Chapter 8
Silver-Based Nano-formulations for Treating Antibiotic-Resistant Microbial Strains

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Abstract Nosocomial infections represent the most severe complications among hospital patients and coworkers upon exposure to antibiotic-resistant microbial strains. Such healthcare-associated infections (HAIs) are one of the leading causes of morbidity and mortality, inflicting enormous social and economic burdens worldwide. Whether a patient's body, hospital floors, or medical textiles and medical devices/instruments, these pathogens find surfaces to attach/grow and deceive the rigorous implementation of adopted hygiene practices. Hence, there is an utmost need to develop new antibiotic alternatives, such as engineered nanoparticles, which could offer a better biocidal response to almost all surfaces while minimizing the antimicrobial resistance crisis. Due to its broad-spectrum features and multitude of mechanisms of action, nano-silver has emerged as the most deserving candidate for designing such antimicrobial strategies. Nano-silver formulations are equally amenable to be utilized as surface coatings, thin films, polymeric layers, hydrogels, nanocomposites, and blend mixtures, other than its usage as a colloidal suspension. Treatments to curb antibiotic overuse by formulating new antibiotics/nano-silver blends are also gaining importance for preventing and controlling microbial infections. The current chapter thus summarizes various nano silver-based antimicrobial strategies, their action mechanism, and efficacy against antibiotics/multidrug-resistant microbial strains.

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V. Kumar et al. (eds.), *Nano-Strategies for Addressing Antimicrobial Resistance*, Nanotechnology in the Life Sciences, https://doi.org/10.1007/978-3-031-10220-2_8

Keywords Multidrug-resistant strains · Silver nanoparticles · Silver nanocomposites · Healthcare-acquired infections · Antibacterial mechanism

8.1 Introduction

The emergence of several new antibiotic resistance bacterial strains poses alarming threats to contemporary advancements in healthcare sector. A wide range of biomedical devices and antibiotics used to treat patients are ineffective in eradicating microbial infections due to the resistance development against them (Jernigan et al. 2020; Thorpe et al. 2018; Cassini et al. 2019; Tacconelli et al. 2018). While bacteria are constantly evolving to develop resistance against new antibiotics, virtually all hospital-related surfaces, e.g., human skin, floor, tiles, sutures, bandages, biomedical implants, surgical instruments, and devices, act as natural "niches" to microbes to attach, grow, and proliferate in the form of biofilm, which eventually protects them against harsh conditions (Høiby et al. 2010). Microbial biofilm provides additional resistance against antibiotics and host immune factors; microbes can survive and multiply quickly over the host surface. Considering the consequences, developing an immediate solution is the current need of the hour, which has gained substantial attention to combat this threat for global health. Researchers have been exploring various materials and approaches to make a universal antimicrobial agent with a broad spectrum of bactericidal activity and its applicability for different applications. The reluctant and robust nature of multidrug-resistant microbes has made most of them ineffective against conventional biocidal agents. A blooming field has suggested some interesting leads, known as nanotechnology (Tacconelli et al. 2018; Gupta et al. 2019; Doughari et al. 2009).

Nanomaterials have witnessed exponential growth in developing new antimicrobial biomaterials with superior characteristics (Sillu and Agnihotri 2020; Agnihotri et al. 2018; Singh et al. 2016, 2021; Chauhan et al. 2019). At the nanoscale, particularly between 10 and 25 nm, materials exhibit remarkable physiochemical attributes such as the high surface area to volume ratio that dictates their antimicrobial potential (Agnihotri et al. 2014). Metal and metal oxide nanomaterials based on silver, gold, copper, zinc oxide, and titania have witnessed their antimicrobial character against various strains (Mukherji et al. 2012; Agnihotri et al. 2015; Yadav et al. 2016; Sirelkhatim et al. 2015). Silver is the most effective nano-weapon due to its innate bacterial killing capacity. It requires low inhibitory concentration to achieve complete bacterial inhibition. Nano-silver has shown its therapeutic arsenal against multidrug-resistant (MDR) strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSA), ampicillin-resistant *Escherichia coli*, and erythromycin-resistant *Streptococcus pyogenes*. The bactericidal activity of silver nanoparticles against biomaterial-associated infections, i.e., *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus*

haemolyticus, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Proteus vulgaris*, *Candida albicans*, and *Propionibacterium acnes*, has also been thoroughly investigated by many scientists.

For biomedical application viewpoints, nano-silver-based antimicrobial therapies can safely be applied during surgical treatments to prevent pathogenic growth without causing any adverse effects on neighboring healthy tissues or interacting with the biomedical apparatus. Although the exact mechanism of antimicrobial action of nano-silver against antibiotic-resistant bacteria is debatable, a series of possibilities, including its direct interaction with the cell wall membrane, production of reactive oxygen species, disrupting cellular respiration machinery, and rupturing signal transduction pathway, have been elucidated as the leading causes (Ahmad et al. 2020; Crisan et al. 2021). The attachment of silver nanoparticles with bacterial cell walls is the first point of interaction, which can be augmented via the functionalization of silver nanoparticles easily. Various studies have established the vital role of nanoparticles' size, as penetration through the cell membrane is a critical step in inhibiting bacterial growth (Agnihotri et al. 2014).

Besides benefits, silver nanoparticles suffer limitations, and their employability for biomedical use is often restricted. It is evident that being extremely unstable, silver nanoparticles experience poor colloidal stability and thus are prone to get aggregated, losing their actual antimicrobial efficacy. Moreover, humans' direct uptake of nano-silver may induce severe cytotoxicity and inflammatory response that worsens the condition for critically ill patients. This issue can be overcome by anchoring silver nanoparticles onto a suitable support material such as porous networks, thin films, hydrogels, polymeric nanocomposites, and even fibrous materials (Agnihotri et al. 2012, 2013, 2015; Agnihotri and Dhiman 2017). These strategies have been investigated to fabricate novel nano-silver-based biomaterials as antimicrobial agents combating MDR (Dhiman et al. 2019). A synergistic impact of host material with nano-silver may result in superior antibacterial surfaces with good cytocompatibility with human cells. Recent advances in the field prove their potential for direct applications in biomedical research as wound healing material, implant surfaces, etc. This chapter thus focuses on two modes of utilizing nano-silver-based formulation in healthcare, either by using the colloidal form or as immobilized/impregnated state in the form of silver-nanobiomaterial.

8.2 Colloidal Nano-silver Formulations

Silver nanoparticles (AgNPs) exist as ultra-smallAg^0 metal in several defined shapes, e.g., spherical, triangular prism, tetrahedral, cubical, prismatic, and octahedral, with various sizes ranging from 5 to 100 nm (de Lacerda et al. 2020; Wahab et al. 2021a). A variety of AgNPs complexes have primarily been employed as a part of the therapeutics to treat various diseases and infections. Also, their non-lethal nature to the human body at low fixation is alluring for obvious medical applications (Talapko et al. 2020). AgNPs are used extensively in food packaging, food

additives, electronics, textiles, household appliances, cosmetics, and water disinfectants, owing to their antibacterial properties (Ferdous and Nemmar 2020). Silver nanoparticles (AgNPs) are shown to be a potential solution against antimicrobial resistance as they present relevant physicochemical characteristics such as stability and good chemical interaction necessary to combat microorganisms (Wahab et al. 2021a).

Despite having a thorough investigation, the exact mechanism of antimicrobial action for AgNPs is still unclear. As per the available literature, three primary hypotheses have been general proposed: (1) degradation of the cell membrane/wall, (2) internalization within the cell and mediating subcellular disruption, and (3) oxidative stress damage (Salleh et al. 2020), as illustrated in Fig. 8.1. Bacterial cell walls and membranes primarily protect them from stress and other harmful localized environments. However, the ultrafine nature of AgNPs and their high surface area help them have better contact with either bacterial cell walls or membranes (Wahab et al. 2021a). AgNPs can easily bind to integral/transmembrane proteins present on the cell wall/membrane of microbes, restricting their vital functions (Wahab et al. 2021a; Salleh et al. 2020; Ijaz et al. 2020). AgNPs attachment may cause severe loss in lipid bilayer integrity and disturb the cell membrane permeability. Reports indicate that such undesirable changes in membrane permeability cause leakage of reducing sugars and proteins across them, leading to cell death. Additionally, the mechanical damage induced through "direct contact" with AgNPs may also result in the extrusion of cytoplasmic fluid. The interactions between AgNPs and cell wall components, especially proteins, inhibit bacterial growth, suggesting that the changes in cell membrane permeability result in the death of bacterial strains (Ghodake et al. 2020).

