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Research review paper

Nanosilver—The burgeoning therapeutic molecule and its green synthesis

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ABSTRACT

Nanotechnology is a field that is burgeoning day by day, making an impact in all spheres of human life. Biological methods of synthesis have paved way for the "greener synthesis" of nanoparticles and these have proven to be better methods due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization. This has motivated an upsurge in research on the synthesis routes that allow better control of shape and size for various nanotechnological applications. Nanosilver has developed as a potent antibacterial, antifungal, anti-viral and anti-inflammatory agent. The recent advancement in the field includes the enzymatic method of synthesis suggesting enzymes to be responsible for the nanoparticle formation. The biomedical applications of silver nanoparticle can be effective by the use of biologically synthesized nanoparticles which minimize the factors such as toxicity and cost and are found to be exceptionally stable. The targeting of cancer cells using silver nanoparticles has proven to be effective, but neither the exact mechanism of action nor the modes of activation of the downstream signaling molecules have been revealed yet. The review illustrates a probable signaling pathway and mechanism by which silver nanoparticles target the cancer cells. The current review also examines the historical background of nanoparticles, role of silver nanoparticles in various biomedical applications and also focusing on better methods of the synthesis of nanoparticles.

Contents

1. Introduction

Silver has known to be a metal that came into use even before Neolithic revolution. Even the Greeks used it for cooking and to keep water safe. The first recorded medicinal use of silver was reported during 8th century (Moyer, 1965a). Silver was known only as a metal till the recent past and it is when the nano era came into existence that

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people started to believe that silver could even be produced at the nanoscale. The intrigue of nanotechnology comes from the ability to control material properties by assembling such materials at the nanoscale. It all started in 1974 when Norio Taniguchi, a researcher at the University of Tokyo, Japan coined the term "nanotechnology" while engineering the materials precisely at the nanometer level (Taniguchi, 1974). The primary driving force for miniaturization at that time came from the electronics industry, which aimed to develop tools to create smaller electronic devices on silicon chips of 40–70 nm dimensions. The use of this term, "nanotechnology" has been growing to mean a whole range of tiny technologies, such as material sciences, where designing of new materials for wide-ranging applications are concerned; to electronics, where memories, computers, components and semiconductors are concerned; to biotechnology, where diagnostics and new drug delivery systems are concerned (Bhatt, 2003; James and Browning, 1999; Sanjeeb and Vinod, 2003; Nathaniel and Mihrimah, 2006; Bohr, 2002).

"Nanotechnology" is the application of science to control matter at the molecular level. It has been well known that living cells are the best examples of machines that operate at the nano level and perform a number of jobs ranging from generation of energy to extraction of targeted materials at very high efficiency. The ribosome, histones and chromatin, the Golgi apparatus, the interior structure of the mitochondrion, the photosynthetic reaction center, and the fabulous ATPases that power the cell are all nanostructures, which work quite efficiently (Goodsell, 2004).

The intrigue in nanomaterial research for regenerative medicine is easy to see and is wide spread. The potential benefits of nanomaterials in biomedical and industrial applications for human health and environment are now accepted in the literature (David et al., 2005; Lanone and Boczkowski, 2006). In the biological context, recent reports focus on the effect of size, shape, bioavailability, uptake and subcellular distribution of such nanomaterials (Sukdeb et al., 2007; Gao et al., 2004). Nanotechnology is the most promising field for generating new applications in medicine. However, only few nanoproducts are currently in use for medical purposes. Silver nanoparticles (AgNPs) have emerged as an arch product from the field of nanotechnology. Silver has gained interest over the years because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activity (Mukherjee et al., 2001; Sondi and Branka, 2004; Chen and Schluesener, 2008). The current review throws light on silver nanoparticles being exploited in medicine for antibacterial, antifungal, anti-viral, anti-inflammatory therapy and also extends an overview of biocompatible methods of synthesis that enhance the usage of these therapies in human system. The review also examines the role of silver nanoparticles in cancer therapy and also covers certain possible formulations of how silver nanoparticles can be exploited in targeted drug delivery.

2. Nanosilver synthesis—an overview

The biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology, has received increasing attention due to a growing need to develop environmentally benign technologies in material synthesis (Huang et al., 2007). Silver nanoparticles can be synthesized through an array of methods like spark discharging, electrochemical reduction, solution irradiation and cryochemical synthesis.

2.1. Traditional methods vs green synthesis of silver nanoparticles

Chemical reduction is the most frequently applied method for the preparation of silver nanoparticles (AgNPs) as colloidal dispersions in water or organic solvents (Tao et al., 2006; Wiley et al., 2005). The reduction of silver ions (Ag^+) in aqueous solution generally yields colloidal silver with particle diameters of several nanometers (Wiley et al., 2005). Initially, the reduction of various complexes with Ag^+ ions lead to the formation of silver atoms (Ag^0), which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of colloidal Ag particles (Kapoor et al., 1994). Controlled synthesis of AgNPs is based on a two-step reduction process. In this technique a strong reducing agent is used to produce small Ag particles, which are enlarged in a secondary step by further reduction with a weaker reducing agent (Lee and Meisel, 1982). Different studies reported the enlargement of particles in the secondary step from about 20–45 nm to 120–170 nm (Schneider et al., 1994; Schirtcliffe et al., 1999; Rivas et al., 2001). Moreover, the initial sol was not reproducible and specialized equipment was needed (Nickel et al., 2000). The syntheses of nanoparticles by chemical reduction methods are therefore often performed in the presence of stabilizers in order to prevent unwanted agglomeration of the colloids.

The biological method of synthesis of nanoparticles have proved to be a better method than the chemical methods due to the large amount of capital involved in production and it involves an energy intensive process. The use of hazardous chemicals such as hydrazine eliminates the method from being an eco-friendly one. Moreover nanocrystalline silver colloids produced by such aqua-chemical routes exhibit aggregation with time, thereby compromising with the size factor upon storage (Kalimuthu et al., 2008; Mukherjee et al., 2008; Parikh et al., 2008). These de-merits recommended the "greener synthesis of nanoparticles" that are environment friendly and involves synthesis at biological pH. Furthermore, due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization.

The green synthesis of AgNPs involves three main steps, which must be evaluated based on green chemistry perspectives, including (1) selection of solvent medium, (2) selection of environmentally benign reducing agent, and (3) selection of nontoxic substances for the AgNPs stability (Raveendran et al., 2003). Amongst these green nanosynthetic routes, biomineralization is an attractive technique. Biomineralization suggests being the best nature friendly method of nanoparticle synthesis. In one of the biomimetic approaches towards generation of nanocrystals of silver, reduction of silver ions has been carried out using bacteria and unicellular organisms. The reduction is mediated by means of an enzyme and the presence of the enzyme in the organism has been found to be responsible for the synthesis (Kalimuthu et al., 2008; Kalishwaralal et al., 2008). Our focus is laid on the biologically synthesized nanoparticles as the particles synthesized by other methods have been found to be anomalous in size and shape and also exhibit certain difficulties when used in therapy.

3. Biological synthesis of silver nanoparticles

3.1. Historical overview

The biological synthesis of nanoparticles germinated from the experiments on biosorption of metals with Gram negative and Gram positive bacterium. The sorption of metals was described using Freundlich adsorption isotherm. Industrially important metals like silver, cadmium, copper etc., were tested to find out if the biological synthesis of these metals were possible or not. Silver was precipitated as discrete colloidal aggregates at the cell surface and occasionally in the cytoplasm (Fig. 1). The electron microscopy results indicated these as aggregates, but these were not known to be nanoparticles during that period. It was in turn inferred that bacterial cells had the capability of binding large quantities of metal. The organisms that were used in testing the sorption of metals include *Bacillus subtilis* and *B. licheniformis* (Gram positive bacteria) and *Escherichia coli* (Gram negative bacteria) (Beveridge and Murray, 1976; Mullen et al., 1989; Doyle et al., 1980; Beveridge and Fyfe, 1985). Cell walls of the Gram positive bacteria were observed to bind larger quantities of several metals than cell envelopes of the Gram negative bacterium (Beveridge and Fyfe, 1985).

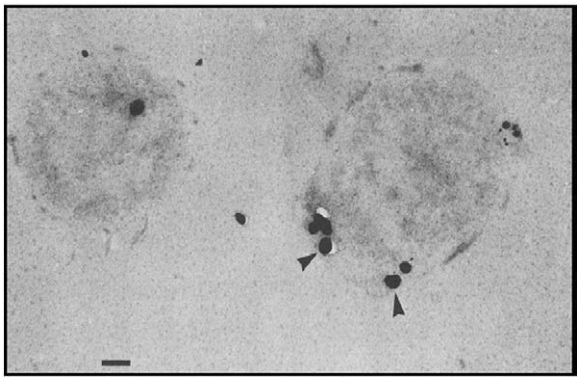

Fig. 1. Transmission electron micrograph of *B. subtilis* cells equilibrated with 1 mM Ag^{+}. Arrows indicate the aggregates of cells formed (Bar 100 nm). Images adapted from (Mullen et al., 1989).

The role of microbial cells in the fate of metals in the environment was not thoroughly examined; however, it was conceived that they represent an important component of metal dynamics (Nies, 1992; White et al., 1997, 1998). The biosynthesis of silver-based crystals came 10 years later when these crystals were identified as nanoparticles which exhibited certain unique properties. The first biosynthesis of nanoparticles was carried out on *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). The TEM of the silver-resistant bacterial strain *P. stutzeri* AG259 cultured in large quantities of silver salts (50 mM $AgNO_3$) showed that the cells can accumulate silver in large quantities. The extent and appearance of the Ag deposition in the periplasm of the bacteria is illustrated in Fig. 2A and B which gives a representative overview of thin sections of a cell culture prepared by removing active cells grown in the dark from an agar plate. The sections demonstrated that most cells exhibited numerous silver-containing particles. Lin et al. (2005) reported biosorption and bioreduction of silver ions by dried *Lactobacillus sp.* A09.

