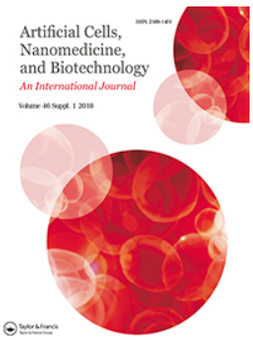

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Pharmaceutical aspects of silver nanoparticles

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ABSTRACT

Silver nanoparticles are particles in the size ranging between 1 and 100 nm. The two major methods used for synthesis of silver nanoparticle are the physical and chemical methods with the disadvantage that they are expensive and can also have toxicity. Biological method is being used as an expedient alternative, as this approach is environment-friendly and less toxic and it includes plant extracts, microorganism, fungi, etc. The major applications of silver nanoparticles in the medical field include diagnostic applications and therapeutic applications, apart from its antimicrobial activity. Due to their nanotoxicity, AgNPs have a several drawbacks too. This review presents a complete view of the mechanism of action, synthesis, the pharmacokinetics of silver nanoparticles, different formulations of AgNPs used in biomedical applications, infertility management, antibacterial effects, skin damage, burns, cancer treatment, etc. and various applications of silver nanoparticles together with the possible toxicological challenge.

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Silver nanoparticles; synthesis; mechanism; formulations; applications; toxicity

Introduction

Nanoparticles (NP) are natural, incidental or manufactured material containing particles, where 50% or more of the particles lie in the size range 1–100 nm [1]. Nanoparticles could be broadly classified as (i) inorganic, and (ii) organic. Inorganic nanoparticles incorporate semi-conductor nanoparticles (like ZnO, ZnS and CdS), metallic nanoparticles (like Au, Ag, Cu and Al) and magnetic nanoparticles (like Co, Fe and Ni); while organic nanoparticles subsume carbon nanoparticles (like fullerenes, quantum dots, carbon nano tubes). Gold and Ag (noble metal) nanoparticles furnish superior characteristics with useful flexibility [2].

Engineered nanoparticles (ENPs) are endowed with exclusive characteristics, such as high surface area to volume ratio, high chemical reactivity, peculiar antimicrobial/fungicidal activity and biocompatible surface properties. These properties are ascribed to small particle size of nanoparticles and a particular size regime [3].

History

Silver has a long history of its usage in different forms and for different purposes. For centuries, the antibacterial properties of silver have been used to fumigate potable water by storage in silver containers [4]. There is anecdotal evidence for the use of nanosilver in ancient Egypt and Rome [5]. The Macedonians used silver plates to improve wound healing and Hippocrates used silver in the treatment of ulcers. In 1520, Paracelsus used silver internally and also applied silver nitrate as a caustic for the treatment of wounds, a practice that continues today [6]. In 1614, Angelo Sala administered silver nitrate internally as a counterirritant, as a purgative and for the treatment of brain infections [6]. C. S. F. Crede is credited with the first scientific publication to describe the medical use of silver in the late nineteenth century. Crede used eye drops containing 1% silver nitrate solution to treat eye infections in new-born [5]. In the United States, colloidal nanosilver, i.e. suspensions of silver particles in liquid, which was registered in 1954 as a biocidal material has been used in medications for nearly one hundred years [5,7]. Use of silver for antimicrobial properties is not a recent development [7].

Synthesis of silver nanoparticle

Silver nanoparticles are generally synthesized by two approaches, (i) "top to bottom" approach and (ii) bottom to top approach.

In *top to bottom* approach, suitable bulk material is broken down into smaller fine particles by size reduction using various techniques like grinding, milling, sputtering, thermal/laser ablation, etc., thus the "top-down" method comprises of mechanical grinding of bulk metals with subsequent stabilization using colloidal protecting agents while in *bottom to top* approach, nanoparticles are synthesized using chemical and biological methods by self-assembly of atoms to new nuclei, which grow into nano size particles while the "bottom-up" methods include chemical reduction, electrochemical methods and sono-decomposition [8,9].

The biggest advantage of "bottom to top" approach method is the production of a large quantity of nanoparticles

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PHYSICAL METHOD	Chemical reduction	BIOLOGICAL METHOD
• Ultrasonication • Irradiation • Microwave • Electrochemical	• Sol gel method • Inert condensation method	• Microorganisms (Bacteria, Fungi, Yeast etc.) • Plants (Angiosperms and gymnosperms)

Figure 1. Different method of synthesis of nanoparticles.

within a short span of time. The major advantage of chemical methods is high yield, contrary to physical methods, which have low yield.

In case of "top to bottom" approach nanoparticles are generally synthesized by evaporation condensation technique with the help of a tube furnace at atmospheric pressure. In this method, the primary material placed centred at the furnace is vaporized into a carrier gas, within a boat. One of the biggest limitations of this method is the imperfections in the surface structure of the product and the other physical properties of nanoparticles are highly dependent on the surface structure in reference to surface chemistry. Hence, cost effective and environment friendly alternate synthetic route was inevitable that culminated into the green syntheses [9].

Green synthesis

Green synthesis of nanoparticles is progressively emerging as a key branch of nanotechnology where the nanoparticles are produced with the help of biological entities like microorganisms, plant extracts or plant biomass and could be a substitute to chemical and physical methods in an eco-friendly manner.

The green synthesis is preferred over physical and chemical methods as the former is environment friendly, cost effective, easily scalable to large scale syntheses, no need to use high temperature, energy and toxic chemicals. The use of plant extracts is potentially beneficial over microorganisms due to the ease of improvement, the less biohazard and strenuous process of maintaining cell cultures as required in microorganism process [8] (Figure 1).

