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Graphene-based metal/metal oxide nanocomposites as potential antibacterial agents: a mini-review

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Antimicrobial resistance (AMR) is a rising issue worldwide, which is increasing prolonged illness and mortality rates in the population. Similarly, bacteria have generated multidrug resistance (MDR) by developing various mechanisms to cope with existing antibiotics and therefore, there is a need to develop new antibacterial and antimicrobial agents. Biocompatible nanomaterials like graphene and its derivatives, graphene oxide (GO), and reduced graphene oxide (rGO) loaded with metal/metal oxide nanoparticles have been explored as potential antibacterial agents. It is observed that nanocomposites of GO/rGO and metal/metal oxide nanoparticles can result in the synthesis of less toxic, more stable, controlled size, uniformly distributed, and cost-effective nanomaterials compared to pure metal nanoparticles. Antibacterial studies of these nanocomposites show their considerable potential as antibacterial and antimicrobial agents, however, issues like the mechanism of antimicrobial action and their cytotoxicity need to be explored in detail. This review highlights a comparative analysis of graphene-based metal and metal oxide nanoparticles as potential antibacterial agents against AMR and MDR.

1. Introduction

Antimicrobial resistance can be described as the resistance generated against specific antimicrobials due to some evolving mechanism in microorganisms that helps to nullify the effect of antimicrobials.[1] AMR includes resistance against antibacterial, antiviral, antifungal, and anti-parasitic drugs whereas antibiotic resistance specifically applies to bacteria only.[1,2] The number of new strains of infectious agents has risen significantly in recent decades and various antimicrobials have been developed to treat them, however, excessive use of antimicrobials over the years has increased the number of drug-resistant pathogen.[1,2] According to the WHO, these drug-resistant pathogens (*e.g.*, bacteria, fungi, viruses, and parasites) are of considerable concern worldwide.[1] Similarly, multi-drug resistance (MDR) where microorganisms generate resistance against more than one drug is also a rising global health risk for patients admitted to hospitals as bacteria-mediated infections may cause various acute or chronic illnesses.[3–5] Therefore, new alternatives have been focused on to overcome this global threat. This and other infection-related problems have motivated researchers to concentrate on the development of novel, inexpensive, and efficient antimicrobial treatment strategies for fighting pathogenic infections.[2,3,6] The nanotechnology-driven solution to overcome the problem of drug resistance for patients and practitioners provides new opportunities in this area. Nanomaterials have tremendous potential in medical fields, however, several queries related to the mechanism of action, significance of size and composition of nanomaterials toward bacterial activity, toxicity criteria, and other issues need to be addressed.[3,6–8] Antibacterial agents may be synthetic, semi-synthetic (chemically modified natural compounds), or of natural origin (plant and animal origin). Among various nanomaterials, metal nanoparticles have been studied tremendously as a potential antibacterial agent and their various mechanism of action are well studied, as shown in Fig. 1. Metal nanoparticles consolidate mostly gold, zinc oxide, titanium oxide; copper oxide, magnesium oxide, and chromium(III) oxide nanoparticles.[8,9] Metal and metal oxide nanoparticles have extraordinary and very much characterized physical and synthetic properties that can be controlled reasonably for desired applications. The effectiveness of any nanomaterial depends on the interaction between the microorganism and the nanoparticles based on criteria like size, shape, surface charge, ion release kinetics, cellular uptake, *etc.* More modest particles with bigger surface-to-volume pro-

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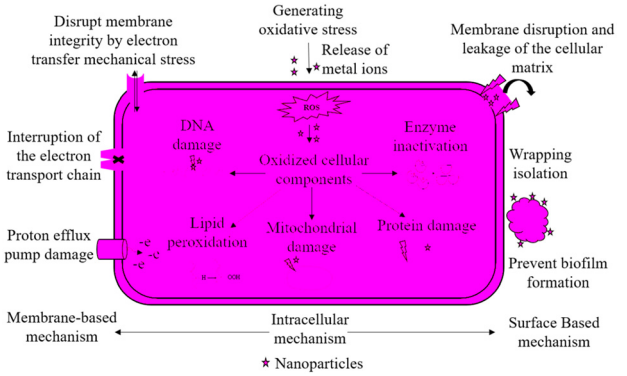

Fig. 1 Shows the possible antimicrobial mechanism of nanoparticles.

