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The progress of silver nanoparticles in the antibacterial mechanism, clinical application and cytotoxicity

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Abstract Nanotechnology is a highly promising field, with nanoparticles produced and utilized in a wide range of commercial products. Silver nanoparticles (AgNPs) has been widely used in clothing, electronics, bio-sensing, the food industry, paints, sunscreens, cosmetics and medical devices, all of which increase human exposure and thus the potential risk related to their short- and long-term toxicity. Many studies indicate that AgNPs are toxic to human health. Interestingly, the majority of these studies focus on the interaction of the nano-silver particle with single cells, indicating that AgNPs have the potential to induce the genes associated with cell cycle progression, DNA damage and mitochondrial associated apoptosis. AgNPs administered through any method were subsequently detected in blood and were found to cause deposition in several organs. There are very few studies in rats and mice involving the in vivo bio-distribution and toxicity, organ accumulation and degradation, and the possible adverse effects and toxicity in vivo are only slowly being recognized. In the present review, we summarize the current data associated with the increased medical usage of nano-silver and its related nano-materials, compare the mechanism of antibiosis and discuss the proper application of nano-silver particles.

Keywords Silver nanoparticles · Mechanism · Application

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Introduction

Nanotechnology is a highly promising field for generating new applications in aerospace engineering, nano-electronics, environmental remediation, medical healthcare and consumer products [1, 2]. Due to their large surface area and high reactivity compared with a bulk solid, nano-sized metal particles exhibit excellent physical, chemical and biological properties [3, 4]. In particular, the properties of nanoparticles, which vary according to their size and shape as well as their chemical environment, have been intensively studied. In addition, there has been increasing interest in the utilization of nanotechnology as a special class of chemotherapy due to its extraordinary physico-chemical properties. An increasing number of nano-products are emerging for medical purposes. The bioactivity of nano-sized metal particles and their biological behavior are research areas of growing interest. In addition to intensive research on novel applications for nanoparticles, concerns have been raised about the potential toxicity risk of nanoparticles when they enter organisms, either directly during the manufacturing processes or indirectly via the environment and food chain [5].

A particularly prominent nano-product is nano-silver. Silver is a white and shiny metallic element positioned 47th in the periodic chart with the chemical symbol Ag, short for "argentums". Pure silver is ideally ductile and malleable and has the highest electrical and thermal conductivity as well as the lowest contact resistance of any metal [6]. There has been 2,000 years for human beings' discovery and use of silver, with applications including jewelry, utensils, monetary currency, dental alloy, photography and explosives [7]. Silver compounds and ions have been extensively used for both hygienic and healing purposes [8]; however, over time, the use of silver compounds and ions as an

anti-infection agent has faded due to the advent of antibiotics and other disinfectants in addition to the poorly understood mechanisms of their toxic effects. Recently, renewed interest has arisen in manufactured silver nano-materials because of their unusually enhanced physicochemical and biological properties and activities compared to their bulk parent materials. A wide range of applications has emerged in consumer products ranging from disinfecting medical devices and home appliances to water treatments [9–11].

Nano-silver particles (AgNPs) are generally smaller than 100 nm and contain 20–15,000 silver atoms and have unusual physical, chemical and biological properties. Due to its strong antibacterial activity, nano-silver has been used as a contraceptive, for the treatment of wounds and burns and even marketed as a water disinfectant or room spray. It is becoming widespread in medical and other fields; however, when using AgNPs for therapeutic and diagnostic purposes, their in vivo fate and toxicity are crucial aspects that need to be evaluated [12]. In general, very few studies of AgNP have been done in whole organisms. New models has been established to study the reproductive and development in *Drosophila melanogaster* and zebra fish [13, 14]. The study of the cytotoxicity, antibacterial mechanisms, bio-distribution, organ accumulation, degradation and possible adverse effects of AgNPs is urgently required. The rapid commercialization of AgNPs requires thoughtful environmental, health and safety research, which would result in an open discussion of the broader societal impacts and urgent toxicological oversight action. AgNPs regulation is still undergoing major changes to encompass environmental, health and safety issues [15]. This review focuses on the major questions associated with the increased medical usage of nano-silver and related nano-materials, along with the proper application of AgNPs.