Mechanism of action of silver nanoparticles

In spite of various theories available the exact mechanism of antimicrobial properties of silver nanoparticles is not yet established. Silver nanoparticles anchor to the bacterial cell wall and penetrate it causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. Formation of free radicals by AgNPs causing the death of the cell is another mechanism of action of silver nanoparticles. The formation of free radicals is confirmed by electron spin resonance studies. When in contact with bacteria, the free radicals have the ability to damage the cell membrane making it porous, which ultimately leads to cell death [10]. In comparison to other salts, silver nanoparticles

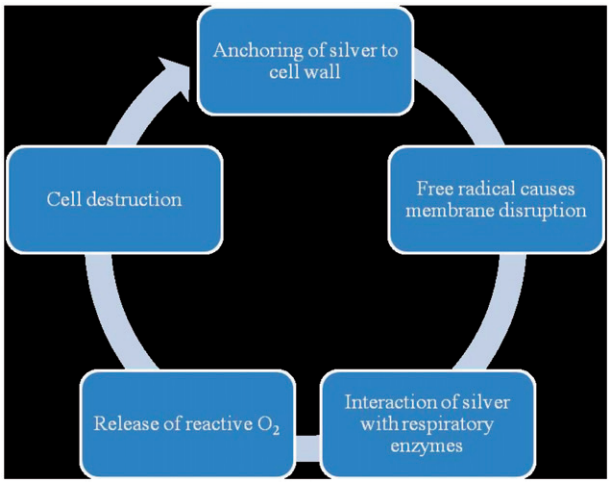

Figure 2. Mechanism of action of AgNPs.

show efficient antimicrobial property because of their large surface area that provides better contact with microorganisms. The release of silver ions by nanoparticles in the bacterial cells enhances/improves their bactericidal activity [11] (Figure 2).

Pharmacokinetics of silver nanoparticles

Predominant accumulation of drugs or NPs in the target tissue is often desirable for enhanced therapeutic effects, and conversely, a high extent of distribution to non-target tissues may cause unwanted toxicity and should be avoided. Therefore, pharmacokinetic and tissue bio-distribution data are crucial for the safe and efficacious biomedical applications of NPs [12].

Absorption

The mechanism of absorption of nanoparticles is more complex than that of small molecules. The nanoparticles that are administered orally can be absorbed by paracellular transport, transcytosis and M cell uptake in the GI tract, while macrophages and lymphatic uptake absorb mainly subcutaneous, intramuscular or inhaled nanoparticles [12,13]. In numerous investigations absorption following oral administration in humans and other mammals has been described qualitatively but only few studies are available that present quantitative data.

In 1980, East et al. inspected silver retention in a 47-year-old woman who already suffered from argyria. Silver retention was found to be 18% of an orally administered dose using radioactive tracer. After oral administration when ionic silver and nanoparticulate silver were compared, the latter was shown to be less bioavailable based on higher faecal excretion and lower absolute levels in organs following head to head investigations [13,14].

Samberg et al. studied for 14 d the infiltration of 20 and 50 nm washed and unwashed AgNPs in porcine skin *in vivo* after topical dosing and observed AgNPs only in the external or exterior layers of the stratum corneum [12,15].

Distribution

In vivo distribution of nanoparticles is dependent on various multiple transport mechanisms, such as opsonization, protein corona formation, mononuclear phagocyte system (MPS) uptake, enhanced permeability and retention (EPR) effect, target-mediated disposition and lymphatic transport [13].

The liver has been described as the primary organ for Ag distribution followed by spleen and kidneys, whether the exposure was oral, intravenous, or subcutaneous or through inhalation. In several cell types deposition of silver in the liver has been detected including the Kupffer cells, hepatocytes and sinusoidal endothelium cells. Deposition of silver was observed in all regions of kidney, including the cortex, medulla, inner medulla, and cortical glomeruli and gender-related difference was reported in the kidney of rats regarding accumulation of silver following repeated oral exposure, with a two-fold inflation in females as compared to males [12].

Metabolism

Silver has been documented to be deposited as particles in tissue, such as the skin epidermis, the glomeruli, and the intestines following oral exposure to both ionic and nanoparticulate silver suspensions. The particle size of these nanoparticles has been described to be 12 nm in diameter in the rat intestine and contain sulphur and selenium apart from silver [16].

Ag^+ can react with GSH, producing H^+ and GS–Ag, which ultimately forms Ag–GSH polymer complexes, followed by partitioning to various tissues. Upon UV-photodecomposition, Ag–thiol complexes can further be reduced to zero-valent AgNPs with slower rates in visible light. Besides thiols, AgNPs can also be sulphidated to produce Ag_2S NPs. In addition, Ag_2S NPs can interact with selenium to produce Ag_2Se NPs and Ag/S/Se argyrial particulate [12].

Elimination

A study reported low excretion of silver in urine is (<0.1% of 24 h intake for both groups) but high in faeces; 63 and 49% of the daily dose for AgNPs and silver acetate groups, respectively, following a 28-day repeated oral exposure to 14 nm PVP-coated AgNPs or silver acetate in rats [12].

Faecal excretion: Following oral administration the faecal excretion rate is reported to be higher for 14 nm AgNP than for ionic silver; namely 63 and 49% for nanoparticles and ionic silver, respectively. This was ascribed to the lower bioavailability of silver nanoparticles [16].

Urinary excretion: East et al. reported that the urinary excretion at 12 h of an orally administered dose of radioactive silver tracer in a woman was in the range of $2–4 \times 1\ 0^{-4}$% [14].

Furchner et al. reported urine excretion of silver following orally administered 110 radionuclide of silver [17]. Van der Zande et al. found that after 8 weeks of post oral dosing of silver or silver nanoparticles (15 and 20 nm), silver was still present in rat brain and testes [18].