portions have more noteworthy antibacterial action.[10–12] Conventional synthesis strategies for metal and metal oxide nanoparticles incorporate reducing and stabilizing toxic chemical agents for people and different species in distinct tropic levels. Consequently, analysts are currently searching for elective methodologies "green union" with the end goal of reducing or wiping out hurtful synthetics during the synthesis of metallic nanoparticles.[12–18]

The process of green synthesis includes either a biological mode of synthesis using biological material as reducing and capping agents or the use of less toxic materials as a substrate to form composites. One of the recently used stabilizing agents as well as a substrate material is graphene oxide (GO). Various researchers have synthesized GO-based metal/metal oxide nanocomposites such as AgNPs–GO, Au–GO, ZnO–GO, *etc.*, and studied their antibacterial efficacy.[19–24] However, GO itself is also studied for its antibacterial activity and also having anticancer, photocatalytic activity.[6] Hu *et al.* 2010 reported the antibacterial activity of two graphene-based materials: graphene oxide (GO) and reduced graphene oxide (rGO) nanosheets, which significantly inhibited *E. coli* bacterial growth. Various mechanisms of antibacterial activity of graphene have been proposed, such as oxidative stress, membrane stress, and electron transfer.[25] Graphene can physically damage bacterial membranes by directly touching their sharp edge.[26] The use of graphene offers various advantages as graphene nanosheets are more easily distributed in the cellular compartments, avoiding aggregation and their subsequent expulsion by gravimetric settling. Graphene nanodispersion phases improve the safety of bio-distribution processes in the cells and minimize the reactive oxygen species (ROS) generation mechanisms.[27,28]

Metallic nanoparticles have shown their potential as antibacterial agents but suffer from several limitations like toxicity, agglomeration, oxidation, stability, cost, *etc*. while the application of GO provides an opportunity to generate less toxic, stable, and homogenously distributed nanoparticles with cost-effectiveness.[11] GO-based metal/metal oxide nanocomposites have shown significant antibacterial activity due to the different mechanisms of killing bacterial cells such as the production of reactive oxygen species, cations release, biomolecule damages, ATP depletion, and membrane interaction caused by metal/metal oxide nanoparticles and membrane stress against bacteria while contacting with the sharp corner basal planes of GO, which results in disruption and damage the cell membranes.[3,6,7,28,29] The fine edges of graphene sheets act as nanoknives, cutting the bacterial cell membrane, which leads to the exposure of intracellular components followed by cell death.[29] The synthesis processes of GO-based metal/metal oxide nanocomposites include both *in situ* and *ex situ* approaches. In the case of *in situ* synthesis simultaneous reduction of metal salts and GO occurred to obtain nanocomposites, while in the *ex situ* approach, metal nanoparticles of the desired size and shape are first synthesized and then deposited onto GO.[21] The metallic and oxide forms of metal nanoparticles also affect the mechanism of action of synthesized nanocomposites.

Therefore, this review summarizes the current efforts in synthesizing graphene-based metal and metal oxide nanocomposites and their potential as new agents to tackle the current challenges of AMR and MDR.

2. Graphene: its oxide and reduced forms

Graphene is an allotrope of carbon as a solitary layer of particles in a 2-D hexagonal cross-section in which one molecule frames every vertex. It is the basic auxiliary component of allotropes, including graphite, charcoal, carbon nanotubes, and fullerenes. The graphene surface is encircled with an electron cloud, capable of behaving as an electron donor and acceptor which enables this material to make extraordinary bonds together and form a new layered structure. The edges of graphene have different bonds, and these spots may have various qualities.[22] Graphene has various beneficial properties like thermal stability, mechanical area, high conductivity, and large surface zone. By giving a thermal, chemical, photochemical, or electrochemical reducing environment, graphene can be changed into other derivatives as shown in Fig. 2.[28,30,31] Graphene and its different forms are utilized as a significant choice to substitute numerous conventional materials for different biomedical applications. It is a one-of-a-kind smart material that has antibacterial action against bacterial species.[32–35]

2.1. Antibacterial properties and mechanism of action

It is demonstrated by various researchers that graphene and its derivatives possess antibacterial activity and can be used as potential antibacterial agents. Particularly, GO, due to its different oxygen-rich functionalities offers additional compound adjustment on the surface which allows it to adhere to the microbial cell surfaces and restrict the uptake of micronutrients from the environment which ultimately leads to cell death.[26,36,37] Further, the antibacterial activity of GO includes both membrane interruption and oxidative stress as shown in Fig. 3. However, the antibacterial activity of graphene GO and

