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Bacteriagenic Silver Nanoparticles: Synthesis, Mechanism and Applications

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Abstract

Silver nanoparticles (AgNPs) have received a tremendous attention due to their significant antimicrobial properties. Large numbers of reports are available on physical, chemical and biological synthesis of colloidal AgNPs. Since there is a great need to develop ecofriendly and sustainable methods, biological systems like bacteria, fungi and plants are being employed to synthesize these nanoparticles. The present review focuses specifically on bacterial-mediated synthesis of AgNPs, its mechanism and applications. Bacterial synthesis of extra- and intra-cellular AgNPs have been reported using biomass, supernatant, cell-free extract and derived components. Extracellular mode of synthesis is preferred over intracellular mode owing to easy recovery of nanoparticles. Silver-resistant genes, *c*-type cytochromes, peptides, cellular enzymes like nitrate reducatse and reducing cofactors play significant roles in AgNP synthesis in bacteria. Organic material released by bacteria act as natural capping and stabilizing agents for AgNPs, thereby, preventing their aggregation and providing stability for a longer time. Regulation over reaction conditions has been suggested to control the morphology, dispersion and yield of nanoparticles. Bacterial AgNPs have anticancer and antioxidant property. Moreover, antimicrobial activity of AgNPs in combination with antibiotics signifies their importance in combating the multidrug-resistant pathogenic microorganisms. Multiple microbicidal mechanisms exhibited by AgNPs, depending upon their size and shape, make them very promising as novel nano-antibiotics.

Keywords

silver nanoparticles (AgNPs); bacteria; synthesis; antimicrobial; mechanism; applications

Introduction

Silver has been used since ancient times for its microbicidal properties. Silver salt and its colloidal formulations have been used to treat ulcers, burns and chronic wounds, sepsis, acute epididymitis, tonsillitis, infections and prevention of eye diseases in infants (Duhamel 1912; Sintubin et al. 2012). But its use was discontinued due to the interfering effects of salt and development of effective new antibiotics (Edward-Jones 2009). However, almost a decade back, nanosilver made a remarkable comeback owing to its high surface-area to volume ratio and size-dependent unique optical, electrical and thermal properties (Schmid 1992). Silver nanoparticles (AgNPs) are now one of the most commercialized nanomaterials having applications in over 200 products such as antimicrobial coatings, medical devices, molecular diagnostics and photonic devices, sensors, textiles, home water purifiers, cosmetics, electronics, household appliances, conductive inks, pastes and fillers (Li et al. 2006; Lin et al. 2011; Sintubin et al. 2012; Wijnhoven et al. 2009).

Biological methods for synthesis of AgNPs, employing microorganisms (Gaidhani et al. 2013, Singh et al. 2013) and plants (Ghosh et al. 2012; Salunkhe et al. 2014), have gained tremendous significance over physical and chemical procedures due to use of non-toxic, biocompatible and environment-friendly substrates and relatively easier synthesis process at ambient conditions (Shedbalkar et al. 2014; Thakkar et al. 2010). Moreover, biomolecules act as natural stabilizers for such nanoparticles, thereby, preventing not only aggregation over course of time, but also an extra stabilization step as observed in chemical methods (Deepak et al. 2011; Gurunathan et al. 2009; Singh et al. 2013). Synthesis using plant extracts is less time-consuming but produce polydisperse AgNPs due to involvement of multiple components, like flavonoids, terpenoids and polyphenols, in reduction of silver ions (Ghosh et al. 2012; Salunkhe et al. 2014). Geographical and seasonal variations may also affect phytogenic synthesis (Singh et al. 2013). Microbial synthesis of nanoparticles does not encounter such variations, although regular maintenance of culture and sterile conditions for nanoparticle synthesis are required (Salunkhe et al. 2014). A number of broad reviews are published containing a sub-section dedicated to microbial synthesis of AgNPs in general (Mohanpuria et al. 2008; Sintubin et al. 2012; Thakkar et al. 2010). However, critical analysis of the enormous information on bacterial AgNPs is still missing. Therefore, this mini-review summarizes the bacteria mediated synthesis of AgNPs and its mechanism with respect to the genetic aspect and role of cell wall, proteins and bacterial enzymes. Optimization, properties and applications of these AgNPs have also been discussed.

Bacterial-mediated synthesis of AgNPs

Nature has devised various processes for synthesis of small-scaled inorganic materials. Biological synthesis of nanoparticles involves natural phenomenon occurring in bacterial, fungal and plant biosystems, thereby, generating biocompatible nanomaterials having therapeutic applications (Mohanpuria et al. 2008). The first report on bacterial-mediated AgNP synthesis came in 1999 when Klaus and coworkers reported the accumulation of AgNPs inside the cells of *Pseudomonas stutzeri* AG259, a bacterium isolated from silver mine

(Klaus et al. 1999). The bacteria exhibited the property to survive in extreme silver rich environment, which might be the possible explanation for accumulation of nanosilver (Klaus et al. 1999). After that series of bacteria, both Gram negative and Gram positive, have been screened for synthesis of AgNPs. In some reports, silver-resistant bacterial strains were employed for nanoparticle synthesis (Parikh et al. 2008; Prakash et al. 2011). Depending upon the location, the synthesis of these nanoparticles can be intracellular or extracellular (Table 1).

Extracellular synthesis

Extracellular synthesis of nanoparticles occurs outside the bacterial cell. These nanoparticles of various shapes, such as spherical, disk, cuboidal, hexagonal, triangular, etc., have been synthesized using cells, culture supernatant or aqueous cell-free extract (Klaus et al. 1999; Oves et al. 2013; Singh et al. 2013; Srivastava and Constanti 2012). The recovery is usually done by high-speed centrifugation (10,000 – 20,000 rpm) of the solution containing nanoparticles. These nanoparticles are collected as a pellet, which can be redispersed in the desired solvent. Extracellular methods of synthesis are advantageous over intracellular synthesis due to the ease of recovery of nanoparticles from the solution.

Synthesis using biomass

On exposure to silver salts, some bacteria produce extracellular AgNPs. There are two possible routes for this- (i) biomolecules released by bacteria into the external medium help in reduction of silver ion to AgNPs, and/or (ii) nanoparticles formed inside the cell are secreted outside. Such synthesis has been demonstrated employing both live (Mahdieh et al. 2012) and dried bacterial biomass (Zhang et al. 2005). Extracellular AgNPs may remain attached to the bacterial cell wall (Parikh et al. 2011), which require mild sonication for recovery. Although silver nitrate salt is mostly used for AgNP production in bacteria, other silver containing nanoparticles, like silver sulfide (Ag_2S) and silver oxide (Ag_2O) nanoparticles, have also been reported (Debabov et al. 2013; Dhoondia and Chakraborty 2012). Parikh et al. (2011) suggested the genus-dependent extracellular synthesis of AgNPs, where production of crystalline AgNPs was observed in genus *Morganella* and closely related members of the Enterobacteriaceae family, including *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Serratia marcescens*, were unable to synthesize AgNPs. However, this was later contradicted by Muthukkumarasamy et al. (2012) and Zaki et al. (2011) showing AgNPs synthesis from *E. coli* biomass.

