Chapter 2
Synthesis, Characterization and Application of Silver-Based Antimicrobial Nanocomposites

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The different types of nanomaterials like copper, zinc, titanium, magnesium, gold, alginate, and silver possess the antimicrobial activity [1–3]. Among the various antimicrobials, silver is most promising because of its inherent properties of high thermal stability, little toxicity to mammalian cells and tissues [4, 5], versatile activity (such as good antimicrobial efficacy against bacteria, viruses, and other eukaryotic microorganisms), and long-term activity [6]. Therefore, the silver-containing materials having excellent antimicrobial activity against a broad spectrum of microbes have attracted much interest of the scientists [7].

The present chapter aims at reviewing the synthesis, characterization, and application of silver-based antimicrobial nanocomposites. Firstly, the common synthetic methods of silver nanomaterials, e.g., physical method, chemical reduction and all kinds of biological approach, are described. Secondly, the bioactivity of silver(I) complexes, silver nanoparticles, silver/inorganic nanocomposites, and silver–polymer nanocomposites are discussed, and especially the different antimicrobial mechanisms of diverse silver-based nanocomposites are emphatically summarized. Thirdly, the applications of the antimicrobial nanocomposites in the medical, pharmaceutical, and food packaging industry, in addition to water disinfection and microbial control, are introduced. Additionally, the toxicology of the antimicrobial nanocomposites is also mentioned. Finally, the current and possible developing trends of the antimicrobial silver-based nanocomposites are prospected.

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2.1 Synthesis of Silver-Based Antimicrobial Nanocomposites

2.1.1 Synthesis of Silver Nanoparticles

The application of nanoparticles varies widely on the basis of their physical properties, like apparent density, surface area, and morphology, which are strongly related to the preparation methods and preparing materials. For example, the antimicrobial activity of silver nanoparticles is closely related to their size and shape [8]. Thus, the control over the size, size distribution, and shape of nanoparticles is an important task in the synthesis of silver-based antimicrobial nanocomposites. Generally, silver nanoparticles can be prepared and stabilized by physical approaches (such as the evaporation–condensation method and laser ablation method), chemical approaches (such as the reduction method), and biological and biotechnological approaches, etc. [9–11].

2.1.1.1 Physical Approach

In the past, mechanical approaches such as homogenization and grinding/ball milling were used to prepare silver nanoparticles for antimicrobial application. But the obtained silver nanoparticles aggregated greatly and showed poor inhibitory and bactericidal effect. In recent physical processes, silver nanoparticles are generally synthesized by evaporation–condensation which could be carried out in a tube furnace. Simchi et al. [12] presented a design of silver nanoparticle synthesis by an inert-gas condensation process. Silver was heated in a small temperature-regulated crucible to produce vapors, then the vapors were rapidly quenched on the surface of a liquid nitrogen tank in a reduced atmosphere of argon gas. They found that source temperature, evaporation rate, and argon pressure greatly affected the average particle size and particle shape.

Laser ablation in liquids has received much attention as a novel nanoparticle-production technique [13]. In general, silver nanoparticles are obtained by irradiating intense laser light onto the metallic bulk material settled in solvents. One advantage of laser ablation compared to other conventional methods for preparing metal colloids is the absence of chemical reagents in the solutions. Therefore, pure colloids, which will be useful for further applications, can be produced by this method. Tsuji et al. [14, 15] carried out laser ablation of silver plates in polyvinylpyrrolidone (PVP) aqueous solutions and found the obtained colloidal silver nanoparticles were more stable than those obtained in neat water. Nanoparticles can also be modified in size and shape by the surfactant coating due to their further interaction with the laser light passing through. The nanoparticles formed by laser ablation in a solution of high surfactant concentration are smaller than those formed in a solution of low surfactant concentration.

Other physical methods, such as ultrasonic-assisted reduction [16], photoinduced synthesis [17], microwave-assisted synthesis [18], irradiation reduction [19], and so on, have been employed to synthesize silver nanoparticles and silver-based nanocomposites. In these methods, silver nanoparticles with various morphologies and excellent antimicrobial activities can be obtained.

2.1.1.2 Chemical Approach

The chemical approach includes chemical reduction (chemical reduction of silver ions in aqueous solutions [20, 21] or non-aqueous solutions [22]), the template method [23, 24], electrochemical reduction [25], the microemulsion method [26], biochemical reduction [27], and so on.

Chemical reduction is the most frequently applied method for the preparation of silver nanoparticles as stable, colloidal dispersions in water or organic solvents. The reduction of silver ions (Ag^+) in aqueous solution generally yields colloidal silver with particle diameters of several nanometers. Initially, the reduction of various complexes with Ag^+ leads to the formation of silver atoms (Ag^0), which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of colloidal silver particles. Commonly used reductants are borohydride, citrate, ascorbate, and elemental hydrogen [28–30]. The use of a strong reductant such as borohydride results in small particles that are somewhat monodispersed, while the use of citrate, a weaker reductant, results in a slower reduction rate and narrower size distribution. Moreover, nanoparticles with different shapes can be easily prepared by controlling the reaction conditions. However, the coalescence of the nanoparticles may lose their characteristic properties. Thus, the most important key in this method is to avoid the agglomeration of silver nanoparticles during the synthesis and preservation procedures. Usually, special organic compounds, such as surfactants, polymers, and stabilizing ligands, are used to passivate the particles and prevent them from aggregating.

To overcome the limitation of chemical reduction and prevent particle aggregation, the reverse microemulsion (reverse micelle) method is introduced to obtain the uniform and size-controlled nanoparticles. This method has the obvious advantage of synthesizing nanoparticles with specific diameter and morphology. The nucleation and growth are restricted within the water cores of inverse micelles. The droplet dimension can be modulated by various parameters, in particular the molar ratio of water and surfactant. Preparation of silver nanoparticles by the microemulsion method has many influencing factors, and the synthesis system also has some characteristics different from other systems.

Currently, in the microemulsion method, some natural compounds such as sodium citrate and reducing sugars are usually used as reducing agents, although the reduction activity is generally low and the reducing reaction takes place under a higher temperature and needs a long time. These reducing agents are mild, inexpensive, and nontoxic. Some of them simultaneously play dual roles as a protective agent and a reducing agent. For instance, silver particles prepared by citrate reduction are nearly spherical, and the crystallites have relatively large diameters (50–100 nm) and a wide range of distributions in size and shape. Citrate serves not only the dual roles of a reductant and stabilizer but also in the role of a template [31].

2.1.1.3 Biological and Biotechnological Approaches

The chemical method allows the preparation of uniform and size-controllable nanoparticles; however, highly deleterious organic solvents with potential risks for environment and biological hazards are employed. It has, therefore, been an increasing awareness towards green chemistry and biological processes leading to the development of an environment-friendly approach for the synthesis of nanoparticles. Unlike other processes in physical and chemical methods, which involve hazardous chemicals, microbial biosynthesis of nanoparticles is an eco-friendly approach. Research in biological and biotechnological approaches provides reliable, cost-effective processes for the synthesis of nanoscale materials.

It has been known for a long time that a variety of nanoparticles are synthesized by biological processes. The biological synthesis of nanoparticles germinated from the experiments on biosorption of metals with Gram-negative and Gram-positive bacteria. A few microorganisms, such as *Pseudomonas stutzeri*, *Klebsiella pneumonia*, *Escherichia coli*, and *Enterobacter cloacae*, have been explored as potential biofactories for synthesis of silver nanoparticles [32, 33]. Moreover, biological reduction is developed as a promising method because of its special advantages such as sufficient material sources, mild reaction conditions, and good dispersion of nanoparticles as well as few chemical additives and poisonous byproducts.

In recent years, fungi such as *Fusarium oxysporum*, *Colletotrichum* sp., *Trichothecium* sp., *Trichoderma asperellum*, *Trichoderma viride*, *Phaenerochaete chrysosporium*, *Fusarium solanii*, *Aspergillus fumigatus*, *Coriolus versicolor*, *Aspergillus niger*, *Phoma glomerata*, *Penicillium brevicompactum*, *Cladosporium cladosporioides*, *Penicillium fellutanum*, and *Volvariella volvaceae* have been explored for silver nanoparticles synthesis. Fungi are more advantageous compared to other microorganisms in many methods [34]. For example, in *Fusarium oxysporum* fungus, the reduction of Ag^+ was attributed to an enzymatic process involving NADH (reduced nicotinamide adenine dinucleotide)-dependent reductase. Hen egg white lysozyme can act as the sole reducing agent and catalyzes the formation of silver nanoparticles in the presence of light. Stable silver colloids formed after mixing lysozyme and silver acetate in methanol, and the resulting nanoparticles are concentrated and transferred to aqueous solution without any significant changes in physical properties [35]. Figure 2.1 is the proposed formation mechanism of silver–lysozyme nanoparticles. In another example, the white rot fungus, *Phanerochaete chrysosporium*, reduced silver ions to form silver nanoparticles, in which a protein was suggested to cause the reduction of silver ions. Environmental SEM analysis revealed that silver nanoparticles were in the size range of 50–200 nm on the surface of the mycelium. This demonstrated the presence of reductase enzymes on the surface of the mycelium, which reduced silver ions to silver nanoparticles. Spent mushroom substrate (SMS) can also be used as a reducer and stabilizer to synthesize stable and monodisperse silver–protein (core–shell) nanoparticles [36].