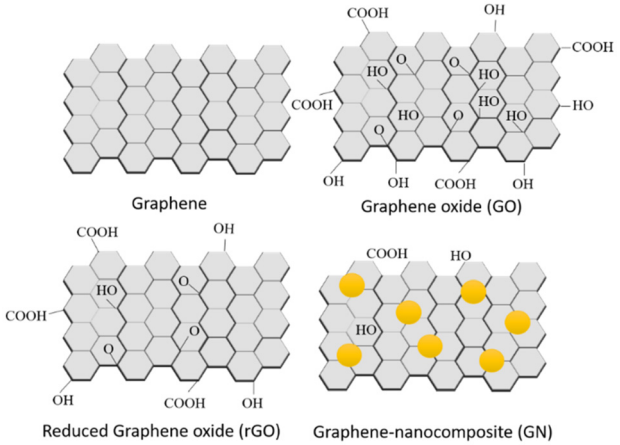

Fig. 2 Chemical structure of graphene, graphene oxide (GO), reduced graphene oxide (rGO), and graphene nanocomposites.

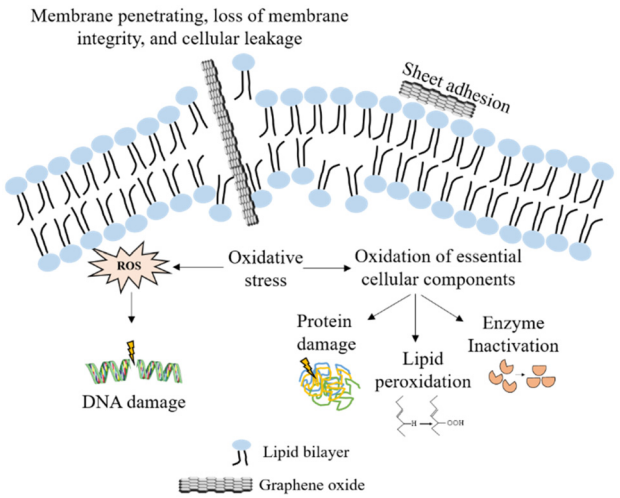

Fig. 3 Possible antibacterial mechanism of graphene.

rGO depends on numerous variables including material properties (*i.e.*, electronic structure, size, and surface synthetic properties) and bacterial cells (*i.e.*, brooding time, fixation), culture medium (*i.e.*, agar-based, broth, salts, pH), temperature and light sources.[7,38] Nanda *et al.*, 2016 described that the antibacterial activity of graphene can be characterized by both physical and chemical methods.[39] It is important to understand the mechanism of action of graphene while interacting with bacterial cells which damage the bacterial membrane by direct contact with its sharp corners and lead to the inactivation of bacteria due to leakage of the intracellular matrix.[6,7,35,40] Since graphene-based materials have sharp edges, this mechanism mode is commonly known as the "insertion mode," which allows microorganisms to penetrate the membrane easily. Additionally, oxidative stress generated due to the overproduction of ROS by GO can cause DNA damage and cellular component dysfunction, which results in bacterial inhibition. It is possible that graphene layer thickness and hydrophilicity affect the degradation of membrane integrity in single-celled microorganisms in this mechanism.[6,7,28,35,40] Kumar *et al.*, 2019, explain the difference between GO and rGO mechanisms such as GO destruct the bacteria by damaging the cell membrane through a chemical reaction whereas rGO induces mechanical stress, which pierces the cell membrane.[6,7,41] The graphene and graphene derivatives have been attributed to different mechanisms, including cell damage by generating oxidative stress and electron transfer mechanisms restricting bacterial growth. In bacterial cells exposed to GO, oxidative interactions play a key role due to the oxidation capacity of graphene material. Oxidative stress can occur through either a reactive oxygen species (ROS)-dependent or a ROS-independent pathway. In the ROS-dependent pathway, the production of ROS can damage cellular components. In the ROS-independent pathway, the production of ROS is not involved, being the charge transfer from the cellular membrane to the graphene surface that induces cell death. Many controlling factors are used to determine the bactericidal efficiency of graphene-like shape, size, and type of bacteria and the chemical factor involves the overproduction of reactive oxygen species (ROS) that was later found to oxidize fatty acids leading to the production of lipid peroxides that stimulate a chain reaction, eventually leading to the disintegration of the cell membrane followed by cell death.[26,28,35] Graphene oxide is preferred in biomedical systems because it has moderately low cytotoxicity toward mammalian cells.

