Nanoparticle-based antiviral strategies to combat the influenza virus (Review)

CLARA PATRICIA RIOS-IBARRA[1], MAURICIO SALINAS-SANTANDER[2], DANIELLE ANNETTE OROZCO-NUNNELLY[3] and JORGE BRAVO-MADRIGAL[1]

[1]Medical and Pharmaceutical Biotechnology Unit, Center for Research and Assistance in Technology and Design of The State of Jalisco (CIATEJ), Guadalajara, Jalisco 44270; [2]Research Department, School of Medicine Saltillo, Universidad Autonoma de Coahuila, Unidad Saltillo, Coahuila 25000, Mexico; [3]Department of Biology, Valparaiso University, Valparaiso, IN 46383-6493, USA

Received October 20, 2023; Accepted January 17, 2024

DOI: 10.3892/br.2024.1753

Abstract. The rapid availability of effective antiviral treatments would be beneficial during the early phases of a pandemic, as they could reduce viral loads and control serious infections until antigenic vaccines become widely available. One promising alternative therapy to combat pandemics is nanotechnology, which has the potential to inhibit a wide variety of viruses, including the influenza virus. This review summarizes the recent progress using gold, copper, silver, silicone, zinc and selenium nanoparticles, since these materials have shown remarkable antiviral capacity against influenza A virus.

Contents

Correspondence to: Dr Jorge Bravo-Madrigal, Medical and Pharmaceutical Biotechnology Unit, Center for Research and Assistance in Technology and Design of the State of Jalisco (CIATEJ), 800 Avenue Normalistas, Colinas de la Normal, Guadalajara, Jalisco 44270, Mexico
E-mail: jbravo@ciatej.mx

Key words: influenza A virus, nanoparticles, antivirals, nanomedicine

1. Introduction

The field of nanotechnology emerged in the mid-20th century to produce new materials, structures and devices on the supramolecular scale, where a nanoparticle (NP) is defined as having a dimension of <100 nm and may encompass a wide variety of shapes (from 0D to 3D) (1). Nanotechnology is likewise proving to be a promising field in the 21st century, with numerous potential novel applications in medical research, including the comprehensive surveillance, control, creation, repair and defense of human biological systems (2). For instance, due to their unique chemical and physical properties, nanomaterials have emerged as novel antimicrobials and have been proposed as a promising antibiotic alternative in the context of the increasing number of multi-resistant bacterial strains that have arisen due to the widespread use of antibiotics (3). Similarly, these materials have become new therapeutic alternatives for viral infections, largely due to the low efficacy of vaccines against this type of pathogen and due to the side effects that certain pharmaceutical products can have, particularly in children (4). Furthermore, nanomaterials have the unique ability to prevent the encapsulated anti-viral drug or agent from deteriorating (5). In the present review article, the available NP-based antiviral strategies were described, specifically for the treatment of the influenza A virus (IAV) (Fig. 1).

2. Influenza A virus (IAV)

IAV is an orthomyxovirus that can adopt either a filamentous or spherical shape; its filamentous form has a dimension of 100 nm in length and 30 nm in diameter (as seen in clinical isolates), while its spherical form has a diameter of ~100 nm (based on laboratory cultures) (6). IAV has a negative-strand RNA genome (3'-5'), which is made up of eight genes that are translated into the following 11 proteins: Hemagglutinin (HA), neuraminidase (NA), matrix 1 (M1), M2, nucleoprotein (NP), nonstructural protein 1 (NS1), NS2 (also known as nuclear protein), RNA-dependent RNA polymerase 1 (PB1) and 2 (PB2) (7) (Fig. 2).

To date, four types of IAV have been described: A, B, C and D. Of these, influenza A and B viruses cause seasonal

influenza (8). Type A viruses have numerous combinations of various HA and NA proteins (9). The high mutation rates of this virus along with its ability to rearrange its genome allow for the appearance of new strains that possess the ability to evade the host's immune system (10).

Acute viral respiratory infection caused by IVA affects individuals of all ages and is associated with high mortality (11). Vaccination is the most effective method of preventing influenza infection and its complications. However, the effectiveness of the influenza vaccine varies each year based on the specific influenza strains in circulation and also due to varying rates of individuals who choose to accept the vaccine in a given season.

Currently, four antiviral drugs targeting the NA surface glycoprotein of the influenza virus are available for the treatment and prevention of the disease: Oseltamivir phosphate (trade name, Tamiflu®), zanamivir (trade name, Relenza®), peramivir (trade name, Rapivab®) and baloxavir marboxil (trade name, Xofluza®) (12). These existing options are up to 60% effective at preventing influenza illness caused by Influenza types A and B.

Regarding vaccines, the following three choices are currently available: Inactivated influenza vaccine, live attenuated influenza vaccine and recombinant influenza vaccine (13).

The existing drugs for IAV often face limitations associated with the emergence of resistant strains, as a result of the constant evolution of the viral genome (14). Therefore, there is a pressing need to develop safe and effective antiviral agents that exert a novel mechanism of action compared to conventional drugs. Due to advances in the field of nanotechnology and its growing applications in drug development, it is now possible to exploit the various biological and antiviral properties of NPs via surface modification (15).

Specifically for the control of viral infections, the usefulness of NPs includes the following mechanisms: As immunity-inducing vaccines, through use in gene silencing, as a drug delivery system and simple NPs for their anti-IAV properties (16). In the following sections, it is first reviewed what is currently known regarding the main types of antiviral NPs (categorized by material) and their applications for the treatment and prevention of IAV, and several conserved IAV protein domains and their roles in pathogenicity are then discussed.

3. Gold nanoparticles (AuNPs)

AuNPs can form stable chemical bonds with *S*-and *N*-containing groups, allowing them to attach to a wide variety of organic ligands or polymers with a specific function (17). AuNPs are biocompatible and can be functionalized with antigens as carriers for the delivery of vaccines (18). However, AuNPs cannot inhibit viruses alone, as they only possess antiviral outcomes when covalently bound to other molecules that bind to the target virus. In this manner, AuNPs can exponentially increase their antiviral effects through multivalent interactions (19). For instance, it has previously been reported that sialic acid-functionalized AuNPs can inhibit influenza virus infection by multivalent interactions (20).

