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Review Article

Applications of Silver Nanoparticles for Medicinal Purpose

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Abstract

Nanoparticles studies have been going on for quite some time now, yet it still holds the scientist's interest as each day something new and something unique could be thought of and applied in different fields. The properties of metallic particles drastically changes into unique nature at the nanoscale dimensions compared to the bulk. Specifically, gold, silver, zink etc. nanomaterials possess unique physicochemical properties which gain a great deal of attention in biomedical applications. In this chapter, we are mainly discussing the different methods of synthesis of nanosilver particles (NSPs). The properties of silver nanoparticles such as broad-acting and potent antibacterial activity are widely investigated. Also highlighting the constrictions that need to be further studied and explored.

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INTRODUCTION

Nanotechnology is rapidly growing by producing nanoproducts and nanoparticles (NPs) that can have novel and size-related physicochemical properties differing significantly from larger matter. The novel properties of NPs have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, environmental remediation and biomedical devices. Among them, silver nanoparticles (Ag-NPs or nanosilver) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macroscale counterparts. Silver has known to be a metal that came into use even before the Neolithic revolution. Even the Greeks used it for cooking and to keep water safe. The first recorded medicinal use of silver was reported during the 8th century. Silver was known only as a metal till the recent past, and it is when the nano era came into existence that people started to believe that silver could even be produced at the nanoscale.

Silver nanoparticles are nanoparticles of silver of between 1 nm and 100n min size. They have unique optical, electrical, and thermal properties and are being incorporated into products that range from photovoltaic to biological and chemical sensors. There are many consumer products and applications utilizing nanosilver in consumer products; nanosilver-related applications currently have the highest degree of commercialization [1-3]. A wide range of nanosilver applications has emerged in consumer products ranging from disinfecting medical devices and home appliances to water treatments. Here we mainly discuss the applications of silver nanoparticles in nanomedicine. Nanomedicine is a branch of

medicine that applies the knowledge and tools of nanotechnology to the prevention and treatment of disease. Nanomedicine involves the use of nanoscale materials, such as biocompatible nanoparticles and nanorobots, for diagnosis, delivery, sensing or actuation purposes in a living organism (Figure 1).

SYNTHESIS ROOTS OF SILVER NPS

Generally, the synthesis of nanoparticles has been carried out using three different approaches, including physical, chemical, and biological methods. In physical methods, nanoparticles are prepared by evaporation-condensation using a tube furnace at atmospheric pressure. Conventional physical methods including spark discharging and pyrolysis were used for the synthesis of NSPs. The advantages of physical methods are speed, radiation used as reducing agents, and no hazardous chemicals involved, but the downsides are low yield and high energy consumption, solvent contamination, and lack of uniform distribution [4,5].

Chemical methods use water or organic solvents to prepare the silver nanoparticles. This process usually employs three main components, such as metal precursors, reducing agents, and stabilizing/capping agents. Basically, the reduction of silver salts involves two stages (1) nucleation; and (2) subsequent growth. In general, silver nanomaterials can be obtained by two methods, classified as "top-down" and "bottom-up". The "top-down" method is the mechanical grinding of bulk metals with subsequent stabilization using colloidal protecting agents. The "bottom-up" methods include chemical reduction, electrochemical methods, and sono-decomposition. The major

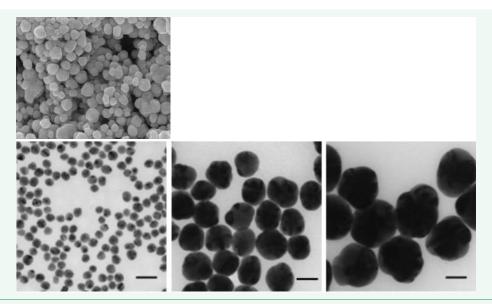


Figure 1 Transmission electron microscopy (TEM) images of silver nanoparticles with diameters of 20 nm 60 nm, and 100 nm respectively. Scale bars are 50nm.

advantage of chemical methods is high yield, contrary to physical methods, which have alow yield. The above-mentioned methods are extremely expensive. Additionally, the materials used for AgNPs synthesis, such as citrate, borohydride, thioglycerol, and 2-mercaptoethanol are toxic and hazardous. Apart from these disadvantages, the manufactured particles are not of expected purity, as their surfaces were found to be sediment with chemicals. It is also very difficult to prepare AgNPs with a well-defined size, requiring a further step for the prevention of particle aggregation. In addition, during the synthesis process, too many toxic and hazardous by-products are excised out [6,7].

