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Toxicological Evaluation of ZnO and $Pb(NO_3)_2$ Synthesized Nonmaterial's Using Peripheral Blood Mononuclear Cell of Human Blood

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Research Article

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ABSTRACT

Background: Use of microorganisms for the synthesis of metal nanoparticles is in the limelight of modern nanotechnology. It is emerges as an ecofriendly and exciting approach, for production of nanoparticles due to its low energy requirement, environmental compatibility, reduced costs of manufacture, scalability and nanoparticle stabilization compared with the physical and chemical synthesis. Biologically synthesized metal nanoparticles are the most efficient miniaturized functional materials that are constructed and engineered to exert specific functions with enormous ability.

Methods: Microorganisms have this extraordinary capacity to form such exquisite nanostructures. This research work reports the biological synthesis of zinc oxide and lead nitrate nanoparticles by using microbes. Microbes play direct or indirect roles in several biological activities because metals present on earth are in constant association with biological components.

Discussion: In the present study, reported microbial synthesis of nanomaterials utilizes of biological components, primarily prokaryotes and eukaryotes such as bacteria and fungi (*Escherichia coli*, *Aspergillus niger*). Both bacterial and fungal cell mass were challenged with two different chemical salts (ZnO, $Pb(NO_3)_2$) and metal nanoparticles were synthesized effectively. Furthermore, the antimicrobial potential of zinc and lead nanoparticles was systematically evaluated.

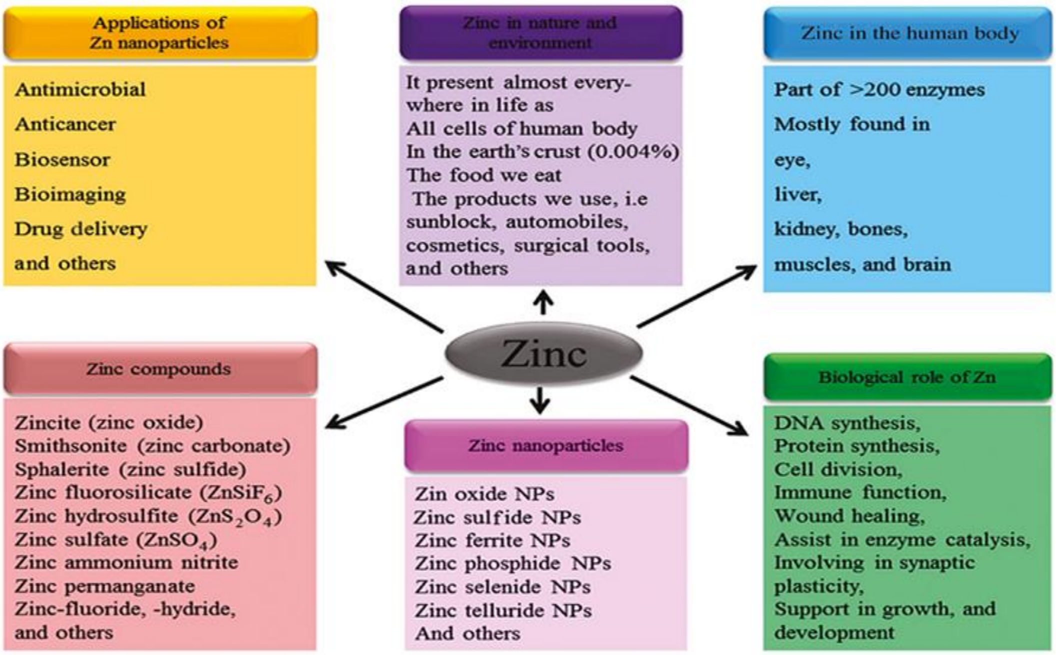
Results: The synthesized nanoparticles could efficiently inhibit various pathogenic organisms, *P. aeruginosa* and *S. aureus*. The bactericidal effect of zinc and lead nanoparticles were compared based on diameter of

inhibition zone in agar diffusion assay, disc method tests and Minimum Inhibitory Concentration (MIC).

INTRODUCTION

In the present era of scientific and technological revolution and global industrial .competitiveness, nanotechnology has emerged from various scientific disciplines/subjects including biological, chemical, physical and engineering sciences. With the help of nanotechnology, useful novel methods now a day are being developed by scientists having potential to probe and manipulate single atom and molecule. In the field of nanotechnology, a nanoparticle (10 m) is the main object in terms of its properties as well as transport. The engineering and sciences of Nano systems are one the fastest growing and most challenging sectors of nanotechnology. This study is presented like a common foundation in order of scientist's interest in biosynthesis of Zinc nanoparticles, its activity, characterization and *in vitro* toxicity assessment. (Figure 1) [1].

Figure 1. Biosynthesis of zinc nanoparticles, its activity, characterization and *in vitro* toxicity assessment.



"Nanotechnology" defined by a scientist Taniguchi is being used in scientific field and research for the last thirty five years. Further, it may also be mentioned that US National Nanotechnology Initiative NNI, in 2000 has defined nanoscience and nanotechnology. A renowned physicist Richard Feynman, first time, given the important concepts and principles of nanoscience and nanotechnology in his scientific lecture/talk "there's plenty of the Room at the Bottom" at an American Physical Society, meeting at Caltech. Biological synthesis of metal nanoparticles seems to be a convenient process as it needs less energy and is environmentally safe. This method will have great scope towards developing useful nanomachines/nanodevices [2].

Continuous scientific research has been successful in the development of nanotechnology in the past decades having a broad range of technologically emerging applications. Now a day, scientists have been able to develop engineered nanostructural materials and nanoparticles which have enormous applications in crucial productive areas such as industry, medicine, cosmetics etc.

Ultimately, nanotechnology can be potentially viable in controlling a matter at the length scale of nanometre. Nanotechnology has significant economic, social, military and environmental implications. A nanometre is defined as one billionth of a meter, almost the width of three/four atoms. As well as the widening of average human hair is near about 25,000 nanometres. It may be emphasized here that nanotechnology may be considered as the technology of engineering tiny machines that projected ability to construct things from the bottom up within personal nano factories using techniques being evolved today to make advanced products materials with enormous applications. Novel properties bearing capability to differentiate nanomaterials from their bulk material are typically developed at their critical length scale below 100 nm". Thus, nanotechnology has potential to revolutionize both science and society [3].

In the area of nanotechnology, materials having extremely small structures can be studied. The “nano” prefix is a Greek word that means “dwarf” and the “nano” word means very small size. The size of a system plays an important role in producing physical phenomena. As the size of the system decreases, the system produces more pronounced physical phenomena. It has also been observed that certain phenomena may not occur during the movement of a system from macro level to micro level while it becomes significant at the nanoscale level for example increase in surface level to volume ratio changes the thermal, catalytic and mechanical properties of the material. The behavior of atoms on the surface of the particle becomes at increasing dominance stage in comparison to behavior of atoms in the interior of the particle during increase in surface area to volume ratio thereby altering the properties of the material such as thermal, catalytic and mechanical property.