AuNPs conjugated with HA-IAV can bind to a virion, blocking its connection with cellular or viral receptors, thereby inhibiting the initiation of the viral cycle (infection); these NPs can also penetrate the cell membrane and inhibit viral genome replication (21). For viral replication and assembly, viruses remodel the membranes of intracellular organelles to create viral replication complexes, and treatment with AuNPs can trigger disruption of subcellular structures by altering the cytoskeleton (22). In addition, AuNPs with an appropriate size of 100 nm can enter lymph nodes and induce antigen-specific T cell-mediated immunity (23).

In recent years, the use of porous gold NPs (PoGNPs) has been explored to combat IAV, since PoGNPs have greater thermal stability compared to AuNPs. This type of NP has the ability to interact with IAV surface proteins by cleaving disulfide bonds, thereby inhibiting the fusion of the viral membrane to its target cell by blocking the viral entry process through conformational deformation of HA (inactivated viruses exhibit lower cellular infectivity) (24).

The absorption, cytotoxicity and *in vivo* biodistribution of AuNPs depend on their size as well as the type of target cell (healthy vs. tumor). For instance, an AuNP that is 50 nm in size results in increased AuNP uptake in HepG2 cancer cells, but decreased AuNP uptake in healthy cells (L02, a normal human liver cell line), whereas in cancer cells, an AuNP that is 5 nm in size promotes apoptosis and the production of reactive oxygen species (ROS) (25). Therefore, when designing an AuNP application, it is critical to identify the ideal particle size in the model being used (26).

In mouse models, it has been reported that intranasal administration of the consensus peptide extracellular domain of M2 (M2e; acetylated-SLLTEVETPIRNEWG-SRSNDSSDC-amidated; molecular weight, 2,736 Da) of IAV conjugated with AuNPs and the CpG oligonucleotide as soluble adjuvant (immunostimulator) (AuNP-M2e+CpG) induces the activation of lung B cells and elevated anti-M2e immunoglobulin G levels in serum (27). Double-stranded RNA analogues have also been proposed as adjuvants using 40-50 bp of polyinosinic-polycytidylic acid (synthetic origin of ~400 bp) electrostatically conjugated with AuNPs and the HA of the influenza virus for the generation of vaccines. This resulted in the stimulation of interleukin 1β (IL-1β), interferon-β (IFN-β) and tumor necrosis factor (TNF-α) production, which activate the innate immune response, induce apoptosis and stimulate macrophages and natural killer cells (28-31).

4. Copper nanoparticles(CuNPs)

CuNPs show much promise due to their low toxicity and good long-term colloidal stability (32). However, CuNPs can become more toxic when they are larger in size (25 vs. 100 nm), and their toxicity is enhanced by a positive charge, which facilitates interactions between cells and NPs. Furthermore, the dissolution of CuNPs depends on the temperature and pH of the solution, which also has a major influence on their toxicity (the lowest toxicity is observed when the pH is between 9 and 11) (33,34). Additional drawbacks are that CuNPs are easily oxidized when exposed to air and that they are unstable in solution (35).

Cu has been proposed to exhibit an antiviral mechanism via inhibition of the attachment and entry of viruses into the host cell through their interaction with cell membrane glycoproteins (36).

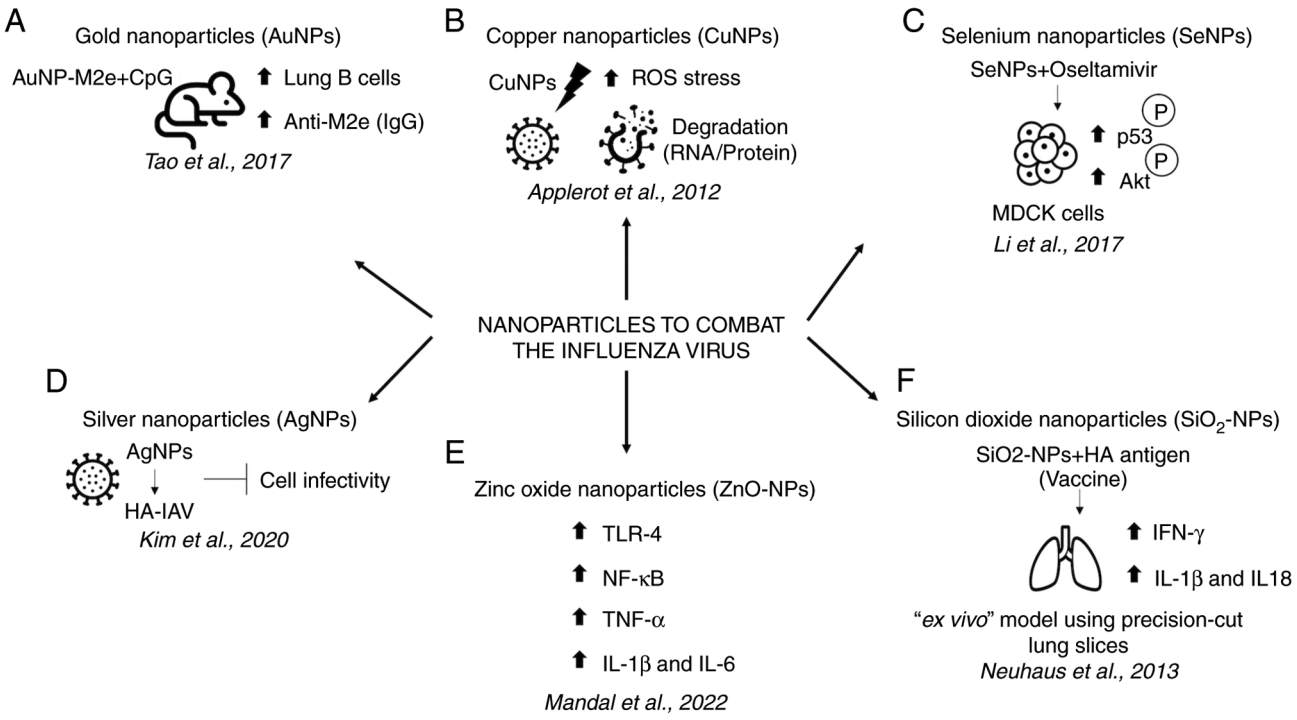

Figure 1. Applications of NPs to combat the influenza virus. (A) AuNPs conjugated with an M2e-IAV peptide and a CpG oligonucleotide induce the activation of lung B cells and increase anti-M2e IgG levels in mice. (B) CuNPs inactivate and degrade viral particles through the generation of ROS, leading to the degradation of the viral genome and proteins. (C) SeNPs increase the antiviral properties of oseltamivir against the H1N1 influenza virus in MDCK cells by activating phosphorylation of p53 and Akt. (D) AgNPs inhibit IAV infection by breaking apart the disulfide bonds contained in HA viral surface proteins. (E) ZnO-NPs stimulate the innate and adaptive immune responses through increased production of TLR-4, NF-κB, TNF-α, IL-1β and IL-6. (F) SiO_2-NPs conjugated with HA-antigen stimulates the production of IFN-γ and reactivates T cells in an '*ex vivo*' model using precision-cut lung slices. NPs, nanoparticles; ROS, reactive oxygen species; IgG, immunoglobulin G; M2e, extracellular domain of matrix protein 2; TLR, Toll-like receptor; HA, hemagglutinin; IAV, influenza A virus.

