THINK INDIA JOURNAL

ISSN:0971-1260
Vol-22-Issue-37-December -2019

MICROBIAL BIOSYNTHESIS OF NANOPARTICLES: A REVIEW

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Abstract:

Nanomaterials and nanoparticles have gained enormous prominence in technological advancements due to their tuneable physical, chemical and biological properties with much enhanced performance over their bulk counterparts because of their unique shape, size and structure. Biological method of nanoparticle synthesis is one of the most prominent as well as cheap, eco-friendly and reliable method against the other physical and chemical methods. The ever-increasing demand in various fields as biomedical, food and agriculture, industrial and environment sectors have forced the synthesis and characterization of wide variety of nanoparticles by using list of biological systems as bacteria, fungi, plant. The present review is designed in order to compile the studies related to produce nanoparticles using bacterial system and its various aspects as characterization, biocompatibility as well as toxicity caused due to nanocomposites. Moreover, this paper systematically examines the recent developments among the different synthesis methods and their uniqueness with respect to type of bacteria and chemicals involved. This paper will contribute in understanding basic mechanism of nanoparticle synthesis using bacteria as a factories and further sustainable research aiming at greater potential applications of environmentally friendly nanomaterials in much healthier way.

Keywords: *Nanoparticles, Green synthesis, Bacteria mediated synthesis, Biocompatibility*

INTRODUCTION

The world we live in today relies very heavily on the development of processes and techniques for the betterment of mankind. In this aspect, nanotechnology has made much advancement and has revolutionized the way we approach our day to day tasks. Having very unique physical and chemical characteristics as a consequence of their size, distribution and morphology, it is defined as the manipulation of matter ranging from 0.1 to 100 nm in size.

In 1959, Richard Feynman presented the nanotechnology and is introduced by Norio Taniguchi, a Professor from Tokyo Science University [1]. The most widely growing field of research due to its applications in multiple fields like biomedical, agriculture, physical science, material science, chemical science, electric fields and optics. Applications range

ISSN:0971-1260
Vol-22-Issue-37-December -2019

from efficient consumption of energy, cleaning the environment and increasing sustainability, to even solving major health concerns across the world.

The basic structure of nanoparticles consists of the *surface layer* - outermost layer functionalized by small metal ions, surfactants or polymer, the *shell layer* - different portion between the outer layer and inner core; *the core* - central portion of any nanoparticle having integrity of density and whole mass [2]. On the basis of their availability and production force i.e. origin, these nanoparticles are divided into three broader categories i.e.

- *Incidental nanoparticles*: produced accidentally and incidentally by welding fumes, combustion process, vehicle engine exhaust and natural phenomenon as lightning, forest fire.
- *Natural nanoparticles*: produced in human bodies, plants, animals and insects.
- *Engineered nanoparticles*: produced by humans under unique conditions for desired specificity and property.

However, the incidental and natural nanoparticles are continuously being formed in the atmosphere, oceans, biological systems but lack stability and specificity to be used as commercial and productive scale [3]

There are several types of nanoparticles based on physical and chemical properties as, metal-based, carbon-based, lipid-based, non-metal-based, polymeric nanoparticles, ceramic and semiconductor nanoparticles are some of them. The nanoparticle synthesized can be zero-dimensional (as nanodots), one dimensional (as graphene), two dimensional (as carbon nanotubes) and three dimensional (as gold nanoparticles). The shape of nanoparticles can be regular like flat, spherical, conical, cylindrical, spiral, tubular, hollow-core, etc. or irregular and size differs from 1 to 100 nm. Due to small size, the nanoparticles show enhanced properties such as greater surface area, stability, high reactivity, sensitivity, strength, etc. [4]

Out of three methods to produce nanoparticles, biological method of nanoparticle synthesis has more advantage over nanoparticles developed by physical or chemical methods because of its cost-effectiveness and eco-friendly nature. The biological method of nanoparticle synthesis involves a number of organisms as bacteria, fungi, plants and animals. But, because of easy manipulation and scale-up, a bacterial system of nanoparticle synthesis is one of the simplest and easiest studied methods. The ability of a microbe to adapt to any harsh/extreme conditions makes them suitable candidates for nanoparticle synthesis. Microbes utilize its enzymes to synthesize nanoparticles having a higher surface area, enhanced catalytic property and improved contacts between metal and respective salt [5].

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The characterization of these nanoparticles shows the effect of controlled synthesis on shape, size and structure by controlling the precursor concentration, temperature and strains of microbes used [6] Magnetic nanoparticles, as well as gold and silver nanoparticles, derived from bacterial strains, are widely used as drug delivery systems, antibacterial, superparamagnetic material, providing biocompatibility and biodegradability at a low price [7].

The following review paper goes in studying the bacterial mediated synthesis of nanoparticles and the conditions & necessary factors affecting their relative properties with respect to their specific characterization methods and tools involved. The study also touches the aspects regarding the biocompatibility of the bacterial mediated nanoparticles and their relative applications among various fields focusing on the methods to fabricate & design materials using these nanoparticles. The paper also tries to give some relative irony about the use of nanocomposites with respect to its toxicity to organism and environment, and the future aspects of nanoparticles and nanotechnology for the welfare of mankind.

CLASSIFICATION AND TYPES OF NANOPARTICLES

The nanoparticles may be classified based on their chemical as well as physical properties i.e. organic and inorganic nanoparticles. Inorganic nanoparticles include semiconductor, magnetic, ceramic nanoparticles and organic nanoparticles include carbon, carbon-based and lipid-based nanoparticles. Among all these two categories, a list of nanoparticles falls based upon their shape, size, morphology, and structure, dimensions, origin and physical/chemical property. Some of their types are the following:

Metal-based nanoparticles:

Having made up of metal precursors, these kinds of nanoparticles exhibit very significant optoelectrical properties due to their localized surface plasmon resonance characteristics. Alkali, as well as alkaline earth metal and transition metals such as Ag, Au and Cu, have large adsorption band of the electromagnetic solar spectrum in the visible zone. The controlled synthesis of these nanoparticles has enormous application in various fields. Gold nanoparticles are used to enhance the electron streaming to obtain good quality scanning electron micrographs, and many more [8].

Carbon-based nanoparticles:

The two major classes of carbon-based nanoparticles are fullerenes and carbon nanotubes (CNTs). Fullerenes which are made up of allotropic forms of carbon atoms

ISSN:0971-1260
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(spherical hollow cage), are arranged in a pentagonal and hexagonal carbon unit and are known for electrical conductivity, high strength, arrangement, electron affinity, and adaptability. Fullerenes are made up of C-60 and C-70, each with a respective diameter of 7.114 nm and 7.648 nm. Carbon nanotubes have an elongated, tubular structure and the size ranges between 1–2 nm in diameter. More similarity to graphite rolled sheet, carbon nanotubes can behave as both conductor as well as semiconductor depending upon the diameter of the arrangements. They are used in the pristine form as well as in nanocomposite form for any commercial purposes such as efficient gas adsorbent, filters and support medium [8].

Lipid-based nanoparticles:

These are effectively used in biomedical applications and it consists of lipid moieties. The diameter usually ranges from 10 to 100 nm and generally spherical in shape containing a lipid core and soluble lipophilic molecules as the matrix. Surfactants or emulsifiers stabilize the external core of the nanoparticles. A very unique and special field of nanotechnology, known as Lipid Nanotechnology designs and synthesizes lipid-based nanoparticles. A wide distribution of applications ranges from drug delivery to RNA release in cancer therapy and drug carrier [8].

Polymeric nanoparticles:

These are organic nanoparticles. The shape of the polymeric nanoparticles is typically nanospheres or nano-capsular and size range from 10 nm to 1µm. Nanosphere has a matrix system which disperses the drug whereas, in the nano capsule drug is incorporated into the cavity. These types of nanoscale molecules are having a solid core which is made up of matrix particles and at the outer boundary of the spherical surface, other molecules are adsorbed [9]. Wide range of applications involves the use in drug delivery; carriers for drugs, sensor, medicine and pollution control [10].

Semi-conductor nanoparticles:

Semi-conductors are the connecting link between metal and non-metal, and due to this property, it has many applications. It has broad bandgaps; allowing nanoparticles to alter their property with the help of bandgaps tuning. Due to their respective band-gap and band edge positions, various semi-conductor nanoparticles are being used in photo optics, electronic device and photo-catalysis [8].

Ceramic nanoparticles:

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These are inorganic non-metallic solid nanoparticles that are synthesized with the help of continuous heating and subsequent cooling. They exist in amorphous, porous polycrystalline, hollow or dense forms having a size less than 50 nm [11]. This type of nanoparticles is having wide application in photo-degradation of dyes, photo-catalysis, and imaging applications [12]. Aluminium oxide and titanium dioxide nanoparticles are used for the design of nanocarriers [13].

NANOPARTICLE SYNTHESIS

There are different methods and aspects to synthesize a nanoparticle. These aspects are- low cost, having a neutral pH, and environment-friendly nature.

Approaches for nanoparticles synthesis:

There are two basic approaches for the nanoparticle synthesis i.e. Bottom-up approach and Top-down approach.

Bottom-up approach: In this method the smaller components are arranged into complex assemblies, i.e. atom by atom or molecule by molecule. Nanoparticle deposition atom by atom leads to the formation of self-assembled atoms/molecules and subsequent clusters, hence forming a unilayer on the substrate surface. The method uses a chemical/physical force working at the nanoscale in order to assemble the basic or smaller units into complex and larger structures.

Top-down approach: This method seeks to slice down or depend on the continuous cutting of bulk material until a nanosized particle is achieved. Various metal and non-metal-based nanoparticles can be produced using this approach.

Non-conventional and Conventional method of synthesis: Over the years, two basic methods of nanoparticle synthesis have been evolved mainly because of their effectiveness and ability to be controlled with respect to the physical and chemical property of nanoparticles thus obtained.

(A) Non-conventional Method: Microfluidic Reactor-Based Synthesis

A microfluidic reactor is used for benchtop material fabrication. It is a miniaturized reactor and helps in the non-conventional nanoparticle synthesis [14]. Nanoparticle synthesis is performed in microreactors which are having a diameter smaller than 1 mm. Microreactors are tubular in design or more commonly lab-on-chip design. Chips are made up of polymers, such as PDMS (Polydimethylsiloxane) or glass. Microfluidic devices have an advantage over

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mass production in terms of reaction yield, improved size and shape distribution; it also helps in the synthesis of various non-spherical particles [15].