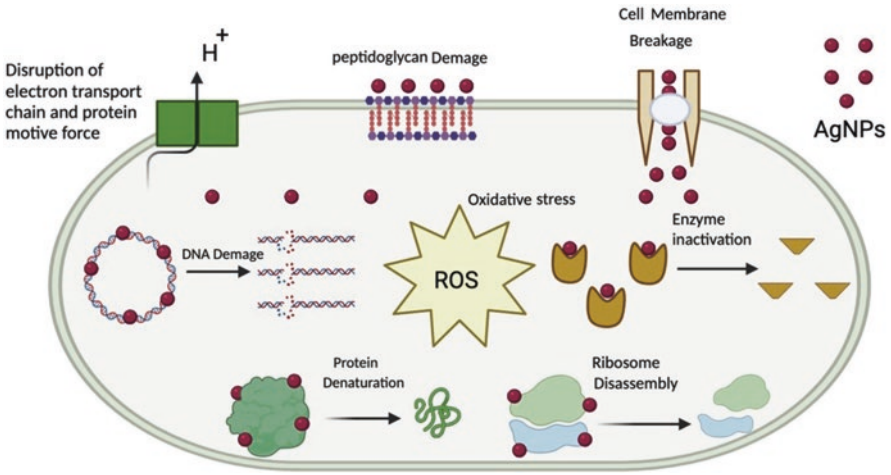

Fig. 8.1 A schematic representation of the probable mechanism of antimicrobial activity of AgNPs. (Reprinted with permission from Wahab et al. (2021a), Elsevier)

A second mechanism, AgNPs may enter the cell through internalization and initiate interaction with subcellular components present, i.e., mitochondria, ribosomes, and nucleus, making them either nonfunctional or disrupting their activities (Wahab et al. 2021a). The ongoing variation in membrane permeability primarily facilitates AgNPs entry into the cell, which interacts with sulfur and phosphorus compounds present in DNA, ultimately resulting in cell death. Furthermore, AgNPs exhibit genotoxic effects that cause severe damages to DNA sequences, where microbes lose their ability to replicate (Wahab et al. 2021a). Another way to inhibit microbial growth is producing reactive oxidative species (ROS) (Salleh et al. 2020). The intercellular ROS prompted by AgNPs may induce lipid damage and leakage of cellular biomolecules and eventually lead to cell apoptosis. The ROS are generated at the particle surface, damaging the cell membrane, protein, and DNA. In a recent study (Khan and Ali 2020), the introduction of AgNPs showed an enhanced quantity of ROS, which subsequently revealed superior antibacterial potential against multidrug-resistant strains such as *Xanthomonas citri*, *S. aureus*, and *Erwinia carotovora*. The mechanism of action also depends on the strain type, i.e., gram-negative bacteria are comparatively more sensitive to AgNPs than gram-positive bacteria (Ahmad et al. 2020). Gram-negative bacteria have more tapered cell walls when compared to gram-positive strains. The thick cell wall of gram-positive bacteria comprises various peptidoglycan coats limiting the permeation of the silver ions (Ag^+) through the cytoplasmic membrane. On the other hand, gram-negative pathogens are mostly consist of only a single peptidoglycan coat. Thus Ag^+ ions easily penetrate into their cytoplasm and cause cell lysis (de Lacerda et al. 2020). While in gram-positive strains, the AgNPs limit the release and uptake of proteins, sugars ions, and other essentials for vital cellular activities (Ahmad et al. 2020). However, regardless of the bacterial cell wall's composition, the penetration of AgNPs will occur, causing cell lysis of both gram-positive and gram-negative bacteria (Ahmad et al. 2020; Salleh et al. 2020).

The routes of AgNPs synthesis also play crucial role in dictating their antibacterial efficacy. Chemical route is often considered to be the most suitable way to synthesize AgNPs as it offers good control on nucleation and growth stages of silver nuclei, resulting in generation of various silver-shaped nanostructures. The ingredients employed in chemical synthesis of AgNPs are referred to as reducing and stabilizing agents. While reducing agent, e.g., trisodium citrate, sodium borohydride ($NaBH_4$), ascorbic acid, and Tollens' reagent, facilitates an instantaneous conversion of Ag ions into silver nuclei (Ag^0), the presence of stabilizing agents like polvinyl alcohol, polyvinylpyrrolidone, polyethylene glycol, and polymethylmethacrylate ensures enough segregation of formed Ag nuclei, thereby controlling shape and size of nanoparticles (Ahmad et al. 2020; Das et al. 2020). For instance, in a study by da Silva et al. (2020), citrate-capped AgNPs were synthesized chemically, and their antibacterial potential was assessed against *Pseudomonas aeruginosa*, one of the most refractory organisms to antibiotic treatment. The local surface plasmon resonance simulation of synthesized AgNPs displayed a significant reduction in bacterial activity. In a similar study, citrate-capped AgNPs were synthesized using silver nitrate ($AgNO_3$) precursor in the presence of sodium citrate as reducing and

protecting agent. The results demonstrated that the capping of citrate on silver surface plays an essential role in enhancing the antibacterial activity of AgNPs against both antibiotic-resistant *S. aureus* and *E. coli* (Kaur et al. 2019). Farouk et al. (2020) synthesized AgNPs (6.8 ± 2.28 nm) using trisodium citrate and sodium borohydride reducing agents. The synthesized AgNPs at various concentrations showed MIC ranging between 0.002 and 0.313μg/mL and MBC of 0.078 and 1.250μg/mL against multidrug-resistant *Salmonella.* Another group of researchers synthesized polyvinyl pyrrolidone (PVP)-coated silver nanowires (25 nm) and silver nanocubes (80 nm) using a modified polyol method. The synthesized NPs were tested for their antimicrobial potential against carbapenem-resistant *Escherichia coli* and vancomycin-resistant *Staphylococcus aureus* strains to treat bloodstream infections in cancer patients. An almost 100% death rate of both the aforementioned strains was achieved using nanowires and nanocubes. They concluded that this nanomaterial could be considered for further biomedical applications to control the growth of multiple drug-resistant strains (Jose et al. 2019). Even though chemical synthesis provides broad control over size and shape; however, it results in chemical sediment on their surfaces, which might be toxic and harmful, thereby increasing AgNPs toxicity towards human cells, discouraging their use (Ahmad et al. 2020; Moradi et al. 2021).

Among all the AgNPs synthesis methods, biological synthesis is the most economical and environmentally sustainable alternative, which could possibly overcome the before-mentioned limitations (Moradi et al. 2021). The reducing and stabilizing agents used in this type of synthesis are molecules produced by bacteria, fungi, yeasts, plants, and algae (Wahab et al. 2021a; Das et al. 2020; Moradi et al. 2021). The typical microbial synthesis process of AgNPs includes incubating the cultured microorganisms with silver precursor salt and monitoring the production of AgNPs visually (Ahmad et al. 2020). In case of the intracellular biosynthesis process, an ultrasonication process is needed to break the cell wall and release the AgNPs (de Lacerda et al. 2020). A plethora of studies suggests that the cellular machinery in microbes mainly aids them in the improved assembly of highly stable AgNPs. In a recent study by (Skóra et al. 2021), *Saccharomyces cerevisiae* was used as a promising tool for synthesizing silver nanoparticles ranging between 17.5 and 20.1 nm. The polydispersity index of 0.397 suggested that the nanoparticles are stable as they do not exhibit any considerable aggregation. The green-synthesized AgNPs showed significant bactericidal activity against multidrug-resistant *S. aureus*, *P. aeruginosa*, *E. coli*, *and C. albicans*. Also, they exhibited biofilm reduction by 53% in *E. coli* and 36% in *P. aeruginosa* for 2 mg/mL AgNPs concentration. The strong inhibition efficacy of AgNPs on migration was observed in cancer cells, suggesting that silver nanoparticles may have a potential function in the inhibition of metastasis. In another study, AgNPs were synthesized using an actinomycete, *Nocardiopsis* sp. *GRG1 (KT235640)* biomass. The relatively well-dispersed AgNPs possessed a size ranging from 20 to 50 nm and showed 91% of inhibition against biofilm-forming methicillin-resistant coagulase-negative *Staphylococcus* at a concentration of 55μg/mL. In conclusion, biosynthesis of AgNPs using microorganisms demonstrates significant antibacterial activity against a variety of antibiotic-resistant microorganisms. However, the pre-synthesis necessities, for

instance, biomass culturing and sometimes low-reaction kinetics, push the need to strive for other bioresources with distinguished privileges (Moradi et al. 2021).

