A DISSERTATION ON

BIOSYNTHESIS OF SILVER NANOPARTICLES BY USING *ASPERGILLUS NIGER* AND ITS APPLICATION

SUBMITTED TO THE

DEPARTMENT OF BIOSCIENCES

INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILLMENT
FOR THE
DEGREE OF MASTER OF SCIENCE
IN BIOTECHNOLOGY

BY

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TO WHOM IT MAY CONCERN

This is to certify that **Mr. Mohd Kamran Khan**, a student of M.Sc. Biotechnology (IV semester), Integral University has completed his four months dissertation work entitled ***"Biosynthesis of Silver Nanoparticles by using Aspergillus Niger and its application"*** successfully. He has completed this work from 2 Feb to 2 June 2022 at the Department of Biosciences, Integral University, under the guidance of **Dr. Salman Khan**.

The dissertation was a compulsory part of his M.Sc. degree. I wish him good luck and a bright future.

Dr. Snober S. Mir
Head,
Department of Biosciences,
Integral University, Lucknow

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IU/DBS/S00192/2022/CIN/221116 **June 2022**

CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Mr. Mohd Kamran Khan**, during the months 2 Feb to 2 June 2022 reported in the present thesis was under my guidance and supervision. The results reported by him are genuine and the script of the thesis has been written by the candidate himself. The thesis entitled ***"Biosynthesis of Silver Nanoparticles by using Aspergillus Niger and its application"*** is, therefore, being forwarded for acceptance in partial fulfillment of the requirements for the degree award of the student of M.Sc. Biotechnology (IV semester), Department of Biosciences, Integral University, Lucknow, (U.P).

Dr. Salman Khan
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ACKNOWLEDGEMENT

First of all, I bow in reverence to the Almighty for blessing me with strong willpower, patience, and confidence, which helped me in completing the present work.

I would like to express my special thanks to ***Dr. Snober S. Mir (Head, Department of Biosciences)*** *for allowing me to join the department laboratory and for providing all the necessary facilities ever since I started my work.*

I would like to express my deep sense of gratitude to ***Dr. Salman Khan (Assistant professor)*** *for their invaluable guidance throughout my dissertation work and academic session. It would have been impossible to complete this work in so short a time without his constant guidance. I wish every trainee and research student were fortunate enough to have such an affectionate guide.*

I gratefully acknowledge ***Ms. Pooja Mishra*** *and* ***Mr. Tabrez Faruqui*** *(Research Scholars) who inspired and encouraged me during various steps of my work although they were busy with their daily tasks but always were available to answer my questions and clear all my doubts.*

My gratitude also goes to all my friends who were fundamental in supporting me during this course of the thesis.

My acknowledgment will be incomplete if I do not mention my parents with whose blessing, I was able to achieve my goal successfully. There are no words to express my feelings toward them. I silently acknowledge my debt to them.

Mohd Kamran Khan **Date**

LIST OF ABBREVIATIONS

AgNPs	Silver nanoparticles
PBS	Phosphate buffer
M	Molarity
mM	Milli Molar
DLS	Dynamic Light Scattering
OD	Optical density
M deg	Milli degree
SPR	Surface Plasma Resonance
TEM	Transmission Electron Microscopy
SEM	Scanning Electron Microscopy
UV-Vis	Ultraviolet-Visible Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
ZP	Zeta potential
nm	Nanometer

Introduction

Nanotechnology

Nanotechnology was first defined by Tokyo Science University Professor Norio Taniguchi in 1974 as *"Nanotechnology mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or by one molecule"*. Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterization, and application of materials and devices whose smallest functional organization, in at least one dimension, is on the nanometre scale or one billionth of a meter. Nanotechnology is the study of extremely small structures. The prefix "nano" is a Greek word that means "dwarf". The word "nano" means very small or miniature size. The ideas and concepts behind nanoscience and nanotechnology started with the talk entitled "There's Plenty of Room at the Bottom" at the American Physical Society Meeting at the California Institute of Technology on December 29, 1959, long before the term nanotechnology was used. Nanotechnology is a scientific movement that has the potential to transform the diagnosis and treatment of disease in the 21st century. The area of investigation is defined by the study, design, manipulation, manufacture, and control of materials or devices by physical or chemical means at resolutions on the order of one billionth of a meter. The potential for a wide range of clinical applications makes a basic understanding of nanotechnology important to physicists. (Gordan et al., 2007) In other words, Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterization, and application of materials and devices whose smallest functional organization in at least one dimension is on the manometer scale or one billionth of a meter. At these scales, consideration of individual molecules and interacting groups of molecules with the bulk macroscopic properties of the material or device becomes important, since it is control over the fundamental molecular structure that allows control over the macroscopic chemical and physical properties. (Silva and Gabriel, 2004) Today's scientists and engineers are finding a wide variety of ways to deliberately make materials at the Nanoscale to take advantage of their enhanced properties such as higher strength, lighter weight, increased control of light spectrum, and greater chemical reactivity than their larger-scale counterparts.

The promise that nanotechnology brings is multifaceted, offering not only improvements to the current techniques, but also providing entirely new tools and capabilities by

manipulating drugs and other materials at the nanometre scale, the fundamental properties, and bioactivity of the materials can be altered. These tools can permit control over the different characteristics of drugs or agents such as (Caruthers et al., 2007).

a. alteration in solubility and blood pool retention time

b. controlled release over short or long durations

c. environmentally triggered controlled release or highly specific site-targeted delivery.

Nanoparticles

Nanoparticles are sized between 1-100nm. The opportunity provided by nanoparticles is that their properties differ from bulk material of the same composition and may be tuned by varying their size, shape, and chemical environment. Nanoparticles (NPs) can be engineered to possess unique compositions and functionalities, which can provide novel tools and techniques that have not previously existed in biomedical research. For example, NPs can be used to image biological processes on the cellular level. They can also be utilized to detect analytes at the attomolar range. (Al Hoff et al., 2010) Nanoparticles can be engineered with distinctive compositions, sizes, shapes, and surface chemistries to enable novel techniques in a wide range of biological applications. The unique properties of nanoparticles and their behavior in the biological milieu also enable exciting and integrative approaches to studying fundamental biological questions. The fact that nanoparticles exist in the same size domain as proteins make nanomaterials suitable for bio tagging or labeling. However, size is just one of many characteristics of nanoparticles that is rarely sufficient if one is to use nanoparticles as biological tags. To interact with biological targets, a biological or molecular coating or layer acting as a bioinorganic interface should be attached to the nanoparticle. Examples of biological coatings may include antibodies, biopolymers like collagen (Sinani et al., 2003), or monolayers of small molecules that make the nanoparticles biocompatible (Zhang Y et al., 2002). In addition, as optical detection techniques are widespread in biological research, nanoparticles should either fluoresce or change their optical properties.

In general, nanoparticles used in the field of biotechnology range in particle size between 10 and 500 nm, seldom exceeding 700 nm. The Nano size of these particles allows various communications with biomolecules on the cell surfaces and within the cells in a

way that can be decoded and designated to various biochemical and physicochemical properties of these cells. Similarly, its potential application in drug delivery systems and non-invasive imaging offered various advantages over conventional pharmaceutical agents. (Mody VV et al., 2009) To utilize nanoparticles at their full throttle, the Nanoparticulate systems must be stable, biocompatible, and selectively directed to specific sites in the body after systemic administration. More specific targeting systems are designed to recognize the targeted cells such as cancer cells. This can be achieved by conjugating the nanoparticle with an appropriate ligand, which has a specific binding activity for the target cells.

Silver Nanoparticles

Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties. These include optical, electrical, and thermal, high electrical conductivity and biological properties. Due to their peculiar properties, they have been used for several applications, including as antibacterial agents, in an industrial, household, and healthcare-related products, in consumer products, medical device coatings, optical sensors, and cosmetics, in the pharmaceutical industry, the food industry, in diagnostics, orthopedics, drug delivery, as anticancer agents, and have ultimately enhanced the tumor-killing effects of anticancer drugs. Recently, AgNPs have been frequently used in many textiles, keyboards, wound dressings, and biomedical devices. Nanosized metallic particles are unique and can considerably change physical, chemical, and biological properties due to their surface-to-volume ratio; therefore, these nanoparticles have been exploited for various purposes. To fulfill the requirement of AgNPs, various methods have been adopted for synthesis. Generally, conventional physical and chemical methods seem to be very expensive and hazardous. Interestingly, biologically-prepared AgNPs show high yield, solubility, and high stability. Among several synthetic methods for AgNPs, biological methods seem to be simple, rapid, non-toxic, dependable, and green approaches that can produce well-defined size and morphology under optimized conditions for translational research. In the end, a green chemistry approach for the synthesis of AgNPs shows much promise. After synthesis, precise particle characterization is necessary, because the physicochemical properties of a

particle could have a significant impact on its biological properties. To address the safety issue of using the full potential of any nanomaterial for human welfare, in nanomedicines, in the healthcare industry, etc., it is necessary to characterize the prepared nanoparticles before application. The characteristic features of nanomaterials, such as size, shape, size distribution, surface area, shape, solubility, aggregation, etc. need to be evaluated before assessing toxicity or biocompatibility. To evaluate the synthesized nanomaterials, many analytical techniques have been used, including ultraviolet-visible spectroscopy (UV-vis spectroscopy), X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), and so on.

Fig. 1. Silver Nanoparticles.

The physicochemical properties of nanoparticles enhance the bioavailability of therapeutic agents after both systemic and local administration and on the other hand, they can affect cellular uptake, biological distribution, penetration into biological barriers, and resultant therapeutic effects. Therefore, the development of AgNPs with controlled structures that are uniform in size, morphology, and functionality is essential for various biomedical applications.

