Review

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Shaina sharma , Anjali Bajaj * , Priyanjali Rajta , Hailemeleak Regassa * , Kasahun Gudeta

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Biomedical Applications of Microbial Mediated Gold and Silver Nanoparticles: Current Prospects

Shaina Sharma [1], Anjali Bajaj [1,*], Priyanjali Rajta [1], Hailemeleak Regassa [1,2,*] and Kasahun Gudeta [3,4]

[1] Faculty of Applied Sciences & Biotechnology, Shoolini University of Biotechnology & Management Sciences, Bajhol, PO Sultanpur, Distt. Solan – 173229 (HP), India, email: shainasharma0612@gmail.com; bajaj6579@gmail.com; rajtapriyanjali2000@gmail.com, haileregg@gmail.com

[2] Raj Khosla Centre for Cancer Research, Shoolini University, Solan, Himachal Pradesh, India

[3] School of Biological and Environmental Sciences, Shoolini University of Biotechnology and Management Sciences, Solan-173229, Himachal Pradesh India; kasahungudeta40@gmail.com

[4] Adama Science and Technology University, Department of Applied Biology, P.O. Box 1888, Adama, Ethiopia

* Correspondence: haileregg@gmail.com; bajaj6579@gmail.com

Abstract: Nanoparticles (NPs) have uniform chemical composition, size, and morphology. Microorganisms are of great interest in Nanoparticle synthesis. The green production of nanomaterials occurs either intracellularly or extracellularly. Gold and silver nanoparticles are mostly synthesised by the enzymatic degradation of metal ions. The produced NPs are characterized by different instruments such as ultraviolet visible, dynamic light scattering, x-ray diffraction, scanning electron microscope, transmission electron microscope, etc. Our review discusses the various biomedical applications of gold and silver nanoparticles synthesized by microbes via intracellular and extracellular mechanisms.

Keywords: biosynthesis; enzymatic activity; green synthesis; gold nanoparticles; silver nanoparticles

Introduction

The production and application of nanoparticles have drastically increased due to the quick advancement of nanotechnology [1]. Two of the most significant types of nanoparticles are silver and gold nanoparticles (AgNPs and AuNPs), which have been utilized extensively for both medical and nonmedical applications due to their specific characteristics of being inert and biocompatibility [2]. Nanotechnology has been involved in technological innovations in health care, environmental protection, electric power transportation, new energy development, computer chips, building materials, and other areas. For conventional medications, it is challenging to manage the release time and position at the same time when developing modern medicines. Nano formulations can boost a drug's bioavailability, increase its capacity to target specific organs, and lessen its toxicity and adverse effects. For instance, nanomedicine delivery methods have been used to introduce new antimicrobial treatments, rheumatoid arthritis drugs, and antineoplastic medications [3]. Nano-drug delivery systems are nanoscale substances like dendrimers, polymeric nanoparticles, liposomes, niosomes, and nanocapsules [4] that have firmly entered the realm of drug delivery to improve drug loading and deliver therapeutic agents in a controlled manner to specific target sites. To properly construct nanodrug delivery systems, it is imperative to comprehend certain recent developments in the field of polymer nanostructures.

Metallic NPs can be synthesized by using microbial enzymes, vitamins, polysaccharides, biodegradable polymers, and various biological systems [5]. Biogenic metal NPs can be made either through bio-reduction, in which metal ions are chemically reduced into their stable forms while the enzymes are oxidized, or through intracellular and extracellular extracts of organisms, in which the

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extracts are mixed with metal salts at room temperature in a reaction that occurs in a few minutes. These environmentally friendly, biologically based processes result in NPs that are affordable, eco-friendly, and safe [6].

The biological production of nanoparticles uses far less energy than chemical or physical synthesis methods. Most microorganisms, including bacteria, fungi, yeast, and algae, can synthesize nanoparticles. The reduction of metal ions into nanoparticles is the basic principle behind nanoparticle synthesis [7]. During Intracellular production, positively charged metal ions are drawn to the microorganism's negatively charged cell wall [8]. Enzymes that bio-reduce metal ions to their equivalent nanoparticles are also found in the bacterial cell wall [9]. The extracellular production pathway relies on the secretion of reductase enzymes by microorganism cells, which leads to the bio-reduction of metal ions to their corresponding nanoparticles [10].

Currently, microorganisms are employed to create a variety of nanoparticles, particularly gold and silver nanoparticles. Silver nanoparticles have a wide range of applications, including in the production of high-performance delicate electronics, antimicrobial agents, fabric cleaners, antireflection coatings, and household cleaners, which also helping to improve the heat transfer from solar energy collectors to their fuel tanks [11]. Gold nanoparticles (AuNPs) have been used as lab tracers in DNA fingerprinting to identify the presence of DNA in a sample, for the detection of aminoglycoside antibiotics such as streptomycin, gentamycin, and neomycin, in immunochemical experiments to identify protein interactions and antimicrobial activities [12], to diagnose cancer and detect cancer stem cells, and to identify various bacterial types [13]. The overall health applications of both gold and silver NPs synthesized from fungi (Table 1) and from bacteria (Table 2) are summarized.

