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Role of metallic nanoparticles to control Avian Influenza Virus in poultry birds

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Abstract

As known from the history of the pandemic, the Avian Influenza virus can potentially be one of the deadliest viruses of poultry. The various strains of the Avian Influenza virus also affect different animal species, including humans. Due to its continually changing genome, this virus has become more resistant to current antiviral medications and vaccinations. The development of new treatments and therapies is therefore desperately needed. The ideal cure has yet to be discovered, even if a new generation of universal vaccinations or anti-influenza medications are being produced. As a result, new control methods must be created. In veterinary medicine, nanoparticle research has gained a lot of interest in the past 20 years as a viable platform that has shown great effectiveness in substituting antiviral medications and conventional techniques. Their peculiar and unique physiochemical properties, including size, shape, charge, and surface area, enable the nanoparticles to interact and penetrate the viral capsid and host cells. Various nanoparticles such as silver, copper, gold, and zinc have demonstrated potential antiviral activity by disrupting viral coats, inhibiting viral replication, and modulate immune response. So, this review article highlights the important methods to synthesize nanoparticles and their specific antiviral mode of action against the Avian Influenza virus.

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1. Introduction

The Avian Influenza virus (AIV) is a lethal virus present globally and has caused AI disease in poultry birds, including chickens and turkeys. This virus also has zoonotic importance as it has also impacted human beings. Several cases have been reported in past decades (Wong and Yuen 2006; Philippon et al. 2020). The most important among all influenza viruses is AIV A which is divided into subtypes, based on its two surface proteins; hemagglutinin (H) and neuraminidase (N). AIV A is divided into two categories based on its virulence - highly pathogenic AI (HPAI) virus and low pathogenic AI (LPAI) virus (Alexander 2000). The HPAI virus group includes H5N1, H5N2, H5N3, H5N6, H5N8, and H7N9 and LPAI viruses are H9N2, H7N3, H6N1, and H7N7. The HPAI viruses have zoonotic importance because they are transferred to humans and cause disease, especially H5 strains. A mortality rate of > 50% has been reported in humans (Wong and Yuen 2006; Kencana et al. 2023).

AIV infection is characterized by one or more signs such as deficiency of energy, decreased appetite, soft-shelled or misshaped shell eggs, declined egg production, nasal discharge, sneezing, coughing, incoordination, diarrhea, inflammation of head, eyelids, comb, wattles, and hocks, purple staining of the wattles, combs, and legs and sometimes sudden death without any clinical sign (Graziosi et al. 2024). The highly virulent species H5N1 had 1,645 outbursts worldwide. From 1996 to 2003, approximately 43 million birds and from 2004-2007, almost 250 million birds were destroyed (Gashaw 2020). During outbreaks of HPAI in the United States of America (USA) from 2014 to 2015, 50 million birds were disposed and it was also known as the worst animal disaster in US history. According to the US Animal and Plant Health Service, almost 879 million dollars were spent on these outbreaks (Johnson et al. 2016; Seeger et al. 2021). Brazil, a major exporter of poultry in the world, witnessed its first HPAI

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(H5N1) case in 2023 (Reischak et al. 2023).

The AIV also had several outbreaks in European countries. For instance, in Italy, in 1999, 16 million birds were culled due to the H7N1 strain at the financial cost of 507 million euros (Sartore et al. 2010; Backer et al. 2015). In the 2003 epidemic of the Netherlands, 30 million birds were killed (Stegeman et al. 2004). The most devasting epidemic that ever occurred in Europe from 2020 to 2021 was due to the HPAI subtype, which affected almost 22.2 million birds in 28 European countries (European Food Safety Authority et al. 2021). From 2005 to 2006, Turkey's HPAI outbreak cost almost 28 million euros (Aral et al. 2010). Similarly, in Asian countries in 2004, there was an outbreak in China that caused a huge loss to the economy and approximately 40 million birds died (Liu et al. 2014). The outbreaks of LPAI from 2004 to 2005 in Pakistan caused economic losses of 35.48 million dollars (Ayaz et al. 2010). Furthermore, 53 laboratories confirmed that 31 human deaths were caused by avian influenza H5N1 species (Cui et al. 2019).

