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Silvernano Particles: A Novel Approach for Therapeutic Applications of Silver.

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ABSTRACT

Nano-size particles of less than 100 nm in diameter are currently attracting increasing attention for the wide range of new applications in various fields of industry. They exhibit properties different than from those of bulk materials, as a result of small particle dimension, high surface area, quantum confinement and other effects. Silver nanoparticles are nanoparticles of silver which are in the range of 1 and 100 nm in size. The major methods used for silver nanoparticle synthesis are the physical and chemical methods, but the synthesis is expensive and can also have toxic substances absorbed onto them. To overcome this, the biological method (biological systems involving bacteria, fungi etc.) provides a feasible alternative. The major applications of silver nanoparticles in the medical field include diagnostic applications and therapeutic applications. In most of the therapeutic applications, it is the antimicrobial property that is being majorly explored. Accordingly, this review presents different methods of preparation silver nanoparticles and application of these nanoparticles in different fields. This review also examines the role of silver nanoparticles in cancer therapy.

Keywords: Nano-particles, anti-microbial, cancer therapy.

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INTRODUCTION

Nanotechnology is the most promising field for generating new applications in medicine. Compared with larger particles of the bulk material, nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology.Most of the unique properties of nanoparticles require not only the particles to be of nano-sized, but also the particles be dispersed without agglomeration.

However, only few nano-products 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. The current review throws light on applications of silver nanoparticles which is being exploited in medicine for antibacterial, antifungal, anti-viral, anti-inflammatory therapy and also discuss the current understanding of the biological actions of the silver nanoparticles. The review also examines the role of silver nanoparticles in cancer therapy and also covers certain methods of silver nanoparticles synthesis[1].

History

Silver has a long history of being an antibacterial agent. Not only did ancient Greek and Roman civilizations use silver to disinfect water and food, but other pioneers also submerged silver coins in water and milk to keep the drinks fresh. Over 120 years ago, in 1889, M. C. Lea reported the synthesis of a citrate stabilized silver colloid. The average diameter for the particles obtained by this method is between 7 and 9 nm. Their size in the nanoscale and the stabilization by citrate are identical to recent reports about nanosilver formation using silver nitrate and citrate. Also the stabilization of nanosilver using proteins has been described as early as 1902. By the 1920s, the U.S. Food and Drug Administration approved silver solution as a type of antibacterial agent[2].

Under the name “Collargol” such a kind of nanosilver has been manufactured commercially since 1897 and has been used for medical applications. Collargol has a mean particle size of 10 nm and as early as 1907 its diameter was determined to be in the nano-range. Other nanosilver preparations were also invented in the next decades, for example the gelatin stabilized silver nanoparticles patented by Moudry in 1953 with 2-20 nm diameter and silver nanoparticle impregnated carbon with a diameter of silver particles below 25 nm. It is important to note that the inventors of nanosilver formulations understood decades ago that the viability of the technology required nanoscale silver, e.g., by the following statement from a patent: “for proper efficiency, the silver must be dispersed as particles of colloidal size less than 250 Å [less than 25

nm] in crystallite size".

In the early part of the 20th century, the commercial sale of medicinal nanoscale silver colloids, known under different trade names such as Collargol, Argyrol, and Protargol, began and over a 50 year period their use became widespread. These nanosilver products were sold as over-the-counter medications and also used by medical doctors to treat various diseases such as syphilis and other bacterial infections.

Silver as a therapeutic agent:

Historically, silver metal has been used widely across the civilizations for different purposes like jewellery, ornamentation, fine cutlery etc. and was considered to impart health benefits to the users. In ancient Indian medical system (Ayurveda) silver has been described as therapeutic agent for many diseases. There is an increasing use of silver as an efficacious antibacterial and antifungal agent in wound care products and medical devices including dental work and catheters. Some other uses are as followed[3,5]

