The Mini-progress of the Application of Silver Nanoparticles

Jingyu Chen[1, †, *], Shiyu Song[2, †, *], Wanqi Zhao[3, †, *]

[1]College of Textile and Clothing Engineering, Soochow University, Suzhou, 215021, China

[2]Department of Materials, University of Manchester, Manchester City, M13 9PL, UK,

[3]College of Resources and Environment, Northeast Agricultural University, Harbin, 150030, China

[†]jychen8797@stu.suda.edu.cn, [†]shiyu.song-2@student.manchester.ac.uk, [†]zwq_victoria@163.com

*These authors contributed equally

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Abstract: Silver nanoparticles (AgNPs) are among the most interesting nanomaterials in biochemistry due to their unique physical properties. AgNPs are types of metallic colloidal nanoparticles, typically 10-100 nm in size, which can take on various shapes. Its special antibacterial and anticancer characteristics are widely reflected in the application. However, its toxicity is easy to be released in the process of use, and reasonable modification methods can solve this problem. This paper first discusses the mechanism and specific application of silver nanoparticles in antibacterial and anticancer areas. We then summarize the toxicity and present the specific modifications with dopamine and 3-MA. The adequate analysis leads to a better understanding of AgNPs, a greater familiarity with the development in applications, which also show a solution to the toxicity problem with appropriate modifications.

1. Introduction

Silver nanoparticles (AgNPs), which are generally smaller than 100 nm and contain about 20–15,000 silver atoms, have different physical, chemical, and biological properties compared with larger parent materials [1]. The visual, thermal, and catalytic properties of silver nanoparticles are strongly affected by their sizes and shapes. Additionally, silver nanoparticles have become the most widely used dream nanomaterials in consumer and medical products, such as textiles, food storage bags, refrigerators, and personal care products because of their board-spectrum capabilities. In previous studies, we can find that silver is a very effective antibacterial agent with low toxicity, which is especially important in treating wounds that have been spread in the past and are rapidly controlled. It is known that drugs which release ions in the ionic form are neutral in biological fluids, and when used over a long period, may cause cosmetic disorders, such as argyria and delayed wound healing [2]. With a wide range of activities, efficiency, and low cost, finding a new and better antiseptic agent is essential. Among the available options, silver nanoparticles have focused on increasing attention and are being nominated as excellent suppliers for therapeutic purposes.

2. Application of AgNPs antibacterial properties

AgNPs can overcome antibacterial-resistant antibodies. Moreover, AgNPs have a larger surface area to volume ratio and crystal surface structure compared with other nanomaterials. Therefore, it is necessary to develop AgNPs as antibacterial agents [3]. Seminal papers reported by Sondi and Salopek-Sondi showed AgNPs antimicrobial activity against Escherichia coli (E. coli). Small particles with a higher proportion of surface-to-volume show higher antibacterial activity than larger ones. Furthermore, the antibacterial activity of AgNPs is also dependent on the shape because AgNPs are interacting in shape with E. Gi-negative organism E. coli. As shown in figure 1a, the effectiveness

of dose-dependent antibacterial activity of biologically synthesized AgNPs in E. coli is determined by the size and shape [3].

2.1. The application of AgNPs in dental medicine

The oral cavity is an active ecosystem normally colonized by pathogenic microorganisms, so dental materials and implants increase the risk of infection by 15% to 70%. The biopsy showed the performance of unique silver nanoparticles when bonded to dental materials such as nanocomposites, acrylic resins, composite resins, adhesives, drugs in coatings, and embedded coatings [4]. They are also used as membranes for tissue regeneration in periodontal treatment, in which smaller silver nanoparticles have increased antibacterial activity against oral anaerobic bacteria [5]. Combining AgNPs with antibiotics can enhance bacterial properties. When inactive antibiotics were combined with AgNPs, they gain potent antibacterial activity against multidrug-resistant bacteria. For example, silver nanoparticles synthesized with a suitable capping agent can promote the inhibition effect against gram-negative bacteria that predominantly cause periodontal infections. Smaller silver nanoparticles exhibit higher antibacterial properties than oral bacteria [6].

