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A review on biogenic nanosilver – an emerging biomedical product

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Abstract

The convergence of nanotechnology and biomedical sciences paves the need for new and effective methods for enhancing the therapeutics and diagnostics. Generally, the use of nanotechnology improves specificity of molecular devices by maximizing the safety and efficiency and by minimizing the hazardous impacts. Silver nanoparticles posses excellent free radical scavenging, antibacterial and larvicidal activities and several works have shown the promising ability of their role in cancer and gene therapies. Further, it is a powerful tool in cell imaging and sensing applications due to their unique biological, optical and chemical properties. Excellent biocompatibility and strong optical absorption of silver nanoparticles enables them to be extensively studied for novel biomedical applications. The goal of this review is to highlight the research carried in implementation of silver nanoparticles for medical therapeutic and diagnostic.

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Introduction

The contemporary technology focuses on the ecofriendly "green processes" in the research areas of engineering and sciences with special concern to nanoscience and nanotechnology. Generally, the size of the molecular device smaller than 1µm on the nano scale is considered as nanomaterial. Nanoparticles are usually smaller than 100 nanometers, contain 20–15,000 of atoms, and exist in a realm that straddles the quantum and Newtonian scales (Wen-TsoLiu 2006). Many different materials can be used to produce nanoparticles in different shapes such as spheres, rods, wires and tubes (Liu, 2006). Nanoparticle application in catalysis and sensors depends on the size and composition of the nanoparticles (Vineet and Sudesh, 2009). The optoelectronic and physiochemical properties of nanoscale matter are a strong function of particle size (Sankar *et al.,* 2004).

The classification of nanostructured materials on the basis of nanostructure dimensions, morphology, composition, uniformity and agglomeration state are well discussed in the literature (Buzea *et al.,* 2007). Nanoparticles can be broadly categorized into two types namely organic and inorganic nanoparticles (Destree and Nagy, 2006). Organic nanoparticles include carbon nanoparticles (fullerenes) and inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles (like gold, silver, iron and copper) and bulk semiconductor nanoparticles (like titanium dioxide and zinc oxide) (Manoj Singh *et al.,* 2011). Inorganic nanoparticles like silver and gold have versatile features like wide availability, rich functionality, good biocompatibility, high specificity, low toxicity and intrinsic optical properties. Of the other metals, silver ions and silver compounds show a toxic effect on some bacteria, viruses, algae and fungi, but without the high toxicity to humans. The ionized form of silver (Ag+) only has antimicrobial properties and its forms strong molecular bonds with other substances such as sulphur, nitrogen and oxygen used by bacteria, depriving them of necessary compounds and eventually leading to their death (Manoj Singh *et al.,* 2011).

Although several nano materials are available currently, silver nanoparticles (AgNPs) is emerging as well as fastest growing product categories in the nanotechnology industry as it exhibits remarkable physical (such as particle aggregation and photoemission, and electrical and heat conductivities), chemical (such as catalytic activity) and biological properties (Figure 1). Besides wide ranging applications, potential environmental risks associated with emerging nanotechnologies are linked to possible bioaccumulation of AgNPs in natural systems via wastewater (Hannah and Thompson, 2008). AgNPs contained in cosmetics, such as sunscreens (Wijnhoven *et al.,* 2009) can contaminate water and soil, contributing to their bioaccumulation in the food chain.

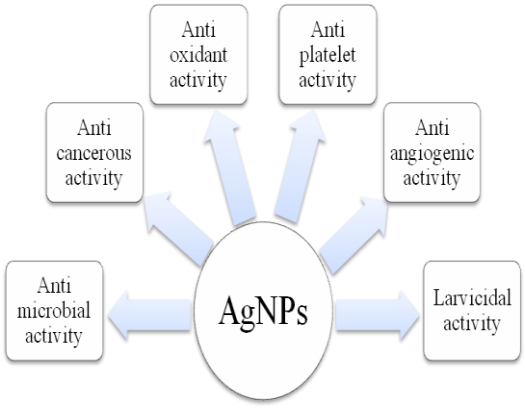

Figure 1. Schematic representation of biological activities of silver nanoparticles

AgNPs find diverse applications in the field of bio labelling, opto-biosensors, cancer cell imaging and drug delivery system, polarizing filters, electrical batteries, staining pigments etc (Jafar *et al.,* 2011a; Hang Xing *et al.,* 2012). It can be exploited in medicine for burn treatment, dental materials, textile fabrics, water treatment, sunscreen lotions, etc.