PHYSICAL SYNTHESIS

For a physical approach, the metallic NPs can be generally synthesized by evaporation-condensation, which could be carried out by using a tube furnace at atmospheric pressure. A thermal-decomposition method was developed to synthesize AgNPs in powder form. The Ag-NPs were formed by decomposition of an ${\rm Ag^{1}}^{+}{\rm oleate}$ complex, which was prepared by a reaction with ${\rm AgNO_3}$ and sodium oleate in a water solution, at a high temperature of 290°C. Average particle size of the Ag-NPs was obtained of about 9.5 nm with a standard deviation of 0.7 nm. This indicates that the Ag-NPs have a very narrow size distribution [8].

There is another method to synthesize metal NPs via a small ceramic heater that has a local heating area. The small ceramic heater was used to evaporate source materials. The results showed that the geometric mean diameter, the geometric standard deviation and the total number concentration of NPs increase with heater surface temperature. The particle generation was very stable because the temperature of the heater surface does not fluctuate with time. Spherical NPs without agglomeration was observed, even at high concentration with high heater surface temperature. The generated Ag-NPs were pure silver when theair was used as a carrier gas. The geometric

mean diameter and the geometric standard deviation of Ag-NPs were in the range of 6.2-21.5 nm and 1.23-1.88 nm, respectively [9].

In summary, the physical synthesis process of Ag-NPs usually utilizes the physical energies (thermal, ac power, arc discharge) to produce Ag-NPs with nearly narrow size distribution. The physical approach can permit producing large quantities of Ag-NPs samples in a single process. This is also the most useful method to produce Ag-NPs powder. However, primary costs for theinvestment of equipment should be considered.

CHEMICAL SYNTHESIS

Among the existing methods, the chemical methods have been mostly used for theproduction of Ag-NPs. Chemical methods provide an easy way to synthesize Ag-NPs in solution. Monodisperse samples of silver nanocubes were synthesized in large quantities by reducing silver nitrate with ethylene glycol in the presence of polyvinylpyrrolidone (PVP), the so-called polyol process. In this case, ethylene glycol served as both reductant and solvent. It showed that the presence of PVP and its molar ratio relative to silver nitrate both played important roles in determining the geometric shape and size of the product. It suggested that it is possible to tune the size of silver nanocubes by controlling the experimental conditions [10].

Spherical Ag-NPs with controllable size and high monodispersity were synthesized by using the polyol process and a modified precursor injection technique. Nearly monodisperse Ag-NPs have been prepared in a simple oleylamine-liquid paraffin system. It was shown that the formation process of Ag-NPs could be divided into three stages: growth, incubation, and Ostwald ripening stages. In this method, only three chemicals, including silver nitrate, oleylamine and liquid paraffin, are employed throughout the whole process. The higher boiling point of 300°C of paraffin affords a broader range of reaction temperature and makes it possible to effectively control the size

of Ag-NPs by varying the heating temperature alone without changing the solvent. Otherwise, the size of the colloidal Ag-NPs could be regulated not only by changing the heating temperature, or the ripening time, but also by adjusting the ratio of oleylamine to the silver precursor [11].

Generally, the chemical synthesis process of the Ag-NPs in solution usually employs the following three main components: (i) metal precursors, (ii) reducing agents and (iii) stabilizing/capping agents. The formation of colloidal solutions from the reduction of silver salts involves two stages of nucleation and subsequent growth. It is also revealed that the size and the shape of synthesized Ag-NPs are strongly dependent on these stages. Furthermore, for the synthesis of monodispersed Ag-NPs with uniform size distribution, all nuclei are required to form simultaneously [12,13] (Figure 2).

PHOTOCHEMICAL SYNTHESIS

The NPs are formed by the direct photoreduction of a metal source or reduction of metal ions using photochemically generated intermediates, such as excited molecules and radicals, which are often called photosensitization in the synthesis of NPs. The direct photoreduction process of AgNO₃ in the presence of sodium citrate (NaCit) was carried out with different light sources (UV, white, blue, cyan, green and orange) at room temperature. A simple and reproducible UV photoactivation method for the preparation of stable Ag-NPs in aqueous Triton X-100 (TX-100) was reported. The TX-100 molecules play a dual role: they act as reducing agent and also as NPs stabilizer through template/capping action. In addition, surfactant solution helps to carry out the process of NPs growth in the diffusion controlled way (by decreasing the diffusion or mass transfer coefficient of the system) and also helps to improve the NPs size distributions (by increasing the surface tension of the solvent–NPs interface) [9,15].