Biogenic synthesis of AgNPs using plants is privileged having a simple, hazardous chemicals-free procedure along with high efficiency and short reaction times. The synthesis method is comparatively nontoxic, reliable, and ecologically supportive (Moradi et al. 2021). The reducing and stabilizing agents naturally available in plants help in synthesizing biocompatible AgNPs. In general, the primary and secondary metabolites like phenols, alkaloids, terpenoids, flavonoids, carbohydrates, and proteins present within the extract are used to reduce Ag^+ into AgNPs (Wahab et al. 2021a; Ijaz et al. 2020). The as-synthesized nanoparticles have been explored for their antibacterial efficacy, for instance, (Choi et al. 2021) employed *Areca catechu* extracts to synthesize AgNPs ranging between 20 and 30 nm. The as-synthesized AgNPs (360μg/mL) showed ZOI of 12.3 ± 0.8 mm, 16.3 ± 1.5 mm, and 17.7 ± 1.2 against vancomycin-resistant *Enterococcus faecalis*, multidrug-resistant *Pseudomonas aeruginosa*, and multidrug-resistant *Acinetobacter baumannii.* They proposed that the AgNPs directly adhered to bacterial cell membranes and caused the subsequent bacterial destruction. Besides, silver ions (Ag^+) may also release from AgNPs restricting the microbial growth. In a similar study, the polyphenol-rich extract of *Origanum vulgare* leaf was used for synthesis of AgNPs (30.20–58.81 nm). The synthesized nanoparticles exhibited significant antibacterial activity against ampicillin-resistant *E. coli* and kanamycin-resistant *E. coli*. It was observed that AgNPs damaged the cell membrane and altered the permeability of the cell membrane, thus disrupting the functioning of the bacterial respiratory chain or the proton FOF1-ATPase, causing cell death. Moreover, AgNPs displayed a genotoxic effect that damages DNA sequences. The shapes of NPs also influence their antibacterial efficacy. AgNPs obtained by reducing plant origin substances extracted from the *O. vulgare* leaves possess round shapes. As per the previous studies, round-shaped AgNPs are more active than the other forms (Hambardzumyan et al. 2020). Similar results have also been referred by (Aghajanyan et al. 2020) by using the *Artemisia annua* extract as reducing agents. When compared with round-shaped AgNPs synthesized using *O. vulgare* leaf extracts, the Artemisia extract's synthesized AgNPs displayed seven to nine times lower activity. These nanoparticles can potentially be applied for various antimicrobial biomedical and biotechnological applications.

In another study, the *Cinnamomum zylinicum* bark extract was employed to biosynthesize AgNPs to combat the MDR gram-negative bacteria *Acinetobacter baumannii*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* strains and gram-positive bacteria *Staphylococcus aureus*. The results indicated that obtained AgNPs when compared with antibiotics are more efficient in inhibiting both gram-positive and gram-negative bacteria, giving a zone of inhibition of 22 mm against *A. baumannii*, 24 mm against *K. pneumonia* and *P. aeruginosa*, and 25 mm against *S. aureus,* respectively. The results are demonstrating the excellent potential of AgNPs in the development of robust antimicrobial products for clinical use (Almalah et al. 2019). Similarly, in a recent study (Maghimaa and Alharbi 2020), AgNPs were synthesized from the aqueous extract of *Curcuma longa* leaf and later coated on cotton fabric. The highest inhibition zone of 24 mm against antibiotic-resistant

P. aeruginosa was displayed, followed by the 22 mm for *S. aureus*. Thus, the AgNPs-loaded cotton fabrics revealed potential as an effective antimicrobial textile fabric for hospital patients and medical workers. Generally, Ag-infused textiles are advertised with the antibacterial effect and have also evidenced reduction of bad odors after sweating. Poly drug, Triphala powder, a combination of powders of three myrobalans, amalaki (*Emblica officinalis*), haritaki (*Terminalia chebula*), and bibhitaki (*Terminalia belerica*), in equal proportion was used for the synthesis of nano-antibacterial agents to combat against biofilm-forming, antibiotic-resistant pathogen. The synthesized AgNPs of hydrodynamic diameter 242.2 nm at various concentrations revealed a 70–86% reduction in the growth rate of biofilm-forming *Klebsiella pneumonia.* The researchers concluded that Triphala-based AgNPs could be incorporated in different formulations to control pathogenic bacteria causing life-threatening infections in humans and animals (Ranjani et al. 2019). The *Tectona grandis* and *Sisymbrium irio* plants were also explored for the biosynthesis of AgNPs with potential antibacterial agents to reduce, spread, and manage the multidrug-resistant bacterial infections (Mickymaray 2019; Rautela et al. 2019). Conclusively, the plant-derived AgNPs possess great antibiotic and antibacterial potential; hence there is a huge interest in the biosynthesis of AgNPs using plants. The most recent studies with their specific key data along with the synthesis routes have been summarized in Table 8.1.

AgNPs in conjugation with existing antibiotics possesses some novel characteristics granting them enhanced antibacterial potential (Mohamed et al. 2020). When attached with antibiotics, AgNPs showed improved stability, selectivity, and functionality (Kingsley et al. 2006). Some studies have also demonstrated that the antibacterial activities of kanamycin, ampicillin, chloramphenicol, and erythromycin were elevated when conjugated with AgNPs against various pathogenic bacteria (Ahmad et al. 2020). For example, (Li et al. 2021a) have synthesized a combination of AgNPs and kanamycin to perform biofilm-triggered on-demand drug release in situ. The combination of kanamycin and AgNPs displayed superior antibacterial activity against multidrug-resistant test strains (*Staphylococcus aureus*, *Streptococcus pneumonia*, *Pseudomonas aeruginosa*, *and E. coli)* than when used separately. In a study by (Anjum et al. 2018), gentamycin-conjugated AgNPs exhibited significant antibacterial activity against antibiotic-resistant *S. pneumoniae* and *S. aureus*. The antibiotic-NPs conjugate system inhibited the cell replication and caused cell lysis. Mohammed et al. (2021) evaluated the combination of various antibiotics with AgNPs to obtain the most efficient antibacterial agent. Among ciprofloxacin, cefotaxime, and ceftazidime, the combination of ciprofloxacin with AgNPs displayed the highest synergistic antimicrobial activity against multidrug-resistant extended-spectrum beta-lactamase (MDR-ESBL) *E. coli.* However, against MDR-ESBL *K. pneumoniae*, combination of AgNPs and cefotaxime was found to be superior, followed by ceftazidime and ciprofloxacin. The least synergistic combination against both the strains was that of ampicillin and AgNPs. Another reason behind the progressive usage of AgNPs-antibiotics conjugate is the toxicity concerns regarding AgNPs. The efforts are made to reduce the effective antimicrobial dose of AgNPs against multidrug-resistant (MDR) pathogens. In a study reported

Table 8.1 Antimicrobial activities of colloidal nano-silver against antibiotic-resistant bacterial strains

Synthesis	Size of NPs	Pathogens tested	Evaluation parameters	Application area	References
Chemical	~5 nm	Methicillin-resistant *Staphylococcus aureus*	MIC: 1–128μg/mL	Antibacterial material for medical applications	Liu et al. (2018)
Biological	~28.30 nm	Methicillin-resistant *staphylococcus Aureus*, methicillin-resistant *Staphylococcus epidermidis*, and vancomycin-resistant *enterococci*	ZoI: 9.6–24.5 mm	Bactericidal support with pharmacodynamic and pharmacokinetic properties	Muthukrishnan et al. (2019)
Plant based	10–78.9 nm	MDR *Acinetobacter baumannii, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*	ZoI: 22–25 mm, and MIC: 2.8–4.5μg/mL	Antibacterial products for clinical use	Almalah et al. (2019)
Chemical	25–30 nm	Vancomycin-resistant *E. coli*	ZoI: 7 ± 0.3 mm	Antibacterial nano-drug complex	Kaur et al. (2019)
Biological	30–50 nm	Methicillin-resistant coagulase-negative *staphylococcus*	ZoI: 15.5–18 mm, and 91% of biofilm inhibition	Drug development and healthcare settings	Rajivgandhi et al. (2019)
Chemical	25 nm, 80 nm	Carbapenem-resistant *E. coli,* vancomycin-resistant *S. aureus*	100% death rate	Biocompatible antimicrobial products for biomedical applications	Jose et al. (2019)
Plant based	242.2 nm	*MDR Klebsiella pneumoniae*	70–86% reduction in bacterial growth rate	Antibacterial formulations for clinical use	Ranjani et al. (2019)
Plant based	30–50 nm	Ampicillin-resistant *E. coli*, kanamycin-resistant *E. coli*	MIC 9.16–18.35μg/mL	Antimicrobial support for biomedical applications	Hambardzumyan et al. (2020)

(continued)

Table 8.1 (continued)

Synthesis	Size of NPs	Pathogens tested	Evaluation parameters	Application area	References
Chemical	20 nm	Antibiotic-resistant *Pseudomonas aeruginosa*	MIC:5–10μg/mL, and 100% cell death	Antimicrobial agent for the infection treatment	da Silva et al. (2020)
Biological	6–24 nm	Multidrug-resistant pathogens, *Escherichia coli and Pseudomonas aeruginosa*	MIC: 6.25–50μg/mL	Antibacterial agents to inhibit multidrug-resistant microorganisms	Akter et al. (2020)
Biological	10–50 nm	Multidrug-resistant *Pseudomonas aeruginosa*	ZoI:15 mm	Antimicrobial for treatment of drug-resistant pathogens	D'Lima et al. (2020)
Chemical	6.8 ± 2.28 nm	MDR *salmonella*	MIC: ≤0.002–0.313μg/mL MBC: 0.078–1.250μg/mL	Antibacterial agents for therapeutic purpose	Farouk et al. (2020)
Biological	4–17 nm	MDR *Staphylococcus aureus* and *Escherichia coli*	Growth inhibition of 97.5–96.7%	Antibacterial material for surface decontamination	Ghodake et al. (2020)
Biological	8–30 nm	Antibiotic-resistant *Vibrio parahaemolyticus* and *salmonella typhimurium*	MIC: 3.12–6.25μg/mL, and MBC: 12.5–25μg/mL	Potent antibacterial material against pathogenic bacterial strains	Huq (2020)
Biological	17.5–20.1 nm	MDR *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Escherichia coli*	53–65% eradication of biofilm	Antibacterial potential for dental implants and wound healing	Skóra et al. (2021)
Plant based	20–30 nm	Vancomycin-resistant *enterococcus faecalis,* multidrug-resistant *Pseudomonas aeruginosa*, and multidrug-resistant *Acinetobacter baumannii*	ZoI: 6.0–17.7 mm, MIC: 5.6–11.25μg/mL, and MBC:11.25–22.5μg/mL	Antibacterial application for treating major cause of nosocomial infections	Choi et al. (2021)

by (Mohamed et al. 2020), the AgNPs-vancomycin combination effect was remarkable on the antibiotic (gentamicin, ampicillin, erythromycin, amoxicillin and vancomycin)-resistant *P. aeruginosa* and *K. pneumonia* strains which can be explained by the high surface-to-volume ratio of the AgNPs and their hydrophobic nature. The AgNPs make transport and entrance of antimicrobial agents inside the bacterial cell more effective by damaging the bacterial cell membrane. Inclusively, the synergistic treatments were more effective than either treatment alone; hence, the utilization of nanoparticles in combination with antibiotic is highly regarded.