AgNPs are known for their antimicrobial properties and potent inducers of apoptosis and inflammation in the liver as compared to silver bulk material. Several studies have demonstrated that AgNPs were cytotoxic against cancer cells and could trigger oxidative stress (Jafar *et al.,* 2011b; Hang Xing *et al.,* 2012). The plasmonic scattering property and the ability of nuclear-targeted silver nanoparticles (NLS/RGD-AgNPs) are used to induce programmed cell death in order to image in real-time the behavior of human oral squamous carcinoma (HSC-3) cell communities during and after the induction of apoptosis. Plasmonic live-cell imaging revealed that HSC-3 cells behave as nonprofessional phagocytes. The induction of apoptosis in some cells led to attraction of and their subsequent engulfment by neighboring cells (AgNPs also possess anti-angiogenic, anti-platelet, acaricidal and genotoxic activities. Nanoparticles of silver have been studied to synthesize composites for use as disinfecting filters and coating materials (Lauren *et al.,* 2011).

AgNPs can be applied for treatment, diagnosis, monitoring, and control of diseases. Numerous research studies have shown the key role of nanoparticles to improve human health, biomedical, pharmaceutical, cosmetic, energy, environmental, catalytic and advanced material applications (West and Halas, 2000; Penn *et al.,* 2003). The integration of nanoparticles with biological molecules has lead to the development of diagnostic devices and important tools in cancel therapy. Silver impregnated dressings and catheters can be more effective than direct surface coating alone, as surface silver can be readily deactivated by protein anions. This impregnation of silver ions would also be beneficial in protecting the inner and outer catheter surfaces against bacterial attachment. Production of AgNPs under nontoxic green conditions is of vital importance to address the growing concerns on the overall toxicity of metallic nanoparticles for medical and technological applications.

Types of AgNPs from chemical synthesis

Silver colloids

Colloidal silver is a suspension of submicroscopic metallic silver particles in a colloidal base. It can be applied topically and/or absorbed into the blood stream sub-lingually, thereby avoiding the negative effects of traditional antibiotics (Chaudhry *et al.,* 2008). The presence of colloidal silver near a virus, fungi, bacterium or any other single celled pathogen disables its oxygen-metabolism enzyme. Colloidal silver have been used in the manufacture of photographic films, cosmetics, preservatives, antibacterial coatings and dietary supplements (Chaudhry *et al.,* 2008).

NanoXact

NanoXact AgNPs are monodisperse, unaggregated nanospheres provided at concentrations of 0.02 mg/ml. Particles are available with diameters ranging from 5 to 100 nm with citrate, tannic acid and PVP surfaces (Mac Cuspie *et al.,* 2011; Gorham *et al.,* 2012).

BioPure silver

BioPure silver nanoparticles are monodisperse, unagglomerated nanospheres which have been concentrated and extensively purified to remove residual reactants. They are provided at concentrations of 1 mg/ml and are available with diameters ranging from 5 to 100 nm with citrate, tannic acid, and PVP surfaces. Further, biopure silver nanoparticle solutions are the ideal formulation for biological and toxicological applications (Martinolich *et al.,* 2012, Mwilu *et al.,* 2013).

OECD-silver

This silver was given an Organization for Economic Cooperation and Development (OECD) guidance. Its formulation has been selected as nanotoxicology standards with PVP and citrate surfaces (OECD, 2002).

Custom silver

Custom silver formulations can be manufactured with custom concentrations, charge, biofunctionalization shells and suspension media (Chhasatia *et al.,* 2008).