2.2. The application of AgNPs in cardiology

In cardiology, for vascular surgery, polymers containing AgNPs are used due to their unique properties. In the past, clinical trials have shown that silver-coated heart valves cause side effects like depression and paravalvular circulation. However, new nanocomposites containing AgNPs and carbon for stents and heart valves have antimicrobial and antibacterial properties, reducing the risk of infection in the first few months after surgery and increasing the likelihood of a positive outcome for the patient [5]. The potential use of AgNPs in heart disease is to use them as a vehicle to deliver drugs to specific locations in the organization. In the USA, for example, at least 15 new drugs have been approved since the 1990s, incorporating nanotechnology into their drug design and delivery systems [7]. Another potential of AgNPs is to stimulate endothelial vasodilatation, improving blood flow in the heart. So, it can be used as a potential antihypertensive agent [8].

2.3. The application of AgNPs in dermatology

Due to the increased resistance of fungal strains, including strains of the skin which prevalence rate in some hospitals has exceeded 10 percent and continues to increase, there is an urgent need for novel antifungals. Therefore, the antimicrobial activity of AgNPs has been tested. In one study, AgNPs were effective against Trichophyton violaceum. Atef et al. also reported the inhibition of silver nanoparticles in Trichophyton mentagrophytes and Candida albicans. However, AgNPs are not against Microsporum canis(M. canis) or Microsporum gypseum. Mousavi et al. also found M. canis to be resistant to silver nanoparticles. Griseofulvin was found to have higher anti-dermatophyte activity than silver nanoparticles by Mousavi et al. However, others have shown that silver nanoparticles have higher efficacy than fluconazole and less antibacterial efficacy griseofulvin [5]. They also showed that the antimicrobial effects of fluconazole and griseofulvin were improved in the presence of silver nanoparticles.

2.4. The application of AgNPs in orthopedic implants

Metallic implants have been widely used in orthopedic fields, inserted in implants and cannot stimulate bone regeneration or inhibit bacterial activity. Therefore, AgNPs as an antibacterial agent are usually used to modify the orthopedic implant surface, achieving antibacterial and osteointegration properties [9]. The incorporation of AgNPs in implants may decrease cytotoxicity by reducing cell uptake of AgNPs. Not only does it benefit osteoblast differentiation and cell-cell communication, but also it can promote osteogenesis with increased cell adhesion, probability, and expression of osteogenic virus. Similar to the effects of osteoblast and osteoclast, AgNPs exert cytotoxic effects on mesenchymal stem cells (MSCs) in quantitative and time-dependent effects. AgNPs can be absorbed into cells and then cause DNA damage, cell death, and functional impairment of MSCs. In another study, AgNPs promoted MSC differentiation even at higher concentrations (4-

20 μM) which can accelerate the recovery of fracture callus and stimulate early closure of the fracture gap in vivo [9].

