REVIEW ARTICLE

A Review Study on Synthesis Methods of AgNanoparticles, Considering Antibacterial Property and Cytotoxicity

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ABSTRACT

Silver nanoparticles (AgNP) have been used in many medical and biological applications due to their unique features and characteristics. One of the most important issues researchers address in nanoscience is finding suitable methods to produce nanoparticles with environmentally friendly and non-toxic properties. The unique chemical, physical and biological properties that AgNP possesses give that impetus to developing their production methods. In recent years, there have been many studies documented for the production of AgNP during the development of green synthesis methods (GSM). The present study describes methods for the GSM of AgNP, their biological properties, and other applications, giving the most appropriate methods to synthesize AgNP.AgNP is one of the essential metallic particles, as they can be manufactured and designed in easy ways, and they are also adjustable because they were used in many fields such as catalysts, ideal biometrics, and photo-controlled delivery Systems. AgNp is beholden as a prospectively for tissue regeneration in bioengineering due to its ability in the delivery system as an ideal gene. The studies examined in the current study demonstrated the ability of AgNP in many medical applications because they possess antibacterial properties, and their toxicity can be reduced according to the recorded reports.

Keywords: Antibacterial, Cytotoxicity, Microwave-assisted green method, Silver nanoparticles.

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INTRODUCTION

In the originated, the term nano has come from Nanos, which means in Greek' dwarf. Therefore we can say nanotechnology or nanoscience can give us a signal or indication for microscopic objects. The nanotechnology and nanoscience branch deals with those characterized by their having dimension in a size range of 1 to 100 nm. Many Nanomaterials were documented and developed for antibacterial activity such as metal halides, metal oxides, and bimetals, including Ag, Au, Al, Ce, Cd, Mg, Ni, Pd, Se, Ti, Y, and superparamagnetic Fe.[1,2]

The distinctive optical, mechanical, magnetic, and electrical properties of silver nanoparticles are the main reason for their use in several fields. The applications of silver nanoparticles (AgNPs) can be classified into four major parts scientific, medical, industrial, and consumer product applications. Scientific applications of AgNPs including enhanced IR absorption spectroscopy, determination of fibrinogens in human plasma, colorimetric sensors for histidine, detection of DNA sequences, glucose sensors for medical diagnostics, optical imaging of cancer, biosensors for detection of herbicides, and optical sensors for zeptomole. Industrial applications of AgNPs include catalysis, electronics, book-binding materials, brochures, envelopes, and business stationery. Consumer product applications of AgNPs are face masks, wet wipes, slippers, pillows, cellular phones, vacuum cleaners, shampoo, washing machines, soap, air filters, and food storage containers. Medical applications of AgNPs are diagnosis, medical devices, coating tools, treatment, drug delivery, medical textiles, contraceptive devices, scaffolds, diabetic socks, wound dressings, medical catheters, and sterilization materials in hospitals, etc.[3-5]

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METHODS TO SYNTHESIS AG NANOPARTICLES

In general, the methods of synthesis nanoparticles are divided into two significant parts bottom-up approach includes green and chemical synthesis methods, and the top-down approach includes physical methods. Green synthesis methods include bacteria, fungi, plant extracts, yeast, enzymes, biomolecules, and microorganism. Chemical techniques involve chemical reduction, sonochemical, microemulsion, photochemical, electrochemical, pyrolysis, microwave, solvothermal, and coprecipitation. Physical methods include pulsed laser ablation, evaporation–condensation, ARC discharge, spray pyrolysis, ball milling, vapor and gas phase, pulse wire discharge, and lithography.[5-7]

Physical Methods of Silver Nanoparticles

Laser ablation and evaporation-condensation are important physical methods used in the preparation of nanoparticles. These methods are characterized by the equal distribution of nanoparticles and are less toxic because they do not use for solvents that cause cytotoxicity. Synthesis of silver nanoparticles using the furnace under atmospheric pressure characterized by defects consumes a large energy bulb. It occupies a large area and causes the temperature of the environment surrounding the source used and takes a long time to settle thermally, leading to a significant waste of time and time. The small ceramic heater has demonstrated to synthesized AgNPS with a local heating source.[8]

The evaporated vapor can cool at a suitable rapid rate because the temperature gradient in the vicinity of the heater surface is very steep compared to that of a tube furnace; this makes possible the formation of small nanoparticles in high concentrations. This physical method can be useful as a nanoparticle generator for long-term experiments for inhalation toxicity studies and as a calibration device for nanoparticle measurement equipment.

AgNP could be synthesized by laser ablation technique (LAT) of metallic bulk materials in solution. The efficiency of nanoparticles prepared in this way depends on several factors, including the duration of the laser pulses, laser wavelengths, effective liquid medium and excision duration. In the LAT has one crucial advantage that doesn't use chemical agents during procedure compare with other methods, that make it useful to produce metal colloids. Moreover, the LAT can produce uncontaminated and pure metal colloids.[9,10] Table 1 shown some physical methods used to synthesized silver nanoparticles.