Halawani et al. (2020) biosynthesized AgNPs from *Rosa damascenes*, and conjugated the same with cefotaxime; the resultant antibiotic-AgNPs conjugate displayed maximum antibacterial activity against methicillin-resistant *Staphylococcus aureus* and cefotaxime-resistant *E. coli* compared to when used separately. Such bio-fabricated AgNPs-antibiotics conjugate provides enormous antibacterial potential against several multidrug-resistant microbes. In a similar study, the combination of AgNPs with a broad-spectrum ciprofloxacin (Cipro-AgNPs) revealed better antibacterial activity when compared to AgNPs and ciprofloxacin separately. The Cipro-AgNPs exhibited zones of inhibition of 33 mm, 35.5 mm, 35.5 mm, and 38.5 mm against pathogenic multidrug-resistant *S. sonnei*, *S. typhi*, *C. amalonaticus*, and *E. coli*, respectively. Hence, it can be suggested that the AgNPs and ciprofloxacin synergistically resulted in better antibacterial activity against pathogens when used in conjugation (Adil et al. 2019).

Even though AgNPs have broad applicability in various fields, certain concerns need to be addressed: First, colloidal AgNPs tend to get aggregated and eventually lose their original antibacterial efficacy. Second, colloidal AgNPs cannot be reused further, making the process uneconomical and unrealistic (Salleh et al. 2020; Agnihotri et al. 2019). In a study performed by (Menazea 2020), the AgNPs were tested in different mediums. The result revealed that these nanoparticles tend to aggregate in an organic medium such as dimethylformamide and tetrahydrofuran solutions. In contrast, immobilized AgNPs represent more stability as they are less prone to aggregation and oxidation when exposed to the aqueous medium. Also, the immobilization of silver nanoparticles on a support matrix further escalates their reusability and minimizes toxic effects associated with their inevitable disposal in the environment (Dhiman and Agnihotri 2020). When immobilized on several organic and inorganic substrates such as graphene, Fe_2O_3, SiO_2, and zinc oxide, silver nanoparticles have displayed enhanced antibacterial performance over long-term use (Agnihotri et al. 2013). Hence, immobilization of AgNPs on a support matrix would allow controlled silver release to realize an effective antimicrobial system against several multidrug-resistant strains.

8.3 Silver Nanocomposites as Antimicrobials

The elimination of limitations and disadvantages associated with colloidal silver nanoparticles has become integral to global research with an aim to promote their reusability, enhancing stability and diversifying their utilization in various applications along with minimizing leaching to reduce ecotoxicological impacts. Alternative strategies such as immobilization and/or incorporation of nanoparticles onto a support matrix are particularly considered to eradicate mainstream issues (Agnihotri et al. 2015; Dhiman et al. 2019; Zheng et al. 2016). The resultant stabilized multi-element structures arising from such strategies are known as nanocomposites. Silver nanocomposites consolidate functionality between silver nanoparticles and matrix materials through enhancing the useful abilities while downplaying the detrimental characteristics. Therefore, the creation of novel silver nano-formulations with modifiable active properties has been enabled by utilization of nanocomposites. In recent years, the unique characteristics of such materials have garnered substantial and widespread interest in extensive applications than single silver nanoparticles.

Most important aspect of nanocomposites is the type of support matrix which is responsible for intended stabilization, and nowadays, there has been availability of wide range of support materials. The incorporation of silver nanoparticles onto some support matrices like electrospun nanofibers, polymeric hydrogels, ceramics, and metal organic frameworks has shown to improve the recovery and reusability of resulting nanocomposites. Other than acting as a template, these support materials may contain some inherent antimicrobial features which work synergistically to enhance antimicrobial performance and broaden their potential applications to combat against the antibiotic resistance in biomedical use. Hence, the current portion will be focused on various silver-based nanocomposites with a support matrix employed for eradicating antibiotic-resistant strains in medical implant-associated infections, biomedical, wound dressings, and other relevant domains.

In biomedical applications, the utility and importance of implants have become indispensable for the replacement and repair of injured tissues/organs. However, implant-associated infections which are caused by bacterial adhesion followed by biofilm formation may result in implant failure, immune system dysfunction, and revision surgeries. Ultimately, the removal of an infected implant followed by a series of antibiotic treatments remains the only way to completely remove infection before implanting new device. As a result, treatments require extended antibiotic therapy and 1000 times higher antibiotic dosage to kill biofilm bacteria than non-sessile planktonic bacteria (Duan and Wang 2006; Xu et al. 2017). This has become progressively difficult with the introduction and increasing amounts of multiple drug/antibiotics-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), which are causing high failure rates of current treatments (Vuong et al. 2016).

The blend composites involving metal/metal oxide nanoparticles along with silver nanoparticles have been investigated for their antibacterial efficacy against antibiotic-resistant bacteria to circumvent bacterial proliferation on implants. The

incorporation of other nano-entity could restrain the cytotoxicity of silver nanoparticles while registering a synergistic impact on the antibacterial potential. For instance, (Van Hengel et al. 2020) employed silver and zinc nanoparticles-biofunctionalized laser-melted titanium implants to prevent implant-associated infections caused by antibiotic-resistant bacteria. The antibacterial activity was determined against MRSA using zone of inhibition, minimal inhibitory concentration (MIC), and minimal bactericidal concentration (MBC). The implants' surfaces containing 75% silver (7–25 nm) and 25% zinc (40–60 nm) nanoparticles demonstrated a complete eradication of both adherent and planktonic bacteria in all experiments performed using murine femora within 24 h. The zone of inhibition for the same composition was around 0.6 cm^2, demonstrating excellent potential for biomedical applications. The addition of Zn reduced the cytotoxicity caused by Ag alone while preserving the synergistic antibacterial behavior against the antibiotic-resistant bacterial infection.

The use of biogenically synthesized silver nanoparticles while designing biomedical coatings could be beneficial to improve hemocompatibility with improved functions. For example, Neethu et al. (2020) fabricated a bionanocomposite coating for the central venous catheter (CVC) using mycogenerated AgNPs and polydopamine. Antibiofilm activity of the fabricated material was investigated against multidrug-resistant *Acinetobacter baumannii*. The antibacterial potency of the surface-modified central venous catheter was evident by the zone of inhibition (ZoI) 23.9 ± 0.8 mm against *Acinetobacter baumannii*. The minimum inhibitory concentration and minimum bactericidal concentration were recorded as 15.6 and 31.2μg/mL, respectively. Study establishes that AgNPs (10–15 nm) at minimum bactericidal concentration were able to disrupt bacterial biofilm on the central venous catheter via lysing the adherent cells. While authors evidenced a significant change in biofilm appearance just after 1 h of treatment, the biofilm was completely removed from catheter's surface after 24 h of treatment. This indicates a promising aspect of using green-synthesized silver nanoparticles in the formation of nano-antimicrobials, which is expected to prevent the infections caused by MDR strains in coming future.

In another study, Guan et al. (2019) fabricated polydopamine (PDA) coatings with silver nanoparticle-loaded TiO_2 nanorods on Ti alloy. The antibacterial activity of the coatings was tested against methicillin-resistant *S. aureus* using disc diffusion and colony-counting methods. Ag-TiO_2/PDA coatings displayed significant antimicrobial action with ZoI of 13.5 mm and bacterial inhibition rates of 95.4%, 88.6%, and 80.1% at 1, 7, and 14 days along with satisfactory biocompatibility against mouse calvarial cells. The authors intended Ag-TiO_2/PDA coatings to combat against infections in orthopedic and dental implants. Silk fibroin derived from *Bombyx mori* silkworm is considered a favorable biomaterial owning to its excellent biocompatibility, biodegradability, mechanical strength, and low inflammatory response. The promotion of tissue integration prior to bacterial adhesion, which prevents colonization of certain bacterial species on the implant, is seen as ideal means to overcome antibiotic resistance in bone tissue engineering. Therefore, a group of authors (Patil and Singh 2019) reported a silk fibroin-based bone tissue scaffold with silver nanoparticles with advanced antimicrobial properties. Silk

fibroin with tyrosine residues that have potent electron-donating capabilities was employed as template for AgNPs biosynthesis, consequent reduction, as well as stabilization. The silk fibroin/AgNPs films at an MIC of 20 nM displayed superior bacterial killing capacity in case of kanamycin-resistant *E. coli* compared to ampicillin-resistant *E. coli* where few colonies could be observed. Nevertheless, AgNPs incorporated presented impressive antimicrobial properties with no detrimental effect on osteogenic differentiation potential of human mesenchymal stem cells.