TEM seemed to be an important method of analysis of silver nanoparticles. But later methods like UV–Vis spectroscopy and X-ray diffraction were also used. The absorption spectrum of the sample was recorded on a UV–Visible spectrophotometer. The AgNPs exhibit a unique peak in the range of 400–460 nm and their synthesis is quantified based on the absorbance value obtained at the peak value. X-ray diffraction was carried out to confirm the crystalline nature of the particles. A comparison of the obtained XRD spectrum with that of the standard confirmed that the silver particles formed were in the form of nanocrystals, as evidenced by the peaks at 2θ values of 39.01°, 46.48°, 64.69° and 77.62°, corresponding to 111, 200, 220, and 311 planes, respectively, for silver.

The synthesis of materials with dimensions on the nanometer scale became a continuing challenge in materials science and in microelectronics. This opened new avenues towards the preparation of nanostructured materials that incorporate silver-based crystalline particles with defined structural, compositional, and morphological properties. Other techniques that were available to prepare this type of material were gas condensation and irradiation by ultraviolet or gamma radiation, which were usually associated with low production rate and high expense (Kreibig et al., 1995; Li et al., 1999). Large-scale production of silver-containing materials by chemical deposition or solution reduction produced particles larger than a few micrometers (Tanja et al., 1999).

3.2. Enzymatic approach for the synthesis of nanosilver

Silver nanoparticles are considered to be an important molecule due to its extensive application in medicinal field. Synthesis of silver nanoparticles using α-NADPH-dependent nitrate reductase and phytochelatin *in vitro* has been demonstrated for the first time

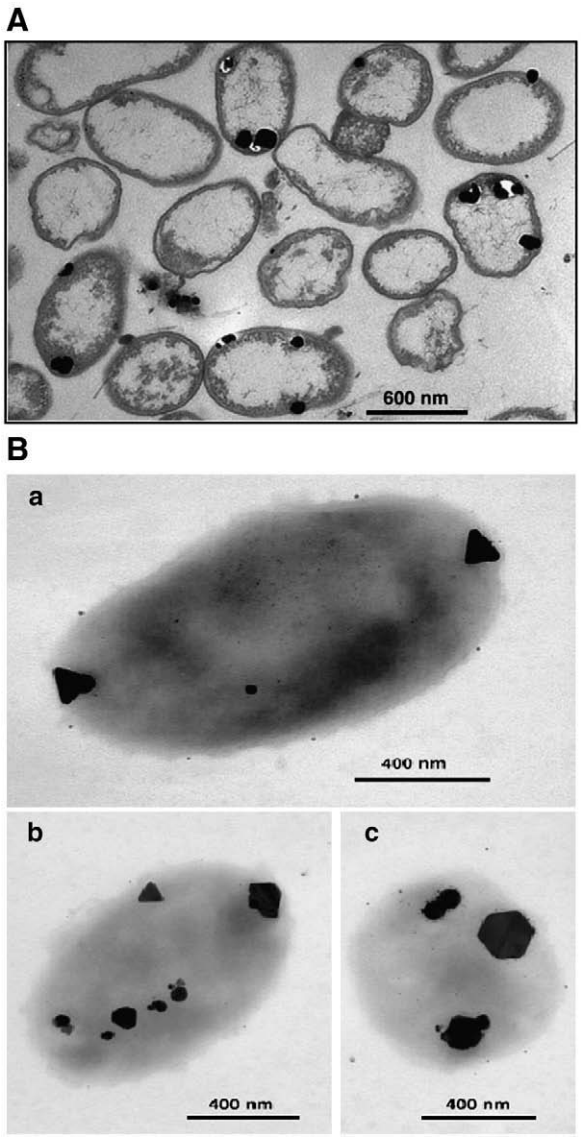

Fig. 2. A: TEM of a thin section of *P. stutzeri* AG259 cells. Large crystalline $Ag^{0}$and Ag_2S particles are deposited between the cell wall and the plasma membrane. Images adapted from (Tanja et al., 1999). B: A variety of crystal topologies; i.e., different morphologies, sizes, and chemical compositions can be produced by *P. stutzeri* AG259. (a) Whole, uncontrasted cell grown on Ag-containing environment with large, triangular, Ag-containing particles at both poles; an accumulation of smaller Ag-containing particles can be found all over the cell. (b and c) Triangular, hexagonal, and spheroidal Ag-containing nanoparticles accumulated at different cellular binding sites. Images adapted from (Tanja et al., 1999).

(Kumar et al., 2007). The organism (*Fusarium oxysporum*) was grown overnight and the supernatant was used to purify the protein nitrate reductase using DEAE and CM column. The synthesis involved the use of silver nitrate (1 mM), phytochelatin (100 μg) α-NADPH (1 mM) and the purified enzyme (100 μg). The TEM analysis confirmed the synthesis of nanoparticles. The use of a specific enzyme in the *in vitro* synthesis of nanoparticles is important because this would do away with the downstream processing required for the use of these nanoparticles in homogeneous catalysis and other applications such as non-linear optics.

Bacterial synthesis of silver nanoparticles has been reported in *B. subtilis*. The synthesis has been reported by use of the culture supernatant and the enzyme nitrate reductase has been found to be present in the supernatant. The exact mechanism of the reduction of metal ions is yet to be elucidated for bacteria. It appears that the reductase together with electron shuttling compounds and other peptides/proteins may be responsible for the reduction of Ag^{+} ions

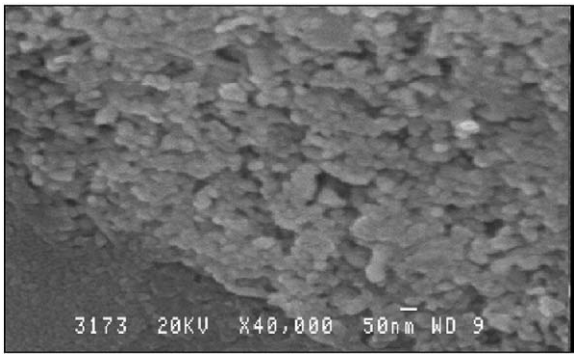

Fig. 3. SEM micrograph of silver nanoparticles formed after reaction of culture supernatant with $AgNO_3$ (1×10^{-3} M) for 24 h (particles at higher resolution shown by scale bar of 50 nm).

and the subsequent formation of silver nanoparticles (Saifuddin et al., 2009).

Among bacteria *B. licheniformis* has been used for both intra and extra-cellular synthesis of silver nanoparticles (Kalimuthu et al., 2008). In *B. licheniformis*, the enzyme is found at the membrane, called as respiratory enzymes. Also in *B. licheniformis* nitrate reductase is found on the membrane that may result in the formation of silver nanoparticles over the invaginated cell membrane (Fig. 3). In all the organisms that synthesize silver nanoparticles nitrate reductase is found to be an integral part of it. Extracellular synthesis of nanoparticles could be highly advantageous from the point of view of synthesis in large quantities and easy downstream processing (Ingle et al., 2008).

Silver nanoparticles seemed to be a defense mechanism of protection from the highly reactive behavior of silver ions (Ag^+) to the cells. The incoming silver ions from the aqueous solution into the cells are converted to reduced state (Ag^0). The synthesis of nanoparticles by this reaction in cells is attributed to the presence of enzyme called nitrate reductase (Fig. 4). The enzyme is found to be present in both aerobes and anaerobes. This enzyme has been exploited from *F. oxysporum*, for the *in vitro* synthesis of silver nanoparticles (Ahmad et al., 2003). This enzyme basically converts nitrate to nitrite and used for the nitrogen cycle in various organisms. The same enzyme during the conversion may shuttle the electron to the silver ions formed in aqueous solutions. Resulting reduced silver may be accumulated and with further reduction of silver ions crystal growth occurs. This mechanism seems to be similar to the formation of magnetic Fe_3O_4 particles by magnetotactic bacteria (Dennis and Richard, 2004).

4. The arsenal of nanosilver synthesizers

Bionanoscience focuses on nanoscale phenomena in biological, biomimicking and bioinspired materials and structures. It focuses on fundamental scientific research to advance nanoscience and nanotechnology as well as biology and medicine. Until now, a wide range of prokaryotes as prospective nanoparticle synthesizers have been witnessed. One major advantage of having prokaryotes as nanoparticle synthesizers is that they can be easily modified using genetic engineering techniques for over expression of specific enzymes, apart from the ease of handling (Kumar et al., 2008; Kalimuthu et al., 2008; Mukherjee et al., 2008; Parikh et al., 2008). However, the use of eukaryotes, especially fungi, is potentially exciting since they secrete large amounts of proteins, thus increasing productivity, and are simple to deal within the laboratory. Moreover the process can be easily scaled up, economically viable with the possibility of easily covering large surface areas by suitable growth of mycelia. Furthermore, downstream processing would be much simpler using fungi. Many microorganisms synthesize silver nanoparticles in both intracellular and extra-cellular systems. Table 1 represents various species synthesizing silver nanoparticles.

