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REVIEW

Silver-Based Nanomaterials as Therapeutic Agents Against Coronaviruses: A Review

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Abstract: Since the identification of the first human coronavirus in the 1960s, a total of six coronaviruses that are known to affect humans have been identified: 229E, OC43, severe acute respiratory syndrome coronavirus (SARS-CoV), NL63, HKU1, and Middle East respiratory syndrome coronavirus (MERS-CoV). Presently, the human world is affected by a novel version of the coronavirus family known as SARS-CoV-2, which has an extremely high contagion rate. Although the infection fatality rate (IFR) of this rapidly spreading virus is not high (ranging from 0.00% to 1.54% across 51 different locations), the increasing number of infections and deaths has created a worldwide pandemic situation. To provide therapy to severely infected patients, instant therapeutic support is urgently needed and the repurposing of already approved drugs is presently in progress. In this regard, the development of nanoparticles as effective transporters for therapeutic drugs or as alternative medicines is highly encouraged and currently needed. The size range of the viruses is within 60–140 nm, which is slightly larger than the diameters of nanoparticles, making nanomaterials efficacious tools with antiviral properties. Silver-based nanomaterials (AgNMs) demonstrate antimicrobial and disinfectant effects mostly by generating reactive oxygen species (ROS) and are presently considered as a versatile tool for the treatment of COVID-19 patients. Other metal-based nanoparticles have been primarily reported as delivery agents or surface modifying agents, vaccine adjuvant against coronavirus. The present review summarizes and discusses the possible effectiveness of various surface-modified AgNMs against animal coronaviruses and presents a concept for AgNM-based therapeutic treatment of SARS-CoV-2 in the near future.

Keywords: silver nanomaterials, coronavirus, silver nanocomposites, antiviral, SARS-CoV

Introduction

Coronaviridae is an emerging family of coronaviruses and comprises two subfamilies, coronavirinae and torovirinae.[1] Coronavirinae is sub-categorized into four genera, namely alpha, beta, gamma, and delta coronaviruses.[2] Until now, humans have been mostly affected by alpha (229E, NL63) and beta genera (OC43, SARS-CoV, HKU1, MERS-CoV and SARS-CoV-2).[3] As a point of fact, the alpha and beta genera infect mammals while the delta and gamma genera mainly infect birds.[4] In 1930, the first bird coronavirus was discovered when domestic chickens were infected by an unknown pathogen named an infectious bronchitis virus (IBV).[5] Later, in 1965, the first human coronavirus was reported[6] with common cold symptoms. In 1968, eight scientists proposed the name "corona" (which means "crown" or "wreath" in Latin) for the newly discovered viruses based on detailed findings of their structures.[7] This structural exploration showed that four types of proteins are present in all coronavirus structures: spike (S), envelope (E), membrane (M), and nucleocapsid (N).[8] A positive sense single-strand ribonucleic acid

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(RNA) (+ssRNA), which is 26–32 kilobases in length, is packed inside an N-protein shell with S-, E-, and M- proteins surrounding it (Figure 1).[9]

The S-protein is believed to be the principle and only receptor-attachable part of each viral body.[10] The S-protein of the SARS virus binds to the angiotensin-converting enzyme-2 (ACE2) receptor while, for the MERS virus, the S-protein binds to host dipeptidyl peptidase-4 (DPP4) receptors.[11]

The recently discovered SARS-CoV-2 genome sequence matches about 79% of previous versions of SARS-CoV and around 50% of MERS-CoV.[12] The newly discovered SARS-CoV-2 was first reported to affect humans in late 2019 with common pneumonia-like symptoms.[13] Based on the degree of infection, other symptoms were also observed including respiratory problems, fever, cough, diarrhea, shortness of breath, dyspnea, kidney failure, and even death.[14] Scientists termed this new virus SARS-CoV-2[15,16] because of its similarity to SARS-CoV and refer to it as a "novel coronavirus" because of its dissimilarity with previously reported coronaviruses.[17] This virus has already infected more than 39 million people of which nearly 1.1 million people have died as of October 18, 2020, a number which continues to increase daily.[18] The World Health Organization (WHO) has proposed that widespread testing is the best way to reduce these numbers.

Repurposing antiviral drugs or drugs used for other types of therapy to provide therapeutic support to SARS-CoV-2 patients is a temporary solution. Targeted therapeutic treatments will take some time to develop and test. Vaccination against the virus is the only treatment that can boost our immune system and grow antibodies that work against SARS-CoV-2. However, a development time of about 12–18 months is needed to prepare a completely new vaccine, although researchers are trying to speed up the development.[19,20] Hence, in this situation, there is an urgent need for better and instant drug support for SARS-CoV-2 patients.

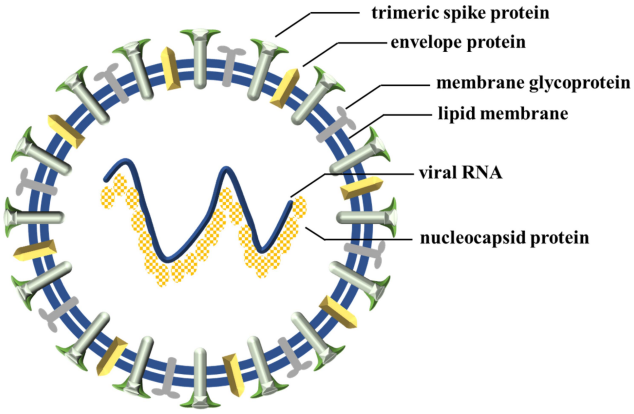


Figure 1 Schematic diagram of coronavirus.