However, Cu has likewise been postulated to inactivate and degrade viral particles primarily through the generation of ROS, leading to the degradation of the viral genome and its proteins (37). An inactivation assay for H1N1 influenza virus (subtype of IAV) revealed that nucleoprotein (protein visualized through western blot) and HA (RNA quantified via reverse transcription-PCR) are imperceptible after 30 min of treatment with CuNPs (38,39). Furthermore, hybrid silver (Ag)-Cu NPs are capable of binding to glycoprotein gp120 at the HIV cell envelope and inhibiting infection in T cells *in vitro* (40).

5. Silver nanoparticles (AgNPs)

The benefits of AgNPs are that they have high yields, good solubility and high stability (41). AgNPs are likewise able to inhibit the production of viral RNA and extracellular virions likely through a specific interaction between the NPs and the viral genome or via direct binding with viral particles (42). However, AgNPs with particle diameters >800 nm are too toxic to be used in cell culture assays (43).

AgNPs adhere to the viral nucleocapsid where they interfere with viral attachment to the cell membrane by binding to sulfur-containing residues on host cell surface glycoproteins (44,45). This blocks virus entry and also effectively inhibits viral nucleocapsids, which are necessary for the proper assembly of viral progeny (46). In addition, within the host cell, AgNPs can interact with viral genomic material and inhibit genome replication of the virus and can also interrupt certain cellular factors, such as kinases required for phosphorylation of several IAV proteins (47-49). At the same time, the NS1-IAV viral protein has been associated with p53 inhibition and the prevention of host cell apoptosis. However, the virus was found to block p53 activity only in the early stages of epithelial cell infection, while apoptosis increased again in the later stages (8-24 h post-infection) (50). In a study using MDCK cells infected with the H3N2 influenza virus, treatment with AgNPs was found to significantly protect cells against viral infection by increasing cell viability, reducing cytotoxicity, inhibiting viral growth and decreasing cell apoptosis. In addition, within the host cell, AgNPs can interact with viral genomic material and inhibit its genome replication (51). In a different study conducted by Li *et al* (52), using the same cell type (MDCK), AgNPs combined with oseltamivir (an antiviral drug that blocks the release of new virions from the cell membrane to provide resistance to influenza A) were found to have the ability to inhibit H1N1 infection. This process can be mediated via signaling pathways involving the accumulation of ROS, along with AKT activation and p53 phosphorylation; therefore, the use of AgNPs during the early stages of infection may be used as an antiviral strategy (52).

6. Silicon dioxide nanoparticles (SiO_2-NPs)

When Selenium (Se)NPs are modified with functional groups such as polyethylene glycol, they can activate targeting ligands, resulting in enhanced disease site accumulation

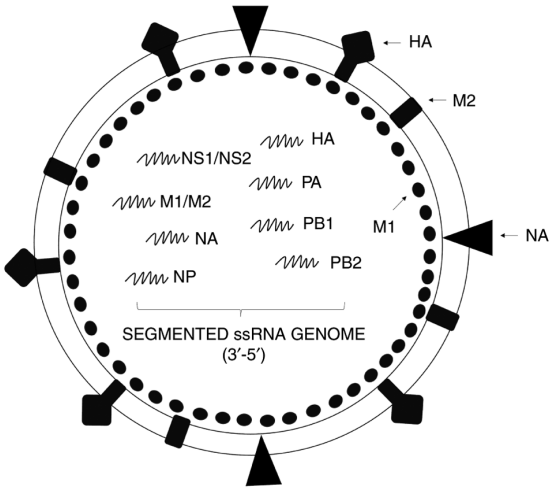

Figure 2. Diagram of the influenza A virus particle. HA and NA are both glycoproteins, which recognize and bind to their respective receptors on target cells during virus-cell fusion. M1 lines the inner surface of the virion, which determines the morphology of the spherical or filamentous virus, resulting in a rigid shell, the virus capsid. M2 is embedded in the lipid envelope, forming tetrameric ion channels that open in response to the low pH of the endosome, allowing a flow of protons toward the virus. The genome of IAV is negative-sense ssRNA, which interacts with a trimetric complex of RNA polymerases: PB1, PB2 and PA protein subunits. NS1 antagonizes the antiviral effect, as mediated by type I interferon, by controlling RNA splicing, inducing or suppressing host apoptotic responses and participating in viral pathogenesis. NS2, which is a known nuclear protein, interacts with M1 and functions in the nuclear export of viral ribonucleoproteins. HA, hemagglutinin; NA, neuraminidase; M1, matrix protein 1; PB1, polymerase basic 1; PA, polymerase acidic protein; NS1, non-structural protein 1; ssRNA, single-stranded RNA.

through controllable surface charge and hydrophobicity of SeNPs in order to modulate cellular uptake, biodistribution and immunostimulation (53). In addition, mesoporous silica NPs can carry antiviral peptides, which help to control the release rate, protection against proteolytic degradation and suppression of binding to serum proteins (54). However, the following negative effects have been observed *in vivo* after acute exposure: Endothelial dysfunction, hemolysis and neurotoxicity (55).

Clinical investigations have demonstrated the antiviral potential of SiO_2-NPs as both a delivery system for small molecules with antiviral activity and for use in vaccines against viruses such as HIV (56). The antiviral success of SiO_2-NPs is largely attributed to their excellent biosafety profile, which includes good tolerability and minimal side effects (57).