- The synthesized silver composites are used as water disinfecting filters.
- Inorganic composites with a slow silver release rate are currently used as preservatives in a variety of cosmetic products.
- Silica gel microspheres containing a silver thiosulfate complex, are mixed into plastics for long-lasting antibacterial protection.
- Metallic silver has also been used for surgical prosthesis and splints, fungicides, and coinage.
- Soluble silver compounds, such as silver salts, have been used for treating mental illness, epilepsy, nicotine addiction, gastroenteritis, stomatitis, and sexually transmitted diseases including syphilis and gonorrhea.
- Silver-nitrate (AgNO3), as eye drops, had been utilized to prevent gonococcal ophthalmic neonatorum in newborns by pediatricians.
- Agents derived from silver, such as silver sulfadiazine (AgSD) cream, have been used by surgeons, as topical treatments to heal burn wounds, when applied directly to the burn site, erythema decreased, while the expression of matrix metalloproteinases (MMPs) increased.
- Silver foil was applied to surgical wounds for improved healing and reduced post-operative infections, while silver and lunar caustic (pencil containing silver nitrate mitigated with potassium nitrate) was used for wart removal and ulcer debridement.

Silver As Silver Nanoparticles

Recent advances in nanotechnology have enabled us to produce pure silver, as nanoparticles, which are more efficient than silver ions (AgSD and AgNO3). The diameters of silver nanoparticle (AgNPs) are generally smaller than 100 nm and contain 20 –15000 silver atoms. Bulk silver is the typical shiny silver colour we are accustomed to, but silver nanoparticles can be a variety of colours, depending on the shape and size. Particles that are approximately spherical and below 20 nm in diameter shows a clear yellow or amber colour. These absorb light very strongly; in fact, a very dilute solution can display a bright colour, **w**hile increase in size leads to amber, brown, and finally a cloudy brownish/green colour, with decreasing efficacy as antimicrobial agents.

In the case of exposing cells or tissue to AgNPs, the active surface of AgNPs would be significantly large compared to silver compounds, and thereby exhibiting remarkably unusual physicochemical properties and biological activities. AgNPs have been increasingly applied in the biomedical, pharmacological fields; also considerable research has been done in clinical medicine.

A. Synthesis of silver nanoparticles

For biological use, the main aim of making AgNPs will be for them to be stable in solution, so that each silver nanoparticle can thoroughly be exposed to the cells in tissue and exert their maximal bio-effects. 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. Many techniques are available to synthesize the silver nanoparticles. Some important methods are

1. Physical method and Chemical method.
2. Biological method (from bacteria and fungi).

Chemical and physical synthesis methods:

Generally, nanoparticles are prepared by a variety of chemical and physical methods such as chemical reduction, photochemical reduction, electrochemical reduction, heat vaporation etc.

Turkevich *et al.* first reported their preparation of AgNPs based on the reduction of silver nitrate with citrate, similar updated methods have also been reported, these reagents could be inorganic such as sodium/potassium borohydrate, hydrazine and salts of tartarate, or organic ones like sodium citrate, ascorbic acid and amino acids capable of being oxidized. Nowadays, AgNPs of different sizes and shapes can be made by using capping agents such as dendrimer, chitosan, ionic liquid, and poly(vinylpyrrolidone) (PVP), based on controlling the growth of silver

nanoparticles through reaction confinement within the matrix or through preferential adsorption on specific crystal facets.

Yen *et al.* reported the production of AgNPs by physical manufacturing. First, silver bulk material was ground into the silver target materials. Then they were vaporized to the atomic level by an electrically gasified method under vacuum then further condensed in the presence of inert gas, and piled up to form AgNPs. The sizes of AgNPs could be effectively managed depending on the evaporation time and electric current used. The AgNPs were collected in a cold trap and centrifuged to obtain the final product. Most of these methods are extremely expensive and they also involve the use of toxic, hazardous chemicals which are not environmental friendly[7-8].

Biosynthesis from bacteria and fungi:

AgNPs can also be synthesized using a reduction of aqueous Ag ions with the culture supernatants of *Staphylococcus aureus*. The supernatant was added separately to the reaction vessel containing silver nitrate. Here supernatant may act both as reducing and capping agents in Ag NPs synthesis. The reduction of Ag+ ions by combinations of biomolecules found in supernatant such as enzymes/proteins, amino acids, polysaccharides and vitamins takes place. But, the mechanism which is widely accepted for the synthesis of silver nanoparticles is the presence of enzyme "Nitrate reductase". Nitrate reductase is an enzyme in the nitrogen cycle responsible for the conversion of nitrate to nitrite. The reduction mediated by the presence of the enzyme in the organism has been found to be responsible for the synthesis. The bioreduction of the silver ions in solution was monitored and the spectra measured in a UV-vis spectrophotometer at a resolution of 1 nm.