Nanoparticles (NPs) have emerged as one of the most important therapeutic materials used in the medical field.[21] Recently, metal,[22–24] metal oxide,[25,26] metal sulfide,[27,28] and inorganic NPs have been developed with proper surface modifications which show promise for important therapeutic applications in various fields.[29] Apart from inorganic NPs, there are also several kinds of organic NPs and among them, some of the most extensively studied are nanospheres,[30] dendrimers,[31] liposomes,[32] micelles,[33] and solid lipid nanoparticles,[34,35] Presently, four organic nanoparticle-based vaccines are in clinical phase trials against COVID-19 and have shown promising results in terms of immunogenicity and safety.[36]

Among inorganic materials, AgNMs have been extensively studied and shown to possess different biological activities, i.e., antibacterial, antifungal, and anticancer. As a result, they are gaining importance in the medical field.[37–41] AgNMs are also being investigated due to their catalytic properties,[42,43] non-toxic nature, high quantum efficiency,[44,45] enhanced conductivity,[46,47] antibacterial properties,[48,49] anticancer properties,[50,51] disinfectant capacity,[52,53] stability,[54] biochemical capacity as sensors,[55,56] water purification properties,[57–60] and straight forward synthesis.[61–65] While AgNMs are still not a well-explored topic in the field of virology, some anti-viral actions against a few known viruses, i.e., human immunodeficiency virus (HIV),[66] H1N1 influenza A virus,[67] hepatitis B virus,[68] herpes simplex virus type1,[69] and monkeypox virus[70] have been reported. Although such nanoparticles are almost undefined and yet to be considered for SARS-CoV-2 therapy, previous results from virologic studies give some hope about their therapeutic use against SARS-CoV-2. Researchers are currently trying to utilize different metals, metal oxides, metal sulfides, and other inorganic nanomaterials for possible therapeutic support against SARS-CoV-2.[71–73] Few AgNMs have been reported as therapeutic agents towards some animal coronaviruses and other metal-based nanomaterials are reported as analytical sensors, vaccine adjuvant, and nanocarriers. This review is primarily focused on the formulation of AgNMs to provide therapeutic support against coronaviruses.

Mechanism of Virus Entry into Host Cells

SARS-CoV-2 transmission occurs mainly through active and passive pathways. In the active pathway, mucus droplets released by an infected person through coughing,

sneezing, or talking directly attack the healthy person whereas, in the passive pathway, the virus attacks through secondary sources such as mucus droplets released by an infected person which are evaporated and end up as dried nuclei, which later attach to objects such as tables, clothes, and door handles and infect a healthy person.[74]

The primary site of SARS-CoV-2 attack is the human respiratory mucosa and the replication of the virus in the organism involves several events such as attachment of the virus to the host cell using ACE2 receptors present on the cell membrane and attachment S-proteins present in the viral capsids, diffusion, uncoating, replication of the virus within the host cell, assembly, and excretion (Figure 2).[75]

Therapeutic Strategies for AgNMs Against Animal Coronaviruses

There are three representative strategies used to develop drugs as therapies against coronaviruses.

Repurposing of "broad spectrum" antiviral drugs which are already on the market for therapeutic purposes. Repurposing therapy has the advantage of a known drug mechanism of action, common dosages, and easy production, but unknown side effects in a new disease and drug efficacy for severe conditions are the disadvantages of this approach.

High throughput screening of drugs already approved for a different therapeutic purpose that may have therapeutic effects against SARS-CoV-2.

Development of new drugs based on genomic information, the pathological features of various coronaviruses, and investigation of the mechanism of their actions against coronaviruses. Several therapeutic pathways, their efficacies, side effects, and efficient drug delivery systems, preferably by nanoparticles, can be investigated.

So far, four types of silver nanoparticles have been reported as possible candidates for antiviral therapy as follows.

(a) Glutathione-capped silver sulfide nanoclusters (GSH-Ag_2S NCs) (Figure 3)
(b) PVP-coated silver nanomaterials (PVP-AgNMs), which include silver nanowires (AgNWs) and silver nanoparticles (AgNPs) (Figure 3)
(c) Silver nanoparticle-anchored graphene oxide nanoparticles (GO-AgNPs)
(d) PDDA-coated PVP functionalized graphene oxide-silver nanocomposites (PDDA-PVP-GO-AgNCs) (Figure 3)

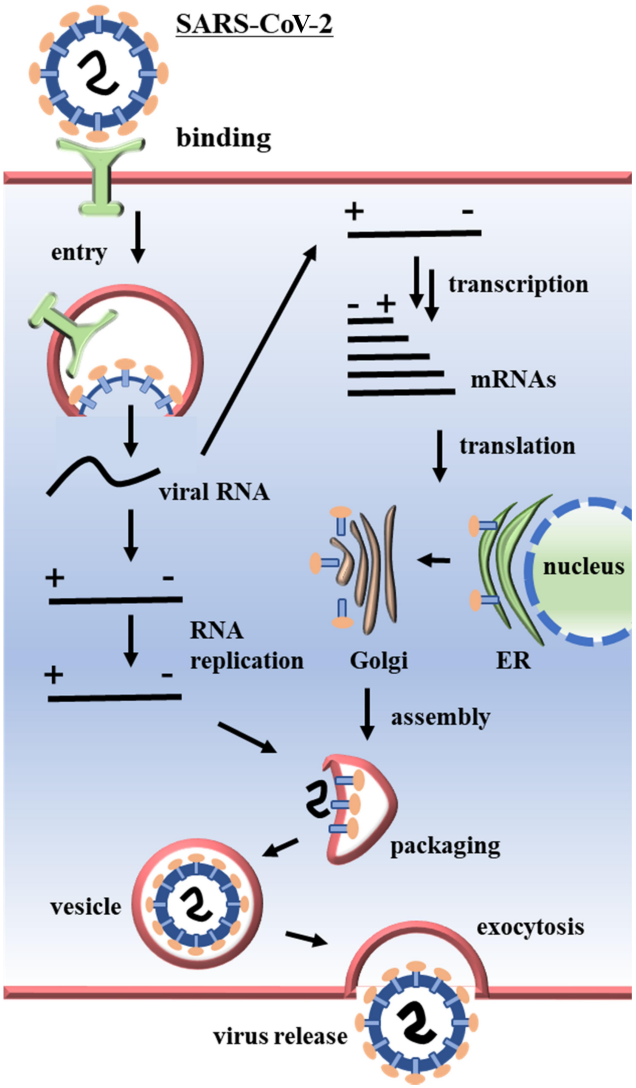


Figure 2 Coronavirus replication mechanism within host cells.

