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REVIEW

Application of Nanotechnology in the COVID-19 Pandemic

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Abstract: COVID-19, caused by SARS-CoV-2 infection, has been prevalent worldwide for almost a year. In early 2000, there was an outbreak of SARS-CoV, and in early 2010, a similar dissemination of infection by MERS-CoV occurred. However, no clear explanation for the spread of SARS-CoV-2 and a massive increase in the number of infections has yet been proposed. The best solution to overcome this pandemic is the development of suitable and effective vaccines and therapeutics. Fortunately, for SARS-CoV-2, the genome sequence and protein structure have been published in a short period, making research and development for prevention and treatment relatively easy. In addition, intranasal drug delivery has proven to be an effective method of administration for treating viral lung diseases. In recent years, nanotechnology-based drug delivery systems have been applied to intranasal drug delivery to overcome various limitations that occur during mucosal administration, and advances have been made to the stage where effective drug delivery is possible. This review describes the accumulated knowledge of the previous SARS-CoV and MERS-CoV infections and aims to help understand the newly emerged SARS-CoV-2 infection. Furthermore, it elucidates the achievements in developing COVID-19 vaccines and therapeutics to date through existing approaches. Finally, the applicable nanotechnology approach is described in detail, and vaccines and therapeutic drugs developed based on nanomedicine, which are currently undergoing clinical trials, have presented the potential to become innovative alternatives for overcoming COVID-19.

Keywords: COVID-19, SARS-CoV-2, antiviral drug, vaccines, nanoparticles, nanotechnology

Introduction

At the end of 2019, viral infectious disease emerged in China, which spread worldwide in months. The World Health Organization (WHO) officially declared that the coronavirus outbreak is turning into a pandemic on March 11, 2020.[1,2] The WHO named this novel coronavirus to SARS-CoV-2 and the disease as COVID-19.[3,4] Coronaviruses (CoVs) are RNA viruses, 27–32 kb in size, and belong to the Coronaviridae family of viruses, which includes the genera *Alphacoronavirus, Betacoronavirus, Gammacoronavirus*, and *Deltacoronavirus*. It is known as "corona" virus because all CoV particles consist of crown-like peplomer spikes. The CoV particles are pleomorphic and approximately 80 to 160 nm in diameter.[5,6] CoVs are known to infect humans and various types of animals. In particular, human coronaviruses (HCoVs), a notable group of CoVs, give rise to several respiratory diseases, including bronchiolitis, pneumonia, and common cold.[7] HCoVs are currently the most instantly evolving viruses because of their high

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genomic nucleotide recombination rates.[8] To date, seven known HCoVs (HCoV-229E, HCoV-NL63, HCoV-HKU1, HCoV-OC43, Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV), and SARS-CoV-2) have been classified. In addition, HCoVs are divided into two categories, the Alphacoronaviruses (including HCoV-NL63 and HCoV-229E) and Betacoronaviruses (including HCoV-HKU1, HCoV-OC43, MERS-CoV, SARS-CoV, and SARS-CoV-2).[9] Virus species belonging to the Coronavirus are very diverse and reported to be the main pathogens causing upper respiratory infections, including cold, along with rhinoviruses. Studies showed that between 30% and 80% of viral colds are infections of rhinoviruses,[10] and approximately 15% are infections of HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1.[11,12] In general, HCoVs have been reported to cause upper respiratory tract infections, including cold symptoms; however, the scope of HCoV infection is not limited to the upper respiratory tract alone. In severe infections, HCoVs tend to attack the lungs directly,[13,14] which is one of the unique characteristics of HCoVs that cause dyspepsia.[15]

HCoVs are one of the top 10 known viruses fatal to human beings, with mortality rates of up to 10% for SARS-CoV and 36% for MERS-CoV.[16–18] Currently, approximately 79.2 million people have already been infected, and 1,754,493 people worldwide die within 354 days of the appearance of SARS-CoV-2 (the WHO on December 27, 2020).

SARS-CoV-2 infection is characterized by serious problems, such as in an incubation period of approximately 2 weeks mild to moderate symptoms develop in infected patients, and a high infection rate. Therefore, vaccines are important, as the data show asymptomatic transmission of SARS-CoV-2.[19–21] Moreover, development of vaccines and therapeutics is the most attractive option to fight SARS-CoV-2 infection and treat infected patients. Globally, scientists and doctors are working continuously to investigate and decipher the exact viral structure, mode of infection, mode of transmission, prevention, immunopathogenic mechanisms, and the most effective treatment strategies.[22]

Furthermore, nanotechnology tools can provide a broader overview of the new vaccine design strategies. For instance, a nano-based formulation for SARS-CoV-2 therapeutics is being developed as a delivery vehicle, along with a novel nano-vaccine metastasis platform and useful nano drugs for treating SARS-CoV-2 infections. Therefore, until now, scientists are working hard to rapidly identify and develop appropriate nano-vaccines and treatment options, including new nano-based technologies.

SARS-CoV

Earlier, SARS-CoV has caused a major pandemic in the new millennium.[23–25] SARS-CoV has been classified as a new virus in the group II CoVs (*Beta*-CoVs) of the Coronaviridae family, which originated from the zoonotic pool of viruses. Owing to its epidemiological association with wild game animals and occurrence of human cases early in the 2003 pandemic,[26] the SARS-CoV strain has been thought to mutate from the bat-related virus through an intermediate civet host (Figure 1A).[27,28] The spike (S) protein in SARS-CoV assembles peplomers, which are located outside the lipid envelope (Figure 1B). Moreover, the S proteins of SARS-CoV, MERS-CoV, and SARS-CoV -2 have surprisingly high amino acid sequence homogeneity with each other (Figure 1C). The S protein directly interacts with the host's cellular receptor, angiotensin-converting enzyme 2 (ACE2).[29] With the binding of the S protein to ACE2, the transmembrane protease serine 2 (TMPRSS2) and furin in the host cell membrane simultaneously cleave the S protein to activate SARS-CoV (Figure 1D).[30,31] The variability of the interaction between the S protein and ACE2 is particularly crucial for cross-species transmission. SARS-CoV is spread by direct contact with the mucous membrane, respiratory droplet nuclei, or fomites.[32] Viral pneumonia with rapid respiratory deterioration is the most representative clinical manifestation of SARS-CoV infection. The major symptoms include chills, fever, muscle pain, discomfort, and nonproductive cough, and rhinitis and sore throat appear less frequently. Rapidly and newly occurring viral infections, such as SARS-CoV infections, are difficult to treat using vaccinology, even though no reagents are required for vaccine development. Moreover, diseases that have not been observed earlier in human beings develop and spread quickly. Novel zoonotic viruses are particularly capricious, as vaccines and remedies focused against formerly derived strains do not work against the strains related to current infectious diseases. Recently, technological advances, such as memory B cell immortalization and phage display, have achieved rapid development of human monoclonal antibodies (hu-mAbs) for SARS-CoV in just a single epidemic year.[33] However, despite the ongoing efforts made by scientists and doctors, there exists no effective