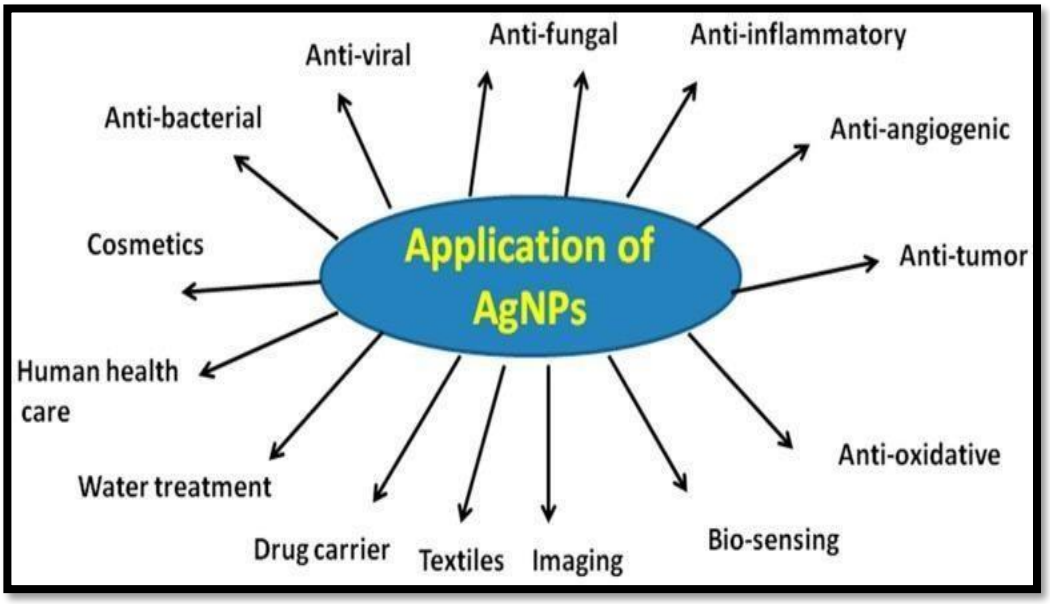

Fig. 2. Applications of Silver Nanoparticles.

Review of literature

Nanotechnology

Nanotechnology deals with materials in the size of 0.1 to 100 nm; however, it is also inherent that these materials should display different properties such as electrical conductance, chemical reactivity, magnetism, optical effects, and physical strength, from bulk materials as a result of their small size. Nanotechnology is the treatment of individual atoms, molecules, or compounds into structures to produce material and devices with special properties. Nanotechnology involves work from top-down i.e. reducing the size of large structures to the smallest structures e.g. photonics applications in Nanoelectronics and nanoengineering, top-down or the bottom up, which involves changing individual atoms and molecules into nanostructures and more closely resembles chemistry biology. This can be used for a broad range of applications and the creation of various types of nanomaterials and nanodevices.

The development in the field of nanotechnology started in 1958 and the various stages are summarized in the above schematic diagram. Different nanomaterials are being produced using copper, zinc, titanium, magnesium, gold, alginate, and silver. Nanoparticles are being used for diverse purposes, from medical treatments, using in various branches of industry, production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes.

Platforms for nanoparticles

There are diverse types of NPs platforms that differ in size, shape, compositions, and functionalities. These nanoparticle platforms are discussed below:

Liposomes: These are the first platform for nanoparticles. In 1965, liposomes were described as a model of cellular membranes. After that, liposomes were used for genetic and drug delivery. Liposomes are vesicles in spherical shape which contain lipids of single or multiple bilayer structures that can assemble themselves in aqueous systems. Liposomes can be used for targeting ligands to upsurge the buildup of diagnostic and therapeutic agents within anticipated cells.

Albumin-bound: NAB uses endogenous albumin trails which transport hydrophobic molecules in the bloodstream. It quandaries with hydrophobic molecules with non-covalent reversible binding. So, this is adapted for drug delivery.

Polymeric: Polymeric NPs formed from biocompatible and biodegradable polymers have been extensively investigated as therapeutic carriers. Polymeric NPs are formulated through block-copolymers of different hydrophobicity. These copolymers spontaneously assemble into a core-shell micelle formation in an aqueous environment. Polymeric NPs have been formulated to encapsulate hydrophilic and/or hydrophobic small drug molecules, as well as proteins and nucleic acid macromolecules (Wang, A. Z., et al., 2008). The NP design can allow for the slow and controlled release of drugs at target sites. Polymeric NPs are usually able to improve the safety and efficacy of the drugs they carry. Functionalizing polymeric NPs with targeting ligands for improved drug delivery has been an important area of investigation since polymeric NPs are unique in their ability to be tailored before particle assembly. The incorporation of targeting ligands on the NPs can lead to their increased uptake along with their cargo, leading to enhanced therapeutic outcomes.

Dendrimers: Another type of polymeric NP is dendrimers. Dendrimers are regularly branched macromolecules made from synthetic or natural elements including amino acids, sugars, and nucleotides. They have a central core, interior layers of branches, and an exterior surface.16 The varied combination of these components can yield dendrimers of well-defined size, shape, and branching length/density. As a result of their unique design, dendrimers can be developed as sensors as well as drug and gene delivery carriers. Dendrimers can be loaded with small molecules in the cavities of the cores through chemical linkage, hydrogen bond, and or hydrophobic interaction. The exterior surface can also be readily modified to produce chemical functional groups for molecular targeting groups, detecting and imaging agents, and therapeutic attachment sites (Mintzer, M. A., et al., 2005).

Iron oxide: Iron oxide NPs are widely studied as passive and active targeting imaging agents as they are mainly superparamagnetic. The superparamagnetic iron oxide NP (SPION) generally has an iron oxide core with a hydrophilic coat of dextran or another biocompatible compound to increase its stability. The most widely used SPIONs consist of a magnetite (Fe_3O_4) and/or maghemite (γFe_2O_3) core. These NPs exhibit size-dependent superparamagnetism, which allows them to become magnetized with the application of an external magnetic field and exhibit zero net magnetization upon removal of the magnetic field. SPIONs have been successfully used as T2-weighted magnetic resonance (MR) contrast agents to track and monitor cells. SPIONs have several advantages over conventional gadolinium-chelate contrast agents including decreased toxicity and increased imaging sensitivity and specificity. SPIONS can also be degraded to iron and iron oxide molecules that are metabolized, stored in cells as ferritin, and incorporated into hemoglobin. Currently, two SPIO agents, ferumoxides (120–180 nm) and ferucarbotran (60 nm) are clinically approved for MRI. SPIONs have also been used in molecular imaging applications such as the detection of apoptosis and gene expression. SPIONs can be functionalized with magnetic, optical, radionuclide, and specific targeting ligands for multimodal imaging. They can also potentially be used as non-invasive diagnostic tools and as drug delivery vehicles (Mahmoudi, M., et al., 2011).

Quantum dot: First discovered in 1980, quantum dots (QDs) are semiconductor particles that are less than 10 nm in diameter. QDs display unique size-dependent electronic and optical properties. Most QDs studied consist of a cadmium selenide (CdSe) core and a zinc selenide (ZnS) cap. The absorption spectra of these particles are very broad and emission is confined to a narrow band. QDs can also emit bright colors, have long lifetimes, high efficiencies, and are stable against photobleaching. They can be generated to have different biochemical specificities and can be simultaneously excited and detected. As a result, QDs have several significant advantages over many organic fluorophore dyes for optical applications. They are widely used in biological research as fluorescence imaging tools for applications such as cell labeling and biomolecule tracking.293028 The small size of quantum dots also enables them to be suitable for biomedical applications such as medical imaging and diagnostics (Michalet, X., et al., 2005).

Gold: Gold NPs offer many size-and-shape-dependent optical and chemical properties, biocompatibility, and facile surface modification. Gold NPs can strongly enhance optical processes such as light absorption, scattering, fluorescence, and surface-enhanced Raman scattering (SERS) due to the unique interaction of the free electrons in the NP with light. These properties have enabled the realization of gold NPs in many applications such as biochemical sensing and detection, biological imaging, diagnostics, and therapeutic applications. Sensing techniques include the use of gold NPs in colorimetric arrays and the use of gold NPs as substrates in SERS to significantly enhance Raman scattering, allowing for spectroscopic detection and identification of proteins and single molecules at the NP surface. Gold NP probes have also been used to detect heart disease and cancer biomarkers. They can also transform absorbed light into heat and therefore, have a high potential for infrared phototherapy (Huang, X., et al., 2006).

Classification of nanomaterials

❖ Nanomaterials can be classified dimension-wise into the following categories:

❖ Nanorods and nanowires have dimensions less than 100nm.

❖ Tubes, fibers, and platelets have dimensions less than 100nm.

❖ Particles, quantum dots, and hollow spheres have 0 or 3 dimensions< 100 nm. Based on phase composition, nanomaterials' indifferent phases can be classified as

❖ The nanomaterial is called single-phase solids. Crystalline, amorphous particles, and layers are included in this class.

❖ Matrix composites and coated particles are included in multi-phase solids.

❖ Multi-phase systems of nanomaterial include colloids, aero gels, ferrofluids, etc.

Classification of nanoparticles

Various characteristics and brief applications of nanosystems

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Synthesis of nanoparticles

Nanoparticles can be synthesized chemically or biologically. Many adverse effects have been associated with chemical synthesis methods due to the presence of some toxic chemicals absorbed on the surface. Eco-friendly alternatives to chemical and physical methods are biological ways of nanoparticle synthesis using microorganisms, enzymes, fungus, and plant or plant extracts.

The development of these eco-friendly methods for the synthesis of nanoparticles is evolving into an important branch of nanotechnology, especially silver nanoparticles, which have many applications.

