Review Article

An Overview on Synthesis, Characterization and Applications of Silver Nanoparticles

Pravalika Sakilam*[1], M. Sunitha Reddy[2]
1. M. Pharmacy, Department of Pharmaceutics, Centre for Pharmaceutical Sciences (CPS), JNTUH-UCEST, JNTUH, Hyderabad, Telangana, India.
2. Professor, HOD, Department of Pharmaceutics, Centre for Pharmaceutical Sciences (CPS), JNTUH-UCEST, JNTUH, Hyderabad, Telangana, India.
***Corresponding author's E-mail:** sakilampravalika@gmail.com

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ABSTRACT

Nanoparticles have a significant impact on a variety of biomedical applications during the past few decades, including diagnostic and medicinal equipment. Nanotechnology is a significant area of contemporary research that deals with creating, producing, and modifying particles having structures between one to one hundred nanometers (nm) in size. Metal nanoparticles for individualized healthcare are very useful Since they have distinct physical and optical properties and biochemical functionality tailored by various size- and shape-controlled parameters. Silver Nanoparticles (AgNPs) in particular have great potential in a broad range of applications as, drug-delivery carriers, antimicrobial agents, imaging probes, biomedical device coatings, and diagnostic and optoelectronic platforms. The main AgNPs production pathways, including physical, chemical, and biological processes, as well as their characterization and applications, are the subject of the current review.

Keywords: Silver Nanoparticles, Nanotechnology, Characterization techniques, Applications.

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INTRODUCTION

Nanoscience has been established recently as a new interdisciplinary science. It can be defined as a whole knowledge of fundamental properties of nano-size objects. The prefix "nano" indicates one billionth or 10^{-9}units.It is widely accepted in the context of nanoscience and nanotechnologies, the units should only be those of dimensions, rather than of any other unit of scientific measurement. It is widely agreed that nanoparticles are clusters of atoms in the size range of 1–100 nm. Frequently, nanometer-size metallic particles show unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts, due to their high surface-to-volume ratio. Thus, these nanoparticles have been the subject of substantial research in recent years.[1,2] Metal nanoparticles have been used in a wide-range, as shapes, sizes, and compositions of metallic nanomaterials are significantly linked to their physical, chemical, and optical properties, technologies based on nanoscale materials have been exploited in a variety of fields from chemistry to medicine.[3,4]

Recently, silver nanoparticles (AgNPs) have been investigated extensively due to their superior characteristics, and their superiority stems mainly from the size, shape, composition, crystallinity, and structure of AgNPs compared to their bulk forms.[5]Efforts have been made to explore their attractive properties and utilize them in practical applications. The potential use in medicine, cosmetics, renewable energies, biomedical devices and bioremediation. Due to their antimicrobial properties, they are used as a bactericidal coating in water filters, wound dressings, dental resin composites, air sanitizer detergents and many other products such as bone cement etc. Also, they are exploited in textile engineering, catalysts, optical sensors and in electronics and optics too.[6] The size of AgNPs can be adjusted according to a specific use, for e.g., AgNPs prepared for drug delivery are mostly greater than 100 nm to accommodate for the quantity of drug to be delivered. With different surface properties, AgNPs can also be formed into various shapes, including rod, triangle, round, octahedral, polyhedral, etc. Silver was a commonly used metal, because of its low toxic properties to the human skin. Silver ions are neutral in water, acid resistant, salt and weak. Good stability against heat and light. The smaller the particle size, the more negligible the influence of gravity. While increasing the surface charge and chemistry allows the repulsive force between particles so that the particles can be dispersed in water easily.[7,8]

METHODS OF SYNTHESIS

Because of how greatly their physical, chemical, electrical, and magnetic properties differ from those of their higher dimensional counterparts and rely on their size and shape, nanostructure materials have gained a lot of attention. To produce materials with regulated shape, size, dimensionality, and structure, numerous processes have been devised. Materials' qualities determine how well they perform.[9] The internal properties are governed by the thermodynamics and kinetics of synthesis and depend on the atomic structure, composition, microstructure, defects, and interfaces. The following are the two key methods for developing nanoparticles:

i. Top-down approach

ii. Bottom-up approach

In top-down approaches, bulk materials are divided to produce nanostructured materials the synthesis techniques are an extension of those that have been used for producing micron- sized particles. Top-down approaches are inherently simpler and depend either on removal or division of bulk material or on the miniaturization of bulk fabrication processes to produce the desired structure with appropriate properties while the bottom-up is an alternative approach, which has the potential of creating less waste and hence the more economical. Bottom-up approach refers to the build-up of material from the bottom: atom-by-atom, molecule-by-molecule, or cluster-by cluster.[10]