Recombinant H1N1/A/California/4/09 influenza virus HA antigen, conjugated to nano-SiO_2 (as an adjuvant), has been used as a vaccine that stimulated the production of IFN-γ and reactivates T cells in an '*ex vivo* precision-cut lung slices' model (58). In this system, silica activates the NLR family pyrin domain containing 3 inflammasome that releases IL-1β and IL-18, which provides an important link between the innate and adaptive immune responses (59-61).

7. Zinc oxide nanoparticles (ZnO-NPs)

ZnO-NPs have the benefit of low toxicity and high UV absorption, making them a good candidate to be used in the biomedical field (62). ZnO-NPs are safe substances and they are easily absorbed by the body. Furthermore, they have demonstrated the ability to inhibit viral protease and polymerase (63). However, certain ZnO-NPs have low penetration and are unsuitable for heterogenous mixtures (64).

ZnO-NPs stimulate the innate and adaptive immune response through the Toll-like receptor 4 signaling pathway, which increases the early activation of NF-κB and triggers the increase of proinflammatory cytokines that keep viral pathogens in check, such as TNF-α, IL-1β and IL-6 (65). In addition, ZnO-NPs can absorb UV-vis light, dissociate water molecules and release Zn^{2+} ions, generating ROS (such as hydrogen peroxide, hydroxyl and superoxide radicals), which alters lipids, proteins, carbohydrates and viral DNA and ultimately leads to viral destruction (66). The release of Zn^{2+} ions specifically prevents viral infection by inactivating virus adsorption/entry, blocking uncoating, preventing replication, assembly and release during the viral life cycle and producing ROS (67). Furthermore, Zn^{2+} ion release causes numerous viruses to adopt zinc finger structures during their replication in the cells they infect, increasing the antiviral activity. For instance, the nucleocapsid protein of HIV-1 takes on a zinc finger conformation after ZnO-NP treatment, reducing the efficiency of the chaperone proteins responsible for rearranging nucleic acids into conformations that are thermodynamically stable for retro-transcription, thus highlighting the potential of zinc as an antiviral agent (68).

8. Selenium nanoparticles (SeNPs)

SeNPs have a high absorption rate, high biological activity and low toxicity, and can be directly absorbed by the human body and converted to organic selenium (69). However, certain SeNPs have poor stability and may be easily converted into gray-black elemental selenium, which may increase their toxicity and result in diminished activity (70). To improve formulation stability and targeted therapeutic effects, the surface of SeNPs could be modified with amino acids, proteins, polysaccharides, folic acid or hyaluronic acid, among others (71).

It has previously been reported that selenium (Na_2SeO_3) can suppress hepatitis B virus replication and transcription in liver cancer cells (72).

Li *et al* (73) observed that adding SeNPs to oseltamivir can increase the antiviral properties against the H1N1 influenza virus in MDCK cells by activating phosphorylation of p53 and Akt.

SeNPs regulate superoxide radicals, thereby possessing the ability to inactivate a virus. For instance, an evaluation carried out in Marc-145 cells infected with porcine reproductive and respiratory syndrome virus treated with SeNPs found a substantial reduction in the rate of apoptosis, as attributed to decreased ROS, lowered phosphorylation levels of c-Jun and degradation of both caspase-3 and poly ADP-ribose polymerase (74).

Furthermore, a study carried out with SeNPs plus amantadine showed reduced NA activity of the H1N1 influenza virus and inhibition of apoptosis in H1N1-infected MDCK cells (75,76). The authors attributed these effects to decreased levels of ROS via activation of the PI3K/Akt pathway, which negatively regulates the JNK pathway through apoptosis signal

Table I. NPs used to combat the influenza virus.

NP type	Size(s), nm	Potential applications	Advantages	Disadvantages	(Refs.)
AuNPs	a) ~12	a) Vector for a peptide vaccine (resulting in anti-M2e immunity against H1N1, H3N2 and H5N1-IAV)	Biocompatible and functionalized antigen carrier	Lack of anti-viral effect by themselves, need multivalent interactions for antiviral outcomes	a) (17-19,27)
	b) ~100	b) Proinflammatory (inducing antigen-specific T cell-mediated immunity)			b) (23)
PoGNPs	c) ~154	c) Antiviral (cleaving disulfide bonds of HA-IAV)			c) (24)
CuNPs	25-100	Antiviral (destruction of H1N1-IAV nucleoprotein and HA)	Low toxicity and long-term colloidal stability when they are smaller in size	Oxidation when exposed to airand unstable in	(32-34,38)
AgNPs	1-400	Antiviral (inhibition of viral penetration of the host cell by preventing binding to H1N1-IAV glycoproteins) Antiviral (inhibition of genome replication)	Inhibition of viral RNA synthesis through a specific interaction between the NPs and the viral genome	solution Particle diameters >800 nm are too toxic for use in *in vitro* assays	(42-44,47-49)
SiO_2-NPs	100-200	Antiviral and anti-inflammatory (strong inhibitory effect on NO, TNF-α and IL-1β against H5N1)	Carriers of antiviral peptides; help to control the release rate, protection against proteolytic degradation	High toxicity in *in vivo* models byacute exposure Endothelial dysfunction, hemolysis and neurotoxicity	(53-55,58)
ZnO-NPs	20-50	Antiviral (reduction of viral load of H1N1 by triggering an increase in proinflammatory cytokines)	Low toxicity and high UV absorption, able to inhibit viral protease and polymerase	Low penetration and unsuitable for heterogenous mixtures	(62-65)
SeNPs	~200	Antiviral (inhibition H1N1-induced apoptosis and reduction of neuraminidase activity when combined with amantadine)	High absorption rate, high biological activity and low toxicity; can be converted to organic selenium	Poor stability and easily converted into gray-black elemental selenium, which could increase toxicity and decrease effectiveness	(69-71,75,76)

NP, nanoparticle; HA, hemagglutinin; NO, nitric oxide; IAV, influenza A virus.

regulating kinase 1, as mediated by Bax and dependent on JNK (77). It should be noted that cells infected with the H1N1 influenza virus displayed an increase in viability after being treated with SeNPs plus amantadine (32.34 vs. 79.6%) (78).