Synthesis using culture supernatant

Culture supernatant of bacteria, grown for 24 to 48 h, is obtained under sterile conditions and incubated at appropriate conditions after exposure to silver salt for synthesis of AgNPs. Even microwave irradiation of silver supplemented culture supernatant has been reported to produce AgNPs (Saifuddin et al. 2009). The fact that supernatant is comprised of nutrient media components and organic molecules secreted by the bacteria during

growth makes it an ideal source for reduction of silver ions to AgNPs. However, one should note that AgNPs synthesized from culture supernatant embed in the organic matrix of media components, which could hinder their colloidal dispersion, characterization, recovery and hence, putative application.

Synthesis using cell-free extract

In view of the above-mentioned difficulty with biomass and supernatant, we have adopted an alternate method of employing cell-free extract (CFE) of bacteria for extracellular AgNP synthesis (Singh et al. 2013). In this method, bacterial biomass is resuspended in sterile distilled water for specific time ranging from 1 to 3 days. CFE, collected after centrifugation and/or membrane filteration, is further challenged with silver salt for AgNP production after optimal incubation (Singh et al. 2013). Solar and microwave irradiation of CFE has been demonstrated to aid in fast synthesis of AgNPs (Boopathi et al. 2012; Wei et al. 2012). This method ensures complete removal of bacterial biomass and media components through repeated washings and enables synthesis of nanoparticles only through organic biomolecules released by cells in aqueous solution due to starvation conditions or by autolysis. Moreover, no downstream processing is required for recovery of nanoparticles.

Intracellular synthesis

Reduction of metal ions by bacterial cells to its nano-form is one of the survival strategies to render the toxic metal ions non-toxic (Klaus et al. 1999). For intracellular synthesis, bacterial cells are added to the culture medium containing silver salt and incubated at appropriate conditions of growth. To avoid the hindrance by media components, grown cells can also be resuspended in sterile distilled water before challenging with silver salt. Table 1 lists few bacteria reported to produce intracellular AgNPs. Periplasmic deposition of triangular and hexagonal nanoparticles was observed in *P. stutzeri* (Klaus et al. 1999). The size of intracellular AgNPs depends on the culture medium used to grow the cells (Srivastava et al. 2013). *Acinetobacter* has been shown to exhibit biocompatibility with its own metal nanoparticles; however, exposure to the corresponding metal salt decreases their cell count (Wadhwani et al. 2014). This could be because of the bacterial self-biomolecules that cover the surface of nanoparticles making them non-toxic to their host. Intracellular method of synthesis requires additional steps to recover the accumulated nanoparticles from cells and therefore, is less preferred. Ultra-sonication of bacterial cells is the most common technique to recover AgNPs (Kalishwaralal et al. 2010). Besides this, heat-treatment like autoclaving, detergents and salts can also be employed to lyse the cells (Fesharaki et al. 2010; Sneha and Yun 2013).

Some bacteria have the ability to produce both extra- and intra-cellular AgNPs simultaneously. These include *Lactobacillus* sp. (Nair and Pradeep 2002), *Aeromonas* sp. SH10 (Mouxing et al. 2006), *Vibrio alginolyticus* (Rajeshkumar et al. 2013) and cyanobacteria, such as *Plectonema boryanum* UTEX 485 (Lengke et al. 2007), *Calothrix pulvinata* and *Anabaena flos-aquae* (Brayner et al. 2007). Such bacteria, besides accumulating AgNPs inside the cell, secrete nanoparticles in the external environment. This leads to a

possibility that these dual-mode synthesizing bacteria can be compelled to switch to one mode of synthesis and it will be interesting to know the environmental conditions/stress and mechanisms responsible for the same.

Synthesis using bacterial-derived components

Microorganisms serve as a good source of biosurfactants having advantages of biodegradation, less toxicity and biocompatibility over their chemical counterparts (Satpute et al. 2010). Biosurfactants from bacteria have been employed for AgNP synthesis, for it enhances synthesis rate and acts as stabilizing agent (Kiran et al. 2010; Kumar et al. 2010). Bacterial enzyme (Deepak et al. 2011), exopolysaccharides (Kanmani and Lim 2013; Morsy et al. 2014), native and reploymerized flagella (Gopinathan et al. 2013), actinorhodin pigment (Manikprabhu and Lingappa 2013), spores (Hosseini-Abari et al. 2013) and polysaccharide bioflocculant (Sathiyanarayanan et al. 2013) have also been reported to synthesize AgNPs. The details have been provided in Table 2.

Silver nanoalloys and nanoconjugates by bacteria

Several material scientists have synthesized bimetallic and even trimetallic silver nanostructures using chemical and physical methods (Mahmoudi and Serpooshan 2012; Venkatesan and Santhanalakshmi 2010). However, only two reports described the bacterial mediated synthesis of bimetallic gold-silver (Au-Ag) nanoparticles (Nair and Pradeep 2002; Govindaraju et al. 2008). *Lactobacillus* sp. isolated from buttermilk assisted the growth of Au-Ag submicronic alloys crystals of 100-300 nm in the periplasmic space (Nair and Pradeep 2002). In another report, interaction of single-cell protein of *S. platensis* with aqueous $AgNO_3$ and $HAuCl_4$ resulted in formation of spherical Au_{core}-Ag_{shell} nanoparticles (Govindaraju et al. 2008). Difference in rate of reduction of the silver and gold ions cause faster formation of AuNPs onto which Ag shell gets assembled (Govindaraju et al. 2008). There are no reports on trimetallic silver nanomaterials from bacteria.

Nano-conjugates and -composites of chemical AgNPs have been synthesized with biomolecules such as DNA (Zheng et al. 2012), polymers like chitosan and polyethylene glycol (Krishna Rao et al. 2012), drugs like penicillin G (Ahmed et al. 2013), etc. These nanostructures may possess certain advantages, such as (i) overall toxicity of AgNPs can be lowered by covering its outer surface, thereby increasing their biocompatibility, (ii) properties of both AgNPs and the linked molecule can be utilized, and (iii) nanoconjugates may also have enhanced properties, as compared to their presursors, owing to interfacial interaction between nanoparticle and linked moiety, making them apt for biomedicinal applications (Dallas et al. 2011). Inspite of these facts, bacteriagenic AgNPs have not been employed yet to form nanoconjugates and there is a scope for the same.