5. Impact of nanosilver on biomedicine

The recent emergence of nanotechnology has provided a new therapeutic modality in silver nanoparticles for use in medicine. Silver powder was believed by Hippocrates, father of modern medicine, to have beneficial healing and anti-disease properties and listed as a treatment for ulcers. But it was mainly silver compounds that actually entered medical practice. Silver compounds were major weapons

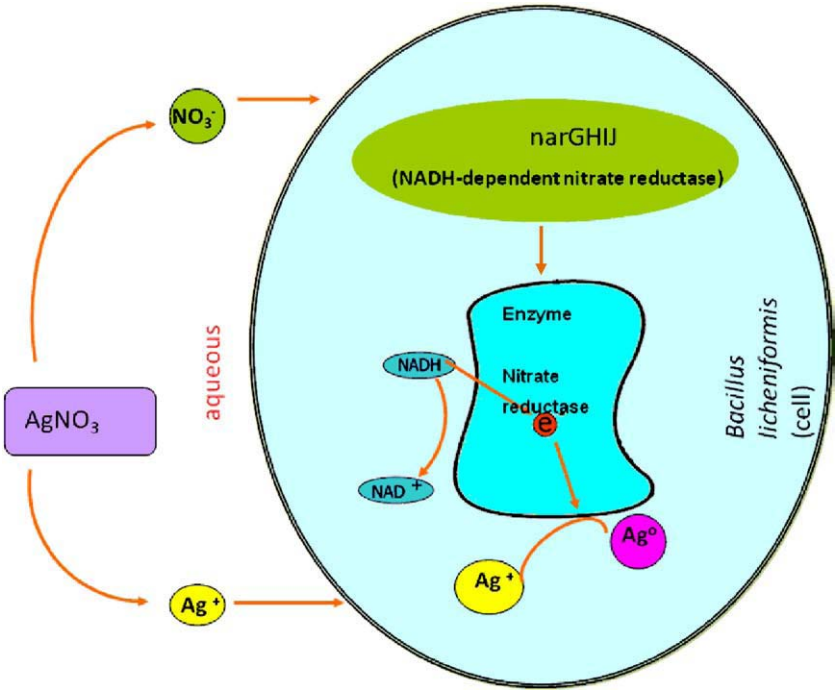

Fig. 4. Possible mechanism for silver nanoparticle synthesis in *B. licheniformis*, involving NADH-dependent nitrate reductase enzyme that may convert Ag^+ to Ag^0 through electron shuttle enzymatic metal reduction process.

Table 1
Various species of microorganisms synthesizing silver nanoparticles.

S. no.	Sources	Biological name	Size	Reference
	Bacteria	*Pseudomonas stutzeri AG259*	200 nm	Tanja et al. (1999)
		Lactobacillus Strains	500 nm	Nair and Pradeep, (2002)
		Bacillus megatherium	46.9 nm	Fu et al. (1999)
		Klebsiella pneumonia (culture supernatant)	50 nm	Ahmad et al. (2007)
		Bacillus licheniformis	50 nm	Kalimuthu et al. (2008)
		Bacillus licheniformis (culture supernatant)	50 nm	Kalishwaralal et al. (2008)
		Corynebacterium	10 to 15 nm	Zhang et al. (2005)
		Bacillus subtilus (culture supernatant)	5–60 nm	Saifuddin et al. (2009)
		Geobacter sulfurreducens	200 nm	Law et al. (2008)
		Morganella sp.	20 ± 5 nm	Parikh et al. (2008)
	Fungus	*Fusarium oxysporum*	5–50 nm	Ahmad et al. (2003)
		Aspergillus fumigatus	5–25 nm	Bhainsa and D'Souza, (2006)
		Aspergillus niger	20 nm	Gade et al. (2008)
		Phanerochaete chrysosporium	100 nm	Vigneshwaran et al. (2006)
		Aspergillus flavus	8.92 ± 1.61 nm	Vigneshwaran et al. (2007)
		Cladosporium cladosporioides	10–100 nm	Balaji et al. (2009)
		Fusarium semitectum	10 to 60 nm	Basavaraja et al. (2008)
		Trichoderma asperellum	13–18 nm	Mukherjee et al. (2008)
	Plant	*Azadirachta indica*	50 nm	Shankar et al. (2004)
		Cinnamomum camphora leaf	55–80 nm	Huang et al. (2007)

against wound infection in World War I until the advent of antibiotics. Metallic silver is subjected to new engineering technologies resulting in extraordinarily novel morphologies and characteristics. Instead of being made "big", metallic silver is engineered into ultra fine particles whose size is measured in nanometers (nm) (Moyer et al., 1965; Wadhera and Fung, 2005; Silver, 2003; Klasen, 2000a,b; Silver et al., 2006). Upon reaching nanoscale, like other nanomaterials and primarily by virtue of extremely small size, silver particles exhibit remarkably unusual physicochemical properties and biological activities. These distinctive properties extend its application in antibacterial, antifungal, anti-viral and anti-inflammatory therapy.

5.1. Nanosilver as a potent bactericidal agent

Microorganisms (bacteria, yeast and fungi) play an important role in toxic metals remediation through reduction of metal ions. Silver nanoparticles have been known to have inhibitory and bactericidal effects and thus extend its application as an antibacterial agent (Chu et al., 1988; Deitch et al., 1987; Margraff and Covey, 1977; Silver, 2003; Atiyeh et al., 2007; Law et al., 2008). The combined effect of silver nanoparticles with antibiotics has proven to be fruitful. Such effects were first observed in *Staphylococcus aureus* and *E. coli* using disk diffusion method. The antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin increased in the presence of AgNPs against both test strains. The same effect was not observed in the case of antibiotics tested. The effects of AgNPs on the antibacterial activity of the aforementioned antibiotics for *E. coli* were lower than *S. aureus*. In contrast, the most synergistic activity was observed with erythromycin against *S. aureus* (Hope et al., 1989).

Bone cement based on polymethylmetacrylate (PMMA) is golden standard for the anchoring of artificial joints. Like all biomaterials PMMA has an elevated risk of infection when implanted into the human body compared to autogenous vital tissue (Breusch et al., 2000; Gristina, 1987). Therefore, the loading of PMMA with antibiotics to reduce infection rate has proven to be a good strategy. Buchholz and Engelbrecht (1970) were the first to load bone cement with antibiotics to reduce infection rates in arthroplasty. The first significant reduction of infection

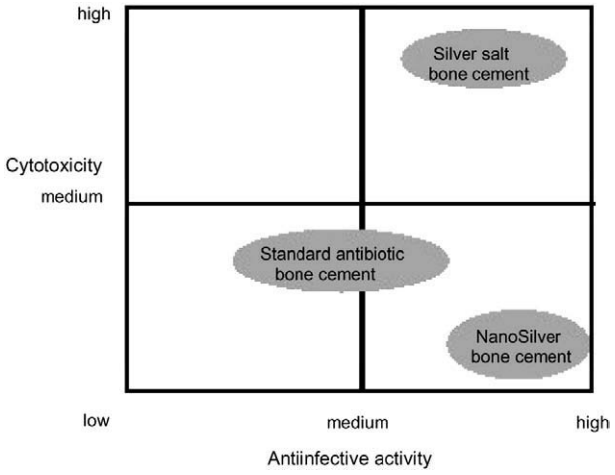

Fig. 5. Overview of cytotoxicity and antibacterial activity. Images adapted from (Alt et al., 2004).

rate by the use of gentamicin loaded bone cement compared to plain PMMA cement was reported by Thierse (1978), Wannske, and Tscherne (1979) confirmed significant difference in antimicrobial activity between antibiotic-loaded and plain PMMA cement in a prospective study. The effect of using silver nanoparticles along with bone cement was found to inhibit the proliferation of the bacterial cells. The increase in concentration of silver increased the antibacterial effect. The cytotoxic levels of the silver nanoparticle bound cement were found to be very less significant and thus it was recommended for treatment of joint arthroplasty (Fig. 5). The most frequently used antibiotics in bone cement are tobramycin or gentamicin (Alt et al., 2004).

Silver has been known to have effective bactericidal properties for centuries. The antibacterial activity of silver nanoparticles can be extended to the Textile Industry as well. The biologically synthesized silver nanoparticles could be of immense use in medical textiles for their efficient antimicrobial function (Vigneshwaran et al., 2006). The sterile cloth and materials play an important role in hospitals, where often wounds are contaminated with microorganisms, in particular fungi and bacteria, like *S. aureus* (Lee et al., 2003). Thus, to reduce or prevent infections, various antibacterial disinfections techniques have been developed for all types of textiles. The silver nanoparticles were incorporated in cotton and silk cloths. Anti-bacterial activity was observed when silver nanoparticles were incorporated in cotton cloth (Durán et al., 2007).

5.2. Nanosilver in antifungal therapy

Fungal infections have become more common in the recent years. In particular, fungal infections are more frequent in patients who are immunocompromised because of cancer chemotherapy, or organ or human immunodeficiency virus infections (Groll et al., 1996). The availability of the antifungal drugs is limited because prophylaxis with antifungals may lead to the emergence of resistant strains. Progress in the development of both topical and systemic antifungal agents lagged, due in part to the intensive research efforts in the area of antibacterial therapy which began in the 1940s following the large-scale production of penicillin and also to the relatively low incidence of serious fungal infections compared with that of bacterial infections. By 1980, members of the four major classes of antifungal agents–polyenes, azoles, morpholines, and allylamines–had been identified, yet the only new drug introduced for the treatment of systemic fungal infections was oral ketoconazole (Kauffman and Carver, 1997). The development of newer antifungal drugs is creating new potential combination therapies to combat the dismal mortality rate. Many studies have shown the antimicrobial effects

Table 2
Antifungal activity of AgNPs derived from comparison of IC_{80} values of AgNPs, Amphotericin B and Fluconazole for various strains.

Fungal strains	IC_{80} (μg/ml)		
	Nano-Ag	Amphotericin B	Fluconazole
C. albicans (4)	2–4	5	10–16
C. tropicolis (2)	7	2–4	13
C. glabrata (4)	1–7	2	10–16
C. parapsilosis (3)	4–25	2	13
C. krusei (1)	13	4	13
T. mentagrophytes (30)	1–4	1–2	20–30

of AgNPs, but the effects of AgNPs against fungal pathogens are mostly unknown.

