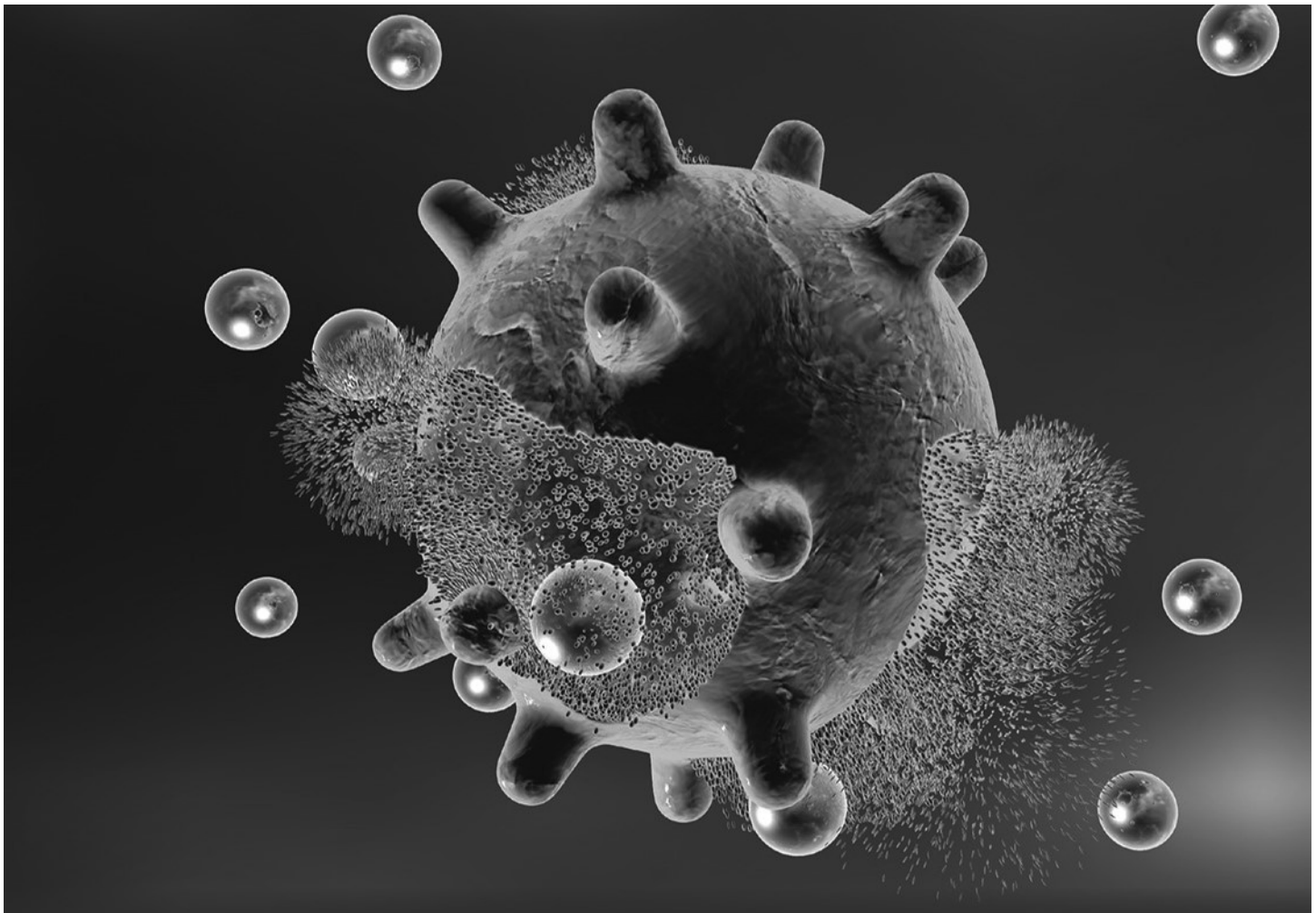


Silver Nanoparticles for Drug Delivery

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CHAPTER 14

Silver nanoparticles for treatment of COVID-19 and other viral diseases

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14.1 Introduction

Viruses are microscopic pathogens that replicate outside living systems only. However, their ability to evolve through genetic mutations continuously possesses a great threat to human health globally. Their infections are usually transmitted both vertically and horizontally with rapid rates of spread and treatment challenges, resulting in various clinical conditions from mild to emergency and mortality situations. The emergence and reemergence of viral diseases have been responsible for most of the global pandemics in the last four decades. Notably among the recently witnessed viral-mediated disease outbreaks worldwide include the human immunodeficiency virus (HIV) in 1981, severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1), firstly appeared in 2002, influenza A subtype H1N1 (swine flu) in 2009, Middle East respiratory syndrome CoV (MERS-CoV) in 2012, Ebola virus in 2013, and the current SARS-CoV-2 [1–4].

Coronavirus 2019 (COVID-19) is a newly emerged viral infection caused by the SARS-CoV-2, a member of the genus *Betacoronavirus*. It shares similar features such as positive-sense, single ribonucleic acid (RNA) strands, and enveloped structure with other members of the subfamily including the MERS-CoV and SARS-CoV-1 [5]. Typical of viruses, SARS-CoV-2 has been developing resistance-inclined genomic mutations to escape host immunity and therapeutic effects, leading to the emergence of its variants of interest/concern including the Mu, Delta, and Omicron. Worrisomely, the variants are associated with more rapid rates of infections than the original [6,7].

Like other members of the family, the pathophysiology of the SARS-CoV-2 involves the binding of the dense spike glycoprotein (S protein) to the host angiotensin-converting enzyme 2 (ACE2) for cell infusion, facilitated by the activation of the S protein by the transmembrane serine protease 2 [5,8]. Upon entry, various intracellular processes continue through the RNA release, polymerase activity to

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generate the nonstructural proteins (NSPs). Other resultant cellular processes include RNA replication, reassembly and budding, and translations, featuring the envelope (E), membrane (M), nucleocapsid (N), and S proteins to produce new matured virions for viral release into biosystem (Fig. 14.1) [9]. These make the structural proteins E, M, N, and S together with the NSPs 1–16 including the RNA-dependent RNA polymerase which remain plausible therapeutic targets for the prevention and treatment of COVID-19.

Undoubtedly, the complexity surrounding the etiology and inadequate understanding of the underlying bioregulation mechanisms significantly hinder the development of effective therapeutics for COVID-19 [10]. The unwavering complications from comorbidities such as hypertension, cardiovascular diseases, diabetes, and renal failure mostly constitute severity and seriously stamp down the clinical success of several medications in patients. These necessitated urgent measures for decreasing the infection and increasing the recovery rates. Despite the concerted scientific efforts being committed globally, no successful candidate has yet been approved as an effective anti-COVID-19 drug. Some renowned antivirals such as remdesivir, ivermectin,

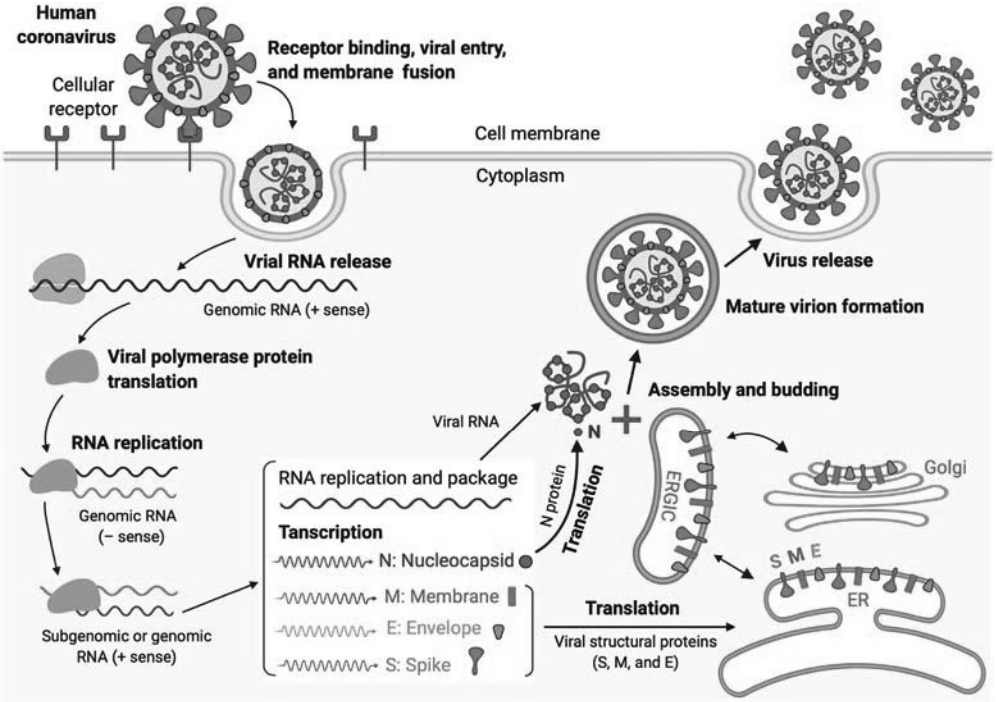


Figure 14.1 Mode of infection and life cycle of coronaviruses from cell entry, replication, and translation to new virus release. *Adapted from S. Jiang, C. Hillyer, L. Du, Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses, Trends Immunol. 41 (2020) 355–359, Licensed under CC BY 4.0.*

interferons, ribavirin, artemisinin, and chloroquine were deployed as repurposable drugs for rescuing the emergency conditions associated with the viral infection. However, some of them have been terminated in clinical trials due to the resistance of the pathogens and various degrees of aftereffects, while the efficacy of others remains being strongly debated within the science community. Incomputable numbers of chemical agents from vast natural resources and synthetic routes are being developed and tested for effective chemotherapeutic effects against COVID-19 and other viruses.