As a microfluidic reactor mixes the reactants at the microscale level, viscosity is the key factor affecting flow rather than inertial forces. In a microfluidic environment, the major mixing occurs through diffusion and laminar flow [16]. For the development of nanoparticles, the reaction temperature is tightly controlled inside the microfluidic reactor. Reducing agents and metal salts at low temperatures (15–20°C) are used for controlling the reaction in a microchannel of the reactor. The synthesis of nanoparticles occurs at much higher temperature i.e. 80–90°C.

As compared with a classical macroscale synthesis of nanoparticles, Microfluidic reactors use small reagent volumes and offer selectivity, environmental friendliness, short reaction time, a small footprint, and improved safety. ZnO nanoparticles have been synthesized using a microfluidic reactor [17].

(B) Conventional Methods

Physically slicing or cutting bulk materials into nano-size is the top-down approach, on the other hand, the bottom-up approach involves the uses of atoms and molecules for fabrication of nanostructures through chemical or biological synthesis. Biological synthesis is also referred to as 'green synthesis' [18].

(i) Physical Methods of nanoparticle synthesis

The physical method of nanoparticle synthesis includes technique as high energy ball milling, arc plasma, thermal evaporation, physical vapour deposition, ultrasonic irradiation, and laser ablation. These physical methods are chemically pure and technically simple; this makes them be used at industrial scale at high production rates. Arc plasma is based upon electrical arc discharge synthesis. It is commonly used for converting bulk materials into nanomaterials via condensation and evaporation [19].

Using thermal evaporation, ZnO thin films and nano ZnO rods were synthesized [20]. Use of ultrasonic irradiation for 75 to 270 min for the synthesis of histidine (capping agent) based ZnO nanoparticles.

(ii) Chemical Methods of nanoparticles synthesis

Microemulsion, sol-gel, precipitation, hydrothermal, solvothermal, and chemical vapour deposition comes under the chemical method of nanoparticles synthesis. Wet-

ISSN:0971-1260
Vol-22-Issue-37-December -2019

chemical synthesis method, based on the physical states of the solid and liquid phases, a commonly used method for nanoparticles synthesis. For controlling the particle size and to prevent agglomeration at industrial-scale, capping agents/stabilizers such as triethylamine (TEA), oleic acid, thioglycerol, and polyethene glycol are used widely in spite of their toxicity.

Using sol-gel synthesis, dodecyl amine (DDA)-capped ZnO nanocrystals was created which is having a low surface density of (25%) due to the hydroxide groups (protons) on the surface of the ZnO nanoparticles. Colloidal suspension (colloid) with dispersed nanoparticles in a suitable solvent is prepared when metal chlorides and metal alkoxides are used as precursors which are made to undergo poly-condensation and hydrolysis reactions. Towards the formation of a continuous inorganic network that contains a liquid phase (gel), the sol is produced.

Metal-oxo or metal-hydroxy polymers are generated in solution when the metal centres connect with oxo (M-O-M) or hydroxy (M-OH-M) bridges leading to the formation of metal oxide. Chemical vapour deposition is a simple yet effective method for the synthesis of photoluminescent ZnO nanoparticles [21].

(iii)Biological Methods of nanoparticles synthesis

A promising cost –effective eco-friendly, safer and rapid alternative to physical and chemical synthesis of nanoparticles is the biological synthesis methods. Studies have been extensively done on microorganism (bacteria, fungi, algae, yeast and phage), DNA, proteins and plant extract for the synthesis of nanoparticles.

The biological entities produce nanoparticles by generating some secondary metabolites. These molecules i.e. secondary metabolites play a vital role in synthesizing and stabilizing the nanoparticle. The extract from these biological organisms can be used to synthesize different sized nanoparticles and with the different chemical composition [22].

There are three approaches for utilizing a biological method for nanoparticle synthesis-1. Using microbes like fungi, yeasts, actinomycetes, bacteria 2. Using extracts from plants and enzymes of plant origin 3. Use of templates such as DNA or membranes.

(iv) Microorganisms Assisted Biogenesis:

Microbes, when in contact with metals, interact with them through their membrane and form nanoparticles. The interaction between the cell and the metal is considered complex

ISSN:0971-1260
Vol-22-Issue-37-December -2019

and yet to be understood completely. Certain microbes separate metal ions by a complex phenomenon which is yet to be understood.

Bacteria such as *Bacillus, Staphylococcus, Sphingobacterium, Halomonas* have been used to synthesize ZnO nanoparticles of diameter 10-95 nm, rod or cubic in shape, multiform, triangular in shape. This ZnO nanoparticle is known for its antimicrobial agents. *Pseudomonas stuzeri Ag259* (Silver mines bacteria) have the capability of accumulating silver both inside and outside of their cell walls. Therefore, intracellular synthesis of different types of silver nanoparticles of various shapes and size ranging around <200nm can be synthesized. *Bacillus cereus* is used to synthesize spherical silver nanoparticles with a diameter of 20-40 nm [23].

Some typical nanoparticles produced by microbes are metallic nanoparticles (gold, silver, alloy, etc), oxide nanoparticles (magnetic and nonmagnetic oxides nanoparticle), sulphide nanoparticles. In addition to those, some other types of nanoparticles are synthesized by microbes like $PbCO_3$, $CdCO_3$, $SrCO_3$, PHB, $Zn_3(PO_4)_2$ [24].

(iv) Plant Extract Assisted Biogenesis:

Because of the presence of the components such as flavonoids, terpenoids and polysaccharides, plant extracts are very well used for the synthesis of various nanoparticles. Chemical compounds like taxol and gibberellins are secreted and extracted from the plant under getting associated with fungus and therefore, the exchange of genes between different species i.e., fungus and plant leads to the generation of different shapes and sizes of nanoparticles.

ZnO nanoparticles and ZnO-Ag nanocomposites have been synthesized using plant extract of *Calotropis Procera, Matricaria chamomilla, Thymus vulgaris, Pelargonium graveolens,* etc.

(v) Bio-Template Assisted Biogenesis:

Using DNA, CdS or any other sulphide nanoparticles can be developed. DNA has the ability to bind to the growing nanoparticle surface. The double-stranded DNA mixed with the proper precursor (cadmium acetate), desired medium-salt and reaction is made to carry out by flowing inert gas like N_2 simultaneously adding Na_2S lead to the synthesis of nanoparticles.

CdS nanoparticles having a size less than 10 nm can be obtained depending on the concentrations of DNA, sodium chloride and cadmium acetate. DNA being negative in charge (due to phosphate group) bonds with the Cd^+ nanoparticle surface which is positively

ISSN:0971-1260
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charged. The same principle can be applied for the utilization of other biological templates as proteins, fats, lipids, carbohydrates, RNA, metabolites for the nanoparticle synthesis.

BACTERIA MEDIATED NANOPARTICLE SYNTHESIS

Bacteria have a significant tendency and ability to interact with and reduce heavy metal ions and hence it becomes one of the most desirable sources for nanoparticle development. In order to persist in an environment, where there is a presence of high level of metals, some bacterial species have evolved various mechanisms to get adapted to them. By modifying or reforming the chemical aspect of the toxic heavy metal rendering it, no longer toxic, results in the synthesis of the nanoparticle of the concerned metal.

On the basis of recent studies, some of the bacteria that are producing nanoparticles belonging to the family **Enterobacteriaceae, Pseudomonadaceae, Micrococcaceae, Bacillaceae, Staphylococcaceae** are listed below-

Name of Organism	Nanoparticle produced
Rhodococcus sp.	Au
Escherichia coli	Pd, Pt
Rhodopseudomonas capsulata	Au
Pseudomonas aeruginosa	Au
Bacillus licheniformis	Ag
Bacillus sphaericus JG-A12	U, Cu, Pb, Al, Cd
Bacillus sp.	Ag
Klebsiella pneumonia	Ag
Escherichia coli	Ag
Enterobacter cloacae	Ag
Lactobacillus sp.	Ag
Enterococcus faecium	Ag

Table 1: Different bacteria and the nanoparticle they produce

The resistant mechanism which is generated against a specific metal leads to the formation of nanoparticle as a by-product and further can be used as a substitute method of nanoparticle production. It was observed by researchers that some bacteria could survive even in very high concentration of metal ion (e.g. *Pseudomonas aeruginosa* and *Pseudomonas stutzeri*) and have the ability to synthesize nanoparticles by intracellular (Pseudomonas, Corynebacterium, Lactobacillus, Bacillus species) as well as extracellular (Pseudomonas and Bacillus species) mechanism. The recent found environment-friendly

ISSN:0971-1260
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nano factories are the microorganisms especially bacteria, because of inexpensive cultivation, easy manipulation, fast grower and easily controllable growth parameters as, pH, temperature, gaseous exchange and media concentration [25].

The nanoparticle of different shape and size produced as a result of changing pH of the growth medium is one of the very important and prime parameters which must be needed to be controlled as size variations among nanoparticles are important for various applications such as catalyst, optics, or anti-microbial [26]. Due to their abundant availability in the environment, their ability to acclimatize to a given environment and their novel ability to reduce toxic compounds and bioremediate them, microbes are usually the best option for environmental research.

In recent researches, it was observed that *Bacillus licheniformis* produces intercellular silver nanoparticles. Colour of the medium changed to dark brown after the addition of silver ions showing the presence of silver nanoparticles. Kalimuthu et al. demonstrated the experimental design for the nanoparticle synthesis; they illustrated that the silver nanoparticles were synthesized intracellularly and were quite dispersed in solution. *Bacillus species* produced nanoparticles when it is sub-cultured in the medium containing with $AgNO_3$. *Bacillus licheniformis* can be used at an industrial scale as it takes only 24 hours for the nanoparticle synthesis, i.e. the high production rate [27].

Another group of scientists added metal ions to the supernatant that was collected by centrifuging the culture of *Enterobacteria* and tested the ability to create metallic nanoparticles using the supernatant. They were able to synthesize extracellular silver nanoparticles in only 5 minutes. Because of the simple downstream processing and increased production rate, extracellular synthesis of nanoparticles is much preferred over intracellular [28].

A variety of Ag^+ ions reducing microbes have been identified for the synthesis of Ag nanoparticles; *Pseudomonas stutzeri* that is isolated from silver mine are tested for the nanoparticle synthesis. *P. stutzeri,* when placed in a concentrated silver nitrate solution, plays an important role in reduction of Ag^+ ions to form Ag nanoparticles of a specific size and topography [29].