Mechanism of silver nanoparticle synthesis

Microbes encounter metals and metalloids of various kinds in the environment and attain certain genetic and biochemical metal-resistance mechanisms to survive (Deshpande et al. 1993; Dhakephalkar and Chopde

1994; Deshpande and Chopade 1994). Some of these mechanisms are extracellular precipitation, extracellular binding and complexation, segregation into complex compounds by thiol-containing molecules, intracellular deposition, alteration in solubility and toxicity by varying redox state of metal ion, cellular efflux pumping system and lack of specific metal transport system (Das and Marsili 2010; Duran et al. 2011). For most of the metals, establishing this resistance and homeostasis involves combinations of above-mentioned mechanisms. Resistance to silver ions has been observed in *E. coli* (Li et al. 1997), *E. cloacae* (Annear et al. 1976), *P. stutzeri* (Slawson et al. 1992) and *A. baumannii* (Deshpande and Chopade 1994). Interestingly, *P. stutzeri* AG259 exhibits intracellular accumulatiom of silver ions as AgNPs (Klaus et al. 1999), whereas silver-resistant *E. coli* mutants display active efflux of silver ions (Li et al. 1997). This can involve reduction of metal ions to elemental metal through cellular machinery (Saklani et al. 2012). Though the mechanistic aspect of nanoparticle synthesis is still poorly understood, various hypotheses have been proposed to elucidate the role of bacterial genes and proteins in synthesis of AgNPs.

Genetics of AgNP synthesis

Accumulation of silver ions may occur in two stages: (i) non-specific and energy-independent attachment to the cell surface, and (ii) intracellular accumulation (Shakibai et al. 2003). *Acinetobacter baumannii* exhibits plasmid mediated silver-resistance rendering bacteria capable of accumulating silver and retaining it by tight binding to a cysteine-rich metalloprotein (Shakibai et al. 2003, Deshpande et al. 1993). Three major gene homologues, namely *silE*, *silP* and *silS* of silver-resistance machinery have been suggested to play significant role in AgNPs production (Parikh et al. 2008). Silver-binding gene homologue (*silE*) encodes a periplasmic silver binding protein (silE) responsible for silver uptake by presenting histidine sites for silver ion binding. On exposure to silver ions, silE-based silver-binding machinery of bacteria gets activated leading to cellular uptake of silver ions. The ions are presented to bacterial silver-reduction machinery where biomolecules, generated by silver-reduction machinery, bind to the ions and reduce them to metallic silver nuclei or seed nanoparticles. These particles undergo growth and assembly to form different shaped (spherical or plate-like) AgNPs followed by their release from the cells via cellular efflux system (Ramanathan et al. 2011) as shown in Fig. 1. Alternatively, bacteria create an extracellular microenvironment during growth in which silver-specific proteins from silver-resistance machinery are released outside the cells. These proteins might reduce silver ions subsequently forming stable extracellular AgNPs (Parikh et al. 2008). Further studies are required to decipher the exact role of *silP* and *silS* genes in AgNPs production. The involvement of periplasmic *c*-type cytochrome (MacA) and outer membrane *c*-type cytochrome (OmcF) in surface reduction of Ag (I) to Ag (0) has been revealed through knockout studies (Law et al. 2008). MacA was involved in electron transfer from the inner membrane to the periplasm while OmcF reduced extracellular electron acceptors. However, complete blockage of silver ion reduction in mutant strains was not obtained (Law et al. 2008) indicating the possibility of multiple electron transfer pathways to Ag (I), which should be investigated further.

Role of cell wall

Cell wall of bacteria also plays a major role in biogenesis of nanoparticles owing to involvement of cell wall components and enzymes. Nucleation of clusters of silver ions during initial phase of AgNP synthesis cause electrostatic interaction between the ions and negatively charged carboxylate groups of cell wall (Wang et al. 2012). Trapped silver ions on bacterial surface are, thereby, reduced by cellular reductases and other redox proteins released by the cells to nanosilver form (Mahdieh et al. 2012; Debabov et al. 2013) as shown in Fig. 1.These nanoclusters can remain diffused through cell wall (Nair and Pradeep 2002). S-layer of bacteria physically masks the negatively charged peptidoglycan sheet of the cell wall and thus, can also be involved in bacteria-metal surface interaction (Prakash et al. 2011). Another potential mechanism across cell wall is conferred in bacteria with electrokinetic potential through generation of transmemebrane proton gradient. This gradient can indirectly drive active symport of sodium along with silver ions from the surroundings. ATP binding employs special silver binding proteins attached to membrane lipids on the external surface of bacteria, which attract the silver ions readily initiating AgNP synthesis (Prakash et al. 2011). Since AgNPs are produced both extra- and intra-cellularly, role of intracellular electron donor and membrane transport system should be investigated for detailed elucidation of mechanism of synthesis.

Enzymes and reducing agents

Silver-reduction machinery involves electron-shuttle enzymatic silver reduction process. NADH generated during bacterial glycolysis and electron transport chain via energy generating reactions creates a cellular reducing environment, due to hydrogen atoms, conducive for synthesis of AgNPs (Jha and Prasad 2010). NADH-dependent enzymes, especially nitrate reductase, have been implicated to play significant role in AgNP synthesis (Kalimuthu et al. 2008). Nitrate ions of $AgNO_3$ salt induce nitrate reducatse. The enzyme gains electron from NADH and oxidizes it to NAD^+ and then itself undergoes oxidation to reduce the silver ions to nanosilver (Fig. 1). Further, nitrate ions (NO_3^-) get converted to nitrogen dioxide (NO_2), followed by nitrogen oxide (NO) and nitrous oxide (N_2O), and ultimately to gaseous nitrogen (N_2) (Karthik and Radha 2012). However, Gaidhani et al. (2013) reported the nitrate-reductase independent synthesis of AgNP in *Acinetobacter*. Nitrogenase and hydrogenase class of reducing enzymes, present in cyanobacteria, can reduce silver ions to form nanosilver (Brayner et al. 2007). Concentration of the cellular nitrogenase dictates the size of AgNPs, where higher concentration in heterocysts leads to rapid formation of larger shaped AgNPs near the cell wall and intermediate content formed small and unaggreagted nanoparticle colloids (Brayner et al. 2007). NfsA, an oxygen-insensitive nitroreductase present in Enterobacteriacae, has also been suggested to reduce $AgNO_3$ to AgNPs (Shahverdi et al. 2007).

High pH and partial pressure of gaseous H_2 are important factors in bacterial-mediated AgNPs production (Prasad et al. 2010). High pH catalyzes the opening of monosaccharide rings to open-chain aldehyde forms that, in presence of silver ions, undergoes oxidation to corresponding carboxylic acid simultaneously reducing silver ions to AgNPs (Sintubin et al. 2009). High pH also activates reductases of oxidoreductase enzymes (Prasad et al. 2010). Besides this, glutathione and thioredoxin systems are significant to maintain the reducing conditions

indirectly and regulate the activity of enzymes (Jha and Prasad 2010). The reducing cofactors generated by activity of various spore-associated enzymes, like glucose oxidase, alkaline phosphatase, lacccase and catalase, can stimulate the biogenesis of AgNPs (Hosseini-Abari et al. 2013). Liu et al. (2012) speculated that to overcome the metal stress, *Gluconacetobacter xylinum* secrets chloride ions from cytoplasm and generates reductases to bioreduce the silver ions to form Ag/AgCl nanoparticles as by-product.