Some other bacterias were also explored AgNPs synthesis like lactic acid bacteria, Pseudomonas stutzeriAG259. Furthermore, Gajbhiyeet al. also reported the use of fungus Alternariaalternata to produce AgNPs. In addition, eukaryotic organisms such as fungi have also been used to grow nanoparticles of different chemical composition and sizes like Verticillumsp.; Fusariumoxysporum and Aspergillusflavus and also with enzymes. These methods are non-expensive and do not involve the use of toxic, hazardous chemicals hence they are environmental friendly procedures [6-7].

B. Silver Nanoparticle Characterization

The size and shape of metal nanoparticles are typically measured by analytical techniques such as TEM, scanning electron microscopy (SEM) or atomic force microscopy (AFM). Measuring the aggregation state of the particles requires a technique to measure the effective size of the particles in solution such as dynamic light scattering (DLS) or analytical disc centrifugation.

However, due to the unique optical properties of silver nanoparticles, a great deal of information about the physical state of the nanoparticles can be obtained by analyzing the spectral properties of silver nanoparticles in solution. The spectral response of silver nanoparticles as a function of diameter, as the diameter increases, the peak plasmon resonance shifts to longer wavelengths and broadens. At diameters greater than 80 nm, a second peak becomes visible at a shorter wavelength than the primary peak. This secondary peak is due to a quadrapole resonance that has a different electron oscillation pattern than the primary dipole resonance. The peak wavelength, the peak width, and the effect of secondary resonances yield a unique spectral fingerprint for a plasmonic nanoparticle with a specific size and shape. Additionally, UV-Visible spectroscopy provides a mechanism to monitor how the nanoparticles change over time. When silver nanoparticles aggregate, the metal particles become electronically coupled and this coupled system has a different spectrathan the individual particles. For the case of a multi-nanoparticle aggregate, the plasmon resonance will be red-shifted to a longer wavelength than the resonance of an individual nanoparticle, and aggregation is observable as an intensity increase in the red/infrared region of the spectrum. Carefully monitoring the UV-Visible spectrum of the silver nanoparticles with time is a sensitive technique used in determining if any nanoparticle aggregation has occurred[9].

For silver nanoparticle solutions that have not agglomerated and have a spectral shape that is identical to the as-received suspension, the UV/Visible extinction spectra can be used to quantify the nanoparticle concentration. The concentration of silver nanoparticle solutions is calculated using the Beer-Lambert law, which correlates the optical density (OD, a measure of the amount of light transmitted through a solution) with concentration. Due to the linear relationship between OD and concentration, these values can be used to quantify the concentration of nanoparticle solutions[9]

APPLICATIONS OF SILVERNANOPARTICLES

As anti-bacterial

Silver returned to prominence recently due to the emergence of antibiotic-resistant bacteria as a result of the overuse of antibiotics. With the advancement of nanotechnology, the interest in the use of the anti-bacterial efficiency of silver nanoparticles has been rekindled. Silver compounds were shown to be effective against both aerobic as well as anaerobic bacteria. Compared with silver compounds, the mechanism for the antimicrobial action of AgNPs may be similar. However, because of the larger surface area to volume ratio, AgNPs may have much better efficiency. The possible mechanisms of action are[10-16]:

