathway.[200] Activation of the RNA interference pathway results in cleavage of the viral RNA at the targeted site, which is critical for combating viral infection.

6.3. Nanobased Immunotherapy against Coronaviruses. Immunotherapy-based NPs have gained attention as a highly effective treatment modality for combating infectious diseases. However, there are still challenges associated with increasing therapeutic efficiency and reducing side effects. Understanding the function of the immune system against infection and the possible approaches to modulate immunity are essential steps toward the design of effective immunotherapy.

6.3.1. Immune Responses against Coronaviruses. The immune responses to CoVs include innate and adaptive immunity. When CoVs encounter the first line of immune defense (i.e., mucus and ciliated cells), the pathogen-associated molecular patterns (PAMPs) on the virus surface alert the innate immune cells to the presence of the invading molecule. This results in the release of type I interferons (IFN-α/β).[201]

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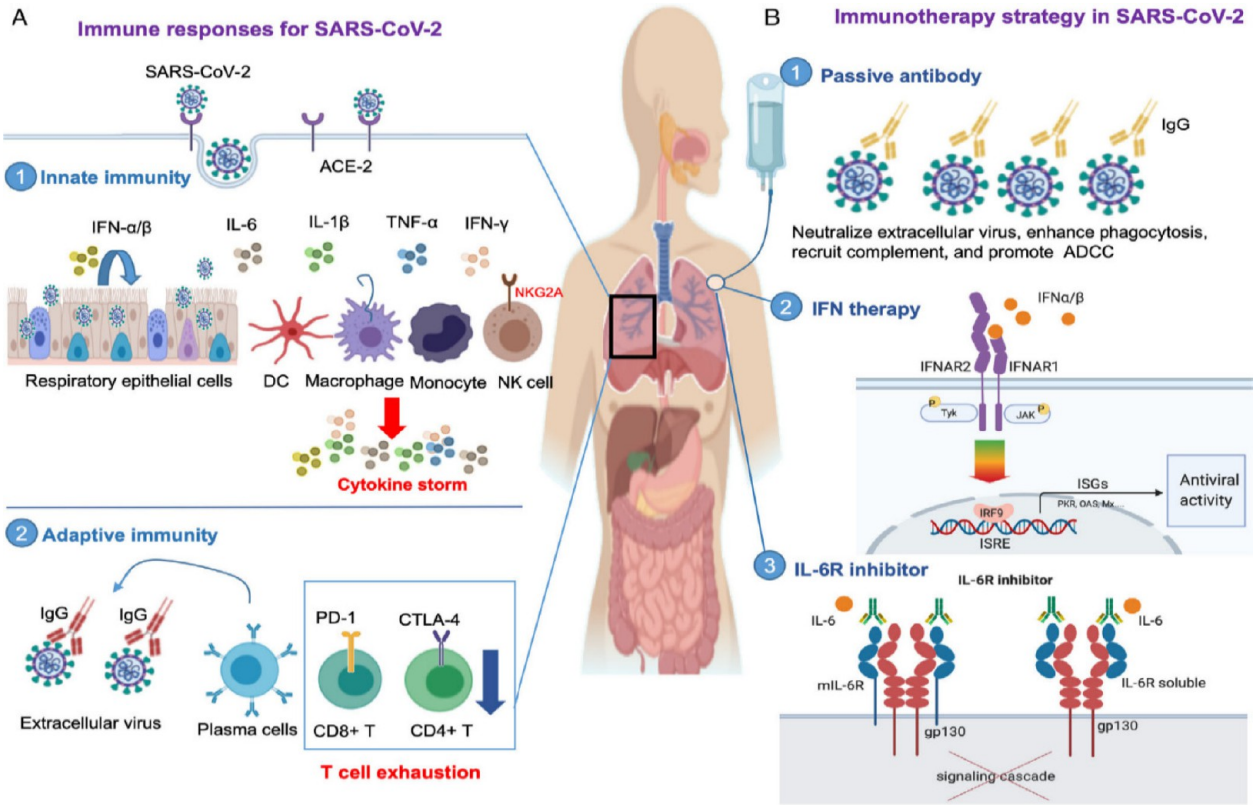

Figure 9. Immune responses and immunotherapy strategy in SARS-CoV-2 infection. (A) Immune response to SARS-CoV-2 involving innate and adaptive immunity. (B) Immunotherapy strategy including passive antibody therapy, interferon α/β, and IL-6 receptor (IL-6R) inhibitor. Abbreviations: IL-6 receptor, IL-6R. Reproduced with permission from ref 215. Copyright 2020 Wiley.

