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# Microbially synthesized nanoparticles as next generation antimicrobials: scope and applications

# 15

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## 15.1 INTRODUCTION

The development of multidrug resistant (MDR) infectious microbes is a major challenge to the healthcare system. Today, more than 70% of bacteria responsible for causing infectious disease are resistant to atleast one of the antibiotics

employed in conventional antimicrobial therapy (Samanipour et al., 2016). Moreover, 80% of microbial infections in humans are caused by biofilm-forming pathogenic organisms. With the growing concern of MDR strains and associated biofilm, attention has been devoted to the development of new and effective antimicrobial agents. In the present scenario, the rapidly growing field of nanotechnology is considered to open new windows to combat and prevent microbial disease. Owing to their high surface area to volume ratio and unique chemical properties, the nanomaterials have emerged as a reliable candidate as a potential anti-infective. Among the nanomaterials, metal and metal oxide nanoparticles exhibiting antimicrobial properties serve as a promising tool in controlling MDR strains.

Many chemical and physical methods are employed for the preparation of metal nanoparticles. However, the use of toxic and expensive chemicals greatly limits its biomedical applicability. On the other hand, a broad range of microorganisms have been studied as potential biofactories for eco-friendly and cost-effective production of metallic nanoparticles, such as silver, titanium oxide, cadmium sulfide, gold, etc. Thus, research in microbial nanotechnology provides a reliable way for large-scale synthesis of nanomaterials (Barapatre et al., 2016). The use of metallic preparations of gold, copper, silver, iron, and lead, as well as alloys in healthcare, can be traced back to ancient civilizations. Microfine metallic powders were used for both external and internal applications, in a wide variety of diseases. Traditionally, gold has been used as an antipruritic medication to suppress itching palms. It influences the immunological response and controls the anaphylactic release of histamine. Iron compounds act as a rejuvenator and as a restorative agent. They are particularly administered in diseases such as iron-deficiency anemia. They also stimulate the functional activity of all the organs and combat a number of diseases (Galib et al., 2011). Silver in various forms including silver metal, silver nitrate, silver sulfadiazine, silver acetate, and silver protein are well known for their antimicrobial properties (McDonnell and Denver Russell, 1999). Copper exerts broad-spectrum biocidal effects on bacterial cells and spores, fungi, and viruses. It damages the microbial envelope, membrane phospholipids, intracellular proteins, and nucleic acids. It is widely recommended in the treatment of ulcers, skin conditions, syphilis, and tuberculosis (Borkow, 2014). Zinc and its salt forms have been employed as therapeutic agents for centuries in topical as well as oral treatment. They are recommended in a number of dermatological disorders and infections including warts, cutaneous leishmaniasis, leprosy, inflammatory dermatoses, melasma, and basal cell carcinoma. Oral zinc, when given with dapsone (adjuvant), results in significant bacterial clearance in patients with lepromatous leprosy. The use of zinc acetate gel, *in vivo*, was found to prevent the sexual transmission of HSV-2 and HIV infection. Due to its anti-biofilm activity on dental plaque forming microorganisms, zinc citrate or acetate are blended in many oral products, including toothpaste (Gupta et al., 2014).

In the last decade, a strong emphasis has been given on the research and applications of nanotechnology to bring advancement in the area of diagnosis and

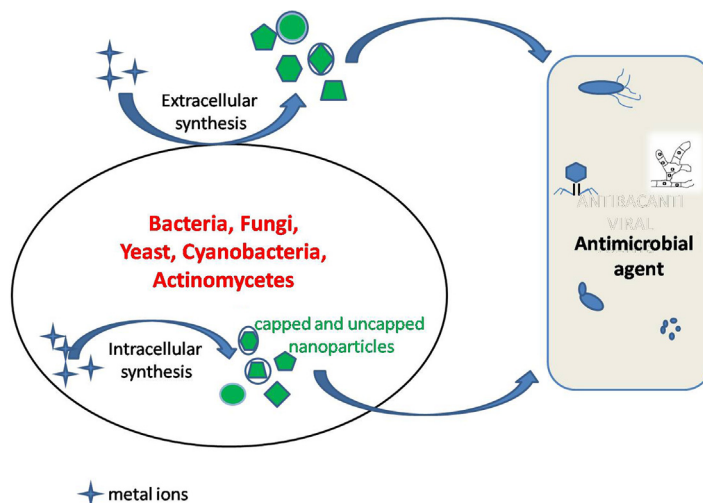
management of diseases. Nanoparticles of size range 100 nm and less have become an area of extensive research due to their antimicrobial properties attributed to their large surface area. Metal nanoparticles like gold, copper, titanium, silver, and zinc nanoparticles have attracted tremendous attention due to their unique physiochemical characteristics, which include antimicrobial, catalytic, electronic, optical, and magnetic properties. Hence, nanoparticles currently play an important part in diagnostics, drug delivery, gene therapy, and tissue engineering (Hamouda, 2012). With the integration of nanoparticles in the development of new health and pharmaceutical products, it is essential to develop eco-friendly and biocompatible techniques for the production of metal nanoparticles. The use of plant extracts, enzymes, bacteria, fungi, and algae provides an environmentally safe route for the production of nanoparticles. The main focus of nanotechnology is to synthesize monodispersed nanoparticles with predictable shape, size, and polymeric coating for potential biomedical applications (Sharma et al., 2015). Metal nanoparticles with inherent antimicrobial property or when coated with an antimicrobial entity on the surface increase their applicability in biomedical devices, textile industries, water treatment, and food packaging. The nanocomposites constituting of metal nanoparticles and polymers provided enhanced synergistic antimicrobial activity (Gutierrez et al., 2010).

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## 15.2 SYNTHESIS OF METAL NANOPARTICLES USING MICROORGANISMS

Large quantities of metal nanoparticles with defined shapes and sizes are synthesized rapidly using various chemical and physical methods, however, being energy intensive and the use of toxic chemicals as the starting material limits its biomedical applicability. These methods are complicated and result in the release of toxic byproducts that are hazardous to the environment and human health (Li et al., 2011). Therefore, an environmentally benign and nontoxic method for nanoparticle synthesis is required to expand its therapeutic applications. Researchers in the field of nanotechnology and nanomedicine have turned to biological systems. In this regard, the microbial cells are considered as promising biofactories for the synthesis of metal nanoparticles. In the past decade, several research groups have successfully synthesized inorganic nanoparticles such as gold, iron, silver, lead, and calcium, either extracellularly or intracellularly using microorganisms like bacteria, cyanobacteria, fungi, actinomycetes, yeast, and algae. A few of the notable examples are shown in Fig. 15.1.

Due to their abundance in the diverse environment and remarkable adaptability, bacteria are most extensively employed for the synthesis of metal-based nanoparticles. The ease of manipulation and control of physical and chemical growth conditions is also an added advantage for the use of bacteria as nanofactories. *Bacillus subtilis*, *Klebsiella pneumonia*, *Escherichia coli*, *Lactobacillus* sp., and



**FIGURE 15.1**

Schematic representation showing microorganism mediated synthesis of metal-based nanoparticles and its antimicrobial application.

*Brevibacterium frigiditolerans* have been employed for the synthesis of spherical Ag-NPs, extracellularly (Velmurugan et al., 2014; Kalpana and Lee, 2013; Gurunathan et al., 2009). Manivasagan et al. (2013) reported the synthesis of bio-fabricated Ag-NPs in size range of 30–90 nm using *Nocardia* sp. MBRC-1. Intracellular synthesis of Ag-NPs was observed when *Ochrobactrum* sp. was used for reduction (Thomas et al., 2014). A similar intracellular synthesis was observed in the synthesis of Au-NPs by *Brevibacterium casei* (Kalishwaralal et al., 2010). A few of the metal-based nanoparticles synthesized using bacteria have been provided in Table 15.1.

A wide range of fungal species have also been reported for the biosynthesis of metal and metal oxide particles (Table 15.2). The large surface area of the fungal mycelium aids in the production of nanoparticles in larger quantity. The advantages of the use of fungi in biosynthesis also lie in the ease of scale-up and downstream processing. Extracellular enzymes and proteins produced by fungi, in some cases, may also act as a reducing and capping agent in the synthesis of nanoparticles.

Algae produce a wide variety of biomolecules and hence are gaining substantial interest in the extracellular synthesis of nanoparticles. A few examples of algal mediated synthesis of nanoparticles are depicted in Table 15.3.

The enzymatic process serves as an easy and inexpensive way to catalyze and synthesize nanoparticles in an aqueous medium at normal temperature, pH, and pressure (Zhang et al., 2011). Numerous microorganisms are reported to produce metallic nanoparticles with properties identical to the ones synthesized chemically

**Table 15.1** List of Metal and Metal Oxide Nanoparticles Synthesized Using Bacteria

Nanoparticles	Bacteria	Size (nm)	Location	Duration	Morphology	References
Ag-NPs	<i>Bacillus subtilis</i> EWP-46	10–20	Extracellular	12 h	Irregular, capped	Velmurugan et al. (2014)
Ag-NPs	<i>Klebsiella pneumonia</i>	15–37	Extracellular	72 h	Spherical, rod, capped	Kalpna and Lee (2013)
Ag-NPs	<i>Escherichia coli</i>	10–90	Extracellular	30 min	Spherical, capped	Gurunathan et al. (2009)
Ag-NPs	<i>Nocardioopsis</i> sp. MBRC-1	30–90	Extracellular	24 h	Spherical, capped	Manivasagan et al. (2013)
Ag-NPs	<i>Ochrobactrum</i> sp.	38–85	Intracellular Extracellular	24 h	Spherical	Thomas et al. (2014)
Ag-NPs	<i>Lactobacillus casei</i> subsp. <i>casei</i>	25–100	Intracellular	12–13 h	Spherical, capped	Korbekandi et al. (2012)
Ag-NPs	<i>Brevibacterium frigoritolerans</i> DC2	50–100	Extracellular	48 h	Spherical	Singh et al. (2015)
Au-NPs	<i>Rhodospseudomonas capsulata</i>	10–500	Extracellular, capped	48 h	Triangular nanoplates	He et al. (2008)
Au-NPs	<i>Deinococcus radiodurans</i>	50–60	Intracellular Extracellular, capped	8 h	Spherical, triangular	Li et al. (2016)
Au-NPs	<i>Geobacillus</i> sp. strain ID17	5–50	Intracellular, capped	16 h	Quasi-hexagonal	Correa-Llanten et al. (2013)
Au-NPs	<i>Marinobacter pelagius</i>	2–10	Intracellular, capped	24 h	Spherical, triangles	Sharma et al. (2012)
Au-NPs	<i>Brevibacterium casei</i>	10–50	Intracellular, capped	24 h	Spherical	Kalishwaralal et al. (2010)
Au-NPs	<i>Thermoscotoductus</i> SA-01	—	Extracellular Intracellular	8 h	Triangular, hexagonal	Erasmus et al. (2014)
Au-NPs	<i>B. subtilis</i>	7.6 ± 1.8	Extracellular Intracellular	1 day	—	Reddy et al. (2010)
Au-NPs	<i>Shewanella oneidensis</i>	2–50	Extracellular, capped	48 h	Spherical	Suresh et al. (2011)
TiO <sub>2</sub> -NPs	<i>Lactobacillus</i> sp.	8–35	Extracellular	—	Spherical	Jha et al. (2009)
ZnO-NPs	<i>Aeromonas hydrophila</i>	57.72	Extracellular capped	12–48 h	Spherical, oval	Jayaseelan et al. (2012)
Fe <sub>3</sub> O <sub>4</sub>	<i>S. oneidensis</i>	40–50	Extracellular	—	Rectangular, rhombic, hexagonal	Perez-Gonzalez et al. (2010)
Fe <sub>2</sub> O <sub>3</sub>	<i>S. oneidensis</i> MR-1	30–43	Intracellular	—	Pseudo-hexagonal rhombohedral	Bose et al. (2009)