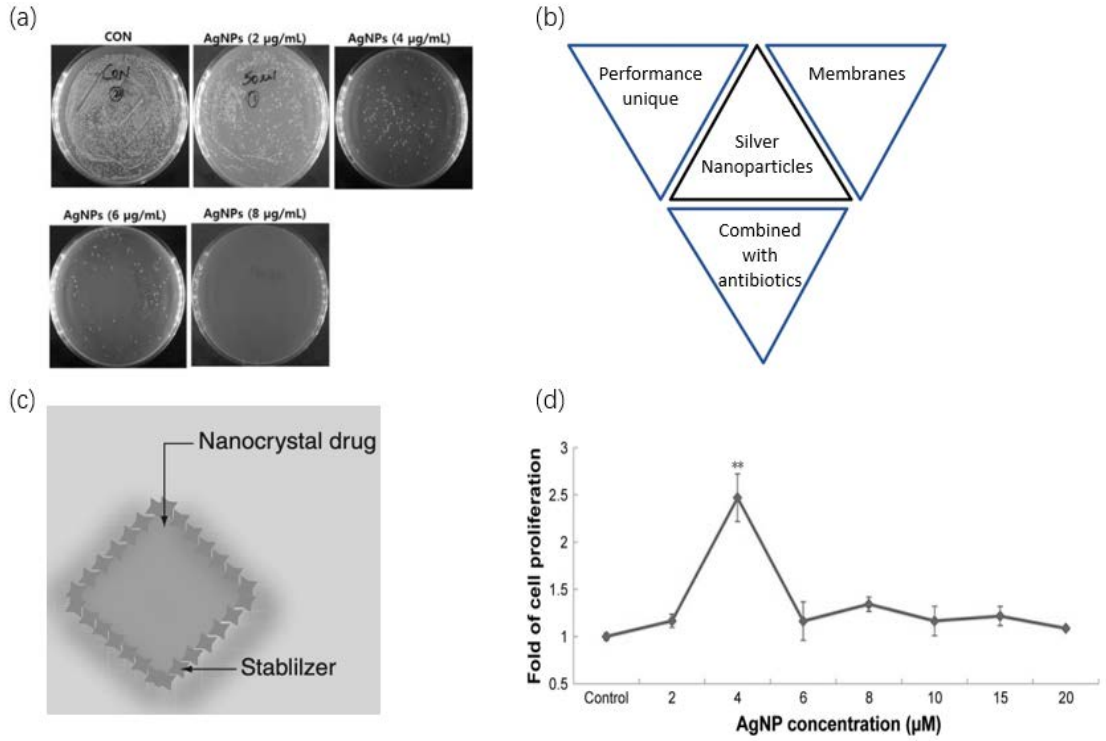

Figure 1. Application of AgNPs antibacterial properties. a) Dose-dependent antibacterial activity of biologically synthesized AgNPs in E. coli. b) The antibacterial application of silver nanoparticles in dentistry. c) Nanotechnology-based drug delivery platform d) AgNPs increase MSC proliferation and do not reduce cell viability at low concentrations.

3. AgNPs inhibit cancer cell growth

The number of new cancer cases has been increasing in the past few decades, which will achieve 24 million in 2035 [10]. The conventional treatment methods for cancer at this stage have frequent side effects and limitations, which influence the patients' recovery and health. For example, malignant tumors can develop multi-drug resistance (MDR), leading to chemotherapy failure [11]. Recently researchers turned their attention to silver nanoparticles (AgNPs), whose unique physicochemical characteristics offer new opportunities for cancer therapeutics.

3.1. Mechanism of the anticancer activity of AgNPs

AgNPs have broad-spectrum anticancer activity via multiple mechanisms. Numerous experiments in vitro and in vivo have proved that AgNPs can decrease the proliferation and viability of cancer cells. The Cytotoxicity of AgNPs could induce the apoptosis or necrosis of cells, which is an important method to fight cancer cells. Both AgNPs and Ag+ can act on the membrane proteins of cancer cells to inhibit cell proliferation by activating signal transduction pathways. They can participate in the mechanism of the signal relay in microorganisms, the cycle of phosphorylation, and dephosphorylation cascade by inhibiting the phosphorylation of proteins from inhibiting their enzymatic activity. It will result in inhibition of bacterial growth like DNA replication, recombination, metabolism, and bacterial cell cycle. In addition, Ag+ can damage DNA. Ag+ caused DNA damage by replacing the hydrogen bonds in the G≡C and A=T base pairs.