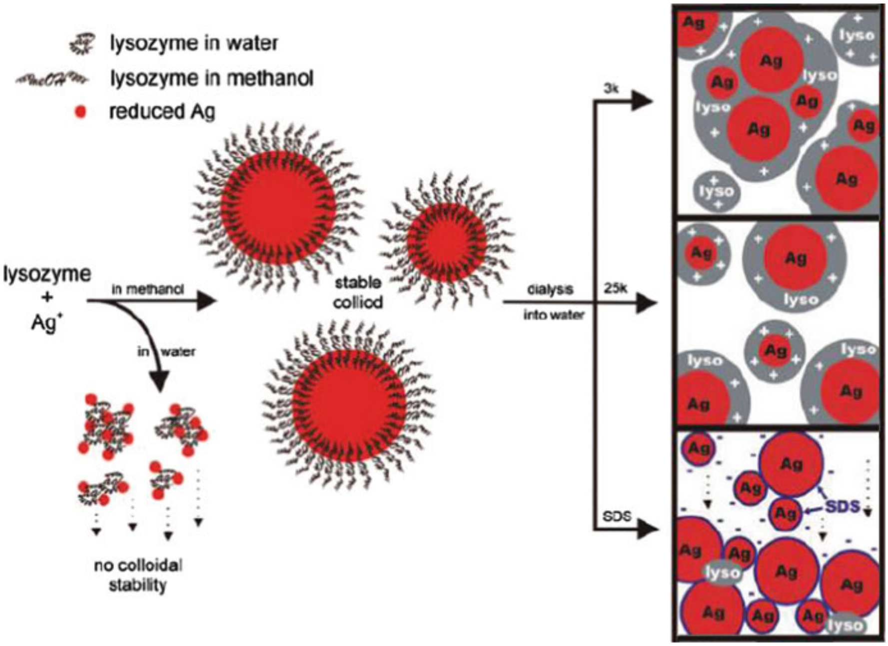

Fig. 2.1 Proposed mechanism of silver–lysozyme nanoparticle formation [35]

Extracts from microorganisms may act as both reducing and capping agents in silver nanoparticles synthesis. The reduction of silver ions by combinations of biomolecules found in these extracts such as enzymes/proteins, amino acids, polysaccharides, and vitamins is environmentally benign. For example, the extract of unicellular green algae *Chlorella vulgaris* was used to synthesize single-crystalline silver nanoplates at room temperature. Proteins in the extract provide the dual function of silver ions reduction and shape control in the nanoparticle synthesis. The carboxyl groups in aspartic and/or glutamine residues and the hydroxyl groups in tyrosine residues of the proteins were suggested to be responsible for the silver ion reduction [37].

Among biological molecules, deoxyribonucleic acid (DNA) is one of the most interesting template systems because of its large aspect ratio (length/diameter) and well-defined sequences of DNA base and a variety of superhelix structures. Sun et al. reported that a silver nanoparticles ring could be successfully fabricated by electrostatic assembling 4-aminothiophenol (4-ATP)-capped silver nanoparticles on a predefined extended circular plasmid pBR322 DNA [38].

Although biological methods are regarded as safe, cost-effective, sustainable, and environmentally friendly processes, they also have some drawbacks in the culturing of microbes, which is time-consuming and difficult to provide better control over size distribution, shape, and crystallinity. In order to improve the rate of synthesis and monodispersity of nanoparticles, factors such as microbial cultivation methods and downstream processing techniques have to be improved and the combinatorial approach such as photobiological methods may be used.

2.1.2 Synthesis of Typical Silver-Based Antimicrobial Nanocomposites

Silver-based antimicrobial nanocomposites deserve special attention due to their unique properties, which differentiate them from other antimicrobial additives. According to the composition of nanocomposites, the silver-based antimicrobials can be placed in four categories: silver–polymer nanocomposites, silver–biopolymer nanocomposites, silver–inorganic compounds, and silver(I) complexes.

2.1.2.1 Silver–Polymer Nanocomposites

Polymer molecules are found to be very effective support for the stabilization of silver nanoparticles [39, 40]. In silver–polymer nanocomposites, the commonly used polymers are polyacrylate [41], poly(amidoamine) [42], polyaniline [43], poly (methyl methacrylate) [44], poly(ethylene oxide) [45], polyrhodanine [46], etc. So far, there are many chemical and physical methods to prepare silver–polymer nanocomposites [47].

One of the in situ syntheses of silver nanoparticles in the polymer matrix involves the dissolution and reduction of silver salts or complexes into the matrix [48]. Generally, the silver cations are first complexed with a certain polymer, and then reduced in situ by using various reducing agents to form the stable colloidal silver–polymer nanocomposites. Here, the polymers acted as templates, stabilizers or protecting agents. For instance, a nanoscale silver cluster protected by sodium polyacrylate (PAA) was prepared through the reduction of aqueous silver nitrate solution containing PAA. The silver nanoparticles in clusters of nanoscale dimension (nanoclusters) could be dispersed into the artificial heterogeneous matrix of polyacrylate and protein (bovine serum albumin, BSA) by adjusting the pH in the acidic region [41]. A series of colloid silver nanoparticles were successfully prepared by in situ reduction and stabilization of hyperbranched poly(amidoamine) with terminal dimethylamine groups [HPAMAM-N$(CH_3)_2$] in water [42]. The schematic procedure for the preparation of Ag-HPAMAMN$(CH_3)_2$ nanocomposite is shown in Fig. 2.2.

Another approach is a system in which simultaneous polymerization and metal reduction occur. For example, core–shell silver–polyaniline nanocomposites have been synthesized by the in situ gamma radiation-induced chemical polymerization method. An aqueous solution of aniline, a free-radical oxidant, and/or silver metal salt were irradiated by γ-rays. Reduction of the silver salt in aqueous aniline leads to the formation of silver nanoparticles which in turn catalyze oxidation of aniline to

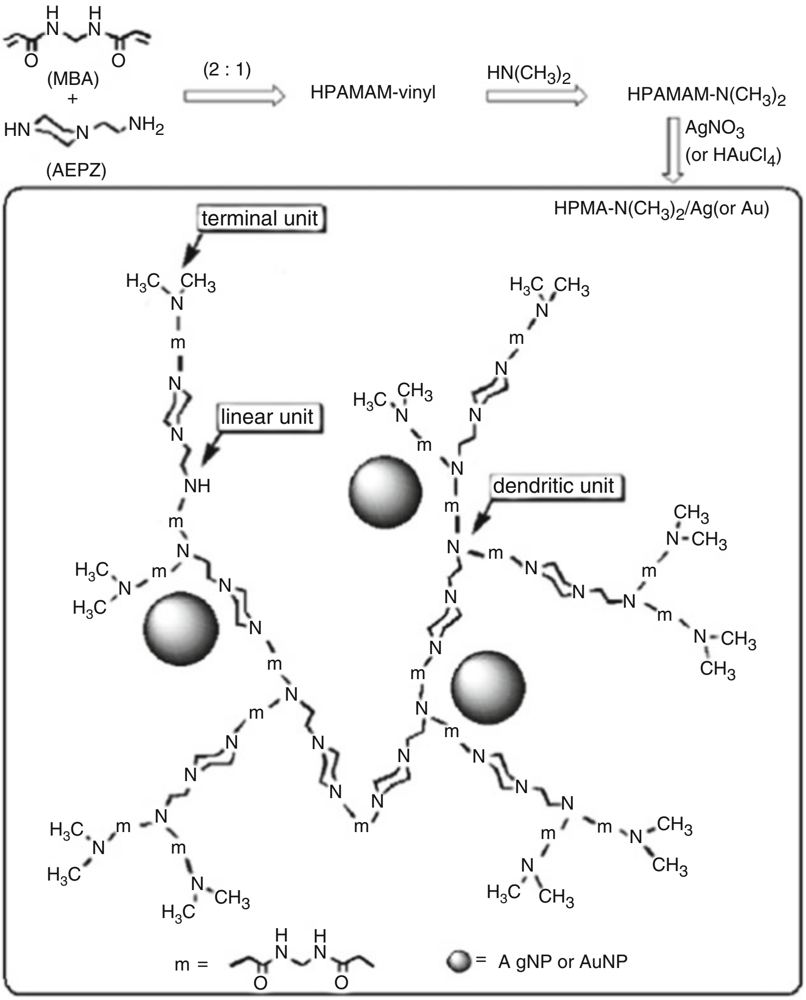

Fig. 2.2 Schematic procedure for the preparation of the Ag-HPAMAM-N$(CH_3)_2$ nanocomposite [42]

polyaniline [43]. Interestingly, in some reactions, silver ions can be used as an oxidant in the chemical oxidation polymerization. For example, during the synthetic process of silver- poly(amidoamine) (Ag-HPAMA) nanomaterials. Silver nanoparticles can be fabricated with HPAMA as the stabilizer and reductant while silver ions act as the oxidant [49]. The schematic plot of preparing Ag-HPAMAM-NH_2 nanocomposites is showned in Fig. 2.3.