**Table 15.2** List of Metal and Metal Oxide Nanoparticles Synthesized Using Fungi

Nanoparticles	Fungi	Size (nm)	Location	Duration	Morphology	References
Ag-NPs	<i>Alternaria alternata</i>	20–60	Extracellular	4 h	Spherical capped	Gajbhiye et al. (2009)
Ag-NPs	<i>Fusarium semitectum</i>	10–60	Extracellular	24 h	Spherical capped	Basavaraja et al. (2008)
Ag-NPs	<i>Humicola</i> sp.	5–25	Intracellular	96 h	Spherical capped	Syed et al. (2013)
Ag-NPs	<i>Fusarium oxysporum</i>	3.4–26.8	Extracellular	5 days	Spherical	Ishida et al. (2014)
Ag-NPs	<i>Arthroderma fulvum</i>	13–18	Extracellular	1 h	Spherical	Xue et al. (2016)
Ag-NPs	<i>Trichoderma viride</i>	5–40	Extracellular	24 h	Spherical rod-like	Fayaz et al. (2010)
Ag-NPs	<i>Aspergillus clavatus</i>	550–650	Extracellular	24 h	Irregular capped	Saravanan and Nanda (2010)
Ag-NPs	<i>Macrophomina phaseolina</i>	5–40	Extracellular	24 h	Spherical Capped	Chowdhury et al. (2014)
Ag-NPs	<i>Coriolus versicolor</i>	10	Extracellular Intracellular	72 h	Spherical Capped	Sanghi and Verma (2009)
Ag-NPs	<i>Candida utilis</i> NCIM 3469	20–80	Extracellular	15 min	Spherical	Waghmare et al. (2015)
Ag-NPs	<i>Schizophyllum commune</i>	51–93	Extracellular Intracellular	24 h	Spherical capped	Arun et al. (2014)
Au-NPs	<i>Neurospora crassa</i>	28–32	Intracellular Extracellular	24 h	Spherical	Castro-Longoria et al. (2011)
Au-NPs	<i>A. clavatus</i>	24.4 ± 11	Extracellular	48–72 h	Triangular, spherical, hexagonal	Verma et al. (2011)
Au-NPs	<i>F. oxysporum</i>	8–40	Extracellular	72 h	Spherical, triangular	Mukherjee et al. (2002)
Au-NPs	<i>Saccharomyces cerevisiae</i>	15–20	Cell wall	< 24 h	Spherical	Sen et al. (2011)
TiO <sub>2</sub> -NPs	<i>F. oxysporum</i>	6–13	Extracellular	—	Spherical	Bansal et al. (2005)
ZrO <sub>2</sub> -NPs	<i>F. oxysporum</i>	3–11	Extracellular	—	Spherical	Bansal et al. (2004)
ZnO-NPs	<i>Candida albicans</i>	15–25	Extracellular	15 h	Quasi spherical	Shamsuzzaman et al. (2013)

**Table 15.3** List of Metal and Metal Oxide Nanoparticles Synthesized Using Algae and Actinomycetes

Nanoparticles	Algae/Actinomycetes	Size (nm)	Location	Duration	Morphology	References
Ag-NPs	<i>Spirogyra varians</i>	~ 17.6	Extracellular	20 min	Quasi-spheres, capped	<a href="#">Salari et al. (2016)</a>
Ag-NPs	<i>Cystophora moniliformis</i>	50–100	Extracellular	30 min	Spherical, capped	<a href="#">Prasad et al. (2013)</a>
Ag-NPs	<i>Pithophora oedogonia</i>	25–44	Extracellular	Few min	Cubical, Hexagonal, capped	<a href="#">Sinha et al. (2015)</a>
Ag-NPs	<i>Chlamydomonas reinhardtii</i>	5–35	Extracellular	10 h and 13 days	Round, Rectangular	<a href="#">Barwal et al. (2011)</a>
Ag-NPs	<i>Caulerpa racemosa</i>	5–25	Extracellular	3 h	Spherical, Triangular	<a href="#">Kathiraven et al. (2015)</a>
Ag-NPs	<i>Streptomyces</i> sp. LK3	5 ± 3.9	Extracellular	96 h	Spherical, Disk	<a href="#">Karthik et al. (2014)</a>
Au-NPs	<i>Streptomyces viridogens</i> strain HM10	8–20	Intracellular	24 h	Spherical rod	<a href="#">Balagurunathan et al. (2011)</a>
Au-NPs	<i>Streptomyces</i> sp.	90	Extracellular	—	Cubical	<a href="#">Vinay Gopal et al. (2013)</a>
Au-NPs	<i>Sargassum muticum</i>	5.42 ± 1	—	30 min	Spherical capped	<a href="#">Namvar et al. (2015)</a>
Au-NPs	<i>Tetraselmis kochinensis</i>	5–35	Intracellular	—	Spherical Triangular	<a href="#">Senapati et al. (2012)</a>
Au-NPs	<i>Stoechospermum marginatum</i>	18.7–93.7	—	10 min	Spherical, triangle hexagonal	<a href="#">Rajathi et al. (2012)</a>
Au-NPs	<i>Ecklonia cava</i>	30 ± 0.25	Extracellular	1 min	Spherical Triangular	<a href="#">Venkatesan et al. (2014)</a>
ZnO-NPs	<i>Anabaena</i> strain L31	80	Extracellular	—	Spherical hexagonal	<a href="#">Singh et al. (2014)</a>
CuO-NPs	<i>Bifurcaria bifurcata</i>	5–45	Extracellular capped	24 h	Spherical	<a href="#">Abboud et al. (2014)</a>
ZnO-NPs	<i>S. muticum</i>	30–57	Extracellular capped	—	Hexagonal	<a href="#">Azizi et al. (2014)</a>



or physically, at ambient temperature, pH, and pressure. However, it is important to study and elucidate the biochemical pathways of the microbes that lead to metal ion reduction and formation of nanoparticles, to exercise a stringent control over size, shape, and constitution of the particles (Gericke and Pinches, 2006). The microorganisms mediated biosynthesis of nanoparticles depends on numerous physical parameters and culture conditions. Hence, standardization of these conditions is necessary for higher yield of nanoparticles with desirable properties. The size of the particle may be controlled by the stringent control of parameters such as temperature, pH, salt content, and time of exposure. For instance, the synthesis medium containing 5 mM of  $\text{AgNO}_3$ , reaction temperature of  $60^\circ\text{C}$ , and pH 10 resulted in the synthesis of Ag-NPs, within in 30 minutes using culture supernatant of *E. coli*. Further, by varying the  $\text{AgNO}_3$  concentration, temperature, and pH the average particle size was controlled between 10 and 90 nm (Li et al., 2011).

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### 15.3 PROPERTIES OF NANOPARTICLES: SIZE AND MORPHOLOGY

Nanoparticles constitute materials with varying dimensions and range in size (in diameter) from a few nanometers to less than 100 nm. Unlike the bulk materials possessing constant physical properties, the nanoparticles exhibit unusual properties different from their bulk counterparts. The surface plasmon resonance and the large surface to volume ratio contribute to the unusual properties of the nanoparticles. The electronic, optical, magnetic, and mechanical properties are greatly influenced by the sizes and shape of the nanoparticles. Tremendous research is directed towards characterization, fabrication, and applications of nanoparticles. The shape and size-dependent chemical and physical properties of the nanoparticles are being extensively studied for various biomedical applications (Li et al., 2011). The nanoparticles are biocompatible to most of the biomolecules and small in size, making them a possible candidate for application as biosensors, drug carriers, and in bioimaging. Another interesting application of nanoparticles in medicine is their use in antimicrobial therapy in combating MDR pathogenic microbes (Li et al., 2011).

The size, shape, and total surface area of the nanoparticles play the central role in the antimicrobial activity. Smaller size particles possessing larger surface to volume ratios are found to have greater antibacterial activity (Duran et al., 2010). The antibacterial property is also influenced by the high particle penetration and total surface area available for interacting with the bacterial cell. Ag-NPs undergo size-dependent interactions with HIV-I and also exhibit shape dependent bactericidal properties against Gram-negative bacteria like *E. coli* (Tak et al., 2015). The truncated triangular silver nanoplates exhibited a comparatively better bactericidal effect as compared with spherical and rod-shaped nanoparticles (Pal et al., 2007).

Different groups of microbes possess the ability to control the shapes and sizes of biologically synthesized nanoparticles. Nanoparticles of various sizes and particle morphologies including spherical, triangular, oval, hexagonal, etc. have been synthesized using microbes. It has been speculated that the particles synthesized intracellularly are usually smaller and monodispersed as compared with those synthesized extracellularly. The intracellular fungal mediated synthesis of gold nanoparticles resulted in nanoparticles of various morphologies and sizes (Gericke and Pinches, 2006). The particles were predominantly spherical in shape and formed in the cytoplasm of the cells. In the case of extracellular synthesis, by tuning the reaction conditions the desired shape and size may be achieved. For example, Gurunathan et al. (2009) reported the synthesis of Ag-NPs and a reduction in particle size by employing optimal a concentration of AgNO<sub>3</sub>, reaction temperature, and pH. The increase in understanding of shape and size-dependent properties of nanomaterials has motivated new developments on the biosynthesis route. Moreover, better control of size and shape has led to new developments in materials science.

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## 15.4 FABRICATION OF NANOPARTICLES

The biomedical uses of metal NPs are limited by their tendency to agglomerate and form large particles postsynthesis. This unusual characteristic of nanoparticles can be avoided by stabilizing and protecting them from self-aggregation using capping agents. Capping reduces the surface energy and maintains the size of the nanoparticles in the range below 100 nm. The choice of capping agent depends on the characteristics of the nanoparticles and its intended application. Amino acids, polypeptides, and tiopronin have been employed as the capping agent for biological applications. Further, the geometry and solubility of the nanoparticles are also influenced by the properties of this capping agent used. Stabilizing agents such as surfactants, polymers, dendrimers, and biomacromolecules are also used for capping the nanoparticles to enhance their stability and prevent further agglomeration (Perni et al., 2014).

