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Silver Nanoparticles as Multi-Functional Drug Delivery Systems

Nadezhda Ivanova, Viliana Gugleva,
Mirena Dobрева, Ivaylo Pehlivanov,
Stefan Stefanov and Velichka Andonova

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Abstract

Nanoparticles can surmount some essential problems of conventional small molecules or biomacromolecules (e.g., DNA, RNA, and protein) used in some diseases by allowing targeted delivery and overcome through biological barriers. Recently, silver nanoparticles have been harnessed as delivery vehicles for therapeutic agents, including anti-sense oligonucleotides, and other small molecules. Silver is the most profit-oriented precious metal used in the preparation of nanoparticles and nanomaterials because of its antibacterial, antiviral, antifungal, antioxidant and unusually enhanced physicochemical properties compared to the bulk material such as optical, thermal, electrical, and catalytic properties. Small silver nanoparticles offer many advantages as drug carriers, including adjustable size and shape, enhanced stability of surface-bound nucleic acids, high-density surface ligand attachment, transmembrane delivery without harsh transfection agents, protection of the attached therapeutics from degradation, and potential for improved timed/controlled intracellular drug-delivery. Plant-mediated synthesis of silver nanoparticles is gaining interest due to its inexpensiveness, providing a healthier work environment, and protecting human health leading to lessening waste and safer products. The chapter presents the essential physicochemical characteristics, antibacterial, and anticancer properties which silver nanoparticles obtained by plant-mediated methods possess, and their application as drug-delivery systems with a critical view on the possible toxicity on the human body.

Keywords: antibacterial activity, anticancer activity, capping agents, plant extracts, reducing agents, surface properties, toxicity

1. Introduction

Nanomedicine is a branch of medicine that uses nanomaterials and applies nanotechnologies in prevention, diagnostics, and treatment of diseases [1]. The broad definition of nanomedicine involves nanoparticles (NPs) as drug delivery systems (DDSs), medical nanosensor, biochips, insulin pumps, needleless injectors, etc. The unique properties of NPs are related to their tiny size (generally between 1 and 100 nm), huge surface area, and surface characteristics. “Nano” DDSs provide targeted delivery of optimal dose with reduced side effects and toxicity. Moreover, NPs solve problems related to drug solubility and bioavailability. These “nano” carriers can protect the drug from the hazardous environment as well as to overcome the biological barriers to entry the drug to the targeted tissues and to deal with drug resistance. They possess organic or inorganic origin and can be prepared from different polymers, metals, ceramics, etc.

Silver is the most profit-oriented precious metal used in the preparation of NPs and nanomaterials. These are known because their antibacterial, antiviral, antifungal, antioxidant, and unusually enhanced physicochemical properties compared to the bulk material such as optical, thermal, electrical, and catalytic properties [2–5]. About 500 tons of silver nanoparticles (AgNPs), used in various industries and everyday life, are produced per year [6, 7]. Rising demand for silver nanomaterials requires the development of eco-friendly synthesis methods. In general, AgNPs can be produced by chemical, physical, and biological methods. Chemical protocols are mainly based on the chemical reduction of Ag^+ ions by organic and inorganic agents, such as sodium borohydride, sodium citrate, sodium ascorbate, elemental hydrogen, N, N-dimethylformamide, polymers, Tollens methods, etc. The reducing agent reduces Ag^+ and leads to the formation of Ag^0 , metallic silver, which agglomerates into oligomeric clusters. These clusters may form colloidal particles of metallic silver. Different surfactants and polymers are used to prevent particles from further agglomeration and protect their shape [8]. The most important physical methods are based on evaporation-condensation technique and laser ablation of silver bulk material in solution. Both physical approaches did not use chemical reagents that may be hazardous to the environment and the human body. Although these require costly specialized equipment, physical methods provide an alternative to environmentally unfriendly and time-consuming chemical protocols.

Biological or so-called “green” methods do not use toxic chemicals in the preparation techniques. Moreover, these methods are based on the usage of bacteria, fungi, algae, and plants to obtain AgNPs characterized by size and shape depending optical, electrical, and antimicrobial properties [8, 9]. These are based on bioreduction of Ag^+ ions in the aqueous medium where the reducing agents are cited above biological sources. Synthesis of AgNPs using living microorganisms (bacteria and fungi) can be performed either intracellularly or extracellularly [10]. The extracellular synthesis is cheaper, less time-consuming, and requires simplified manufacturing technology compared to the intracellular synthesis. Studies used culture supernatants of pathogenic and nonpathogenic microorganisms like *A. flavus*, *B. indicus*, *B. cereus*, *Bacillus* strain CS 11, *E. coli*, *P. proteolytica*, *P. meridiana*, *S. aureus*, etc. [10–12]. The drawbacks of bacterial synthesis of AgNPs are related to the selection and cultivation of suitable bacterial strain,

a mandatory stage of purification, the poor understanding of the mechanisms governing the nanoparticle formation which hinders scaling laboratory process in the industry as well as the requirements of highly aseptic conditions and their maintenance [13].

Plant-mediated green synthesis of AgNPs is gaining immense popularity because of its eco-friendly nature, accessibility, economy, execution-simplicity, and the possibility of large-scale production. Many studies have used different plant extracts such as *Azadirachta indica*, *Crocus sativus* L., *Calliandra haematocephala*, Neem leaves, *Madhuca longifolia*, grape seed extract, *Andean blackberry* fruit extract, geranium leaf aqueous extract, marigold flower, etc. [7, 14–19]. The rich phytochemical composition of the extracts used implies its complex action, for example, as reducing, stabilizing, and capping agents. The AgNPs thus obtained can be exploited as DDSs for different active pharmaceutical ingredients.