Nanoparticles

A particle with microscopic size having atleast one dimension or more of the order of 100 nm or less is defined as nanoparticle. Nanoparticles are of highly scientific interest as they serve as a bridge between atomic/molecular structures and bulk materials. A bulk material exhibits constant physical properties and there is no effect of size in its physical properties/behavior while in case of nano scale the size plays a significant role showing size dependent properties/behavior, in their research study has clearly demonstrated that nanoparticles produce size dependent properties/behavior which is different from fine particles. The results of this study confirm that the properties/ behavior of particles significantly change at their nano scale level. The unique significant physical as well as chemical properties are found in nanoparticles. These significant behaviors of nanoparticles make them useful in developing biological and chemical sensors having varied applications in crucial sectors including health and medicine.

There are two extensive types of nanoparticles which include organic nanoparticles and inorganic nanoparticles. Carbon nanoparticles come under organic nanoparticle for example fullerenes. Metal nanoparticles, magnetic nanoparticles containing metals like copper, silver, zinc, gold, lead etc. As well as semiconductor nanoparticles such as zinc oxide, titanium dioxide come under inorganic nanoparticles [4].

Inorganic nanoparticles exhibiting superior material properties along with functional versatility have been found better diagnostic tools for medical imaging and disease treatment. The inorganic nanoparticles because of their small size features as well as unique physical properties have attracted clinicians and physicians in diagnosis and treatment of diseases in the present time. This group of nanoparticles is being considered as better diagnostic tool in comparison to usually available chemical imaging, drug and drugs agents in Medical Science.

It is interesting to highlight that inorganic metal and its oxide nanoparticles such as $Pb(NO_3)_2$ and ZnO nanoparticles gave wide range of industrial and commercial applications. These inorganic nanoparticles are easily available and they have significant versatile features like rich functionality, wide availability, good biocompatibility, and controlled release of drugs and their capability of targeted delivery. Inorganic nanoparticles possessing these significant properties have immense potential in biomedical field with special interest to cellular drug delivery and their cellular responses in disease conditions. Inorganic nanoparticles due to their specific features as mentioned above have attracted interest in the study pertaining to optical properties of composites too. In addition, inorganic metal nanoparticles are being extensively used in toxicological impacts assessment (Figure 2) [5].

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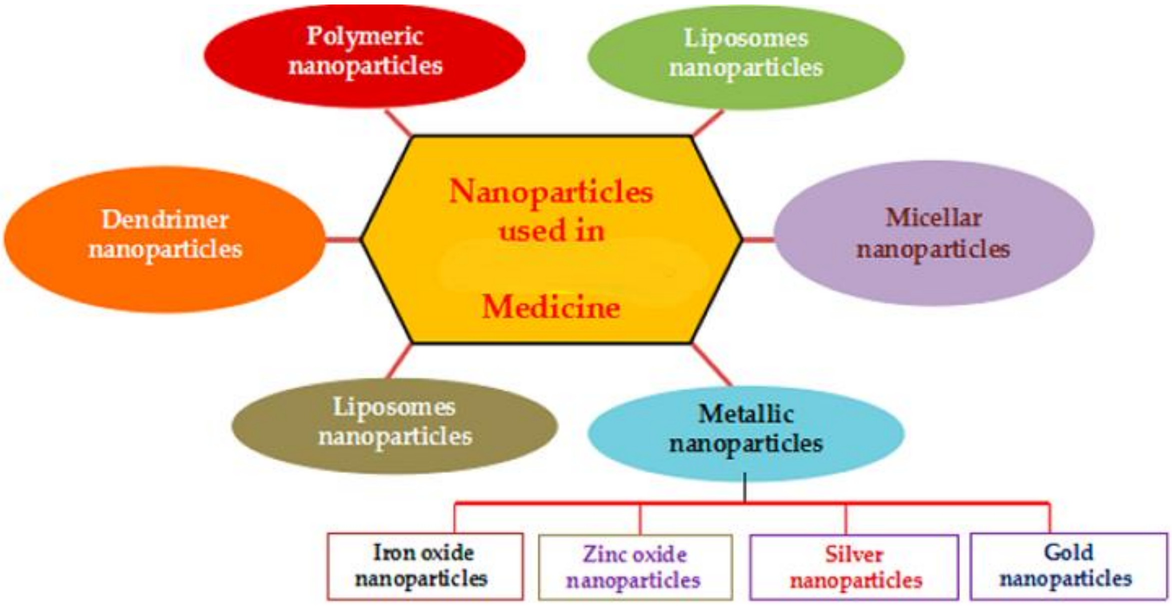
Figure 2. Nanoparticles is being considered as better diagnostic tool in comparison to usually available chemical imaging, drug and drugs agents in medical science.



Metal nanoparticles

Metal nanoparticles contain a highly specific surface area and their surface atoms exhibit a high fraction. Metal nanoparticles present significant physicochemical properties like optical properties, electronic properties, catalytic activity, magnetic properties and antibacterial properties which make them useful tools in novel methods of synthesis. Zinc oxide and lead nitrate nanoparticles are being widely used as these metal oxide nanoparticles have varied applications. They are used in important areas including piezoelectric, optical, gas sensing and magnetic etc. Metal nanoparticle ZnO has also been found to exhibit high absorption ability and pronounced catalytic activity. Thus ZnO nanoparticle is used in manufacturing of important useful products like sunscreens, ceramics processing and rubber processing. ZnO nanostructure is also used in waste water treatment. ZnO nanoparticle has ability to counteract fungal infections and act as fungicide.

Due to vast applications of metal nanoparticle in various sectors, synthesis of metal nanostructure has become important area of research in the field of material science and technology. Although the biological, chemical and physical methods for the synthesis of Ag (silver) and Au (gold) NPs have been reported in scientific literature, but much work has not been done on the aspect covering the biological synthesis of lead nitrate and zinc oxide nanoparticles using microbes like *Escherichia coli* (bacteria) and *Aspergillus niger* (fungus) and its impacts on human health. Research efforts are also needed to explore more potential applications of these metal nanoparticles [6].

MATERIALS AND METHODS

Recent scientific developments and research efforts towards the synthesis of nanoparticles could be able to give better control over chemical and physical properties of nanometre scale particles. Traditionally physical, chemical and biological methods only were used for the production of nanoparticles. Advanced researches with multidisciplinary approach being conducted by renowned scientists and technologists throughout the world in the emerging and frontal area of “nanotechnology” has led to the development of useful and novel nanomaterials. New kinds of nanomaterials are coming up day by day in scientific world which has resulted constant changes in classifications of nanomaterial.