Synthesis of silver nanoparticles

Top-down approach

Mechanical grinding/milling: The top-down approach is an extension of traditional methods to produce large quantities of fine and/or nanocrystalline powders. These processes generally involve high-energy dry/wet milling, with the addition of milling aids, and typically use milling times from several hours up to many days [(Schwarz, J. A., at al 2004), (Zhang, D. L. 2004), (Koch, C. C. 2003), (DeCastro, C. L., 2002),(Suryanarayana, C. 2001)]. The advantages include relatively simple operation, ease of scalability, and convenience to form slurries in various liquid matrices. In recent years, much improvement was made to the milling instruments and the quality of grinding media in the <0.1 mm diameter range, which has led to significant progress in the use of this technique for the production of a wide range of metal oxide nanoparticles. However, the top-down approach still suffers from difficulties in ensuring that all the particles are milled properly. This disadvantage becomes more serious as the hardness of metal oxide materials increases. The drawback typically results in a wide particle size distribution having a long ‘tail’ on the larger particle side, representing the un-milled precursors in the final commercial products. In addition, longer milling times will result in more milling impurities. The removal of these impurities, and/or any grinding aids which were used in the processing can cause subsequent problems.

Bottom-up approach

Vapour phase technique

Vapour phase techniques create nanoparticles by the rapid solidification of a liquid or vapor in a gaseous medium. This has been achieved by methods ranging from burning precursors to more elaborate vapourization or plasma-based synthesis methods. Particle size, agglomeration, and size distribution are controlled by the vaporization rate and the flow of the newly formed particles. Since the melting temperature of metal oxides is normally extremely high, corresponding metals are often used as the precursors which are vapourised using resistance, electron beam, laser, or electric arc at a temperature beyond the melting point of the material, until a sufficient rate of atomization is achieved. The technique is advantageous for producing metal oxide nanoparticles with high purity and high crystallinity, due to the fewer sources of contaminants and the high temperatures involved. However, the method suffers from the inevitable trade-off between particle size/quality and throughput. Increasing production rates will make it increasingly difficult to control particle growth and prevent agglomeration, due to high-temperature operations and the lack of a solid medium that hinders agglomeration.

Liquid phase technique

Liquid phase processes have been widely used in industry to make conventional micron-scale powders, and have proven to be economical for many materials. The advantages are the ability to control particle sizes, shapes, and stoichiometry in a precise manner, as well as flexibility in reaction paths. To date, the smallest commercial nanoparticles such as quantum dots are produced using liquid-phase techniques. Most of the recent developments in the technique have revolved around the improvement of the stability of an inherently unstable system, by the use of Commercial-scale production of inorganic nanoparticles various polymers, vesicles, gels, or microemulsions that constrain the growth of the particles. An additional process to remove those additives is normally required. However, the difficulty in scaling up the production lies in achieving a stable and uniform reaction environment in large chemical baths to ensure the uniform quality of the resulting nanoparticles. An increase in production rate requires high particle

concentration which causes particle agglomeration during particle growth. Moreover, the resulting particles are often hydroxides or other types of salts and hence additional processes to decompose the salts into oxides are necessary, which introduces the chance of particle sintering. As a result, nanoparticles in commercial-scale production have characteristics of spherical shapes, a narrow size distribution of primary particles but high degrees of agglomeration. Due to the chance of agglomeration that is inherent to vapor and liquid phase methods, mechanical grinding is often employed at the final production stage of those nanoparticles (Tsuzuki, T. 2009), (Park, J., Joo, J., at al 2007), (Eastoe, J., at al 2006), (Boldyrev, V. V. 1996), (PG, M. 1995).

Solid-phase technique

Mechanochemical processing

The technology uses high-energy dry milling to mechanically induce chemical reactions to occur at low temperatures in a ball mill. Milling of precursor powders leads to the formation of a nanoscale composite structure of the starting materials which react during milling or subsequent heat treatment to precipitate nanoparticles of the desired phase within a solid matrix. By carefully controlling the volume ratio between nanoparticle and salt matrix phases, the precipitated nanoparticles can be separated from each other by the solid matrix. The nanoparticles can be further heat treated in the solid matrix that prevents temperature-induced agglomeration from occurring. Then the nanoparticles are collected simply by selective removal of the matrix phase. The technique has advantages such as relatively simple operation and eases to create a uniform reaction environment that leads to uniform size and shape of nanoparticles. Of significance is the fact that this technique allows the formation of nanoparticles separated by a solid matrix during the particle growth stage, leading to agglomeration-free nanoparticles. However, additional processes to remove the solid matrix and by-product phases increase production costs and a chance of contamination. The characteristics of mechanochemically produced nanoparticles are near-spherical shapes, very narrow size distribution, and low levels of agglomeration (Senna, M. 2001), (Tsuzuki, T. 2009).

Biosynthesis: Mechanism

Biosynthesis of nanoparticles by microorganisms is a green and eco-friendly technology. Diverse microorganisms, both prokaryotes, and eukaryotes are used for the synthesis of metallic nanoparticles viz. Silver, gold, platinum, zirconium, palladium, iron, cadmium, and metal oxides such as titanium oxide, zinc oxide, etc. These microorganisms include bacteria, actinomycetes, fungi, and algae. The synthesis of nanoparticles may be intracellular or extracellular according to the location of nanoparticles. **Intracellular synthesis of nanoparticles by fungi**: The method involves the transport of ions into microbial cells to form nanoparticles in the presence of enzymes. As compared to the size of extracellularly reduced nanoparticles, the nanoparticles formed inside the organism are smaller. The size limit is probably related to the particle nucleating inside the organism.

1. **Extracellular synthesis of nanoparticles by fungi**

It has more applications as compared to intracellular synthesis since it is void of unnecessary adjoining cellular components from the cell. Mostly, Fungi are known to produce nanoparticles extracellularly because of the reduction and capping of nanoparticles.

2. **Microbes for the production of nanoparticles**

Both unicellular and multicellular organisms produce inorganic material either intra or extracellularly. The ability of microorganisms like bacteria and fungi to control the synthesis.

Fungi

Fungi contain enzymes and proteins, which have the capabilities of reducing metal ions into nanoparticles and then behaving as a stabilizer for nanoparticles. Fungi produce a large number of proteins, due to which the conversion of metal salts into metal nanoparticles is very fast. *A. fumigates* and Phoma sp. Can be used for the extracellular synthesis of silver nanoparticles. Gold nanoparticles have been synthesized using *Cylindrocladium floridanum* and *A. niger*. Magnetite nanoparticles have been synthesized from *F. oxysporum* and *Verticillium sp.*

Aspergillus Niger: In 1917, a food chemist named James Currie made a promising discovery: any strain of the filamentous mold *Aspergillus niger* would produce high concentrations of citric acid when grown in a sugar medium. This tricarboxylic acid, which we now know is an intermediate of the Krebs cycle, had previously been extracted from citrus fruits for applications in food and beverage production. Two years after Currie's discovery, industrial-level production using *A. niger* began, the biochemical fermentation industry started to flourish, and industrial biotechnology was born. A century later, citric acid production using this mold is a multi-billion dollar industry, with *A. niger* additionally producing a diverse range of proteins, enzymes, and secondary metabolites. The fungus *Aspergillus niger* is a type of mold, which can sometimes be attributed to the cause of some cases of pneumonia. It is also the causative agent of 'black mold' on the outsides of certain foods, such as apricots, onions, grapes, etc - therefore making Aspergillus niger a food 'spoilage' organism. These are classed as 'Conidiophores' - an organism that forms filaments or hyphae, otherwise known as conidia (the a-sexual method of fungal reproduction). The name 'Aspergillus' comes from the Latin word 'aspergillum', which roughly translates to 'holy water sprinkler', referring to the shape of these sprinklers being very similar to how these fungi appear when viewed under a microscope.

Green synthesis of silver nanoparticles

Green synthesis refers to the recruitment of biogenic matter including plant extracts, biopolymers, and microbial sources like bacteria, fungi, algae, and yeast for nanomaterials fabrication. The development of biocompatible, non-toxic, and eco-friendly methods for the synthesis of AgNPs is a topic of concern in green synthesis.

The advancement of green synthesis of AgNPs is progressing as a key branch of nanotechnology, where the use of biological entities like microorganisms, plant extract, or plant biomass for the production of AgNPs could be an alternative to chemical and physical methods in an eco-friendly manner. In the case of biological synthesis of AgNPs, the reducing agent for reducing Ag+ ions and the stabilizing agents for preventing aggregation of AgNPs is replaced by molecules produced by living organisms. Green synthesis of AgNPs possesses the following advantages over traditional chemical

methods. 1. Green synthesis is simple and usually involves a 1-pot reaction. 2. It is amenable to scale-up 3. Toxicity-associated hazardous chemicals are eliminated. 4. Green biological entities can be used as reducing and capping agents. 5. Finally the process is cost-effective, requires a little intervention or input of energy, uses renewable resources, and environmentally friendly methods and it is not necessary to use high pressure, energy, and temperature. Toxic chemicals. Green synthesis of AgNPs involves three main steps based on the green chemistry perspective. 1. Selection of a biocompatible and nontoxic solvent medium. 2. Selection of environmentally benign reducing agents, and 3. Selection of nontoxic capping and stabilizing agents for stabilization of AgNPs which prevents aggregation of AgNPs.

Green Synthesis of Silver Nanoparticles Using Aspergillus niger:

1. People who become infected with drug-resistant microorganisms usually spend more time in the hospital and require a form of treatment that uses two or three different antibiotics and is less effective, more toxic, and more expensive.

2. Nanoparticles (NP) are usually clusters of atoms in the size range of 1–100 nm. It is understood that the properties of a metal NP are determined by its size, shape, composition, crystallinity, and structure. As an important metal, silver nanoparticles (AgNPs) have several applications, from electronics and catalysis to infection prevention and medical diagnosis

3. For example, AgNPs could be used as substrates for Surface-Enhanced Raman Scattering (SERS) to probe single molecules, and also useful catalysts for the oxidation of methanol to formaldehyde. AgNPs have been known as excellent antimicrobial and anti-inflammatory agents, and thus were used to improve wound healing. To date, several physical and chemical strategies have been employed for the synthesis of AgNPs.