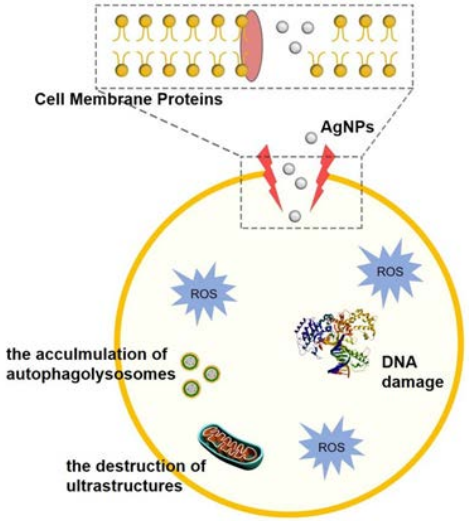

Figure 2. The mechanism of the anticancer activity of AgNPs. The several paths above may achieve the AgNPs anticancer effect: acting on the membrane proteins, DNA damage, the accumulation of autophagolysosomes, the destruction of ultrastructures, and ROS.

Autophagy-induced cell death is identified as an important mechanism of AgNPs anticancer activity. Nanoparticle-induced autophagy is a critical cell degradation process. AgNPs induce the continuous accumulation of autophagolysosomes in human ovarian cancer cells by increased autophagosome formation or in a PtdIns3K-dependent fashion to promote autophagy and cell death [12]. These autophagosomes subsequently fuse with lysosomes to form autolysosomes, leading to the degradation of the engulfed contents by lysosomal enzymes [13]. The destruction of ultrastructures such as cell membranes and intracellular organelles leads to cell apoptosis and necrosis. The higher the concentration of AgNPs and the longer the exposure time, the more severe the damage to the cell ultrastructure. AgNPs exposed cells undergo morphological changes of cytoplasmic organelle damage and undergo different death modes: apoptosis, necrosis, and autophagy. AgNPs lead to a high concentration of reactive oxygen species (ROS), which produce oxidative stress, contributing to autophagy, apoptosis, and necrosis of cancer cells [14]. AgNPs distributed in tumor cells via endocytosis can result in autophagy and apoptosis through various ROS-mediated stress responses. In addition, AgNPs-induced formation of ROS may affect cellular signal transduction pathways, which may participate in the activation of apoptosis. For example, the mitochondrial function can be inhibited by AgNPs via disrupting the mitochondrial respiratory chain, suppressing ATP production. Excessive ROS induced by AgNPs may ultimately lead to DNA damage in a concentration-dependent manner. The mechanism of toxicity of apoptosis induced by AgNPs is well established in various cell lines such as human lung cancer cells, ovarian, and breast cancer [15]. As a result, AgNPs are often used in cancer therapy as the excellent potential properties of physical, chemical, and biological.

3.2. Anticancer applications of AgNPs

Gopinath et al. observed that AgNPs not only induce apoptosis but also sensitize cancer cells. AgNPs induced alterations in human glioblastoma cell morphology, decreased its viability and metabolic activity, and increased oxidative stress leading to mitochondrial damage and increased production of reactive oxygen species (ROS), ending with DNA damage of human glioblastoma cells [16]. Albanese and her team studied a kind of AgNPs embedded into a specific polysaccharide (EPS) which works effectively on human breast and colon cancer cell lines (Figure3a). In particular, EPS induced a significant decrease of cell motility and MMP-2 and MMP-9 activity and a significant increase in ROS generation, which, in turn, supported cell death in both human breast and colon cancer cells mainly through autophagy and to a minor extend through apoptosis [17]. Yuan investigated that AgNPS worked effectively in human cervical cancer cells companying with Camptothecin (CPT) [15]. In this application, AgNPs can modulate the Pgp activity and enhance

chemotherapeutic efficacy in multidrug-resistant cancer cells to efficiently destroy human cervical cancer cells. Biologically synthesized AgNPs capable of altering cell morphology of cancer cells. The novel combinational formulation of AgNPs and CPT has an inhibitory effect on the proliferation of HeLa cells and overcome chemoresistance by enhancing cytotoxicity and apoptosis (Figure3b). Thus, it confirms that AgNPs can be combined with other chemical drugs to treat cervical cancer. With all this being said, AgNPs have shown excellent effects and good prospects in cancer treatment. AgNPs may also be used in synergistic or other anticancer treatments for different cancers. Anti-cancer or effects and further research is needed.