Drug delivery aspects of silver nanoparticles

Different formulations

- Patil and Kumbhar synthesized silver nanoparticle via green synthesis using extract of *Lantana camara L.* leaves and found these NPs to exhibit dose dependent antioxidant potential comparable to that of standard ascorbic acid. AgNPs also showed significant antimicrobial activity against Gram positive *Staphylococcus aureus* than Gram negative *Pseudomonas aeruginosa* and *E. coli* comparable with standard, Ciprofloxacin [19].
- Jha et al. synthesized AgNPs from *Ocimum tenuiflorum* extract followed by study of AgNP loaded multi-walled carbon nanotubes (MWCNT) with mammalian sperm to evaluate the increased targeting potential for the development of portable diagnostic tool for the infertility management. AFM demonstrated the loading of AgNP inside MWCNT as surface height of MWCNT increased from 22 to 32 nm, which in turn assured the encapsulation of 10 nm size of AgNP inside the tube [20].
- Kumar et al. reported green synthesis of AgNP by *Jatropha curcas* and *Lannea grandis*, which further demonstrated low MIC and low minimum biofilm eradication concentration against *C. albicans* biofilm. The formulation developed was stable and cytotoxic against goat blood RBC and it could be further used for treatment of *C. albicans* associated infection [21].
- Bilal et al. synthesized AgNPs loaded chitosan-alginate construct from methanolic extract of *E. helioscopia* and antibacterial activities against six clinically pathogenic strains including *S. aureus, P. aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii, Morganella morganii* and *Haemophilus influenza* were investigated. All construct exhibited excellent biocompatibility for normal cell line, i.e. L929 and anti-cancer efficacy against HeLa cells. Thus, the newly engineered construct could be a useful candidate for biomedical applications [22].
- Castangia et al. synthesized grape-silver nanoparticles stabilized by phospholipids vesicles, which inhibited proliferation of *S. aureus* and *P. aeruginosa* providing safeguard of keratinocytes and fibroblast against oxidative stress that could be used as topical formulation for skin damages [23].
- Loo et al. investigated interaction of silver and curcumin NPs against Gram positive and Gram negative bacteria and their 100 μg/mL concentration distorted matured bacterial biofilms. This formulation could be used for its sustained antibacterial effects [24].
- Ibrahim and Hassan developed silver nanoparticle functionalized cotton fabric using green synthesis, which displayed good qualitative and quantitative antibacterial activity against *E. coli* and *S. aureus* and support that this property could be further utilized in manufacturing antimicrobial finishing and textiles [25].
- Jadhav et al. synthesized antibacterial silver nanoparticles using extract of *Ammannia baccifera*. AgNPs gel (0.025% w/w) when compared with marketed 0.2% w/w silver nitrate gel displayed equal zone of inhibition against all pathogenic bacteria responsible for infections in burns.

The formulated AgNPs gel could be used as an efficient and better substitute in burns by promoting cellular growth and relieving pain [26].

- Khan et al. described the method of synthesis of nanoparticle using *Heliotropium crispum* plant extract. The antibacterial action of AgNPs was found to be species independent and strictly strain dependent as both Gram negative *P. aeruginosa* (PA) and *A. baumannii* (AB) and Gram positive Multiple Drug Resistant *S. aureus* (MRSA) exhibited differential inhibition zones and decrease in bacterial viability [27].
- Kajani et al. synthesized AgNP using extract of *T. baccata* plant, which showed better anticancer activity than the previously reported ones due to the synergistic role of Taxus compound in the nanoparticle cytotoxicity. It was concluded that the biogenic synthesis of AgNPs in combination with targeted therapy of tumours may give rise an alternative approach for efficient treatment of cancers with fewer side effects [28].
- Sadat et al. developed high drug-loaded Imatinib-loaded silver nanoparticle for potent bioavailability and decreasing dose frequency which is important in antitumor drug delivery to breast cancer cells, which was incited by apoptosis rather than necrosis. They concluded that green synthesized silver nanoparticles are promising sustained release system to the Imatinib and will be potentially useful for controlled drug delivery [29].
- Djahaniani et al. prepared silver nanoparticles using *Tribulus longipetalus* leaf extract and tested against the microorganism *S. aureus, Bacillus cereus, E. coli, K. pneumoniae, B. subtilis* and *S. typhimurium*. These nanoparticles were found to have proficient antimicrobial action against all bacterial species, except for *K. pneumoniae*. The highest inhibition zone (12.0 mm ±1) and the lowest MIC value (256 μg/mL) were achieved for *B. subtilis*, which indicated this microorganism as the most subtle species to polar and non-polar extracts [30].
- Govindarajan et al. reported mosquitocidal silver nanoparticle using aqueous leaf extract of *A. indica* and suggested that the synthesis of nanoparticle using *A. indica* may be considered for the development of newer and safer mosquito larvicides [31].
- Jannathul and Lalitha described the synthesis of silver nanoparticles using the aqueous extract of *Alternanthera sessilis* as a reducing agent by sonication, espousing green chemistry principles. The cytotoxic effect of biosynthesized silver nanoparticles was studied by MTT assay against breast cancer cells (MCF-7 cell line) and the NPs exhibited significant cytotoxic activity with IC_{50} value 3.04 μg/mL compared to that of standard cisplatin. The data obtained in the study revealed the potent therapeutic value of biogenic silver nanoparticles and the scope for further development of anticancer drugs [32].
- Marslin et al. biosynthesized cream formulation of silver nanoparticle using *Withania somnifera* extract and concluded that WS-AgNP cream had higher antimicrobial activity, hence could be used in low doses and less toxic for patient in comparison with $AgNO_3$ counterparts [33].
- Sahana et al. formulated antibacterial cold cream using biosynthesized silver nanoparticle from flower extract of *Cassia auriculata* as a reducing agent. The cold cream containing the flower extract alone showed a minimum inhibitory effect on the pathogens whereas the bactericidal cold cream containing the NPs synthesized from the flower extract showed an excellent antibacterial activity [34].
- Veerakumar et al. synthesized AgNP using *Heliotropium indicum* plant leaves and displayed larvicidal effects on *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* vector mosquito species. The LC_{50} and LC_{90} values of *H. indicum* aqueous leaf extract appeared to be effective against *A. stephensi* followed by *A. aegypti* and *C. quinquefasciatus* further ensuring the potential to be used as an ideal eco-friendly approach for the control of mosquito vector species [35].
- Rajakumar and Rahuman utilized aqueous extract from *Eclipta prostrata* to synthesize AgNP possessing larvicidal activity. The results conveyed that the AgNPs have the capability to be used as an ideal eco-friendly way for the control of the *Culex tritaeniorhynchus* and *A. subpictus* and claimed being the first report on the mosquito larvicidal activity of synthesized AgNPs against vectors [36].
- Kuberan et al. prepared AgNPs by green synthesis using *Carica papaya* latex extract as a bio-reductant and clearly suggested that CPAgNPs could be developed as nano drug formulation to combat against bacterial infection and breast cancer chemotherapy [37].
- Goyal et al. synthesized AgNP using β-glucan replacing conventional reducing agents with biocompatible and structural compatible sugar molecules. An efficient nanoemulsions delivery method for AgNP was developed wherein DOX was encapsulated in nanoemulsions containing AgNP and displayed antibacterial effects together with antitumor efficacy, which is quite useful in treatment of cancer cells, while preventing microbial infection. The effective loading of drug was about 15–30% [38].
- Manukumar et al. developed thymol-loaded chitosan AgNP by novel route using chitosan as reducing agent and thymol as capping agent, which is highly efficacious against multiple food-borne pathogens and is potent in controlling the human diseases induced by both Gram negative and Gram positive bacteria [39].
- Thapa et al. embedded graphene oxide in silver nanoparticles (GO-AgNP) using glucose as reducing agent. By covalent conjugation of MTX to GO-AgNP via amide bond the targeting of folate receptors expressing cancer cells could be achieved and their combination may be used synergistically for treatment of cancer [40].
- Catanzano et al. formulated wound healing bio functional hydro gel using alginate and hyaluronic acid containing ultra-small silver nanoparticles as antimicrobial constituent and were fabricated using $CaCO_3$ and glucono- δ-lactone [41].
- Alves et al. formulated antimicrobial thermo-responsive gel by interaction of AgNP with PVP and PVA polymers and thereafter incorporated in CUR/P407 (1:2) solid dispersion into a polymer dispersion of 20% P407 and