Several antiviral drugs have been used over the years to control AIV. These antiviral drugs are divided into several types based on mechanism of action such as neuraminidase inhibitors, M2 ion channel blockers, RNA polymerase inhibitors (Mtambo et al. 2021; Sarker et al. 2022) inosine monophosphate (IMP) dehydrogenase inhibitors (De Clercq 2007), and Interferons (Meng et al. 2011). Drugs that are included in neuraminidase inhibitors are oseltamivir (Kongkamnerd et al. 2012), zanamivir (Kode et al. 2019), peramivir (McLaughlin et al. 2015), and A-315675 (Abed et al. 2008). M2 ion channel blockers are amantadine (Lashkov et al. 2023) and rimantadine (Musharrafieh et al. 2020). The RNA polymerase inhibitors include pyrazine (Furuta et al. 2017), flutamide (Pagadala 2019), pyrimidine (Abu-Zaied et al. 2021), and pyrimidinyl acyl-thiourea (Torrence 2007). Ribavirin and viramidine belong to IMP dehydrogenase inhibitors (Leyssen et al. 2008). Interferons are used in combination with ribavirin against AIV (De Clercq and Neyts 2007). These antivirals were more effective when researchers used them in combination to reduce the infection rate. For example, oseltamivir, when mixed with ribavirin (Ilyushina et al. 2008), has shown good results. Similarly, interferon, rimantadine, and ribavirin triplet combinations have shown better results in reducing the intensity of infection (Hayden 2013). However, excessive, frequent, and under-dosing of these antiviral drugs has led to the development of resistance. Table 1 shows different antiviral drugs, their mode of action, resistance, year of resistance, and level of resistance.

Due to the development of resistance to antiviral drugs, scientists used vaccines to control AIV. Vaccinations whose primary goal is to prevent influenza disease are categorized into 4 types: inactivated whole influenza virus, *in vivo* articulated HA proteins, *in vitro* articulated HA proteins, and nucleic acid vaccines (Swayne 2009; Abbas et al. 2024). More than 113 billion doses of these vaccines were used from 2002 to 2010. Among all vaccines, 95.5% of vaccines were inactivated, and 4.5% were live R-virus vaccines (Swayne 2012). China, Egypt, Indonesia, Vietnam, and Hong Kong utilized vaccination up to 99%, while Russia, North Korea, Sudan, Netherlands, Mongolia, and Pakistan used less than 1% of AIV vaccines. AI vaccines are required to be re-evaluated over time to determine their efficacy against the strains that are found presently in the field (Swayne and Kapczynski 2016).

AIV has several dimensions, as mentioned above, it impacts the global economy on a vast level. The virus has many strains that are now going through genetic mutations that have rendered already produced vaccines ineffective. Another factor that is involved in the AIV vaccine failure is the genetic drift. To overcome the resistance, researchers have shifted to alternatives to eliminate AIV effectively. Nowadays, many alternatives, including micronutrients, vitamins, minerals, herbs, plant extracts, essential oils, and nanoparticles have been used against AIV (Munir et al. 2023; Mwafy et al. 2023). Among all, nanoparticles (NPs) have just attracted much attention as antiviral agents. In drug delivery, personal hygiene, and industry, NPs have useful benefits (Ibrahim et al. 2024). Their new properties such as small dimensions and large surface area improve the stability and solubility of therapeutic agents and improve their activity in target treatment (El-Qabbany et al. 2024). The effects of influenza on the poultry industry are the central theme of this study. This review article gives a detailed discussion of different NPs, their synthesis, and their mode of action as antivirals against AIV.