Biological activity of AgNPs

AgNPs – a bactericidal agent

Plants or their extracts have been explored in the synthesis of AgNPs using silver ions as substrate. In ancient times silver was used as a preservative and also to reduce inflammation and prevent infection of wounds. AgNPs are non-toxic and non-tolerant disinfectant. Bactericidal property of these nanoparticles relies on their stability in the growth medium, since it imparts greater retention time for bacterium – nanoparticle interaction (Petrus *et al.,* 2011). The major mechanism through which silver nanoparticles manifest antibacterial properties is by anchoring to "building elements" of the bacterial membrane, causing structural changes and degradation and finally, cell death. Incorporation of AgNPs on to the bacterial membrane can also lead to loss of DNA replication and protein denaturation (Huda *et al.,* 2010). Formation of "pits" on the bacterial surfaces indicates the treatment of bacteria with silver ions (Sondi and Branka, 2004). The effect of AgNPs is dose dependent and is more pronounced against gram negative organisms than gram-positive ones (Petrus *et al.,* 2011).

The callus and leaf extract of *Citrullus colocynthis* are effective against biofilm forming bacteria and harmful human pathogens (Satyavani *et al.,* 2011). The leaf extract of *Moringa oleifera* shows maximum zone of inhibition towards *Staphylococcus aureus* (15 mm) and *Candida tropicalis* (13 mm) (Prasad and Elumalai, 2011). The fruit extract of *Carica papaya* were used in antibacterial assays on human pathogenic *Escherichia coli* and *Pseudomonas aeruginosa* by standard disc diffusion method (Jain, 2008). Agar dilution method can also be employed to study the bactericidal activity of silver nanoparticles on *P. aeruginosa* (Kora and Arunachalam, 2011).

Biogenic Silver nanoparticles could be of great interest in medical textiles for their efficient antimicrobial function (Vigneshwaran *et al.,* 2006). Cotton cloths incorporated with silver nanoparticles in hospitals helps to reduce or prevent infections.

Anti viral effects of AgNPs

It is generally known that Ag, in various forms, inactivates viruses by denaturing enzymes via reactions with sulfhydra, amino, carboxyl, phosphate, and imidazole groups. Interactions between viral biomolecules and silver nanoparticles emphasize the use of nanosystem for prevention of infection and antiviral therapies. Virucidal AgNPs bind with disulfide bond regions of CD4 binding domain in viral envelope glycoprotein gp 120 of HIV-1 (Jose *et al.,* 2005).

Monkey Pox Virus (MPV) is a big threat to human life and therapeutic agents against this virus are under development. Silver-based nanoparticles have driven its use in inhibiting infections of the MPV. Plaque reduction assay evaluates the dose dependent effect of test compound concentration on the mean number of plaque-forming units. This study demonstrates that AgNPs of approximately 10 nm inhibit MPV infection in vitro, supporting their use as an antiviral therapeutic (Thirumalai Arasu *et al.,* 2010). Inhibition of MPV plaque formation could be due to the physical interference of virus–host cell binding by nanoparticles and the endocytosis of metal-based nanoparticles by cultured cells leading to disruption of intracellular pathways that could ultimately attenuate viral replication (James *et al.,* 2008). Silver nanoparticles also inhibit the in vitro production of Hepatitis-B Virus (HBV) RNA and extra-cellular virions. Anti-viral mechanism occurs by direct interaction between these nanoparticles and HBV double-stranded DNA or virus particles (Lut *et al.,* 2008).

Anti-cancerous activity of AgNPs

Nano encapsulated therapeutic agents drive its use to selectively target the tumor agents. AgNPs from various plant extracts possess cytotoxic ability against cancer cell lines. The cytotoxic effects of silver are due to the physicochemical interaction between the silver atoms and the functional groups of intracellular proteins, nitrogenous bases and phosphate groups of DNA. AgNPs synthesized from calli and leaf extract of *Citrullus colocynthis* reported anti-tumor effects on human epidermoid larynx carcinoma (HEp-2) cell line (Satyavani *et al.,* 2011). Viability of tumor cells is known by MTT assay, in which AgNPs mediate a concentration and time dependent increase in toxicity. AgNPs affect the metabolic activity of the cell and increases the chances of apoptosis induction. Caspase 3, a molecule that plays a vital role in apoptotic pathway of the cell, mediates this process. Activation of Caspase 3 causes cell death which was further supported by cellular DNA fragmentation (Satyavani *et al.,* 2011).