Chien et al. (2018) strived to create an antibacterial material targeted for prosthetic joint implants (metal or ceramic), the failure of which has become increasingly common due to infections arising from antibiotic-resistant pathogens. The silver nanoparticles were confined to mesoporous silica (MS-AgNPs), and the composite powder was evaluated for its antibacterial activity against methicillin-resistant *S. aureus*. The diameters of the inhibition zones (ZoI) were found to be in the range of 15.7–17.6 mm (Fig. 8.2) with increasing composition ratios of silica/Ag of 100:1 (MS/Ag1), 100:5 (MS/Ag5), and 100:10 (MS/Ag10). Additionally, time-killing assay demonstrated that only composites in the MIC range of 5–20 mg/mL were able to effectively inhibit the growth of MRSA.

Wounds have the tendency to get infected which makes the overall healing process more complicated and impeded with the potential risk of body fluid loss and acute inflammation (Wahab et al. 2021b; Potara et al. 2011). Few bacterial infections require external medication and, more so, dressing pads impregnated with a broad-spectrum antibacterial agent that has a higher bacterial killing rate and curing (Konop et al. 2016). In the context of treating antibiotic-resistant strain infections, AgNPs-bio composites could be an excellent antibacterial material and play a key role in designing materials for wound dressing applications because of their biocompatibility, good flexibility, tear resistance, and broad-spectrum antimicrobial activity (Kumar et al. 2018).

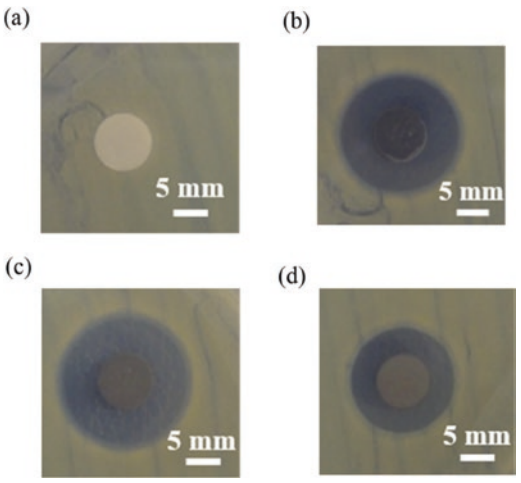

Fig. 8.2 Inhibition zone images of (**a**) mesoporous silica (MS), (**b**) MS/Ag1, (**c**) MS/Ag5, and (**d**) MS/Ag10 against methicillin-resistant *Staphylococcus aureus* (MRSA). (Reprinted with permission from Chien et al. (2018), Elsevier)

Stojkovska et al. (2019) produced an eco-friendly, non-sticky, and bioactive honey-based AgNPs/alginate hydrogel dressing with the aim to target multidrug-resistant bacterial strains causing nosocomial wound infections. The resulting nanocomposites molded into different forms of microbeads, microfibers, and discs were tested for their antibacterial efficacy against multi-resistant hospital strains of *A. baumannii*, *P. aeruginosa*, and *S. aureus*. At the total released silver concentration of ~9μg/mL, the hydrogels exhibited about 99.9–100% bacterial reduction. Bassous and Webster (2019) engineered highly sophisticated and biocompatible polymeric nanovesicles called polymersomes via hydrophobic interactions through self-assembly. The hydrophobic corona of such polymersomes was then employed to incorporate antibacterial formulations consisting of silver nanoparticles and antimicrobial peptides (PR-39, AMPs). These structures were functionalized to aid with wound healing or immunomodulation and combat antibiotic-resistant bacterial infections, caused especially by methicillin-resistant *S. aureus* (MRSA). In vitro testing on MRSA showed that ratio of AgNPs to AMPs of around 1:5.8 corresponding to ≈11.6μg/mL of silver nanoparticles and 14.3×10^{-6} M of peptides worked synergistically to yield 100% MRSA inhibition over time frame of 23 h. These AMP/AgNPs polymersomes could potentially replace antibiotic therapies in the clinical setting on account of its bacteriostatic activity coupled with nominal cytotoxicity towards native human dermal fibroblast cells.

The hybrid composites consisting of natural polymers and metal nanoparticles are promising candidates for biomedical applications. Owing to its various unique features such as biodegradability, biocompatibility, bioactivity, cell adhesion, and ability to form any structure, chitosan has been used widely for biomedical applications. The incorporation of silver nanoparticles with chitosan has been investigated for their antibacterial efficacy. For instance, (El-Aassar et al. 2021) used chitosan-based-crosslinked gelatin/polyvinyl pyrrolidone-embedded silver nanoparticles for formulating novel wound dressing to eliminate multidrug-resistant microbes. Skin healing properties and ability of blood coagulation were among the major aspects for using gelatin, while mechanical strength governed by biocompatible polyvinyl pyrrolidone justifies its role in the composite dressing material. The authors also addressed the use of crosslinker (glutaraldehyde) for modulating mechanical strength and hydrophilicity of the wound dressing. Composite membranes with silver nanoparticle (9.99 nm) concentration ranging from 10μg/mL to 150μg/mL were employed to determine the antibacterial activity against multidrug-resistant microbe strains, i.e., *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*. Zone of inhibition (ZoI) was measured to analyze the bactericidal efficiency; at highest concentration of silver nanoparticles (150μg/mL), the widest ZOI of 3.12 cm was found for *S. typhi*, followed by *B. subtilis* (2.78 cm), *S. aureus* (2.55 cm), and *E. coli* (1.97 cm). The detailed investigation of mode of mechanism revealed reduction in resistance genes expression, i.e., *β-lactamase*, *mecA*, and *erm*. The formulated composite membrane resulted in 50% reduction in expression of *mecA* and 97% reduction in expression of *erm* in *S. aureus* (MRSA). In gram-negative bacterial strain *E. coli*, complete elimination of *β-lactamase* and 63% reduction of *erm* expression were reported. Additionally,

observed 12-fold less cytotoxicity of the composite membrane as compared to the control demonstrated its biocompatibility for direct wound application.

Recently, a chitosan-sericin hybrid nanocomposite film containing a unique blend of AgNPs and antibiotic moxifloxacin was prepared for wound dressing applications (Shah et al. 2019). The preliminary experiments performed on Sprague-Dawley rats (burn wound model) for 7 days revealed faster healing pattern in wounds indicated by successful fibrosis, collagen reorganization, and mild epidermal regeneration, while the presence of AgNPs ruled out the chances for contamination. Additionally, the films displayed highest antibacterial activity (ZoI, 37.25–50.75 mm) against all clinical isolates of MRSA. Shah et al. (2018) fabricated functional chitosan silver nanocomposite (CSN) films through in situ chemical reduction using $NaBH_4$. The developed nanocomposite films exhibited significant antibacterial activity against clinical isolates of MRSA strains with ZoI ranging between 12.67 and 24.73 mm as the content of silver increased. This biocidal efficacy was found to be equally and/or even effective than commercially available dressing products such as Aquacel Ag®, Bactigras®, and Kaltostat®. Thus, these nanocomposite films were said to have potential to control antibiotic resistance in healthcare applications such as water disinfection, wound dressing, etc.

Bacterial proliferation control and prevention are essential not only for medical supplies like bandages and dressings but also in case of clothes and domestic use textiles. Such products could easily serve as the niche for obnoxious microbial growth (Karwowska 2017). Therefore, in order to limit contamination from pathogenic microbes and drug-resistant microorganisms, various ventures are being undertaken for development of fibers and textiles with "self-cleaning properties." Nowadays, nanoparticle-based coatings and impregnation particularly involving nano-silver are most recommended for fabric modifications (Dastjerdi and Montazer 2010). Recently, a group of authors to meet the challenge of drug-resistant bacteria made use of highly advocated antimicrobial photodynamic therapy (Chen et al. 2019). In this study, silver nanoparticles (0.91 mg per 100 cm^2 of fabric) combined with a potent photosensitizer, zinc phthalocyanine, were conjugated on a cellulose fabric. The composite material presented highly efficient photodynamic biocidal effect against methicillin-resistant *S. aureus* with 99.96% kill efficacy within minutes under light illumination. Antibacterial mechanism revealed that two active components (AgNPs and photosensitizer) worked synergistically for photoinactivation of bacteria. The photosensitizer activated the silver nanoparticles into silver ions under light illumination, while at the same time, AgNPs enhanced the ROS generation premeditated by the photosensitizer. Moreover, modified cellulose fabrics still displayed about 99% bacterial killing efficacy when used repeatedly for five washing cycles.