Various analytical instruments are used to characterize nanoparticles such as scanning electron microscopy, transmission electron microscopy, high resonance transmission electron microscopy, Fourier transfer infrared ray spectroscopy, dynamic light scattering, UV–visible spectral analysis, polydispersity index, particle size and zeta potential studies, field emission scanning electron microscopy, and atomic force microscopy [14,15]. According to various literature reviews and research reports on the biosynthesis and characterization of other metallic or semiconductor nanoparticles for biological purposes has not been as successful as that of silver and gold nanoparticles [16]. The general synthesis mechanism and optimizers that maintain the synthesis process and their characterization are shown in Figure 1.

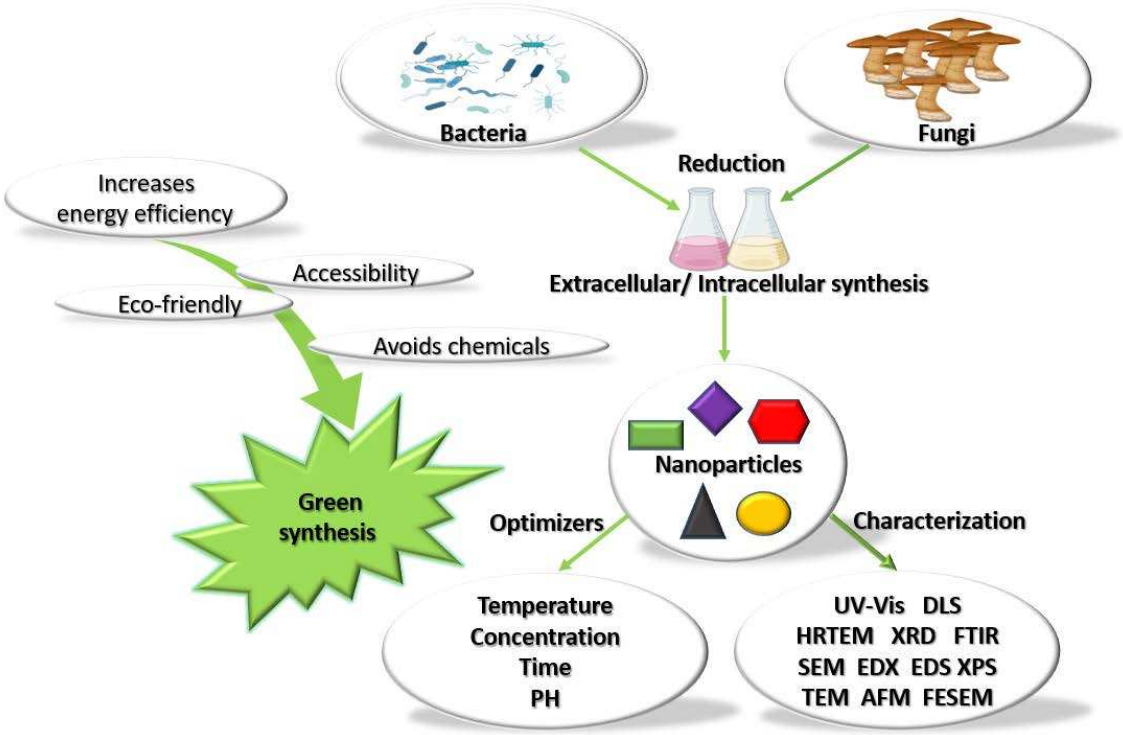

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Figure 1. The creation and evaluation of environmentally friendly nanoparticles from sources including bacteria and fungi. The green synthesis of nanomaterials improves energy efficiency, affordability, and accessibility and avoids the use of chemicals in the synthetic process. The microorganisms that produce these nanomaterials are bacteria and fungi, which degrade metal ions through enzymatic activity either extracellularly or intracellularly. We used a variety of techniques, including UV–Vis, DLS, HRTEM, XRD, FTIR, SEM, EDX, EDS, XPS, TEM, AFM, and FESEM, to study the characteristic sizes and shapes of the formed nanomaterials. The production process is primarily dependent on temperature, concentration, time, pH, and other factors.

Mechanism of Microbial mediated nanoparticles synthesis

Gold and Silver NPs Synthesis

Microbes have the capacity to adsorb metal ions and reduce them into nanoparticles using enzymes produced by metabolic processes. Depending on where they are produced, classified as either intracellular or extracellular nanoparticles [17]. Specific ions are carried into the negatively charged cell wall via the intracellular mechanism, and when they meet positively charged metals, they are electrostatically drawn through the cell wall. The poisonous metals are then transformed into non-toxic metal nanoparticles by the enzymes found in the bacteria' cell walls [18]. The extracellular process uses enzyme-mediated synthesis, such as that of hydroquinone or nitrate reductase, which is produced by many fungi or prokaryotic organisms and transforms metallic ions into metallic nanoparticles [19].

Gold nanoparticles made from *Rhodomonas capsulate* a variety of detoxifying mechanisms, such as vacuole compartmentalization, metal binding, or volatilization, which involves making metals volatile. In cases when they are exposed to high toxic metals, microorganisms will employ a variety of mechanisms to get rid of the metals to survive [20]. Active metal ion efflux across the cell membrane, toxic metal ion reduction to non-toxic metal ions, and metal ion accumulation within the cells are all part of the process. Ion pumps, carrier-mediated transport, endocytosis, ion channels, or lipid permeation are some of the mechanisms through which heavy metals like gold, silver, lead, nickel, and so on, ingress. Small ion-binding molecules called siderophores are chelating agents that chelate heavy metals and facilitate their uptake as well as their transit out of microscopic organism cells. Metal detoxification is the primary function of molecules like glutathione, which is derived from peptide (phytochelatin) binding metals or Metallothioneines (MTs), a cystien-rich protein, low molecular weight, isolated from *Syneococcus sp., Pseudomonas putida, Cyanobacterium,* and *E. coli* [21].