Recent studies have been done on *E. coli* for the synthesis of Ag nanoparticles because of its low generation time and easy cultivation. 1mM of $AgNO_3$ is inoculated over *E. coli* culture, lead to the Ag nanoparticles formation in only 10 minutes. The colour changes from yellow to reddish-brown, confirming the synthesis and presence of silver nanoparticles.

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The intensity of the colour is directly proportional to nanoparticle production and efficacy of produced silver nanoparticle can be tested based upon the zone of inhibition (MIC) [30].

Strains of *Bacillus as* (*B. subtilis, B. amyloliquefaciens, B. cecembensis, B. incidus*) have been studied for the nanoparticle synthesis. The cellular extract of *B. amyloliquefaciens* and silver nitrate helps in the production of circular and triangular crystalline Ag nanoparticles, which showed antimicrobial activity. The properties of nanoparticles developed depend upon the extract concentration, light intensity, addition of sodium chloride [31].

The cellular extract of *E. coli and Enterobacter cloacae*, when used for the Ag nanoparticle synthesis, took only 5 minutes for the nanoparticles to be formed after Ag^+ ions were in contact with cellular filtrate. Even very low concentrations of metal ions (Au^+, Ag^+) can be converted to metal nanoparticles with the help of Lactobacillus strain present in buttermilk. *Enterobacterer sp.* has been used for the synthesis of mercury nanoparticles. A pH of 8.0 and a low concentration of mercury precursor uphold the synthesis of uniform-sized, spherical, mono-dispersed and intracellular Hg nanoparticles.

Magnetotactic bacteria (*Magnetospirillum magnetotacticum*) synthesize iron oxide, iron sulphide or both nanoparticles intracellularly. *Klebsiella pneumoniae* was analysed for its ability to produce Selenium nanoparticles by utilizing Selenium chloride. The highest reduction ability was seen on tryptic soy agar (TSA). The nanoparticles synthesized from *K. pneumoniae* was in the range of 100 to 550 nm. For the reduction of selenite to elemental selenium, *E. coli* and *P. stutzeri* can also be used [32].

Clostridium thermoaceticum have the tendency to precipitate CdS on the surface of the cell and also in the medium using $CaCl_2$ when cysteine hydrochloride is present, where cysteine is the sulfur source. *Klebsiella pneumoniae* if interacted with calcium ions in the medium it will synthesize CdS nanoparticles of 20-200 nm size on the cell surface. *E. coli* if incubated with $CdCl_2$ and Na_2SO_4 will produce CdS nanoparticles intracellularly [33].

Owing to the high enzyme production and non-pathogenic nature, *Lactic acid bacteria* (LAB) is preferred for the synthesis of ZnO nanoparticles. The electro-kinetic potential in these bacterial systems helps them to attract heavy metal ions for nanoparticle synthesis by both oxidation and reduction processes. The thick peptidoglycan layer of cell wall acts as a site for bio-reduction and biosorption. Crystalline, spherical and highly pure ZnO nanoparticles with size ranging from 7-19 nm are synthesized by *Lactobacillus plantarum* [34].

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Gold nanoparticles of size ranging between 15-30 nm, can be synthesized using *E. coli* as the bacterial system. The size and shape were not homogenous and most of them were spherical to few triangles as well. Members of *Bacillus sp. (B. subtilis, B. megaterium)* and *Rhodopseudomonas capsulata* also contribute to Au nanoparticle synthesis of by a reduction process [25].

MECHANISM OF NANOPARTICLE SYNTHESIS BY BACTERIAL SYSTEM

Experiments have proven that bacteria produce various biomolecules, enzymes, protein and other that play a vital role in the reduction process of compounds to form nanoparticles. These biomolecules attribute to the synthesis of mono and poly-dispersed nanoparticles of different shape, size, structure, complexity and distributions. The stability of nanoparticle is defined by a protein secreted by bacteria which acts as a capping agent.

Not all bacteria produce nanoparticles as the production depends upon the metabolic processes and enzymatic activity of bacteria. Thus, for the synthesis of nanoparticle, selection of suitable bacterial system is very crucial. The basic mechanism for synthesis states that; in order to survive in a high concentration of toxic metal or salts, bacteria develop a resistance against the metallic ions in the environment and are responsible for its reduction to a nanoscale level resulting from the nanoparticle synthesis of respective metal or precursor.

Bacteria produce nanoparticles either by intracellular or extracellular mechanism;

Intracellular mechanism of bacterial nanoparticles synthesis

In the intracellular pathway of nanoparticle synthesis, the cell wall of bacteria and charged ions play an inevitable role. Intracellular mechanism of nanoparticle synthesis involves transportation of the metal ions on the bacterial cell with the help of certain enzymes and coenzymes.

The cell wall of bacteria has an active site for binding of metal ions. Evidence has shown that a high concentration of metal ions and heavy metals exhibit more threat to the microbes, which will increase the affinity of bacteria towards ions by gripping and trapping the ions due to electrostatic interactions. The reason behind this interaction is the presence of negative charge on bacterial cell wall (due to carboxylate group of polypeptides, cysteine, and specific enzymes) that attracts the metal ions [35]

Furthermore, NADH-dependent reductase acts as an electron carrier, transfers the electron which helps in reduction of trapped ions into elemental atoms. NADH-dependent reductase is embedded in the cell membrane. As a result, the nuclei develop to form

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nanoparticles and accumulate them in the cytoplasm or in the periplasmic space. The proteins and amino acids (cysteine, tryptophan and tyrosine) that naturally exist inside the bacterial cells are stabilizes the newly synthesized nanoparticles [36].

A group of researchers demonstrated the intracellular mechanism of nanoparticle synthesis involving three steps i.e. trapping, bio-reduction and capping. The presence of metal nanoparticles inside the cell is confirmed by TEM analysis.

Some of the *Bacillus sp.* and *Rhodococcus sp.* produces intracellular Au and Ag nanoparticles of size 5-15 nm by the reduction process. *Bacillus subtilis* has the potential to reduce Au^{3+} ions to nanoparticles that are octahedral ranging between 5-25 nm size intracellularly [36].

Extracellular mechanism of nanoparticle synthesis by bacteria

The number of studies has shown that extracellular nanoparticle synthesis and metal ions reduction to form nanoparticles is due to the nitrate reductase complex. Extracellular synthesis is an enzyme-mediated synthesis whereas, these enzymes may be located on the plasma membrane or released into the growth medium as an extracellular enzyme.

The conversion of nitrate to nitrite is catalysed by the enzyme nitrate reductase. For example, the first step in the extracellular synthesis is bio-reduction of metal ions which is initiated by transfer of electrons by NADH and NADH-dependent reductase that acts as an electron carrier. This leads to the reduction of Zn^{2+} to Zn^{0}. After this, nucleation of Zn^{0} occurs and they get aggregated. Finally, they are capped and stabilized with a capping agent [37].

Species like *E. coli, B. licheniformis, P. aeruginosa, Rhodopseudomonas capsulate, Enterobacter cloacae, Lactobacillus garvieae* are already explored for the synthesis of Gold and silver nanoparticles production extracellularly.

Microorganism	**Nature of Organism**	**Nanoparticles**	**Size/Shape**	**Application**
Micrococcus yunnanensis	Gram +	Au	53.8 nm/ Spherical	Antibacterial, Anticancer
Myobacterium sp.	Gram +	Au	5-55 nm/ Spherical	Anticancer
Escherichia coli	Gram -	Cd & Te	2-3.2 nm/Round	Antibacterial activity
Escherichia coli	Gram -	Au	20-30 nm/Hexagonal, Triangle	Antibacterial activity

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Bacillus licheniformis	Gram + mesophilic	Ag	50 nm/NA	Antimicrobial activity
Corynebacterium glutamicum	Gram +	Ag	5-50 nm/Irregular	Antimicrobial activity
Rhodopseudomonas capsulate	Phototrophic	Au	10-20 nm/Round	Antibacterial activity
Pseudomonas aeruginosa	Gram -	Au	15-30 nm/NA	Antibacterial activity

Table 2: Different types of strains and their respective nanoparticles and characteristics

FACTORS AFFECTING THE BIOSYNTHESIS OF METALLIC NANOPARTICLES

In the case of bacterial synthesis of nanoparticles, it is important to note that each bacterial family and the species within it have slightly varying methods of producing Nanoparticles. While the general procedure may remain the same, there are certain factors that affect not only the production method and efficiency but also the characteristics and properties of the produced nanoparticles themselves.

The major bacterial groups that are looked at for nanoparticle biosynthesis include *Lactobacillus sp.*, *Bacillus sp.*, *Corynebacterium sp.* and *Pseudomonas sp.*, which further include certain bacterial species for producing specific nanoparticles. For example, the production of silver nanoparticles have been achieved by using *Pseudomonas stutzeri (109)* wherein silver ions were converted into silver nanoparticles [38], or the use of *Pseudomonas aeruginosa* to synthesize gold nanoparticles in an extracellular fashion [39]

It is important to note that these species were specifically chosen due to certain properties of theirs, but for them to effectively produce these desired nanoparticles, factors such as pH, temperature and reaction mixture concentration etc. are very crucial. This will be further elaborated in the next section. In the bacterial synthesis of metallic nanoparticles, the most important factors are the conditions provided to the bacteria. This primarily includes the aforementioned pH, temperature, chemical concentration, the type of metallic salt used, the type of media used, raw material concentration, and the specific protocols used [40].

It is difficult to distinguish specifically which factors are ideal for bacterial culturing as compared to factors for obtaining an ample yield of certain sized nanoparticles [42] and can be assumed that these factors overlap and affect one another. The following section of the article will further elaborate on the importance of these aspects.

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(i) ***pH-*** Since the advent of biological synthesis of nanoparticles, the effect of pH has been widely tested and observed. It has been established that any alterations to the pH of the chemical solution and the bacterial environment, alter the texture and size properties of the generated nanoparticles. In many cases, pH changes led to varying shapes of nanoparticles. For example, a variety of studies carried out by He et al, using the bacterium '*Rhodopseudomonas capsulata'* outlined the effect of changing pH and salt concentration on gold nanoparticles. At a pH of 6, the bacteria produced 10-20 nm-sized spherical nanoparticles but produced both spherical and overlapping triangular plates at a pH of 4 [42].

