Chapter

Antimicrobial Efficacy of Biogenic Silver and Zinc Nanocrystals/Nanoparticles to Combat the Drug Resistance in Human Pathogens

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Abstract

The emergence of biogenic nanomaterials as novel antimicrobials introduces a new paradigm in human health care. Based on the recent reports of the World Health Organization, infectious diseases pose one of the greatest health challenges. Increased multi-drug resistance prevalence among human pathogens, due to the inefficiency of commercially available antimicrobial drugs in the market is a great threat to humans. The poor solubility, stability and side effects of the antibacterial therapy prompted the researchers to explore new innovative strategies for developing new antimicrobials. Recently, biogenic nanoparticles have proven their effectiveness against multidrug-resistant (MDR) pathogens as an alternative to conventional antibiotics. Biogenic nanoparticles such as silver nanoparticles (AgNPs) and Zinc Oxide nanoparticles (ZnONPs) are easy to produce, biocompatible, provide enhanced uptake and are eco-friendly. Moreover, the capping of the biogenic nanocrystals provides an active surface for interaction with biological components, facilitated by free active surface functional groups to enhance their efficacy and delivery. Inorganic nanocrystals (AgNPs and ZnONPs) are effective both as nano-bactericides and as nanocarriers against sensitive and MDR) pathogens. The present chapter focuses on the utilization of the recent nanosystems to combat drug resistance in human pathogens. Nanomedicine represents a new generation of potiential antimicrobial candidates capable of combating the drug resistance in various pathogenic organisms.

Keywords: Antimicrobials, MDR, AgNPs, ZnONPs, nanocrystals, biogenic nanoparticles

1. Introduction

Nanotechnology is regarded as a new discipline that has a significant influence on human life in several respects with various applications [1]. Current nanoscience research is based on nanoparticle synthesis. Metal nanoparticles are used for a

wide range of applications including biosensors, organic marking, cancer therapy, textiles, household and industrial applications etc. Silver nanoparticles AgNPs are mostly used in wound dressings, care of the eye, oral hygiene, biomaterials of bone substitutes, antimicrobial and anti-inflammatory drugs as well as in the coating of catheter products as anti-inflammatory and antimicrobial agents [2]. Silver is a stable and non-hazardous antibiotic agent used for centuries [3]. Most antimicrobials have many disadvantages including low stability, environmental toxicity and the lack of specificity towards the target microorganisms [4]. Few other antimicrobials are extremely irritating and expensive to develop [5].

Silver has the unusual property of binding cellular components that are far larger than nuclear acids [6]. AgNPs may be synthesized employing physical, chemical and biological processes. The majority of the physical and chemical processes of synthesizing nanoparticles have many disadvantages such as low yield, strong reducing agents, energy-intensive mechanisms, uneven particle size and aggregate instability, hazardous waste production, difficulty to scale up and expensive organo-metallic precursors are required [7]. Biological approaches for the synthesis of nanoparticles are regarded as more stable and efficient [8]. For several nanoparticles like gold, silver, platinum and palladium, titanium dioxide, magnetite and cadmium sulphide, the most possible bio-factories are bacteria. Bacteria-mediated AgNPs synthesis is preferred in comparison with other techniques.

Furthermore, bacteria mediated AgNPs are simpler to grow and environment friendly. Both intracellular (biomass) as well as extracellular (cell extracts) synthesis of silver nanoparticles can be performed. Intracellular approaches include the release of synthesized nanoparticles through ultrasonication and additional reactions with specific detergents. It is therefore essential that the AgNPs are synthesized with extracellular methods because of their easy downstream processing that supports large-scale development [7, 9, 10]. There is now a prevalence of multiple tolerance to antibiotics by various clinical infections and pathogens of the urinary tract, caused by excess antibiotics and by an accumulation of antibiotics in the system. This kind of resistance is exhibited by *Staphylococcus* sp., *Streptococcus* sp., *Klebsiella* sp., *Enterococcus* sp., *Proteus* sp., *Pseudomonas* sp. and *E. coli* due to their biofilm-forming potentials [11]. The use of antimicrobial silver nanoparticles will eliminate the multiple-drug resistance, which is a suitable option for antibiotics [12]. Biofilm formation has been regarded as the global barrier in avoiding catheter-related infections in the field of medicine [13]. The conversion of nanoparticles into therapeutic agents, however, involves a detailed knowledge of the physicochemical particularities, results *in vitro* and *in vivo*, biodistribution, pharmacokinetics and pharmacodynamics, apart from the suitable methods of their synthesis [14].