- At nanometer scale silver provides an extremely large surface area for contact with bacteria. There are formation of 'pits' on the cell surface, and accumulation of the nanoparticles on the cell surface, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell.
- The electron spin resonance spectroscopy studies suggested that there can be release of silver ions by the nanoparticles, and these ions can interact with the thiol groups of many vital enzymes and inactivate them. Then the generation of reactive oxygen species, which are produce possibly through the inhibition of a respiratory enzyme by silver ions and attack the cell itself.
- AgNPs can have a sustained release of Ag+ once inside the bacterial cells (in an environment with lower pH), which may create free radicals and induce oxidative stress, thus further enhancing their bactericidal activity. Furthermore, a recent study showed that yeast and *E. coli* were inhibited at a low concentration of AgNPs, study of mechanisms revealed that free radicals and oxidative stress were responsible for the antibacterial activities.
- Silver is a soft acid, and there is a natural tendency of an acid to react with a base, in this case, a soft acid to react with a soft base. The cells are majorly made up of sulfur and phosphorus which are soft bases. The action of these nanoparticles on the cell can cause the reaction to take place and subsequently lead to cell death.
- The interaction of the silver nanoparticles with the sulfur and phosphorus of the DNA can lead to problems in the DNA replication of the bacteria and thus terminate the microbes.
- It has also been found that the nanoparticles can modulate the signal transduction in bacteria. Phosphorylation of protein substrates in bacteria influences bacterial signal transduction. Dephosphorylation is noted only in the tyrosine residues of gram-negative bacteria. The phosphotyrosine profile of bacterial peptides is altered by the nanoparticles.

As anti-inflammatory

Nanocrystalline silver dressings were introduced commercially as antimicrobial dressings in 1998 and these have found to improve wound healing, which may result from potent anti-inflammatory activity. This unusual activity of nanocrystal is said to occur due to its small size.

Nanocrystalline silver has unique dissolution behaviour, releasing silver oxide (Ag0) into solution. This species (Ag0) is said to exhibit anti-inflammatory activity and this was illustrated when it was tested on animal models. Silvernanoparticles 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.

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 treatments. 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. Inflammation was induced by means of DNCB and the control was compared to that of the ones treated with Saline (0.9%), AgNO3 (0.5%) and nanocrystalline silver. Day by day observations were made and it was observed that the wound healing was much more significant.

Nadworny*et al.* explored the effect of AgNPs using a porcine model of contact dermatitis, while Bhol and Schechter utilized AgNPs in a rat model of ulcerative colitis. The findings confirmed that AgNPs had direct anti-inflammatory effects and improved the healing process significantly when compared with controls.[17]

As virucidal

The mechanism of action of AgNPs as an antiviral and virucidal has been studied against several enveloped viruses. Virucidal agents differ from virustatic drugs in that they act directly and rapidly by lysing viral membranes on contact or by binding to virus coat proteins.

A virus is a sub-microscopic infectious agent that can only multiply in living cells of animals, plants, or bacteria. Viruses are about 1/100th the size of bacteria and consist of a single- or double stranded nucleic acid (DNA or RNA) surrounded by a protein shell called a capsid. Some viruses have an outer envelope composed of lipids and proteins. The viral capsid proteins bind to the host cellular surface specific receptors. This attachment can induce the viral envelope protein to undergo changes that results in the fusion of viral and cellular membranes and may lead to an infection. For this reason there is a high interest studying possible mechanisms of binding nanoparticles to the viral capsid and inhibit the later fusion.

Recently, it has been suggested that nanoparticles bind with a viral envelope glycoprotein and inhibit the virus by binding to the disulfide bond regions of the CD4 binding domain within the gp120 glycoprotein, as demonstrated in vitro.[19]

Silver nanoparticles undergo a size-dependent interaction with HIV-1, nanoparticles ranging from 1 to 10 nm attached to the virus, and their surface chemistry can modify their interactions with viruses, tested with silver NPs that had three different surface chemistries: foamy carbon, poly (N-vinyl-2-pyrrolidone) (PVP), and bovine serum albumin (BSA). Differences have been observed in HIV-1 inhibition and can be justified because BSA and PVP are directly bounded to the nanoparticle surface and are totally encapsulated, while the foamy carbon silver nanoparticles have fundamentally a free surface area, which exhibit higher inhibitory effect and cytotoxicity as they are able to have stronger interactions[20-21]

There are also other studies that analyse the interaction of silver nanoparticles with hepatitis B virus. The effects of silver nanoparticles on hepatitis B virus (HBV) have been reported using an infection model HepAD38 human hepatoma cell line. The binding affinity of NPs with different sizes (10 and 50 nm) for HBV DNA and extracellular virions resulted very high and could also inhibit the production of HBV RNA and extracellular virions in vitro, which was determined using a UV-VIS absorption titration assay.