Biosynthesis and fabrication of metal and metal oxide nanoparticles by the aid of intracellular and extracellular functional groups and enzymes from plants, bacteria, fungi, algae, and viruses have recently gained much interest (Siddiqi and Husen, 2016). Bacteria such as *Rhodospseudomonas capsulate*, *Deinococcus radiodurans*, *Geobacillus* sp., *B. subtilis*, and *B. casei* have been documented for the synthesis and capping of Au-NPs (He et al., 2008; Li et al., 2016; Correal-Llanten et al., 2013; Reddy et al., 2010; Kalishwaralal et al., 2010). Perni et al. (2014) documented the synthesis of L-cysteine capped Ag-NPs using cell extract of *E. coli*. The Ag-NPs prepared exhibited remarkable antibactericidal activity against *Staphylococcus aureus* and *E. coli*. Spherical shaped Ag-NPs were prepared using exopolymer isolated from the culture supernatant of *Ochrobactrum*

*rhizosphaerae*. The exopolymer responsible for synthesis and capping of the Ag-NPs was characterized and found to be glycolipoprotein (GLP) in nature. The purified exopolymer was successfully employed to prevent agglomeration and maintain the stability of the Ag-NPs. Further, significant antibacterial activities of the GLP–AgNPs conjugate, more prominent than antibiotic (ciprofloxacin), was observed against *Vibrio cholera*. Owing to their small size and high surface-to-volume ratio, the GLP–AgNPs ruptured the cell wall and caused cell lysis in the test pathogen, *V. cholera* (Gahlawat et al., 2016)

Metal and metal oxide nanoparticles have been also been extracellularly produced and fabricated using algal species such as *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, *Fucus vesiculosus*, *Ecklonia cava*, *Spirulina platensis*, *Oscillatoria willei*, *Sargassum muticum*, *Stoechospermum marginatum*, etc. (Siddiqi and Husen., 2016). Iron oxide nanoparticles of  $18 \pm 4$  nm size were prepared using an aqueous extract of the brown alga, *S. muticum* at 25°C. The reduction in the FeCl<sub>3</sub> to Fe<sub>3</sub>O<sub>4</sub> nanoparticles was brought about by the polysaccharides present in the algal extract (Mahdavi et al., 2013). In some cases of fungal mediated production of nanoparticles, the biosynthesis of nanoparticles is followed by fabrication of the nanoparticles by stabilizing/capping agents. The capping agents are usually the extracellular proteins from fungal mycelia. The presence of natural capping agents terminates the postproduction steps of capping required for medical applications of the nanoparticles. Silver nanoparticles are well studied and reported for their profound antimicrobial property on several multidrug-resistant bacteria. The extracellular biosynthesis of Ag-NPs using cell-free filtrate of phytopathogenic fungus, *M. phaseolina* (Tassi) Goid was reported by Chowdhury et al. (2014). The synthesized Ag-NPs nanoparticles were found to be naturally coated and stabilized by 85 kDa protein. Arun et al. (2014) reported the extracellular and the intracellular synthesis of biofabricated Ag-NPs by a mushroom fungus, *Schizophyllum commune*, when grown in culture medium supplemented with AgNO<sub>3</sub>. The Ag-NPs exhibited remarkable antibacterial and anti-dermatophytic activity against *Pseudomonas* sp., *B. subtilis*, *K. pneumonia*, *Trichophyton mentagrophytes*, *E. coli*, *Trichophyton simii*, and *Trichophyton rubrum*. Similarly, Jain et al. (2011) demonstrated a cost-effective and eco-friendly synthesis of biofabricated Ag-NPs using the cell-free extract of *Aspergillus flavus* NJP08. The presence of extracellular capping protein on the Ag-NPs was revealed using Fourier transform infrared (FTIR) spectroscopy. The SDS–PAGE profiles showed the presence of two intense bands of 32 and 35 kDa confirming the presence of extracellular proteins, which might be responsible for the synthesis and stabilization of Ag-NPs. A cost-effective extracellular synthesis of nano bowl-shaped silver nanoparticles using *Trichoderma viride* was documented by Chitra and Annadurai (2013). The presence of two protein bands of 45 and 39 kDa indicated the presence of capping agent on the Ag-NPs. Further, the synthesized Ag-NPs exhibited a considerable bactericidal activity against *Klebsiella planticola* (2277) and *B. subtilis* (3053).

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## 15.5 ANTIMICROBIAL ACTIVITY OF METAL-BASED NANOPARTICLES

The control of infectious diseases has become a major challenge to the healthcare system due to the rise in the number of microorganisms resistant to the conventional antibiotics. In recent times, the advancement in the field of nanotechnology presented the metallic NPs with wide antimicrobial functionality with a profound influence on human health. Nanoparticles (NPs) of a dimension ranging from 10 to 1000 nm exhibit unique physiochemical properties and biological activities. Microbially synthesized metal and metal oxide nanoparticles use multiple mechanisms to kill and/or inhibit the growth of pathogenic microbes, thereby making them promising antimicrobial agent to revolutionize the currently available antimicrobial therapy. Different metallic NPs, including gold (Au), silver (Ag), titanium (Ti), zinc (Zn), copper (Cu), and magnesium (Mg), are known for their efficient antimicrobial properties (Schrofel et al., 2014). These NPs are stable when stored for long duration and can withstand harsh processing conditions including high temperature and pH, without getting inactivated (Ranghar et al., 2014).

The shape and size of nanoparticles greatly influence its antimicrobial activity. Particles with size ranging from 1 to 10 nm have shown to possess higher bactericidal activity compared with its larger counterparts. As a result, small size with improved biocompatibility is widely used in the biomedical field (Allaker and Memarzadeh, 2014). Moreover, NP acts on a wide range of microbial targets to exert their antimicrobial mechanisms. They may directly disrupt the cell membrane or indirectly damage the cellular components by generating free radicals. The free radicals generated in turn cause damage and inhibit the synthesis of DNA, protein, and other cellular components. Nanoantimicrobial agents serve as a cost-effective and nontoxic alternative to the conventional antibiotics in controlling infectious diseases. Metal and metal oxide NPs provides new hope in fighting infectious diseases caused by MDR stains. In addition, nanoparticles loaded with antibiotics when efficiently administered improve the pharmacokinetics and therapeutics of the drug. Some of the metal and metal oxide NPs exhibiting antimicrobial property are described below.

### 15.5.1 SILVER NANOPARTICLES (AG-NPS)

Since time immemorial, the medicinal properties of silver have been recognized and silver-based formulations have been employed in the treatment of various injuries, burn wounds and infections. Silver salts and their derivatives are well reported for their broad spectrum antagonistic activity against fungi, bacteria, and viruses. According to the recent studies, Ag-NPs have been suggested as a potential medium for delivery of antimicrobial agents, in disinfecting filters and as coating materials (Ravishankar and Jamuna, 2011).

Several mechanisms were proposed to justify the antimicrobial property of Ag-NPs. It is believed that the positive charge of  $\text{Ag}^+$  ion of the Ag-NPs causes electrostatic interaction with the negatively charged bacterial cell membrane, thereby contributing to its antimicrobial property (Kim et al., 2007). It is also suggested that Ag-NPs exhibit high affinity for the sulfur-containing protein present inside the cells as well as in the bacterial membrane. Subsequently, the bacterial cell in contact with Ag-NPs takes up the  $\text{Ag}^+$  ions, thereby resulting in inhibition of the cellular functions and respiratory enzymes (Kim et al., 2007; Ravishankar and Jamuna, 2011). Ag-NPs affect the permeability of the plasma membrane and cell viability (Chaloupka et al., 2010). It was also suggested that the  $\text{Ag}^+$  ion released from Ag-NPs interacts with the phosphorus-containing elements such as DNA, causing a damaging effect on DNA replication and protein synthesis (Ravishankar and Jamuna, 2011; Chaloupka et al., 2010; Gogoi et al., 2006). In fungi,  $\text{Ag}^+$  of the Ag-NPs inhibits DNA replication and the respiratory chain as well. At lower concentration of Ag-NPs, the  $\text{Ag}^+$  ions have been reported to inhibit respiratory chain enzymes, which resulted in uncoupled respiratory electron transport from oxidative phosphorylation. Due to the high affinity of the  $\text{Ag}^+$  for the sulfhydryl (thiol) groups present on the membrane-bound respiratory enzymes, the  $\text{Ag}^+$  interferes with the electron transport chain and transfer of energy through the membrane (Bard and Holt, 2005; Allaker and Memarzadeh, 2014). At higher concentration,  $\text{Ag}^+$  ions have been shown to cause a detrimental effect on the cytoplasmic components and nucleic acids of the cell (Dibrov et al., 2002; Lara et al., 2010). Like most of the metal NPs, Ag-NPs also have the ability to generate intracellular reactive oxygen species (ROS) in bacterial cells. They show better antimicrobial properties mediated by the production of ROS like  $\text{H}_2\text{O}_2$ , which exert metabolic stress inside the cell, subsequently causing cell death (Ravishankar and Jamuna, 2011).

Furthermore, the size and shape play a crucial part in imparting antimicrobial property to the Ag-NPs. Hence, the antibacterial activity may be regulated by controlling particle size of the Ag-NPs. The large surface–volume ratio facilitates interaction of the Ag-NPs with the cell membrane and allows easy penetration inside the cell, thereby causing complete damage to cell viability (Blecher et al., 2011). Ag-NPs of  $\sim 10$  nm a diameter readily form pores in the bacterial cell wall. Consequently, the cytoplasmic content gets released out of the cell, ultimately leading to cell death. Ag-NPs of size less than 20 nm (in diameters) increases the permeability of bacterial cells by attaching to sulfur-containing proteins of the bacterial cell membranes (Morones et al., 2005; Gogoi et al., 2006; Ravishankar and Jamuna, 2011). Ag-NPs with triangular and truncated shape readily release  $\text{Ag}^+$  and hence exhibit greater antimicrobial activity (Blecher et al., 2011).

Silver nanoparticles, either alone or in combination with other antimicrobial agents, show remarkable synergistic effects against numerous Gram-positive and Gram-negative bacterial pathogens. For instance, Ag-NPs exhibits enhanced antimicrobial effects against *E. coli* and *S. aureus* when administered in combination with amoxicillin, erythromycin, penicillin G, and vancomycin (Fayaz et al., 2010;

Blecher et al., 2011). Silver nanoparticles also exhibit antagonistic effect against drug-resistant bacteria, fungi, and viruses. The broad spectrum antimicrobial activity of Ag-NPs may be attributed to the multiple mechanisms of antimicrobial action exhibited by Ag-NPs. Lara et al. (2010) determined the bactericidal efficacy of Ag-NPs against ampicillin-resistant *E. coli*, multidrug-resistant *Pseudomonas aeruginosa*, and erythromycin-resistant *Streptococcus pyogenes*. It was revealed that the bacteriostatic action of Ag-NPs resulted due to inhibition in the cell wall, protein and nucleic acid synthesis.