Chemical Methods

A common chemical method to produce silver nanoparticles is the chemical reduction method using organic and organic reducing agents. A few chemical reducing agents such as N, N-dimethylformamide (DMF), sodium borohydride (NaBH4), polyethylene glycol, and Tollens reagent, are used to reduce silver ion from its salts in non-aqueous and non-aqueous solutions. As mentioned previously, the reducing agents are used to reduce the silver ions (Ag+) and to create the metallic silver (Ag0) by following the agglomeration into the oligomeric clusters. The oligomeric clusters then develop the metallic colloidal silver particles. These particles are required to use the protective agents to ensure the dispersive nanoparticles are stable throughout the metal nanoparticle preparation and to protect the nanoparticles from being absorbed or bind to the nanoparticles' surfaces. It is crucial for surfactants such as thiols, amines, acids, and alcohol, for the surface of the particles to have stable particle growth and protect the particle from sedimentation, agglomeration, or losing their surface features. Tollens method is a one-step process and has been applied to synthesize the silver nanoparticles in controlled size. However, in the modified Tollens method, the silver ions are reduced by the saccharides with ammonia, yielding silver nanoparticle

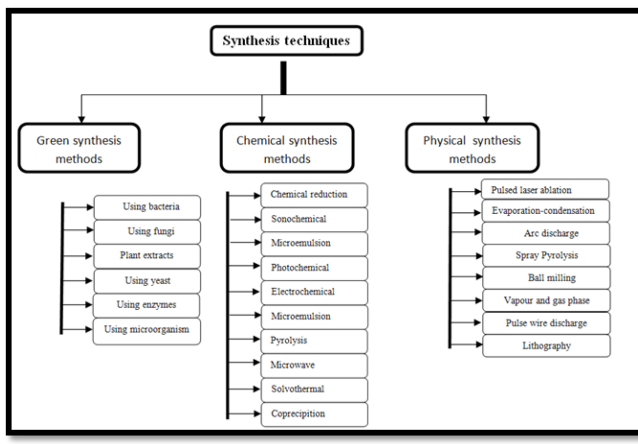

Figure 1: Synthesis methods of silver nanoparticles

Table 1: Physical methods of silver nanoparticles with some applications

Physical methods	*Shape & Size*	*Applications*	*Ref*
Pulsed laser ablation	10 nm, spherical	useful to monitor the ablation process	[13]
	10 nm	Antimicrobial	[14]
	10–40 nm, spherical	Plasmon resonance	[15]
	20 nm, spherical	Anti-microbial	[16]
Arc discharge	18 nm	Antimicrobial	[17]
	10 nm, spherical	Elimination of toxic p-nitrophenol	[18]
	33 & 220 nm	Electronic packaging	[19]
Spray pyrolysis	35 nm, spherical	Pasmonic properties	[20]
Ball milling	28 nm, spherical		[21]
Pulse wire discharge	19–35 nm	Water treatment	[22]

films between 50 to 200 nm, silver hydrosol between 20 to 50 nm, and different silver nanoparticles.[11,12] Table 2 presents the chemical methods to synthesize the silver nanoparticles.

Green Synthesis Methods

In recent years, researchers have focused on developing methods used to synthesize silver nanoparticles in green chemistry, which is based on reduction, stenosis, and stability. The process of synthesizing silver nanoparticles in the green chemistry method is done by using environmentally friendly and non-toxic biological materials. The use of natural extracts of existing biological materials or organisms (enzymes, vitamins, amino acids, and polysaccharides) are environmentally moderate but very sophisticated in chemical terms. Previous studies were demonstrated the successful synthesis of AgNPs by using biological and microorganisms systems. Green synthesis used many biological sources such as bacteria, fungi, plant extracts, yeast, enzymes, biomolecules, and microorganism.

Bacteria Mediated to Synthesis Silver Nanoparticles

Previous studies had documented many types of bacteria to produce silver nanoparticles, such as Aeromonas sp. THG-FG1.2,[30] Bacillus methylotrophic,[31] Pseudomonas putida NCIM 2650,[32] Vibrio alginolyticus,[33] Lactobacillus spp.,[34] Endosymbiotic Bacterium,[35] Penicillium glabrum (MTCC 1985),[36] Novosphingobium sp.HG-C3,[37] and Rhodococcus spp.[38] Pseudomonas stutzeri AG259 was used to synthesize the silver nanoparticles from bacteria, and the size of the obtained silver nanoparticle was 200 nm.[39] Singh *et al*.[30] reported that the synthesis of AgNPs was successfully done by using Aeromonas sp. THG-FG1.2 to obtain the silver nanoparticles with the size between 8 to 16 nm and spherical. Serratia nematodiphila was used to produce the silver nanoparticle with the size between 10 to 31 nm and spherical.[40] Actinobacteria Streptomyces spp. 211A and Cyanobacteria Spirulina platensis were used to produce silver nanoparticles by using $AgNO_3$ as