Synthesis and Characterization of AgNMs

AgNMs have already been successfully tested for antiviral activity in many studies.[76] AgNMs are synthesized by various physical and chemical methods that apply common and simplified techniques.

Physical Methods

The basic and key feature of the physical methods employed for the synthesis of AgNMs are processes that include evaporation, condensation, laser ablation, electric irradiation, gamma irradiation, and lithography, which do not use chemicals such as redox reagents, polymers, and electrolytes of colloid stabilizers. Methods such as matrix isolation, gas flow cold trap, gas flow solution trap, and the pulse photo acoustic (PA) technique are used to study the synthesis of AgNPs.[77]

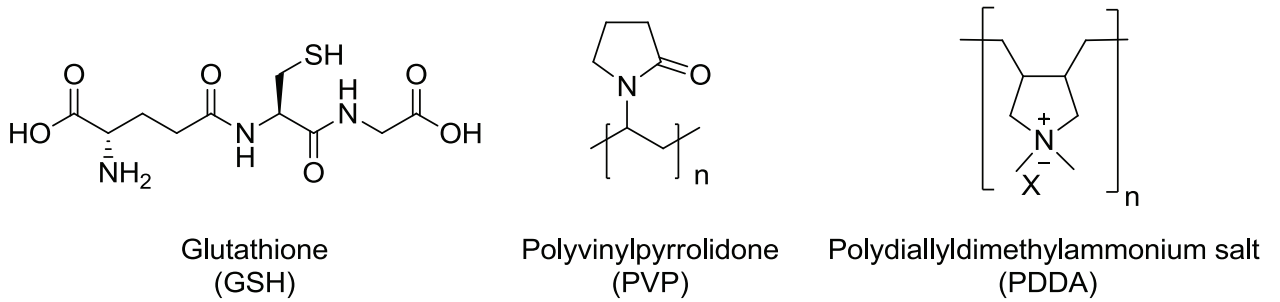


Figure 3 Chemical structures of organic capping agents.

Chemical Methods

There are several chemical methods used for the synthesis of AgNMs of different shapes and sizes based on the preparation method. Herein, we are interested in the synthesis of four different kinds of AgNMs used against coronavirus.

Preparation of GSH-Ag_2S NCs

A suitable capping agent is the key factor used to control the size, stability, and morphology of Ag_2S NCs. Therefore, glutathione (a small peptide that contains three amino acids) was used as a capping agent to prevent the growth of large NCs. The water solubility of GSH-Ag_2S NCs is due to the presence of a multiplicative functional group in glutathione. Du et al reported the synthesis of GSH-Ag_2S NCs, following the steps mentioned in Figure 4.[78]

Preparation of PVP-AgNMs

Commercially available AgNMs supplied from the Institute for Health and Consumer Protection (IHCP) were used in a study by Lv et al. They used four AgNMs:AgNPs (NM-300) (average size of <20 nm), two kinds of AgNWs (60 nm and 400 nm in diameter), and silver colloids (approximately 10 nm).[79] The stabilizing agent for the AgNPs (NM-300) was a mixture of 7% ammonium nitrate, 4% polyoxyethylene glycerol trioleate, and 4% Tween20. The stabilizing agents for the two AgNWs were <0.5 wt% PVP and 2 wt% PVP for the silver colloids.

Preparation of GO-AgNPs

Chen et al synthesized GO-AgNPs to investigate their antiviral activity against Feline coronavirus (FCoV) coronaviruses. The GO-AgNPs were synthesized through a number of steps starting with commercially available graphite powder following Hummer's method (Figure 5).[80]

Preparation of PDDA-PVP-GO-AgNCs

Du et al synthesized GO-AgNCs using commercially available GO and silver nitrate ($AgNO_3$) following the three steps mentioned below.

Preparation of PDDA-coated PVP functionalized graphene oxide: PVP-functionalized GO was obtained simply by adding PVP to a GO solution followed by centrifugation. To obtain PDDA-PVP-GO, PDDA and KCl solutions were mixed followed by PVP-capped GO (Figure 6).

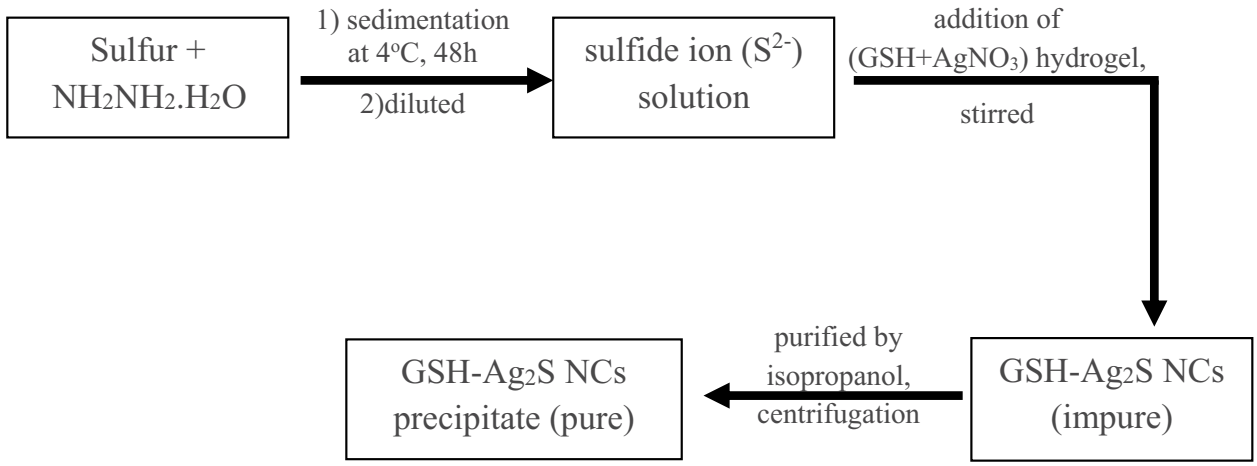


Figure 4 Flowchart for stepwise synthesis of GSH-Ag_2S NCs.