2. Synthesis of nanoparticles

In broader terms, nanoparticles can be synthesized either by (i) Top- down approach, or (ii) Bottom- up approach [15]. Based on the reaction conditions and operation, these two classes can be further categorized as physical, chemical and biological methods [15].

i. Top- down approach

In this method, larger molecules are broken down into smaller units which are then transformed into suitable nanoparticles [15] According to a study, the synthesis of the spherical magnetite nanoparticles that uses

natural iron oxide is performed through top- down method which produces particle sizes ranging from 20 nm to 50 nm [15].

ii. Bottom- up approach

This method works in reverse to the top- down approach as nanoparticlessynthesised using this approach are formed from smaller and relatively simpler substances that form clusters and are subsequently converted into desired nanoparticles. This technique is also known as building up approach. Sedimentation and reduction technique fall under this category which include sol gel, green synthesis, spinning and biochemical synthesis [15].

3. Green synthesis of nanoparticles

Green synthesis of nanoparticles refers to the synthesis of nanoparticles through biological routes such as those with the help of microorganisms, enzymes, fungus plants or using various plant products [16, 17] Conventional physical or chemical methods of nanoparticle synthesis often produce byproducts that are hazardous to the environment which is one of the key reasons to opt for a more suitable alternative, that is, the green synthesis or green technology [16]. Other aspects by which green synthesis is more superior than the physical and chemical methods are that they are cost efficient and consume less energy [16].

Bottom- up approach is employed in biological- based synthesis of nanoparticles that requires the use of stabilizing and reducing agents [16]. The process of biologically synthesizing nanoparticles is basically divided into three steps: (i) the choice of a suitable solvent medium used, (ii) the choice of a suitable reducing agent that is eco- friendly and environmentally benign, and (iii) the choice of a non- toxic capping agent that can stabilize the synthesized nanoparticles [16].

Prokaryotes as well as eukaryotes are used in the green synthesis of metallic nanoparticles such as silver, gold, platinum, iron, and metal oxides such as zinc oxide and titanium oxide [17].

3.1 Biological components for green synthesis

Bacteria: Prokarytic bacteria and actinomycetes are widely used in the synthesis of metal and metal oxide nanoparticles as they have the potential to reduce metal ions and therefore, are suitable candidates for the preparation of nanoparticles [18] . The fact that it is relatively easier to manipulate bacteria is a key point in employing them in nanoparticle synthesis [18].

Fungi: Another popular choice for the biological synthesis of metal and metal oxide nanoparticles is fungi as they behave as better biological agents because they have diverse intracellular enzymes [18]. It is also reported that fungi can comparatively synthesize more amounts of nanoparticles than bacteria which could also be because of the fact that fungi have various enzymes/proteins/reducing components on the surface of their cells [18].

Yeast: Saccharomyces cerevisiae has found to be quite effectively employed in the synthesis of silver and gold nanoparticles as reported in numerous studies [18].

Plants: The most simple, efficient, cost effective and feasible method of biosynthesis of metal and metal oxide nanoparticles is using plants and plant extracts as biological agents. Biomolecules such as carbohydrates, proteins and coenzymes extracted from plants are employed to reduce metal salt into nanoparticles [18].