Table 2: Chemical methods of silver nanoparticles with some applications

Chemical methods	*Size and shape*	*Applications*	*Ref*
Chemical reduction	10–20 nm , spherical	Antimicrobial	[23]
Sonochemical	50–100 nm	Antimicrobial	[24]
Microemulsion	50 nm, spherical	Antimicrobial	[25]
Photochemically	3.5 nm , Spherical	Biocompatibility antibacterial	[26]
Electrochemical	50 nm	Biosensors	[27]
Microwave	8.5 nm	Sensors	[28]
Solvothermal	50 nm, hexagonal	Surface-enhanced Raman scattering	[29]

Table 3: Bacteria mediated to synthesis silver nanoparticles

Bacteria	*Size*	*Morphology*	*Ref*
Kinneretia THG-SQ14	15–20	Mono-disperse, FCC, spherical	[30]
Aeromonas sp. THG-FG1.2	8–16	Face Centered Cubic (FCC), spherical	[30]
Bacillus methylotrophicus	10–30	Spherical	[31]
Endosymbiotic Bacterium	10–60	Cubic, spherical, hexagonal, crystalline, oval	[44]
Novosphingobium sp.HG-C3	8–25	Spherical and crystalline	[37]
Rhodococcus spp.	5–50	Spherical	[38]
Exiguobacterium mexicanum	5–40	–	[45]
Bacillus strain CS 11	45±0.15	FCC, spherical	[46]
Actinobacteria	5–50	Spherical	[47]
Serratia nematodiphila	10–31	Crystalline and spherical	[40]
Vibrio alginolyticus	50–100	Spherical	[33]
Escherichia coli DH5α	10–100	Spherical	[48]
Pseudomonas putida NCIM 2650	70	Spherical	[32]
Nocardiopsis sp.MBRC-1	45±0.15	Spherical	[42]
Lactobacillus spp.	2–20	Spherical	[34]
Lactobacillus casei subsp. Casei	25–50	Spherical	[49]
S1–S10	25–30	–	[50]
Cyanobacteria Spirulina platensis and actinobacteria Streptomyces spp. 211A	7–15	–	[41]
Shewanella oneidensis	4±1.5	Spherical	[51]
Bacillus sp.	5–15	Crystalline	[52]

a precursor, and the size of the obtained AgNPs was between 7 to 15 nm.[41] Thamilselvi and Radha[42] reported the synthesis of silver nanoparticles using Pseudomonas putida NCIM 2650 and obtained the size of 70 nm in a spherical shape. Table 3 displays the synthesis of silver nanoparticles from the organisms.

The presence of nitrate reductase enzyme is extensively recognized as a mechanism for the biosynthesis of silver. In the process of vitro synthesis of silver, bacteria are used with alpha-nicotinamide adenine dinucleotide phosphate (NADPH). It functions to remove the step in the downstream process that is needed in other circumstances.[43]

Besides, the bacteria continue to grow after the AgNPs have developed. However, the drawback of using bacteria as nano factories is the synthesis rate whereby it becomes slow, and the number of sizes and shapes is limited compared to the conventional methods. Thus, the synthesis for the AgNPs was investigated onto the fungi-based nano factories and chemical reactions such as plant extracts materials.[5]

Fungi Mediated to Synthesis Silver Nanoparticles

In comparison to bacteria, fungi can create more nanoparticles as they can discharge a high amount of protein that can be meaningful to the productivity of the nanoparticles. The followings are the mechanism of silver nanoparticles using fungi: Ag+ ions are trapped at the surface of the fungal cells, and the following silver ions are reduced by the enzymes that exist in the fungal system. Extracellular enzymes such a snapthoquinones and anthraquinones are reported to facilitate the process of reduction. For instance, *F. oxysporum* is the NADPH-dependent nitrate reductase, and a process of extracellular shuttle quinine is accountable for the formation of nanoparticles. Although the exact mechanism involved in the production of silver nanoparticles was not mentioned, yet the situation above is accountable throughout the process.

Many studies reported the synthesis of silver nanoparticles from fungi such as *Trichoderma viride*,[53] *Phoma glomerata*,[54] *Fusarium semitectum*,[55] *Alternaria alternate*,[56] *Penicillium citrinum*,[57] *Hemath*,[58] Endophytic fungus *Penicillium sp*.,[59] and Endophytic bryophilous.[60] Shelar and Chavan[61] reported the synthesis of AgNPs by using Trichoderma harzianum. The obtained AgNPS was 34.77 nm in size and spherical shape. Ghazwani[62] also reported the synthesis of silver nanoparticles with spherical shape but with the size of 20 nm. The process used three different fungal, *Alternaria solani*, *F. oxysporum*, and *Aspergillus niger*. Ellipsoid and spherical shapes silver nanoparticles were successfully synthesized with a range of size between 1–50 nm by using Fusarium semitectum.[63] The most common shape for silver nanoparticles was spherical, and the size was from 1–109 nm. Table 4 tabulated several studies related to the use of fungi in the synthesis of silver nanoparticles.