Fe (III)-reducing bacteria, such as *Geobacter sp., Magnetospirillum magnetotacticum*, and others, can be used for bioremediation of toxic metals like Fe (III) through reduction, where iron is actively taken up by the cell and re-oxidized to hydrous oxide (low density) to Fe (III) oxide (ferrihydrite), which is of high density. In the last phase, the Fe (III) ions are reduced, and the dehydration that occurs inside the magnetosome vesicles results in the production of magnetite. Iron is stored in the vesicles by an intracellular protein called ferritin, which keeps it in a soluble, non-toxic state. The created nanoparticles have high purity, minimal crystalline flaws, narrow row size, mono-dispersive, etc. [22].

The extracellular synthesis mechanism is remarkably facilitated by thermophilic bacteria. The number of nanomaterials produced by these extracellular systems, which reduce the downstream processing of these metals, can be replaced with an eco-friendly alternative. Multi-drug resistant (MDR) microorganisms have created antibacterial drugs that are effective to gram positive or negative bacteria. It is well-known that the very thin peptidoglycan coating of gram-negative bacteria's cell walls makes them vulnerable to the effects of nanoparticles [23].

The extracellular creation of both gold and silver nanoparticles can benefit greatly from the use of thermophilic bacteria. The number of nanomaterials produced by these extracellular systems, which reduce the downstream processing of these metals, can be replaced with an eco-friendly alternative. Multi-drug resistant (MDR) microorganisms have created antibacterial drugs that are effective to gram positive or negative bacteria. It is well-known that gramme negative bacteria have a relatively thin peptidoglycan coating on their cell walls, making them vulnerable to the effects of nanoparticles [24].

Fungi are commonly employed because they secrete higher quantities of enzymes that may be worked with in a lab and have numerous useful applications. The filamentous fungus has distinct

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benefits over other microorganisms like bacteria and algae because they have a high metal tolerance and the capacity for bioaccumulation. They are useful for scaling up, managing biomass, downstream processing, and ensuring economic viability. They also release extracellular enzymes, whose manufacture at a large scale is simple [25]. Active biomolecules generated by fungi regulate the biochemical makeup, shape, and size distribution of the nanoparticles. They took up the gold ions and caused the intracellular production of gold nanoparticles. Reducing sugars, proteins such as ATPase, glyceraldehyde-3-phosphate dehydrogenase, and 3-glucan binding proteins are some of the active molecules in play; these are crucial to the fungi's cells' ability to use energy. Gold nanoparticles were discovered to be accumulated in the cell vacuoles of the Au-fungal cells [26].

Silver nanoparticle synthesis

Metal and other organic nanoparticles have been produced in large quantities by bacteria. The nitrate reductase enzyme is crucial in the process of turning nitrate into nitrite in the bacterial bio-reduction of silver. The lepton is transferred to the silver ion during this bio-reduction, which produces AgNPs by regenerating nitrate into a cluster [27]. The stability and characteristics of synthesised AgNPs are determined by variables including temperature, pH, and bacterial species. Temperature preferences vary across bacteria, for instance, *Arthrobacter kerguelensis* and *Phaeocystis antarctica*. Bacteria like *Acinetobacter calcoaceticus, Bacillus amylo liquefaciens, B. flexus,* and *Staphylococcus aureus* have been noted for both intracellular and extracellular production with different forms including spherical, disk-shaped, cuboidal, and triangular [28].

Other bacteria used for silver NPs synthesis include cyanobacteria, Proteus mirabilis, Enterobacter cloacae, E. coli, Bacillus licheniformis, Lactobacillus fermentum, Klebsiella pneumoniae, and Pseudomonas stutzeri. Metals and other inorganic nanoparticles have been widely synthesised by bacteria [29].

Fungi have also been utilised to make AgNPs much as bacteria. Fungi secrete more proteins than bacteria, which allows them to produce more nanoparticles. This mechanism of mycosynthesis includes the trapping of silver ions at the fungal cell surface and their subsequent reduction to silver nanoparticles (AgNPs) under the catalysis of the NADPH-dependent nitrate reductase enzyme [30]. To produce nanomaterials, fungi are chosen above other microorganisms. Fungi including *Aspergillus flavus, A. fumigates, Fusarium oxysporum, F. acuminatum, F. culmorum, F. solani, Metarhizium anisopliae, Phoma glomerata, Phytophthora infestans, Trichoderma viride,* and *Verticillium sp*. have been used to synthesise AgNPs both intracellularly and extracellularly [31].