Moreover, supercritical carbon dioxide (sc-CO_2) has been attracting interest as a polymerization and processing medium, primarily driven by the need to replace conventional solvents with more environmentally benign and economically viable systems [50, 51]. It possesses many advantages like non-flammability, high diffusivity, low cost, low viscosity, compressibility, etc. [52]. And the product can easily

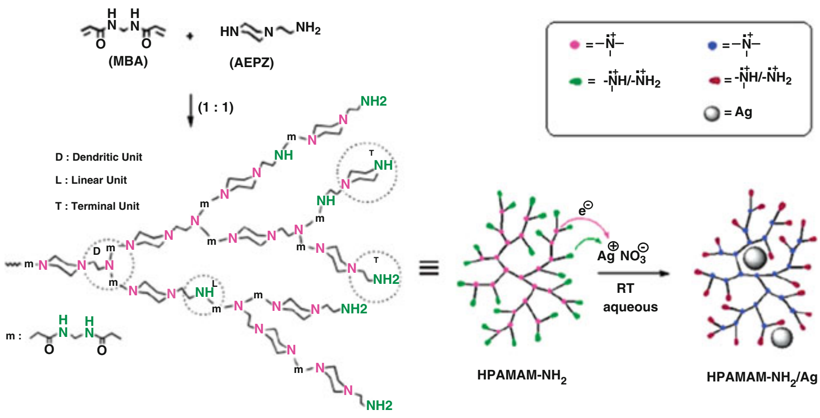

Fig. 2.3 Schematic plot of preparing Ag-HPAMAM-NH_2 nanocomposites [49]

be separated by depressurizing CO_2. Many chemicals are not soluble in CO_2, but they can easily be emulsified in sc-CO_2 by selecting proper fluorinated or siloxane-based surfactants/stabilizers. Shiho and De Simone pioneered the dispersion polymerization of methyl methacrylate in sc-CO_2. They used the CO_2-soluble fluorinated homo-polymer, poly(dihydroperfluorooctyl acrylate) (PFOA) as a stabilizer for the polymerization reaction [53]. Kamrupi et al. [54] have elaborately explained one simple, effective and green route to synthesize silver–polystyrene nanocomposite particles in water-in-sc-CO_2 medium. Silver nanoparticles are synthesized by chemical reduction of silver nitrate using sodium borohydrate as a reducing agent and polydimethylsiloxane (PDMS) as a stabilizer in the water-in-sc-CO_2 medium. This work represents a simple, reproducible and universal way to prepare a variety of metal–polymer nanocomposite particles. The shematic diagram of the apparatus for preparing the silver nanocomposite by the above-mentioned menthod is displayed in Fig. 2.4.

2.1.2.2 Silver–Biopolymer Nanocomposites

To date, polysaccharide biopolymers, which are generally non-toxic and available from renewable agricultural sources, can integrate with inorganic nanoparticles, and the research on the hybrid systems of silver–biopolymers is a dramatic activity in current bio-nanoscience. Characteristic macromolecular and supramolecular properties of these biopolymers make them become the good controlled environments for growth of metallic and semiconductor nanocrystals [55]. For example, silver–cellulose colloidals are obtained by immersing bacterial cellulose into the silver nitrate solution through chemical reduction [56].

The glycogen biopolymer from bovine liver has been used as stabilization agent for the growth of silver nanoparticles [57]. The nanoparticles encapsulated in

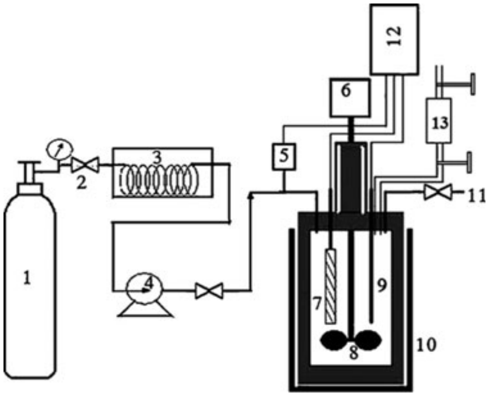

Fig. 2.4 Schematic diagram of the apparatus *1* Carbon dioxide cylinder, *2* back pressure valve, *3* refrigeration unit, *4* high-pressure liquid pump, *5* back pressure gauge, *6* motor for mechanical stirrer, *7* cooling unit, *8* mechanical stirrer, *9* heating probe, *10* SFE Vessel, *11* vent, *12* digital display unit, *13* specially designed dropper [54]

glycogen with various contents of silver are prepared by two different procedures that include fast (using microwave radiation) and slow (conventional) heating of the reaction mixtures.

Starch is the most extensively used biopolymer for the stabilization of the growth of metallic and semiconductor nanoparticles. Stable silver nanoparticles have been synthesized by using soluble starch as both the reducing and stabilizing agents [58]. The silver nanoparticles embedded in soluble starch have been proved to be an environmentally benign and renewable material. The use of a silver–starch nanocomposite like soluble starch offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications.

Chitosan possesses many unique properties, including antimicrobial characteristics; hence, it has been used in various applications, such as medical, pharmaceutical, textile, water treatment, food, cosmetics, packaging, etc [59–61]. Silver nanoparticles can be formed by reduction of corresponding metal salts with a reductant such as $NaBH_4$ in the presence of chitosan, and then chitosan molecules are adsorbed onto the surface of as-prepared silver nanoparticles in the cross-linking process to form the corresponding silver–chitosan nanocomposites [62].

Silver-impregnated polymer nanofibers have been developed as a new class of biomedical materials by releasing biocidal silver with a protective polymer barrier against infection [63–65]. Kong et al. [66] reported the fabrication of novel silver–polyrhodanine nanofibers and their antimicrobial efficacy. Silver ions are reduced to silver nanoparticles by oxidizing rhodanine monomer and simultaneously complexed with the rhodanine due to coordinative interactions, resulting in the formation of silver nanoparticle-embedded polyrhodanine nanofibers. The synthesized nanofiber is found to have excellent antimicrobial activities.

The other polysaccharide biopolymers such as poly(L-lactide) [67] and gum arabic [68] are also proving to be good stabilization agents for silver nanoparticles. To sum up, silver nanoparticles synthesized within the biopolymer are biocompatible and hydrophilic, which could be important for their application in biology and medicine.

2.1.2.3 Silver–Inorganic Compounds

In recent years, the use of inorganic–inorganic hybrid antimicrobial agents has attracted interest because of their industrial and medical applications. The key advantages of inorganic antimicrobial agents are improved safety and stability [69]. As can be observed, the integration of silver into nanocomposites and bimetallic nanoparticles is limitless. With each combination, silver is being used to generate nanoparticles with new characteristics.

Hydroxyapatite (HA) has been widely used for bone repair and substitute because of its good biocompatibility and the high silver ions exchange rate. The hydroxyapatite nanoribbon spherites have large surface, good bio-consistency and high physical/chemical activities [70]. The silver nanoparticles were controlled to be synthesized under the cooperation of the ethylenediamine and the cetyltrimethyl ammonium bromide. Then, under the electrostatic effect of the silver nanoparticles and with the strong adsorbability of the hydroxyapatite nanoribbon spherites, the two substances were combined to form special nanocomposite spheres [71].

The photocatalytic activity of anatase titanium dioxide (TiO_2) was discovered by Fujishima and Honda. This photodecomposition of organic compounds is also useful for killing bacteria. The antimicrobial activity of silver nanoparticles was enhanced when it was incorporated into an Ag/TiO_2 nanocomposite, and would result in higher toxicity levels in the ecosystem in the event of release to the environment [72]. Silver-hydroxyapatite/titania nanocomposite thin films were coated on commercially pure titanium by a modified dipping method, and the antimicrobial effect of this thin film on Gram-positive material proved to be excellent [73].

A combination antimicrobial property is achieved when silver nanoparticles are combined with other metal nanoparticles or oxides acting as a shell or a core to form bimetallic nanoparticles. For example, silver colloids are synthesized using polymer polyvinylpyrrolidone (PVP) as protecting agent, and the silica shell is then coated by means of the Stober process to fabricate Ag@SiO_2 core shell particles. The shell thickness can be easily controlled by the amount of tetraethyl orthosilicate (TEOS) [74]. The preparation process is described in Fig. 2.5.

Carbon nanofibers (CNFs) have some inherent advantages, such as excellent chemical stability and mechanical stability. As a substrate for an antimicrobial system, its nanofibrous structure can offer higher cell adherence compared with other structures. In addition, considering their other functional properties, they have been used for the fillers in polymer composites or used as the biosensors [75]. It is anticipated that the composite of carbon materials and silver nanoparticles can contribute to antimicrobial efficiency. One-dimensional (1D) carbon nanomaterials wrapped by silver nanoparticles were fabricated via a facile and environmentally benign route with the assistance of supercritical carbon dioxide [76].

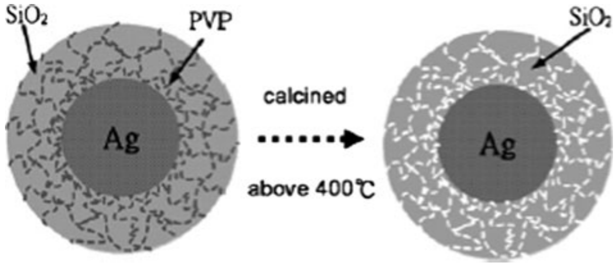

Fig. 2.5 Schematic illustration of the porous characteristics of an Ag@SiO_2 core shell particle [74]

2.1.2.4 Silver(I) Complexes

Silver(I) complexes have long been known to show powerful antimicrobial activity. A great number of silver(I) complexes have been prepared by impregnating silver salts into the corresponding chemical compound. Rowan et al. [77] provided the synthesis and structures of the related silver(I) complexes containing non-functionalized imidazole ligands. The imidazole complex salt $[Ag(imH)_2]NO_3$ has been prepared by reacting $AgNO_3$ with imH (ca. 1:4 molar ratio) and in the $[Ag(imH)_2]^+$ cation, the metal is bonded to the imine N atoms of two neutral imidazole ligands in a similar fashion to that found in the core structure of $[Ag_2(imH)_4](salH)_2$. Chen et al. [78] synthesized thiourea chitosan (TU-CTS) by the reaction of chitosan with ammonium thiocyanate, which was easy to dissolve in 1% acetic acid solution. Ag^+(TU-CTS-Ag^+) complex was obtained by mixing TU-CTS and $AgNO_3$, which greatly enhanced the antimicrobial activity of chitosan as well as the stability of Ag^+.

2.2 Bioactivity of Silver-Based Antimicrobial Nanocomposites

Silver compounds have also been exploited for their medicinal properties for centuries. They were popular remedies for tetanus and rheumatism in the nineteenth century and for colds and gonorrhea before the advent of antibiotics in the early part of the twentieth century [79]. Silver-based medical products, ranging from typical ointments and bandages for wound healing to coated stents, have been proven to be effective in retarding and preventing bacterial infections. Among the properties of silver compounds and silver-based nanocomposites, the bioactivity is most important for biomaterials (such as antimicrobial materials). Therefore, the knowledge about the bioactivity of silver-based antimicrobial nanocomposites will be summarized here.