Given that none of the other conditions was altered, this indicated a direct correlation between the 2 aspects i.e. pH and size of the nanoparticles [43]. Based on the study by Soni and Prakash, varying the pH, while maintaining other parameters have led to changes in the size of silver nanoparticles synthesized by *C. tropicum* and *F. oxysporum.* Herein, the pattern indicated an inversely proportional relationship between pH and size; as the pH was increased, the absorbance value by the colourimeter increased, thereby indicating the production of larger sized nanoparticles [44]. Depending upon the specific bacterial strain being used, we can ascertain that varying the pH, alters the shape and size of the nanoparticles produced.

(ii) ***Temperature -*** It has been established that the temperature is just as important as the pH, as the bacteria react differently under varying stress conditions. While physical and chemical methods of NP synthesis require higher temperatures, lower temperatures are needed for biological synthesis; ideally, room temperature to a maximum of 100 ºC is the range that bacteria are exposed to [45].

This is because, in order to maintain the viability of the specific bacterial strains, we must create conditions within which they can survive. Based on the study by Gericke and Pinches on green synthesis of Au nanoparticles, the temperature showed a direct correlation between the formation rates of the nanoparticles [46]. Therefore, an ideal balance must be found between the temperatures ideal for the bacteria, versus temperatures suited for a high rate of NP production.

(iii) Methodology - The green synthesis of nanoparticles produces particles that are largely benign, non-toxic and environmentally friendly; therefore, the risk of pollution (especially that of metallic Nanoparticles) is reduced by a large extent. This furthermore reduces that chance of bioaccumulation and subsequent bio-magnification which could prove harmful to the flora and fauna that depend on that environment for survival. These benefits have

ISSN:0971-1260
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increased their use in today's research, and are proving as the stepping stone to viable large-scale production of nanoparticles for a variety of applications [40].

(iv) Time of reaction **-** The incubation period of bacteria in the reaction mixture affects the type of nanoparticle that is produced, as well as its quality and viability [47]. As mentioned by Darroudi et al, the study explained an increase in reaction time subsequently increasing the concentration of Ag nanoparticles. However, a change in the absorbance value beyond the 3-hour incubation time period signifies size reduction of produced nanoparticles. This helps us establish that nanoparticle synthesis varies greatly from strain to strain, but also by the incubation time of the culture in the metallic salt solution.

(v) Microbial resistance **-** The selection of the microbe for nanoparticle synthesis is extremely important. In the case of using bacterial strains, they must be capable of withstanding the potentially toxic effects of the metallic salt solutions that they are exposed to, in order for them to survive and subsequently convert the metal from the solution into specific metallic nanoparticles. By exposing the bacterial culture to a slow increasing gradient of the metallic salt solution, any toxic or lethal effects on the bacteria can be avoided, while increasing their resistance to the solution. This would eventually help researchers in being able to use these metallic salt solutions in large volumes and concentrations to obtain a larger yield of the desired metallic nanoparticles.

(vi) Pressure **-** While this may not always be possible on a small lab-scale setup, it can be managed at a medium to large scale setup at a pilot or industrial level. This aspect can directly impact the shape and size of the nanoparticles and is an essential feature of green synthesis by reducing the potential toxicity of the metal nanoparticles [48].

It can be deduced that a higher degree of pressure during the incubation phase can help increase a reduction rate of metal ions to lower oxidation states, thereby saving time as well as reducing their potential toxicity in their applications. Bacterial nanoparticle synthesis, using ambient pressure conditions is ideal for the fastest reduction rate of metal ions [49].

(vii) Particle characteristics; shape and size **-** Obtaining nanoparticles that are of varying shapes and sizes are influential towards the synthesis process in the way that the conditions that are established during the production methodology, rely on the theoretical type of nanoparticles wish to be obtained. By knowing these eventual characteristics, the other aforementioned aspects can be varied accordingly.

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There is a direct correlation between the nanoparticle size and their melting point [50], which is useful as a lower melting point would help save cost in temperature manipulation.

PHYSIOCHEMICAL CHARACTERIZATION OF NANOPARTICLES

Upon production of the nanoparticles from the bacterial sources, it is of utmost importance to characterize the various features of the nanoparticles; in order to confirm what the expected results were. Characterization is not an easy process and certainly not without its own hurdles. Many features of the desired nanoparticle can are analysed such as; particle size, shape, zeta potential, crystalline structure, pore size and porosity, surface area, surface interactions, adsorption potential, solubility and orientation [51].

A large variety of techniques exist that allow one to carry out a reliable analysis of the physical as well as chemical properties. These include some notable examples such as Transmission Electron Microscopy, Scanning Electron Microscopy, Nuclear magnetic resonance, X-Ray Diffraction, Fourier transform infrared spectroscopy and many more [52]. The following sections will further elaborate on the features of these tests. For the scenario of utilizing Microbial biosynthesis, there are a variety of the internal factors as well as external factors that can potentially alter the features of the nanoparticles and therefore these tests gain further importance. It is necessary to ascertain that the benefits of green synthesis of nanoparticles have come through. The features that are of notable importance include nanoparticle formation, morphological study, analysis of surface charge and potential magnetic properties, and surface interaction and crystalline structure. Accordingly, the various tests and methods can be further categorized as per the following subsections.

Nanoparticle formation

This feature will help provide information about the structural properties, nanoparticle stability and their aggregation behaviour [53].

UV-Visible Spectroscopy: The main feature of UV-Visible spectroscopy is that it measures 'Plasmon resonance' and analyses the effect of electromagnetic waves on the fluctuations of electrons [51]. Especially for metallic nanoparticles, the incident light/EM wave creates a resonance with the electrons within the nanoparticles thereby emitting energy at specific absorbance bands; which primarily depend on the nanoparticle size [54]. By doing so, it helps gather basic data about the size, shape and nanoparticle aggregation [55].

Determining morphological properties and size characteristics

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The following techniques are useful in observing and determining the size, shape basic morphology, atomic microstructures, surface texture and the overall nanoparticle size distribution [56-57]

(i) ***Transmission electron microscopy (TEM)*** **-** In itself, TEM can help identify similar characteristics as UV-Vis spectroscopy; however, it can provide more data about the nanoparticle morphology than the former technique [58].

The samples are prepared in solution, at an ultra-thin scale to allow for the transmission of electrons through the sample, by placing them on copper grids that are layered with carbon. This aqueous sample is then dried using liquid nitrogen and a mercury lamp, after which exposure to a beam of electrons creates an image [59]. This image can then be assessed to verify not only the formation of nanoparticles but also their basic structural characteristics by observing the crystal structure at the atomic scale.

(ii) High-resolution transmission electron microscopy (HR-TEM) **-** While TEM can provide a great resolution, the high resolution of HR-TEM can be used to view the internal and surface atomic arrangement to a greater extent. We can even observe the lattice microstructures present; fringes, defects, vacancies, glide planes etc. [61]. Notably, the procedure for sample preparation remains the same.

(iii) Scanning electron microscopy (SEM) **-** This technique is used mainly for direct visualization of the nanoparticles' surface for morphological and size-based studies. This microscope provides 3 images for analysis; primary and secondary electron images with x-ray maps [61].

Unlike the process for TEM, SEM requires the sample to be dried/lyophilized and subsequently coated with either graphite or a metal/metal alloy that is conductive. The coated sample is then exposed to a high-intensity beam of electrons which then relays the information to a detector as a spaced-out 2-D image [62]. It provides results about the surface and crystalline morphology, orientation, and even chemical analysis. Although time-consuming and costly, it provides invaluable results for characterization.

(iv) Atomic force microscopy (AFM) **-** Although similar to TEM and SEM in providing morphological data, it is different and superior as it generates a 3-dimensional image to obtain more accurate results about the volume and particle size [63]. It does so by using a probe-tip which carries out a sub-micron analysis of the nanoparticle [64]. AFM provides the

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advantage of allowing the sample to be analysed in a liquid phase, and not having to expose the samples to potentially harsh treatment [65]

(v) Dynamic light scattering (DLS) – This technique uses light, this technique is also known as Photon-correlation spectroscopy and is used to measure the size distribution of Brownian spherical particles in colloids. The exposure of nanoparticles to the light beam alters its wavelength in correlation to the size. In turn, device computationally measures the particle's diffusion coefficient to relay information about nanoparticle size distribution and motion [66].

Analysis of Surface Interactions and Properties

By observing the surface properties of the nanoparticles, it is possible to determine their surface charge and subsequent stability their primary structure and functional groups, obtain data about the varying elements that contribute to the chemical composition and ascertain the presence of specific coatings on the NP surface [67-68].

(i) Zeta Potential – Zeta-sizer Nanomachine - The Zeta potential test is an indirect method of nanoparticle characterization which analyses the surface charge of the nanoparticles as well as their stability. By measuring the difference between the shear surface and the Helmholtz plane, the analysis can help predict how stable the nanoparticle is in a colloid suspension.

If the tests result in a high negative or positive value, it indicates that the nanoparticle is quite stable and less aggregated, and further highlights the degree of hydrophobicity on the NP surface [67].

(ii) Fourier transform infrared spectroscopy (FT-IR) - This technique is useful for instantaneous identification of any functional groups within the nanoparticle structure [68]. Utilizing the infrared spectrum wavelengths, the vibrational frequencies of the intra-molecular bonds are recorded as an absorption value. This helps generate peaks of absorbance at certain wavelengths which create a 'fingerprint' for the nanoparticles being characterized, as well as provide some indication as to the present functional groups.

It should be noted, that prior to analysis, a KBr control sample/pellet must be placed within the machine and then again when testing the nanoparticle sample [61].

(iii) X-ray photoelectron spectroscopy (XPS) - XPS is primarily used for potentially magnetic nanoparticles, and similar to FT-IR, helps to obtain information about the bonds within the nanoparticle, the various elements present, and the structural characteristics [69].

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(iv) Thermal gravimetric analysis (TGA) - Similar to XPS, TGA also works mostly for magnetic nanoparticles to approximate their binding capacity and efficiency. This works by a confirming the presence of certain polymers either on or within the nanoparticle itself [69].

Observing the crystalline structure

The benefits of crystal structure analysis include structural forms of various crystals and to further verify the composition of the elements within the nanoparticle itself [70].

