American Journal of Sciences and Engineering Research

E-ISSN -2348 – 703X, Volume 7, Issue 3,

Biological Synthesis of Sliver-Nanoparticles from Fungi and Its Antibacterial Activity: Review

Ehab Y. Jabber[1], Salim S. Jaafar[2] , Safa M Dyaa[3]

University of Babylon, DNA research center, Babylon, Iraq

Abstract: *In the field of nanotechnology, the use of various biological units instead of toxic, chemicals for the reduction and stabilization of nanoparticles, has received extensive attention. Among the many possible bio resources, biologically active products from fungi,represent excellent scaffolds for this purpose. Since fungi are very effective secretors of extracellular enzymes and number of species grow fast and therefore culturing and keeping them in the laboratory are very simple. They are able to produce metal nanoparticles and nanostructure via reducing enzyme intracellularly or extracellularly. The focus of this review is the application of fungi in the green synthesis of inorganic nanoparticles. Meanwhile the domain of biosynthesized nanoparticles is somewhat novel; the innovative uses in nano medicine in different areas including the delivery of drug, cancer therapy, antibacterial, biosensors, and MRI and medical imaging are reviewed. The proposed signaling pathways of nanoparticles induced apoptosis in cancerous cells and anti-angiogenesis effects also are utilization of eukaryotes like yeast and fungi in the biosynthesis of nanoparticles (NPs) and their uses.*

Keywords: *Nanoparticles, Nanotechnology, Silver Nanoparticles, Antibacterial activity.*

I. Introduction

Silver has been recognized as a nontoxic, safe inorganic antibacterial agent used for centuries. Silver nanoparticles are used vastly today in biomedical, health care, food agriculture, industrial, electronics and environmental field. In medicine, the antimicrobial action of nanoparticles have the ability to destroy the wide spectrum of pathogens and multidrug resistance bacteria. Silver nanoparticles have used as a novel antimicrobial agent like antibacterial, antiviral, antifungal and anti-inflammatory agents. It acts as antimicrobial agent for preventing biofilm attachment, penetration bacterial biofilm or delivering antimicrobial agents. Moreover, silver nanoparticles can be synthesized by variety of different methods such as physical, chemical and biological. The last one is most favorable method because it safety, less toxicity and ecofriendly to environment while the chemical and physical methods are very costly, may contains a poisonous and dangerous materials (Chojniak *et al.*, 2017).

Antimicrobial materials used in the clinical setting today are beset by significant shortfalls, including weak antimicrobial activities, risk of microbial resistance, difficulty in monitoring and extending the antimicrobial functions, and difficulty in functioning in a dynamic environment. Thus, effective and long-term antibacterial and biofilm-preventing materials constitute an immediate need in medicine and dentistry (Beyth *et al.*, 2015).

II. Nano biotechnology

Nanotechnology is the science and technology of small things i.e.ˋ Nano ʼ. This term originate from Greek word meaning ˋ dwarf ʼ. This term was firstly used by ˋ Richard Feynman ʼ in 1959. Therefore, nanotechnology term was used from last 53 years. Nanotechnology is responsible for the production and study of metal

Received-28-04-2024, Accepted- 10-05-2024

nanoparticles. These structures present several applications that highlight antimicrobial activity, and this property is an important tool in combating microorganisms resistant to conventional drugs (Shenashen *et al.,* 2014).

Nanobiotechnology can be efficiently used to treat various infectious diseases (Radovic-Moreno *et al* .,2012).

When in comparison with bacteria, fungi can produce larger amounts of nanoparticles because they can secret larger amounts of proteins which directly translate to higher productivity of nanoparticles **(**Mohanpuria *et al*., 2008**)**. The mechanism of silver nanoparticle production by fungi is said to follow the following steps: trapping of Ag^+ ions at the surface of the fungal cells and the subsequent reduction of the silver ions by the enzymes present in the fungal system **(**Mukherjee *et al*., 2001).

Several microorganisms from bacteria to fungi have been reported to synthesize inorganic materials either intra- or extracellular, and thus to be potentially utilized as eco-friendly Nano factories (Mohanpuria *et al*.,2008).

Nanotechnology is a broad field of science that represents the design, synthesis, characterization and application of materials at nano scale level which can be used across various fields such as chemistry, biology, physics, material science, medicine, etc. (Jassim *et al.*, 2016) and nanoparticles (NPs) are viewed as fundamental building blocks of nanotechnology (Vastrad and Goudar, 2016).

Nanotechnology is responsible for the production and study of metal nanoparticles. These structures present several applications that highlight antimicrobial activity, and this property is an important tool in combating microorganisms resistant to conventional drugs (Shenashen *et al*., 2014).

Nanoparticles (NPs) are particles in the size range of 1–100 nm and form building blocks of nanotechnology (Alghuthaymi *et al*., 2015).

Biosynthesis (green synthesis) of Nano silver has received extensive attention due to the growing need for environmentally friendly synthesis methods that use eco-friendly reducing and capping agents, such as protein, peptides, carbohydrate, various species of bacteria, mold and yeast (Nam *et al*., 2008; Balaji *et al*., 2009 ; Sintubin *et al*., 2012; nisha *et al* ., 2013).

Different synthetic NSP routes lead to variable sizes, shapes, morphology, and even stability. Generally, these methods can be classified into three broad categories: physical, chemical, and biological (or green) synthesis, the possible mechanism of biological synthesis includes enzymatic and non-enzymatic reduction (Anil *et al*., 2007).

The nanoparticles synthesized by biological process have higher catalytic reactivity and greater specific surface area (Seo *et al*., 2006). Green synthesis of nanoparticles is an easy, efficient and eco-friendly approach, where most researchers are looking at the eco-friendly and green synthesis of nanoparticles paves the way for the researchers around the world to explore the ability of various herbs in synthesizing nanoparticles. Nanoparticles synthesized by chemical method are not eco-friendly (Savithramma *et al* ., 2011).

The biological synthesis of nanoparticles depends on three factors, including the solvent, the reducing agent and the non-toxic material , the use of biological molecules for the synthesis of Ag-NPs is eco-friendly, pollution-free and biological methods seem to provide controlled particle size and shape , which is an important factor for various biomedical applications (Gurunathan *et al*., 2014).

The biological activity of Ag-NPs depends on factors including surface chemistry, size, size distribution, shape, particle morphology, particle composition, coating/capping, agglomeration, and dissolution rate, particle reactivity in solution, efficiency of ion release, and cell type, and the type of reducing agents used for the synthesis of Ag-NPs are a crucial factor for the determination of cytotoxicity (Carlson *et al*., 2008).

Silver nanoparticles (Ag-NPs) effectively inhibit microorganisms without causing significant cytotoxicity also; Ag-NPs are nontoxic in mice and guinea pigs when administered by oral, ocular, and dermal routes. These conflicting results reveal the difficulty of pinpointing the overall toxicity of Ag-NPs to humans because the tremendous variation in particle size, particle aggregation, and concentration or coating thickness (in implants) of Ag-NPs has different profiles of silver release and bioactivity. In short, much longer and detailed studies have to be carried out to seriously consider Ag-NPs potential toxicity in humans (Sharma *et al*., 2009; Murphy *et al*., 2015).

Fungi are regarded as the most important organisms that produce nanoparticles extracellular, because of their secretory components, which are involved in the reduction and capping of nanoparticles (Gade *et al*., 2008).

Silver nanoparticles (Ag-NPs) have been known to have inhibitory and bactericidal effects. Resistance to antimicrobial agents by pathogenic bacteria has emerged in last years and is a major health problem (Nanda and Saravanan, 2009).

The filamentous fungi possess some advantages over bacteria in nanoparticles synthesis, as most of the fungi are easy to handle, required simple nutrient, possess high wall-binding capacity, as well as intracellular metal uptake capabilities (Sanghi and Verma ,2009).