4. However, concern has been raised about the toxicity of chemical agents used in AgNPs synthesis. Thus, it is essential to develop a green approach for AgNPs production without using hazardous substances for human health and the environment.

5. Fungi have been known to secrete much higher amounts of bioactive substances,

which made fungi more suitable for large-scale production

6. The extracellular biosynthesis using fungi could also make downstream processing much easier than the large number of active substances secreted by fungi that played important roles as reducing agents and capping agents in the reaction bacteria.

7. AgNPs have the potential to serve as an alternative to antibiotics and to control microbial infections such as those caused by multidrug-resistant pathogens.

Green synthesis refers to the recruitment of biogenic matter including plant extracts, biopolymers, and microbial sources like bacteria, fungi, algae, and yeast for nanomaterials fabrication. The development of biocompatible, non-toxic, and eco-friendly methods for the synthesis of AgNPs is a topic of concern in green synthesis.

Characterization of nanoparticles

UV/Vis Spectrophotometer: Ultraviolet-visible spectrophotometer refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, atoms and molecules undergo electronic transitions. Absorption spectroscopy is complementary to fluorescence spectroscopy, in that fluorescence deals with the transition from the excited state to the ground state, while absorption measures transition from the ground state to the excited state.

Fourier transform infrared spectroscopic analysis (FTIR): Infrared spectroscopic analysis is used to determine the chemical functional groups in the sample. It is the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. Different functional groups absorb characteristic frequencies of IR radiation. Using various sampling accessories, IR spectrometers can accept a wide range of sample types such as gases, liquids, and solids. Thus, IR spectroscopy is an important and popular tool for structural elucidation and compound identification.

Dynamic Light Scattering (DLS): It is also known as quasi-elastic light scattering, a noninvasive, well-established technique for measuring the size and size distribution of molecules and particles typically in the submicron region. The principle of DLS states that the particles suspended within a liquid undergo Brownian motion. The larger the particle, the slower the Brownian motion will be. DLS monitors the Brownian motion with light scattering. One of the most popular light scattering techniques because it allows particle size down to 1mm diameter. Simple DLS instruments that measure at a fixed angle can determine the mean particle size in a limited range.

Scanning electron microscopy(SEM): This electron microscopy-based technique determines the size, shape, and surface morphology with direct visualization of the nanoparticles. Therefore, scanning electron microscopy offers several advantages in morphological and sizing analysis. However, they provide limited information about the size distribution and true population average. During the process of SEM characterization, the solution of nanoparticles should be initially converted into a dry powder. This dry powder is then further mounted on a sample holder followed by coating with a conductive metal (e.g. gold) using a sputter coater. The whole sample is then analyzed by scanning with a focused beam of electrons. Secondary electrons emitted from the sample surface determine the surface characteristics of the sample. This electron beam can often damage the polymer of the nanoparticles which must be able to withstand vacuum. The average mean size evaluated by SEM is comparable with results obtained by dynamic light scattering. In addition, these techniques are time-consuming, costly, and frequently need complementary information about sizing distribution. **Transmission electron microscopy(TEM)**: Experimental difficulties in studying nanostructures stem from their small size, which limits the use of traditional techniques for measuring their physical properties. Transmission electron microscopy techniques can provide imaging, diffraction, and spectroscopic information, either simultaneously or serially, of the specimen with an atomic or a sub-nanometer spatial resolution. TEM operates on a different principle than SEM, yet it often brings the same type of data. The sample preparation for TEM is complex and time-consuming because of its requirement to be ultra-thin for electron transmittance. High-resolution TEM imaging, when combined with nano diffraction, atomic resolution electron energy-loss spectroscopy, and nanometer

resolution X-ray energy dispersive spectroscopy techniques, is critical to the fundamental studies of importance to nanoscience and nanotechnology. During the TEM characterization, nanoparticle dispersion is deposited onto support grids or films. After dispersion, they are fixed using either a negative staining material (phosphotungstic acid or derivatives, uranyl acetate, etc., or by plastic embedding). This is done to make nanoparticles withstand the instrument vacuum and facilitate handling. Alternatively, the nanoparticles sample can also be exposed to liquid nitrogen temperatures after embedding in vitreous ice. When a beam of electrons is transmitted through an ultra-thin sample it interacts with the sample as it passes through The surface characteristics of the sample are obtained. TEM imaging mode has certain benefits compared with the broad-beam illumination mode.

Antimicrobial/ antifungal activities

In recent years, Nobel metal NPs have been the subject of research interest because their unique properties such as electronic, optical, mechanical, magnetic, and chemical properties are significantly different from the bulk material. The physical and chemical properties of NPs are functions of their size/ shape and are therefore different as compared to size-independent constant physical properties of bulk material. Properties of AgNPS are significantly different from bulk material. These are large surface-to-volume ratios leading to a large fraction of surface atoms, high surface energy, spatial confinement, and reduced imperfections. The size, shape, and surface morphology of AgNPs play a vital role in controlling their properties. The antibacterial properties of AgNPs are associated with their oxidation and liberation of Ag+ ions to the environment making them an ideal biocidal agent. It is expected that a large surface area to volume ratio and a high fraction of surface atoms AgNPs lead to high antimicrobial activity as compared to bulk silver metal. Moreover, the small size of AgNPs facilitates the penetration through cell membranes to affect intracellular processes from inside. Additionally, excellent antibacterial properties exhibited by AgNPs are due to their well-developed surfaces which provide maximum contact with the environment.

The formation of free radicals from the surface of AgNPs may be considered to be another mechanism by which the cells die. Free radicals can damage the cell membrane and make it porous which can ultimately lead to cell death. Gram+ve bacteria are made of thick cell walls containing peptidoglycan so that AgNPs do not affect easily. AgNPs easily penetrate Gram -ve bacteria due to the structure of the cell wall of that thin lipid layer, so AgNPs easily enter the cell and disturbs it.

1. They are considered a novel and potential alternative to standard antibiotic drugs since they have great potential against the increasing multidrug resistance in pathogenic bacteria and fungi. Development and synthesis of these nanomaterials for use as an alternate antibiotic therapy straightly depend on some physical and chemical properties such as their size, shape, concentration, and zeta potential. Therefore, the properties of nanoparticles should be considered and given more attention when designing antimicrobial AgNPs.

2. One of the most important physicochemical properties that affect antimicrobial activity is size. Typically, smaller nanoparticles have relatively increased stability and enhanced antimicrobial activity. This is due to the larger surface area of smaller nanoparticles, which provides a higher interaction area and ascendant intracellular penetration.

3. The size of nanoparticles should be lower than 50 nm to have an effective antimicrobial activity Nanoparticles in the range of 10–15 nm sizes have superior antimicrobial activity.

4. The colloidal AgNPs synthesized by using maltose and lactose had higher antibacterial and bactericidal activity against tested Gram-positive and Gram-negative bacteria than those synthesized by using glucose and galactose.

5. The shape is also a crucial Physico-chemical property regarding antimicrobial activity. AgNPs with different shapes has been shown to exhibit different antimicrobial activity due to displaying various degrees of interaction with the cell membrane and hence causing the membrane damage.

6. Another important factor is concentration.

7. Zeta potential is another Physico-chemical property that has an effect on antimicrobial activity since the interaction between nanoparticles and the cell membrane is based on electrostatic adhesion.

8. The reducing and capping materials of the green synthesized AgNPs are derived from the biological extracts containing various naturally occurring compounds and biomolecules such as alkaloids, terpenoids, phenolics, vitamins, coenzymes, carbohydrates, and enzymes, and proteins. Therefore, these molecules increase the probability of attachment and action of AgNPs on the microbial cells.

9. Although antimicrobial activity of AgNPs alone or in combination with standard antibiotic drugs has been proved against a wide range of microorganisms including Gram-negative and Gram-positive bacteria, and fungi, still a little is known about the precise mechanism of their mode of antimicrobial action.

A. Limited new antifungal targets are explored and fungi are also developing resistance mechanisms through mutation arch. In such scenarios, silver nanoparticles which have already been proved to exert antibacterial action are often tried against fungal infections. Due to a limited antifungal arsenal, nanoparticles are thought to serve as carriers of antifungals.

B. If the reducing agent itself serves antifungal action and deposits over the nanoparticle surface as a capping agent, it would offer synergistic antifungal action. Antifungal drug molecules can also be successfully adsorbed on the surface of biogenic silver and thus can offer an efficient strategy for antifungal drug delivery.

C. Green synthesized AgNPs are also reported to exhibit antifungal activity due to their bio- coating in combination with their size.

D. It has been established by food poisoning techniques that the growth of following fungi like *Bipolaris spicifera, Fusarium oxysporum, and Aspergillus niger* Therefore AgNPs may be used as a good antifungal drug to control several pathogenic fungi.

Application of AgNPs

Compared to bulk materials, AgNPs have multiple unique properties, which make them suitable for applications in the medical industry, food packaging bioengineering, catalysis, and environmental sciences (Zhao, X., et al., 2018).

1. Applications in medicine

The applications of AgNPs against pathogenic microorganisms, cancer, and HIV have been extensively explored. Here, we briefly focus on the functions of AgNPs as antiviral, antimicrobial, antitumor, and anti-inflammatory agents.

2. Antiviral agents

Many chemical AgNPs have been successfully used to inhibit viruses, including HIV-1, hepatitis B virus, Monkeypox virus, Tacaribe virus, herpes simplex virus, and respiratory syncytial virus (Khandelwal, N., et al., 2014). For example, AgNPs have dose-dependent anti-retrovirus activity and exhibit high potency at 50mM (98%) to inhibit HIV-1 replication. AgNPs (5 mM) reduced the percentage of apoptotic Hut/CCR5 cells from 49 to 35%, by inhibiting viral replication [86]. It was also demonstrated that AgNPs undergo a size-dependent interaction with HIV-1, and nanoparticles exclusively in the range of 1–10nm could attach to the virus. The exposed sulfur-bearing residues of the glycoprotein knobs are attractive sites for nanoparticle interaction. Due to this interaction, AgNPs were able to inhibit the binding of the virus to host cells. AgNPs could exert anti-HIV activity at an early stage of viral replication, most likely as a virucidal agent or as an inhibitor of viral entry. AgNPs binding to gp120 prevented CD4-dependent virion binding, fusion, and infection, which acted as an effective virucidal agent against cell-free viruses and cell-associated viruses.