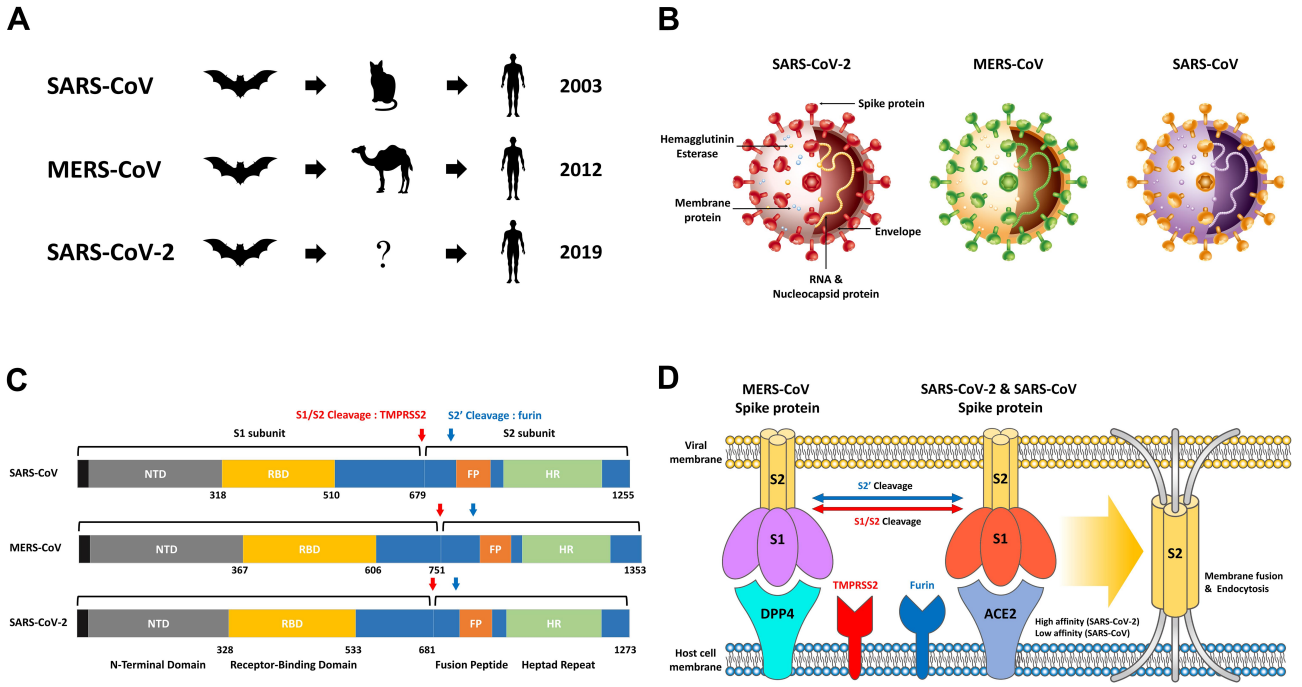

Figure 1 SARS-CoV, MERS-CoV, SARS-CoV-2 overview. (**A**) The origin of SARS-CoV, MERS-CoV and SARS-CoV-2 is widely known as bats as native hosts. While SARS-CoV and MERS-CoV have been shown to be intermediary hosts in civets and camels, SARS-CoV-2 can infect humans through an as-yet unknown intermediate host. After animal infection, SARS-CoV-2 has spread rapidly worldwide to date, mainly through continuous human-to-human transmission. (**B**) Schematic structure of SARS-CoV-2, MERS-CoV and SARS-CoV including Hemagglutinin Esterase, Membrane protein, RNA & Nucleocapsid protein, Envelope, and Spike protein. (**C**) Comparison of the S proteins of SARS-CoV, MERS-CoV and SARS-CoV-2. NTD, RBD, FP, HR and Cleavage site by TMPRSS2 and furin. (**D**) The MERS-CoV, SARS-CoV, and SARS-CoV-2 S proteins bind to ACE2 and DPP4, which act as receptors present in host cells. In order for the S2 domain in the virus to be fused to the host cell membrane to cause endocytosis, the process of cutting at two sites (S1/S2 and S2') through the proteases Furin and TMPRSS2 is essential. SARS-CoV-2 has a much higher affinity than binding affinity to SARS-CoV S protein and ACE2, resulting in a high infection rate.

treatment for SARS. Antibiotics are not effective against viruses, and antiviral drugs do not show much benefit. RNA interference (RNAi) has been proposed as a new therapeutic strategy for SARS-CoV because it can degrade specific mRNAs. In addition, the inhibition of SARS-CoV by RNAi in cultured cells and animal tissues has been reported.[34–43]

In an experiment using Vero cells, plasmid-based siRNAs designed specifically for the viral RNA polymerase have been shown to inhibit the SARS-CoV cytopathic effect.[41] In addition, a study demonstrated the suppression of the expression of S and nucleocapsid (N) proteins when RNAi is applied to SARS CoV-infected cells.[36] In primate cells, siRNA duplexes targeting SARS-CoV genomic RNA have been found to block viral replication and infection.[34,40]

Since developing effective vaccines and achieving social immunity takes too long, the therapeutic strategy using broadly neutralized hu-mAbs targeting SARS-CoV is also a useful direction to follow at present.[44] The 80R, a hu-mAb targeting SARS-CoV, has been reported to neutralize the civet (SZ3) S protein effectively in vivo.[45] In the case of the hu-mAb m396, it has been reported to neutralize SARS-CoV equipped with GD03 S protein (icGD03-S) completely.[46] Furthermore, animal experimental data have already been accumulated, which show that hu-mAbs can protect against SARS-CoV infection.[44] Additionally, there have been some notable advances in development of small molecules targeting SARS-CoV and the design of polypeptides. However, no effective results have been published strengthening the applicability of new concepts, such as nanotechnology-based development of SARS-CoV vaccines.

The SARS-CoV epidemic is characterized by vulnerabilities in the elderly population, with a mortality rate of 25–55% among people aging 65 years and higher.[47–50] Existing research suggests that the influenza virus vaccine for a young adult population is very inefficient in the elderly population at 17–53% compared with the record high level of 70–90%, which seems to be the result of an aging-related immune system dysfunction.[51] Currently, in order to overcome some deficiencies pertaining to the aging immune system, co-administration of adjuvants (MF59, CpG DNA) or cytokines (IL-2) that completely

activate Antigen-presenting cells (APC)/Th cells during vaccination has been attempted, and consequently, the probability of achieving a successful preventive effect has increased.[52–54] Therefore, if a basic strategy aimed at developing vaccines for the elderly is continually developed, it will make a significant contribution to the research on effective SARS-CoV vaccines.

MERS-CoV

Middle East respiratory syndrome (MERS) is a novel virus-associated infectious disease identified in elderly male patients with breathing difficulties in Saudi Arabia in mid-2012.[55] Most of the early MERS infections occurred in West Asia,[55] and later MERS spread to Southeast Asia, North America, Europe, and North Africa.[56–62]

MERS-CoV has also been classified as a new virus of the group II CoVs (*Beta*-CoVs) in the Coronaviridae family.[63] In order to enter host cells, MERS-CoV, similar to SARS-CoV, requires a receptor for the S protein. In the case of MERS-CoV, candidate proteins that act as receptors have been identified as tetopeptidases, such as dipeptidyl peptidase 4 (DPP4).[64] When the S protein interacts with DPP4, the next step is the activation of the infection by TMPRSS2 and furin expressed on the host cell membrane (Figure 1D).[65,66] MERS-CoV has shown little variation, except for a single mutation during the infection process between human populations. It has also been reported that this single mutation is independent of the process of binding to DPP4. Another characteristic of MERS-CoV is its ability to bind DPP4 of multiple species. Therefore, it is possible to infect other animals with MERS-CoV, except camels and humans (Figure 1A).[64,65]

About 60% of all MERS cases are estimated to occur via human-human transmission of MERS-CoV,[67] and for the remaining cases, the cause of MERS-CoV infection has not been identified. Furthermore, the risk of virus transmission has been reported to be significantly reduced for the second case.[67] Research has shown that MERS is fatal to the elderly and patients with underlying diseases, such as kidney or lung disease, chronic heart disease, high blood pressure, and diabetes.[68–73] One of the most persuasive animals is the one-humped camel (*Camelus dromedarius*) that propagates MERS-CoV to humans, as antibodies that neutralize MERS-CoV have been detected in camel herds in the Middle East and Africa.[74–83] Humans with MERS usually begin to develop symptoms after an incubation period of 2 weeks, which include respiratory infections, fever, shortness of breath, and dry or productive cough.[68] To date, MERS-specific therapeutic drugs have not been developed. In vitro studies have shown that some potential drugs are effective for treating MERS; however unfortunately, most of these drugs have not been proven effective using animal models closely related to humans. Therefore, clinical treatment of MERS comprises symptomatic treatment and supportive care.

Unlike other host animals, replication of MERS-CoV is impossible in mice. This is because, mouse DPP4 (mDPP4) has two amino acid sequences different from that of human DPP4 (hDPP4), which prevents the binding of the S protein in MERS-CoV.[84] Therefore, the mouse model has been developed as a strategy for replacing mDPP4 with hDPP4 or a mutant of mDPP4 so that it can bind to the viral S protein.[85] Initially developed mouse models have frequently failed to reproduce the disease observed in humans with MERS-CoV infection. However, recently, several transgenic mouse models have been reported to reproduce the human disease caused by MERS-CoV infection relatively well. Therefore, it is thought that the development of such a mouse model can contribute greatly to testing the efficacy of the candidate MERS-CoV vaccine.

Since DPP4 is a specific receptor for MERS-CoV, it represents a good strategic target for designing therapeutic agents. The therapeutic agents targeting DPP4, such as DPP4 and DPP4 antagonists and specific antibodies, mainly inhibit the interaction or binding between DPP4 and MERS-CoV receptor-binding domain (RBD), thereby suppress the MERS-CoV infection. In developing therapeutics against MERS, it is essential to consider the function and structure of the S protein. Therefore, specific regions of the S1 and S2 subunits, RBD, and N-terminal domain related to S proteins are the main targets. Almost all MERS-CoV-neutralizing antibodies have been designed to target RBD. In particular, RBD-specific monoclonal antibodies have stronger neutralizing activity than that of antibodies made with other targets.

SARS-CoV-2

In Wuhan, being the most largely populated in central China, patients began to develop severe pneumonia due to an unknown cause at the end of 2019.[86] Rapid research resulted in identifying the cause of the disease to be a type of coronavirus. To date, the number of infected people continues to increase rapidly.

SARS-CoV-2 belongs to the genus *Betacoronavirus*, and its sequence is 79% identical to the genomic sequence of SARS-CoV.[87] Similar to other human betacoronaviruses, it is estimated that more than 90% of the genes in the SARS-CoV-2 genome match with those from bats, and there are several candidates for intermediate hosts existing before transmission to humans, but these are still unknown.[88,89] Until now, there has been a strong hypothesis that the transmission occurred to humans by accident, such as that for SARS-CoV. Public health measures that can be used to control the transmission of SARS-CoV-2 across individuals are as much as passive approaches, such as isolation, social distancing, and refraining from small indoor gatherings. Moreover, there is a high likelihood of another major crisis occurring in the near future, as there is no vaccine or specific treatment available for SARS-CoV -2 even after an alleviation of the situation.