The size of the synthesised AgNPs was approximately 25±12 nm. Freeze-dried *Phoma sp.* 3.2883 mycelia with a size of 71.06± 3.46 nm were also used to make the AgNPs. *Fusarium oxysporum* was also used to synthesise AgNPs inside of cells. As bio-transformants, catalysts, and electron donors for the creation of silver nanoparticles, silver salt, fungal biomass, and glucose are utilised in this process. With a size range of 25–50 nm or 100 nm, respectively, the synthesised spherical AgNPs were either single or aggregated. Probably because of the enzymes found in the cell wall, a reduction was made in the negatively charged cell wall. Freeze-dried *Phoma sp.* 3.2883 mycelia with a size of 71.06±3.46 nm was also used to make the AgNPs [32].

Fungi and bacteria have been used to synthesize gold and silver NPs with different size ranges; for instance, 5-15 nm with *Rhodococcus sp*., 8-14 nm with *Fusarium oxysporum,* <10-25 with *Plectonema boryanum,* 8-12 nm with *Sargassum wightii,* 15 nm with *Yarrowia lipolytica,* 10-50 nm with *Brevibacterium casei,* 32 nm with *Neurospora crassa,* 50-70 nm with *Ureibacillus thermosphaericus,* 12±5 nm with *Shewanella oneidensis,* 50-70 with *Pseudomonas fluorescens,* 10 nm with *Marinobacter Pelagius,* and 10-20 nm with *Geobacillus sp* [33].

Biomedical applications of gold and silver NPs

Antibacterial activity

The nontoxic nature and unique nanorelated properties of AgNPs and AuNPs endow them with tremendous antibacterial efficiency [34,35]. The intrinsic chemical and physical properties of AgNPs

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and AuNPs, such as their nanoscale size, dispersion, stability, and low propensity to aggregate, increase the effectiveness of their activity [36]. These nanoparticles are more effective than silver and gold ions in terms of antipathogenic activity [37]. Biosynthesized silver and gold nanoparticles have an unconventional antimicrobial effect against multiple drug resistant (MDR) microbes [38]. Due to their physicochemical properties and intrinsic bactericidal effect, these nanoparticles are the most used antimicrobial agents and are applicable for both gram-positive and gram-negative bacteria in modern antimicrobial applications [39]. The mechanism of their antibacterial activity occurs by the interaction between the nanoparticles and the bacterial cell membrane in which the nanoparticle directly penetrates the cell, which leads to dysfunction in the normal metabolism and cellular functions of the bacteria and leads to structural damage and cell death [40].

The intrinsic antibacterial effectiveness of AgNPs and AuNPs also helps when they are incorporated in wound and burn dressings. Nanoparticles are used in the pharmaceutical design of materials such as coatings for medical devices, antibacterial clothes, and burn ointments that are primarily resistant to mutation. Numerous investigations have shown that the stability of nanoparticles significantly affects their toxicity. Different capping agents, such as PEG (polyethylene glycol) or PVP (polyvinylpyrrolidone), are used to preserve nanoparticle stability [41]. The physicochemical characteristics of the nanoparticles, such as shape, size, concentration, and colloidal state, sustain their antibacterial activity. The combination of antimicrobial silver nanoparticles with natural or synthetic polymers produces a synergistic effect, helps to eliminate or reduce microbial contamination and colonization [42].

Silver cations, which are responsible for disrupting the physiological activity of bacteria and leading to their death, which mainly occurs due to their cation binding to the thiol groups of the bacterial proteins [43]. These nanoparticles attach to the cell and penetrate to the cell barrier and release intracellular metallic silver ion, which leads to impairments in cellular respiration and permeability [44,45]. These nanomaterials exert their antibacterial effects through disruption and destabilization of extracellular polymeric substances within the biofilm matrix or interference with bacterial signalling molecules [46].

Table 1. Biomedical applications of silver and gold NPs synthesized from fungal strains.

Species	Microorganism	Mechanism	Nanoparticle	Size (nm)	Characterization	Applications	Ref
Verticillium sp.	Fungi	Intracellular	Silver	40-50	UV-VIS spectroscopy, FTIR, XRD, FESEM-EDX, and TEM-SAED	wound healing activity, cytotoxic properties against human keratinocyte	[47]
Fusarium oxysporum	Fungi	Extracellular	Silver alloy	8-14	TEM	Anti-viral, anti-bacterial, anticancer, anti-fungal, anti-parasite, antibacterial	[48]
Aspergillus fumigatus	Fungi	Extracellular	Silver	5-25	FTIR, SEM, EDX, DLS, UV-Vis Spectroscopy	Drug delivery	[49]
Aspergillus flavus	Fungi	Extracellular	Silver	12.5± 5.1	UV-Vis spectroscopy, FT-IR, TEM, SEM-EDX, and XRD	Antibacterial & anticandidal activity	[50]
Aspergillus flavus	Fungi	Extracellular	Silver	12.5± 5.1	UV-Vis spectroscopy, FT-IR, TEM, SEM-EDX, and XRD	Antibacterial & anticandidal activity	[51]
Trichoderma viride	Fungi	Extracellular	Silver	0.1–10.0	UV-visible spectroscopy, FTIR, SEM, EDX	anticancer and immunostimulatory	[52]
Aspergillus flavus	Fungi	Extracellular	Silver	<35	UV-Vis spectrophotometer, Zeta potential, Zeta sizer, FT-IR, and XRD	antibacterial activity against K. pneumoniae, E. coli, E. cloacae, S. aureus, S. epidermidis, and Shigella sp.	[53]
Fusarium oxysporum	Fungi	Extracellular	Silver	30–36	UV–vis, SEM, XRD, FTIR	against multidrug-resistant bacteria, and the development of antimicrobial textile finishes.	[54]
Fusarium oxysporum	Fungi	Extracellular	Gold	22-30	UV–vis, FT-IR, XRD, and TEM	antibacterial activity against MDR, *E. coli, P. aeruginosa, B. cereus,* and MRSA	[55]
Fusarium oxisporum	Fungi	Extracellular	Gold	8-12	UV–vis, FT-IR, XRD, SEM and TEM	Antibacterial	[56]
Neurospora crassa	Fungi	Intra/extracellular	Gold	32.0	UV–vis, FT-IR, XRD, AFM, DLS, EDS, SEM and TEM	Biomedical	[57]
Aspergillus sydowii	Fungi	Intra/extracellular	Gold	8.7–15.6	UV–vis, TEM, lattice fringes in high resolution, SAED, and EDXA	Biomedical applications	[58]