2. Nanomedicine and nanoparticles

In modern-day science, nanomedicine is rapidly expanding, and nanotechnology is used to generate nanostructures for targeted and controlled release of nano drugs at particular sites. NPs have diameters in the range of 1 to 100nm (Khan et al. 2019; Mehwish 2023), and due to different global perspectives, no single universal definition of NPs exists. The Environmental Protection Agency (EPA) states that "NPs can exhibit unique properties dissimilar than the equivalent chemical compound in a larger dimension" (Ravindran et al. 2017). According to the US Food and Drug Administration (USFDA), "materials that have

Table 1 Drugs, mode of action, and their resistance by AI Virus

Class	Salt	Mode of action	Resistance	Level of resistance	Year of resistance	Reference
NA Inhibitor	Oseltamivir	Blocking the neuraminidase enzyme on the surface of the virus	Virus Mutation	R292K, E119G, in H5N1 due to H274Y mutation in the N gene	1999-2002	(Torrence 2007; Kim et al. 2018)
NA Inhibitor	Zanamivir	Blocking the neuraminidase enzyme on the surface of the virus	Virus Mutation	E119D, E119G, T325N	2009	(Yates et al. 2016)
RNA polymerase inhibitor	Pyrazine	Binds directly to viral NP	___	___	___	(Li et al. 2023)
IMP dehydrogenase inhibitor	Ribavirin	Halt the synthesis and capping of the viral RNA and mRNA	___	___	___	(Bi et al. 2016)
M2 ion channel blocker	Amantadine	Blocks the release of H+ ions inside endosomes	Virus mutation	Glutamic acid substituted on glycine 34 of M2 protein	2000-2004	(Hay 1989; Ilyushina et al. 2005)

at least one dimension in the range of ~ 1 to 100 nm and exhibit dimension-dependent phenomena constitute NPs" (Jeevanandam et al. 2018; Abbas et al. 2024).

NPs exhibit different properties in terms of their size in nanometers, which facilitates their diffusion, larger surface area for effective drug delivery, and modified shape to increase their therapeutic effects (Zein et al. 2020). Because of their particular properties, NPs have medicinal properties and are used as therapeutic agents. They have antioxidants, antibacterials, antivirals, antifungal, and antiparasitic properties (Khan et al. 2023; Mustafa et al. 2024). Other than this, they are used for effective and targeted drug delivery, as antidepressants in nanomedicine, immunosuppressant during organ transplants, and immuno-modulators during chronic diseases (Asghar et al. 2024; Hayat et al. 2024). Humans had already been exposed to NPs around 4000 years ago when ancient Egyptians processed ~5 nm diameter lead sulphide for hair dye (Walter et al. 2006). Because of their exceptional properties that make them more therapeutically effective, NPs have been used as potential candidates for the treatment of viral infections. The nanomaterials, most notably silver and gold, play both roles of effective drug-delivery vehicles as well as direct antiviral agents interfering with viral infectivity (Azam et al. 2023; Akhreim et al. 2024). The nanosized nature allows the delivery of site-specific distribution and cellular internalization, addressing deficiencies of traditional antiviral drugs such as limited solubility and bioavailability. Additionally, because of their extensive surface area, they can be functionalized with antiviral medication that improves treatment and interaction with the pathogen and reduces the possible side effects (Bahrun et al., 2025).

3. Methods used for NPs synthesis

NPs are produced by two important methods that include top-down and bottom-up approaches (Ahmad et al. 2016). These methods vary primarily in their initial material and the mechanism through which NPs are modelled. The top-down approach starts with bulk material and breaks it down into nanosized particles, while the bottom-up approach assembles NPs from atoms or molecules, allowing more detailed control over size, shape, and surface properties (Abid et al. 2022). The bottom-up approach is centered on the principle of building NPs from the atomic or molecular level, also known as the 'wet method' because the procedure is passed out in a liquid-phase environment (Thiruvengadathan et al. 2013; Ayuk et al. 2017). In this method, ions, atoms, or molecules go through controlled chemical reactions that take the lead to the nucleation and growth of NPs. Joint synthetic techniques falling into this grouping include block copolymer synthesis, which allows the development of NPs with well-defined shapes and sizes. Newly, microbial production has gained status as an eco-friendly and workable bottom-up procedure that uses fungi, bacteria, or plant extracts to produce NPs (Annamalai et al. 2021).

