REVIEW ARTICLE

Biosynthesis of Gold and Silver Nanoparticles and their applications

ABSTRACT

The synthesis of nanocrystals is in the limelight in modern nanotechnology. Biosynthesis of nanoparticles by using different methods is currently under exploitation. Nanoparticles can be synthesized by Bacteria , Virus , Fungi , Algae and Plants.. Among the use of living organisms for nanoparticle synthesis, plants have found application particularly in metal nanoparticle synthesis. Use of plants for synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as this eliminates the elaborate process of maintaining cell cultures. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extracellularly using plants or their extracts and in a controlled manner according to their size, dispersity and shape. Plant use can also be suitably scaled up for large-scale synthesis of nanoparticles. In view of this, we have reviewed here the use of plants or their extracts in the synthesis of silver and gold nanoparticles for various human applications..The marked difference of shape control between gold and silver nanoparticles was attributed to the comparative advantage of protective biomolecules and reductive biomolecules. The polyol components and the water-soluble heterocyclic components were mainly responsible for the reduction of silver ions or chloroaurate ions and the stabilization of the nanoparticles, respectively.

Key words: Biosynthesis, Gold, Silver, Nanoparticles

INTRODUCTION

The study of nanomaterial has been emerging dramatically throughout the world in the 21st century due to their incredible applications in all spheres of human life [1]. Proliferation of nanotechnology has opened up novel fundamental and applied frontiers in materials science and engineering, such as quantum dots, surface-enhanced Raman scattering (SERS) and nanobiotechnology [2]. In the last decade, biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology has received increasing attention due to a growing need to develop environmentally benign technologies in material synthesis [3]. Metal nanoparticles have been extensively studied due to their specific characteristics such as catalytic activity, optical properties, electronic properties, antimicrobial properties and magnetic properties [4]. Synthesis of noble metal nanoparticles, in particular silver nanoparticles (AgNPs) synthesis using natural organism has become a major research area in the field of nanotechnology. This may due to their simplicity of procedures, stability of nanoparticles, and their potential applications in chemical sensing, biological imaging, antimicrobial, gene silencing, drug delivery [5]. Recently, several studies have reported natural polymers such as chitosan, starch and tannic acid as reducing agents for the synthesis of silver and gold nanoparticles [6,7]. A vast array of biological resources including plants, algae, fungi, yeast, bacteria, and viruses has been studied so far for the intra and extracellular synthesis of silver, gold, platinum and titanium nanoparticles in different sizes and shapes [8].

BACTERIA MEDIATED SYNTHESIS OF NANOPARTICLES

Soil is an extensively explored ecological niche for sources that the Pseudomonas stutzeri AG259, isolated from a silver mine, produced silver nanoparticles of well-defined size and distinct morphology within the periplasmic space of the bacteria [9]. In recent study various bacterial strains such as Bacillus amyloliquefaciens, Acinetobacter calcoaceticus, Escherichia coli and Bacillus megaterium could effectively induce the synthesis of silver nanoparticles [10]. Biosynthetic methods can be categorized into intracellular and extracellular synthesis according to the place where nanoparticles are formed. Of which, the extracellular synthesis of nanoparticles is still continually emerging in order to understand the mechanisms of synthesis, easy downstream processing and rapid scale-up processing. For these reasons, a bacterial system could prove to be a potential source for the extracellular synthesis of metal nanoparticles instead of physical and chemical procedures [11].