Like SARS-CoV, SARS-CoV-2 binds to the receptor ACE2 using the RBD of the S protein.[90,91] Subsequently, the processes of fusion of cell membranes and entry of the virus into the host cell occur, similar to the mechanisms underlying other virus infections. The process by which TMPRSS2 and furin activate the S protein plays a critical role in SARS-CoV-2 infection and its spread throughout the patient's body. Therefore, the host and host cell affinity depend on the amino acid sequence and distribution of ACE2, TMPRSS2, and furin (Figure 1D).[92,93] In addition, in smokers or people with heart diseases, ACE2 levels are higher than that in healthy people, thereby increasing the susceptibility to SARS-CoV-2 infection and fastening the disease progression.[94,95]

SARS-CoV-2 infection is not limited to any particular class, and people of all age groups are vulnerable. The virus is mainly transmitted through droplets from symptomatic patients; however, there are many cases of infection from asymptomatic people, wherein the virus is transmitted even before the symptoms appear.[96] SARS-CoV-2, present in the droplets from symptomatic patients, can usually survive on the contact surface for several days but is easily degraded by commonly available disinfectants, such as hydrogen peroxide and sodium hypochlorite.[97] This droplet can cause infection via its inhalation through the respiratory tract during conversation with a SARS-CoV-2-infected individual or by touching the mucous membrane area with the hand that touched a surface contaminated by the droplet. In general, infection is caused by droplets containing SARS-CoV-2 at less than 2 m, and the risk of airborne transmission has not been reported. SARS-CoV-2 can survive for up to 3 h in droplets, and it has been known to have a survival period of about 4 h on copper compared with other metals and materials.[98]

Initially, after SARS-CoV-2 infection, symptoms such as dry cough, fever, and fatigue appear.[99] Although not common, symptoms such as body aches, headaches, conjunctivitis, diarrhea, and sore throat may also appear.[100] Currently, respiratory symptoms caused by SARS-CoV-2 vary widely, ranging from mild to severe hypoxia due to acute respiratory distress syndrome (ARDS).[99] Epidemiological studies have shown that the incidence rate is significantly lower in children, and the mortality rate is very high in the elderly population.[101–103] As the mortality rate in more severely ill patients increases, the disease can also be fatal to the elderly population. When infected with SARS-CoV-2, macrophages and monocytes move to the site of infection, and T and B cells together induce an immune response and begin to remove virus particles.[99] In most healthy individuals, this immune response is used as a defense mechanism against viral infection; however, in patients with immunomodulatory disorders, a cytokine storm occurs, leading to severe organ failure, damaging multiple organs. This can also lead to death.[104]

When SARS-CoV-2 was first discovered, the most commonly searched genome was clade L, corresponding to NC_045512.2. In early 2020, the first mutant virus clades S and O appeared to have been identified. Clades V and G have appeared around the same time in mid-January. Subclades GH and GR have been reported one month after appearance of clade G. In general, clades S and GH have been observed in America, including the United States, and G and GR clades are widespread in Europe. While the appearance of clade G (including GH and GR) continues to increase gradually, that of clades L and V is gradually declining. The most common clades of the SARS-CoV-2 genome currently spread worldwide are the G clades and their derivative GH and GR clades (Figure 2). In particular, the GR clade (Nucleocapsid RG203KR mutations and the combination of the spike D614G) with high infectivity is currently the most common form of SARS-CoV-2 across the globe.[105] According to a recently published study, the high mortality associated with the G clade (including GH and GR) is due to carrying the D614G mutation in the S protein, which causes SARS-CoV-2 to enter the cells at a rate more than double and be more resistant to anti-serum neutralization.[106,107]

Clade	Amino acid mutation	Viral Functional change
G	NSP3 : F106F NSP12b : P314L S : D614G	Intensification of viral infection Increased fatality rate
GH	Clade G + ORF3a : Q57H	Intensification of viral infection Increased fatality rate
GR	Clade G + N : RG203KR	Intensification of viral infection Increased fatality rate
L	The reference genome NC_045512.2	The original lineage
O	Unidentified	Unidentified
S	NSP3 : S76S ORF8 : L84S	Unidentified
V	NSP6:L37F ORF3a : G251V	Unidentified

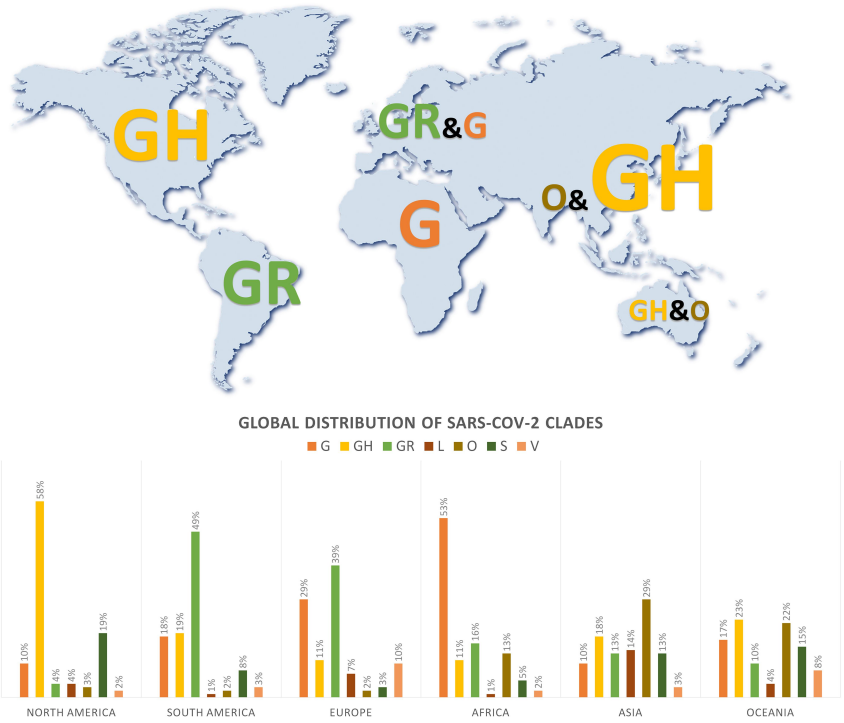

Figure 2 Global distribution of SARS-CoV-2 Clades (26 June 2020). The distribution of clades by continent (North America, South America, Europe, Africa, Asia, and Oceania) was plotted as a percentage and the major clades of each continent were indicated. Amino acid mutations are indicated for each clade, and the resulting changes in the function of SARS-CoV-2 are summarized using a table.

Therapeutic Strategies

Antiviral Drugs

Currently, COVID-19 has increasing number of disease determinants worldwide without availability of approved treatment options, and thus, researchers are urgently developing effective vaccines and treatments. In addition, attempts of using existing medicines that have been approved for other uses may benefit COVID-19 patients to a limited extent. In vitro studies have shown that several drugs approved for other applications have some effect on SARS-CoV-2, but the results were different in small-scale non-randomization trials. These include remdesivir, which was developed as an experimental drug against Ebola virus (EBOV) during the Ebola epidemic in West Africa, chloroquine (CQ) and hydroxychloroquine (HCQ) for malaria, and lopinavir/ritonavir (LPV/r), which is used as an acquired immunodeficiency syndrome (AIDS) treatment.

First, remdesivir, a nucleotide analog prodrug that inhibits the function of the viral RNA polymerases, has been reported to reduce SARS-CoV-2 infection remarkably in Vero cells (Figure 3).[108,109] Another study has found that expanded access to remdesivir in severely ill COVID-19 patients improves clinically in 36 of 53 patients.[110] However, trials in severely ill COVID-19 patients in China have shown statistically insignificant clinical results.[111]

Second, HCQ and CQ are representative drugs for the treatment and prevention of malaria. HCQ and CQ have been shown to be effective against SARS-CoV-2 infection in an in vitro study (Figure 3).[112] However, in a prospective randomized trial on COVID-19 patients in China, there was no effect of HCQ on the patients compared with those receiving conventional treatment. Rather, it has been found that a patient in the HCQ treatment group developed a serious illness.[113] In clinical adjuvant therapy trials on SARS-CoV-2-infected patients, two high- and low-dose patients among patients administered 50 different CQ doses have shown a 50% lower mortality rate than that of low-dose patients.[114]

Finally, LPV/r is an antiretroviral drug, which is a protease inhibitor used for treating human immunodeficiency virus (HIV). In vitro studies have reported that LPV/r displays the effect of inhibiting SARS-CoV-2 replication (Figure 3).[109] In addition, another clinical study has reported the administration of LPV/r and ribavirin in patients to reduce the risk of death and ARDS caused by