Graphene oxide (GO), a graphene derivative, has numerous attributes that are different from those of graphene. Its derivatives can be synthesized using various methods as mentioned in Table 1. Hummers' strategies are accepted universally to form GO *via* the oxidation of graphite to acquire hydrophilic groups on a superficial level. After the oxidation cycle, the subsequent step was viewed as chemical exfoliation and oxidation of layered glass-like graphite to get one multi-layer GO sheet, which can be accomplished using ultra-sonication.[42,43] In GO, sp^2-bonded carbon molecules are arranged in a solitary, two-dimensional hexagonal shape and the edges possess oxygen-containing functional groups such as hydroxyl, carbonyl, carboxylic, and epoxy. Colloidal GO offers several chances for functionalization with particles, polymers, and metal nanoparticles and expands its different applications.[44–47] These groups act to restrain electron movement that makes the nuclear layer hydrophilic and extends interlayer separation.[41] The oxygen-containing functional groups can trap the microbes and it is dispersible in fluid arrangements which makes it convenient to use as a feasible antibacterial agent.[39,48,49] Graphene is exceptionally hydrophobic and doesn't break up in hydrophilic dissolvable sheets therefore graphene oxide is usually utilized as another option.[50]

Similarly, reduced graphene oxide (rGO) is another derivative with less oxygen content than GO. rGO can be achieved *via* reducing GO by chemical, thermal, or physical methods to decrease the oxygen content, while graphene oxide is a material generated by the oxidation of graphite which prompts expanded interlayer dividing and functionalization of the

Table 1 Methods of synthesis of graphene and its derivative

S. no	Material	Synthesis method	Explanations	Ref.
1	Graphene	Micromechanical exfoliation & chemical vapor deposition	Synthesized from graphite and has low production efficiency	52
2	GO	Chemically exfoliation	GO chemically exfoliated from graphite oxide (GtO)	38
		Modified Hummers' method	Graphene oxide (GO) sheets were prepared by bath-sonicating graphite oxide particles prepared *via* a modified Hummers' method	62
		Modified hummers method	The water-soluble GO was prepared by oxidizing pristine graphite	63
		Modified Hummers' method	Oxidation of graphite using strong oxidizing agents such as $KMnO_4$ and $NaNO_3$ in H_2SO_4/H_3PO_4	7, 22, 39, 41 and 52
		(Modified Hummers—Offerman method is known as the early method Brodie, Staudenmaier, Hoffman)	Using strong acids (nitric and/or sulfur) and potassium chlorate. Uses a mixture of concentrated H_2SO_4, $NaNO_3$, and $KMnO_4$ respectively	64 and 65
		Micromechanical exfoliation	Reduced graphene oxide rGO to GO by (ultrasonication) oxidation of graphite	66
3	rGO	Micromechanical exfoliation	Thermal annealing or chemical treatment can eliminate functional groups on GO to generate rGO	38
		Oxidation, intercalation, exfoliation, & reduction	Graphene oxide is reduced using ascorbic acid as a reducing agent	51
		Thermal reduction, chemical reduction and electrochemical reduction	Reduction of GO by thermal treatment doesn't require shows of chemical agents; chemical reducing agents have been reported to be effective at synthesizing rGO, such as ascorbic acid, sugars, amino acids, and even microorganisms; this reduction is exclusively driven by electron exchange between GO and the electrodes of a typical electrochemical cell respectively	52
		Thermal reduction methods	Transformation of GO into rGO is going in a wide range of temperatures. Reduction by irradiation [microwave, infrared visible, or ultraviolet (UV)] in a vacuum, inert, or reducing atmosphere	65

basal planes of graphite.[51] rGO has enhanced self-aggregation cycles and lower solubility yet improved conductivity, light absorption properties, and mechanical strength. The nature of rGO relies upon the number of cycles and the nature of reducing agents.[52] The structural and functional properties of GO and rGO can be characterized by UV-vis spectroscopy, Fourier transforms infrared (FTIR) spectroscopy, and X-ray diffraction patterns (XRD).[35,53,54]