Furthermore, AgNPs inhibited post-entry stages of the HIV-1 life cycle. The AgNPs-coated PUC is capable of inactivating a broad spectrum of microbial infectivity, including HIV-1 and HSV- 1/2. Moreover, no significant toxic effects were observed when human HeLa, 293T, and C8166 T cells were exposed to AgNPs-coated PUC for three hours. This antimicrobial method would provide a safe and efficient way to disrupt different sexually transmitted infections, including HIV-1 and HSV infections, at the mucosal surface.AgNPs synthesized by fungi has also been found to have excellent antiviral

properties. For example, AgNPs synthesized by *Aspergillus* spp. showed inhibitory activity on bacteriophage viral strains, in a concentration-dependent manner, with the range of 30–210 ppm, resulting in a plaque number decrease to 2 plaque-forming units (PFU), and total inhibition of the viral growth at nanoparticle concentrations of 210–240 ppm [90]. AgNPs, prepared by *Aspergillusochraceus*, were reduced from the initial value of 80 to 32 PFU after half an hour, compared to 78 PFU in the control. Small-sized AgNPs (3–10 nm), synthesized by *Aspergillus niger*, also showed excellent antiviral activity at 8–12 ppm and total inhibition of viral growth in the host bacterial strain, *E. coli*. AgNPs produced by *F. oxysporum*and *Curvularia* species had excellent antiviral activity (80–90% inhibition) against HSV-1 and HPIV-3 viruses, at 10 µg/mL. The IC_{50} value of chemical AgNPs against HIV-1 ranged from 0.44 to 0.91 mg/mL, and completely inhibited HSV-2 replication at 100 µg/mL. This suggests that fungal-AgNPs have higher antiviral activity than chemical AgNPs.

Antimicrobial Agents

Multidrug resistance is an important problem caused by the extensive use of chemical antimicrobial agents. An alternative way to overcome drug resistance is desperately needed. AgNPs have been used for decades as an antimicrobial agents in biomedical applications (Marambio- Jones, C., et al., 2010). Nano-sized Ag can expand the contact surface with the microorganisms, which consequently enhances the antimicrobial activity. The antimicrobial activity of AgNPs has been observed when using *Aspergillus terreus*, *Pestalotiopsis* sp., and *Pimeleacolumellifera*. *Pallida*, *Aspergillus clavatus*, *Trichoderma harzianum*, *Penicillium aculeatum*, *Candida albicans*, Fusarium verticillioides, andEmericellanidulans for synthesis. The produced AgNPs could inhibit Gram-positive (*Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis*) and Gramnegative (*Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonasfluorescens*, *Pseudomonas aeruginosa*, and *Salmonella typhi* bacterial pathogens. In addition, these AgNPs were also shown to inhibit fungal pathogens, such as *Candida albicans* (Ma, L., et al., 2017). The fungal-derived AgNPs showed higher antimicrobial activity against bacteria and fungi than silver ions. The diameters of the inhibition zones of AgNPs biosynthesized by *Penicillium aculeatum* were reported as 12.17 ± 1.04mm (*E. coli*), 15.33 ± 0.76mm (*P. aeruginosa*), 12.83 ± 0.58mm (*S. aureus*), 16.17 ± 1.04mm (*B.*

subtilis), and 18.17 ± 1.26mm (*C.albicans*), which were significantly higher than that of $AgNO_3$ 9.00 ± 1.00mm (E. coli), 13.33 ± 0.58mm (*P. aeruginosa*), 11.00 ± 0.50mm (*S. aureus*), 12.50 ± 1.50mm (*B. subtilis*), and 14.33 ± 1.04mm (*C. Albicans*), proving that AgNPs have higher antimicrobial activity than $AgNO_3$ (Ma, L., et al., 2017). AgNPs produced by *P. chrysogenum* MUM 03.22 exhibited higher biological activity (MIC value ¼ 2.89 μg/mL) than fluconazole, but lower activity than terbinafine, itraconazole, and chemical-AgNPs (MIC values ¼ 1.19 μg/mL). Such differences can be partially explained by the diversity in nanoparticle size. Smaller chemical AgNPs exhibited lower MIC than Bio-AgNPs. In addition, the capping may also be involved in the difference. Chemical AgNPs only covered with PVP exhibited higher biological activity than AgNPs with biological capping, produced by fungi. Multiple mechanisms underlying the anti-microbial activity of AgNPs have been determined, as follows:

- AgNPs become attached and then penetrate the cell membrane, accessing the bacterial cells.
- AgNPs inactivate the sulfhydryl groups in the cell wall and disrupt the enzymes and lipids in the cell membrane, which finally results in cell lysis and the release of Kþ from the cytoplasm. AgNPs were also proposed to bind and inactivate the key functional enzyme groups.
- AgNPs attack the respiratory chain and disrupt ATP production, forming reactive oxygen species (ROS) and eventually leading to cell death. Proteomic investigations suggest that the expression of a panel of envelope and heat shock proteins is up-regulated, which constitutes direct evidence of damage to the outer membrane of bacterial cells and intracellular ATP depletion (Duncan, T. V. 2011).

The nanoparticles bind to proteins or DNA and damage them by inhibiting replication. The electrostatic attraction between the negatively charged cell membranes and positively charged nanoparticles is responsible for the interactions between nanoparticles and bacterial cells.

Anticancer agents

The anti-cancer activity of AgNPs has been tested against different cancer cell lines using the MTT method (Devanesan, S., et al., 2017). Results indicated that AgNPs could reduce cell viability in a time- and dose-dependent manner. AgNPs selectively killed cancer cells

by suppressing cell proliferation and inducing cell cycle arrest during the G2/M phase, as well as apoptosis. The IC50 of AgNPs synthesized using various methods against MCF-7 breast cancer cell lines was reported in the range of 10–30 µg/mL. However, the IC50 of AgNPs synthesized by *Deinococcusradiodurans* was 7–8 µg/mL, which suggests that these nanoparticles were more effective against MCF-7 human breast cancer cell lines than AgNPs synthesized by other methods. More importantly, chem-AgNPs were less potent in cancer treatment than bio-AgNPs, since the IC50 of chem-AgNPs was 2.8- fold that of bio-AgNPs (25 µg/mL) (Han, J. W., et al., 2014). Therefore, AgNPs might be developed as effective antitumor chemotherapeutic agents or as combination nano-drugs for future anti-cancer therapies.

- **Anti-inflammatory agents**

Fungal extracts have been reported to have anti-inflammatory activity (Joel, E. L., & Bhimba, B.2011), and AgNPs synthesized by the extract of *Penicillium* (Ps-AgNPs) could inhibit the denaturation protein that leads to inflammation. AgNPs have anti-inflammatory activity, which is due to fungal nanoparticles inhibiting the release of lysosomal RBC to various levels. PsAgNPs can also inhibit the release of neutrophil lysosomal content at the inflammation site. Proinflammatory cytokines such as TNF-a, IL-1b, and IL-6 play major roles in wound healing, by protecting against the potential invasion of a variety of microorganisms (Wen, L., et al., 2016). Downregulation of proinflammatory cytokines can alleviate wound inflammation and enhance wound closure. Wounds treated with AgNPs, synthesized by the endophytic fungus Orchidanthachinensis, can significantly lower the levels of TNF-a and IL-6 throughout the healing process (Wen, L., et al., 2016).

The wound area of the fungal AgNPs treated group was 10% of the original size on day 14 and 5% on day 21 (Wen, L., et al., 2016), whereas, the wound area of patients treated by chemical AgNPsdecreased to 31.23% of the original size on day 12 and 3.34% on the day. This suggests that fungal AgNPs have higher anti-inflammatory activity than chemical AgNPs, within a short treatment period.

- **Applications in foods**

Food quality control has become a very common problem throughout the world (De Moura, M. R., et al., 2012). AgNPs have unique physicochemical properties that could be

used in food manufacturing, packaging, and storage. Additionally, AgNPs could be developed into detectors, which can detect the presence of molecular contaminants, microorganisms, and gases or respond to changes in environmental conditions (Duncan, T. V. 2011). Electrochemical detection based on nanomaterial sensors is another popular method that has been applied in the food industry. Apart from these, the excellent antimicrobial activity of AgNPs could have great potential applications in food packaging to prevent bacterial contamination in foods. AgNPs biosynthesized by *Aspergillus niger*, *Emericellanidulans*, and *Aspergillus terreus* have antimicrobial activity against a broad range of Gram-negative and Gram-positive pathogenic bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, Staphylococcus *aureus*, *Shigella boydii*, *Acinetobacter baumanni*, *Shigella sonnei*, and *Salmonellatyphimurium*.

- **Applications in catalysis**

Recently, increasingly more attention has been placed on AgNPs that can be used as catalysts in various processes. For instance, AgNPs could be applied as catalysts in redox reactions (Merga, G., et al., 2007). Methylene blue (MB) is frequently used to colorize products in the textile industry, as a kind of inexpensive and efficient dye, but it tends to cause pollution and harm the environment. AgNPssynthesized with the cell filtrate of *Penicillium oxalicum* showed the excellent catalytic property in reducing MB, by completing the catalytic degradation reaction at ambient temperature. Another example is 4-nitrophenol, which can cause many health problems, and the photoreduction of nitrophenol to aminophenols is the main solution to the problem. But the photo-reduction reaction is slow and incomplete. Among the various amended methods, it has been shown that reduction reactions could accelerate in the presence of AgNPs. AgNPs synthesized by Dilleniaindica bark extract showed enhanced free-radical scavenging ability and excellent catalytic activity when used in the reduction of 4-nitrophenol at room temperature.