displayed good antioxidant activity. The Minimum Inhibitory Concentration (MIC) values were efficacious for Gram negative bacteria than for Gram positive ones and antibacterial activity of curcumin was alleviated in presence of AgNP for use as hydro gel [42].

- Petr Paril et al. investigated the antifungal effects of copper and silver NPs against wood- rotting fungi. Nanosilver treatment exhibited very low mass loss and high efficiency against *T. versicolor* fungi in comparison to *Poria placenta* decaying [43].
- Mugade et al. synthesized mannan sulphate capped silver nanoparticles, which appear a promising topical agent with increased wound healing properties due to faster uptake of mannose receptor and increase site specific delivery. These stable MS-AgNPs exhibited enhanced cytocompatibility, targeting potential and cellular uptake in murine macrophages, human skin fibroblasts and human keratinocytes [44].
- Yamada et al. immobilized silver nanoparticles on the surface of yttria stabilized zirconia (YSZ) and tested for antibacterial activity against *S. aureus, S. mutans, E. coli* and *A. actinomycetemcomitans*, which were found to be concentration dependent on AgNPs whereas excellent antimicrobial activity against *E. coli* was observed and no cytotoxic effects on L929 cells were detected at coating concentrations below 2.5 mM. Further, AgNP-coated YSZ can be potentially used to control dental caries and periodontal disease [45].
- Azizi et al. developed a novel nano-composite with the aim of making specific targeting of silver nano particles as a drug for tumour cells and developing new anticancer agents. Albumin coated silver nanoparticles (ASNPs) were synthesized, and their anti-cancerous effects were evaluated against MDA-MB 231, a human breast cancer cell line. The morphological changes of the cells were observed by inverted, florescent microscopy and also by DNA ladder pattern on gel electrophoresis revealing that the cell death process occurred through the apoptosis mechanism. It was found that ASNPs with a size of 90 nm and negatively charged with a zeta-potential of about −20 mV could be specifically taken up by tumour cells. The LD_{50} of ASNPs against MDA-MB 231 (5 μM) was found to be 30 times higher than that for white normal blood cells (152 μM) suggesting ASNPs as a good candidate as chemotherapeutic drug [46].
- Sobral-Filho et al. produced fine-tuned gold and silver nanoshells via an entirely reformulated synthesis which yielded ultra monodisperse samples, with polydispersity indexes (PI) as low as 0.02. A library of nanoshell samples with localized surface plasmon resonances (LSPR) spanning across the visible range was synthesized. A cell labelling experiment, targeting different subcellular compartments in MCF-7 human breast cancer cells, exhibited that the monodisperse nanoparticles could be used as a multiplex platform for single cell analysis at the intracellular and extracellular level. Antibody-coated gold nanoshells targeted the plasma membrane, while silver nanoshells coated with a nuclear localization signal (NLS) targeted the nuclear membrane. A fluorescence counter-staining experiment displayed the excellent selectivity and specificity of each type of nanoparticle for its designed subcellular compartment. A time-lapse photodegradation experiment affirmed the improved stability of the nanoshells over fluorescent labelling and their potential for long-term live cell imaging [47].
- Dojčilović et al. studied the interaction of the tryptophan functionalized Ag nanoparticles and live *Candida albicans* cells by synchrotron excitation deep-ultraviolet (DUV) fluorescence imaging at the DISCO beamline of Synchrotron SOLEIL. DUV imaging showed that incubation of the fungus with functionalized nanoparticles resulted in significant increase in the fluorescence signal. The analysis of the images disclosed that the interaction of the nano particles with (pseudo) hyphae polymorphs of the diploid fungus was less prominent than in the case of yeast cells or budding spores. The results of time-integrated emission in the mentioned spectral ranges suggested that the nanoparticles infiltrate the cells, and majority of the nanoparticles adhere to the surface of cell [48].
- Rajabnia and Meshkini investigated the effect of different concentrations of adenosine 5′-triphosphate (ATP) as a stabilizing agent on the physicochemical and biological behaviour of AgNPs. Cellular viability studies in osteosarcoma cells (Saos-2), breast cancer cells (MCF-7 and T47D), and leukaemia cells (K562) suggested that ATP-capped silver nanoparticles (ATP@AgNPs) possess high-antitumor efficacy compared with the naked ones. Moreover, the cytotoxicity induced by ATP@AgNPs proceeds from the perturbation of intracellular oxidative status, leading to the induction of apoptosis [49].
- Wildt et al. introduced a novel technique, nanoparticle associated cytotoxicity microscopy analysis (NACMA), which integrates fluorescence microscopy detection using ethidium homodimer-1, a cell permeability marker that binds to DNA after a cell membrane is compromised (a classical dead-cell indicator dye), with live cell time-lapse microscopy and image analysis to concomitantly enquire silver nanoparticle accumulation and cytotoxicity in L-929 fibroblast cells. Studies conducted on 10, 50, 100 and 200 nm silver nanoparticles disclosed size dependent cytotoxicity with particularly high cytotoxicity from 10 nm particles. In addition, NACMA results, when combined with transmission electron microscopy imaging, reveal direct affirmation of intracellular silver ion dissolution and possible nanoparticle reformation within cells for all silver nanoparticle sizes [50].
- Swanner et al. focussed on determining the properties of the nano material that are important to retain or enhance Triple-negative breast cancer (TNBC) selective response. The increased sensitivity of TNBC cells as compared to non-cancerous cells was found to be independent of nanoparticle size, and TNBC cell lines (MDA-MB-231, BT-549, SUM-159) were more sensitive to AgNP exposure than luminal A (MCF-7) or non-cancerous breast (MCF-10A, 184B5). Remarkably, AgNP treatment significantly slowed TNBC tumour growth *in vivo* with no apparent systemic toxicity. AgNPs were functionalized with folic

