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Silver bullet: A new shine to antimicrobial agent, a preface to silver nanoparticles

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Abstract
This review article summarizes the use of silver as an antimicrobial, its mechanism of action, general introduction of silver nanoparticles, synthesis, toxicity, its medical application. Silver is a health additive added in customary traditional Chinese and Ayurvedic medicine. Its action is antibiotic and it is non selective toxic biocide. Silver has been effective against all organism tested and used to treat numerous infection and non infectious condition. Silver is incorporated in wound dressing and used as antibacterial coating in medical devices. Silver ion is bioactive and mainly responsible for bactericidal action. This review also covers the major use of silver in form silver nanoparticle as it has broad spectrum bactericidal and fungicidal activity. Bactericidal nanoparticles are gaining importance because the size of the nanoparticles is similar to biological molecules and are used in *in vitro* and *in vivo* biomedical studies. Metallic nanoparticles are better nanoparticles with best antibacterial properties as these increase chemical activities due to crystallography surface structure. Nanoparticle of silver brings the significant improvement in the antibacterial activity.

Keywords: silver, antimicrobial, antibacterial, silver nanoparticles, silver nanoparticle synthesis, toxicity, application

Introduction
Silver is known for its medical properties for over 2000 years [1]. It has long history in human healthcare and medicine. Silver was used in wound dressing which could be traced back to 18th century during which silver nitrate was used in treatment of ulcers, post surgical infections, in dentistry etc. [2] due to the various side effect of silver nitrate colloidal silver was introduced in and was widely used. Colloidal silver usually contains metallic silver particles suspended in solution with less than 10% ionized silver [6]. Silver was effectively used in wound dressing till 1920, the introduction of antibiotics in 1940 replaced the use of silver and its related compound. The resistant strain of the bacteria helped the emergence of silver. It was used in combination with sulfonamide to create a broad spectrum silver based antibacterial agent [2]. More recently silver is used in wound dressing with varying level of silver, a variety of silver based dressing have become available e.g. Acticoat, Actisorb which offer better therapeutic action. There are advances in creating medical grade silver technology for the synthesis of new, safer and bio available silver compounds. Silver based vascular and urinary catheters are also clinically used. Silver and its compounds are highly toxic to microorganism. Nitrate form of silver is generally used to induce anti microbial effect [1], but when silver nanoparticles are used the microbes are exposed to wider surface, silver nanoparticles have use against microbes its action is not fully understood, there are various mechanism through which silver nanoparticles act.

Mechanism of action
The complete mechanism through which silver acts is still unknown. The few mechanism understood are:

1. **Protein inactivation:** Silver inhibits the bacterial growth by binding with the thiol group in enzymes causing the deactivation of enzymes. Silver structures stable S-Ag bonds with thiol-containing compounds within the semi-permeable membrane layer that are present in trans-membrane energy generation and particle transport. Silver can take part in synergistic oxidation reactions that result in the formation of disulfide bonds (R-S-S-R). Silver will this by catalyzing the response between chemical element molecules within the cell and H atoms of thiol teams: water is discharged as a product and 2 thiol groups become covalently bonded to one another through a disulfide bond. The silver-catalyzed formation of disulfide bonds prompts to alter the form of cellular enzymes and have an effect on their function. The silver-catalyzed formation of disulfide bonds results in changes in super molecule structure and also the inactivation of key enzymes, required for internal respiration. It is hypothesized that silver ion bind with 30S ribosomal subunit deactivating the complex and inhibiting the translation of proteins.
2. **DNA association:** Another hypothesis was proposed by Klueh (2000). The hypothesis proposed that Ag^+ enters the cell and get inserts between the purine and pyrimidine base pairs and disrupts the hydrogen bonding between the two anti-parallel strands and denaturing the DNA molecule, this has yet to be proved, it has been demonstrate that silver ion particles do associates with DNA once they enter the cell.
3. **Action of silver nanoparticles:** The exact mechanism is still unknown but hypothesis suggest that silver nanoparticles have ability to anchor to cell wall of bacteria and penetrate it, causing the structural changes in the cell membrane by forming pits on the cell surface and causing the

accumulation of silver nanoparticles [26].