A major disadvantage of using microbes for the synthesis of silver nanoparticles is a long process that can be time-consuming compared to plant extracts. Due to this, plant extracts are a feasible choice in the process of synthesizing silver nanoparticles.

Plants Mediated to Synthesis Silver Nanoparticles

Plant extracts are chosen to synthesize silver nanoparticles due to many advantages such as its availability, safe, and non-toxic. In many cases, it has a wide range of metabolites that can reduce the silver ions and faster than the microbes in the synthesis process. The main mechanism of this process is the plant-assisted reduction due to the presence of phytochemicals, which comprises amides, aldehydes, carboxylic acids, flavones, ketones, and terpenoids. Phytochemicals that are accountable

Table 4: Fungi mediated to synthesis silver nanoparticles

Fungi	*Size (nm)*	*Morphology*	*Ref*
Trichoderma harzianum	34.77	Ellipsoid & spherical	[61]
Aspergillus niger, Fusarium oxysporum and *Alternaria solani*	20	Spherical	[62]
Fusarium semitectum	1–50	Ellipsoid & polydispersed spherical	[63]
Endophytic fungus Penicillium sp.	25 and 30	Spherical	[59]
Schizophyllum commune	51–93	Spherical	[64]
Penicillium citrinum	109	Uniform spherical	[57]
Endophytic bryophilous	25–50	Ellipsoidal	[60]
Pestalotia sp.	12.40	Polydispersed and spherical	[65]
Trichoderma reesei	5–50	Random	[66]
Trichoderma viride	5–40	Spherical	[53]
Endophytic fungus Aspergillus clavatus	10–25	Polydispersed, Hexagonal, & spherical	[67]
Filamentous fungus Penicillium sp.	58.35 ± 17.88	–	[58]
Phoma glomerata	60–80	Spherical	[68]
Penicillium fellutanum	5–25	Spherical	[70]
Alternaria alternate	32.5	Polydispersed and spherical	[56]
Fusarium semitectum	10–60	Crystalline and spherical	[71]
Fusarium acuminatum	13	Spherical	[72]

for the immediate reduction of ions are flavones, organic acids, and quinones, as they are known to be water-soluble phytochemicals. Past researches have proven that xerophytes encompass emodin and anthraquinone that had gone through the process of tautomerization that can result in the formation of silver nanoparticles. As for mesophytes, there are three types of benzoquinones which are cyperquinone, diechequinone, and remain. Generally, phytochemicals are advised to be applied directly in the process of ions reduction and the silver nanoparticles formation.[73]

Previous studies documented the synthesis of silver nanoparticles with a wide range of plants such as *G. officinalis*,[74] *Euphorbia tirucalli*,[75] *Elephantopus scaber*,[76] *Indigofera tinctoria*,[77] *Alysicarpus monilifer*,[78] *Rheum palmatum*,[79] *Crocus sativus L*,[80] and *Excoecaria agallocha*.[81] The leaves of *G. officinalis* were used to produce the silver nanoparticles with 23 nm and spherical.[74] Kalaiselvi *et al*.[75] successfully reported the synthesis of silver nanoparticles from the latex of *Euphorbia tirucalli*. AgNPs with the size between 20–30 nm and in spherical shape were investigated.

Past literature reported the different sizes and shapes for AgNPs such as irregular flower, worm-like, dendritic structures,[82] hexagon, spheres (Arokiyaraj *et al*., 2017), polydispersed, spherical,[83] rectangular, oval,[84] cubical,[85] and triangular[86] with a wide range of sizes from 1 to 100 nm.

Biosynthesis is almost similar to the bottom-up approach, whereby the significant reaction that occurs is the reduction or oxidation. The requirement for the biosynthesis of nanoparticles was demanded as the cost of the physical and chemical methods is high. Normally, the chemical synthesis method can result in the existence of toxic chemicals that can be harmful to medical applications. However, different outcomes were obtained when the nanoparticles were biosynthesized using the green synthesis method. Thus, to reduce the cost of nanoparticles synthesis, the scientists decided to use microbial enzymes and plant extracts due to their antioxidants or reducing properties. In general, green synthesis has many advantages compared to physical and chemical methods. Green synthesis methods are cheaper, eco-friendly, and convenient to be scaled up for large-scale synthesis. Moreover, this method