In our everyday lives, paper and related products such as books, magazines, office paper, wallpaper, medical records, bank notes, and food items packaging paper are widely used. Since these materials do not have any inherent antimicrobial characteristics, their circulation and distribution in various environments to multiple people can cause alarming rates of transmission of numerous infectious diseases (Angelakis et al. 2014). Furthermore, re-emergence of previously well-controlled

infectious diseases as well as development of new strains of bacteria resistant to currently available antibiotics can arise from such contamination (Ma et al. 1994). Thus, the demand of papers with the ability for inhibition and/or prevention of attachment, establishment, and proliferation of microbes on their surfaces has risen. Consequently, to address this need, many attempts have been made by either preparing antimicrobial pulp for paper or by directly modifying the paper with antimicrobial agents (Amini et al. 2016). Paper with such defense characteristics can be very useful in different applications, including packaging and filtering, etc. Islam et al. (2018), inspired from the biology of the marine mussel, devised an effective method for AgNPs immobilization on cellulose paper (CP). The strategy first involved the succinic acid surface activation of cellulose paper which otherwise lacks any functional groups or provides specific chemical reactivity. Further functionalization of cellulose paper was carried out by dopamine conjugation via coupling reaction which was proven useful in utilizing the tethered catechol groups to effectively immobilize the AgNPs. The antimicrobial properties of the final composites were found to be dependent upon immersion time in ammoniacal $AgNO_3$ solution. The Ag-Dopa-CP at 8 h immersion displayed higher values of ratio between the diameter of the zone of inhibition and the diameter of Ag-Dopa-CP disk (dia_{ZOI}/dia_{disk}) at 2.25, 1.98–2.11 against antibiotic-resistant strains of *Vibrio parahaemolyticus* and *Enterococcus faecalis.* Excellent antimicrobial properties along with exceedingly low leaching of AgNPs from the paper surface would obviously facilitate the use of the fabricated Ag-Dopa-CP as a packaging material. With exceedingly low leaching of AgNPs at 2.6μg/mL (0.21% of total silver content), such modified papers could be employed as antimicrobial packaging materials.

Despite the advancements in many fields in the last several decades, provision of sufficient methods for treatment of freshwater and wastewater to eradicate organic matter, pathogenic microbes, and unwanted chemicals is still among the top vital goals to be accomplished (Lemire et al. 2013). Various traditional approaches have been employed in the past for treatment and still are in effect such as filtration (suspended or microparticle removal), advanced oxidation (organic component degradation), and filtration with UV exposure (disinfection of water from microorganisms). However, there is a requirement for multifunctional systems to decompose moieties and function against multidrug-resistant microbes present in water. Thus, composites of AgNPs provide innovative formulations with multitasking ability against various microorganisms as silver can form nanocrystalline providing better efficacy in water disinfection than regular filters (Deshmukh et al. 2019). These composite systems are feasible and economical as AgNPs can treat water by degrading the organic substances and killing the antibiotic-resistant microbiological species both in one step without affecting the physicochemical properties of water (Villanueva et al. 2014).

Naz et al. (2019) prepared silver nanoparticles (10–15 nm)-embedded graphene oxide (AgNPs-GO) nanocomposites through one-pot synthesis technique which exhibited enhanced photocatalytic and antibacterial activities with improved biocompatibility. The synergism between AgNPs and GO not only completely eradicated the growth of methicillin-resistant *S. aureus* for up to 24 h but also degraded

97–99% of toxic organic dyes such as rhodamine B, methylene blue, and commercial dye, AY, within 12–24 minutes time frame. Furthermore, the nanocomposites with doses up to 160 ppm were nontoxic with ~85% cell viability for human corneal epithelial cells. Similarly, (Guo and Tian 2019) loaded TiO_2 and AgNPs onto graphene oxide to form a novel nanocomposite material for antibiotic resistance control in wastewater systems. The bactericidal capacity of the composites with concentration ranging from 10 to 100 mg/L revealed that growth inactivation rates between 66.6 and 97.8% could be achieved within 10 minutes against tetracycline-, gentamicin-, streptomycin-, kanamycin-, and ampicillin-resistant *E. coli* strains under simulated sunlight irradiation.

Ullah et al. (2018) synthesized water-soluble graphene oxide-based composite loaded with an aminoglycoside antibiotic, tobramycin (TOB), and silver nanoparticles. In the composite TOB/GO/AgNPs, tobramycin served a dual role in assisting the reduction/decoration of silver nanoparticles and creation of synergism for enhanced antibacterial efficacy. The nanocomposite visually reduced the bacterial colonies of drug-resistant *E.coli* pathogen to a significant number compared to pristine individual counterparts (Fig. 8.3). The synergistic effect of the three components where silver nanoparticles produced ROS while GO cuts membrane by physical contact and tobramycin inhibits protein synthesis was collectively attributed to improved antibacterial activity. Analogous antimicrobial performance of silver-based nanocomposites against multidrug-resistant bacterial pathogens has been summarized in Table 8.2.

The broad-spectrum antibacterial activity of AgNPs compared to other common antibiotics will bring benefits to general biomedical applications. Rafael et al. (2019) synthesized a thermosensitive hydrogel based on Pluronic® F127 loaded with AgNPs for in situ chirurgic biomedical applications. The silver nanoparticles with average size of around 22 nm were incorporated into the hydrogel in an attempt to circumvent antibiotic resistance and provide postoperative infection prophylaxis. At MIC of ≥0.125 mM, drug-resistant clinical isolates of *P. aeruginosa* and *S. epidermidis* showed susceptibility towards the loaded hydrogels. Although, it was revealed through in vitro experiments that AgNPs inside the hydrogel demonstrated constrained antibacterial activity and therapeutic effect due to slower silver ion release. However, this sustained release from a positive perspective of clinical applications might allow such hydrogels to have prolonged prophylactic effects over time.

In another report, the effect of zinc oxide and silver nanoparticles (ZnO-AgNPs) on the biofilm of methicillin-resistant *Staphylococcus aureus* (MRSA) and *icaA* gene expression was investigated (Shakerimoghaddam et al. 2020). The composite (ZnO-AgNPs) had reduced minimum inhibitory concentration of 60.8μg/mL as compared to the MIC values of ZnO (393.2μg/mL) and Ag (179.8μg/mL). The reduction in the MIC could be attributed to the increased permeability of silver nanoparticles into the bacterial biofilms and increased free-electron production from zinc oxide due to the combinational effect. The complete inhibition of biofilm was observed at MIC concentration of the ZnO-Ag nanoparticles. Also the expression of *icaA* gene which affects the initial binding of bacteria and biofilm formation was significantly decreased (2.98-fold) under the influence of nanoparticles at

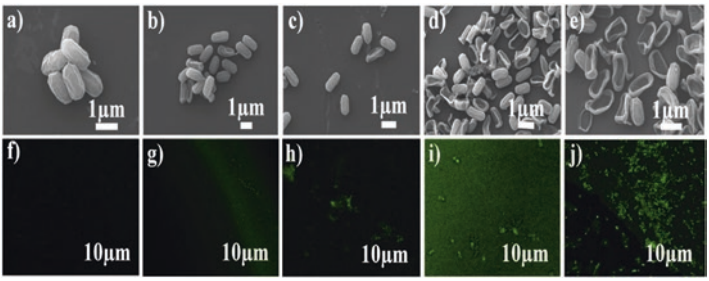

Fig. 8.3 SEM image analysis of treated multidrug-resistant *E.coli* with (**a**) PBS, (**b**) GO, (**c**) tobramycin, (**d**) only AgNPs, and (**e**) TOB/GO/AgNPs. The intracellular production of ROS in (**f**) control, (**g**) GO, (**h**) tobramycin, (**i**) only AgNPs, and (**j**) TOB/GO/AgNPs. (Reprinted with permission from Ullah et al. (2018), Elsevier)

sub-MIC (1/2 MIC). The observed results demonstrated that nanoparticles synergistically prevent early attachment and biofilm formation, thus proving to be a potent antimicrobial agent for biomedical applications.

Recently, antibacterial activity of silver nanoparticles-decorated and mesoporous silica-coated single-walled carbon nanotubes was evaluated against drug-resistant bacteria (Zhu et al. 2020). The MIC and MBC of the composite were determined against multidrug-resistant bacteria *Escherichia coli* and *Staphylococcus aureus*. The MIC of composite (SWCNTs@mSiO_2-TSD@Ag) against gram-negative *E. coli* was 120μg/mL, which was equivalently better than both SWCNTs@mSiO_2-TSD and commercial AgNPs. Similar results were obtained in case of gram-positive bacteria *S. aureus* where MIC of composite was recorded at 140μg/mL. The improved antibacterial activity could be due to two reasons: First, the mesoporous silica due to its hydrophilic nature induced dispersibility, thus increasing the contact area with bacterial cell walls. Second, the small size and uniform distribution of silver nanoparticles (2.78 ± 0.70 nm) in the pores of silica have superior antibacterial potential. Authors further investigated the bacterial growth kinetics in presence of the composite and reported that even at MIC/2 concentration, the propagation of *E. coli* is restrained for the first 12 h, and using the MIC concentration of composite (120μg/mL) resulted in complete inhibition of the growth of *E. coli* up to 24 h. The morphological observations revealed the damage on bacterial cell membranes after treatment with composite material. The release of Ag^+ ions was attributed as the mode of action as the release was faster on the first day, and then followed a sustainable release till 11 days. The cumulative release of silver ions was found to be five times higher in case of composite material as compared to silver nanoparticles. Exposure of Ag^+ to the bacterial cytoplasmic matrix leads to irreversible damage to DNA, proteins, and lipids resulting in death of the bacteria. The cytotoxicity assay of 150μg/mL SWCNTs@mSiO_2-TSD@Ag demonstrated 71% cell viability, which was in the safer limit. The superior bacterial killing, wound healing characteristics, as well as excellent biosafety of the composite present it as an excellent means for treating clinical drug-resistant infections.