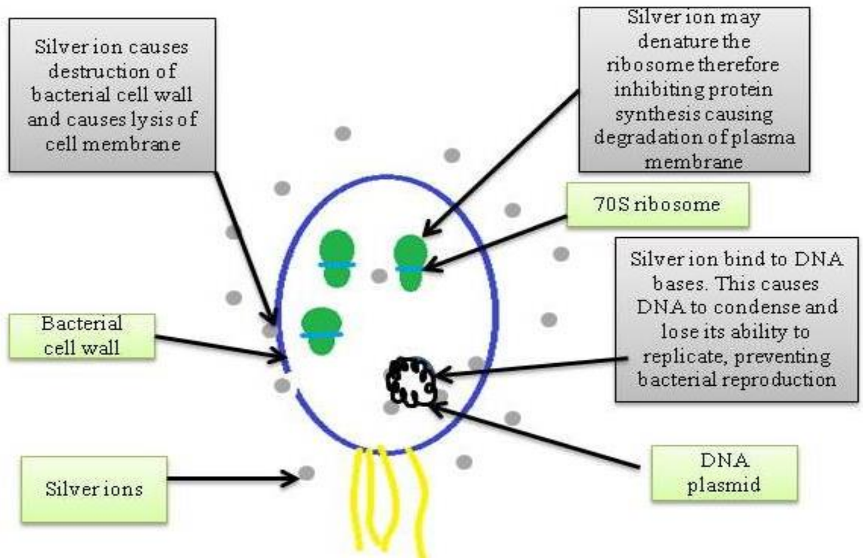

Fig 1: Mechanism of action of silver ions

Different forms of silver effective at microbial inhibition

1. Silver salts- silver salts. For example: silver nitrate ($AgNO_3$)
2. Silver zeolites
3. Silver nanoparticles

Silver nanoparticles

Nanotechnology is delivering nano product and nanoparticles that can have novel and size related physicochemical properties varying from substantial large particle. Nanoparticles size is of similar with the biological molecules and structures can be useful for both *in vitro* and *in vivo* studies. In all nanoparticles metallic nanoparticles are best with anti-bactericidal properties. Silver is transition metal element with atomic number-47 and atomic mass 107.87.It is a non selective toxic biocide. Silver kills some 650 different disease organisms (Gupta *et al*.). Silver nanoparticles have attracted interest due its unique physical, chemical, biological properties. Silver nanoparticles have distinctive physicochemical properties including high electrical, thermal conductivity, chemical stability. Silver nanoparticles were also found to cause destabilization of outer membrane, collapse of plasma membrane potential and depletion of intracellular ATP. It is an efficient physicochemical system with antimicrobial activities. The silver nanoparticles are generally in the range of 1-100 nm. Silver nanoparticles exhibit broad spectrum bactericidal and fungicidal activity [4].