Table 5: Plants mediated to synthesis silver nanoparticles

Plant names	*Part of plant*	*Size*	*Morphology*	*Ref*
G. officinalis	Leaves	23 nm	Spherical	[74]
Euphorbia tirucalli	Latex	20–30 nm	Spherical & cubic	[75]
Passiflora edulis f. flavicarpa	Leaves	20 & 50 nm	Cubic	[87]
Annona reticulata	Leaves	6–8 nm	Cubic	[88]
Dodonaea viscosa	Leaves	15, 18, 12, and 20 nm	Spherical, irregular flower, worm-like, and dendritic structures	[82]
Elephantopus scaber	Leaves	37.86 nm	Cubic	[76]
Prosopis juliflora	Bark	1–50 nm	Spherical	[89]
Indigofera tinctoria	Leaves	16.46 nm	Spherical, crystalline	[79]
Givotia moluccana	Leaves	55 nm	Cubic	[90]
Crocus sativus L	Leaves	15 nm	Spherical	[80]
Rheum palmatum	Root	121 nm	Hexagon & spheres	[79]
Calliandra haematocephala	Leaves extract	70 nm	Spherical and FCC	[91]
Prunus japonica (Rosaceae)	Leaves extract	26 nm	Hexagonal, spherical, crystalline and irregular	[92]
Terminalia arjuna	Bark extract	2–100 nm	Spherical	[93]
Turbinaria ornata (Turner)	Seaweed aqueous extract	20–32 nm	Spherical and crystalline	[94]
Catharanthus roseus	Leaves extract	20 nm	Crystalline, FCC and spherical	[95]
Althaea officinalis radix	Hydroalcoholic extract		Polydispersed and spherical	[83]
Solanum nigrum andclitoria ternatea	Leaves extract	23 nm	Spherical	[96]
C. olitorus Linn and I.batatas Lam	Leaves extract	37.9 & 67.3 nm	Crystalline	[97]
Eucalyptus globulus	Leaves extract	1.9–4.3 and 5–25 nm	Spherical	[98]
Pistacia atlantica	Seeds extract	10–50 nm	Spherical	[99]
Thevetia peruviana	Latex extract	10–30 nm	Spherical	[100]
Solanum tuberosum	Potato infusion	10–12 nm	Crystalline, FCC and spherical	[101]
Aloe vera	Leaves extract	70 nm	Spherical, rectangular, cubical and triangular	[85]
Peach gum	Peach gum powder	23.56 nm	FCC	[102]
Premna serratifolia L.	Leaves extract	22.97 nm	Cubic	[103]

does not require high energy, pressure, temperature, and toxic chemicals.

ANTIBACTERIAL PROPERTIES OF AG NANOPARTICLES

Silver nanoparticles were investigated for their activities that are known to be antibacterial against a wide range of gram-negative, gram-positive, as well as against antibiotic-resistant strains. Gram-negative genera include Vibrio, Salmonella, Pseudomonas, Escherichia, and Acinetobacter. Acinetobacter species linked to the disease that afflicts the patient during hospital treatment in the medical care unit. Gram-positive genera include Streptococcus, Staphylococcus, Listeria, Enterococcus, Clostridium, and Bacillus. Meanwhile, antibiotic-resistant bacteria involve strains such as Enterococcus faecium, vancomycin-resistant Staphylococcus aureus, and methicillin-resistant.[3]

Antibacterial silvers are used in many applications such as coating-based usages, textiles, and plastic products. Nano-silver particles have also been used in many applications due to their antibacterial properties. Several studies had been conducted on the silver nanoparticles against bacteria, and the outcomes have proven their high activity as an antibiotic. It has been proven that the nanoparticles of Ag are potential to be anti-microbial agents against drug-resistant bacteria. Based on previous studies on silver nanoparticles, the cause of bacteria-killing is due to the penetration of its cellular wall. The researchers hypothesized that the silver nanoparticles had stimulated laminates and lesions in the bacteria's cell membrane, which leads to the disintegration of the bacteria. Studies have also shown that silver can interact with enzymes that control the metabolic process for the cells that disrupt its functioning and cell death.[5,104] Several studies had provided a few causes that can significantly impact the effectiveness of silver nanoparticles against bacteria, such as the size, shape, concentration, and type of bacteria. Among the different shapes of synthesized silver nanoparticles that had been reported are pseudospherical,[105] Cubic,[106] Spherical,[107] ellipsoidal,[108] Hexagonal,[109] Platelets,

Table 6: Sizes and shapes with their antibacterial activities of silver nanoparticles

