Applied Microbiology: Theory & Technology
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Special Column on Microbial Nanotechnology
Review

Synthesis and Characterization of Nanoparticles for Antimicrobial Applications-A Review

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Received: 20 February 2023; **Revised:** 4 May 2023; **Accepted:** 10 May 2023

Abstract: Nanotechnology is an interdisciplinary science developed since the 1970s and has found tremendous commercial applications owing to their unique properties. Nanoscale materials are of the order of 1-100 nm and offer extremely advantageous optical, electronic and structural properties that are characteristic due to size-controlled features than their bulk materials. Biological methods are alternative sources of nanoparticle synthesis compared to physical and chemical techniques. Microorganisms can be used for production of different kinds of nanoparticles which are highly suitable for many industrial applications. This review provides an overview of nanotechnology, with a brief discussion of the development of nanotechnology since the ancient world and highlights the biogenic approaches of mono- and bi-metallic nanoparticle biosynthesis by different microorganisms. The mechanisms of intracellular and extracellular biosynthesis of metal nanoparticles by microorganisms is illustrated. The classical microscopic and spectroscopic techniques used for investigating the nanoparticle characteristics are also described in detail with hints for practical analysis. Meanwhile, the applications of metal nanoparticles as antimicrobial agents are summarized. In conclusion, this review includes a final outlook in the field of Microbial Nanotechnology.

Keywords**:** ecofriendly approach, green synthesis, microorganisms, nanoparticle, nanobiotechnology

1. Introduction

The 20th century has witnessed the early beginnings and rapid development of a new and emerging field of science known as Nanotechnology, which has revolutionized the technology advancements in materials science, physical and chemical sciences, medical and pharmaceutical sciences and disease biology. Physical and chemical techniques have been employed for the synthesis of nanoparticles but these methods are energy-intensive, costly and are detrimental to the environment. Microorganisms are particularly useful resources as they are cheap, safe and can be scaled up for large-scale production, and result in non-toxic byproducts which are beneficial in many ways to the environment. Microorganisms are viable sources and offer a favorable environment for nanoparticle synthesis [1]. Microbial Nanotechnology is an emerging science for green synthesis of metallic nanoparticles with promising applications in

DOI: https://doi.org/10.37256/amtt.4120232530

agricultural, clinical, engineering, energy and environment sectors [2]. The desired size and shape of nanoparticles can be obtained through optimum production in a minimal culture time. Improved stability of nanoparticles and optimization of specific microorganisms for suitable applications still remain as challenges to be addressed in the future [3]. This review is aimed to briefly describe the milestones in Nanotechnology evolution since ancient years and to discuss the potentials of a number of microorganisms such as bacteria, fungi, yeasts and microalgae in the biogenic production of metal nanoparticles. The review also highlights the classical characterization techniques of nanoparticles used to evaluate their therapeutic actions and includes the applications of metal nanoparticles in antimicrobial treatment. The review, therefore, consolidates the biogenic production of nanoparticles and the promising applications of Microbial Nanotechnology for pursuit of novel therapeutic and commercial potentials of the microbial world.

2. Nanotechnology

In the recent years, Nanotechnology has become the most significant area of research, which deals with the manufacturing of new materials, creating new processes and novel applications [4]. Nanotechnology is an interdisciplinary science related to biological and engineering technologies, for the synthesis of environment-friendly biogenic nanoparticles. Nanotechnology combines knowledge from diverse dimensions of science with a plethora of applications in physics, chemistry, biology, and medicine.

Nanoparticles are materials of one or more dimensions in the order of 100 nm or less and have attracted great attention due to their unusual and fascinating properties and applications. Recently, nanotechnology has focused on the development of “clean” and “green” technologies which have various significant environmental benefits and has been known as “green technology”. The nanoparticles made from green innovations are eco-friendly, energy-efficient, minimize waste, and curtail greenhouse gas emissions. These nanoparticles have several advantages because of their unique size and shape properties. Green synthesis of nanoparticles removes harmful chemicals and pollutants from the environment and does not disturb the ecosystem with conservation of natural resources [5-6].

3. Nanotechnology in ancient world

Nanoparticles and structures have been used by humans since the fourth century AD. Some of the examples of nanotechnology in ancient world are:

• In the 4th century, Roman glass cage cup made of a dichroic glass named Lycurgus cup is considered one of the famous examples of ancient glass industry, consisting of nanoparticles of 50-100 nm diameter with Silver (Ag): Gold (Au) in the ratio 7:3 containing in addition about 10% copper (Cu) dispersed in a glass matrix [7-8].

• During the 7th-19th centuries, glowing, glittering “luster” ceramic glazes (Islamic glass, Metallic luster and Luster ceramics) used in the Middle East, and later in Europe, contained Ag or copper (Cu) or other nanoparticles and stained-glass windows in medieval church [9].

• In 13th-18th century, “Damascus” saber blades, cementite nanowires and carbon nanotubes were used to provide strength, resilience, and the ability to hold a keen edge [10].

The Italians employed nanoparticles in creating Renaissance pottery during 16th century [11].

These colors and material properties were produced intentionally for hundreds of years. Medieval artists and forgers, however did not know the cause of these surprising effects.

4. The nano revolution

Nanotechnology is not evolution, but, a revolution in science, medicine and technology. It can be distinguished from all other scientific and industrial revolutions in many ways. In fact, for the first time in human history, man has been able to change the fundamental properties of matter, such as band gaps and luminescence as well as customize materials with desirable attributes, manipulate nanoscale objects such as atoms and molecules and fabricate and build nanodevices. These are the fundamental characteristics of nano revolution [12]. The first characteristic is due to the quantum size effects, by which the properties of a material change with its size in the nanometer regime. The second characteristic is made possible by the invention of high-resolution transmission electron microscopy (HR-TEM), scanning probe microscopy (SPM), scanning tunneling microscopy (STM) and atomic force microscopy (AFM) techniques [13]. The third characteristic is a result of the developments of various nanofabrication techniques such as nanoimprint lithography (NIL) using electron beams or X-rays and due to a physical phenomenon known as ‘‘quantum confinement’’ effect [14].