Peptides

Peptides received major attention for synthesis and stabilization of AgNPs after Naik et al. (2002) demonstrated the biogenic formation of AgNPs employing silver binding peptides. Peptides interact with preformed silver nanocluster in the solution and generate a reducing environment around them leading to the reduction of silver ions and formation of polydisperse AgNPs. Peptides containing amino acids like arginine, cysteine, lysine, methionine, glutamic acid and aspartic acid are involved in recognition and reduction of silver ions to silver crystals (Naik et al. 2002; Nam et al. 2008). Physiological conditions also play a significant role in dictating the metal-peptide interfical interactions. For example, tyrosine undergoes conversion to semi-quinone structure under alkaline conditions through ionization at phenol group, which reduces silver ions (Selvakannan et al. 2004). Tryptophan is converted to transient tryptophyl radical at high pH, which donates electron to reduce silver ions (Si and Mandal 2007). Peptides with disulfide linkage can also be used for peptide-coated AgNP synthesis (Graf et al. 2009).

Optimization

Although biological methods are regarded as safe and biocompatible, it is difficult to control morphology of nanoparticles (Wadhwani et al. 2014). In chemical methods, only a single reducing agent is responsible for reduction of metal ions to nanoparticles, and hence, well-defined monodisperse nanoparticles are easy to obtain (Li et al. 2012). On the other hand, several factors like enzymes, amino acids, media components, cofactors, etc. interact with metal ions differently, thereby, forming polydisperse nanoparticles in bacteriagenic synthesis. However, in our study we have shown that morphology of bacterial AgNPs can be controlled through regulating various over physicochemcial parameters, such as culture age, silver ion concentration, temperature and incubation time, and it is possible to produce size-controlled AgNPs through appropriate combination of these factors (Singh et al. 2013). Various combinations of these parameters result in formation of large aggregated particles to smaller monodispersed spherical AgNPs. These factors also affect the yield of AgNPs (Singh et al. 2013). Other factors that affect morphology and productivity of AgNPs are inoculum size, nutrient medium, pH, enhancers, etc. Both single-factor optimization and statistical response-surface methodology have been adopted to obtain size-controlled AgNPs and maximize synthesis yield (Deepak et al. 2011; Debabov et al. 2013; Gaidhani et al. 2013; El-Naggar and Abdelwahed 2014).

Characterization techniques

Preliminary detection of synthesis in laboratory is usually done by visual observation for color change. Reaction medium turns reddish-brown or borwn during synthesis of AgNPs (Shahverdi et al. 2007; Singh et al. 2013). Change of color from purple to deep brown was observed for Au_{core}-Ag_{shell} nanoparticles at different molar ratios of $AuCl_4$ and $AgNO_3$ (Govindaraju et al. 2008). However, various analytical techniques such as UV-Vis spectroscopy (Shahvedi et al. 2007), FTIR (Duraisamy and Yang 2013), energy dispersive X-ray analysis (Gaidhani et al. 2013), X-ray diffraction (Dhoondia and Chakraborty 2012), electron microscopy (Klaus et al. 1999; Prakash et al. 2011; Oves et al. 2013), atomic force microscopy (Sadhasivam et al. 2010), dynamic light scattering (Singh et al. 2013) and zeta potential (Krishnaraj and Berchmans 2013) are oftenly used to determine nature, surface properties, composition, purity, stability and morphology of any nanoparticles. Other techniques to characterize nanoparticles are atomic absorption spectroscopy (Zhang et al. 2005), X-ray photoelectron spectroscopy (Rajeshkumar et al. 2013), neutron activation analysis (Kiran et al. 2010) and thermogravimetric analysis (Shanmugasundaram et al. 2013). Some of the electron micrographs of bacteriagenic AgNPs are represented in Fig. 2.

Properties of AgNPs

It is well known that properties of nanomaterials are quite distinct from their bulk counterparts (Shedbalkar et al. 2014). Nanoparticles, mono- or multi-metallic, have unique physical, chemical, optical, dielectric, electrical, thermal, mechanical, catalytic, and biological properties due to their composition and size-dependent characteristics (Schmid 1992). A single surface plasmon resonance (SPR) peak, between 400 to 440 nm, generally corresponds to 2-100 nm spherical AgNPs. However, anisotropic particles can give rise to two or more SPR bands depending on their morphology (He et al. 2002). SPR peak also depends upon the size and metal composition (mono- or bi-metallic) of nanoparticles (Govindaraju et al. 2008; Singh et al. 2013). However, in bacterial-mediated synthesis of AgNPs, the major focus has been given to their colloidal stability.

Stability of nanoparticles can be obtained by either electrostatic/ charge stabilization or polymeric stabilization (Sperling and Parak 2010). Electrostatic stabilization is based on the formation of a charged layer through adsorption of ionic groups present in medium to the surface of nanoparticles, thereby, creating a repulsive force among each other. This does not allow the aggregation to occur (Sperling and Parak 2010). Two different mechanisms are included under polymeric stabilization. Firstly, steric stabilization is achieved by attaching macromolecules to the surface of nanoparticles, and secondly, depletion stabilization is imparted by macromolecules that are free in dispersion medium. Combination of steric and depletion stabilization is very common in presence of high amount of free polymer interacting with colloidal nanoparticles in the medium (Kraynov and Müller 2011). Aggregation of AgNPs over time is one of the common problems in chemical methods of synthesis; therefore, stabilizers like polyvinylpyrrolidone, ammonia, citrate, gelatin, cellulose and starch are externally added (Khan et al. 2013; You et al. 2013). However, in bacteriagenic synthesis, the cell itself secretes natural organic components in form of proteins and other biomolecules. These coat the

nanoparticles or form an organic matrix to embed them, thus, preventing their aggregation and providing stability for prolonged period (Singh et al. 2013; Duraisamy and Yan 2013).

Applications of AgNPs

Antimicrobial property of AgNPs is well documented and has been extensively used in health industries, textile coatings, surgical masks, bandages, disinfectant, water treatment and other environmental applications (Li et al. 2006; Lin et al. 2011; Perelshtein et al. 2008). Besides these, use of chemically synthesized AgNPs has been reported in optics, biosensors, catalysis, and diagnosis (Li et al. 2006; Sintubin et al. 2012; Wijnhoven et al. 2009). Despite finding applicability in numerous fields, only a "limited type" of applications has been investigated for bacterial AgNPs so far. It is important to note that following applications are not exclusive to bacterial AgNPs.