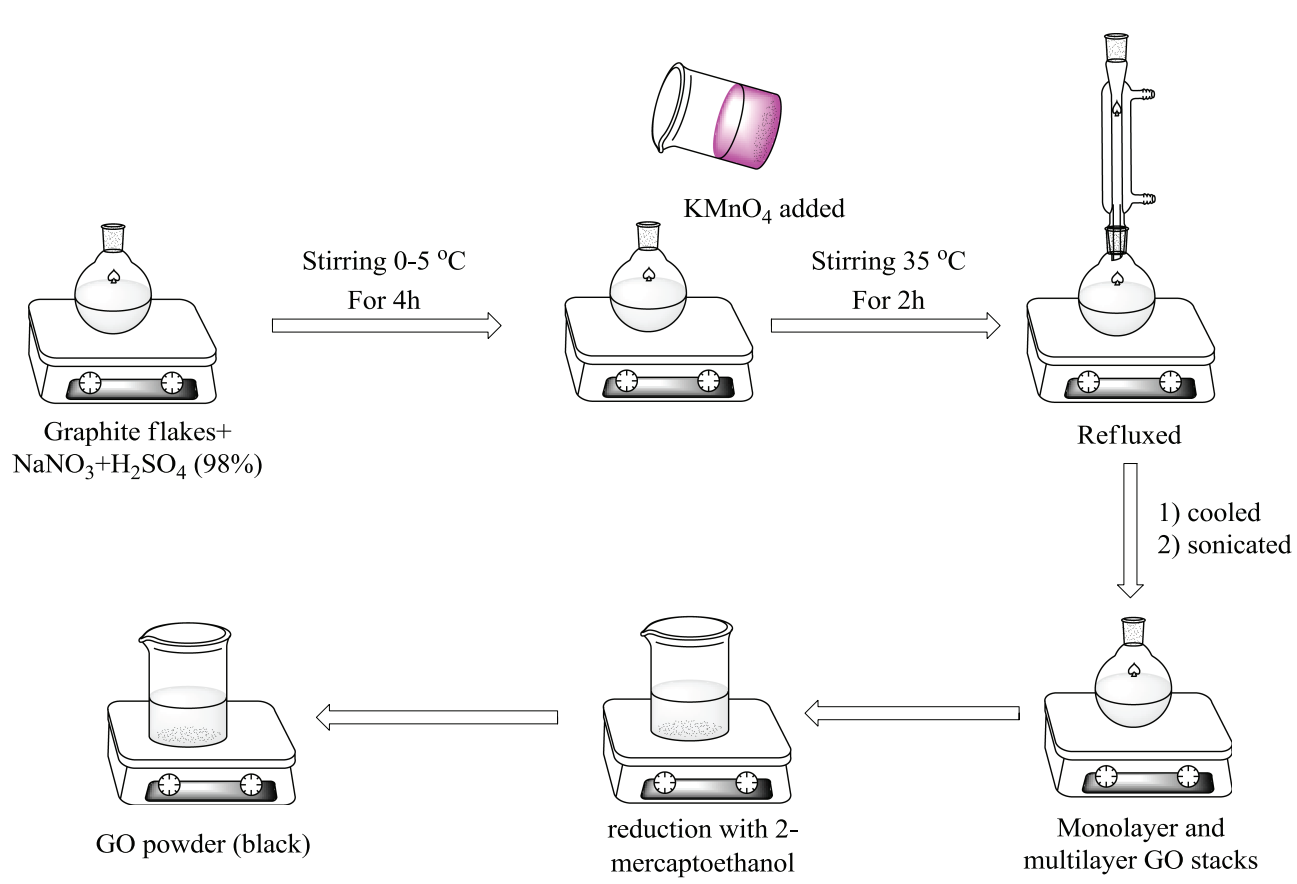


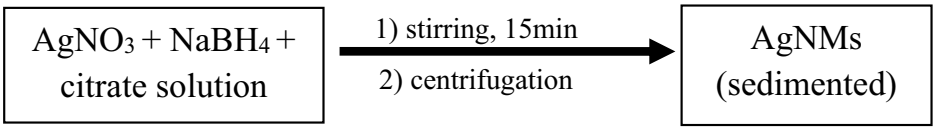
Figure 5 Flowchart for stepwise synthesis of GO-AgNPs.

Synthesis of PDDA-PVP-GO-AgNCs: The AgNPs were synthesized by reducing $AgNO_3$ with $NaBH_4$, followed by the addition of a citrate solution under vigorous stirring. The PDDA-PVP-GO suspension was then injected into the silver nanoparticle suspension and was kept overnight to obtain the PDDA-PVP-GO-AgNCs (Figure 6).[81]

Step-I: Synthesis of GO

Sonication of commercially available GO.