Table 1. Ingenious founders of Nanotechnology

Year	Scientist	Discovery	Ref.
1857	Michael Faraday	Colloidal nanoparticles	[16]
1908	Gustav Mie	Light scattering nanoparticles	[17]
1928	Edward Synge	Near field optical microscope	[18]
1931	Max Knoll and Ernest Ruska	TEM	[19]
1936	Erwin Muller	Field electron microscope	[20]
1947	William Shockley, Walter Brattain, John Bardeen	Semiconductor transistor	[21]
1951	Erwin Muller	Field-ion microscope	[22]
1953	James Watson and Francis Crick	DNA	[23]
1956	Arthur Von Hippel	Molecular Engineering	[24]
1958	Leo Esaki	Electron tunneling	[25]
1959	Richard Feynman	Introduction of the concept of Nanotechnology-“There’s Plenty of Room at the Bottom: An Invitation to Enter a New Field of Physics”	[26]
1960	Charles Plank and Edward Rosinski	Zeolites and catalysis	[27]
1963	Stephen Papell	Ferrofluids	[28]
1965	Gordon Moore	Moore’s law- The number of transistors on a microchip doubles every two years, though the cost of computers is halved	[29]
1970	Eiji Osawa	Existence of C60 molecule in icosahedron form	[30]
1974	Norio Taniguchi	First coined the term Nanotechnology	[31]
1974	Mark A. Ratner and Arieh Aviram	Molecular electronics	[32]
1977	Richard P. Van Duyne	Surface Enhanced Raman Spectroscopy (SERS)	[33]
1980	Jacop Sagiv	Self-Assembly Monolayers (SAMs)	[34]
1981	Gerd Binnig and Heinrich Rohrer	Scanning Tunneling Microscope (STM)	[35]
1981	Alexey Ekimov	Nanocrystalline Quantum Dots in a glass matrix	[36]
1981	Eric Drexler	Molecular Engineering	[37]
1982	Nadrian Seeman	The concept of DNA Nanotechnology	[38-39]

Table 1. (cont.)

Year	Scientist	Discovery	Ref.
1983	Louis Brus	Colloidal Quantum Dots	[40, 41]
1985	Richard Smalley, Robert Curl and Harold Kroto	Discovery of Buckminsterfullerenes C-60	[42]
1986	Gerd Binnig, Christoph Gerber and Calvin F. Quate	Atomic Force Microscope (AFM)	[43]
1987	Dimitri Averin and Konstantin Likharev	Single-Electron Tunneling (SET) transistor	[44]
1992	Charles T. Kresge	Mesoporous silica MCM-41	[45-46]
1993	Sumio Iijima and Donald Bethune	Carbon nanotubes	[47-48]
1996	Chad Mirkin and Robert Letsinger	S-Adenosyl Methylation (SAM) of DNA + gold colloids	[49]
1997	Zyvex	Foundation of the first molecular Nanotechnology company	[50]
1998	Cees Dekker	Transistor using carbon nanotubes	[51]
1999	Chad Mirkin	Dip-pen Nanolithography (DPN)	[52]
2000	Mark Hersam and Joseph Lyding	Feedback-Controlled Lithography (FCL)	[53]
2001	Carlo Montemagno	Molecular nanomachines: molecular motor (rotor) with nanoscale silicon devices	[54]
2002	Cees Dekker	Carbon nanotubes functionalized with DNA	[55]
2003	Naomi Halas	Gold nanoshells	[56-57]
2004	Andre Geim and Konstantin Novoselov	Graphene	[58-59]
2004	Xu et al.	Fluorescent Carbon dots	[60]
2005	James Tour	Nanocar with turning buckyball wheels	[59, 61]
2006	Paul Rothemund	DNA origami	[62]
2007	J. Fraser Stoddart	Artificial molecular machines: pH-triggered muscle-like	[63]
2008	Osamu Shimomura, Martin Chalfie and Roger Y. Tsien	Nobel Prize in Chemistry for the discovery and development of the green fluorescent protein, GFP	[64]
2009	Nadrian Seeman	DNA structures fold into 3D rhombohedral crystals	[65]
2010	IBM	Ultra-fast lithography to create 3D nanoscale textured surface	[66]
2011	Leonhard Grill	Scanning tunneling microscope (STM) describes the electronic and mechanical properties of individual molecules and the polymer chains	[67]
2016	Jean-Pierre Sauvage, Sir J. Fraser Stoddart and Bernard L. Feringa	Nobel Prize in Chemistry for the design and synthesis of molecular machines	[68]

The developments in nanotechnology research have progressively increased, mainly due to new and desirable properties of nanomaterials. The nano revolution is expected to impact every aspect of human activities other than science and industrial technology. The applications of nanomaterials in biotechnology, agriculture, environment and biomedicine unites the fields of biology and material science. Nanoparticles are essentially beneficial with unique properties leading to a wide-range of applications [15]. Table 1 lists the cascade of developments in Nanotechnology and its ingenious founders from its early years.

5. Biogenic approach towards nanoparticle synthesis

Nanoparticles can be synthesized by various physical, chemical, biological and other hybrid methods [69]. The physical and chemical methods require cost-intensive equipments and high energy conditions [70]. The nanoparticles synthesized by means of biogenic approach present good polydispersity, nanoregime dimensions and improved stability. It also facilitates synthesis at physiological pH, ambient temperature and pressure, and low-costs of production. Several microorganisms are capable of nanoparticles synthesis both intracellularly or extracellularly.

Biological synthesis of Nanoparticles from microbial sources is advantageous, because of rapid synthesis, controlled toxicity, control of size characteristics and ecofriendly approach. A large number of microbial sources are available for nanoparticle synthesis from fungi, yeast and bacteria. The biological process is an acceptable green route and does not require high energy and is environment friendly. The main interest is the production of nanoparticles from a cheap resource with a facile approach, ease of production, increased biomass and size uniformity. Though numerous chemical methods are available for nanoparticle production, huge problems are often experienced with product stability, control of the crystal growth and aggregation of particles upon long term exposure [71]. Microbial sources are mostly utilized among various bio-methods of nanoparticle production [72-75]. Bio-based synthesis of nanoparticles using microorganisms has been recently discussed by Hossain et al. [76] for silver nanoparticles. Bacteria, fungi, yeasts and algae transport metals from their culture environment and convert them into elemental nanoparticles which may be accumulated intracellularly or secreted extracellularly into the culture medium. By biogenic synthesis, the presence of capping agents on the surface of nanoparticles enable reduction in further purification steps largely [77].