In the event of an acute infection, other immune cells, including natural killer (NK) cells, alveolar macrophages, monocytes, and neutrophils, are activated. This produces a large amount of pro-inflammatory cytokines (IFN, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6), resulting in a condition known as the cytokine storm that severely impairs the respiratory epithelial cells.[5] The innate immune cells use pattern recognition receptors (PRRs) such as retinoic acid-inducible gene I-like receptors, Toll-like receptors, and nucleotide-binding and oligomerization domain-like receptors to detect PAMPs and generate an appropriate immune response.[202] Subsequent interactions between PAMPs and PRRs stimulate phagocytosis by macrophages and dendritic cells and induce intracellular molecular pathways to express pro-inflammatory cytokines (i.e., type I interferons (IFN-I), IFNα/β; and type II interferon (IFN-II), IFN-γ) and chemokines (i.e., CCL-2 and CXCL-10). The IFN-I blocks the replication of viruses through multiple pathways.[203] The infected cells that express major histocompatibility complex I induce NK cells to produce IFN-γ and stimulate apoptosis via antibody-dependent cellular cytotoxicity.[204]

In SARS-CoV-2 infection, extensive production of antibodies was observed along with reduction in $CD4^+/CD8^+$ T cells.[205,206] In this infection, macrophages and dendritic cells have an essential role in mounting specific immune responses. These cells remove virus particles through phagocytosis and IFN-I secretion, with subsequent priming of the adaptive immune responses.[207] In additions, IFN-I inhibits the replication of viruses through upregulation of interferon stimulated genes, including protein kinase R (PKR) and 2′-5′-oligoadenylate synthase (OAS)/RNase L.[203] These important components of the protein synthesis machinery block the synthesis of proteins via phosphorylation of OAS/RNase L and eukaryotic initiation factor 2 subunit-α (eIF2α), resulting in degradation of the viral ssRNA and impairment of viral replication.[208] The IFN-I promotes $CD8^+$ T cell priming, induces B cell activation and antibody production, and eventually stimulates NK cells and macrophages to halt viruses. Several studies have reported that MERS-CoV expresses NS4a protein to block the activation of PKR and OAS/RNase L in the innate immune responses.[209−211] The response of the immune system to SARS-CoV-2 infection is shown in Figure 9A. Researchers suggested that using IFN-α as a pretreatment approach prior to infection with SARS-CoV could induce the expression of IFN-related genes and signaling pathways.[212] An in vitro study reported that IFN-α could restrain SARS-CoV infection.[213] A more recent study also indicated that pretreatment of cells with IFN-I resulted in a significant decrease in SARS-CoV-2 replication. These initial findings suggest that IFN-1 possesses antiviral activity against SARS-CoV-2.[214] However, more clinical trials are required to validate these findings.

6.3.2. Immunotherapy Strategies against Coronaviruses. Humoral immunity is crucial for inhibiting viral infection through the activation of B cells for antibody generation.[216] Antibodies recognize and mediate the killing of the virus-infected cells via several pathways, including phagocytosis, opsonization, neutralization, and activation of the classical complement pathway, as well as mediating antibody-dependent cellular cytotoxicity.[217] As such, the virulence of virus and the host immune response should be balanced to successfully overcome the viral infection. Although the host's inflammatory

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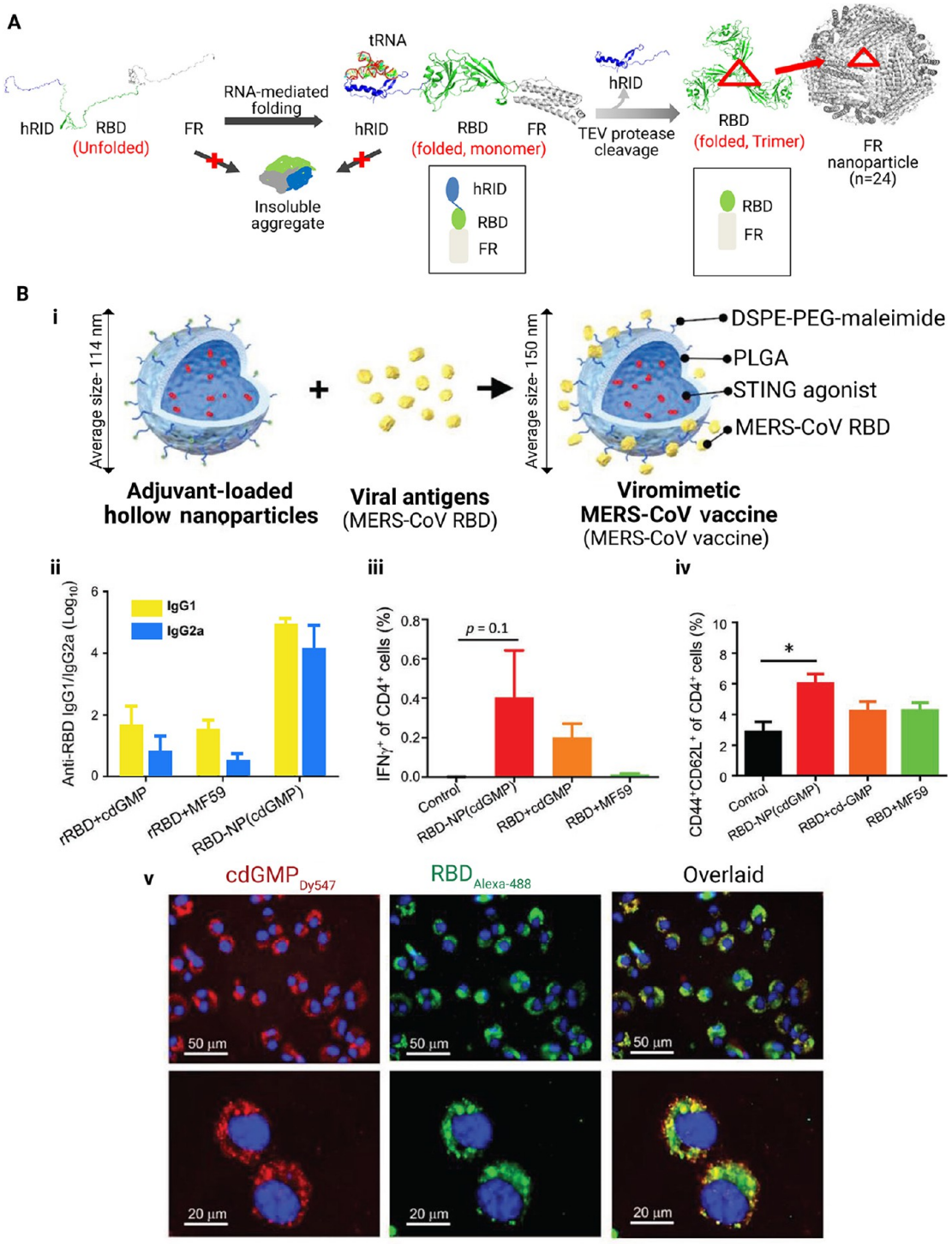

