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REVIEW

Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives

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Abstract
In recent years the outbreak of re-emerging and emerging infectious diseases has been a significant burden on global economies and public health. The growth of population and urbanization along with poor water supply and environmental hygiene are the main reasons for the increase in outbreak of infectious pathogens. Transmission of infectious pathogens to the community has caused outbreaks of diseases such as influenza (A/H_5N_1), diarrhea *(Escherichia coli),* cholera *(Vibrio cholera),* etc throughout the world. The comprehensive treatments of environments containing infectious pathogens using advanced disinfectant nanomaterials have been proposed for prevention of the outbreaks. Among these nanomaterials, silver nanoparticles (Ag-NPs) with unique properties of high antimicrobial activity have attracted much interest from scientists and technologists to develop nanosilver-based disinfectant products. This article aims to review the synthesis routes and antimicrobial effects of Ag-NPs against various pathogens including bacteria, fungi and virus. Toxicology considerations of Ag-NPs to humans and ecology are discussed in detail. Some current applications of Ag-NPs in water-, air- and surface- disinfection are described. Finally, future prospects of Ag-NPs for treatment and prevention of currently emerging infections are discussed.

Keywords: silver nanoparticles, antimicrobial effects, toxicology, disinfectant, infectious diseases

Classification numbers: 2.05, 4.02, 5.02

1. Introduction

Nanotechnology is rapidly growing by producing nanoproducts and nanoparticles (NPs) that can have novel and size-related physico-chemical properties differing significantly from larger matter [1]. 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 [2–4]. Among them, silver nanoparticles (Ag-NPs or nanosilver) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts [5]. Ag-NPs have distinctive physico-chemical properties, including a high electrical and

thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and non linear optical behavior [6]. These properties make them of potential value in inks, microelectronics, and medical imaging [7]. Besides, Ag-NPs exhibit broad spectrum bactericidal and fungicidal activity [8] that has made them extremely popular in a diverse range of consumer products, including plastics, soaps, pastes, food and textiles, increasing their market value [9–11]. To date, nanosilver technologies have appeared in a variety of manufacturing processes and end products. Nanosilver can be used in a liquid form, such as a colloid (coating and spray) or contained within a shampoo (liquid) and can also appear embedded in a solid such as a polymer master batch or be suspended in a bar of soap (solid). Nanosilver can also be utilized either in the textile industry by incorporating it into the fiber (spun) or employed in filtration membranes of water purification systems. In many of these applications, the technological idea is to store silver ions and incorporate a time-release mechanism. This usually involves some form of moisture layer that the silver ions are transported through to create a long-term protective barrier against bacterial/fungal pathogens [9–11].

There are many consumer products and applications utilizing nanosilver in consumer products; nanosilver-related applications currently have the highest degree of commercialization. A wide range of nanosilver applications has emerged in consumer products ranging from disinfecting medical devices and home appliances to water treatments. According to the Project on Emerging Nanotechnologies (PEN, (http://www.nanotechproject.org) over 1300 manufacturer-identified, nanotechnology-enabled products have entered the commercial market place around the world. Among them, there are 313 products utilizing nanosilver (24% of products listed), this has made nanosilver the largest and fastest growing class of NPs in consumer products applications. According to the report of silver nanotechnology commercial inventory published in 2008, the health and fitness markets were found to be the biggest emergence of products utilizing nanosilver (131 records) compared to other categories such as appliances (15), medical applications (10), and electronics and computers (8). The worldwide market incorporating nanotechnology continues to grow on a rapid and consistent basis. With the world annual rate of increase ∼25%, the commercial nanotechnology industry value is predicted to increase significantly from $91 billion by 2009 to $1 trillion by 2015 and $3 trillion by 2020 (http://www.nanotechproject.org). Despite the historic use of nanosilver in health-related fields, however, the future prospect of silver nanotechnologies applications for other product fields, i.e., for environmental disinfections remains to be unexploited.

Because of their widespread applications, the scientific community and industry has paid special attention to the research topic of Ag-NPs. Figure 1 shows a statistical data analysis of the trend in published research papers in this area. These databases were collected up to 30 September, 2012 from 'ISI Web of Science' using the keyword 'silver nanoparticle'. It was found that there are a total of 18825 records. During the 10 years from 2001 to 2011, the number of published papers has grown by nearly 93% (from 247 articles by 2001 to 3603 articles by 2011). Figure 2(a) illustrates the topic of research areas on Ag-NPs. It includes chemistry (55%), materials science (40.4%), physics (27.3%), engineering (5.7%), polymer science (4.6%), optics (4.1%), spectroscopy (3.6%), electrochemistry (3.0%), molecular biochemistry (2.6%) and other topics (22.1%). Chemistry and materials science are now the largest in the research areas of Ag-NPs. In addition, the data analysis also includes published papers from 96 different countries. Figure 2(b) illustrates the breakdown of articles by countries. It indicates that China has the most published articles on the Ag-NPs topic with a total of 4434 (23.6%), followed by USA 3809 (20.7%), India 1842 (9.8%), South Korea 1331 (7.1%), Japan 1283 (6.8%), Germany 1079 (5.7%), France 770 (4.1%), Taiwan 669 (3.6%), Spain 540 (2.8%), Russia 539 (2.8%) and elsewhere around the world. China and USA are now the largest countries in published papers concerning Ag-NPs. It is emphasized that a large number of practical applications utilizing Ag-NPs in consumer products are being developed in parallel with study of these syntheses and properties.

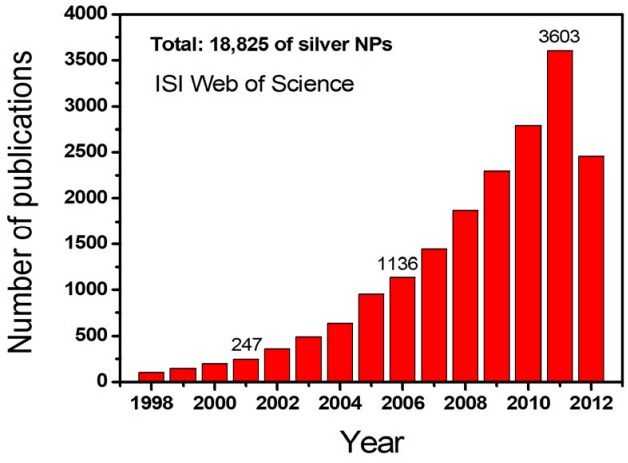

Figure 1. The trend in published research articles on the topic of silver nanoparticles. These databases were collected from 'ISI Web of Science' using the keyword 'silver nanoparticle', up to 30 September, 2012.

In recent years a growing number of outbreaks of infectious diseases have emerged. For an example, in early May 2011, an outbreak of diarrhea disease caused by an unusual serotype of Shiga-toxin–producing *Escherichia coli* (O104 : H4) began in Germany with a large number of cases of diarrhea with 3167 without the hemolytic–uremic syndrome (16 deaths) and 908 with the hemolytic–uremic syndrome (34 deaths) [12]. These infectious diseases have not only occurred in developing countries with low levels of hygiene and sanitation, but have also been recognized in developed countries. Food and waterborne pathogens are the main factors for the outbreak of these diseases, the transmission of these pathogens endangering public health. The outbreak of re-emerging and emerging infectious diseases are a significant burden on global economies and public health [13]. Their emergence is thought to be driven largely by socio-economic, environmental and ecological factors. To prevent further spread of the infectious pathogens, disinfection methods should be done properly to eliminate these pathogens from infected environmental areas, and effective treatments should

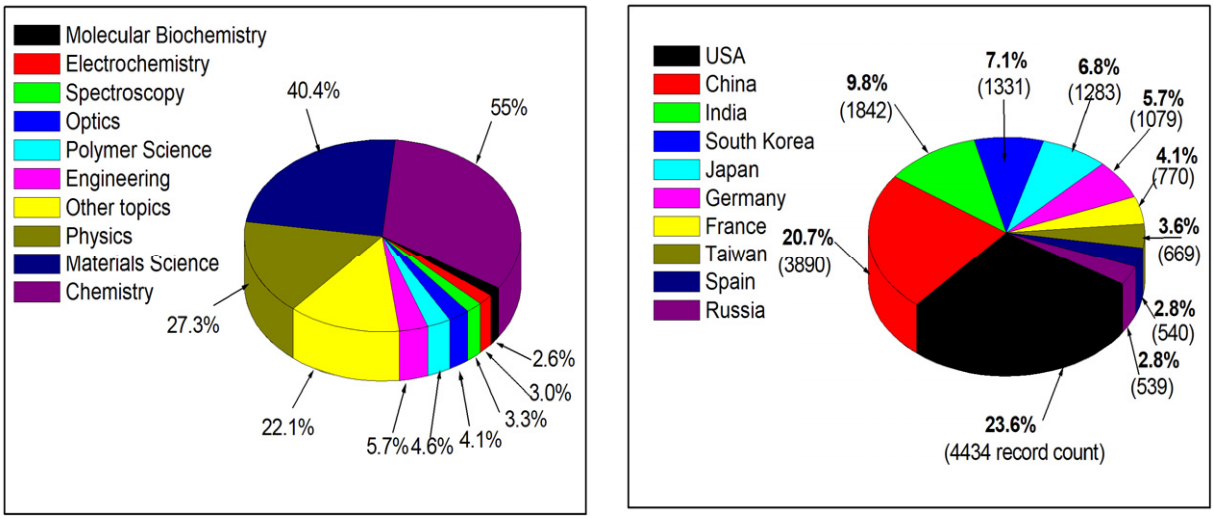

Figure 2. Database analyses are divided according to (a) research areas and (b) countries/regions.

also be carried for patients in hospitals and in the community. Particularly, the noble metal Ag-NPs are drawing increasing attention for potential prevention of bacterial/fungal and viral infections due to their well-documented antimicrobial and disinfectant properties. The generation of stable and efficient Ag-NPs forms offers an advanced perspective in the field of environmental hygiene and sterilization.

This paper aims to review synthesis routes and antimicrobial effects of Ag-NPs against various pathogens including bacteria, fungi and viruses. Next, toxicology considerations of Ag-NPs to humans and ecology are discussed in detail. Some current applications of Ag-NPs for environmental treatments are described. Future prospects of Ag-NPs for treatment and prevention of currently emerging infections are discussed.