- **Applications in agriculture**

Farmers are facing many challenges in agriculture, including the sustainable use of natural resources, environmental pollution, and insect pests. With the development of nanotechnology, several techniques, such as nano-scale carriers and fabricated xylem

vessels, became available for farming. Precision farming is an efficient way to solve these problems, which allows for precise control on a nanometer scale (Dasgupta N, et al.). Presently, water contamination is an urgent problem needed to be solved in agriculture. Pathogenic microorganisms, heavy metal ions, and chemicals are the major contaminants. Biologically synthesized nanomaterials, from fungi like *S. cerevisiae*, accumulate heavy metals mainly Hg and Pb. AgNPs can kill microbes, which could be exploited during water cleaning. As the size of AgNPs decreases, the atoms present on the surface increase, this results in a pronounced increase in chemical reactivity, related to some specific conditions. Therefore, this process can be used for the decomposition of many toxic compounds, such as pesticides that take a long time to degrade under normal conditions (Dasgupta N, et al.). It is also suggested that AgNPs synthesized by *Penicillium notatum* would be proper to develop a biological process for crop pest control. AgNPs could reduce the fecundity of pests at low concentrations, which could have less impact on the plants and ecosystem compared to other chemical insecticides (B et al., 2014).

Objectives

Objectives

❖ Biosynthesis of silver nanoparticles using *Aspergillus Niger*.

❖ Characterization of synthesized Silver Nanoparticles using UV/Vis spectroscopy, Zeta Potential, DLS, TEM, and FTIR.

❖ To check the Antibacterial potential of *Aspergillus Niger* synthesized SNPs.

Material and methods

Material

Silver nitrate was purchased from Sigma Aldrich and used as received. Microbiological media and ingredients were purchased from Hi-Media, India. All solvents and chemicals were of analytical grade and used as obtained from Merck and Sigma Aldrich (St. Louis, MO, USA).

MG-YP Media

- Malt extract (0.3%)
- Glucose (1.0%)
- Yeast extract (0.3%)
- Peptone (0.5%)

Methods

In vitro synthesis of silver nanoparticle

Microorganism and growth

A.niger was maintained on PDA slants (potato 20% w/v, dextrose 2% w/v, and agar 2% w/v) at 25 °C. The fermentation was carried out by inoculating a fungal mass of 1 cm diameter, from a 7-day-old PDA slant into 100 mL of liquid MGYP medium (0.3% w/v malt extract, 1.0% w/v glucose, 0.3% w/v, yeast extract, and0.5%w/v peptone) in a 500 mL Erlenmeyer flask, followed by incubation at 26 ± 1 °C on a rotary shaker (200 rpm) for 96 h. The mycelium was collected by centrifugation (6000 rpm, 20 min at 10 °C), washed extensively with distilled water under aseptic conditions, and used for further studies.

Extracellular synthesis of silver nanoparticles

20 g (wet weight) of the mycelia were incubated in 100 mL of sterile distilled water containing 1 mM silver oxide in a 500 mL Erlenmeyer flask for96 h under shaking (200 rpm) at room temperature. The samples were collected at fixed time intervals and subjected to UV-Vis spectroscopy to trace the formation of nanoparticles. At the end of fermentation, the unbound proteins were removed by precipitation with 2 volumes of absolute ethanol and the nanoparticles were collected for further characterization.

Results

Results

Biological Synthesis of F-AgNPs and Characterizations

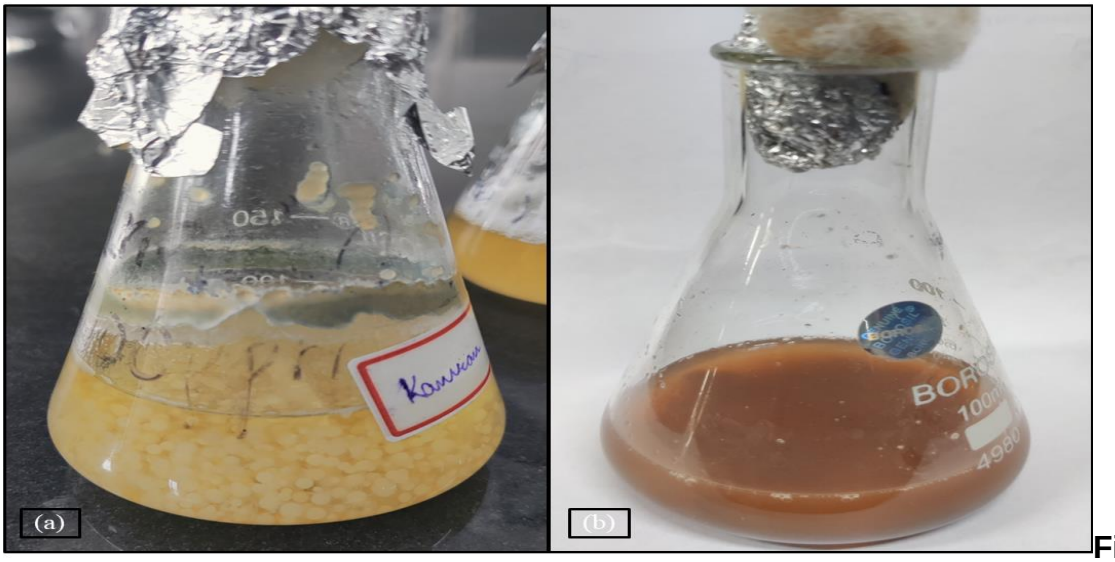

Fig. 4 (a) Flask contains Aspergillus Niger growth (b) After incubation of (48 hours), the change of color of the reaction mixture at the end of the incubation (48h) from white to brown indicates the formation of Silver Nanoparticles.

Characterization

UV/VIS spectroscopy

UV/Vis spectrophotometric measurements were performed on a Shimadzu dual-beam spectrophotometer operated at a resolution of 1nm in the quartz cuvette. Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry (UV- Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, atoms and molecules undergo electronically.

To observe the optical property of biosynthesized silver nanoparticles, samples were periodically analyzed for UV- vis spectroscopic studies at room temperature operated at a resolution of 1nm between 250and 800nm ranges. Absorption spectroscopy is

complementary to fluorescence and deals with transitions from an excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

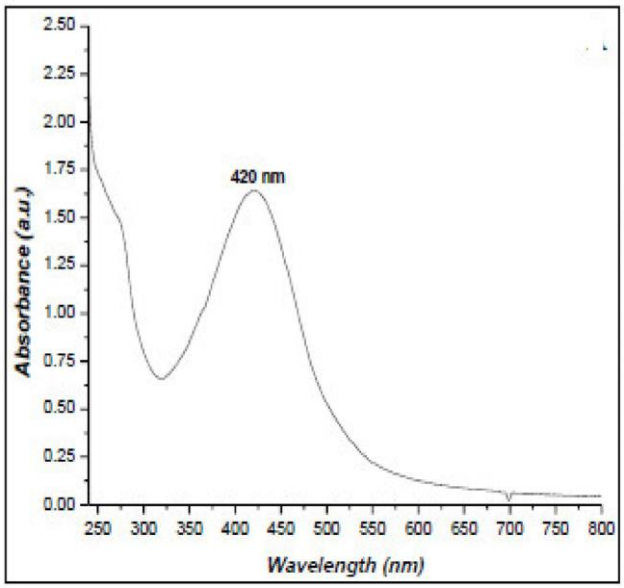

Fig. 5. UV-Visible Spectroscopy of The F-AgNPs show a distinct and broad absorption band centered at 420 nm.

The polydispersity without agglomeration and spherical shape of F-AgNPs were revealed by the TEM micrograph. The average size of F-AgNPs was found to be 15±1 nm after TEM analysis (Figure2). The extent of single-crystallinity under HR-TEM analysis was found to be quite remarkable in the as-synthesized particles in ambient conditions. The lattice planes exhibited a spacing of ~2.36 Å for the given Ag nanoparticles for the lattice planes {111} with cubic phase geometry. The hydrodynamic diameter of F-AgNPs was 47 nm (Figure3 (a)) with a particle distribution index of 0.447, and they were found to be highly stable with a zeta potential of 20.03 mV (Figure 3 (b) D).

FTIR measurements were accomplished to identify the presence of various functional groups in the biomolecules responsible for the bio-reduction of Ag^{+} and cap-ping/stabilization of silver nanoparticles (Figure 4). The band at 3472 cm^{-1} in the spectrum corresponds to O-H stretching vibration indicating the presence of alcohol and phenol. The band at 2912 cm^{-1} and the 2867 cm^{-1} region arising from the C-H

stretching of the aromatic compound were observed. The band at 1768 cm^{-1} was assigned for (C-C) stretching (non-conjugated). The band at 1642 cm^{-1} in the spectrum corresponds to C-N and C-C stretching, indicating the presence of protein. The band at 1450 cm^{-1} was assigned to N-H stretching vibrations present in the amide linkages of proteins. These functional groups have a role in the stability/capping of Ag NPs. The bands at 1450 and 1062 cm^{-1} were assigned to N-H and C-N (amines) stretching vibrations of proteins, respectively.

FTIR spectrum of plant aqueous extract: the bands at 3429, 2931, 2861, 1631, 1436, 1342, and 1124–911 cm^{-1} correspond to polyphenols, carboxylic acids and their derivative (C=O), N-H stretching, and C=N stretching of aliphatic amines.