Before the sudden outbreak of SARS-CoV-2 in 2019, the existing viral diseases were among the most life-threatening maladies affecting humans with limited treatment strategies. Similarly to the COVID-19 situation, the clinical successes of various topical chemotherapeutic agents against the preexisting viruses are challenged by incessant viral resistance, ceaseless emergence of novel strains, toxicity, and suboptimal delivery of drugs to targets [11]. These make the treatment of viral diseases to generally remain tedious.

Nanoparticles (NPs) are materials with particle sizes ranging from 1 to 100 nm in diameter, and sometimes including larger particles (up to 500 nm), designed through nanotechnology for various applications. Controlled discharge to targets, enhanced cell/membrane permeability, luminescence, controlled stability, easy functionalization, ease of size modification, polarity, and high surface/volume ratio are among the vital features that promote the application of NP-structured materials in the biomedical field. Other factors include biocompatibility, ability to cross the blood-brain and blood-air barriers, strong interactions with biomolecules, and tenability. These multifaceted characteristics support the utility of NPs in bioimaging, biosensing, drug release, regenerative medicine, catalysis, microbial treatment, and disinfection. The interesting performances of NPs against microbial infections attracted their explorations against viral diseases, the focus of this chapter. The intended applications are selectively considered during preparation. For instance, silver nanoparticles (AgNPs) can be prepared by physical, chemical, and biological methods. The biological method otherwise referred to as the green synthesis involves the use of biogenic materials such as plants, fungi, bacteria, and algae in a bottomup approach. It is most preferred if the intended application for the NPs is within the biomedical field, especially in drug-related designs. This is because, in addition to biocompatibility, cost-effectiveness, easy scalability, and environmental safety, NPs synthesized via the biological route are considerably safer and are usually associated with less toxic byproducts [12]. Several antiviral agents have been modified into various NP formulations as micelles, dendrimers, liposomes, nanogels, nanocapsules, nanorods, nanospheres, nanosheets, and solid lipid NPs through the method (Fig. 14.2). The NPs mostly demonstrate improved bioactivities compared to the parent nano-free materials [11,13,14].

The incorporation of nanotechnology into pharmaceutical designs enhances the stabilization of the chemotherapeutic agents and their adequate delivery/release to pharmacological sites. Additionally, the highly reactive surface and mechanistic destructive ability of AgNPs on lipid layers and viral protein RNAs are mainly considered for their

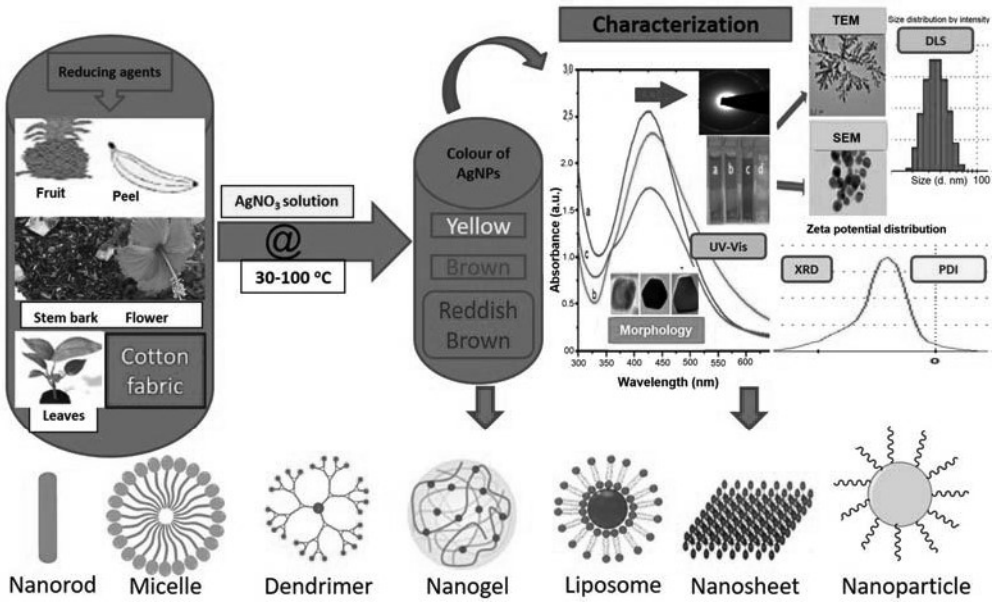


Figure 14.2 Illustration of basic preparation, characterization, and formulations of silver nanoparticles.

incorporation into personal protective equipment (PPE) such as face masks, safety goggles, garments, surface disinfectants, and mouth/nasal rinse for prevention against viral infections. For instance, the results from a randomized trial conducted on health workers that are in contact with COVID-19 patients in Mexico support the protective efficiency of ARGOVIT-AgNPs against SARS-CoV-2 infection. Through the mouth and nasal rinse, the agent showed strong inhibitory potency in vitro cell-cultured SARS-CoV-2 and 84.8% protective efficacy in 231 participants [15–18]. Drawing shreds of evidence from interesting activities of AgNPs and some metal complexes, the development of hybrid nanomaterials consisting of functional NPs such as AgNPs and some metal-organic frameworks has been suggested. These hybrid NPs are expected to exhibit synergistic antiviral effects for improving the efficacy of some PPE, respirators, and surgical euipments used for the prevention of COVID-19 [19]. These make the technology to remain invaluable in various key aspects of viral disease management such as diagnosis, prevention, vaccine, and drug development.

14.2 Silver nanoparticles for diagnosis of COVID-19 and other viral diseases

Silver-based nanobiosensors have been identified as effective agents for detecting viral antigens-nucleic acid sequence, proteins, or host antibodies expressed during viral

infections. Their selective viral detections are achieved by the immobilization of biorecognition molecules on the surface of nanomaterials with optical and electrochemical transducer systems [20]. In a recent study, AgNP aggregation induced by pyrrolidinyl peptide nucleic acid was employed to develop a paper-based colourimetric assay for detecting DNA of MERS-CoV and human papillomavirus (HPV). The oligonucleotide detections were indicated by the color change of AgNP with detection limits recorded as 1.53 and 1.03 nM for MERS-CoV and HPV, respectively. The method was suggested as a simpler, faster, selective, and sensitive DNA biosensor alternative to traditional colourimetric approaches for detecting MERS-CoV and HPV [21]. Single-particle inductively coupled plasma mass spectrometry (SP-ICP-Ms) is an assay used for the diagnostic analysis of highly infectious pathogens. Silver NP probes were also designed in SP-ICP-Ms for simultaneous and homogeneous diagnosis of viral diseases with similar infection modes such as the SARS-CoV-2 and influenza (H3N2). The tool displayed enhanced pulse signal intensity and reduction in frequency and was recorded with faster analysis and improved efficiency in detecting biomolecules such as nucleic acid, protein, and cell [22]. These shreds of evidence summarily highlight the advanced performances of diagnostic equipment when incorporated with AgNP-based nanotechnology.

14.3 Nanoparticles for vaccine development against COVID-19 and other viral diseases

Progressively, the emergence of vaccines and monoclonal antibodies for mitigating the viral infectivity, replication, and transcription has doused the tensions of COVID-19, with a significant reduction in daily new cases and death tolls. Although, most of the available vaccines are not specifically AgNP-based, but contain other products of nanotechnology. The vaccines, Oxford-AstraZeneca, Pfizer/BioNTech, Moderna, Jansen Pharmaceutical, and Sinovac are among the popular approved ones currently in use, reported with an efficacious provision of active acquired immunity to SARS-CoV-2 ranging between 70% and 97%. Several others are still in clinical trial stages. Relevantly, most of the approved vaccines and those in clinical trials are designed with lipid nanoparticles (LNP) in different forms. These include the LNP-encapsulated messenger RNAs (mRNAs) encoding for monoclonal antibodies against the SARS-CoV-2, self-amplifying RNA encoding the viral glycoprotein or the receptor-binding domain (RBD), and the Novavax which has an NP-designed full length recombinant SARS-CoV-2 glycoprotein. The nano vaccines are designed to activate the major histocompatibility complex class I (MHC-I) proteins and class II (MHC-II) counterparts. The respective proteins function by presenting antigens to both helper T cells with CD4 + receptors and cytotoxic T cells with CD8 + receptors for the production of antiviral antibodies [10,23].