The review paper is divided into seven parts. Section 1 provides an introduction of studies and current application fields of Ag-NPs. In section 2, the synthesis routes for production of the different Ag-NPs are presented. Various techniques for synthesis of Ag-NPs are discussed. In section 3, the antimicrobial properties of Ag-NPs against fungus, bacteria and virus are described. Potential mechanisms for antimicrobial activity of Ag-NPs are discussed. In section 4, the use of Ag-NPs for some environmental treatments applications developed is given. In section 5, toxicological considerations of the different Ag-NPs to human health and ecology are discussed in detail. In section 6, future prospects for the use of silver-based NPs in environmental treatments containing infectious pathogens are described. Finally, some concluding remarks are given in section 7.

2. Synthesis of silver nanoparticles

2.1. Chemical synthesis

Currently, many methods have been reported for the synthesis of Ag-NPs by using chemical, physical, photochemical and biological routes. Each method has advantages and disadvantages with common problems being costs, scalability, particle sizes and size distribution. Among the existing methods, the chemical methods have been mostly used for production 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) [14], 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.

Spherical Ag-NPs with a controllable size and high monodispersity were synthesized by using the polyol process and a modified precursor injection technique [15]. In the precursor injection method, the injection rate and reaction temperature were important factors for producing uniform-sized Ag-NPs with a reduced size. Ag-NPs with a size of 17 ± 2 nm were obtained at an injection rate of 2.5 ml s^{-1} and a reaction temperature of 100 °C. The injection of the precursor solution into a hot solution is an effective means to induce rapid nucleation in a short period of time, ensuring the fabrication of Ag-NPs with a smaller size and a narrower size distribution.

Nearly monodisperse Ag-NPs have been prepared in a simple oleylamine-liquid paraffin system [16]. It was shown that the formation process of Ag-NPs could be divided into three stages: growth, incubation and Oatwald 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.

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 monodispered Ag-NPs with uniform size distribution, all nuclei are required to form at the same time. In this case, all the nuclei are likely to have the same or similar size, and then they will have the same subsequent growth. The initial nucleation and the subsequent growth of initial nuclei can be controlled by adjusting the reaction parameters such as reaction temperature, pH, precursors, reduction agents (i.e. $NaBH_4$, ethylene glycol, glucose) and stabilizing agents (i.e. PVA, PVP, sodium oleate) [17–19].

2.2. 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. However, in the case of using a tube furnace at atmospheric pressure there are several drawbacks such as a large space of tube furnace, great consumption energy for raising the environmental temperature around the source material and a lot of time for achieving thermal stability. Therefore, various methods of synthesis of Ag-NPs based on the physical approach have been developed.

A thermal-decomposition method was developed to synthesize Ag-NPs in powder form [20]. The Ag-NPs were formed by decomposition of a Ag^{1+}–oleate complex, which was prepared by a reaction with $AgNO_3$ and sodium oleate in a water solution, at 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.

In another work Jung *et al* [21] reported an attempt 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 were observed, even at high concentration with high heater surface temperature. The generated Ag-NPs were pure silver, when air 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.

Tien *et al* [22] used the arc discharge method to fabricate Ag-NPs suspension in deionized water with no added surfactants. In this synthesis, silver wires (Gredmann, 99.99%, 1 mm in diameter) were submerged in deionized water and used as electrodes. The experimental results show that Ag-NPs suspension fabricated by means of arc discharge method with no added surfactants contains metallic Ag-NPs and ionic silver. With a silver rod consumption rate of 100 mg min^{-1}, yielding metallic Ag-NPs of 10 nm in size and ionic silver obtained at concentrations of approximately 11 ppm and 19 ppm, respectively.

More recently Siegel *et al* [23] reported on the development of an unconventional approach for the physical synthesis of gold-NPs and Ag-NPs. The notable metallic NPs were synthesized by direct metal sputtering into the liquid medium. The method, combining physical deposition of metal into propane-1,2,3-triol (glycerol), provides an interesting alternative to time-consuming, wet-based chemical synthesis techniques. From this method, both Au-NPs and Ag-NPs possess round shape with average diameter of about 3.5 nm with standard deviation of 1.5 and 2.4 nm, respectively. It was observed that the NPs size distribution and uniform particle dispersion remains unchanged for diluted aqueous solutions up to glycerol-to-water ratio 1 : 20.

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 investment of equipment should be considered.

2.3. Photochemical synthesis

The photo-induced synthetic strategies can be categorized into two distinct approaches, that is the photophysical (top down) and photochemical (bottom up) ones. The former could prepare the NPs via the subdivision of bulk metals and the latter generates the NPs from ionic precursors. The NPs are formed by the direct photoreduction of a metal source or reduction of metal ions using photo-chemically generated intermediates, such as excited molecules and radicals, which is often called photosensitization in the synthesis of NPs [24, 25].

The direct photo-reduction process of $AgNO_3$ in the presence of sodium citrate (NaCit) was carried out with different light sources (UV, white, blue, cyan, green and orange) at room temperature [26]. It was shown that this light-modification process results in a colloid with distinctive optical properties that can be related to the size and shape of the particles. A simple and reproducible UV photo-activation method for the preparation of stable Ag-NPs in aqueous Triton X-100 (TX-100) was reported [27]. 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 co–efficient of the system) and also helps to improve the NPs size distributions (by increasing the surface tension at the solvent–NPs interface).

In another study, the Ag-NPs were synthesized in an alkalic aqueous solution of $AgNO_3$/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 [28]. 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 photo-induced 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 [25].

2.4. Biological synthesis

As mentioned above, when Ag-NPs are produced by chemical synthesis, three main components are needed: a silver salt (usually $AgNO_3$), a reducing agent (i.e. ethylene glycol) and a stabilizer or aping agent (i.e. PVP) to control the growth of the NPs and prevent them from aggregating. In case of the biological synthesis of Ag-NPs, the reducing agent and the stabilizer are replaced by molecules produced by living organisms. These reducing and/or stabilizing compounds can be utilized from bacteria, fungi, yeasts, algae or plants [29].

A facile biosynthesis using the metal-reducing bacterium, *Shewanella oneidensis*, seeded with a silver nitrate solution, was reported [30]. The formation of small, spherical, nearly monodispersed Ag-NPs in the size range from ∼2 to 11 nm (average size of 4 ± 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 study, the use of the fungus *Trichoderma viride* (*T. viride*) for the extracellular biosynthesis of Ag-NPs from silver nitrate solution was reported [31]. 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 an airborne bacteria (*Bacillus* sp.) and silver nitrate [32]. The biogenic NPs were observed in the periplasmic space of the bacterial cells, which is between the outer and inner cell membranes.

Also, the Ag-NPs were produced by using the *Lactobcillus* spp. as reducing and capping agent. Sintubin *et al* [33] were carried with different *Lactobcillus* species to accumulate and subsequently reduce Ag^+. The result showed that only the lactic acid bacterial were confirmed to have the ability to produce Ag^0. In addition, both particle localization and distribution inside the cell were dependent on *Lactobcillus* species. The mean diameter of the biogenic Ag-NPs produced by this method varied with the *Lactobacillus* spp. used. The smallest NPs were produced by *L. fermentum* and had a diameter of 11.2 nm. The recovery of silver and the reduction rate were pH dependent.

On the other hand, Naik *et al* [34] have demonstrated the biosynthesis of biogenic Ag-NPs using peptides selected by their ability to bind to the surface of silver particles. By the nature of peptide selection against metal particles, a 'memory effect' has been imparted to the selected peptides. The silver-binding clones were incubated in an aqueous solution of 0.1 mM silver nitrate for 24–48 h at room temperature. The silver particles synthesized by the silver-binding peptides showed the presence of silver particles 60–150 nm in size.

In summary, 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 bioreduces the metal ions to NPs [35]. When microorganisms are incubated with silver ions, extracellular Ag-NPs can be generated as an intrinsic defense mechanism against the metal's toxicity. Other green syntheses of Ag-NPs using plant exacts as reducing agents have been performed [36, 37]. This defense mechanism can be exploited as a method of NPs synthesis and has advantages over conventional chemical routes of synthesis. However, it is not easy to have a large quantity of Ag-NPs by using biological synthesis. Characteristics of synthesis routes of the Ag-NPs are summarized in table 1.

3. Antimicrobial effects of Ag-NPs

3.1. Antibacterial effects

The Ag-NPs have been demonstrated as an effective biocide against a broad-spectrum bacteria including both Gram-negative and Gram-positive bacteria [43], in which there are many highly pathogenic bacterial strains. Table 2 summarizes the exhibited antibacterial activities of Ag-NPs, the data were collected from recent publications.

In 2004 Sondi and Salopeck-Sondi [44] reported the antimicrobial activities of Ag-NPs against the growth of *E. coli* on Luria–Bertani agar plates. In this study, the *E. coli* bacterial strain served as a model of Gram-negative bacteria. Results showed that the growth inhibition of *E. coli* was dependent on the concentration of Ag-NPs and the initial concentration of cultivated bacteria. The growth inhibitory concentrations were found to be about 50–60 and 20 $\mu g\,cm^{-3}$ for 10^5 CFU and 10^4 CFU of *E. coli,* respectively. Noticeably, the bacterial cells were damaged and destroyed along with the accumulation of Ag-NPs in the bacterial membrane. Morones *et al* [45] have also used different types of Gram-negative bacteria to test the antibacterial activities of Ag-NPs in the range of 1–100 nm. It was reported that the antibacterial activity of Ag-NPs against Gram-negative bacteria divided into three steps: (i) nanoparticles mainly in the range of 1–10 nm attach to the surface of the cell membrane and drastically disturb its proper functions, such as permeability and respiration; (ii) they are able to penetrate inside the bacteria and cause further damage by possibly interacting with sulfur- and phosphorus-containing compounds such as DNA; (iii) nanoparticles release silver ions, which will have an additional contribution to the bactericidal effect of Ag-NPs. In addition, Kim *et al* [46] have used a model of both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria to investigate the antibacterial activities of Ag-NPs. Their studies revealed that *E. coli* is inhibited at a low concentration of Ag-NPs (3.3 nM), and ten times less

Table 1. Characteristics of synthesis routes of the Ag-NPs.