Silver nitrate is still a common antimicrobial used in the treatment of chronic wounds. Such a way they prevent infection, heals the wounds and anti-inflammatory infection (Taylor *et al*., 2005).

The science of NPs synthesis by fungi is referred to as myco nanotechnology, which has a great demand and considerable potential, partly due to the wide range and diversity of fungi (Tyagi , 2016).

Nitrate reductase is an enzyme in the nitrogen cycle responsible for the conversion of nitrate to nitrite, the reduction mediated by the presence of the enzyme in the organism has been found to be responsible for the synthesis (Duran *et al.*, 2005).

Silver nanoparticles are usually synthesized from bacteria and fungi. The latter posses more advantages which include filamentous fungal tolerance towards metals, their high binding of capacity and intracellular uptake of metals (Dias *et al.*, 2002).

Among the different microbes used for the synthesis of NPs fungi are efficient candidates for fabrication of metal NPs both intra-and extracellular (Gade *et al*., 2010). These organisms are characterized by their fastidious growth, ease of handling and fabrication property (Saha *et al*., 2011).

Ag-NPs have been among the most commonly used nanomaterial's in our health care system for hundreds of years. Ag-NPs have become of intense interest in biomedical applications figure (1), because of their antibacterial, antifungal, antiviral, and anti-inflammatory activity (El-Badawy *et al*., 2010).

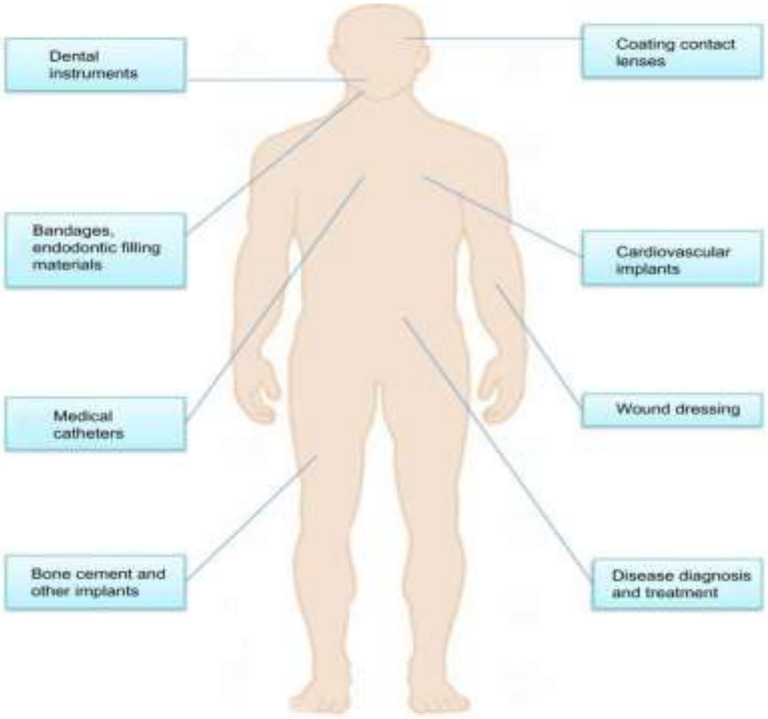

Figure (1) Biomedical applications of Nano silver particles in human health care (Zhong *et al*., 2010).

Synthesis of Ag-NPs produces toxic waste, such as ammonia (Panacek *et al.*, 2009).
Biosynthesis of nanoparticles of different elements is reported from both pathogenic and nonpathogenic fungi (Vigneshwaran *et al.*, 2007).

III. Characterization of Nps

Cell-free filtrate of some fungal isolates exhibited a gradual change to brown color when each was incubated with silver nitrate salt and maintained under dark conditions. The color of the culture filtrate with silver nitrate salt changed to intense brown after 24 hr of incubation whereas the control (without silver nitrate salt) did not exhibit any color change (Gitanjali and Ashok, 2015).

The color changes can be attributed to the surface plasmon resonance (SPR) phenomenon of deposited Ag-NPs as in figure (2).

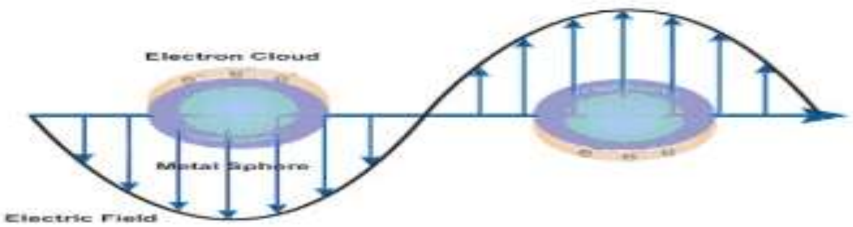

Figure (2) Surface plasmon resonance of silver nanoparticles with specific wavelength of incident light (Mulvaney , 1996).

UV-Visible Spectrometry

The technique of spectrophotometry is generally used for the qualitative and quantitative estimation of biomolecules such as proteins, sugar, carbohydrates, amino acids, nucleic acids and vitamins, etc. the UV– visible spectroscopy has proven to be very useful for analyzing of different nanoparticles such as gold and silver nanoparticles (Ramezani *et al.*, 2008).

Remarks of strong broad surface plasmon peaks at visible regions (400– 600 nm) has been well documented for various metal nanoparticles, with sizes ranging widely from 2-100 nm. UV–visible spectrophotometer includes from two devices, namely a spectrometer for producing light with different wavelengths, and a photometer for measuring the intensity of light. The tools are arranged so that liquid in a cuvette can be placed between the spectrometer beam and the photometer. Further information is available about the mentioned spectroscopic method and this technique has been well described by various authors (Hesse *et al.*, 2008).

X-Ray Diffraction (XRD)

X-ray crystallography is a powerful technique used to study the three-dimensional structure of crystals including macromolecules such as protein and nucleic acids. The technique is also known as the x-ray diffraction technique. There are a number of methods for studying three dimensional structure, but x-ray crystallography is the most effective of these techniques at present. Certainly the other techniques complement crystallography and have a valued place in the set of tools that we use for studying the structure of molecules. A microscope works in two stages. First, light strikes the object and is diffracted in various directions. The lens collects the diffracted rays and reassembles them to form an image (Figure 3). With x-rays, we can detect diffraction from molecules, but we have to use a computer to reassemble the image. It's not that simple, but that's the essence of the method. (Sapsford *et al.*, 2011).

X-ray diffraction is also defined as one of the primary analytical techniques used for phase identification and crystalline structure determinations in solid-state materials. Non-amorphous materials are composed of repeating regular planes of atoms that form a crystal lattice. In this method, a monochromatic X-ray directed onto a sample and the interaction between these planes of atoms and X-rays lead to diffracted rays being emitted. Depending on the atomic composition of the crystalline lattice and their arrangement, various minerals diffract X-ray beams differently. These diffracted X-rays are then collected and processed, and by comparison of the sample diffraction pattern with standard reference patterns and measurements, the material identification will be allowed (Kohler and Fritzsche , 2004).