The chapter presents the essential physicochemical characteristics, antibacterial, and anticancer properties, which AgNPs obtained by plant-mediated methods possess, and their application as DDSs with a critical view on the possible toxicity on the human body.

2. Plant-mediated synthesis of silver nanoparticles

It is well known that plant extracts have a rich phytochemical composition including phenolics, saponins, terpenoids, flavonoids, catechins, tannins, enzymes, proteins, polysaccharides, etc. All of these biomolecules take place in a very complicated mechanism of reduction and stabilization of Ag⁺ ions to form AgNPs. For example, Li et al. suggested a recognition-reduction-limited nucleation and growth model to explain the possible formation mechanism of AgNPs in *Capsicum annuum* L. extract [19]. According to the authors, the proteins which have amine groups played a reducing and controlling role during the formation of AgNPs in the solutions, and that the secondary structure of the proteins changed after reaction with Ag⁺ ions. In another study, Mirgorod and Borodina, based on the surface-enhanced Raman spectroscopy data, stated that the NPs were formed as a result of a redox reaction between flavonoids and Ag⁺ ions as well as there are flavonoids near the surface of the AgNPs, which react complexly with Ag⁺ ions and with the NPs [20]. Ahmed and co-workers described different approaches of syntheses of AgNPs and protocols employed for their synthesis in detail [21].

It is important to note that technological parameters such as temperature, pH, the concentration of Ag⁺ ions, duration of the obtaining process, phytochemical composition of the extract used, mechanical stirring, microwave assistance, etc., are crucial both for nanoparticle preparation and for their characteristics and fate [6, 7, 14–19]. These parameters affect not only the process of reduction of Ag⁺ ion and formation of metallic silver but also its agglomeration into oligomeric clusters which may form colloidal particles with specific features. Amin and co-workers found that the time of reaction, temperature, and volume ratio of methanol extract from *Solanum xanthocarpum* berry to AgNO₃ could accelerate the reduction rate of Ag⁺ ions and affect the AgNPs size and shape [22]. The NPs were found to be about 10 nm in size, mono-dispersed in nature, and spherical in shape.

Surface functionality of nanomaterials is crucial for their applicability, compatibility, and safety. Generally, surface behavior defines how a nano-entity will interact with biosystems, environment, etc. [23]. AgNPs are characterized with variable morphology—size, shape, surface area, purity/coating—and related electrochemical and electromagnetic properties—charge, zeta potential, redox potential, surface plasmon resonance, and conductivity [24, 25]. A change or intentional attempt to control these essential characteristics is an essential tool in tailoring AgNPs for specific purposes and might be highly sought on several accounts: (1) increased stability; (2) increased selectivity; (3) increased therapeutic or diagnostic potency; (4) enhanced catalytic activity; (5) reduced toxicity; and (6) reduced reactivity [23, 25]. Surface functionalization of AgNPs may be determined by the synthesis pathway chosen (one-step functionalization) or additional treatment after isolation (multi-step functionalization).

3. Surface properties of silver nanoparticles

3.1. Purity on the surface of “green” synthesized AgNPs

The “green” synthesis of AgNPs using plant extracts often results not only in deliberate, but also inevitable surface functionalization because every component in the total aqueous plant extract (being reducers, stabilizers, or concomitant constituents) has a certain affinity to the silver surface [16, 24, 26]. After isolation and purification, surface remain only those components which can bind the strongest is “attached” to the AgNPs. Sorption, or so-called “attachment,” might occur due to chemical (chemisorption) or physical (physisorption) phenomenon. Chemisorption, in the case of AgNPs, happens via ionic, covalent, or coordinate-covalent chemical bonds. S-containing molecules (some amino acids, peptides, and proteins) possess the highest affinity to the silver surface because of the strong Ag-S bond and hence are the first to be considered for interaction [17, 26–28]. Next, N and O atoms from amide, amino, hydroxyl, phenol, carboxyl, and carbonyl groups are targets for complex formation with Ag⁺ ions and thus also very likely to be absorbed on the surface [7, 15, 16, 18, 25–30]. The latter exist in most primary and secondary metabolites in plants (phenolic acids, polyphenols, flavanoids alkaloids, glycosides, polysaccharides proteins, etc.) and are found to be present on AgNPs’ surface by many researchers [7, 15–18, 24, 28, 31]. Physisorption arises due to Van der Waals forces, and though is much weaker compared to chemisorption; it is non-specific and can affect every polarized unit in the AgNP’s surrounding. Knowing that the electric potential of colloidal silver can be considerable, this explains the significant role of physisorption for the surface functionalization of “green” synthesized AgNPs. It has to be noted, that regardless the mechanism, biomolecules participating in Ag⁺ ion reduction, are more likely to enter in an interaction with the silver surface because of their initial intimate contact with the arising particles [7, 15–18, 24, 31].

A question may arise whether this heterogenic and uncontrollable “impurity” on AgNPs’ surface following “green” synthesis with plant extracts is only advantageous or does it have any weak sides. In fact, this highly depends on the particles’ designation. The presence of tannins, proteins, polysaccharides, flavonoids, and lipids has been proven to benefit stability, increases

AgNPs' catalytic, antibacterial, and antioxidant activity and reduces toxicity by passivation of the surface [7, 14–16, 21, 31–34]. However, the “coating” of AgNPs reduces their size and agglomeration rate, as well as some researchers, suggest that this may have an adverse effect on cytotoxicity [33]. Furthermore, for surface-selective analytical techniques (such as surface-enhanced Raman spectroscopy, SERS), where the use of AgNPs provides promising results as enhancers, a “clear” surface is required that allows access to targeted analytes [26]. In this regard, the use of pure natural reducers (e.g., the flavonoids quercetin, chrysin, apigenin, luteolin, etc.) might be preferable instead of the total plant extract [25, 26, 28, 35]. However, if the presence of multicomponent and unpredictable adherence on the AgNPs is unwelcome, still the need for a “capping” agent exists. Sugars and polysaccharides, proteins and proteoglycans as glucose, galactose, mannose, chitosan, sodium alginate, glucans, gelatin, and others are commonly used as coatings for the purpose [17, 27, 35, 36]. These are most often being included in the reduction media during synthesis, whereas the mechanisms of their attachment to the surface follow the above-described principles [27, 35].