Methods	Precursors	Reducing agent or solvent	Stabilizer or surfactant	Particle morphology and size	Influencing factors or features	Ref.
Chemical synthesis	$AgNO_3$	Trisodium citrate	Trisodium citrate	Nanospheres 30–60 nm	Concentration of silver ion	[38]
	$AgNO_3$	Ethylene glycol	Poly(vinyl pyrrolidone) (PVP)	Nanocubes $\sim$ 50–115 nm	Temperature, concentration of $AgNO_3$ and PVP	[14]
	$AgNO_3$	$NaBH_4$	Dodecanoic acid (DDA)	Nanospheres $\sim$7 nm	Highly concentrated silver	[39]
	$AgNO_3$	Ethylene glycol	PVP	Nanospheres 17 ± 2 nm	Heating rate, reaction temperature and injection rate	[15]
	$AgNO_3$	Paraffin	Oleylamine	Nanospheres 10–14 nm	Temperature, ripening time and concentration of OLA and silver ion.	[16]
	$AgNO_3$	Thermal decomposition	Sodium oleate	Nanosilver powder 9.5 ± 0.7 nm	Decomposition temperature	[13]
Physical synthesis	Ag target	ac power		Nanospheres 6.2–21.5 nm	Temperature	[14]
	Ag foil	Ion beam	Silica	Nanospheres 2.2 ± 0.3 to 5.2 nm	Concentration of Ag	[40]
	Ag wires	Electrical arc discharge, water		Nanospheres $\sim$10 nm	Silver rod consumption rate	[15]
	$AgNO_3$	Electrical arc discharge	Sodium citrate	Nanospheres 14–27 nm	Arc current, duration arc	[41]
	Ag target	Sputtering current, glycerol and water		Nanospheres 3.5 ± 2.4 nm	Deposition time, sputtering current	[23]
	$AgNO_3$	TX-100, UV	TX-100	Nanospheres 30 nm	Concentration of TX-100 and Ag ion	[27]
Photochemical synthesis	$AgNO_3$	Carboxymethy lated chitosan (CMCTS), UV	CMCTS	Nanocubics 2–8 nm	pH, concentration of CMCTS	[28]
	$AgNO_3$	Sodium citrate, light sources	Sodium citrate	Ag colloids	Irradiation time, light source	[26]
	Triangular Ag nanoplate	UV, water	PVP	Nanospheres, thick round plates	Duration of UV irradiation	[42]
Biological Synthesis	$AgNO_3$	Peptide	Peptide	Hexagonal, spheres and triangular 60–150 nm	Nature of peptide	[34]
	$AgNO_3$	*Bacillus* sp.	*Bacillus* sp.	Nanospheres 5–15 nm	Aerobic conditions	[32]
	$AgNO_3$	*Lactobcillus*	*Lactobcillus*	Nanospheres 6–15.7 nm	pH, *Lactobcillus* species	[33]
	$AgNO_3$	*Shewanella oneidensis*	Proteins	Nanospheres 2–11 nm	pH, Ag ion concentration	[30]
	$AgNO_3$	Fungus *T. viride*	*T. viride*	Nanospheres, rod 5–40 nm	pH, temperature Ag ion concentration	[31]
	$AgNO_3$	*Cassia angustifolia*	*C. angustifolia*	Nanospheres, rod 9–31 nm	pH, temperature, Ag ion concentration	[36]
	$AgNO_3$	*Daucus carota*	*D. carota*	Nanospheres, 20 nm	Reducing agent, absorbing species	[37]

Table 2. Antimicrobial effects of Ag-NPs.

Characterization of Ag-NPs					
Type	Particle size	Surface stability	Microbial strains	Major outcomes	Ref.
1. Antibacterial effects					
Ag-NPs powder	12 nm	None	*E. coli*	Growth inhibitory concentration: 50–60 μg cm^{-3} (10^5 CFU), and 20 μg cm^{-3} (10^4 CFU)	[44]
Ag-NPs powder	1–100 nm	Carbon matrix	*E. coli, V. cholera, P. aeruginosa* and *Salmonella typhus*	Growth inhibitory concentration: 75 μg ml^{-1} *P. aeruginosa* and *V. cholera* were more resistant than *E. coli* and *S. typhus*	[45]
Ag-NPs	13.4 nm	None	*E. coli* and *S. aureus*	*Minimum* inhibitory concentration: >3.3 nM (*E. coli*) and >33 nM (*S. aureus*)	[46]
Ag-NPs in aqueous media	10–15 nm	None	*E. coli*, ampicillin-resistant *E. coli*, multi-drug resistant *Salmonella typhi* and *S. aureaus*	Growth inhibitory concentration: 25 μg ml^{-1} for *E. coli,* ampicillin-resistant *E. coli* and multi-drug resistant strains of *S. typhi* Undetermined data for *S. aureus*	[47]
Ag-NPs in different shapes	Different sizes and shapes	None	*E. coli*	Growth inhibitory concentration: 1 μg (truncated triangular particles) 50–100 μg (spherical particles) >100 μg (rod-shape particles)	[48]
Ag-NPs in PDDA	26 nm	Polymers/ surfactants	Standard strains, and isolated from human clinical samples	Minimum inhibitory concentration: 1.69–13.5 μg ml^{-1} 1 μg ml^{-1} SDS-modified Ag-NPs	[49]
Ag-NPs in culture media	100 nm	NA	Drug-resistant bacteria: erythromycin-resistant *S. Pyogenes,*-ampicillin-resistant *E. coli* O157 : H7, and multidrug-resistant *P. aeruginosa* Drug-susceptible bacteria: *Streptococcus* sp., *E. coli*, and *P. aeruginosa*	Minimum inhibitory concentrations (in average): 79.4 nM for drug-resistant bacteria and 71.5 nM for drug-susceptible bacteria Minimal bactericidal concentrations (in average): 83.3 nM for drug-resistant bacteria and 74.3 nM for drug-susceptible bacteria	[56]
Ag-NPs powder	NA	NA	*E. coli* and *S. aureus*	Minimum inhibitory concentration: 100 μg ml^{-1}	[75]
Ag-NPs	9–10 nm	Oleate ions	*E coli* and *V. cholerae*	Minimum inhibitory concentration: ~3 μg ml^{-1} for both bacterial strains	[55]
Ag-NPs	8–50 nm	SDS	*E. coli, P aeruginosa* and *S. aureus*	Minimum inhibitory concentration: <7 ppm	[50]

Table 2. Continued.

Characterization of Ag-NPs					
Type	Particle size	Surface stability	Microbial strains	Major outcomes	Ref.
2. Antifungal effects					
Ag-NPs	~ 3 nm	None	44 strains of 6 fungal species from clinical isolates and ATCC strains of *T mentagrophytes* and *C. albicans*	IC_{80}: 1–7 μg ml^{-1}	[60]
Ag-NPs-Coated plastic catheters	3–18 nm	NA	*C. albicans*	Growth inhibition of Ag-NPs - coated catheter (80–120 nm in thick) was almost complete for *C. albicans*	[62]
Ag-NPs	25 nm	None, and SDS	*Candida* spp.	Minimum inhibitory concentration: 210 μg ml^{-1} for naked Ag-NPs 50 μg ml^{-1} for Ag-NPs modified with SDS	[63]
Ag-NPs	~ 5 nm	None	*C. albicans* and *Candida glabrata*	Minimum inhibitory concentration: 0.4–3.3 μg ml^{-1}	[64]
Ag-NPs	28.2–100 nm	None	*T. rubrum*	*Minimum* inhibitory *Concentration:* 10 μg m1^{-1}	[65]
3. Antiviral effects					
Ag-NPs	1–10 nm	Carbon, PVP and BSA	HIV-1	Ag-NPs undergo a size-dependent interaction with HIV-1 (1–10 nm), and inhibit the virus from binding to host cells	[67]
Ag-NPs	10, 50 and 800 nm	None	HBV	Inhibition of HBV replication (Ag-NPs,10 nm)	[69]
Ag-NPs	NA	PVP, recombinant RSV fusion (F) protein, and BSA	RSV	44% inhibition of syncytial virus infection for PVP-coated Ag-NPs	[70]
Ag-NPs	10–80 nm	None, or polysaccharide coating	Monkeypox virus (MPV)	Ag-NPs of approximately 10 nm inhibit MPV infection *in vitro*	[71]
Ag-NPs	11.2 nm	Biogenic Ag^0	MNV-1	Addition of 31.25 mg biogenic Ag^0 m^{-2} on the filter caused a 3.8-log decline of the virus as compared with a 1.5-log decrease by the original filter	[72]
Ag-NPs	10 nm	None	H1N1 influenza A virus	Efficient inhibitory activity on H1N1 influenza A virus	[73]

IC_{80}: 80% inhibitory concentration; SDS: sodium dodecyl sulfate; PDDA: poly (diallyldimethylammonium) chloride; PVP: poly (*N*-vinyl-2-pyrrolidone); BSA: bovine serum albumin; NA: not available.

than the minimum inhibitory concentration on *S. aureus* (33 nM). In another report, Shrivastava *et al* [47] described the strong antibacterial potency of novel Ag-NPs in the range of 10–15 nm with increased stability against some strains of non-resistant and drug-resistant bacteria. It was concluded that the antibacterial effect is dose-dependent and is more pronounced against Gram-negative than Gram-positive bacteria; it was also independent of acquisition of resistance by the bacteria against antibiotics. It was also suggested that the major mechanism in which Ag-NPs manifested antibacterial properties was by anchoring to and penetrating the bacterial cell wall, and modulating cellular signaling by dephosphorylating putative key peptide substrates on tyrosine residues.

In a comparative study of the effect of the Ag-NPs in different shapes on the Gram-negative bacterium, Pal

et al [48] have demonstrated that Ag-NPs undergo shape-dependent interaction with *E. coli*. Truncated triangular silver nanoplates with a {111} lattice plane as the basal plane displayed the strongest biocidal action, compared with spherical and rod-shaped nanoparticles, and with ionic silver.