Step-II: Synthesis of AgNPs



Step-III: Synthesis of PDDA-PVP-GO-AgNCs

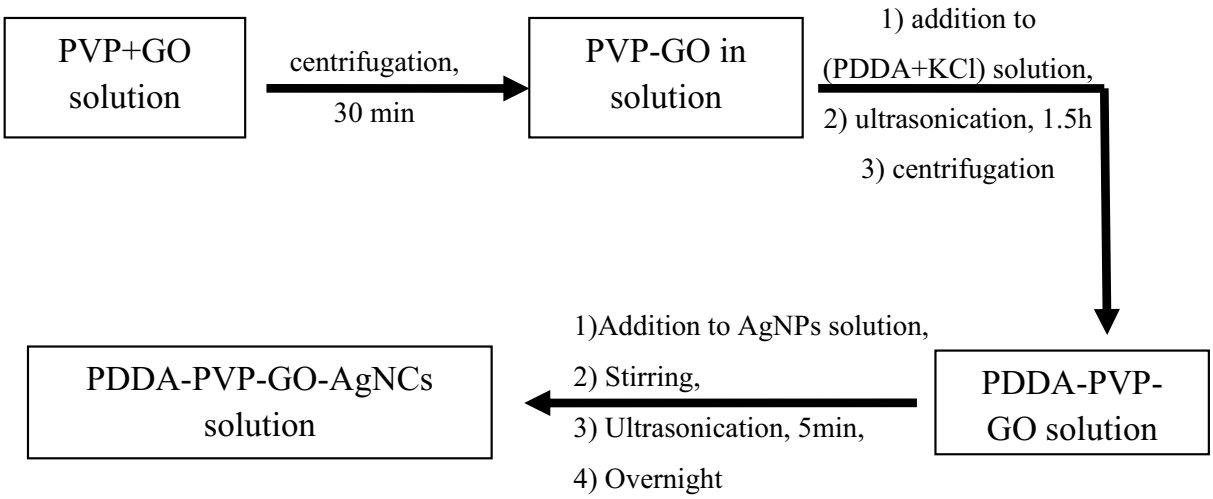


Figure 6 Flowchart for stepwise synthesis of PDDA-PVP-GO-AgNCs.

Characterization of the AgNMs

Characterization is an essential component of synthesized AgNMs. The size, shape, surface charge, crystal structure, and surface chemistry have been studied by different techniques. The UV-visible absorption (UV-Vis) and dynamic light scattering (DLS) data support the formation of AgNMs at room temperature. Topographical imaging, including the size, shape, impurities, and surface stability of respective nanomaterials, was evaluated by scanning electron microscopy (SEM), atomic force microscope (AFM) surface enhanced raman spectroscopy (SERS), and transmission electron microscopy (TEM). Further, the presence of surface modifiers was confirmed using Fourier transform infrared spectroscopy (FTIR) data or UV-Vis spectral analysis. The structures for the crystalline nanomaterials were confirmed by powder X-ray diffractometry (XRD). The thickness of the GO sheets and the layer number were evaluated by AFM analysis. The deposition of AgNMs on GO sheets was confirmed by thermogravimetric analysis (TGA).

In summary, the characterization techniques used for the four AgNMs are as follows.

(a) **GSH-Ag_2S NCs:** UV-Vis, FTIR, HRTEM, XRD, DLS.
(b) **PVP-AgNMs:** Environmental scanning electron microscopy (ESEM), TEM, and nanoparticle tracking analysis (NTA).
(c) **GO-AgNPs:** HRTEM, FESEM, XRD, X-ray photoelectron spectroscopy (XPS), AFM, and TGA.
(d) **PDDA-PVP-GO-AgNCs:** UV-Vis, FTIR, DLS, XPS, SERS, and TEM.

Biological Screening for Antiviral Assays

In this regard, prior studies have been carried out to investigate the antiviral activities of AgNMs against coronaviruses including porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), and FCoV. The antiviral assays for the nanomaterials are generally screened according to the following procedures.[76,82]

Cell Viability Assays

The cell viability in virology is the percentage of cells which survived after applying an antiviral agent. The cell viability assay is carried out using techniques including Resazurin reduction, tetrazolium reduction, caspase or mitochondrial activity, flow cytometry, and ATP assays. In this study, the optimum concentration of NMs was used to demonstrate a viricidal effect in virus-infected cells.

Plaque Assays

The plaque assay determines the infectivity of a virus and estimates the antiviral ability of functionalized NMs. Herein, the zone of infected cells (plaque) is formed after the virus progeny is released by the cell at room temperature. The plaque-forming titer for the virus stock is represented as plaque-forming units per milliliter (PFU/mL) and the PFU value denotes the antiviral ability of the functionalized NMs.

Indirect Immunofluorescence Assay (IFA)

IFA helps to determine the inhibitory impact of NMs on the expression of viral antigens via antigen–antibody interactions. The virus inhibitory effect can be determined by a comparative analysis of the fluorescence intensity of the NM-treated cells and the control experiment (NM non-treated cells).

Western Blot

After treating the virus with functionalized NMs, the protein degradation is recorded by qualitative detection. The first step is denaturation, followed by gel electrophoresis characterization, and finally generation of an antibody and binding to a proper target. Indirect detection of binding to a proper target protein can be performed by approaches such as staining, immunofluorescence, and radioactivity.

Real-Time Quantitative Polymerase Chain Reaction (qRTPCR)

RT-PCR or the quantitative real-time polymerase chain reaction (qRTPCR) involves the polymerase chain reaction (PCR), which is basically the amplification of targeted DNA/RNA. This procedure is the most useful approach to explore viral DNA/RNA and can detect the gene sequence of host cells to test viral infectivity. Hence, after applying functionalized NMs, PCR provides an indirect investigation of the ability of being antiviral in nature.

The biological screening for the four AgNMs is summarized as follows.