4. Applications of nanoparticles as antimicrobial agents

Nanoparticles have an antimicrobial activity that is capable of overcoming typical resistant mechanisms, including inactivation of the enzymes, reduction of cell permeabilities, modification of target sites/enzymes, and increasing efflux by excessive expression of efflux pumps to escape antimicrobial activity [19, 20]. In addition, NPs combined with antibiotics have a synergistic impact on bacteria, prevent biofilm formation and are used in combating multidrug-resistant organisms [20, 21]. Many features of the nanoparticles provide alternatives to conventional antibiotics. First, with the high volume-surface ratio of nanoparticles, the interaction area with the target species is increased. NP can function as nanoscale molecules that communicate with bacterial cells, regulate penetration of cell membranes and interfere with molecular pathways [22–24]. Secondly, nanoparticles may enhance the inhibitory effects of antibiotics. Saha et al. [25] reported that gold nanoparticles conjugated with ampicillin, streptomycin, or kanamycin could lower the minimum inhibitory concentrations (MICs) of the antibiotic counterparts against both gram-negative and gram-positive bacteria. Gupta et al. [26] have shown a synergistic impact on multi-drug-resistant *Escherichia coli* infections through functionalized gold nanoparticles and fluoroquinolone-based antibiotics.

The complexity of the physicochemical properties, including the scale, form, chemical changes, solvent and environmental factors may influence the antibacterial properties and interfere with the bacteria during the preparation of nanoparticles [27]. Finally, antibiotic and nanoparticle combinations have diverse antimicrobial pathways for overcoming antibiotic resistance [28]. Thus, nanoparticles are regarded as next-generation antibiotics.

Nanoparticles, primarily metallic, have proved effective against gram-positive and gram-negative bacteria in both in vitro and in vivo studies [29]. Even though the antimicrobial mechanisms that are based on the size, shape, zeta-potential, ligands, and material used are not fully understood [28–30] some of the currently accepted mechanisms include (1) disruption of membrane potential and integrity through direct contact with bacteria; (2) activation of the host immune responses; (3) inhibition of biofilm formation; (4) generation of reactive oxygen species (ROS); and (5) induction of intracellular effects to inhibit RNA and protein synthesis [21, 27]. Nanoparticle coatings on implantable devices, urinary catheters, wound dressings, bone cement, or dental implants/materials can function as nanoparticle-based antibiotic delivery systems [31, 32]. Furthermore, nanoparticles can be used as vectors to transfer and deliver higher doses of drugs to infected sites [21]. Thus, the combination of NPs and antimicrobial agents may be beneficial in fighting the ongoing crisis of antimicrobial resistance [20]. The combination of nanoparticles and antimicrobials could thus help to combat the current anti-microbial resistance crisis [20].

Nanoparticles with antimicrobial activity that combat *E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa, and Enterobacter species* [24, 27, 33, 34] include the nanoparticles containing silver (Ag), gold (Au), zinc (Zn), copper (Cu), Titanium (Ti), magnesium (Mg), nickel (Ni), cerium (Ce), selenium (Se), aluminum (Al), cadmium (Cd), yttrium (Y), palladium (Pd), or superparamagnetic iron [24]. It has been reported that among various metallic nanoparticles and their oxides, silver or its ionic form is most toxic to bacteria [35]. Silver nanoparticles (AgNPs) find numerous uses as they possess several mechanisms of antibacterial activity [36], high biocompatibility, and functionalized potential and are easily detectable [37]. Even though AgNPs are difficult to functionalize with biomolecules and antibiotics, Ag–gold (Au) alloys provide an alternative, since they combine

the antimicrobial effects of Ag with the effectiveness of functionalization and the stability of Au in the form of bimetallic NPs [20]. Furthermore, Ag–Au NPs functionalized with tetracycline have been shown to have a synergetic effect, which is attributed to the generation of ROS [38].

Antibiotic resistance is often associated with biofilm formation and an active quorum sensing machinery within a bacterial cell. Biofilm plays a crucial role by evading the action of antibiotics that hinder their entry in the cell membrane surface as a result rapid dissemination of resistance may occur. Almost all bacteria posses biofilm and it may remain in an inert state by modulating the epigenetic changes within its genome allowing them to develop adaptive resistance as a result. Exopolysaccharide capsular fractions, extracellular polymeric substances (EPS), also provide structural integrity that works as a barrier, restrict the antibiotic action by modulating the cellular integrity, further promote antimicrobial resistance. Several evidences have shown the capability of nanoparticle to disrupt the cell membrane, hinder biofilm formation and dispute the active quorum-sensing systems. The design of biologically active nanoparticle has to be made in such a way that it must circumvent the barrier function to elicit their activity. The most predominant and promising strategy could be to interfere with the cellular signaling mediated by quorum sensing molecules such as Acyl homoserine lactone (AHLs) for Gram-negative bacteria and peptides of Gram-positive bacteria. (**Figure 1**).