Antimicrobial activity

Bacteriagenic AgNPs are extensively tested for their antimicrobial activity against broad range of microorganisms including Gram-negative and Gram-positive bacteria, fungus, yeast and microbial biofilms (Abdeen et al. 2014; Gaidhani et al. 2013; Manivasagan et al. 2013; Singh et al. 2013). There are various methods to evaluate the inhibition of microbes in response to exposure to AgNPs. These include determining (i) zone of inhibition by disc-diffusion and agar well-diffusion method, (ii) minimum inhibitory concentration (MIC) by broth macro-dilution and micro-dilution assay, (iii) minimum bactericidal concentration (MBC), (iv) growth pattern, and (v) time-kill curve (Priyadarshini et al. 2013; Zhang et al. 2014). Although diffusion techniques are mostly preferred, but these are labor-intensive and calculation disparities between these methods make it more difficult to compare the various published data (Allahverdiyev et al. 2011). MIC and MBC are easy to assess despite different concentration units such as μg/ml, mg/L or ppm and provide accurate information with respect to susceptibility of a microorganism. Both growth and time-kill curve studies for bacteriagenic AgNPs are rare.

Concentration-dependent increase in growth-inhibition of pathogen is usually observed on exposure to AgNPs (Priyadarshini et al. 2013); however, the response varies form species to species depending upon morphology of AgNPs, type of strain, microbial inherent sensitivity and cell wall composition acting as barrier to AgNP penetration (Abdeen et al. 2014; Duraisamy and Yang 2013; Singh et al. 2013). Pourali et al. (2013) reported the decrease in antibacterial activity of AgNPs synthesized by bacteria after heating at 100°C and 300°C for 2 h. The plausible reason was aggregation among nanoparticles after heat treatment, which increased the size of AgNPs causing loss in their penetrating capability. Enhanced effect of bacterial AgNPs on antimicrobial activity of antibiotics has also been observed with display of inter- and intra-group variations in degree of synergism (Manikprabhu and Lingappa 2013; Shahverdi et al. 2007). This depends on the interaction of nanoparticles with a given antibiotic and the tested pathogen (Ghosh et al. 2012). We pursued a similar study

to determine the efficacy of the combination of bacterial AgNPs and antibiotics as per the MIC breakpoints provided in the CLSI guidelines for the first time (Singh et al. 2013). MIC breakpoints define the resistance and susceptibility of microorganisms to a given antibiotic and are important when potency of antibiotics is considered. Interestingly, *A. baumannii* AIIMS7, which exhibited resistance to ten of 14 antibiotics, became susceptible to seven of them in presence of AgNPs. The synergistic effect of drug and AgNPs was so profound that it lowered the MIC and MBC by upto 2000 folds (Singh et al. 2013). Such studies are important when current scenario of antimicrobial resistance among microorganisms is concerned.

Unlike commercial antibiotics, AgNPs do not exhibit their effects in a single specific way. Combinations of mechanisms such as disruption of cellular morphology, inactivation of vital cellular enzymes and proteins, DNA condensation (Li et al. 2011), loss of DNA replication, depletion of ATP (Kumar et al. 2008), protein denaturation (Sondi and Salopek-Sondi 2004), inhibition of ribosome interaction, accumulation at lethal concentration in cell (Dror-Ehre et al. 2009), generation of reactive oxygen species (ROS), oxidative stress and modulation of cellular signaling (Braydich-Stolle et al. 2005) make AgNPs ideal for targeting a broad range of microorganisms (Fig. 3). This also indicates that to protect themselves from AgNPs, microbes would have to develop a number of mutations simultaneously. Combination of AgNPs with antibiotics reduces the toxicity of both towards human cells by decreasing the required dosage with enhanced microbicidal properties (Allahverdiyev et al. 2011). Moreover, such combinations restore the ability of drug to kill bacteria that have acquired resistance to them (Singh et al. 2013). Thus, a different approach of using bacteriagenic AgNPs alone and in combination can act as effective novel antimicrobials to sensitize resistant pathogens. However, care should be taken to avoid the constant exposure of these nanoparticles to microorganisms since study on *E. coli* suggested that the bacteria could evolve to acquire AgNP resistance on its continuous exposure for 225 generations through genetic mutations (Graves et al. 2015).

Anti-biofilm activity

Biofilm is a structured consortium of bacteria encased in self-produced hydrated matrix consisting of proteins, DNA and polysaccharides (Sahu et al. 2014). Bacterial biofilms adhere to implanted medical devices, cause chronic infections and tissue damage. It is important to note that more than 80% of microbial infections in human are caused by biofilms (Davies 2003). These exhibit upto 1000 folds resistance to antibiotics (Gaidhani et al. 2014), and therefore, pose a problem in medicine. One of the promising strategies to deal with bacterial biofilms can be the use of AgNPs that can penetrate through biofilm to eradicate them. Inhibition of biofilm formation and disruption of preformed biofilms can be achieved employing bacterial AgNPs (Gaidhani et al. 2013). Exposure to AgNPs leads to reduction in microbial biomass and surviving cells, and also inhibition of exopolysacchride and protein production (Zhang et al. 2014). It has been suggested that though EPS blocks antibiotic molecules, small sized nanoparticles can penetrate through biofilm layers (Gaidhani et al. 2013), implying the potency of AgNPs to eradicate biofilms.

Cytotoxicity

Development of cost-effective and biocompatible method to treat cancer is indispensable due to toxic and undesirable side effects of currently available chemopreventives and chemotherapeutic agents (Azim et al. 2011). Dose-dependent reduction in cell viability has been observed in HeLa cervical cancer (Manivasagan et al. 2013; Shanmugasundaram et al. 2013), MDA-MB-231 breast cancer (Gurunathan et al. 2013), A549 adenocarcinoma lung cancer (Kumar et al. 2014) and HEP2 (Namasivayam et al. 2011) cell lines after treatment with bacterial AgNPs. Nanosilver cause morphological changes, such as loss of membrane integrity, cell shrinkage and reduced cell density, activate lactate dehydrogenase and caspase 3 and generate intracellular ROS induced apoptosis in cancer cells (Manivasagan et al. 2013; Gurunathan et al. 2013). Cytotoxicity of bacteriagenic AgNPs on cancer cells is an important finding, but one should also consider their effect on normal healthy cells. To act as anti-cancer agents, it is a prime requisite that AgNPs do not harm other body cells. Bacterial components adhered to the surface of AgNPs may impart immunogenicity during *in vivo* applications. However, biocompatible nature of such AgNPs has been shown against VERO cell lines, erythrocytes and splenocytes (Oves et al. 2013; Namasivayam et al. 2011). Furthermore, 3T3-L1 adipocyte and L929 fibroblast cells exhibit good proliferation on polycaprolactam coated with bacterial AgNPs (Veluchamy et al. 2012). This action specificity of AgNPs towards tumorogenic cells warrants further investigation.

Other applications

AgNPs synthesized by bacteria exhibit excellent larvicidal potency against dengue vector, *Aedes aegypti* (Debabov et al. 2013) and acaricidal activity against *Rhipicephalus microplus* and *Haemaphysalis bispinosa* (Karthik et al. 2014), indicating their application as potent insecticide. These also have anti-oxidant (Shanmugasundaram et al. 2013) and anti-coagulative (Kalishwaralal et al. 2010) activity. Their application in wastewater treatment (Sathiyanarayanan et al. 2013) and as a bionanocatalyst for reduction of 4-nitrophenol in presence of sodium borohydride (Otari et al. 2014) has also been reported.