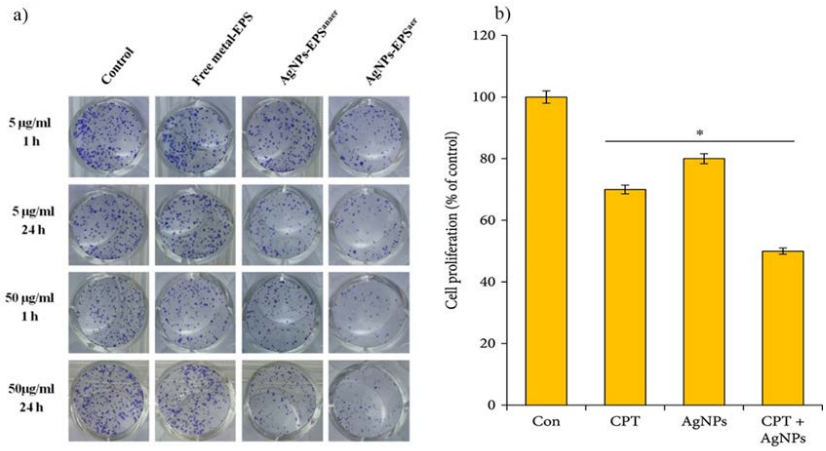

Figure 3. Certification of AgNPs Anticancer Properties. a) Cytotoxic effect of AgNPs-EPS in SKBR3 cells evaluated by clonogenic assay; b) The comparison of cell proliferation treated by CPT, AgNPs, or a combination of CPT and AgNPs.

4. Toxicity mechanisms of AgNPs

Any biomaterial for human use must be evaluated for its biosafety. At present, cytotoxicity assessment is listed in the first place in all biological evaluation standards of biomaterials at home and abroad. Cytotoxicity of biomaterials is a simple cell killing behavior caused by biomaterials, which is independent of the cell death process of apoptosis or necrosis. Nano-silver materials developed were widely used in medicine and other fields due to their good properties, but it was gradually found to have certain toxicity. Silver nanoparticles (AgNPs) are important nanomaterials and play an important role in nanoscience and nanotechnology, especially in nanomedicine, including potential applications in cancer diagnosis and treatment [16]. However, the high bioactivity of AgNPs is potentially harmful to the exposed tissues and cells, and it has been gradually found that AgNPs also have certain toxicity. The cytotoxic mechanism of AgNPs in vitro and in vivo has been demonstrated [18].

With the widespread use of AgNPs, a large amount of silver is released into estuaries or Marine aquatic systems every year, and these silver nanomaterials will cause irreversible harm to plants, aquatic organisms, and humans when they enter the environment [19, 20]. Silver ions deposited on the skin can cause silver poisoning, and the deposited area will turn livid. Clinically, local deposition of silver in soft tissues such as skin, liver, and spleen will cause allergic reactions and have certain toxicity to blood vessels and the gastrointestinal tract [21]. Inhalation of AgNPs over a long period, even at very low doses, can cause changes in lung function and inflammation [22].

Recently, it has been found that AgNPs can enter cells through the "Trojan horse" effect, bind to glutathione in cells, consume glutathione in vivo, and increase the vulnerable points of oxygen free radicals in cells, demonstrating the toxic effect of AgNPs on cells themselves [23, 24]. Under the condition of the weak acid, the toxicity inhibition of silver nanoparticles is the strongest. Researchers speculated that the cytotoxicity of silver nanoparticles might be due to their ability to cause oxidative stress in cells [25]. On the one hand, silver nanoparticles can enter the biological body in the form of nanoparticles (rather than the ionic state after the nanoparticles are dissolved), which will generate

protein adsorption and form silver nanoparticles covered with protein membranes, through which silver nanoparticles covered with protein membranes interact with cells. On the other hand, silver nanoparticles can be slightly dissolved in water and release Ag+ and Ag0, which have antibacterial activity, leading to the binding of silver nanoparticles to cells in a complex form and have certain toxic effects on cells.