Aqueous extract from the leaf of *Melia azedarach* synthesize AgNPs and its cytotoxic effects are studied against in vitro HeLa cell lines and in vivo Dalton's ascites lymphoma (DAL) cell line in mice model (Sukirtha *et al.,* 2012). Cytotoxicity of AgNPs against in vitro HeLa cell lines shows dose-response activity. Some studies shows that biosynthesized AgNPs have the potential to interfere genes associated with cell cycle progression, inducing DNA damage and leading to apoptosis of cancer cells (Sanpui *et al.,* 2011). Cytotoxicity against in vivo DAL mice model shows increased life span in a dose dependent manner. Acridine orange and ethidium bromide staining are employed to investigate the mode of cell death.

Larvicidal activity of AgNPs

Insecticides that are available in the market are synthetic chemical products and their persistent applications lead to ecological imbalance and elimination of non-target organisms in the environment.

Extracts or essential oils from plants may serve as alternative sources of mosquito larval control agents due to their rich source of bioactive compounds. AgNPs from *Mimosa pudica* shows larvicidal activity against the *Anopheles subpictus, Culex quinquefasciatus*, and *Rhipicephalus microplus* (Jayaseelan *et al.,* 2011; Sampath *et al.,* 2011). Tetracyclic triterpenoids isolated from the methanolic extract of fresh leaves of *Azadirachta indica* shows its larvicidal effects against *Anopheles stephensi* (Siddiqui *et al.,* 2003). Fifty percent hydroethanolic extracts of *Bonninghausenia albiflora* whole plant, *Calotropis procera* root, *Citrus maxima* flower, *Acorus calamus* rhizome, and *Weidelia chinensis* whole plant shows acaricidal effects on *Rhipicephalus microplus* (Ghosh *et al.,* 2011). AgNPs synthesized from peel extract of *Musa paradisiaca* shows larvicidal activity against the larvae of *Haemaphysalis bispinosa, Hippobosca maculata*, *Anopheles stephensi* and *C. tritaeniorhynchu* (Jayaseelan and Rahuman, 2012). Dose-response bioassay of plant extract and synthesized AgNPs demonstrates the larvicidal activity. AgNPs from the aqueous leaf extract of *Ocimum canum* reported larvicidal activity against *Hyalomma anatolicum anatolicum* and *Hyalomma marginatum isaaci* (Jayaseelan and Rahuman, 2012).

Applications of AgNPs

AgNPs in cell imaging

Many metal nanoparticles have unique size dependent and shape dependent optical properties and these unique properties can be exploited for use in cellular imaging and delivery. Compared to other metal nanoparticles, AgNPs easily form stable nanostructures and are able to bind different targeting molecules. AgNPs react with amino-compounds due to formation of silver–nitrogen bond; this surface chemistry paves way for conjugation of nucleic acids, polypeptides or cellular ligands. Some of the key factors to consider when imaging live cells include nanoparticle concentration, charge, size, and surface modifications.

For many cells, the key mechanisms of nanoparticle internalization include clathrin-mediated endocytosis, caveolin-dependent endocytosis, macropinocytosis and phagocytosis that are temperature and energy dependent (Thurn *et al.,* 2007). The key variables for the mechanism of nanoparticle uptake include the size of the nanoparticle, the charge of the nanoparticle surface and the cell type being used.