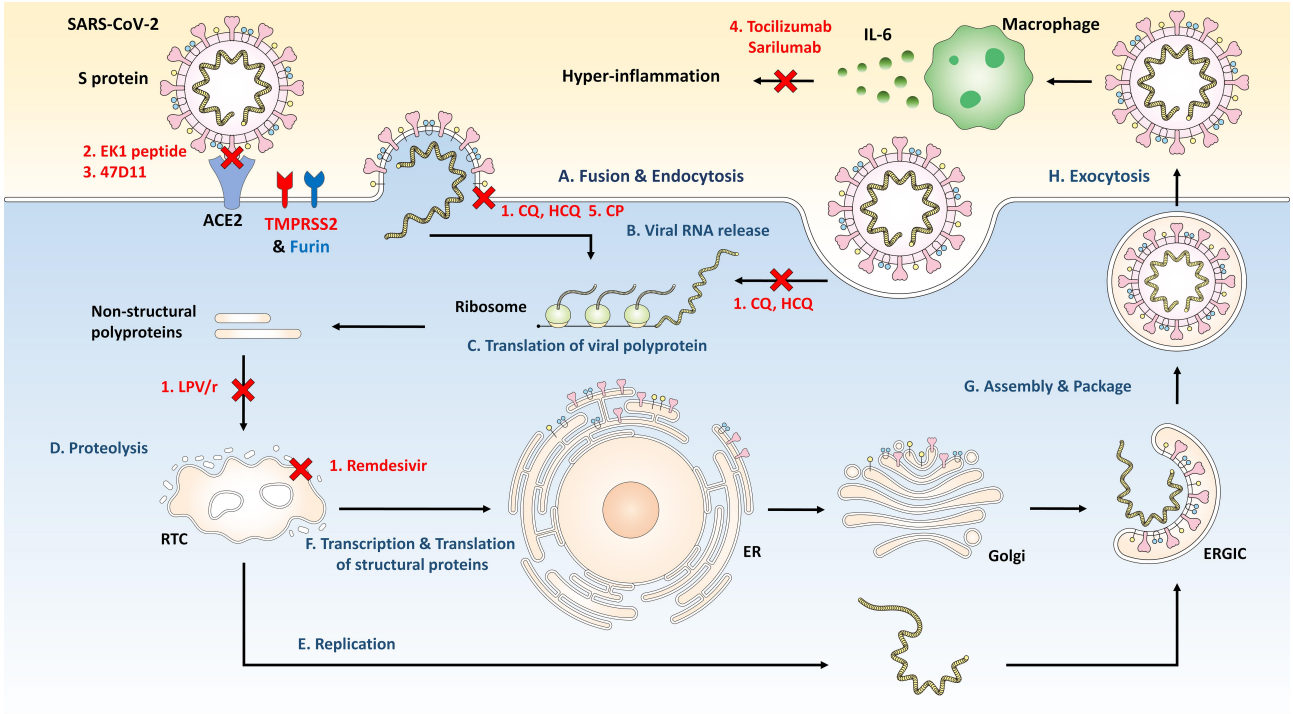

Figure 3 The SARS-CoV-2 life cycle and potential targets by antiviral agents as therapeutic strategies. (A) SARS-CoV-2 entry in target cell through endocytosis or interaction of S protein and ACE2. (B) Releasing SARS-CoV-2 genomic RNA. (C), (D) Viral polyproteins are translated and cleaved to form a replication transcription complex (RTC). (E) Genomic and subgenomic RNA replication. (F) Subgenomic RNAs produced through the transcription are translated into viral structural proteins inserted in endoplasmic reticulum (ER). (G) The viral nucleocapsid, assembled viral genomic RNA and structural proteins, bud into the lumen of the ER-Golgi intermediate cavity (ERGIC). (H) Exocytosis of SARS-CoV-2. 1. Antiviral drugs; chloroquine (CQ), hydroxychloroquine (HCQ), lopinavir/ritonavir (LPV/r), and remdesivir. 2. S protein and ACE2 interaction inhibitors; EK1 peptide. 3. Neutralizing antibodies; 47D11. 4. Immunotherapy (Anti-interleukin (IL)-6 Drugs); tocilizumab and sarilumab. 5. Convalescent plasma therapy; Convalescent plasma (CP).

SARS-CoV.[115] However, the clinical effect of LPV/r against SAR-CoV-2 is yet to be confirmed.

The S Protein and ACE2 Interaction Inhibitors

The S protein is associated with the binding to host cell receptors and membrane fusion. Therefore, inhibitors that interfere with this process are used to prevent virus transmission from infected patients. In particular, interfering with the interaction of ACE2 with certain motifs in the S2 subunit of the S protein of SARS-CoV-2, which is involved in the virus fusion with the host cell, may be effective. According to recent in vitro research, the EK1 peptide, a pan-CoV fusion inhibitor, inhibits receptor-mediated infection and fusion between SARS-CoV-2 particles and host cell membrane, thereby prevents the formation of 6-helix bundles through interaction with heptad repeat 1, which is located in the S2 subunit of the S protein of SARS-CoV-2 (Figure 3).[116]

Neutralizing Antibodies

Unlike vaccines, monoclonal antibodies provide immediate protection; therefore, administering purified monoclonal antibodies with neutralizing capacity could be another SARS-CoV-2 treatment strategy. Currently, the development of effective neutralizing antibodies mainly focuses on the S protein immobilized on SARS-CoV-2. Two potent neutralizing camelid single-domain antibodies against SARS-CoV and MERS-CoV isolated from llama can cross-react with SARS-CoV-2, disrupting the receptor binding interface.[117] Recently, it was confirmed that the 47D11 hu-mAb that binds to the S protein RBD can neutralize SARS-CoV-2 infection (Figure 3).[118] The S309 antibody, also known as the SARS-CoV monoclonal antibody, also potently inactivates SARS-CoV-2 by acting on the S protein.[119] Therefore, the use of various monoclonal antibody cocktails, which can target the listed non-RBD and RBD simultaneously, can be a good alternative for effective and safe COVID-19 prevention and treatment.

Immunotherapy

Excessive cytokine serum levels (cytokine storm) leading to multiple organ damage in severely ill COVID-19 patients are closely related to ARDS following exacerbation of COVID-19. Therefore, prevention and treatment of cytokine storms can be a good alternative that can interfere with COVID-19 progression. Clinical studies have shown that the main cause of inflammation is an increase in the levels of IL-6.[103] The complex produced by binding of IL-6 with soluble IL-6 receptor (sIL-6R) or membrane IL-6 receptor (mIL6R) activates the inflammatory response through interaction with gp130. Tocilizumab (monoclonal antibody against IL-6) can block the signal transduction that triggers the inflammatory responses by selectively acting on sIL-6R and mIL-6R (Figure 3).[120] A recent study reported that HCQ and CQ can block the development of proinflammatory cytokines, such as IL-6, which are involved in the generation of cytokine storms.[121] However, the cost and safety aspects can hinder the use of tocilizumab in COVID-19 treatment. Based on a recently published study, sarilumab, another IL-6 receptor antagonist, may aid in rapid recovery in severely ill COVID-19 patients characterized by systemic hyperinflammation (Figure 3).[122]

Convalescent Plasma Therapy

Convalescent plasma (CP) therapy is another effective method; however, the CP should be used within at least 2 weeks post recovery to ensure high neutralizing antibody titers.[123] According to a recent study, SARS-CoV-2 obtained from patients with severe respiratory disease can be neutralized by serum from several other patients (Figure 3).[124] In another study, it was thought that the clinical status of five severely ill COVID-19 patients, who were administered CP containing neutralizing antibodies, would improve.[125] Even today, many clinical trials are testing CP for COVID-19 treatment globally.

Preventive Vaccination Strategies

To develop effective SARS-CoV-2 vaccines, a multifaceted strategic approach to vaccine development is being attempted worldwide. Since the genomic and structural information of SARS-CoV-2 has become available much faster than that of other HCoVs, there is a possibility of rapid vaccine development.[91,126–129] Moreover, data generated from vaccine development for SARS-CoV and MERS-CoV, which have been studied so far, are also helpful for developing a vaccine candidate for SARS-CoV-2.[130,131]

Inactivated or Live-Attenuated Vaccines

Inactivated or live-attenuated vaccines have advantages, such as stimulation of pattern recognition receptors and high immunogenicity. The viruses are alive and replicable but non-toxic. However, due to the risk of live viruses, long-term surveillance is required for assessing the safety of the vaccine. Several inactivated virus vaccines are currently being developed against SARS-CoV-2, and the first clinical trials by Sinovac Biotech, Beijing, China, have recently begun (Figure 4). More recently, recombinant SARS-CoV-2 has been synthesized from viral DNA fragments using synthetic genomics.[132,133] Based on these findings, it is possible to approach a slightly more rapid generation of live-attenuated vaccines against SARS-CoV-2. Additionally, Codagenix, Farmingdale, NY, USA is exploring vaccine candidates against SARS-CoV-2 using a "codon-optimized off" strategy for virus attenuation (Figure 4).[134]

Recombinant Vaccines

Recombinant vaccines allow live viruses to retain some additional genes derived from pathogens through genetic manipulation, thereby translating the target protein and triggering the desired immune response.[135] The advantages of recombinant vaccines are sufficient target protein expression, prolonged stability, and induction of strong immune responses.[136]

Vaccinia virus vector-based vaccines are currently being evaluated for use in many clinical trials based on the studies that have shown that they can induce very strong immune responses to foreign antigens.[137,138] Another advantage of the vaccinia virus vector-based vaccine could be the availability of a large-scale manufacturing method, as in the case where Bavarian Nordic A/S produced and provided large amounts of its own smallpox vaccine IMVAMUNE® to the US government.[139]

The broad spectrum viral affinity and infectivity in dividing and non-dividing cells has made it possible to use a wide range of adenovirus (Ad) vectors to our advantage. Among the human Ad sera identified to date, human Ad serotype 5 (Ad5), which can be easily produced at high titers, is the most widely studied gene transfer vector.[140,141] However, pre-existing immunity against Ad induced in many people who have already been exposed to the Ad serotype is a disadvantage of Ad vectors.