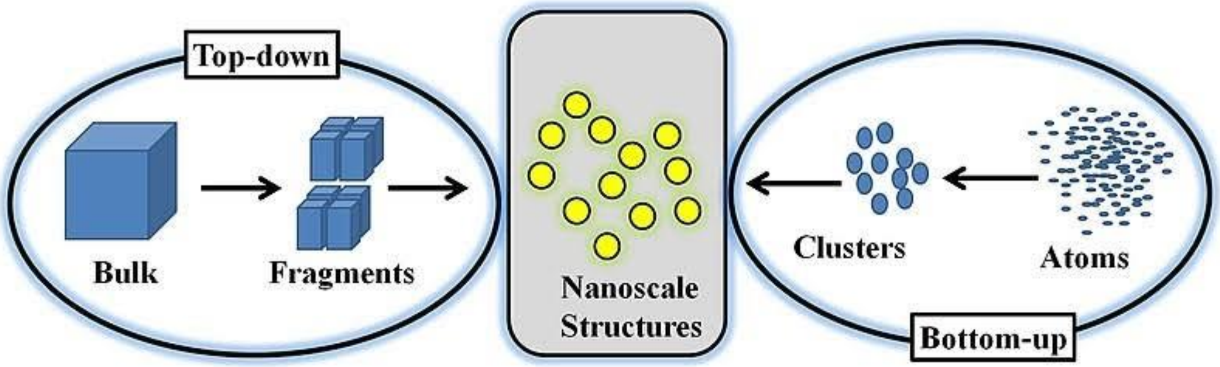

Figure 1: Schematic representation of ‘top-down’ and 'bottom-up’ approaches for synthesis of nanoscale materials.

There are two routes to prepare the metal nanoparticles, the first one is a **Physical approach** that utilizes several methods such as Evaporation/Condensation and Laser ablation. The second method is the **Chemical approach** and it involves reducing the metal ions in solution under circumstances that will eventually lead to the production of tiny metal aggregates or clusters.

1. Physical Approach

In physical processes, metal nanoparticles are generally synthesized by **Evaporation–condensation**, which could be carried out using a tube furnace at atmospheric pressure. The source material within a boat centered at the furnace is vaporized into a carrier gas. However, the generation of silver nanoparticles (AgNPs)using a tube furnace has several drawbacks, because a tube furnace occupies a large space, consumes a great deal of energy while raising the environmental temperature around the source material, and requires a lot of time to achieve thermal stability.[11] AgNPs have been synthesized with **Laser Ablation** of metallic bulk materials in solution. The characteristics of these metal particles formed and the ablation efficiency strongly depend upon many parameters such as

- Wavelength of the laser impinging the metallic target,
- Duration of the laser pulses,
- The laser fluence,
- Ablation time duration and
- The Effective liquid medium, with or without the presence of surfactants.

One of the advantages of laser ablation compared to other conventional methods for preparing metal colloids is the absence of chemical reagents in solutions. Therefore, pure colloids, which will be useful for further applications, can be produced by this method.

Thermal decomposition has been used to synthesize Ag-NPS in powder form. It involves the reaction of $AgNO_3$ with sodium oleate to prepare a Ag^+ oleate complex and then its decomposition to give Ag-NPS with average size of 9-5nm.[11,12]

2.Chemical Approach

One of mostly used methods for Ag-nps production are chemical methods, as they provide an easy way to synthesis Ag-nps in solution. Generally, the process of chemical synthesis of Ag-Nps in solution usually involves three main components:

- Metal precursors: Silver nitrate
- Reducing agents: Sodium Citrate, Sodium Ascorbate, Sodium Borohydride, Elemental Hydrogen, Tollens Reagent, N,N-Dimethylformamide (DMF), And Poly (Ethylene Glycol)-Block Copolymers.
- Stabilizing/capping agents**:** Poly Vinyl Alcohol, Poly Vinyl Pyrrolidone, Poly Ethylene Glycol, Poly Methacrylic Acid, And Polymethacrylate.[13]

