Antiviral and Antimicrobial Applications of Silver Nanoparticles

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Purpose

The ongoing COVID-19 pandemic has generated an urgent and severe need for personal protective equipment (PPE) and improved antiviral technologies. NovaCentrix is committed to help address this need through its expertise in manufacturing and processing of metallic nanoparticle materials. NovaCentrix recognizes that this contribution must proceed from accurate scientific understanding in order to be impactful. **Thus, the following white paper summarizes the antiviral and antimicrobial properties of silver nanoparticles (AgNPs) from an academic perspective.** The contents of this white paper are based on recent findings from research published in peer-reviewed journals. The language of this document is intended to be accessible to STEM professionals with a non-clinical background.

Scope

To evaluate the practical utility of AgNP materials as antiviral/antimicrobial agents in PPE and other clinical applications, several fundamental considerations must be addressed:

1. The **physical mechanisms** associated with antiviral/antimicrobial activity must be sufficiently understood to design functional products.
2. The interaction of AgNPs with their **immediate environment** must be considered appropriately in product design to ensure efficacy.
3. **Quantitative measurements** of antimicrobial/antiviral properties must be available to assess the value of AgNP-enabled products.
4. Antiviral/antimicrobial functionality must be achievable without presenting significant **risk to human health**.

The remainder of this document summarizes current understanding related to the above considerations.

Technical Summary

1. Are metallic nanoparticles useful as antiviral and antimicrobial agents?

There is significant evidence that certain metals and metal oxides exhibit antiviral and antimicrobial properties in nanoparticle form. Silver is arguably the most successful and popular choice among published literature [1-3]. Copper [4, 5], copper oxide [6, 7], zinc oxide [8-10], and gold nanoparticles [2, 11] have also been validated as effective antiviral/antimicrobial agents.

2. Are AgNPs effective against all viruses, or only certain ones? Are AgNPs proven to be effective against coronavirus specifically?

The antiviral properties of AgNPs are general-purpose, in the sense that they are applicable to a wide range of viruses and microbes [2, 12, 13]. The efficacy of antiviral AgNPs have been confirmed directly for various strains of human influenza A virus [7, 14], avian influenza virus [7], African swine fever virus [13], feline calicivirus [14], Tacaribe virus [15], vaccinia virus (VACV) [16], human immunodeficiency virus 1 (HIV-1) [17, 18], herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), monkeypox virus (MPV), and hepatitis B virus (HBV) [2, 13, 18]. Demonstrations with COVID-19 specifically have not yet appeared in peer-reviewed literature at the time of this writing. However, the antiviral efficacy of AgNP has been tested against other types of coronaviruses, including transmissible gastroenteritis virus (TGEV), a type of porcine coronavirus [19], and feline coronavirus (FCoV) [20].

3. What physical mechanisms are responsible for antiviral properties of AgNPs?

Several possible antiviral mechanisms for AgNPs have been suggested and investigated in recent literature. Ionic silver (Ag^+) may play a significant role by interfering with cellular proteins, metabolic pathways, and DNA by virtue of its bonding with phosphorous and sulfur and generation of reactive oxygen species [6, 21, 22]. Some researchers have hypothesized that Ag^+ competes for receptors that mediate viral entry, thereby neutralizing infection and replication [9]. Stimulation of the human immune response by AgNPs and ionic silver has also been suggested as a means of preempting an expected viral infection [23].

On the other hand, ionic silver alone may not account for the antiviral activity of AgNPs. Significant disruption of viral entry has been observed even when the dissolved Ag^+ concentration remains negligible [15, 16], and entry of entire nanoparticles into host cells has also been observed in numerous studies [16, 19, 23]. One possibility is that AgNPs alter the entire uptake landscape of host cells in such a way as to interfere with viral entry. [16, 23]. Another possibility is that AgNPs inhibit cell apoptosis after viral entry, thereby disrupting the cycle of viral infection [19].

It should be recognized that the size and surface chemistry of AgNPs are also influential with respect to antiviral activity. For example, AgNPs have been shown to be most effective for a binding-based inactivation mechanism against human immunodeficiency virus (HIV-1) at a diameter of 10 nm or less [23]. Several studies have also suggested that capping agents used to stabilize AgNP suspensions can play an essential role in antiviral and antimicrobial properties [2, 12, 22, 24]. For electrostatically dispersed AgNPs, viruses and bacteria can be attracted and subsequentially immobilized on the charged particle surfaces.