(i) ***X-ray diffraction (XRD)*** **-** This is the foremost used technique for analysing the crystalline structure of nanoparticles both for identification and quantitative assessment. It may also be used to ascertain the elemental composition. A powdered sample is exposed to incident x-ray beams which create specific angles of diffraction based on the nanoparticle's structure, surface and lattice composition. Similar to the result visualization in FT-IR, XRD also creates peaks based on the diffracted x-rays that are detected, which correspond to the size of the particle. Using the Scherrer formula, the particle size can be calculated [61, 70]

Potential magnetic properties

These methods are important in the analysis of magnetic nanoparticles. They help in determining the degree/intensity of the nanoparticle's properties [69]. They quite clearly are not applicable to all types of metallic nanoparticle and are therefore used in specific cases.

(i) Vibrating sample magnetometry (VSM) **-** While techniques such as XPS and TGA are quite specific for magnetic nanoparticles, VSM only provides general clues as to the magnetization of the nanoparticles. Based on a range of 3 to -3 Tesla, a magnetic field is utilized and the response of the nanoparticles is recorded at low temperature and room temperature to obtain VSM curves [69]. This curve provides information as to the degree of magnetic properties of the sample.

Specific techniques in certain cases

These techniques are important for analysing nanoparticles, that are dissolved in an aqueous phase or in a colloid suspension; helps in determining the elements present within the nanoparticles like the aforementioned methods. Given that, they are most useful in determining the interaction between the nanoparticle and the liquid/mobile phase; it is possible to study the effect of these nanoparticle being dissolved in a body of water.

There are even more specific features that can be analysed and will be elaborated in this section. It is very specific to note that the following techniques may not directly be used

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for characterization of nanoparticles, but they can supplement the process by even carrying out separation and filtration in certain cases.

(i) Chromatographic techniques **-** These techniques are useful for the separation of nanoparticles that are found in a liquid phase. Size exclusion chromatography and Hydrodynamic chromatography can separate nanoparticle based on size and hydro-dynamicity respectively [71]

Given that nanoparticles can range anywhere between 1-100 nm, the columns that are used can separate anywhere between 5-1200 nm sized nanoparticles. This large range also allows for the observation of the various sizes and their aggregation.

Herein, while chromatographic columns use stationary and mobile phases, another technique of 'Field flow fractionation' uses only an open phase and causes particle separation based on the type of force applied [73].

(ii) Hyperspectral imaging **-** This method can be used to identify the variety of nanoparticles created in the environment and to further help in the analysis of these nanoparticles in a liquid phase. It can also be used to study the surface interactions as well as the spectral properties of nanoparticle [73].

(iii) Energy dispersive X-rays (EDX) **-** EDX is most useful for the characterization of nanoparticles produced by biosynthetic methods. The sample containing the nanoparticles is subjected to a beam of electrons that releases X-rays, which are in turn detected to identify the elemental composition of the nanoparticle. Given that each element would emit a different x-ray, the observed pattern would also help quantify the element and its characteristics [68].

(iv) Small-angle X-ray scattering (SAXS) **-** SAXS is a generic technique that uses X-rays to analyse the structural properties of nano-sized solid and liquid particles. Its biggest advantage is that is non-destructive and therefore can be safely used to establish the specific elements and their concentrations within a given sample [72].

(v) Preparative Ultracentrifugation **-** This technique is useful for the fractionation of minute particulates, separation of TEM and AFM substrate nanoparticles, and for their harvesting as well. This technique is an effective and low-cost method, which can, in turn, increase the efficiency of TEM and AFM characterization methods [74].

BIOCOMPATIBILITY OF NANOPARTICLES SYNTHESIZED WITH BACTERIAL PARTICIPATION

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The term biocompatibility readdressed as with reference to medical therapy, the capability of a biomaterial to perform its desired functions, without evoking any systemic effect or undesirable effect in the beneficiary or the recipient of that therapy, still giving rise to most appropriate advantageous tissue or cellular response [75].

The size of nanoparticles is about 1-100 nm or less but not more than that providing the ability to easily penetrate the cellular membranes, spread along the lymphatic and vascular vessels and then to nerve cell synapses and be selectively accumulated in a certain cellular structure at the same time. In biomedical applications, the development of nanoparticles is undergoing a considerable evolution or expansion. Having a very distinctive size, shape and structure, the analysis of the biomedical application of nanotubes is currently being undertaken. Different nanotubes which are used for biomedical application are boron nitride nanotubes, titanium dioxide nanotubes, silicon dioxide nanotubes, carbon nanotubes, and organic nanotubes. Among all of these the widely used is the carbon nanotubes.

Biocompatibility of nanoparticle

The biomedical applications of these nanoparticles involve entering the body and coming in direct contact with the tissues and cell, making biocompatibility of these nanoparticles a serious area to explore.

(i) Hemocompatibility

Nanoparticles are being used as a vector for various applications such as drug delivery, biosensor, gene delivery, etc which involves direct contact of blood with nanoparticles. According to the recent study and experiments including haemolysis, blood cell aggregation, coagulation behaviour, the parameters have laid down regarding the compatibility of blood by nanoparticle in *in vitro* conditions. Haemolysis is being considered as the most reliable and simple method for measuring the hemocompatibility of materials [76].

Chouhan and Bajpai underwent the haemolysis assay to judge hemocompatibility of PHEMA nanoparticles [77].This experiment was performed on the surface of the particles already prepared to suggest the average level of biocompatibility. It has been observed that most of the studies have been meant to comprehend the hemocompatibility of foreign materials making it one of the important parameters for compatibility determination. Many factors including physiochemical features such as surface area, surface charge,

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hydrophobicity, hydrophilicity etc. control the response of blood in contact with the nanomaterial, hence playing a decisive role in their response [78].

(ii) Histocompatibility

The biocompatibility of various types of nanoparticles like superparamagnetic iron oxide (SPION), carbon nanoparticles (CNT), gold nanoparticles, mesoporous silica particles etc. in the application as targeted drug delivery marks a mechanism of histocompatibility analysis.

SPION, in general, does not show a toxic effect in vitro or in vivo. No immunostimulatory effects were observed in primary human macrophage, when the cell was exposed to 30 nm of dextran-coated SPION. When primary peritoneal macrophages from mice and rats were exposed to 60 nm and 20 nm dextran-coated SPIONs, a reduced production of proinflammatory cytokines and an elevated secretion of anti-inflammatory cytokines were observed [79].

In a macrophage cell line of murine origin, an increase in proinflammatory cytokines was observed and upon exposure to dextran-coated SIPON, a decreased phagocytic function observed in the cells [80]. However, SIPONs when administered *in vivo* to rats, did not cause any side effects neither induce any of the oxidative stress.

When the nanomaterial surface is modified with PEG, a marked decrease in the cytotoxicity of dendrimer (causes generation-dependent toxicity) is observed. Anionic dendrimers are less toxic that cationic dendrimers. Formation of pores in membranes induced by cationic dendrimers leads to disruption [81]. Cationic dendrimer can also induce apoptosis caused by mitochondrial dysfunction. A substantial change in morphology and haemolysis of RBCs in a generation-dependent manner can be caused by cationic dendrimers. Anionic dendrimers do not exhibit any such effects as the cationic dendrimers. Cationic dendrimers are shown to exhibit caspase-dependent apoptosis and negatively influence proliferation in a murine macrophage cell line model [82].

Glucosamine-conjugated dendrimers inhibit the formation of proinflammatory cytokines in human dendritic cells and macrophages treated with LPS. Inhibitory effect showed by dendrimer conjugate on toll-like receptor 4 (TLR4), a receptor that triggers LPS-induced stimulation of immune-competent cells [83]. A good degree of biocompatibility is demonstrated by silica nanoparticles [84]. Without affecting the cell survival, the silica-coated nanoparticles have confirmed to enter the cell.

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FACTORS AFFECTING BIOCOMPATIBILITY

A recent study on the uptake of oxide nanoparticles by human pneumonocytes under *in vitro* condition determine that the effect of aggregation of nanoparticles is based on their size [85]. The magnetic nanoparticle with variation in the size and their surface characters can lead to distinct cellular responses. As in the case of aggregation of magnetic nanoparticle, the large particles would produce a higher cellular response [86].

Under the same provided concentration, the cellular uptake of the smaller nanoparticles was significantly low as compared to larger nanoparticles [85]. It was reported that the uptake rate of spherical gold nanoparticle of 14 nm diameter was lower than that of particles whose diameters were lesser than 50 nm. The size of Au and Ag nanoparticles is reported to be affected by the pH of the environment in which it is formed. At pH 7, particle aggregation is much more obvious contributing significantly to increased nanoparticle size and stability [87]. The renal clearance was shown higher in the case of smaller inorganic nanoparticles [88].

(i) Effect of size and shape

Being an important parameter size drastically affects the performance of the synthesized nanoparticles [2]. Many of the properties largely depend on influencing the size and shape of the particle, for example, cycling time, pathways through which the nanoparticles enter the cells, the ability to overcome the biological barrier, etc. These parameters also influence the particles during blood transportation, mainly in small vessels and tumour vessels [89]. The size and shape correlation factors, such as aspect ratio or geometric structure affects the interaction and transportation between the cells and the particles.

Iron oxide nanoparticles have been synthesized in many shapes like nano octahedrons, nano-hexagons, nanorods, nanotubes nanowires, nanoflowers, nanorings, nano capsules, nanotubes, and nanoplates. The biocompatibility study of iron oxide nanoparticles was carried out by using them in human A549 lung tumour cells at a concentration of the range of 10-1000μg mL^{-1} [90]. After the incubation for 24 hrs, CCK-8 assay disclosed the result that > 90% of the cell survived; proposing that these nanoparticles were not harmful, but safe to the cells within the specified range of tested concentration

(ii) Effect of surface charge

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Various biological applications and performance of nanoparticles which depends upon solubility, stability, biodistribution, cytotoxicity, the surface charge is the deciding property of nanoparticles [91]. Only the particles carrying positive charge can be ingested by the cells when dispersed in the culture medium.

There can be an elimination of the electrostatic difference between positive and negative charge particle only if the particle is connected to protein [92]. In determining the particle-to-cell attraction, protein-coated particles are not very important although they may help in identifying antigens or receptors. Reduced cellular uptake was shown by the negatively charged and the polyethene glycol (PEG)-functionalized particles whereas effective cellular internalization was shown by the positively charged particles [93].