The antifungal effects of silver nanoparticles and their mode of action were investigated. Antifungal effects on fungi tested with low hemolytic effects against human erythrocytes were observed. Although antifungal drug resistance does not seem to be as much of a problem as resistance to antibacterial agents in bacteria, one long-term concern is that the number of fundamentally different types of antifungal agents that are available for treatment remains extremely limited. This is because fungi are eukaryotic organisms with a structure and metabolism that are similar to those of eukaryotic hosts. Therefore, there is an inevitable and urgent medical need for antibiotics with novel antimicrobial mechanisms. Though the biocidal effect and mode of action of silver ion are known, nevertheless, the antifungal effects and the mode of action of AgNPs against fungi have remained mostly unknown (Kim et al., 2008a).

Amphotericin B, an antifungal agent used to treat serious systemic infections was used as a positive control to compare with AgNPs (Hartsel and Bolard, 1996). AgNPs showed significant antifungal activity against *T. mentagrophytes* and *Candida* species. Towards all fungal strains, AgNPs exhibited similar activity with amphotericin B, but more potent activity than fluconazole. However, this compound exhibited less potent activity than amphotericin B for *C. parapsilosis* and *C. krusei*. The effect of AgNPs, amphotericin and fluconazole on the organisms are described using the IC_{80} range and are tabulated in Table 2. The serum-induced mycelia were significantly inhibited from extending and forming in the presence of AgNPs, but the mycelia formed was normal in the absence of AgNPs (Kim et al., 2008b). The hemolytic activity was evaluated by the percentage of hemolysis at the concentration range of 1.25–10 μg/ml of AgNPs. AgNPs caused 6% lysis of erythrocytes at a concentration of 10 μg/ml, while amphotericin B caused 10% lysis at the same level. These results strongly support silver nanoparticle being a potent antifungal agent.

The ability of AgNPs to disrupt the fungal envelope structure was documented using Transmission Electron Microscope. The treated fungal cells showed significant damage, which was characterized by the formation of a "pit" in their cell walls and pores in their plasma membrane (Fig. 6A, B and C). The intracellular physiology is elicited by the study on the effects of cell cycle in the fungal cells. The percentage of cells in the G_2/M phase increased by 15%, while that in the G_1 phase significantly decreased by about 20% in the presence of AgNPs. The cells were cultured in the presence or absence of AgNPs and their DNA content was determined via flow cytometry by staining with propidium iodide (PI). PI is a DNA-staining dye that intercalates between the bases of DNA or RNA molecules (Tas and Westerneng, 1981).

5.3. Anti-viral nanosilver and its HIV interaction

Nanomaterials with such excellent properties have been extensively investigated in a wide range of biomedical applications, in particular regenerative medicine. Silver nanoparticles have been used to exhibit the antimicrobial efficacy against viral particles. Monkeypox virus (MPV), an orthopoxvirus similar to variola virus, is the causative agent of monkeypox in many species of non-human primates (James et al., 2008). Silver nanoparticles have been shown to exhibit promising cytoprotective activities towards HIV-infected T-cells; however, the effects of these nanoparticles towards other kinds of viruses remain largely unexplored.

Monkeypox virus is a human pathogen with a clinical presentation similar to that of smallpox (James et al., 2008). MPV is considered a big threat to human life and therefore research is being carried out to develop drugs and therapeutic agents against this virus. The versatile properties of silver nanoparticles have driven its use to provide resistance against viruses. The surface plasmon resonance and large effective scattering cross section of individual silver nanoparticles make them ideal candidates for molecular labeling, where phenomena such as surface enhance Raman scattering (SERS) can be exploited. The research on the nanoparticle–HIV-1 interaction demonstrated that silver nanoparticles inhibited HIV-1 infectivity *in vitro* by binding to the disulfide bond regions of the CD4 binding domain within the gp120 glycoprotein subunit. Binding of these nanoparticles to the gp120 subunit appeared to be size dependent as particles greater than 10 nm were not observed attached to the viral envelope (Elechiguerra et al., 2005). Thus the use of silver-containing nanoparticles as an anti-viral therapeutic may be a new area of developing nanotechnology-based anti-viral therapeutics.

The recent reports suggest that Ag-PS-10 and $AgNO_3$ were effective at reducing MPV-induced plaque formation *in vitro* at concentrations ranging from 12.5 to100 μg/ml. This induced the reduction in HIV-1 infectivity and this reduction was considered to be size dependent, as only silver-containing nanoparticles ranging from 1 to 10 nm in diameter established a strong enough physical interaction with the gp120 glycoprotein of HIV-1 virions to inhibit viral binding to a host cell.

Although these experiments indicate that Ag-PS-10 and $AgNO_3$ decrease MPV plaque formation, the mechanism by which this inhibition occurs is not known and has been proposed that it could involve blockade of host cell binding, disruption of host cell biochemical pathways, or both. Poxvirus entry into a host cell can occur by endocytosis or direct fusion with the plasma membrane, which is followed by a regulated sequence of events leading to viral replication.

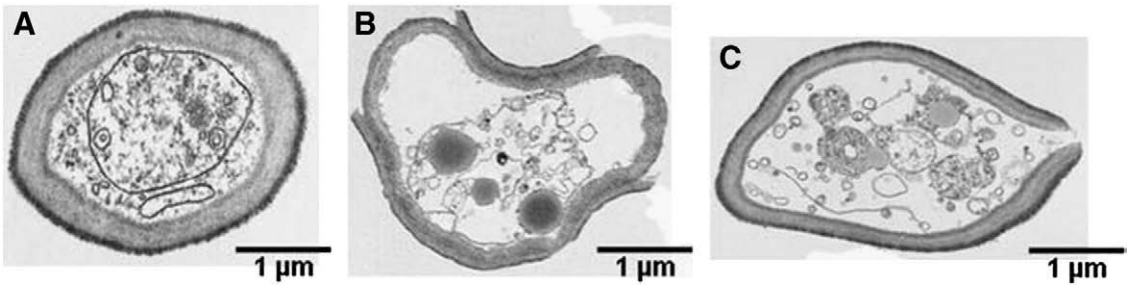

Fig. 6. Transmission electron micrograph of *C. albicans* cells. (A) Control with no AgNPs, (B) treated with 170 μg/ml of AgNPs, (C) 400 μg/ml of AgNPs. Images adapted from (Kim et al., 2008b).

The specific cellular receptors or proteins involved in poxvirus fusion with the plasma membrane and subsequent cellular entry are not known. The experiments reveal that inhibition of MPV plaque formation could be due to the physical obstruction of virus–host cell binding by nanoparticles and the internalization of metal-based nanoparticles by cultured cells and the subsequent changes in cellular biochemistry suggest there is also a possibility for disruption of intracellular pathways that could ultimately attenuate viral replication (James et al., 2008).

Silver nanoparticles could also inhibit the *in vitro* production of HBV RNA and extra-cellular virions. It has been proposed that the direct interaction between these nanoparticles and HBV double-stranded DNA or viral particles is responsible for their anti-viral mechanism (Lut et al., 2008). Many other studies were involved to study the viral–nanoparticle interaction. One of them is embedding nanoparticles with foamy carbon matrix and this embedding prevents coalescence during their synthesis. TEM analysis shows that the nanoparticles tend to be agglomerated inside the foamy carbon matrix, although a significant fraction of the population is released from this matrix by the energy provided from the ultra-sonic bath (Fig. 7A). These released nanoparticles are mainly free surface nanoparticles, and only those nanoparticles released from foamy carbon matrix interact with the HIV-1 cells (Fig. 7B and C). The TEM results indicated that these particles were smaller in size (about 16 nm). It also revealed that the sample is composed of several morphologies including multi-twinned nanoparticles with five-fold symmetry, i.e. decahedra and icosahedra, truncated pyramids, octahedral and cuboctahedral nanoparticles, among others.

PVP-coated nanoparticles were synthesized by the polyol method using glycerine as both reducing agent and solvent. This method involves a metal precursor being dissolved in a liquid polyol in the presence of a capping agent such as PVP (Bonet et al., 1999). PVP is a linear polymer and stabilizes the nanoparticle surface via bonding with the pyrrolidone ring. Infrared (IR) and X-ray photoelectron spectroscopy (XPS) studies have revealed that both oxygen and nitrogen atoms of the pyrrolidone ring can promote the adsorption of PVP chains onto the