Synthesis of Silver Nanoparticles

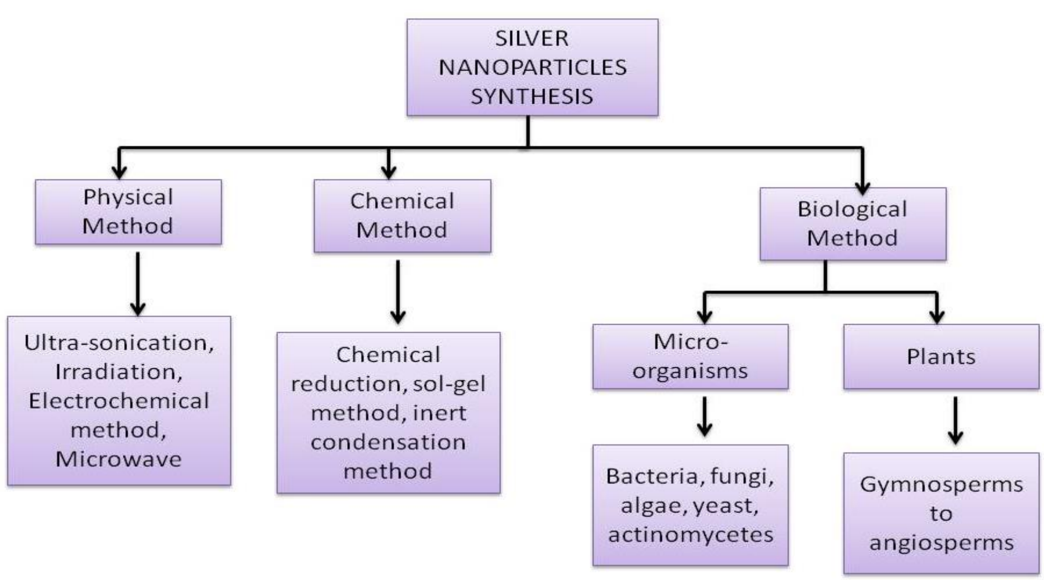

Fig 2: Silver nanoparticles synthesis

1. Chemical and Physical synthesis of silver nanoparticles

The simplest method includes the chemical reduction of the metal salt $AgBF_4$ by $NaBH_4$ in aqueous solvent. The nanoparticles which are obtained are of size between 3 to 40nm, which are characterized by transmission electron microscopy (TEM) and UV visible absorption spectroscopy to evaluate the quality. Another method involves sono chemical reduction of an aqueous silver nitrate solution in an atmosphere of argon–hydrogen. The particles re characterized by TEM, X-ray diffraction, absorption spectroscopy. A microwave synthesis of silver nanoparticles involves the reduction of silver nanoparticles using variable frequency microwave radiation as against conventional method. The method yields higher concentration of silver nanoparticles with the same temperature and exposure [1].

2. Biological synthesis of silver nanoparticle

Chemical and physical method of synthesis of silver nanoparticle is expensive and includes the use of toxic and hazardous chemicals. The need for the environmental and economically safe way to synthesize these nanoparticles led to the search of new method which involves the biomimetic production of silver by biological method. The three major sources of synthesizing silver nanoparticles involves bacteria, fungi and plant extract [12].

A. Silver synthesizing bacteria

The nitrate reductase enzyme converts nitrate into nitrite. In *in vitro* synthesis of silver utilizing microorganism the presence of alpha nicotinamide adenine dinucleotide phosphate reduced kind (NADPH) – dependent nitrate enzyme would take away the downstream procedure step. During reduction nitrate is converted into nitrite and electron is transferred to the silver ion. This is observed in Bacillus licheniformis which secrete NADPH and NADPH dependent enzymes like nitrate reductase that converts Ag^+ to Ag^0 [11].

Table 1: Silver nanoparticles synthesized from silver synthesizing bacteria [1].

S. No.	Bacteria	Particle size (nm)
1.	*P. stutzen*	200
2.	*Bacillus megaterium*	46.9
3.	*Plectonema boryanum*	1-200
4.	*Enterobacter cloacae*	50-100
5.	*Escherichia coli*	5-25

B. Silver synthesizing fungi

Fungi can synthesize nanoparticles because they secrete large amount of proteins which directly translate to higher productivity of silver nanoparticles. It includes the trapping of Ag+ ions at the surface of fungal cell, causing reduction of the silver ions by the enzyme present in the fungal molecular system. The enzymes like naphtha quinones and anthro quinones helps in the reduction of silver ions. Exact mechanism involved is still not known but the above mentioned phenomenon is responsible for the process but the process is very slow [16].

Table 2: Silver nanoparticles synthesized from silver synthesizing fungi [1].

S. No.	Fungi	Particle size (nm)
1.	*Verticillium sp.*	25
2.	*F.oxysporum*	20-50
3.	*Aspergillus fumigates*	5-25
4.	*Aspergillus flavus*	7-10
5.	*Fusarium solani*	5-35

C. Silver synthesizing plants

The advantage using plant extracts for silver nanoparticles synthesis is that they are easily available and non toxic in nature. The main is plant assisted reduction by phytochemicals like terpenoids, flavones, ketones, amides etc. Past examination associated studies explains that Xerophytes contain emotions an anthraquinone that undergoes tautomerization prompting the formation of silver nanoparticles. Phytochemicals are included directly in reduction of ions and formation of silver nanoparticles [23].