Metallic nanoparticles have been synthesized from different microorganisms in varying sizes and shapes either intracellularly or extracellularly. Silver nanoparticles have been synthesized by *A.flavus* [78], *A.fumigatus* [79], *B.cereus* [80], *B.licheniformis* [81] and *Fusarium oxysporum* [82] in spherical shapes upto 50 nm and by *Phaenerochaete chrysosporium* [83] in pyramid forms upto 200 nm. Other metallic nanoparticles of mercury, palladium, uranium, cadmium telluride and selenium have also been synthesized from *Enterobacter* sp [84], *Desulfovibrio desulfuricans* [85], *Pyrobaculum islandicum* [86], *E.coli* [87] and *Shewanella* sp. [88] respectively in spherical shapes. Bullet-shaped, rectangular, Rhombic and hexagonal shaped metal oxide nanoparticles of Fe_3O_4, $FePO_4$ nanopowder, spherical and tetragonal $BaTiO_3$ have been synthesized from different bacterial, fungal and yeast strains in intracellular and extracellular fractions [89-94]. The third type of sulfide nanoparticles, such as CdS, FeS and ZnS have been synthesized from bacteria such as *E.coli* [95], *Lactobacillus* [96], *Rhodobacter sphaeroides* [97], fungi such as *Fusarium oxysporum* [98] and yeasts such as *Schizosaccharomyces pombe* and *Candida glabrata* [99]. Table 2 indicates the nanoparticle production from various bacteria. Table 3 represents nanoparticle production from fungi and Table 4 shows nanoparticles produced by yeasts, molds and algae as cited from literature. Figure 1 illustrates the biosynthesis pathways of Nanoparticle production by microorganisms.

Table 2. Nanoparticles produced by bacteria

Nanoparticle	Size (nm)	Morphology	Bacteria	Synthesis pattern	Ref.
Silver (Ag)	2-4	Ribbon-shaped	*Acetobacter xylinum*	Extracellular	[100]
	6.4	ND	*Aeromonas* sp SH10	Extracellular	[101]
	50	ND	*Bacillus licheniformis*	Extracellular	[102]
	5-15	Spherical	*Bacillus* sp.	Intracellular/Periplasmic space	[103]
	20-50	Hexagonal	*Lactobacillus* sp.	Intracellular	[104]
	20	Spherical	*Morganella* sp.	Extracellular	[105]
Gold (Au)	1.9 ± 0.8	Spherical	*Bacillus megatherium* D01	Extracellular	[106]
	ND	Spherical	*Escherichia coli* DH5α	Intracellular/Cell surface	[107]
	> 100	ND	*Lactobacillus* sp.	Intracellular	[104]
	10	Cubic	*Plectonema boryanum* UTEX485	Intracellular/Membrane vesicles	[108]
	10-20 50-400 50-60	Spherical Triangular nanoplate Spherical nanowires	*Rhodopseudomonas capsulate*	Extracellular	[109]
	5-15	ND	*Rhodococcus* sp.	Intracellular	[110]
	< 10	ND	Sulfate-reducing bacteria	Intracellular/Cell envelope	[111]
Selenium (Se)	300	Nanospheres	*Sulfurospirillum barnesii, Bacillus selenitireducens,*	Extracellular	[112]
Tellurium (Te)	10	Nanorods	*Bacillus selenitireducens*	Extracellular	[113]
Titanium (Ti)	40-60	Spherical	*Lactobacillus* sp.	Extracellular	[114]
Magnetite	10-50	Fine grained super paramagnetic magnetite crystals	*Geobacter metallireducens* GS-15	Extracellular/anaerobic condition	[115]
	10-40	Quasi-spherical	*Actinobacter* sp.	Extracellular	[116]
Fe_3O_4	40-50	Octahedral prism	*Aquaspirillum magnetotacticum*	Intracellular	[117]
	40 × 40 × 60	Parallel	*Magnetotactic bacterium* MV-1	Intracellular	[118]
	47.1	Cubo-octohedrons	*Magnetospirillum magnetotacticum*	Intracellular/Membrane bound	[119]
	50	Cubo-octahedral	*Magnetospirillum magnetotacticum* (MS-1)	Intracellular	[120]
Fe_3S_4, FeS_2	7.5	ND	Magnetotactic bacterium	Intracellular	[121]
FeS	2	Octahedral/ Cubo-octahedral	Sulfate-reducing Bacteria	Intracellular/Cell surface	[122]
	5-200	ND	*Klebsiella pneumonia*	Intracellular/Cell surface	[123]

Table 3. Nanoparticles produced by fungi.

Nanoparticle	Size	Morphology	Fungi	Synthesis pattern	Ref.
Silver (Ag)	20-60	Polydisperse/spherical	*Alternaria alternata*	Extracellular	[124]
	5-27	Spherical	*Amylomyces rouxii*	ND	[125]
	20	Spherical	*Aspergillus niger*	Extracellular	[126]
	1-20	Spherical	*Aspergillus terreus*	Extracellular	[127]
	2.5	Spherical	*Aspergillus terreus* CZR-1	Extracellular	[128]
	10-100	Spherical	*Cladosporium cladosporioides*	ND	[129]
	25-75; 444-491	Spherical/ellipsoidal	*Coriolus versicolor*	Intra- and extracellular	[130]
	5-50	Spherical	*Fusarium oxysporum*	Extracellular	[131]
	10-80	Spherical	*Fusarium semitectum*	Extracellular	[132]
	16.23	Spherical	*Fusarium solani* (USM-3799)	ND	[133]
	5-40	Spherical	*Macrophomina phaseolina*	Cell-free filtrate	[134]
	58.35 ± 17.88	ND	*Penicillium brevicompactum* WA2315	ND	[135]
	25 ± 2.8	Spherical	*Penicillium nagiovense* AJ12	Cell-free filtrate	[136]
	5-25	Spherical	*Penicillium fellutanum*	Extracellular	[137]
	10-100	Mostly spherical	*Penicillium strain* J3	ND	[138]
	5-200	Pyramidal	*Phanerochaete chrysosporium*	ND	[139]
	60-80	Spherical	*Phoma glomerata*	ND	[140]
	35-48	Polydisperse and spherical	*Rhizopus nigricans*	ND	[141]
	25-30	Quasi-spherical	*Rhizopus stolonifer*	ND	[142]
	5-50	ND	*Trichoderma reesei*	Extracellular	[143]
	5-40	ND	*Trichoderma viride*	Extracellular	[144]
	13-18	Nanocrystalline	*Trichoderma asperellum*	Extracellular	[145]

Table 3. (cont.)