The simplest method involves the **Chemical Reduction of the metal salt** AgBF4 by NaBH4 in water. The process produces silver nanoparticles in the size range of 3 to 40nm which are characterized by transmission electron microscopy (TEM) and UV-Visible spectroscopy technique to evaluate their quality. Another method involves the reduction of silver nitrate with Ethylene glycol in the presence of poly vinyl pyrrolidone (pvp) producing large quantities of silver nanocubes and is called **Polyol Process**. A modified polyol process using precursor injection technique was used to synthesize spherical Ag-nps with a controllable size and high monodispersity.[14]

Uniform and size controllable silver nanoparticles can be synthesized using **Micro-Emulsion Techniques**. The nanoparticles preparation in two-phase aqueous organic systems is based on the initial spatial separation of reactants (metal precursor and reducing agent) in two immiscible phases.[15]The interface between the two liquids and the intensity of inter-phase transport between two phases, which is mediated by a quaternary alkyl-ammonium salt, affect the rate of interactions between metal precursors and reducing agents. Metal clusters formed at the interface are stabilized, due to their surface being coated with stabilizer molecules occurring in the non-polar aqueous medium, and transferred to the organic medium by the inter-phase transporter.[16]

3. Biological Approach (Green Synthesis)

Green synthesis of silver nanoparticles is preferred because this method is environmental, commercial and single step method and doesn't need elevated temperature, pressure, force and deadly chemicals. Different materials like leaf extract, bark, root, stem, leaf, fungi etc are used for the synthesis of nanoparticles.[17] Apart from these small molecules like vitamins and amino acids are also used as an alternative of chemical methods for the preparation of not only AgNP but some other nano-particles also. Many of the studies report that these AgNPs are prepared without use of any toxic and hazardous chemicals and also cost-effective. In the preparation of AgNPs different type of bacteria are used these are; Lactobacillus strains, *Brevibacterium casei*, *Pseudomonas stutzeri*, *Escherichia coli* and fungi that is used is Fusarium oxysporum.

Biological synthesis includes main 3 components these are solvent, reducing agent and non-toxic material.[18] In the biological method we are able to synthesize nano-particle of controlled size and shape, which is one of the most important requirements for preparation of nanoparticle. Availability of amino acids and proteins is the main advantage of biological method as in this ecofriendly material is used which is less toxic towards both environment and humans. Apart from all these other advantages of AgNPs are availability of vast resources, less time involvement, stability etc. As we know size and shape are most important characteristics of AgNPs, this is because the biological activity of AgNPs depend upon this only. If we see in the literature small size nanoparticles are found to be more effective as well as they have better properties.[19,20]

CHARACTERIZATION

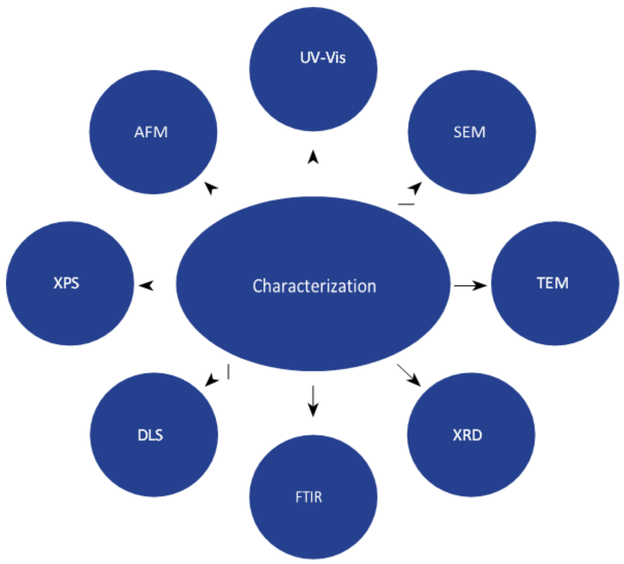

Figure 2: Schematic Representation of Characterization Methods of AgNP's.