The mechanism of the nanoparticle often dealt with the synthesis of N-acetylated homoserine lactonase proteins (AiiA) capable of modulating signals by preventing the stimuli from reaching its cognate receptor site, as a result, the intracellular signaling affects the communication. The activity of quorum quenching could be enhanced by coupling the drug moieties into a nanoformulation. Enormous alternatives are available, capable enough to elicit its activity by employing dendrimers, chitosan and liposome coating to give effective drug delivery. Siddhardha et al. [39], have recently evaluated the anti-biofilm property of the chrysin-loaded chitosan nanoparticles and their role in combating infections caused by *S.aureus*. Chrysin, a plant flavone constituent of *Orocylumineicum vent* is well-established for its biological properties. However, its therapeutic attributes have

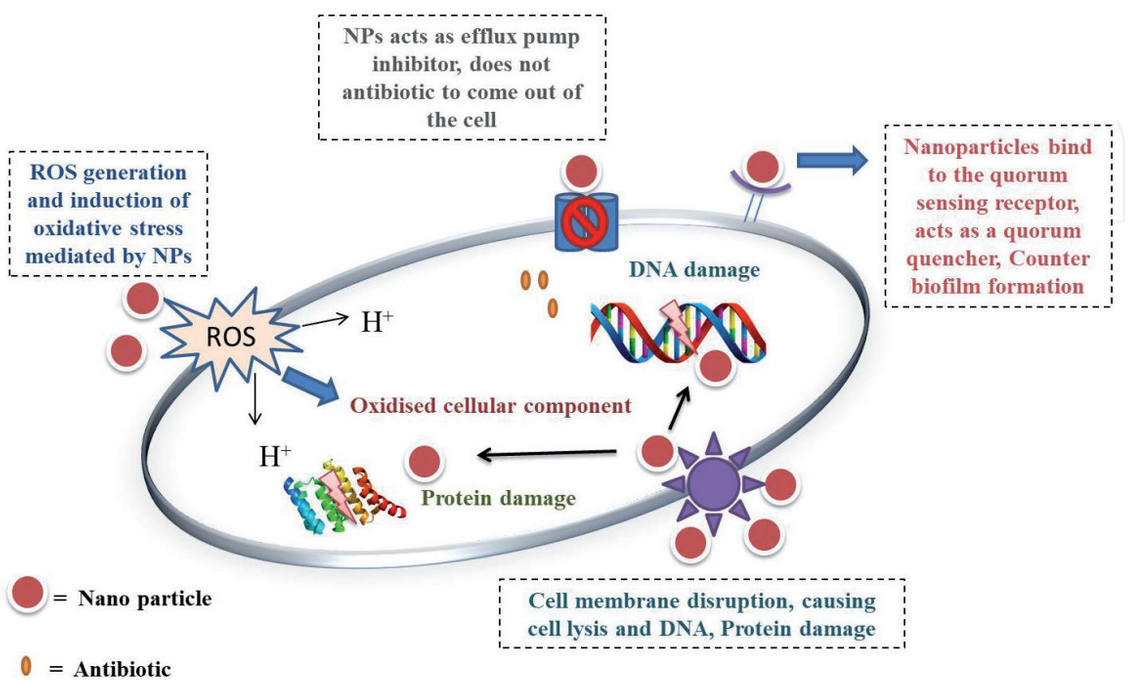

Figure 1.
Mechanism of antimicrobial activity by nanoparticles.

not been fully deciphered due to its poor solubility and less bioavailability. In their study, chrysin has been encapsulated into chitosan derived nanoparticles using TPP as a linker.