Sayes et al. [26] reported that the cytotoxicity of nanomaterials could be explored through the study of cell morphology and cell membrane structure. As a result, on the one hand, the influence of nano-silver on cell attachment morphology can be directly observed by microscope, and the cytotoxicity of nano-silver can be qualitatively evaluated through the change of cell morphology. On the other hand, cell mortality can be measured by LDH activity detection method, the effect of nano-silver on cell membrane fluidity can be measured by DPH fluorescence labeling method, and the structural integrity of cell membrane can be observed by TEM to evaluate the cytotoxicity of nano-silver

Therefore, the evaluation of cytotoxicity of silver nanoparticles to human cells can provide an experimental basis for the biosafety evaluation of silver nanoparticles to a certain extent. Due to the cytotoxicity of silver nanoparticles, surface modification is needed to change the cytotoxicity of silver nanoparticles.

5. Modification method of AgNPs

In the existing technology, the surface modification method of silver nanoparticles usually adopts the addition of surfactants to form nanoparticles with a certain morphology and guide the growth. In existing technology on modification of nano silver method to a certain extent, some deficiencies, such as physical method preparation of nano silver on equipment demand, are higher. Hence, the preparation cost is relatively high, the chemical methods used in many of the reducing agents is toxic, protection requires a lot of surfactants generated particles stability is poorer, prone to reunite, uneven distribution of particle size. Thus, the use of nanometer silver is limited.

Dopamine is a kind of low molecular weight catecholamine that can oxidize and self-polymerize when exposed to air under weakly alkaline and oxidizing conditions. The oxidized dopamine molecules adhere to the surface of the substrate and form a polydopamine membrane. In addition to supertransport materials, dopamine can adhere to the surface of almost all materials, forming a polydopamine membrane. Based on this property of dopamine, dopamine can also act as a reducing agent to reduce silver ions to elemental silver and form "silver species" precursors to grow silver nanoparticles on the substrate.

Nanomaterials are pretreated with dopamine and then reduced on the composites by microwave heating in situ to form nano-silver materials with excellent properties, UV resistance, electrical conductivity, antimicrobial properties, water repellency, and nano effect. This kind of material has a wide application prospect in the fields of administration, medical and military.

Geisler-Lee et al. [27] found that the addition of L-cysteine can significantly reduce the uptake of AgNPs and AgNO3 by plants [28]. Thus, the toxic effect of AgNPs on cells was reduced. Cysteine reduced the absorption rate constant of AgNO3 and alleviated the toxicity of AgNO3 to wheat.

Inhibition of autophagy by 3-methyladenine (3-MA) can reduce cell apoptosis and DNA damage induced by silver nanoparticles. This study deepens the understanding of the cytotoxicity mechanism of silver nanoparticles and provides a strategy to reduce the toxicity of silver nanoparticles for better application of this material. Moreover, the conversion rate of silver nanoparticles increased significantly with the increase of solution pH and cumulative illumination intensity [29]. The acute toxicity of silver ions and nano-silver to macrofleas cells showed that the toxicity of silver to macrofleas was significantly reduced by reducing silver ions to nano-silver from organic matter in an aqueous solution under light conditions.