Kvitek *et al* [49] have reported that the antibacterial activity of Ag-NPs is also dependent on surface modifications (surfactant/polymers). In their study, different types of surfactants/polymers (sodium dodecyl sulfate-SDS and polyoxyethylenesorbitane monooleate-Tween 80), and one polymer (polyvinylpyrrolidone-PVP 360) were used. These stabilized Ag-NPs were tested with some bacterial strains including *S. aureus, E. faecalis*, *E. coli* and *P. aeruginosa*, and other strains isolated from human clinical samples such as *P. aeruginosa*, methicillin-susceptible *S. epidermidis*, methicillin-resistant *S. epidermidis*, methicillin-resistant *S. aureus*, vancomycin-resistant *E. faecium* and *K. pneumonia.* The obtained results showed the minimum inhibitory concentrations (MICs) of Ag-NPs in the range of 1.69–13.5 μg ml^{-1}, depending on bacterial strains, and the use of surfactants/polymers. Specifically, the antibacterial activity of the Ag-NPs was significantly enhanced when modified by SDS where the MIC decreased under the 'magical value' of 1 μg ml^{-1}. Furthermore, Guzman *et al* [50] have reported the results of antibacterial activities of synthesized Ag-NPs against *E. coli, P. aeruginosa* and *S. aureus* around 14.38, 6.74, and 14.38 ppm, respectively.

In our studies, Ag-NPs were synthesized by different techniques and tested with several bacterial strains such as *E. coli*, *S. aureus*, *V. cholera,* etc. The low MICs were found against these bacterial strains [51–55]. Especially, oleic acid stabilized-Ag-NPs showed the MIC against *E. coli* as low as 1 μg ml^{-1} [51].

Recently, the increasing number of drug-resistant bacteria has become a major challenge endangering human health. Ag-NPs have been also demonstrated as an effective biocide against these drug-resistant strains [43, 49, 56, 57]. In a study, Lara *et al* [56] have tested the antibacterial activities of commercial Ag-NPs (100 nm), and results revealed a minimum inhibitory concentration (on average) at 79.4 nM for drug-resistant bacteria such as Erythromycin-resistant *Streptococcus. pyogenes* (66.7 nM), ampicillin-resistant *E. coli* O157:H7 (83.3 nM) and multidrug-resistant *P. aeruginosa* (83.3 nM), and at 74.3 nM for drug-susceptible tested bacterial strains. This study also showed the MICs at 100 nM for methicillin-resistant *S. aureus* (MRSA), and at 200 nM for drug-susceptible *S. aureus*.

To date, there have been many studies on the effects of Ag-NPs against different bacterial strains. Although some articles proposed different ways to explain the growth inhibition and death of bacterial cells acted on by Ag-NPs [44, 46, 48], but the exact antibacterial mechanism of Ag-NPs has not been fully understood. In a recent review, Jones and Hoek [43] summarized three most common antibacterial mechanisms of Ag-NPs as follows: (i) uptake of free silver ions followed by disruption of ATP production and DNA replication, (ii) Ag-NPs and silver ion generation of reactive oxygen species (ROS) and (iii) Ag-NPs' direct damage to cell membranes. However, further investigations are still needed to demonstrate more clearly this mechanism, especially our question concerning the affinity of Ag-NPs to sulfur- and phosphorus-containing proteins of bacteria, and the effects of this affinity to the functions of bacterial proteins [54, 55].

Of course, with observed excellent antibacterial properties, Ag-NPs have been suggested as effective broad-spectrum biocides against a variety of drug-resistant bacteria, and a potential candidate for use in pharmaceutical and medical products in order to prevent the transmission of drug-resistant pathogens in different clinical environments [58].

3.2. Antifungal effects

Fungi are increasingly recognized as major pathogens in critically ill patients, especially nosocomial fungal infections [59]. Although the antibacterial activities of Ag-NPs are well-known, the antifungal activities of this material have not yet been studied adequately (table 2). This section aims to review the most important properties of Ag-NPs against common fungal strains.

Kim *et al* [60] studied the antifungal activities of Ag-NPs against a total of 44 strains of six fungal species from clinical isolates and ATCC strains of *Trichophyton mentagrophytes (T. mentagrophytes)* and *Candida albanicans* (*C. albanicans*). Results showed 80% inhibitory concentration (IC_{80}) from 1 to 7 μg ml^{-1}. The antifungal activity of Ag-NPs against *C. albanicans* could be exerted by disrupting the structure of the cell membrane and inhibiting the normal budding process due to the destruction of the membrane integrity [61]. In another publication, Roe *et al* [62] have tested the antifungal activity of plastic catheters coated with Ag-NPs (∼100 nm thick), and results showed that the growth inhibition was almost complete for *C. albicans*. More recently, Pamacek *et al* [63] investigated the antifungal activity of Ag-NPs prepared by the modified Tollens process. Results also revealed the minimum inhibition against *C. albicans* growth at 0.21 mg^{-1} using naked Ag-NPs, and 0.05 mg l^{-1} using Ag-NPs modified with sodium dodecyl sulfate (SDS). Additionally, Ag-NPs effectively inhibited the growth of the tested yeasts at the concentrations below their cytotoxic limit against the tested human fibroblasts determined at a concentration equal to 30 mg l^{-1} of Ag-NPs.

Other publications also reported MICs of Ag-NPs from 0.4 to 3.3 μg ml^{-1} against *C. albicans* and *C. glabrata* adhered cells and biofilm [64], and at 10 μg ml^{-1} against *Trichophyton rubrum (T. rubrum)* [65]. In summary, Ag-NPs have also been revealed as potential biocide against fungal strains, and could help to prevent fungal infections for protection of human health.

3.3. Antiviral effects

In recent years, there was an increase in reported numbers of emerging and re-emerging infectious diseases caused by viruses such as SARS-Cov, influenza A/H_5N_1, influenza A/H_1N_1, Dengue virus, HIV, HBV, and new encephalitis viruses etc. These viral infections are likely to break out into highly infectious diseases endangering public health [66].

Ag-NPs have shown effective activities against microorganisms including bacteria and fungi as mentioned above. However, the antiviral activities of Ag-NPs are still

open questions to researchers. Very few papers have been found that investigate the effects of Ag-NPs against viruses (table 2). As the first report, Elechiguerra *et al* [67] have investigated the interaction between Ag-NPs and HIV-1. It was reported that Ag-NPs undergo a size-dependent interaction, with NPs exclusively in the range of 1–10 nm attached to the virus. It was also suggested that Ag-NPs interact with the HIV-1 virus via preferential binding to the exposed sulfur-bearing residues of the gp120 glycoprotein knobs, resulting in the inhibition of the virus from binding to host cells. This mechanism was then demonstrated by Lara *et al* [68]. In this article, it was reported that Ag-NPs exert anti-HIV activity at an early stage of viral replication, most likely as a virucidal agent or as an inhibitor of viral entry. The Ag-NPs bind to gp120 in a manner that prevents CD4-dependent virion binding, fusion, and infectivity, acting as an effective virucidal agent against cell-free virus (laboratory strains, clinical isolates, T and M tropic strains, and resistant strains) and cell-associated virus. Besides, Ag-NPs inhibit post-entry stages of the HIV-1 life cycle.

In another study, Lu *et al* [69] investigated the effects of Ag-NPs of different sizes (10, 50 and 800 nm) on the hepatitis B virus (HBV), and using a HepAD38 cell line as infection model. Their study showed that only Ag-NPs could inhibit production of HBV RNA and extracellular virions *in vitro*. Furthermore, Sun *et al* [70] have utilized Ag-NPs conjugated with (*N*-vinyl-2-pyrrolidone) (PVP), recombinant respiratory syncytial virus (RSV) fusion (F) protein, and bovine serum albumin (BSA) in order to study the inhibition of RSV infection in HEp-2 cell culture. The obtained results revealed that PVP-coated silver nanoparticles, which showed low toxicity to cells at low concentrations, inhibited RSV infection by 44%, a significant reduction compared to other controls. It was concluded that when the PVP-coated Ag-NPs mixed with RSV, they would bind to the G proteins on the viral surface and interfere with viral attachment to the HEp-2 cells resulting in the inhibition of viral infection. Monkeypox virus was tested with Ag-NPs in different sizes and surface coatings. The preliminary results showed that both polysaccharide-coated Ag-NPs (25 nm) and non-coated Ag-NPs (55 nm) exhibit a significant ($P \leqslant 0.05$) dose-dependent effect of test compound concentration on the mean number of plaque-forming units (PFU), and Ag-NPs of approximately 10 nm inhibit MPV infection *in vitro* [71].

De Gusseme *et al* [72] produced the *bio*-Ag-NPs (called biogenic Ag^0), and tested the antiviral activities of both biogenic Ag^0 and ionic Ag^+ against murine norovirus 1. Remarkably, the obtained results showed that in the disinfection assay with ionic Ag^+, only a small decrease in genomic copies was detected. Exposure to biogenic Ag^0 did not result in a significant decrease in genomic copies as well. In contrast, the plaque assays demonstrated that the infectivity of MNV-1 was completely inhibited. It was suggested that the inactivation mechanism of MNV-1 is the interaction of biogenic Ag^0 with (the thiol groups of) the MNV-1 capsid proteins, making the RNA accessible and rendering the virus particle noninfectious. This interaction might possibly be favored by smaller nanoparticles.

More recently, Xiang *et al* [73] investigated the inhibitory effects of Ag-NPs on H1N1 influenza A virus *in vitro*. Their study revealed that Ag-NPs have efficient inhibitory activity on H1N1 influenza A virus, which can rapidly inhibit H1N1 influenza A virus hemagglutination of chicken RBCs. In addition, Ag-NPs could also reduce H1N1 influenza A virus induced apoptosis toward MDCK cells. However, the authors also proposed to clarify how Ag-NPs inhibit H1N1 influenza A virus infectivity as well as the application of Ag-NPs to be an effective drug against influenza.

In summary of the antiviral effects of Ag-NPs, most publications have suggested that Ag-NPs could bind to outer proteins of viral particles, resulting in inhibition of binding and the replication of viral particles in cultured cells. Although the antiviral mechanism of Ag-NPs has not been fully known yet, Ag-NPs are still suggested as potential antiviral agents in the future [74].

4. Silver nanoparticles in environmental treatments

4.1. Air disinfection

Bioaerosols are airborne particles of biological origins including viruses, bacteria, fungi, which are capable of causing infectious, allergenic or toxigenic diseases. Particularly, indoor air bioaerosols were found to accumulate in large quantities on filters of heating, ventilating, and air-conditioning (HVAC) systems [76]. It is found that outdoor air pollution and insufficient hygiene of an HVAC installation often resulted in the low quality of indoor air. Moreover, the organic or inorganic materials deposited on the filter medium after air filtration contribute to microbial growth. The WHO estimated that 50% of the biological contamination present in indoor air comes from air-handling systems, and the formation of harmless micro-organisms such as bacterial and fungal pathogens was found in air filters. It is important to note that most of these pathogens produce mycotoxins which are dangerous to human health. To reduce the microbial growth in air filters, the integration of antimicrobial Ag-NPs in air filters has been proposed and developed.