The topic of physical mechanisms for antiviral activity remains under active investigation in the research community. Depending on the virus, host cell, and environment, it is possible than more than one of the mechanisms summarized above may be relevant. Furthermore, because of the wide variation and proprietary nature of AgNP surface chemistry across commercial suppliers, observed

antiviral/antimicrobial performance may not always be replicable across studies that use different AgNP sources.

*4. How are the antiviral properties of AgNPs affected by the **ambient environment** in which they are used?*

A critical consideration for the use of nanoparticle silver as an antiviral agent is whether a wet environment is required for effectiveness. The vast majority of available studies, including those cited here, consider only interactions between viruses and AgNPs in solution or in humid environments. Based on the available evidence, the antiviral mechanisms described above may not be relevant in dry air environments. A study by Ortí-Lucas and Muñoz-Miguel on the antimicrobial efficacy of a commercially-available silver coating provides some evidence that silver is less effective in dry environments The study found that the antimicrobial coating was effective along bed and sink surfaces, where the risk of droplet-borne contamination was highest, relative to other surfaces tested over a three-month period [25].

5. What size nanoparticles are required to achieve antiviral/antimicrobial properties?

Various studies report beneficial effects for particles ranging from 1 nm to 100 nm. These studies do not suggest strict limits on the range of efficacious nanoparticle size. However, they do delineate between the functionality provided by small particles versus ions. The majority of papers surveyed here use nanoparticles in the range of 10-25 nm, with suggestion from some authors that 1-10 nm may be the optimal size for some antiviral mechanisms [22].

6. What is the advantage provided by metallic nanoparticles versus other antiviral agents?

The effectiveness of silver against a wide range of viruses is an essential feature for any PPE, where protection against only a narrow subset of viruses or microbes is not of practical use. Antiviral and antimicrobial silver can also provide much-needed disinfectant capabilities in situations where conventional options are ineffective or infeasible [3, 5]. The broad-spectrum effectiveness of silver is especially advantageous against novel viral strains and the risk of resistance by mutation [2]. For protection against bacteria specifically, AgNPs provide a useful alternative to antibiotics, whose overuse and misuse has emerged as a modern medical dilemma [3, 11].

The benefit provided by nanoparticle suspensions of silver, relative to ionic silver, is the persistence of its antiviral/antimicrobial benefit [22]. This may be ensured, for example, by the gradual release of Ag^+ at nontoxic levels. Since the utility of AgNPs spans multiple fields and applications [3], the potential for an efficient and robust supply chain is strong despite the high cost of bulk silver. The industry outlook for environmentally friendly synthesis of AgNP materials is also favorable [1].

*7. What **types of products** have previously benefitted from antiviral/antimicrobial AgNPs?*

AgNPs have been incorporated into face masks and air filters for clinical applications [7] [3, 6, 21, 26-28], as well as water filtration membranes made by the phase-inversion process [21, 29]. For filtration applications, the antiviral/antimicrobial functionality of AgNP relates to anti-biofouling and passive decontamination over several hours or days. It is important to recognize that in this context, AgNPs do not necessarily enhance removal or inactivation of viruses in the fluid that passes through the filter. Rather, the silver treatment ensures that the filter material is far less hospitable to viruses and/or microbes, thereby alleviating the consequences of accidental or unwanted contamination.

AgNPs have been incorporated into a wide range of additional products for purposes of passive decontamination. These products include wound dressings [2], clothing [30], and permanent surface

coatings [25]. Other authors have suggested the use of nanoparticle silver as an *in vivo* antiviral treatment, either alone or in conjunction with other medications [3, 13].

8. From a manufacturing standpoint, how have AgNPs been incorporated into personal protective equipment (PPE) and other antiviral/antimicrobial products?

AgNPs and other metallic nanoparticles have been deposited onto textiles by various methods, including aerosol deposition [26, 27], radiochemical synthesis [28], and wet pickup followed by immobilization by a siloxane emulsion treatment [30]. To help prevent unwanted removal from the fabric, other studies have incorporated the metallic nanoparticles into the textile fibers themselves, for example, by electrospinning of an AgNP-impregnated polymer solution [7].