2.2.1 Silver Salts and Silver(I) Complexes

The silver ion exhibits broad-spectrum biocidal activity toward many different bacteria, fungi, and viruses, and is believed to be the active component in silver-based antimicrobials. Simple silver salts have been known for centuries to exert anti-infective properties, and proven to have significantly low cytotoxicity. However, overdoses of all the forms of silver can lead to complications generally known as argyria. The delivery system becomes more stable when a positively charged silver ion is complexed to negatively charged ions ($AgCl$, $AgNO_3$, Ag_2SO_4). The standard is 0.5% silver nitrate and the most popular silver salt solution used for typical burn wound therapy. Concentrations exceeding 1% silver nitrate are toxic to the tissues. Ionic silver solutions are highly bactericidal and have a beneficial effect in decreasing wound surface inflammation. However, the solutions are unstable and can produce typical black stains when exposed to light, and are therefore extremely impractical. On the other hand, nitrate is toxic to wounds and cells, and appears to decrease healing thus offsetting to some degree the antimicrobial effect of silver. Moreover, the reduction of nitrate to nitrite can cause cell damage, which is most likely the reason for the impaired re-epithelialization when used in partial thickness burns or donor sites.

Silver(I) complexes have been reported to show a different antimicrobial spectrum against microorganisms compared to the spectra of the ligand itself and the hydrated silver(I) ion [80]. Amongst the few silver(I) complexes, silver sulfadiazine complex(AgSD) is commonly prescribed today for the treatment of burns and wounds [81]. AgSD, introduced by Fox [82] in the 1970s, is usually used as a 1% water-soluble cream [83] and the additive in polymer or biopolymer composites [84, 85].

It has recently been reported that silver(I) complexes with hard donor atoms [i.e., complexes with silver(I)-N and silver(I)-O bonds] exhibit an excellent and wide spectrum of antimicrobial activities [86]. Yesilel et al. [87] have synthesized five new silver(I)–saccharinate complexes. In the complex with the molecular structure of $[Ag_2(sac)_2(tmen)_2]$ (sac = saccharinate, tmen = N,N,N', N'-tetramethylethylenediamine), the sac ligand acted as a bridge to connect the silver centres through N or O atom in the imino or carbonyl groups, leading to the formation of an eight-membered bimetallic ring with a chair conformation (Fig. 2.6). The complexes showed a better antimicrobial activity against the studied wild-type and clinical bacterial isolates than the corresponding silver salts.

2.2.2 Silver Nanoparticles

Silver nanoparticles have been proved to be most effective nanomaterials due to the good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms. The nanoparticles synthesized by inert gas condensation and

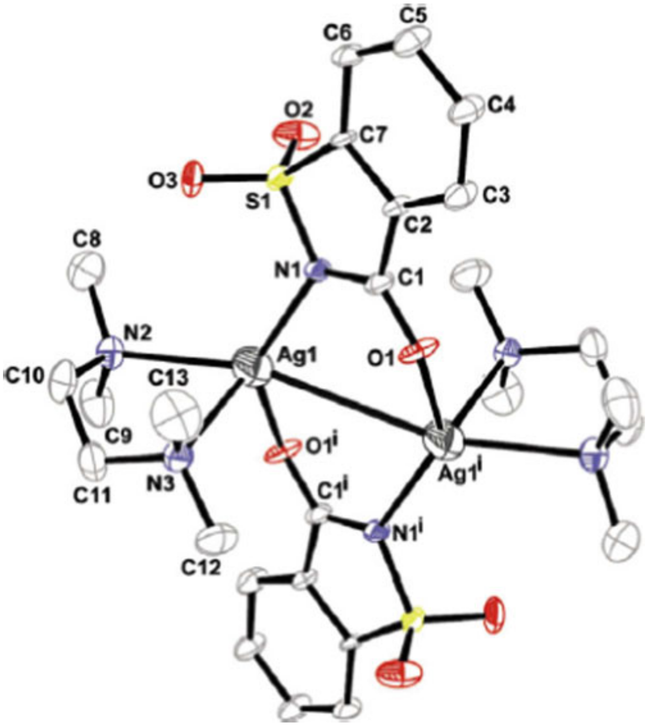

Fig. 2.6 Dimeric unit in $[Ag_2(sac)_2(tmen)_2]$ with the atom-labeling scheme and 50% thermal ellipsoids. H atoms have been omitted for clarity [Symmetry code: (i) 1-x, 1-y, 1-z] [87]

co-condensation techniques were observed to exhibit high antimicrobial activity at low concentrations. It is thought that the antimicrobial activity is related to their specific surface area, and the larger specific surface area generally leads to higher antimicrobial activity [88].

Morones et al. [89] studied the effect of silver nanoparticles on Gram-negative bacteria using high-angled annular dark field microscopy (HAADF) and TEM. The effect of different concentrations of silver nanoparticles with the size of about 16 nm on the growth of bacteria demonstrated that there was no significant bacterial growth observed at a concentration above 75 μg/mL. The HAADF images show that the smaller sized nanoparticles (~5 nm) depicted more efficiently antimicrobial activity. Panacek et al. [90] found the silver nanoparticles synthesized by a one-step protocol have high antimicrobial and bactericidal activity on Gram-positive and Gram-negative bacteria including multiresistant strains such as methicillin-resistant *Staphylococcus aureus*. They found that antimicrobial activity of silver nanoparticles was size dependent, and that nanoparticles 25 nm in size possessed the highest antimicrobial activity.

Pal et al. [91] investigated the antimicrobial properties of silver nanoparticles of different shapes, and found that silver concentration for the inhibition of bacterial growth by spherical nanoparticles was 12.5 μg/mL, while it was 1 μg/mL by the truncated triangular nanoparticles. Therefore, they concluded that the antimicrobial efficacy of silver nanoparticles is shape dependent.

Shahverdi et al. [92] investigated the combination effects of silver nanoparticles with antibiotics. It was observed that the antimicrobial activity of antibiotics like penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin increased in the presence of silver nanoparticles against *E. coli* and *S. aureus*. Ingle et al. [93] investigated the use of *Fusarium accuminatum*, isolated from infected ginger, for the synthesis of silver nanoparticles and analyzed its antimicrobial activity against human pathogenic bacteria. Results revealed that the antimicrobial activity of silver nanoparticles is 2.4–2.9 times that of silver ions.

2.2.3 Silver/Inorganic Nanocomposites

The incorporation of silver nanoparticles into solid inorganic materials in a controlled way can facilitate the application of the nanoparticles or combine the properties of the nanoparticles and the inorganic matrixes to produce some novel properties that are beyond those of the individual component alone.

Recently, it was reported that doping TiO_2 with silver greatly improved the photocatalytic inactivation of bacteria and viruses. Reddy et al. [94] demonstrated that 1 wt% silver in TiO_2 reduced the reaction time required for complete removal of 10^7 cfu/mL *E. coli* from 65 to 16 min under UV light irradiation. Silver nanoparticles in TiO_2 are believed to enhance photoactivity by facilitating electron–hole separation and/or providing a larger specific surface area for the adsorption of bacteria. Additionally, Ag/TiO_2 nanocomposites can be excited by the visible light due to the plasmon effect of silver nanoparticles, then produce the electron–hole pairs which are capable of destroying the bacteria [95]. Therefore, Ag/TiO_2 nanocomposites show promising antimicrobial activity under visible light irradiation.

Kim et al. [96] synthesized hybrid structures of Ag/SiO_2 nanocomposites by the one-pot sol–gel method to prevent aggregation of Ag nanoparticles and increase their antimicrobial abilities. The antimicrobial properties of Ag/SiO_2 nanocomposites annealed below Tammann temperature were excellent because silver nanoparticles were formed on the surface of SiO_2 nanoparticles without aggregation, while they were drastically decreased above Tammann temperature due to the growth of silver crystals. The above-mentioned nanocomposites annealed at the different temperatures of 25°C and 800°C could more effectively kill the Gram-negative bacteria than Gram-positive bacteria, because of the positive charged silver nanoparticles reacting easily with the former bacteria rather than the latter.

Ag/carbon nanocomposites were prepared by impregnating carbon materials (such as carbon fibers) into silver ions solution and then reducing the silver ions to metallic form [97, 98]. For example, silver ions were absorbed into a carbon matrix and then exposed to the fungus *Aspergillus ochraceus* to form intracellular silver nanoparticles, and finally the prepared nanocomposites were heat treated in a nitrogen environment. The experimental results revealed that Ag/carbon nanocomposites possess excellent antimicrobial properties against both bacteria and viruses and have sustained activity due to silver nanoparticles firmly embedded into the carbonaceous matrix. It is anticipated that the composites of 1D carbon nanomaterials (carbon nanofibers or carbon nanotubes) and silver nanoparticles can contribute to antimicrobial efficiency [99]. Carbon nanofibers or nanotubes wrapped by silver nanoparticles (Ag/CNF or Ag/CNT) were fabricated via a facile and environmentally benign route with the assistance of supercritical carbon dioxide. The minimum inhibitory concentration (MIC) for Ag/CNF or Ag/CNT nanocomposite against *E. coli* is 0.5%, indicating that both the nanocomnposites possessed the good antimicrobial activity [76]. Figure 2.7 shows the antimicrobial activities of Ag/CNT nanocomposite.

Ag/graphene oxide (Ag/GrO) nanocomposites were prepared by the chemical reduction of $AgNO_3$ in graphene oxide (GrO) suspension [100]. The size and shape of silver nanoparticles on the surface of GrO sheets was controlled by changing the concentration of $AgNO_3$ solution. The as-prepared Ag/GrO nanocomposites can efficiently inactivate Gram-negative bacteria such as *E. coli* and *P. aeruginosa*.