Nanoparticles were further characterized and assessed for their ability as an anti-biofilm compound against *Staphylococcus aureus*. In sub-Minimum Inhibitory Concentration, (MICs) these nanoparticles exhibited increased anti-biofilm efficacy against *S. aureus* as compared to its counterparts, chrysin and chitosan, remarkably reduced in the cell surface hydrophobicity index and exopolysaccharide (EPS) production indicated by the inhibitory effect of nanoparticles on the primary stages of biofilm formation. However, growth curve analysis further showed that at a sub-MIC point nanoparticles did not exert any bactericidal effect against *S. aureus*. During the last few years, various nano-mediated delivery systems such as poly(lactic-co-glycolic acid) (PLGA), fusogenic liposome molecules, solid lipid nanoparticles, also known as SLNs, are physiological lipids dispersed in aqueous surfactant and lipid-polymer hybrid have proven to be promising vehicles to act as a nanocarrier. Commercially available Protein, and lipid conjugated polymeric substance viz. Intron A and AmBiosome have become the most predominant nano platform to evaluate their efficacy in terms of countering the biofilm formation within pathogenic bacteria. In one of the recent studies, sodium lauryl sulfate (SLSs) have been shown to act as anti quorum sensing agents (Quorum quencher) by downregulating the pyocyanin production in *P. aeruginosa* strain.

5. Silver nanoparticles and their antibacterial efficacy

Silver nanoparticles have been identified as an excellent antibacterial agent with potential medical applications. Green nanoparticle synthesis has emerged as a viable alternative to overcome the limitations of traditional methods of nanoparticle synthesis [40]. Green synthesis of silver nanoparticles (AgNPs) employs plant constituents such as sugars, fats, flavonoids, alkaloids, polyphenols have become an alternative to the rational chemical formulation. To date, green synthesis of AgNPs is carried out by using fruit extract of *Emblica officinalis*, leaves extract of *Citrus limon*, *Azadirachta indica*, *Coffea Arabica*, *Acalypha indica*, *Camellia sinensis*, a root extract of *Phoenix dactylifera*, *Morinda citrifolia*, inflorescence extract of *Mangifera indica* etc. [41]. Phytochemicals exhibit various antimicrobial activities and they act as reducing or stabilizing agents in plants and the co-occurrence of these natural compounds shape the biogenic AgNPs morphology.

Paradoxically Mechanism of action of AgNPs for eliciting antibacterial efficacy is a controversial topic. Several research papers have shared certain assumptions such as Ag ions get released by the AgNPs and submerged around the cell surface affecting the penetration barrier of the cell wall or cell surface by dismantling the cellular permeability. It has been reported that AgNPs can affect DNA replication while interacting with thiol moieties of protein molecule initiate protein deactivation with the concomitant liberation of reactive oxygen species (ROS) [42].

Other hypotheses suggest that the receptor musking capabilities of AgNPs due to their size which might restrict the biofilm formation in *E.coli* and *S.aureus* by disputing the quorum sensing machinery. The dissolution status of AgNPs strongly affects their antibacterial efficacy depends on synthetic and intrinsic characteristics of surrounding media. The coexistence of particle size and morphology on the liberation of Ag + described, by the Ostwald–Freundlich equation. The presence of smaller AgNPs with quasi-spherical shape are more prone to Ag + release, due to their greater surface ratio. This quality further explains the lower silver content of aggregated nanoparticles, which is relative to isolated nanoparticles. Certain

capping agents are used to modulate the AgNPs surface, which in turn, may cause their dissolution behavior. Sometimes, the surrounding media itself can influence the release of Ag + ions. The co-occurrence of inorganic or organic components in a growth medium can affect the solvation potential of silver nanoparticles by augmenting with nascent AgNPs or coupling with silver ions. Studies have also shown that AgNPs release Ag + faster in acidic condition than they do in neutral solution [43].

Certain Gram-negative bacteria are more susceptible to AgNPs because of their hallmark cellular architecture, which is the presence of an outer membrane coating over the polymeric peptidoglycan layer. On the other hand, the cell wall of gram-negative bacteria is much narrower than that of gram-positive bacteria, hence, the thick cell wall might reduce the penetration of NPs into cells. Moreover, the antibacterial effects of AgNPs on gram-negative and gram-positive cell emphasize that uptake of AgNPs is crucial to elicit its antibacterial effect.