Table 3: Silver nanoparticles synthesized from silver synthesizing plant [1].

S. No.	Plant	Particle size (nm)
1.	*Medicago sativa*	2-20
2.	*Azadirachta indica*	50
3.	*Aloe vera*	15-20
4.	*Cinnamomum camphora leaf*	55-80
5.	*Cinnamomum zeylanicum bark*	50-100

Biological properties of silver nanoparticles

Silver nanoparticles are used in medicine due to its antibacterial, antifungal, antiviral, anti-inflammatory, osteoinductive effect and property of wound healing.

1. **Antibacterial properties of silver nanoparticles**: Antibiotics bind to specific chemical targets of bacteria, this binding specifically narrows the number of bacterial species that are vulnerable to a specific antibiotic later on multiple dosing bacteria grows resistant towards the antibiotics. Silver is used as an alternate as a result of it's an antimicrobial agent that targets a broad spectrum of gram positive, gram negative and antibiotic resistant strains. Because of larger surface to mass ratio silver nanoparticles provide larger active surface, higher solubility and chemical reactivity. Antibacterial action of silver nanoparticles is dependent on the surface area with nanoscales having larger surface area. Silver nanoparticles show antimicrobial action towards *S. aureus*, *S. epidermidis, Enterococcus faecalis*, *Enterococcus faecium, E. coli, P. aeruginosa* and *K. pneumonia* [13].
2. **Antifungal properties of silver nanoparticles**: The repetitive long term administration of antifungal drugs leads to the fungal resistance, generally by *Candida species*. Silver nanoparticles exhibits antifungal activity towards *C. tropicalis, C.krusei, C.glabrata, C.albicans*. Silver nanoparticles synthesized from aqueous raspberry extract acted as growth inhibitor against *Cladosporium cladosporioides* and *Aspergillus Niger*. Higher

concentration of silver nanoparticles induces reduction in fungal rate [17].

3. **Antiviral properties of silver nanoparticles:** Silver nanoparticles act as broad spectrum agent against a variety of viral strains. The antiviral activity of silver nanoparticles has been studied against HSV-1, HSV-2, Hepatitis. The mode of action of silver nanoparticles occurs during the early phases of viral replication [3].
4. **Anti-inflammatory properties of silver nanoparticles:** Silver nanoparticles coated wound dressing decreases the level of proinflammatory cytokines transforming growth factors-(TGF) beta and tumor necrosis factor (TNF) alpha [20].
5. **Osteoconduction and Osteoinduction of silver nanoparticles based materials**: AgNP (silver nanoparticles) implant induced osteogenesis while suppressing the bacterial survival in contaminated rat femoral canals with no cytotoxicity. The composite grafts of AgNP/PLGA display the osteoconductive properties as they did not inhibit adherence, proliferation. These findings require more thorough research but they promise therapeutic materials for orthopaedic surgery [23].

Table 4: Literature surveys and their interpretation regarding silver nanoparticles