For safety and efficacy of every nanoparticle it is very essential to monitor the physiochemical properties. So, to check or evaluate the functional properties and properties of synthesized nanoparticles, depiction is very important. There are many techniques that are used for the characterization of these nanoparticles those are XRD, FTIR, DLS, UV-Visible spectroscopy, TEM, SEM, XPS, so with the use of these technique we are able to determine various characteristic features of synthesized nanoparticles. [21]

1. UV-Visible spectroscopy

This UV-Visible methodology is used to assess the stability of synthesized nanoparticles in addition to being one of the easiest and most reliable ways for the first characterization of produced nanoparticles. The synthesized AgNPs in this process can interact with a specific light wavelength. The sample does not need to be calibrated if the suspension is colloidal. Therefore, it is determined that this method is easy to use, accurate, sensitive, effective, and selective in a wide range of nanoparticles. The Surface Plasmon Resonance (SPR) band, which is often caused by coupled fluctuation of AgNPs electrons in resonance with light wave, is a result of the free movement of electrons in AgNPs since the valence band and conduction band are discovered to be quite close to each other in the research. Particle size, the dielectric medium, and the chemical environment are the three factors that have the most influence on how well AgNP is assimilated from samples.[22]

2. Scanning Electron Microscopy (SEM)

To learn much more about nanotechnology and nanosciences many techniques are used which include electron microscopy and Scanning electron microscopy. SEM is a technique that is used to determine the morphology of the particles. AS SEM is a surface imagining method so with the use of SEM we can determine the particle size, particle size distribution, surface morphology, and nanomaterial shapes. With the use of this method, we can find the morphology of particles and then we can either draw a histogram or can also count the number of particles either manually or by using some other software.SEM and EDX are combined to analyze the chemical composition of silver powder and identify its morphology. SEM has the drawback of not allowing to detect particle's internal structure, but it also has the benefit of allowing to assess particle's purity and degree of aggregation.[23]

3. Transmission Electron Microscopy (TEM)

TEM is one of the most frequently used and an important technique which is used for the characterization of particles. With this technique we can find quantitative dimension of particle size, particle size distribution and particles morphology characters. The proportion of distance between objective lens and specimen and the distance between that objective lens and image plane will help to determine the magnification of TEM. TEM is able to provide better resolution and better analysis then SEM. Limitation of TEM is that it requires high rate of vacuum, sample section should be very thin and sometimes time consuming also.[24]

4. X-Ray Diffraction Spectroscopy (XRD)

Here, the study of both molecular as well as crystalline structure, determination of quantitative movement in chemical species, degree of crystallinity, particle size etc. can be done by using this technique. Structural features of wide range of compounds like glasses, superconductors, inorganic catalysts, polymers can be done with the use of this XRD technique. When light falls on the crystal it results into formation of many patterns of diffraction and then those patterns are able to reflect the physio-chemical properties of the structure of crystal. In case if specimen is in powder then the beams that are diffracted they typically come from sample and then that beam will be able to reflect physiochemical structure of the product. Basically, this method is primary or main technique to identify the crystalline nature of the product. Apart from this, this method is used for phase identification measurements, conduction of qualitative analysis, and also to determine imperfection in the structure in many of the streams like pharmaceutical, environmental, geological, and sometimes forensic science also. But the Disadvantage of this technique is that there is difficulty in growing the crystals.[25]

5. Fourier Transform Infrared Spectroscopy (FTIR)

With the use of FTIR it becomes possible to determine the small changes in absorbance i.e. up to 10^{-3}, which will eventually help in performance of difference spectroscopy which will be then helpful for determination of little combination bands of functionally dynamic residues that left from the large absorption bands of proteins.[26] This method provides accuracy as well as reproducibility and also we are able to determine that whether biomolecules are involved in the synthesis of nanoparticles. Apart from FTIR is non-invasive technique. FTIR is also used in some other aspects like authentication of functional molecules that are graft onto silver, gold, carbon nanotubes etc. This method is also able to provide strong data, rapid collection of data, large signal-to noise ratio and also a smaller amount of sample heat up. Overall if we see FTIR is simple, accurate, valuable, cost effective, non-invasive technique that is used to confirm the function of biomolecules.[27]