The antimicrobial effect of Ag-NPs on bacterial contamination of activated carbon filters (ACF) was studied [76]. The results showed that Ag-deposited ACF filters were effective for the removal of bioaerosols. The antibacterial activity analysis of Ag-coated ACF filters indicated that two bacteria of *Bacillus subtilis* and *E. coli* were completely inhibited within 10 and 60 min, respectively. It was found that silver deposition did not influence the physical properties of ACF filters such as pressure drop and filtration efficiency, however, the adsorptive efficacy was decreased by silver deposition. Therefore, the authors also suggested that the amount of Ag-NPs on the ACF filters needs to be optimized to avoid excessive reduction of their adsorptive characteristics and to show effective antimicrobial activity.

In a recent work [77], the authors generated Ag-coated CNT hybrid nanoparticles (Ag/CNTs) using aerosol nebulization and thermal evaporation/condensation processes and studied their applicability to antimicrobial air filtration. CNT and Ag-NPs aerosols mixed together and attached to each other, forming Ag/CNTs. The antimicrobial activity of Ag/CNT-coated filters was tested against two bacteria of Gram-positive *S. epidermidis* and Gram-negative *E. coli*. It

was found that when Ag/CNTs were deposited on the surface of an air filter medium, the antimicrobial activity against tested bacterial bioaerosols was enhanced, compared with the deposition of CNTs or Ag-NPs alone, whereas the filter pressure drop and bioaerosol filtration efficiency were similar to those of CNT deposition only. It was reported that the surface area of Ag-NPs was enhanced by CNTs. This is the main reason for the higher antimicrobial filtration efficacy of Ag/CNTs compared with that of pure Ag-NPs.

Polymer air filters made of polypropylene and silver nitrate ($AgNO_3$) were examined for bacterial survival [78]. This study showed that the addition of antibacterial $AgNO_3$ agent to filters was effective in preventing bacteria from colonizing filters. The presence of an antimicrobial $AgNO_3$ compound in the air filters caused a decrease in the amount of bacteria, which was observed in the case of both Gram-negative and Gram-positive strains of *Micrococcus luteus, Micrococcus roseus, B. subtilis, Pseudomonas luteola*. The clear reduction in living bacterial cells in silver treated filters made the technology of antimicrobial filter treatment really necessary for the future.

4.2. Water disinfection

4.2.1. Drinking water disinfection. Water is one of the most important substances on Earth and is essential to all living things. About 70% of the Earth is covered with water, but only 0.6% is suitable for human consumption. Safe drinking water is an important health and social issue in many developing countries [79]. According to the WHO, at least 1 billion people do not have access to safe drinking water. Contamination of drinking water and the subsequent outbreak of waterborne diseases are the leading cause of death in many developing nations [80]. Moreover, the spectrum and incidence of some infectious diseases are increasing worldwide, therefore, there is an enormous need for treatments to control the microbial contamination of water and decrease the number of waterborne diseases. Significant interest has arisen in the use of Ag-NPs for water disinfection.

The chemically produced nanosilver (*chem*-Ag-NPs) can be uniformly decorated onto porous ceramic materials to form a Ag-NPs–porous ceramic composite by using 3-aminopropyltriethoxysilane (APTES) as a connecting molecule [81]. This composite can be stored for long periods and is durable under washing without loss of NPs. The sterilization property of Ag-NPs–porous ceramic composite as an antibacterial water filter was tested with *E. coli*. It was found that at a flow rate of $0.01\,l\,min^{-1}$, the output count of *E. coli* was zero when the input water had a bacterial load of $\sim 10^5\,CFU\,ml^{-1}$. It also proved that the connection between the *chem*-Ag-NPs and the ceramic bases on the coordination bonds between the $–NH_2$ group at the top of the APTES molecule and the silver atoms on the surface of the NPs. This kind of connection ensured that the *chem*-Ag-NPs were tightly fixed to the interior channel walls of the porous ceramic so that they can release a sufficient quantity of silver ions for antibiosis. Such Ag-NPs–porous ceramic composites were successfully tested in drinking water purification [82]. Additionally, the *chem*-Ag-NPs can be coated on common polyurethane (PU) foams by overnight exposure of the foams to *chem*-Ag-NPs colloid [83]. The NPs are stable on the foam and are not washed away by water. Morphology of the foam was retained after coating. The NPs binding is due to its interaction with the nitrogen atom of the PU. At a flow rate of $0.5\,l\,min^{-1}$, after few seconds the output count of *E. coli* was nil when the input water had a bacterial load of $10^5\,CFU\,ml^{-1}$.

Also, the *chem*-Ag-NPs were successfully formed on to the macroporous methacrylic acid copolymer beads for disinfection of water [84]. This showed that the *chem*-Ag-NPs formed on these copolymer beads by chemical reduction method were stable under water washing and their stability was due to the interaction of the *chem*-Ag-NPs with the $–COO^-$ carboxylic functional group on the copolymer beads. Polymeric microspheres containing *chem*-Ag-NPs displayed highly effective disinfection against two gram-negative bacteria (*E. coli, P. aeruginosa*) and two gram-positive bacteria (*B. subtilis, S. aureus*). The *chem*-Ag-NPs bound copolymer beads performed efficiently in bringing down the bacterial count to zero for all the tested strains. The bacterial adsorption/adhesion tested revealed that copolymer beads containing *chem*-Ag-NPs do not have any adsorption/adhesion of bacterial cell.

Recently a new class of polyethersulfone (PES) hybrid ultrafiltration membranes bending with modified halloysite nanotubes (HNTs) loaded with the *chem*-Ag-NPs for water purification was reported [85]. The results of antibacterial activity tests showed that the hybrid membrane had a good antibacterial property, and the antibacterial rates against *E. coli* and *S. aureus* were about 99.9 and 99.8%, respectively. Noticeably, this novel hybrid ultrafiltration membrane was observed to exhibit both organic antifouling and antibacterial properties by addition of the *chem*-Ag-NPs.

Along with the use of the *chem*-Ag-NPs for bacterial disinfection in water, some reports for use of the biologically produced nanosilver (*bio*-Ag-NPs) for virus disinfection in water were also given [72]. Nico Boon *et al* [79] has reported a method for production of the *bio*-Ag-NPs referred to as biogenic Ag^0 by using lactic acid bacteria as reducing agent. It was shown that the bacterial cell wall served as a microscale carrier matrix for the NPs and this unique association of the NPs with bacterial carrier matrix prevented them from aggregating, and therefore they are very promising for disinfection technologies. The antiviral properties of *bio*-Ag-NPs were tested with murine norovirus 1 (MNV-1), a model organism for human noroviruses. The *bio*-Ag-NPs was also applied to an electropositive cartridge filter (NanoCeram) to evaluate its capacity for continuous disinfection. It was found that the addition of $31.25\,mg$ *bio*-Ag-NPs m^{-2} on the filter ($135\,mg$ *bio*-Ag-NPs kg^{-1} filter medium) caused a 3.8-log decline of virus, as compared to 1.5-log decrease in original filter. Furthermore, the *bio*-Ag-NPs were also incorporated in polyvinylidene fluoride (PVDF) polymeric membranes for investigation of continuous virus disinfection in water [86]. It showed that the virus inactivation activity of the *bio*-Ag-NPs-immobilized membranes was successfully demonstrated at low and high membrane fluxes, and was most probably related to the slow release of Ag^+ from the membranes. The results of this work also indicated that the membrane structure affected the release rate of Ag^+ from the *bio*-Ag-NPs, the Ag^+ concentration in the filtrate could

be further decreased and the depletion of Ag^+ from the material should be controlled. Therefore, this technique can be effectively applied for the treatment of limited volumes of contaminated water but not for long-term drinking water production.

In summary, the silver-based NPs are very ideal for use in water disinfection. The silver-based NPs can be incorporated to core materials and polymeric membranes to disinfect the water contaminated with the bacteria and viruses. The application of silver-based NPs is of utmost importance to prevent outbreaks of waterborne diseases related to poor treatment of drinking water. Moreover, the addition of silver-based NPs could prevent bacterial/viral attachment and biofilm formation in filtration medium [87]. Most results suggested the release of Ag^+ to be the main mechanism for both above outcomes.

4.2.2. Groundwater and biological wastewater disinfection. The impact of Ag-NPs on microbial communities in wastewater treatment plants was evaluated [88]. It was found that original wastewater biofilms are highly tolerant to Ag-NP treatment. With an application of Ag-NPs of $200\,mg\,l^{-1}$, the reduction of biofilm bacteria measured by heterotrophic plate counts was insignificant after 24 h. Biofilm can provide physical protection for bacteria under Ag-NP treatment, and extracellular polymeric substances (EPS) may play an important role in this protection. Susceptibility to Ag-NPs is different for each microorganism in the biofilm microbial community. This study showed two suggestions: (i) Ag-NPs could impact wastewater biofilm microbial community structures, depending on the characteristics of each strain, e.g., its ability to produce EPS and growth rate, and the community interactions among these strains; and (ii) the effects of Ag-NPs on planktonic cells were different to those on wastewater biofilms. Biofilm bacteria treated as isolated pure culture are much more sensitive to Ag-NPs, compared with mixture of bacteria in the biofilm.

The contamination of groundwater sources by pathogenic bacteria poses a public health concern to communities who depend totally on this water supply. Very recently, novel cost-effective filter materials coated with *chem*-Ag-NPs were developed for the disinfection of groundwater [89]. It was revealed that the *chem*-Ag-NPs were successfully deposited on zeolite, sand, fibreglass, anion and cation resin substrates. The performance of these substrates as antibacterial water filter system was tested for the removal of pathogenic bacteria of *E. coli, S. typhimurium, S. dysenteriae and V. cholerae* in groundwater. The results revealed the highest bacteria removal efficiency by the Ag/cation resin filter, with complete (100%) removal of all targeted bacteria, and the lowest by the Ag/zeolite filter, with an 8–67% removal rate. This study suggested that the filter system with Ag/cation resin substrate can be used as a potential alternative cost-effective filter for the disinfection of groundwater and production of safe water source.