3.2. The surface area of AgNPs

The active surface area of AgNPs is determined by their size, shape, and agglomeration rate. Reaction conditions as pH, temperature, extract volume and concentration, reactants ratio, and time define the dimensions and degree of crystal growth and thus affect the size and shape of the silver aggregates [16, 24, 25, 31].

The polydispersity of the resulting AgNPs is a disadvantage of the “green” synthesis with plant extracts, which is likely due to the uncontrollable deposition of different compounds on the surface and the heterogeneity of the reaction media. In this regard, the use of an o/w microemulsion-upgraded method has shown good results [18]. Post-synthesis agglomeration may lead to enlargement of the aggregates and eventually to colloid instability. Here is the role of the “cap” on AgNPs' surface, which is aimed to overcome the attractive forces between the particles and increase physical stability. A large surface area is desirable because it provides greater catalytic and antibacterial efficacy due to the increased Ag⁺ release from the surface which is a fundamental mechanism of AgNPs' antibacterial action [25, 32]. However, this precise mechanism, proven by many, is also related to increased oxidative stress and cytotoxicity [33, 35]. Furthermore, AgNPs smaller than 10 nm can pass through the nuclear pores and interact with chromosomes and DNA. Thus such particles are proper for gene therapy and diagnostics, but dangerous regarding genotoxicity [33]. On the one hand, each intervention leading to suppression of particles' agglomeration and reducing their size is welcome concerning stability and potency in catalysis, antibacterial therapy, and diagnostics. On the other hand, the same intervention can be potentially hazardous concerning increased toxicity of the NPs obtained [25, 33, 35].

The shape of AgNPs has also been demonstrated to have an impact on toxicity [34, 37]. For example, wire-shaped AgNPs have shown higher toxicity compared to spherical NPs [37], whereas another study testifies that plate-shaped AgNPs' toxic potential exceeds those of wires and spheres [34].

3.3. Electrochemical and electromagnetic properties of AgNPs

The charge and zeta potential of AgNPs occurring in suspension are main factors determining the stability of the colloidal system and depend highly on the synthesis of variables as well. Among them, pH of the reaction media and the type of coating are crucial [31, 35]. Zeta potential (ζ) is the potential occurring between the surface of AgNPs and the surrounding liquid phase and is an important measure for the stability of colloidal systems. Values beyond $\zeta = \pm 30$ mV are usually taken as a requirement for colloid's endurance [31]. Adjustment of pH during synthesis is considered an electrostatic approach for stabilization of colloids (by changing the type and quantity of the electric charge), whereas the coating aims to diminish the attractive forces in a steric way [31, 35]. AgNPs obtained by reduction with plant extracts most often are negatively charged [7, 15, 17, 18, 27, 29, 35]. The negative zeta potential can be considered an advantage because increased cellular uptake and subsequent cytotoxicity are found for positively charged AgNPs [23, 33].

The presence of "capping" agents on the surface is essential for the stability of colloidal systems, but they also affect the so-called "redox potential" of AgNPs, that is, their ability to acquire electrons and be reduced [38]. Low redox potential is needed for oxidation on the surface and Ag^+ release and therefore promotes higher antibacterial activity and toxicity [33]. In some cases, the immobilization of AgNPs in slightly permeable "cap" may lead to loss of ability for oxidation and antibacterial properties [35].

The surface plasmon resonance (SPR) is a characteristic optical property of AgNPs due to resonant oscillation of electrons on the surface caused by irradiation with light [39]. This electromagnetic phenomenon results in an intense peak in the violet-blue sector of the visible spectrum [7, 15, 18, 24, 26]. The latter depends strongly on surface functionality (size, coating, etc.) and is considered proof for successful AgNPs synthesis [7, 15, 18, 24, 26, 39].

3.4. Association of AgNPs in complexes and delivery vehicles

A few attempts to incorporate "green" synthesized AgNPs in the structure of liposomes, cyclodextrins, nanoemulsions, and hydrogel beads are reported. Such approaches give the opportunity for targeted delivery, better compatibility, and lower toxicity [35, 40]. For example, one-step synthesis of AgNPs-stabilized liposomes have shown improved stability, compatibility, and antibacterial properties of resulting vesicles compared to AgNPs alone, also giving the opportunity for dermal delivery [40]. Other studies report that association of AgNPs with β -cyclodextrin improves their catalytic activity [25], whereas kappa-carrageenan hydrogel beads of "green" synthesized AgNPs have been found to deliver Ag^+ in a desirable controlled manner [41].

3.5. Functionalization by conjugation

Next level surface functionalization is the conjugation of AgNPs with bioactive molecules. This approach, unlike all of the above mentioned, can not only change but also lead to entirely new functions. The conjugation of oligonucleotides to metal nanoparticles' surface is widely

researched for targeted gene therapy and bio-diagnostics. However, the attachment of DNA sequences on AgNPs surface has been challenging due to the lower stability of the complex. Few successful reports are available from the past years with disulfide or sulfhydryl inserted DNA [42, 43].