Silver nanoparticles, smaller than 10 nm can directly alter cell permeability, enter bacterial cells and cause cell damage by employing the abovementioned strategies. Most of the noxious biofilm formers, rapidly develop exopolymeric substances which protect bacteria from both Ag + and NPs activity by hindering their transport. Researchers have observed that 100% reduction or mortality of bacteria did not occur in the biofilm state, whereas, AgNPs with the same concentration, enabled their effect by killing all planktonic cells. However, the polymeric biofilm hinders the use AgNPs, due to its complicated cellular architecture. Diffusion coefficients of AgNPs are generally related to size, shape and physicochemical characteristics, which governs their mobility and bioavailability in biofilm structure. The diffusion coefficient decreases with increasing molar mass make it harder for larger AgNPs to counter biofilm formation. Inter and Intracellular transport through bacterial biofilm can be impacted for particles >50 nm. Chemical constituents of NPs can also arouse adsorption augmentation of AgNPs in the biofilm, by interfering with their diffusion profile. Lastly, electrostatic intricacy among bacteria and AgNPs could influence charged nanoparticles' penetration through biofilm [44].

Silver is being considered to be a potent antimicrobial agent. Silver nanoparticles (AgNPs) are the most promising inorganic NPs employed to treat a variety of bacterial infections. Synthesis of AgNPs could be achieved by green syntheses such as using plant or microbial extracts. AgNPs mediated cell death, causing cell wall disruption has become one of the most striking opportunities for the antimicrobial researcher to evaluate its efficacy *in vitro.* AgNPs are capable of producing their activity inactivating the respiratory chain followed by ROS production with a process of oxidation of the bacterial cellular constituents. The cell permeability increases upon treating with AgNPs, cause depolarization of the cell wall. AgNPs have been found to show synergized effect with different antibiotics that resulted in better efficacy. Lara et al. [45] have demonstrated the efficacy of AgNPs against multidrug-resistant nosocomial pathogenic bacteria such as *P.aeruginosa*, ampicillin-resistant *E.coli* O15:H7 and MDR *Streptococci* strain. It has been reported that Ag-bearing nanocrystals are sensitive against a variety of Gram-positive and Gram-negative bacteria such as Vancomycin-resistant *Enterococci*, *S. mearcesens*, counter the biofilm formation in MTP assays.

Antimicrobial peptides (AMPs) prove to be one of the key elements for the defense strategy against various high-density bacterial infections. Unfortunately, AMPs are sparingly soluble and have possess poor enzymatic stability, often get churned into pieces before they cross the biological barrier due to their low permeability. Immobilization of AMPs onto NPs can be an exciting way for drug delivery. Polymyxin B is the most used AMP, as it has a good antibacterial activity, it modulates the LPS in the outer membrane present within Gram-negative cells. It has been

demonstrated recently that AgNPs coupled with polymyxin-B removed endotoxin contamination from the surgical solutions and downregulated the biofilm formation on the blades used for surgical procedures.

6. Zinc oxide nanoparticles and their antibacterial efficacy

Zinc oxide nanoparticles are utilized in food as additives, supplements, containers, and packaging; in the energy sector as fuels and catalysts; in consumer electronics, semiconductors, and air filters; in biomedical engineering; and in drinking water.

Plant constituents such as *Cassia tora*, *Calotropis gigantean*, *Hibiscus sp*, *Corymbia citriodora* etc. have been used for green synthesis of ZnNPs [46, 47]. These plants for example, *Catharanthus roseus* produce secondary metabolites, containing more than 200 terpenoid based indole alkaloids, notable for their antimicrobial and anticancer activity [48].