acid (FA) to exploit the folate receptor α (FRA), which is over expressed in approximately 80% of TNBC. In comparison to non-targeted AgNPs, FA-AgNPs significantly increased the cytotoxic effects of the prepared AgNPs against TNBC *in vitro*. The authors concluded that AgNPs exerted significant anti-cancer activity towards TNBC cells *in vitro* and *in vivo* [51].

- Pimentel et al. reported the synthesis of AgNPs (3.89 ± 0.90 nm) through the polyol method, the generation of AgNP nanocarriers and the bioconjugation protocol of the nanocarrier with soybean agglutinin (SBA). The free AgNPs, the AgNP nanocarriers, and the SBA-bioconjugated AgNP nanocarriers were tested for cytotoxicity in breast cancerous (MDA-MB-231and MCF7) and non-cancerous (MCF 10A) cells, using the MTT assay. AgNPs demonstrated cytotoxic activity *in vitro*, the non-cancerous cells (MCF 10A) being more sensible than the cancerous cells (MDA-MB-231 and MCF7) showing LD_{50} values of 128, 205 and 319 μM Ag, respectively; the nanoencapsulation diminished the cytotoxic effect of AgNPs in non-cancerous cells, alleviating the effect on the cancer-derived cells, whereas the SBA-bioconjugation allowed AgNP cytotoxic activity with a similar behaviour to the nanocarriers [52].
- Kravets et al. demonstrated the application of luminescent silver nanoparticles as imaging agents for neural stem and rat basophilic leukaemia cells and further studied the experimental size dependence of the extinction and emission spectra for silver nanoparticles. The nanoparticles were functionalized with fluorescent glycine dimers. Spectral position of the resonance extinction and photoluminescence emission for particles with average diameters ranging from 9 to 32 nm was inspected. The nanoparticles were able to penetrate cell membranes of rat basophilic leukaemia and neural stem cells fixed with paraformaldehyde. Additionally, toxicity studies were performed and it was found that towards rat basophilic leukaemia cells, luminescent silver nanoparticles had a toxic effect in the silver atom concentration range of 10–100 μM [53].
- Verma et al. formulated wound dressing carbopol based hydro gel using sericin and chitosan capped AgNP, which displayed higher antibacterial activity and were found to be non-irritant, potential wound healer, biocompatible by *in vivo* studies [54].
- Borrego et al. tested the antiviral activity of AgNP formulated as Argovit against Rift valley fever virus, which represents an important zoonotic pathogen and potential biological weapon. Silver nanoparticles combined with the virus will help to plan a more effective application of Argovit against viral infections both prophylactically and therapeutically [55].
- McLaughlin et al. prepared sprayable formulation of AgNPs by exchanging citrate capping agents with LL37-SH peptide thereby forming antimicrobial composite that was acting as an anti-infective and anti-biofilm barrier against *P. aeruginosa* infection without having any toxic or anti-proliferative effects on human skin fibroblasts and when used as in vivo wound model, the composite remained in the affected area without infiltrating other tissue and organ [56].
- Chen et al. formulated multifunctional nano carrier based on multi-walled carbon nanotubes (MWCNTs) decorated with gold/silver core–shell nanoparticles (Au@Ag NPs) and fluorescein isothiocyanate (FITC) for tracing the intracellular drug release process. The nano carrier, the Au@Ag NPs adsorbed on the surface of MWCNTs were labelled with the pH-dependent SERS reporter 4-Mercaptobenzoic acid (4-MBA) for SERS based pH sensing. Fluorescent doxorubicin (DOX) was used as the model drug, which can be loaded onto MWCNTs via π–π stacking and released from the MWCNTs under acidic condition. The drug release dynamics were enquired, 2-D colour-gradient pH mapping were plotted, which were calculated from the SERS spectra of 4MBA further indicating that the designed nanocarrier have a great potential in intraceable drug delivery, cancer cells imaging and pH monitoring [57].
- Yen et al. presented a platform for multiplexed pathogen detection using multi-coloured silver nanoplates, which requires no external excitation source and permits multiplexed analysis in a single channel, facilitating integration and manufacturing. Rapid point-of-care (POC) diagnostic devices are needed for field-forward safeguarding or screening of severe acute systemic febrile illnesses. Multiplexed rapid lateral flow diagnostics have the capability to differentiate among multiple pathogens, thereby facilitating diagnosis and ameliorating patient care [58].
- Tutaj et al. synthesized AgNP using antifungal agent amphotericin B acting both as reducing as well as capping agent. An excellent anti-fungal activity of AmB-AgNPs was ascribed to the synergistic effect of antifungal activity of amphotericin-B and antimicrobial properties of silver [59].
- Li Hui et al. reported on silver decahedral nanoparticles (Ag10NPs)-based FRET (fluorescence resonance energy transfer) sensor for target cell imaging. Fluorophores-functionalized aptamers (Sgc8-FITC) were bound with Ag10NPs via the SH group on the aptamer to form Ag10-Sgc8-FITC. Then, quencher-carrying strands (BHQ-1) were hybridized with Sgc8-FITC to form an Ag10NPs-based FRET sensor (Ag10-Sgc8-F/Q). The sensor interplayed with membrane protein tyrosine kinase-7 (PTK-7) on the CCRF-CEM (CCL-119, T-cell line, human acute lymphoblastic leukaemia) cell surface to acquire fluorescence imaging of CCRF-CEM cells. The addition of CCRF-CEM cells resulted in many sensors binding with cells membrane and the displacement of BHQ-1, thus disordering the FRET effect and the intensified fluorescence intensity of FITC. It was found that Ag10NPs largely alleviated the fluorescence intensity of FITC. The results also suggested that the Ag10NPs-based FRET sensor (Ag10-Sgc8-F/Q) was not only predominant to the bare FRET sensor (Sgc8-F/Q) and sensor Ag-Sgc8-F/Q but also highly responsive and particular for CCRF-CEM cells imaging [60].
- Appadurai and Rathinasamy developed formulation with improved antimitotic, apoptotic and antiproliferative