An exciting field of study is the AgNPs potential as drug-delivery carriers [29, 30, 44]. Hypotheses suggest that AgNPs can be used as vehicles to transport drug molecules to target zones and thereby improve therapeutical efficacy; furthermore, express synergism with synthetic antibiotics regarding antibacterial properties. These assumptions have been tested by several scientist in the field, who report successful conjugation of tetracycline (multiple hydroxyl, phenol, and amide groups), glycopeptide antibiotic vancomycin (multiple amide, phenol, and hydroxyl groups), and the immunosuppressant azathioprine (S-atom and basic N-atoms in heterocycle) [29, 30, 44].

4. Antibacterial activity of silver nanoparticles

Since ancient times elemental silver and its compounds have been used as antimicrobial agents. AgNPs synthesized by different methods were widely tested and had been proved effective against over 650 microorganisms including bacteria (both Gram-positive and Gram-negative), fungi, and viruses [21, 45]. Multiple mechanisms of antibacterial action of AgNPs are considered, but most studies simplified to three primary mechanisms: (1) adhesion of AgNPs onto the surface of cell wall and membrane; (2) penetration of AgNPs inside the cell and damaging of intracellular structures (mitochondria, vacuoles, and ribosomes), and biomolecules (protein, lipids, and DNA); and (3) generation of reactive oxygen species (ROS), leading to induced cellular toxicity, and oxidative stress [21, 45, 46]. According to Prabhu et al. and Dakal et al., modulation of signal transduction pathways is also a distinct mechanism of antimicrobial action of AgNPs [45, 47].

The adhesion of AgNPs onto the surface of the cell wall is facilitated by the positive surface charge of the AgNPs, and the occurred electrostatic attraction between AgNPs and the negatively charged cell membrane of microorganisms [48]. The interaction of Ag⁺ ions with the proteins containing sulfur, presented in the bacteria cell wall, irreversibly disrupted the bacterial cell wall [49]. The damage of cell membranes by AgNPs causing structural changes renders bacteria more permeable and disturbs the respiratory function [45, 46]. Morones et al. demonstrated the existence of silver in the membranes of treated bacteria as well as in the interior of it by transmission electron microscopy (TEM) analysis [50]. The composition and thickness of the cell wall also influence the antimicrobial potential of AgNPs [45, 48]. In Gram-negative bacteria such as *E. coli*, *Pseudomonas*, *Salmonella*, the cell wall consists of a layer of lipopolysaccharide, followed by a thin layer of peptidoglycan (3–4 nm). The cell wall in Gram-positive bacteria such as *Staphylococcus*, *Streptococcus*, *Bacillus* is mainly composed of a thick layer of peptidoglycan (30 nm thickness) [48, 51]. Hence, AgNPs exhibit greater antimicrobial effect against Gram-negative bacteria regardless of their resistance level as compared to Gram-positive bacteria [49].

It has also been proposed that Ag⁺ ion enters the cell and interacts with the sulfur and phosphorus of the DNA, which can lead to problems in the DNA replication of the bacteria and cell death [47].

The antibacterial potential of AgNPs has related also with the generation of free radicals and ROS and consequent increase in oxidative stress in cells. Silver ion can interact with the thiol groups of many vital enzymes, inactivate them and generate ROS. An excessive amount of generated free radicals lead to direct damage to mitochondrial membrane causing necrosis and eventually cell death [52].

The antimicrobial effect of AgNPs depends on various parameters including discussed above size, shape, zeta potential, dose, and colloidal state [15, 46, 49]. AgNPs having a size in the range of 10–100 nm showed strong bactericidal potential against both Gram-positive and Gram-negative bacteria [50, 51]. Depending on the size of the NPs, the large surface area comes in contact with the bacterial cells to provide a higher percentage of interaction than bigger particles [51, 53].

The effect of shape on the antibacterial activity of AgNPs has been studied by Pal et al. [54]. The AgNPs of different shapes (triangular, spherical, and rod) were tested against *E. coli*. According to the authors, triangular NPs are more active than spherical NPs, which are again more active than rod-shaped AgNPs against *E. coli*. This could be due to their larger surface area to volume ratios and their crystallographic surface structures [54]. Rout et al. synthesize AgNPs of different shapes (i.e., spherical, triangular, and rod) by using Mulberry (*Morus rubra L.*) leaves extract and studied their antibacterial activities against *E. coli* in both liquid systems and on an agar plate. High reactivity of the truncated triangular NPs has also been observed in comparison to spherical and rod-shaped particles [55].

Sondi and Salopeck-Sondi investigated the antibacterial activities of AgNPs against *E. coli* on Luria-Bertani agar plates and reported that the antibacterial activity of AgNPs was dose-dependent [56]. AgNPs in colloidal form, that is, suspended nano-sized Ag particles have shown enhanced antimicrobial potential over AgNPs alone. Colloidal AgNPs produced by green synthesis are characterized with controlled size, high stability, and improved antibacterial activity which is examined in different studies by directly exposing bacteria to AgNPs [45, 57].

Okafor et al. produced AgNPs by green synthesis from aloe, geranium, magnolia, and black cohosh extracts and studied their antibacterial activity on different species of bacteria: three Gram-negative and three Gram-positive bacteria [58]. The overall results indicated that the AgNPs showed antibacterial activity at doses of 2 and 4 ppm towards the Gram-positive and Gram-negative test bacteria. Aloe extract NPs showed the highest antibacterial activity, followed by black cohosh and geranium NPs with the lowest inhibition. The high antimicrobial effect of the aloe produced AgNPs may be due to a combination of the AgNPs and the aloe bioactive molecules (quinines and other aromatic compounds), which in combination enhanced the inactivation or growth inhibition of the bacteria species. In another study, Zhang and co-workers also reported that aloe-produced NPs have a high inhibitory growth in *E. coli* at low concentrations [59].