However, the top-down method entails physically or mechanically shrinking bulk material to the nanoscale (Arole and Munde 2014). This method is usually employed when exact control over shape is less important. These methods include laser ablation, which uses high-energy lasers to vaporize solid marks into NPs (Pustovalov 2025); milling, where bulk material is crushed into fine particles (El-Eskandarany et al. 2021), and spark ablation, which creates NPs by electrical discharges (Kohut et al. 2017). Though efficient, top-down methods may create NPs with surface weaknesses or irregular morphology. After the formation, the synthesized NPs are typically isolated and transferred for further applications. The isolation of NPs involves centrifugation, filtration, and washing to remove unnecessary substances, impurities, and compounds. The obtained NPs are then transferred to another media depending on their specific use such as drug delivery and catalysis. Various methods for the synthesis of NPs are shown in the Fig. 1.

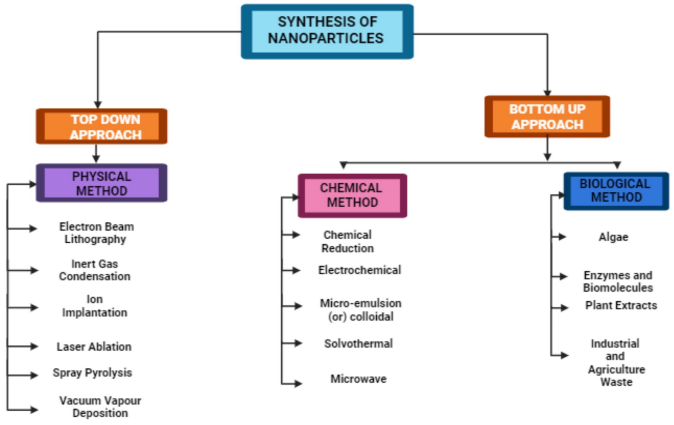

***Fig. 1**: Various methods used for synthesis of metallic NPs*

4. NPs as antiviral agents

Viral diseases are the most challenging task to deal with, due to the rapid mutation of viruses most of the antiviral drugs are not effective due to the development of resistance (Irwin et al. 2016). Viral diseases can also be prevented by the use of vaccines but their use is also limited because of mutation in the viral gene into a different strain (Milovanovic et al. 2017). Diseases such as AIV, Newcastle Disease, Fowl Pox, and many more that affect poultry are viral in nature (Mustafa et al. 2023). Not only poultry suffer from viral diseases, but in humans 60% of diseases have roots in viral agents (Hussain et al. 2022; Kausar et al. 2023). To cure humans and for food safety, the control of these viral diseases is inevitable. NPs inhibit the virus attachment and entry into the cell by binding with cell membrane glycoproteins (Ermini and Voliani 2021; Ali et al. 2024), but predominantly they generate ROS which disgraces the viral proteins and genome (Applerot et al. 2012; Rukh et al. 2024). Specifically, silver NPs effectively inhibited HIV-1 and HIV-1/2 when they were coated on polyurethane condoms (Mohammed et al. 2012). Ag NPs have shown efficacy against the H3N2 influenza virus (Xiang et al. 2013). Graphene oxide inactivated the endemic GIT AIV H9N2 (Song et al. 2015). Zinc oxide NPs showed antiviral activity against herpes simplex virus type 1 (HSV-1) when they were coated on polyethylene glycol (Burman et al. 2013; Anjum et al. 2023). They inhibit the genomic copy numbers of HSV-1 at the rate of ~ 92% and also show efficacy in the reduction of virus titer (Tavakoli et al. 2018; Maqsood et al. 2023). The usage of NPs will have a high efficacy rate in the treatment and may play an important role in the eradication of infectious diseases. NPs enhance the effect of antiviral drugs and also increase their half-life (Milovanovic et al. 2017). NPs' antioxidant effects in reducing AI inflammatory responses and viral infections are becoming increasingly prominent within studies. Mesoporous silica NPs have viricidal effects against H5N1 via virus inactivation and inflammation reduction of pro-inflammatory cytokines. Selenium NPs during H1N1 infection bypass inflammation and cell death, causing potentially increased antioxidant defense and