The NP-incorporated vaccines in clinical trials as of March 226, 2022 include the SK SARS-CoV-2 recombinant NP vaccine, SARS-CoV-2 rS NP vaccine, ICC and qNIV NP vaccine-2, NVX-CoV2373, CoV2 SAM (LNP), and SARS-CoV-2 Spike Ferritin NP (SpFN) for COVID-19. Others are the Epstein-Barr virus gp350 Ferritin NP for Ebola, Respiratory Syncytial Virus (RSV) F vaccine for RSV, Ebola virus Glycoprotein vaccine for Ebola, Universal influenza A vaccine, Recombinant Trivalent NP influenza vaccine and NanoFlu™ for influenza, and T cell priming peptide vaccine for dengue (https://clinicaltrials.gov/). The shreds of evidence highlight the invaluable applicability of NPs in the prevention of COVID-19 and other viral pandemics through vaccine development. Even though various degrees of adverse events from mild such as swelling, pain, fatigue, headache, flu-like illness to severe such as hemolytic anemia, transverse myelitis, and myalgia have been commonly recorded in patients against some vaccines [10]. However, these are subjects of further scientific efforts having provided glimpses of hope and health relief globally. Moreover, the daily scientific search for effective therapeutics for prevention and treatment remains ceaseless.

14.4 Mechanisms of antiviral activities of silver nanoparticles

Generally, the exact mechanism of antiviral activities of AgNPs is yet to be fully understood. The reported mechanisms from various studies are hypothetically traceable to the ability of AgNPs to function on viral surfaces and physically inhibit cell entry and other life-dependent processes through interactions with host cells. The breakdown of the pathways of antiviral effects of AgNPs on viral targets includes the interaction with viral particles such as the RNA, genome, chromatin, DNA, etc, facilitated by their characteristic large surface area and chemical functionality. Furthermore, the denaturation of enzymes, inhibition of protein synthesis, oxidative potential, activation of proinflammatory cytokines, interferon-stimulated gene, and nucleic acid-binding protein have been reported as mechanisms of impeding viral replication by AgNPs [4,17,24]. For instance, the viral S-protein known for cell receptor binding and internalization of CoVs (MERS-CoV and SARS-CoV-1/−2) contains conserved disulfide (S-S) bonds playing critical roles in stabilizing the RBD for infectivity. Studies have shown that residues 319–541 of the CoV RBDs express four distinct S-S bonds: firstly, in Cys480-Cys488 located in the loop, pivotal for ACE2 interaction. The other three, Cys336-Cys361, Cys379-Cys432, and Cys391-Cys525, are located on the opposite side of the RBD [25–27]. In nonenveloped viruses such as the polyomavirus family, the S-S bonds also perform important functions in the compactness and stabilization of viral capsids for assembly and disassembly pathways [28]. Interestingly, AgNPs have been demonstrated to destabilize the sulfhydryl group-rich proteins by cleaving their S-S bonds through selective binding, thereby deactivating the respective viral infectivity [17].

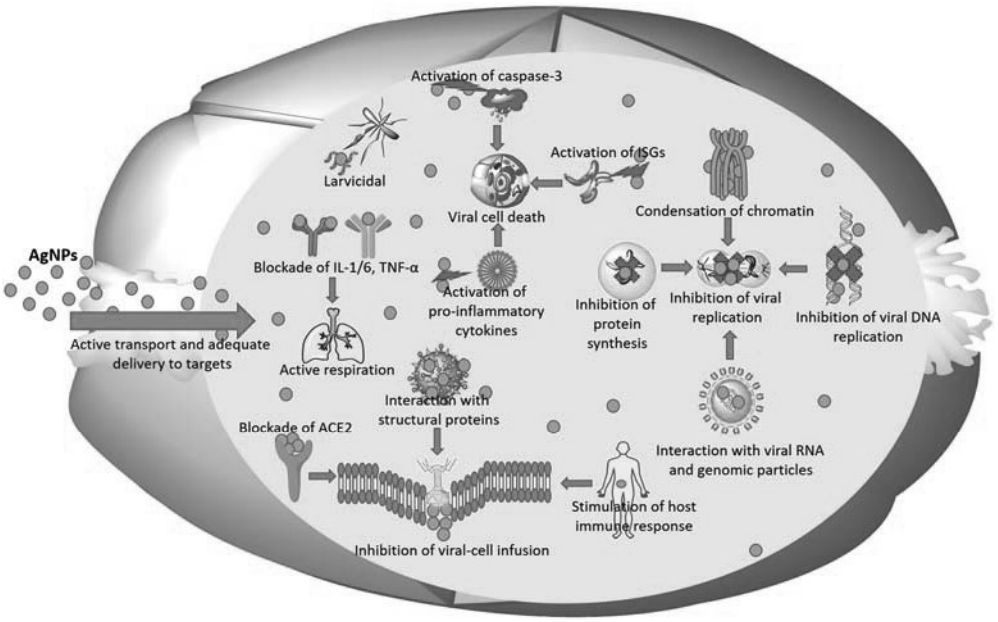


Figure 14.3 Multitarget-directed mechanisms of antiviral activities of silver nanoparticles.

Additionally, a weakened immune system associated with ageing or preexisting comorbid illnesses has been earmarked as a cause of the severity of COVID-19 and other viral infections. As such, the application of antiinflammatory agents for stimulating the innate immune response has been shown to ameliorate the severity. Efficient nanocarriers such as AgNPs can aid adequate delivery of the agents to relevant immune cells such as the inflammatory macrophages, dendritic cells, granulocytes, and T cells for the blockade of interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α), and other cytokines [29]. The multitarget-directed mechanisms of pharmacology exhibited by AgNPs as suggestively summarized (Fig. 14.3) undoubtedly promote their effective antiviral activities against COVID-19 and preexisting viral infections with complex etiology.

14.5 Silver nanoparticles for treatment of COVID-19

Several silver-modified NPs of chemical materials have been recently reported with potent antiviral effects against the current SARS-CoV-2 and are suggestively amenable for future therapeutic development for COVID-19 and its emerging variants [30]. The established intriguing efficacy of AgNPs against several strains of pathogenic infections including the drug-resistant types attracted their deployment for the urgent rescue missions demanded by COVID-19. Whilst the search continues, some active

AgNPs have been discovered through various experimental models and are being proposed for clinical trials as promising therapeutic agents for COVID-19.

Progressively, some of the AgNPs are currently in clinical trials as therapeutics for COVID-19. An example of such with a "recruiting" status is colloidal silver, a dietary supplement anciently applied to treat microbial and viral infections [31]. Several other AgNPs have been experimented at preclinical levels with intriguing antiviral activities against the SARS-CoV-2. Although, the deep understanding of the underlying mechanisms of antiviral action of AgNPs remains at the infantry. However, the release of Ag^+ to provoke damage to specific viral genome and disruption of the viral membrane has been inclusively identified as common mechanisms of antiviral effects of AgNPs [32].

Biosynthesized AgNPs of methanolic extracts of ginger (*Zingiber officinale*) and strawberry (*Fragaria ananassa*) were evaluated for cytotoxicity and anti-SARS-CoV-2 activities on VERO cells using 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. The 50% maximal inhibitory concentration (IC_{50}) values of 0.0340 and 0.0989 μg/mL recorded for the respective ginger and strawberry AgNPs indicate strong antiviral activities against the SARS-CoV-2. Interestingly, they were observed to be less toxic with a 50% cytotoxic concentration (CC_{50}) of 0.252 and 0.202 μg/mL for the respective AgNPs. Among the phytochemicals identified in the extracts using liquid chromatography-mass spectrometry, neohesperidin was demonstrated with strong inhibitory binding to the viral NPS16 and human AAK1, suggesting the possible mechanisms of actions of the AgNPs [8].

Polyvinylpyrrolidone-capped AgNPs of 10 nm diameters were tested in vitro for antiviral activities against SARS-CoV-2. The NPs at concentrations of 1—10 ppm potently protected the VeroE6/TMPRSS 2 cells from SARS-CoV-2-mediated cell death. The luciferase-based pseudovirus entry assay indicated the effective disruption of the viral integrity by the NPs to inhibit the viral entry in a concentration/dose-dependent manner. Interestingly, the NPs were only observed to be toxic to mammalian VeroE6/TMPRSS 2 and Calu-3 cells at ≥ 20 ppm, a concentration that doubly exceeds the effective dose, demonstrating the systemic tolerability and biocompatibility of the NPs (Fig. 14.4). Although, proper disposal of the agents is encouraged to avoid environmental and ecosystem disturbance [33]. Similarly, glutathione-capped Ag_2S nanoclusters reportedly inhibited CoV modeled on the porcine epidemic diarrhea virus efficiently, indicated by a 3-folds reduction in viral titer just after 12 hours of treatment. The mechanism study showed that the NPs remarkably block the synthesis of negative-stranded RNA and viral budding, and upregulated the IFN, ISGs, and the expressions of proinflammatory cytokines [34].