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Antioxidant activity

The term oxidative stress refers to a phenomenon that occurs when an excess of oxidants, such as reactive oxygen species (ROS) or nitrogen species (RNS), or organic compounds containing sulfur produce alkyl sulfinyl radicals (RS·), disrupt the equilibrium between the cellular antioxidative defence system and oxidants [59]. For instance, transition metal ions in their lower oxidation states do not exist as oxidant species by themselves but can operate as prooxidants by causing the oxidation of other substances to produce RNS or ROS. Naturally, the presence of oxidants also results in oxidative changes in biological systems on a molecular level, which causes damage and, ultimately, speeds up cellular death [60].

Using the stable radical DPPH, the ability of AgNPs and vitamin C to scavenge free radicals was evaluated. One millilitre of silver nanoparticles at concentrations of 10, 20, and 100 mg/ml was combined with 1 ml of freshly prepared DPPH solution in methanol [61], and the results showed 24.28% antioxidant activity; in another study, in the concentration range of 160-960 mg/ml, the percent DPPH inhibition by the AgNPs was 24-78%, and in the concentration range of 20-120 mg/ml, the percent ABTS inhibition was 22-96% [62].

In the gold nanoparticle study, different doses of gold nanoparticles (10 mg/ml-60 mg/ml) were added to 1 ml of 0.1 mM DPPH in methanol. When an antioxidant combines with DPPH in solution, the violet solution turns into an inert liquid, which shows that the antioxidant has successfully counteracted the free radicals. The results of this assay are determined by measuring the absorbance spectrophotometrically [63]. It has dose-dependent antioxidant activity with an IC_{50} value of 165.0 g/ml; the percent inhibition increased along with the nanoparticle concentration. An inhibition rate of 35-96% was predicted for green-synthesized gold nanoparticles in the concentration range of 80-480 mg/ml, and the DPPH IC_{50} value was 256 mg/ml [64]. An antioxidant at a concentration of 10 mg/ml provided the best antioxidant activity, and as the concentration increased, the activity declined [65].

The scavenging activities of gold nanoparticles greatly outperformed those demonstrated by their precursor salts and increased in a dose-dependent manner. The lowest evaluated concentration of biosynthesized gold nanoparticles had a percent scavenging activity of 15.85±0.49; however, when the concentration was increased to 500 g/ml, the scavenging ability improved to 60±1.82 [66]. In another study, the protective capping of gold nanoparticles with different amino acid residues and surface-bound proteins appeared to be the primary contributor to the improved free radical scavenging activity, as 233.75 g/ml was the determined IC_{50} value [67].

Anticancer activity

The abnormal development of cells and tissues and disease-related death are both caused primarily by cancer. Cancer is still treated with surgery, chemotherapy, and radiation, which frequently kill or manipulate healthy cells [68]. The advent of nanotechnology has accelerated the design of drugs and the development of cancer imaging. In a CO_2 incubator, HepG-2 and A559 cells were exposed to various concentrations of silver nanoparticles (1-100 mg/ml), and the cells were then cultured for 24 hr with 5% CO_2. The lowest number of nanoparticles that inhibited these cancer cell lines was 1 g, whereas 100 and 50 g were found to have the highest inhibitory concentration [69]. Silver nanoparticle therapies against liver cancer appear to be more effective than those against lung cancer. In Hep G2 and A549 cells, the amounts of nanoparticles needed to cause 50% cell death were determined to be 50 g and 100 g, respectively [70]. When the toxicity of silver nanoparticles was examined in the Vero cell line and the Hep2 cell line, their respective cytotoxic doses were 86 g and 107 g. However, it is not fully understood how nanoparticle inhibitory agents work to suppress cancer cell lines [71].