Nanoparticle	Size	Morphology	Fungi	Synthesis pattern	Ref.
	12 ± 5	Spherical, triangular, hexagonal	*Alternaria alternate*	Extracellular	[146]
	24.4 ± 11	Triangular, spherical and hexagonal	*Aspergillus clavatus*	Extracellular	[147]
	50-500	Spherical, Nanoplates, Nanowalls, spiral plates, aggregates			
	10-20	Polydispersed	*Aspergillus niger*	Extracellular	[148]
	12.8 ± 5.6	Spherical, Elliptical			
	10-60	Various shapes, mostly spherical	*Aspergillus oryzae var. viridis*	cell-free filtrate (biomass), Mycelial surface	[149]
Gold (Au)	8.7-15.6	Spherical	*Aspergillus sydowii*	Extracellular	[150]
	29 ± 6	Spherical	*Aureobasidium pullulans*	Intracellular	[151]
	60-80	Non spherical	*Candida albicans*	Cell-free extract	[152]
	10-60	Spherical, triangular and hexagonal	*Penicillium brevicompactum*	Extracellular	[153]
	20-80	Spherical, triangular, hexagonal	*Penicillium rugulosum*	ND	[154]
	30-50	Spherical	*Penicillium* sp	Cell filtrate	[155]
	15	Spherical	*Saccharomyces cerevisiae*	Cell wall, Cytoplasm	[156]
Gold/Silver (Au/Ag)	8-14	Quasi-spherical	*Fusarium oxyporum*	Extracellular	[157]
Platinum (Pt)	10-100	Rectangular, triangular, spherical hexagonal, pentagonal and squares	*Fusarium oxysporum*	Extra-and intracellular	[158]
Zinc (Zn)	100-200	Irregular, some spherical	*Fusarium* spp.	Intracellular	[159]
Mercury (Hg)	20.5 ± 1.82	ND	*Aspergillus versicolor*	Surface of mycelia	[160]
Zinc oxide	54.8-82.6	spherical	*Aspergillus terreus*	Extracellular	[161]
Fe_3O_4	20-50	Irregular, quasi-spherical AND	*Fusarium oxysporum*	Extracellular	[162]
	100-400; 20-50	Cubo-octahedral, quasi-spherical	*Verticillium* sp.	Extracellular	[162]
$PbCO_3$, $CdCO_3$	120-200	Spherical	*Fusarium oxysporum*	Extracellular	[163]
$SrCO_3$	10-50	Needlelike	*Fusarium oxysporum*	Extracellular	[164]

Table 4. Nanoparticles synthesized by Yeasts, Molds and Algae.

Nanoparticle	Size (nm)	Morphology	Yeast, molds and microalgae	Synthesis pattern	Ref.
Silver (Ag)	10-30	ND	*Desmodesmus* sp. (KR 261937)	Intracellular and extracellular synthesis	[165]
	5 to 13	Spherical shape, face centered cubic crystals	*Fusarium oxysporum*	Extracellular	[166]
	5-25	spherical shape, face centered cubic crystals	*Humicola* sp.	Extracellular	[167]
	3-35	spherical shape, highly crystalline cluster	*Scenedesmus* sp. (IMMTCC-25)	Intracellular synthesis and extracellular synthesis	[168]
Gold (Au)	25-60	spherical	*Penicillium brevicompactum* KCCM 60390	Extracellular	[153]
	5-35	spherical and triangular shape	*Tetraselmis kochinensis*	Intracellular synthesis	[169]
Zirconium (Zr)	ND	Irregular mesoporous	Yeast	ND	[170]
$Zn_3(PO_4)_2$	10-80	Rectangular	Yeast	Extracellular	[171]
Cadmium telluride (CdTe)	2.0-3.6	cubic	*Saccharomyces cerevisiae*	Extracellular	[172]
Tellurium (Te)	60.80	oval to spherical shape	*Aspergillus welwitschiae* KY766958	ND	[173]
Gold/Silver (Au/Ag)	9-25	ND	Yeast	Extracellular	[174]
Chitosan	90.8	spherical shape, amorphous structure	*Trichoderma harzianum* SKCGW008	Extracellular	[175]
ZnS	12-24	spherical	*Aspergillus flavus*	Extracellular	[176]
PbS	35-100	cubic crystal	*Aspergillus flavus*	Extracellular	[177]

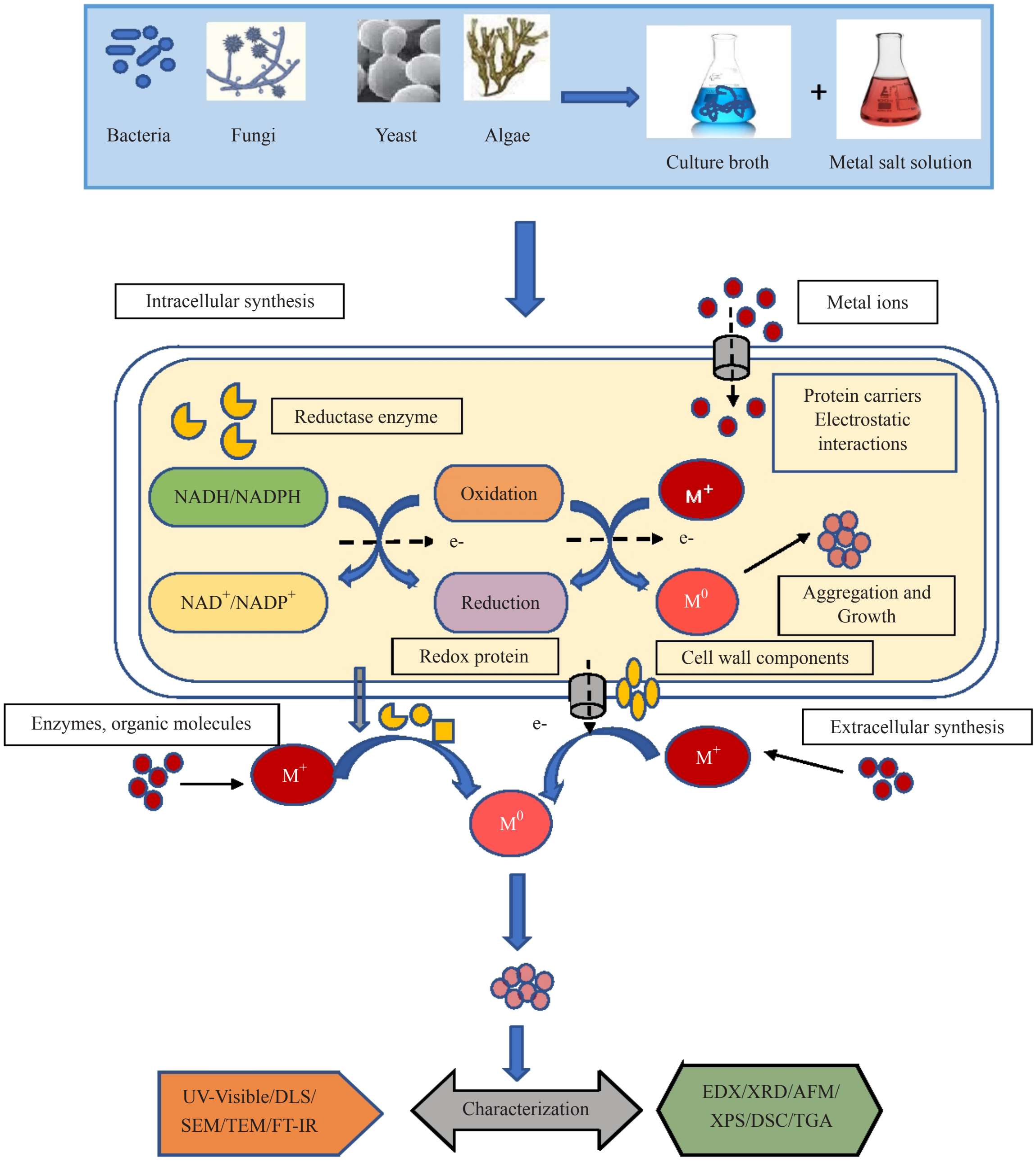


Figure 1. Intracellular and Extracellular production of Nanoparticles by microorganisms [178].