Silver type	*Shape*	*Size*	*Bacterial type*	*Inhibition zone or MIC*	*Ref*
AgNPS	Spherical	7 nm	*E.coli & S. aureus*	6.25 & 7.5 μg/mL	
	Spherical	29 nm	*E.coli & S. aureus*	13.02 & 16.67 μg/mL	
	pseudospherical	89 nm	*E.coli & S. aureus*	11.79 & 33.71 μg/mL	[105]
AgNPS	Not reported	16 nm	*E.coli*	25 μg/mL	[115]
AgNPS	Cubic	5–40 nm	*E.coli*	8-12 mm	[106]
AgNPS	Spherical	10.78 nm	*B.cereus & B.subtilis S, aureus &, P. aeruginosa*	9 & 12 mm 15 & 6 mm	[107]
AgNPS	Cubic	18.2 nm	*E.coli & S. aureus*	12.7 &11.3 mm	
$AgNO_3$	-	-	*E.coli & S. aureus*	9.5 & 10 mm	[116]
AgNPS	Spherical	55–83 nm	*K. pneumoniae & M. luteus*	18 & 11 mm	[114]
AgNPS	Spherical	15 nm	*P. aeruginosa & S.aureus*	11 & 19 mm	[117]
AgNPS	Spherical	20–90 nm	*E.coli*	5 mm	
AgNP-PVA	Spherical	20–90 nm	*E.coli*	1.5-2.5 mm	[118]
AgNPS	Spherical	16 nm	*E.coli & S. aureus*	31 & 27 mm	
AgNPS	Ellipsoidal	20 nm	*E.coli & S. aureus*	23 & 21 mm	[108]
AgNPS	Spherical	46 mm	*S. aureus & B. cereus* *Pseudomonas & B. subtilis*	14 & 13 mm 18 & 20 mm	[119]
AgNPS	Spherical	10–25 nm	*S. enteric & S. aureus*	15 & 11 mm	[120]
AgNPS	Spherical	7.3 nm	*P. aureginosa*	15.3 mm	[121]
AgNPS	Hexagonal	15 nm	K. pneumonia & S. aureus P. aeruginosa & P. vulgaris	23 & 27.5 mm 26 & 14 mm	[109]
AgNPS	Cubic	53.2 nm	*K. pneumonia &, M.flavus,* *P. aeruginosa & B.pumilus*	8.5 & 9 mm 10.5 & 10 mm	[122]
AgNPS	Spherical	40 nm	*E.coli*	20 mm	
AgNPS	Triangular	40 nm	*E.coli*	24 mm	
AgNPS	Hexagonal	40 nm	*E.coli*	10 mm	[111]
AgNPS	Spheres	40–80 nm	Not reported	Not reported	
	Cubic	140–180 nm	Not reported	Not reported	
	Platelets	20–60 nm	Not reported	Not reported	
	Rods	120–180 nm	Not reported	Not reported	[110]

Rods,[110] and Triangular.[111] Meanwhile, the different sizes of silver nanoparticles were also reported ranges from 1–500 nm in size due to different synthesis methods and were investigated against a wide range of bacteria such as E.coli, S. aureus,[105] V. cholera,[112] B. subtilis,[113] B.cereus, B.subtilis, P. aeruginosa,[107] K. pneumonia, and M. luteus.[114] Table 6 presents the different sizes and shapes with their antibacterial activities of silver nanoparticles.

Mechanism of Action of Silver Nanoparticles on Bacteria

Interaction between the silver nanoparticles and the cell surface of different bacteria can occur physically. In gram-negative bacteria, several studies had reported the bond and growth of AgNPs to the surface of the bacteria. Silver nanoparticles act as the anchor to the bacterial cell wall and eventually enter it, causing changes to the cell membrane structure. This renders the bacteria to be more permeable. The accumulation of the AgNPs causes gaps in the bilayer of the cell membrane. The permeability of the cell increases and eventually causes the cell to die.[123] Studies on electron spin resonance spectroscopy reported the development of free radicals from the silver nanoparticles when interacting with the bacteria causing the cell to die.

Similarly, when the activity of the silver nanoparticles and the free ions combines, it creates a vigorous antibacterial activity from the broad spectrum. Besides, the nanoparticles might also release the silver ions, and these ions can interact with the thiol groups from various enzymes, which can deactivate them.[124] Once the bacterial cells are in contact with the silver, the silver ions get absorbed, consequently hindering the cells functions and causing damages to the cells. Besides that, reactive oxygen species (ROS) also get generated. ROS is free-forming radicals that possess dominant bacterial action and are produced by inhibiting respiratory enzymes from the silver ions attacking the cells by themselves. Silver ions damage its structure when it enters the microbial body. Ribosomes may cause inhibition to the protein synthesis due to its denatured state, causing the translation and transcription to be blocked by the genetic materials or bacterial cells. When the AgNPs have been treated, the protein synthesis is altered, and the proteomic data exhibits the gathering of immature precursors

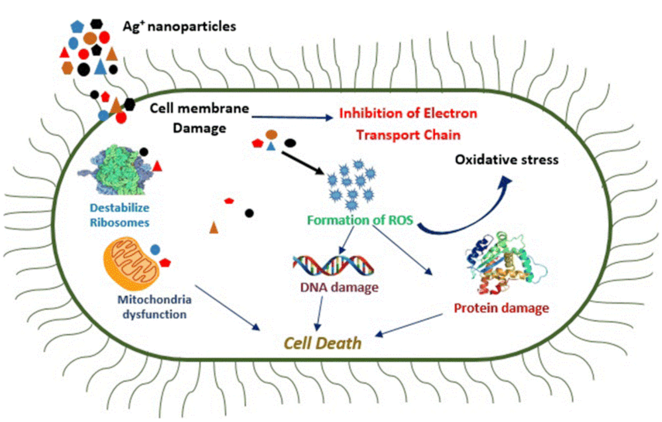

Figure 2: The Proposed Scheme of the Mechanism of Action of Silver Nanoparticles on Bacteria.[127]

in the membranes proteins. Consequently, the composition of the outer membrane undergoes destabilization.[125]