9. What is the standard methodology for ***quantitative measurements*** *of antiviral/antimicrobial properties of AgNPs?*

Several standard methods for evaluating antiviral and antimicrobial efficiency can be found in recent literature. The most common are bioassays conducted *in vitro*, *i.e.* measurements of cell cultures carried out in a petri dish, test tube, or other controlled environment outside a living organism. Antimicrobial efficiency and toxicity to humans are measured by observing cultures of those types of cells [4]. Antiviral efficiency, by contrast, typically requires observation of the host cell targeted by the virus, in order to infer the rate of viral replication and spread [31].

- The colony counting method (CCM) measures the number of colony forming units in the petri dish, and computes the efficiency as the ratio of colonies for a given sample versus the colonies for a control group [26].
- The disk diffusion method (DDM), *a.k.a.* Kirby-Bauer method, involves placing a microbe in a petri dish that contains antibiotic agent at specific locations. The test then measures the radius of non-growth around each antibiotic-treated area. Larger areas of non-growth imply a more effective antibiotic (or, conversely, a more successful bacteria colony) [26, 32].
- The colorimetric MTT assay [31] leverages the reduction of optically clear tetrazole 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to form a purple dye. The rate of reduction correlates to the metabolic activity of a microbe colony. Thus, by characterizing the absorbance of the material at a specific wavelength (570 nm), it is possible to infer the rate of reduction of MTT and thus the rate of microbe activity in a given sample.
- The optical density (OD) method measures the reduction optical transmission due to light scattering by a growth of bacteria. Typically, a subscript denotes the specific wavelength of light used for the measurement, for example, OD_{60} for 600 nm [4].

It is critical to note the procedures used for bioassay sample preparation in order to interpret the significance of published results correctly. In particular, most studies on antiviral and antimicrobial filter materials involve placing the filter material directly on a petri dish for observation over the course of several hours or days. These studies are concerned primarily with activity of viruses and bacteria immobilized on the filter material, rather than filtration efficiency.

10. What are the ***risks to human health*** *from materials that contain AgNPs?*

There is a general consensus among the literature surveyed here that AgNPs exhibit antiviral properties at concentrations that do not harm human health. This ensures that a safe range of silver concentration can be defined for therapeutic use, depending on the form of the silver and mode of delivery [12]. Nevertheless, at sufficiently high concentrations, silver is known to be toxic to humans

[33]. Exposure is possible through skin contact, inhalation, and ingestion. Accumulation of AgNPs has been observed in lung tissue for rats, and the liver and kidneys have also been identified as target organs for silver toxicity. There is also evidence that AgNPs can cross the blood-brain barrier as well as the blood-testes barrier [2].

Just as the mechanisms for antiviral and antimicrobial activity are not yet certain, the primary mechanisms for AgNP toxicity in humans are also somewhat unclear. In particular, more work is required to determine whether the harmful effects of AgNPs derive entirely from dissolved Ag+, or whether the nanoparticles themselves have some additional deleterious effect [2]. According to OSHA 71, the recommended threshold limit value on a timeweighted average (TLV-TWA) for silver in particulate for is 0.1 mg m^{-3} [34]. Under normal breathing (500L per hour) and work environment (8 hours per day), this limit corresponds to inhalation of about 400µg of silver particles per day.

NovaCentrix AgNPs

AgNPs manufactured and sold by Novacentrix have been shown to exhibit antiviral and antimicrobial properties in numerous studies. These studies include the following:

- A 2008 study by Zodrow et al. on polysulfone ultrafiltration membranes [21].
- A 2008 study by Carlson et al. on the effect of silver nanoparticles on alveolar macrophages [23].
- A 2010 study by Speshock et al on the interaction of silver nanoparticles with Tacaribe virus [15].
- A 2013 study by Trefrey and Wooley on *Vaccinia virus* (VACV) replication in monkey kidney cell lines and human HeLa cervical cell lines [16].
- A 2018 review by Hoseinnejad et al. on antimicrobial nanoparticle treatments for food packaging applications [11].

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