Table 8.2 Antimicrobial activities of silver-based nanocomposites against antibiotic-resistant bacterial strains

Silver-based nanocomposites (NCs)	Size of NPs	Pathogens tested	Evaluation parameters	Application area	References
Zeolites/AgNPs	ND	Methicillin-resistant *S. aureus*	MIC: 0.019–0.750 mg/mL	Antibiotic resistance control in medicine	Golubeva et al. (2018)
Dopamine-modified cellulose paper/AgNPs discs	50–60 nm	Antibiotic-resistant *E. faecalis*, *V. parahaemolyticus*, *S. marcescens*	ZoI_{dia}/ZoI_{disc}: 1.66–2.52	Antimicrobial packaging material	Islam et al. (2018)
Chitosan/AgNPs film	ND	Clinically isolated MRSA strains, *S. aureus*, *P. aeruginosa*	ZoI: 11.40–36.83 mm	Multifunctional material for antibiotic resistance control in wound dressing, water disinfection, drug delivery	Shah et al. (2018)
Tobramycin/silver nanospheres/graphene oxide	5 nm	Multidrug-resistant *E. coli*	Colony counting; visual bacterial reduction	Hybrid material to combat MDR bacteria	Ullah et al. (2018)
Mesostructured silica (MS)/AgNPs	2–80 nm	Methicillin-resistant *S. aureus*	MIC: 5–20 mg/mL ZoI: 15.1–17.6 mm	Antibacterial material for resistant pathogens in prosthetic joint infection	Chien et al. (2018)
Polydopamine (PDA)/AgNPs-loaded TiO_2 nanorods	50–100 nm	Methicillin-resistant *S. aureus*	ZoI: 13.5 mm; Inhibition rates: 95.4%–96.7%	Antibacterial orthopedic and dental Ti alloy coatings	Guan et al. (2019)
Chitosan/sericin/AgNPs/moxifloxacin	18.39–96.93 nm	Methicillin-resistant *S. aureus*, 2 clinical isolates of MRSA	ZoI: 37.25–50.75 mm	Antibacterial films for wound healing	Shah et al. (2019)
Alginate/AgNPs/honey hydrogel	5–10 nm	*Multidrug-resistant A. baumannii*, *P. aeruginosa*, *S. aureus*	99.9–100% bacterial reduction	Antibacterial wound dressing	Stojkovska et al. (2019)

Polymersome nanocapsules (PsNPs)/PR-39 peptides/AgNPs	118–136.9 nm	Methicillin-resistant *S. aureus* (MRSA)	100% bacterial growth inhibition	Materials for alternate antibiotic therapies in clinical setting and wound healing	Bassous and Webster (2019)
β-Carboxyphthalocyanine zinc (PS)/AgNPs/cellulose fabric	~100 nm	Methicillin-resistant *S. aureus* (MRSA)	99.96% kill efficacy under light illumination	Wound healing and biomedical textile antibacterial material	Chen et al. (2019)
Pluronic® F127/AgNPs hydrogel	20.06–22.25 nm	Extremely drug-resistant (XDR) clinical isolates of *P. aeruginosa*, *S. epidermidis*	MIC: ≥0.125 mM	Antibacterial product for broad prophylaxis of postoperative infections	Rafael et al. (2019)
Ag/graphene oxide	10–15 nm	*E.coli,* methicillin-resistant *S. aureus*	100% growth inhibition	Antibiotic resistance control material for wastewater treatment	Naz et al. (2019)
AgNPs/silk fibroin scaffold	5–12 nm	Ampicillin and kanamycin-resistant *E. coli*	MIC: 20 nM	Bone tissue engineering	Patil and Singh (2019)
TiO_2/ag/graphene oxide	ND	Tetracycline, gentamicin, streptomycin, kanamycin, and ampicillin-resistant *E. coli* strains	Inactivation rates: 66.6–97.8%	Antibiotic resistance control material for wastewaters	Guo and Tian (2019)
Porous titanium functionalized with silver nanoparticles	7–25 nm	*Methicillin-resistant S. aureus (MRSA)*	ZoI 0.6 cm^2	Treatment of implant-associated infections (IAIs)	Van Hengel et al. (2020)
AgNPs and polydopamine coatings	10–15 nm	*Acinetobacter baumannii*	ZoI 2.39 cm	Development of antibacterial surfaces	Neethu et al. (2020)
ZnO-AgNPs	20 nm	*Methicillin-resistant S. aureus (MRSA)*	MIC 60.8μg/mL	Antimicrobial agent	Shakerimoghaddam et al. (2020)

(continued)

Table 8.2 (continued)

Silver-based nanocomposites (NCs)	Size of NPs	Pathogens tested	Evaluation parameters	Application area	References
Silver nanoparticles-decorated and mesoporous silica-coated single-walled carbon nanotubes	2.78 ± 0.70 nm	*E. coli* and *Staphylococcus aureus*	MIC 120–140μg/mL	Treatment of clinical drug-resistant infections	Zhu et al. (2020)
Fungal chitosan-silver nanoparticle nanoconjugates	13.8–68.9 nm	*S. Typhimurium* and *S. aureus*	MIC 3.50–4μg/mL	Antimicrobial agent	Alsaggaf et al. (2020)
Graphene oxide and silver nanoparticle hybrid composite	9–12 nm	*P. aeruginosa*	Incubation time: 0–10 min (kill efficiency 20–100%)	Antibacterial agents	Lozovskis et al. (2020)
Silver-microfibrillated cellulose bio-composite	140 nm	*S. aureus* and *P. aeruginosa*	MIC (125–1500 ppm)	Development of biomedical instruments and therapeutics	Garza-Cervantes et al. (2020)
Chitosan-based-crosslinked gelatin/polyvinyl pyrrolidone-embedded silver nanoparticles	9.99 nm	*P. aeruginosa, S. typhi*, *S. aureus*, *E. coli*, and *B. subtilis*	ZoI 0.33–3.12 cm	Wound dressing	El-Aassar et al. (2021)
Silver covalently bound to cyanographene	10 and 28 nm	MRSA and ESBL (extended-spectrum *β*-lactamases-producing *Klebsiella pneumoniae*)	MIC 0.2–3.4 mgL^{-1}	Broad-spectrum antibacterial agents	Panáček et al. (2021)
Nano-silver-decorated biodegradable mesoporous organosilica nanoparticles	2–8 nm	*E. coli, P. aeruginosa, S. aureus,* and *E. faecalis*	MIC (12–48μg/mL) MBC (12–48μg/mL)	New generation of antibacterial materials to kill antibiotic-resistant bacteria	Li et al. (2021b)
Silver nanoparticles in nanoporous carbon nitride	4–8 nm	Multidrug-resistant *E. coli*	MIC (16μg/mL)	Potential for biomedical applications including medical devices, food packaging, disinfectant, and wound dressing applications	Wahab et al. (2021b)
Ag/80S bioactive nanocomposite	2.26–5.66 nm	Carbapenem-resistant *K. pneumoniae*	MIC (2.5 to5.0 mg/mL)	Novel antimicrobial agent	Yang et al. (2021)

In another study, (Alsaggaf et al. 2020) used phyco-synthesized silver nanoparticles and nano-fungal chitosan composites against drug-resistant bacterial pathogens of *Salmonella Typhimurium* and *Staphylococcus aureus*. The ZoI and MIC of the composite were found to be 25.9 ± 1.5 mm and 3.50μg/mL against gram-negative strain (*S. Typhimurium*) and 23.8 ± 1.4 mm and 4.0μg/mL against gram-positive strain (*S. aureus*). The morphological observations after 4 h of treatment revealed shrunken and distorted bacterial cells with defects on their surfaces. The complete lyses of bacterial cells were observed after 8 h of treatment. The electrostatic interaction could be the reason for superior antibacterial activity as the positive-charged fungal chitosan provides favorable microenvironment for composite docking onto the bacterial surface, facilitating the interactions of silver nanoparticles with bacterial cell membranes. The fabricated composite could be utilized as antimicrobial agent in biomedical field.