Metabolic activity can be slowed down by silver nanoparticles that can enter cell membranes via ion channels. Protein aggregation is caused by the partial unfolding of proteins due to the interaction between the active silver nanoparticles and functional groups on intracellular proteins, enzymes, and nitrogen bases in DNA [72]. By inhibiting the functions of many signalling proteins and the apoptotic

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signalling pathway, silver nanoparticles have the potential to be anticancer agents [73]. The silver nanoparticle-mediated reduction in the percent viability of a cancer cell line was based on the doses administered. HCT-116, PC-3, MCF-7, A-549, and Hep-G2 cells were the most susceptible to the toxicity of different concentrations of the tested AgNPs generated from microbes, whereas CACO, HEP-2 and HELA cells were the most resistant after 24 hr of exposure [74]. With over 80% inhibition of MCF-7 cells, silver nanoparticles at a very low concentration demonstrated extremely significant activity. The IC_{50} value of these silver nanoparticles was less than 10 g/ml, and at greater concentrations (10-100 g/ml), no discernible change in cancer cell suppression was observed [75]. The HCT-116 and Hep-G2 cell lines were the most vulnerable to the toxicity of the AgNPs, whereas Caco2 cells were the most resistant [76].

Upon increasing the dosage of biogenic AgNPs compared to the untreated control, the substantial anticancer activity and cell viability of HepG2 cells were both considerably reduced. Additionally, it was shown that various silver nanoparticle concentrations had no toxic impact on healthy human renal cell lines but may specifically kill malignant cells [77]. By triggering apoptosis and lowering DNA synthesis in cancer cells, AgNPs greatly suppressed the growth of human liver cancer Hep-G2 cells. It has also been demonstrated to inhibit angiogenesis, which is critical during the development of tumours [78]. To date, however, there has not been enough research on the mechanism of toxicity caused by AgNPs and how they affect normal human cell lines. The functional groups of intracellular proteins were coated on silver nanoparticles and may have a role in their cytotoxicity and the destruction of malignant cells [79]. The AgNP concentration is positively correlated with their toxicity to Hep-G2 cells. When silver nanoparticles come into direct contact with Hep-G2 cells, an increase in cytotoxicity, the production of reactive oxygen species (ROS), the induction of apoptosis, and mitochondrial damage result, and enhance cellular oxidative stress to kill malignant cells [80]. The control cells had a normal appearance and were adhered to the surface; in contrast, the cells exposed to silver nanoparticle solution shrank and lost their ability to adhere to the surface while maintaining their normal form. The silver nanoparticles and their associated capping group may have caused the cell shape to become altered and induced cytotoxicity by stimulating the necrosis process after their entry into cells [81].

Gold nanoparticles are perfect for biological applications due to their unique physicochemical features [82]. AuNPs decrease harm to healthy cells and reduce the possibility of adverse consequences. As a new drug for cancer treatment, AuNPs exhibit aggregation and size-dependent lethal action against many types of cancer cells. It is also influenced by the nanoparticle dosage [83]. However, a description of the mechanism of action is still preliminary. Several researchers have noted that AuNPs are internalized by cells; nevertheless, the interactions between AuNPs and cells vary in different ways [84]. Most crucial to a cell's ability to internalize gold nanoparticles are their surface characteristics. The gold nanoparticles and cells having opposing charges is what causes the nanoparticles to be taken up and internalized [85]. Gold nanoparticles are positively charged, whereas lipids in cancerous and normal cell membranes, particularly phosphate groups, are negatively charged [86]. Gold nanoparticles can also enter cells by endocytosis. According to studies in which very small gold nanoparticles were endocytosed and aggregated inside HeLa cells [87].

When HeLa cells were treated with biologically produced gold nanoparticles, comparable outcomes were observed. It was discovered that apoptosis was caused by activation of the caspase cascade, which includes caspases 3, 8, and 9 and cell cycle arrest in the G2/M phase [88]. In A549 cells, caspase-mediated apoptosis was also detected by the increase in the activities of caspase 9 and caspase 3/7 and a substantial drop in the level of ATP, as well as significant increases in the protein concentrations of p53 and Bax. When the concentration of the generated AuNPs was between 2 and 400 mg/ml, the vitality of HeLa cells, breast cells, and normal cells was between 98 and 67%, 97 to 58% and 98 to 60%, respectively [89]. HeLa cells, breast cells, and normal cells all had a high percent viability when in the presence of a low concentration of gold nanoparticles, but as the nanoparticle concentration rose, the percent cell viability fell [90]. As a positive control, the widely used anticancer medication mitomycin C (400 mg/ml) was utilized, which resulted in 17, 19, and 19% viability of HeLa cells, breast cells, and normal cells, respectively [91].

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Normal fibroblasts showed less of a reduction in viability after treatment with gold nanoparticles, indicating that the gold nanoparticles had a less harmful effect on normal cells. HeLa cells demonstrated 67% cell viability after treatment with 400 mg/ml gold nanoparticles, whereas breast cancer cells demonstrated 58% viability and normal cells demonstrated 60% viability [92]. The morphological properties of cancer cells change or are altered when treated with various concentrations of gold nanoparticles [93]. Breast cancer cells treated with silver nanoparticles displayed morphological alterations, including cell clumping, cell rupture, suppression of cell proliferation, and loss of membrane stability. [94]. Additionally, nanoparticles cause apoptosis through a variety of mechanisms, including the production of ROS, activation of the caspase-3 cascade, alterations in the expression of apoptotic proteins, and cell cycle arrest, which reduces membrane blebbing, cell growth, nuclear fragmentation, and chromatin condensation [95]. By downregulating Bcl-2 and activating caspase-9 and caspase-3/7, gold nanoparticles induced caspase-mediated death in A549 cells. Gold nanoparticles upregulated the expression of Bax, caspase-3, and caspase-9 while downregulating the expression of Bcl-2 and Bcl-xl in PANC-1 cells [96]. The cytotoxic impact of metal nanoparticles is dose- and time-dependent and relies on the type of cell, shape of the nanoparticle, capping agents, and nanoparticle size [97].