Recently, the antibacterial efficacy of Zn NPs was assessed for *Staphylococcus aureus* MTCC 9760, *Pseudmonas aeruginosa* MTCC 424, *Streptococcus pyogenes* MTCC 1926, *Escherichia coli* MTCC 40, *Bacillus cereus* MTCC 430 and *Proteus mirabilis* MTCC 3310. The green newly synthesized Zn NPs exhibited promising antibacterial activity against both Gram-positive and Gram-negative bacteria. These results elucidated a rapid, cost-effective, environment friendly and convenient method for ZnO NPs synthesis, which could be used as a potential antimicrobial agent against drug-resistant microbes [49].

Zinc nanoparticles minimize the use of toxic substances in NPs fabrication and modulate the antibacterial efficacy and biomedical applications. Several studies have reported the efficacy of ZnO NPs against various pathogenic bacteria. It has been observed that the shape and size of ZnO NPs dependent on several physicochemical characteristics such as reaction kinetics, annealing temperature and pH. ZnONPs exhibit a strong antimicrobial efficacy for *E. coli* and *S. aureus* without UV illumination at NP concentration of 0.025 mg mL^{-1} after 8 h of incubation [50].

Green synthesis of Zn NPs could be initiated by rapid mixing of the aqueous solution of 0.01 M zinc acetate dehydrate with above mentioned any of the leaf extracts. This process is further followed by constant stirring till the appearance of white suspension. The pH needs to be adjusted till the ZnO NPs precipitate is completely dissolved. Finally, the spectra exhibites an absorption band with a resolution of 1.0 nm between 350 and 500 nm, which confirms the formation of ZnO NPs. Physicochemical parameters regulate the green synthesis of inorganic metal NPs. Hence, several physicochemical parameters are optimized such as pH, temperature; reaction time and concentration of the metal ions [51].

Zinc oxide NPs are characterized for their bactericidal activity by affecting planktonic cell growth and their proliferation. Like AgNPs the mechanism is to produce ROS with an adjunct to its Zn^{2+} release in a liquid medium, which hinders the enzymatic activity and normal physiological process within a healthy bacterial cell. The stability of these oxides is achieved by their high density, high melting point, high thermal conductance and conductivity. Besides ZnO, rare-earth NPs have become one of the alternative treatment choices and their oxide forms differ in chemistry from the main group elements as well as transition metals because the 4*f* orbitals of these elements are buried deep within the atom and the 4*d* and 5*p* electrons shield the 4*f* orbitals from the atom's environment and thus owing to the unique characteristics of these elements in terms of catalytic, magnetic and electronic properties which can be exploited by preparing nanoparticles from their oxide forms. Sarwar and his coworkers [52] have recently demonstrated that the

nanoformulation of ZnO has caused significant oxidative stress to *Vibrio cholera*, with a concominant downstream process of DNA degradation, protein leakage, membrane depolarization and fluidity as a result.

Moreover, ZnO NPs impregnated with antibiotics have shown better efficacy to act as an antimicrobial agent against almost every noxious biofilm formers under the group of ESKAPE pathogens viz. *S. aureus*, *Proteus* sp., *Acinetobacter* sp., *P. aeruginosa*, and *E. coli*, whereas the antibiotic alone found to be resistant towards them, showed a synergistic effect under these circumstances.

7. Nanoparticle based alloys

Nanoparticle alloys made by the combination of Ag and Au may.be used to enhance the effects of a drug. Even tough they have antimicrobial effects when used alone, once they are used in combination their effects get enhanced [30, 53]. It has been reported that AgNPs face difficulties in functionalizing with biomolecules and drugs, to overcome this limitation in monometallic NPs, alloy/bimetallic NPs have been introduced which possess improved electronic, optical and catalytic properties [54, 55]. AuNPs are considered to be good vectors for the delivery of pharmacologic compounds. The combinatorial effect of nanoparticle alloys increases efficacy- in biological media, gold enhances the functionalization improves the stability along with the antimicrobial activity of silver [56, 57]. Fakhri et al. [38] found that antibiotic (tetracycline) along with the bimetallic nanoparticles (Au-AgNPs.) showed a synergetic effect, with greater antimicrobial activity. Baker et al. [58] in their study have reported antimicrobial activity of bimetallic AgAuNPs against *Pseudomonas veronii* strain AS41G inhibiting *Annona squamosa L.* They also reported their synergistic effect with standard antibiotics against the bacitracin resistant strains of *Bacillus subtilis, E. coli*, and *K. pneumoniae* [58]. Zhao et al. [59] have signified the antibacterial activity of bimetallic NPs like AuPtNPs against drug resistant bacteria.