9. IAV conserved sequences and NPs

The HA protein of IAV is an excellent target for the development of new treatments, largely because it stimulates the production of neutralizing antibodies during natural infection or vaccination. It should be noted that the HA2 subunit (stem domain) is more conserved compared to the HA1 subunit (globular head domain) (79). The conserved regions are very relevant as possible treatment targets, since effective antivirals can be designed on them (80). The use of NPs as antivirals could exert more targeted effects if the NPs are coupled to molecules capable of recognizing these targets. This has already been achieved for diagnostic purposes, to detect various viruses in samples, including influenza (81). Likewise, AuNPs have been designed to which molecules capable of acting as receptors for the influenza virus have been coupled, inhibiting its hemagglutinating capacity and thus its infectious capacity (45). These capabilities have been achieved with the successful coupling of the viral HA receptor glycans onto the NPs (82). However, it would also be relevant to use NPs coupled with broad-spectrum influenza virus neutralization antibodies as a means of recognition (83). The neutralization capacity of these antibodies has revealed the presence of epitopes that are conserved among various subtypes of influenza, both group 1 and group 2 of IAV (84) and even influenza B virus (IBV) (85). Therefore, the use of these antibodies would facilitate a specific action of the NPs on viruses or cells that express HA, a product of viral infection, on their membrane, with the great advantage of the broad recognition capacity of influenza subtypes.

M2 is a tetrameric membrane protein with pH-controlled selective proton channel activity, which promotes the entry of virus constituents into the cytosol of the infected cell. Specifically, a 54-amino-acid sequence within the M2 protein has previously been found to localize to the cytosol, where it has a key role in the assembly and release of viral particles by interacting with the M1 protein. In addition, M2e is highly conserved among various IAVs, making it an important antigenic target for developing a universal influenza vaccine (86). The coupling of M2e to the surface of AuNPs has shown to be very useful as an immunogen; it has been successfully tested in a murine model to avoid the lethal consequences of infection with influenza virus A/PR/8/34 (H1N1) (87), which has shown the relevance of the use of NPs as carriers of relevant antigens for possible universal vaccines.

10. Conclusions and future directions

In the present review, the antiviral activities of six NPs were described, which may serve as an aid for the development and manufacture of novel and safe biopharmaceuticals targeted against the influenza virus (Table I). NP medicines offer high storage and transport stability. However, it is necessary to optimize the biocompatibility of each medication and to consider their inhibitory effects on resistant viral strains (88). The following parameters should be carefully considered when designing nanomedicines: Particle composition, charge, size, surface functionalization, stability, morphology and cellular target (89). To date, only a small number of clinical trials have been conducted using metal NPs (90). Most of the data available have been acquired via *in silico*, *in vitro* and *in vivo* assays (91). This is largely due to the fact that metal NPs exhibit immunogenicity and inflammation after systemic administration, which causes damage to the cardiovascular system via oxidative stress, systemic inflammation, endothelial dysfunction, thrombosis and arrhythmia (92). Therefore, these limitations need to be considered and future research is warranted to address these limitations before safely incorporating metal NPs into future clinical trials.

Acknowledgements

Not applicable.

Funding

Financial support was provided by the Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT) [Ciencia de Frontera (grant no. 1715645 to JBM) and postdoctoral fellowship (2022-1) (grant no. 2306495 to CPRI)].

Availability of data and materials

Not applicable.

Authors' contributions

CPRI, MSS, DAON and JBM conducted the review of the literature and wrote the manuscript. CPRI, MSS, DAON and JBM reviewed, edited and provided expert opinion on the manuscript. All authors conceptualized the review paper and have read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Khan I, Saeed K and Khan I: Nanoparticles: Properties, applications and toxicities. Arab J Chem 12: 908-931, 2019.
2. Haleem A, Javaid M, Singh RP, Rab S and Suman R: Applications of nanotechnology in medical field: A brief review. Glob Health J 7: 70-77, 2023.
3. Wang L, Hu C and Shao L: The antimicrobial activity of nanoparticles: Present situation and prospects for the future. Int J Nanomedicine 12: 1227-1249, 2017.
4. Sekiya T, Ohno M, Nomura N, Handabile C, Shingai M, Jackson DC, Brown LE and Kida H: Selecting and using the appropriate influenza vaccine for each individual. Viruses 13: 971, 2021.