(a) **GSH-Ag_2S NCs:** Cell viability, plaque assay, IFA, western blot, attachment assay, penetration assay, release analysis
(b) **PVP-AgNMs:** Cell viability, antiviral activity, qRTPCR, western blot, IFA, flow cytometry analysis
(c) **GO-AgNPs:** Virus inhibitory assay, cytotoxicity
(d) **PDDA-PVP-GO-AgNCs:** Plaque assay, virus entry assay

Antiviral Activity of AgNMs

Various inorganic nanomaterials have been studied related to coronavirus and among them, the most effective nanomaterials were silver-related nanomaterials. AgNMs have a broad spectrum as potential virucidal agents and drug carriers. They have been successfully applied against HIV, influenza virus, and hepatitis virus.[64] The key steps of action of AgNMs involve the inhibition of virus entry into the cells and the generation of radicals (reactive oxygen species) by interacting with biomolecules, causing disruption of the cell membrane and reacting within the cell prompting DNA and RNA damage. Few AgNMs have been reported to exhibit antiviral properties against coronaviruses (only animal coronaviruses from the beta genus). The antiviral evaluation of these materials is discussed in the following section.

Effect of Ag_2S NCs on PEDV-Infected Vero Cells

Du et al showed that Ag_2S NCs with a glutathione coating at a concentration of 46 μg/mL were successful in preventing viral infection and resulted in retention of more than 90% cell viability of Vero cells, even after 48 h of infection by PEDV.[81] A plaque reduction assay showed that, at a definite concentration (46 μg/mL), the Ag_2S NCs had

very good antiviral effects against viral replication compared to a negative control. They also showed that smaller (2.5±0.6 nm) Ag_2S NCs had better antiviral efficacy than larger NCs (4.1±1.5 nm) since their small size enables them to penetrate deeper than larger nanoparticles.[79] Ag_2S NCs were observed to have a 3.0 log-fold reduction in viral titers at 12 hpi, suggesting a high efficacy against PEDV infections. Ag_2S NC treatment was observed to block viral negative-strand RNA synthesis and prevent viral budding. Ag_2S NCs were also observed to inhibit PEDV infections by producing IFN-stimulating genes (ISGs) and proinflammation cytokines in Vero cells (Table 1).

Effect of AgNMs on TGEV-Infected ST Cells

Lv et al observed that PVP-coated AgNMs at concentrations of 25 and 50 μg/mL were highly toxic to ST cells and showed 80% cell viability at 12.5 μg/mL.[79] qRTPCR showed that TGEV 3CLpro and S-X were the preferential targets of AgNMs and AgNWs. The inhibitory effects of AgNPs and AgNWs were examined by IFA to observe the amount of TEGV in ST cells after treatment with both and it was observed that both types of AgNMs can significantly reduce the amount of TGEV in ST cells, whereas only TGEV infection of host cells resulted in cell apoptosis. The results of the analysis by annexin V-FITC and a PI dual staining kit suggested that the rate of apoptosis of the virus control (without AgNMs) was 10.07%, but pretreatment with AgNMs decreased the cell apoptosis to 5.33% (AgNPs), 4.97% (AgNW60), and 4.93% (AgNW400) (Table 1).

Effect of GO-AgNPs Towards FCoV-Infected Fcwf-4 Cells

Chen et al studied the antiviral activity of graphene oxide (GO) sheets and GO sheets coupled with silver nanoparticles (GO-AgNPs) against FCoV. They reported that when the concentrations of GO and GO-Ag nanomaterials were less than 1.5625 mg/mL, the cell viability was 90% and the cytotoxicity concentrations 50% (CC_{50}) values were 17.4 mg/mL for GO and 19.7 mg/mL for GO-Ag nanoparticles.[83] They also observed that GO-Ag had a greater antiviral effect on FCoV-infected cells compared to GO. The effective inhibitory concentration of GO-Ag nanoparticles was 0.1 mg/mL. They hypothesized that the reactive oxygen species produced by the GO-Ag nanoparticles damaged the viral RNA or blocked the host cell's receptors. In addition, pretreatment with GO-Ag nanoparticles led to physical or chemical interactions between GO sheets and the coronavirus envelope, resulting in decreased infectivity (Table 1).

Effect of PDDA-PVP-GO-AgNCs on PEDV-Infected MARC-145 Cells

MARC-145 cells were cultured with PDDA-PVP-GO-AgNCs (0.5–4.0 μg/mL) for 24 and 48 h. At the optimum concentration of 4.0 μg/mL, the viability was reported to be over 85% but at 8.0 μg/mL, the viability decreased to below 80%. Additionally, an inhibitory effect of PVP-GO-AgNCs was observed on PEDV-infected MARC-145 cells and the inhibitory rate increased with increasing nanocomposite concentration.[81] According to Du et al, the possible mechanism was the inhibition of viral entry (Table 1).

Table 1 AgNMs Used as Antiviral Agents Toward Coronaviruses and Their Mechanisms

Type of CoV	Host	NMs Used as Antiviral Agent	Size (nm)	Cultured Cell Used	Applied Conc. of NMs	Antiviral Mechanism
PEDV	Pig	GSH-Ag_2S NCs	2.5 ±0.6, 4.1 ±1.5	Vero cells	23–184 μg/ml	Prevent -ssRNA synthesis, inhibit viral binding
		PDDA-PVP-GO-Ag nanocomposites	17 ±3.4	MARC-145 cells	0.5–8.0 μg/ml	Prevent viral entry
TGEV	Pig	PVP-AgNWs	60–400	ST cells	3.125–50 μg/ml	Disable cell apoptosis
		PVP-Ag colloids	~10			
		PVP-AgNPs	<20			
FCoVs	Cat	GO-Ag NPs	5–25	fcwf-4	0.390625–50 mg/ml	Inhibition of viral entry