2.2.4 Silver–Polymer Nanocomposites

Silver–polymer nanocomposites, providing the antimicrobial efficacy with a sustained release of silver, have been synthesized with a great deal of effort to

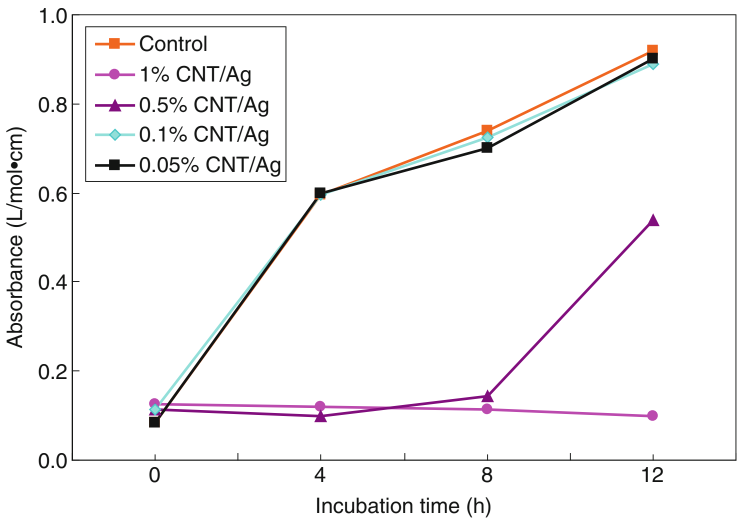

Fig. 2.7 Growth inhibition by Ag/CNT nanocomposites of various concentrations against *E. coli* [76]

prevent diseases in public health hygiene [101, 102]. Due to the stabilization of macromolecules, silver nanoparticles in a polymer matrix are more difficult to aggregate while the antimicrobial activity of silver–polymer nanocomposites is usually higher than that of silver nanoparticles and silver complexes at the same silver concentration [103, 104]. Kong et al. [105] prepared poly(methyl methacrylate) (PMMA) nanofiber containing silver nanoparticles by radical-mediated dispersion polymerization and investigated its antimicrobial activity against both Gram-negative and Gram-positive bacteria. The results showed that the silver–polymer nanofiber had an enhanced killing rate and effective antimicrobial activity than that of $AgNO_3$. Figure 2.8 shows the plot of % reduction versus contact time (min) of different silver compounds on *E. coli*.

Silver-loaded acetate hollow fibers also show good antimicrobial activity. Composite membranes containing a 50-nm-thick chitosan layer on a poly(acrylic acid)/poly(ethylene glycol) diacrylate layer were found to exhibit potent antimicrobial activity towards Gram bacteria, and the antimicrobial activity of the membrane improved with increasing chitosan content [106]. In a similar way, nanocomposite membranes incorporating other functional (e.g., catalytic, photocatalytic, and antimicrobial) nanoparticles into water treatment membranes can be developed.

A recent study has reported on in vitro antimicrobial activity and in vitro cell compatibility of poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) nanofibers loaded with metallic silver particles of a size of 5–13 nm [107]. Of the results, only silver-containing PHBV nanofibrous scaffolds showed a high antimicrobial activity and an inhibitory effect on the growth of both *S. aureus* and *Klebsiella pneumoniae* bacteria (Fig. 2.9). Moreover, the nanofibrous scaffolds having silver nanoparticles

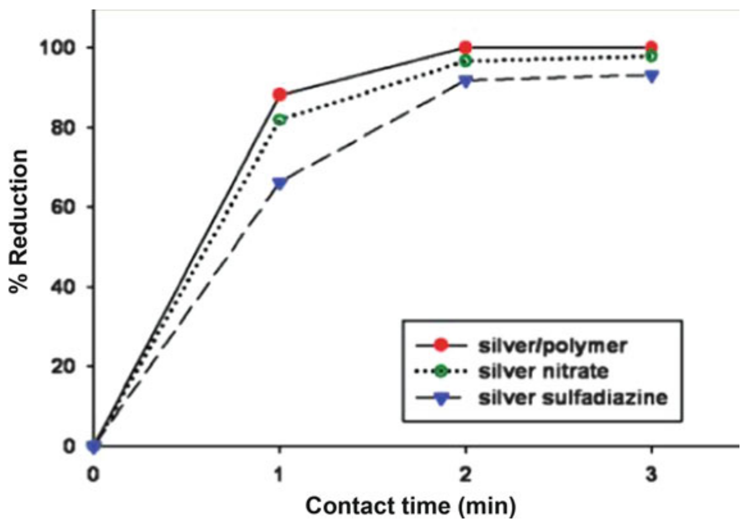

Fig. 2.8 The plot of % reduction versus contact time (min) of different silver compounds on *Escherichia coli* [105]

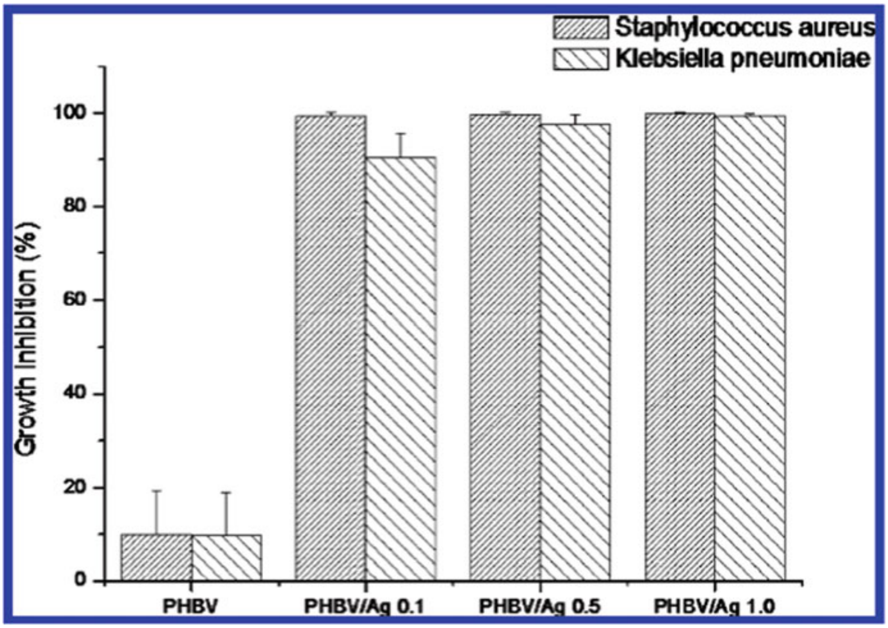

Fig. 2.9 Growth inhibition of PHBV nanofibrous scaffolds with different amounts of silver against *Staphylococcus aureus* and *Klebsiella pneumoniae* [107]

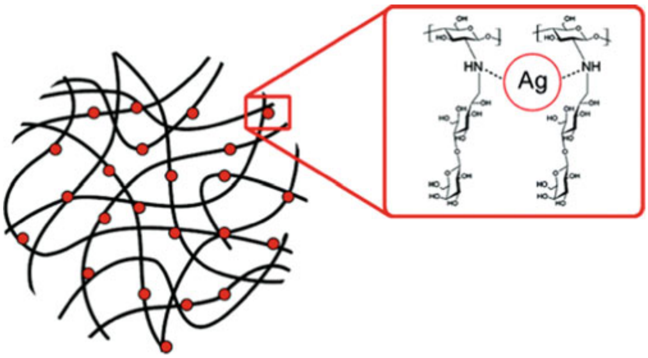

Fig. 2.10 Schematic representation of the polymeric chains of Chitlac providing the nitrogen atoms for the coordination and stabilization of silver nanoparticles [108]

<1.0% were free of in vitro cytotoxicity. Thus, the prepared nanofibrous scaffolds were considered to have a potential to be used in joint arthroplasty.

Travan et al. [108] prepared antimicrobial non-cytotoxic silver–polysaccharide nanocomposites with the polymeric chains of Chitlac providing the stabilization of silver nanoparticles (the schematic representation of the nanocomposites is shown in Fig. 2.10). The results show that these nanocomposite systems display a very effective bactericidal activity toward both Gram-positive and Gram-negative bacteria. However, the hydrogel does not show any cytotoxic effect toward three different eukaryotic cell lines. This novel finding could advantageously contribute to responding to the growing concerns on the toxicity of nanoparticles and facilitate the use of silver–biopolymer composites in the preparation of biomaterials.

2.3 Antimicrobial Mechanisms of Silver-Based Nanocomposites

The study of antimicrobial mechanisms of silver-based nanocomposites offers a valuable contribution to nanobiotechnology. The antimicrobial nanocomposites with their unique chemical and physical properties could be synthesized on the basis of the antimicrobial mechanisms. Therefore, the diverse silver-based nanocomposites could be used to fight infections and prevent spoilage in a broader biological field.

The possible antimicrobial mechanism of silver ions, silver nanoparticles, and silver-based nanocomposites on the microbes has been suggested according to the morphological and structural changes found in the bacterial cells. In general, the antimicrobial mechanism of silver materials is linked with the interaction between silver atoms (ions) and thiol groups in the respiratory enzymes of bacterial cells [109].

One antimicrobial mechanism of silver ions has been reported with silver ions reacting with proteins by attaching the thiol group and then inactivating the proteins [110, 111]. The detailed explanation for the mechanism can be seen in Sects. 1.1–1.3 of Chap. 1

Another mechanism is believed to be that silver ions interact with three main components of the bacterial cell to produce the bactericidal effect: (1) the peptidoglycan cell wall and the plasma membrane, causing cell lysis; (2) the bacterial (cytoplasmic) DNA, preventing DNA replication; and (3) the bacterial proteins, disrupting protein synthesis (Fig. 2.11) [112].