Silver is a soft acid that can respond to the soft base. Cells made up of sulfur and phosphorus are known to have soft bases. Also, DNA is a major component that comprises sulfur and phosphorus. Thus, if the nanoparticles act on these soft bases, it causes damages to the DNA cell and eventually leads to cell death.[126]

CYTOTOXICITY OF SILVER NANOPARTICLES

Nanotoxicology is a sub-branch of toxicology associated with the toxic potentials of nanoparticles (NPs) and their hostile impacts on living organisms and the environment. The NPs nanotoxicity relies on their doses and distinctive features of their chemical and physical properties. Now, silver nanoparticles are applied for many applications to serve various purposes, specifically scientific applications such as anti-microbial and pharmaceutical applications, food packaging processes, textile industry, and water purification systems. However, information relating to their nano-toxic potentials is still insufficient and requires several considerations of AgNPs, such as their size, shape, surface, and stability. Several studies, such as in-vivo, *in-vitro*, and in-silico studies, highlighted the AgNPs' nano-toxic potentials. This section evaluates the common properties of NPs related to nanotoxicology, such as the shapes, sizes, the status of agglomeration, dose, and surface area.[128]

When the nanoparticles undergo green synthesis, they are assembled using biological systems such as bacteria, fungi, plant extracts, and yeast. Other colloidal metal nanoparticles such as platinum (PtNPs) and gold (AuNPs) also applied the green synthesis method. However, for this study, AgNPs become the main focus due to their bacterial properties. The use of green synthesis onto the AgNPs had transformed the field of nanoparticle synthesis. Green synthesis is widely utilized due to its various reasons such as easily attainable, sparsely distributed, easy and safe to handle, have a wide range of metabolites, minimizes the amount of waste, and requires less energy and cost. The green synthesis method for AgNPs involves selecting the medium of the solvent, reducing agents, stabilizing the non-toxic compound/materials, and avoiding the accumulations of the nanoparticles. By adopting this technique, the nanoparticles exhibited low toxicity which can be used to encapsulate the drug molecules. Generally, the field of nanomedicine related to AgNPs is further examined throughout the whole world.

Many *in-vitro* studies have documented the cytotoxicity of silver nanoparticles. In previous studies, many researchers had focused on the toxicity of the silver nanoparticles based on the concentrations. Several studies have been demonstrated various range of non-toxic concentrations such as 10 µg/mL,[119] 25 µg/mL,[129] 50 µg/mL,[130] 40 µg/mL,[131] 100 µg/mL,[132] 160 µg/mL, 320 µg/mL,[133] and 300 µg/mL.[134] Many reported researches mentioned that the toxicity of AgNPs is less when it is synthesized using natural material to reduce the cytotoxicity of silver nanoparticles.[135] Table 7 presents some studies about the cytotoxicity of AgNPs.

According to previous studies, silver nanoparticles can be toxic in high concentrations, which indicated the possibility of using the silver nanoparticles in the human body with a control amount to obtain its unique properties and avoid the possible side effects by using a few ratios of it.

APPLICATIONS OF SILVER NANOPARTICLES

Nanotechnology has succeeded in providing solutions to various challenges in science and engineering in various sectors. The nanoscale structure can be used anywhere and for all applications. The properties of the material depend primarily on the size of the nanoparticles.[139] Silver nanomaterials are reasonable metallic silver particles with at least one dimension less than 100 nm. The story behind the discovery of nano-silver is not new, as it dated back more than 100 years. In the past, silver nanoparticles were produced by carrying them out through the passage with positive electric current through the suspended silver rods in the water. Through this technique, the obtained sizes of the silver nanoparticles were between 15–500 nm and were used to treat bacterial infections before the discovery of penicillin in 1928. When the silver converted from natural size to the size of the nanoparticles, it has caused an increase in the physical, chemical, and optical properties of silver. Due to the increase of the surface area, this leads to an increase of effectiveness against bacteria and fungi as it penetrates the cell wall of bacteria, which eventually stops the cellular metabolism and the cell proliferation.[140,141]

