



## NANOBIOTECHNOLOGY- AN ERA OF REVOLUTION IN SCIENCE AND RESEARCH

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**Nanotechnology** is a recently developed era of scientific research, which refers to the ability to create and manipulate biological and biochemical materials, devices, and systems at nano level (Kholoud *et al.* 2010). From the last few years, the synthesis of metal nanoparticles is an important topic of research in modern material science and the whole research in circling around it. A major number of scientists are contributing in this field to bring up its wider application for society welfare. The term nanotechnology was coined by Professor Norio Taniguchi of Tokyo Science University in the year 1974 to describe precision manufacturing of materials at the nanometer level (Taniguchi, 1974). Nanotechnology is a developing interdisciplinary field of research interspersing material science, bionanoscience, and technology. Nanoparticles are often referred to as particles with a maximum size of 100 nm. Nanoparticles exhibit unique properties, which are quite different than those of larger particles. (New applications of nanoparticles and nanomaterials are emerging rapidly (W. Jahn 1999; H. S. Naiwa 2000; C. J. Murphy 2008). Nanotechnology concerns with the development of experimental processes for the synthesis of nanoparticles of different sizes, shapes and controlled dispersity. New properties of nanoparticles related to variation in specific characteristics like size, shape and distribution have been demonstrated. Nanotechnology can improve our understanding of living cells and of molecular level interactions. A reliable and eco-friendly process for synthesis of metallic nanoparticles is an important step in the field of nanotechnology. Nowadays, a large number of noble metals have been fabricated for synthesis of nanoparticles but Silver has acquired a large attention due to its diverse applications.

Silver-containing materials were also employed in textile fabrics, as food additives, and in package and plastics to eliminate microorganisms. Oligodynamic silver having antimicrobial efficacy extends well beyond its virotoxicity and it has lethal effects spanned across all microbial domains (Prabhu *et al.*, 2010) Because of such a wide range of applications, numerous methods concerning the fabrication of silver nanoparticles, as well as various silver-based compounds containing ionic silver (Ag<sup>+</sup>) or metallic silver (Ag<sup>0</sup>) have been developed.

**Why- Silver Nanoparticles:** Silver has been given a great preference from ancient times due to high antimicrobial properties. A number of nanoparticles based therapeutics have been approved clinically for infections, vaccines and renal diseases. The application of silver nanoparticles in drug delivery, drug discovery





and new drug therapies have declare war on many dead full diseases and they use the body natural transport pathway and natural mechanism of uptake of the drug by the diseased cells. But with the advent of antibiotics progress, the medical applications of silver as antimicrobial were declined (Castellano *et al.* 2007). Silver nanoparticles having size in the range of 10–100 nm showed strong bactericidal potential against both Gram-positive and Gram-negative bacteria (Morones *et al.* 2005). Generally, silver does not adversely effect viable cells and does not easily provoke microbial resistance. Hence, silver has been incorporated into plastics in various forms (e.g., catheters, dental material, medical devices and implants, and burn dressings) to protect against microbial contamination.

Due to rapid emergence of these multi drug resistant microorganisms, its a great threat to ecological systems. There is a quick need to get rid and to become as faster as these micro-organisms develop their rate of resistance.

Historical review reveals silver being used to treat maladies. Prior to the 1800s, silver was used for treating epilepsy, venereal infections, acne, and leg ulcers. Silver foil applied to surgical wounds was known to improve wound healing and reduce post operative infections, and silver pencils were used to remove warts and to debride ulcers. In the late 19th century, 1% AgNO<sub>3</sub> solution was instilled into conjunctiva sacs to reduce post partum eye infections. In the late 1960s Moyer and Monafio introduced silver nitrate 0.5% solution for burn wound treatment (Demling and DeSanti, 2001; Dunn and Edwards-Jones, 2004). However, silver nitrate dressings are labour intensive as they needed to be applied several times a day or re-moistened 2 hourly. The potency of silver as an antimicrobial was found to be related to the amount and rate of free silver released onto the wounded. In the late 1960s, Fox introduced silversulfadiazine cream for burn wound management. This dramatically revolutionized the management of burn wounds by reducing the incidence of burn wound infections. Silversulfadiazine cream has a relatively short action, its penetration of the burn eschar is poor and it forms a pseudo-eschar. Both silver nitrate dressings and silversulfadiazine cream require a high frequency of dressing changes (Joy and Fiona, 2006).