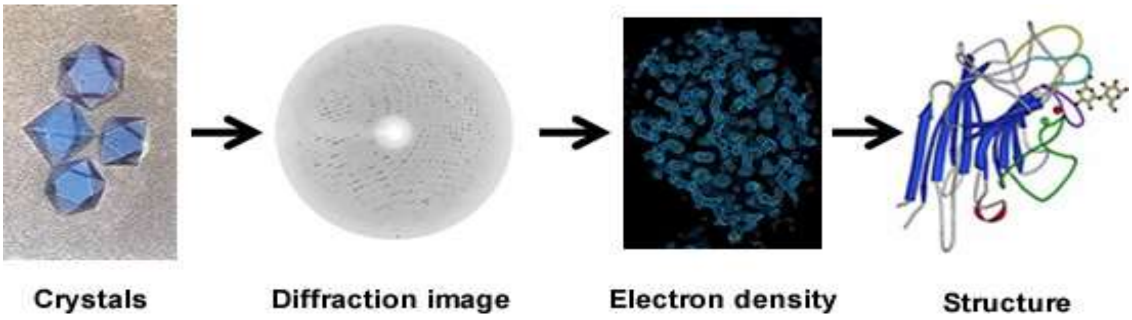

Figure (3) Different steps in x-ray crystallography—crystal, x-ray diffraction pattern, and the final three-dimensional structure of protein.

Scanning Electron Microscope (SEM)

Electron microscopy based technique determines the size, shape and surface morphology with direct visualization of the nanoparticles, therefore scanning electron microscopy offer 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, 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. Whole sample is then analyzed by scanning with a focused fine beam of electrons. Secondary electrons emitted from the sample surface determine the surface characteristics of the sample (Jores *et al.*,2004).

Electron beam can often damage the polymer of the nanoparticles which must be able to withstand vacuum (Molpeceres *et al.*, 2000).

Different kinds of detectors are used to collect signals, and using secondary and backscattered electrons the image is formed. Secondary electrons have relatively low energy and are a surface-sensitive signal. This signal causes high-resolution topographic information and SEM imaging. Backscattered electrons possess higher energy than secondary electrons and come from deeper regions and therefore give good phase contrast information. Because all points were mentioned, the SEM image could fprovide us two main types of data, one is the topographic structure of the surface and the second one is the distinction of different phases in the sample (Poole and Owens 2003).

Energy-dispersive X-ray spectroscopy (EDX)

It is an analytical technique used for elemental composition determination of a sample. The sample undergoes an electron beam bombardment and the surface comprising atoms eject their outer-shell electrons. Then, after the higher-state electrons filled the resulted vacancies, the energy difference between these two states of electrons is emitted as an X-ray beam. The emitted X-rays were detected and analyzed for their energy. Since the X ray energy is a distinctive characteristic of each element; it could be used to componential analysis of samples (Kohler and Fritzsche 2004). The EDX spectrum recorded in the spot profile mode from one of the densely populated silver nanoparticles region on the surface of the film. The Nano crystallites were analyzed using Quanta 200 FEG (Devi *et al.*, 2012)

Fourier Transform Infra-Red (FTIR Spectroscopy)

Fourier Transform Infra-Red spectroscopy (FTIR) is able to provide accuracy, reproducibility, and also a favorable signal-to-noise ratio. By using FTIR spectroscopy, it becomes possible to detect small absorbance changes on the order of 10−3, which helps to perform difference spectroscopy, where one could distinguish the small absorption bands of functionally active residues from the large background absorption of the entire protein (Gerwert ,1999; Zscherp and Barth, 2001).

Infrared (IR) or Fourier transform infrared (FTIR) spectroscopy has a large application range, from the analysis of small molecules or molecular complexes to the analysis of cells or tissues. The imaging of tissues is one of the recent developments of infrared spectroscopy, taking advantage of infrared microscopy and of the use of synchrotron IR radiation. It is used for the mapping of cellular components (carbohydrates, lipids, proteins) to identify abnormal cells (Levin and Bhargava, 2005; Petibois and Deleris , 2006).

IV. Application of nanoparticles in medicine

A list of some of the applications of nanomaterials to biology or medicine is given below:

- Fluorescent biological labels
- Drug and gene delivery
- Bio detection of pathogens
- Detection of proteins
- Probing of DNA structure
- Tissue engineering
- Tumour destruction via heating (hyperthermia)
- Separation and purification of biological molecules and cells

we briefly focus on the functions of Ag-NPs as antiviral, antimicrobial, antitumor, and anti inflammatory agents**.** Where one use of silver ion or metallic silver as well as silver nanoparticles can be exploited in medicine for burn treatment, dental materials, coating stainless steel materials, water treatment, sunscreen lotions, etc. (Duran et al.,2007). Nanoparticles play an important role in medication, biomedical, biological and pharmaceutical field by using as novel antimicrobial, drug delivery (Loureiro *et al*., 2016). Some of uses of nanoparticles in this field are early diagnostic of diseases which also can be used as nutrient level determination in the body. In addition to use as therapeutic agents duo to their antibacterial properties as dressing wound, covering catheter and antimicrobial agents (AshaRani *et al*., 2008) and that most important aspect of nanoparticles application.

Examples of biological coatings may include antibodies, biopolymers like collagen, or monolayers of small molecules that make the nanoparticles biocompatible (Priyadarshana *et al* ., 2015).

Nanoparticles as antimicrobial agents:

At present day, antibiotic resistant bacteria increase and become a serious problem to the health in the world, among them are biofilm and multidrug-resistant bacteria. So it need to develop a novel effective antibacterial. The evolution of nanoparticles create a new antibacterial options and a promising platform to control bacterial diseases as alternative medicine. Nanoparticle is very suitable candidate to carry out antimicrobial operation, as a result to its small size (Beyth *et al*., 2015).

Application of silver nanoparticles

Silver nanoparticles Ag-NPs are one of the most commonly used nanoparticles both in everyday life, and in research laboratories. Ag-NPs are of interest because of the unique properties (e.g. size and shape depending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cosmetic products, and electronic components (Klaus- Joerger *et al*., 2001).

Silver nanoparticles have been shown to interact with bacterial membrane proteins, intracellular proteins, phosphate residues in DNA, and to interfere with cell division, leading to bacterial cell death (Sondi and Salopek-Sondi, 2004; Xu *et al*., 2004)

Silver nanoparticles have been well known for its strong inhibitory and bactericidal effects and can effectively use for the treatment of various infectious diseases (Afreen *et al*., 2011).

Silver nanoparticles (Ag-NPs) accumulation on the cell membrane creates gaps in the integrity of the bilayer which predisposes it to a permeability increase and finally bacterial cell death (Rajeshkumar and Malarkodi, 2014**).**

The applications of these biosynthesized nanoparticles in a widespread spectrum of potential areas are presented including cancer treatment, targeted drug delivery, gene therapy and DNA analysis, antibacterial agents, biosensors, separation science, enhancing reaction rates, and magnetic resonance imaging (MRI)(Li *et al*., 2011).

The Ag-NPs effects on antiviral, most publications have suggested that Ag-NPs could bind to outer proteins of viral particles, resulting in inhibition of binding and the replication of viral particles in cultured cells. Although the antiviral mechanism of Ag-NPs has not been fully known yet, Ag-NPs are still suggested as potential antiviral agents in the future (Galdiero *et al*., 2011).

Silver nanoparticles (Ag-NPs) have several characteristics that make it currently among the most widely used nanoparticle in science. One highly useful characteristic is its antimicrobial property (Morones *et al*., 2005).

Nanoparticles are now being increasingly utilized for medical applications and are of great interest as an alternative approach to control infectious agents offering broad spectrum activities against bacteria, fungi and viruses (Seil and Webster, 2012).

Recent studies have shown that metal based nanoparticles are one of the most promising therapeutic agents owing to their unique physico chemical and biological properties (Franci *et al*., 2015).

Researchers in the field of health have obtained favorable results by applying these substances against infectious agents. One of these nanoparticles is Ag-NPs. Due to their unique properties, Ag-NPs are considered an appropriate candidate for preventing and controlling the pathogenic microorganisms (Yu *et al*., 2011).

Synthesis of nanoparticles

Various methods can be employed for the synthesis of NPs, but these methods are broadly divided into two main classes i.e. (1) Bottom-up approach and (2) Top-down approach (Wang and Xia, 2004) as shown in figure 4 (Iravani, 2011). These approaches further divide into various subclasses based on the operation, reaction condition and adopted protocols.