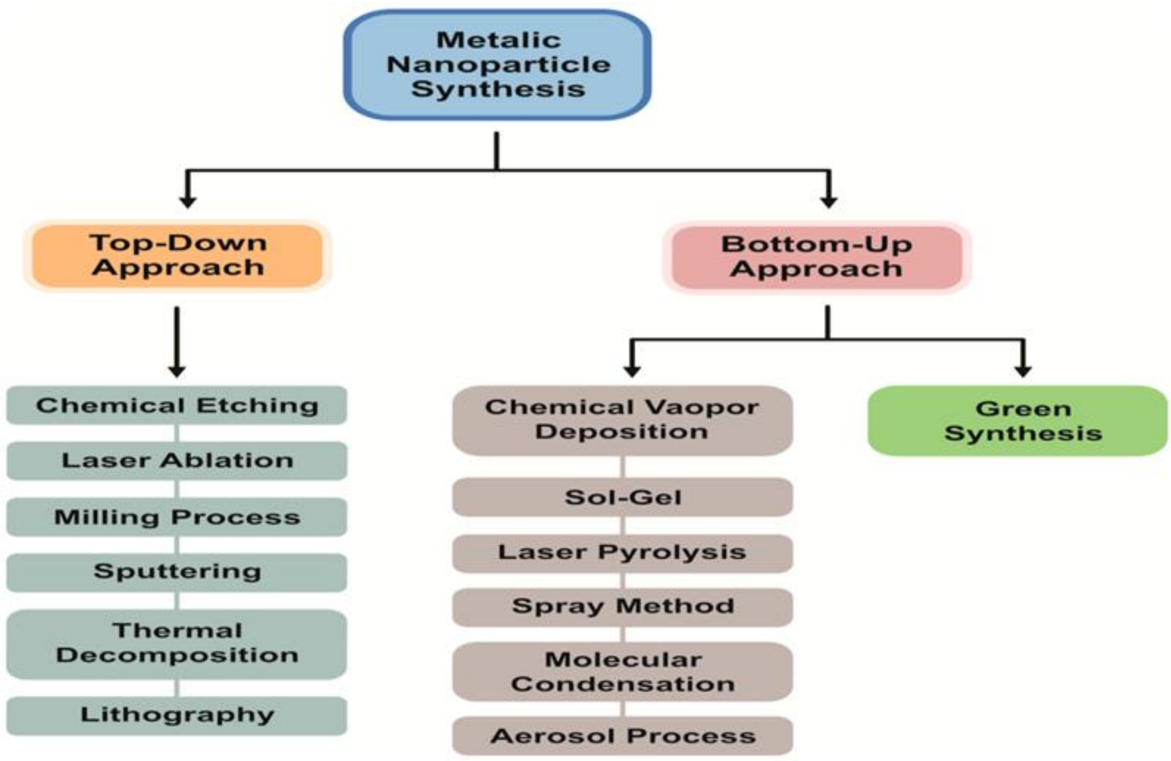
Nanoparticles can be classified in three categories like nano clusters, nanopowders and nano crystals.

- **Nano clusters:** Nano clusters are semi crystalline nanostructures having dimensions within 1 10 nm and a narrow size distribution.
- **Nano powders:** Aggregation of non-crystalline nanomaterials having dimensions between 10-100 nm results in formation of nano powders.
- **Nano crystals:** Nano crystals are single crystalline nanomaterials with dimensions between 100–1000 nm.

Synthesis of metallic nanoparticles

For synthesizing metallic nanoparticles two techniques namely top down method and Bottom up method is generally used. Among these two methods, bottom up approach appears to be more advantageous since it can produce nanoparticles having homogenous structure and better short and long ordering (Figure 3) [7].

Figure 3. Techniques of nanoparticles synthesis.



In the Top down approach, scientists try to formulate nanoparticles using larger ones to direct their assembly. In this approach nanomaterials are synthesized by removing crystals planes present on the substrate. Thus, in this method the building blocks are removed from the substrate in order to form nanomaterials. This method involves the use of larger initial structure which can be externally controlled during the nanostructure synthesis. Ball millings, etching through the mask, application of severe plastic deformation are few examples of top down approaches.

The main principle of this method is that the nanomaterials are synthesized onto the substrate by stacking atoms onto each other giving rise to crystal planes. Crystal's planes further stack on to each other giving formation of nanomaterials. Thus it can be said that in a bottom up approach the building blocks are added onto the substrate to form the nanomaterials.

A bottom up approach involves two phenomena miniaturization of materials components (up to atomic level) self-assembly process giving rise to production of nanostructures. It may be emphasized that the physical forces operating at nano scale level are used to combine basic units into larger stable structures during self-assembly. Quantum dot production during epitaxial growth and synthesis of nanoparticles from colloidal dispersion are few examples of a bottom up approach.

Physical and chemical method for nanoparticles synthesis

Some physical and chemical methods, make nanoparticles interesting and special, generally used for synthesis of nanoparticles are as under:

- Chemical reduction, Sol gel technique, Laser ablation, Solve thermal synthesis and Inert gas condensation
- Biological Method for Synthesis of Metal Nanoparticles

As physical and chemical methods generally used for the synthesis of nanoparticles appear to be costly and economically non-viable, scientists are developing cheaper processes for development of nanoparticles using microorganisms which are available in nature. Biosynthesis of nanomaterials is largely unexplored area which requires in depth research. It is interesting to mention that nature is reservoir of a large number of microorganisms and phytochemicals useful for survival of whole universe and protecting of mankind. Nature has inherent capability to devise processes for the synthesis of nano and micro length scaled inorganic materials. This has opened a new vista of research before scientists and technologists. Biosynthesis of nanoparticles generally involves oxidation or reduction process like a kind of bottom up approach. In this process, the microbial enzymes/plant phytochemicals play important role in reduction of metal compounds in their respective nanoparticles.

Actually, microorganisms or phytochemicals bearing antioxidant or reducing potential are responsible in this process according to the scientific study conducted. The biosynthesis of nanoparticles should follow the principle of Green Chemistry. Main steps involved in the process are:

- The choice of the solvent medium used for the synthesis
- The choice of an environmentally benign reducing agent
- The choice of a nontoxic material for the stabilization of the nanoparticles

Since bio organism is involved in biosynthesis process, biosynthetic pathway for production of nanoparticles follows the principles of Green chemistry as bio organism used as reducing agent and capping agent is eco-friendly.