VIRUS MEDIATED SYNTHESIS OF NANOPARTICLES

Viruses are unicellular organisms that hijack the replication machinery of the host cell and suspend most endogenous cellular activity. Their structure consists of nucleic acid, either DNA or RNA, which is surrounded by a protein shell that may or may not contain a lipid envelope. Viral genomes can be non-segmented, consisting of a single nucleic acid molecule, or segmented, consisting of more than one nucleic acid molecule. The nucleic acid molecules of a virus can be contained within a single virus or separated into multiple viruses. Viruses do not express their own ribosomal RNA. Viruses hold great promise in assembling and interconnecting novel nanosized components, allowing developing organized nanoparticle assemblies. Due to their size, monodispersity, and variety of chemical groups available for modification, they make a good scaffold for molecular assembly into nanoscale devices. Virus based nanocomposites are useful as an engineering material for the construction of smart nano-objects because of their ability to associate into desired structures including a number of morphologies. Viruses exhibit the characteristics of an ideal template for the formation of nano-conjugates with noble metal nanoparticles. As nanoscale assemblies, viruses have sophisticated yet highly ordered structural features, which, in many cases, have been carefully characterized by modern structural biological methods. More recently, other pathogens such as plant viruses, bacteriophages and viruses are increasingly being used for nanobiotechnology purposes because of their relative structural and chemical stability, ease of production, and lack of toxicity and pathogenicity in animals or humans [12]. Biological scaffolds (viruses) hold great promise in assembling and interconnecting novel nanosized components, allowing such organized assemblies to interface with well-developed technologies such as lithography as nanotechnology develops [13]. The cowpea mosaic virus (CPMV), for example, due to its size, monodispersity, and variety of chemical groups available for modification, makes a good scaffold for molecular assembly into nanoscale devices. The tobacco mosaic virus (TMV) was also used as bio-template, which has the shape of a linear tube, for assembly of various kinds of nanoparticles inside and outside the tubes. One can assemble gold nanoparticles onto the surfaces of polypeptide nanotubes while controlling their assembly position on the biomolecules using the specific affinities of the polypeptide sequences [14].

FUNGI MEDIATED SYNTHESIS OF NANOPARTICLES

Cell mass or extracellular components from fungi, such as Fusarium oxysporum, Aspergillus flavus, Aspergillus clavatus, and Penicillium brevicompactum [15,16] have been utilized for the reduction of silver ions to AgNPs. Filamentous fungi possess some distinctive advantages over bacteria due to high metals tolerance, wall binding capacity, and intracellular metal uptake capabilities [17]. Previously, Vigneshwaran et al. [18] also showed that the use of Aspergillus flavus resulted in the accumulation of silver nanoparticles on the surface of its cell wall when incubated with silver nitrate solution for 72 hr. The average particle size was found to be 8.92 nm. The intracellular synthesis of gold nanoparticles produced by V. luteoalbum [19] showed morphologies of spherical, hexagonal and rods in the size range of 8.92~25 nm. Fungi are more advantageous compared to other microorganisms in many ways. Fungal mycelial mesh can withstand flow, pressure, agitation and other conditions in bioreactors or other chambers compared to plant materials and bacteria. These are fastidious to grow, easy to handle and easy for fabrication. The extracellular secretions of reductive proteins are more and can be easily handled in downstream processing. Since the nanoparticles precipitated outside the cell is devoid of unnecessary cellular components, it can be directly used in various applications [20].

YEAST MEDIATED SYNTHESIS OF NANOPARTICLES

Among the eukaryotic microorganism, yeast has been exploited mainly for the synthesis of semiconductors. Candida glubrata produced intracellularly monodispersed spherically shaped peptide bound CdS quantum crystallites of 20 Å by neutralizing the toxicity of metal ions by forming metal-thiolate complex with phytochelatins [21] and Schizosaccharomyces pombe also produced wurtzite-typed hexagonal lattice structured CdS nanoparticles in mid-log phase in the range of 1~1.5 nm [22]. Kowshik et al. [23] first reported the synthesis of fcc structured CdS nanocrystallites exhibiting quantum semiconductor properties using yeast, Torulopsis sp. which intracellularly produced in the vacuoles with a dimension of 2~5 nm in spherical morphology when incubated with Pb2+ exhibiting λmax of 330 nm in UV-Vis spectrophotometer. These nanoparticles were used to fabricate diode heterojunction with poly (p-phenylenevinylene). In addition, baker's yeast, S. cerevisiae was also reported to biosorb and reduces Au+ to elemental gold in the peptidoglycan layer of the cell wall in situ by the aldehyde group present in reducing sugars [24]. Similarly, Pichia jadinii intracellularly formed gold nanoparticles of spherical, triangular and hexagonal morphologies throughout the cell mainly in the cytoplasm, of size 100 nm in 24 hr .