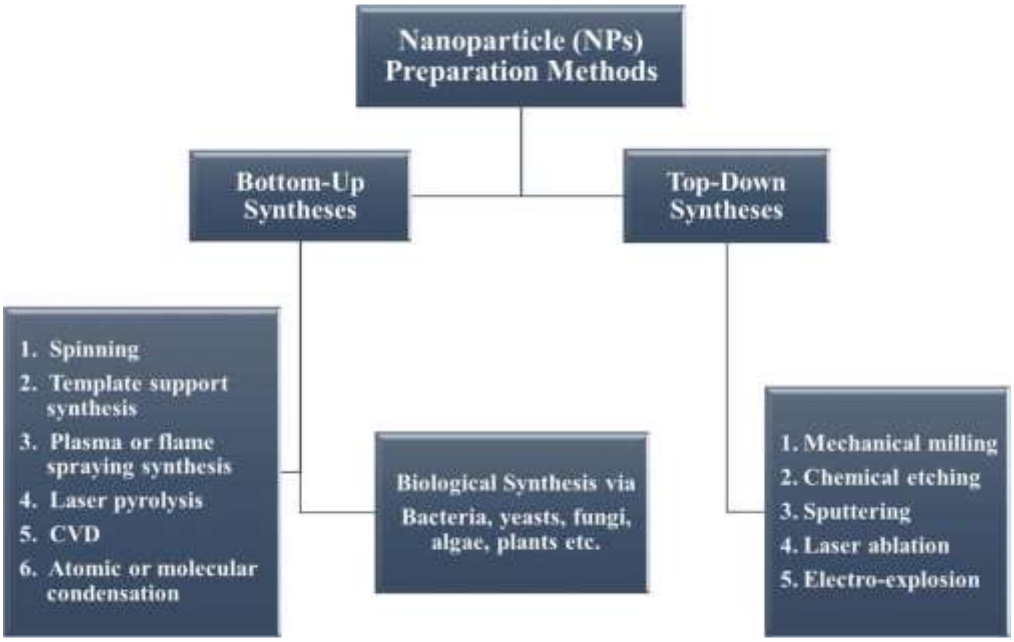

Figure 4. Typical synthetic methods for NPs for the (a) top-down and (b) bottom-up approaches

- Top-down syntheses

In this method, destructive approach is employed. Starting from larger molecule, which decomposed into smaller units and then these units are converted into suitable NPs. Examples of this method are grinding/milling, CVD, physical vapor deposition (PVD) and other decomposition techniques (Iravani, 2011). This approach is used to synthesized coconut shell (CS) NPs. The milling method was employed for this purpose and the raw CS powders were finely milled for different interval of times, with the help of ceramic balls and a well-known planetary mill. They showed the effect of milling time on the overall size of the NPs through different characterization techniques. It was determined that with the time increases the NPs crystallite size decreases, as calculated by Scherer equation. They also realized that with each hour increment the brownish color faded away due to size decrease of the NPs. The SEM results were also in an agreement with the X-ray pattern, which also indicated the particle size decreases with time (Bello *et al.*, 2015).

One study revealed the spherical magnetite NPs synthesis from natural iron oxide (Fe2O3) ore by top-down destructive approach with a particle size varies from ∼20 to ∼50 nm in the presence of organic oleic acid (Priyadarshana *et al.*, 2015). A simple top-down route was employed to synthesize colloidal carbon spherical particles with control size. The synthesis technique was based on the continuous chemical adsorption of polyoxometalates (POM) on the carbon interfacial surface. Adsorption made the carbon black aggregates into relatively smaller spherical particles, with high dispersion capacity and narrow size distribution as shown in Fig. 5 (Garrigue *et al.*, 2004). The powerful laser irradiations generate well-uniform NPs having good oxygen vacancies (Zhou *et al.*, 2016). The average size of the Co3O4 was determined to be in the range of 5.8 nm ± 1.1 nm.

- Bottom-up syntheses

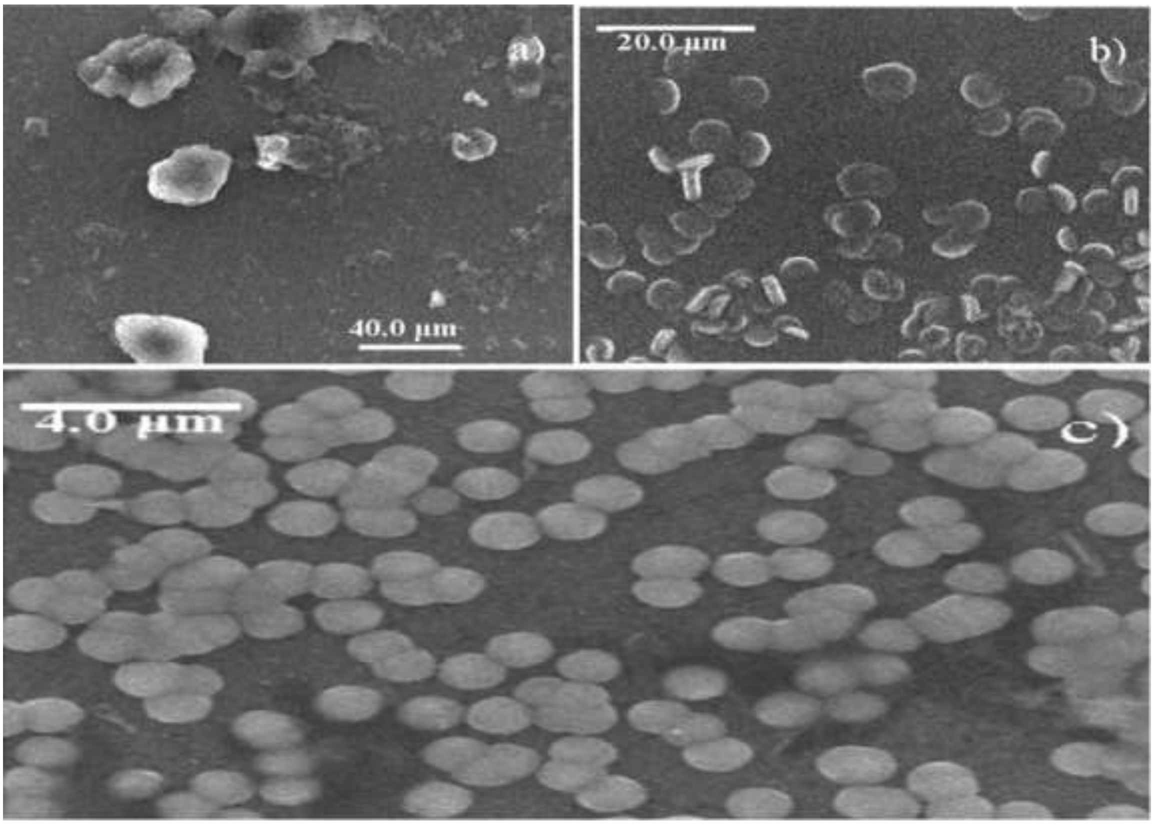

Figure 5. SEM images of (a) The untreated carbon black, (b) and (c) 10 min and 1 h ultra-sonication in POM solution (Garrigue *et al.*, 2004).

This approach is employed in reverse as NPs are formed from relatively simpler substances, therefore this approach is also called building up approach. Examples of this case are sedimentation and reduction techniques. It includes sol gel, green synthesis, spinning, and biochemical synthesis. (Iravani, 2011). synthesized TiO2 anatase

NPs with graphene domains through this technique (Mogilevsky *et al*., 2014). They used alizarin and titanium isopropoxide precursors to synthesize the photoactive composite for photocatalytic degradation of methylene blue. Alizarin was selected as it offers strong binding capacity with TiO2 through their axial hydroxyl terminal groups. The anatase form was confirmed by XRD pattern. The SEM images taken for different samples with reaction scheme are provided in figure 6. SEM indicates that with temperature elevation, the size of NPs also increases (Mogilevsky *et al*., 2014).