6. Dynamic Light Scattering (DLS)

For study of the biological activities by using radiation scattering technique, physiochemical parameters or evaluation of synthesized nanoparticle is an important parameter. Particle size ranging from submicron to one nanometre can be determined using DLS. This method is basically depends upon the interaction between light and particles. It is the most commonly used technique in measurement of particle size and particle size distribution. DLS measures that light which is scattered from a laser which will be able to pass through a colloid, and will mainly rely on Rayleigh that is scattering from that of nanoparticles are on the edge. Then, the modulation of the scattered light force that is acting as a purpose of time is analyzed, and the hydrodynamic size of particles can be resolute. It has been observed that size that is obtaining from DLS is better as compared to TEM, this is possibly will be due to Brownian Motion.[28]

7. X-Ray Photoelectron Spectroscopy (XPS)

To estimate empirical formulae this XPS technique is used. The other name of XPS is Electron Spectroscopy for Chemical Analysis (ESCA). This technique is generally used under high vacuum conditions. With this XPS method we are able to find, identify and characterize the specific groups of macromolecules aromatic rings. It also helps in giving the access of the information regarding qualitative, quantitative and speciation that concerns about the surface of sensor.[29]

8. Atomic Force Microscopy (AFM)

To find dispersion and aggregation of nanomaterials AFM is used generally. Three more different scanning modes are available apart from their size, shape, and structure and these three modes are known to be as contact mode, non-contact mode and intermittent sample contact mode. The communication of nanomaterials with their supported lipid bilayer can be characterized using AFM which cannot be done by other electron microscopy techniques. Limitation of AFM is that due to the size of canilever there is overestimation of lateral dimensions of the sample, so to remove error it should require much more attention.[30]

APPLICATIONS

Silver has been used extensively from last 5000 years for its antibacterial nature. Ag is preferred as nanoparticle for the reason that it has antibacterial property and non-toxic to human beings. Either killing or reducing the growth of bacteria without affecting surrounding cells is known as antibacterial activity. Apart from this activity,AgNPs are also used extensively for their antiviral activity, antifungal, antiangiogenic, anti-inflammatory, drug carrier, anti-oxidative activities.

These are also used in-

- Cosmetics,
- Water Treatment,
- Pollution degradation
- Biosensors
- Imagining Purpose(Diagnostic tools) and
- Textiles.
- Food and Agriculture.

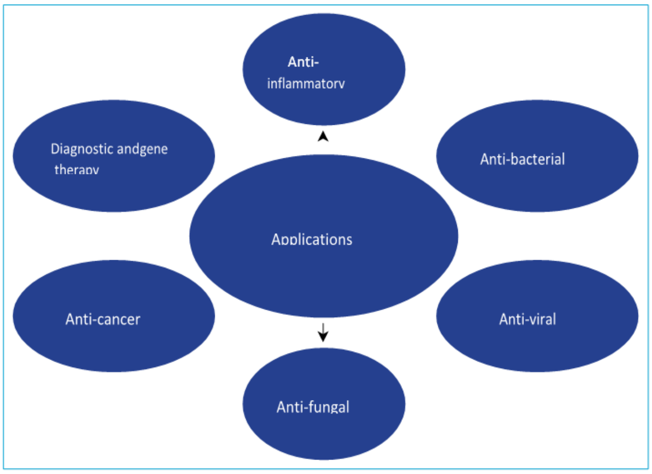

Figure 3: Various Medical Applications of Silver Nanoparticles.

1. Antimicrobial Activity

Researchers found out the functions of Ag NPs as antimicrobial agents against both fungi and bacteria after discovering that silver metal has been utilized as an antiseptic for wound healing. Ag NPs have a great deal of promise against bacteria and fungi that are multi-drug resistant, and because their antibacterial mechanisms vary depending on the species, so does their susceptibility. Methicillin-resistant Staphylococcus aureus, methicillin-resistant Staphylococcus epidermidis, and Streptococcus have been shown to exhibit the strongest antibacterial activity. It has been reported that due to differences in the cell wall structure and quantity of the functional groups on the cell surface of the various bacteria, the chitosan-Ag colloid has better bactericidal efficacy against Escherichia coli but it has very poor activity on C. albicans. Ag NPs have enhanced the effect of commonly used drugs such as Amphotericin B, Nystatin, and Fluconazole.[31,32]

Activity of AgNps on microbes-

1) These particles have the ability to penetrate microbial membrane and make it porous.

2) AgNps release silver ions; these ions can interact with the thiol groups of many vital enzymes and inactivate them.

3) AgNps produce reactive oxygen species (ROS) which make oxidative stress to cells and this was investigated by electron spin resonance spectroscopy.