5. Sarkar J, Das S, Aich S, Bhattacharyya P and Acharya K: Antiviral potential of nanoparticles for the treatment of Coronavirus infections. J Trace Elem Med Biol 72: 126977, 2022.
6. Moreira EA, Yamauchi Y and Matthias P: How influenza virus uses host cell pathways during uncoating. Cells 10: 1722, 2021.
7. Krammer F, Smith GJD, Fouchier RAM, Peiris M, Kedzierska K, Doherty PC, Palese P, Shaw ML, Treanor J, Webster RG and García-Sastre A: Influenza. Nat Rev Dis Primer 4: 3, 2018.
8. To J and Torres J: Viroporins in the influenza virus. Cells 8: 654, 2019.
9. Park JE and Ryu Y: Transmissibility and severity of influenza virus by subtype. Infect Genet Evol 65: 288-292, 2018.
10. Hutchinson EC: Influenza virus. Trends Microbiol 26: 809-810, 2018.
11. Gaitonde DY, Moore FC and Morgan MK: Influenza: Diagnosis and treatment. Am Fam Physician 100: 751-758, 2019.
12. Centers for Disease Control and Prevention (CDC): Antiviral drugs for seasonal influenza. CDC, Atlanta, GA, 2022.
13. Javanian M, Barary M, Ghebrehewet S, Koppolu V, Vasigala V and Ebrahimpour S: A brief review of influenza virus infection. J Med Virol 93: 4638-4646, 2021.
14. Wieczorek K, Szutkowska B and Kierzek E: Anti-influenza strategies based on nanoparticle applications. Pathogens 9: 1020, 2020.
15. Ghaffari H, Tavakoli A, Moradi A, Tabarraei A, Bokharaei-Salim F, Zahmatkeshan M, Farahmand M, Javanmard D, Kiani SJ, Esghaei M, *et al*: Inhibition of H1N1 influenza virus infection by zinc oxide nanoparticles: Another emerging application of nanomedicine. J Biomed Sci 26: 70, 2019.
16. Kheirollahpour M, Mehrabi M, Dounighi NM, Mohammadi M and Masoudi A: Nanoparticles and vaccine development. Pharm Nanotechnol 8: 6-21, 2020.
17. Ielo I, Rando G, Giacobello F, Sfameni S, Castellano A, Galletta M, Drommi D, Rosace G and Plutino MR: Synthesis, chemical-physical characterization, and biomedical applications of functional gold nanoparticles: A review. Molecules 26: 5823, 2021.
18. Salazar-González JA, González-Ortega O and Rosales-Mendoza S: Gold nanoparticles and vaccine development. Expert Rev Vaccines 14: 1197-1211, 2015.
19. Bowman MC, Ballard TE, Ackerson CJ, Feldheim DL, Margolis DM and Melander C: Inhibition of HIV fusion with multivalent gold nanoparticles. J Am Chem Soc 130: 6896-6897, 2008.
20. Papp I, Sieben C, Ludwig K, Roskamp M, Böttcher C, Schlecht S, Herrmann A and Haag R: Inhibition of influenza virus infection by multivalent sialic-acid-functionalized gold nanoparticles. Small 6: 2900-2906, 2010.
21. Mikhailova EO: Gold nanoparticles: Biosynthesis and potential of biomedical application. J Funct Biomater 12: 70, 2021.
22. Li F, Huang Q, Zhou Z, Guan Q, Ye F, Huang B, Guo W and Liang XJ: Gold nanoparticles combat enveloped RNA virus by affecting organelle dynamics. Signal Transduct Target Ther 8: 285, 2023.
23. Wang C, Zhu W, Luo Y and Wang BZ: Gold nanoparticles conjugating recombinant influenza hemagglutinin trimers and flagellin enhanced mucosal cellular immunity. Nanomedicine 14: 1349-1360, 2018.
24. Kim J, Yeom M, Lee T, Kim HO, Na W, Kang A, Lim JW, Park G, Park C, Song D and Haam S: Porous gold nanoparticles for attenuating infectivity of influenza A virus. J Nanobiotechnology 18: 54, 2020.
25. Xia Q, Huang J, Feng Q, Chen X, Liu X, Li X, Zhang T, Xiao S, Li H, Zhong Z and Xiao K: Size- and cell type-dependent cellular uptake, cytotoxicity and in vivo distribution of gold nanoparticles. Int J Nanomedicine 14: 6957-6970, 2019.
26. Bimler L, Song AY, Le DT, Murphy Schafer A and Paust S: AuNP-M2e + sCpG vaccination of juvenile mice generates lifelong protective immunity to influenza A virus infection. Immun Ageing 16: 23, 2019.
27. Tao W, Hurst BL, Shakya AK, Uddin MJ, Ingrole RS, Hernandez-Sanabria M, Arya RP, Bimler L, Paust S, Tarbet EB and Gill HS: Consensus M2e peptide conjugated to gold nanoparticles confers protection against H1N1, H3N2 and H5N1 influenza A viruses. Antiviral Res 141: 62-72, 2017.
28. Tazaki T, Tabata K, Ainai A, Ohara Y, Kobayashi S, Ninomiya T, Orba Y, Mitomo H, Nakano T, Hasegawa H, *et al*: Shape-dependent adjuvanticity of nanoparticle-conjugated RNA adjuvants for intranasal inactivated influenza vaccines. RSC Adv 8: 16527-16536, 2018.
29. Aarreberg LD, Esser-Nobis K, Driscoll C, Shuvarikov A, Roby JA and Gale M Jr: Interleukin-1β induces mtDNA release to activate innate immune signaling via cGAS-STING. Mol Cell 74: 801-815.e6, 2019.
30. Johnston SC, Lin KL, Connor JH, Ruthel G, Goff A and Hensley LE: In vitro inhibition of monkeypox virus production and spread by interferon-β. Virol J 9: 5, 2012.
31. Puthothu B, Bierbaum S, Kopp MV, Forster J, Heinze J, Weckmann M, Krueger M and Heinzmann A: Association of TNF-alpha with severe respiratory syncytial virus infection and bronchial asthma. Pediatr Allergy Immunol 20: 157-163, 2009.
32. Sun Y, Jiang X, Liu Y, Liu D, Chen C, Lu C, Zhuang S, Kumar A and Liu J: Recent advances in Cu(II)/Cu(I)-MOFs based nano-platforms for developing new nano-medicines. J Inorg Biochem 225: 111599, 2021.
33. Grigore ME, Biscu ER, Holban AM, Gestal MC and Grumezescu AM: Methods of synthesis, properties and biomedical applications of CuO nanoparticles. Pharmaceuticals (Basel) 9: 75, 2016.
34. Chang YN, Zhang M, Xia L, Zhang J and Xing G: The toxic effects and mechanisms of CuO and ZnO nanoparticles. Materials (Basel) 5: 2850-2871, 2012.
35. Ali ZI, Ghazy OA, Meligi G, Saleh HH and Bekhit M: Radiation-induced synthesis of copper/poly(vinyl alcohol) nanocomposites and their catalytic activity. Adv Polym Technol 37: 365-375, 2018.
36. Ermini ML and Voliani V: Antimicrobial nano-agents: The copper age. ACS Nano 15: 6008-6029, 2021.
37. Applerot G, Lellouche J, Lipovsky A, Nitzan Y, Lubart R, Gedanken A and Banin E: Understanding the antibacterial mechanism of CuO nanoparticles: Revealing the route of induced oxidative stress. Small 8: 3326-3337, 2012.
38. Ha T, Pham TTM, Kim M, Kim YH, Park JH, Seo JH, Kim KM and Ha E: Antiviral activities of high energy E-beam induced copper nanoparticles against H1N1 influenza virus. Nanomaterials (Basel) 12: 268, 2022.
39. Puchkova LV, Kiseleva IV, Polishchuk EV, Broggini M and Ilyechova EY: The crossroads between host copper metabolism and influenza infection. Int J Mol Sci 22: 5498, 2021.
40. Sportelli MC, Izzi M, Kukushkina EA, Hossain SI, Picca RA, Ditaranto N and Cioffi N: Can nanotechnology and materials science help the fight against SARS-CoV-2? Nanomaterials (Basel) 10: 802, 2020.
41. Gurunathan S, Park JH, Han JW and Kim JH: Comparative assessment of the apoptotic potential of silver nanoparticles synthesized by Bacillus tequilensis and Calocybe indica in MDA-MB-231 human breast cancer cells: Targeting p53 for anticancer therapy. Int J Nanomedicine 10: 4203-4223, 2015.
42. Lu L, Sun RWY, Chen R, Hui CK, Ho CM, Luk JM, Lau GK and Che CM: Silver nanoparticles inhibit hepatitis B virus replication. Antivir Ther 13: 253-262, 2008.
43. Galdiero S, Falanga A, Vitiello M, Cantisani M, Marra V and Galdiero M: Silver nanoparticles as potential antiviral agents. Molecules 16: 8894-8918, 2011.
44. Mori Y, Ono T, Miyahira Y, Nguyen VQ, Matsui T and Ishihara M: Antiviral activity of silver nanoparticle/chitosan composites against H1N1 influenza A virus. Nanoscale Res Lett 8: 93, 2013.
45. Feng F, Sakoda Y, Ohyanagi T, Nagahori N, Shibuya H, Okamastu M, Miura N, Kida H and Nishimura S: Novel thiosialosides tethered to metal nanoparticles as potent influenza A virus haemagglutinin blockers. Antivir Chem Chemother 23: 59-65, 2013.
46. Morris D, Ansar M, Speshock J, Ivanciuc T, Qu Y, Casola A and Garofalo R: Antiviral and immunomodulatory activity of silver nanoparticles in experimental RSV infection. Viruses 11: 732, 2019.
47. Ratan ZA, Mashrur FR, Chhoan AP, Shahriar SM, Haidere MF, Runa NJ, Kim S, Kweon DH, Hosseinzadeh H and Cho JY: Silver nanoparticles as potential antiviral agents. Pharmaceutics 13: 2034, 2021.
48. Meineke R, Rimmelzwaan G and Elbahesh H: Influenza virus infections and cellular kinases. Viruses 11: 171, 2019.
49. Trefry JC and Wooley DP: Silver nanoparticles inhibit vaccinia virus infection by preventing viral entry through a macropinocytosis-dependent mechanism. J Biomed Nanotechnol 9: 1624-1635, 2013.
50. Tripathi S, White MR and Hartshorn KL: The amazing innate immune response to influenza A virus infection. Innate Immun 21: 73-98, 2015.