8. Synergistic effects of NPs with antibiotics

For overcoming antibiotic resistance, NPs can be customized and packaged with various antimicrobial agents. NPs use several mechanisms to combat bacterial resistance. If NPs are used in combination with antibiotics, there is very less chance that bacteria can develop resistance [60, 61]. Thus, NPs in combination with antibiotics can be a promising strategy to overcome bacterial resistance. Additionally, NPs are effective antimicrobial delivery systems, and may reduce the dosage and toxicity of antibiotics [24]. Very low concentrations of AgNPs and antibiotics have been synergistically found to inhibit various pathogens such as *S. aureus*, *P. aeruginosa*, *A. baumannii* strains etc. [62–64]. Similarly, synergistic effects Ag, Au, and ZnO NPs along with antibiotics have been found to be effective against *S. aureus*, *E. faecium*, *E. coli*, *A. baumannii*, and *P. aeruginosa* [24]. The efficacy of antibiotics along with functionalized NPs may help in reversal of antimicrobial resistance and may also enhance the antimicrobial effects of various antibiotics [24].

9. Nanotoxicity

Although there is an immense progress in the field of nanotechnology, but the consequent health effects that are related to the exposure to nanoparticles remain largely unexplored. Researchers have started to characterize the risks associated

with nanoparticle exposure. [65]. Impact of NPs on beneficial bacteria in humans should be of high concern [66, 67]. It has been reported that NPs can cause hemeolysis thus, may impair blood coagulation [68]. The precise mechanism of toxic effects of NPs is not so clear, but it has been observed that nanoparticles larger in size pose a greater threat on human health. In most of the invitro studies conducted on AgNPs, it has been observed that AgNPs are more toxic for cell lines [69, 70]. Deposition of AgNPs has been observed in many organs such as liver, lungs, spleen which has been linked to organ damage [24]. In blood and urine of burn patients elevated levels of Ag have been reported, this may be due to leaching from Ag wound dressing (Acticoat®),which is composed of Ag nanocrystals, into the blood stream [67, 71].

Neurotoxicity has been associated with Al2O3 NPs which interact with cellular components [33]. CuONPs have been observed to induce oxidative damage. CuO NPs induce hepatotoxicity and nephrotoxicity through the generation of free radical-mediated oxidative stress [20, 72, 73]. DNA damage has been associated with ZnO or TiO2 NPs thus, limiting their use [24]. Hagens et al. [74] have reported that NPs that are administered intravenously could get accumulated in bone marrow, colon, lung, liver, spleen as well as lymphatic system. Inhalation of NPs has been associated with cytotoxicity in the lungs [75].

In various *in vivo* studies, it has been reported that there is lethal toxicity associated with the use of NPs [29, 76–78]. Nevertheless, the assessment of toxicity at the cellular level as well as mode of administration is crucial for clinical use of NPs [67].

10. Conclusion

Silver and Zinc NPs have the ability to interact with and influence the growth of a variety of microorganisms. Therefore, Ag and Zn NPs could be employed as a broad-spectrum antibiotic agents to combat bacterial infections. Plant extracts are used to make Ag and Zn NPs, which is cost-effective and eco-friendly. The antibacterial action of Ag and Zn NPs appears to be attributed to their structure, ultrasmall size, and increased surface area, which allows them to damage and pass the bacterial membrane that is responsible for intracellular damage. It has been described that NPs in combination with antibiotics can be a promising strategy to overcome bacterial resistance. Synthesis of Ag and Zn NPs via green-synthesis methods and mechanisms of action against bacteria have been elucidated. We have also shed light on toxicity associated with the use of NPs. Ultimately, it can be conluded that both Ag and Zn sNP have broad-spectrum antibiotic activity against bacteria, making them prospective therapeutic agents for bacterial infections and multidrug-resistant pathogens.

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