Applications in the clinic

The antimicrobial spectrum of AgNPs is broader than that of common antibiotics. Most researchers normally select *Escherichia coli* (G−) and *Staphylococcus aureus* (G+) to study the inhibition of bacteria by AgNPs [16–18]. Polysulfone ultrafiltration membranes incorporated with AgNPs were found to exhibit antimicrobial properties towards a variety of bacteria, including *E. coli* K12, *Pseudomonas mendocina* KR1, and the MS2 *bacteriophage* [19]. AgNPs were found to exhibit a high antifungal activity against pathogenic Candida species, at very low concentrations and with no cytotoxic effects on human fibroblasts [20]. The explosion of SARS, HIV and other viruses have perplexed numerous scientists, who are now focusing on the study of nanoparticles in an attempt to find a magical method with which to fight these viruses. It has been confirmed that AgNPs exert anti-HIV activity at an early stage of viral replication, by binding to gp120 in a manner that prevents CD4-dependent virion binding, fusion, and infectivity, thus acting as an effective virucidal agent against cell-free and cell-associated viruses. Furthermore, AgNPs inhibit the post-entry stages of the HIV-1 life cycle [21].

Surgical application

AgNPs are fully used in surgical fields, such as urology, dentistry, general surgery and orthopedics [22–24]. Hospital-acquired, or nosocomial, infections, such as those observed with orthopedic fixation and artificial joint surgery, are normally hard to avoid infections. The utilization of silver nanoparticles in a slow release dressing would allow controlled bacteriostasis [23]. Poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) nano-fibrous scaffolds that contain AgNPs have shown not only good antibacterial activity but also good in vitro cell compatibility with the potential to be used in joint arthroplasty [25]. Nano-silver bone cement was also shown to inhibit the proliferation of *S. epidermidis* without in vitro cytotoxicity; this has yet to be confirmed in vivo but it's use in total joint arthroplasty is highly anticipated [26]. Surgical equipment, such as scalpels and surgical scissors, coated with nano-silver also may have excellent antibacterial properties.

Wound therapy

Wounds such as burns (particularly extensive burns) require a great deal of external medicines, which have the potential to produce side effects on the body. There are a variety of drugs for surface wounds, but these usually have a limited curative effect. Sulfadiazine silver has been widely used for the treatment of burns and wound infections, but its use typically leads to an allergic reactions or systemic toxicity. The emergence of AgNPs could offset these deficiencies. Wound dressings coated with AgNPs could be a new kind of anti-infective dressing with no toxic components. When used for second degree burns, it could decrease the incidence of infection and accelerate healing time [27].

To treat large-scale burns and refractory wounds, an increasing number of scientists have begun to devote themselves to tissue engineering of the skin. Some products are already being used, but they often show difficultly in resisting infection and are associated with other risk factors. Adding AgNPs into biological materials can inhibit the growth of pathogens. A cross-linked chitosan coated Ag-loading nano-SiO_2 composite (CCTS–SLS) was shown to exhibit high antibacterial activity against *E. coli* and *S. aureus* [28]. Sudheesh Kumar and co-workers [29]

developed novel β-chitin/nano-silver composite scaffolds for wound healing applications using a β-chitin hydrogel containing AgNPs. They found that the composite scaffolds were bactericidal and showed good blood clotting ability. Cell attachment studies showed that the cells were well attached to the scaffolds, suggesting its potential as a wound dressing material. Incorporation of silver into alginate fibers was also shown to increase antimicrobial activity and improve the binding affinity for elastase, matrix metalloprotease-2, and the proinflammatory cytokines tested [30].

Prospective uses of AgNPs

Anti-inflammatory uses

Nano-crystalline silver dressings were introduced commercially as antimicrobial dressings in 1998. In vivo and in vitro studies demonstrated that these dressings improve wound healing [31–33], which may result from their potent anti-inflammatory activity. The anti-inflammatory activity of a topical nano-crystalline silver cream was assessed using a murine model of allergic contact dermatitis. Researchers found that nano-crystalline silver more noticeably reduced erythema in a concentration-dependent manner when compared with Steroids and immunosuppressant. It also suppresses the expression of TNF-α and IL-12 and induces apoptosis of inflammatory cells [34, 35]. This activity may be due in part to the induction of apoptosis and the suppression of MMP activity. Nano-crystalline silver suppresses the production of the proinflammatory cytokines TNF-α, IL-8, TGF-β and IL-12 [35–37]. Due to the similarities between human and pig skin, these results suggest that AgNPs may have a beneficial impact on the treatment of human skin inflammatory conditions. AgNPs released into the blood and circulation were shown to be taken up by peripheral blood mononuclear cells, causing apoptosis and inhibiting the expression level of interleukin-5 (IL-5), interferon-γ (INF-γ), and tumor necrosis factor-α (TNF-α) [38, 39].