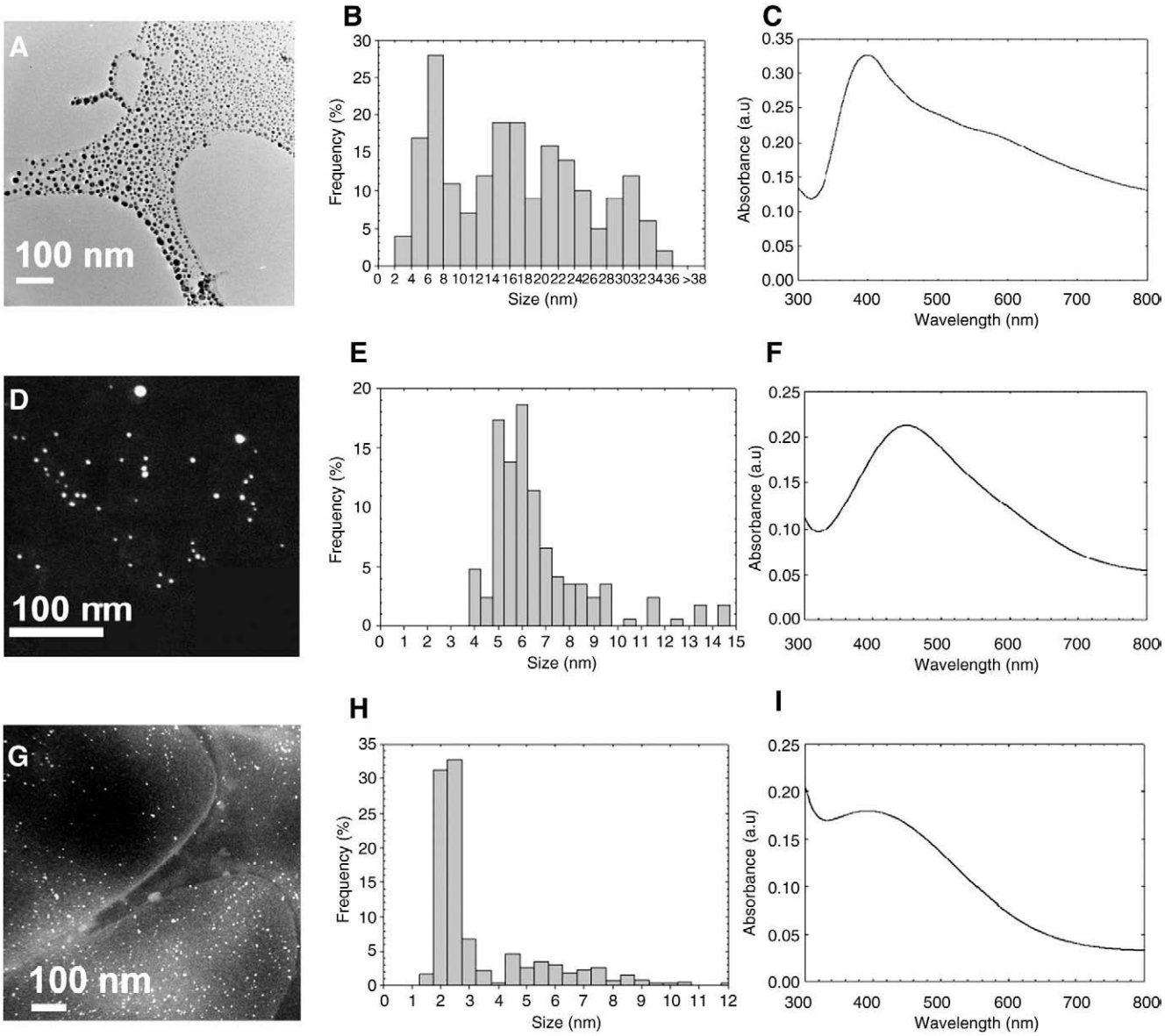

Fig. 7. TEM image of free surface silver nanoparticles released from the foamy carbon matrix by dispersing the as-received powder in de-ionized water by ultra-sonication. B) Size distribution of free surface nanoparticles measured by TEM analysis. C) UV–Visible spectrum of carbon-coated silver nanoparticles. Images adapted from (Elechiguerra et al., 2005). D) HAADF image of PVP-coated silver nanoparticles. E) Size distribution of PVP-coated nanoparticles measured by TEM analysis. F) UV–Visible spectrum of PVP-coated silver nanoparticles. Images adapted from (Elechiguerra et al., 2005). G) HAADF image of BSA-coated silver nanoparticles. H) Size distribution of BSA-coated nanoparticles measured by TEM analysis. I) UV–Visible spectrum of BSA-coated silver nanoparticles. Images adapted from (Elechiguerra et al., 2005).

surface of silver (Fig. 7D) (Wiley et al., 2005). The sample size distribution was obtained from high angle annular dark field (HAADF) images (Fig. 7E and F) (Elechiguerra et al., 2005). Silver nanoparticles directly conjugated to BSA protein molecules were synthesized in aqueous solution (Fig. 7G). Serum albumin is a globular protein, and is the most-abundant protein in blood plasma. Bovine serum albumin (BSA) is a single polypeptide chain composed of 583 amino acid residues (Peters, 1996). Several residues of BSA have sulfur, oxygen, and nitrogen bearing groups that can stabilize the nanoparticle surface. The strongest interactions with silver likely involve the 35 thiol-bearing cysteine residues. By using sodium borohydride, a strong reducing agent, BSA stabilizes nanoparticles via direct bonding with these thiol-bearing cysteine residues, and provides steric protection due to the bulkiness of the protein. The sample size distribution was obtained from HAADF images (Fig. 7H and I) (Elechiguerra et al., 2005).

High angle annular dark field (HAADF) scanning transmission electron microscopy was employed to study the interaction of silver nanoparticles with HIV-1. HAADF images are primarily formed by electrons that have undergone Rutherford backscattering (James and Browning, 1999). As a good approximation, lighter elements appear dark and heavier elements appear bright. Due to a large difference in atomic number, silver nanoparticles are easily distinguished from the organic matter that composes the virus. The presence of silver was independently confirmed by Energy Dispersive X-ray Spectroscopy (EDS). The sizes of nanoparticles bound to the virus were exclusively within the range of 1–10 nm (Elechiguerra et al., 2005).

Both the spatial arrangement of nanoparticles and the size dependence of interaction can be explained in terms of the HIV-1 viral envelope, and can provide insight into the mode of interaction between the virus and nanoparticles. The exterior of the HIV-1 virus is comprised of a lipid membrane interspersed with protruding glycoprotein knobs, formed by trimers consisting of two subunits: the gp120 surface glycoprotein subunit is exposed to the exterior, and the gp41 transmembrane glycoprotein subunit spans the viral membrane and connects the exterior gp120 glycoprotein with the interior p17 matrix protein (Ghosh et al., 2008). The main function of these protruding gp120 glycoprotein knobs is to bind with CD4 receptor sites on host cells. Numerous cellular proteins are also embedded within the viral envelope (Forster et al., 2000). However, the protruding gp120 glycoprotein knobs are more exposed to the exterior, and should be more accessible for potential nanoparticle interactions. These correlate with the structural model for HIV-1 proposed by Arthur et al. (1992). The observed darker contrast at these sites could indicate the locations of the glycoprotein knobs. Presuming that the most attractive sites for interaction are the sulfur-bearing residues of the gp120 glycoprotein knobs, there are only a limited number of bonds that a nanoparticle can form. This limited number of stabilizing sites can explain why larger nanoparticles are not observed to attach to the virus.

5.4. Silver nanoparticles and its anti-inflammatory effect

Despite the many billions of dollars that have been spent on immunological research, few effective anti-inflammatory drugs have emerged. An urgent need for new drugs exists, as many inflammatory diseases are inadequately responsive to current medications (Charles, 2008). Moreover, in developed countries, the incidence of some inflammatory diseases, such as asthma, has increased markedly over recent decades (Maria et al., 2002). The incidence of some autoimmune diseases, including type-1 diabetes, is also on the rise, further suggestive of links between inflammatory diseases and aspects of modern lifestyles (Wright et al., 2002). The challenge for the development of new anti-inflammatory drugs has been to find appropriate targets that are essential in the inflammatory process but are mostly dispensable for host defense against pathogens. The development of several new drugs is poised to revolutionize the treatment of inflammatory diseases. Therapeutics that selectively alters cell migration represents a particularly promising class of the new anti-inflammatory drugs. The pharmaceutical industry has targeted various types of molecules to subdue inflammatory diseases. Drugs that disrupt cell migration appear particularly promising in clinical trials and in many animal models of inflammatory disease.

Nanocrystalline silver dressings were introduced commercially as antimicrobial dressings in 1998 and these have found to improve wound healing (Wright et al., 2002), which may result from potent anti-inflammatory activity. This unusual activity of nanocrystal is said to occur due to its small size (Bhol et al., 2004). Nanocrystalline silver has unique dissolution behavior, releasing Ag^0 into solution (Demling and Burrell, 2002; Fan and Bard, 2002). This species (Ag^0) is said to exhibit anti-inflammatory activity and this was illustrated when it was tested on animal models (Mizushima et al., 1965). Au^0 nanoparticles suppress the activity of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) while relieving rheumatoid arthritis symptoms, indicating it may have an anti-inflammatory effect (Abraham and Himmel, 1997). The crystal structure (face-centered cubic) and Pauling covalent radii for silver and gold are equal. Thus, silver and gold can replace each other in crystal lattices, suggesting that Au^0 and Ag^0 clusters should be identical physically, and therefore may have similar biological activity (Bayler et al., 1996). The treatment of murine infected burns with silver nanoparticles was found to increase the rate of healing and decrease the scarring in comparison with silver sulfadiazine. This was accompanied by increased expression of IL-10, vascular endothelial growth factor, and interferon-γ, with reduced IL-6 expression. In a porcine infected wound model, nanocrystalline silver treatments enhanced tissue regeneration while decreasing erythema and edema relative to silver nitrate ($AgNO_3$) treatments (Tian et al., 2007; Wright et al., 2002). Nanocrystalline silver treatments were also found to increase the polymorphonuclear cell apoptosis while the matrix metalloproteinase (MMP) levels remained low, suggesting an anti-inflammatory effect.

In dinitronitrofluorobenzene-induced mouse ear rashes, an emollient cream-based nanocrystalline silver treatment significantly reduced erythema, edema, and expression of IL-12 and TNF-α, while increasing apoptosis in inflammatory cells. The anti-inflammatory effect of silver nanoparticles was checked on porcine skin, an excellent model of human skin (Bhol et al., 2004; Moyer, 1965b). Inflammation was induced by means of DNCB and the control was compared to that of the ones treated with Saline (0.9%), $AgNO_3$ (0.5%) and nanocrystalline silver. Day by day observations were made and it was observed that the wound healing was much more significant.