Ahmed et al. synthesized AgNPs using *Azadirachta indica* aqueous leaf extract and studied their antibacterial activity towards both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacterial strains compared with control and plant extract alone [7]. According to the authors, AgNPs showed effective antimicrobial properties compared to others due to their vast surface area providing better contact with the cell wall of microorganisms. Also, Bagherzade et al. synthesized AgNPs using an extract of saffron (*Crocus sativus* L.) [14]. The biosynthesized AgNPs showed a significant antibacterial effect against *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. flexneri*, and *B. subtilis*.

Gomathi et al. obtained spherical shaped AgNPs using *Datura stramonium* leaf extract and studied their antibacterial activity against *E. coli* and *S. aureus* using well diffusion technique [32]. The authors reported that AgNPs exhibited greater antibacterial activity against *E. coli* than *S. aureus*, due to the variation in cell wall membrane of these bacteria. In another study, spherical-shaped AgNPs with dimensions of 50–100 nm were observed using *Alternanthera dentate* aqueous extract and were tested against *E. coli*, *P. aeruginosa*, *K. pneumonia*, and *E. faecalis* by agar diffusion method [60]. The authors reported that the antibacterial effect of AgNPs was size- and dose-dependent and was more pronounced against Gram-negative bacteria than Gram-positive bacteria.

Antimicrobial activity of AgNPs with various antibiotics has been studied, and the synergistic antibacterial effect has been found. The bactericidal potential of AgNPs synthesized from the leaf extract of *Murraya koenigii* singly and in combination with antibiotics (gentamycin, ampicillin, and streptomycin) against the pathogenic bacteria, namely *E. coli*, *S. aureus*, and *P. aeruginosa* was studied [61]. The authors reported that AgNPs in combination with gentamycin showed the maximum activity against *E. coli* with an increase in fold area 4.06, while tetracycline combination with NPs showed maximum activity against *S. aureus*. The authors concluded that the activity of standard antibiotics was significantly increased in the presence of AgNPs and that can be used against antibiotic-resistant pathogens effectively.

5. Silver nanoparticles as anticancer drug delivery systems

Over the past years, nanomedicine created new horizon in the future development of anticancer strategies. Conventional cancer treatment such as chemotherapy, radiotherapy, or surgery has its limitations associated with drugs toxicity, unpredictable side effects, drug resistance problems, and lack of specificity. AgNPs overcome these disadvantages by reducing side effects and enhancing the efficiency of cancer therapy. One of their distinguishing features is the ability to cross various biological barriers and to provide targeted delivery of drugs. Green synthesis of AgNPs together with specific delivery of anticancer drugs to tumor tissues offers an innovative approach for improving cancer treatment [62].

5.1. Anticancer activity of biologically synthesized AgNPs

The anticancer activity of biologically synthesized AgNPs has been studied using both *in vitro* and *in vivo* models. The reported results suggested that cytotoxicity of AgNPs may be

influenced by particle size, shape, and surface chemistry. Several authors have claimed that increasing AgNPs concentration the viability of tumor cells decreases [63, 64].

The effect of time and concentration of AgNPs on inhibition of cell viability and membrane leakage are evaluated with a variety of methods [65, 66]. Usually, MTT assay, quantification of ROS, RT-PCR, and western blotting techniques are used for the assessment of AgNPs ability to inhibit cellular growth and mediate cell death [65–68]. *In vitro* cytotoxic activity in a dose-dependent manner was estimated for green synthesized AgNPs from different plants—*Vitex negundo* L., *Acalypha indica*, *Euphorbia nivulia*, and *Premna serratifolia* [63]. MCF-7 (human breast adenocarcinoma) cell lines were treated with AgNPs obtained by the use of *Erythrina indica* and *Andrographis echiooides* extracts. In both cases, the growth of cancer cells was inhibited following AgNPs concentration-response relationship [63]. Similar results were found in other studies [65, 67]. The AgNPs obtained by use of *Artemisia marshalliana* Sprengel extract and *Ganoderma neo-japonicum* Imazeki extract had a confirmed cytotoxic potential on human gastric cancer AGS cell line and MDA-MB-231 human breast cancer cell. The authors found that the cytotoxic activity of AgNPs was time- and dose-dependent as well as the size of NPs and the temperature of the preparation process.

Dependence on anticancer activity of AgNPs on human cancer cell lines has been found, according to the source for the synthesis of NPs as well as on the type of the cell lines [69]. Extracts from fruits, leaves, seeds, and roots of *Citrullus colocynthis* produced AgNPs with different size and alteration in ID₅₀ on various cell lines. The toxicity assay of biologically synthesized AgNPs using seaweed *Ulva lactuca* showed potential cytotoxicity of AgNPs against tumors. For human colon cancer, HT-29 cell lines ID₅₀ was 49 µg/ml whereas its value reached 12.5 µg/ml in human liver cancer Hep G-2 cell lines.

One of the significant drawbacks of conventional anticancer therapy is drug-mediated toxicity in healthy cells. AgNPs synthesized from plants have the potential to avoid this problem by offering selective toxicity to cancer cells. AgNPs produced using leaf extract of *Podophyllum hexandrum* Royle induced cytotoxicity to cervical carcinoma cells. The reported results proved that AgNPs could selectively inhibit the cellular mechanism of *HeLa* by DNA damage and caspase-mediated cell death [70]. In another study, the cytotoxicity of AgNPs towards cancer cells was estimated comparing human myeloblastic leukemia cells HL60 and cervical cancer cells *HeLa* to normal peripheral blood mononuclear cells (PBMC) [66]. *Sargassum vulgare* had been used for the green synthesis of AgNPs. It was found that HL60 cells were affected by AgNP-mediated toxicity while the normal PBMC suffered less damage.