The Ag-NPs can also be synthesized in an alkali aqueous solution of AgNO₃/carboxymethylated chitosan (CMCTS) with UV light irradiation. CMCTS, a water-soluble and biocompatible chitosan derivative, served simultaneously as a reducing agent for silver cation and a stabilizing agent for Ag-NPs in this method. It also revealed that the diameter range of as-synthesized Ag-NPs was 2-8 nm and they can be dispersed stably in the alkalic CMCTS solution for more than 6 months. In summary, the main advantages of the photochemical synthesis are: (i) it provides

the advantageous properties of the photoinduced processing, that is, clean process, high spatial resolution, and convenience of use, (ii) the controllable in situ generation of reducing agents; the formation of NPs can be triggered by the photo-irradiation and (iii) it has great versatility; the photochemical synthesis enables one to fabricate the NPs in various mediums including emulsion, surfactant micelles, polymer films, glasses, cells, etc [16].

BIOLOGICAL SYNTHESIS

In thebiological synthesis of Ag-NPs, the reducing agent and the stabilizer as in chemical methods are replaced by molecules produced by living organisms. These reducing and/or stabilizing compounds can be utilized by bacteria, fungi, yeasts, algae or plants. A facile biosynthesis using the metal-reducing bacterium, Shewanellaoneidensis, seeded with a silver nitrate solution, was reported [17]. The formation of small, spherical, nearly monodispersed Ag-NPs in size range from ~ 2 to 11 nm (average size of 4 \pm 1.5 nm) was observed. The Ag-NPs exhibit useful properties such as being hydrophilic, stable, and having a large surface area. This bacterially based method of synthesis is economical, simple, reproducible, and requires less energy when compared to chemical synthesis routes.

In another method, the use of the fungus Trichodermaviride (T. viride) for the extracellular biosynthesis of Ag-NPs from silver nitrate solution was reported. In this regard, T. Viride proves to be an important biological component for extracellular biosynthesis of stable Ag-NPs. The morphology of Ag-NPs is highly variable, with spherical and occasionally rod-like NPs observed on micrographs. The obtained diameter of Ag-NPs was in the range of from 5 to 40 nm. In another study, stable Ag-NPs of 5-15 nm in size were synthesized by using airborne bacteria (Bacillus sp.) and silver nitrate [18] (Figure 3).

The biological method provides a wide range of resources for the synthesis of Ag-NPs, and this method can be considered as an environmentally friendly approach and also as a low-cost technique. The rate of reduction of metal ions using biological agents is found to be much faster and also at ambient temperature and pressure conditions. In biological synthesis, the cell wall of the microorganisms pays a major role in the intracellular synthesis of NPs. The negatively charged cell wall interacts electrostatically with the positively charged metal ions and reduces the metal ions to NPs. When microorganisms are incubated with silver ions, extracellular Ag-NPs can be generated as an intrinsic defence mechanism against the metal's toxicity.

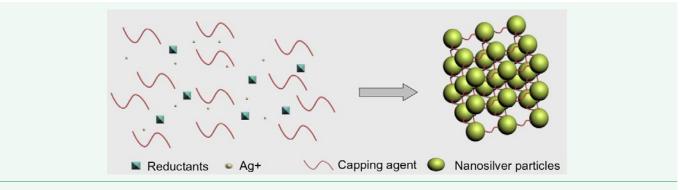


Figure 2 Chemical synthesis of nanosilver particles [14].

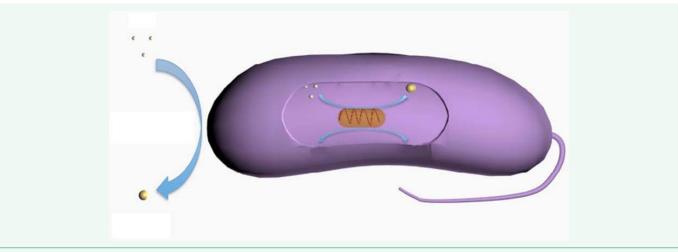


Figure 3 Biological (or green) synthesis of nanosilver particles [14].

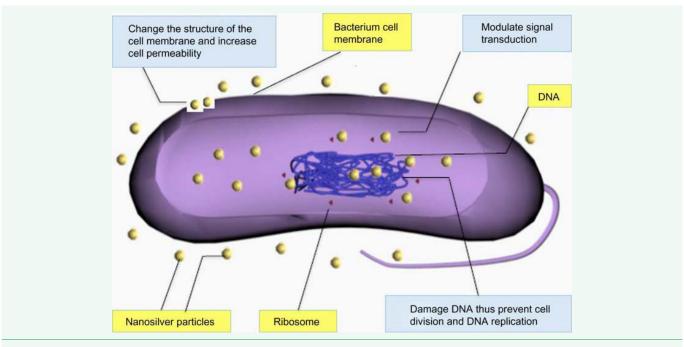


Figure 4 Antibacterial mechanism of nanosilver particles [14].