It is further added that chemical synthesis involves use of chemical compounds, which may be toxic in nature, absorb on the surface thereby producing adverse effects to human beings in disease conditions effecting disease diagnosis and medical treatment. Such type of condition does not arise in biosynthetic pathway for synthesizing nanoparticles due to its eco-friendly nature and bio competitiveness behavior for medical and pharmaceutical applications. Thus biosynthetic path way is advantageous over chemical methods for the production of nanomaterials. Biosynthesized nanoparticles being eco-friendly in nature does not produce any kind of adverse effects/reactions in medical applications.

Microbial synthesis

Microbial synthesis of nanoparticles involves application of microorganisms. This approach is eco-friendly in nature and is being extensively used for the synthesis of nanoparticles. Biological components like prokaryotes and eukaryotes are major source for microbial synthesis of nanoparticles. Several important biological processes in nature are governed by microbes. There is ample evidence to say that there is constant association between biological components and metals as well as non-metals existing on earth. Bacteria are said to be present abundantly in biosphere and any slight change in climate could adversely affect their life processes thereby affecting microbial synthesis of nanoparticles too. Microbial cells used for the production of nano sized materials have opened an eco-friendly approach for the production of metallic nanoparticles.

Even though the efforts which directed towards the biological synthesis of nano materials are new, the interaction between metals and microorganisms has been well documented as well as the ability of microbes to accumulate or extract metals are employed in biotechnological commercial processes such as bioremediation and bioleaching. Fungi and bacteria are known to synthesize inorganic metal nanoparticles either intra cellularly/extra cellularly [8].

Research has focused heavily on prokaryotes (*E.Coli*) as a means of synthesizing metallic nanoparticles. *E.Coli* is presented in abundant in our biosphere and it can adapt to extreme environmental conditions /survive in adverse environmental conditions. Therefore, bacteria have been considered to a best candidate for microbial synthesis of metal nanoparticles and allied studies. Bacteria has a tendency to grow fastly even in extreme environmental conditions. Bacteria can be easily manipulated and cultivated in our environment. Various biological factors like temperature, oxygenation, medium and time of incubation necessary for growth condition for bacteria can be controlled.

It has been observed that the change in pH of growth medium during incubation facilitate the biosynthesis of nanoparticles and nanoparticles of differing size and shape are obtained. Since nanoparticles have opened a wider application in industry, environment and health, suitable nanoparticles with varying sizes are required to meet the demand. This has necessitated for the proper control measure of growth conditions as mentioned above of bacteria for the production of nanomaterials in experimental/lab settings. Few examples of bacteria being used for the production of inorganic metal nanoparticles are given below:

Magneto tactic bacteria are used in synthesis of magnetic nanoparticles single layer bacteria are used to produce gypsum and calcium carbonate layers.

Further, the research studies have shown that some microorganisms have capability to survive at high concentration of metal ions because they have resistance showing ability towards metals. It has been documented that the mechanisms of metal resistance properties of microbes involve various biological processes including biosorption, alteration of toxicity and solubility via oxidation or reduction, efflux systems, bioaccumulation, precipitation or extra cellular complexation of metals as well as lack of specific transport systems of nanomaterials. One example of the microorganism showing metal resistance behaviour is Pseudomonas stutzeri AG 259. This microorganism can grow and survive even at high concentration of metal ions in silver mines have ability to produce silver nanoparticles.

Another example also suggested here for the production of gold nanoparticles via bacteria. It is an alternative method for gold nanoparticle synthesis. The bacterium Rhodopseudomonas capsulata have ability to synthesize gold nanoparticles extracellularly of respective size 10-20 nm and suggested that through the use of an enzyme NADH Dependant Reductase, these nanoparticles were produced. The NADH Dependant Reductase is an enzyme that has been shown in the past to be important in metal biosynthesis.

The microorganism, 'fungi' are also an alternative biological species for the production of metal nanoparticles. Fungus has ability to biosynthesize various metal oxide nanoparticles. The mechanisms of formation of nanoparticles employing fungi have been studied for very few nanoparticles and documented in scientific literature. Fungi have received significant interest for the production of metallic nanoparticles over bacteria due to the fact that fungi have certain advantages in comparison to bacteria. The ease of scaling up and downstream processing, the economic feasibility and the presence of mycelia offering an increased surface area, are important advantages to consider.

Mukherjee also suggested that because fungi *Aspergillus niger* secrete significantly higher amounts of proteins than bacteria, this would amplify the nanoparticle synthesis productivity. Although fungi can be used to synthesize nanoparticles having uniformity in their size and shape, fungi can also be helpful in producing nanoparticles with well-defined dimensions. Fungi have ability to secrete more amounts of proteins (such as glycosylated proteins) being responsible for producing large amount of nanoparticles [9].

Thus, fungi can serve as a rich source for producing nanoparticles in large quantities in comparison to bacteria. Few examples of them such as *Fusarium oxysporum* and *Aspergillus niger* have been largely studied by leading scientists with a view to generate inorganic metal nanoparticles. A lot of works have been done especially towards their silver nanomaterial creation. Pure silver nanoparticles (Ag NPs) were synthesized at 5-15 nm size range and it was informed that they were capped in order to stable them through proteins secretion of fungus. Although the most important advance in the fungal synthesis of organic nanomaterial was that *A. niger* and *F. oxysporum* produced these nanoparticles extracellularly, in contrast to all previous research which reported only the intracellular production of Ag and AuNPs.

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Instrumentation techniques

Some instrumentation techniques are highly useful for nanomaterials characterization such as Electron Microscopy and Optical Microscopy. These advanced microscopic techniques characterized nanomaterials on the basis of their morphology, size and surface charge. Electron microscopy includes Transmission electron microscopy and Scanning electron microscopy as well as Optical microscopy includes UV-Visible spectroscopy. UV-Visible spectroscopy is a highly useful technique which allows estimation of nanoparticles size, aggregation and concentration level.

Optical microscopy of UV visible spectrophotometry

UV-Visible Spectroscopy is a highly reliable and useful technique in the study of nanomaterials. This technique is used to characterize the nanoparticles formation and to monitor nanomaterial synthesis and its stability. In this method the desired reaction mixture/incubated culture medium is subjected for UV-Visible spectrophotometric measurements. Many molecules absorb ultraviolet/visible in this techniques.

When visible or ultraviolet light of certain frequency passed through the samples, the percentage of transmittance light radiations could be determined. This spectrophotometer analyses records the intensity of absorbance or Optical Density (OD) as a wavelength function. The absorption of the sample is directly proportional to the absorbing species concentration (Beer's law) [10].