Disease causing microbes that have become resistant to drug therapy are an increasing public health problem. Therefore there is an urgent need to develop new bactericides (Yaminini, 2011). Antibiotic resistance profiles lead to fear about the emergence and re-emergence of multidrug-resistant (MDR) pathogens and parasites (Tenover 2006). The development of or modification in antimicrobial compounds to improve bactericidal potential is a priority area of research in this modern era (Humberto *et al.* 2010). The most widely accepted application of silver nanoparticles is in medical industry. The bactericidal potential of silver nanoparticles against the MDR bacteria can be used for the treatment and prevention of drug-resistant microbes (Rai, M.K. 2011). The antimicrobial property of silver has attained great attention due to its oligodynamic effect on microbes. Because of such a wide range of applications, numerous methods concerning the fabrication of silver nanoparticles, as well as various silver-based compounds





containing ionic silver (Ag<sup>+</sup>) or metallic silver (Ag<sup>0</sup>) have been developed (Awwad, 2012).

**Best of all nanoparticles: Silver Nanoparticles:** Scientists have been working a lot on fabrication of different nanoparticles by using different noble metals. Different types of nanomaterials like copper, zinc, titanium, magnesium, gold (Gu *et al.*, 2003), alginate (Ahmad *et al.*, 2005) and silver have come up but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms (Gong *et al.*, 2007). Also gold nanoparticles have been exploited as antimicrobial agents, mainly as a tool to deliver other antimicrobials or in order to enhance the photodynamic killing of bacteria (Pissuwan, *et al.*, 2009) but the effects of silver nanoparticles against fungal pathogens are mostly unknown; silver nanoparticles (Stefania, G. 2011). Silver is an effective antimicrobial agent exhibits low toxicity (Farooqui *et al.*, 2010). The antibacterial activity of silver species has been well known since ancient times (Shrivastava *et al.*, 2007).

Silver is a naturally occurring precious metal, most often as a mineral ore in association with other elements. It has been positioned as the 47th element in the periodic table, having an atomic weight of 107.8 and two natural isotopes 106.90 Ag and 108.90 Ag. It is proved by various research work done that silver nanoparticles is having potentiation among all other nanoparticles in medical therapy.

Nanotechnology provides a good platform to modify and develop the important properties of metal in the form of nanoparticles (Rai, 2011). In the 19th century, silver nitrate was used to treat the burns, and it was believed that silver nitrate allows epithelisation and promotes crust formation on the surface of wounds (Castellano *et al.* 2007). Oligodynamic silver having antimicrobial efficacy extends well beyond its virotoxicity and it have lethal effects spanned across all microbial domains (Prabhu *et al.*, 2010). Silver nanoparticles of 5– 20 nm diameter can inhibit HIV-1 virus replication. (Sun *et al.* 2005; Humberto *et al.* 2010). In 1884, aqueous silver nitrate drops were used to prevent the transmission of *Neisseria gonorrhoeae* from infected mothers to children during childbirth (Silvestry-Rodriguez *et al.* 2007). Silver nitrate in the form of 5% solution was used for the treatment for burns, stating that this solution have strong bactericidal potential against *Staph. aureus*, *Ps. aeruginosa* and *E. coli* and does not interfere with epidermal proliferation (Bellinger and Conway 1970).

**Fabrication of Silver Nanoparticles:** There are various ways for fabrication of nanoparticles. Chemical approaches are most popular methods for synthesis of nanoparticles. But it could not avoid toxic effects in the surrounding (Satyavani, 2011). Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable (Geetalakshmi, 2010). Different biological methods are gaining recognition for the production of silver nanoparticles (Ag-NPs) due to their multiple applications. Microorganisms are considered as potential biofactory for synthesis of metallic nanoparticles such as cadmium sulfide, gold and silver (Sastry *et al.*, 2003). Different microorganisms such as bacteria, fungi, and yeasts can be used as nanofactories for the biosynthesis of nanoparticles (Sable, N. 2012) According to





various studies performed fungi are also considered as potential source for fabrication of nanoparticles. Near about 20 different fungi has been investigated for the synthesis of metal nanoparticles( Sable,N. 2012). *Verticillum* sp. reduces metal ions into Au and Ag nanoparticles (Mukherjee *et al.* 2002). *Fusarium oxysporum* produces high stable gold, silver and platinum nanoparticles (Mukherjee *et al.* 2002, Riddin *et al.* 2006). Other reports of nanoparticles synthesis by fungi includes by *Aspergillus niger* (Gade *et al.* 2008), *Fusarium acuminatum* (Ingle *et al.* 2009). There have been several reports on the synthesis of Ag-NPs using medicinal plants such as *Basella alba*, *Helianthus annuus*, *Saccharum officinarum*, *Oryza sativa*, *Sorghum bicolour*, *Zea mays* [19], *Aloe vera* [20], *Medicago sativa* (Alfalfa) [21], *Capsicum annuum* [22], *Magnolia kobus* [23], *Cinnamomum camphora* leaf [24], and *Geranium* sp. [25] from (Zargar, Mohsen, 2011). Living plants (Torresdey *et al.* 2002, 2003) are used for synthesis of gold and silver nanoparticles, part of a plant like from geranium leaf broth (Shivshankar *et al.* 2003, 2004, 2005) or by fruits (Li *et al.* 2007) from (Sable, 2012).