Table 2. Biomedical applications of silver and gold NPs synthesized from various bacterial strains.

Species	Microorganism	Mechanism	Nanoparticle	Size (nm)	Characterization	Applications	Ref
Bacillus licheniformis	Bacteria	Extracellular	Silver	10-30	FTIR, XRD	antimicrobial activity, against human breast adenocarcinoma cells	[98]
Morganella sp.	Bacteria	Intra/extracellular	Silver	10-50	UV–vis spectrophotometer TEM, SEM-EDX, FT-IR, XRD	antibacterial activity	[99]
***Sphingobium sp.* MAH 11^T**	Bacteria	Extracellular	Silver	7–22	SAED and XRD	antimicrobial agent against *E. coli* and *S. aureus*	[100]
Brevibacterium casei	Bacteria	Extracellular	Silver	42–92	UV-Vis, DLS, and TEM	anticancer, antibacterial, antidiabetic agents, bioimaging, and biosensing	[101]
Pseudomonas aeruginosa	Bacteria	Extracellular	Silver	30–70	TEM, XRD, and FT-IR	Anti-cancer against thyroid cancer cells	[102]
Bacillus subtilis	Bacteria	Extracellular	Silver	3-20	UV–Vis spectroscopy, TEM, and FT-IR	anti-microbial	[103]
Enterobacter aerogenes	Bacteria	Extracellular	Silver	47.22 – 105.0	UV–vis, SEM, XRD, FTIR	antimicrobial activity against Multidrug resistance, treat many oral cavity diseases	[104]
Vibrio sp.	Bacteria	Extracellular	Silver	32.67–107.18	UV–vis, SEM, XRD, FTIR, DLS, AFM, zeta potential, FESEM	antibacterial activity against Escherichia coli and Staphylococcus aureus	[105]
Pseudoduganella	Bacteria	Extracellular	Silver	8-24	TEM, SAED, XRD, FTIR	antimicrobial activity against multidrug-resistant	[106]
Rhodococcus sp.	Bacteria	Extracellular	Gold	30-120	AFM. DLS, SEM, EDX	antimicrobial activity against *Micrococcus luteus Escherichia coli* bacteria.	[107]
Actinobacteria	Bacteria (*R. erythropolis*,*R. ruber* cells)	Extracellular	Gold	30–120, 40–200, respectively	AFM, DLS, SEM, EDS	Antimicrobial	[108]
Brevibacterium casei	Bacteria	Extracellular	Gold	10-50	UV–vis, FT-IR, XRD, SEM and TEM	Antibacterial	[109]
Ureibacillus thermosphaericus	Bacteria	Intra/extracellular	Gold	50-70	UV–vis, FT-IR, SEM and TEM	Biomedical, sensor, catalysis, diagnostic and pharmaceutical applications.	[110]

Shewanella oneidensis	Bacteria	Intra/extracellular	Gold	12.0	UV–vis, FT-IR, XRD, AFM, DLS, EDS, SEM and TEM	Targeted drug delivery, cancer treatment, gene therapy, antimicrobial agent, biosensors, and imaging.	[111]
Pseudomonas fluorescens	Bacteria	Intra/extracellular	Gold	50-70	UV–vis, FT-IR, XRD, AFM, DLS, EDS, SEM and TEM	Biomedical applications	[112]
Geobacillus sp.	Bacteria	Intracellular	Gold	10-20	UV–vis, FT-IR, XRD, AFM, DLS, EDS, SEM and TEM	Immune response regulation	[113]
Ureibacillus thermosphaericus	Bacteria	Extracellular	Gold	50-70	UV–vis, FT-IR, XRD, AFM, DLS, EDS, SEM and TEM	Antimicrobial, anti-inflammatory, Targeted drug delivery, cancer treatment, gene therapy, biosensors, and imaging.	[114]
Shewanella oneidensis	Bacteria	Extracellular	Gold	12.0	UV–vis, FT-IR, XRD, AFM, DLS, EDS, SEM and TEM	Highly performance transistors, oscillators, in catalysis, biosensors, drug delivery, and as therapeutic agents	[115]
Paracoccus haeundaensis **BC74171**	Bacteria	Extracellular	Gold	20.93 ± 3.46	UV–vis, SEM and TEM	Antioxidant, antiproliferation activity on cell lines	[116]
Marinobacter Pelagius	Bacteria	Intra/extracellular	Gold	10.0	UV–vis, FT-IR, XRD, AFM, DLS, EDS, SEM and TEM	Antibacterial, antifungal, anti-inflammatory, Targeted drug delivery, cancer treatment, gene therapy, biosensors, and imaging.	[117]
Geobacillus sp.	Bacteria	Extracellular	Gold	10-20	UV–vis, FT-IR, XRD, AFM, DLS, EDS, SEM and TEM	Anticancer	[118]

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Other applications

Imaging: Imaging is one of the best methods with which to diagnose cancer. Currently, the instruments we use to diagnose cancer are costly and have negative impacts on health [119]. To compensate for these side effects, current research has used the concept of nanotechnology. Nanotechnology changes the overall approach and helps to detect the disease at an early stage [120]. Gold and silver nanoparticles are very helpful for biosensing and bioimaging cancer due to their light absorption and light scattering properties. These nanoparticles reach cancerous cells and stealthily escape detection by the immune system [121]. Nanoparticles interact with the input signal during Raman imaging, dark field microscopy, photoacoustic imaging, and computed tomography to give a better image without affecting the patient [122].