S. No.	Researcher	Year	Microorganisms on which experiment performed	Silver nanoparticles	Interpretation and Result
1.	DVM *et al*.	2007	*E. coli, yeast, S. aureus*	Low concentration of silver nanoparticles was used	On yeast and *E. coli* silver nanoparticles exhibited antimicrobial activity but on *S. aureus* mild antimicrobial action was seen
2.	Ping Li, Juan Li	2015	*E. coli*	silver nanoparticles with amoxicillin antibiotic was used	Bactericidal action of amoxicillin with silver nanoparticles was seen and synergistic action was recorded against *E. coli*
3.	SK Gogoi *et al*.	2006	recombinant *E. coli*	Sodium borohydride synthesized silver nanoparticles	Silver nanoparticles above a certain concentration were bactericidal and reduces the size of treated bacteria
4.	Sharma *et al*.	2009	Gram positive and gram negative bacteria	silver nanoparticles and its modified form by polymers and surfactant	Antibacterial activity was observed.
5.	Anh Tuan *et al*.	2010	*S. aureus*	Oleic acid stabilized silver nanoparticles	High antibacterial activity was seen
6.	Wang *et al*.	2006	*E. coli*	Silver ion exchanged wit titanium phosphate film	Silver ions were effective in prohibiting the growth of *E. coli* and can be used as coatings
7.	M Liong *et al*.	2009	Gram positive and gram negative bacteria	silver nanocrystals encapsulated in mesoporous silica nanoparticles	Silver nanocrystals possess antibacterial activity
8.	Panacek *et al*.	2009	*Candida sp*.	silver nanoparticles prepared by Tollens process	Antifungal activity was seen and MIC(minimum inhibitory concentration) and MFC (Minimum fungal concentration) was found to be 0.21mg/l
9.	Leung *et al*.	2006	*E.coli, S. aureus*	nanoparticles consisting of titanium dioxide and silver nitrate	1/128 and 1/512 were the MIC of silver nanoparticles against *E.coli* and *S.aureus* respectively
10.	M. Guzman *et al*.	2011	*E. coli, S. aureus, Pseudomonas aeruginosa*	By Kirby-Bauer method, silver nanoparticles was synthesized	Antibacterial activity was seen against *E.coli, S.aureus, Pseudomonas aeruginosa.*
11.	Muthuswamy, Krishnamurthy	2001	*E. coli*	silver nanoparticles synthesized the water soluble organics of *Curcuma longa* tuber and extract	MBC (minimum bactericidal concentration) for *E.coli* BL-21 strain was 50mg/l.
12.	Musarrat *et al*.	2010	*Shigella dysenteriae type 1, S. aureus, Citrobacter sp., E. coli, P. aeruginosa, B. subtilis, C.albicans, Fusarium oxysporum.*	silver nanoparticles synthesized from the roots of date palm (*Phoenix dactylifera*)	Antimicrobial activity was seen against *Shigella dysenteriae type 1, S.aureus, Citrobacter sp., E. coli, P. aeruginosa, B. subtilis, C. albicans, Fusarium oxysporum.*
13.	Mubarakali *et al*	2011	clinically isolated human pathogens	silver nanoparticles synthesized from *Mentha piperita* leaves extract	Antimicrobial activity was seen against clinically isolated human pathogens
14.	Krishnaraj *et al*	2009	*Vibrio. cholera, E. coli*	silver nanoparticles biologically synthesized from *Acalypha indica* leaf extract	Antimicrobial activity was seen and MIC (minimum inhibitory concentration) was found to be 10ug/ml for both pathogens.
15.	Kalimuthu *et al*	2010	*P.aeruginosa, S.epidermidis*	biologically synthesized silver nanoparticles	Anti-bio-film formation activity was seen when tested *in vitro* during 24 hour treatment. Silver nanoparticles resulted in more than 95% inhibition of bio-film formation
16.	Shivaji *et al*.	2011	*Arthrobacter kergulesis, A. gangotriensis, B. indicus*	silver nanoparticles synthesized from psychrophilic bacteria	Antibacterial activity was seen and the lowest concentration is 2ug/ml at which they arrest the bacterial growth.

17.	Mirzajani *et al.*	2011	*S.aureus* PTCC1431	silver nanoparticles	The concentration of silver nanoparticles above 8ug/ml results in the release of muramic acid into the medium that cause cell wall destruction
18.	Sondi *et al.*	2004	*E. coli*	silver nanoparticles	*E.coli* cells treated with silver nanoparticles found to be accumulated in bacterial membrane which results in the increase of permeability and later in cell death
19.	Thombre *et al.*	2016	*haloarchaea*	silver nanoparticles synthesized from *Cinnamomum tamala* or Indian bay leaf	Antibactericidal action against extremely resistant *haloarchaea* was seen and MIC for it was found to be between 300-400 ug/ml