51. Xiang D, Zheng Y, Duan W, Li X, Yin J, Shigdar S, O'Connor ML, Marappan M, Zhao X, Miao Y, *et al*: Inhibition of A/Human/Hubei/3/2005 (H3N2) influenza virus infection by silver nanoparticles in vitro and in vivo. Int J Nanomedicine 8: 4103-4113, 2013.
52. Li Y, Lin Z, Zhao M, Xu T, Wang C, Hua L, Wang H, Xia H and Zhu B: Silver nanoparticle based codelivery of oseltamivir to inhibit the activity of the H1N1 influenza virus through ROS-mediated signaling pathways. ACS Appl Mater Interfaces 8: 24385-24393, 2016.
53. Yang Y, Zhang M, Song H and Yu C: Silica-based nanoparticles for biomedical applications: From nanocarriers to biomodulators. Acc Chem Res 53: 1545-1556, 2020.
54. Häffner SM, Parra-Ortiz E, Browning KL, Jørgensen E, Skoda MWA, Montis C, Li X, Berti D, Zhao D and Malmsten M: Membrane interactions of virus-like mesoporous silica nanoparticles. ACS Nano 15: 6787-6800, 2021.
55. Maurer-Jones MA, Lin YS and Haynes CL: Functional assessment of metal oxide nanoparticle toxicity in immune cells. ACS Nano 4: 3363-3373, 2010.
56. Cheng K, El-Boubbou K and Landry CC: Binding of HIV-1 gp120 glycoprotein to silica nanoparticles modified with CD4 glycoprotein and CD4 peptide fragments. ACS Appl Mater Interfaces 4: 235-243, 2012.
57. Tng DJH and Low JGH: Current status of silica-based nanoparticles as therapeutics and its potential as therapies against viruses. Antiviral Res 210: 105488, 2023.
58. Neuhaus V, Schwarz K, Klee A, Seehase S, Förster C, Pfennig O, Jonigk D, Fieguth HG, Koch W, Warnecke G, *et al*: Functional testing of an inhalable nanoparticle based influenza vaccine using a human precision cut lung slice technique. PLoS One 8: e71728, 2013.
59. Yazdi AS, Guarda G, Riteau N, Drexler SK, Tardivel A, Couillin I and Tschopp J: Nanoparticles activate the NLR pyrin domain containing 3 (Nlrp3) inflammasome and cause pulmonary inflammation through release of IL-1α and IL-1β. Proc Natl Acad Sci USA 107: 19449-19454, 2010.
60. AbouAitah K, Swiderska-Sroda A, Kandeil A, Salman AMM, Wojnarowicz J, Ali MA, Opalinska A, Gierlotka S, Ciach T and Lojkowski W: Virucidal action against avian influenza H5N1 virus and immunomodulatory effects of nanoformulations consisting of mesoporous silica nanoparticles loaded with natural prodrugs. Int J Nanomedicine 15: 5181-5202, 2020.
61. Wang H, Chen L, Li R, Lv C, Xu Y and Xiong Y: Polydopamine-coated mesoporous silica nanoparticles co-loaded with Ziyuglycoside I and Oseltamivir for synergistic treatment of viral pneumonia. Int J Pharm 645: 123412, 2023.
62. Lakshmipriya T and Gopinath SCB: 1-Introduction to nanoparticles and analytical devices. In: Nanoparticles in Analytical and Medical Devices. Gopinath SCB and Gang F (eds.) Elsevier, pp1-29, 2021.
63. Attia GH, Moemen YS, Youns M, Ibrahim AM, Abdou R and El Raey MA: Antiviral zinc oxide nanoparticles mediated by hesperidin and in silico comparison study between antiviral phenolics as anti-SARS-CoV-2. Colloids Surf B Biointerfaces 203: 111724, 2021.
64. Singh TA, Sharma A, Tejwan N, Ghosh N, Das J and Sil PC: A state of the art review on the synthesis, antibacterial, antioxidant, antidiabetic and tissue regeneration activities of zinc oxide nanoparticles. Adv Colloid Interface Sci 295: 102495, 2021.
65. Poon WL, Alenius H, Ndika J, Fortino V, Kolhinen V, Meščeriakovas A, Wang M, Greco D, Lähde A, Jokiniemi J, *et al*: Nano-sized zinc oxide and silver, but not titanium dioxide, induce innate and adaptive immunity and antiviral response in differentiated THP-1 cells. Nanotoxicology 11: 936-951, 2017.
66. Mandal AK, Katuwal S, Tettey F, Gupta A, Bhattarai S, Jaisi S, Bhandari DP, Shah AK, Bhattarai N and Parajuli N: Current research on zinc oxide nanoparticles: Synthesis, Characterization, and biomedical applications. Nanomaterials (Basel) 12: 3066, 2022.
67. te Velthuis AJW, van den Worm SHE, Sims AC, Baric RS, Snijder EJ and van Hemert MJ: Zn(2+) inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. PLoS Pathog 6: e1001176, 2010.
68. Imani SM, Ladouceur L, Marshall T, Maclachlan R, Soleymani L and Didar TF: Antimicrobial nanomaterials and coatings: Current mechanisms and future perspectives to control the spread of viruses including SARS-CoV-2. ACS Nano 14: 12341-12369, 2020.