4) AgNps release Ag+ and this creates free radicals which have the ability to damage the cell membrane and make it porous and lead to cell death.

5) Silver is a soft acid, cells are majorly made up of sulfur and phosphorus which are soft bases, so soft acids react with soft bases. DNA has sulfur and phosphorus as its major components; the nanoparticles interact with it and other soft bases in the cell destroy the DNA and cause cell death.

6) It's noted that AgNps Alter the phosphotyrosine profile of bacterial peptides of gram-negative bacteria which leads to signal transduction inhibition.[33]

2. Antiviral Activity:

In the whole world, viral infections are found to be very common, so it's very important to make antiviral agents that results in showing prominent results. AgNPs are found to be prominent in showing such results this is due to their very small size and their shape also. It has been observed that silver is found to be relatively non-toxic towards humans as well as animals and found to be effective against viruses. In case of HIV AgNPs found to give permissible result. AgNP acts as anti-viral in this case at an early stage of viral replication, mainly acts as virucidal or may be inhibit the entry of virus. AgNP will bind to gp120 and will prevent CD-4 dependent virion binding and infectivity which will result in acting as an effective virucidal agent against cell free virus. Apart from this AgNPs they inhibit post-entry stages of HIV-1 life cycle.[34]

3. Anticancer Activity:

Cancer is basically an uncontrolled growth of cells in specific area in a body. Every 1 in 3 person in the world is suffering from one or another type of cancer. There are many treatments for patients who are suffering from cancer but the side effects of these treatments are very harsh and the process is also very painful. So it is required to find effective, cheap and sensitive molecules for the treatment of cancer. Several studies have been done to know the promising result of AgNPs. It is found to be most suitable as well as an alternative for other cancer treatments. They have ability to target specific cells or tumour at that site only by encapsulation of therapeutic agent in nanoparticle and then used as drug delivery system. After 6 hours of exposure to *Albizia adianthifolia* leaf extract synthesized AgNPs (AA-AgNPs), A549 cells showed 21% and 73% cell viability, respectively, and normal peripheral lymphocytes showed 117 and 109 percent viability. This means that AgNPs aren't harmful to normal PLs cells. At 43 g/mL of AA-AgNPs, 50 percent cell inhibition of A549 cells was achieved, and cell death was induced by the generation of ROS, resulting in apoptosis. After 48 hours of Hoechst staining, MCF-7 cells treated with Sesbania Grandiflora mediated AgNPs (20 g/mL) display nuclear condensation, cell shrinkage, and fragmentation. These changes mean that DNA repair has been enabled as a result of the cleavage.[35]

4. Antifungal Activity

Persons having less immunity are more prone to fungal infections. To overcome the fungus related diseases it is found that this process is found to be very tedious in nature. AgNPs are found to be prominent against many diseases that are caused due to fungi. By inhibiting conidial germination, biologically synthesized AgNPs showed good antifungal activity against *Bipolaris sorokiniana*. Indoor fungal species such as *Penicillium brevicompactum*, *Aspergillus fumigatus*, *Cladosporium cladosporoides*, *Chaetomium globosum*, *Stachybotrys chartarum*, and *Mortierella alpine* cultured on agar media are also inhibited by AgNPs.[36]

5. Neurosurgical catheters

Catheters are used to drain excess cerebrospinal fluid (CSF) in neurosurgery, which can cause cerebral hypertension and brain damage. They can be fully implanted and used as shunts to divert CSF, or be external; both of these cases must be protected from bacterial infection so AgNps become established in everyday neurosurgical. Catheter-associated ventriculitis (CAV) is inflammation of the ventricles of the brain. External ventricular drain Catheters with AgNps were used to determine whether NS is beneficial in preventing CAV. Finally, the study showed that AgNps is potentially beneficial in the prevention of CAV and is promising for using in humans, with no evidence of toxicity.[37]

6. Silver nanoparticles as biosensors

AgNps have a unique advantage that can be utilized for detecting various abnormalities and diseases in the human body including cancer. The plasmonic properties of nanosilver also make it an excellent candidate for bioimaging as they can be used to monitor dynamic reactions. It can be conjugated to the target cells and then be used to absorb light and convert it to thermal energy; the thermal energy can lead to thermal ablation of the target cells.[38]

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