6. Techniques for characterization of nanoparticles

To pre-determine the drug nanoparticle interactions with cell surface receptors and the release properties *in vivo*, characterization techniques by microscopy and spectroscopy are required. There is no standardized procedure for a particular order of techniques to characterize nanoparticles. There is also no FDA approved regulatory protocols to characterize nanoparticles [179]. Various spectroscopic and microscopic techniques are available to assess suitable characteristics and evaluate the potential of the nanoparticles for biological applications. Recently Palani and Elangovan [180] have discussed the microbial-mediated synthesis of Cu nanoparticles and have characterized by various techniques for different kinds of applications. We list below some of the classical and recently developed characterization techniques which are the basic requirements for any nanoparticle study in the present years.

6.1 *Particle size analysis*

The nanoparticle size is a critical factor while considering its therapeutic potentials. It has been proposed that the nanoparticle-targeted therapeutic delivery can be improved by controlling the size parameter [181] which is primarily determined by the preparation technique [182]. Size of the nanoparticle is measured by dynamic light scattering (DLS) technique or photon-correlation spectroscopy (PCS). In this technique, the Brownian motion of nanoparticles in colloidal suspension is determined which is based on the nanoparticle sizes [183]. When the laser beam from the instrument hits a nanoparticle in the dispersion, light is scattered at varying intensities which depend on the different nanoparticle sizes. Thereby, size measurements are obtained using the Stokes-Einstein equation. DLS can measure particle sizes from 20-200 nm [184]. Based on their sizes, the nanoparticles may be classified as monodisperse when the sizes are uniform or polydisperse when there are size differences among the nanoparticles. The DLS technique can also measure the Polydispersity Index (PDI) which gives specific information on the aggregation behavior of nanoparticles. Lower the PDI, the lesser the nanoparticle aggregation [185]. The measurement of Nanoparticle sizes is made usually in water, a universal dispersant or in phosphate-buffered saline (PBS) in case of nanoparticles which need to be physiologically stable. However, in PBS, nanoparticles tend to aggregate which may reflect an increase in nanoparticle sizes. In the dispersant medium, it is necessary to suspend the nanoparticles prior to measurement using a bath or probe sonicator to get reasonable accuracy. The nanoparticle size may be displayed as histograms or linear graphs in nanometer scale.

6.2 *Surface charge or zetapotential measurement*

Zeta potential indicates the charge on the particle surface [186] and the extent of the surface hydrophobicity [183]. It determines interactions of nanoparticles with the surrounding physiological environment. Zeta potential indicates of the stability of the nanoparticles in colloidal suspension [187]. Zeta potential values predict the aggregation tendency of nanoparticles. Nanoparticles showing high positive or negative zeta pontential values are considerably regarded as stable without aggregation in solution [183]. Values close to $\pm$ 30 mV represent stable nanoparticle suspensions. In practical conditions, zeta potential values are usually negative and lower and indicate increased stability of the nanoparticles [187-189]. Zeta potential values can be measured using a Zetasizer instrument which evaluates particle size, zetapotential and molecular weight of the nanoparticles in suspension.

6.3 *Transmission Electron Microscopy (TEM)*

The shape and size of the nanoparticles can be evaluated using a Transmission electron microscope (TEM). Recently images can be obtained with a High Resolution (HR)-TEM. TEM provides morphological observation with an atomic scale resolution as shown in Figure 2 [3]. Characterization of nanoparticles by TEM has been the ‘gold’ standard method for all types of nanomaterials.

Biological nanoparticles are liable to be destroyed by the high vacuum condition and the strong impact of electrons which impinge on cellular structures. Hence, biologically-derived nanoparticle specimens need to be prepared by staining with Osmium tetroxide and Uranyl actetate, prior to imaging. Other nanoparticles such as carbon nanotubes or nanorods, polymeric and metallic nanoparticles require no pretreatment and can be imaged as such.