Figure 10. (A) Schematic of Middle East respiratory syndrome-coronavirus receptor-binding domain (RBD) nanoparticles (MERS-CoV RBD-FR NPs) using the chaperna-mediated hRID fusion partner. The hRID facilitated folding of the aggregation-prone RBD-FR through interaction with RNA. The monomer of RBD-FR forms a properly folded trimeric structure by cleaving hRID with tobacco etch virus (TEV) protease. Eight trimers assembled into MERS-CoV-like NPs. Red triangles indicated the RBD trimer on the FR NPs. Reproduced with permission from ref 243. Copyright 2018 Frontiers. (B) Schematic of the preparation of viromimetic NP vaccine: (i) Hollow poly(lactic-*co*-glycolic acid) (PLGA) NPs with encapsulated adjuvant and surface maleimide linkers were prepared. Recombinant viral antigens were conjugated to the surface of NPs via thiol-maleimide linkage. (ii) MERS-CoV RBD-specific IgG1 and IgG2a titers in immunized mice on day 35 postvaccination (n = 6). (iii) $CD4^+$ T-cell responses against MERS-CoV RBD in immunized mice were determined by intracellular cytokine staining on day 7 after boosting (n = 3). (iv) Frequency of central memory ($CD44^+CD62L^+$) $CD4^+$ T cell in the draining lymph nodes of immunized mice, 28 days after boosting (n = 3). (v) Cellular distribution of Dy-547-labeled cyclic diguanylate monophosphate (cdGMP) (red) and AlexaFluor-488 labeled recombinant MERS-CoV RBD antigen (green) in JAWS II cells following 24 h of incubation with RBD-NP (cdGMP). Abbreviations: Middle East respiratory syndrome-coronavirus receptor-binding domain (RBD) nanoparticles, MERS-CoV RBD-FR NPs; poly(lactic-*co*-glycolic acid), PLGA; nanoparticles, NPs; tobacco etch virus, TEV; cyclic diguanylate monophosphate, cdGMP. Reproduced with permission from ref 245. Copyright 2019 Wiley.

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responses in the early stages of infection is essential, the severe inflammatory responses at the late stages of the viral infection aggravate the clinical manifestations.[218] For this reason, immunotherapy strategies that enhance viral clearance and minimize the hyper-inflammatory responses should be used to overcome coronaviruses infection.[219] Immunotherapy against SARS-CoV and MERS-CoV infections is classified into three approaches: passive antibody therapy, interferon α/β and IL-6 receptor inhibition (Figure 9B).[215]

Passive antibody therapy includes the administration of antibodies from recovered patients to new patients involved with the same infection.[220] Neutralizing antibodies may be isolated from individual convalescent plasma or developed as monoclonal antibodies through immortalizing B-cell repertoires of the convalescent plasma.[221] Several issues should be regulated to improve the efficacy of passive antibody therapy. These issues include administered antibody titer, plasma administration time, and accurate convalescent plasma screening for blood-borne pathogens.[220] The use of monoclonal antibodies is preferred in comparison with the other approaches in blocking the attachment of viruses. This because of the unique properties of monoclonal antibodies, including purity, specificity, low risk of blood-borne pathogen contamination, and safety.[222] Monoclonal antibodies comprising different polyclonal antibodies are capable of recognizing different epitopes on the viral surface and holds promise in overcoming virus infection. Targeting the S protein as the key neutralizing antibodies inducer has also been considered for the treatment of SARS-CoV-2.[223]