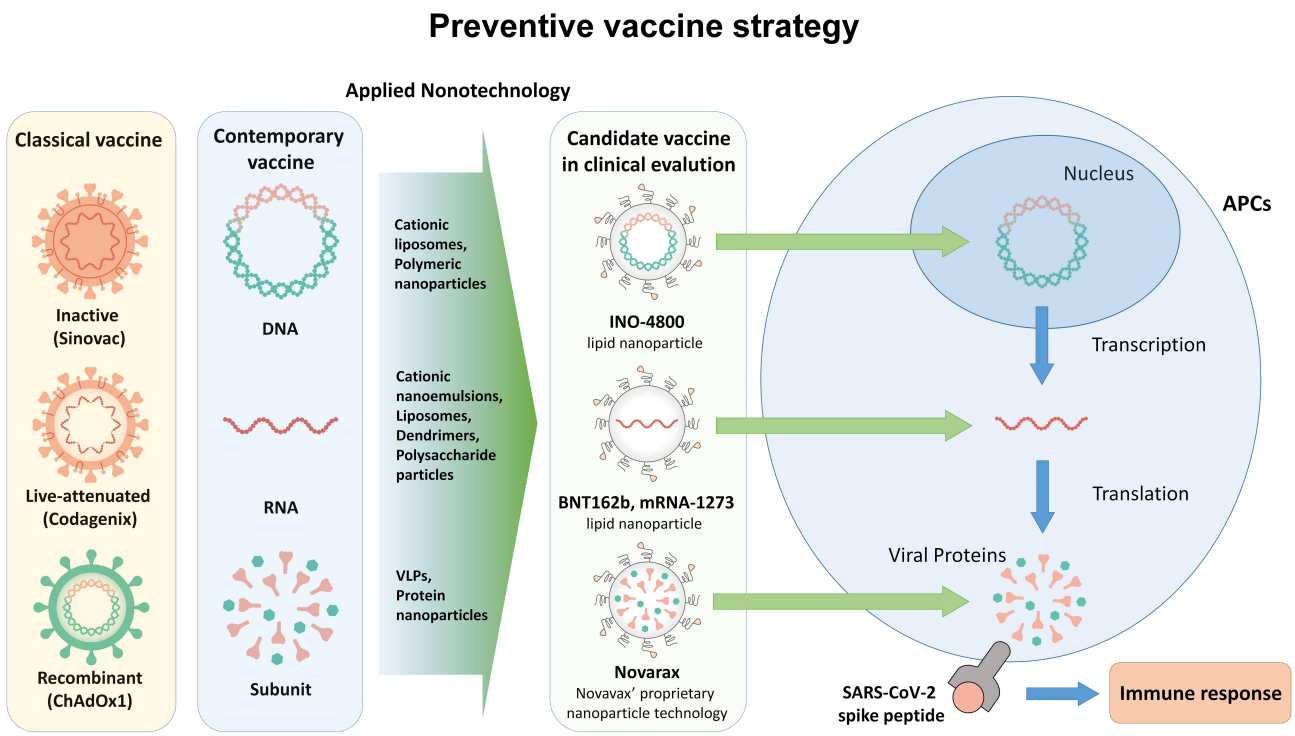

Figure 4 Classical vaccine, modern vaccine and nanotechnology applied vaccine against SARS-CoV-2. Types of classic vaccines and representative candidate vaccines in clinical trials, Nanoparticles applicable to contemporary vaccines using DNA, RNA, and subunits, representative candidate vaccines in clinical trials, and mechanism of action of nanotechnology-based vaccines in APC.

Adeno-associated virus (AAV) is a non-pathogenic, low immunogenic, vector-enclosed, single-stranded DNA virus. AAV has both the characteristics and advantages of Ads. AAV vectors require a very efficient large-scale production method, such as the baculovirus system, which has been developed because of their low titer production efficiency.[142,143] AAV is better than Ad when continuous transgene expression is required for treatment.[144,145] The capsid modification vector is an alternative to overcome the low immunogenicity of AAV vectors. Mixed capsids generated from different serotypes provide effective gene transfer and tropism to host cells.[146] Since AAV vectors often require integration with the host genome for viral gene expression, genotoxicity risk assessment must be considered when using AAV vectors.[147]

ChAdOx1 nCoV-19 (AZD1222), an Ad-based recombinant vaccine developed at the University of Oxford, Oxford, UK, was found to be resistant in the Phase I/II COV001 trial, and a strong immune response to SARS-CoV-2 was generated in all participants (Figure 4).[148] Almost all participants receiving AZD1222 showed a four-fold increase in the antibody neutralizing activity against the SARS-CoV-2 S protein.[148] In addition, no serious side effects were reported with the use of AZD1222.[148]

Application of Nanotechnology in COVID-19 Therapeutics

Scientists in the field of nanomedicine have steadily conducted research on linking the gene delivery ability of various nanosystems and viral vectors to high infectivity. Nanomedical researchers have studied the molecular mechanisms of vectors to develop delivery systems that can be used in a variety of fields.[149,150] Nanoparticles (NPs) and viruses act at the same scale, which makes the nanotechnology approach very powerful in vaccine development and immunoengineering.[151] NPs are tools that can reproduce the structural and functional properties of viruses, and nanomedicine can be the best alternative to innovative vaccine development technologies.[151–153] From the perspective of vaccine technology development, the present time, wherein SARS-CoV-2 is a major threat worldwide, is most important, and nanotechnology and nanomedicine are presented as new therapeutic technologies and approaches that can have a clinical impact.[154–157]

Theranostic Nanoparticles

Recently, the application of NPs has emerged as groundbreaking in the medical field and allows accurate diagnosis

and specific treatment of several diseases at once. The small size, low toxicity, electrical charge, and chemical plasticity make it possible to overcome several barriers encountered in various routes of administration of a generic drug. Treatment with NPs can target the SARS-CoV-2 entry and life cycle. The S protein is the most important factor in preventing the entry of SARS-CoV-2 via the first process of membrane fusion. Thus, therapeutic NPs can be designed to pre-block SARS-CoV-2 entry by inhibiting the S protein from binding to host cells. Since the introduction of nanotechnology in the treatment of general viral infections, nanomedicines that can effectively treat viruses, including Influenza A and B viruses (IAV and IBV),[158–160] EBOV,[161] HIV1 and HIV2,[162–165] Herpes simplex virus type 1 and 2 (HSV1 and HSV2),[166–169] hepatitis B virus (HBV),[170–176] hepatitis C virus (HCV),[177] and human norovirus (HuNoV),[178] have been developed and commercialized in various ways (Table 1). In particular, a few months ago, the first SARS-CoV-2 therapeutic drug, dexamethasone has been developed using nanotechnology. It has been reported of treating infections caused by SARS-CoV-2 using an anti-edema and anti-fibrotic mechanism, and effective delivery and treatment can be expected using various nano-formulating dexamethasones.[179]

Intranasal Delivery Therapy

Currently, many studies are being conducted on developing a method for delivering nanoparticles into the nasal cavity as a safe and more effective countermeasure against viral infection and treatment.[180] Since SARS-CoV-2 initiates infection on the mucosal surface of the eye or nasal cavity, mucosal therapy is the most important strategy for treating such infectious diseases. Delivery through the nasal cavity is not only simple and inexpensive but also non-invasive, and the NP is rapidly absorbed due to the cavity's abundant capillary plexus and large surface area.[181] The properties of the NPs, such as the surface charge, size, and shape, are important factors to be considered while optimizing the method of delivery to the nasal cavity and play a critical role in effective and safe treatment.[182] Studies have been conducted using small animals to evaluate the system that is delivered to the lungs by administering NPs to the nasal cavity. Therefore, findings of these animal studies cannot be easily generalized to humans. To date, three types of NPs (organic, inorganic, and virus-like NPs) have been designed with delivery capabilities that are suitable for therapeutic purposes, which can also be administered intranasally for effective delivery.