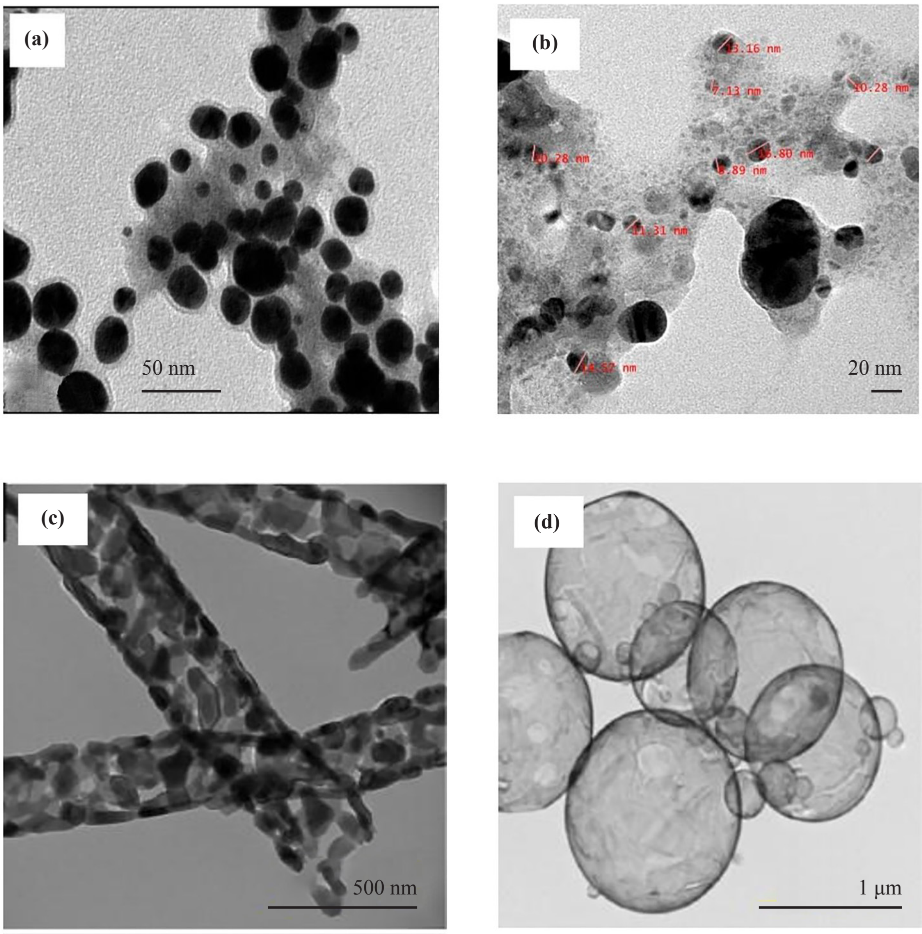


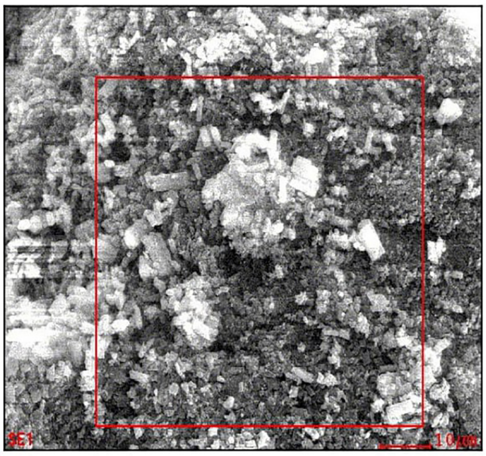
Figure 2. TEM micrographs showing differences in characteristics of (a) bacterial synthesized gold nanoparticles [3]; (b) biogenically synthesized silver nanoparticles [190]; (c) hollow TiO_2 nanotubes [191] and; (d) hollow TiO_2 nanospheres synthesized by electrospinning [191]

6.4 *Scanning Electron Microscopy (SEM)/Energy dispersive X-ray analysis (EDX)*

SEM is a versatile technique to characterize nanoparticles with respect to morphology, size and shape of nanomaterials. Similar to HR-TEM, HR-SEM provides information on the characteristic features of the nanoparticle sample by high resolution imaging. SEM images provide surface topological features with high magnification and large field depth in correlation with the surface electron density of the nanoparticles as depicted in Figure 3 [192]. SEM analysis also offers knowledge about the nanomaterial purity and the degree of aggregation [193]. An electron gun, made of Tungsten filament is used for emission of an electrons beam. In the case of a Field emission (FE)-SEM, a Field emission gun (FEG) of cold-cathode type Tungsten single crystal emitter is used [194]. In this microscopy also, biologically derived nanoparticles need pretreatment by staining with Osmium tetroxide and several steps of dehydration with gradient concentrations of alcohol, usually ethanol. After pretreatment, the nanoparticles are sputter-coated with gold and placed on a stub and imaged at appropriate magnifications. Scanning electron micrographs should be in the nanometer scale before acquiring the sample images.

EDX works in integration with SEM and cannot provide data without the SEM instrument. EDX spectrum is obtained from a SEM image and gives an account of the elemental composition of the nanomaterial analysed. This provides the accurate element identification and its percent composition present in the nanomaterial. The peaks in the EDX spectrum corresponds to the energy levels which receive more X-rays during the electron transfer process from the outer shell to the gap formed in the inner shell with lower energy level. The amount of energy released as X-rays are unique to the atoms of an element which enable the identification of the element. The peak length is proportional to the concentration of the element in the sample. However, in an EDX spectrum, H-atom cannot be identified as the amount of X-rays emitted by the H-atom is very less and it is not within the detectable range [194].

(a)



(b)

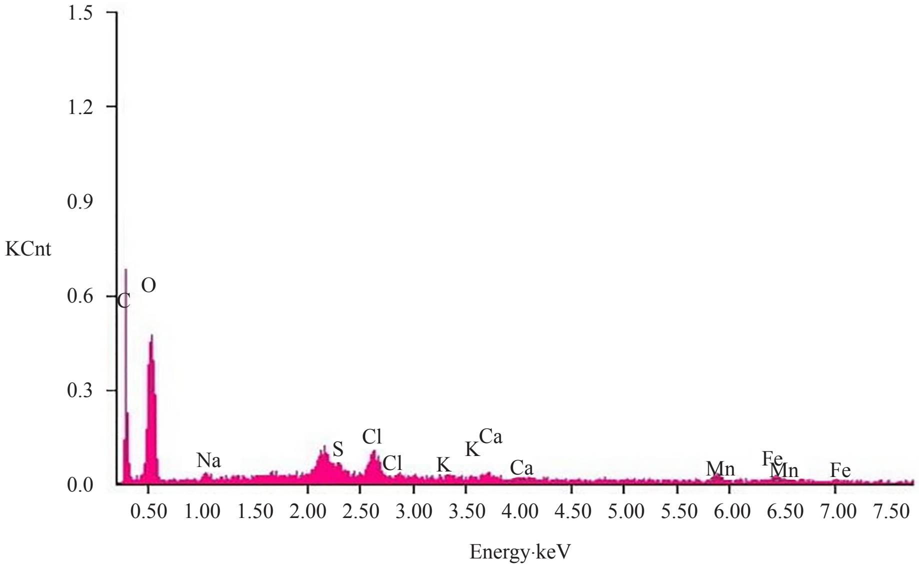


Figure 3. HR-SEM image of nanoparticles (a) and its corresponding EDX spectrum (b) showing different elemental composition (unpublished data).

6.5 *Fourier Transform-Infrared (FT-IR) spectroscopy*

An FT-IR spectrum can provide information on the molecular structure of the nanomaterial due to vibration properties of the molecules. When a molecule is exposed to infrared radiation, it absorbs infrared energy at particular frequencies which are characteristic of that molecule. Hence, based on the IR spectrum of percent transmittance againt wave number, each frequency band corresponds to a specific molecular or functional group of the nanomaterial. Hence, the chemical structure of the molecule can be identified. The IR spectrum is an inverse spectrum or is obtained as a reverse peak [194]. From the respective peaks, alkane, alkene, alkyl, phenolic, hydroxyl, benzyl and several other chemical bonds can be identified which provide a clear picture about the molecular structure in whole. FT-IR spectroscopy is therefore a valuable tool for the characterization of the nanoparticles.