It may be interesting to emphasize that unique optical properties are found in metal nanoparticles. Due to this unique property, metal nanoparticles have ability to interact with distinct wavelengths of light. Beside this, UV Vis spectroscopy is selective and sensitive for various types of nanoparticles and it is also fast, simple and easy to use. For the measurement of sample it requires only a short time period and finally particle characterization of colloidal suspensions does not require calibration. The valence band and conduction band of metal nanoparticles lie closer to each other where electrons can move freely. Movement of these free electrons gives rise to a surface Plasmon Resonance (SPR) absorption band, occurring because of the collective oscillation of metal nanoparticles electrons in resonance with light wave. The absorption of metal nanoparticles depends on the dielectric medium, chemical surroundings and size of particle. The observation of this peak assigned to the surface plasmon which is well documented for different metal nanoparticles with 2 to 100 nm size range.

Electron microscopy

Transmission Electron Microscope (TEM) as well as Scanning Electron Microscope (SEM) respectively, provides a way to observe nanoparticles directly, with the former method being better for morphological examination. In this method, structural information of a specific compound/molecule is easily obtained through electron differentiation. By this method, particles in even smaller size could be easily detected. In this way this method appears to be an improved validation of other methods.

Transmission electron microscopy

Transmission Electron Microscopy (TEM) is a valuable and frequently used technique and highly useful for the study of lattice image and size of the particle. This study mainly came in progress to know about the morphology and particle size distribution of nanomaterial's and being useful to obtain quantitative measures of size distribution, morphology and size of the particle. Beside this, TEM shows the crystal structure and shape of the particles.

In TEM there is an electron source at the uppermost top of the microscope, one meter long column is attached for vacuum, allows following down the electron. Electron gun, Electron lens, specimen and image forming system are different components of the microscope used for imaging. It has resolving power of 1 nm and provide 2D image of the sample. The magnification of TEM is mainly determined by the distance ratio between the objective lens and the specimen and the distance between its image plane and objective lens.

This method has two more advantages over SEM such as TEM has ability to provide good spatial resolution. It has capability to give additional analytical measurements of the sample. There are some disadvantages which include requirement of high vacuum and thin sample section. In addition, preparation of sample is a time consuming process and appears to be another vital aspect of transmission electron microscopy. In this technique, sample preparation is an important aspect of characterization with a view to obtain a potentially highest quality image.

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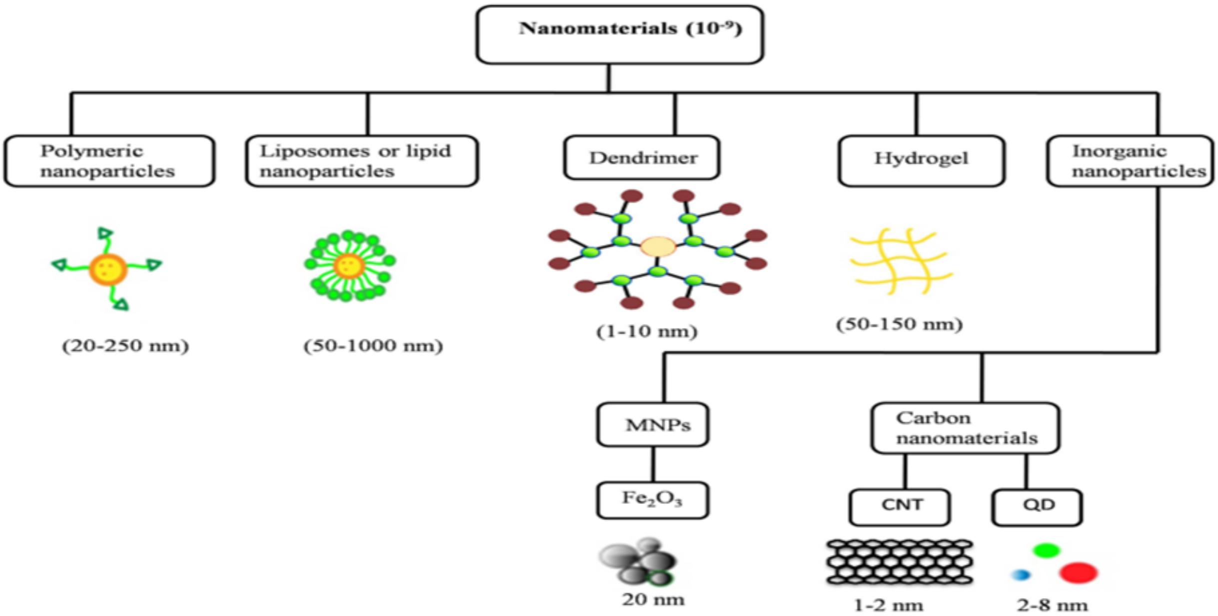
Scanning electron microscope

In this method thin beams of electrons are used which gets scattered along each successive point of the specimen. These scattered electrons are measured by detectors which surround the sample. When the sample is scanned the secondary electrons are collected by the detector and surface topography of the sample is obtained with display of an image. SEM is also useful to determine the purity as well as size and morphology of the sample. That's why we can say here that electron microscope also included Scanning Electron Microscope (SEM) (Figure 4) [11].

Figure 4. SEM with modern high resolution can distinguish the morphology of nanomaterials even below the 10 nm size level.



Because SEM is a kind of electron microscope, it images the sample surface by scanning it using a high energy beam of electron in a faster scan pattern. The complete information about the topography composition of the sample's surface as well as other properties can be obtained through SEM. Research studies have shown that when upcoming electrons interact with the sample's atoms it can generate the sample producing signals that inform about topography and also other characteristics such as electrical conductivity of the sample. SEM is a kind of surface imaging method among several electron microscopy techniques. This method is absolutely capable of resolving size distributions, various particle sizes, shapes and surface morphology of the newly synthesized materials at the micro as well as nanoscale levels. Moreover SEM with modern high resolution can distinguish the morphology of nanomaterials even below the 10 nm size level.

Antimicrobial agent

Scientists and technologists have been involved in conduct of advanced research in the field of nanomaterials in the last few decades owing to the applicability of materials at nanostructure level in different sectors of public utility. These continuous R and D efforts have created a strong base for nano science and nanotechnology in India and abroad. Nano science and nanotechnology has occupied a significant position in biomedical field. Now a day, Nano medicine sector has opened a more clinically beneficial subject for diagnosis and treatment of diseases.

In the present era of antibiotic resistance of pathogens, drugs/antibiotics available in our modern system of medicine do not prove to be effective in treating microbial diseases/infections.

Some metal nanoparticles can be used to treat microbial infections and work as an alternative to antibiotics. The distinct properties like small size and high surface to volume ratio present in metal nanoparticles are responsible for exhibiting bactericidal properties of metal nanoparticles. It has been observed that this distinct behavior allows nanomaterials to closely interact with microbial membranes not only because of the release of metallic ions in solution.

The cell membrane of bacteria covered with another outer membrane layer known as cell wall. It is significant for various lives processing as well as survival of bacteria. The cell wall of bacteria essentially made up of peptides and polysaccharides named peptidoglycan. Bacteria contain widely distributed two different kinds of cell wall ie., gram negative and gram positive.