It has been proven that biologically synthesized AgNPs show substantial anticancer activity with less toxic manner compared to particles whose preparation involves some toxic and expensive chemicals. The production AgNPs through green chemistry approach via *Cleome viscosa* plant extract offers another solution for optimizing anticancer treatment. Anticancer activity was *in vitro* evaluated against human cancer cell lines PA1 (Ovarian teratocarcinoma cell line) and A549 (Human lung adenocarcinoma) [68]. The results concluded that green synthesized AgNPs could inhibit cancer cells growth and provide great potential in the treatment of cancer.

To determinate the anticancer efficacy of biologically synthesized AgNPs and to fully apprehend the mode of programmed cell death three critical parameters need to be taken into consideration: (1) DNA fragmentation; (2) structural changes in the cell morphology; and (3) Annexin V binding and caspase activation. Upregulation of apoptosis is only one of the possible mechanisms for antiproliferative activity of biosynthesized AgNPs that was proven in many studies [67, 71, 72]. AgNPs could elicit cell death through ROS generation, membrane leakage, activation of caspases, and DNA damage [65, 66, 72].

5.2. AgNPs for targeted drug delivery

AgNPs represent an alternative therapeutic strategy as DDSs in curing cancer because these can provide passive or active targeting to tumor tissue. Accumulation of drugs at desired sites increases the efficacy of anticancer therapy *in vivo*. Receptor-mediated endocytosis can facilitate cellular uptake of drugs. This kind of active targeting relays on molecular recognition. Suggested approach for optimizing biogenic AgNPs properties is surface functionalization with specific targeting molecules or coating with biocompatible and biodegradable polymers [73, 74]. For example, AgNPs obtained by use of various concentrations of *Setaria verticillata* seed extract were loaded with hydrophilic anticancer drugs, doxorubicin (DOX), and daunorubicin (DNR). The significant loading (80.50%) and capacity (40.25%) efficiency of DOX-AgNPs and DNR-AgNPs presented them as future novel DDSs [64].

Drug delivery into the cells by endocytosis depends on the size of NPs. Spherical-shaped AgNPs were extracted from *Aerva javanica* plant and conjugated with the anti-cancer drug gefitinib. Scanning transmission electron microscopy (STEM) images determinates average size of 5.7 nm. The apoptotic potential of gefitinib-AgNPs has been compared to gefitinib alone. Reduction of cell viability of breast cancer cells MCF-7 treated with conjugated gefitinib-AgNPs was significant. Delivery of gefitinib using AgNPs optimizes its effectivity and reduces side effects [75].

The variety of green synthesized AgNPs exhibiting anticancer activity offer new treatment opportunities. Their specific features as nanocarriers benefit the development of DDSs with unique properties and biocompatible profile.

6. Silver nanoparticles as photoactivated drug delivery vectors

Nanoparticles can surmount some essential problems of conventional small molecules or bio-macromolecules (e.g., DNA, RNA, and protein) used at some diseases by allowing targeted delivery and overcome through biological barriers [76]. Noble metal NPs have specific high developed photophysical properties which contribute to their potential as photoactivated drug delivery vectors [77]. AgNPs have been used extensively as biological sensors which take advantage of plasmon resonance (PR) to enhance detection of specific targets. Noble metal nanoparticle-based sensors benefit from the extreme sensitivity of localized surface

plasmon resonance (LSPR) spectra to environmental changes. Application of metal nanoparticles is not limited to molecular detection. Recently, AgNPs have been harnessed as delivery vehicles for therapeutic agents, including antisense oligonucleotides and other small molecules. Small metal NPs offer many advantages as drug carriers, including adjustable size and shape, enhanced stability of surface-bound nucleic acids, high-density surface ligand attachment, transmembrane delivery without harsh transfection agents, protection of the attached therapeutic from degradation, and potential for improved timed/controlled intracellular release. The photophysical properties of AgNPs may potentially bring these to the forefront of drug delivery, enabling targeted delivery, spatiotemporally controlled (photo-)release, and delivery confirmation via imaging [78].

AgNPs in the diameter range of ~2–100 nm exhibit SPR spectra in the visible region, which are tunable and dependent on particle shape, size, environment, and interparticle distance. AgNPs have unique properties which make them a desirable alternative particle type in many cases. AgNPs are the strongest light scatterers of the noble metal particles, and it is reported that the light scattering cross section of AgNP is ~10 times greater than that of a similarly sized gold NPs. The extinction (light absorption and scattering) band of AgNPs is due to free conduction electron oscillations, and bound electron movements also contribute to the optical spectra. Thus enhancement of absorption/emission of light by molecules near the AgNPs surface is dependent on particle size and proximity or overlap of the resonance (SPR) spectra with the absorption/emission bands of the molecular species [78].

Mie Theory has calculated the light absorption and scattering properties for AgNPs of different sizes. For larger particle sizes (~50–60 nm), the scattering efficiency (Q_{sca}) is higher (≈ 5). The AgNPs in this size range scatter light at or above the solid metal surface, but the scattering efficiency increases even higher to 5.8 for size 70–80 nm while maintaining surface PR in the UV to the visible range. This characteristic is ideal for traditional and red-shifted photocleavable compounds typically used as photo-caging compounds [78].

The generality of current nanoscale delivery systems are polymeric in their essence. The studies of metallic NPs have shown their suitability for delivery of various therapeutic agents including small molecules, antisense oligonucleotides, and siRNAs. Nanoscale silver is one of the optically active surface-enhancing substrates available. AgNP-based single delivery platforms incorporate solutions to both intracellular detection and external control over surface-tethered drug release via chemical photothermal or photochemical triggers [77].