The use of IFNs may overcome viral infection by promoting the expression of interferon stimulated genes that encode antiviral proteins and cytokines.[224] Such antiviral proteins exert antiviral effects by either the hindering viral replication or inducing the adaptive immune system. According to reported experimental investigations, IFNα and IFNβ possess potent antiviral activities that restrict SARS-CoV and MERS-CoV replication.[225,226]

Cytokines are other potential targets for efficient immunotherapy of coronaviruses. Among the cytokines, IL-6 is considered more important in the treatment of SARS-CoV-2. This is because overexpression of IL-6 is associated with the severity of inflammatory cytokine storm.[227] It has been proposed that targeting of IL-6 and its receptor (IL6R) through the use of immunosuppressive drugs such as tocilizumab and chimeric monoclonal antibody such as siltuximab can overcome cytokine storms and reduce the clinical manifestations in SARS-CoV-2 patients.[228]

The use of the described immunotherapy approaches, alone or in combination with other drugs, has been proposed for treating patients with SARS-CoV-2 infection.[229] Notably, all immunotherapy efforts against SARS-CoV-2 mostly involve the use of polyclonal antibody via plasma therapy, polypeptide hormone for T cell maturation, neutralizing antibodies, ACE2 immunoadhesin, immunoglobulins, and monoclonal antibody against IL-6.[230] In spite of the extensive attempts in the development of monoclonal antibody-based passive immunotherapy for combating CoV infections, no monoclonal antibody is available to date. The major limitation is that large-scale production of monoclonal antibodies is difficult, expensive, and time-consuming.[231] Designing and developing advanced platforms and materials are essential in providing immunotherapy at a reasonable cost in a short time period.

Nanoparticle formulations are promising platforms for overcoming the hurdles associated with immunotherapy.[232] For example, nanoparticulate forms of antigens and other immunomodulatory agents can modulate the function of immune components through enhancing multivalent receptor cross-linking, regulating intracellular processing, inducing cytosolic delivery, targeting the innate immune system, and reducing the toxicity associated with immunomodulators.[233] In additions, nanomaterials have the potential to incorporate several antigens on their surface for more effective activation of the immune system. Thus, nanomaterials are not only therapeutic carriers but may possess immunomodulatory properties themselves, acting as potential immune adjuvants. To date, an extensive range of nanomaterials such as dendrimers, liposomes, carbon nanotubes, polymer-based materials, and inorganic NPs have been investigated as potential platforms for immunological applications.[234] Nanomaterials such as PLGA and liposomes can activate $CD8^+$/$CD4^+$ T cells and promote antigen cross-presentation for effective antigen delivery.[235,236] Moreover, inorganic NPs such as AuNPs can interact with dendritic cells, promoting the expression of pro-inflammatory cytokines (i.e., IL-1, IL-6, IL-12, IFN-α, and TNF-α), and the down-regulation of anti-inflammatory factors (i.e., transforming growth factor (TGF)-β1 and IL-10).[237,238] Gold nanoparticles also activate T cells-related immune responses and increased the phagocytic activity of dendritic cells. Despite the progress of experimental application of nanomaterials in immunotherapy, there are relatively few fundamental investigations on the use of NP-based immunotherapy against CoVs.

6.4. Nanobased Vaccines against Coronaviruses.

Because of their specificity and capacity to induce immune memory, vaccines are the preferred defense tools against infectious diseases, compared to chemotherapeutical drugs.[239] Some of the current vaccines utilize either delivered or expressed viral proteins to induce neutralizing antibodies against CoVs. These antibodies inhibit viral entry by binding to the M, E or S proteins of CoVs.[240] The use of nanobased therapeutic agents against different types of CoVs has been perceived as a potential solution based on the immunostimulatory effects of NPs.[241]

Gold nanoparticles conjugated with TGEV were used for stimulating the protective immune response against CoV in immunized mice and rabbits.[242] The use of this antigen-colloidal gold complex resulted in the activation of macrophages and immunity against TGEV, with induction of IFN-γ production and higher titers of neutralizing antibody in vaccinated animals. Proliferation of T cells was amplified ten-fold following immunization with the antigen-colloidal gold complex, compared with the free antigen response. On the basis of this result, virus-conjugated AuNPs were suggested as a potential antiviral vaccine.[242]

Ribonucleic acid conjugated to ferritin-based NPs have been proposed as a potent molecular chaperone.[243] The use of this NP-based vaccine against MERS-CoV induced $CD4^+$ T cells and promoted the production of TNF-α and IFN-γ (Figure 10). In another study, an immunogenic vaccine against MERS-CoV was introduced using a heterologous prime-boost method.[244] Using a recombinant adenovirus serotype 5 that encodes the MERS-CoV spike gene (Ad5/MERS) and spike protein NPs, female BALB/c mice was immunized three times with the prime-boost vaccination. The homologous immunization with spike protein NPs successfully induced higher