PROPERTIES OF SILVER NANOPARTICLES

Numerous shapes of nanoparticles can be constructed depending on the application at hand. There is growing interest in utilizing the optical properties of silver nanoparticles as the functional component in various products and sensors. Silver nanoparticles are extraordinarily efficient at absorbing and scattering light and, unlike many dyes and pigments, have a colour that depends on the size and the shape of the particle. It has become increasingly popular as antibiotic agents in textiles and wound dressings, medical devices, and appliances such as refrigerators and washing machines. They are traditionally defined as particles with overall dimensions below 100 nm, but the term 'nanosilver' is also becoming widely adopted, especially in the context of commercial products that contain nanomaterials with a large fraction of silver. Different properties

of silver nanoparticles are toxicity is antifungal, antibacterial, anti-inflammatory and toxicity etc [19].

Since silver nanoparticles undergo dissolution releasing silver ions, which is well-documented to have toxic effects, there have been several studies that have been conducted to determine whether the toxicity of silver nanoparticles is derived from the release of silver ions or from the nanoparticle itself. Several studies suggest that the toxicity of silver nanoparticles is attributed to their release of silver ions in cells as both silver nanoparticles and silver ions have been reported to have similar cytotoxicity [20]. NSPs may have potential toxicities at some concentrations and can cause various health problems if used improperly. Thus, it is necessary to address the biosafety of SNPs in human health. NSPs have been reported to be cytotoxic to several types of cells, including human peripheral blood mononuclear cells,

human alveolar epithelial cell line, murine and human alveolar macrophage cell line, neuroendocrine cells, rat liver cell line, and mouse germline cells. Although the details of the toxic mechanism are unclear, it suggests that NSPs are ionized in the cells, which leads to activate ion channels and changes the permeability of the cell membrane to both potassium and sodium, interaction with mitochondria, and induction of the apoptosis pathway via the production of reactive oxygen species, which leads to cell death [21].

Animal and human studies indicate that it is difficult to remove silver completely once it has been deposited in the body; however, nanosilver can be excreted through the hair, urine, and faeces. There is no consensus on nanosilver's toxicity to humans, and most toxicity investigations of silver nanoparticles are based on in vitro cellular experiments and relatively short-term animal experiments.

NSPs have a broad antibacterial effect on a range of Gramnegative and Gram-positive bacteria and antibiotic-resistant bacteria strains [22]. Antimicrobial efficacy of NSPs depends on their size and concentration. Normally, a high concentration leads to more effective antimicrobial activity, while particles of small sizes can kill bacteria at a lower concentration. Apart from size and concentration, the shape also influences the antimicrobial efficiency of NSPs (Figure 4).

Nanosilver is an effective antifungal agent against a broad spectrum of common fungi. NSPs can inhibit the growth of Candida albicans, Candida glabrata, Candida parapsilosis, Candida krusei, and Trichophytonmentagrophytes effectively. It can disrupt cellular membrane and inhibit the normal /budding process; however, the exact mechanisms of action of nanosilver against fungi are still not clear. Silver nanoparticles are also an antiviral agent against HIV-hepatitis B virus, respiratory syncytial virus, herpes simplex virus type 1, and monkeypox virus. It has been observed that NSPs have higher antiviral activity than silver ions, due to species difference as they dissolve to release Ag0 (atomic) and Ag+ (ionic) clusters, whereas silver salts release Ag+ only [23].

APPLICATIONS OF NANO SILVER PARTICLES IN NANOMEDICINE

Nanomedicine is the medical application of nanotechnology. Nanomedicine ranges from the medical applications of nanomaterials and biological devices to nanoelectronic biosensors and even possible future applications of molecular nanotechnology such as biological machines. Current problems for nanomedicine involve understanding the issues related to toxicity and environmental impact of nanoscale materials. Some of the applications of NSPs in nanomedicine are the following:

Cancer treatment

The application of NSPs in cancer is divided into diagnostic and therapeutic purposes. NSPs are capable of adsorbing cytosolic proteins on their surface that may influence the function of intracellular factors, and that they can regulate gene expression and pro-inflammatory cytokines. NSPs could alter the regulation of more than 1000 genes. Among several genes,

metallothionein, heat shock protein, and histone families were significant. Recently, autophagy-induced cell death has been another identified mechanism for the anti-cancer activity of NSPs. Autophagy induced by nanoparticles is a critical cellular degradation process, and elevated autophagy could promote cell death. Our recent findings show that NSPs are capable of inducing autophagy through the accumulation of autophagolysosomes in human ovarian cancer cells (figure). Therefore, autophagy can have a dual function; at lower levels, it can enhance the cell survival, and at elevated levels, it can cause cell death. Therefore, the use of autophagy inhibitors or autophagy protein-5 (ATG5)-small interfering RNAs (siRNA) enhanced NSPs induced cell death in cancer cells [24].