activity of plumbagin (PBL) by interacting AgNPs with the anticancer agent PLB, which will further enhance the internalization of PLB. Hence, AgNP could be a promising and potent drug delivery system for enhanced activity of PLB in cancer treatment [61].

- Perez-Diaz et al. prepared chitosan gel loaded with AgNPs to check the anti-biofilm capacity and application in chronic wounds with respect to standard silver sulphadiazine. The results showed that the developed formulation could be used for prevention and treatment of infections in chronic wounds as it completely inhibit the formation of biofilm and kill bacteria in established biofilm [62].
- Rath et al. explored the wound healing capacity of collagen nanofiber mats containing silver nanoparticle. In *in vivo* study, the wound healing rate of composite nanofiber mats was found to be higher owing to their intrinsic antibacterial, anti-inflammatory, controlled drug release profile and haemostatic properties compared with plain collagen nanofiber [63].
- Vankayala et al. reported that sensitization and formation of singlet O_2 is strongly dependent on the morphologies of gold and silver nanostructures. Furthermore, it also demonstrated *in-vitro* morphology dependent sensitization behaviour of silver nanoparticles in the photodynamic cancer treatment. The results indicated that metal nano particles with certain morphologies were potentially very promising dual functional nano materials with capabilities of simultaneously serving as near infrared (NIR) activatable photodynamic therapy and photothermal therapy reagents for cancer treatments [64].
- Acosta-Torres et al. developed silver nanoparticle containing acrylic resin and added to PMMA formulation, as a biocompatible nontoxic antifungal agent for denture base, which would decrease adherence of most common oral pathogen, i.e. *C. albicans*. The results demonstrated that PMMA-silver nanoparticles are a suitable means of producing nontoxic materials with antimicrobial properties for use in dentistry and inhibition of *Candida albicans* on denture resins that could play a significant role in preventing the development of denture stomatitis [65].
- Drescher et al. developed a method for quantification of the number of metal nanoparticles at the single-cell level on the basis of matrix-matched calibration wherein the Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was utilized for spatially resolved bio imaging of the distribution of silver and gold nano particles in individual fibroblast cells upon different incubation experiments. The results provided insight into nano particle/cell interactions and had implications for the development of analytical methods in tissue diagnostics and therapeutics [66].
- Stevens et al. reported the development of safer central venous catheters (CVC) whose coating contains N-vinylpyrrolidone and n-butyl methacrylate wherein silver nanoparticle and heparin were embedded imparting the simultaneous antimicrobial and antithrombogenic action to the CVC [67].
- Boca et al. reported the performance of newly synthesized chitosan-coated silver nanotriangles (Chit-AgNTs) with strong resonances in near-infrared (NIR) to operate as photothermal agents against a line of human non-small lung cancer cells (NCI-H460). The hyperthermia experiments were conducted by excitation of nanoparticles-loaded cells at 800 nm wavelength from a Ti:Sapphire laser. The results revealed a novel class of biocompatible plasmonic nanoparticles with high potential to be implemented as effective phototherapeutic agents in the battle against cancer [68].
- Zhang et al. demonstrated that the nanostructures comprising silver cores and dense layers of Y_2O_3:Er separated by a silica shell is an excellent model system to study the interaction between upconversion materials and metals in nanoscale. Finally, the nano particles are potentially interesting as fluorescent labels in, for instance (single particle), imaging experiments or bioassays which require low background or tissue penetrating wavelengths [69].
- George et al. showed the proficient antibacterial activity of silver nanoparticle-encapsulated cyclodextrin and commended the use of the system in future biological and biomedical applications [70].
- Lee et al. characterized the transport of single silver nanoparticles into an *in vivo* model system (zebrafish embryos) and their effects on early embryonic development at single-nanoparticle resolution in real time was investigated. It was found that single Ag nano particles (5–46 nm) were transported into and out of embryos through chorion pore canals (CPCs) and exhibited Brownian diffusion (not active transport), with the diffusion coefficient inside the chorionic space (3×10^{-9} cm^2/s) $\sim$26 times lower than that in egg water (7.7×10^{-8} cm^2/s). In contrast, nano particles that were trapped inside CPCs and the inner mass of the embryos, showed restricted diffusion [71].
- Lee et al. investigated the dependence of the sensitivity of the surface plasmon resonance (frequency and bandwidth) response to changes in their surrounding environment and the relative contribution of optical scattering to the total extinction, on the size and shape of nano rods and the type of metal, that is, Au vs. Ag. On the other hand, a greater enhancement in magnitude and sharpness of the plasmon resonance band was observed in nano rods with higher Ag concentration, which gives better sensing resolution despite similar plasmon response. Furthermore, Ag nano rods had an additional advantage as better scatterers compared with Au nano rods of the same size [72].