Pre-Clinical Efficacy Studies of AgNPs

Although AgNMs are widely known for their in vitro viricidal activity, only a few in vivo studies have been reported. PVP-coated AgNPs have been reported as a potential vaginal microbicide preventing HIV-1 infection transmission.[84] Morris et al evaluated the antiviral and immunomodulatory effects of PVP-coated AgNPs in respiratory syncytial virus (RSV) infections. Bagg and Albino common strain of laboratory (BALB)/c mice were inoculated with RSV pre-incubated with AgNPs and they observed significant reductions in viral titer in the lung tissues as compared to untreated mice infected with RSV.[85] With regards to influenza infections, Xiang et al showed that AgNPs were successful in preventing A/Human/Hubei/3/2005 (H3N2) influenza virus infection in an in vivo mice model by destroying their morphologic structures in a time-dependent manner.[86] In addition, AgNPs, when administered intranasally, resulted in the inhibition of virus growth in lungs and the development of lung lesions, which led to significantly enhanced survival benefits in mice. Zhang et al reported that BALB/c mice inoculated with Rhesus rotavirus resulted in Biliary atresia. They observed that mice treated with AgNPs showed a significant increase in survival rates that led to a reduction in jaundice, restoration of liver enzymes and bilirubin metabolism to normal levels, and improved body weight. Additionally, the viral load decreased and upregulation of TGF-β mRNA transcripts was observed upon treatment with AgNPs.[87]

However, Stebounova et al showed that silver nanoparticles when inoculated in mice at subacute concentrations resulted in minimal pulmonary inflammation or cytotoxicity.[88] Further, the effect of longer-term exposures in mice have yet to be analyzed based on higher lung burdens of AgNPs, resulting in the underscoring of eventual chronic effects.

From the above pre-clinical data, it can be concluded that AgNMs showed positive changes in mice health status. Also, in an in vivo study, AgNMs showed almost no toxicity. Therefore, the application of AgNMs in an advanced clinical study can be safely performed in the future.[37,89]

Mechanism of Action of AgNMs

Nanomaterial-based therapeutics offer a versatile tool for antiviral therapy researchers. AgNMs exhibit unique therapeutic efficacy, pharmacokinetics, and superior biological functions as antimicrobial agents. Recently, the antiviral properties of AgNMs have been reported in many studies which inhibit the replication of HIV-1, a receptor of the Tacaribe virus (TCRV), and monkeypox virus (MPV). The mode of action of AgNMs (against coronavirus infection) remains unclear but it has been reported that Ag^+ ions participate in the generation of oxidative stress, induction of antibody responses, cytokine production, and inhibition of viral RNA synthesis by blocking the interactions between virus and ACE-2 cell receptors or glycoprotein120 (gp120). AgNMs interact with the gp120 subunit of the viral envelope glycoprotein to inactivate the virus before host cell binding.[74,76,90,91]

AgNMs work as an adjuvant to improve the immune response of the vaccine. It has been reported that the addition of AgNMs with the influenza vaccine efficiently induced an immune response in infected mice. However, the AuNP performance was not as good compared to AgNMs.[92]

Moreover, AgNPs and chitosan conjugates were also tested against H1N1 influenza A virus and it was observed that synergistically, the conjugates showed remarkable antiviral effect against the virus compared to AgNMs or chitosan alone.[75]

AgNMs as Vaccines

Vaccines are biological particles which facilitate building of acquired immunity against infectious diseases. To fight against infectious diseases, vaccination is one of the most cost-efficient and simple methods. Typically, a vaccine consists of virus-like particles (VLP), attenuated viruses, or protein-subunit antigens, which stimulate an immune response against infectious diseases. With the advent of nanotechnology, efforts were made to determine the immunoactivity of natural and engineered NMs.[93]

Several researchers reported the impact of AgNMs on the inflammatory response.[94] Silver was reported to react with immune cells and affect the suppression or stimulation of various pathological conditions. It was reported that an increase in the size of AgNMs resulted in an increase in inflammatory cytokine secretion in rat alveolar macrophages,[95] toxicological effects on macrophage U937,[96] and also elicited an immune response in macrophages.[97] Park et al reported that the repeated oral administration of AgNPs leads to an increased level of cytokine production, inflammatory cell infiltration, and B cell distribution in mice.[98] AgNPs were also used as adjuvants and showed effects in vitro and in vivo using model antigens of oval albumin (OVA) and bovine serum albumin (BSA).[99]

Therapeutic Effect of Oral Inhalation of Silver Nanoparticles

The antibacterial[100–102] and antiviral[103] properties of silver nanoparticles provide a promising regimen in the fight against COVID-19. Colloidal silver solution[104] inhalation is reported to be a promising therapy to tackle the aggravation of respiratory system infections. For antiviral applications, the nanoparticle size should be in the range of 3–7 nm. It was reported that the antibacterial effect of an ionic silver solution in water[105] has a much higher minimum inhibitory concentration (MIC) than a colloidal silver solution. However, in the case of an antiviral effect, as was observed in the case of HIV, a colloidal silver solution was 10 times more potent than an ionic silver solution.[66]

For the oral inhalation of colloidal silver nanoparticles, it is important to determine the MIC level. A study showed that the MIC is very sensitive to the nanoparticle size.[106] Smaller particles contain a greater number of particles at a specific weight fraction, producing a higher particle density that can interact with the pathogen best at low concentrations (MIC value).

It has already been shown that silver nanoparticles 10 nm or smaller are much more effective than particle sizes of 25–50 nm against HIV.[107] Smaller AgNPs are easily permeable and hence highly incorporated into the cells, thus exert more toxicity in cells.[108–110] It was observed that nanoparticles that are bound to the virus were exclusively within the range of 1–10 nm. While the HIV particle size is ~120 nm, the SARS virus size varies between ~90–100 nm. The MIC of AgNPs for HIV was observed to be 10 µg/ml. Since SARS has a similar size as HIV, it is expected that it can also be susceptible to AgNPs at a similar MIC value.