a) **Synthesis of Ag-Nanoparticles by Bacteria:** The biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology has received increasing attention due to its environmentally benign technologies (V., Deepak, 2011). A surprising thing in synthesis of nanoparticles is that the enemies are helping its own enemies. Resistance of bacteria to bactericides and antibiotics has increased in recent years due to the development of resistant strains( Sondi, I., *et al.*, 2004). Silver nanoparticles having size in the range of 10– 100 nm showed strong bactericidal potential against both Gram-positive and Gram-negative bacteria (Morones *et al.* 2005). A novel biological method for synthesis of silver nanoparticles using *Vericillum* was proposed by Mukherjee *et al.* 2001. The first evidence of synthesizing silver nanoparticles was established in 1984 using the microorganism *Pseudomonas stutzeri* AG259, a bacterial strain that was originally isolated from silver mine (Haefeli *et al.* 1984; Zhang *et al.* 2005; Nair and Pradeep 2002). Bacteria isolated from marine water was identified as *Bacillus subtilis* strain GQ413935 on the basis of 16s rDNA sequences and obtained silver nanoparticles of standard size to check its antimicrobial property. The morphology of the nanoparticles is typically spherical whereas Saifuddin *et al.* [2009] found highly variable, with spherical and occasionally triangular nanoparticles from *B. subtilis*. Studies using culture supernatants of bacteria like *Pseudomonas proteolytica*, *Pseudomonas meridiana*, *Arthrobacter kerguelensis*, *Bacillus indicus*, etc., were also proven its property to form extracellular nanoparticles very effectively (Shivaji *et al.* 2011). The formation of extracellular and intracellular silver nanoparticles by bacteria such as *Pseudomonas stutzeri*, *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Salmonella typhus*, and *Staphylococcus aureus* has been investigated (Lengke *et al.*, 2007).





- b) Synthesis of Ag-Nanoparticles by Fungi:** Fungi are potent contributors in the fabrication of silver nanoparticles. Fungi were found to be capable of reducing the metals ions into their corresponding nanometals either in intracellularly or extracellularly depending on the position of the reduction enzymes. Kowshik *et al.* [5] reported the conversion of 3mM silver nitrate solution to nanosilver by *Fusarium oxysporum* in an aqueous medium due to the change in color of the reaction mixture from pale yellow to dark brown. In *Fusarium oxysporum* fungus, the reduction of Ag<sup>+</sup> ions was attributed to an enzymatic process involving NADH dependent reductase (Ahmad *et al.*, 2003). The biosynthesis of Ag NPs using *Aspergillus clavatus* (AzS-275) has been investigated. *A. clavatus*, an endophytic fungus isolated from sterilized stem tissues of *Azadirachta indica* A. Juss. synthesized Ag NPs and were found to be extracellular, polydispersed spherical or hexagonal particles ranging from 10 to 25 nm in size (Verma *et al.*, 2010).
- c) Synthesis of Ag-Nanoparticles by Plants:** The use of environmentally benign materials like plant leaf extract (Parashar *et al.* 2009), bacteria (Saifuddin *et al.* 2009) , fungi (Bhainsa and D'Souza 2006) and enzymes (Willner *et al.* 2007) for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness. Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process (Jose *et al.* 2005; Lok *et al.* 2006). The reducing silver ions present in the aqueous solution of silver nitrate by the help of *Cleome viscosa* extract (Yamini, 2011), Mulberry leaves extract ( Awwad, A. M. And Salem, N.M. 2012), by the root extract of the *Trianthema decandra* plant ( Geetalakshmi and Sarada, 2010), seeds of *Catharanthus roseus* ( Malabadi, B.R. *et al.*, 2012). *Cinnamomum canphora* leaf (Huang *et al.*, 2007). Using green tea, *Camellia sinensis* extract as reducing agent for silver nanostructures in aqueous solution at ambient conditions (Nestor *et al.*, 2008). Plant extracts from live alfalfa, the broths of lemongrass, geranium leaves and others have served as green reactants in Ag NP synthesis (Torresdey *et al.*, 2003; Shankar *et al.*, 2003; Shankar *et al.*, 2005). The reaction of aqueous AgNO<sub>3</sub> with an aqueous extract of leaves of a common ornamental geranium plant, *Pelargonium graveolens*, gave Ag NPs after 24 h (Shankar *et al.*, 2003). A vegetable, *Capsicum annum* L., was also used to synthesize Ag NPs (Li *et al.*, 2007).