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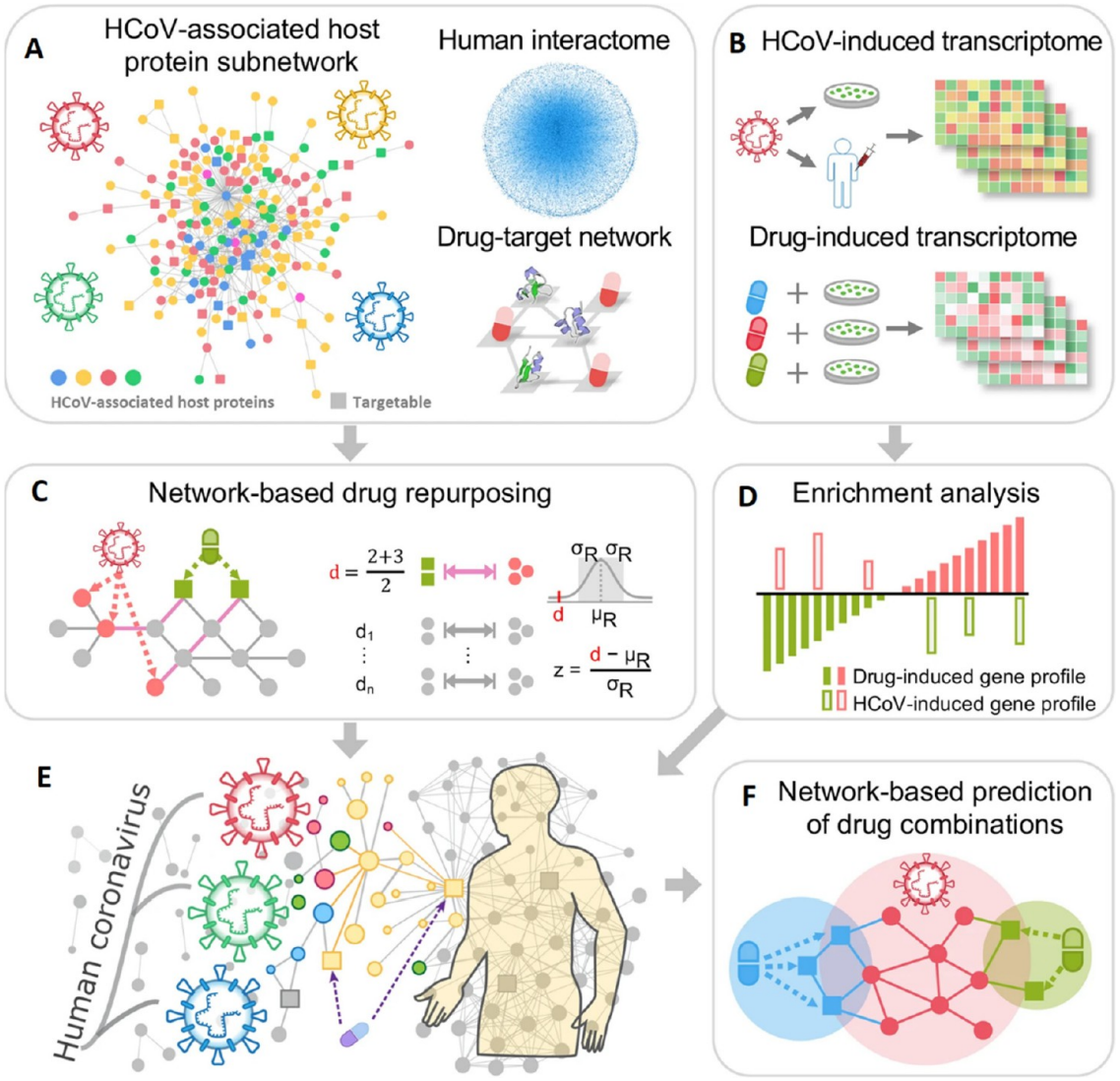

Figure 11. Network-based methodology constructed on a protein−protein network. (A) Human coronavirus (HCoV)-associated host proteins collected from the literature are pooled to generate a pan-HCoV protein subnetwork. (B) Screening of potential repurposable drug candidates via analyzing the network proximity between targeted drugs and proteins associated with HCoV. (C, D) Validation of network-based predictions using gene set enrichment analysis. (E) Network-based prediction of optimized drug combination using complementary exposure pattern. (F) Hypothetical illustration of the network-based methodology to explore protein−protein interactions constructed on the human interactome. Abbreviations: human coronavirus (HCoV). Reproduced with permission from ref 252. Copyright 2020 Springer Nature.

antibody titers. However, Th1 immune response was not generated by spike protein NPs. Only the Th2 immune response was elicited with the induction of neutralizing antibodies. A heterologous one-stage Ad5/MERS prime and two-stage spike protein NP boost appear to be more effective than the homologous prime-boost regimen in providing more durable immunogenicity and balance of Th1/Th2 responses.[244]