Anti-angiogenic agents

Research focusing on the anti-angiogenic use of AgNPs found that they could act as an anti-angiogenic molecule by targeting the activation of the PI3K/Akt signaling pathways, demonstrating a potential use in diabetic retinopathies [40]. AgNPs can also inhibit VEGF-and IL-1β-induced permeability, thus acting as a potent anti-permeability and anti-angiogenic molecule. Through the targeting of the *Src* and PI3K/Akt signaling pathways, it also offers potential targets in the inhibition of ocular related diseases [41–43]. The present study provides insight into promising potential applications of AgNPs on diabetic retinopathy by preventing retinal vascular hyperpermeability.

However, AgNPs as an anti-angiogenic molecule may prevent the development of new vascular cells, which is the key to wound regeneration [40, 42]. Additional studies need to be performed to confirm this and also to guarantee its safety as an anti-angiogenic molecule. A product with stable concentrations and well-proven properties is required.

Antineoplastic agents

Tumors are the most frequently occurring disease, and they are still a therapeutic challenge to researchers and medical practitioners. Nanotechnology provides a new way to capture tumors by playing the role of the weapon to kill the tumor cells directly, either by inducing cell physiological disorders or as the carrier of specific agents. It is widely accepted that AgNPs are cytotoxic and may lead to cellular apoptosis. The underlying mechanism of this effect is not clear, but it is known that they can kill cells in a manner similar to chemotherapeutics, giving AgNPs the potential to act as antineoplastic agents. Wang et al. studied the effects of protein-conjugated silver sulfide nano-crystals of different shapes on the proliferation of different cancer cells. They found AgNPs that protein-conjugated Ag_2S nano-crystals offered greater inhibition on the viability of C6 glioma cells and human hepatocellular carcinoma (Bel-7402) cells [44]. Sur et al. [45] performed a cytotoxicity study using modified AgNPs and revealed that only naked AgNPs affect the viability of A549 cells. Cancer cells are highly susceptible to and do not recover from damage due to AgNPs-induced stress. AgNPs found to be acting through intracellular calcium transients and chromosomal aberrations are believed to play key roles in cytoskeleton deformations that ultimately inhibit cell proliferation [46]. AgNPs as anti-angiogenic molecules may therefore inhibit the proliferation of tumors [40]. This has the potential of providing new tools, which could be used for novel applications in clinical cancer diagnosis and treatment and other therapeutic applications [45].

Gynecology and reproductive medicine

Broad spectrum AgNPs are an efficient antibacterial and sterilization compounds, and they have been used for gynecological inflammatory diseases such as vaginitis, cervicitis, and cervical erosion. The AgNPs application of nano-silver in the treatment of gynecological diseases is a recent development, but for unknown reasons, its use has not yet been widely accepted. As mentioned previously,

AgNPs are a highly reactive species, readily attacking RNA and DNA, which may be the basis of its mechanism of cytotoxicity and antibiosis [47]. Reproduction is a complex biological process that is especially sensitive to environmental insults; therefore, AgNPs may be particularly toxic to the reproductive system. Many elements, including ultrafine particles, have a robust effect on the germ line and embryo, although until now, few studies have demonstrated their reproductive toxicity. AgNPs have been shown to induce a significant decline in spermatogonial stem cell proliferation, although this effect has also been shown to be dependent on their size and coating [48]. Moreover, in vitro exposure to AgNPs induces apoptosis and retards early post-implantation development after transfer to host mice, demonstrating that AgNPs have the potential to induce embryo cytotoxicity [49]. Therefore, for contraceptive applications, condoms have been coated with AgNPs.

The applications of AgNPs in medicine are unlimited in the aforementioned fields, but their potential cytotoxicity cannot be denied. Studies on the mechanisms of AgNP cytotoxicity are as important as research on their application. I believe that with the continuous improvement of new technologies, AgNPs have broad prospects for development and practical applications for only by fully understanding the disadvantages, can we make better use of the benefits.