A study by Nanda and Saravanan (2009) revealed that *S. pyogenes*, Methicillin-resistant *S. aureus* (MRSA), and *Staphylococcus epidermidis* (MRSE) are significantly susceptible to Ag-NPs. However, it shows a moderate antibacterial activity against Gram-negative pathogenic bacteria, *K. pneumonia* and *Salmonella typhi*. Morones et al. (2005) revealed the antibacterial efficacy of Ag-NPs against Gram-negative bacteria, *P. aeruginosa*, *S. typhi*, *E. coli*, and *V. cholera*. Feng et al. (2000) documented the morphological changes resulting in treatment with Ag-NPs in Gram-positive and Gram-negative bacteria. The cytoplasmic membrane was found to detach from cell walls and the DNA condensed in the middle of the cell. A differential antagonistic activity of Ag<sup>+</sup> observed in Gram-negative and Gram-positive bacteria resulted due to the variation in the thickness of peptidoglycan layer present in the cell wall (Sondi and Salopek-Sondi, 2004). Raheman et al. (2011) reported the extracellular synthesis of Ag-NPs by an endophytic fungus, *Pestalotia* sp., isolated from leaves of *Syzygium cumini*. The synthesized Ag-NPs exhibited antibacterial efficacy against *S. typhi* (ATCC-51812) and *S. aureus* (ATCC-25923).

Various studies have revealed the potential of Ag-NPs as antifungal agent. Ag-NPs were found to effectively inhibit the growth in numerous fungal pathogens such as *Aspergillus fumigatus*, *Candida albicans*, *Cladosporium cladosporioides*, *Chaetomium globosum*, *Penicillium brevicompactum*, *Stachybotrys chartarum*, and *T. rubrum* (Allaker and Memarzadeh, 2014). The antifungal effects of spherical Ag-NPs may be attributed to their damaging effect on the fungal mycelia. Ag-NPs showed significant antifungal efficacy against dermatophytes, *Candida* sp. and *T. mentagrophytes* (Ravishankar and Jamuna, 2011). Ag-NPs have been found to possess antiviral property against hepatitis B virus and human immunodeficiency virus type 1 (HIV 1) (Lara et al., 2010). The above-mentioned reports suggest the possible use of Ag-NPs as a potential antifungal agent in medical devices and healthcare products.

### 15.5.2 GOLD NANOPARTICLES (AU-NPS)

The use of gold and gold-based agents in the treatment of infectious diseases can be traced back to 5000 years ago in Egypt. Gold is considered as inert and shows comparatively lesser antimicrobial activity to that of silver and copper. However, owing to its high surface-to-volume ratio and remarkable binding efficacy, gold is exceptionally suitable for attaching ligands and enhancing its chemical or

photothermal functionality (Allaker and Memarzadeh, 2014). In addition, Au-NPs also possess unique shape and size dependent optical and electronic characteristics (Li et al., 2011).

Au-NPs exert their antimicrobial mechanisms by targeting multiple biological pathways. They generate pores on the cell wall, which in turn leads to leakage of cytoplasmic content and cell death. It also generates photocatalytic ROS, which damage the cellular components of bacteria and viruses. Au-NPs affect the membrane potential and decrease cellular ATP levels by strongly inhibiting ATPase activity. Au-NPs also inhibit binding of tRNA to the ribosome subunit (Schrofel et al., 2014). They prevent unwinding of DNA and inhibit the transcription process (Rudramurthy et al., 2016).

Recent studies have been directed towards conjugation of Au-based nanoparticles with photosensitizers for antimicrobial photodynamic therapy (APDT). On exposure to infrared (NIR) radiation, the Au-based nanomaterials with characteristic NIR absorption destroy the bacterial cell by photothermal heating. Au nanorods fabricated with photosensitizers were found to effectively destroy MRSA via NIR photothermal radiation and photodynamic antimicrobial chemotherapy (Kuo, 2009; Pissuwan et al., 2009). Gold nanorods conjugated with toluidine blue O (a hydrophilic photosensitizer) exhibit dual function against methicillin-resistant *S. aureus* agents and kill the bacteria by hyperthermia and photodynamic inactivation (Gil-Tomas, 2007; Perni, 2009). Light absorbing Au-NPs fabricated with specific antibodies have also been employed to photothermally target *S. aureus* (Zharov, 2006). Khan et al. (2012) determined the possible use of Au-NPs fabricated with methylene blue for prevention of biofilm formation by nosocomial fungus, *C. albicans*.

Au-NPs are doped with biomolecules such as polysaccharides, proteins, antibodies, and oligonucleotides to develop functional moieties for various applications (Rudramurthy et al., 2016). Various studies have revealed the possibility of conjugating gold nanoparticles with antibiotics to enhance their antibacterial efficacy. Rai et al. (2009) reported the synthesis of spherical Au-NPs fabricated with cefaclor, a second generation antibiotic. It was found that the amine group of cefaclor acts as reducing as well as capping agent in the synthesis of Au-NPs. The cefaclor fabricated Au-NPs were found to exhibit better antibacterial activity against both Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*) as compared with gold nanoparticles and cefaclor, taken separately. The synergistic activity was studied and it was revealed that Cefaclor suppresses the synthesis of the peptidoglycan layer of the bacterial cell wall, thereby making the cell wall porous. The Au-NPs also generated holes in the cell wall, causing the leakage of cytoplasmic contents and ultimately leading to cell death. Au-NPs may also inhibit the uncoiling and transcription of DNA by binding to the DNA of bacteria. Coating of aminoglycosidic antibiotics with Au-NPs significantly increases their antibacterial activity against a range of Gram-positive and Gram-negative bacteria (Grace and Pandian, 2007; Saha, 2007). The antibacterial efficacy of vancomycin against vancomycin-resistant enterococci (VRE) was profoundly enhanced when

coated with Au-NPs (Gu et al., 2003). Recently, Fayaz et al. (2011) used non-pathogenic fungus, *T. viride*, to synthesize Au-NPs, at room temperature. The synthesized Au-NPs when conjugated with vancomycin by the ionic interaction efficiently suppressed the growth of vancomycin-resistant *S. aureus*. It was found that the Au-NPs penetrate inside the bacterial membrane resulting in the death of the cells. Stable biofabricated Au-NPs coated with antibiotics, rifampicin, vancomycin ciprofloxacin, and gentamycin significantly enhanced the inhibitory effect of the antibiotics against *S. epidermidis* and *Staphylococcus haemolyticus* (Ahangari et al., 2013). Brown et al. (2012) found that Au-NPs conjugated ampicillin (Au-NPs–AMP) exert bactericidal effect against numerous MDR bacteria, including MRSA, *Enterobacter aerogenes*, *P. aeruginosa*, and *E. coli*. It is speculated that the coating of ampicillin molecules on the surface of Au-NPs allows the Au-NPs–AMP to suppress the beta-lactamases expressed by the bacterial pathogens. Further, Au-NPs–AMP also hampers the proper functioning of transmembrane efflux pump, which is responsible for efflux of drug from the bacterial cell. Hence, Au-NPs may be used to coat the surface of a wide variety of medical devices and implants.

### 15.5.3 COPPER OXIDE NANOPARTICLES (CUO-NPS)

Copper oxide nanoparticles are relatively cheap, photocatalytic, and stable in regard to their chemical and physical properties. The potential application of CuO-NPs as anti-infective agents lies in their extremely high surface area and desirable crystal morphologies (Kwak and Kim, 2005; Stoimenov, 2002). A few mechanisms contributing to the antimicrobial property of the CuO-NPs have been proposed. CuO-NPs may adhere to the bacterial cell walls, owing to their positive charge. CuO-NPs interact with carboxyl and amine groups present on the surfaces of microbial cells. Therefore, bacteria with higher density of these ionic groups on their cell surfaces, such as *B. subtilis*, have higher affinity for and are highly susceptible to CuO-NPs (Blecher et al., 2011; Huh and Kwon, 2011). However, Gram-negative bacteria like *Proteus* spp. and *P. aeruginosa* are less susceptible to the positively charged CuO-NPs (Rupareli et al., 2008).

In living microorganisms, copper is a structural component for numerous enzymes. Hence, a relatively high concentration of  $\text{Cu}^{++}$  is required to generate toxic effects against microbial pathogens. When administered in high dose,  $\text{Cu}^{++}$  ions aid in the formation of ROS, which thereby interact with DNA and intercalate nucleic acid strands. The release of  $\text{Cu}^{++}$  may also disrupt the amino acid synthesis in many microbes (Huh and Kwon, 2011; Rupareli et al., 2008; Esteban et al. 2009). In addition, the generation of ROS may also result in oxidative stress-induced membrane damage in bacteria (Raffi et al., 2010; Pramanik et al., 2012; Weitz et al., 2015).

CuO-NPs exhibit broad range of microbicidal activity against both bacteria (*S. aureus*, *E. coli*, and *Listeria monocytogenes*) and fungi (especially *S. cerevisiae*) (Blecher et al., 2011). The highly ionic CuO nanoparticles effectively target a



wide range of bacterial pathogens associated with nosocomial infections. However, a significantly high dose of CuO-NPs is required to generate an antimicrobial effect (Ren et al., 2009). CuO-NPs show profound antibacterial activity against Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative bacteria (*E. coli*) (Chatterjee et al., 2012). Khashan et al. (2016) documented the antibacterial activity of CuO-NPs against *S. aureus*, *E. coli*, *Proteus vulgaris*, and *P. aeruginosa*. CuO-NPs in combination with fluconazole showed synergistic antifungal effect against pathogenic yeast, *C. albicans* (Raffi et al., 2010).

#### 15.5.4 ALUMINUM OXIDE NANOPARTICLES (Al<sub>2</sub>O<sub>3</sub>-NPS)

Alumina (also identified as aluminum(III) oxide) is thermodynamically stable and possesses a corundum-like structure, with hexagonal close-packed structure formed by oxygen atoms with two thirds of the octahedral sites in the lattice filled by Al<sup>3+</sup> ions (Martinez Flores et al., 2003). At near-neutral pH, they carry a positive charge on its surface. The electrostatic interaction together with the hydrophobic interactions and polymer bridging between the particles and negatively charged bacterial cells results in the adhesion of nanoparticles on the cell surfaces (Li and Logan, 2004). The antimicrobial activity of Al<sub>2</sub>O<sub>3</sub>-NPs resulting due to the release of metal ions has also been studied (Mukherjee et al., 2011). Al<sub>2</sub>O<sub>3</sub>-NPs generates ROS, which disrupt the bacterial cell wall, causing oxidative damage to cell membrane and eventually leading to cell death (Ravishankar and Jamuna, 2011).