The toxicity is predominantly related mainly to the shape, size and concentration of the nanoparticle. At higher concentrations of about 80μg/ml the cytotoxic activity exhibited by magnetic nanoparticles was higher than at the lower concentrations [86]. The structure of nanoparticle specifically the core material determines the toxicity of nanoparticle. The easiest and simplest mechanism for causing toxicity is the leakage of the toxic materials mainly by nanoparticle decomposition which can be further mitigated using inorganic core being embedded in a cross-shaped polymer or structurally stable on the surface.

NANOFABRICATION AND 3-D STRUCTURE GENERATION

It is a technique of designing and manufacturing devices at the nanometre of dimension. Nanofabrication is further divided into three major areas: thin films, lithography, and etching.

Three-Dimensional Structure Generation by Nanofabrication

(i) Three-Dimensional Network Structure with Multilevel Porosity

Three- dimensional networks with multilevel porosity have known to be shown by zinc oxide nanoparticle [90]. International Union of Pure and Applied Chemistry - IUPAC defines pores in terms of size i.e. micropores (<2 nm), mesopores (2 nm–50 nm) and macropores (>50 nm) [94]. Micropores are also known by the term “nanopore”. Bimodal (micro-meso, meso-micro or micro-macro) or Trimodal (micro-meso-macro or meso-meso-macro) usually show multiscale or multilevel porosity [95]. There is some structure with high porosity, multiscale and are interconnected with a large surface area and low density, and are known as hierarchically porous structured materials.

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Microporous, mesoporous, and microporous (nano porous) channel modalities are designed to meet specific performance goals using a hierarchically porous material model. Via light scattering, multiple internal reflections of nanoparticle aggregates, potential harvesting, and layer-by-layer structures, a network of ZnO nanoparticles are tuned to provide catalysis [96].

Zinc oxide structures by the one-pot hydrothermal method are used to synthesize nanostructure having a specific surface area of 36.4 m^2/g and pore size of 3–40 nm. Hierarchically porous ZnO microspheres are capable of adsorbing highly efficient dye as congo red, therefore, used to remove anionic organic dyes and components from wastewater. Hierarchically porous ZnO nanoparticle assemblies can also be applied to tissue engineering and drug delivery besides having the property of gas sensing and photocatalytic degradation [97]. For antibacterial drug delivery, sub-micrometric or micro-metric aggregates of spherical nanoparticles which were loaded with ibuprofen showed effectiveness in preventing the growth of *S. aureus* > *C. albicans* > *K. pneumonia*.

(ii) Nanofabrication Techniques

Conventional method of nanofabrication

Hierarchically porous materials can be fabricated by a variety of procedures. The procedure includes colloidal crystal templating, emulsion templating, bioinspired processing, dual surfactant templating, polymer templating, selective leaching, freeze-drying, replication, zeolitization, phase separation, sol-gel control, breath figures and post-treatment [98-100]. Further, the fabrication process is categorized under 4 different methods, basic, chemical, replication-related chemical and physical-chemical methods:

- The basic method includes (surfactant templating, sol-gel control, replication, and post-treatment)
- The chemical method includes (emulsion templating, zeolitization, phase separation, and self-formation)
- The replication-related chemical method includes (colloidal templating, polymer templating and bioinspired processing)
- The physical-chemical method includes (supercritical fluids, breath figures, freeze-drying, and selective leaching) [101]

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The hierarchically porous materials from metal alkoxide and surfactant in a solvent are produced through self-formation in chemical fabrication via a spontaneous phenomenon. The advantages of this method include direct production, simplicity, and facile scale-up.

Non-Conventional methods of nanofabrication

a) *Bio-templating* - Using a biological structure to help and guide the assembly of an inorganic material or either replicate the morphological characteristics and functionality of biological species is the aim of biotemplating. To lead the array or assembly of inorganic nanoparticles, bio templating replicates the functional and morphological characteristics of biological species or makes use of biological structures [102]. In order to obtain the required morphology, natural scaffolds, proteins, microorganisms, biopolymers are being used as bio templates. Bio templates should be removed during purification after the synthesis of nanoparticles.

In order to sense acetic acid in an aqueous mixture, ZnO nanoparticles films were designed using albumen as a bio template. With the help of the biomimetic method desired crystal structure was obtained using gelatin as a bio template [103].

Microorganisms are used as bio template for bottom-up fabrication of biologically stimulated heterogeneous nanostructures as they are more attractive, cost-effective and versatile bio templates [104]. For the development of ZnO nanomaterials, a variety of bacteria and viruses have been used as bio templates. For the formation of hollow ZnO sphere, macerozymes species have been used further which was used to detect acetone [104-105]

b) Nanofabrication via Self-Assembly

Based on the conventional covalent, hydrogen, metallic, ionic, and coordinate bond there occurs the formation of organized structure or pattern. These bonds are constructed from weaker forces like π–π and hydrophobic, van der Waals and Casimir, electrical or optical forces, magnetic, and colloidal and capillary forces [107]

A self-assembled 3-D network structure consisting of ZnO nanoparticles and aggregates was made by dripping ZnO nanoparticles-hexane suspension in Si-H_2O. The subsequent evaporation of hexane, a hierarchical, trimodal porosity network was developed on solid Si-H_2O by ZnO nanoparticle. With the help of a hydrothermal technique using O-phthalic acid, self-assembly of hexagonal, grid-like ZnO lamellae was reported [106-107]

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The interlinked self-assembly of ZnO nanoparticle in ethanol let to the development of grid-like ZnO lamellae. This was further coated onto a gold electrode containing alumina ceramic tube after air drying at 60°C for 1 hr and heated on an electric furnace for at least 1.5 hr at 350°C. On Indium-Tin oxide substrate, monolayer-based and self-assembled hydrothermal fabrication of ZnO nanorods were reported [108].

Self-assembled porous and precursor-directed ZnO nanosheets that serve as a high-performing semiconductor substrate for surface-enhanced Raman scattering, having a distinctive parallelogram morphology were designed. Further following an alkalization reaction and self-assembly where di-dodecyl-dimethyl-ammonium bromide was used as a surfactant.

APPLICATIONS OF NANOPARTICLES SYNTHESIZED WITH BACTERIAL PARTICIPATION

With the onset of nano and microtechnologies, nanoscale fabrications of devices and configurations having specific singularities of supramolecular assembly have become very recurrent. Nanomaterials explore much-enhanced reactivity and thus shows better efficacy when equated to their bulkier complements due to their high surface-to-volume ratio. In addition, these nanoscale materials offer the unique potential to leverage very distinctive surface chemistry which succour in targeting specific molecule of interest.

This inception has increased the relative potential of nanoparticles for a novel juncture in a vast assortment of fields such as biomedical, food and agriculture, industrial and environment.

Biomedical applications of bacterial mediated nanoparticles

Nanoparticles are being exploited various biomedical applications such as targeted drug delivery, bioimaging and biosensors, photoablation therapy, magnetic hyperthermia, gene therapy have steadily increased over the last few years. This is primarily because of their excellent attributes such as biocompatibility,non-toxicity, chemical stability, high magnetic susceptibility and high saturation magnetisation [109].

(i) Targeted drug delivery

The concept of targeted drug delivery is based on the fact that it is the method to direct the specific drug to the targeted tumour site and eventually enhancing the quantity of drug to be delivered at the tumour site and subsequently reducing any of its side effects. There are certain goals in using nanoparticles in delivering drugs have the following goals: faster

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production, specific drug targeting the delivery site and its subsequent delivery, maintaining biocompatibility during the application process and through-out the therapy [110].

These magnetic particles of nanoscale size conjugated with specific drug enter cells due to influx occur by endocytosis. An external magnetic field helps to guide the nanoparticle-drug complex to the specific delivery site once it is administered, or diffused through the cell membrane's lipid bilayer. Changes in pH, enzyme activity, temperature, osmolality, the diffusion coefficient, the release mechanism of drugs and the rate of biodegradation are the main factors which govern the release rate of the drug [111].

The activity of many enzymes to maintain homeostasis in the body, as well as a vital role in humoral and cellular immunity, which protects cells against cancer, the deficiency of Zn in the body, causes the initiation and propagation of cancer cells via DNA mutation and p53 disruption [91]. Through ROS generation, Zinc oxide nanoparticles synthesized from families as Enterobacteriaceae, Pseudomonadaceae, Staphylococcaceae, having enhanced permeability and retention (EPR) effects, can kill cancer cells [112]

After microwave-triggering, VP-16 (etoposide) loaded into beta-cyclodextrin having functionalized iron oxide QDs (quantum dots), coated with ZnO and doped with Er^{3+} and Yb^{3+}, shows antitumor activity in MCF-7 cells [91]. The iron oxide nanoparticles synthesized from Desulfobacteriaceae, functionalized with paclitaxel, rhodamine B isothiocyanate and propargyl folate could specifically target and induce apoptosis of cancerous cells that overexpress folate-receptors with higher efficacy [113].

The blood-brain barrier (BBB) and the extracellular matrix of the brain often hinder the passage of therapeutics systemically delivered and thereby limit the distribution and half-life of locally delivered agents. It has been inferred that encapsulating paclitaxel with polymeric nanoparticles produced from bacterial enzymes increases the uptake of the drug in rat brain [114]. The conjugation of specific peptides (less than 10nm) with the carbon and metal-based nanoparticles shows immunologically directed targeting. The immuno-modulatory nature of ZnO nanocomposites defines great adjuvant (enhance antigen processing) capability when integrated into the vaccine formulation and/or as an immune-stimulating adjuvant to activate or enhance immunity [115]. ZnO nanocomposites deliver an antigen (peptides) to both MHC-I and MHC-II compartment of the adaptive immune system, thereby inducing very significant and simultaneous elevation of CD4 and CD8 T-cells specific to ZnO-associated antigens [116].

(ii) Bioimaging

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Bioimaging spans the non-invasive visualization of subcellular structures, complete cells over tissues to whole multicellular organisms. Totally different from traditional imaging techniques, these techniques produce information about the cellular anatomy which is connected to the functional data, as suggested by magnetic and electric fields, mechanical movement, and its metabolism. Molecular Bioimaging, Biomedical Imaging, Bioimaging in Drug discovery and Computational Bioimaging are four broad categories of bioimaging.

Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET) Computed Tomography (CT), and ultrasound are some techniques that can capture high quality and high-resolution images of internal organs. Contrast agents may be used help to identify the tissue or organ of interest despite identifying healthy tissue from diseased ones. Over the years, these contrasting agents as gadolinium or IV contrast dye (iodine-based) have been developed due to its low retention time, low imaging time and less toxicity. MRI works basically on the principle of radiofrequency pulses and nuclear magnetic resonance. Magnetic iron oxide nanoparticles are being used as contrast agents for magnetic resonance imaging (MRI) owing to its ability to reduce and shorten T2 relaxation times in the spleen, liver, and bone marrow [117].

Semiconductor quantum dots of transparent nanoparticles have unique optical & electronic property i.e. to fluorescence under light sources. In the core-shell configuration i.e. inner core structure & outer shell made of different components, the photoluminescent quantum yield of the core emission is boosted and shielded from [118]. The imaging time and the biocompatibility of contrast agents are suggested to be increased by the use of Core-shell nanoparticles [119]. The semiconductor quantum dots (QDs) such as ZnS, ZnSe and CdS in combination with carbon dots, that have unique photo-luminescent properties, are said to be used in PET and NAT imaging as well as ultrasound imaging [120].

To enhance bioimaging, a ‘glow nanohybrid’ was described where surface functionalized ZnO nanorods and a highly fluorescent CNP are chemically grafted [121]. It serves a dual purpose i.e. it can be anti-bacterial (*Staphylococcus aureus*, *Escherichia coli*) as ZnO has an efficient antibacterial property and due to the presence of highly fluorescent nanoparticles it can also detect bacteria. This can be detected under a fluorescent microscope as it emits bright green light under UV. The development of new imaging mode called magnetomotive photoacoustic imaging, with advanced contrast enhancement using core-shell nanohybrid of iron oxide and gold nanoparticles, offering a much greater contrast have been reported [122]

(iii) Biosensors

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A biosensor is an analytical device which converts a biological, chemical, or biochemical signals into an electrical signal. A biosensor contains three essential components-

- The bio-element or bio-receptor composed of enzymes, antibodies, nucleic acids, cells or tissues.
- The transducer that can be optical, electronic, electrochemical, gravimetric, piezoelectric or pyroelectric.
- The electronic unit that contains the processor, amplifier and display.

The bioreceptor identifies the specific target, analyte or substrate of interest (cells, tissues, proteins, carbohydrates, antibodies, nucleic acids, microorganisms), the transducer transforms the captured signal into an electrical signal that can be more easily quantified.

Over the years many kinds of nanoparticles, such as metal and metal-oxide, semiconductor and carbon nanoparticles, have been used for developing biosensors. Among all of these, because of unique optical properties gold nanoparticles are the most preferred and is one of the major players in biosensor designing [123-124]. The detection of even very low concentrations of DNA (as low as 200pM) in the sample by conjugating gold nanoparticles with thiolate DNA and identifying the occurrence of the additional plasmonic bands by hybridization is possible [125]. Glucose biosensors based on gold nanoparticles have a detection limit of 0.18μM [126].

Silver nanoparticles synthesized from families as Enterobacteriaceae, Pseudomonadaceae, because of their catalytic property and electro-compatibility with carbon nanotube film, can be used to develop H_2O_2 biosensors based on direct electrochemistry of haemoglobin (Hb) via hybrid sol-gel technique [127].

Magnetic nanoparticles-based biosensors are being used to detect enzymes, nucleic acids, drugs, pathogens, proteins and tumour cells with the most efficient sensitivity and have occupied a remarkable application in diagnostic magnetic resonance (DMR) [128]. Magnetic resonance imaging (MRI) or Nuclear magnetic resonance (NMR) uses magnetic nanoparticles as proximity sensors to modulate the transverse relaxation time of neighbouring water molecules whose signals are then quantified.

The development of new and novel technologies is being used currently to design biosensor devices, which has a monolayer of self-assembled metal particles, nanolithography,

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and vacuum evaporation. These characters of metal clusters play as the fundamentals for the formulations of new promising plasmon resonance biosensor systems (SPR-biosensors).

(iii) Photoablation therapy

The photoablation or laser ablation is the process of irradiating a solid surface with a laser beam enabling the removal of the material from it. The absorbed laser energy heats the material and then it evaporates or sublimates at low laser flux. However, the material is typically converted to a plasma at high laser flux.

Photoablation therapy is classified into two major categories-

i. ***Photodynamic therapy (PDT)*** - Photodynamic therapy uses photosensitisers which are non-toxic light-sensitive compounds and these become toxic upon exposure to light at a specific wavelength [129].

ii. ***Photothermal therapy*** – In Photothermal therapy to irradiate tumour cells a near-infrared (NIR) light source. Hyperthermia is created by the light energy which can lead to cell death.

In order to inhibit the malignant glioma cells' growth, photoexcitation of TiO_2 nanoparticles was investigated [130]. Clinical application of TiO_2 as the photosensitiser agent is very limited to the fact that it utilizes ultraviolet light for its activation and in doing so UV is capable of damaging the healthy cells and tissues. UV light is used to induce photodynamic activities from graphene oxide/TiO_2 hybrid nanoparticles that are prepared by self-assembly method. Most recent advancements in the class of nanoparticles namely up-converting nanoparticles which on proper encapsulation of photosensitizer molecules show much appreciable results compared to its counterparts [131]. Multifunctional nanoparticles (MFN nanoparticles) based on UCN nanoparticles with the very effective combination of optical and magnetic property produces a prominent role in multimodality imaging and treatment.

(iii) Magnetic hyperthermia

Heat has been used as a potential treatment for disease for more than 5000 years, when Hippocrates, gave a very famous comment on hyperthermia: "those diseases which medicines do not cure, the knife cures; those which the knife cannot cure, fire cures; and those which fire cannot cure, are to be reckoned wholly incurable."

The infected/diseased area is raised to a temperature of 41–46 °C which kills the cancerous cells without causing damage to the healthy cells. This is due to higher temperature

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sensitivity of the tumour cells, which undergo cellular apoptosis when heated called hyperthermia effect, termed as thermoablation.

The main advantage of magnetic hyperthermia (MHT) is creating a temperature differential between the healthy and tumour tissues. Hyperthermia is administered in combination with chemotherapy and radiotherapy in cancer treatment. Furthermore, it is non-invasive and leads to minimal damage to normal cells. Therefore this has become a promising cancer therapy option.

Food & agricultural applications of bacterial nanoparticles

Nanotechnology is definitely set to revolutionize food and agriculture industry by the innovating new practises like enhancing the ability of plants to absorb nutrients, precision farming techniques, disease detection and control, efficient and targeted use of inputs, withstand environmental pressures, operative systems for processing, advanced storage and packaging of various products [132].

Nanoparticles and precision farming techniques

Precision farming is an approach where inputs are utilised in very much controlled and precise quantity to get increased average yield when compared to traditional counterparts of the same. GPS soil sampling, variable-rate technology (VRT), computer-based applications and remote sensing technology are some of the technologies involved in precision farming. Use of silver nanoparticles-based sensors serve one the prominent example of involvement of nanotechnology in precision farming allows increased productivity in agriculture by providing exact information, thus assisting the farmers.

Biology or biological samples are used in the bioanalytical nanosensors. Nano smart dust, gas sensors etc. may be used in identifying the dust in the air and the number of pollutants. The use of nano-encapsulation method for using pesticides and herbicides can reduce the dosages, without any serious loss in efficiency [133]

Food processing, packaging and storage

The nanomaterials can be used as flavour, preservatives, colour additives or carriers for food supplements. TiO_2 nanoparticles are frequently used as food additives for white sauces, gum, candy, cake icing and several puddings.

Nano-coatings are produced for fruit coverings to prevent weight loss and shrinkage of the fruit [134]. The use of ZnO quantum dots can detect various pesticides as aldrin,

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glyphosate, tetradifon because of the higher affinity of pesticides having a strong leaving group and ZnO quantum dots (Saho et al, 2018). A very fine layer of an edible hybrid nano-film can be used as a barrier to gas and moisture and to prevent spoilage by microbes and enhance the storage life of the respective food product [72]

Disease detection and control

Adverse effects on human health, on pollinating insects, on domestic animals, direct and indirect effect on ecosystems can be caused due to the uncontrolled use of pesticides on food. Cautious use of nanomaterials and nanoparticles help in ease down the problems generated by the overuse of chemical fertilizers and pesticides as they can act with enhanced toxicity and sensitivity.

According to a report metal oxide nanoparticle such as ZnO, is reported to efficiently inhibit fungal growth on *Aspergillus niger, Fusarium graminearum, Aspergillus flavus.* Nano-based several diagnostic kits to detect any biomolecule, not only increases the speed of detection as well as power and extent of detection of diseases. Various metal, as well as non-metal nanoparticles, are having antibacterial as well as antifungal property.

Industrial applications of bacterial mediated nanoparticles

Termed as one of the most promising tools, nanotechnology is used for the creation of materials which are multifunctional and having high performance for various industrial applications. The nanomaterials from natural sources exhibit exceptional characteristics of strength as well as great biocompatibility and hence can be used as promising materials for industrial purposes.

The surface coating based on nanoparticles for maritime components

There is always a permanent constant attack of the marine ecosystem as biological species, saltwater and temperature fluctuation on the massive engineered structures such as marine and ships platforms as well as offshore jetties and rigs.

The coating of nanoparticles as ZnO, SiO_2 based epoxy have been developed to overcome biofouling and corrosion. The nanoparticle based epoxy coatings could be a promising solution to enhance the performance of maritime components, due to its synergistic effects produced by various nanoparticles and the anti-fouling system that results in a combination of the large surface area of nanomaterials, roughness, hydrophobicity and anticorrosion properties. Nanoparticle-based systems can provide an excellent adhesion and transparency

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Application in consumer goods production

Textile industries: Wrinkle-free well as water- and- stain repellent clothes use fine engineered nanofibers in the production line, which make these clothes to be washed at low frequency as well as low temperature. ZnO nanoparticles and nanorods are being integrated into the clothes because of their antibacterial activity and UV protection property. Tiny carbon nanoparticles along with fine cotton fibre make a fine composite of clothes which guarantee complete surface protection from the electrostatic charge for the wearer. SiO_2 nanoparticles are added as a coating material for the fine fibre which is abrasion-resistant.