7. TRANSLATING RESEARCH INTO CLINICAL PRACTICE

Translating archived knowledge acquired from the laboratory into clinical trials is a crucial and challenging stage for safe and tangible combat against COVID-19.[246] To date, developing anti-COVID-19 drugs have encountered challenges because of their side effects to the lung and heart. A smart technology is therefore required for the design and fabrication of rational drugs that only target SARS-CoV-2 with minimal side effects.[247] Drug repurposing is an effective drug discovery strategy based on the use of existing drugs. Such a strategy shortens the time and reduces the cost compared to de novo drug discovery. *In silico* pharmacology performed on a computer or via computer simulation is a smart, revolutionary technology for evaluating approved medicine, reducing the regulatory costs of innovation and decreasing the time for marketing of biomedical products. Such a "virtual" process is indispensable in contemporary drug discovery research for translating drugs into clinical trials.[248,249] The combination of in silico strategy and large drug-related databases facilities the selection of appropriate repurposed drugs by screening their side effects on different organs. Drug-repurposing strategies have recently been performed by computational modeling on the interaction and mechanism of potential drugs with the host cells and SARS-CoV-2[250] Computer modeling offers a platform for visual assessment and analysis of the molecular mechanisms involved in the entrance, replication, and

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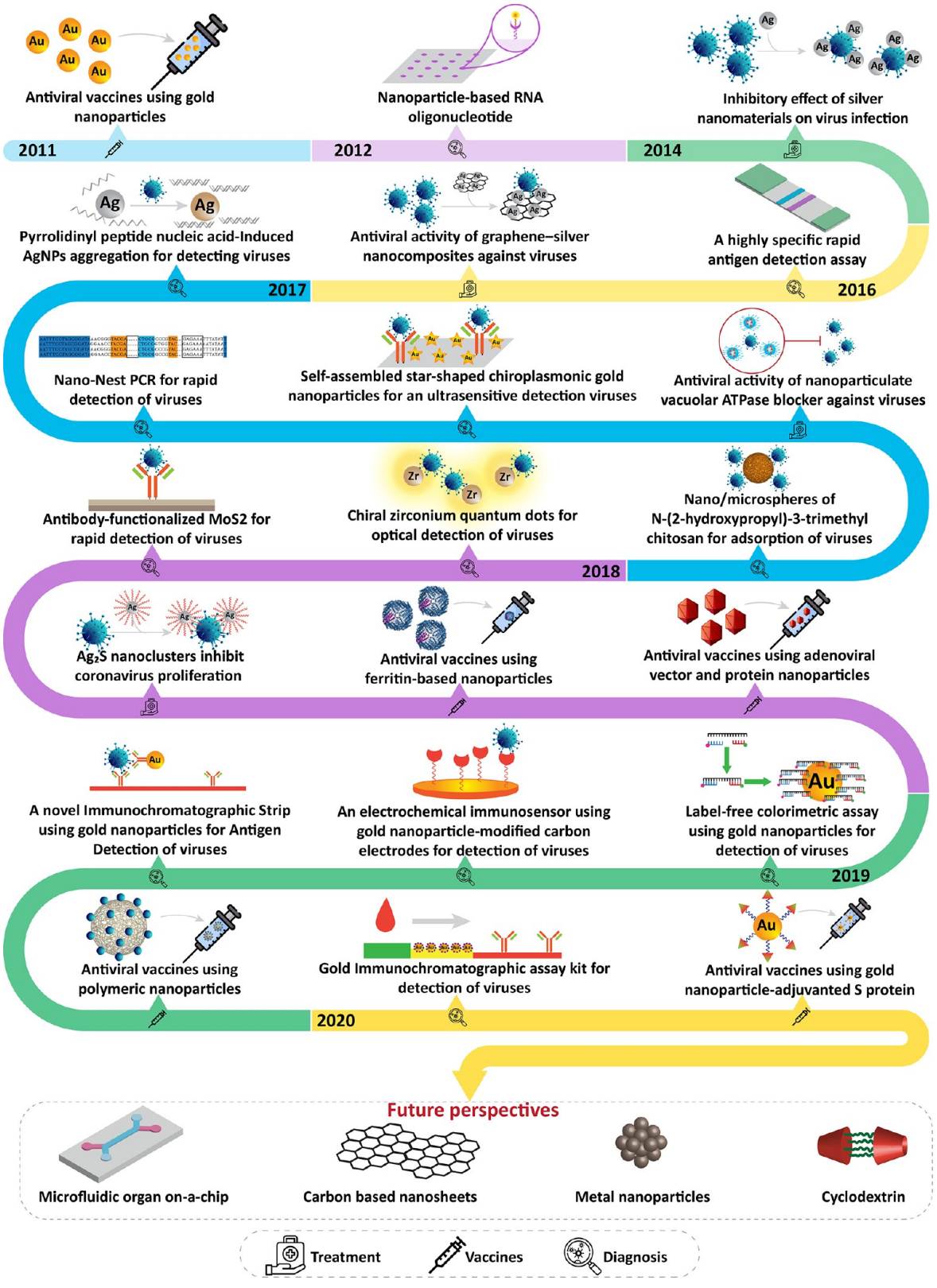

Figure 12. Potential use of nanobiotechnology for biosensing, nanomedicine, and nanovaccine components against coronaviruses.

transcription of virus molecules as well as their interactions with host cells, immune response, and the potential mechanisms of cell recovery.