minimally reduced tissue damage (Iqbal et al. 2024). Several strains of influenza are efficiently blocked by anionic gold NPs, possibly due to host cell antioxidant defenses. The multi-strain activity of gold and Ag NPs indicates that they can be used as broad-spectrum antivirals and are suitable for preparing against pandemics, particularly for drug-resistant strains (Ielo et al. 2021). However, safety tests and long-term consequences are essential before being applied clinically (Altaf et al. 2024). The antiviral mechanism of NPs is given in Fig. 2.

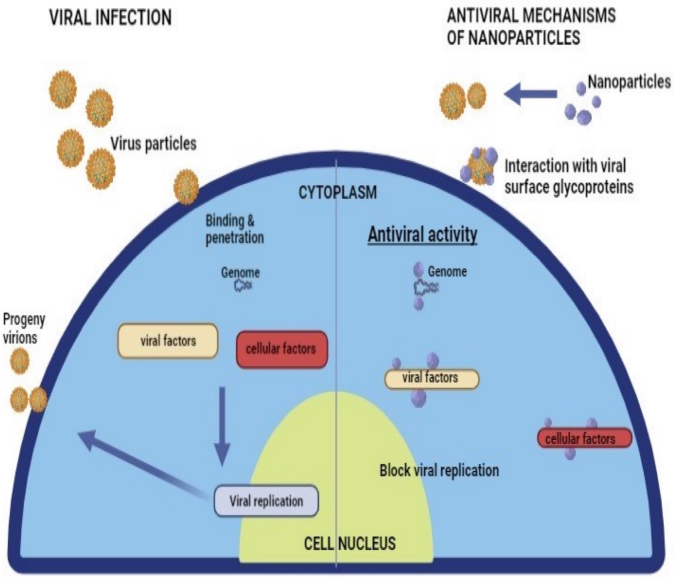

***Fig. 2**: Antiviral mechanism of action of various NPs against infectious viruses*

5. NPs as an antiviral against the AI virus

Nanotechnology emerged as a promising field in human and veterinary medicines. NPs have shown great efficacy as antivirals, especially against the AIV. For instance, gold NPs (Au NPs) have multivalent interactions as they can bind the HA protein of AIV, inhibiting the attachment of the virus to the cellular surface and also interacting with viral genome replication, which results in inhibition (Mikhailova 2021). Now, another form of gold nanoparticle, the porous gold NPs (PG NPs), have higher efficacy than Au NPs for the reason that PG NPs have high thermal stability (Kim et al. 2020). Silver NPs (Ag NPs) used along with an antiviral drug oseltamivir, against whom resistance has been developed, resulted in the inhibition of infection caused by the H1N1 strain (Li et al. 2016). Copper NPs (Cu NPs) inactivated the H1N1 strain of AIV in an assay in which nucleoprotein and HA were undetectable after 30 minutes of treatment (Puchkova et al. 2021; Ha et al. 2022; El-Hamaky et al. 2023). Selenium NPs (Se NPs), used along with the antiviral drug amantadine, reduce the NA activity of H1N1 AIV and inhibit the apoptosis in cells that are infected by H1N1 (Liu et al. 2022). Epigallocatechin gallate (EGCG) combined with zinc sulfate and Ag NPs destroy the H5N1 strain, probably due to the inhibition of polymerase, protease, HA, and NA activity (Saadh and Aldalaen 2021; Aslam et al. 2023). The same combination also inhibits the H9N2 strain (Saadh et al. 2021). When zirconia NPs (ZrO2 NPs) are charged positively, they restrain the inflammatory activity of cytokines induced by infection of the H5N1 strain and boost the innate immunity in early infection (Huo et al. 2020). NPs have played an effective role in treating AI strains either directly or indirectly with combinations. Table 2 gives some of the important NPs and their doses against various strains of AVI.