5.4.1. Apoptosis detection

Apoptosis is a form of cell death with unique morphological and biochemical hallmarks. It is involved in eliminating inflammatory cells from inflamed tissues. Hence, compounds that induce apoptosis, such as noble metals, may be beneficial in the treatment of inflammatory diseases. Various gold-containing compounds, used as anti-inflammatory agents in the treatment of rheumatoid arthritis, induce apoptosis in cells including T-cells and macrophages through multiple mechanisms (Serhan and Savill, 2005; Rigobello et al., 2002). The induction of apoptosis by nanocrystalline silver at the dermal cells suggests a highly discriminatory process, related to the unique silver species released (e.g., Ag^0), that is different from the indiscriminate activity of Ag^+. Silver ions can interfere with the respiratory chain at the cytochromes and can interact with the electron transport chain to activate the intrinsic signaling pathway to apoptosis through the activation of downstream pro-caspases. Saline-treated pigs demonstrated some apoptosis in the epidermis and upper dermis (Fig. 8D,E and F). $AgNO_3$-treated pigs showed high levels of apoptosis near tissue surfaces. Keratinocytes, as well as some inflammatory cells and fibroblasts, show apoptotic staining, suggesting that $AgNO_3$ induced cell death in all the cell types it contacted (Fig. 8G, H and I). In contrast, animals treated with nanocrystalline silver demonstrated increased inflammatory cell apoptosis compared with all other groups (Fig. 8J, K and L). This apoptosis

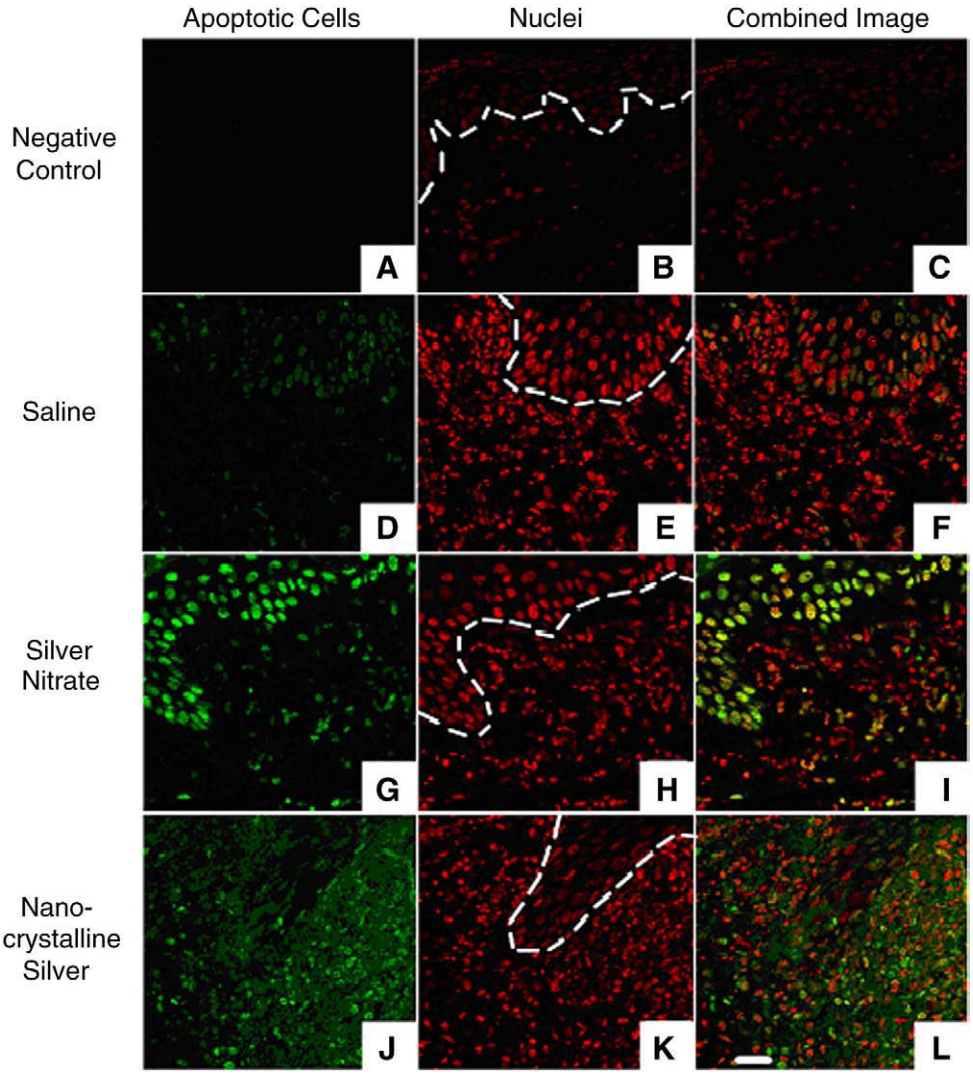

Fig. 8. Representative fluorescence images and quantification of immunohistochemical detection of apoptotic cells after 24 h of treatment. Dashed white lines delineate the epidermal/dermal junction. From left to right the columns show staining for apoptotic cells (green) and nuclei (red); and the combined image (overlapping staining for apoptosis and nuclei appears yellow). A–C, Negative control. D–F, saline-treated. G–I, $AgNO_3$-treated. J–L, Nanocrystalline silver treated. Images adapted from (Nadworny et al., 2008). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was in the dermis and did not target keratinocytes. This significant effect reveals the unique nature of silver nanoparticles in wound healing. Nanocrystalline silver has anti-inflammatory activity and this is due to the induction of apoptosis in inflammatory cells, and suppression of MMP activity. Nanocrystalline silver suppresses the production of proinflammatory cytokines TNF-α, IL-8, and TGF-β, and may impact others, including IL-12. Overexpression of MMPs including MMP-2 and MMP-9 contributes to tissue injury and inflammation. Therefore, MMP inhibition has been suggested as a therapeutic approach to controlling inflammation and the similarities between the model system and human skin suggests the use of silver nanoparticles for as better anti-inflammatory agent (Nadworny et al., 2008).

5.5. Nanosilver in cancer therapy

The integration of nanotechnology with biotechnology and medicine means the ability to uncover the structure and function of biosystems, which intrinsically have an organizational level at the nanoscale (Kairemo et al., 2008). Nanotechnology maybe translated into nanomedicine thereby referring to treatment and curing of diseases at a molecular scale. Indeed, the use of nanoparticles (100 nm or smaller) for delivery and targeting of therapeutic and diagnostic agents is at the forefront of projects in cancer medicine. The targeting and accumulation of drugs to specific sites where the agent is released provides a means to reach high drug concentration at a designated area with far less systemic side effects (John et al., 2008).

Cancer remains one of the world's most devastating diseases and current cancer treatments include surgical intervention, radiation and chemotherapeutic drugs, which often also kill healthy cells and cause toxicity to the patient. Both the field of drug design and cancer imaging are going to gain from a development that is generally regarded as a fundamental breakthrough in medicine, namely the introduction in nanotechnology (Peer et al., 2007). Progression of tumors has been attributed to the cumulative accumulation of multiple alterations throughout the genome, which is manifested by genomic instability (Viswanathan et al., 2003). Ubiquitously targeting cells within a tumor is not always feasible because some drugs cannot diffuse efficiently and the random nature of the approach makes it difficult to control the process. This lack of control may induce multiple-drug resistance (MDR), a situation where chemotherapy treatments fail in patients owing to resistance of cancer cells towards one or more drugs. MDR occurs because transporter proteins that expel drugs from cells are over-expressed on the

surface of cancer cells. Expelling drugs inevitably lowers the therapeutic effect and cancer cells soon develop resistance to a variety of drugs.

The emerging trend of using nanoparticles as drug carriers has exploited the potential of nanoparticles to revolutionize cancer therapy. Silver has been now recognized as a developing therapeutic molecule and will surely extend its use as a drug carrier. Silver nanoparticles can be used for both active and passive targeting of drugs. Silver nanoparticles have recently emerged as an attractive candidate for delivery of various payloads into their targets. The payloads could be small drug molecules or large biomolecules, like proteins, DNA or RNA. Efficient release of these therapeutic agents is a prerequisite for effective therapy. The release could be triggered by internal (e.g. glutathione (GSH), or pH or external (e.g. light stimuli) stimulus. This binding may be achieved by attaching targeting agents (ligands) to the surface of the nanocarrier by a variety of conjugation chemistries. Nanocarriers will recognize and bind to target cells through ligand–receptor interactions, and bound carriers are internalized before the drug is released inside the cell. In general, when using a targeting agent to deliver nanocarriers to cancer cells, it is imperative that the agent binds with high selectivity to molecules that are uniquely expressed on the cell surface. It is also possible to increase binding affinity and selectivity to cell surface targets by engineering proteins that detect a specific conformation of a target receptor (Ghosh et al., 2008).

The use of nanoparticles as a carrier has become widespread. But yet another aspect of nanoparticles that needs attention is their resplendent property of acting as a drug by itself. The biggest question that arises when such an aspect is laid focus is the mechanism by which the particles evade the cancer cells. The answer is yet to be revealed. Many attempts have been made to use silver nanoparticles as an anti-cancer agent and they have all turned up positive. The next milestone would be the discovery of the mechanism of action. The possible mechanism by which silver nanoparticles target cancer cells is illustrated in Fig. 9.