Cosmetics industry: ZnO or TiO_2 nanoparticles in sunscreen used to block UV rays in order to minimize white patches on the skin due to UV damage. Liposome mediated nanoparticles present in anti-ageing creams used to deliver proteins from stem cells to a target site. Silver nanoparticles used in baby lotions used to provide optimum nutrient by penetrating deep in the body.

Aerospace and vehicle manufacture: Use of carbon nanotubes as their applications include higher tensile strength, lower weight, icing mitigation, removal of CO_2 and electromagnetic shielding on aircraft thereby contributing to efficient wing materials and lubricants [135]. The self-healing property of several nanoparticles makes them more efficient for longer-term sustainable weather coating material or designing of wheels, body, electronic fibre system [136].

TOXICITY OF NANOCOMPOSITES

Nanoparticles of less than 10nm are similar to gas and can easily enter human tissue disrupting the cells' normal biochemical environment [137]. Nanoparticles disrupt the liver, spleen, brain, heart, in addition to gastrointestinal tract and lungs after oral exposure and inhalation [138-139].

The components of the immune system get activated in order to clear these nanoparticles from the body causing an allergic kind of immediate situation. About 700-days has been estimated to be the half-life of nanoparticles inside the lungs, making it a major threat to the lungs. Toxicity is inversely proportional to the nanoparticle size and combined with the fact that large-sized chemical particle is less toxic to humans as compared to nanoparticles of the same chemical composition, makes a major threat to humanity [140].

Different nanoparticles and their relative toxicity

Aluminium oxide nanoparticles

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Their toxic effects include increased oxidative stress, a threat to cell viability, disturbing mitochondrial function, altering the tight junction protein expression of the blood-brain barrier (BBB) [141].

Aluminium oxide nanoparticles cause a significant toxic effect at concentrations of as low as 10 and concentrations as high as 400 μg/mL, on the viability of mammalian cells [142]. Aluminium oxide nanoparticles also give rise to genetic disease by causing genotoxic effect and cytotoxicity effect result in damaging the DNA without causing any mutagenic effects [143]

Gold nanoparticles

Having very distinct physicochemical properties possesses the ability to easily bind to thiol and amine group i.e. easy functionalization capability. Gold nanoparticles are relatively considered safe due to the presence of non-toxic and inert core.

Differently sized (4, 12, and 18 nm) gold particles having different capping agents were investigated in order to check their cytotoxicity against a cell line of leukaemia [144]. Spherical gold nanoparticles entering the cell are non-toxic to cellular functions and side chain-dose with stabilizers can be used to determine the level of cytotoxicity related to gold nanoparticles [145].

Copper oxide nanoparticles

These nanoparticles are used as anti-microbial reagents, semiconductors, intrauterine contraceptive devices, heat transfer fluids, etc [146] which has led to severe impairment in kidney, liver and spleen. The highly reactive ionic copper after interacting with the gastric juice when administered orally goes to accumulate in the kidney. Even (50 nm) copper oxide-induced oxidative stress and disturbs cell membrane integrity making it cytotoxic and genotoxic [147].

Titanium oxide nanoparticles

The nanoparticles made of titanium dioxide carry some health hazards or effects like DNA damage, the effect on experimental animals, lung inflammation and genotoxicity [148]; Titanium dioxide (<100 nm) leads to the formation of DNA adducts and causes oxidative stress [149]. Besides all these effects, it also causes effects on the liver, spleen, kidney, myocardium, lipid, glucose homeostasis, and disturb immune functions.

Zinc oxide nanoparticles

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The major toxic effect caused by the zinc nanoparticles is increased oxidative stress, cytotoxicity, cell membrane damage [150]. Complete cell death occurred when rodent fibroblast cells, as well as human mesothelioma cells, were exposed to high concentration (49 mg/mL) of zinc oxide nanoparticles. Under the in-vitro study, change in cellular morphology, alteration in mitochondrial activity (human hepatocytes), alteration in embryonic kidney cells, DNA damage, have been reported as a major effect of ZnO nanoparticles.

A decrease in the viability of the human dermal fibroblast cells on exposure to (20 nm) zinc oxide nanoparticles stating genotoxic potential and cytotoxicity effect of zinc oxide nanoparticles.

Iron oxide nanoparticles

Iron oxide nanoparticles have been used in broad applications such as drug delivery, diagnostic fields and in biomedical, these nanoparticles get accumulated in various organs like organs of the reticular endothelial system and liver [151].

It has been studies that under in-vivo condition iron oxide nanoparticles enter the cell, stay in the cell organelles as endosomes/lysosomes. After decomposition, these particles get released into cytoplasm further contributing to the cellular iron poll.

It has also been observed that magnetic iron oxide nanoparticles get accumulated in various organs such as spleen, liver, brain, and lungs after inhalation. Hence showing their tendency to cross the blood-brain barrier [150]. It has been shown by the shreds of evidence that the iron oxide nanoparticles show their effect, in formation of inflammation, by disrupting the coagulation system of blood and through cell lysis also leading to a reduction in cell viability which is the most common effect.

FUTURE PROSPECTS

Difficulty in purification steps and incomplete understanding on the mechanisms of synthesis are the major drawbacks related to the biosynthesis of nanoparticles using bacteria. The major problems usually faced in the nanoparticle biosynthesis, is to controlled size and shape of the nanoparticle in order to achieve the uniform monodispersed nanoparticles in solution phase. In order to produce well-characterized nanoparticles, there are some distinct aspects to be considered in future as follows.

Selecting Best Bacteria

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Researchers have targeted some essential intrinsic properties of the bacteria in order to select the best out of them. Properties include such as enzyme activities, growth rate, and biochemical pathways. Depending on the application of the resulting nanoparticles, a good candidate for nanoparticles production must be chosen. For example, one may require to produce nanoparticle in less time or require to produce nanoparticles with specific shapes and smaller sizes; demanding entirely different bacterial systems [152] (Iravani et al, 2011;

Selecting biocatalyst state

Bacterial enzymes (biocatalyst) are the dominant agents during the synthesis. Either a whole cell, purified enzyme or a crude enzyme can be used as biocatalyst. It was seen that the rate of reaction can be increased by taking cell extract of the cell or using culture supernatant however lacking long term stability. Most of the reactions are bio-reduction reactions that are responsible for nanoparticle formation. There is a requirement of coenzymes that need to be supplied in a stoichiometric amount in bio-reduction processes. Coenzymes include NADH, NADPH, FAD. Being a highly expensive whole cell is preferred as during the pathway in the whole live cell the coenzyme will be recycled [153] (Korbekandi et al, 2009).

Optimal conditions for the enzyme activity and cell growth

By the synthesis of biomass in large amounts, a greater amount of enzyme production can be accomplished. Growth condition optimization is very important and other factors should also be optimized such as buffer strength, nutrients, inoculum size, pH, temperature, light, mixing speed, etc. At the beginning of the growth due to the presence of substrates and some related compounds, there will be an increase in the activity. In the case of making the use of whole cells and crude enzymes harvesting time is essential [153]

Optimizing the reaction conditions

To prevent any detrimental reactions and in order to provide a cleaner medium for improved, excellent, effortless and simple analysis, the cells need to be harvest to remove any unwanted residual metabolites and nutrients. The production rate and the yield are essentially issued to be undertaken to use the bacteria for the nanoparticle production hence in the reaction mixture there is a need to optimize the bio-reduction reactions.

Many other conditions need to be optimized such as the electron donor, its concentration, the biocatalyst concentration, mixing speed, exposure time, pH, the substrate concentration, buffer strength, temperature, and the light. Microwave irradiation, visible light and boiling are some complementary factors that could disturb the size, morphology and the

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rate of reaction. Highly stable nanoparticles with desired sizes and morphologies can be formed by the optimization of all the above parameters [152, 154]

Extraction and purification processes

Additional methods like reacting with specific detergents or ultrasound treatment may be essential in order to release the intracellularly produced nanoparticles further which can be useful in the restoration of the precious and rare metals from the metal leachates and mine wastes. In different chemical reactions, bio matrixed metal nanoparticles can be used as a catalyst. In order to extract the synthesized nanoparticle from the cell, various physicochemical methods can be used such as heating processes, osmotic shock, freeze-thawing, etc. There are some chemical materials such as surfactants or organic solvents which can be applied for both stabilization and extraction of nanoparticles but still have some limitations i.e. they are toxic, expensive and hazardous. For efficient extraction and purification of the nanoparticles, centrifugation can be used but even for only those that are produced extracellularly, but here aggregation may happen.

Stabilization of the produced nanoparticles

Nanoparticles displayed compelling stability that is being produced by the eco-friendly bio-based approaches without any aggregation at RT even for many weeks. The presence of protein and enzymes secreted by the microorganism provided stability to the nanoparticles.

Scaling up to industrial scale

Biosynthesis of nanoparticles can be enhanced by the optimization of the reaction condition. For the production of well-characterized and highly stable nanoparticle, the biological protocol can be used when aspects such as genetical and inheritable properties, optimal reaction condition, type of organism, the optimal condition for enzymatic activity and cell growth, and biocatalyst selection have been considered. By altering the above-mentioned conditions size and morphology of the nanoparticles synthesized can be controlled [152, 154]

CONCLUSION

Nanoscience and nanotechnology have indeed fascinated an undeniable interest over the last few years owing to its impact on many research and scientific areas such as energy, electronics, medicine, pharmaceutical industries, and space industries. Nanotechnology is a very important area of research in nanosciences by creating particles of nano size of varying

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sizes, morphologies and mono-dispersed nature. Owing to this property, there is an increasing need to synthesize eco-friendly, non-toxic, clean, reliable, green experimental protocols for nanoparticle synthesis [152].

Different bacteria possess distinct and remarkable efficiencies to reduce metal ions and are considered as one of the greatest options for synthesis of various nanoparticles. For example, some species of bacteria have developed the ability to respond to certain defence mechanisms to quell stresses like heavy metal toxicity. It is also well observed that some of bacterial species could resist, survive and grow even at higher concentrations of metal ion (e.g., *Pseudomonas stutzeri* and *Pseudomonas aeruginosa*)

There are numerous types of nanoparticles as, silver nanoparticles, gold nanoparticles, magnetite nanoparticles, palladium and platinum nanoparticles, selenium and tellurium nanoparticles, zinc oxide nanoparticles, titanium and titanium Dioxide nanoparticles, cadmium sulphide nanoparticles, zinc sulphide nanoparticles are some of them produced and synthesized using the bacterial system of nanoparticle production.

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