Cy5-avidin conjugates covalently bound on the 20 nm silver particles work as the molecular imaging reagents. T-lymphocytic *PM*1 cell lines were surface-biotinylated by EZ-link sulfo-NHS-biotin and then fluorescently labeled by the avidin-metal complexes. Scanning confocal microscopy was used to record the cell images. The cell images show the binding of avidin-metal complexes on the cell surfaces showing with no influence from the steric hindrance of metal particle. This binding displays stronger emission signal, shorter lifetime, and better photostability relative to the metal free avidin conjugate (Zhang *et al.,* 2009). This approach can be used to estimate the precise amount of target molecule on the cell surface at the single cell level (Zhang *et al.,* 2009). ^{125}I-isotope of iodine is used in radiolabeling mainly for *in vitro* assays, and it has recently been used *in vivo* for small animal imaging. A rapid method was developed for radiolabeling of AgNPs with iodine-125 in order to track *in vivo* tissue uptake of AgNPs by biodistribution analysis and single-photon emission computerized tomography (SPECT) imaging. Radiolabeled silver nanoparticles were intravenously injected in Balb/c mice, and the *in vivo* distribution pattern obtained by SPECT imaging revealed uptake of the nanoparticles in the liver and spleen (Adrian and Jan, 2010). The radioactivity in the tissues of liver and spleen are due to the uptake of ^{125}I-Ag NPs and not free iodine-125. Free iodine is taken up rapidly by the thyroid gland, salivary gland, and stomach (Lundh *et al.,* 2006). High uptake of AgNPs in the liver implicates AgNPs-associated toxicity towards hepatic tissue.

Liver-specific accumulation of AgNPs is beneficial for the treatment of antibiotic-resistant bacterial liver infections.

Aptamer based silver nanoparticles are used in intracellular protein imaging and single nanoparticle spectral analysis. The aptamer acts as a biomolecule specific recognition unit and AgNPs acts as an illuminophore (Stanley and Mirunalini, 2011). The scattering property of plasmonic nanoparticles has been used in biological imaging and cancer diagnostic applications. Nuclear-targeted silver nanoparticles (NLS/RGD-AgNPs) induce programmed cell death in order to image the behavior of human oral squamous carcinoma (HSC-3) cell communities during and after the induction of apoptosis. Plasmonic live cell imaging revealed cellular attraction, clustering, and bystander killing after incubation with low concentrations of apoptosis-inducing AgNPs. At high AgNP concentrations, the degree of attraction and clustering is diminished. Plasmonic live-cell imaging and nuclear-targeting AgNPs had the ability to investigate intercellular responses to external stimuli in a cellular community without the use of fluorescent dyes and expensive microscopic equipment (Lauren *et al.,* 2011).

AgNPs in diagnostics

Early detection of disease remains a primary goal of the medical community. Nanotechnology holds great promise for enabling the achievement of this goal. Silver nanoparticles have unique plasmon-resonant optical scattering properties that are driving its use as signal enhancers, optical sensors, and biomarkers. AgNPs provides extremely high spatial resolution and specificity in many labeling applications and they are needed in terms of their interactions with cellular receptors extra – intra cellular proteins, organelles and DNA (Carlson *et al.,* 2008). Aggregation based chemical sensing has been used to detect enzymatic reactions where adenosine triphosphate (ATP) is consumed,

such as ATP dephosphorylation by calf intestine alkaline phosphatase and peptide phosphorylation by protein kinase (Carlson *et al.,* 2008). As ATP is consumed in the enzymatic process, nanoparticles begin to aggregate (Wei *et al.,* 2008). Colorimetric assays have been accomplished for the detection of histidine, pesticides and Yb3+ ions (Xiong and Li, 2008; Xiong *et al.,* 2008; Han *et al.*, 2009).

Silver nanoparticles can effectively provide biological labeling in live cells due to their intense optical properties that can be readily detected by a high illumination system. By administering a critical, low concentration of AgNPs in neuroblastoma cells, the physical blockage of cellular functions occurring after internalization, prevention of extracellular protein or nutrient binding between the nanoparticles and cell culture media may be avoided. Key factors to consider for the optimization of AgNPs as biological labels include modifications to their size, shape, functionalization, mechanism of uptake and concentration for targeted applications. AgNPs can be used as potential biological labels, even if the surface is chemically modified with a biocompatible material (Amanda *et al.,* 2008). The SERS pH sensors are prepared by labeling the AgNPs with Raman active dyes that are sensitive to specific analytes such as hydrogen ion or large biological molecules. This sensor was sensitive to pH changes in the range of 6 to 8. The nanoparticle sensors were delivered into living chinese hamster ovary cells for the intracellular pH measurement. The SERS spectrum showed the pH surrounding the nanoparticle to be below 6, which is consistent with the particles present inside a lysosome (Kim *et al.,* 2009).