Applications

Due to unique properties of silver nanoparticles, such as size and shape which depend on optical, electrical and magnetic properties, they are of immense interest and can be subsumed into antimicrobial applications, biosensor materials, composite fibres, cryogenic superconducting materials, cosmetic products and electronic components. Nanoparticles have numerous applications in different fields, such as

medical imaging, nano-composites, filters, drug delivery and hyperthermia of tumours [73].

(a) Antimicrobial activity of AgNPs

AgNPs have been used tremendously as anti-bacterial agents in the health industry, food storage, textile coatings, numerous environmental applications, as an antibacterial agent from fumigating medical devices and home appliances to water treatment cotton fibre [74]. Smaller AgNPs have a greater binding surface and show more bactericidal activity when compared to larger AgNPs. The reason for the sensitivity of Gram positive and Gram negative bacteria towards AgNP is because of variation in thickness and molecular composition of the membrane structures. Bactericidal activity is apparently due to alteration in the bacterial cell wall structure as a result of interactions with embedded AgNPs, leading to enhanced membrane permeability and finally death. AgNPs also react with sulphur and phosphorus-rich biomaterials, such as proteins or DNA, or membrane protein, which affect the respiration, division and ultimately survival of cells. Upon entering the bacterial cell wall, silver ions (as part of AgNPs) can enter into cells, leading to the aggregation of damaged DNA and exert effect on protein synthesis [75].

(b) AgNPs in cancer control

Since AgNPs can disrupt the mitochondrial respiratory chain, they could be expanded to instigate the reactive oxygen species (ROS) production, ATP synthesis and finally DNA damage; they can perform well in cancer therapeutics. *Sesbania grandiflora* leaf extract mediated AgNPs exhibited cytotoxicity to MCF-7 cancer cells instigating ROS production resulting in oxidative stress and caspase-mediated synthesis with further changes in morphological attributes including hampering of membrane integrity, cell growth reduction, cytoplasmic condensation, etc.

G. mangiferae extracts mediated AgNP synthesis are highly biocompatible with IC_{50} values of AgNPs were 63.37, 27.54 and 23.84 μg/mL against normal African monkey kidney (Vero), HeLa (cervical), and MCF-7 (breast) cells, respectively, should be probed or examined as promising candidates for a variety of biomedical/pharmaceutical and agricultural applications. The alcoholic flower extract of *Nyctanthes arbor-tristis* mediated AgNP can be used for molecular imaging and drug delivery and AgNP were slightly toxic to L929 cells even at highest concentration, i.e. 250 μg/mL. MCF-7 cells treated with either AgNPs or cisplatin demonstrated decreased Bcl-2 expression and increased Bax expression, pointing out the embroilment of mitochondria in the mechanism of death induced by AgNPs. *Rosa indica* mediated AgNP synthesis may be used in vast range of therapeutic anticancer application and act as radical scavenger and induce apoptosis in HCT-15 cells and the ROS generation [75].

(c) Antioxidant activity of AgNPs

The highest recorded radical scavenging activity is 64.81% at a concentration of 500 μg/mL is by AgNP synthesized using leaf extracts of *Leptadenia reticulate* whose extract bolster dose dependent DPPH radical scavenging activity. The potential of antioxidants to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals is probably due to their capability to donate hydrogen and easily incorporate electrons; the latter is possible due to the presence of host lipophilic radicals. As compared to nanoparticles, the DPPH radical scavenging activity of $HAuCl_4$ and $AgNO_3$ was negligible, which may be due to salt conditions or weaker solubility of metal oxides [75].

(d) Antidiabetic activity of AgNPs

Tephrosia tinctoria stem extracts mediated AgNP synthesis was evaluated for control of blood sugar levels. AgNPs scavenged free radicals, reduced the levels of enzymes that bring about the hydrolysis of complex carbohydrates (α-glucosidase and α-amylase), and as a result of which there is an increase in consumption rate of glucose [73].

(e) Different field applications of AgNPs

Nanotechnology is rapidly developing nano products and nanoparticles (NPs) that can have peculiar and size-correlated physicochemical properties which are quite different from larger material. These unique characteristics of NPs have been utilized in several possible applications in medicine, cosmetics, biomedical and environmental remediation [76].

The pharmaceutical industry seems to be one of the largest beneficiaries of AgNPs; employing these nano materials as antimicrobial and anti-fungal preparations. Silver has been used anciently for burns, wounds and bacterial infections; the utility of AgNP in these treatments is better appreciated in modern context due to growing overwhelming antibiotic resistance in bacteria. AgNPs have been shown to improve efficiency of cancer treatments by increasing effectiveness of drug delivery and producing anti-tumorogenic effects, which display great capability in cancer therapeutics. Studies concentrating on the therapeutic applications of AgNPs in the gastrointestinal tract have displayed that gastric cells can be sensitized to radiation by the use of AgNP and they may bypass the stomach and instead release the drug in small intestine [75].

As antimicrobial agents silver nanoparticles have been used largely in the health industry, food storage, textile coatings and a number of environmental applications. *Indigofera aspalathoides* extract mediated silver nanoparticles were assessed in wound-healing applications following excision in animal model. *Chrysanthemum morifolium* extract mediated AgNPs were added to clinical ultrasound gels, which are used with an ultrasound probe, and were found to possess bactericidal activity, aiding to the sterility of the instrument [77].

Toxicity

Nanosilver can apparently cause ill effects on humans as well as on the environment is suggested by different studies and reports. From industrial wastes tons of silver are let out into the environment thereby causing toxicity in the environment. Free silver ions have ill or harmful effects on humans and all living beings including permanent bluish-grey discoloration

of the skin (argyria) or the eyes (argyrosis), and exposure to soluble silver compounds may produce toxic effects like liver and kidney damage; eye, skin, respiratory and intestinal tract irritations; and untoward changes in blood cells. Numerous reports suggest that nano silver cannot differentiate between the microbes that are harmful or useful to the ecology. Only limited studies have been performed to evaluate the toxicity of nanosilver. It was demonstrated by *in vitro* toxicity assay of AgNP that rat liver cells on low level exposure elicit oxidative stress and damaged mitochondrial function. By interrupting mitochondrial function and causing leakage through the cell membrane, silver nanoparticles manifested to be toxic to *in vitro* mouse germ line stem cells. On male reproductive system they adversely affect sperm cells by crossing the blood–testis barrier and get deposited in the testes. The target organ in mouse for the nanosilver is liver as revealed by *in vivo* studies on the oral toxicity on rats. The higher occurrence of bile duct hyperplasia, with or without necrosis, fibrosis and pigmentation in the study animals revealed that when the nanoparticles are stored for a long span of time there is let out of silver assuring that aged nanosilver is more toxic than new nanosilver [10].