Antibacterial mechanism

Some scientists believe that when dissolved in water, AgNPs convert to silver ions (Ag+), which can then be used to kill pathogens [50, 51]. However, Simon Silver and his colleagues found that some microbes were resistant to Ag^+ and defined the genes involved in bacterial resistance, which indicated AgNPs' independent role of antibiosis [52, 53]. Many researchers attribute the highly efficient antibacterial effect of AgNPs to their ultrafine size and larger surface area, by which AgNPs can easily destroy the membrane, pass through the microbial body, then convert to silver ions (Ag^+) in cytoplasm to damage the intracellular structure as secondary result. AgNPs undergo a shape-dependent interaction with the gram-negative bacterium *E. coli*, so it has been speculated that AgNPs with the same surface area, but with different shapes, may also have different effective surface areas in terms of active facets [54]. Choi et al. [55] observed that spherical and hexagonal AgNPs were adsorbed onto the bacterial cell surface, causing cell surface depression when viewed under the electron microscope. Nano-scale sized particles can enter pathogens, combine with their protein groups and then kill them. Recent literature has reported considerable changes in bacterial cell membranes upon silver ion treatment, which may be the cause or consequence of the subsequent cell death. It is possible that the physical contact between AgNPs and the cell wall of a bacterium is sufficient to trigger a cytotoxic signal; therefore, highly concentrated AgNPs may disrupt the membrane integrity of the local bacterial membrane [56]. The most widely known bactericidal mechanism of AgNPs is their interaction with the thiol groups of the L-cysteine residue of proteins, and as a result, the consequent inactivation of their enzymatic functions [57, 58]. The inhibitory effect of AgNPs is therefore due to their sorption to the negatively charged bacterial cell wall, deactivation of cellular enzymes, and disruption of membrane permeability [57]. AgNPs are therefore more reactive with their increased catalytic properties and can be more toxic than their bulk counterpart. AgNPs (<5 nm) can easily pass through the microbial body and block the translation of their transcriptase by attacking the genetic material. Ag^+ released by AgNPs could induce a massive proton leakage through the Vibrio cholerae membrane, which results in complete deenergization and, with a high degree of probability, cell death [7].

Cytotoxicity of silver nanoparticles

It has been widely accepted that a high concentration of AgNPs can cause apoptosis in human cells. Because of this, researchers prefer to expose single cells to a considerable amount of AgNPs, while observing the changes in cell morphology. Silver nanoparticles showed higher cytotoxicity than silver micro-particles with manifestations such as more severe morphological abnormalities, more cells arrested in the G2/M phase and more cells undergoing apoptosis [59]. These results indicated that the mechanism of AgNPs cytotoxicity in vitro was related to their nano-size. Toxicity induced by silver ions was studied using $AgNO_3$ as the Ag^+ source. Researchers found that AgNP-treated cells have limited exposure to Ag^+, despite the potential release of Ag^+ from AgNPs in cell culture [60]. This has encouraged studies of the independent role of AgNPs. Studies in the past decade have demonstrated that the electromagnetic, optical, and catalytic properties of noble-metal [61]. The interaction between AgNPs and cells is a process of invasion, similar to its sterilization mechanism. Both of the AgNPs and Ag^+ that resourced from AgNPs contributed to the whole process of cytotoxicity in different ways. It is most probable that AgNPs simply provide a perfect surface outside the mitochondria for the univalent reduction of oxygen to superoxide from electrons flowing through the electron transport chain most likely at the flavoprotein level. The Ag^+ on the other hand binds to proteins and nucleic acid, and interferes with function.

Generation of reactive oxygen species (ROS) by AgNPs

ROS are a group of short-lived reactive oxidants, including the superoxide radical, hydroxyl radical (–OH), hydrogen peroxide (H_2O_2), and singlet oxygen. ROS may be generated by external and internal factors, and oxidative stress results from an imbalance between ROS generation and cellular defensive functions, including those of antioxidant enzymes and antioxidants. Intracellular ROS generation by AgNPs has been clearly shown. AgNPs have been found to induce an increase in the respiration rate, thus generating intracellular ROS during this process [62–64]. Ingestion of nano-silver during the larval stage of *D. melanogaster* showed major dose, size, and coating-dependent effects on each of the aspects of life history effects on reproduction, development and fertility. However these above effects of AgNPs could be partially or fully reversible by vitamin C [14]. Thus, ROS generation and oxidative stress can be used as a paradigm to assess AgNPs toxicity.