Among antiviral activities, the capacity of AgNPs to inhibit an influenza virus was determined in a MDCK cell culture and was demonstrated that with AgNPs at 0.5 μg/ml concentration viral infectivity was reduced. Nanosilver may interfere with the fusion of the viral membrane, inhibiting viral penetration into the host cell.[18]

In another report Sun and colleagues showed that AgNPs were superior to gold nanoparticles for cyto-protective activities toward HIV-1-infected Hut/ CCR5 cells. It is generally understood that Ag, in various forms, inactivates viruses by denaturing enzymes via reactions with sulfhydra, amino, carboxyl, phosphate, and imidazole group. However, it is necessary to design in vivo studies with silver NPs are necessary to design therapeutics and vaccines that can specifically target viruses in order to increase therapeutic benefit and minimize adverse effects.

As antiplatelet agent

Thrombotic disorders have remained a significant problem, use of anticoagulant and thrombolytic therapy may sometimes lead to serious bleeding complications. As platelets play a central role in thrombotic disorders, the focus has now shifted to regulating and maintaining these cells in an inactive state. Recently, Shrivastava*et al.* demonstrated that AgNPs could effectively inhibit integrin-mediated platelet functional responses like aggregation, secretion,

adhesion to immobilized fibrinogen or collagen and retraction of fibrin clot in a dose-dependent manner. *In vivo* studies using mouse models has also supported the anti-platelet properties of silver nanoparticles. The results had shown, significant inhibition of platelet functions with a relatively low dose of AgNPs, combined with the lack of cell lysis, raise the hope for its use as an anti-platelet therapeutic agent[22].

In cancer treatment

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 areawith far less systemic side effects[24.]

Progression of tumors has been attributed to the cumulative accumulation of multiple alterations throughout the genome, which is manifested by genomic instability. 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 fromcells 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.

As a drug carrier:

The emerging trend of using nanoparticles as drug carriers has exploited the potential of nanoparticles to revolutionize cancer therapy. Silver also has been now recognized as a developing therapeutic molecule. 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 nano-carrier by a variety of conjugation chemistries. Nano-carriers 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 nano-carriers 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.

Physical property consideration:

Due to toxicity of silver nanoparticles towards the body cells, the targeting of silver nanoparticles towards cancer cells involves certain limitations. These can be overcome by altering the particle size, curvature of shape, homogenicity of protein coating etc. In case of particle size, nanoparticles of larger size have proven to be toxic to the cells. Since 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. 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. Another reason could be, if higher amount of CTAB surfactant molecules adsorbed onto the rod-shaped nanoparticle surface during synthesis, the serum protein may not be able to bind onto the silver nanoparticle surface efficiently. Also, non-homogeneous protein coating on the surface of the rod-shaped silver nanoparticles, the ligands may not bind the receptors on the cell surface as strongly (due to a lack of multivalent binding). This would affect the uptake of the nanoparticles.

Binding of silver nanoparticles towards cancer cell:

The other question that 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.

Inhibition of phosphorylation:

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. The biogenesis of carrier vesicles is an important aspect of membrane transport along the secretory pathway. The mechanism by which carrier membranes identify their correct target and undergo docking and fusion, requires the knowledge of the membrane associated proteins. The targeting of nanosilver without ligands have shown to inhibit the PI3/Akt phosphorylation, 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.

Role 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 pro-apoptotic 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 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.

Anti-angiogenic activity:

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 (Banumathiet al., 2009). VEGF signaling often represents a critical rate-limiting step in physiological angiogenesis. 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. 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 inhibition of some/all of the kinases in the downstream

signaling could evolve silver nanoparticles as an anti-angiogenic agent.

The role of silver nanoparticles as an anti-cancer agent should open new doors in the field of medicine. The design of smart multifunctional nano-systems 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[23].

Nano-silver in diagnosis and imaging.