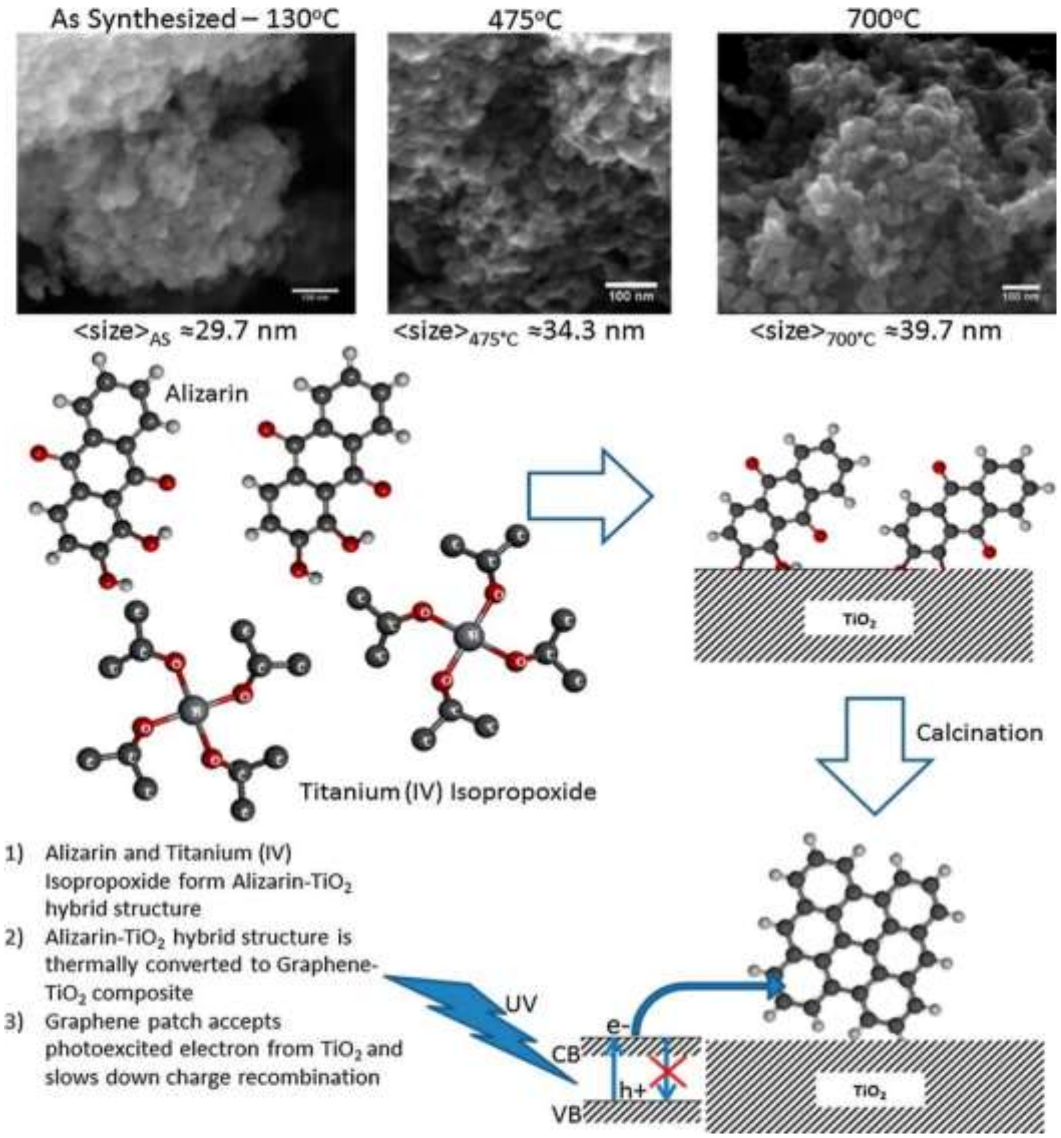

Figure 6. Synthesis of TiO2 via bottom-up technique. SEM images showing the TiO2 NPs (Mogilevsky *et al.*, 2014).

Well-uniform spherical shaped Au nanospheres with monocrystalline have been synthesized via laser irradiation top-down technique (Liu *et al*., 2015a, Liu *et al*., 2015b). Liu et al. selectively transform the octahedra morphology to spherical shape by controlling the laser treatment time and other reaction parameters. Fig. 7 provides the SEM and TEM of the prepared Au nanospheres, which showed average diameter of 75 ± 2.6 nm of Au nanospheres (red column Fig. 7e) and 72 ± 3.1 in edge length of Au octahedra per particle (blue column Fig. 7f).

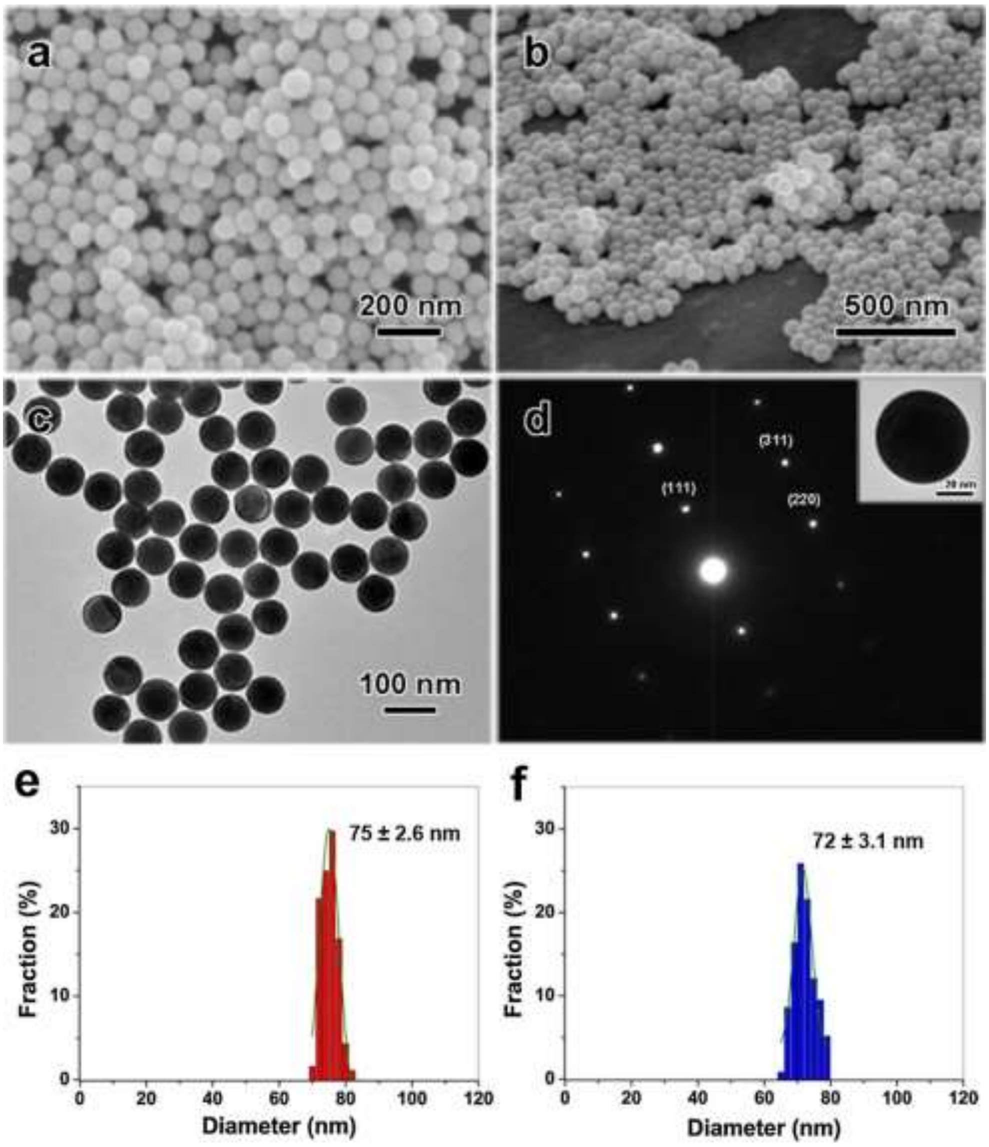

Figure 7. SEM for Au nanospheres (a) top view, (b) tilted view, (c) TEM image of Au nanospheres (d) SAED pattern (inset: TEM of single Au particle), € and (f) size distribution spectra of spherical and octahedral Au NPs.