When the reaction of bacterial cells occurs with the gram stain, the name of cell wall originated as gram negative and positive. Gram stain is a long employed test in order to classify the bacterial species. In case of Gram positive bacteria, the cell wall is made up of many layers of peptidoglycan which make it hard and thick. In contrast, the cell wall of gram negative bacteria is made up of some layers of peptidoglycan, make it thin. For example, the interaction of copper nanoparticles with gram negative bacteria, nanoparticle's surface directly affects the cell wall of bacteria resulted the rupture of membrane and killing of bacteria [13].

Metal nanoparticles have been developed and used to treat microbial infections for centuries. These metal nanoparticles have been proved to be safe and nontoxic. In addition few metal nanoparticles have been developed recently which exhibit significant antibacterial, antifungal and antiviral properties. These can be safely used as antibacterial, antiviral and antifungal agent in disease conditions. For example, Ag (silver) works as a biocide in suspension and solution but especially in nano particulate form it behaves as a potential antimicrobial agent. Ag nanoparticle is extensively being used in consumer and medical products also. Account for these properties, metal at their nano size level use in continuously increased number of medical and consumer products applications.

Since metal nanoparticles have been found to exhibit strong antimicrobial effect and have ability to kill bacteria causing infections resulting into generation of dreadful diseases, research has been focused in recent years towards developing metal nanoparticles as antimicrobial products and understanding their biochemical mechanism of action. Metal oxide nanoparticles with unique distinct behavior and properties have been found to show significant biocide property against Gram negative as well as Gram positive bacteria. However, no more reports are available to control some pathogenic bacteria isolated from environments using microbial mediated ZnO and $Pb(NO_3)_2$ metal nanoparticles.

Use of biological organisms such as few microorganisms including bacterial as well as fungal biomass could become an alternative to biological methods than physical and chemical methods for the development of nanostructured material in an eco-friendly way. Accordingly, the present study focused to determine the antimicrobial property of metal oxide nanoparticles synthesized by using few microorganism, against some pathogenic bacteria as *Pseudomonas aeruginosa* (gram negative) and *Staphylococcus aureus* (gram positive) bacteria.

Nano toxicology

Although recent development in nanotechnology resulting to development of variety of nanomaterials having diverse applications in daily life has attracted the interest of common people, much research work and scientific efforts are further required to meet the demand of public considering the changing scenario of society infrastructure, industrialization and global competitiveness requiring the need of new materials, whether it may be in health, medicine, industry, cosmetics etc to be used for the welfare of society and common people.

The extensive development of nanotechnology has given successful path in production of nanoparticles having applications in diverse areas like clinics, biomedicine, industry and cosmetics in general. It has been observed that the performance of products can increase when nanoparticles are incorporated in them but particles with nano sized structure can induce toxic effects or undesirable adverse effects too.

At the nano meter length scale, the reduced size of particle increases the number of various essential properties such as their surface atoms/molecules as well as their surface area exponentially. This will lead to complex bio physicochemical interactions when it exposed to physiological environments. Although nanoparticles exhibit unique significant beneficial properties, toxic effects of these nano products are also to be looked seriously. In this context, it may be further added that toxic effects of metal and metal containing nanoparticles have been studied and documented.

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It is interesting to emphasize that the minute/nanostructure level size of a particle plays significant role and appears to be responsible in exerting undesirable side effects/toxic effects by a particular nanomaterial as the particles with nanostructure size can easily penetrate the basic biological structures due to which normal physiological functions of biological structures are get disturbed allowing them to produce toxic effects such as tissue inflammation as well as altered cellular redox balance along with the increased production of Reactive Oxygen Species (ROS), causing abnormal function, tissue damage and cell death etc.

Experimental studies conducted on animal model and clinical studies on human subjects have clearly shown that inhaled small particles at nanostructure level can be removed less efficiently in comparison to larger particles through the mechanism of macrophage clearance in the lung which causes pulmonary damage. Further inhaled nanoparticles can also be trans located through lymphatic system, nervous system and circulatory system to most organs and tissues, including the brain [14].

Toxicity of metal nanoparticles

From a toxicological point of view many studies have been focused on nanomaterials especially on metal oxide nanoparticles. For example zinc oxide and lead nitrate nanoparticles have been considerably studied because these nanoparticles are frequently used in cosmetics, sunscreens and paints. ZnO nanoparticles when come in contact with skin have ability not to penetrate it, thus these nanostructured materials have been asserted not to possess a potential risk to public health when it applied on undamaged skin. However, metal oxide nanoparticles have semiconductor properties due to this reason the electronic and industrial applications have received much attention with other exposures routes as well as risks at hand.

Size of particles plays a key role in producing toxicity in a biological system. However, significant increases in solubility of metal oxide nanoparticles have also been reported with decreasing particle size. The solubility of a material also greatly influences the toxicity of different NPs. The dissolution rate is dependent on a particle's size, its chemical and surface properties, and is further influenced by the surrounding media. Reported that more soluble NPs like ZnO and $Pb(NO_3)_2$ shows higher acute toxicity than NPs of much low solubility.

ZnO NPs are used in paints, sunscreens, food packaging, solar cells, drug delivery and other therapeutics. The wide applications are because of its high optical transparency, large band gap, near UV emission and antimicrobial properties. The growing use of NPs has also enhanced the likelihood of exposure of NPs to humans and environment. Toxicity of ZnO nanoparticles have been reported in bacteria, human cells and also *in vivo* models. On entering the biological system, the toxicity of nanoparticles occurs due to their higher surface to volume ratio which provides an enhanced available area for physical and chemical interaction with the cells. The increase in reactive surface area is the reason for toxicological insult by the production of ROS and oxidative stress.

Lead is continuously to be of concerns to human health because it is used in ceramics fabrication and in metal field in different countries. At low concentration, the toxic effect of Pb causes several neurological, behavioral and metabolic disorders. In Nano toxicology, many research studies conducted on metal concentration indicated that even low concentration of lead level in human blood associated with diminished skeletal growth, impairment in the psychological progress and disturbances in the cardiovascular function. Lead poisoning results from the food ingestion or contamination of water with lead. It can also enter into the body through the inhalation of lead based paint, leaded petrol, soil and dust. Hence, to understand the nano lead chemical and physical properties and its toxicity compare to lead metal. Lead metal affecting very nearly on all organs as well as system in body. There is ample evidence to indicate that consumption of food and water contaminated with lead, occasional ingestion of contaminated soil, dust, or lead based paint have been found major causative factors for lead poisoning [15].