Nanosilver also has toxic effects on aquatic animals and hinders osmoregulation in fish by interacting with the gills of fish and impedes basolateral Na^+–K^+-ATPase activity suggesting that the release of nano silver into the environment has to be heedfully reviewed [10].

The toxicity of silver nanoparticles is affected by a number of factors such as particle size, shape and surface chemistry, crystallinity, capping agents, environmental factors, such as ionic strength, pH and the presence of ligands, divalent cations and macromolecules. Hence, to reach final conclusion on its toxicity it is mandatory to carry out more studies to check the toxic effects of nanosilver has *in vivo* [76].

a) Oral toxicity

In mammals and in humans, the orally administered silver has been manifested in the range of 0.4–18 and 18%, respectively. Silver seems to be allocated to all the organs probed, with the maximum being seen in intestine and stomach. Silver induces a blue-grey discoloration in the skin termed argyria [16]. Increased plasma alkaline phosphatase and decreased plasma urea were seen following ionic silver oral administration in the form of silver acetate (9 mg of silver/kg of bw/day) or nanoparticulate silver (9 mg of 14 nm particles/kg of bw/day) after 28 d oral analysis. Enhanced serum cholesterol levels were noted at the 125 mg/kg of bw/day dose and above for males and were noted for females following the oral administration of 500 mg/kg of bw/day [78].

b) Neurotoxicity

Silver induced neurotoxic effects occur via secondary molecules that are released from periphery, even in the absence of silver in the brain extracellular fluid. Hadrup and Lama stated the effects of the ionic and 14 nm nanoparticulate silver for 28 d on neurotransmitters in rats, and noticed changes in noradrenaline, dopamine and 5-HT concentrations in brain at doses of 2.25 mg/kg of bw/day of nanoparticulate silver and 9 mg/kg of bw/day of ionic silver [16]. Mirsattari et al. observed that following oral administration of large amounts of colloidal silver, myoclonic status epilepticus was observed in man [79].

c) Immunotoxicity [13]

After feeding high doses of 13 nm nanoparticulate or 2–3.5 micro particulate silver, lymphocyte infiltration was documented in mice by Cha et al. [80]. Havarinasab et al. reported an autoimmune condition in H-2 s mouse strain following 0.5 g of silver nitrate/L in the drinking water for 10 weeks [81]. Van der Zande et al. reported no sign of immunotoxicity following the oral ingestion of 90 mg/kg of bw of silver nanoparticles (15 and 20 nm) or 9 mg/kg of bw/day of ionic silver for 28 d [18]. Hadrup and Lama also demonstrated the decrease in thymus weight following the oral ingestion of nanoparticulate and ionic silver for 28 d [16].

d) Environmental toxicity

In the aquatic environment, the response of nanotoxins including AgNP has a considerable impact. With the help of algal growth inhibition assays, various studies probed AgNP toxicity to primary producers. These studies included: LC_{50} Median lethal concentration, EC_{50} Median effective concentration, IC_{50} Median Immobilizing Concentration. Silver barbs, zebra fish and god fish have been employed in toxicity evaluation of *in vivo* fish studies. Aquatic ecotoxicological studies exploit primary fish cells and continuous fish cell lines [5].

e) Reproductive toxicity

Following oral administration of AgNPs (∼15 nm in size) for 2–4 weeks, at 30 or 300 mg/kg/d caused changes in histopathology including inflammation, apoptosis and degenerated follicles in the ovaries of female rats. Male and female mice fertility was checked by intravenously injecting with AgNPs and it was concluded that that their gene expressions were down regulated resulting in debilitated development of spermatocytes and oocytes. Oral AgNPs (15 nm) exhibit ovarian toxic potential at 30 mg/kg/d. Intravenous AgNPs (20 nm) lowers the number of follicles and augment embryonic deaths at 0.5 mg/kg [1].

Conclusion and future prospects

Silver has been most broadly studied and used since early times to fight against infections and hinder spoilage in comparison to other antimicrobial agents. Silver is also found to be non-toxic to humans in minute concentrations. The resistance developed by microorganism in silver in less as compared to antibiotics as a broad range of microorganisms is being targeted by silver.

The uses of silver nanoparticles are varied and many, but the most utilized and desired aspect is their antimicrobial capacity and anti-inflammatory capacity. The toxicity induced

by silver nanoparticles at various degrees leads to their pitfall. It is suggested that higher concentrations of silver nanoparticles are toxic and can cause various health problems and can induce various ecological problems if released into the environment. Varied applications of silver nanoparticles is found in the form of wound dressings, coatings for medical devices, silver nanoparticles impregnated textile fabrics, etc., as there is uninterrupted release of silver ions and the devices can be coated by both the outer and inner side hence, alleviating its antimicrobial efficacy.

However, there are some issues, which need to be addressed, such as, the exact mechanism of interaction of silver nanoparticles with the bacterial cells, how the surface area of nanoparticles influence its killing activity, use of animal models and clinical studies to get a better understanding of the antimicrobial efficiency of silver dressings, the toxicity if any of the silver dressings, etc. Hence, care has to be taken to utilize this marvel well and in a good, effective and efficient way, understanding its shortcoming and taking utmost care that it does not cause any harm to an individual or the environment.

On the whole, the silver nanoparticles due to their unique properties of silver and nano size appear to be promising in pharmaceutical, biomedical and allied fields provided safety data is generated to prove their safety and simultaneously ruling out their toxicity.

Disclosure statement

No potential conflict of interest was reported by the authors.

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