Although, some AgNPs were designed not for direct pharmacological effects on the SARS-CoV-2. However, they suppress severity by ameliorating the comorbidities. For instance, synthetic mucoadhesive AgNP chips experimented successfully as a local

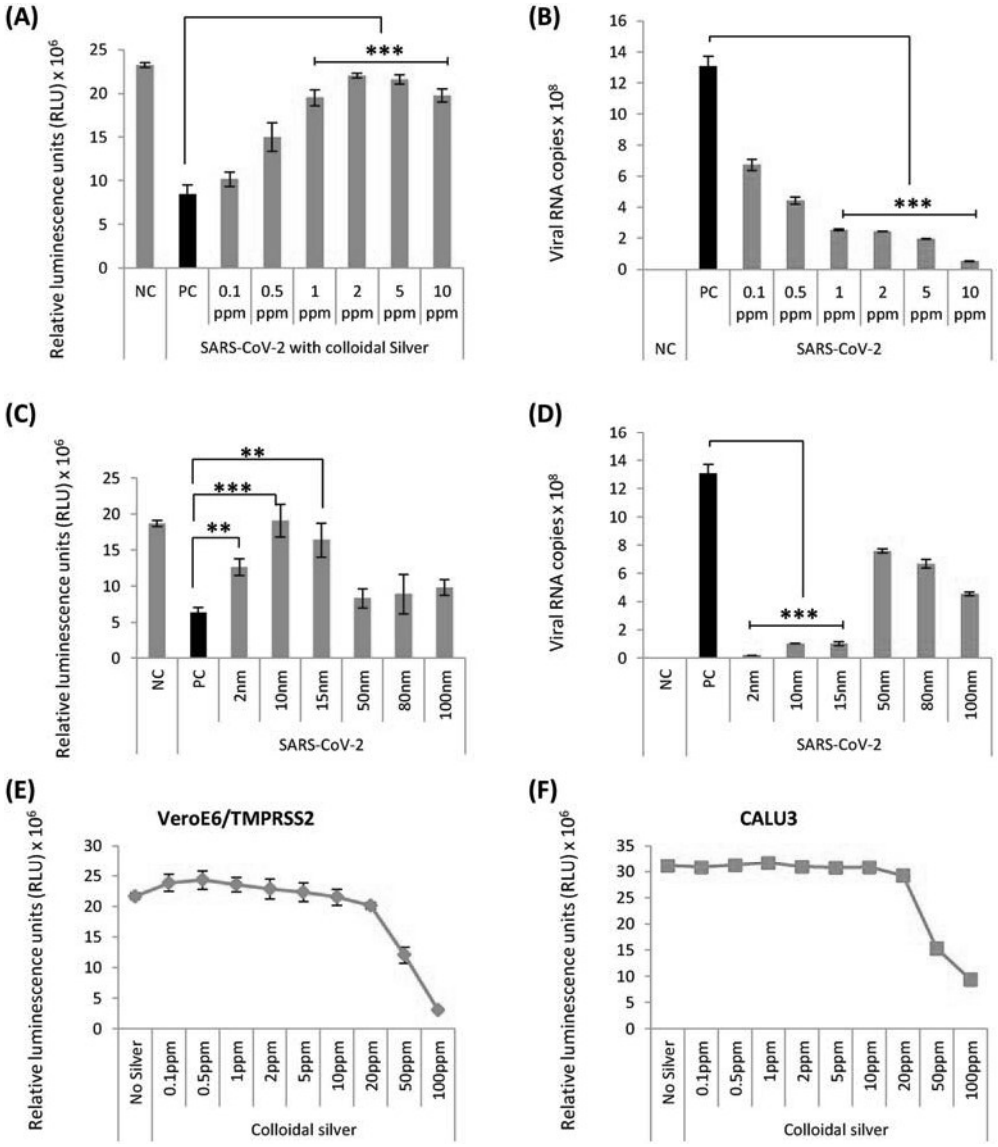


Figure 14.4 Concentration and dose-dependent antiviral effects of naked AgNPs on SARS-CoV-2. (A) Colloidal silver rescues VeroE6/TMPRSS 2 cells from SARS-CoV-2-mediated cell death in a concentration-dependent manner. Error bars obtained from triplicate testing, *P* value_0.005 (***). (B) Concentration-dependent inhibition of SARS-CoV-2 replication in Calu-3 cells by colloidal silver. Error bars obtained from triplicate testing, *P* value_0.001 (***). (C) Silver NPs exhibit size-dependent antiviral action against SARS-CoV-2 in VeroE6/TMPRSS 2 cells. Error bars obtained from triplicate testing, *P* value_0.005 (***). (D) Size-dependent viral inhibition of SARS-CoV-2 by AgNPs in Calu-3 cells. Error bars obtained from triplicate testing, *P* value_0.001 (***). (E) Cytotoxicity exhibited by serial concentrations of colloidal silver on mammalian VeroE6/TMPRSS 2 cells. (F) Cytotoxicity is exhibited by serial concentrations of colloidal silver on Calu-3 cells. *Adapted with permission from S.S. Jeremiah, K. Miyakawa, T. Morita, Y. Yamaoka, A. Ryo, Potent antiviral effect of silver nanoparticles on SARS-CoV-2, Biochem. Biophys. Res. Commun. 533 (2020) 195—200., Copyright (2020) Elsevier Inc.*

drug delivery system against periimplantitis, a bacterial infection comorbid in COVID-19 conditions [35].

Inhalation delivery of nanomedicine was hypothetically proposed as a first-line intervention for respiratory infections primarily at the bronchial tree and upper respiratory system. Silver-based nanoparticles colloids (NPCs) with particle sizes of 2–10 nm and large negative Z-potential selectively bind to viruses with predominately positively charged S-proteins, however less effective against the negatively charged counterparts [36]. Thus, the design of NPCs with Ag or any other metal/oxide as a construction ingredient to attain the set properties could be effective in the safe delivery of nanomedicine at effective doses by inhalation.

Despite the interesting activities displayed by AgNPs against several viral diseases including the multidrug-resistant types, the toxicity concerns to human and environmental systems remain a major stumbling block to their real-time clinical application. Shreds of experimental pieces of evidence have shown that the toxicity studies of AgNPs on human subjects are still at the preliminary stages for a reasonable conclusion [37]. Generally, comprehensive scientific efforts are necessarily required in this direction for transforming the promising efficacy of AgNPs against viral disease into reality.

14.6 Silver nanoparticles for treatment of other viral diseases

Bioactive molecules designed into nanostructures usually display enhanced strain-specific targeting amenable to overcoming treatment-resistant viruses and emerging viral infections [38]. Silver NPs suitably demonstrate strain-specific targeting against several other viral diseases including rhesus rotavirus (RRV), influenza, herpes simplex virus (HSV), RSV, Chikungunya, hepatitis C virus (HCV), norovirus, and enterovirus, supporting their potentials for antiviral therapeutic designs.

14.6.1 Rotavirus

RRV is a characteristic double-stranded member of the family *Reoviridae*. The virus is highly contagious and commonly associated with diarrhea among children. It was generally believed that every child in the world would be infected with the virus at least once by the age five. Although, the ongoing development of vaccines has ameliorated such unpleasant frequency, however curative agents for treating viral disease are much needed. Some synthesized AgNPs were evaluated at different concentrations against the Simian rotavirus SA11 cell lines in vitro using an MTT assay. The AgNPs demonstrated strong antiviral effects, indicated by a reduction in viral titer by 0.5–2.75 $\log_{10}$ $TCID_{50}$, corroborated by CC50 of 346–747 μg/mL against the mosquitocidal *Bacilli* cells [39]. Further research on the cytotoxic effects of bioactive AgNPs of *Bacillus subtilis* (expressing rotavirus) on human liver cancer (HepG-2) cells, African green monkey

kidney epithelial (MA104) cells, and lung cancer (A549) cells produced CC_{50} scores of 6.4, 78.9, and 212.5 mg/mL, respectively. The results showed that the MA104 cell line was much protected from rotavirus upon incubation with the AgNPs, with a significant reduction in viral titer by 3.25 $\log_{10}$ $TCID_{50}$ [40]. In another study, RRV (MMU 18006 strain) was inoculated into MA104 cells to evaluate the antiviral activities of some AgNPs using plaque assay in vivo rotavirus mouse model of biliary atresia, a viral infection in neonatal conditions. The almost total reduction in biliary epithelial cells was recorded on days 9 and 12 post-RRV-inoculation, showing effective induction. This overturned upon treatment, with a significant indicative reduction in jaundice and weight loss, and an increase in rates of survival in the AgNP-treated mice group (Fig. 14.5). Upregulation of the TGF-β mRNA and reduction in viral loads were suggested mechanisms of action of the NPs [41]. The overall results indicate the potency of AgNPs to protect the mice and enhance their survival against biliary atresia.

14.6.2 Influenza virus

Influenza virus, also known as flu, is a nonsegmented negatively sensed RNA genome virus commonly hosted by mammals, livestock, and wild aquatic birds. It is primarily categorized as A, B, and C, with subtype A and its various subtypical lineages being the most virulent, ubiquitous, and mostly responsible for influenza outbreaks [42]. Experimental evidence indicates the potential of AgNPs to treat these virulent viral infections. For instance, the antiviral effects of some AgNPs were evaluated against the influenza A virus using sulforhodamine B assay. Slight inhibitory effects (4.18%–5.97%) were observed at lower doses (0.005–0.15 M). The effects became apparent with an increase in the concentration and attained an equilibrium state at 0.25 M, recording 15.12% inhibition. Although, no precise mechanism was succinctly proposed for the activities. However, none of the tested doses was found to be cytotoxic to uninfected MDCK cells [43], supporting the systemic tolerability of the AgNPs and posing them as promising candidates for further study.