Since metal nanoparticles produce considerable toxic effects or undesirable adverse effects on biological systems/products during their manufacturing stage or even at their disposal stage, studies to understand the biochemical basis of toxicity of metal nanoparticles and its abatement measures are of great concern in the field of metal oxide nanoparticles. Different methods are available in order of toxicity assessment imposed through nanoparticles on to the various organisms. The methods for toxicity assessment can be categorized as *in vivo* and *in vitro*.

RESULTS

In vivo and *in vitro* toxicity assessment

In vivo tests are time consuming, expensive, and involve ethical issues. Investigated the reliability of in vitro systems at predicting the pulmonary toxicity *in vivo* manner of fine ZnO particles and ZnO nanoparticles in rats, and concluded that *in vitro* cell culturing systems do not precisely forecast the nanomaterial. Toxicologists have explored the impacts of a variety of nanomaterials in animal experiments. However, *in vivo* studies with nanomaterial's, unlike studies involving chemicals or compounds, are interlaced with many challenges. The *in vivo* dose used for experiments should be derived from the quantity of nanoparticles exposed in the actual scenario.

When *in vivo* treatment is given for any test substance, it should be ensured that the vehicle is isotonic and nontoxic, and that the nanoparticle is well dispersed in the vehicle. Since nanoparticles are very susceptible to agglomeration owing to their increased relative surface area, they may not form a stable suspension in the physiological solutions suitable for *in vivo* exposure. The poor dispersion of nanoparticles during *in vivo* exposure negatively affects their biological distribution and subsequent activity. Therefore, the results from such studies may be misleading and will differ from study to study.

In vitro experimentation has always been the first choice for toxicologists, since it is time and cost effective. Although it cannot replace animal experimentation completely, but it does help to ensure that they are only used when absolutely necessary, and it sometimes provides mechanistic information on the toxicity of nanoparticles after studies.

The risk assessments of different aspects of nanotechnology are still in its early stages. Therefore, most of the studies pertaining to nanoparticle toxicology that has been carried out so far have been preliminary and confined to the classical *in vitro* toxicity assessment methods established for drugs and chemicals. Due to these properties, nanoparticles interfere with normal test systems, and this interference has been well documented in the literature. Examples of such properties include: large surface area and leading to increased adsorption capacity; different optical properties that interfere with fluorescence or visible light absorption detection systems.

The study about *in vitro* toxicity assessment of nanoparticles was under taken to find out the recent deficient knowledge of the cellular response of nano sized particle exposure. The study evaluated the acute toxic effects of metallic oxide nanoparticles (ZnO, $Pb(NO_3)_2$) proposed for future use in industrial production methods using *in vitro* in human blood. The use of metal oxide nanoparticles are widely distributed towards the coating industry and paint and also in cosmetics, whereas the knowledge about their potential interactions with human being is necessary.

The aim of the study is to investigate the result about human health, when commercially available nanomaterials may affect various human blood cells. Various types of nanoparticles synthesized by biological methods were evaluated for their potential toxicity. It may be noted that some manufactured nanomaterials are redox active while some transport across cell membranes. These properties/actions of nanoparticles on biological system are of great concern towards the toxicity studies of these nanoparticles.

Peripheral blood mononuclear cell

Although inflammatory effects of nanoparticles (*in vitro* and *in vivo*) have been studied by leading scientists and results are well documented in scientific literature, there is little knowledge about the mechanism by which nanoparticles interact with human system and produce effects. Studies on cell lines which are reproducible and standardized assays have thrown some light in this direction. However, it is unclear how well these results translate to primary human cells.

Only some study has examined the effect of nanomaterial on human Peripheral Blood Mononuclear Cells (PBMC). A peripheral blood mononuclear cell is any peripheral blood cell having a round nucleus. These cells consist of lymphocytes (T cells, B cells, NK cells) and monocytes, in case of humans, the major part of the peripheral blood mononuclear cell population is made up of lymphocytes, followed by monocytes, and only a few percentages of dendritic cells. With the help of gradient centrifugation of whole blood, these peripheral blood mononuclear cells could be extracted. This centrifugation technique will separate the whole blood sample in to three distinct layer ie., plasma, a top layer, followed by a PBMCs layer and polymorph nuclear cells (such as neutrophils and eosinophil) and erythrocytes, a bottom fraction.

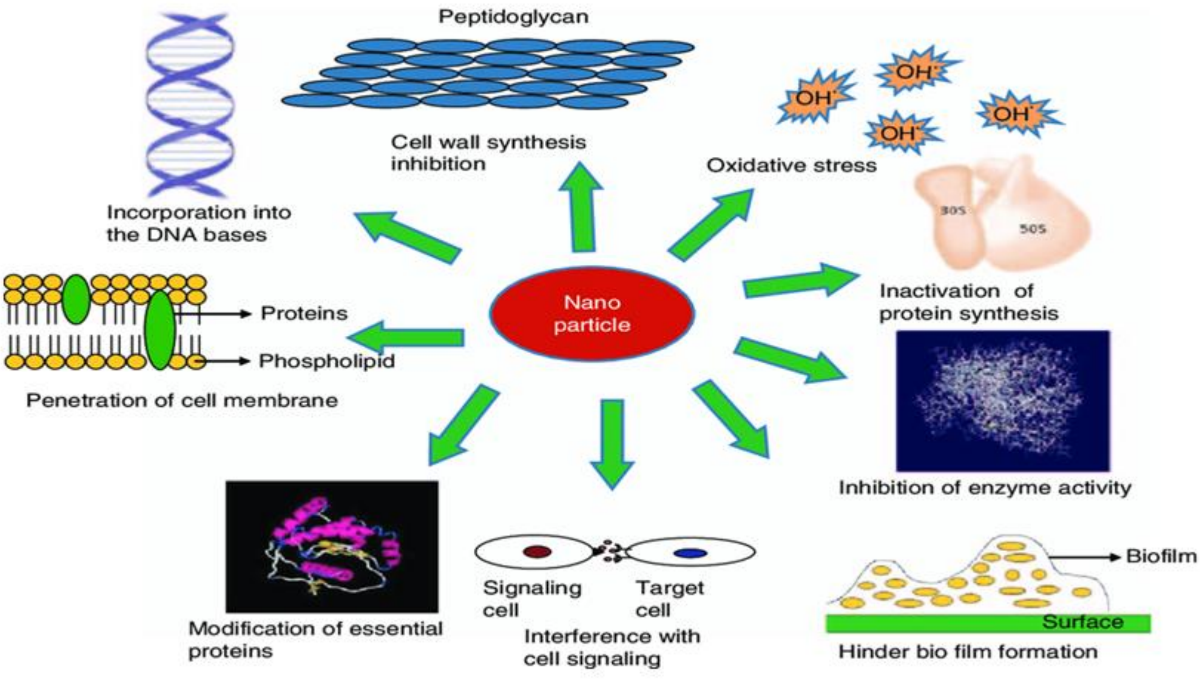
Cell viability

The toxic effect of nanoparticles is measured by applying biological technique "Cell viability". Many studies have been focused towards Carbon Nanoparticles/Nanotubes (CNTs). Among different nanoparticles carbon nanoparticles are frequently used for studying toxicity and assessment of viability/lethality of cells. Only few studies have been developed towards the toxicity assessment of metal oxide nanoparticles. In this present study, two different metal oxide nanoparticles have been exposed to human blood to examine their toxicity employing cell viability technique.