The antimicrobial efficacy of Al<sub>2</sub>O<sub>3</sub>-NPs is greatly influenced by its shape, surface charge, and particle size. The Al<sub>2</sub>O<sub>3</sub>-NPs when attached to the bacterial cell surface causes structural deformities to the cell wall. These nanoparticles cross the cell membrane and this eventually results in loss of membrane integrity, as a consequence of intracellular oxidative stress. Al<sub>2</sub>O<sub>3</sub>-NPs show higher mutagenicity and sensitivity against *Pseudomonas fluorescens* compared with its bulk counterpart (Balasubramanyam et al., 2010). Likewise, nanoalumina also exhibits a better antibacterial effect of towards *E. coli*, *B. subtilis*, and *P. fluorescens* as compared with the bulk materials. The nanoparticles significantly decrease the extracellular protein content of the bacteria. It was found that Al<sub>2</sub>O<sub>3</sub>-NPs at a concentration of 1000 µg mL<sup>-1</sup> show a mild antibacterial effect against *E. coli*. The interaction of the nanoalumina with the bacterial cells results in distortion in cell morphology and agglomeration of particles in the cell wall (Mukherjee et al., 2011). Ansari et al. (2014) investigated the antibacterial efficacy of Al<sub>2</sub>O<sub>3</sub>-NPs against multidrug-resistant strain of *E. coli* isolated from clinical sample. It was found that Al<sub>2</sub>O<sub>3</sub>-NPs exert structural modification in the phospholipids layer, subsequently resulting in loss of amphiphilic properties of the cell wall, destruction of the membrane and leakage of cellular components. It is suggested that Al<sub>2</sub>O<sub>3</sub>-NPs interact with L-α-phosphatidyl-ethanolamine (PE) and lipopolysaccharides (LPS) of the bacterial cell wall through hydrogen bonds and ligand exchange. The binding of NPs to the surface of cell membrane results in the

formation of irregular-shaped pores and perforation on the bacterial cell surfaces. It is proposed that the  $\text{Al}_2\text{O}_3$ -NPs might have caused adverse effects by interacting with the intracellular biomolecules and eventually triggering cell death. Ansari et al. (2015) suggested that  $\text{Al}_2\text{O}_3$ -NPs may be exploited as a potential antimicrobial agent to target extended-spectrum, nonextended-spectrum and metallo- $\beta$  lactamases strains of *P. aeruginosa*.

$\text{Al}_2\text{O}_3$ -NPs disrupt the cell membranes and exhibits mild inhibitory effect on microbial growth when administered at high concentration. The antibacterial effect of alumina NPs ( $10\text{--}1000\ \mu\text{g mL}^{-1}$ ) on the growth *E. coli* was reported by Sadiq et al. (2009). The inhibitory effect was attributed to the interactions between the nanoparticles and bacterial cell surface due to opposite surface charge. It is revealed that  $\text{Al}_2\text{O}_3$ -NPs can enter inside the cytoplasm of *E. coli* and exert damaging effects. The extracellular protein content was decreased in cells on treatment with  $1000\ \mu\text{g mL}^{-1}$  alumina.  $\text{Al}_2\text{O}_3$ -NPs when conjugated with other metals enhance its antibacterial behavior. According to Bala et al. (2011),  $\text{Al}_2\text{O}_3$ -NPs exhibit enhanced antimicrobial effects against *E. coli* and *S. epidermidis* in conjugation with silver. Similar observations were made in case of  $\text{TiO}_2\text{--Ag}$  and  $\text{Al}_2\text{O}_3\text{--Ag}$  nanocomposite. The antimicrobial efficacy of alumina nanoparticles indicates their potential use in the development of effective therapeutic agents against the infections caused by MDR strains.

### 15.5.5 IRON OXIDE (IO-NPS)

The biological compatibility of the magnetic IO-NPs makes them an attractive candidate for implementation in biomedicine. Like other metal oxide nanoparticles, IO-NPs also exhibit antimicrobial activity by generation of free radicals. The mechanism of antimicrobial action for  $\text{Fe}_3\text{O}_4$ -NPs is believed to be as a result of oxidative stress generated due to the release of ROS such as hydroxyl radicals ( $\text{OH}^\cdot$ ), superoxide radicals ( $\text{O}_2^\cdot$ ), hydrogen peroxide, and singlet oxygen ( $^1\text{O}_2$ ) (Behera et al., 2012). These resulting free radicals in turn cause depolymerization of polysaccharides, DNA damage, lipid peroxidation, and/or inactivation of enzymes (Ranghar et al., 2014).

The antibacterial activity of IO-NPs has been determined against various bacteria including *P. vulgaris*, *S. aureus*, *E. coli*, *S. epidermidis*, and *Xanthomonas* sp. (Tran et al., 2010; Prabhu et al., 2015; Lee et al., 2008). According to Tran et al. (2010), IO/PVA generates ROS leading to the growth inhibition in *S. aureus*. The  $\text{Fe}^{2+}$  reacts with oxygen to produce  $\text{H}_2\text{O}_2$ , which in turn reacts with ferrous irons via Fenton reaction to generate hydroxyl radicals, which damage the cellular constituents. The small size of the nanoparticles also contributes to its antimicrobial property. Nanoparticles ranging in size from 10 to 80 nm readily penetrate inside the cell membrane of *E. coli* and react with intracellular oxygen, thereby resulting in oxidative stress and disruption of the cell membrane. Prabhu et al. (2015) reported that ROS generated by iron oxide nanoparticles significantly inhibited the pathogenic bacteria like *P. vulgaris*, *S. aureus*, *Xanthomonas* sp.,

and *E. coli*. Chen et al. (2008) demonstrated that immunoglobulin G-bound Fe<sub>3</sub>O<sub>4</sub>/titania core/magnetic shell NPs successfully suppress the growth of MDR pathogenic bacteria, that is, *S. pyogenes*, MRSA, and *Staphylococcus saprophyticus*.

### 15.5.6 ZINC OXIDE NANOPARTICLES (ZNO-NPS)

Zinc is an important trace element in the human body and nano ZnO has gained considerable attention due to its biocompatibility and stability under harsh processing conditions (Allaker and Memarzadeh, 2014). Moreover, ZnO-NPs show selective toxicity towards targeted bacteria and exert minimal toxicity to human cells (Ravishankar and Jamuna, 2011). ZnO-NPs possess potent antimicrobial properties and use several mechanisms to combat pathogen, thereby making it unlikely to develop resistance against them. ZnO-NPs are thought to adhere strongly to the cell membranes and damage both the lipids and proteins components of the bacterial membrane, which subsequently leads to increase in permeability of cell membrane and cell death (Hajipour et al., 2012). The generation of ROS and release of Zn<sup>2+</sup> ions are few of the mechanisms proposed to describe the antimicrobial efficacy of ZnO-NPs. ZnO-NPs induce the release of ROS, such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radicals (OH<sup>-</sup>). The generated H<sub>2</sub>O<sub>2</sub> on the surface of ZnO-NPs penetrate inside the bacterial cells and damage the cell membrane (Allaker and Memarzadeh, 2014; Padmavathy and Vijayaraghavan, 2008). However, the negatively charged OH<sup>-</sup> and superoxides are unable to enter the cell membrane and remain on the surface of the cell. The release of Zn<sup>2+</sup> ions also damages the cell membrane and the intracellular components (Brayner et al., 2006; Blecher et al., 2011; Huh and Kwon, 2011). It was observed that polyvinyl alcohol (PVA) coated ZnO-NPs increases the permeability of the cell membrane and imposes oxidative stress on entering the cytoplasm (Huh and Kwon, 2011).

The antimicrobial efficacy of ZnO-NPs is significantly governed by their shape, size, crystal structure, and dispensability. ZnO-NPs in different form such as nanorods, nanoplates, flowers, needles, dandelions, helixes, rings, springs, and snowflakes have been synthesized (Yamamoto, 2001). The generation of H<sub>2</sub>O<sub>2</sub> increases with the increase in surface area of the ZnO-NPs. It is presumed that with decrease in particle size, the ZnO particles per unit volume increases, resulting in overall increase in surface area and release of hydrogen peroxide (Ravishankar and Jamuna, 2011). According to Vidic et al. (2013), ZnO nanostructures of 100 nm in diameter exhibit significant antibacterial efficacy against *B. subtilis* and *E. coli*. Jin et al. (2009) reported that ZnO nanoparticles of ~12 nm in size inhibited the growth of *E. coli* by disrupting the cell membrane and increasing its permeability. Pati et al. (2014) indicated the potential application of ZnO-NPs as antibacterial agent against *S. aureus*. The destruction of membrane integrity, decrease in cell surface hydrophobicity, and downregulation in the transcription of oxidative stress resistance genes were observed on exposure

of the bacterial cell to ZnO-NPs. Liu et al. (2009) investigated the antibacterial efficacy of ZnO-NPs against verocytotoxigenic *E. coli* strain O157:H7. A considerable destruction in the membrane integrity and leakage of intracellular contents was observed without much effect to nucleic acid (Liu et al., 2009). ZnO-NPs also possess significant inhibition efficacy against methicillin resistant strains of *S. aureus* and *Streptococcus agalactiae* (Hajipour et al., 2012).

### 15.5.7 TITANIUM DIOXIDE-CONTAINING NANOPARTICLES (TiO<sub>2</sub>-NPS)

Titanium dioxide (TiO<sub>2</sub>) is a well established photocatalytic antimicrobial agent. The anatase form of nano TiO<sub>2</sub> when irradiated with UV light excitation brings about maximum antimicrobial activity of TiO<sub>2</sub>-NPs. This photocatalysis process leads to oxidation of the polyunsaturated phospholipid present in the lipid membrane, causing loss of respiratory activity and subsequent cell death (Allaker and Memarzadeh, 2014).

Titanium dioxide-containing nanoparticles employ two antimicrobial mechanisms. In the photocatalysis process, TiO<sub>2</sub>-NPs generate ROS when exposed to UV light at wavelength lower than 385 nm. The ROS generated including hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radicals (\*OH) act on the phospholipids present in the bacterial cell resulting in site-specific DNA damage. It also damages the cell membranes of the bacteria, thereby increasing membrane permeability, hampering the process of oxidative phosphorylation, and sometimes resulting in cell death (Rudramurthy et al., 2016; Pelgrift and Friedman, 2013). The antimicrobial effect of TiO<sub>2</sub> photocatalyst against fungi and bacteria in suspension has been reported. The findings have led to the development of photocatalytic methods for combating pathogenic viruses and bacteria using TiO<sub>2</sub>-NPs in aqueous media (Ravishankar and Jamuna, 2011). It has been suggested that TiO<sub>2</sub>-NPs in combination with UV irradiation may also be employed as an effective way to decrease the disinfection time and eradicate pathogens (Ravishankar and Jamuna, 2011). Researchers have reported the light-induced biocidal property of engineered TiO<sub>2</sub>-NPs in controlling *Aspergillus niger* and *E. coli* (Rudramurthy et al., 2016). Hence, the photocatalytic activity of TiO<sub>2</sub>-NPs may be exploited in the development of antibiofilm coatings.