The emergence of resistance significantly hampers clinical successes of several antiviral medications such as zanamivir (ZNV), a neuraminidase inhibitor usually applied for the prevention and treatment of influenza A and B infections. To enhance the efficacy, an AgNP surface was decorated with the drug and evaluated the drug-resistant H1N1 virus. The nano design aided an adequate delivery to the target where the AgNP-ZNV significantly inhibited the neuraminidase activity and reversed the viral resistance. The NPs effectively protected the MDCK cell against the apoptosis induced by the H1N1 virus. Other proposed mechanisms of the antiviral actions include the activation of caspase-3, chromatin condensation, DNA fragmentation, and the downregulation of the H1N1 virus-induced reactive

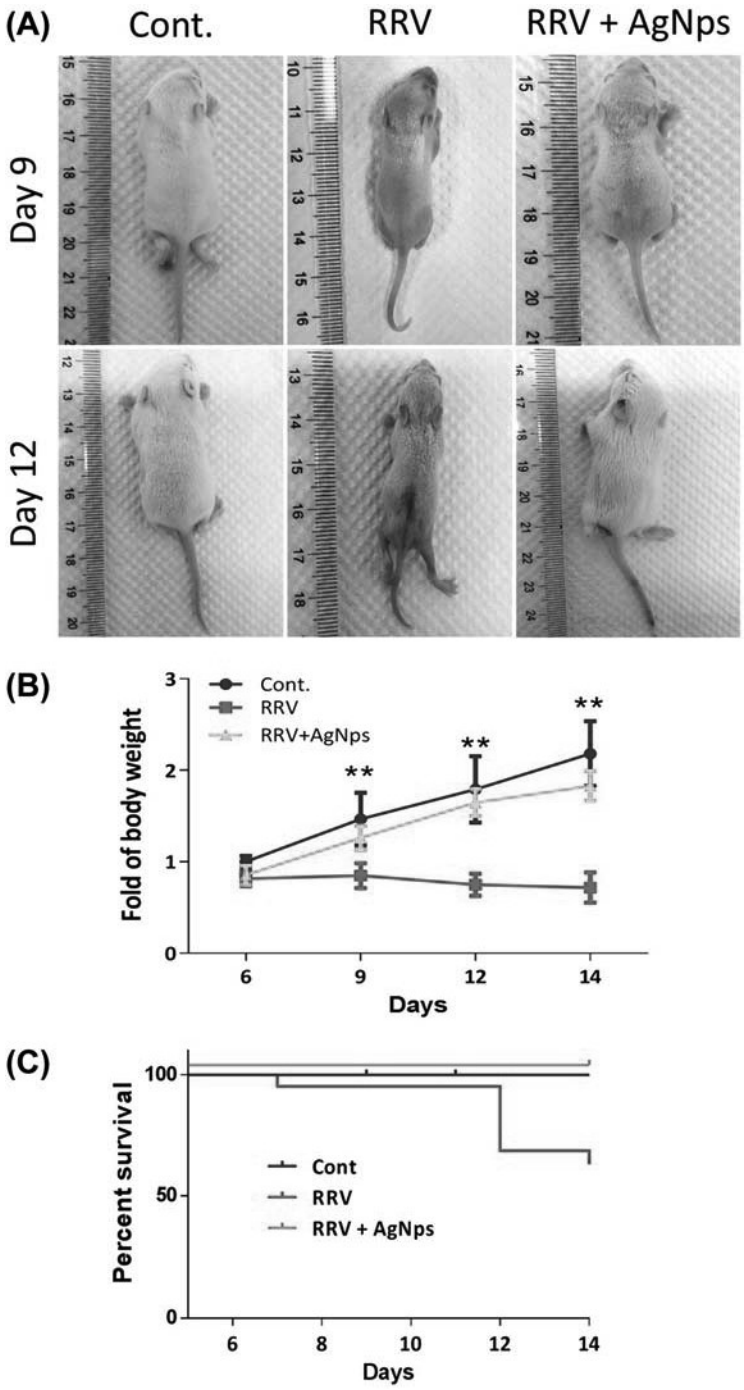


Figure 14.5 Effect of AgNps on biliary atresia (BA) syndrome in rhesus rotavirus-induced BA mouse model. (A) The physical appearance of mice on days 9 and 12 after virus inoculation (RRV) alone

(*Continued*)

oxygen species (ROS) and P38/P53 signaling pathways [42]. The study further emphasizes the multitarget-directed mechanisms of antiviral activities exhibited by AgNP-incorporated biomaterials.

In another study, antiviral effects of epigallocatechin gallate (EG) were observed in combination with $ZnSO_4$ and AgNP against H5N1 avian flu. Separately, 1.5 mg/mL of $ZnSO_4$ and AgNP induced reductions in the viral titer by 5.7 $\log_{10}$ and 5.6 $\log_{10}$, respectively. When EG-AgNP was combined with $ZnSO_4$, the viral reduction significantly increased by 7.6 $\log_{10}$ with no recognized toxicity [44]. These further buttress the hypothesis of hybrid NP design to improve efficacy through synergistic pharmacology as previously postulated.

14.6.3 Herpes simplex virus

Human alphaherpesvirus, mostly called HSV 1 and 2, are causative pathogens of genital and oral herpes, popular sexually transmitted infections in humans. They are primarily transmitted through oral-oral and oral-genital contacts. The symptoms of HSV include blisters or sores on genitals and mouth/lips, although some carriers have asymptomatic HSV without noticeable episodes of herpes. The viral infections are oftentimes complicated with neurodegenerative diseases, contributing to its complexity, yet with limited therapeutic options. *Oscillatoria* sp. and *Spirulina plantesis* were used as capping agents to synthesize Ag_2O/AgO NPs, which were experimented with interesting antiviral efficacy against the HSV in vitro Vero cells. The NPs induced up to 90% reduction in cytopathic effect (CPE) of HSV-1 with proposed mechanisms such as the inhibition of viral-host entry and agglutination of viral glycoprotein [45]. Furthermore, some biosynthesized AgNPs of *Neochloris aquatic*, freshwater microalgae expressed antiviral and antiproliferative effects on cervical adenocarcinoma (HeLa), laryngeal cancer (Hep-2), and HSV cell lines. The cells experienced morphological changes, leakages of cytoplasmic content, and permeabilization of cell membranes at micromolar IC_{50} values [46]. Similar pharmacological actions were shown by biosynthesized AgNPs and *Melaleuca alternifolia* where the CPE of HSV-1 and HSV-2 were reduced by 44.00% and 45.04%, respectively. The viral entry and replication were suggestively inhibited by the NPs through apoptotic-inclined binding

◀ and at days 3 and 6 after injection with AgNps (RRV + AgNps). (B) Weight of each group at different time points after injection with AgNps was recorded; the y-axis indicates the fold increase of weight, which was calculated relative to the weight of the control group on day 6. $^{**}P < .01$, $n = 16$, 18, and 17 in Cont, RRV, and RRV + AgNps group, respectively. (C) The survival curve of each group at different time points was recorded. *Adapted with permission from R. Zhang, Z. Lin, V.C. Lui, K.K. Wong, P.K. Tam, P. Lee, et al., Silver nanoparticle treatment ameliorates biliary atresia syndrome in rhesus rotavirus inoculated mice, Nanomed.: Nanotechnol. Biol. Med. 13 (2017) 1041–1050 Copyright 2016, Elsevier Inc.*

interactions of the NPs with the viral envelope glycoprotein, DNA and RNA. Although, more molecular studies were suggested to advance the understanding of the mechanisms [47]. In an in vivo experiment, the immune responses of mice infected with vaginal HSV-2 were reportedly triggered when treated with AgNPs of tannic acid (TA-AgNPs). The enhanced immune responses were related to the activation of the plasma cells, interferon-gamma + /effector-memory CD8 + T cells (T lymphocytes), B cells (B lymphocytes), and natural killer (NK) cells, as observed in the TA-AgNPs-treated compared to the NaCl-treated group. Upon treatment, the animals were also observed with low viral titers and significant improvement in clinical scores (Fig. 14.6) [48]. The study revealed the potential of the AgNPs to amplify the host immune response against viral infectivity.

In addition, AgNPs prepared by Haggag and coworkers have been experimented with strong inhibitory activities against the HSV-1, indicated by an IC_{50} of 5.13 μg/mL [49]. Oftentimes HSV appears as a complication with tumor growth. An example of such is the human oncogenic γ-HSV, Kaposi sarcoma-associated HSV (KHSV) with currently no specific treatment. The limited efficacy of several cytotoxic drugs against the viral-complicated tumor has resulted in several mortalities. Searching for effective therapy, silver NPs were recently documented with experimental cytotoxicity against KHSV-infected cells by reactivating the viral lytic replication through the induction of autophagy and ROS generation. Other proposed mechanisms for the interesting activities against the viral-related tumor include the interaction with the KHSV virion particles to block its entry, inhibition of its colony formation, and suppression of its growth in a xenograft mouse model [50]. The presence of multifunctional TA-AgNPs in a mucoadhesive hydrogel also reportedly enhanced the antiviral effects of the gel in vitro immortal human keratinocyte cells and in vivo murine model of HSV-2-genital infection. The hydrogel exhibited pseudoplastic and thixotropic features that boost their cytotoxicity against the virus. Mechanistically, it mitigated viral attachment, blocked its cell entry, and minimized its cell-to-cell transmission [51]. The volume of convincing pieces of evidence from experimental studies further supports the therapeutic propensity of AgNPs against HSV infections including the cancer-complicated types and their worthiness for translation into real-time anti-HSV drugs upon further assessments.