The targeting of silver nanoparticles towards cancer cells involves certain limitations. The first question that arises in one's mind is regarding the toxicity of silver nanoparticles towards the body cells. The question is inevitable from the discussion as nanoparticles of larger size have proven to be toxic to the cells. Larger size nanoparticles also do not bind with the receptors, thus reducing the targeting efficacy. One way to overcome this limitation is to use particles of smaller size (5–20 nm). This particle size range has proven to be less toxic to the cells and also exhibit higher binding affinity towards the receptors. Similar results have been obtained when the particles were checked for interaction with HIV-1 (Elechiguerra et al., 2005). One reason could be the difference in the curvature of the different-shaped nanoparticles. For example, the rod-shaped nanoparticles can have larger contact area with the cell membrane receptors than the spherical nanoparticles when the longitudinal axis of the rods interacts with the receptors. This, in effect, could reduce the number of available receptor sites for binding. A second reason could be the amount of CTAB surfactant molecules adsorbed onto the rod-shaped nanoparticle surface during synthesis. If the CTAB was still on the surface, the serum protein may not be able to bind onto the silver nanoparticle surface efficiently. Also, the protein coating on the surface of the rod-shaped silver nanoparticles may not be homogeneous. In such a case, the ligands on the surface of the rod-shaped silver nanoparticles may not bind to receptors on the cell surface as strongly (due to a lack of multivalent binding). This would affect the uptake of the nanoparticles. The other question that

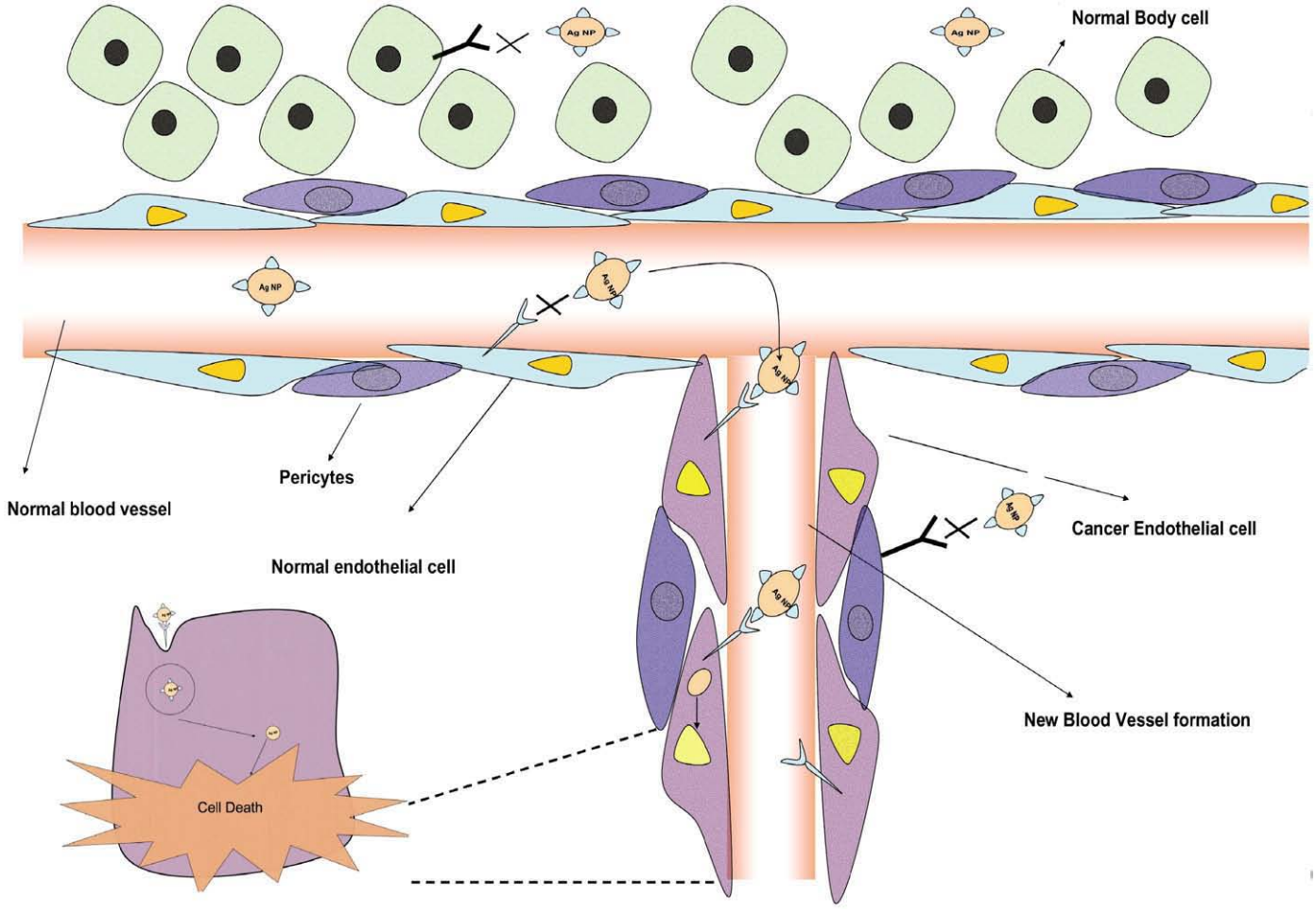

Fig. 9. Possible mechanism for targeted delivery of silver nanoparticles in cancer therapy.

would arise is the specific binding of silver nanoparticles towards cancer cells but not the other body cells. One possible reason could be due to morphological differences between cancer cells and the other body cells. The cancer cells are different in pore size when compared to the other cells and so a size controlled targeting of silver nanoparticles can prove effective in the case of cancer treatment. Mechanisms that govern size- and shape-dependent intracellular uptake of nanoparticles can be speculated. Clearly, nonspecific adsorption of serum proteins mediates the uptake of the nanoparticles. The presence of these proteins on the surface of the nanoparticles dictates uptake half-life, rates, and amount.

The role of silver nanoparticles can be further exploited by exploring the signaling pathways triggered by silver nanoparticle inside the cancerous cells. Growth and maturation of a functional vascular network are complex and still incompletely understood processes involving orchestrated activation of vascular progenitors in the early stages of embryonic development followed by vasculogenesis and angiogenesis. These processes require a tightly regulated activation of several growth factors and their receptors. Protein and lipid transport along the secretory pathway in eukaryotes involves the selective packaging and delivery of cargo from one compartment to the next. This is accomplished by carrier vesicles and tubulovesicular structures that bud from a given donor compartment and fuse with a specific target compartment (Gurunathan et al., 2000). The biogenesis of carrier vesicles is an important aspect of membrane transport along the secretory pathway (Gurunathan et al., 2002). The mechanism by which carrier membranes identify their correct target and undergo docking and fusion, requires the knowledge of the membrane associated proteins (Gurunathan et al., 2000). The signaling model proposed explains the possible targets of nanosilver and also its targeted delivery using a tissue specific ligand (Fig. 10). The targeting of nanosilver without a ligand have shown to inhibit the PI3/Akt phosphorylation (data not shown), thus preventing cell survival. The uptake of silver nanoparticle inside the cell could be mediated by the binding to an unknown receptor. The inhibition of PI3/Akt phosphorylation has a sustained effect on the cell survival and so the next big concern would be the role of silver nanoparticles in apoptosis.

Apoptosis is a tightly regulated and at the same time highly efficient cell death program which requires the interplay of a multitude of factors. Apoptotic pathways involve the activation of the downstream of mitochondrial proapoptotic events. Our study so far focused on the effect of silver nanoparticles on Caspase-3 activation, the final downstream molecule of the Caspase cascade. Silver nanoparticles have shown to be effective in triggering the activation of Caspase-3 molecule and thus resulting in the mediation and amplification of the death signal. The activation of the Caspase cascade could be through any of the three possible ways which include; granzyme B mediated activation, death receptor mediated activation and the apoptosome mediated activation which includes the release of cytochrome c. The study on Caspase-3 alone gives a very transparent idea making the discussion very ambiguous and so examining the other caspases would be the next level of interest. There is yet another point of discussion which involves the evidence for the molecule leading to only programmed cell death and not necrosis, although both the mechanisms result ultimately in cell death. The IC_{50} values would determine the level of toxicity to the cells and so for apoptosis to occur the toxicity effect must be less significant. The Caspase-3 activation makes it evident that it leads to cleavage of Caspase substrates, resulting in the fragmentation of the DNA. Thus, the triggering of the death inducing signal forms an important area of interest in the trafficking of the nano molecule.

Angiogenesis is an important biological process not only under physiological conditions but also in a variety of diseases including cancer, diabetic retinopathy and rheumatoid arthritis (Banumathi et al., 2009). VEGF signaling often represents a critical rate-limiting step in physiological angiogenesis. The successful implementation of this process depends upon the balance of growth promoting factors