69. Shahabadi N, Zendehcheshm S and Khademi F: Selenium nanoparticles: Synthesis, in-vitro cytotoxicity, antioxidant activity and interaction studies with ct-DNA and HSA, HHb and Cyt c serum proteins. Biotechnol Rep (Amst) 30: e00615, 2021.
70. Zhang T, Qi M, Wu Q, Xiang P, Tang D and Li Q: Recent research progress on the synthesis and biological effects of selenium nanoparticles. Front Nutr 10: 1183487, 2023.
71. Ferro C, Florindo HF and Santos HA: Selenium nanoparticles for biomedical applications: From development and characterization to therapeutics. Adv Healthc Mater 10: 2100598, 2021.
72. Cheng Z, Zhi X, Sun G, Guo W, Huang Y, Sun W, Tian X, Zhao F and Hu K: Sodium selenite suppresses hepatitis B virus transcription and replication in human hepatoma cell lines. J Med Virol 88: 653-663, 2016.
73. Li Y, Lin Z, Guo M, Xia Y, Zhao M, Wang C, Xu T, Chen T and Zhu B: Inhibitory activity of selenium nanoparticles functionalized with oseltamivir on H1N1 influenza virus. Int J Nanomedicine 12: 5733-5743, 2017.
74. Kopel J, Fralick J and Reid TW: The potential antiviral effects of selenium nanoparticles and coated surfaces. Antibiotics (Basel) 11: 1683, 2022.
75. Liu X, Chen D, Su J, Zheng R, Ning Z, Zhao M, Zhu B and Li Y: Selenium nanoparticles inhibited H1N1 influenza virus-induced apoptosis by ROS-mediated signaling pathways. RSC Adv 12: 3862-3870, 2022.
76. Xu T, Lai J, Su J, Chen D, Zhao M, Li Y and Zhu B: Inhibition of H3N2 influenza virus induced apoptosis by selenium nanoparticles with chitosan through ROS-mediated signaling pathways. ACS Omega 8: 8473-8480, 2023.
77. Banoth B and Cassel SL: Mitochondria in innate immune signaling. Transl Res 202: 52-68, 2018.
78. Li Y, Lin Z, Guo M, Zhao M, Xia Y, Wang C, Xu T and Zhu B: Inhibition of H1N1 influenza virus-induced apoptosis by functionalized selenium nanoparticles with amantadine through ROS-mediated AKT signaling pathways. Int J Nanomedicine 13: 2005-2016, 2018.
79. Khanna M, Sharma S, Kumar B and Rajput R: Protective immunity based on the conserved hemagglutinin stalk domain and its prospects for universal influenza vaccine development. Biomed Res Int 2014: 546274, 2014.
80. Cilento ME, Kirby KA and Sarafianos SG: Avoiding drug resistance in HIV reverse transcriptase. Chem Rev 121: 3271-3296, 2021.
81. Draz MS and Shafiee H: Applications of gold nanoparticles in virus detection. Theranostics 8: 1985-2017, 2018.
82. Zhang Z, Schepens B, Nuhn L, Saelens X, Schotsaert M, Callewaert N, De Rycke R, Zhang Q, Moins S, Benali S, *et al*: Influenza-binding sialylated polymer coated gold nanoparticles prepared via RAFT polymerization and reductive amination. Chem Commun (Camb) 52: 3352-3355, 2016.
83. Corti D, Cameroni E, Guarino B, Kallewaard NL, Zhu Q and Lanzavecchia A: Tackling influenza with broadly neutralizing antibodies. Curr Opin Virol 24: 60-69, 2017.
84. Joyce MG, Wheatley AK, Thomas PV, Chuang GY, Soto C, Bailer RT, Druz A, Georgiev IS, Gillespie RA, Kanekiyo M, *et al*: Vaccine-induced antibodies that neutralize group 1 and group 2 influenza A viruses. Cell 166: 609-623, 2016.
85. Dreyfus C, Laursen NS, Kwaks T, Zuijdgeest D, Khayat R, Ekiert DC, Lee JH, Metlagel Z, Bujny MV, Jongeneelen M, *et al*: Highly conserved protective epitopes on influenza B viruses. Science 337: 1343-1348, 2012.
86. Kang SM, Song JM and Compans RW: Novel vaccines against influenza viruses. Virus Res 162: 31-38, 2011.
87. Nesovic LD, Roach CJ, Joshi G and Gill HS: Delivery of gold nanoparticle-conjugated M2e influenza vaccine in mice using coated microneedles. Biomater Sci 11: 5859-5871, 2023.
88. Chen L and Liang J: An overview of functional nanoparticles as novel emerging antiviral therapeutic agents. Mater Sci Eng C Mater Biol Appl 112: 110924, 2020.
89. Nagy A and Robbins NL: The hurdles of nanotoxicity in transplant nanomedicine. Nanomedicine (Lond) 14: 2749-2762, 2019.
90. Tagami T and Ozeki T: Recent trends in clinical trials related to carrier-based drugs. J Pharm Sci 106: 2219-2226, 2017.
91. Gopal J, Muthu M and Sivanesan I: A comprehensive survey on the expediated anti-COVID-19 options enabled by metal complexes-tasks and trials. Molecules 28: 3354, 2023.
92. Abukabda AB, Stapleton PA and Nurkiewicz TR: Metal nanomaterial toxicity variations within the vascular system. Curr Environ Health Rep 3: 379-391, 2016.