14.6.4 Respiratory syncytial virus

RSV is a respiratory system viral infection that affects mostly infants and has no specific treatment. Shreds of evidence from experimental studies have shown the promising therapeutic potentials of AgNPs against the virus. In an instance, some AgNPs were assessed in vitro for therapeutic effects on RSV infection utilizing two epithelial A549 and Hep-2 cell lines in a plaque test at doses of 0−50 g/mL. The maximum action

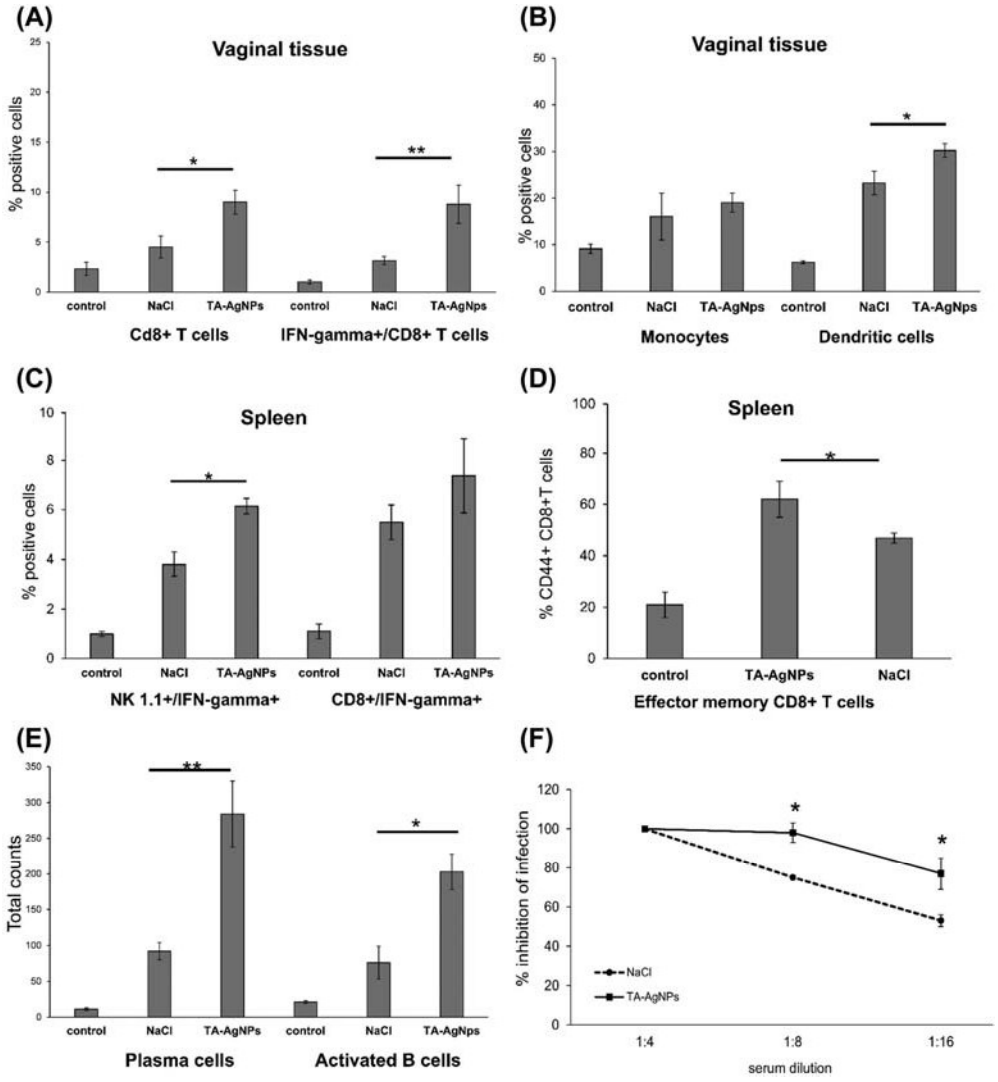


Figure 14.6 Antiviral activity of TA-AgNPs through the stimulation of T and NK cells' response. (A) TA-AgNPs boosted the percentage of CD8 + T cells and CD8 + /IFN-gamma + T cells. (B) Monocytes and dendritic cells were enhanced by TA-AgNPs. (C) TA-AgNPs induced an increase in the percentage of NK1.1/IFN-gamma + cells and CD8 + /IFN-gamma + T cells. (D) TA-AgNPs improved the effector memory of CD8 + T cells (E) TA-AgNPs enhanced the response of B cells after re-challenge in the vaginal tissue. (F) Intravaginal treatment with TA-AgNPs of HSV-2 infection induced better seroneutralization titers. The means are expressed as mean ± SEM for $n = 20$; *represents significant differences with $P \leq 0.05$, while ** mean $P \leq 0.01$. *Adapted from P. Orłowski, A. Kowalczyk, E. Tomaszewska, K. Ranoszek-Soliwoda, A. Węgrzyn, J. Grzesiak, et al., Antiviral activity of tannic acid modified silver nanoparticles: potential to activate immune response in* Herpes genitalis, *Viruses 10 (2018) 1—15, Licensed under CC BY-4.0.*

was seen at 50 g/mL, indicating significant dose-dependent suppression of RSV replication and prevention of viral infusion into host cells. When evaluated in vivo mice infected with RSV, the AgNPs enhanced a decrease in proinflammatory chemokines and cytokines, induced lung tissue activation, increased chemoattractant CXCL1, granulocyted colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and neutrophils [52]. The promising anti-RSV potential shown with multitarget-directed mechanisms and minimal toxicity after 24 hours of exposure to the lowest and highest doses (10 and 50 g/mL respectively), suggesting the potential of the AgNPs for prophylaxis and treatment of RSV, amenable for therapeutic design. In another investigation, nanocomposites prepared from AgNP and graphene oxide (GO) were evaluated as replication suppressors against porcine reproduction and RSV (PRRSV). The AgNP-GO composites inhibited the PRRSV quantitatively by 59.20% suggestively through the prevention of viral entry and stimulation of IFN-α and ISGs which directly triggered the effective inhibition of viral proliferation [53]. The study further reiterates the postulated idea of hybrid nano design to conquer viral resistance.

14.6.5 Chikungunya virus

Chikungunya virus (CHIKV) is a single-stranded, positive-sense RNA arthropod-borne member of the genus alphavirus. Its infection usually induces fever and other illnesses including arthritis and maculopapular rashes. Its recurrent emergence across various continents raises much concern about serious health threats, especially with currently limited therapeutic or vaccine options. Raising the hope, AgNPs of *Citrus limetta* peels showed interesting antiviral efficacy when tested against CHIKV, indicated by a substantial decrease in CHIKV viral titer and viral RNA level. Using the plaque-reduction and qRT-PCR analyses, the NPs were reported with >90% viral inhibition at the tested concentrations, 0.05, 0.1, and 0.2 mg/mL, with a concentration-dependent reduction in the viral mRNA genome. The NPs also recorded a micromolar IC_{50} as 11.73 μg/mL and an SI value (CC_{50}/IC_{50} ratio) of 145.7, indicating its safety to normal cells [54]. Similarly, some biosynthesized AgNPs of extracts from *Andrographis paniculata, Phyllanthus niruri*, and *Tinospora cordifolia* were evaluated for anti-CHIKV activities in vitro Vero cells using MTT assay. AgNPs of *A. paniculata* were observed to be effective at a maximum nontoxic dose (MNTD) of 31.25 μg/mL while others were recorded with 125 and 250 μg/mL, respectively. Consistently, AgNP of *A. paniculata* also showed the highest CPE and significant increment in cell viability from 25.69 to 66.86 and subsequently 80.76% upon treatment on CHIKV-infected cells at half MNTD and MNTD, respectively [55]. Moreover, some AgNPs of *Carica papaya* also displayed impressive antiviral effects against CHIKV with percentage inhibition of 39 and 52 at half MNTD and MNTD of 62.5 and 125 μg/mL, respectively. The increment of 14% in cell viability upon treatment with half MNTD of the AgNPs supports the anti-

CHIKV efficacy of the NPs. The pieces of evidence from various scientific investigations indicate the promising therapeutic potential of AgNPs for therapeutic development against CHIKV. More interestingly, the capping agents were derived from vast, safe, cost-effective, and environmentally friendly natural sources for ease of further studies. Although, more experimental validations are required on the toxicity of the AgNPs to healthy cells [56].

14.6.6 Hepatitis C virus

HCV is a viral infection prominently identified in the liver. It is characterized by chronic symptoms and complications including liver damage, liver cancer, and cirrhosis. More seriously, it exhibits high resistance to treatment [57], although appropriate preventive measures and treatment at early stages usually ameliorate its severity. To explore the therapeutic possibility, some AgNPs of *Amphimedon* extract in different media were assessed *via* in vitro experiments against the HCV NS3 helicase and protease activity. The AgNPs of petroleum ether exhibited more activity against the NS3 helicase with IC_{50} recorded as 0.11 ± 0.62 μg/mL relative to the AgNPs of total extract, $AgNO_3$ and ribavirin with respective IC_{50} values observed as 1.52 ± 1.18, 77.72 ± 4.57, and 4.66 ± 0.29 μg/mL. Consistently, the AgNPs of petroleum displayed the most potent inhibition against the NS3 protease with IC_{50} of 2.38 ± 0.57 μg/mL while others including the drug references have an IC_{50} value range of 4.77 ± 0.26—52.67 ± 0.33 μg/mL [57]. Similarly, AgNPs of extracts from cyanobacteria, *Spirulina platensis*, and *Nostoc linckia* were found effective at nontoxic doses when evaluated against the HCV. The respective AgNPs were recorded with interesting antiviral activities between 48.334% and 64.976%, competitively with a renowned antiviral drug, ribavirin which showed 66.673% [58]. Although, the mechanisms of impressive activity remain less understood, however the AgNPs of the extracts from *Amphimedon*, *Spirulina platensis*, and *Nostoc linckia* represent promising therapeutic agents for HCV, amenable for future medicinal designs.