Similar to the resistance developed by various microorganisms against antibiotics, resistance against silver nanoparticles is also evident in some of the bacteria, imposing a greater threat. The resistance against silver is induced via secretion of flagellin, which triggers coagulation of silver nanoparticles, thus restraining their antibacterial activity. The feasible solution for this is to prevent the nanoparticle agglomeration, and silver nanoparticles-based composites are the best suitable candidate. For instance, (Panáček et al. 2021) used densely functionalized graphene (cyanographene) for effective covalent attachment of silver nanoparticles and investigated its use against silver and antibiotic resistance bacterial strains. In case of silver resistance strains (*Escherichia coli* and *Pseudomonas aeruginosa*), silver covalently bound to cyanographene (GCN/Ag) was able to fully eradicate bacteria even at 30-fold lower concentration as compared to only silver required to kill all bacterial cells. The MIC of composite was found to be 1.9 mg/L against *P. aeruginosa* and 3.4 mg/L against *E. coli*. The composite was even more effective against multidrug-resistant bacterial strains, where MIC of 0.5 mg/L against ESBL (extended-spectrum *β*-lactamases-producing *Klebsiella pneumoniae*) and 1.9 mg/L against MRSA (methicillin-resistant *Staphylococcus aureus*) was observed. Cell membrane damage was evident of contact killing mechanism. The composite remained efficient antibacterial activity after 60 bacterial generations, as compared to colloidal AgNPs which lost their activity after 20 generations. The cytocompatibility of GCN/Ag in healthy human cells was also tested, with 60% cell viability composite demonstrated excellent cytocompatibility. The limited silver release was another important factor of the composite where accumulative leaching of only 0.27 mg/L was recorded even after 6 months of storage in water. The effective anchoring of silver nanoparticles onto other materials could also be used to produce broad-spectrum antibacterial agents and restrain the leaching of silver into environment.

In another report, graphene oxide and silver nanoparticle hybrid composite (GO-Ag HN) was investigated for antimicrobial activity against antibiotic resistance *Pseudomonas aeruginosa* strain (Lozovskis et al. 2020). The authors observed that with increasing incubation time period from 0 to 10 minutes with hybrid nanocomposite, the abundant growth of bacterial strain decreased from 80% to 0%. Similar

results were obtained via counting of colony-forming units in similar incubation period. The structural changes in the bacterial cell membrane before and after incubation period revealed appearance of some bulges, sags, and pores on the surface of cells. The change in the cell membrane surface indicates membrane damage that increases permeability and membrane leakage and results in cell lysis. The superior antibacterial efficacy of hybrid nanocomposite against antibiotic-resistant *P. aeruginosa* can be attributed to toxicity of graphene oxide, which increases after addition of silver nanoparticles (9–12 nm). Sharp edges of graphene oxide formed in the final composite may have caused physical damage to the cell membrane upon direct contact with bacteria. Furthermore, material characterization demonstrated that the edges of graphene oxide sheets were densely decorated with silver nanoparticles, causing high penetration of silver nanoparticles into the bacterial cell. These obtained results indicated that GO-Ag HN is a promising antibacterial agent with futuristic potential.

Multidrug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* were used to determine antibacterial activity of silver-microfibrillated cellulose bio-composite (Garza-Cervantes et al. 2020). The MIC of gram-positive *S. aureus* was found to be 1500 ppm and gram-negative *P. aeruginosa* was 125 ppm. The high concentration of bio-composite required for complete inhibition of gram-positive bacteria can be attributed to the thicker peptidoglycan layer that could prevent the penetration of bio-composite into the cell cytoplasm. The study highlights the potential of the bio-composite to be applied in the future development of biomedical instruments and therapeutics. Wahab et al. (2021b) investigated the silver nanoparticles-incorporated nanoporous carbon nitride (NCN@Ag) as antibacterial agent against multidrug-resistant *Escherichia coli* pathogens. The highly dispersed AgNPs containing NCN@Ag sample demonstrated superior bactericidal effect as compared to NCN, signifying the role of in situ-incorporated AgNPs to improve the antibacterial activity. The MIC value of NCN@Ag was recorded at 16μg/mL, which was 64-fold lower than the MIC value of NCN (1024μg/mL) for the complete inhibition of multidrug-resistant *E. coli*. Small sizes of NPs (4–8 nm), as well as higher surface-to-volume ratio of NCN@Ag, governed a larger contact area with infectious pathogens which contributed towards the enhanced antibacterial efficacy. The possibility of the presence of silver nanoparticles on the outer surface of pore channels further plays a significant role via direct contact with bacterial cell membrane. In future the similar approach can be used at a wider scale via employing other nanoporous materials with high surface area, large pore volumes, and accessible porosity. The application area of such materials can be expended in various biomedical applications such as medical devices, food packaging, disinfectant, and wound dressing applications.

Porous materials impose as a better attachment matrix for silver nanoparticles owing to their uniform pore sizes, large pore volumes, and large surface areas. Mesoporous materials have the pore size appropriate to act as a reactor for nanoparticle formation and improve their use as antimicrobial agents. For instance, (Yang et al. 2021) used mesostructured materials to confine silver nanoparticles (Ag/80S) and investigated its antibacterial potential against carbapenem-resistant *Klebsiella*

pneumonia. Material characterization revealed 7.5 nm mesopore size and high surface area of 307.6 m^2/g and uniform distribution of silver nanoparticles through the composite. MIC values of the composite were recorded in the range of 2.5–5 mg/mL, with low cytotoxicity. The zone of inhibition study against *K. pneumonia* resulted in 18 mm of inhibition zone for Ag/ 80S disk, whereas no zone of inhibition was observed for 80S alone. The morphological analysis of bacterial cells treated with Ag/80S revealed high pore deformations in membrane of cells treated with 1% Ag/80S as compared to untreated or 0.5% Ag/80S. High surface area of the composite resulted in convenient interaction between silver nanoparticles and membrane surface, where silver nanoparticles penetrated the bacterial membranes and triggered cell death. A time-dependent increase in ROS levels was also recorded which is in accord with increasing interaction between silver nanoparticles and cell membrane surface. Nano-silver-decorated mesoporous organosilica nanoparticles (Ag-MONs) were explored for their antibacterial capacity against various antibiotic-resistant bacterial strains (Li et al. 2021b). Gentamicin-loaded nano-silver-decorated mesoporous organosilica (Ag-MONs@GEN) nanoparticles were also investigated for antibacterial potential and as delivery agent of gentamicin and nano-silver. Ag-MONs@GEN significantly inhibited the growth of antibiotic-resistant *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis* in a dose-dependent manner. The MIC values of Ag-MONs@GEN, against *E. coli*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*, were 12, 12, 24, and 48μg/mL, respectively. The effect was more prominent in gram-negative bacteria than for gram-positive bacteria owing to the selective killing effects of both GEN and nano-silver on gram-negative bacteria. At minimum bactericidal concentration, Ag-MONs@GEN exhibited complete inhibition of the bacterial colonies of *Escherichia coli* (24μg/mL), *Pseudomonas aeruginosa* (12μg/mL), *Staphylococcus aureus* (24μg/mL), and *Enterococcus faecalis* (48μg/mL). The excellent antibacterial effects on resistant bacterial strains demonstrated the synergistic antibacterial capacity of Ag-MONs@GEN as compared to Ag-MONs and GEN alone. The cytotoxicity evaluation revealed excellent biocompatibility with IC_{50} values of 313.6 ± 15.9 and 295.7 ± 12.3μg/mL, in L929 and HUVECs cells. The study can be used in future to develop simultaneous platforms for drug delivery and antibacterial agents with enhanced antibacterial ability against antibiotic-resistant bacteria. Golubeva et al. (2018) modified zeolites comprised of Rho, Beta, and paulingite structures with silver nanoparticles and clusters (3–6 wt.%). The modified structures displayed excellent antimicrobial activity against MRSA which increased with increment in silver content, ultimately demonstrated by decreasing MIC from 0.750 to 0.019 mg/mL. Under identical conditions of silver concentration, type of zeolite matrix and conformation of silver particles gained the controlling aspect of antimicrobial activity. For instance, Rho zeolite displayed higher antibacterial activity on account of stabilized Ag_8 nanoparticles and clusters compared to the least antimicrobial activity in Beta zeolite. These silver zeolites were found to be suitable for antibiotic resistance control in medicine for their pronounced biological activity towards drug-resistant strains and selective cytotoxicity towards tumor cells.

8.4 Conclusions

The need for hygienic living conditions prompts new challenges for the development of affordable and efficacious antimicrobial materials that should be environmentally friendly and absolutely nontoxic towards human beings. The development of antibiotic resistance among individuals is developing at a fast pace, which creates several complications during medical treatments and/or surgical procedures. In the context of reducing the risk of microbial infections, several antibacterial nanoformulations of AgNPs and its associated composites based on supporting matrices have been developed by the researchers. Encouragingly, the existing antimicrobial therapies including antibiotics can work efficiently in combination with different silver nanoparticles for treatment complications either due to antibiotic-resistant or multidrug-resistant microbial strains. Recent investigations and various studies pertaining to antibacterial and biocompatibility activities of AgNPs have provided evidence that these activities may be influenced by many factors such as types of nano-formulations, films, coatings, and hydrogels and concentration or volume ratio of each constituent. By controlling and manipulating such parameters with extensive research on AgNPs cytotoxicity can help these materials earn a marketable commercially available status. The wide applications of silver nanoparticles and their composites towards controlling antibiotic resistance consolidated in this chapter would provide a reference value for future research to construct and implement nano-silver-based biomaterial designs in biomedical applications.

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