Another research group investigated a deep-learning Dense Fully Convolutional Neural Network (DFCNN) model for screening established drugs against SARS-CoV-2 infection.[251] In this approach, RNA sequences were collected from the Global Initiative on Sharing All Influenza Data (GISAID) database to investigate the 3D protein sequences and protein–ligand interactions via homology modeling. Drug screening was

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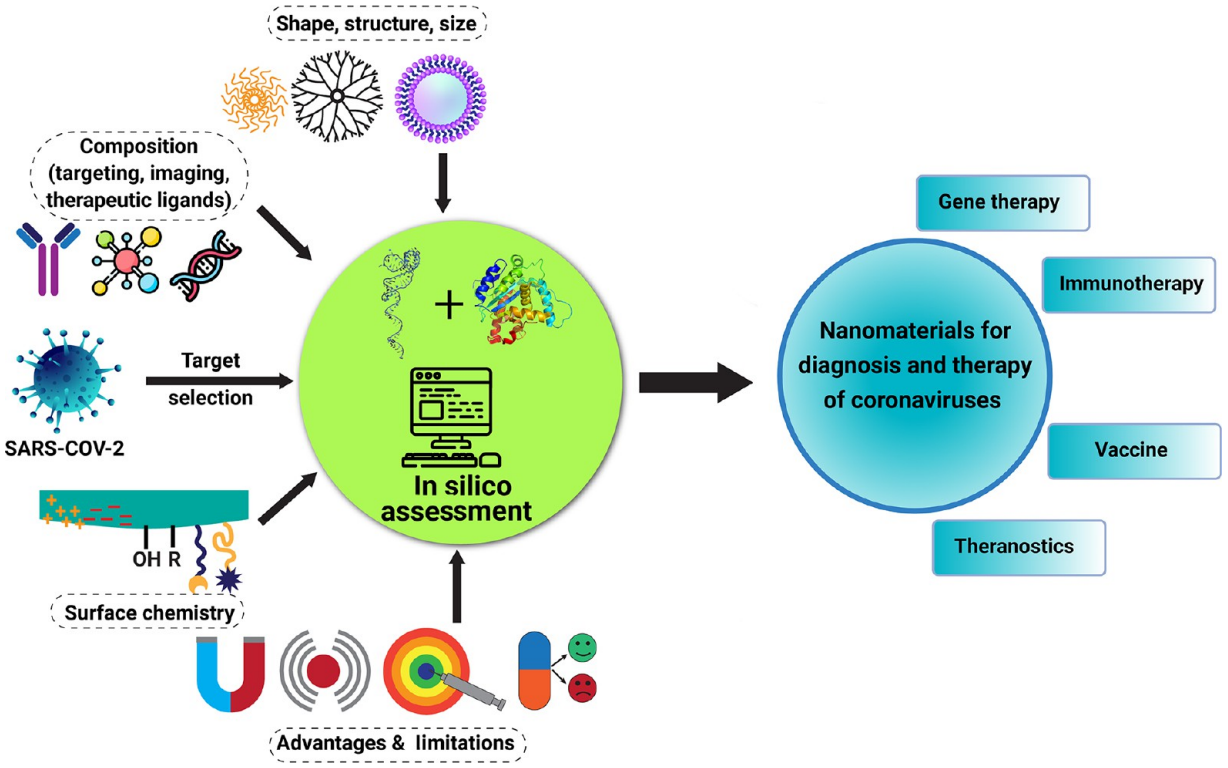

Figure 13. Rational design of nanomaterials using in silico assessments for effective diagnosis and treatment of CoV infections.

performed without using docking or molecular dynamics. This modeling successfully recognized chemical ligands (meglumine, vidarabine, adenosine, D-sorbitol, D-mannitol, sodium gluconate, ganciclovir, and chlorobutanol) and peptide drugs (combination of isoleucine, lysine, and proline) from the databases to aid scientists in identifying molecules that can combat SARS-CoV-2 in a shorter time period.

In another interesting work, an advanced pharmacology network-based approach was developed to evaluate a rational drug for effective treatment of COVID-19 infection (Figure 11).[252] In this work, phylogenetic analysis of 15 human CoV whole genomes with coronavirus infection was performed. Using network proximity analyses of drug targets and human CoV−host interactions in the human interactome,[16] potential anti-CoV repurposable drug candidates such as melatonin, mercaptopurine, and sirolimus were identified and further validated by enrichment analyses of drug−gene signatures in human cell lines. In addition, three potential drug combinations were identified through a "Complementary Exposure" pattern, including (i) sirolimus plus dactinomycin, (ii) mercaptopurine plus melatonin, and (iii) toremifene plus emodin. The study provides an excellent role model for the rapid identification of therapeutic drugs for combating SARS-CoV-2 infections.