4.3. Surface disinfection

4.3.1. Silver-nanoparticle-embedded antimicrobial paints. Developing bactericidal coatings on surfaces has attracted increasing interest to protect human health and the environment. Among them, Ag-NPs-embedded paints are of particular interest owing to their potential bactericidal activity. John *et al* [90] described an environmentally friendly chemistry approach to synthesize metal NPs-embedded paint, in a single step, from common household paint. The naturally occurring oxidative drying process in oils, involving free-radical exchange, was used as the fundamental mechanism for reducing metal salts and dispersing metal NPs in the oil media, without the use of any external reducing or stabilizing agents. These well-dispersed metal NPs-in-oil dispersions can be used directly on different surfaces such as wood, glass, steel and different polymers. The results showed that the surfaces coated with silver-nanoparticle paint showed excellent antimicrobial properties by killing both gram-positive human pathogens (*S. aureus*) and gram-negative bacteria (*E. coli*).

4.3.2. Antimicrobial surface functionalization of plastic catheters. Ag-NPs were used as antimicrobial coatings in medical devices to reduce nosocomial infections at hospitals. Catheters were coated with Ag-NPs [62]. Silver release from the catheters was determined *in vitro* and *in vivo* using radioactive silver. Silver-coated catheters showed significant *in vitro* antimicrobial activity and prevented biofilm formation against pathogens (*E. coli, Enterococcus, S. aureus, coagulase-negative staphylococci, P. aeruginosa and C. albicans*); most of them involved in catheter-related infections. These catheters are non-toxic and are capable of targeted and sustained release of silver at the implantation site. Because of their demonstrated antimicrobial properties, they may be useful in reducing the risk of infectious complications in patients with indwelling catheters.

4.3.3. Antimicrobial gel formulation for topical use. In addition, Ag-NPs were also used in therapeutics, especially for treating burn wounds. In order to develop this test, a gel formulation containing Ag-NPs (S-gel) was developed [91]. The antibacterial spectrum of S-gel was found to be comparable to that of a commercial formulation of silver sulfadiazine, albeit at a 30-fold less silver concentration. As part of toxicity studies, localization of Ag-NPs in Hep G2 cell line, cell viability, biochemical effects and apoptotic/necrotic potential were assessed. It was found that Ag-NPs get localized in the mitochondria and have an IC_{50} value of $251\,\mu g\,ml^{-1}$. Further, it was obvious that Ag-NPs induced apoptosis at concentrations up to $250\,\mu g\,ml^{-1}$, which could favor scarless wound healing. Acute dermal toxicity studies on Ag-NPs gel formulation (S-gel) in Sprague-Dawley rats showed complete safety for topical application. These results clearly indicated that Ag-NPs could provide a safer alternative to conventional antimicrobial agents in the form of a topical antimicrobial formulation.

4.3.4. Antimicrobial packing paper for food preservation. In addition to the above described applications of Ag-NPs as antimicrobial coatings for household paints, biomedical and therapeutic fields, Ag-NPs-coated paper could be useful for preventing microbial growth for longer periods in food preservation by providing a reservoir for slow releasing of

ionic silver from the surface to the bulk as well as preventing growth on the surface itself. A simple method to develop coating of colloidal silver on paper using ultrasonic radiation was reported, called the sonochemical coating [92]. It was revealed that by varying the precursor concentrations and reaction times, the thickness of the Ag-NPs coating and the particle size can therefore be controlled to a great extent. Moreover, these Ag-NPs- coated papers have been shown to possess microbiocidal properties against the Gram-negative *E. coli* as well as against the Gram-positive *S. aureus* bacteria. The results showed that such Ag-NPs-coated paper has potential application in the food industry as a packing material with a long shelf life and antifouling properties.

4.3.5. Silver-impregnated fabrics for clinical clothing Progressive contamination of clinical clothing with a mixture of bacteria from the wearer and the environment is a common occurrence. Furthermore, bacteria such as *Enterococcus* and *Staphylococcus* spp. can survive for more than 90 days on clothing worn by health care workers and surgical scrub suits (scrubs) may be contaminated by bacteria even when freshly laundered. Moreover, these bacteria can be transferred from nurses' uniforms to patient bedding, and bacteria cultured from the front of surgical scrubs preoperatively have subsequently been isolated from infected surgical wounds; hence the role of clinical clothing in the epidemiology of nosocomial bacterial infections. Freeman *et al* [93] investigated the effect of silver impregnation of surgical scrub suits on surface bacterial contamination during use in a veterinary hospital. It was found that silver-impregnated scrubs had significantly lowered bacterial colony counts (BCC) compared with polyester/cotton scrubs. The results showed that silver impregnation appeared to be effective in reducing bacterial contamination of scrubs during use in a veterinary hospital.

5. Toxicology of silver nanoparticles to humans and the ecology

Nanotechnology has been rapidly growing with utilization in a wide range of commercial products throughout the world. However, there is still a lack of information concerning the increase of human, animal and ecological exposure to NPs including Ag-NPs and the potential risks related to their short- and long-term toxicity. This section is devoted to reviewing the possible risks of Ag-NPs to mammalian cells *in vitro* and *in vivo*, and the toxic effects of Ag-NPs are summarized in table 3.

5.1. In vitro tests

To understand the toxicity of different NPs *in vitro*, Braydich-stolle *et al* [94] have assessed the suitability of a mouse spermatogonial stem cell line to assess toxicity of silver (Ag-15 nm), molybdenum (MoO_3-30 nm), and aluminum (Al-30 nm) NPs in the male germ line. Results showed a concentration-dependent toxicity for all types of tested NPs. Ag-NPs were the most toxic (5–10 $\mu g\,ml^{-1}$), and reduced mitochondrial function drastically and increased membrane leakage. This cell line was suggested as a valuable model with which to assess the cytotoxicity of NPs in the germ line *in vitro*. In addition, Hussain *et al* [95] have used the *in vitro* rat-liver-derived cell line (BRL 3A) to evaluate the acute toxic effects of metal/metal oxide NPs including silver (Ag; 15, 100 nm), molybdenum (MoO_3; 30, 150 nm), aluminum (Al; 30, 103 nm), iron oxide (Fe_3O_4; 30, 47 nm), and titanium dioxide (TiO_2; 40 nm). Results showed that mitochondrial function decreases significantly in cells exposed to Ag-NPs at 5–50 $\mu g\,ml^{-1}$. Fe_3O_4, Al, MoO_3 and TiO_2 had no measurable effect at lower doses (10–50 $\mu g\,ml^{-1}$), while there was a significant effect at higher levels (100–250 $\mu g\,ml^{-1}$). Lactate dehydrogenase (LDH) leakage significantly increased in cells exposed to Ag-NPs (10–50 $\mu g\,ml^{-1}$). Other NPs have been tested to displayed LDH leakage only at higher doses (100–250 $\mu g\,ml^{-1}$). The study showed the significant depletion of reduced glutathione (GSH) level, reduced mitochondrial membrane potential and increase in reactive oxygen species (ROS) levels, which suggested that cytotoxicity of Ag (15, 100 nm) in liver cells is likely to be mediated through oxidative stress. In addition, Calson *et al* [96] have investigated evaluating size-dependent cellular interactions of biologically active Ag-NPs (Ag-15, Ag-30 nm, and Ag-55 nm). After 24 h of exposure, results showed that viability metrics significantly decreased with increasing dose (10–75 $\mu g\,ml^{-1}$) of Ag-15 and Ag-30 nm. With a more than ten-fold increase of ROS levels in cells exposed to 50 $\mu g\,ml^{-1}$ Ag-15 nm, it was suggested that the cytotoxicity of Ag-15 nm is likely to be mediated through oxidative stress; this mechanism was then supported by the study on human hepatoma cells [97]. Moreover, Arora *et al* [98] have studied the interaction of synthesized Ag-NPs with HT-1080 (human fibrosarcoma) and A431 (human skin/carcinoma) cells *in vitro*. Results showed that a concentration of Ag-NPs was safe in the range from 1.56 to 6.25 $\mu g\,ml^{-1}$, and some effects appeared when concentrations $>6.25\,\mu g\,ml^{-1}$. In another study, the authors have also investigated the *in vitro* interactions of 7–20 nm spherical Ag-NPs with primary fibroblasts and primary liver cells isolated from Swiss albino mice. Upon exposure to Ag-NPs for 24 h, IC_{50} values for primary fibroblasts and primary liver cells were 61 and 449 $\mu g\,ml^{-1}$, respectively. It was suggested that although Ag-NPs seem to enter the eukaryotic cells, cellular antioxidant mechanisms protect the cells from possible oxidative damage [99]. In relation to the genotoxicity of Ag-NPs following exposure to mammalian cells, Ahamed *et al* [100] have examined the DNA damage response to polysaccharide surface functionalized (coated) and non-functionalized (uncoated) Ag-NPs in two types of mammalian cells: mouse embryonic stem (mES) cells and mouse embryonic fibroblasts (MEF). Results showed that the different surface chemistry of Ag-NPs induce different DNA damage response: coated Ag-NPs exhibited more severe damage than uncoated Ag-NPs. AshaRani *et al* [101] have investigated the cytotoxicity and genetoxicity of starch coated Ag-NPs to normal human lung fibroblast cells (IMR-90) and human glioblastoma cells (U251). The results indicated mitochondrial dysfunction, induction of ROS by Ag-NPs which in turn set off DNA damage and chromosomal aberrations. A possible mechanism of toxicity was proposed which involves disruption of the mitochondrial

Table 3. Toxicology of Ag-NPs to mammalian.