The generally accepted view is that mitochondrial damage is the basis of the mechanism of early apoptosis caused by AgNPs. While the specific mechanism remains unknown, mitochondria are known to be the major site of ROS production within the cell. During oxidative phosphorylation, oxygen is reduced to water by the addition of electrons, in a controlled manner, through the respiratory chain [65]. Some of these electrons occasionally escape from the chain and are accepted by molecular oxygen to form the extremely reactive superoxide anion radical (O_2^-), which is further converted to hydrogen peroxide (H_2O_2) and in turn may be fully reduced to water or partially reduced to a hydroxyl radical (OH^-), one of the strongest oxidants in nature [64]. Toxic agents increase the rate of superoxide anion production, either by blocking electron transport or by accepting an electron from a respiratory carrier and transferring it to molecular oxygen without inhibiting the respiratory chain [66]. Inhibition of the respiratory chain is expected to cause a decrease in ATP synthesis. Deposition of AgNPs in the mitochondria can alter their normal function by disrupting the electron transport chain, ultimately resulting in the production of ROS and a low ATP yield. Ingestion of nano-silver during the larval stage of *D. melanogaster* affecting the phenotype which could be reversible with antioxidants mean that the ROS may be the primary effect and causes many secondary problems such as protein damage, DNA damage, and lipid peroxidation [14]. It can also block processes such as signal transduction cascades, protein ubiquitination and degradation, and the disruption of the cytoskeleton, which is the major mechanism for bacterial killing by many drugs and antibiotics. Both nano-titanium dioxide and nano-silver have these effects mentioned above but titanium dioxide is a kind of inert substance insoluble in water and would not produce any chemical reaction in body [67–70]. It indicates that not only the independent role of nanoparticle but the active physicochemical property of AgNPs' causes the secondary damage in vivo. Hsin et al. [63] reported that nano-silver-induced apoptosis was associated with JNK activation. Ahamed et al. [71] reported the toxic effects of well-characterized polysaccharide coated 10 nm AgNPs on heat shock stress, oxidative stress, DNA damage and apoptosis in *D. melanogaster*. Their results indicated that AgNPs induce heat shock stress, oxidative stress, DNA damage and apoptosis. At high concentrations of ROS, all of the above processes are activated in combination with enhanced damage to the building blocks of the cell, leading to apoptosis or even necrosis [64].

DNA damage by Ag^+

ROS are highly reactive and result in oxidative damage to proteins and DNA. Hence, it is indispensable to investigate the genome stability within cells showing significantly higher ROS production [68, 72]. DNA damage by AgNPs has been further studied using comet assays and cytokinesis-blocking. Extensive and dose-dependent DNA damage was observed after treatment of cells with AgNPs. Chromosomal abnormalities are a direct consequence of DNA damage, such as double-strand breaks and disrepair of strand breaks, resulting in chromosomal rearrangement [65]. As a result of oxidation, monomer Ag transforming to Ag^+ would play a crucial role in binding with intracellular biological groups. Fourier transform infrared spectroscopy and capillary electrophoresis were used to analyze the Ag^+ binding mode, the binding constant, and the polynucleotide structural changes within the Ag-DNA and Ag-RNA complexes. The spectroscopic results showed that in the type I complex formed with DNA, Ag^+ binds to the guanine N7 atom at a low cation concentration and to the adenine N7 atom at higher concentrations, but does not bind to the backbone phosphate group [47]. In regards to the cell cycle, Ag^+ can induce G1 arrest, and Ag^+ at higher concentrations induce a complete blockage in the S phase with the induction of cellular apoptosis [73]. Asharani et al. [65] reported that starch-coated Ag^+ induced G2/M phase arrest and DNA damage in human glioblastoma cells and human fibroblasts. Another recent study observed G1 arrest in mouse lung epithelial cells exposed to C60 and SWCNT. S phase arrest was also observed in human lung epithelial cells exposed to carbon black coated with benzo(*a*)pyrene [74, 75]. A typical result of compounds that inhibit DNA synthesis is perturbation of the cell cycle preceded by a reduction of cell viability and a subsequent inhibition of population growth and an accumulation of cells in the S phase, leading to cell death.

AgNPs can cross the cell membrane via a free shuttle or by damaging the membrane' integrity through bonding with thiol-containing proteins. The AgNPs can then access the mitochondria to interfere with the respiratory chain, resulting in a large number of superoxides and nano-silver ions, both of which have the ability to attack the cell nucleus and damage genetic material and other structural organelles. In addition, the apoptotic gene, *bax*, is able to pass through the nuclear membrane and trigger cellular apoptosis (Fig. 1).