Nanomaterials have been used for diagnosis, treatment, and prevention of cancer using photo-based therapeutic approaches. The nanostructures are more capable of destroying the cancer cells than noncancer cells at low irradiation power density. According to this property, scientists developed sensitive and specific detection aptamer-based Ag-Au shell-core nanostructure-photothermal therapy in which the nanostructures were able to target the cells with high affinity and specificity. Several laboratories have addressed the enhancement of the therapeutic use of NSPs as nanocarriers for targeted delivery, chemotherapeutic agents, and as enhancers for radiation and photodynamic therapy. Recently, photo-based nanomedicine has gained much importance for cancer treatment among several approaches. NSPs can be used as nanocarriers for desired drugs for cancer treatment. However, there is a need to address the challenges and limitations of using nanoparticles for cancer therapy; these include physiological barriers, limited carrying capacity, enhanced permeability and retention effect (EPR), the variability of nanoparticles, and regulatory and manufacturing issues [25].

Therapeutic application

Fungal infections have become more common in the recent years. In particular, fungal infections are more frequent in patients who are immune -compromised because of cancer chemotherapy or organ or human immunodeficiency virus infections. The antifungal effects of silver nanoparticles and their mode of action were investigated. Antifungal effects on fungi tested with low hemolytic effects against human erythrocytes were observed. Although antifungal drug resistance does not seem to be as much of a problem as resistance to antibacterial agents in bacteria, one long-term concern is that the number of fundamentally different types of antifungal agents that are available for treatment remains extremely limited. These are because fungi are eukaryotic organisms with a structure and metabolism that are similar to those of eukaryotic hosts. Therefore, there is an inevitable and urgent medical need for antibiotics with novel antimicrobial mechanisms. Though the biocidal effect and mode of action of silver ions are known, nevertheless, the antifungal effects and the mode of action of NSPs against fungi have remained mostly unknown [26,27].

Wound dressing

Robert Burrell developed the world's first commercially available nanosilver product to treat various wounds in the clinic,



including burns, chronic ulcers, toxic epidermal necrolysis, and pemphigus NCP-loaded wound dressings significantly reduced the healing time by an average of 3.35 days and increased bacterial clearance from infected wounds compared to silver sulfadiazine, with no adverse effects [28-30].

Biodiagnostic applications

NSPs can be used for bio-diagnosis, where plasmonic properties of NSPs strongly depend on size, shape, and dielectric medium that surrounds it. Zhou et al., developed a silver nanoparticle array biosensor for clinical detection of serum p53 in head and neck squamous cell carcinoma. NSPs are also employed to produce dual-imaging/therapy-immuno target nanoshells to locate cancer cells and can absorb light and selectively destroy targeted cancer cells through photothermal therapy [31,32].

Catheters

Much research has been conducted to investigate NSPs as antibacterial materials for coating catheters, including central venous catheters and neurosurgical catheters. Because of their superior antibacterial properties and lack of observed toxicity, NSPs can decrease the incidence of bacterial infection and complications after surgery, thus they have been widely accepted for use in medical catheters [33,34].

CONCLUSION

Nanobiotechnology is an emerging field that has made its contribution to all spheres of human life. The biological synthesis of nanoparticles has paved for better methodologies and approaches in the medicinal field. Hence the development of better experimental procedures for the synthesis of nanoparticles of different chemical compositions, sizes, shapes and controlled polydispersity is vital for its advancement. Silver NPs have gained considerable interest because of their unique properties, and proven applicability in diverse areas such as medicine, catalysis, textile engineering, biotechnology, nanobiotechnology, bioengineering sciences, electronics, optics, and water treatment. It represents a prominent nanoproduct and is already widely used in medical applications, including wound dressing, diagnosis, and pharmacological treatment. Since the shape, size, and composition of NSPs can have significant effects on their function and possible risks to human health, extensive research is needed to fully understand their synthesis, characterization, and possible toxicity.

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