It has been reported that the mode of antimicrobial action of silver nanoparticles is similar to that of silver ions. However, silver nanoparticles show more efficient antimicrobial properties because of their much larger specific surface area than that of ordinary solid silver salts. The nanoparticles firstly get attached to the cell membrane and also penetrate the bacteria, then interact with the sulfur-containing proteins in the bacterial membrane as well as with the phosphorus-containing compounds like DNA, and finally destroy the respiratory chain and lead the bacterial cells to death. In addition, the silver nanoparticles release silver ions in the bacterial cells, which can further enhance their bactericidal activity [89, 113]. Sondi et al. [114] reported on antimicrobial activity of silver nanoparticles against *E. coli* as a model for Gram-negative bacteria. From the SEM micrographs, the formation of aggregates composed of silver nanoparticles and dead bacterial cells were observed. It was also observed that the silver nanoparticles interacted with the building elements of the bacterial membrane and caused damage to the cell. The TEM

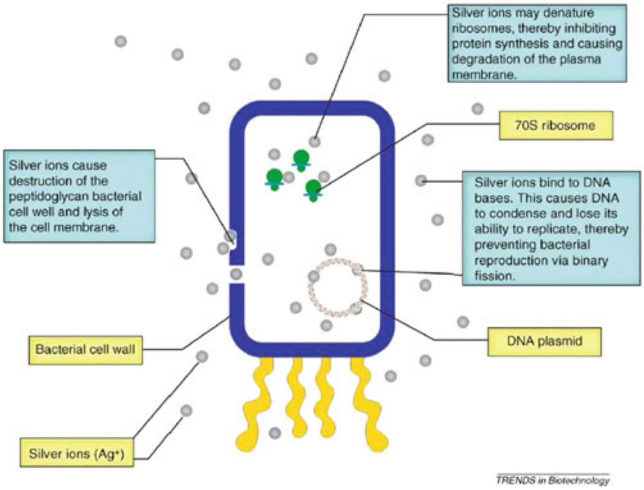

Fig. 2.11 Mechanisms of the antimicrobial activity of silver ions [112]

analysis and EDAX study confirmed the incorporation of silver nanoparticles into the membrane, which was recognized by the formation of pits on the cell surface.

However, because those studies include both positively charged silver ions and negatively charged silver nanoparticles, it is insufficient to explain the antimicrobial mechanism of positively charged silver nanoparticles. Therefore, we expect that there is another possible mechanism. Amro et al. [115] suggested that metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change the membrane permeability, which is caused by progressive release of lipopolysaccharide molecules and membrane proteins. Although their inference involved some sort of binding mechanism, the mechanism of the interaction between silver nanoparticles and components of the outer membrane is still unclear.

Another mechanism of the growth-inhibitory effects of silver nanoparticles on microorganisms has been speculated by the Electron Spin Resonance (ESR) spectroscopy study of silver nanoparticles [116]. It has been revealed that the growth inhibition of bacterials may be related to the formation of free radicals from the surface of silver. Uncontrolled generation of free radicals can attack membrane lipids and then lead to a breakdown of membrane function [117]. Kim et al. [118] suggested that the antimicrobial mechanism of silver nanoparticles is related to the formation of free radicals and subsequent free radical-induced membrane damage. They confirmed that the antimicrobial activity of silver nanoparticles and silver nitrate was influenced by NAC (N-acetylcysteine), and also suggested that free radicals, probably derived from the surface of silver nanoparticles, were responsible for the anti-microbial activity through ESR.

The silver nanoparticles with smaller size possess a larger surface area to contact with the bacterial cells, and hence will have a higher antimicrobial activity than those bigger nanoparticles [119]. Panacek et al. [90] prepared a series of silver nanoparticles by the reduction of the $Ag(NH_3)^{2+}$ complex cation by saccharides, and found that Ag nanoparticles synthesized using disaccharides (such as maltose and lactose) have a much higher antimicrobial activity than those synthesized using monosaccharides (such as glucose and galactose). They investigated the sizes of the silver nanoparticles by TEM and concluded that silver nanoparticles prepared from disaccharide have a smaller size than those from monosaccharide. Therefore, they thought that the observed antimicrobial activity of silver nanoparticles is related to their size.

The antimicrobial efficacy of silver nanoparticles also depends on the shapes of the nanoparticles, which can be confirmed by studying the inhibition of bacterial growth by silver nanoparticles with different shapes [91]. Silver nanoparticles with different shapes (triangular, spherical, and rod) were tested against typical bacterials such as *E. coli*. The {111} facets of silver crystals have high-atom-density, which is favorable to the reactivity of silver. A triangular nanoplate has a high percentage of {111} facets whereas spherical and rod-shaped silver nanoparticles predominantly have {100} facets. According to Pal et al. [91], truncated triangular nanoparticles show bacterial inhibition with silver content of 1, 12.5 μg/L for the spherical nanoparticles, and 50–100 μg/L for the rod-shaped nanoparticles.

Enhanced antimicrobial activities have been reported in silver nanoparticles modified by surfactants, such as SDS and Tween 80, and in polymers, such as PVP 360 [120]. The results are presented in Fig. 2.12. The antimicrobial activity was significantly enhanced for most of the species when silver nanoparticles were modified by SDS while not significant when nanoparticles were modified by Tween 80. This result can be explained as follows [121]: (1) SDS provides more stability for silver nanoparticles than Tween 80, resulting in the decrement of nanoparticle aggregation; and (2) SDS is an ionic surfactant and may have the ability to penetrate or disrupt the cell wall, particularly of Gram-positive strains. The antimicrobial activities of PVP-modified silver nanoparticles were significant because the polymer is most effective in stabilizing particles against aggregation.

2.4 Applications of Silver-Based Antimicrobial Nanocomposites

It has been mentioned that microorganisms with resistance to the antimicrobial activity of silver are exceedingly rare [122, 123]. For decades, silver-based nanocomposites have been used extensively as antimicrobial agents in a number of areas, including medical and pharmaceutical, textile and fiber, coating and paint, food storage, and environmental applications.

2.4.1 Applications in the Medical and Pharmaceutical Industry

Silver has been used for the treatment of medical ailments for over 100 years due to its natural antimicrobial and antifungal properties [124]. Silver ions, silver

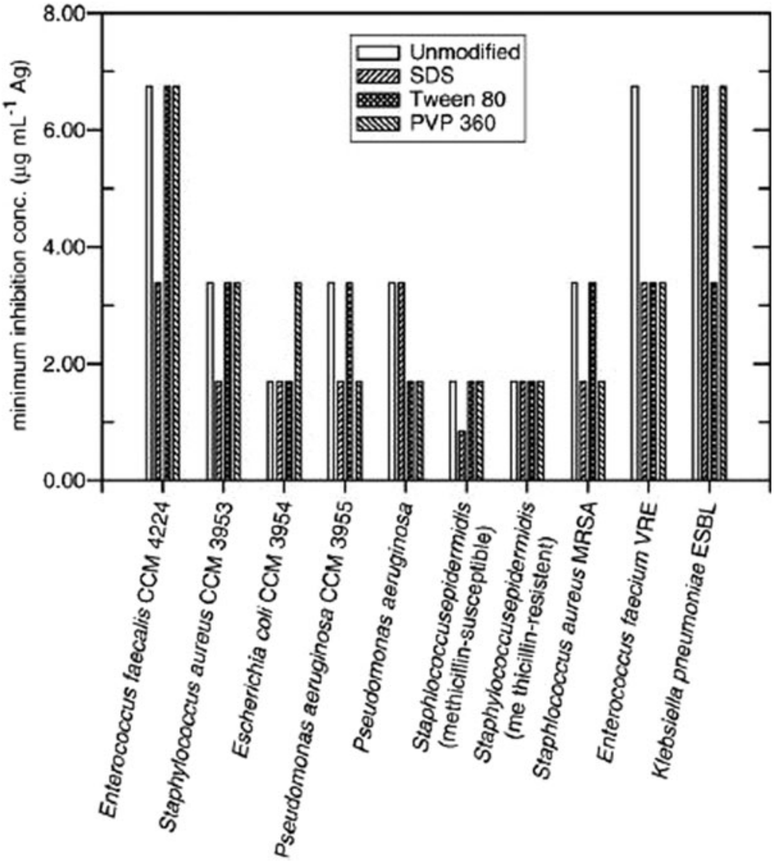

Fig. 2.12 A plot of minimum inhibition concentration (MIC) of the Ag nanoparticles prepared by the modified Tollens process with D-maltose and consequently modified by addition of SDS, Tween 80, and PVP 360 in a concentration of 1% (w/w) [120] (Data used with permission from the American Chemical Society)

compounds and polymer-supported silver nanoparticles have been widely used in various biomedical fields, such as wound dressing materials, body wall repairs, disinfecting medical devices, tissue scaffolds, antimicrobial filters [63], and so on.

2.4.1.1 Wound Dressing Materials

Silver has been used extensively for the treatment of burns, with silver ions or silver nanoparticles incorporated into bandages for use in large open wounds [125]. Many silver-coated and silver-containing dressings are now available for the treatment of wounds [126].

Silver sulfadiazine has been widely accepted as the standard treatment for burn injuries in both animals and humans [127]. It produces better healing of burn wounds due to its slow and steady reaction with serum and other body fluids [128].

The use of silver in the past has been restrained by the need to produce silver as a compound, thereby increasing the potential side effects. Nanotechnology has provided a way of reducing pure silver nanoparticles. This system also markedly increases the release rate of silver. The silver nanoparticles are reported to show better wound healing capacity, better cosmetic appearance, and scarless healing when tested using an animal model [129].