Conclusions and future prospects

Bacteria serve as one of the promising candidates for biosynthesis of AgNPs through cells, supernatant and aqueous extract. Combination of bacterial source with non-toxic green reducing agents such as starch, glucose and citrate will aid in development of new biochemical synthesis methods. This may help in synthesis of new morpho-types of AgNPs having enhanced therapeutic applications. Multi-metallic nanoparticles have more benefits owing to enhanced composition-based properties and effective applications. However, bacterial-mediated bimetallic nanoparticles are rarely studied. Formation of spherical-shaped AgNPs, which has been majorly reported, is a thermodynamically favorable reaction. But production of other shapes, like triangle, cube and hexagon, is also possible by regulating physicochemical conditions and will be fascinating to study the interaction of reducing agent with silver ions for the same. Moreover, currently all the experiments are being

conducted on laboratory scale; hence, it is necessary to look for scale-up methods of production. Though mechanism of both extra- and intra-cellular synthesis is yet not fully understood, but silver-resistance machinery plays a significant role in AgNP biogenesis in bacteria. There are multiple electron transport pathways in a bacterial cell for reduction of silver ions, which need to be elucidated along with involvement of other enzymes and reducing cofactors. Small AgNPs are known to exhibit greater antimicrobial potency due to high surface-area to volume ratio, although no such size-dependent study has been pursued with bacteriagenic AgNPs. Furthermore, anti-mycobacterial and anti-viral activity of these nanoparticles needs to be explored. Redeeming the profound synergistic effect of bacterial AgNPs on activity of antibiotics, it can be concluded that surface functionalization of these AgNPs with drugs to form nanoconjugates will open avenues for newer generation of antimicrobials. Besides this, there are many other important applications like disinfection, sensors and catalysis that have been reported with chemical AgNPs but not yet studied for bacterial ones. It is of paramount importance to focus on developing environmental, biomedical and nanomedicinal applications of bacterially synthesized AgNPs.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1 Bacterial-mediated synthesis of silver nanoparticles

Bacteria	Shape	Size (nm)	Location	Reference
Gram-negative				
Acinetobacter calcoaceticus	spherical	8-12	extracellular	Singh et al. 2013
		4-40	extracellular	Gaidhani et al. 2013
Aeromonas sp.	-	6.4	extracellular and intracellular	Mouxing et al. 2006; Wang et al. 2012
Bordetella sp.	-	63-90	extracellular	Thomas et al. 2012
Enterobacter aerogenes	spherical	25-35	extracellular	Karthik and Radha 2012
Escherichia coli	spherical	42.2-89.6	extracellular	Gurunathan et al. 2009
Geobacter sulfurreducens	-	-	extracellular	Law et al. 2008
Gluconobacter roseus	-	10	extracellular	Krishnaraj and Berchmans 2013
Idiomarina sp.	-	25	intracellular	Seshadri et al. 2012
Klebsiella pneumonia	spherical	15-37	extracellular	Duraisamy and Yang 2013
	-	5-32	extracellular	Shahverdi et al. 2007
Morganella sp.	quasi-spherical	10-40;	extracellular	Parikh et al. 2011
	nanoplates	50-450 [a]	extracellular	Ramanathan et al. 2011
Proteus mirabilis	spherical	10-20	extracellular and intracellular	Samadi et al. 2009
Pseudomonas aeruginosa	spherical, discs	6.3±4.9	extracellular	Srivastava and Constanti 2012
	spherical	8-24	extracellular	Kumar and Mamidyala 2011
	quasi-spherical	5-25	intracellular	Otaqsara 2011
Pseudomonas stutzeri AG259	triangle, hexagons	200 [a]	cell poles	Klaus et al. 1999
Rhodobacter sphaeroides	spherical	3-15	extracellular	Bai et al. 2011
Rhodopseudomonas palustris	spherical	5-20	extracellular	Chun-Jing and Hong-Juan 2011
Salmonella typhimurium	-	5-150 [a]	extracellular	Ghorbani 2013
Shewanella oneidensis MR-1	spherical	2-16 (Ag_2S)	extracellular	Debabov et al. 2013
Stenotrophomonas maltophilia	cuboidal	93	extracellular	Oves et al. 2013
Vibrio alginolyticus	spherical	50-100	extracellular and	Rajeshkumar et al. 2013

			intracellular	
Xanthomonas oryzae	spherical, triangles, rods	14.86	extracellular	Narayanan and Sakthivel 2013
Yersinia enterocolitica	-	10-80	extracellular	Pourali et al. 2012
Gram positive				
Bacillus sp.	-	5-15	extracellular and periplasmic space	Pugazhenthiran et al. 2009
Bacillus licheniformis	spherical	50	intracellular	Sriram et al. 2012
Bacillus subtilis	triangle, hexagon	-	extracellular	Kannan et al. 2011
	polydisperse	20-60 (AgCl)	-	Paulkumar et al. 2013
Bacillus thuringiensis	-	43.52-142.97	extracellular	Banu et al. 2014
Brevibacterium casei	spherical	10-50	intracellular	Kalishwaralal et al. 2010
Corynebacterium SH09	-	10-15	extracellular	Zhang et al. 2005
Enterococcus faecalis	-	10-80	extracellular	Pourali et al. 2012
Exiguobacterium sp.	spherical	5-50	extracellular	Tamboli and Lee 2013
Geobacillus stearothermophilus	spherical	5-35	extracellular	Fayaz et al. 2011
Lactobacillus mindensis	spherical	2-20 (Ag_2O)	extracellular	Dhoondia and Chakraborty 2012
Lactobacillus sp.	-	500 (AgNP); 100-300 (Ag-Au) [a]	extracellular and intracellular	Brayner et al. 2007
Pedicoccus pentosaceus	-	-	intracellular	Sintubin et al. 2009
Rhodococcus sp.	spherical	10-15	extracellular	Otari et al. 2014
Staphylococcus epidermidis	-	10-80	extracellular	Pourali et al. 2012
Streptococcus thermophiles	spherical	28-122 [a]	extracellular	El-Shanshoury et al. 2011
Thermoactinomyces sp.	spherical	20-40	extracellular	Deepa et al. 2013
Ureibacillus thermosphaericus	spherical	10-100	extracellular	Juibari et al. 2011
Actinobacteria				
Nocardiopsis sp.	spherical	45±0.15	extracellular	Manivasagan et al. 2013
Streptomyces hygroscopicus	spherical	20-30	extracellular	Sadhasivam et al. 2010
Cyanobacteria				
Anabaena flos-aquae	-	40±4.2 (i);	extracellular, inside	Brayner et al. 2007