Treatment Using Organic NPs

Lipid nanoparticles (LNs) are biocompatible due to their lipid properties; hence, they can be selectively applied in fields such as biomedical science. Among the various LNs, liposomes in the form of spherical capsules, which are hydrophilic on the inside and consist of a phospholipid bilayer on the outside, are most suitable for intranasal delivery.[183] The advantages[184–188] and disadvantages[189–192] obtained by using liposomes have been summarized (Table 2). Using lipid-coated mesoporous silica nanoparticles, a form of LNs, an antiviral molecule ML336, which is unstable and highly hydrophilic against Venezuelan equine encephalitis virus (VEEV), has been delivered into VEEV-infected mice. The suitability, cycle time, and viral titer have been shown to improve.[193] Drug candidates in the form of nucleic acids, such as siRNA, have a significant limitation of being unstable during systemic circulation.[194–196] However, transporting siRNA using LNs can target specific organs and has the great advantage of preventing degradation during systemic circulation.[197]

Polymer nanoparticles (PNs) are an effective choice of delivery systems because their properties and functions can be adjusted according to their specific application. Conjugation of a therapeutic compound to chitosan-made PNs can improve penetration of the mucosal tissue and the persistence of PNs in the mucosal environment.[198,199] Antibody-drug conjugates using auristatin are used for relatively safe treatment of blood cancer by eliminating the risk of high toxicity but have a critical disadvantage of the drug payload being too low. To overcome this limitation, nanoparticle-drug conjugates of monomethyl auristatin E, developed using PN technology, enable the availability of a large amount of auristatin payload, and have high safety.[200] In addition, in the case of accurin PNs encapsulating the Aurora B kinase inhibitor AZD2811, the toxicity has been observed to reduce significantly and the efficacy has been found to increase compared with that before introducing PNs, which has been shown to cause decisive side effects in a Phase 2 clinical trial.[201]

Dendrimer nanoparticles (DNs) have strong interactions with viruses. The resulting system improves antiviral activity and has a powerful effect in preventing infection in the host. In addition, effective cases have been reported, wherein DNs are used as a treatment for viral infectious

Table 1 Conventional and Nanomedical Treatments for Major Viruses

Viruses	Characteristics of Viruses	Disease	Medications for the Treatment	Nano Technology Applied Medications: Antiviral Mechanism
IAV and IBV	Sudden high fever, headache, and muscle pain Antigenic drift	Influenza (flu)	Oseltamivir, zanamivir, peramivir, baloxavir marboxil	STP702 (Fluquit™):[158] RNA interference Titanium dioxide (TiO_2) nanoparticles:[159] Photocatalytic inactivation of the virus Oseltamivir-modified silver nanoparticles:[160] Inhibition the activity of H1N1 influenza virus through ROS-mediated signaling pathways
EBOV	A lethal viral hemorrhagic fever	Ebola virus disease (EVD) or Ebola hemorrhagic fever (EHF)	None	TKM-130803:[161] RNA interference
HIV1 and HIV2	Targeting CD4-positive T cells to attack the immune system, resulting in acquired immunodeficiency syndrome (AIDS)	AIDS	24 approved drugs belonging to the class of Nucleoside Reverse transcriptase inhibitors (NRTIs), The non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase inhibitors, Tat TAR interaction inhibitors	Indinavir loaded Solutol® HS15 nanocapsules:[162] Enhancing drug distribution DermaVir:[163] DNA-based therapeutic HIV vaccine Doravirine (MK-1439):[164] Non-nucleoside reverse transcriptase inhibitors VivaGel®:[165] Blocking the interaction between viral spike proteins and the human cell proteins
HSV1 and HSV2	Herpes simplex	Herpes	Acyclovir Valacyclovir Famciclovir Herpes DNA polymerase inhibitors Helicase primase inhibitors	Acyclovir-loaded nanoparticles:[158,159] Enhancing drug distribution Silver nanoparticles:[160] inhibit HSV-1 infections by blocking the attachment VivaGel®:[161] Blocking the interaction between viral spike proteins and the human cell proteins

(*Continued*)

Table 1 (Continued).

Viruses	Characteristics of Viruses	Disease	Medications for the Treatment	Nano Technology Applied Medications: Antiviral Mechanism
HBV	No subjective symptoms	Hepatitis B	Lamivudine Adefovir Dipivoxil Entecavir Telbivudine Tenofovir disoproxil Tenofovir alafenamide	Interferon (IFN)-α:[170] inhibit HBV replication Pegylated IFN (Pegasys®):[171] unknown Lamivudine (Epivir®):[172] inhibit the reverse transcriptase of HBV Adefovir (Hepsera®):[173] blocking reverse transcriptase Entecavir (Baraclude®):[174] inhibits reverse transcription, DNA replication and transcription in the viral replication process Telbivudine (Tyzeka®):[175] impair DNA replication Tenofovir (Viread®):[176] inhibit the reverse transcriptase of HBV
HCV	A high genetic diversity	Hepatitis C	Peginterferon α-2a (Pegasys®) Peginterferon α-2b (Pegasys®)	PEGylated IFN and Ribavirin:[177] Enhancing Ribavirin's antiviral effect
HuNoV	Nausea, vomiting, watery diarrhea, and abdominal pain	Norovirus infection	None	Gold/copper sulfide (AuCuS) core-shell Nanoparticles:[178] degradation of the RNA and destruction of the capsid
SARS-CoV-2	Fever, cough, and difficulty breathing	COVID-19	HCQ and CQ, Lopinavir/ritonavir, Umifenovir, Camostat mesylate (TMPRSS2 inhibitor), Tocilizumab, Meplazumab	Nano-formulating dexamethasone:[179] anti-oedema activity and anti-fibrotic effects

Table 2 Advantages and Disadvantages of Nanomedicine on Therapeutic Strategies for COVID-19

NPs	Advantages	Disadvantages
Liposomes	Reduced toxicity[184] Selective target specificity[185] Enhancement of drug activity against pathogens[186,187] Improved pharmacokinetics and pharmacodynamics[188]	Low drug entrapment[189] Difficulty of sterilization[190] Short shelf life due to instability[191] Rate of removal from the bloodstream[192]
Polymer Nanoparticles (PNs)	High stability[203,204] Various preparation methods[205,206] Control and persistence of drug release[204,207] Adjustability of chemical and physical properties[208] Suitability for hydrophilic and hydrophobic drugs[203]	Difficult scalability[213] Inadequate toxicological assessment[214]
Dendrimer Nanoparticles (DNs)	High cell penetration[209,210] High structural homogeneity[209] High miscibility and solubility[211] Controllable synthesis and degradation[209,210,212]	High production cost[209] Difficulty of clinical application in basic research[215] The need for quality management improvement[210]
Gold Nanoparticles (GNs)	High biocompatibility[210] Controllable particle size[210] Convenience of synthesis and conjugation of various bioactive agents[210]	Nanoparticle aggregation[211] Impossible biodegradation[220,221] High cost of large-scale production[222]
Virus Like Particles (VLPs)	Stabilization by disulfide bonds[227,228] Produced by cell-free protein synthesis[227,229] Small molecule, nucleic acid and protein loading capacity[230,231] Functionalization of antibody fragment display for specific cell targeting[232,233]	Low stability[227] Phagocytic avoidance[234] Extravasate from blood vessel[235]
Cell-Derived Vesicles	Low inherent toxicity[246] Low apparent risk of aneuploidy[247] Low immune rejection[248]	Promoting metastasis formation in tumor cells[249–252] contribution to tumor cell survival[253,254]

diseases, such as influenza virus and HIV.[202] The advantages[203–212] and disadvantages[209,210,213–215] obtained by applying PNs and DNs are summarized (Table 2).

Treatment Using Inorganic NPs

The ability of gold nanoparticles (GNs) to induce an immune response by antigen-presenting cells easily is attractive for use in vaccine development. GNs have the advantage of being easily transformed for delivery through the nasal cavity.[216] It also has the advantage of activating the immune response associated with CD8+ (cytotoxic) T cells by spreading to the lymph nodes.[217] The advantages[218] and disadvantages[219–222] of applying GNs to nanomedicine have been separately summarized (Table 2).

Treatment Using Virus-Like Nanoparticles

Virus-like nanoparticles (VLPs) are capsids, comprising virus-derived structural proteins and adjuvants. VLPs can generate a potential immunogenic epitope, resulting in higher immunogenicity. Furthermore, since VLPs are small, they can act as adjuvants, and changing adjuvants can induce a much more effective immune response than viruses.[223–225] As a result of intranasal delivery of VLPs using influenza virus, it has been found that VLP functions as a vaccine by producing a very large number of T cells and antibodies that can induce various types of immune reactions to improve immunity and prevent further infection.[226] The advantages[227–233] and disadvantages[227,234,235] of applying VLPs in terms of drug delivery or treatment have been summarized separately (Table 2).