ALGAE MEDIATED SYNTHESIS OF NANOPARTICLES

Algae are eukaryotic aquatic oxygenic photoautotrophs, which produce its food through photosynthesis using sunlight producing oxygen as their by-products. Their photosynthesis machinery has been evolved from cyanobacteria via endosymbiosis. They are predominant primary producers in many aquatic environments. Among various algae, Chlorella sp. was found to accumulate various heavy metals such as cadmium, uranium, copper, and nickel. Chlorella vulgaris is a single-celled green algae belonging to phylum Chlorophyta, and the extracts of C. vulgaris showed anti-tumor properties [25]. The dried algal cells were found to have a strong binding ability towards tetrachloroaurate (III) ions to form algal-bound gold, which was subsequently reduced to form Au(0). Nearly 88% of algal-bound gold attained metallic state and the crystals of gold were accumulated in the interior and exterior of cell surfaces with tetrahedral, decahedral and icosahedra structures. Though chemical synthesis produces nanoparticles more rapidly with well-controlled shape, size and dispersity, the use of toxic and expensive chemicals as reducing and capping agents restricts its use in biomedical applications. In that case, optimizing the conditions like pH, temperature and metal ions (solute) concentration for expediting the biological synthesis of nanoparticles with narrow size and shape is mandatory. To date, only very few reports have been documented on the optimization in biological processes. A 28-kDa "gold shape-directing protein (GSP)" present in the extract of green algae, C. vulgaris, was used in the bioreduction and in the synthesis of size/shape controlled distinctive triangular and hexagonal gold nanoparticles. With an increase in the concentration of GSP, gold plates with lateral sizes up to micrometers were produced . The study of heavy metal biosorption by various algae showed that brown algae are superior compared to other autotrophs and algae [26].

PLANTS MEDIATED SYNTHESIS OF NANOPARTICLES

Indeed, a number of bacteria, fungi and yeast have been well-known for the synthesis of non-toxic noble nanoparticles. However the microbial mediated synthesis of nanoparticles is not industrially feasible as it requires expensive medium and maintenance of highly aseptic conditions. Hence, exploration of the plant systems as the potential bio-factories has gained heightened interest in the biological synthesis of nanoparticles. Hence, exploration into plant systems has been considered to be a potential bioreactor for synthesis of metal nanoparticles without using toxic chemicals [27].

MECHANISM OF NANOPARTICLE FORMATION

In producing nanoparticles using the intracellular and an extracellular extract of organisms, the extract is simply mixed with a solution of the metal salt at room temperature. The reaction is complete within minutes. Nanoparticles of silver, gold and other metals have been produced previously [28]. The nature of the living extract, its concentration, the concentration of the metal salt, the pH, temperature and contact time are known to affect the rate of production of the nanoparticles, their quantity and other characteristics [29]. In addition to the individual synthesis of either silver or gold nanoparticles, plants have been reported for their potential for both silver and gold nanoparticle synthesis.[30] Sun-dried biomass of Cinnamomum camphora leaf when incubated with aqueous silver or gold precursors at ambient temperature produces both silver nanoparticles (55–80 nm) and triangular or spherical gold nanoparticles. The marked difference in shape of gold and silver nanoparticles could be attributed to the comparative potential of protective and reductive biomolecules from leaf extracts.[31] The polyol and water-soluble heterocyclic components were mainly responsible for the reduction of silver ions or chloroaurate ions.6 Neem (Azadirachta indica) leaf broth has also been used for the extracellular synthesis of pure metallic silver, gold and bimetallic Au/Ag nanoparticles.[32]

Use of neem leaf extract for nanoparticlesynthesis has an advantage in terms of the rapid formation of stable silver and gold nanoparticles at higher concentrations.[33]The silver and gold nanoparticles were polydisperse, with a large percentage of gold particles exhibiting an interesting flat, platelike morphology, while silver nanoparticles formed in the mixtures were spherical, polydisperse and of 5 to 35 nm in diameter.[34]

This characteristic of competitive reduction of Au^{3+} and Ag^{+} ions by neem leaf extract leads to the synthesis of bimetallic Au core–Ag shell nanoparticles ranging in size from 50 to 100 nm.[35]