1. Wound dressing: Medical grade silver containing dressing approved by FDA/EPA includes Silvadene (Marion Laboratories), Curad silver (Beiersdorf AG), Actisorb 220(Johnson & Johnson) [6].
2. Endotracheal tubes: Endotracheal tubes are used by patients needing ventilator-assisted breathing. Silver coatings on the inside of endotracheal tubes delay the appearance of bacteria on the insides of these tubes [26].
3. Surgical mask: Surgical masks coated with silver nanoparticles were capable of a 100% reduction in viable *E. coli* and *S. aureus* cells after incubation [16].
4. Silver nanoparticles are used in bone cements that are used in bone replacement therapy [11].
5. Nanosilver is also used in biosensing for biosensing large number of protein [21].
6. Nanosilver also used in bioimaging using its plasmonic properties [1].
7. Axenohl (Axen30) is silver dihydrogen citrate disinfectant which kills sensitive strain bacteria in 30 seconds and resistant strains, such as MRSA, in 2 minutes [6].

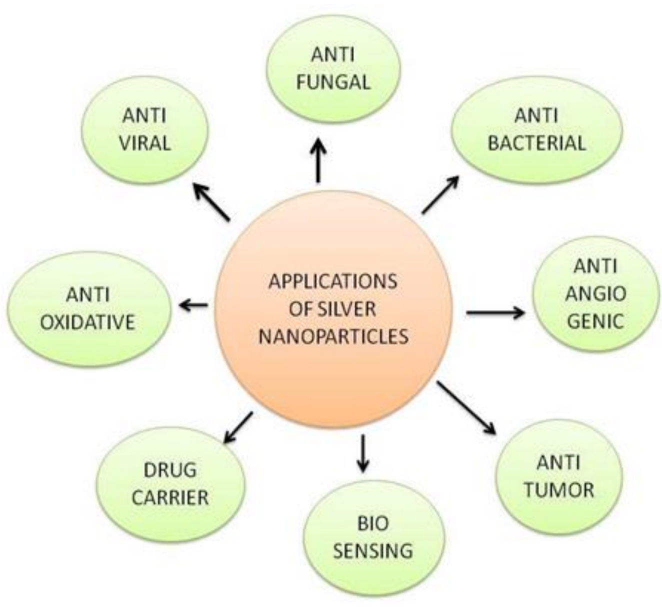

Fig 3: Applications of silver

Toxicity of silver

The major side effect known of silver include the condition of Argyria which is identified by the development of bluish grey skin known to occur at the lengthy exposure to silver [6]. Also silver wound dressing which release low level of silver ions are more dangerous in terms of resistance if silver ion concentration is sub-lethal. Faster acting dressing present less risk because organism is most likely to be killed therefore eliminating the enrichment of the resistant population through growth and division. Asbestos is less cytotoxic than silver nanocrystals. Nanosilver will induce the proliferation and multiplication protein expression by peripheral blood mononuclear cells. Nanosilver also shows severe toxic effects on the male reproductive system [1].

Conclusion

The resistance towards the antibiotics is a major issue and alarming concern. There is need for a cheap broad active agent that can be used against micro-organisms. Silver is an excellent antimicrobial agent and has been used for a long time, but there is need for silver MIC (minimum inhibitory concentration) level and breakpoints to be developed, studied and standardized. The antimicrobial properties of silver are as a result of its ionised form, Ag+, and its capability to cause injury to cells by interacting with thiol-containing proteins and deoxyribonucleic acid. Silver nanoparticle may be a variety of silver which may be used widely as a result of its simple production, high antimicrobial action, and ability to be incorporated into varied product. With the ever increasing number of antibiotic-resistant strains of bacteria and silver's low toxicity to humans, the use of silver as an antimicrobial agent are relevant to many fields of study and industry. Recently it has been concluded that silver nanoparticles acts bactericidal, virucidal, and promotes wound healing. Nonetheless conclusive and decisive safety is not studied widely, additional testing of silver nanoparticles needs to be considered and studied before they can be utilized in clinical applications. Additional studies also demonstrate that silver nanoparticles can cause ecological problems and cause disturbance in ecosystem. So there is need to synthesize the silver nanoparticle in an effective and efficient way.

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