Organism	Size(nm)	Author(year)
<b>BACTERIA:</b>		
<i>Pseudomonas stutzeri</i> AG259	<b>200</b>	Tanja <i>et al.</i> (1999)
<i>Bacillus megaterium</i>	<b>46.9</b>	Fu <i>et al.</i> (1999)
<i>Lactobacillus</i> Strains	<b>500</b>	Nair and Pradeep (2002)
<i>Corynebacterium</i> sp.	<b>10-15</b>	Zhang <i>et al.</i> (2005)
<i>Klebsiella pneumonia</i> (culture supernatant)	<b>50</b>	Ahmad <i>et al.</i> (2007)
<i>Staphylococcus aureus</i>	<b>1-100</b>	Nanda and Saravanan (2009)
<i>Lactic acid bacteria</i>	<b>11.2</b>	Sintubin <i>et al.</i> (2009)





<i>Brevibacterium casei</i>		Kalishwaralal <i>et al.</i> (2010)
<b>FUNGI:</b>		
<i>Verticillium sp.</i>	<b>25 ± 12</b>	Mukherjee <i>et al.</i> (2001)
<i>Fusarium oxysporum</i>	<b>5-50</b>	Ahmad <i>et al.</i> (2003)
<i>Aspergillus fumigatus</i>	<b>5-25</b>	Bhainsa and D'Souza (2006)
<i>Aspergillus flavus</i>	<b>8.92 ± 1.61</b>	Vigneshwaran <i>et al.</i> (2007)
<i>Fusarium semitectum</i>	<b>10-60</b>	Basavaraja <i>et al.</i> (2008)
<i>Cladosporium cladosporioides</i>	<b>10-100</b>	Balaji <i>et al.</i> (2009)
<i>Trichoderma viride</i>	<b>5-40</b>	Fayaz <i>et al.</i> (2010)
<b>PLANTS:</b>		
<i>Azadirachta indica</i>	<b>50</b>	Shankar <i>et al.</i> (2004)
<i>Cinnamomum camphora leaf</i>	<b>55-80</b>	Huang <i>et al.</i> (2007)
<i>Cinnamomum camphora Leaf</i>	<b>5-40</b>	Huang <i>et al.</i> (2008)
<i>Phyllanthus amarus</i>	<b>18-38</b>	Kasthuri <i>et al.</i> (2009)
<i>Carica papaya</i>	<b>60-80</b>	Mude <i>et al.</i> (2009)
<i>Coriandrum sativum leaf extract</i>	<b>26</b>	Sathyavathi <i>et al.</i> (2010)

Referred from Deepak V, 2011.

**Mode of action Of Silver nanoparticles on Microbes:** A lot of research has been done to check the antimicrobial action of various microbes. Ag-nanoparticles have already been tested in various field of biological science, drug delivery, water treatment and an antibacterial compound against both Gram (+) and Gram (-) bacteria by various researchers . The exact mechanism which silver nanoparticles employ to cause antimicrobial effect is not clearly known and is a debated topic (Sukumuran Prabhu, 2012). The bactericidal potential of nanoparticles is also influenced by their shapes, which is shown by studying the bacterial growth inhibition by differentially shaped nanoparticles (Morones *et al.* 2005). They analysed the interaction of silver nanoparticles with bacteria by growing the bacterial cells up to mid-log phase, measuring OD at 595 nm, studied the effect of different concentrations of silver on bacterial growth and concluded that concentration up to 75 lg ml)1 was sufficient for bacterial growth but above that, there was no significant bacterial growth (Morones *et al.* 2005). The truncated triangular nanoparticles show bacterial inhibition with silver content of 1 µg. While, in case of spherical nanoparticles total silver content of 12.5 µg is needed. The rod shaped particles need a total of 50 to 100 µg of silver content (Pal *et al.*, 2007). Thus, the silver nanoparticles with different shapes have different effects on bacterial cell.