More recently, solvent-exchange method is used to achieve limit sized low density lipoprotein (LDL) NPs for medical cancer drug delivery purpose by Needham et al. In this method nucleation is the bottom approach followed by growth which is the up approach. The LDL NPs . were obtained without using phospholipid and possessed high hydrophobicity, which is essential for drug delivery applications (Needham *et al*., 2016).

The mono dispersed spherical bismuth (Bi) NPs were synthesized by both top-down and bottom-up approaches (Wang and Xia, 2004). These NPs have excellent colloidal properties. In the bottom-up approach bismuth acetate was boiled within ethylene glycol, while in top-down approach the bismuth was converted into molten form and then the molten drop was emulsified within the boiled diethylene glycol to produce the NPs. The size of the NPs obtained by both methods was varied from 100 nm to 500 nm (Wang and Xia, 2004). The details of this study are provided in figure 8. Green and biogenic bottom-up synthesis attracting many researchers due to the feasibility and less toxic nature of processes. These processes are cost-effective and environmental friendly, where synthesis of NPs is accomplished via biological systems such as using plant extracts. Bacteria, yeast, fungi, Aloe vera, tamarind and even human cells are used for the synthesis of NPs. Au NPs have been synthesis from the biomass of wheat and oat (Parveen *et al*., 2016) and using the microorganism and plant extracts as reducing agent (Ahmed *et al*., 2016).

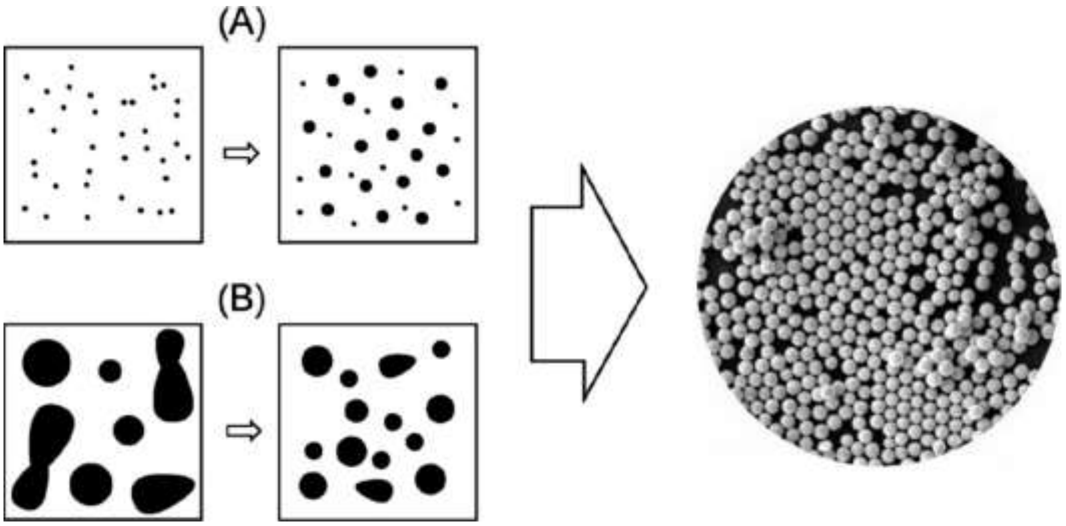

Figure 8. (A) Bottom-up approach: A molecular precursor is disintegrated to simpler metal atoms that grow into colloids. (B) Top-down approach: Large drops of a metal broken into smaller drops.

Biosynthesis of Nanoparticles by Fungi

Fungi have excellent potential for the production of many compounds that can be used in different applications. Around 6,400 bioactive substances are known to be produced by microscopic filamentous fungi (ascomycetes and imperfect fungi) and other fungal species (Bérdy, 2005).

These organisms are widely used as reducing and stabilizing agents, due to their heavy metal tolerance and capacity to internalize and bioaccumulate metals. Furthermore, fungi can be easily cultivated on a large scale ("nanofactories") and can produce nanoparticles with controlled size and morphology (Khan *et al*., 2017).

Fungi have advantages over other microorganisms, in that they produce large quantities of proteins and enzymes, some of which can be used for the fast and sustainable synthesis of nanoparticles (Alghuthaymi *et al*., 2015).

The mechanism of biogenic synthesis of nanoparticles using fungi may be intracellular or extracellular. In the case of intracellular synthesis, the metal precursor is added to the mycelial culture and is internalized in the biomass. Consequently, extraction of the nanoparticles is required after the synthesis, employing chemical treatment, centrifugation, and filtration to disrupt the biomass and release the nanoparticles (Rajput *et al*., 2016; Molnár *et al*., 2018).

In extracellular synthesis, the metal precursor is added to the aqueous filtrate containing only the fungal biomolecules, resulting in the formation of free nanoparticles in the dispersion. This last method is most widely used, since no procedures are required to release the nanoparticles from the cells (Sabri *et al*., 2016; Costa Silva *et al*., 2017). Nonetheless, the nanoparticle dispersion must be purified in order to eliminate fungal residues and impurities, which can be achieved using methods such as simple filtration, membrane filtration, gel filtration, dialysis, and ultracentrifugation (Qidwai *et al*., 2018; Yahyaei and Pourali, 2019).

Antibacterial activity of silver nanoparticles

Ag-NPs have a broad antibacterial effect on a range of Gram-negative and Gram-positive bacteria and antibiotic-resistant bacteria strains (Kim *et al* ., 2007).

Antimicrobial efficacy of Ag-NPs depends on their size and concentration. Normally, a high concentration leads to more effective antimicrobial activity, while particles of small sizes can kill bacteria at a lower concentration. Apart from size and concentration, the shape also influences the antimicrobial efficiency of Ag-NPs.

The antimicrobial efficacy of silver nanoparticles (SNPs) has been demonstrated through several studies, although only a few anticancer studies have been conducted in this regard, But the food and drug administration (FDA) approved its usage in human body (Newmeyer *et al.*, 2003; Sanpui *et al*., 2011).

Silver nanoparticles (Ag-NPs) are could be used as potential antimicrobial, especially in emergent situations such as treating burns and healing of wounds (Mazurak *et al*., 2007).

Ag-NPs seem to be alternative antibacterial agents to antibiotics and have the ability to overcome the bacterial resistance against antibiotics. Therefore, it is necessary to develop Ag-NPs as antibacterial agents. Among the several promising Nano materials, Ag-NPs seem to be potential antibacterial agents due to their large surface-to-volume ratios and crystallographic surface structure (Zhang *et al*., 2016).