14.6.7 Human immunodeficiency virus

HIV primarily attacks the host's immune system and oftentimes results in acquired immunodeficiency syndromes if left untreated. It remains one of the significant viral threats to human well-being with limited therapeutic options. As such, preventive strategies against its transmission remain the better probable approach for its control. The only few available anti-HIV drugs including the highly active antiretroviral therapy are also challenged with hypersensitivity and various contraindications, unpleasant side effects, and toxicities. The effects of constant mutation, drug escape mechanisms, and incessant resistance developed by the virus cannot be underestimated. Due to the large implications of the immune cells in the pathogenesis, they are mostly recognized

alongside viral integrase enzymes as therapeutic targets for mitigating the HIV-host infusion and replication [59,60]. From experimental studies, Ag nanorods of sodium 2-mercaptoethan sulfonate (Ag-MES) were reported with potent inhibitory effects on HIV and HSV-1. The application of 5 μg/mL of the NPs induced up to 50% inhibition of the growth of HIV virion and the entire cells subsequently become inhibited (by 100%) when the concentration of the NPs was increased to 10 μmol/mL. Although, Ag-MES were demonstrated to be more sensitive to HIV cells, however they also inhibited HSV growth by 90%, indicating their therapeutic potential on both viruses. Similarly, AgNPs of curcumin were evaluated for immunomodulatory-based antiretroviral activities on ACH-2 cells infected with HIV-1. The NPs displayed interesting anti-HIV effectively through the inhibition of long terminal repeat, IL-1β, IL-6, p24 antigen, nuclear factor-kappa B, and TNF-alpha quantitatively by 54%–79%. The NPs inclusively inhibited the HIV-1-induced proinflammatory responses and interestingly showed no significant toxicity [61].

14.6.8 Dengue

Dengue virus (DEN-2) is a mosquito-borne, single positive-stranded RNA virus of the family *Flaviviridae*. It causes dengue fever, prominently within the tropical and subtropical regions of the world. Mosquitoes of the family Diptera: *Culicidae* constitute major public health significance due to their involvement in the transmission of various viral/arthropod-borne diseases including Zika, yellow fever, Chikungunya, and DEN-2. Specifically, *Aedes* species (*Aedes aegypti* and *Aedes albopictus*) are the vectors responsible for the transmission of DEN-2 during blood-feeding [37,62]. Despite the widespread and significant health challenge of DEN-2, no effective therapeutic candidate is yet approved for its treatment. Thus, preventive strategies remain critical in the effective control and management of the disease with a scientific focus directed at the vectors [62,63]. In the vector-dependent life cycle of the virus, the mosquito carrier transmits the virus to an uninfected human during blood-feeding, making him a new host. The virus enters the host bloodstream and the circulatory system. An uninfected mosquito becomes a new carrier by feeding on the viral human host, then laying eggs which develop at various stages to produce a new adult mosquito (Fig. 14.7). As such, effective control of the mosquito reproduction cycle represents a plausible approach to controlling viral transmission.

Due to their multifaceted bioactivities including biocidal, AgNPs have been experimentally shown with potent larvicidal effects on the intermediates forms between the eggs and adult mosquitos, i.e. larvae and pupae. In a reported study, AgNPs of pulp extract of *Cassia fistula* fruit were evaluated for larvicidal and pupicidal effects on *A. albopictus* at larvae (I–IV instar) and pupae stages. Half-maximum lethal concentration (LC_{50}) values in the range of 8.3–17.3 mg/L were recorded on the developing

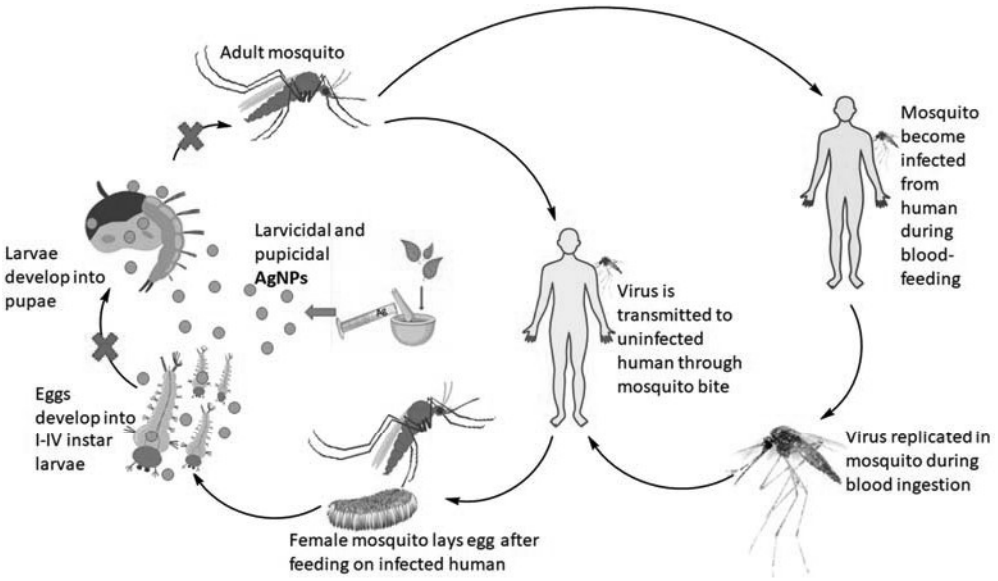


Figure 14.7 Life cycle of mosquito, transmission of viral diseases, and larvicidal/pupicidal effects of silver nanoparticles.

mosquitoes after 24, 48, and 72 hours of treatment with the NPs. Acetylcholinesterase and α-/β-carboxylesterase was implicated biomarker enzymes upon evaluating the systemic effects of the NPs on the fourth larvae of *A. albopictus* [64]. Similarly, very strong larvicidal effects were recorded when green synthesized AgNPs of *Annona glabra* (An-AgNPs) were investigated on third instar larvae of *A. aegypti* and *A. albopictus*. After 24 hours of treatment with the NPs, LC_{50} values of 5.29 and 2.43 mg/L were recorded against the respective vectors, whereas the leaf extract of *A. glabra* produced 5.94 and 5.00 mg/L, respectively [65]. Similarly, AgNPs of aqueous extract of *Solanum mammosum L.* exhibited very strong larvicidal effects on *A. aegypti*, indicated by LC_{50} of 0.06 ppm whereas the crude extract was recorded with LC_{50} of 1631.27 ppm after a 24-hours treatment [37]. Biosynthesized AgNPs of extracts from *Moringa oleifera* seed also demonstrated antiviral activities in vitro DEN-2 cells by reducing the viral titer loads from 7 $\log_{10}$ to 3.8 $\log_{10}$ $TCID_{50}$/mL after a single treatment with the NPs at 20 μL/mL. The DEN-2 yielded 5.8 $\log_{10}$ PFU/mL in the control but remained at 1.4 $\log_{10}$ PFU/mL 6 hours after treatment with the NPs 20 μL/mL. Interesting larvicidal and pupicidal effects were also recorded on *A. aegypti* quantitatively by LC_{50} values of 10.24—21.17 ppm against I instar larvae and pupae, respectively [63] Silver AgNPs of *Bruguiera cylindrical* also inhibited the E protein of DEN-2 and downregulated its E gene effectively upon treatment with 30 μg/mL of the NPs. The NPs were highly cytotoxic on the I-IV instar larvae and pupa with LC_{50} values of 8.935, 11.028, 13.913, 22.443, and 30.698 ppm, respectively. Interestingly, the NPs displayed no effect on the goldfish

predation of *A. aegypti*, supporting its selectivity against the nontargets [62]. Green-synthesized AgNPs of leaf extracts of *Ambrosia arboscens* also exhibited a significantly higher larvicidal activity against third instar larvae of *A. aegypti*. Quantitatively, LC_{50} and LC_{90} values of 0.28 and 0.43 ppm respectively were recorded when the larvae were exposed to 0.2—0.5 ppm of the AgNPs. However, the crude extract was observed with much lower toxicity against the larvae, indicated by the LC_{50} and LC_{90} values of 1844.61 and 6043.95 ppm, respectively, at the treatment concentration range of 1500—6000 ppm for the same duration [66]. The shreds of evidence support the potency and applicability of green synthesized AgNPs in the control and management of DEN-2 via the disruption of the vector/mosquito life cycles. Again, it channels a plausible alternative route for effective controlling of the disease carrier mosquito, boycotting the challenges of pollution, environmental disturbance, human allergy, and pesticide-resistance development associated with the pesticide method.