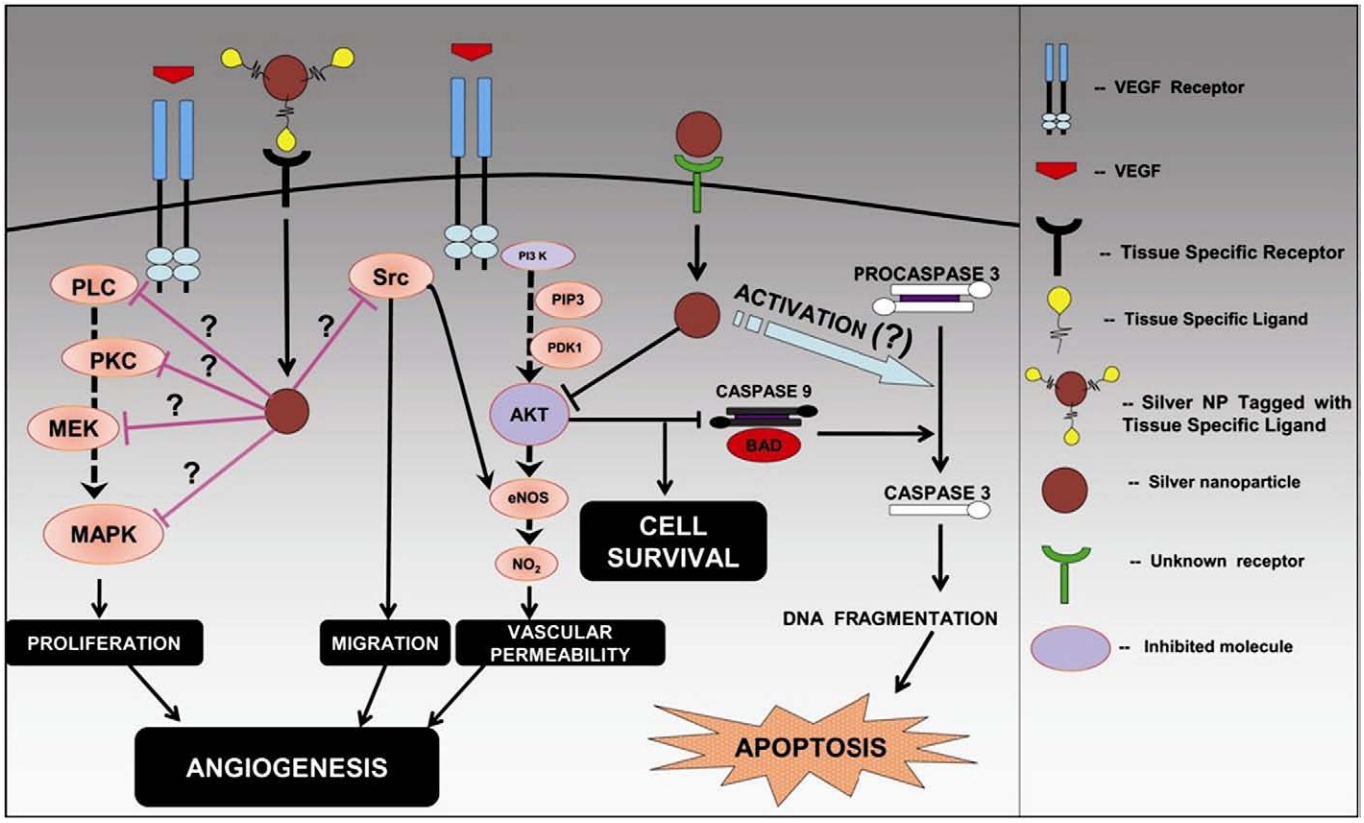

Fig. 10. Possible signaling pathways induced by silver nanoparticles when bound to an unknown as well as VEGF receptor.

and growth inhibitory factors. These receptors are transmembrane tyrosine kinases which upon binding of their ligands to the extracellular domain of the receptor activate a cascade of downstream proteins after the dimerization and autophosphorylation of the intracellular receptor tyrosine kinases (Otrock et al., 2007). There are several secreted glycoproteins and among them VEGF-A are secreted by tumor cells. It is a tumor-secreted cytokine with critical importance in both normal and tumor-associated angiogenesis. VEGF-A exerts its biologic effect through interaction with cell surface receptors. Dimerization of receptors leads to their activation and subsequent autophosphorylation on certain tyrosine residues, which in turn triggers intracellular signaling cascade mediated by several effectors, which are able to recognize and dock at phosphorylated tyrosine residues of the activated receptors (Kowanetz and Ferrara, 2006). The cancer cells can be targeted using silver nanoparticle conjugated with a tissue specific ligand that can activate any of the VEGF receptors and express its effect on any of the downstream molecules. The illustration explains that all the molecules that are represented could be a possible target and depending upon the targeting its impact on proliferation, migration and vascular permeability can be studied. The inhibition of some/all of the kinases in the downstream signaling could evolve silver nanoparticles as an anti-angiogenic agent. However, more studies are required to augment such a prediction and also exploring such a pathway will serve as a benchmark not only in cancer but also other diseases involving similar signaling mechanisms.

The role of silver nanoparticles as an anti-cancer agent should open new doors in the field of medicine. The design of smart multifunctional nanosystems for intracellular imaging and targeted therapeutic applications requires a thorough understanding of the mechanisms of nanoparticles entering and leaving the cells. For biological and clinical applications, the ability to control and manipulate the accumulation of nanoparticles for an extended period of time inside a cell can lead to improvements in diagnostic sensitivity and therapeutic efficiency. This when revealed completely would eliminate the use of expensive drugs for cancer treatment. In general, silver nanoparticles should serve as one of the best ways of treating diseases that involve cell proliferation and cell death.

6. Conclusion and future prospects

Nanobiotechnology is an emerging field that has made its contribution to all spheres of human life. The biological synthesis of nanoparticles has paved for better methodologies and approaches in the medicinal field. Hence the development of better experimental procedures for the synthesis of nanoparticles of different chemical compositions, sizes, shapes and controlled polydispersity is vital for its advancement (Bhattacharya and Mukherjee, 2008). There is an ever-increasing need to develop environmentally benign processes in place of synthetic protocols involving toxic ingredients. As a result, researchers in the field of nanoparticle synthesis and assembly are focusing their attention on biological systems (Shankar et al., 2004; Vigneshwaran et al., 2007). Rapid and green synthetic methods using extracts of bio-organisms have shown a great potential in AgNPs synthesis (Saifuddin et al., 2009). Bacterial synthesis of nanoparticles are more often reported due to the fact that bacteria are easy to handle and can be manipulated genetically without much difficulty. Considering these advantages, a bacterial system could prove to be an excellent alternative for the synthesis of AgNPs. This is an economical, efficient, eco-friendly and simple process. A progress in this area will give new green paths in the development of controlled shape and size AgNPs. Custom designed biomolecules can then be made to synthesize AgNPs, which will in turn fill the gap between biological synthesis and biometric synthesis. Moreover, the synthesis of AgNPs with a high yield and in a wide range of shapes has always been challenging tasks.

Silver nanoparticles have been widely used as a novel therapeutic agent extending its use as antibacterial, antifungal, anti-viral and anti-inflammatory agent. So far the chemically synthesized nanoparticles have been used for these approaches, but the recent reports suggest that biologically synthesized nanoparticles exercise numerous advantages over the chemically synthesized ones. The toxicity effects caused due to the metal ion in the cells are reduced in the case of nanoparticles. The nanoparticles synthesized from microbes are exceptionally stable and the stability is likely to be due to capping with proteins secreted by the microbe. Using metal-accumulating microorganisms as a tool for the production of nanoparticles, and their assembly for the construction of new advanced materials, is a completely new technological approach. The concentration of $AgNO_3$ does have an effect on the synthesis of particles. The effect of concentration of $AgNO_3$ was studied in *Morganella sp.* (Parikh et al., 2008). The concentration of $AgNO_3$ does have a role in size dependent synthesis of the particles. It is speculated that particle size and shape are dependent on various conditions, such as the culture supernatant, nanoparticle type, and reaction temperature and reaction-mixture composition. This may also be because $AgNO_3$ forms a coat on growing particles, thereby preventing their aggregation and, thus, yielding particles of nanoscale size. This shows that silver ions, by their dispersive action, have a role in controlling the growth of AgNPs. The increase in size of the particles does increase the toxicity of the particles. The smaller size particles are attributed to the stability, catalytic activity and enhanced adherence to the cells. Previous reports suggest that the increase in concentration of AgNPs has resulted in the increase in cell death (Kalishwaralal et al., 2009; Hsin et al., 2008). The effects of AgNPs on size dependent toxicity with various concentrations already explained earlier (Carlson et al., 2008) suggest that a size controlled synthesis is very much necessary when it comes to cellular interactions. The extra-cellular synthesis offers a great advantage over an intracellular process of synthesis makes it possible to harness and immobilize/deposit onto desired solid support for the use of different practical purposes (Basavaraja et al., 2008; Balaji et al., 2009). The recent reports suggest nitrate reductase as the enzyme responsible for the synthesis of silver nanoparticles (Kumar et al., 2007). Research is being carried out to identify different enzymes responsible for synthesis in different microbes and also find out the mechanism involved in the production silver nanoparticles.

The advancements that have taken place have been able to address some of biggest questions regarding human life. Great strides are already being made in creating nanoparticle systems for targeted drug delivery. Nanoenabled drug delivery has already been successful in delivering drugs to specific tissues within the body, and promises capabilities that will enhance drug penetration into cells, as well as other means to improve drug activity. A very promising prospect of nanoparticles is its use in targeted drug delivery and also "multi-targeting", which is essential in the case of several diseases (Woodleand and Lu, 2005). The targeted delivery has the potential to develop dual specificity for the specific targets which is a very promising aspect in the case of cancer. Newer drugs are being developed that interfere with pathways that are specifically activated in cancer cells. The delivery of anti-neoplastic drugs to cancer cells or cancer-associated tissues such as tumor vasculature can be selectively increased by associating the drugs with molecules that bind to antigens or receptors that are either uniquely expressed or over-expressed on the target cells relative to normal tissues. This allows specific delivery of drugs to the cancer cells. The design of smart multifunctional nanosystems for intracellular imaging and targeted therapeutic applications requires a thorough understanding of the mechanisms of nanoparticles entering and leaving the cells (Devika et al., 2006). The use of silver nanoparticles for cancer therapy will surely emerge as one of the novel approaches and the evidence of molecular mechanism of targeting is surely going to take the field to great heights. The ability of PEG coated particles to target neovasculature has opened new doors in finding a cure for Diabetic Retinopathy. The targeted delivery of siRNA has proven to be augmenting the pathological (VEGF) process (Woodleand and Lu, 2005). Recently, a new PCR driven method for identification of a unique set of sequences that bind to silver and cobalt

nanoparticles from a phage peptide display library has been developed. There is an enormous interest in exploiting nanoparticles in various biomedical applications since their size scale is similar to that of biological molecules (e.g., proteins, DNA) and structures (e.g., viruses and bacteria). These advancements are on a promising note that the field will be at its pinnacle in the years to come and also make a mark for itself in the scientific arena.

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