	Ag-NPs			Toxicology of Ag-NPs to mammalians		
Cell lines/ organisms	Particle size	Surface stability	Dose	Exposure time	Major outcomes	Ref.
1. *In vitro*						
Mouse spermatogonial stem cell line (C18–4)	15 nm	None	EC_{50}: 8.75 μg ml^{-1}	48 h	Concentration-dependent toxicity Reduced mitochondrial function drastically and increased membrane leakage	[94]
Rat liver derived cell line (BRL 3A)	15 and 100 nm	None	5–50 μg ml^{-1}	24 h	Depletion of reduced glutathione (GSH) level Reduced mitochondrial membrane potential and increase in reactive oxygen species (ROS) levels	[95]
Peripheral blood mononuclear cells (PBMCs)	1–1.25 nm	None	>15 ppm	72 h	A significant cytotoxic effect on PBMCs	[109]
Rat alveolar macrophages	15, 30 and 55 nm	None	10–75 μg ml^{-1}	24 h	A size-dependent toxicity Mechanism of toxicity was found to be largely mediated through oxidative stress	[96]
HT-1080 (human fibrosarcoma) and A431 (human skin/ carcinoma) cells	7–20 nm	None	6.25–60 μg ml^{-1}	24 h	Reduced cell viability, oxidative stress, DNA fragmentation and higher caspase-3 activity	[98]
Primary mouse fibroblasts and liver cells	7–20 nm	None	25–100 μg ml^{-1}	24 h	Reduced cell viability, oxidative stress and apoptosis	[99]
Mouse embryonic stem (mES) cells and Mouse embryonic fibroblasts (MEF)	25 nm	None, and polysch arite	50 μg ml^{-1}	4–72 h	Induced DNA damage and apoptosis. Coated Ag NPs produced more severe effects than uncoated	[100]
Human lung fibroblast cells (IMR-90) and human glioblastoma cells (U251)	6–20 nm	Starch	25–400 μg ml^{-1}	24–72 h	A concentration-dependent cytotoxicity (low metabolic activity) Genotoxicity (DNA damage and chromosomal aberrations) Cell cycle arrest in Ag-NPs treated cells	[101]
Human Mesenchymal stem cells (hMSCs)	100 nm	None	>5 μg ml^{-1}	7 days	Cytotoxic effects on hMSCs at high concentrations Induce cell activation (as analyzed by the release of IL-8) at high but nontoxic concentrations	[102]
Human hepatoma cells	5–10 nm	None	0.2–2 μg ml^{-1}	28 h	Oxidative stress is primarily responsible for the cytotoxicity of Ag-NPs	[97]

Table 3. Continued.

Cell lines/ organisms	Ag-NPs		Toxicology of Ag-NPs to mammalians			Ref.
	Particle size	Surface stability	Dose	Exposure time	Major outcomes	
Mouse fibroblasts and liver cells	7–10 nm	None	>1 mg l^{-1}	24 h	Cell proliferation and chemotaxis were decreased while IL-8 release was increased	[105]
Mouse Peritoneal macrophage cell line (RAW264.7)	68.9 nm	Fetal bovine serum (FBS)	0.2–1.6 ppm	24–96 h	Ag-NPs were ionized in the cells to cause cytotoxicity by a Trojan-horse type mechanism	[106]
Rat liver mitochondria	40 and 80 nm	None	2 and 5 μg mg^{-1} protein	25 min	Cause impairment of mitochondrial function Uncoupling effect on the oxidative phosphorylation system	[123]
HaCaT cells	9.8–48.8 nm	None	0.006–333 μM	24 h – 7 days	A relatively short time of contact with Ag NPs causes a long-lasting inhibition of cell growth	[107]
A549 human lung carcinoma epithelial-like cell line	30–50 nm	0.2% PVP	2.5–15 μg ml^{-1}	24 h	Cell death was primarily due to a dose-dependent increase in necrosis/late apoptosis at 2.5–15 μg ml^{-1} Ag NPs	[113]
RAW 264.7, L929 mouse fibroblast, D3 murine embryonic stem cell line and mouse embryonic fibroblasts (MEF-LacZ)	20, 80 and 113 nm	None	0.1–100 μg ml^{-1}	16 h	The potency of silver in the form of nanoparticles to induce cell damage compared to silver ions is cell type and size dependent	[112]
Human mesenchymal stem cells (hMSCs)	<50 nm	None	10 μg ml^{-1}	24 h	Cyto- and genotoxic potential of Ag-NPs in hMSCs at significantly higher concentrations as compared to antimicrobial effective levels	[104]
NT2- human testicular embryonic carcinoma cell line, and primary testicular cells (C57BL6 mice)	20 nm	None	10–100 μg ml	24 h, 48 h	Apoptosis, necrosis and decreased proliferation in a concentration- and time-dependent manner	[108]
Human monocytic cell line THP-1	24 nm	None	5–50 μg ml^{-1}	24 h	>12.5 μg/ml^{-1} caused significant macrophage death of approximately 50%	[114]
Human intestinal cell line (Caco-2)	20, 40 nm	Peptide	5–100 μg ml^{-1}	4–48 h	Induced decreasing adherence capacity and cytotoxicity, the formation of reactive oxygen species	[115]
2. *In vivo*						
Sprague-Dawley rats	18 nm	None	1.73×10^4 cm^{-3}, 1.27×10^5 cm^3 1.32×10^{-6} cm^3	Inhalation: 6 h per day, 5 days per week for 4 weeks	No significant changes in body weight, the hematology and blood biochemical values	[116]

Table 3. Continued.

	Ag-NPs			Toxicology of Ag-NPs to mammalians		
Cell lines/ organisms	Particle size	Surface stability	Dose	Exposure time	Major outcomes	Ref.
Male C57BL/6 mice	22.18 nm	None	1.91×10^7 cm^{-3}	Inhalation: 6 h per day, 5 days per week, during 14 days	Potential neurotoxicity and immunotoxicity	[118]
Male (6 weeks old) C57Bl/6 mice	5 and 22 nm	None	3.3 mg m^{-3}	Inhalation: 4 hours/ day, 10 days	40 h inhalation of 3.3 mg m^{-3} nanosilver induced minimal pulmonary toxicity or inflammation	[119]
Sprague-Dawley rats	60 nm	None	30, 300, and 1000 mg kg^{-1}	Ingestion: 28 days	No significant changes in body weight Dose-dependent changes in alkaline phosphatase activity, cholesterol level and slight liver damage	[120]
Adult-male C57BL/6N mice	25 nm	None	100 500 and 1000 1000 mg kg^{-1}	Injection : sacrificed after 24 h	Produce neurotoxicity by generating free radical-induced oxidative stress and by altering gene expression, producing apoptosis and neurotoxicity	[121]
Porcine skin	20, 25, 35, 50 and 80 nm	None, and carbon	0–1.7 μg ml^{-1}	Skin exposure: 14 consecutive days	Focal inflammation, specifically Intracellular and inter cellular epidermal edema, after 14 days	[122]

EC_{50}: half maximal effective concentration.

respiratory chain by Ag-NPs leading to production of ROS and interruption of ATP synthesis, which in turn causes DNA damage. It was anticipated that DNA damage is augmented by deposition, followed by interactions of Ag-NPs to the DNA leading to cell cycle arrest in the G2/M phase. Notably, Greulich *et al* [102] have reported the influence of spherical Ag-NPs (diameter about 100 nm) on the biological functions of human mesenchymal stem cells (hMSCs). Results showed a concentration-dependent activation of hMSCs at nanosilver levels of 2.5 μg ml^{-1}, and cytotoxic cell reactions occurred at Ag-NPs concentrations above 5 μg ml^{-1}. Kittler *et al* [103] have demonstrated that the toxicity of Ag-NPs increases during storage because of slow dissolution under release of silver ions. The NPs could release up to 90% of their weight depending on the functionalization as well as on the storage temperature. The release of silver was suggested as a considerably increased toxicity of Ag-NPs which had been stored in dispersion for several weeks toward human mesenchymal stem cells due to the increased concentration of silver ions. In another publication, Hackenberg *et al* [104] also reported cytotoxic effects of Ag-NPs (46 ± 21 nm) at concentrations of 10 μg ml^{-1} for all test exposure periods to hMSCs. Interestingly, Kawata *et al* [105] have reported the *in vitro* toxicity of Ag-NPs at non-cytotoxic doses to HepG2 human hepatoma cells. Results showed that Ag-NPs accelerate cell proliferation at low doses (<0.5 mg/l^{-1}). However, only Ag-NPs exposure exhibited a significant cytotoxicity at higher doses (>1.0 mg l^{-1}) and induced abnormal cellular morphology, displaying cellular shrinkage and acquisition of an irregular shape. Moreover, Park *et al* [106] have showed cytotoxicity to mouse peritoneal macrophage cell line (RAW264.7) by increasing sub G1 fraction, which indicates cellular apoptosis. Ag-NPs decreased intracellular glutathione level, increased nitric oxide secretion, increased TNF-α in protein and gene levels, and increased gene expression of matrix metalloproteinases (MMP-3, MMP-11 and MMP-19). It was suggested that Ag-NPs were ionized in the cells to cause cytotoxicity by a Trojan-horse type mechanism. To investigate the effects of Ag-NPs on skin, Zanette *et al* [107] have performed their study on the human-derived keratinocyte HaCaT cell line model. Ag-NPs caused a concentration- and time-dependent decrease of cell viability, with IC_{50} values of 6.8 ± 1.3 μM (MTT assay) and 12 ± 1.2 μM (SRB assay) after 7 days of contact. Results also demonstrated that on HaCaT keratinocytes, a relatively short time of contact with Ag-NPs causes a long-lasting inhibition of cell growth, not associated with consistent Ag-NPs internalization. Recently, Asare *et al* [108] have investigated the cytotoxic and genotoxic effects of nanoparticles such as Ag-NPs (20 nm) and submicron- (200 nm) size, and titanium dioxide nanoparticles (TiO_2-NPs; 21 nm) in testicular cells. The results suggested

that silver nano- and submicron-particles (Ag-NPs) are more cytotoxic and cytostatic compared to TiO_2-NPs, causing apoptosis, necrosis and decreased proliferation in a concentration- and time-dependent manner. The 200 nm Ag-NPs in particular appeared to cause a concentration-dependent increase in DNA-strand breaks in NT2 cells, whereas the latter response did not seem to occur with respect to oxidative purine base damage analyzed with any of the particles tested. There are some more publications demonstrating the cytotoxicity and genotoxicity of Ag-NPs tested *in vitro* to peripheral blood mononuclear cells (PBMCs) [109]; mouse lymphoma cell line (L5178Y thymidine kinase $(tk)^{+/-}$-3.7.2C cells) and human bronchial epithelial cells (BEAS-2B) [110]; monocytic cell line THP-1 (ATCC 202) [111]; human mesenchymal stem cells [104]; RAW 264.7 murine peritoneal macrophage cell line, L929 mouse fibroblast, D3 murine embryonic stem cell line and mouse embryonic fibroblasts (MEF-LacZ) [112]; human lung carcinoma epithelial-like cell line [113]; primary testicular cells [108]; human monocytic cell line [114]; and human intestinal cell line [115].