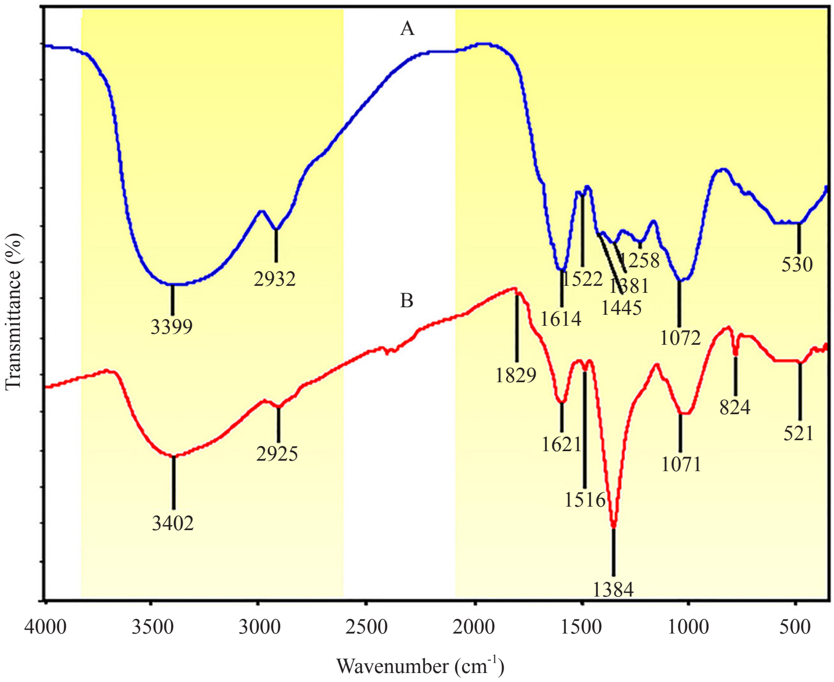


Figure 4. FT-IR spectrum of Entada spiralis extract (a) and the silver nanoparticles synthesized from the extract (b) showing differences in characteristics of the nanoparticles [195].

In addition, FT-IR spectroscopy can be used to analyse bacterial biomolecules such as organic functional groups of bacterial proteins and further provide information on capping and functionization of the metal oxide nanoparticles, presented as an example in Figure 4 [196]. Conventional FT-IR spectrum is obtained from a sample which has been derivatized with potassium bromide (KBr). This procedure is replaced in the recent years by Attenuated Total Reflectance (ATR-IR) spectroscopy which does not require prior sample preparation. Nanomaterials identified by FT-IR offer structural differences in molecular structure of biomolecules [197]. The peaks in a FT-IR spectrum are attributed to the biological components present in the synthesis of nanoparticles. The differences in the particle size produce different wavenumber and frequencies in the spectrum [198].

6.6 *Atomic Force Microscopy (AFM)*

AFM also referred as Scanning Probe microscopy (SPM) has been used significantly to study surface morphology at nanometer resolution and for force measurements. Since the advent of Nanotechnology in early 1990's, AFM has been used as an important technique for the characterization of nanomaterials to provide information on the size and surface morphology of nanoparticles in both 2- and 3-dimensional images. As an example, a three-dimensional AFM image of nanoparticles with accurate size measurement is shown in Figure 5. AFM uses a probe tip of atomic scale and the attractive or repulsive forces between the tip and the sample surface is measured [198]. AFM provides information on topography, sample size, size distribution, shape and aggregation state of nanoparticles, similar to SEM. AFM can be operated under various conditions such as air, liquid and vacuum [3]. Sample preparation is an important step for imaging by AFM. There are two widely used modes of AFM imaging, namely, contact mode and the tapping mode [199]. The most important advantage of AFM is that it is non-destructive and requires no sample treatment for analysis. Therefore, biologically derived nanomaterials can be investigated by AFM. Compared to DLS and SEM, AFM gives an accurate measurement of the size of the nanoparticle. The advantage of AFM over SEM and TEM is that it analyses 3-D images and can calculate particle height and volume [200]. While DLS, and SEM provide a higher value of nanoparticle size, AFM provides exact size value of the sample studied [183]. Considering TEM, the 'gold'standard for nanoparticle analysis, AFM replaces this technique as a more sophisticated tool in the characterization of nanomaterials.

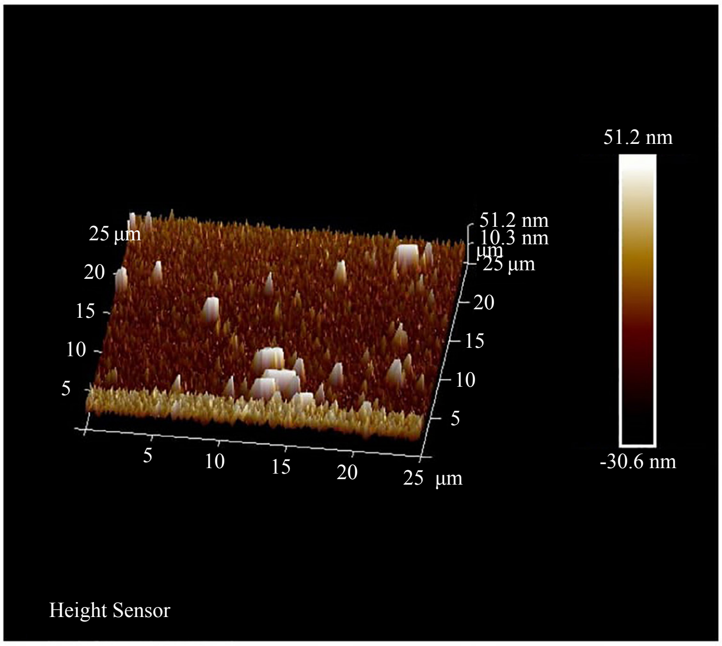


Figure 5. The 3-D AFM image of nanoparticles showing accurate size measurement of 51.2 nm (unpublished data).

7. Antimicrobial applications of nanoparticles

Since 1500 B.C., metals such as copper salts have been used as antibacterial agents [201]. With the advent of Nanotechnology, the use of metal and metal oxide nanoparticles in antimicrobial applications in the biomedical and industrial fields have gained increased attention. Metallic nanoparticles which are highly ionic are desired candidates due to their increased surface areas and a number of reactive sites with unusual crystal morphological structures [202]. The significant features of using metallic nanoparticles for antimicrobial applications are an increase in antibacterial and antifungal activities [203], functionality [201], extended antimicrobial activity at minimal dosages and broad-spectrum inhibitory activity due to specific dimensions and shapes [204]. Metallic and metal oxide nanoparticles represent the most studied antimicrobial nanoparticles to date [191]. During the past two decades, different types of metal and metal oxide nanoparticles have been tremendously used for antimicrobial applications such as silver, copper, zinc oxide, titanium oxide, copper oxide and nickel oxide nanoparticles with differences in antimicrobial activities based on composition, methods of surface modification, intrinsic physical and chemical properties and the targeted microbial species [205]. Correa et al. [191] have observed that the antimicrobial agents can be classified as bacteriocidal if the percent lethality was above 90% at 6h and bacteriostatic if the percent lethality was below 90% at 6 h which can be considered as the basis of determining the efficiency of antimicrobial agents.