Nanocrystalline silver dressings, creams, and gel effectively reduce bacterial infections in chronic wounds [130, 131]. For example, the electrospun silver/poly (vinyl alcohol) fiber web show efficient antimicrobial property as a wound dressing [132, 133]. The silver/poly(N-vinyl-2-pyrrolidone) composite functioned as a bactericide applied in complicated cases of infected burns and purulent wounds. Pape et al. [134] developed an activated carbon fiber after-treated with silver nanoparticles. Yeo et al. [135] applied silver nanoparticles to produce antimicrobial as-spun mono-filament yarns. Fernández et al. [136] have developed silver nanoparticles on cellulose fibers to be used as the absorbent pad. They immersed fluff pulp and nano-structured lyocell fibers in silver nitrate and the subsequent transformation to silver nanoparticles were done by physical (thermal or UV) or chemical (sodium botohydride) methods. Fu et al. [137] have produced multi-layer composite films from heparin/chitosan/silver for biomedical applications. Khundkar et al. [138] presented an evaluation of the evidence available regarding the use of Acticoat™ (registration certificate number: SFDA 20043640127, made by Smith & Nephew, UK) in burn wounds. The studies show that the non-charged form of silver (Ag^0) in Acticoat™ reacts much more slowly with chloride and thus is deactivated less rapidly in wounds and does not need a carrier. In vitro studies confirm that Acticoat™ provides a sustained release of silver, lasting for a few days. In contrast, the silver ions released from silver nitrate are deactivated within a few hours.

2.4.1.2 Tissue Scaffolds

Bone cement is used for the secure attachment of joint prostheses in, for example, hip and knee replacement surgery. Silver nanoparticles has been used as an antimicrobial additive to poly(methyl methacrylate) (PMMA) bone cement. Ag-PMMA bone cement has been suggested as a means to decrease the incidence of resistance through its multifaceted mechanism of action, and has further shown impressive in vitro antimicrobial activity and low cytotoxicity [139].

Considering the large surface and strong adsorption properties of hydroxyapatite nano-ribbon to adsorb bacteria and high bioactivity of silver nanoparticles, the hydroxyl apatites nano-ribbon spherites containing silver nanoparticles have been successfully prepared by Liu et al. [140]. Hydroxy apatite/silver composite coating has been designed particularly for reducing bacterial infections after implant placement [141]. Bioactivity, dissolution range, and resorption properties, which are close to those of natural bones, have presented in hydroxyapatite, so this material and its composites are the excellent candidates for wide applications in coating artificial joints and tooth roots. Vojislav et al. [142] synthesized the monophasic silver-doped hydroxyapatite nanopowders with high crystallinity and examined their antimicrobial and hemolytic activities. The atomic force microscopic studies illustrate that silver-doped hydroxyapatite samples cause considerable morphological changes of microorganism cells which might be the cause of cell death. Hemolysis ratios of the silver-doped hydroxyapatite samples were below 3%, indicating the good blood compatibility and promising biomaterials for tissue scaffolds. Kumar et al. [143] developed novel β-chitin/nanosilver composite scaffolds for wound healing applications using β-chitin hydrogel with silver nanoparticles. The prepared β-chitin/nanosilver composite scaffolds were bactericidal against *E. coli* and *S. aureus* and also showed good blood-clotting ability. Cell attachment studies using vero (epithelial) cells showed that the cells were well attached on the scaffolds.

2.4.1.3 Implantable Devices

Silver-impregnated medical devices like surgical masks and implantable devices show significant antimicrobial efficacy [144]. For example, poly(ethylene terephthalate) (PET) has been widely used in cardiovascular implants because of its excellent mechanical properties and moderate biocompatibility. However, the infection and thrombogenicity commonly exist in this kind of cardiovascular implants, which can lead to significant morbidity and mortality [145]. Fu et al. [137] has successfully prepared natural polyelectrolyte multilayer films containing nanosilver on aminolyzed PET film via a layer-by-layer fashion. The multilayer films containing well-dispersed nanosilver were effective in killing *E. coli*, and had good anticoagulation activity and low cell toxicity. The above investigated films with biocompatibily may have good potentials for surface modification of medical devices, especially for cardiovascular implants.

2.4.1.4 Disinfecting Medical Devices

The high incidence of infections caused by the use of implanted biomedical devices has a severe impact on human health and health care costs. Many studies suggest a strong antimicrobial activity of silver-coated medical devices [146]. These devices, such as dressings, heart valves, central venous catheters and urinary catheters, have been proved to effectively reduce the infections [147].

The silver-coated silicone catheters are effective at reducing the incidence of catheter-associated urinary tract infections and resistant organisms in an acute care hospital. In a randomized clinical study, a silver alloy-coated central venous catheter reduced the infection rate by 50% although there was no reduction of catheter colonization or of catheter-associated sepsis. Akiyama and Okamoto [148] first described the use of a silver-coated urinary catheter in an uncontrolled study of 102 patients in which no episodes of bacteriuria occurred. Other research has also shown that there was a statistically significant difference in the occurrence of bacterial infection (defined as $>10^5$ organisms/mL) in patients treated with a silver alloy-coated catheter as compared to those treated with a standard device.

2.4.1.5 Biomedicine

Silver nanoparticles have been used to exhibit antimicrobial efficacy against viral particles. The recent emergence of nanotechnology has provided a new therapeutic modality in silver nanoparticles for use in medicine.

Monkeypox virus (MPV), an orthopoxvirus similar to variola virus, is the causative agent of monkeypox in many species of non-human primates [149]. Silver nanoparticles have been shown to exhibit promising cytoprotective activities towards HIV-infected T cells; however, the effects of these nanoparticles towards other kinds of viruses remain largely unexplored. Recently, a study revealed the potential cytoprotective activity of silver nanoparticles toward HIV-1 infected cells [150]. Thus, the use of silver nanoparticles as an anti-viral therapeutic may be a new area of developing nanotechnology-based anti-viral therapeutics.

In addition, many attempts have been made to use silver nanoparticles as an anti-cancer agent. The discovery of an anti-cancer mechanism would be a milestone for cancer treatment. The key to the mechanism is the specific binding of silver nanoparticles towards cancer cells but not the other body cells. One possible reason for this result could be because of the morphological differences between cancer cells and the other body cells, e.g., the morphology of cancer cells is more favorable to the anti-cancer activity of silver nanoparticles [151].

2.4.2 Antimicrobial Food Packaging

Antimicrobial packaging is a promising form of active food packaging material to prevent bacterial infection in foodstuffs. If silver-based antimicrobial nanocomposites are incorporated into packaging materials, the microbial contamination can be controlled by reducing the growth rate of microorganisms. Since the antimicrobial silver can be released from the package during an extended period, the activity can also be extended into the transport and storage phase of food distribution [152].

Many efforts have been made to load and/or to incorporate silver nanoparticles into acceptable packaging materials such as filter paper, low density polyethylene (LDPE), and poly(methyl methacrylate) (PMMA). Also, numerous biodegradable materials, e.g. polysaccharides (such as starch, chitosan, alginate, and konjak glucomannan) have been used to fabricate silver nanoparticle-based composite films. For example, silver zeolite has been developed as the most common antimicrobial agent incorporated into plastics and used in food preservation, disinfection and decontamination of products [153]. The zeolite, in which some of surface atoms are replaced by silver atoms which are laminated as a thin layer (3–6 mm) in the surface of the food-contact packaging polymers, continuously releasing silver ions when silver nanoparticles are exposed to the aqueous solution from the food [154].

Recently, some studies focus on making the filter paper with degradable nature as an attractive alternative for eco-friendly packaging materials. Tankhiwale et al. [155] developed ordinary filter papers as antimicrobial packaging material by grafting vinyl monomers like acrylamide on paper macromolecules followed by loading of silver nanoparticles. This newly developed material shows a strong antimicrobial property against *E. coli*.

2.4.3 Water Disinfection and Microbial Control

The presence of bacteria is the main indication of water contamination. A World Health Organization (WHO) investigation showed that 80% of disease is due to contaminated drinking water [156]. To overcome this promblem, chemical agents and physical treatments including silver-based nanocomposites are commonly used.

Silver nanoparticles are of great value to waste water treatment and to biological systems. The inhibitory effects of silver nanoparticles on microbial growth were evaluated at a treatment facility using an extant respirometry technique. The nitrifying bacteria were susceptible to inhibition by silver nanoparticles, which have detrimental effects on the microorganisms in waste water treatment [157]. For example, Fe_3O_4 containing silver nanoparticles can be used for water treatment [158].

Basri et al. [159] prepared silver-filled asymmetric polyethersulfone (PES) membranes by a simple phase inversion technique. The silver nanoparticles were formed in PES membranes when polyvinylpyrrolidone (PVP) was added during dope preparation. The improved silver dispersion on membrane surfaces was able to enhance the antimicrobial activity against *E. coli* and *S. aureus*, and the investigated results confirmed that the Ag-PES membrane can inhibit almost 100% bacterial growth in rich medium, indicating the potential of Ag-PES to be used in antimicrobial applications especially in water treatment.

The water-related diseases like diarrhea and dehydration can be reduced by improving the quality of the drinking water. Lv et al. [160] prepared silver nanoparticle-decorated porous ceramic composites by fixing silver nanoparticles to the interior walls of porous ceramic channels with the aminosilane coupling agent, 3-aminopropyltriethoxy silane (APTES), as a connecting bridge. On-line tests show that, at a flow rate of 0.01 L/min, the output count of *E. coli* was zero when the input water had a bacterial load of ~10^5 colony-forming units (CFU) per millilitre.

Combined with low cost and effectiveness in prohibiting the growth of *E. coli*, such materials should have wide applications in drinking water treatment.

To sum up, as antimicrobial agents, silver-based materials were applied in a wide range of applications from medical devices to water treatment. Therefore, the silver nanoparticles with their unique chemical and physical properties are proving an alternative for the development of new antimicrobial agents.