The antimicrobial action of Ag-NPs is linked with four well-defined mechanisms: **(1)** adhesion of Ag-NPs onto the surface of cell wall and membrane, **(2)** Ag-NPs penetration inside the cell and damaging of intracellular structures (mitochondria, vacuoles, ribosomes) and biomolecules (protein, lipids, and DNA) **(3)** Ag-NPs induced cellular toxicity and oxidative stress cause by generation of reactive oxygen species (ROS) and free radicals, and **(4)** Modulation of signal transduction pathways. Besides these four well-recognized mechanisms, Ag-NPs also modulate the immune system of the human cells by orchestrating inflammatory response, which further aid in inhibition of microorganisms as in figure 2-8. (Tian *et al.,* 2007).

V. Reference

1. Vigneshwaran, N., Ashtaputre, N. M., Varadarajan, P. V., Nachane, R. P., Paralikar, K. M. and Balasubramanya, R. H. (2007). Biological synthesis of silver nanoparticles using the fungus Aspergillus flavus. Materials letters, 61(6), 1413-1418.
2. Xu, X. H. N., Brownlow, W. J., Kyriacou, S. V., Wan, Q. and Viola, J. J. (2004). Real-time probing of membrane transport in living microbial cells using single nanoparticle optics and living cell imaging. Biochemistry, 43(32), 10400-10413.
3. Vastrad, J. V. and Goudar, G. (2016). Green synthesis and characterization of silver nanoparticles using leaf extract of Tridax procumbens. Oriental Journal of Chemistry, 32(3), 1525-1530.
4. Verma, V. C., Kharwar, R. N. and Gange, A. C. (2010). Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus Aspergillus clavatus. Nanomedicine, 5(1), 33-40.
5. Wang, Z., Chen, J., Yang, P. and Yang, W. (2007). Biomimetic synthesis of gold nanoparticles and their aggregates using a polypeptide sequence. Applied Organometallic Chemistry, 21(8), 645-651 .
6. Yu, B., Leung, K. M., Guo, Q., Lau, W. M. and Yang, J. (2011). Synthesis of Ag–TiO2 composite nano thin film for antimicrobial application. Nanotechnology, 22(11), 115603
7. Zhang, X. F., Liu, Z. G., Shen, W. and Gurunathan, S. (2016). Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. International journal of molecular sciences, 17(9), 1534 .
8. Zhong, W., Xing, M. M. and Maibach, H. I. (2010). Nanofibrous materials for wound care. Cutaneous and ocular toxicology, 29(3), 143-152 .
9. Zscherp, C. and Barth, A. (2001). Reaction-induced infrared difference spectroscopy for the study of protein reaction mechanisms. Biochemistry, 40(7), 1875-1883 .
10. Sharma, V. K., Yngard, R. A. andLin, Y. (2009). Silver nanoparticles: green synthesis and their antimicrobial activities. Advances in colloid and interface science, 145(1-2), 83-96.
11. Sharma, D., Kanchi, S. and Bisetty, K. (2015). Biogenic synthesis of nanoparticles: A review. Arabian journal of chemistry.
12. Shenashen, M. A., El-Safty, S. A. and Elshehy, E. A. (2014). Synthesis, morphological control, and properties of silver nanoparticles in potential applications. Particle and Particle Systems Characterization, 31(3), 293-316 .
13. Sintubin, L., Verstraete, W. and Boon, N. (2012). Biologically produced nanosilver: current state and future perspectives. Biotechnology and Bioengineering, 109(10), 2422-2436 .
14. Sondi, I. and 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
15. Taylor, P. L., Ussher, A. L. and Burrell, R. E. (2005). Impact of heat on nanocrystalline silver dressings: Part I: Chemical and biological properties. Biomaterials, 26(35), 7221-7229 .

16. Tian, J., Wong, K. K., Ho, C. M., Lok, C. N., Yu, W. Y., Che, C. M. and Tam, P. K. (2007). Topical delivery of silver nanoparticles promotes wound healing. ChemMedChem: Chemistry Enabling Drug Discovery, 2(1), 129-136 .
17. Tyagi, P.K. (2016) Production of metal nanoparticles from biological resources. Int J Curr Microbiol Appl Sci 5, 548–558. Uskokovic, V. (2008) Nanomaterials and nanotechnologies: approaching the crest of this big wave. Curr Nanosci 4, 119–129.
18. Gurunathan, S., Han, J. W., Kwon, D. N. and Kim, J. H. (2014). Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. Nanoscale research letters, 9(1), 373
19. Gade, A., Ingle, A., Whiteley, C., Rai, M. (2010). Myogenic metal nanoparticles: progress and applications.Biotechnol. Lett, 32,593–600.
20. Gade, A. K., Bonde, P., Ingle, A. P., Marcato, P. D., Duran, N. and Rai, M. K. (2008). Exploitation of Aspergillus niger for synthesis of silver nanoparticles. Journal of Biobased Materials and Bioenergy, 2(3), 243-247.
21. Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G. et al. (2015). Silver nanoparticles as potential antibacterial agents. Molecules, 20(88), 56-74.
22. Gitanjali,B., Shelar, and Ashok, M. (2015). Myco-synthesis of silver nanoparticles from Trichoderma harzianum and its impact on germination status of oil seed. International quarterly Journal of Biology & Life sciences, 3(1):109-113.
23. Gerwert, K. (1999). Molecular reaction mechanisms of proteins monitored by time-resolved FTIR-spectroscopy. Biological chemistry, 380(7-8), 931-935 .
24. Galdiero, S., Falanga, A., Vitiello, M., Cantisani, M. Marra, V. and Galdiero,M. (2015).Silver Nanoparticles as Potential Antiviral Agents. Molecules, 16(10):8894-8918 .
25. Jores, K., Mehnert, W., Drechsler, M., Bunjes, H., Johann, C. andMäder, K. (2004). Investigations on the structure of solid lipid nanoparticles (SLN) and oil-loaded solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy. Journal of Controlled Release, 95(2), 217-227
26. .Jassim, A. M. N., Farhan, S. A.and Dadoosh, R. M. (2016). Green synthesis of silver nanoparticles using seed aqueous extract of Abelmoschus esculentus and study of their properties. Advances in Environmental Biology, 10(4), 51-67
27. .Huang, Z., Zheng, X., Yan, D., Yin, G., Liao, X., Kang, Y. and Hao, B. (2008). Toxicological effect of ZnO nanoparticles based on bacteria. Langmuir, 24(8), 4140-4144 .
28. Hesse, M., Meier, H. and Zeeh, B. (2008). Spectroscopic methods in organic chemistry. Thieme Medical Pub.
29. Kumar, S., Mukherjee, M. M. and Varela, M. F. (2013). Modulation of bacterial multidrug resistance efflux pumps of the major facilitator superfamily. International journal of bacteriology, 2013.
30. Kohler, M. and Fritzsche, W. (2008). Nanotechnology: an introduction to nanostructuring techniques. John Wiley and Sons * .
31. Kim, J. S., Kuk, E., Yu, K. N., Kim, J. H., Park, S. J., Lee, H. J. and Kim, Y. K. (2007). Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine, 3(1), 95-101 .
32. Duran, N., Marcato, P. D., Alves, O. L., De Souza, G. I. and Esposito, E. (2005). Mechanistic aspects of biosynthesis of silver nanoparticles by several Fusarium oxysporum strains. Journal of nanobiotechnology, 3(1), 8 .
33. Devi, L. S. andJoshi, S. R. (2012). Antimicrobial and synergistic effects of silver nanoparticles synthesized using soil fungi of high altitudes of eastern Himalaya. Mycobiology, 40(1), 27-34.
34. Dias, M. A., Lacerda, 1. C. A., Pimentel, P. F., De Castro, H. F. and Rosa, C. A. (2002). Removal of heavy metals by an Aspergillus terreus strain immobilized in a polyurethane matrix. Letters in applied microbiology, 34(1), 46-50.