6. Limitations

Although NPs have shown efficacy against the AI virus but there have been concerns about their use as they exhibit an immunogenicity response (Abukabda et al. 2016; Nooraei et al. 2021), and when used systemically, they cause inflammation, which further affects the cardiovascular system because of oxidative stress, endothelial dysfunction, systemic inflammation, arrhythmia, and thrombosis (Shi et al. 2023). Moreover, the higher costs and very complex mechanism of synthesis of NPs limit their use in the poultry sector, especially in low-income settings (Ying et al. 2022). Furthermore, they may accumulate in the tissues of broiler chicken, which can cause toxicity and disturb the food chain, especially when humans consume the poultry (Kulak et al. 2018). Additionally, a lack of *in vivo* studies and insufficient clinical trials on poultry birds make it tough to fully understand their distribution, pharmacokinetics, and immune reactions. Higher authorities and regulatory bodies have not given proper guidelines about their dosage to use them in poultry birds. However, more research is required for their accurate and precise administration to overcome viral diseases, especially AIV in Poultry.

7. Future prospects

Future research on metallic NPs against AIV should pay attention to developing targeted delivery methods and the production of nano-vaccines to enhance their efficacy and immune responses in poultry. Nano-based biosensors may also be used for early detection to overcome the outbreak. In the future to get rid of tissue and environmental toxicity issues, NPs of plant origin should be

Table 2 Various NPs and their doses against Avian Influenza virus strains

Nanoparticle	Source	Dose	Method	Strain of AIV	Reference
Selenium NPs	Ascorbic acid + Na_2SO_3	1 mg/kg	Chemical	H5N1	(Yehia et al. 2022)
Epigallocatechin gallate (EGCG) + silver and zinc sulphate NPs	EGCG solution + Silver nitrate	50µM / 1.3ml/kg ($ZnSO_4$)	Chemical	H9N2	(Saadh et al. 2021)
AgNPs	Noble elements	20-0.0002 µg/ml	Biological	H1N1	(Naumenko et al. 2023)
ZnO-NPs	Zinc oxide powder	75 µg/ml	Physical	H1N1	(Ghaffari et al. 2019)
Porous Gold NPs	HAuCl4 + Anilinism ion	0.2mg/ml	Surfactant-free emulsion	H1N1, H3N2, and H9N2	(Kim et al. 2020)
Mesoporous Silica NPs		150-75 µg/ml	Biphasic stratification	H5N1	(AbouAitah et al. 2020)

synthesized because they are more biocompatible, biodegradable, and easily penetrate at a specific site. During the preparation of NPs, the size, composition, charge, stability, morphology, surface functionalization, and cellular target should be considered very carefully. Future strategies should also focus on the combination of different metallic NPs with conventional AI antiviral drugs or vaccines to achieve synergistic protection. While doing all this one should be careful about the cost-effectiveness and scalable production of these NPs for widespread commercial use in the poultry industry.

8. Conclusion

Historically AIV is a global disease and it has been treated with different types of antiviral drugs and prevented with vaccines. However, these steps are not valuable as the virus can mutate with time, and as far as AIV is concerned its genetic drift is very high compare to other virus families. AIV not only affects the poultry birds but also the humans and has a very crucial role in global economic losses. Some of the effective measures have been taken to tackle its effects and one of them is NPs (NPs). Several NPs such as AgNPs, AuNPs, and ZiO_2NPs at different doses are used against different strains of AIV and have shown better potential.

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