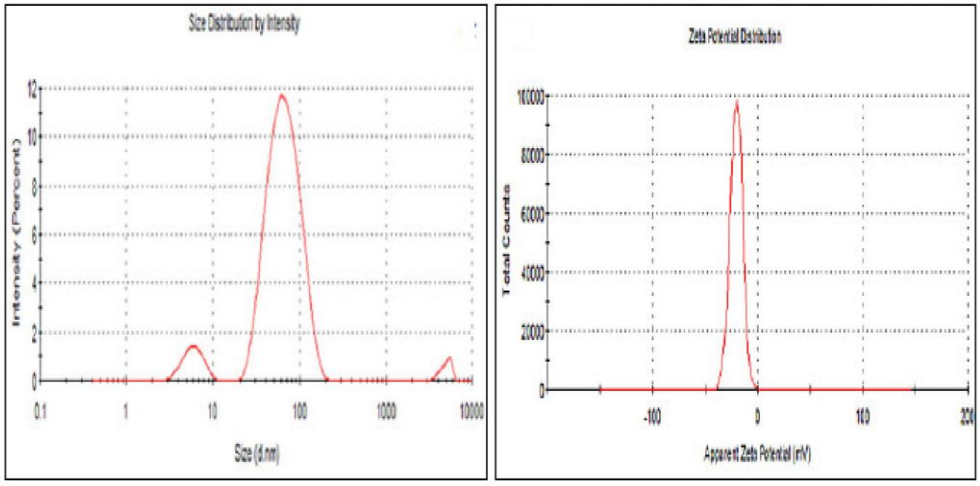

Fig. 7. (A) Dynamic light scattering (average size 47 d.nm); (B) zeta potential (−20.3 mV).

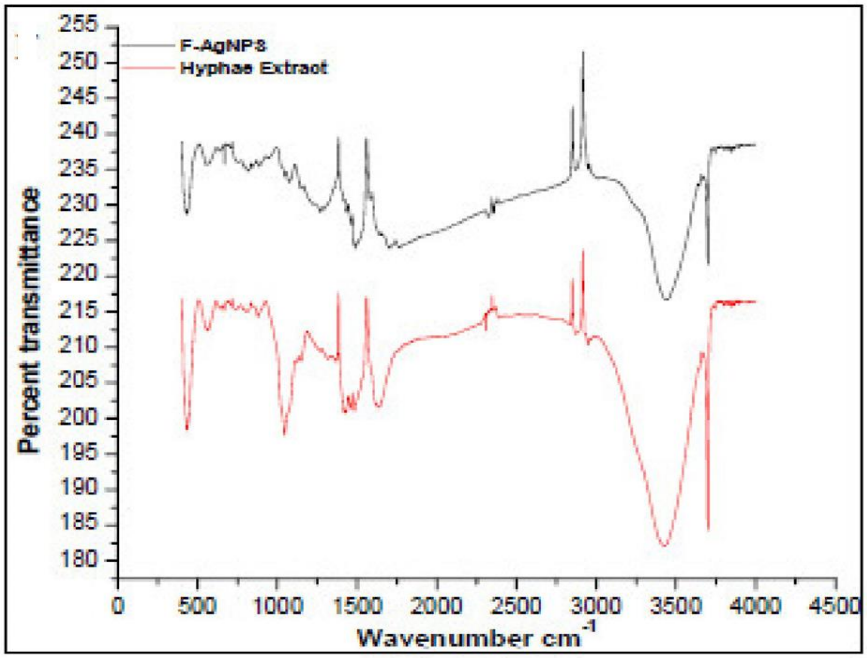

Fig. 8. FTIR analyses.

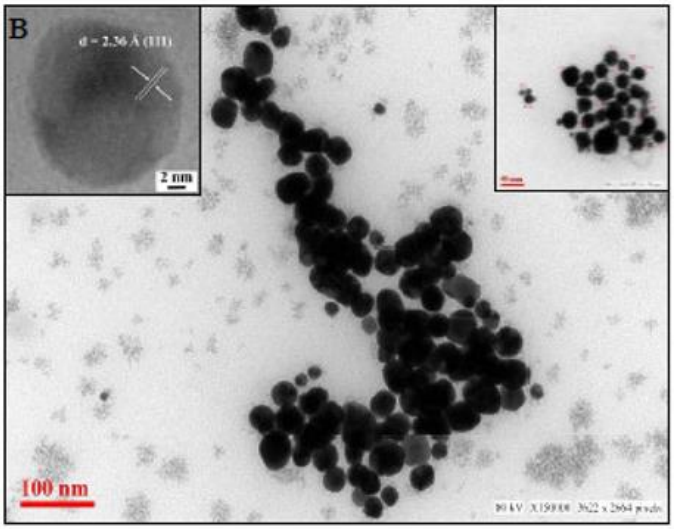

Fig. 6. Transmission electron microscopy analysis (average size ~15 nm).

Antibacterial of F-AgNPs

The intrinsic antibacterial potential of silver nanoparticles is well known, and their mode of action has been explained broadly through direct and ion-mediated destruction mechanisms. Generally, AgNPs are found to be more active against Gram-negative bacteria than Gram-positive due to differences in the cell wall structures of these microbes. However, some biogenic AgNPs contradict this finding

or show variable effectiveness among bacterial groups. The antibacterial action of F-AgNPs against various bacteria was found to be satisfactory. They produced zones of inhibition against *Staphylococcus aureus, Klebsiella pneumonia*, *Salmonella abony*, and *Escherichia coli* of 3, 2.9, 3.1, and 3.2 mm, respectively (Figure 6). Further, their MIC_{50} values against *Staphylococcus aureus, Klebsiella pneumonia*, *Salmonella abony*, and *Escherichia coli* were 10.3, 12.5, 22.69, and 16.25 µg/mL, respectively (Figure 7), suggesting a broad-spectrum effect. The bactericidal effect of F-AgNPs is due to their adherence to the surfaces of bacterial cell walls and interactions with sulfur-containing proteins. They cause irreversible changes in sulfur-containing proteins' structures; reduce the compactness of lipid bilayer; and alter the permeability of the cell membrane, which leads to leakage of cellular contents, including ions, proteins, sugars, and the cellular energy reservoir. These particles and silver ions can damage cellular structures (e.g., ribosomes), and biomolecules such as proteins, lipids, and DNA, by interacting with them, and hence, disturb their maintenance and activities. Inhibition of ATP synthesis is one of the strategies of AgNPs against *S. aureus* and *P. aerogenosa*. The unique intrinsic surface properties of F-AgNPs lead them to them reacting with cellular components. Generally, silver ions (released from F-AgNPs) and F-AgNPs can quickly bind to thioenzymes/proteins, deactivate some of the housekeeping enzymes, disturb the proton pumps, and make transport nonspecific across the cell membrane through interfering with membrane proteins or the phospholipid bilayer. The leakage of different ions, including H^+, causes fungal cell disruption. Similarly, the particles can also disrupt the natural architecture of DNA by cross-linking with DNA bases, combining with DNA bases to form cross-links, and substituting the hydrogen bonds adjoined to nitrogen in purines and pyrimidines.

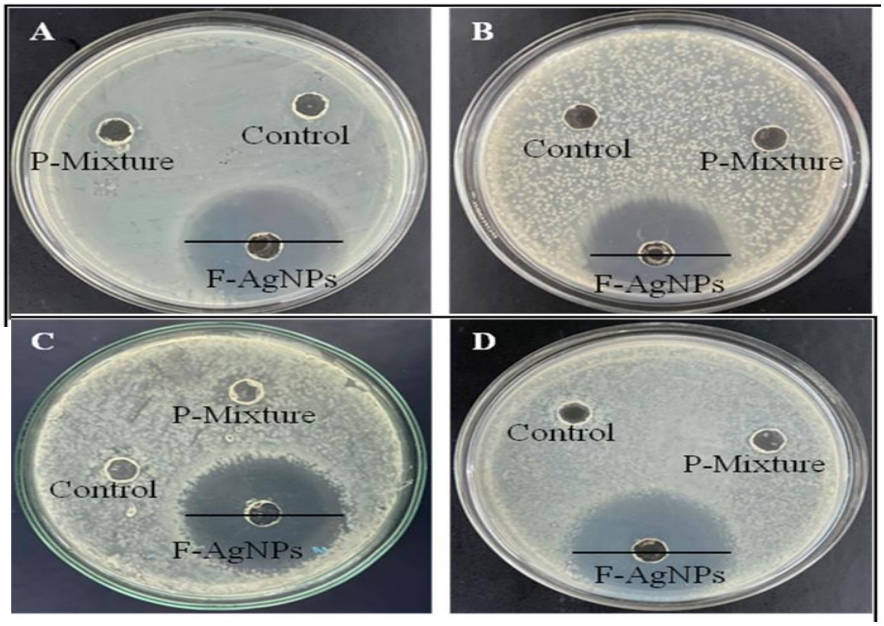

Fig. 9. Images showing the zones of inhibition of F-AgNPs and fungus isolated protein mixture (P-mixture) against (**A**) *Staphylococcus aureus*, (**B**) *Klebsiella pneumonia*, (**C**) *Salmonella abony,* (**D**) *Escherichia coli.*

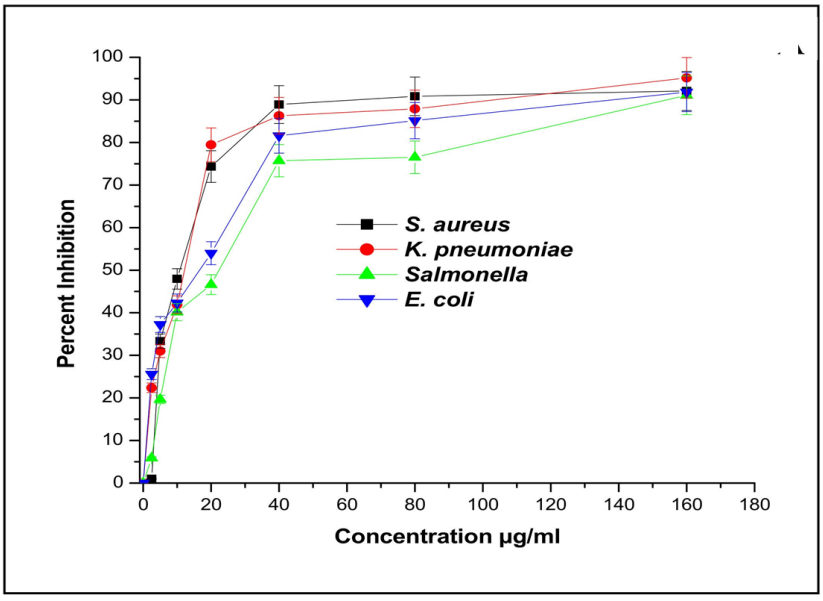

Fig. 10. Shows MICs (8µg/ml) values of F-AgNPs (96-well plate method).