Light-responsive systems are of great interest in the field of drug delivery and gene therapy, owing to the capability of external, spatiotemporal control over the delivery, and activation of therapeutics coupled with such systems. Electromagnetic radiation triggers light-responsive DDSs, typically in the UV, visible, and near-infrared (NIR) range. These systems are based upon photosensitive compounds which can be incorporated into a drug delivery vehicle, or coupled to the drug itself (“caging” compounds), and may switch to an active or inactive state upon electromagnetic irradiation within a specific frequency range. Caged compounds are potent tools for spatiotemporal control over drug activity in living systems. Photocleavable groups have been used to cage, or inactivate, various biomolecules, including nucleotides, proteins, and nucleic acids, for controlled, on-site photo-activation. Uncaging via light irradiation

allows rapid, spatially, and temporally defined release of a biomolecule at intended tissues or even within a specific intracellular compartment [78].

AgNPs with the size of 60–80 nm decorated with thiol-terminated photolabile DNA oligonucleotides were used as photo-activated drug delivery vectors [77]. *In vitro* assays showed efficient photo-activation of surface-tethered caged ISIS2302 antisense oligonucleotides with internal photo-cleavable linkers. These nanocarriers have several advantages such as protection against nucleases, efficient photorelease, and enhanced cellular uptake when compared to commercial transfection agents. The light-induced release of anti-sense oligonucleotides for silencing ICAM-1 (intracellular adhesion molecule-1) has potential application in the wound healing, where inflammation is a significant criterion such as in Crohn's disease.

7. Toxicity assessment of silver nanoparticles

Nanotechnology has been rapidly growing with utilization in a wide range of commercial products throughout the world. However, there is still a lack of information concerning the increase of human, animal, and environmental exposure to NPs including AgNPs and the potential risks related to their short- and long-term toxicity. However, some studies have already been made.

7.1. *In vitro* tests

AgNPs have emerged as an important class of nanomaterials for a wide range of industrial and medical applications that have potential risks to human health. *In vitro* studies reported, that AgNPs produced toxicity targeted a variety of organs including the lung, liver, brain, vascular system, and reproductive organs. AgNPs induced the expression level of genes involved in cell cycle progression and apoptosis. Possible mechanisms of AgNP toxicity include induction of ROS, oxidative stress, DNA damage, and apoptosis [79].

To understand the toxicity of NPs *in vitro* different tests have been assessed. Testing silver (Ag – 15 nm), molybdenum (MoO₃–30 nm), and aluminum (Al – 30 nm) NPs on mouse spermatogonial cell line have been determined concentration-dependent toxicity for all types. AgNPs were the most toxic (5–10 µg ml⁻¹), and reduced mitochondrial function drastically and increased membrane leakage [80]. Similar conclusions have been made testing the toxic effects of the metal/metal oxides NPs mentioned above on rat liver-derived cell line (BRL 3A). Results showed that mitochondrial function decreases significantly in cells exposed to AgNPs at (5–50 µg ml⁻¹). Fe₃O₄, Al, MoO₃, and TiO₂ had no measurable effect at lower doses (10–50 µg ml⁻¹), while there was a significant effect at higher levels (100–250 µg ml⁻¹) [81].

Generally, in *in vitro* tests, the mechanism of AgNPs-mediated cytotoxicity is mainly based on the induction of ROS. Notably, exposure to AgNPs causes a reduction in GSH, elevated ROS levels, lipid peroxidation, and increased expression of ROS responsive genes; it also leads to DNA damage, apoptosis, and necrosis. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction, Alamar Blue (Invitrogen, Carlsbad, CA) reduction, and

lactate dehydrogenase (LDH) leakage were used as parameters for cytotoxicity assessment. Toxicity of different AgNPs was compared to that of various corresponding concentrations of Ag⁺ ions. Based upon the IC₅₀ values determined by three cytotoxicity assays, AgNPs and Ag⁺ ions did not exhibit a dramatic difference in cytotoxicity [82]. The cytotoxicity and genotoxicity of AgNPs are size-, concentration-, and exposure time-dependent. The cell viability was determined by MTT and CB assay in macrophage (*RAW 264.7*, *J774.1*), pulmonary epithelial (*A549*), renal epithelial (*A498*), hepatic (*Hep G2*), and neuronal (*Neuro 2A*) cell lines. AgNPs showed a concentration-dependent reduction in cell viability after 72 h incubation in all cell lines. *A498* and *RAW 264.7* cells appeared to exhibit the highest sensitivity to the toxic effects of AgNPs and showed significant reduction in cell viability at 1 and 3 g/ml AgNP-concentration, respectively. On the other hand, *A549* cells were least sensitive to cytotoxic effects of AgNPs. The internalization of NPs can induce stress response(s) due to stimulation of free radical production, which in turn, stimulates inflammatory signaling pathways. Hence, the production of reactive nitrogen species (RNS), ROS, and cytokines following AgNPs exposure was determined. AgNPs significantly increased nitrite release by *RAW 264.7* cells at the highest concentration following 72 h incubation. AgNPs also stimulated ROS production in a concentration-dependent manner after 24 h incubation. Inflammatory cytokine (tumor necrosis factor- α [TNF- α] and interleukin-6 [IL-6]) production was significant at 10 and 100 g/ml while 1 g/ml showed no effect on cytokine production. Free radical production has been demonstrated to bear a direct correlation with cytotoxicity of NPs. However, the involvement of other mechanisms cannot be ruled out. Therefore, to determine the contribution of free radicals in AgNP cytotoxicity, cells were incubated with AgNPs in the presence of various antioxidants. Surprisingly, the most potent antioxidants like superoxide dismutase (SOD) and catalase showed no significant protection from AgNPs cytotoxicity. Therefore, two cell membrane ROS scavengers—Trolox (water-soluble vitamin E analog) and tempol (broad-spectrum antioxidant and SOD mimetic)—were investigated. In line with observations in SOD and catalase-treated cells, Trolox and tempol also failed to protect cells from AgNPs cytotoxicity. On the other hand, weak antioxidants like N-acetylcysteine (NAC), methionine and cysteine abrogated the cytotoxic effect of AgNPs. The relative ineffectiveness of potent antioxidants suggests that free radical-dependent mechanisms do not significantly influence cytotoxicity of AgNPs [83]. Other studies showed that p53 protein expression level increased within 4 h after the cells were exposed to AgNPs. The up-regulated expression patterns of p53 protein in two types of mammalian cells by AgNPs exposure suggest that the p53 could be an excellent molecule marker to assess the genetic nanotoxicity. The results suggest the different surface chemistry of AgNPs have different effects on genotoxicity [84]. Beer et al. concluded that free Ag⁺ ions in AgNPs preparations play a considerable role in the toxicity of AgNPs suspensions [85]. While the contribution of the free Ag⁺ ion to the measured toxicity of AgNPs suspensions is an essential determinant for the toxicity, a combined effect of Ag⁺ ion and AgNPs appears for lower concentrations of Ag⁺ ions. These data indicate that the amount of Ag⁺ ions in AgNPs preparations should be routinely measured and reported in toxicological work. They advise that the supernatant of AgNPs suspensions should be used as an additional standard control to make reliable statements of the toxicity of AgNPs and to discriminate between Ag⁺ ions toxicity and AgNPs-induced toxicity [85].