		25.2±2.5 (e)[b]	heterocyst and vegetative cell	
Calothrix pulvinata	-	15±0.8 (i); 10±1.2 (e)[b]	extracellular, inside heterocyst and vegetative cell	Brayner et al. 2007
Microcoleus sp.	-	44-79	extracellular	Sudha et al. 2013
Oscillatoria willei	spherical	100-200 [a]	extracellular	Ali et al. 2011
Plectonema boryanum	-	1-15, 1-40 and 5-200[a,c]	extracellular and intracellular	Lengke et al. 2007
Spirulina platensis	spherical	7-16 (AgNP); 17-25 (Ag-Au)	extracellular	Govindaraju et al. 2008
Archaebacteria				
Halococcus salifodinae	-	12 and 22[d]	intracellular	Srivastava et al. 2013

[a] size ≥100 nm but reported as nanoparticle

[b] (i)- intracellular; (e)- extracellular

[c] size depend on temperature

[d] size depend on culture medium

- Not reported

Table 2 Bacterial component mediated synthesis of silver nanoparticles

Bacterial component	**Bacteria**	**Shape**	**Size (nm)**	**Reference**
Actinorhodin pigment	*Streptomyces coelicolor*	irregular	28-50	Manikprabhu and Lingappa 2013
Bioflocculant	*Bacillus subtilis* MSBN 17	spherical	60	Sathiyanarayanan et al. 2013
Biosurfactant	*Brevibacterium casei* MSA19	-	-	Kiran et al. 2010
Cellulose	*Gluconacetobacter xylinum*	-	5-40	Liu et al. 2012
Exopolysaccharide	*Lactobacillus rhamnosus* GG ATCC 53103	spherical, triangular, rod and hexagonal	2-15	Kanmani and Lim 2013
Extracellular polysaccharide/Matrix	*Nostoc commune*	spherical	15-54	Morsy et al. 2014
Flagellin	*Salmonella typhimurium*	-	3-11	Gopinathan et al. 2013
Rhamnolipids	*Pseudomonas*	spherical	15.1±5.8	Kumar et al. 2010

	aeruginosa BS-161R			
Spores	*Bacillus athrophaeus*	polydisperse	5-30	Hosseini-Abari et al. 2013
URAK enzyme	*Bacillus cereus* NK1	spherical	50-80	Deepak et al. 2011

- Not reported

Figure Legends

Fig. 1 Proposed mechanism of bacterial mediated synthesis of AgNPs. **a** cellular uptake of silver ions and activation of silver reduction machinery, **b** electron shuttle system involving various cofactors and enzymes, **c, d** intra- or extracellular localization of AgNPs, **e** electrostatic interaction between silver ions and cell wall components, **f** reduction through extracellular enzymes and other organic molecules released in solution

Fig. 2 Electron micrographs of bacterial AgNPs. **a** SEM image of extracellular AgNPs by *Streptomyces hygroscopicus* (Sadhasivam et al. 2010), **b** FESEM micrograph of cuboid AgNPs produced by *Stenotrophomonas maltophilia* OS4 extracellularly (Oves et al. 2013), TEM images of **c** AgNPs precipitated on surface of *Plectonema boryanum* UTEX 485 (Lengke et al. 2007), **d** intra- and extra-cellular synthesis of AgNPs by *Bacillus* sp. (Pugazenthiran et al. 2009), **e** intracellular synthesis of triangular and hexagonal AgNPs by *Pseudomonas stutzeri* AG259 (Klaus et al. 1999), **f** spherical AgNPs in *Anabaena* cells (Brayner et al. 2007), **g** extracellular spherical AgNPs synthesized by *Acinetobacter calcoaceticus* (Singh et al. 2013), and **h** silver nanoplates by *Morganella psychrotolerans* (Ramanathan et al. 2011)

Fig. 3 Anti-microbial action of AgNPs. Nanosilver **a** attach to cell membrane creating pores to cause cellular leakage, **b** distort cellular morphology, **c** break dsDNA, **d** inhibit DNA replication, **e** interact with 30S ribosome, **f** inactivate vital enzymes, **g** denature protein, **h** modulate cellular signaling, **i** generate ROS, which acts on DNA and cell membrane, **j** release silver ions, which affect the normal functioning of membrane proteins, and **k** accumulate inside the cells in lethal concentrations