35. Duran, N., Marcato, P. D., De Souza, G. I., Alves, O. L. andEsposito, E. (2007). Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. Journal of biomedical nanotechnology, 3(2), 203-208.
36. Bansal, V., Rautaray, D., Ahmad, A. andSastry, M. (2004). Biosynthesis of zirconia nanoparticles using the fungus Fusarium oxysporum. Journal of Materials Chemistry, 14(22). 3303-3305 .
37. Bar, H., Bhui, D. K., Sahoo, G. P., Sarkar, P., Pyne, S. andMisra, A. (2009). Green synthesis of silver nanoparticles using seed extract of Jatropha curcas. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 348(1-3), 212-216.
38. Carlson, C., Hussain, S. M., Schrand, A. M., K. Braydich-Stolle, L., Hess, K. L., Jones, R. L. andSchlager, 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.
39. Balaji, D. S., Basavaraja, S., Deshpande, R., Mahesh, D. B., Prabhakar, B. K. andVenkataraman, A. (2009). Extracellular biosynthesis of functionalized silver nanoparticles by strains of Cladosporium cladosporioides fungus. Colloids and surfaces B: biointerfaces, 68(1), 88-92
40. Alghuthaymi , M. A., Almoammar, H., Rai, M., Said-Galiev, E. andAbd-Elsalam, K. A. (2015). Myconanoparticles: synthesis and their role in phytopathogens management. Biotechnology and Biotechnological Equipment, 29(2), 221-236 .
41. Anil Kumar, S., Abyaneh, M.K., Gosavi, S.W. and et al. (2007). Nitrate reductase- mediated synthesis of silver nanoparticles from AGNO3. Biotechnology Letters, 29(3). 439 445.
42. Afreen, Rathod, V. and Ranganath, E. (2011). Synthesis of mono dispersed silver nanoparticles by Rhizopus stolonifer and its antibacterial activity against MDR strains of Pseudomonas aeruginosa from burnt patients. International Journal of Environmental Sciences, 1(7), 1583-1592.
43. Senapati, S (2005) Biosynthesis and Immobilization of Nanoparticles and their Applications. Ph.D. thesis, University of Pune.
44. Seo, W. S., Lee, J. H., Sun, X., Suzuki, Y., Mann, D., Liu, Z. and Dai, H. (2006) .FeCo/graphitic-shell nanocrystals as advanced magnetic-resonance-imaging and near- Infrared agents. Nature materials, 5(12), 971.
45. Riddin, T. L., Gericke, M. and Whiteley, C. G. (2006). Analysis of the inter-an Extracellular formation of platinum nanoparticles by Fusarium oxysporum sp. Lycopersici Using response surface methodology. Nanotechnology, 17(14), 3482.
46. Sanpui, P., Chattopadhyay, A. and Ghosh, S. S. (2011). Induction of apoptosis in Cancer cells at low silver nanoparticle concentrations using chitosan nanocarrier. ACS Applied materials and interfaces, 3(2), 218-228
47. Saha, S., Chattopadhyay, D., Acharya, K. (2011). Preparation of silver nanoparticles by bio-production using Nigrospora oryzae culture filtrate and its antimicrobial Activity. Digest Journal of Nanomaterials and Biostructures (DJNB), 6(4).
48. Savithramma, N., Rao, M.L., Devi, P.S. (2011). Evaluation of antibacterial efficacy of Biologically synthesized silver nanoparticles using stem barks of Boswellia ovalifoliolata Bal. And henry and Shorea tumbuggaia. Roxb. J. Bil. Sci.
49. Mazurak, V. C., Burrell, R. E., Tredget, E. E., Clandinin, M. T..and Field, C. J .(2007). The effect of treating infected skin grafts with Acticoat™ on immune cells. Burns, 33(1), 52-58.
50. Sanghi, R. andVerma, P. (2009). Biomimetic synthesis and characterisation of protein Capped silver nanoparticles. Bioresource technology, 100(1), 501-504.
51. Sapsford, K. E., Tyner, K. M., Dair, B. J., Deschamps, J. R. and Medintz, I. L. (2011) .Analyzing nanomaterial bioconjugates: a review of current and emerging purification and Characterization techniques. Analytical chemistry, 83(12), 4453-4488.
52. Ramezani, N., Ehsanfar, Z., Shamsa, F., Amin, G., Shahverdi, H. R., Esfahani, H. R. M .
53. And Shahverdi, A. R. (2008). Screening of medicinal plant methanol extracts for the Synthesis of gold nanoparticles by their reducing potential. Zeitschrift fur Naturforschung B, 63(7), 903-908.

54. Rajeshkumar, S. and Malarkodi, C. (2014). In vitro antibacterial activity and mechanism of silver nanoparticles against foodborne pathogens. Bioinorganic chemistry and Applications, 2014.
55. Radovic-Moreno, A. F., Lu, T. K., Puscasu, V. A., Yoon, C. J., Langer, R.and Farokhzad, O. C. (2012). Surface charge-switching polymeric nanoparticles for bacterial Cell wall-targeted delivery of antibiotics. ACS nano, 6(5), 4279-4287.
56. Poole, C.P. J.r. and Owens, F.J. (2003). Introduction to nanotechnology. Wiley, New York.
57. Petibois, C. and Deleris, G. (2006). Chemical mapping of tumor progression by FT -IR imaging: towards molecular histopathology. TRENDS in Biotechnology, 24(10), 455-462.
58. Panacek, A., Kolar, M., Vecerova, R., Prucek, R., Soukupova, J., Krystof, V. and Kvitek, L. (2009). Antifungal activity of silver nanoparticles against Candida spp. Biomaterials, 30(31), 6333-6340.
59. Newmeyer, D. D. and Ferguson-Miller, S. (2003). Mitochondria: releasing power for Life and unleashing the machineries of death. Cell, 112(4), 481-490.
60. Nanda, A. and Saravanan, M. (2009). Biosynthesis of silver nanoparticles from Staphylococcus aureus and its antimicrobial activity against MRSA and MRSE. Nanomedicine: Nanotechnology, Biology and Medicine, 5(4), 452-456.
61. Narayanan, K. B. and Sakthivel, N. (2010). Biological synthesis of metal nanoparticles by Microbes. Advances in colloid and interface science, 156(1-2), 1-13.
62. Mukherjee, P., Ahmad, A., Mandal, D., Senapati, S., Sainkar, S. R., Khan, M. I. and Sastry ,M. (2001). Bioreduction of AuCl4− ions by the fungus, Verticillium sp. And surface trapping of the gold nanoparticles formed. Angewandte Chemie International Edition, 40(19), 3585- 3588.
63. Mulvaney, P. (1996). Surface plasmon spectroscopy of nanosized metal particles. Langmuir, 12(3), 788-800.
64. Murphy, M., Ting, K., Zhang, X., Soo, C. and Zheng, Z. (2015). Current development of Silver nanoparticle preparation, investigation, and application in the field of medicine. Journal of Nanomaterials, 2015, 1-5.
65. Nam, K. T., Lee, Y. J., Krauland, E. M., Kottmann, S. T. and Belcher, A. M. (2008) .Peptide-mediated reduction of silver ions on engineered biological scaffolds. Acs Nano, 2(7), 1480-1486.
66. Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T. and Yacaman, M. J. (2005). The bactericidal effect of silver nanoparticles. Nanotechnology, 16(10), 2346-2353.
67. Molpeceres, J., Aberturas, M. R. andGuzman, M. (2000). Biodegradable nanoparticles as A delivery system for cyclosporine: preparation and characterization. Journal of Microencapsulation, 17(5), 599-614.
68. Mohanpuria, P., Rana, N. K. and Yadav, S. K. (2008). Biosynthesis of nanoparticles :Technological concepts and future applications. Journal of nanoparticle research, 10(3), 507-517.