Application of Other Metal, Metal Sulfide, and Metal Oxide-Based Nanoparticles Against Coronavirus

Apart from AgNMs, there are also other metal, metallic oxide and metallic sulfide nanomaterials which are effective in the treatment of coronaviruses. These nanomaterials are utilized as various agents other than therapeutic treatments like nano-carriers, vaccine adjuvants, and analytical sensors. Gold nanoparticles (AuNPs) stand out because of their antimicrobial, photonic, electrical, and catalytic properties. AuNPs were used against coronavirus as a vaccine carrier towards TGEV-infected mice[111] and as a vaccine adjuvant towards SARS-CoV-infected BALB/c mice.[112] AuNPs are also used as an electrochemiluminescence towards MERS-CoV infected cells,[113] as a chiroimmunosensor towards IBV-infected cells,[114] and as an immunochromatographic strip towards IBV.[115] Wang et al synthesized AuNPs as an electrochemiluminescence for studying HCoV and towards PEDV as a non-nest PCR material.[116] Other nanomaterials like MoS_2 nanosheets,[117] zirconium quantum dots,[118] ferritin-based nanoparticles,[119] and magnetoplasmonic nanoparticles were also used as chemosensors and vaccine carriers. Khaiboullina et al used TiO_2 nanoparticles as a surface modifier.[120] In their in vitro study, they showed effectivity against alpha coronavirus HCoV-NL63, which is highly similar to the SARS-CoV-2 (Table 2).

Toxicity of Silver Nanoparticles

It can be hypothesized that silver nanoparticle toxicity is due to the attachment of the AgNMs directly to the viral protein surface.[76] Hence, proper surface modification can be carried out by investigating the exact interacting site. It has been shown that silver nanomaterials are broad-spectrum antiviral agents and can efficiently reduce viral infectivity when applied to cultured cells.[76] It has been reported that these nanomaterials are highly cytotoxic to mammalian cells because of their interaction with biomolecules that generate reactive oxygen species by interfering with defensive antioxidant mechanisms, thus posing harmful effects that damage lipids, proteins, and DNA through oxidation.[121] An in vitro study revealed that the toxicity level of such nanomaterials varies depending on the dose. Although the use of nanomaterials is still debatable due to their toxicity or side effects on normal human cells, silver nanomaterials provide a promising means to carry antiviral or other drugs throughout the body. In order to reduce toxicity, surface modifications of the nanoparticles need to be made so that the metal surfaces do not directly attach to cells. Also, their concentration in the interior of a cell should not be high within a particular cell compartment. Although the progression of nanoparticle research is presently ongoing, the detailed mechanisms of nanomaterial actions are not clear yet, which requires improvements with respect to their safety in order to optimize clinical advancements.

Conclusion

Experimental findings suggested that small nanoparticles show greater effectiveness than larger nanoparticles. Hence, AgNPs smaller than 20 nm are more efficient than AgNWs (60 nm and 400 nm) in PEDV-infected cells. Smaller (2.5±0.6 nm) nanomaterials were also

Table 2 Some Representative Nanomaterials Other Than AgNMs Used as Antiviral Agents Toward Coronaviruses and Their Mechanisms

Nanoparticle	Virus/ Antigen	Targeted Living System	Host	Purpose of Use/Acts as
Gold nanoparticle	Swine TGEV	Mice/Rabbit	Pig	Nano carrier of vaccine[111]
	SARS-CoV	BALB/c mice	Human	Vaccine adjuvant[112]
	MERS-CoV	-	Camel	Electrochemiluminescence (analytical sensor)[113]
	HCoV	-	Human	Electrochemiluminescence (analytical sensor)[113]
	IBV	-	Chicken	Chiroimmunosensing (analytical sensor)[114]
	IBV	-	Chicken	Immunochromatographic strip (analytical sensor)[115]
	PEDV	-	Pig	Nano-nest PCR (analytical sensor)[116]
MoS_2 nanosheet	IBV	-	Chicken	Immunosensing (nanosensor for diagnosis)[117]
Zirconium QDs and magnetoplasmonic nanoparticles	IBV	-	Chicken	Photoluminescence (nanosensor)[118]
Ferritin-based nanoparticle	MERS-CoV	Female BALB/C mice	Camel	Vaccine[119]
TiO_2 nanoparticle	HCoV	-	Human	Surface modifier agent[120]

observed to be more efficacious compared to larger ones (4.1±1.5 nm), such as Ag_2S NCs in the case of TGEV-infected cells. In the case of GO-Ag NPs, they are effective against FCoV-infected cells at a diameter of 5–25 nm but no size dependent experiments have been carried out with these NPs. A GO-Ag nanocomposite with a diameter of 17±3.4 nm was also very efficient against the PEDV coronavirus. Although Ag and Ag-related nanomaterials are well-explored antiviral and antibacterial agents, there are no such clinically approved antivirals known currently. The recent pandemic demands continuous therapeutic advancements and hence, such nanomaterials or nanoclusters should be under consideration as an alternative research topic for therapies aimed against SARS-CoV-2, as they are still less studied than other types of antiviral therapy.

Future Perspectives

It has been around ten months since SARS-CoV-2 was declared a pandemic by the WHO and the search for targeted therapies is far from providing therapies that can be used in the clinical setting. The repurposing of some antiviral drugs is in clinical trial throughout the world and has provided some prospective results that have been published recently. In this scenario, the use of nanoparticles is considered to be a suitable alternative therapy. AgNMs are good and effective antimicrobials, although their mechanisms of action are not clear yet. Their antiviral activity against some viruses has been extensively studied and has been found to be good enough to expand this research area to include nanotherapies effective against coronaviruses. Although some research has been carried out for nanoparticle use against a few animal coronaviruses, the respective nanomaterials have still not been studied in order to identify their effects against human coronaviruses. With respect to the SARS-related coronaviruses which are raging across the world currently, the use of nanomaterials in personal protection equipment (PPE) including face masks by coating PPEs with these various nanomaterials is becoming popular and safe.

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Disclosure

The authors report no conflicts of interest for this work.

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