AgNPs in therapeutics

Nanotechnology provides a patient-friendly alternative to injection, which may be able to accelerate therapeutics for infectious disease and cancer. Nano delivery traverse membrane boundaries and can be readily absorbed into the bloodstream based on their size.

Size controlled targeting of AgNPs towards cancer cells can prove effective in the case of cancer treatment. AgNPs are reported to show better wound healing capacity, better cosmetic appearance and scarless healing when tested using an animal model (Rashid *et al.,* 2009).

Cancer therapy

The emerging trend of using nanoparticles as drug carriers has exploited the potential of nanoparticles to revolutionize cancer therapy. AgNPs can be used for both active and passive targeting of drugs. The interactions between immune cells and tumor cells were visualized using optical microscope in the case of targeting. When using a targeting agent to deliver nanocarriers to cancer cells, the agent that binds with high selectivity to molecules is important (Dan Peer *et al.,* 2007). It is also possible to enhance binding affinity and selectivity to cell surface targets by manipulating proteins that detect a specific conformation of a target receptor (Shahverdi *et al.,* 2007).

Gene therapy

Gene therapy refers to the transfer of genetic material to specific target cells of a patient for altering a particular disease state. Vectors are usually viral but several nonviral techniques are being used for gene delivery. A variety of nanoparticles such as nanoliposomes, gelatin nanoparticles, calcium phosphate nanoparticles, dendrimers and various composites can be used for nonviral gene delivery (Jain, 2008).

Vaccination

Nanoparticles are potential delivery systems for DNA vaccines (Akin *et al.,* 2007). Components of the immune system recognize particles more efficiently than soluble proteins. Nanoencapsulating potent viral antigens in biodegradable polymer nanospheres for controlled release can induce the production of protective and neutralizing antibodies.

This controlled release vaccine delivery technology delivers different types and combinations of vaccines including whole inactivated virus particles, DNA plasmids and/or antigens (Bhupinder, 2012).

Cell therapy

Cell therapy is the prevention or treatment of human disease by the administration of cells that have been selected, multiplied and pharmacologically treated or altered outside the body. Now that stem cell-based therapies are in development, in which nanotechnology plays an important role in tracking stem cells *in vivo* (Zheng Wang *et al.,* 2009). Nanobiotechnology is well suited to optimize the generally encouraging results already achieved in cell transplantation (Halberstadt *et al.,* 2006). The small size of nanomaterial constructs provides an increasing number of options to label, transfect, visualize, and monitor cells/tissues used in transplantation. Nanoparticles are by their nature well suited to interact with cells. AgNPs may come into close contact with body tissues, including human mesenchymal stem cells (hMSC). Quantitative determination of the uptake of AgNPs by flow cytometry revealed a concentration-dependent uptake of the particles indicating clathrin-dependent endocytosis and macropinocytosis as the primary uptake mechanisms (Greulich *et al.,* 2011). Antibodies (10 nm) and viruses (100 nm) can easily interact with cells and transport across cell membranes.

Conclusion

Applications of silver nanoparticles are beginning to show an impact on the practice of novel medicine. Increasing awareness towards green chemistry and biological processes has led to the development of non toxic nanoparticles. It is to be expected that future advancements in this method will be directed towards the improvement of more robust and reproducible techniques with a maximum efficiency. The nanoparticle size, surface area, and surface fictionalization are major factors that influence the biocompatibility.

New types of nanoparticles are developed daily and cellular manipulations with these nanoparticles are devoted to enhancement of new therapies and imaging tools. The major trend in nanomaterials is to make them multifunctional and controllable by external signals or by local environment thus essentially turning them into nano-devices.

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