Combining data generated on the mechanisms of COVID-19 infections with in silico models enables virologists, immunologists, clinicians, and computational biologists to collaborate in understanding the accurate molecular mechanisms of SARS-CoV-2 infection. This approach provides a useful guide for the development of advanced and efficient nanomedicine against the COVID-19 pandemic.

8. CHALLENGES AND FUTURE PERSPECTIVES

Nanotechnology is rapidly becoming a vivid player in antiviral therapy for combating coronaviruses. Nanomaterials have been developed specifically to improve the delivery of biotherapeutics across physiological barriers, thereby resolving the classical challenge of low bioavailability.[253] Nanomaterials possess various physicochemical and biological benefits. These benefits include reduced particle sizes that facilitate delivery through natural barriers, larger surface areas for higher drug loading, adjustable surface charge to facilitate drug entry across charged cell membranes, capability to anchor to targeting ligands to increase the specificity of the destined target, superior solubility and pharmacokinetic properties that result in longer circulation times, better accumulation, controlled/sustained release, and improved efficacy caused by either entrapping drug agents and protecting them from the physiological environment or surface modifications for targeting purposes.[254−256]

The application of nanomaterials as drug carriers, however, is not free from challenges. One of the most eminent challenge is their degradation prior to reaching the target. Nanoparticles, for example, are degraded in the gastrointestinal tract when they are administrated orally. Nanoparticles are not always successful in crossing the mucus barrier, which results in reduced or nonabsorption.[257] Other challenges associated with the use of nanomaterials include interactions with biological molecules that result in opsonization, phagocytosis by macrophages that reduces their plasma half-life,[258] nonspecific absorption which induces apoptosis of the cells that absorb them, and disruption of their cell membranes.[259]

An ideal nanocarrier for proficient antiviral treatment needs to possess several attributes. These attributes include: (1) excellent clinical outcome, as therapeutic devices are required to be effective, available, targeted, safe, and affordable; (2) the

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nanocarrier needs to improve the efficacy of drug delivery, reduce intake rate and time, decrease side effects, and reduce the cost of therapy; (3) the nanocarrier should possess an appropriate fabrication design that permits targeted drug delivery in a sustained released manner. Hybrid nanosystems have the potential to meet the requirements for nano-manufacturing and shape/size configurations. In addition, the nanomaterials used for fabricating the designated compositions should be biodegradable, biocompatible, and nontoxic. In this regard, polymers offer tremendous potential for chemical surface modifications. More complex challenges are associated with nanocarrier shape because this property is associated with NP size and surface charge. Polymer-based nanomaterials such as polyethylene glycol and poly(lactide-*co*-glycolide) are close-to-ideal candidates because of their flexibility to uptake various charges, capacity to be fabricated in different shapes and sizes for enhancing the permissibility of the composition, and reduced clearance to prolong circulation time.[260] Polymeric nanomaterials are likely to emerge as the materials of choice for the development of vaccine and drug carriers for single-dose and needle-free delivery.[261]

Metal NPs such as AgNPs, AuNPs, MNPs, and their related compositions may be used as alternative candidates for the delivery of therapeutic agents against CoVs.[262] Because the size of the devices influences their biodistribution and rate of uptake, a nanocarrier has to be used in the nanometer size range (e.g., <200 nm).[263] For cyclodextrin drug delivery systems such as hydroxypropyl beta-cyclodextrins (HPβCD), the use of carbon-based nanosheets may overcome formulation challenges of antiviral drugs by improving solubility and bioavailability.[264,265] Likewise, they may be used as safe and efficient adjuvants in vaccines for coronaviruses. Figure 12 summarizes the trend of nanobiotechnology against CoVs.

In the grand scheme of things, the applications of nanoplatforms for the detection of human coronaviruses have yet remained unresolved for nanotechnology researchers. Colorimetric sensing, electrochemiluminescence, immunosensing, photoluminescence, and chiroimmunosensing, as well as electrochemical sensors, are potential techniques to detect coronaviruses. Various nanobased vaccines have demonstrated the potential to induce a more potent immune response. However, further investigations on the interaction of virus particles with host cells are required to tackle the application of smart NPs against the mutated versions of highly contagious SARS-CoV2 (Figure 13).

As of February 2021, eight COVID-19 vaccines based on different technologies have been approved or authorized for emergency use. They are the mRNA vaccines BNT162 from Pfizer/BioNtech and mRNA-1273 from Moderna, the chimpanzee adenovirus-based AZD1222 (Covidshield) vaccine from Oxford-Astra Zeneca, the Ad26-based viral vector vaccine from Johnson &Johnson, the virus-inactivated Covaxin vaccine from Indian Barhat Biotech, the CoronaVac vaccine from Sinovac Biotech, China, and the human adenovirus-based Sputnik V vaccine from the Gamaleya National Center of Epidemiology and Microbiology, Russia.[266,267] Although more than 250 other vaccines are in various stages of development, the emergence of new SARS-CoV2 variants[268] with possible highly transmissibility demonstrate the urgency of developing new vaccine formulations with high effectiveness.

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Notes

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