Treatment Using Cell-Derived Vesicles

Cell membrane nanovesicles and exosomes have been reported to have the ability to bind and neutralize bacterial toxins by previous studies.[236–238] In addition, recently, the development of cell membrane nanovesicles containing proteins having the same structure and activity as native

cells is being made by biomimetic synthesis technology that includes the synthesis and display of proteins on the cell surface.[239–241] Cell membrane nanovesicles, built to display high levels of ACE2 and abundant cytokine receptors, are nanodecoys that can compete with host cells for viral and cytokine binding. Studies have shown that nanodecoy significantly inhibited the replication and infection of SARS-CoV-2 and efficiently binds and neutralizes inflammatory cytokines such as IL-6 and GM-CSF.[242,243] Therefore, a treatment method using cell membrane nanovesicles can be an effective alternative to SARS-CoV-2 and cytokine storms.

Exosomes are tiny nanovesicles with a size of 30nm to 150nm, secreted for all types of cell-to-cell communication, and are emerging nanomaterials in recent cell regeneration, treatment, and diagnostic research.[244] It has already been reported that exosomes containing the S protein of SARS-CoV induced an accelerated neutralizing antibody titer by priming with a vaccine of the S protein of SARS-CoV and then increasing with an adenovirus vector vaccine.[245] Therefore, this strategy using exosomes has the potential to be sufficiently applied to treatment for SARS-CoV-2. The advantages[246–248] and disadvantages[249–254] of applying cell-derived vesicles to nanomedicine have been separately summarized (Table 2).

Pulmonary Delivery Using NP Inhalation Aerosols

If the advantage of drug delivery through the nasal cavity is to act on the mucous membrane area where the infection occurs, then the lungs are an important organ for drug delivery because they are another target for treating SARS-CoV-2 infection, which infects primarily through the respiratory tract (the upper airways and lung).[255–257] Therefore, the use of inhaled aerosols is suggested as an effective non-invasive mode of administration. Additionally, the delivery of inhalable NPs to the lungs overcomes disadvantages, such as side effects caused by high drug concentrations in the serum with conventional oral or intravenous drug administration methods. Various nanotechnologies have been applied to develop NPs that can function as lung inhalation aerosols. These respirable NPs can be encapsulated by microparticles manufactured down to five microns to fit the aerodynamic size range or agglomerate into an aerodynamic size range. Most NPs are delivered directly to the lungs either by spraying colloidal dispersions or via dry powder inhalers and pressurized metered dose inhalers in solid form.[255]

The LNs mentioned previously are also one of the most widely studied NPs for effective delivery of drugs into the lungs.[258] Due to the unique advantages of LNs, which include their production from substances present in the lungs, such as components of lung surfactants, they are the highest priority candidates for delivering therapeutics to the lungs.[259] Liposomes are generally liquid, and the application of aerosol through a nebulizer has mainly been attempted to deliver them to the lungs in the early days;[260] however, drug stability and nebulizer leakage have been pointed out as the disadvantages.[261] Therefore, to compensate for these shortcomings, various studies have been conducted on the development of liposome formulations in the form of dry powder.[262–264] Moreover, cationic liposomes, which have the advantage of self-assembly with nucleic acids, are attracting the most attention as systems that deliver genes to the lungs and are known to be suitable for transporting peptides and substances having high molecular weight.[265]

PNs play important roles in drug delivery to the lungs by achieving efficient delivery of drugs, maintaining the stability of drugs, and controlling the release of drugs.[266] Currently, cationic LNs have many clinical advantages over PNs; however, cationic PNs are one of the important carriers for pulmonary delivery of genes.[267,268]

Although studies on delivery through the nasal cavity using DNs have already been mentioned above, many studies on pulmonary delivery using DN are also being conducted for delivering DNA drugs to the cell nucleus, with properties similar to that of liposomes.[269] Research has already focused on drug delivery to the lungs as one of the applications of the method of delivering high molecular weight substances into the body using DNs.[270,271] In order to use DNs effectively while delivering drugs to the lungs, additional studies are needed that consider aspects such as the biocompatibility and cytotoxicity.

Nanotechnology-Based Diagnosis

Nano biosensors have the advantage of selectively detecting all types of analytes by combining the excellent electrical and optical properties of nanomaterials with biological or synthetic molecules used as receptors.[272] Using these advantages, various methods of detecting SARS-CoV-2 are being studied.[273]

Currently, using a Silicon-on-insulator nanowire sensor made using complementary metal-oxide-semiconductor compatible technology, the SARS-CoV-2 antibody can be detected in 5–15 minutes with an expected sensitivity of 10^{-12}–10^{-15} M.[274]

In the case of applying Graphene, the detection of SARS-CoV-2 in clinical samples was attempted with a sensor

produced by coating the graphene sheet of the field-effect transistor with a specific antibody against the SARS-CoV-2 spike protein.[275] As a result of the study, it was possible to detect SARS-CoV-2 spike protein at a concentration of 1 fg/mL in phosphate-buffered saline and 100 fg/mL clinical transport medium.[275]

The SARS-CoV-2 biosensor using thiol-modified antisense oligonucleotides-capped GNs can diagnose positive COVID-19 cases with the naked eye through color change within 10 minutes from total RNA isolated from infected biosamples.[276] As another application method for GNs, the glycan bond between the polymer-stabilized multivalent GNs bearing sialic acid derivative and the S protein of SARS-CoV-2 was identified using a glyconanoparticle platform. Applying these characteristics has the advantage of building a low-cost detection platform that can be detected in less than 30 minutes with a lateral flow diagnostic device.[277]

Nanotechnology-Based Vaccine Development

Subunit Vaccines

Subunit vaccine candidates are required to enhance immunogenicity effectively by eliciting an immune response when co-administered with molecular adjuvants using specific parts of the structural components of SARS-CoV-2. Therefore, developing a vaccine that targets the subunit of the SARS-CoV-2 S protein is a top priority. This is because membrane fusion and receptor-binding sites are present on the S protein.[278] Vaccines based on the S protein inhibit viral infection by activating antibodies that prevent viral binding and subsequent membrane fusion.[279] The SARS-CoV-2 S protein, which interacts with ACE2, is a notable candidate sufficient for both vaccine and therapeutic development.[87,124,280] In addition, NPs similar to immunogenic viruses have been developed and produced with the Novavax® proprietary recombinant nanoparticle vaccine technology with the S protein (Figure 4).[281] The University of Queensland, Brisbane, Australia is also developing a new SARS-CoV-2 subunit vaccine using a "molecular clamp" technology that pre-blocks the binding of viral proteins.[282] As an alternative, the development of subunit vaccines using NPs, such as VLPs and protein NPs, is also actively underway. A higher binding affinity of RBD in SARS-CoV-2 for ACE2 than that of RBD in SARS-CoV has been found.[283] Therefore, the RBD-based SARS-CoV vaccine can help prevent SARS-CoV-2 infection and be important for SARS-CoV-2 vaccine development. Moreover, RBD-based vaccines are effective in preventive and therapeutic strategies and are currently being developed by many research institutes and multinational pharmaceutical companies.[134] RBD-based vaccines also have the advantage of minimizing host immunity enhancement.[279]

Nucleic Acid Vaccines

When viruses enter the host cell by infection, the antigen encoded by the nucleic acid is expressed, which induces a cell-mediated reaction with the antibody. Based on this principle, nucleic acid vaccination is another effective immunization method that uses artificially synthesized nucleic acids to elicit an immune response, such as that induced by live-attenuated vaccines. The improved immunogenic properties that mimic the infectious process are the potential advantages of mRNA vaccines. To maximize the effect, several mRNAs are mixed into a single vaccine.[134,284] An RNA vaccine candidate against SARS-CoV-2 is now known as mRNA-1273 (Moderna, Cambridge, MA, USA) (Figure 4). This vaccine comprises a synthetic mRNA strand such that the binding site for ACE2 can be translated to the previously modified SARS-CoV-2 S protein.[285] After inoculation with intramuscular injection, a specific antiviral response to the SARS-CoV-2 S protein is induced. Moreover, the synthesis of nucleic acid vaccines does not require viruses, unlike conventional vaccines made of small subunits of inactivated or live pathogens.[285] Therefore, as the safety is guaranteed, only the passing of the Phase I trial for mRNA-1273 will help a continuous evaluation of efficacy to progress quickly.[285] mRNA-1273 is designed based on the LN platform; however, new nanotechnology is being introduced for the effective delivery of nucleic acid vaccines. In the case of mRNA-based vaccines, not only LNs but also DNs and PNs are being used for effective delivery and high stability.[286,287] BNT162b1, under development by Pfizer, New York, NY, USA, is a codon-optimized mRNA vaccine encoding the SARS-CoV-2 RBD (Figure 4). This vaccine uses the RBD antigen to which the trimerization domain of T4 fibritin has been added to increase immunogenicity.[288] Coalition for Epidemic Preparedness Innovation had begun developing vaccines as soon as the first gene sequence was released through partnership with a group developing vaccines using a novel platform. As a result, the mRNA-based SARS-CoV-2 candidate progressed to the human clinical trial stage.[289] In addition, INO-4800, developed by Inovio Pharmaceuticals, Inc., Plymouth Meeting, PA, USA, is a candidate DNA vaccine among nucleic acid vaccines (Figure 4). Similar to RNA vaccines, INO-4800 is a nucleic acid vaccine that can induce an immune response by being translated into proteins within human cells. Compared with

conventional vaccines, nucleic acid vaccines have great advantages in terms of production cost and purification methods. Furthermore, the nucleic acid-only structure also prevents the production of misfolded proteins that can occur in recombinant vaccines.[290,291] However, the immunogenicity of nucleic acid vaccines is greatly influenced by the amount of plasmid injected into the cell and the appropriate administration interval and route. Through nanotechnology, NPs, including cationic liposomes, DNs, or PNs, have been applied to the development of nucleic acid-based vaccines to enhance the delivery efficacy and stability.[286,287]