The unique optical, mechanical, magnetic, and electrical properties of silver nanoparticles are the main reason for their use in several fields. The applications of AgNPs can be classified into four major parts; scientific, medical, industrial, and consumer product applications. The scientific applications of AgNPs comprise the enhanced IR absorption spectroscopy, evaluating the fibrinogens in human plasma, colorimetric sensors for histidine, detecting DNA sequences, glucose sensors for medical diagnostics, optical imaging of cancer, biosensors for detection of herbicides, and optical sensors for zeptomole. Meanwhile, the industrial applications of AgNPs include catalysis, electronics, book-binding materials, brochures, envelopes, and business stationery. In contrast, the consumer product applications of AgNPs are face masks, wet wipes, slippers, pillows, cellular phones, vacuum cleaners, shampoo, washing machines, soap, air filters, and food storage containers. In the medical applications of AgNPs, the applications include medical devices, coating tools, treatment, drug delivery, medical textiles, contraceptive devices, scaffolds, diabetic socks, wound dressings, medical catheters, and sterilization materials in hospitals.[3,4,138]

The use of silver nanoparticles in the medical field is due to several properties: its antibacterial properties,[142] anti-fungal,[143] anti-inflammatory,[144] anticancer, antioxidant,[145] anti-biofilm,[146] surface plasmon resonance,[147] and metal-enhanced fluorescence.[148] Due to these properties, silver nanoparticles are used widely for medical applications. Furthermore, due to their antibacterial properties, AgNPs have been commonly utilized in the health industry, food storage, textile coatings, and other environmental applications.[149] Although it has been used for many years, the nanoparticles' toxicity is still vague and unclear. Silver nanoparticles have been used extensively in medicine and home applications due to their anti-microbial properties. Silver sulfadiazine creams are used to avoid infections at the burn site, and in Appliance Company, silver will be integrated into their washing machine.[150]

Table 6: Cytotoxicity studies of silver nanoparticles

Cytotoxicity test	*AgNPs size*	*Cell viability*	*Synthesis methods*	*Concentration AgNPs (µg/mL)*	*Ref*
MTT, MCF-7 breast cancer cell	46 nm	88%, 67%, 67%, 66%, 60%, 45% and 47 %.	Green synthesis method	10, 20 , 30, 40, 50, 60, 70 and 80	[119]
MTT & LDH 3T3 mouse embryo fibroblast cell line	37–71 nm	93 % & 47 %	Green synthesis method	25 & 50	[129]
MTT, HeLa	78 nm	88–55 %	Green synthesis method	50–400	
MTT, HBL	78 nm	89–66%	Green synthesis method	50–1000	[136]
Alamar Blue assay against human neonatal skin stromal cells (hSSCs)	3.2 to 16 nm	From 92 to 88 %	Green synthesis method	2, 4, 6, 8, and 10	[135]
Alamar Blue assay, colon cancer cells (HT115)	3.2 to 16 nm	From 88 to 85 %	Green synthesis method	2, 4, 6, 8, and 10	[135]
MTT splenocytes cells	10–70 nm	From 98 to 67 %	Green synthesis method	From 10 to 40	[137]
MTT, MDA-MB-231 cells	20–30 nm	100, 88, 85, and 67 %	Green Synthesis methods	1, 10, 50 and 100	[130]
MTT, HT-29 and & MCF-7	2–15 nm	From 100 to 58 % From 100 to 83 %	Green Synthesis methods	10 to 100	[132]
MTT human lung epithelial A549 cells	12 nm	105, 105, 103, 100, 98 and 97 %	Green Synthesis methods	0.5, 1, 2.5, 5, 10, 25, 50	[138]
CAM and WST assays, NIH3T3 Cells.	2–40 nm	110, 110, 105, 100, 97, 95, and 92 %	Green Synthesis methods	5, 10, 20, 40, 80, 160 and 320	[133]
MTT assays, fibroblast cell line L-929	75 nm	100, 100, 84, 77, 63, 55, and 45 %	Green Synthesis methods	100, 200, 300, 400, 500, 600 and 700	[134]

CONCLUSION

In general, many initiatives have been conducted for the establishment of green synthesis. Green synthesis is leading over the chemical and physical methods as this method is known to be cost-effective, eco-friendly, and easy to scale up for large-scale synthesis. Indeed, nature presents a delicate and inventive method in producing a competent miniaturized functional material. Awareness towards green synthesis and the use of natural routes to produce AgNPs attracted many researchers to create eco-friendly methods for various organisms ranging from a straightforward bacterium to a complex one such as complex eukaryotes to synthesized NPs with preferred sizes and shapes. Nevertheless, the establishment of microorganisms and vast scale of formula tend to be difficult than others. Due to the low synthesis rate and limited sizes and shapes, the study intends to utilize plant extracts. The production of AgNPs using plants can lead to many advantages over other biological entities, which can solve the slow route using the microorganisms and maintain their culture as they can lose their capabilities in the productions of NPs. Other benefits of using plant extracts in the production of NPs are it is known to have a hygienic working environment, less waste, and tend to be a stable product. Several important aspects of nanotechnology should be highlighted when synthesizing the AgNPs using green synthesis. Overall, AgNPs have been developed for the present and the future era for various applications such as agriculture, biosensors, cardiovascular implants, dentistry, medicine, and therapeutics. Implants, dentistry, medicine, therapeutics, biosensors, agriculture, and many more.

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