2.5 Toxicology of Silver-Based Antimicrobial Nanocomposites

With increasing public knowledge about health care in the world, people are more and more concerned about the rise of possible subsequent diseases caused by new technologies, including nanotechnology and the application of nano-materials especially through inhalation during manufacturing or usage.

Silver-based nanocomposites have been introduced as materials with good potentiality to be extensively used in biological and medical applications. The therapeutic property of silver has shown that it is a safer antimicrobial agent in comparison with some organic antimicrobial agents [161, 162], principally due to its antimicrobial activities having low toxicity to human cells [163].

Some evidence has proved the safety of the application of silver-based materials. There are very few reports in the literature of silver toxicity despite large exposures to silver in the treatment of burn wounds [164]. Skin-innoxiousness of silver nano-colloidal solution especially in the case of smaller nanoparticles has been demonstrated via the skin irritation test performed on rabbits [165]. Hendi et al. [166] reported that silver nanoparticles can promote wound healing and reduce scar appearance in a dose-dependent manner. Furthermore, the experimental results show that silver nanoparticles act by decreasing inflammation, and there were no side effects on the liver and kidney functions through a rat model. The potential benefits of silver nanoparticles in all wounds can therefore be enormous. Nontoxicity of the interaction of silver nanoparticles and the membrane surface has been proved by growing human fibroblasts on various concentrations of silver nanoparticles [167]. Jeong et al. applied a silver–sulfur (Ag/S) composition and observed that addition of sulfur enhances the antimicrobial properties and the stability of silver ions. They emphasized that the Ag/S composite is nontoxic even though sulfur is a toxic agent [168]. Ag/S nano-colloidal solution (SNSE) has also been used by Ki et al. for functionalization of wool [169].

Silver nanoparticles in most studies are suggested to be nontoxic. But some reports state that the biological activity of silver nanoparticles can be detrimental. Many nanoparticles are small enough to have access to skin, lungs, and brain [170]. Exposure of metal-containing nanoparticles to human lung epithelical cells generated reactive oxygen species, which can lead to oxidative stress and cellular damage. Nanoparticles and reactive oxygen production have an established link in vivo. Therefore, it is important to study the potential for the application of silver nanoparticles in the treatment of diseases that require maintenance of circulating drug concentrations or targeting of specific cells or organs [171, 172]. Burd et al. [173] studied the cytotoxicity of five silver nanoparticle-impregnated commercially available dressings, and found that three of the silver dressings showed cytotoxicity effects in keratinocytes and fibroblast cultures. Braydich-Stolle et al. reported the toxicity of silver nanoparticles on C18-4 cells, a cell line with spermatogonial stem cell characteristics [74]. Both studies showed that the cytotoxicity of silver nanoparticles to mitochondrial activity increased with the increase in their concentration.

The comprehensive biologic and toxicologic information of silver nanoparticles is explored by Ahamed et al. [174], while the sources of their exposure and associated risk to human and environmental health are discribed schematically in Fig. 2.13.

The potent anti-inflammatory properties of silver ions and nanoarticles on a wound have been demonstrated histologically. Most of the reports are purely descriptive in nature identifying the decrease in erythema and increased healing. However, it must be realized that not all silver is anti-inflammatory, and that the anti-inflammatory properties depend on the delivery vehicle, the available concentration and species of silver, and the duration of release [175, 176]. Investigation of bio-innoxiousness of silver revealed that smaller-sized silver particles are less toxic to skin than larger ones at the same concentration. Although a small irritation has been reported by applying the colloidal silver with 30-nm particle size, colloidal silver with a particle size of 2–3 nm has been known to be innoxious [177].

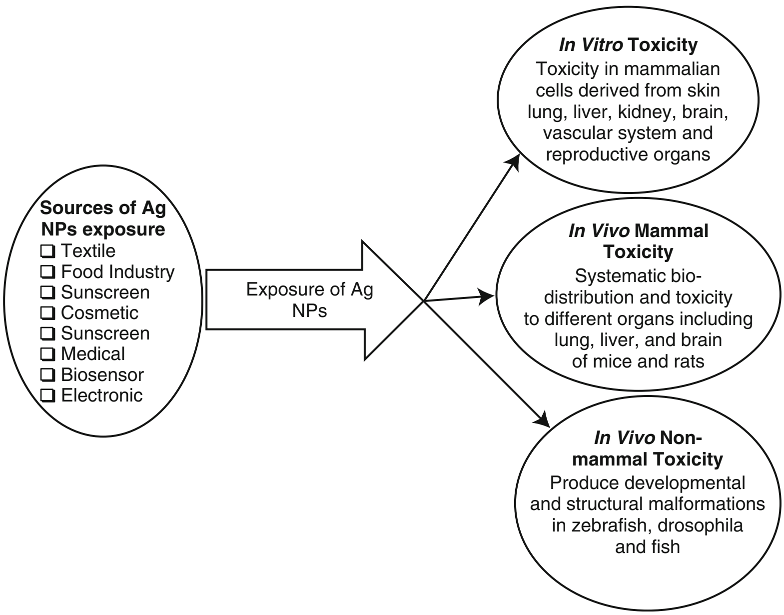

Fig. 2.13 Sources of silver nanoparticles exposure and its known adverse effects [174]

Argyria, a permanent disorder caused by silver deposition in the skin's microvessels in patients who are exposed to chronic silver toxicity [178], is only seen following large oral or inhaled intakes of silver dust or colloidal silver over an extended period of time, and has never been reported as a result of topical application. In addition, prolonged topical application of silver sulfadiazine cream can induce argyria even though it has never been reported as a result of topical application except locally [179]. In vitro studies showed that silver sulfadiazine is cytotoxic but that the cytotoxicity can be reduced by controlling the delivery of the active agent [180].

Additionally, silver nanoparticles are suggested to be hazardous to the environment due to their small size and variable properties [181]. The environmental risk of silver nanoparticles was recently investigated by determining released silver from commercial clothing (socks). The sock material and wash water contained silver nanoparticles of 10–500 nm diameter. The fate of silver in waste water treatment plants (WWTPs), which could treat a high concentration of influent silver, was also examined. The model suggested that WWTPs are capable of removing silver at concentrations much more than expected from the silver nanoparticles-containing consumer products. However, silver concentrations in the biosolids may exceed the concentration (5 mg/L), established by the USEPA. This may restrict the fertilizer application of biosolids to the agricultural lands.

Reactive oxygen species (ROS) generation and oxidative stress appear to be two possible mechanisms of silver nanoparticles toxicity [182]. Depletion of glutathione and protein bound sulfhydryl groups and changes in the activity of various antioxidant enzymes indicative of lipid peroxidation have been implicated in oxidative damage [183]. Oxidative stress occurs when generation of ROS exceed the capacity of the anti-oxidant defense mechanism. ROS and oxidative stress elicit a wide variety of physiologic and cellular events including stress, inflammation, DNA damage and apoptosis [184]. A number of investigators reported that cytotoxicity, DNA damage and apoptosis induced by silver nanoparticles were mediated through membrane lipid peroxidation, ROS and oxidative stress [185]. Mitochondria appear to be sensitive targets for silver nanoparticles toxicity. Asharani et al. suggested that the disruption of the mitochondrial respiratory chain by silver nanoparticles increased ROS production and interruption of ATP synthesis, thus leading to DNA damage. Interaction of silver nanoparticles with DNA led to cell cycle arrest at the G2/M phase [186]. Possible mechanisms of silver nanoparticle-induced toxicity are described in Fig. 2.14.

From the above studies, it can be concluded that longer-term studies and monitoring of humans exposed to silver nanoparticles are imperative to evaluate any potential toxicity. Care must also be taken in the use of silver-based antimicrobials in everyday applications so that the burden of silver ion and nanoparticles exposure does not exceed subtoxic levels. Furthermore, the environmental impact of silver ions and nanoparticles, which leach into the water system, must be considered to prevent ecological disaster.

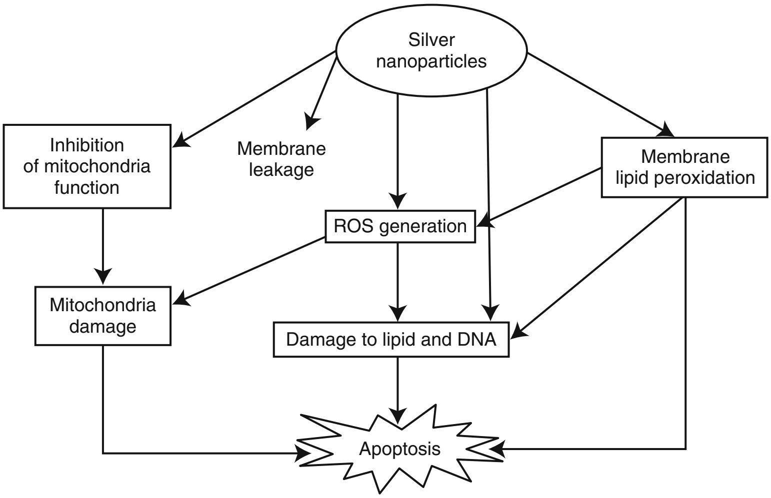

Fig. 2.14 Possible mechanism for silver nanoparticle-induced toxicity [186]

2.6 Future Perspectives

Silver-based nanomaterials exhibit remarkable biological and antimicrobial properties in many fields, and silver nanoparticle dressings are now the new gold standard for medical application. Implantable medical devices, such as neurosurgical and venous catheters, have greatly benefited from the broad antimicrobial activity of silver nanoparticles by reducing both patient infection and dependence on antibiotic use and the associated costs.

The application of silver-based nano-antimicrobials in biomedical and therapeutic applications has developed a wide area of nanotechnology, but the possible side effects of silver ion and nanoparticles have not been completely studied, and hence, detailed studies are needed before the introduction to the market of products related to nano-antimicrobial materials.

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