However, control over the shape and size of gold and silver nanoparticles has been obtained with the use of Aloe vera leaf extract as reducing agent.[36] The extract volume used for the synthesis of nanoparticles and temperature during the reaction had a great impact on the synthesis of characteristic nanoparticles.[37]

CHARACTERIZATION OF NANOPARTICLES

Microscopic techniques

Scanning electron microscopy, transmission electron microscopy and atomic force microscopy are mainly used for morphological studies of nanoparticles. Before morphological studies are carried out, there is need to standardize the synthesis of nanoparticles using plants or their extracts.[38] The formation of various nanoparticles from their different salts gives characteristic peaks at different absorptions that can be monitored using UV-vis spectroscopy. For example, silver nanoparticles formation from silver ions show an absorption peak around 450 nm, while gold nanoparticles show an absorption peak around 550 nm. [39]Similarly, several other metal nanoparticles give characteristic absorption peaks. A progressive increase in the characteristic peak with increase in reaction time and concentration of plant extracts with salt ions is a clear indicator of nanoparticle formation. UV-vis absorption spectra show peaks characteristic of the surface plasmon resonance of nanosized particles. [40]

The X-ray diffraction (XRD)

The X-ray diffraction (XRD) technique is used to establish the metallic nature of particles. X-rays are electromagnetic radiation with typical photon energies in the range of 100 eV–100 keV. For diffraction applications, only short-wavelength X-rays (hard X-rays) in the range of a few angstroms to 0.1 Å (1–120 keV) are used.[41] Because the wavelength of X-rays is comparable to the size of atoms, they are ideally suited for probing the structural arrangement of atoms and molecules in a wide range of materials. The energetic X-rays can penetrate deep into the materials and provide information about the bulk structure.[42]

Fourier transform infrared (FTIR) spectroscopy

However, Fourier transform infrared (FTIR) spectroscopy is a chemical analytical technique, which measures infrared intensity versus wavelength (wavenumber) of light. It is used to determine the nature of associated molecules of plants or their extracts with nanoparticles.[43] Based upon the wavenumber, infrared light can be categorized as far infrared (4–400 cm−1), mid infrared (400–4000 cm−1) and near infrared (4000–14 000 cm−1). Infrared spectroscopy detects the vibration characteristics of chemical functional groups in a sample.[44]When an infrared light interacts with matter, chemical bonds will stretch, contract and bend. As a result, a chemical functional group tends to adsorb infrared radiation in a specific wavenumber range regardless of the structure of the rest of the molecule.[45] For example, the C O stretch of a carbonyl group appears at around 1700 cm−1 in a variety of molecules. Hence, the correlation of band wavenumber position with chemical structure is used to identify a functional group in a nanoparticle associated molecule in a sample [46]. The wave number positions where functional groups adsorb are consistent, despite the effect of temperature, pressure, sampling, or change in molecular structure in other parts of the molecules. A FTIR spectrometer obtains infrared spectra by first collecting an interferogram of a sample signal with an interferometer, which measures all of the infrared frequencies simultaneously.[47] A FTIR spectrometer acquires and digitizes the interferogram, performs the FT function, and outputs the spectrum. This technique has been used in the characterization of silver and gold nanoparticles and their associated molecules from plant extracts in various studies.[48]

Raman spectroscopy

Raman spectroscopy is a widely used tool to characterize material composition, sample temperature, and strain from analysis of the material-specific phonon mode energies. It requires very little sample preparation and a rapid, non-destructive optical spectrum is easily achieved. Raman spectroscopy is conventionally performed with green, red or near-infrared lasers.[49]These wavelengths are below the first electronic transitions of most molecules, as assumed by scattering theory. The situation changes if the wavelength of the exciting laser is within the electronic spectrum of a molecule. In that case the intensity of some Raman-active vibrations increases by a factor of 102 –104. This resonance enhancement or resonance Raman effect can be quite useful.[50] The selection of nanoparticles for achieving efficient contrast for biological and cell imaging applications, as well as for photothermal therapeutic applications, is based on the optical properties of the nanoparticles.[51]It has been described by the use of Mie theory and the discrete dipole approximation method to calculate absorption and scattering efficiencies and optical resonance wavelengths for three commonly used classes of nanoparticles: gold nanospheres, silica–gold nanoshells, and gold nanorods.[52]The calculated spectra clearly reflect the wellknown dependence of nanoparticle optical properties, viz. the resonance wavelength, the extinction cross-section, and the ratio of scattering to absorption, on the nanoparticle dimensions.[53]

APPLICATIONS

Production of nanoparticles can be achieved through different methods.