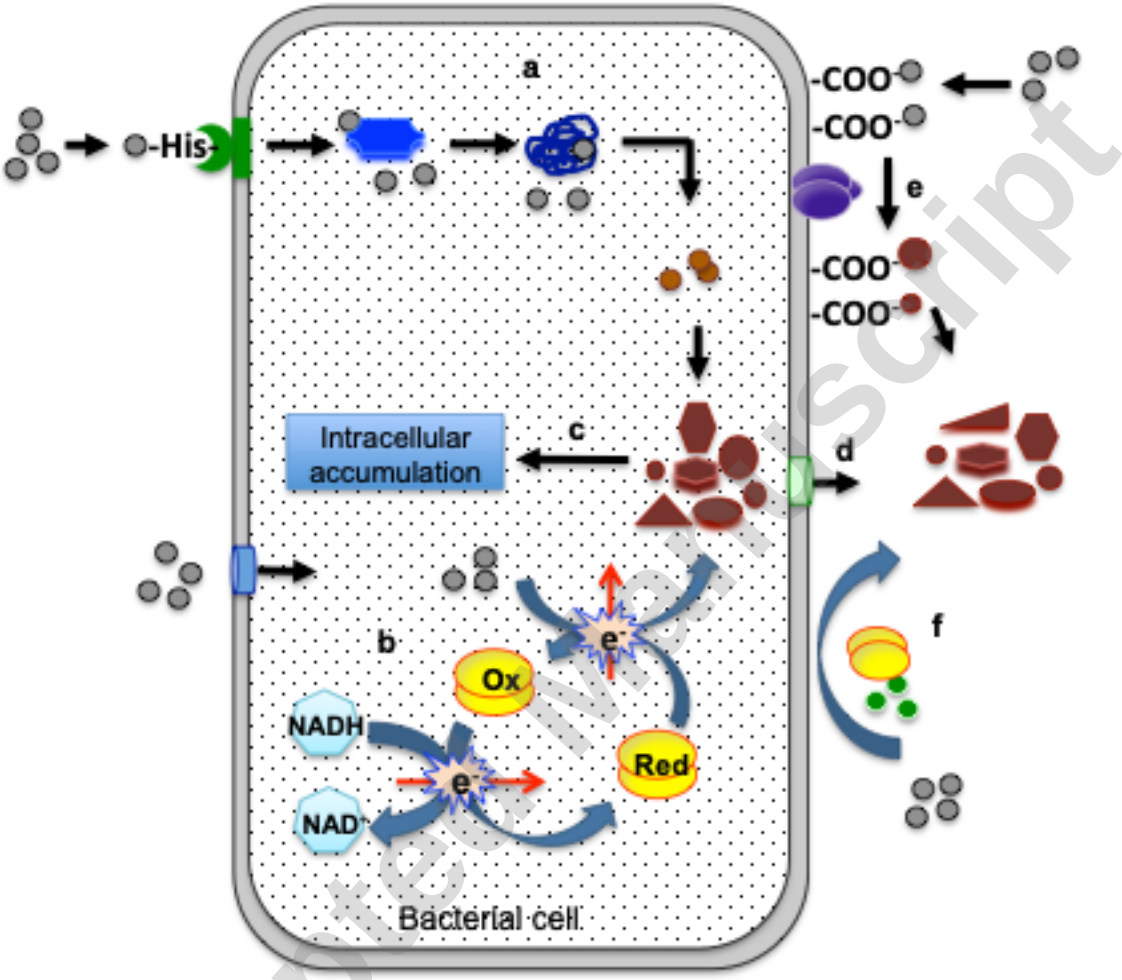

Fig. 1

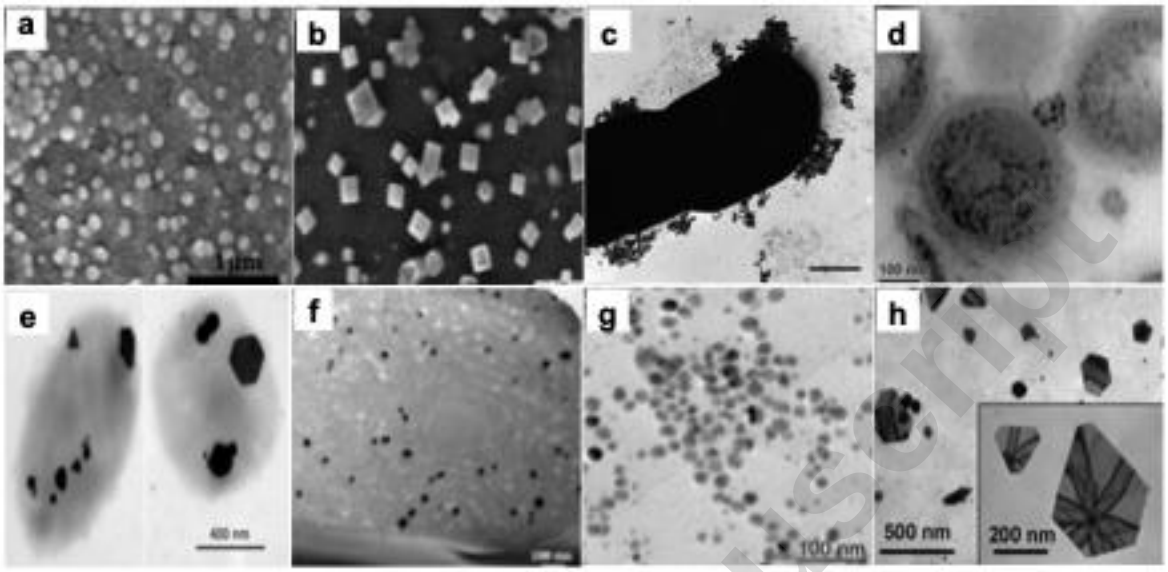

Fig. 2

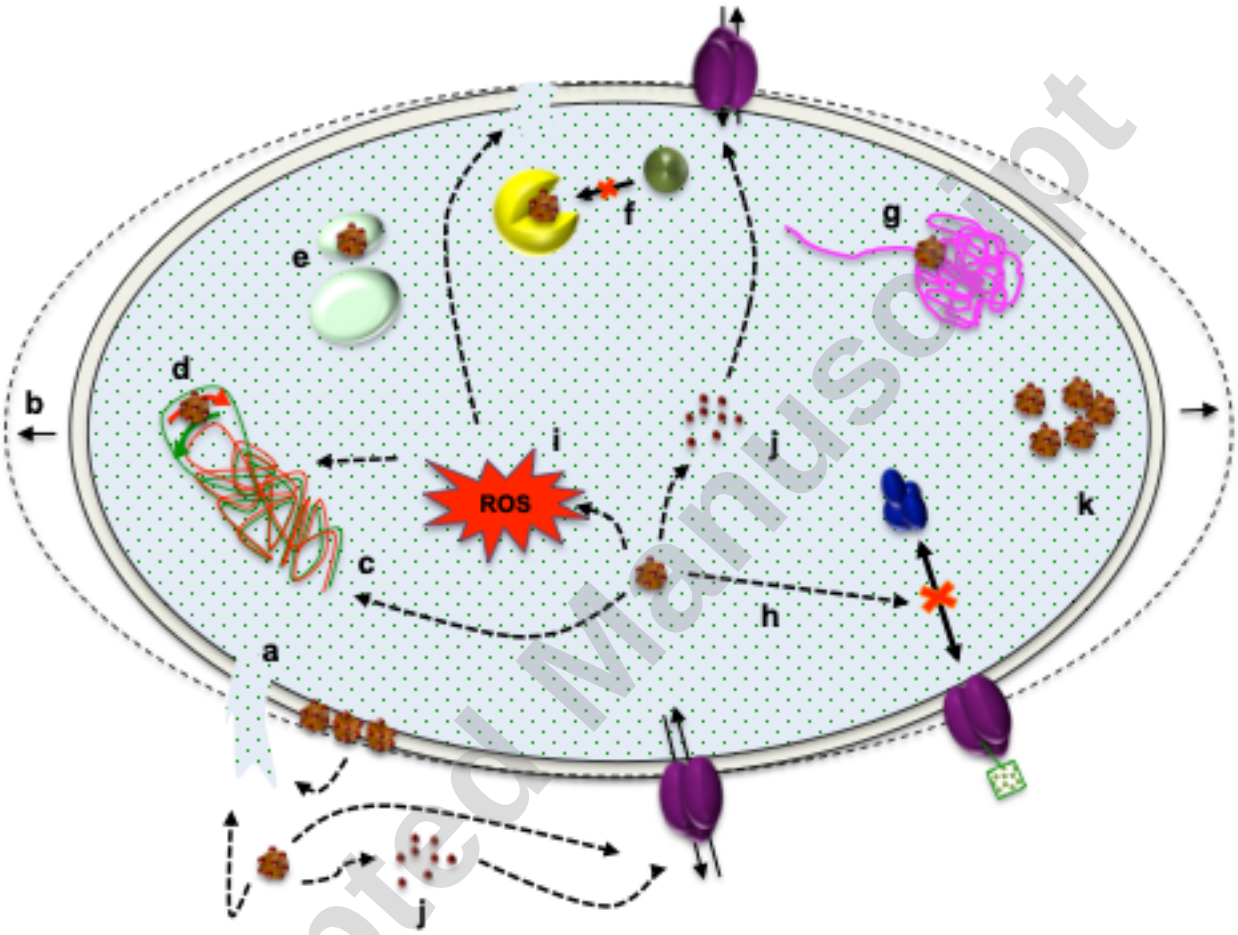

Fig. 3