In the emerging field of nano science and technology, a significant area of research deals with the production of nanostructured material of various sizes, controlled monodispersity and different chemical compositions. The engineering and science of nano systems is one of the fastest growing and most challenging sectors of nanotechnology. Indeed, the shape controlling properties of nanoparticle is a current addition in to the demand list being made up of novel emerging production methods.

In view of sustainable development of society and making environment free from hazardous chemicals being used in helm of various human activity, there is a growing need to the development of environmentally benign production processes of nanoparticle that avoid the use of any toxic chemicals in to the synthesis protocol. Considering above observations in view the present study has been conducted towards synthesis of ZnO and $Pb(NO_3)_2$ nanoparticles, their characterization and antibacterial activity against few pathogenic bacteria. The study also deals with toxicological evaluation of these synthesized nanomaterials using peripheral blood mononuclear cell of human blood. It is hoped that the present study might be useful in developing novel nanomaterials having applications in important sectors such as health, medicine, environment and industry (Figure 5) [16].

Figure 5. Synthesis of ZnO and $Pb(NO_3)_2$ nanoparticles, their characterization and antibacterial activity against few pathogenic bacteria.



DISCUSSION

Metallic nanoparticles have great potential in many different industries. The need for a process to synthesize such nanoparticles in a reliable and easy way is becoming more pressing. Current physical and chemical methods involve high temperatures and toxic chemicals that are not only dangerous to the environment but costly too. Here performed biological systems have been investigated in an endeavour to provide are source efficient, sustainable and cheap method of production.

CONCLUSION

In summary, a concise overview of the microorganisms' uses such as bacteria and fungi to biosynthesize metal NPs has been described, with shake flask methods currently in vogue. The production of metal oxide nanoparticles through bacteria (*E.coli*) and fungus (*A.niger*) has a considerable potential over traditional methods of synthesis. The biological synthesis of nanomaterials, technology has to be scaled up to check the cost effectiveness. The process of synthesis is eco-friendly, rapid, followed biological approach mechanism. *E.coli* bacteria have a high growth rate and are relatively cheap to cultivate in comparison of other biological systems. Bacteria have few advantages over fungi because it can be manipulated easily, make them as the chassis of choice for the near term bio production of nanoparticles. Alternatively, fungus *A. niger* have the advantage of producing very high yields of secreted proteins, which may increase nanoparticle synthesis rate.

The microorganism fungi have mycelia which provide an increased surface area to fungus species in comparison of bacteria. Thus this increased area could be helpful to support the interaction of fungal reducing agent with metal ions and enhancing the conversion of ions to metal oxide nanoparticles. Fungi also have the advantage of ease of downstream processing when nanoparticles are produced, allowing for a more efficient way of extracting nanoparticles from them. Scalability, another factor for consideration in the content of commercial production of nanoparticles, gives fungi the edge as the chassis of choice in order of long term development as they can be used more easily in large scale reactors than bacteria. Since fungus *A.niger* has ability to secrete much larger amounts of proteins then bacteria *E.coli,* thereby considerably enhancing the productivity of biosynthetic approach of production and characterization of metal oxide nanoparticles.

Competing interests

A concise overview of the microorganism's uses such as bacteria and fungi to biosynthesize metal NPs has been described, with shake flask methods currently in vogue. The production of metal oxide nanoparticles through bacteria (*E.coli*) and fungus (*A.niger*) has a considerable potential over traditional methods of synthesis.

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REFERENCES

1. Guo Z, et al. Biosynthesis of gold nanoparticles using the bacteria Rhodopseudomonas capsulate. Mater Lett. 2007;61:3984–3987.
2. Binupriya AR, et al. Bioreduction of trivalent aurum to nano crystalline gold particles by active and inactive cells and cell free extract of Aspergillus oryzae, J Hazard Mater. 2010;177:539–545.
3. Yousaf SA, et al. 2008. Effect of heating environment on fluorine doped tin oxide thin films for solar cell applications. Int Nucl Informat system. 2011.
4. Valenti G, et al. Variable Doping Induces Mechanism Swapping in Electrogenerated Chemiluminescence of Ru (bpy)32+ Core−Shell Silica Nanoparticles. J Am Chem Soc. 2016;138(49):15935–15942.
5. Chaiti R, et al. Recent advances of metal–metal oxide nanocomposites and their tailored nanostructures in numerous catalytic applications. J Mater Chem. 2017;5: 9465 9487.

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6. Rudge S, et al. Adsorption and desorption of chemotherapeutic drugs from a magnetically targeted carrier. J Control Release. 2001;74:335–340.
7. Zhao G, et al. Multiple parameters for the comprehensive evaluation of the susceptibility of Escherichia coli to the silver ion. Biometals. 1998;11:27 34.
8. Kruis FE, et al. Sintering and evaporation characteristics of gas phase synthesis of size selected PbS nanoparticles, Mater Sci Eng B. 2000;69:329 334.
9. Han M, et al. Quantum dot tagged micro beads for multiplexed optical coding of biomolecules. Nat Biot. 2001;19:631–635.
10. Bharde A, et al. Bacterial Aerobic Synthesis of Nanocrystalline Magnetite Nanoscience Group, Materials Chemistry Division. J Am Chem Soc. 2005;127(26):9326–9327.
11. Slawson R, et al. Silver accumulation and resistance in Pseudomonas stutzeri. Arch Microbiol. 2004:158 (6): 398–404.
12. Johnston C, et al. Gold biomineralization by a metallophore from a gold associated microbe, Nat Chem Biol. 2013;9:241 243..
13. Ahmad A, et al. Fungus Mediated Syntheses of Silver Nanoparticles and Their Immobilizaation in the Mycelial Matrix. Nano Letters. 2001;1:515 519.
14. Korbekandi H, et al. Optimization of biological synthesis of silver nanoparticles using Fusarium oxysporum. Iran J Pharm Res. 2013;12:289–298.
15. Sapsford KE, et al. Analyzing nanomaterial bioconjugates: A review of current and emerging purification and characterization techniques. Anal Chem. 2011;83:4453–4488.
16. Sastry M, et al. pH Dependent changes in the optical properties of carboxylic acid derivatized silver colloidal particles. Colloids Surf A Physicochem Eng Asp. 1997;127:221–228.