The silver nanoparticles showed efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. Due to the nano size of nanoparticles, they get attached to the cell membrane and also penetrate inside the bacteria. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their





bactericidal activity (Morones *et al.*, 2005;; Sondi and Salopek-Sondi, 2007; Song *et al.*, 2006). Shrivastava *et al.* 2008 proposed that silver nanoparticles modulate the phosphotyrosine profile of putative bacterial peptides that can affect cellular signalling, which leads to growth inhibition in bacteria.

**Synergistic activity of silver nanoparticles and antimicrobials:** It is worldwide known that silver ions play a crucial role in antimicrobial activity. But it is also true that silver can show good antimicrobial efficacy along with antimicrobial agents. Enhanced antimicrobial activities of commonly used antibiotics were observed in combination with the mycosynthesized AgNPs (Devi, L.S. and Joshi, J.S., 2012).

Synergistic effects also reported by Shahverdi *et al.*, 2007 who reported the increase in antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin in combination with the mycosynthesized AgNPs against *E. coli*, *Pseudomonas aeruginosa* and *S. aureus*. Fayaz *et al.* 2010 also reported the increase in the antibacterial activities of ampicillin, kanamycin, erythromycin, and chloramphenicol in combination with AgNPs against *S. typhi*, *E. coli*, *S. aureus* and *Micrococcus luteus*.

Silver nanoparticles, as well as antimicrobial peptides (AMPs), can be used to fight infectious diseases. Since AMPs are known to permeabilize bacterial membranes and might therefore help silver nanoparticles to access internal target sites (Rudan, S., *et al.*, 2009). Their data thus showed that silver nanoparticles together with all antimicrobial peptides tested exhibit at least an additive effect. They found complete synergy when silver nitrate and silver nanoparticles were used together with polymyxin B and gramicidin S. The combination of silver nanoparticles and polymyxin B showed the most pronounced antibiotic synergy against gram-negative bacteria. The combination of silver nanoparticles with polymyxin B is thus a promising candidate for a new treatment for infections caused by gram-negative pathogens

Therefore, it can be concluded that AgNPs alone or their formulations in combination with commonly used antibiotics can be used as effective bactericidal agents.

#### **Application of Silver nanoparticles:**

**Silver has been considered as the best antimicrobial tool in the field of medicine.** Silver in the form of various compounds and bhasmas have been used in Ayurveda to treat several bacterial infections since time immemorial (Rai, M.K. *et al.*, 2012). Due to development of multidrug resistivity in microbes the development of ormodification in antimicrobial compounds to improve bactericidal potential is a priority area of research in this modern era (Humberto *et al.* 2010). Silver has various applications as discussed so far. Silver nanoparticles having size in the range of 10–100 nm showed strong bactericidal potential against both Gram-





positive and Gram-negative bacteria (Morones *et al.* 2005). Silver in ion or metallic can be exploited in medicine for burn treatment, dental materials, water treatment, sunscreen lotions, etc. (Duran *et al.*, 2007). Researchers have also recommended the use of silver and copper ions as superior disinfectants for wastewater generated from hospitals containing infectious microorganisms (Lin *et al.*, 1996). Silver has been used extensively for the treatment of burns (George *et al.*, 1997). Silver with AgSD ( sulfadiazine) incorporated into bandages for use in large open wounds (Wright *et al.*, 1999).

Nanocrystalline technology appears to give the highest, sustained release of silver to a wound without clear risk of toxicity (Leaper, 2006). Silver zeolite is used in food preservation, disinfection and decontamination of products (Matsuura *et al.*, 1997; Nikawa *et al.*, 1997). Matsumura *et al.* (2003) suggested two possible modes of action of silver zeolite: (i) when bacterial cells come in contact with silver zeolite, cells take in silver ions, which ultimately damage the bacterial cell; and (ii) generation of reactive oxygen species, by which inhibition of respiratory enzyme takes place with the help of silver ions leads to bacterial cell damaging.

**Conclusion:** Bactericidal efficacy of silver nanoparticles was investigated by many researchers and their effective potential against broad range of microbes was proved. Among the various modes of synthesis of silver nanoparticles, biological synthesis is of immense use . The antimicrobial efficacy of the nanoparticles depend on the shapes of the nanoparticles also, this can be confirmed by studying the inhibition of bacterial growth by differentially shaped nanoparticles. Nanotechnology provides a good platform to overcome the problem of resistance, with the help of the silver nanoparticles. Silver nanoparticles of size 10–100 nm have strong bactericidal potential against both Gram-positive and Gram-negative bacteria.

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