NP-Based Vaccines

Unlike SARS-CoV, MERS-CoV has been utilized multiple times to introduce nanotechnology into vaccines or therapeutic research. Importantly, it has been recently reported that VLPs are suitable for the development of vaccines or treatments for symptoms of MERS-CoV infection. Nano-sized VLPs, which have the characteristic function of the virus, have the advantage of being better delivered through the lymph and capillaries than other small vaccines.[292–294] In addition, it has the effect of reducing the systemic inflammatory response, and similar to viruses, has the advantage of being able to very easily enter cells.[293] Furthermore, the delivery of many antigens makes the antigen-presenting cell functioning more effective. Therefore, the synthesized complex recognized by the T cell receptor increases the vaccine's immunogenicity and efficacy, thereby ensuring patient safety.[293] Nano-sized VLPs entering the host cell are directly involved in B cell activation and boosting the immune system.[292,295] Indeed, the characteristics of these synthetic nano-sized VLPs are principle to developing vaccine platforms.[296–298] Nano-sized VLPs have also been reported to overcome viruses by increasing the immune response effectively in animal experiments.[281,299,300]

Recently, the MERS-CoV S protein has been synthesized using silkworm larvae. This has then been applied to the nano-sized VLPs,[223] which exhibit native conformational epitopes produced via incubation with surfactant and cell membrane vesicles.[239] In another study, the development of nano-sized VLPs capable of acting as a nanocarrier in red blood cells has been achieved by single compression of red blood cells through a 1-μm filter.[301] MERS-CoV nano-sized VLPs have been synthesized using the recombinant S, membrane, and envelope proteins, tested in animal models, and linked to having increased immunogenicity.[223] Nano-sized VLPs have a wide range of applications, can enhance vaccine safety and effectiveness, and have tremendous advantages that can be utilized for specific purposes. Since these findings have been derived for the S protein commonly present in MERS-CoV and SARS-CoV, they can be effectively applied for treating SARS-CoV-2 infection.

Inactivation of SARS-CoV-2 in the External Environment Using Nanotechnology

SARS-CoV-2 is activated at temperatures ranging from 1 to 35°C and is easily inactivated under UV, highly alkaline, or acidic conditions.[302] In addition, the degree of stability of SARS-CoV-2 varies greatly depending on the components that make up the surface of the infectious particle, and SARS-CoV-2 can be easily inactivated with commonly available disinfectants.[303] The activation of SARS-CoV-2 in aerosols and on surfaces is similar to that of SARS-CoV; therefore, surface treatment using NPs that have been proven effective against SARS-CoV will be sufficiently applicable to SARS-CoV-2.[98] The use of nanotechnology can provide alternatives more effective than conventional disinfection protocols for viruses used in general or medical settings that typically rely on chemical, physical, and biological strategies. Moreover, by using NPs, one can freely control the release rate of metal ions, which have proven to be antibacterial, on the surface of substances requiring antibacterial action. Because NPs can accumulate in cells owing to their nature, they can overcome the disadvantages of antimicrobial substances or metal ions that easily leak out of cells. Silver, which has been used as an antibacterial agent since ancient times, is now applied to paints and food trays.[304–306] Silver nanoparticles (Ag-NPs) have already been proven to display antiviral effects against various viruses.[307–310] Ag-NPs exert antiviral activity by dissolving and releasing Ag^+ ions with microbial toxicity. Ag^+ ions can interact with proteins present on the surface of the virus or infiltrate and accumulate in host cells, disrupting the function of proteins that play an important role in virus replication, such as enzymes involving thiols.[308,311] Another antiviral function of Ag-NPs has been hypothesized, wherein they competitively interfere with virus binding to host cells because of their physical interactions with the viral surface, depending on their size.[307] As a result, it has been found that Ag-NPs with a size of about 10 nm show the strongest physical interaction and antiviral effect compared with that of particles in other sizes.[308] In addition, Ag-NPs have an antiviral effect of damaging the virus structure using reactive oxygen species that are released after binding to the virus surface. Ag-NPs have already been applied to and used in medical equipment.

When applied to face masks and air filters, they can be used to inactivate SARS-CoV-2 via the antiviral effect of Ag^+ ions. Currently, it has been reported that bacteriophage MS2 from dust can effectively be blocked by applying Ag-NPs to filters.[312]

Copper, which has recently been proven to exhibit antiviral effect against HuCoV-229E, may be a suitable candidate for the inactivation of SARS-CoV-2.[313] When a virus incubates on a surface coated with Cu, the virus genome is degraded and inactivated.[313] This antiviral mechanism involves the inactivation of virions by disrupting the function of certain viral proteins using hydroxyl radicals produced by Cu^{2+} ions present on the surface of the material and inactivation by direct contact with the surface.[314] Similarly, studies have reported that SARS-CoV-2 is easily deactivated on the surface of Cu-loaded materials.[98] Furthermore, Cu is far more advantageous in terms of economy than Ag, and it can easily be used to produce PNs and has excellent stability. Therefore, the development and application of NPs with Cu or copper oxide (CuO) is the most suitable strategy to inactivate SARS-CoV-2 in the external environment. For example, in an experiment using a mask containing CuO-NPs, the influenza virus has been shown to be inactivated remarkably.[315]

Graphene derivatives (GDs), together with metal NPs, can effectively inactivate viruses.[316] The antiviral mechanism of GDs involves electrostatic interactions, wherein the negative charge on the coated surface of the GDs promotes its binding to the positively charged viral particles.[317] When GDs are applied to antibodies against viruses using nanotechnology, they show excellent effects on rotavirus and influenza virus infections.[318–320] In addition, this characteristic of GDs can also be applied to the prevention, diagnosis, and treatment of SARS-CoV-2, according to recent studies.[321]

Iron oxide nanoparticles (IONPs) have already proven antibacterial activity through many studies.[322,323] It has also been approved by the US Food and Drug Administration (FDA) for the treatment of anemia because of the excellent biocompatibility of IONPs.[324] The interaction between IONPs and the S protein of SARS-CoV-2 has been identified in recent studies and the potential antiviral activity of IONPs has been reported.[325] In addition, the ability of IONPs to produce ROS can be applied to inactivate SARS-CoV-2 in the external environment.[326,327]

Conclusion

In the past, treatment and vaccine candidates for SARS and MERS have not been fully researched and developed, as they have not been recognized for adequate investment and effectiveness due to the significantly lower infection rates than that for COVID-19. However, unlike the case of SARS or MERS, COVID-19 has been a worldwide threat for almost a year. Research and development using innovative methods, such as nanotechnology, is essential to end this pandemic effectively in a short time. Various treatments using nanotechnology have been developed and commercialized for common viral infections, such as IAV and IBV,[158–160] EBOV,[161] HIV1 and 2,[162–165] HSV1 and 2,[166–169] HBV and HCV,[170–177] and HuNoV.[178] The accumulated advancements in these virus-fighting nanotechnologies can play an important role in taking SARS-CoV-2 treatment and vaccine development to the next level. The tedious COVID-19 pandemic, which has not yet been put to end, is now moving in the direction of overcoming the virus in a step-by-step fashion with the help of nanomedicine. Currently, several companies are moving away from traditional SARS-CoV-2 treatment and prevention strategies and using nanotechnology to develop various types of vaccines and therapeutics and conduct clinical evaluations. For example, dexamethasones, a COVID-19 therapeutic agent that has introduced via various nano-formulations, has led to a big turn in the treatment of COVID-19.[179] In addition, the clearance of Phase 3 clinical trials of the liposomal mRNA vaccine (BNT162b) developed by Pfizer can be considered a great achievement of nanomedicine.[328] Moreover, the technology that deactivates SARS-CoV-2 in the external environment using nanomaterials, such as Ag-NPs,[307–310] NPs with Cu or CuO,[314] and GDs,[316] and diagnostic technology that can quickly detect SARS-CoV-2 without the use of expensive equipment by applying GNs,[329] are also contributing towards the prevention and control of COVID-19. Nonetheless, owing to the complex situation caused by COVID-19, it is believed that the existing platform needs to be modified in order for the research in various fields globally to be more efficient. Therefore, nanotechnology and nanomedicine can be suitable alternatives to this change in the research and development paradigm.

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Disclosure

The author declares that they have no conflicts of interest for this work.

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