7.2. *In vivo* tests

The most significant problem to understand is the real impact of AgNPs on human health and animals. There are several *in vivo* studies on cytotoxicity and genotoxicity of AgNPs reported. Due to the ultra-small sizes of AgNPs, they have high mobility in different environments, and humans are easily exposed via routes such as inhalation, ingestion, skin, etc. AgNPs can translocate from the route of exposure to other vital organs and penetrate into cells.

Inhalation toxicity of AgNPs has been investigated on Sprague–Dawley rats over a period of 28 days. Results showed that the male and female rats did not show any significant changes in body weight relative to the concentration of AgNPs during the 28-day experiment. There were also no significant changes in the hematology and blood biochemical values in either the male or female rats. Whereas, some investigators have reported that lungs are primary target tissues affected by prolonged inhalation exposure to AgNPs [86]. Lee et al. have reported AgNPs exposure modulated the expression of several genes associated with motor neuron disorders, neurodegenerative disease, and immune cell function, indicating potential neurotoxicity and immunotoxicity associated with AgNPs exposure [87]. Minimal pulmonary inflammation or cytotoxicity of mice was found after 10 days of AgNPs exposure. Gastrointestinal toxicology caused by AgNPs (60 nm) exposure via ingestion has also been tested over a period of 28 days in Sprague–Dawley rats. Results showed that the male and female rats did not show any significant changes in body weight relative to the doses of AgNPs during the 28-day experiment. Some significant dose-dependent changes were found in the alkaline phosphatase and cholesterol values in either the male or female rats, seeming to indicate that exposure to over more than 300 mg of AgNPs may result in slight liver damage. Results suggested that AgNPs do not induce genetic toxicity in male and female rat bone marrow *in-vivo* [88]. Ahamed et al. indicated that AgNPs produce reproductive failure, developmental malformations, and morphological deformities in some non-mammalian animal models. Common causes of AgNPs-induced toxicity include oxidative stress, DNA damage, and apoptosis [79].

Generally, very few papers on the *in vivo* toxicology of AgNPs were found, so further investigation is needed in this field to evaluate precisely the real impact of AgNPs in commercial products to humans and animals.

8. Conclusion

Plant-mediated synthesis of AgNPs has revealed new horizons in drug-delivery. On the one hand, this approach of nanoparticle preparation is preferable due to its economic, accessible, eco-friendly nature, and simplicity of execution. On the other hand, the rich phytochemical composition of plant extracts performs a multi-functional role in the synthesis process of AgNPs as reducing, stabilizing, and the surface-active agent. The AgNPs thus obtained are usually characterized by tiny sizes, monodispersity, and stability of colloidal system because of the capping properties of some of the biomolecules in the extract. Despite their excellent antibacterial, antiviral, antifungal, anticancer, antioxidant, and unusually enhanced

physicochemical properties compared to the bulk material, the AgNPs could be used as vehicles to transport drug molecules (such as oligonucleotides, DNA, siRNA, etc.) to targeted tissues and cells and thereby to improve therapeutic efficacy. Moreover, AgNPs could express synergism with different antibiotics regarding enhanced antibacterial properties. In this regard, the AgNPs might be used as multi-functional drug carriers having great potential in targeted drug-delivery, minimizing side effects, and improving therapeutic efficacy. However, there is still a lack of information concerning the increase of human, animal, and environmental exposure to AgNPs and the potential risks related to their short- and long-term toxicity. Further profound investigations are needed for their safe inclusion as DDSs in commercially available products for the prevention and treatment of life-threatening diseases.

Conflict of interest

The authors have declared no conflicts of interest for this article.

Author details

Nadezhda Ivanova, Viliana Gugleva, Mirena Dobрева, Ivaylo Pehlivanov, Stefan Stefanov and Velichka Andonova*

*Address all correspondence to: andonova_v@abv.bg

Department of Pharmaceutical Technologies, Faculty of Pharmacy, Medical University – Varna “Prof. Dr. Paraskev Stoyanov”, Varna, Bulgaria

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