14.6.9 Norovirus

Oftentimes, the viral diseases exist as water- or food-borne through contaminations, posing a great threat of rapid spread. Notably, norovirus is a highly contagious contamination-related viral disease usually causing diarrhea, nausea, and vomiting. Adhering to personal and environmental hygiene, especially during food preparation, and minimal contact with an infected person are effective prevention measures to curb its outbreaks. Moreover, the development of therapeutic interventions against the disease is still imperative. Some AgNPs, Ag30—SiO_2 particles were tested against the bacteriophage Ms2 (*Emesvirus zinderi*) and murine norovirus (MNV) in four varieties of water from different sources at 5°C and 20°C. The NPs exhibited higher temperature-dependent inhibition of the bacteriophage Ms2 in distilled water than in pipe-borne water with more than $4\log_{10}$ viral reduction at 20°C, while no apparent effect could be observed with surface/groundwater. Similar activities were recorded in favor of the AgNPs against MNV [67]. Consistently, an AgNP-coated glass filter showed a filtration efficiency of 99.99% against the Ms2, tunavirus (T1), and *Escherichia* virus T4 [68]. The shreds of evidence validate the promising antiviral potentials of the AgNPs for the effective treatment of water-borne viral diseases such as norovirus and water treatment against viral infection.

14.6.10 Enterovirus

Enterovirus 71 (EV71) has been linked to a variety of neurological disorders, as well as hand, foot, and mouth disease, particularly in children. The lack of effective treatment strategies constitutes widespread. Small interfering RNAs (siRNAs) have been earmarked with the potential for anti-EV71. However, their inability to cross cell membranes limits their onward application. Consequently, nanotechnology was applied to

improve their cell penetration as anti-EV71 agents. Some surface decorated AgNPs of polyethyleneimine (PEI) and siRNA were examined for cytotoxic effects on EV71 in vitro Vero cells using MTT assay. The treatment of Vero cells with EV71 showed the viability of 25% and subsequently increased to 40% then 62% upon treatment with ordinary AgNPs and AgNPs of PEI, respectively. Interestingly, cell viability increased to 85% upon treatment with a composite AgNPs of PEI and siRNA and the viral effects on the cells such as morphological change, reduction in cell numbers, and loss of cell-to-cell contacts induced by EV71 were adequately corrected by the NPs. The proposed mechanisms for the intriguing anti-EV71 actions include the inhibition of EV71 cell infusion, inhibition of ROS, prevention of DNA fragmentation, condensation of chromatin, and activation of caspase-3, P53, and AKT [69].

14.7 Silver nanoparticles in clinical trials for COVID-19 and other viral/microbial diseases

Only handful numbers of AgNPs are currently available in clinical trials (https://clinicaltrials.gov/) for COVID-19 and other viral diseases as of March 27, 2022. These include the mouthwash and nose rinse of AgNP solution and colloidal silver being evaluated for the prevention and treatment of COVID-19, respectively. Another is Nowarta110 in phase I/II for safety and efficacy against plantar wart, a disease caused by HPV with respective "completed," "recruiting," and "completed" status. Other AgNP-based agents currently in clinical trials are for nonviral microbial infections such as fungal and bacterial syndromes (Table 14.1). The unprogressive evaluations of many AgNPs in clinical trials could be traced to the incomprehensive preclinical studies, especially on their toxicity and environmental concerns.

14.8 Limitations of silver nanoparticles for treatment of COVID-19 and other viral diseases

The concerns of systemic and environmental toxicity remain major stumbling blocks against the real-time biomedical utility of AgNPs for the treatment of diseases. For instance, the strong oxidative effects of AgNPs induce the release of Ag^+ ions, which in turn pose unpleasant biological responses including cytotoxicity, genotoxicity, and other direct effects on human health including cell apoptosis. Similarly, the presence of Ag^+ ions in soil and water environments is hypothesized as being harmful indiscriminately to both desired and unwanted microorganisms [70]. Another concern is the imminent possibility of resistance to AgNPs by microorganisms, commonly to newly emerged therapeutic agents for treating microorganism infections [71]. Although, the properties support their use in the development of biocidal, water-purifying, and environmental disinfecting agents. However, it beleaguered their

Table 14.1 Silver nanoparticles currently in clinical trials.

AgNP material	Condition	Trial title	Status
Mouthwash and nose rinse of AgNP solution	COVID-19	Evaluation of silver nanoparticles for prevention of COVID-19	Completed
Colloidal silver	SARS-CoV-2	Colloidal silver, treatment of COVID-19	Recruiting
Nowarta110 (Fig extract with colloidal silver)	Plantar warts	A phase I/II double-blind safety and efficacy of Nowarta110 in patients with plantar warts	Completed has results
AgNP antimicrobial gel	Food infection fungi, bacteria	Topical silver nanoparticle for microbial activity	Unknown
AgNPs	Aspergillosis	Thyme and carvacrol nanoparticle effect on fungi	Completed
AgNPs	Multidrug resistant bacteria	Silver nanoparticles in multidrug-resistant bacteria	Completed
Nanosilver fluoride, silver diamine fluoride	Microbial colonization, dental caries	The antibacterial effect of nanosilver fluoride on primary teeth	Not yet recruiting

biomedical applications and necessitated advanced design processes to transform their interesting biological activities into utility.

14.9 Conclusions

Viral diseases have been posing a health threat to humans for time immemorial. Viral-mediated outbreaks and pandemics are being globally experienced indefatigably. Notably, the recently witnessed viral pandemics include influenza, Ebola, HIV, SARS-CoV, MERS-CoV, and the current SARS-CoV-2. Due to the scarcity of effective therapeutics against the preexisting viruses, the sudden emergence and reemergence of new strains usually overwhelm the global healthcare systems. Moreover, the available antiviral drugs are mostly challenged by incessant resistance development by the pathogen, inadequate efficacy, poor delivery to targets, and unpleasant after effects. Nanotechnology advertently aids the design of functional and nonfunctional biomaterials from natural and synthetic routes with improved efficacy, ability to overcome viral resistance, and good delivery to targets amenable for biomedical applications. Among the most commonly explored nano-fabricated materials for

the prevention and treatment of viral diseases including the drug-resistant types, AgNPs remain popular due to their intriguing activities at experiment levels. The fascinating properties that support their antiviral efficacy specifically include permeability, easy functionalization for efficient drug delivery, oxidative potentials, ability to inhibit viral entry, replication and transcription processes, stimulate viral proinflammatory cytokines and preserve host immunity, and other mechanisms implicated in viral/host therapeutic pathways. The exhibition of multitarget-directed mechanisms of action by AgNPs also promotes their suitability for curbing antiviral resistance and effective management of comorbidities that are usually associated with complex viral infections such as COVID-19. Interestingly, these have attracted the continuous exploration of AgNPs for preventive and curative efficiency against several viral diseases affecting humans. These include rotavirus, influenza, HSV, RSV, chikungunya, HCV, HIV, norovirus, dengue, enterovirus, and the current SARS-CoV-2. Mostly, AgNPs demonstrate more interesting antiviral potencies at various in vitro and in vivo experimental stages when compared to conventional antiviral drugs or other nano-free agents. Thus, the NP types are gaining arrays of scientific attention and are being suggested for full translational studies into the real-life application as antiviral therapeutics. As such, some of them including the colloidal silver, AgNP mouthwash and nose rinse solution, and Nowarta100 are currently in clinical trials for efficacy in preventing and treating COVID-19 and Planta warts caused by SARS-CoV-2 and HPV, respectively.

Some viral diseases such as Chikungunya, dengue, and Zika are understandably arthropod-/mosquito-borne and pose great public health threats with limited therapeutic options. These make the effective controls of the implicated vectors remain the most plausible preventive measures against such diseases. To mitigate the unpleasant effects of pesticides in this regard such as pollution, human allergy, and pesticide-resistance development, AgNPs of natural products reportedly experimented with very high potency against the developmental forms of the vectors of the viral diseases, specifically *A. aegypti* and *A. albopictus*. These indicate the worthiness of AgNPs in replacing pesticides for effective control of viral disease vectors.

Furthermore, the preparation of AgNPs through green chemistry using natural products from plants and microorganisms has afforded cost-effective, vast, easy, and environmentally safe approaches. Especially, AgNPs synthesized by the biological method are reportedly more suitable and compliant for biomedical applications including viral disease prevention and treatment. In addition, the design of AgNPs in hybrid form incorporating some bioactive metal complexes could afford synergistic effects and improved activities amenable for curbing viral resistance and complications.

Despite the interesting reports of efficacious antiviral activities in favor of AgNPs, incomprehensive preclinical studies especially, in vivo approaches to observe their toxicity-related physiological side effects are among the factors that retard their

progression to clinical evaluations. In addition, the mechanisms of their antiviral activities are yet to be succinctly understood. Limited studies have also been carried out on their pharmacokinetics and pharmacodynamic properties required for future drug candidates. The concerns of systemic accumulation and environmental safety of nano-sized Ag and Ag^{+} due to their daunting toxicity remain to be guaranteed. As such, more scientific efforts are required in adequate discharging of the NPs from the biosystem and their remediation in the environments. Moreover, issues of imminent resistance development by microorganisms, commonly associated with newly emerged antipathogenic drugs/prodrugs have been identified with the propensity of AgNPs as antivirals. These channels the pathways for future scientific exploration and advanced designs to mitigate the challenges whilst enhancing the antiviral efficacy of AgNPs. Summarily, AgNPs remain indispensable biomaterials for effective prevention, treatment, and control of viral diseases including the current COVID-19 and other emerging strains upon further study.

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