Silver nanoparticles are the most extensively studied metal nanoparticles and are an interesting class of antimicrobial nanoparticles for applications in pharmaceutical, medical, food packaging and textile industries and water treatment plants [206]. The particle size of nanoparticles is also considered as a significant factor affecting the antibacterial activity [207]. Monodispersed and smaller sized CuO nanoparticles showed increased antibacterial activity against both Gram-negative and Gram-positive bacterial strains. It has been demonstrated that spherical Cu nanoparticles exhibited strong bactericidal activity against Gram negative as well as Gram positive bacteria [208]. Further studies showed that irradiation of TiO_2 nanospheres with UV-A rays for 60 min increased its antibacterial activity towards methicillin-resistant *Staphylococcus aureus* strains than the non-irradiated commercial TiO_2 nanoparticles [209]. The shape of the metallic nanoparticles also affects antimicrobial potential. CuO nanorods showed good antimicrobial activity against *E.coli*, *S.flexneri* and *S.aureus* [210]. Cu nanoparticles have demonstrated better antibacterial activity than silver and gold nanoparticles [211]. These nanoparticles exhibited broad-spectrum antibacterial activities against *S.aureus*, *Salmonella enteric*, *Campylobacter jejuni*, *E.coli* and *Listeria monocytogenes* [212]. Silver nanoparticles showed high antibacterial activities againt Gram-negative *E.coli* and Gram-positive *Micrococcus luteus* bacterial strains with zones of inhibition of 5.5 ± 0.2 mm to 6.5 ± 0.3 mm and 7.0 ± 0.4 mm to 7.7 ± 0.5 mm respectively [213]. The antimicrobial activity of Cu nanoparticles enhanced in a composite of carbon nanotubes using multi-walled carbon nanotubes (MWCNT) which increased the surface area of Cu nanoparticles with a subsequent reduction in bacterial colonies of *E.coli* strain than with Cu nanoparticles alone. The percent kill of bacterial colonies were also 75% ± 0.8 with Cu-MWCNT nanoparticles while only 52% ± 1.8 was observed for Cu nanoparticles alone [214].

The synergistic activities of two metals as bimetallic nanoparticles can be exploited for their cumulative biological potentials in antimicrobial applications. These nanoparticles are highly reactive and exert strong interactions [215]. In comparison to 25% and 50% antimicrobial efficiencies of gold and silver nanoparticles, about 80% antimicrobial efficiency was obtained with bimetallic nanoparticles against *Candida albicans*, *S.aureus* and *P.aeruginosa* [216]. TiO_2/ZnO nanoparticles supported into 4A zeolite possessed optimum antibacterial activities with *S.aureus*, *P.fluorescens*, *Listeria monocytogenes* and *E.coli* O57:H7 bacterial sp. The doping of A4 zeolite forming a nanocomposite with TiO_2/ZnO nanoparticles resulted in controlled release of nanoparticles which increased the antibacterial efficiency against *E.coli* O157:H7 strain with the highest zone of inhibition of 10.73 ± 0.04 mm [217]. A nanocomposite of ZnO-CuO containing fluoride ions exhibited good antibacterial activity against *S.mutans* which could possibly find application for preventing bacterial growth in dental implants [218].

Drug resistance of microorganisms to antibacterial agents is of current interest as the number of pathogens resistant to several antibiotics has risen over the past years. Metal nanoparticles are the preferred choice to overcome drug resistance in microbial organisms due to their unique physical and chemical characteristics [219]. Antimicrobial nanoparticles target several metabolites and tend to reduce or eliminate the evolution of drug-resistant microorganisms [220]. The common resistance mechanisms of microorganisms that evade the antimicrobial action of antimicrobial agents are modification or inactivation of enzymes, decreased membrane permeability and overexpression of efflux pumps which efflux out antimicrobial agents. Metal nanoparticles have the ability to overcome these resistance mechanisms and promote antimicrobial action [221]. The nanoparticles when conjugated with antibiotics show synergistic effects in antibacterial activity by preventing formation of biofilm and elmininating multi-drug resistant organisms [222].

The antimicrobial actions of metal nanoparticles occur as a result of formation of ROS, membrane permeability, metal ion release, inhibition of protein function, DNA damage and changes in expression of metabolic genes of different types of microorganisms. Studies have demonstrated that metal nanoparticles exert increased antibacterial activity towards Gram-positive bacteria than Gram-negative bacteria because of differences in cell wall structure and the negative charges which which cause strong or slight attraction of the nanoparticles and lead to collapse of cell wall structure resulting in cell death [191]. However, a study on silver nanoparticles reflected that antimicrobial activity was not affected by bacterial cell structure and it showed similar antibacterial effects on both Gram-positive and Gram-negative bacteria [213]. In the case of Zirconium nanoparticles, direct contact of nanoparticles by adhesion to bacterial cell membrane resulted in penetration of the nanoparticles into the cells followed by ion-mediated killing of the bacterial cells [223].

8. Conclusions

Nanoparticles are endowed with unique characteristics than their bulk counterparts due to the increased surface area and improved electrical, electronic and optical properties which make them ideal candidates for varied applications. Biological synthesis of nanoparticles is often desired from plants and microorganisms as these processes are less energy consuming, produce reduced harzardous wastes and are environmentally friendly. In this review, we have discussed the historical aspects of Nanotechnology and its early developments and have focused on the different microorganisms which produce nanoparticles by 'green' technology. The important characteristics of the nanoparticles produced by the bacterial and fungal microorganisms have been tabulated from previous literature. In addition, the different characterization techniques for assessing nanoparticles are briefly described. The developments in the preparation methods of metal nanoparticles has led to their applications as antimicrobial agents in various sectors. It is expected that there will be a strong demand for metallic nanoparticles as antimicrobial agents in the future which prompt a lot of investigations of antimicrobial nanoparticles. Further, the rise in pathogenic infections due to drug resistance require new and efficient metallic and metal oxide nanoparticles to be explored for use in antibacterial surfaces in the medical sector. It should also be considered that safety issues comply with the random use of metallic nanoparticles in various applications as human ingestion or release into the environment may restrict the development and application of these nanoparticles. Hence, it is recommended that regulatory bodies provide appropriate safety measures in the use and discard of nanoparticles without harmful effects on human health and environmental concerns.

Acknowledgements

The authors would like to acknowledge Dr. Valli Nachiyar for her guidance and advice during all the stages of writing this article. The authors also thank the Director, Centre for Nanoscience and Technology, Anna University, Chennai and the HOD of Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai for their kind consent to publish this review.

Conflict of interest

The authors declare no competing financial interest.

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