In summary, the toxicity of AgNPs can most likely be attributed to two different processes. The increased surface area provided by the AgNPs is conducive to the univalent reduction of oxygen to superoxide via electrons coming from the respiratory chain of the mitochondria. The source of electrons is most likely at the flavin level of the electron transport chain. In addition, Ag^{+} is released from the AgNPs and has been demonstrated to bind to proteins and nucleic acids interfering with their respective functions. This is an area where future research may reveal ways to reverse AgNP toxicity. AgNPs are toxic to both microorganisms and human cells, but when exposed to similar concentrations, different reactions may be observed. Therefore, the question arises, why does such variation exist? We speculate that due to the simplified structure of prokaryotic and acellular microorganisms, AgNPs can easily pass through the cell wall and gain access to the genetic material. On the contrary, eukaryotic cells have a multi-layer membrane, which includes the mitochondrial membrane and karyotheca, both of which have the ability to act as a barrier. In addition, the eukaryotic immune system provides further protection from cellular damage. A difference exists though, when comparing normal versus cancerous cells. AgNPs are potentially toxic and antibiotic, and in this case, neither human cells nor other organisms would be able to avoid their attack.

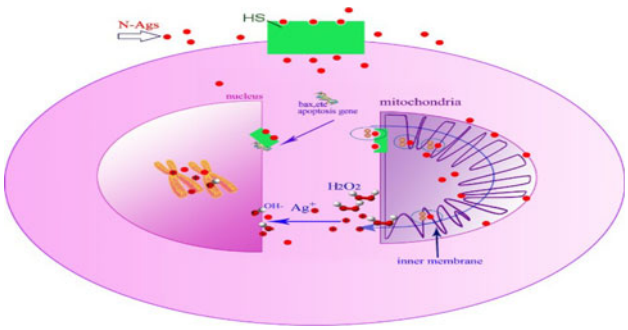

Fig. 1 AgNPs cross the cell membrane, access the mitochondria and subsequently interfere with the respiratory chain. This results in a large number of superoxides and nano-silver ions, both of which can attack the cell nucleus and damage genetic material and other structural organelles. In addition, the apoptotic gene, *bax*, is able to pass through the nuclear membrane, triggering cell apoptosis

Conclusion and future perspectives

AgNPs are a new form of antimicrobial agent. To date, there have been no reports on a resistance effect when used in clinical applications, and they have been found to be superior when compared to conventional drugs. Most of the drugs associated with nano-silver rely on its antimicrobial properties, and while its toxic effects cannot be ignored, they should also not be overstated. Both of the two sides of species would be effective for human beings if they are properly applied. This material has the ability to attack both pathogens and normal cells, but cancer cells are also targeted by AgNPs. When exposed to the proper concentration of nano-silver, all of these microorganisms are fragile, but there are several reasons that lead us to conclude that normal cells are more resistant. First, eukaryotic organisms possess an immune system with the ability to defend against outside attack. In addition, due to the unique structure of pathogens and cancer cells, AgNPs possess a natural preference towards them, although these conclusions still require additional research. Second, the properties of nano-materials differ from those of bulk materials of similar composition, allowing them to execute the novel activities mentioned above, although evaluation of the safety of nano-materials is urgently required. The assessment of the toxicity of nano-materials is a relatively new and evolving field. Most nano-toxicology studies have focused on mechanistic understanding through the use of in vitro models, with early reports demonstrating that high levels of AgNPs are lethal to eukaryotic cell-based systems [76–78]. Pure silver is more easily modified then traditional antimicrobial agents. It has been demonstrated that ROS generation and oxidative stress are the primary method of cytotoxicity, which can also be used as a paradigm to assess NP toxicity [79]. However, according to present studies, the mechanism of cytotoxicity is still unclear and much work needs to be performed. Standard toxicological tests are still needed to assess the risks of AgNPs. The use of nano-silver as a treatment for cancer has been proposed, although this use remains in the experimental stage. A standardized format for clinical utility that focuses on cytotoxicity and the lowest dose necessary to obtain the best therapeutic effect is required [80]. Several research labs are focusing on the development of methods for reducing the toxicity of nanoparticles [81]. The elucidation of the risks posed by silver nanoparticle utilization is necessary not only for the protection of human health and environmental integrity but also to aid industry and regulatory bodies in maximizing the applications of these materials [12]. In short, the only way to industrialize AgNPs and ultimately bring them into clinical use is to clarify both the toxicity mechanisms at the molecular level and the interaction between the body and AgNPs.

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