Discussion and conclusion

Discussion

In this study, *A.niger* was used for the synthesis of AgNPs. Cell walls, cell membranes, enzymes, proteins, and other organic molecules play important roles in the synthesis of AgNPs. Silver ions are reduced from AgNO3 by enzymes on the cell surface and stabilized by fungal proteins and peptides. The mechanism of AgNPs synthesis is linked to cell wall polymers or electron shuttle quinones. The reduction of Ag occurs under the canalization by a nitrate-dependent reductase. AgNPs are stabilized by fungal proteins and peptides due to electrostatic attraction between free amine groups or cysteine residues in the proteins with carboxylate groups. The electrostatic interactions on the cell surface trap the Ag and further reduce it, to form silver nuclei, through the action of enzymes in the cell wall, which leads to the formation of AgNPs on fungal cell-free filtrate.

F-AgNPs were characterized by using physical techniques, including UV–visible spectroscopy, zeta potential, DLS, TEM, and HR-TEM. The particles were found to be polydispersed and quasispherical in shape under TEM. They had an average size of 15 nm. The well-dispersed particles were found to have consistent crystallinity with cubic phase geometry under HR-TEM. The presence of different functional groups on the surfaces of biosynthesized F-AgNPs was confirmed by FTIR. The particle distribution index was found to be 0.447 with a hydrodynamic diameter of ~47 d.nm, and the high value of zeta potential (−20.3 mV) revealed the stability of the nanoemulsion. These particles were found to be active against *Staphylococcus aureus, Klebsiella pneumonia*, *Salmonella abony*, and *Escherichia coli* with MIC_{50} 10.3, 12.5, 22.69, and 16.25 µg/mL, respectively

Conclusions

Now, it can be concluded that the biogenic nanoparticles we used have several medicinal properties from the source(s) used in their biosynthesis that are distinct from the intrinsic properties of metal nanoparticles. The F-AgNPs clearly showed antibacterial, which were also shown by *Aspergillus sp*. Therefore, the metal nanoparticles received several metabolites, enzymes, and proteins from their redox source and mimic their properties, though outdoing the parent source due to the accumulation of the compounds at the nanoscale, which allows them to act synergistically. This study could be extended by finding the mechanisms of action for these F-AgNPs.

References

References

Saini, R., Saini, S., & Sharma, S. (2010). Nanotechnology: the future medicine. Journal of cutaneous and aesthetic surgery, 3(1), 32.

Silva, G. A. (2004). Introduction to nanotechnology and its applications to medicine. Surgical neurology, 61(3), 216-220.

Caruthers, S. D., Lanza, G. M., & Wickline, S. A. (2011). -Theragnostic Nanoparticles with the Power to Diagnose, Treat, and Monitor Diseases. In Bionanotechnology II (pp. 108-121). CRC Press.

Lin, P., Moore, D., & Allhoff, F. (2009). What is nanotechnology and why does it matter?: from science to ethics. John Wiley & Sons.

Sinani, V. A., Koktysh, D. S., Yun, B. G., Matts, R. L., Pappas, T. C., Motamedi, M., ... & Kotov, N. A. (2003). Collagen coating promotes biocompatibility of semiconductor nanoparticles in stratified LBL films. Nano Letters, 3(9), 1177-1182.

Gurunathan, S., Park, J. H., Han, J. W., & Kim, J. H. (2015). Comparative assessment of the apoptotic potential of silver nanoparticles synthesized by Bacillus tequilensis and Calocybe indica in MDA-MB-231 human breast cancer cells: targeting p53 for anticancer therapy. International journal of nanomedicine, 10, 4203.

Li, W. R., Xie, X. B., Shi, Q. S., Zeng, H. Y., Ou-Yang, Y. S., & Chen, Y. B. (2010). Antibacterial activity and mechanism of silver nanoparticles on Escherichia coli. Applied microbiology and biotechnology, 85(4), 1115-1122.

Mukherjee, P., Ahmad, A., Mandal, D., Senapati, S., Sainkar, S. R., Khan, M. I., ... & Sastry, M. (2001). Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. Nano letters, 1(10), 515-519.

Chernousova, S., & Epple, M. (2013). Silver as antibacterial agent: ion, nanoparticle, and metal. Angewandte Chemie International Edition, 52(6), 1636-1653.

Li, C., Zhang, Y., Wang, M., Zhang, Y., Chen, G., Li, L., ... & Wang, Q. (2014). In vivo real-time visualization of tissue blood flow and angiogenesis using Ag2S quantum dots in the NIR-II window. Biomaterials, 35(1), 393-400.

Sondi, I., & Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of colloid and interface science, 275(1), 177-182.

Li, L. S., Hu, J., Yang, W., & Alivisatos, A. P. (2001). Band gap variation of size-and shape-controlled colloidal CdSe quantum rods. Nano letters, 1(7), 349-351.

Gordon, A. T., Lutz, G. E., Boninger, M. L., & Cooper, R. A. (2007). Introduction to nanotechnology: potential applications in physical medicine and rehabilitation. American journal of physical medicine & rehabilitation, 86(3), 225-241.

Sharma, V. K., Yngard, R. A., & Lin, Y. (2009). Silver nanoparticles: green synthesis and their antimicrobial activities. Advances in colloid and interface science, 145(1-2), 83-96.

Gurunathan, S., Kalishwaralal, K., Vaidyanathan, R., Venkataraman, D., Pandian, S. R. K., Muniyandi, J., ... & Eom, S. H. (2009). Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli. Colloids and Surfaces B: Biointerfaces, 74(1), 328-335.

Lin, P. C., Lin, S., Wang, P. C., & Sridhar, R. (2014). Techniques for physicochemical characterization of nanomaterials. Biotechnology advances, 32(4), 711-726.

Pleus, R. (2012). Nanotechnologies-Guidance on Physicochemical Characterization of Engineered Nanoscale Materials for Toxicologic Assessment. ISO: Geneva, Switzerland.

Murdock, R. C., Braydich-Stolle, L., Schrand, A. M., Schlager, J. J., & Hussain, S. M. (2008). Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. Toxicological sciences, 101(2), 239-253.

Gurunathan, S., Han, J. W., Kim, E. S., Park, J. H., & Kim, J. H. (2015). Reduction of graphene oxide by resveratrol: A novel and simple biological method for the synthesis of an effective anticancer nanotherapeutic molecule. International journal of nanomedicine, 10, 2951.

Sapsford, K. E., Tyner, K. M., Dair, B. J., Deschamps, J. R., & Medintz, I. L. (2011). Analyzing nanomaterial bioconjugates: a review of current and emerging purification and characterization techniques. Analytical chemistry, 83(12), 4453-4488.

Carlson, C., Hussain, S. M., Schrand, A. M., K. Braydich-Stolle, L., Hess, K. L., Jones, R. L., & Schlager, J. J. (2008). Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. The journal of physical chemistry B, 112(43), 13608-13619.

Jo, D. H., Kim, J. H., Lee, T. G., & Kim, J. H. (2015). Size, surface charge, and shape determine therapeutic effects of nanoparticles on brain and retinal diseases. Nanomedicine: Nanotechnology, Biology and Medicine, 11(7), 1603-1611.

Staquicini, F. I., Ozawa, M. G., Moya, C. A., Driessen, W. H., Barbu, E. M., Nishimori, H., ... & Arap, W. (2011). Systemic combinatorial peptide selection yields a non-canonical iron-mimicry mechanism for targeting tumors in a mouse model of human glioblastoma. The Journal of clinical investigation, 121(1), 161-173.

Duan, X., & Li, Y. (2013). Physicochemical characteristics of nanoparticles affect circulation, biodistribution, cellular internalization, and trafficking. Small, 9(9-10), 1521-1532.

Panáček, A., Kolář, M., Večeřová, R., Prucek, R., Soukupová, J., Kryštof, V., ... & Kvítek, L. (2009). Antifungal activity of silver nanoparticles against Candida spp. Biomaterials, 30(31), 6333-6340.

Albanese, A., Tang, P. S., & Chan, W. C. (2012). The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annual review of biomedical engineering, 14(1), 1-16.

Zodrow, K., Brunet, L., Mahendra, S., Li, D., Zhang, A., Li, Q., & Alvarez, P. J. (2009). Polysulfone ultrafiltration membranes impregnated with silver nanoparticles show improved biofouling resistance and virus removal. Water research, 43(3), 715-723.

Wong, K. K., Cheung, S. O., Huang, L., Niu, J., Tao, C., Ho, C. M., ... & Tam, P. K. (2009). Further evidence of the anti-inflammatory effects of silver nanoparticles. ChemMedChem: Chemistry enabling drug discovery, 4(7), 1129-1135.

Gurunathan, S., Lee, K. J., Kalishwaralal, K., Sheikpranbabu, S., Vaidyanathan, R., & Eom, S. H. (2009). Antiangiogenic properties of silver nanoparticles. Biomaterials, 30(31), 6341-6350.

Sriram, M. I., Kanth, S. B. M., Kalishwaralal, K., & Gurunathan, S. (2010). Antitumor activity of silver nanoparticles in Dalton's lymphoma ascites tumor model. International journal of nanomedicine, 5, 753.