Generally, in *in vitro* tests, the mechanism of Ag-NPs-mediated cytotoxicity is mainly based on the induction of reactive oxygen species (ROS). Particularly, exposure to Ag-NPs causes reduction in GSH, elevated ROS levels, lipid peroxidation and increased expression of ROS responsive genes, it also leads to DNA damage, apoptosis and necrosis. The cytotoxicity and genotoxicity of Ag-NPs are size-, concentration- and exposure time-dependent.

5.2. In vivo tests

The most important question here is on the real impact of Ag-NPs to human health and animals. There are several *in vivo* studies on cytotoxicity and genotoxicity of Ag-NPs reported to demonstrate this question (table 3). Due to the ultra-small sizes of Ag-NPs, a great mobility is conferred in different environments, and humans are easily exposed via routes such as inhalation, ingestion, skin, etc. Ag-NPs can translocate from the route of exposure to other vital organs and penetrate into cells. For inhalation toxicity of Ag-NPs, Ji *et al* [116] have investigated the inhalation toxicity of Ag-NPs on Sprague-Dawley rats over a period of 28 days. The rats were exposed to the Ag-NPs for 6 h per day, 5 days per week, for a total of 4 weeks. Results showed that the male and female rats did not show any significant changes in body weight relative to the concentration of Ag-NPs during the 28-day experiment. There were also no significant changes in the hematology and blood biochemical values in either the male or female rats. Whereas, some investigators have reported that lungs are major target tissues affected by prolonged inhalation exposure to Ag-NPs [117]. In another publication, Lee *et al* [118] have reported Ag-NPs exposure modulated the expression of several genes associated with motor neuron disorders, neurodegenerative disease, and immune cell function, indicating potential neurotoxicity and immunotoxicity associated with Ag-NPs exposure. Minimal pulmonary inflammation or cytotoxicity of mice was found after 10 days of Ag-NPs exposure [119]. For gastrointestinal toxicology caused by Ag-NPs exposure via ingestion, Kim *et al* [120] have tested the oral toxicity of Ag-NPs (60 nm) over a period of 28 days in Sprague-Dawley rats. Results showed that the male and female rats did not show any significant changes in body weight relative to the doses of Ag-NPs during the 28-day experiment. But, some significant dose-dependent changes were found in the alkaline phosphatase and cholesterol values in either the male or female rats, seeming to indicate that exposure to over more than 300 mg of Ag-NPs may result in slight liver damage. It was suggested that Ag-NPs do not induce genetic toxicity in male and female rat bone marrow *in vivo*. There are some publications found concerning the studies on toxicology of Ag-NPs to organism via skin exposure or injection [121, 122].

Generally, very few papers on the *in vivo* toxicology of Ag-NPs were found, so further investigation is needed in this field to evaluate exactly the real impact of Ag-NPs in commercial products to humans and animals. Furthermore, the impact of Ag-NPs to environment and ecology has been discussed in detail by Ahamed *et al* [8]. In this review, the authors have indicated that Ag-NPs produce reproductive failure, developmental malformations and morphological deformities in a number of non-mammalian animal models. Common causes of Ag-NPs-induced toxicity include oxidative stress, DNA damage and apoptosis.

6. Future prospects

6.1. Powerful disinfectant for control and prevention of microbial infections

Due to the potent activities of Ag-NPs, they can be promisingly used in treating infectious pathogens and preventing microbial infections. Very recently, our research group demonstrated an excellent disinfectant ability of colloidal Ag-NPs for the prevention of gastrointestinal bacterial infections [55]. The reports revealed that the Ag-NPs colloid showed an enhancement of antibacterial activity and long-lasting disinfectant effect as compared to conventional chloramin B (5%) disinfection agent. Additionally, the Ag-NPs displayed long-term antibacterial effect as compared with two other disinfectants of sodium hypochlorite (NaClO) and phenol (C_6H_5OH) [124]. With observed advantages such as long-lasting effect and enhanced bactericidal activity, the Ag-NPs are promising for environmental treatments contaminated with gastrointestinal bacteria and other infectious pathogens. More importantly, the powerful disinfectant activity of Ag-NPs will open a new generation of silver-containing disinfection products for controlling and preventing further outbreak of diseases (e.g. diarrhea and cholera). However, the emerging questions on the disinfectant ability of Ag-NPs against viral infections (e.g. A-H5N1 influenza virus, enterovirus 71, Rota virus etc.) need to be clarified. Further investigations on evaluation of disinfectant efficiency of silver-based NPs for real environmental contaminations are needed.

6.2. Magnetic disinfectant for treatment of waterborne diseases

Ag-NPs can be promisingly used in core/shell magnetic disinfectant systems for effective disinfection of drinking

water. The magnetic disinfectant includes magnetic oxide NPs as the core (i.e. Fe_3O_4) and Ag-NPs as the shell [125]. Importantly, these core/shell nanostructures can be successfully removed from the medium by using an external magnetic field, which provides a mechanism to prevent uncontrolled waste disposal of these potentially hazardous nanostructures. This indicates that the nanocomposite disinfectant can be recovered from water solution for reuse through magnetic separation and the waste and possible contamination of the environment by disinfectant are avoided [126]. Fe_3O_4–Ag and Fe_3O_4–SiO_2–Ag core/shell nanocomposites exhibited excellent antibacterial effect and enhanced stability against bacterial pathogens [127, 128]. The main characteristic of magnetic disinfectant is its effectiveness against waterborne pathogens using low concentrations of silver, which makes it economic to use. Hence, the magnetic disinfectant can be possibly used in disinfection and biomedical applications where they can be exploited for a targeted delivery of an antimicrobial agent and its subsequent removal by means of an external magnetic field.

6.3. Effective sorbent and catalyst for removal of environmental pollution

A novel concept is proposed to synthesize a new class of composites featuring magnetic, molecular sieve and metallic NPs properties. These multi-functional materials have potential applications as recyclable catalysts, disinfectants and sorbents. The magnetic property enables effective separation of the spent composites from complex multiphase systems for regeneration and recycling, safe disposal of the waste and/or recovery of loaded valuable species. The zeolite molecular sieve provides a matrix which supports a remarkably new, simple, efficient and economical method to make stable, supported Ag-NPs by silver ion exchange and controlled thermal reduction [129]. Each component has a specially designed function, which makes this novel composite practically operational, sustainable, economical and environmentally friendly [130]. A new class of magnetic zeolite composites with surface-supported Ag-NPs as sorbents for mercury removal was tested. The produced nanocomposite has potential applications as a catalyst, a bacterial disinfectant for municipal water, or a sorbent for the removal of elemental mercury from the flue gases of coal-fired power plants, a pressing environmental concern. Hence, the Ag-NPs embedded in complex composite systems can be promisingly used as sorbent and catalyst for removal of environmental pollution.

6.4. Advanced surface disinfections

Nanosilver-containing solutions are of particular interest to industry for surface disinfection applications. Disinfection methods include spraying, wiping, dipping in solutions. Nanosilver-based disinfectant solutions are very useful for decontamination of surfaces, tools in kindergartens, schools, workplaces, for different types of equipment and computers, toys, all kinds of furniture and the like, in industrial and domestic, and public utilities. This will open new opportunities to develop nanosilver-based consumer products for surface disinfection such as spray bottles for providing long-lasting protection, disposable wipes for disinfecting hands and to maintain daily personal hygiene, etc. In this regard, nanosilver-containing products are very promising for surface disinfection of contaminated environmental areas and/or the product can be sprayed on surfaces and materials in order to inhibit the pathogen development and to improve human health and safety.

6.5. Environmentally friendly silver-based nanocomposites

As mentioned in section 5, it has been concluded that Ag-NPs have the potential to cause health and ecotoxicity issues in a concentration- and size-dependent manner. In order to overcome this problem, new approaches for development of silver-containing nanocomposites were proposed. A recent approach is to develop the hybrid nanostructure between carbon-based nanomaterials such as carbon nanotubes (CNTs) [130] or graphene (Grp) [131] and Ag-NPs. These silver-decorated nanohybrids constitute an interesting class of antibacterial materials. By decorating Ag-NPs on carbon nanomaterials, the toxicity of Ag-NPs will be reduced, thus helping in preventing potential contamination to the environment [132]. It is noteworthy that these silver-decorated nanohybrids are very different from isolated and dispersed colloidal Ag-NPs, and it appears that the strong interactions between the Ag-NPs and the CNTs or Grp surfaces make the Ag-NPs less toxic due to the fact that they are not able to release themselves into the environment. Another important class of antibacterial silver-based nanomaterials is composed of inorganic–inorganic and organic–inorganic nanocomposites. The silver-based nanocomposite on inorganic support (i.e., Ag/TiO_2) [133] or that on polymer matrix (i.e. Ag/PVA) [17] are advanced functional materials composed of Ag-NPs dispersed inside the polymeric matrix and/or coated by polymer/inorganic carrier, thus forming a core/shell structure. This nanocomposite structure inhibited effectively the release of silver ions from NPs, thus reducing the generation of ROS and toxicity of Ag-NPs [134–136]. As a result, these nanocomposites can be environmentally friendly when used for developing novel products applications.

7. Conclusions

Ag-NPs are one of the most attractive nanomaterials for commercialization applications. They have been widely used for antimicrobial, electronic and biomedical products. In this review, we provide a comprehensive understanding of the Ag-NPs from synthesis methods, antimicrobial effects and possible toxicology considerations of Ag-NPs to both humans and ecology. The emphasis is also placed on the disinfectant ability of Ag-NPs nanomaterials with respect to environmental treatments. An overview of some current applications for use of Ag-NPs in disinfectant applications was given and discussed. Importantly, some future prospects for use of Ag-NPs-based nanomaterials for treatments of infectious diseases were discussed. A new class of nanosilver-containing disinfectant nanoproducts will be promising for advanced environmental treatments including air disinfection, water disinfection, surface disinfection and

personal hygiene; this will help to prevent the further outbreak of diseases. For silver-based NPs to be used in the field of infectious diseases treatment, however, further investigation is needed to determine how to safely design, use, and dispose of products containing silver without creating a new risk to humans or the environment.

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