However, the major drawback in the using TiO<sub>2</sub>-NPs is the need to provide UV light illumination to activate the photocatalyst and initiate the killing of the bacteria and viruses. In recent years, it was found that visible light absorbing photocatalysts of Ag/AgBr/TiO<sub>2</sub> efficiently inhibit *E. coli* and *S. aureus* (Ravishankar and Jamuna, 2011). It has been found that silver coated TiO<sub>2</sub> material exhibits enhanced photocatalytic and antibactericidal property relative to TiO<sub>2</sub> alone (Ranghar et al., 2014). Recently, it is observed that TiO<sub>2</sub>-NPs also show antimicrobial efficacy in absence of UV irradiation, suggesting their involvement in other bactericidal mechanism (Pelgrift and Friedman, 2013).

TiO<sub>2</sub>-NPs damages viruses, bacterial cell walls, and spores. They show highest antibacterial efficiency for *E. coli*, followed by *P. aeruginosa*, *S. aureus*, *Enterococcus faecium*, and *C. albicans* (Huh and Kwon, 2011).

### 15.5.8 MAGNESIUM OXIDE NANOPARTICLES (MGO-NPS)

MgO-NPs are usually considered safe for humans and exhibit great stability under severe processing conditions. Unlike TiO<sub>2</sub>, MgO-NPs do not need to be photoactivated to trigger its inherent antimicrobial activity. Multiple strategies have been suggested to justify the antibacterial property of Mg-ONPs, a few of which include generation of lipid peroxidation, ROS generation, enzyme inhibition, alkaline effects, and electrostatic interactions. The production of ROS results in lipid peroxidation in the bacterial cell envelope, which in turn facilitates the entry of the NPs through the plasma membrane. Consequently, the cytoplasmic pH drops, which raises the membrane potential causing the intracellular cytoplasmic components to exude out of the cell. The strong electrostatic interaction between the MgO-NPs and the bacterial cell surface also contributes to the antibacterial activity. The alkaline effect on the surface of MgO-NPs, due to the formation of a thin layer of water molecules, damages the bacterial cell membrane when it comes in contact with MgO-NPs, ultimately resulting in cell death (Rudramurthy et al., 2016).

The size of the MgO-NPs influences its antimicrobial property as it alters the surface energy. MgO-NPs with size less than 15 nm in diameter exhibit enhanced bactericidal property as compared with its large and aggregated counterparts (Huang et al., 2005). MgO-NPs were found to show strong bactericidal effect against *Salmonella* and *E. coli* O157:H7. It was found that treatment with MgO-NP causes damage and deformation to the bacterial cell membrane, causing effluence of cytoplasmic constituents and eventually cell death (Jin and He, 2011). MgO-NPs were found to exhibit antagonistic effect against both Gram-negative and Gram-positive bacteria such as *Bacillus megaterium*, *S. aureus*, *P. aeruginosa*, and *E. coli*. In addition, they also exhibit biocidal activity towards spores and thus, may be employed as an effective disinfectant against a wide range of pathogens (Rudramurthy et al, 2016).

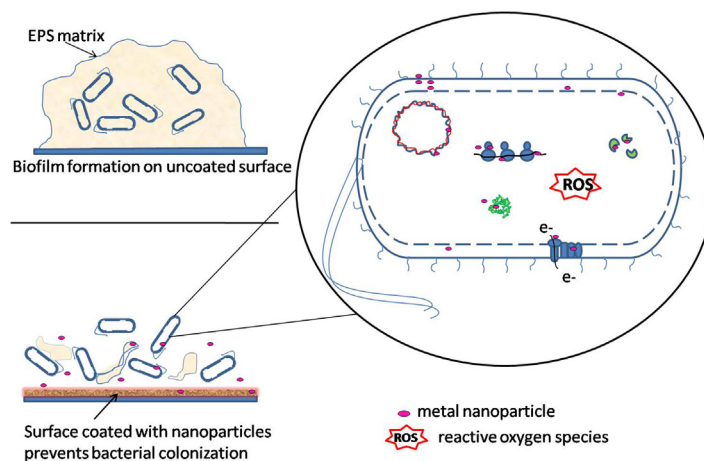
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## 15.6 ANTIBIOFILM ACTIVITY OF METAL-BASED NANOPARTICLES

The formation of biofilm is an important mode of growth in pathogens related to chronic infections, affecting millions of people worldwide (Shakibaie et al., 2015). The production of exopolysaccharide (EPS) leading to the subsequent formation of biofilm is one of the major virulence determinants contributing to the development of MDR strains of pathogenic organisms. EPS matrix consisting of

polysaccharides, proteins, eDNA, and cellular debris makes the pathogen inaccessible to the host defense system and antibiotics. Hence, the biofilm matrix confers an increase in antibiotic resistance in the case of biofilm-associated diseases like gingivitis, otitis media, and cystic fibrosis. They are responsible for many nosocomial as well as chronic infections associated with medical devices and surgical implants (Chen et al., 2013; Pelgrift and Friedman, 2013). The difficulty in eradication of these pathogens lies in the fact that the biofilms are highly resistant and inaccessible to the antimicrobial agents.

Nanoparticles have the ability to invade the biofilm matrix and gain access to inside the microbial cells. As depicted in Fig. 15.2, they attach to the bacterial cell wall, infiltrate inside the cell membranes, and accumulate inside cells, resulting in a continuous liberation of ions inside the cell (Ansari et al., 2013). Nanoparticles have received tremendous attention for their ability to inhibit/disrupt biofilm formation for possible clinical applications. Ag-NPs effectively prevented biofilm development in extended spectrum  $\beta$ -lactamases producing *Klebsiella* spp. and *E. coli* by restricting bacterial growth and production of EPS. The antibiofilm property of the Ag-NPs may be exploited towards the development of Ag-NPs based coatings for medical devices and implants. These NPs may also be used in the management of various infections caused by drug-resistant biofilm forming bacteria (Ansari et al., 2013). Among the leading nosocomial pathogens, *S. aureus*, *P. aeruginosa*, and *Proteus mirabilis* are capable of colonizing medical devices and catheters resulting in biofilm-related infections of the urinary and lower respiratory tract. Selenium nanoparticles (Se-NPs) prepared using *Bacillus* sp. MSh-1 were found to exhibit antibiofilm efficacy against biofilm producing clinically isolated nosocomial pathogens. The nanoparticles



**FIGURE 15.2**

A diagrammatic illustration showing common mode of action of metal-based nanoparticles against biofilm forming bacteria.

significantly suppress the biofilm formation in *S. aureus*, *P. mirabilis*, and *P. aeruginosa*, by 42%, 53.4%, and 34.3%, respectively. It was also revealed that the effect of Se-NPs on the development of biofilm was considerably determined by the temperature and pH (Shakibaie et al., 2015). Selenium nanoparticles (Se-NPs) synthesized using Actinobacterium, *Streptomyces minutiscleroticus* M10A62 was able to completely inhibit biofilm formation in six *Acinetobacter* species within 48 hours of incubation. The inhibition of biofilm formation was achieved with 3.2  $\mu\text{g}$  concentration of Se-NPs indicating the direct interaction of target bacteria with the nanoparticles resulted in inhibition of biofilm at low concentration (Ramya et al., 2015). Ag-NPs prevent *E. coli*, *S. aureus*, *C. albicans*, and *P. aeruginosa* from forming biofilm on plastic catheters (Pelgrift and Friedman, 2013). Ag-NPs produced by the lignin-degrading fungus *Emericella nidulans* and *A. flavus* inhibited biofilm formation in Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa* and *E. coli*) bacteria. At a concentration of 0.5–64  $\mu\text{g mL}^{-1}$ , the Ag-NPs significantly decreased 74%–84% biofilm formation as compared with fungal cell-free filtrate (devoid of Ag-NPs) showing 4%–6% antibiofilm activity (Barapatre et al. 2016). Zero-valent bismuth nanoparticles completely inhibited growth and formation of biofilm by *Streptococcus mutans* (Hernandez-Delgadillo et al., 2012). Selenium and tellurium nanoparticles prepared using *Stenotrophomonas maltophilia* SeITE02 and *Ochrobactrum* sp. MPV1 were found to possess antibacterial and antibiofilm activity against *P. aeruginosa* PAO1, *E. coli* JM109 and *S. aureus* ATCC-25923 (Zonaro et al., 2015).

Metal oxide nanoparticles also exhibit profound antibiofilm activity. For example, thin film composite (TFC) membranes coated with  $\text{TiO}_2$ -NP damages the cell membrane of *E. coli*, thereby preventing the adherence of bacteria onto the surface of the TFC membrane and inhibiting biofilm formation (Blecher et al., 2011). According to Hajipour et al. (2012),  $\text{ZnO}$ -NPs coated glass surfaces prevented biofilm formation by *E. coli* and *S. aureus* by generating ROS. Metal nanoparticles conjugated to photosensitizers also resulted in an increase in their antibiofilm efficiency. Photoactivated Au-NPs bound to methylene blue were found to inhibit biofilm formation in *C. albicans* (Khan, 2012). Magnetite ( $\text{Fe}_3\text{O}_4$ ) nanoparticles coated with *Rosmarinus officinalis* essential oil exhibit a significant inhibitory effect on the formation of biofilm on catheters by *C. albicans* and *Candida tropicalis*. Reports suggested that the antibiofilm activity of the NPs is governed by the dimension and size of the nanoparticles. The high surface to volume ratio plays a vital role in imparting antibiofilm property to the nanoparticles. Thus, the decrease in the size of the NPs corresponds to increase in their activity. Small sized  $\text{MgF}_2$ -NPs coated glass surfaces inhibited adherence of bacteria, *S. aureus* and *E. coli*, to surfaces and subsequent biofilm formation (Pelgrift and Friedman., 2013). The colonization and growth of *Mycobacterium smegmatis* biofilm on membranes was decreased by over 98.7% when coated with Ag-NPs, with an average diameter  $12.6 \pm 5.7$  nm at a concentration of 100  $\mu\text{M}$ . The biofilm formation by *S. aureus* and *P. aeruginosa* was notably arrested by 65% and

88%, respectively, in presence of starch-stabilized nanoparticles ( $\sim 20$  nm in diameter), even at very low concentrations of 1–2 mM (Markowska et al., 2013).