Early diagnosis of any disease condition is vital to ensure that early treatment is started and perhaps resulting in a better chance of cure. For example, in patients undergoing general anaesthesia for surgery, the risk of developing pulmonary complications will be lowered if any sub-clinical upper respiratory tract viral infections can be detected prior to surgery. Surface-enhanced Raman spectroscopy (SERS) has emerged as a powerful analytical tool that extends the possibilities of vibrational spectroscopy. SERS differs from standard Raman scattering in that the incoming laser beam interacts with the oscillations of plasmonic electrons in metallic nanostructures to enhance the vibrational spectra of molecules adsorbed to the surface. In a recent study, SERS was used to obtain the Raman spectra of the respiratory syncytial virus (RSV), using substrates composed of silver nanorods. It was shown in this study that the four virus strains tested were readily detected at very low detection limits.

In terms of detecting cancer, Au–Ag nanorods were used in a recent study as a nano-platform for multivalent binding by multiple aptamers, so as to increase both the signal and binding strengths of the aptamers in cancer cell recognition. The molecular assembly of aptamers on the nanorods was shown to lead to a 26-fold higher affinity than the original aptamer probes. Thus, these nanorod–aptamer conjugates are highly promising for use in specific cell targeting, as well as having the detection and targeting ability needed for cell studies, disease diagnosis, and therapy[25].

Nano-silver in therapeutics:

Wound dressing.

Wound healing is regarded as a complex and multiple-step process involving integration of activities of different tissues and cell lineages. Acticoat®, is the first commercial dressing made

up of two layers of polyamide ester membranes covered with nano-crystalline silver ions, has been studied extensively. Acticoat® has been shown to have the lowest MIC and MBC values, and the fastest Kill kinetics against the five bacteria tested in in vitro studies. The sustained release of silver particles should minimize the likelihood of bacteria developing resistance to silver. In a randomized prospective clinical study involving 30 patients with each group of patients having comparable burn wound size, depth and location, the wounds were either treated with silver nanoparticles dressing or a gauze soaked in 0.5% silver nitrate solution. The frequency of burn wound sepsis, as well as secondary bacteraemia, were were found to be less in patients treated with silver nanoparticles than in those treated with the control. Sibbald et al. conducted a prospective study to evaluate the use of silver nanoparticles dressing on a variety of chronic non-healing wounds. The study concluded that silver nanoparticles dressing on a variety of chronic non-healing wounds. The study concluded that silver nanoparticles dressing have a beneficial effect of protecting the wound site from bacterial contamination. Compared with other silver compounds, AgNPs seem also to promote healing and achieve better cosmetics after healing. . Along with burn wounds, now there is increasing evidence for the use of silver nanoparticles in the treatment of chronic wounds, such as leg ulcers, diabetic foot ulcers and pressure ulcers [26]

Silver-impregnated catheters.

Central venous catheters.

Central venous catheters (CVC) are widely used in hospital practice, However use of CVCs is associated with potential infective complications like catheter-related bloodstream infection. Previous studies have suggested that impregnation of catheters with antibiotics could decrease the rates of colonization of catheters, but the increasing use of antibiotic-impregnated catheters could lead to bacterial resistance. A new generation of silver-impregnated catheters based on the use of an inorganic silver powder, on which silver ions are bonded with an inert ceramic zeolite, has become available for clinical use. In a recent study comparing these silver-impregnated catheters with standard catheters in terms of incidence of catheter-related blood stream infections, it was shown that overall colonization rate was significantly lower in the silver-impregnated CVC tips[27].

Ventricular drainage catheters.

Insertion of temporary external ventricular drainage (EVD) is a commonly used procedure in intensive care patients for the management of acute occlusive hydrocephalus. However, an important complication of external cerebrospinal fluid (CSF) drainage isbacterial colonization of

the catheter, resulting in ventriculomeningitis and encephalitis. The availability of silver-impregnated ventricular catheters since 2004 resulted in a pilot study addressing their clinical efficacy in neurological and neurosurgical patients requiring external CSF drainage. Thestudy found that CSF cultures performed at least three times a week yielded 25% more positive cultures in the control group compared to 0% in the treatment group using silver catheters[28].

Silver in orthopaedics.