Relevant experiments show that the absorption peak strength of the generated silver nanoparticles increases with the increase of the interval time. With the increase of the interval time, more and more silver nanoparticles are generated [30], and the trend of the generation of silver nanoparticles is

consistent with that of PDA. Therefore, it can be preliminarily inferred that during the synthesis of PDA-Nano Ag, the reduced ability of PDA formed by the polymerization of DA under alkaline and aerobic conditions is stronger than that of DA. It enables the rapid and large amount of nano-silver to be produced, thus reducing the toxicity of silver. In addition, some studies have shown that the surface of nano-silver reduced by dopamine in situ has obvious cytotoxicity, which can be attributed to the rapid leaching of silver and the accumulation of silver in the culture medium [31]. For example, Zhang et al. [32] observed that the dopamine-loaded nano-silver coating had inhibitory effects on the adhesion and proliferation of osteoblasts, so it was speculated that it might have a better effect. It is of guiding significance for the construction of dopamine-modified silver coating on biomaterials.

The catechol group of dopamine has a good binding ability to metals, and the polydopamine composite layer has a strong reducing ability to metal ions. After soaking in the metal salt solution, the modified material with a polydopamine layer deposited on the surface will reduce the positive metal ions from the solution and deposit them on the material's surface, thus achieving metallization of the material surface without electroplating [33]. SERS signals were generated based on Raman molecular labeled silver nanoparticles, and the PDA layer was wrapped on the surface of the nanoparticles. Organic dyes were adsorbed to generate fluorescence signals by using the self-polymerization of DA. When used in cell imaging, good fluorescence and SERS signals could be obtained respectively by selecting appropriate excitation wavelength for fluorescence /SERS dual imaging. The composite nanoparticles can also be used for controlled drug release. According to the adhesion, reducing, and chelating properties of polydopamine, the targeted modification of composite nanoparticles can be further carried out. So the composite silver nanoparticles have potential application prospects in tumor targeting, disease diagnosis, and controlled drug release.

Due to their special physical and chemical properties, nano-silver materials have a wide range of applications in many fields such as catalysis, optics, electronics, surface tensile reinforcement, biomedical, and biosensors. These unique properties of nanomaterials are closely related to their size and shape, so it is very important to prepare nanomaterials with controllable morphology. Although the preparation methods of nanomaterials are increasingly diversified, the achievements in the controllable preparation of nanomaterials are still very limited. Therefore, it is of great significance and broad application prospect to find new synthesis methods to prepare silver nanostructures with adjustable size and morphology.

By optimizing nanomaterials' physical and chemical properties, nanomaterials can improve the direct effect of nanometer materials and biological and nanomaterials in organism stay, transformation, and discharge status. Mastery of this process will make people know more about reducing the toxicity of nanomaterials, and better use of its potential value, to provide medical, nanoscience, and technology in areas such as low toxicity even nontoxic nanomaterials.

6. Conclusion

With the rapid development of nanotechnology, more and more nanoproducts have come into people's life. While enjoying the great convenience of nanoproducts to people's lives, people are also confronted with some threats to health brought by them. To sum up, the nano silver anti-bacterial anti-cancer properties while can cause some damage to the body of each system, including the digestive system, respiratory system, reproductive system, nervous system, etc. However, most silver nanoparticle toxicity studies use animal and plant tests and in vitro culture cell test conclusions. Different test models will affect the toxic effect of silver nanoparticles. The special physical and chemical properties of silver nanoparticles will also affect the experimental results. Therefore, it is far from enough to extrapolate from the experimental results to the person only by relying on the conclusion of toxicology research. More evidence from epidemiological investigation and its risks and benefits should be considered comprehensively. At present, the studies on the toxicity of silver nanoparticles are mainly confined to the analysis of its biological distribution, cell damage, organ system damage, and other pathological studies. Still, the research on the mechanism is rarely carried out. This suggests that there is still a long way to go in future research on the toxicity mechanism and

modification of silver nanoparticles. It is necessary to explore the mechanism of the toxic effect of nano-silver and innovate new modification methods as soon as possible, facilitating the further application and development of nano-silver materials and laying a foundation for the establishment of risk assessment and biosafety assessment of nano-silver products.

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