Drug delivery: Chemotherapy is one of the treatment methods for cancer, but due to its great side effects, such as anaemia, hair loss, infertility, skin damage, and weight loss after treatment, it is not considered the best solution; hence, nanoparticles have been used in targeted drug delivery systems (TDDSs). TDDSs are a current technique in medication administration by which drugs are delivered to the targeted system with low toxicity and tissue damage [123].

Photothermal therapy: PTT has fewer side effects on tissues during treatment. PTT releases localized heat to the tumour during the primary stages of early metastasis [124]. The optical properties of gold and silver nanoparticles are excellent, which helps to attract light at a specific wavelength, and electron-phonon interactions immediately convert this light into heat. The duration of irradiation, size and shape of the nanoparticles, and laser power are factors that determine the temperature of the surrounding area [125].

Radiotherapy: In addition to chemotherapy and surgery, radiotherapy has become the main method that is utilized in 50% of patients. Effective radiotherapy treatment relies on cellular uptake and targeted tumour attack [126]. For the eradication of tumours, ionizing radiation is used by generating free radicals directly in the tumour [127]. Controlling the radiation dosage is essential to lessen normal tissue damage. The interaction between the electromagnetic wave and the nanoparticles causes the emission of secondary electrons, which leads to the disruption of cellular organelles, such as the mitochondria [128].

Catheter modification: Among catheter-related infections, 82% are caused by methicillin-resistant *Staphylococcus aureus* strains. *S. aureus* genes are modified during the biofilm development and bacterial dispersion processes. Silver nanoparticles in clinically useful materials modify 1D and 2D surfaces, such as thin polymer films, cotton fabrics, wound pads, and artificial and natural fibres [129]. Gram-positive and gram-negative bacterial biofilms were inhibited by central venous catheters coated with silver nanoparticles due to their ability to bind to bacterial cells, which is influenced by the surface area available for interaction. Catheters modified with silver nanoparticles are useful for reducing dialysis-related infections [130]. Methicillin-resistant *Staphylococcus aureus* urinary tract infections can be prevented by using silver/gold-coated catheters for urinary catheterization [131].

Dental applications: One of the most common conditions in the oral cavity worldwide is dental caries. The clinical impact of dental caries can be eliminated by nanotechnology [132]. The intrinsic biocompatibility of DBMs (dental barrier membranes) are used mainly for alveolar bone reconstruction [133]. Nanoparticles prevent dental-related biofilm formation. Dental implant contamination can be prevented by using antimicrobial mouthwash, prophylactic antibiotics, and proper tooth brushing techniques. Mainly, silver nanoparticles are used in implantology, endodontic and restorative dentistry, and dental prostheses [134]. Silver nanomaterials have a great role in nanomaterial-related biomedical, regenerative, and restorative multifunctional biomedicine [135]. Silver/gold-based nanostructures embedded in dental materials are very helpful for preventing bacterial infection or producing bactericidal effects [136]. Caries is prophylactically treated by the compound diamine fluoride in nanosilver form. Decreasing the size of the nanoparticle, which in turn increases the size of the contact surface, helps to improve the antimicrobial effect and prevent black staining of the teeth [137]. The antimicrobial and physicomechanical effects can be improved

by adding AgNPs/AuNPs to acrylic resin, which helps to lessen bacterial colonization and contamination [138,139].

Wound healing: Patient morbidity is related to wound infection, which is becoming a major clinical problem [140]. The intrinsic physicochemical features of silver and gold nanoparticles help protect against wound infections [141]. The biocidal activity of these nanoparticles against gram-positive and gram-negative bacteria is highly efficient [142]. The gold/silver ions that penetrate the cell mix with structural and enzymatic proteins [143]. These ions are responsible for the absorption of wound dressings and eradication of the bacteria that are present in the exudate. Various secondary infections related to delayed diabetic wound treatment can be managed by proper using of silver nanoparticles and silver carriers [144].

5. Conclusion and future directions

Numerous new technologies can benefit from metal nanoparticles. Gold and silver nanoparticles can be synthesized by bacteria and fungi. These microbes can be processed and purified physiochemically for biomolecular chemical analysis. The pharmaceutical, biological, and drug delivery sectors use gold NPs. The inherent antibacterial and anticancer properties of AgNPs make them one of the more significant nanoparticle types. In addition, AgNPs have been studied for use in several biomedical applications, including wound dressings, bone cements, bio-diagnosis, catheters, antioxidants, etc.

A variety of microbe-producing enzymes, metabolites, and proteins contribute to the conversion of silver and gold ions into silver and gold nanoparticles; nevertheless, the cellular, molecular, and biochemical mechanisms by which different microorganisms produce nanoparticles are still poorly understood. The specific synthesis parameters, enzymes, genes, and other biomolecules that mediate the mechanisms by which AgNPs and AuNPs are microbially synthesized must be identified through more investigations.

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