Nanoparticles are coated and functionalized with various antimicrobial agents to enhance their antibiofilm activity. Phosphatidylcholine-coated Au nanoparticles doped with gentamicin exhibited efficient antagonistic activity against biofilm formation and established biofilm in both Gram-positive and Gram-negative bacteria (Mu et al., 2016). Bimetallic Au–Ag nanoparticles conjugate prepared using  $\gamma$ -proteobacterium, *Shewanella oneidensis* MR-1 remarkably arrested the biofilm production in Gram-positive bacteria (*S. aureus* and *E. faecalis*) as well as Gram-negative (*P. aeruginosa* and *E. coli*) bacteria, at 10  $\mu$ M concentration (Ramasamy et al., 2016). It was found that curcumin, an anti-quorum sensing agent that is known to display antibiofilm properties when fabricated with Ag-NPs exhibited profound synergistic effect against both Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacterial pathogens. The synergistic effect of the conjugated nanoparticles resulted in disruption of 50% of the preformed biofilm, at 100  $\mu$ g mL<sup>-1</sup> (Loo et al., 2016).

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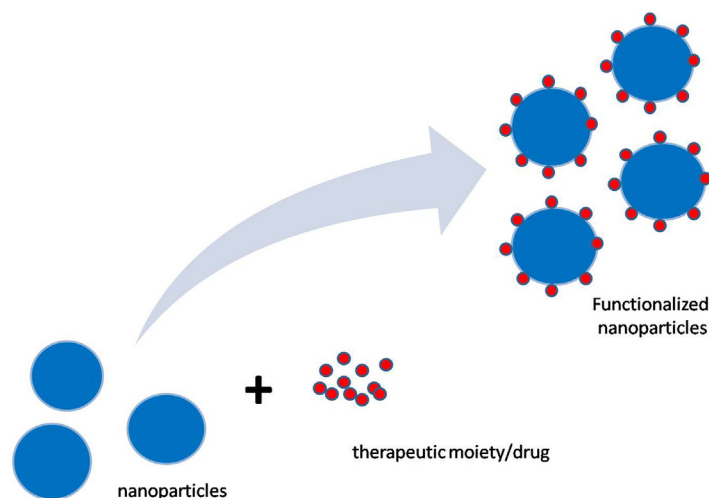
## 15.7 FUNCTIONALIZATION OF METAL NANOPARTICLES

Combinatorial drug therapy is expected to exhibit higher potency to combat microbial resistance as a result of the synergistic effects provided by the use of multiple drugs. The surface properties of NPs are highly flexible and can be easily manipulated using ligand engineering to impart desired properties to the NPs. Nanocomposites prepared by conjugating the nanoparticles with antibodies may be used against the antigen present on the surface of the target microbe (Fig. 15.3).

For example, Au-NPs fabricated with antibodies against protein A were used to selectively target *S. aureus*. In another instance, the IgG in IgG–Fe<sub>3</sub>O<sub>4</sub>–TiO<sub>2</sub> magnetic nanoconjugate targets several types of bacteria, including *S. pyogenes*. Understanding the interactions of nanoparticles with the target pathogen and its biofilm mode plays a vital role in designing NP-based antimicrobial agents. Moreover, the use of NPs loaded antimicrobial agent aids in the controlled and sustainable release of the drug molecule, contributing to marked improvement in their biocompatibility and efficiency in mammalian cells (Gupta et al., 2016). According to recent reports, multiple antimicrobial agents may be fabricated within the same nanoparticle, thus making it multifunctional. The incorporation of multiple drugs in a nanoparticle results in higher antimicrobial efficacy enhanced potency and most likely overcome the existing problem of multidrug resistance in pathogenic microbes (Pelgrift and Friedman., 2013) (Table 15.4).

Among all metal nanoparticles, Au-NPs have been extensively studied and considered as a highly efficient drug delivery system due to their facile synthesis process, ease in functionalization, low toxicity, and high biocompatibility. The





**FIGURE 15.3**

Schematic representation showing functionalization of metal-based nanoparticles for therapeutic application.

nontoxic and nonimmunogenic Au-NPs serves as an ideal carrier for drug delivery. Nanocomposites of Au-NPs doped with antibiotics have been employed for the selective photothermal killing of pathogenic microorganisms. Gold NPs themselves are considered inert and do not affect bacterial growth, however, conjugating antibiotics with Au-NPs significantly increases their antibacterial activity. Gold NPs synthesized using nonpathogenic fungus, *T. viride*, may be conjugated with vancomycin to form Vancomycin-bound biogenic gold nanoparticles (VBG-NPs). The VBG-NPs exhibited remarkable antimicrobial efficacy against drug-resistant *E. coli* and *S. aureus*. The antibacterial activity is proposed to be as a result of the nonspecific binding of VBG-NPs to the transpeptidase of Vancomycin-resistant *S. aureus* strain (Fayaz et al., 2011). Au-NPs have also been shown to efficiently deliver and increase the concentration of drug at the targeted site. Hence, they are used as drug carriers to selectively and specifically target cells, thereby increasing the efficiency of the drug in treating acute infections, without causing adverse effects (Shahverdi et al., 2007). Au-NPs when coated with Cefaclor, a second-generation  $\beta$ -lactam antibiotic, and ampicillin results in a profound increase in antibacterial activity against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria (Yin et al., 2015).

Nanoparticles may be effectively used in conjugation with antibiotics to enhance their antagonistic efficacy against various pathogens. Brown et al. (2012) determined that Au-NPs and Ag-NPs functionalized with ampicillin act as an effective broad-spectrum bactericidal agent against both Gram-positive and Gram-negative bacteria. The conjugation of ampicillin onto the surfaces of

**Table 15.4** A Summary of Various Nanoparticles Conjugated With Antibiotics and Their Role as Antibacterial Agents

Nanoparticles	Drug Conjugate	Target	Activity	Reference
Ag-NPs	Ciprofloxacin, gentamycin, rifampicin, vancomycin	<i>Staphylococcus epidermidis</i> <i>Staphylococcus haemolyticus</i>	Antibacterial	<a href="#">Roshmi et al. (2015)</a>
Ag-NPs Au-NPs	Ampicillin	<i>E. coli</i> , <i>V. cholerae</i> , <i>Enterobacter aerogenes</i> , MRSA, <i>Pseudomonas aeruginosa</i>	Antibacterial	<a href="#">Brown et al. (2012)</a>
Au-NPs	Vancomycin	<i>E. coli</i>	Antibacterial	<a href="#">Fayaz et al. (2011)</a>
Au-NPs	Gentamicin	<i>E. coli</i> K12	Antibacterial	<a href="#">Burygin et al. (2009)</a>
Ag-NPs	Penicillin G, amoxicillin, erythromycin, clindamycin, vancomycin	<i>E. coli</i> , <i>Staphylococcus aureus</i>	Antibacterial	<a href="#">Shahverdi et al. (2007)</a>
Au-NPs	Streptomycin, gentamycin, neomycin	<i>S. aureus</i> , <i>Micrococcus luteus</i> , <i>E. coli</i> , <i>P. aeruginosa</i>	Antibacterial	<a href="#">Grace and Pandian (2007)</a>
Au-NPs	Ampicillin, streptomycin, kanamycin	<i>E. coli</i> DH5 $\alpha$ , <i>M. luteus</i> , <i>S. aureus</i>	Antibacterial	<a href="#">Saha et al. (2007)</a>
Au-NPs	Kanamycin	<i>Y. pestis</i> CO92, <i>S. epidermidis</i> , <i>P. aeruginosa</i> PA01, <i>E. aerogenes</i> , <i>S. bovis</i>	Antibacterial	<a href="#">Payne et al. (2016)</a>
Ag-NP	Imipenem, gentamycin, vancomycin, ciprofloxacin	<i>P. aeruginosa</i> , <i>M. luteus</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>A. baumannii</i> , <i>E. faecalis</i> , <i>Bacillus</i> spp., <i>K. pneumoniae</i>	Antibacterial	<a href="#">Naqvi et al. (2012)</a>

Ag-NPs and Au-NPs results in significant increase in their intrinsic antibacterial activity, which resulted in the antibacterial activity against MRSA, *P. aeruginosa*, *E. coli*, *V. cholera*, and *E. aerogenes*. Gold nanoparticles synthesized using *Bacillus* sp. when functionalized with antibiotics (gentamycin, ciprofloxacin, rifamycin, and vancomycin) inhibited the growth of *S. epidermidis* and *S. haemolyticus* (Roshmi et al., 2015). Gold nanoparticles possess a large surface to volume ratio, thereby providing a large surface area for a number of drug molecules to get attached onto its surface. When these antimicrobial agents come in a close proximity with a nanogold core, it is believed to affect the growth of the microorganisms more effectively (Burygin et al., 2009).

Gold nanoparticles act as an efficient vehicle for drug delivery systems. The in vitro antibacterial activities of Au nanoparticles capped with aminoglycosidic antibiotics showed profound antibacterial efficiency against both Gram-positive and Gram-negative bacteria, for example, *Micrococcus luteus*, *P. aeruginosa*, *S. aureus*, and *E. coli* (Grace and Pandian, 2007). Au-NPs when conjugated with kanamycin, streptomycin, and ampicillin enhance its bactericidal efficacy. The antibiotic-capped Au-NPs showed greater bactericidal activity against *E. coli* DH5 $\alpha$ , *M. luteus*, and *S. aureus* (Saha et al. 2007). Au-NPs conjugated with an antibiotic, kanamycin, exhibited dose-dependent broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria, including bacteria resistant to kanamycin. It was indicated that the increased efficacy might have resulted due to the damage of the bacterial cell envelope, consequently resulting in the leakage of intracellular components and cell death (Payne et al., 2016).

Fungi have been found to be relatively more efficient to bacteria in the synthesis of nanoparticles. Extracellularly synthesized silver nanoparticles (Ag-NPs) using culture filtrate of *A. flavus* have been successfully conjugated with conventional antibiotics. Shahverdi et al. (2007) reported the synthesis of Ag-NPs using the culture supernatant of *K. pneumoniae*. The antibacterial efficacy of amoxicillin, penicillin G, vancomycin, erythromycin, and clindamycin against *S. aureus* and *E. coli* were significantly enhanced in the presence of Ag-NPs. The highest effect was found in case of vancomycin, amoxicillin, and penicillin G against *S. aureus*. The synergistic effect of antibiotics in conjugation with biologically synthesized Ag-NPs significantly increased the susceptibility of the test pathogens. The combined effect of Ag-NPs and antibiotics, imipenem, gentamycin, and ciprofloxacin was notably exhibited against *S. aureus*, *E. coli*, *Bacillus* spp., *K. pneumoniae*, *P. aeruginosa*, and *M. luteus*. Antibiotic–AgNPs conjugates were prepared in which an Ag-NP core was surrounded by antibiotic molecules. Thus, the antibiotic concentration was considerably increased at the target sites, leading to the destruction of the bacteria cell. Moreover, if a bacterium develops resistance to one of them, the other bactericidal agent would kill the bacteria (Naqvi et al., 2012).