Chemical approaches are the most popular methods for the production of nanoparticles. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol. Since noble metal nanoparticles such as gold, silver and platinum nanoparticles are widely applied to human contact areas, there is a growing need to develop environmentally friendly processes of nanoparticle synthesis that do not use toxic chemicals.[54] Therefore, biological methods of nanoparticle synthesis using plant or plant extract in addition to microorganisms have been suggested as possible ecofriendly alternatives to chemical and physical methods.[55] Using plants for nanoparticle synthesis can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures. It can also be suitably scaled up for large-scale synthesis of nanoparticles. These reasons make the biological synthesis of nanoparticles more valuable, though people are thinking that their use is similar to that gained by chemical methods.[56]

GOLD NANOPARTICLES:

The use of gold nanoparticles dates back to the 16th century, for bothmedical and staining purposes. Thereafter, gold nanoparticles have found application in analytical methods such as **colorimetric techniques** for the determination of heavy metal ions in aqueous solutions.[57]

Biosensors

Gold nanoparticles also used in the field of sensors.48,49 In biology, gold nanoparticles are used for the development of biosensors, DNA labels,26,50 and in medicine.51 However, spherical gold nanoparticles have been used to generate functional electrical coatings.[59]

Medical applications

In medical applications, gold nanoparticles have been used in treating **B-chronic lymphocytic leukemia** (CLL). CLL is an incurable disease predominantly characterized by apoptosis resistance. Earlier CLL treatment was with anti-VEGF antibody; however, treatment was found to be more effective when VEGF antibody wasattached to the gold nanoparticles.[60] Apoptosis with gold–AbVF was higher than with the CLL cells exposed only to VEGF antibody or gold nanoparticles. Non-coated gold nanoparticles alone were able to induce some level of apoptosis in CLL B-cells. Thus gold nanoparticles could be used for treating CLL.29 Gold nanoparticles have also been used in the Carter-Wallace home pregnancy test 'First Response'. [61]

SILVER NANOPARTCILES:

Ecology and medicine:

Owing to the distinct biological activity of silver nanoparticles, they have found application in ecology and medicine. Silver nanoparticles showed good antimicrobial activity and therefore can be used for purification in water-filtering apparatus.[62]

The interaction of metallic nanoparticles with biomolecules, microorganisms and viruses is another expanding field of research. It was noticed that silver nanoparticles undergo a size-dependent interaction with HIV-1. Nanoparticles ranging in size from 1 to 10 nm readily interact with the HIV-1 virus via preferential binding to gp120 glycoprotein knobs.[63]This specific interaction of silver nanoparticles inhibits the virus from binding to host cells, demonstrated by in vitro study. Hence, silver nanoparticles could find application in preventing as well as controlling HIV infection.[64]

Silver nanoparticles also find application in topical ointments and creams used to prevent infection of burns and open wounds.[65]

Medical devices:

Another widely used application is in medical devices and implants prepared with silver-impregnated polymers.

Sporting Equipments

In addition, silver-containing consumer products such as colloidal silver gel and silver-embedded fabrics are now used in sporting equipment.[70] Hydrophobic Ag–Au composite nanoparticles show strong adsorption and good electrical conducting properties, and therefore can be used in enzyme electrode design.

Biosensors

Current response of the glucose biosensor in the presence of Ag–Au nanoparticles has been reported to be much higher than that without nanoparticles. These nanoparticles can assist electron transfer between the enzyme and the bulk electrode surface. In these composite particles, current response of electrodes with molar ratios silver (50)–gold (50) and silver (25)–gold (75) was higher than that with silver (75)–gold (25) molar ratio.[71] Gold and silver nanoparticles have also found application in surfaced-enhanced Raman spectroscopy (SERS).

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