Artificial joint replacements have become the gold standard treatment for many arthritic diseases. Like all biomaterials, bone cement based on polymethylmetacrylate (PMMA) has an elevated risk of infection when implanted into thehuman body. Indeed, an increasing number of joint infections with multi-resistant bacteria mean that an adequate prophylaxis against these organisms is necessary. Recent studies have been carried out to evaluate bone cement loaded with nanosilver. Here, nanosilver-loaded bone cement could be shown to have high antibacterial activity against all tested strains including methicillin-resistant *Staphylococcus aureus*(MRSA). Furthermore, the nanoparticles did not seem to have cytotoxicity to osteoblasts grown *in vitro*.

For many years, ultra high molecular weight polyethylene (UHMWPE) has been the material of choice for fabrication of bearing inserts for joint replacement components. A major problem with the longevity of UHMWPE was wear and concomitant debris generation, which can activate macrophages, with subsequent inflammation, and eventual failure of the artificial joints. In one study, incorporation of silver nanoparticles was demonstrated to lead to both physical and chemical stabilization of the polymer surface layer toward friction oxidation and degradation. This procedure was further shown to significantly decrease the process of polymer/metal tribochemical debris formation and at the same time enhances UHMWPE biocompatibility and antimicrobial activity. Taken together, it would appear that silver nanoparticles could play a significant role in the next generation of biomaterials in orthopaedics[29].

Surgical mesh:

For general surgery, surgical implants are often unavoidable. Surgical meshes are commonly used for bridging large wounds, as well as acting as reinforcements to tissue repair. However, being foreign material, they do carry a risk of infection. Indeed, it has been estimated that one million nocosomial infections are seen each year in patients with implanted prosthetic materials. The use of silver nanoparticles polypropylene mesh has been studied recently. Similar to other studies using silver nanoparticles, the results showed that silver nanoparticles polypropylene mesh had significant bactericidal efficacy against *S.aureus*. Furthermore, it was shown that silver nanoparticles could continue to diffuse off the mesh and had sustained activity. These results

clearly warrant further *in vivo* studies to determine whether silver nanoparticles-coated polypropylene mesh can decrease the prosthetic infection rate and the host inflammatory response in the clinical setting [29].

Future Opportunities and Challenges

Nanotechnology has received much attention from scientists and journalists in the last few years raising hopes of revolutionary developments in a wide range of technologies on an increasingly small scale, dramatic improvements to standards of living, and solutions to a variety of environmental, medical and communications problems. Silver nanoparticles have already been applied as drug delivery systems with great success. Nanoparticles provide massive advantages regarding drug targeting, delivery and release and with their potential for combine diagnosis and therapy and one of the major tools in nanomedicine. Also in biosciences, nanoparticles are replacing organic dyes in the applications that require high photo-stability as well as high multiplexing capabilities.

There are many technical challenges in developing the following techniques:- virus- like systems for intracellular systems, architecting of biomimetic polymers, control of sensitive drugs, functions of active drug targeting, bioresponsive triggered systems, systems interacting with the person (body smart delivery), nanochips for nanoparticle release, carriers for advanced polymers for the delivery of therapeutic peptide / proteins. Improvements in the development of novel silver nanoparticles-containing products are continuously sought.

There are some developments in directing and remotely controlling the functions of nanoprobes, for example driving magnetic nanoparticles to the tumour and then making them either to release the drug load or just heating them in order to destroy the surrounding tissue. The major trend in further development of nanomaterials is to make them multifunctional and controllable by external signals or by local environment thus essentially turning them into nano-devices. In particular, there is an increasing interest towards the exploitation of silver nanoparticles technology in the development of bioactive biomaterials, aiming at combining the relevant antibacterial properties of the metal with the peculiar performance of the biomaterial.

CONCLUSION

The advance in nanotechnology has enabled us to utilize particles in the size of the nanoscale. Living organisms have huge potential for the production of nanoparticles/nanodevices of wide applications. By using the organisms from simple bacteria to highly complex eukaryotes in the reaction mixture, the production of nanoparticles/nanodevices with desired shape and size can be

obtained. Though nano-biotechnology is at its infancy but various examples through which this technology and their use have been explained in this article would attract the attention of peoples towards its applications. However, the elucidation of exact mechanism of nanoparticles production using living organisms needs much more experimentations.

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