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## 15.8 NANOPARTICLES IN ANTIMICROBIAL PHOTODYNAMIC THERAPY

APDT has been suggested as a promising nonantibiotic approach to control and treat numerous infectious diseases caused by both Gram-positive and Gram-negative bacteria. APDT involves the use of a photosensitizer (PS), usually a nontoxic dye in combination with a harmless visible light. The irradiation of the PS generates ROS that selectively destroy microbial cells and biofilm. ROS such as hydroxyl radicals ( $\text{HO}^\bullet$ ), superoxide ( $\text{O}_2^-$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) cause the oxidation of biomolecules, that is, proteins, lipids, and DNA inside the cell. It is also responsible for the damage to the cell wall and increases in membrane permeability, which results in leakage of cytoplasmic components. Since the microbial inactivation is a multitarget process, the possibility of development of resistant strains is minimal. Owing to the differences in the cell wall structures, the Gram-negative bacteria are found to be less susceptible to APDT as compared with the Gram-positive ones. Microorganisms such as yeasts, fungi, and viruses were also found to be susceptible to APDT (Yin et al., 2015).

Delivery of drug to the target site is essential to provide maximum bioavailability and therapeutic effect. Nanoparticles are used as a drug carrier to enhance the solubility of hydrophobic drugs, decrease side effects, minimize enzymatic degradation, prolong their circulation, and increase their bioavailability. It was established that the antimicrobial activity was greatly enhanced when PS was covalently bound to nanoparticles. At present, inorganic metal oxide (ZnO,  $\text{TiO}_2$ , and MgO) nanoparticles have gained much interest as antimicrobial agents due to their high biocompatibility and stability. A significant bactericidal activity of ZnO, Ag, and  $\text{TiO}_2$  in the presence of UV light against *P. aeruginosa* and MRSA has been documented. The use of nanoparticles aids in the attachment and uptake of PS by the microbial cells and they are more easily exposed to the lethal ROS. APDT holds promising applications in self-sterilizing urinary catheters, antibacterial surgical, and dental implants. APDT may also be successfully employed in the management of periodontitis, root canal disinfection, gingivitis, oral candidosis, burn wounds, and acne (Perni et al., 2011; Yin et al., 2015).

Exploitation of metals and metal oxide nanoparticles, particularly those that produce ROS when exposed to ultraviolet (UV) light, such as zinc oxide (ZnO) and titanium dioxide ( $\text{TiO}_2$ ), are finding increasing application in antimicrobial therapy. These nanoparticles act as the PS itself or interact with the PS either by conjugation or the nanoparticles. Titanium dioxide nanoparticles ( $\text{TiO}_2$ -NPs) are the most common PS and their ability to act as a photocatalytic antimicrobial agent is well established. The high reactivity of nanobased  $\text{TiO}_2$  is extensively exploited for their bactericidal activity in the development of filters and coatings of glasses, ceramics, alumina, and polymers. As a photosensitizer, it possesses photocatalytic properties that allow the destruction of pathogenic microbes. When irradiated with UV light of wavelength less than 385 nm,  $\text{TiO}_2$  results in the

photocatalytic generation of active free hydroxyl radicals ( $\text{OH}^-$ ), which catalyze the killing of both Gram-positive and Gram-negative bacteria. It also brings about photocatalytic oxidation of biofilm components on  $\text{TiO}_2$ -coated surfaces and prevents biofilm formation. Suspensions containing  $\text{TiO}_2$  were found possess bactericidal effect on *E. coli*, *P. aeruginosa*, *Acinetobacter*, planktonic cells, and spores of *Bacillus cereus*. It has been suggested that in an aqueous medium, nanostructured  $\text{TiO}_2$  irradiated with UV light may be employed as an efficient way to eliminate virus such as hepatitis B, influenza A/H1N1, influenza A/H3N2, polio virus type 1, SARS, and coronavirus.  $\text{TiO}_2$  can successfully kill fungi, including *A. niger*, *C. albicans*, and *Cryptosporidium parvum* (Ravishankar and Jamuna, 2011; Yin et al., 2015).

Gold nanoparticles are generally considered to be biologically inert but they can be engineered to impart chemical or photothermal functionality. Au-NPs in conjugation with PS have been employed to attain greater bactericidal effect through APDT. The biologically inert Au-NPs absorb the UV-light and generate a significant photothermal effect, which damages the microbial cell. Gold nanoparticles have been shown to possess intrinsic antibacterial activity against *E. coli*, VRE, MRSA, and *P. aeruginosa*. Au-nanoparticles enhance the antimicrobial properties of PS such as toluidine blue O and methylene blue. According to Narband et al. (2008) the hydrophilic photosensitizer, toluidine blue O when fabricated on the surface of Au nanoparticles results in photodynamic inactivation and hyperthermia against methicillin-resistant *S. aureus*. The enhancement in the lethal properties might be the result of higher light capturing ability of the PS when adsorbed on the surface of the Au nanoparticles. Au nanorods coated with PS destroy MRSA cells by synergistic antimicrobial and photothermal effect. *S. aureus* can be inhibited using laser activated Au-NPs fabricated with antibodies. Au-nanoparticles were used against *C. albicans* biofilms using methylene blue-induced APDT (Yin et al., 2015).

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## 15.9 NANOPARTICLES FOR ANTIMICROBIAL DRUG DELIVERY

A wide range of antimicrobial drugs are available and prescribed to treat infectious diseases caused by various fungi, bacteria, and viruses. However, the inefficient delivery of these conventional drugs to the target site results in inadequate therapeutic effects and undesirable side effects. Other limitations include difficulty in administration due to their low solubility, lack of target specificity, cytotoxic to host tissues, and rapid degradation and clearance from the body (Ranghar et al., 2014). Delivering the right dose of drug precisely and safely to the specific target at the right time is the main goal in the designing of an ideal drug delivery system (Li et al., 2011). In this regard, NPs have emerged as promising drug delivery systems. It is well reported that the metal-based nanoparticles serve as an effective antimicrobial agent against common pathogens. Owing to their small

size, they are highly reactive and readily taken up by the host as well as microbial cells (Zhang et al., 2010). The surface properties of the NPs can be tuned to attain controlled release of the pharmacologically active agent at a specific site at a therapeutically optimal rate and dose (Ranghar et al., 2014). The drug molecules are generally loaded to the nanoparticles adsorption, physical encapsulation, or chemical conjugation to increase the therapeutic index and pharmacokinetics of the drugs (Zhang et al., 2010). Many advantages of nanoparticles based drug delivery have been recognized, including improvement in solubility of the drugs, increased circulation lifetime, controlled release of drugs, specificity, and selectivity of the target (Zhang et al., 2010). The use of nanoparticles as carrier allows delivery of optimum doses of a drug to the targeted site, thereby reducing the risk of resistance mechanisms and minimizing toxicity to patients (Pelgrift and Friedman, 2013). They also increase the stability of a variety of therapeutic agents such as oligonucleotides and peptides. The surfaces of Au-NPs can be readily functionalized with ligands containing phosphines, thiols, and amines, which exhibit high affinity for gold surfaces (Li et al., 2011). When administered, the drug-loaded nanoparticles can enter the host cells via endocytosis. Once inside the host cell, these nanoparticles release drugs during the course of time, that then kill the intracellular pathogen (Zhang et al., 2010). The accumulation of required dose of the drug at the site of infection eliminates the intracellular bacteria before the development of resistance. Nanoparticles may be fabricated with antibodies to specifically target antigen present on the surface of the targeted microbe. These nanoparticles based antimicrobial drug delivery systems help in distinguishing the target microbes or infectious cells from healthy cells, allowing specific targeting ability. For example, Au-NPs coated with antibodies against protein A have been successfully employed to selectively kill *S. aureus*. Functionalized TiO<sub>2</sub> magnetic NPs are used to target several types of bacteria, including *S. pyogenes* (Pelgrift and Friedman, 2013). Aptamers also exhibit in vitro antimicrobial activity against beta-lactamase producing Gram-positive and Gram-negative bacteria. They also possess antiviral efficacy against reverse transcriptase of HIV and inhibits replication of Vaccinia virus (Pelgrift and Friedman, 2013). Due to the above-mentioned reasons, numerous nanoparticle-based drug delivery systems have been approved to treat a variety of diseases and many others are currently under various stages of clinical trials.

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## 15.10 CONCLUSION

The ability to form biofilm and development of drug resistance in many pathogenic microorganisms greatly reduced the efficacy of currently available antibiotics, leading to a serious problem in public health management. Hence, the use of nanoparticles serve as a novel strategy and a potential alternative to antibiotics in dealing with the serious medical scenario of the development of MDR strains.

This chapter discusses the synthesis of nanoparticles by various microorganisms, the synthesis mechanisms, and their potential applications to solve the problem of drug resistance in numerous pathogens. Over the last decade, there has been a tremendous development in the field of microorganism mediated synthesis of nanoparticles and their applications. Owing to the rich biodiversity, microorganisms as a biofactory serve as an environmentally safe and sustainable method for the synthesis of nanoparticles. Actinomycetes, yeast, algae, fungi, and bacteria have been successfully employed for the enzymatic synthesis of safe and stable metal and metal oxide nanoparticles. The ease of cultivation and manipulation of microorganisms also gives the advantage to manipulate the synthesis rate and the associated parameters. The synthesis process takes place either extracellularly or intracellularly by the aid of microbial enzymes. The biosynthesized nanoparticles naturally capped with protein/lipid layer imparts stability and physiological solubility to the nanoparticles, making them suitable for biomedical applications. Metals and metal oxide nanoparticles possess remarkable bactericidal, fungicidal, and antiviral properties, demonstrating their potential as a promising tool in antimicrobial therapy. The potency of these nanoparticles is significantly governed by physical characteristics including shape, size, and stability. Different metallic NPs, such as silver, gold titanium, copper, and magnesium, are known for their antimicrobial efficacies. Many nanoparticles exhibit bactericidal properties by generating ROS and in some cases penetrate inside the cell membrane and release metal ion. Due to their small, controllable size and property, functionalized nanoparticles have emerged as an effective carrier in drug delivery. The recent progress and the ongoing research in functionalization gave way to new avenues for commercial applications of functionalized nanoparticles in medicine and healthcare. Nanoparticles show a significant increase in biocompatibility and activity when conjugated with other materials. Nanoparticles fabricated with antibiotics, dyes and antibodies exhibit enhanced antimicrobial activity. The nanoparticles exhibiting antimicrobial property are being explored for their application in prosthetic device coatings, catheters, ointments and gels, and in dental implants. Further, the application of nanoscale antimicrobials in the management of life-threatening diseases, facilitated by their fascinating microbicidal and drug delivery capabilities, is of ongoing interest. Further, research on microbially synthesized nanoparticles possessing unique optical, physicochemical, and electronic properties is of great significance for application in medicine. Thus, nanoparticles in the field of antimicrobial chemotherapy have gained much attention as an eco-friendly agent to fight microbial resistance and prevent diseases.

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