Recently, another 2D material black phosphorus (BP) is also become a choice of researchers to be used as novel antibacterial agent. Here, it is important to compare graphene and black phosphorus with respect to their antibacterial potential. The graphene-based materials have large surface area and unique physical properties which leading to their bactericidal activity towards wide range of bacteria.[55,56] Graphene nanosheets possesses significant features such as tunable-zero bandgaps, enabling metallic behaviour, easy and diverse surface functionalization, easy experimental preparation and significant catalytic properties which enhanced its antibacterial efficacy.[56] Similarly, the black phosphorus nanosheets have significant biocompatibility and specific surface area, which enable these to combat bacteria through various mechanisms like physical disruption of bacterial membranes and photothermal therapies.[57] However, BP yet not well studied and explored therefore, shows limitations like low preparation efficiency, complex synthesis procedure *i.e.*, need of specific environment for synthesis such as high temperature and high pressure without oxygen, requires expensive auxiliary materials or solvents, high costs, cannot achieve mass production and oxidative degradation, which limits its widespread application.[57,58] Another 2D material is Carbon nitride (C_3N_4), composed of carbon and nitrogen atoms arranged in a hexagonal lattice structure, similar to graphene. Graphitic carbon nitride (g-C_3N_4), as a polymeric semiconductor, is promising for ecological and economical photocatalytic applications because of its suitable electronic structures, together with the low cost, facile preparation, and metal-free feature.[59] It has significant photocatalytic activity with excellent physico-chemical properties and have considerable antibacterial activity, however, detailed studies needs to be done to claim it as a significant antibacterial agent.[60] Compared to both these 2D materials graphene has much explored and proved to have significant antibacterial properties accredited to its tunability including shape, size, and orientation, which makes it a versatile candidate for tailored antibacterial solutions.[61]

3. Graphene-based metal nanocomposites

Metal nanoparticles are frequently used in fields like medicine, physics, mechanics, pharmaceutics, and others, are continuously using metal nanoparticles. In the last few years, metal nanoparticles have had great potential and are being

used in the healthcare sector.[67] Metal and metal oxide nanoparticles exhibit different physiochemical properties. They are different from their native bulk compounds in several aspects and have received substantial research attention due to their exceptional electrical, optical, magnetic, and catalytic properties.[68,69] These methods for nanoparticle synthesis can be categorized into two main classes: bottom-up methods and top-down methods depending on the starting material of nanoparticle preparation.[67] It has been demonstrated that metallic nanoparticles are a promising alternative to antibiotics, due to their small particle size, large surface area, and physical, mechanical, and chemical properties.[69] However, these have several limitations like toxicity, agglomeration, oxidation, stability, cost, *etc*.[70] The issues of toxicity and agglomeration can be overcome by introducing a substrate like graphene oxide. GO/rGO provides an opportunity to generate less toxic, stable, and homogenously distributed nanoparticles with cost-effectiveness.[6] Graphene oxide is the most suitable material to disperse and stabilize metals utilizing its large specific surface area and abundant oxygenated functional groups. GO/rGO sheets act as a support for the growth and stabilization of metal nanoparticles as oxygen-containing groups act as anchoring sites for the attachment of metal nanoparticles and GO/rGO produces stable dispersions in water. The synthesis of graphene-based metal nanoparticle hybrids using various reducing agents and stabilizing agents (to prevent metal nanoparticle agglomeration and control its structure) *via* different physical and chemical approaches has been reported in Table 2.[66] These include gold, and silver since their antibacterial effects have been described.[9,40] The antibacterial properties of graphene and its derivatives are explained in an earlier section in detail. The antibacterial mechanism of metal nanoparticles are as follows: (1) disruption of the bacterial cell membrane; (2) generation of ROS; (3) penetration of the bacterial cell membrane; (4) nonoxidative stress; (5) interruption of electron transport chain (6) prevention of biofilm formations; and (7) induction of intracellular antibacterial effects, including interactions with DNA and proteins.[71–74] These antimicrobial mechanisms of metal nanoparticles halted cellular processes such as DNA replication by damaging DNA, protein leakage and protein damage, and ribosome disassembly. It has been demonstrated that these nanocomposites of GO/rGO and metal nanoparticles work synergistically to enhance their properties, such as higher antimicrobial, catalytic activities, and thermal conductivity.[50] The synthesis of composites nanoparticle composites has opened up new avenues for both the materials for conducting new research and applications.[21,63,66,75] The synergistic properties of these hybrid materials have proven to be useful in a variety of applications (electronics, catalysis, electrochemical biosensing, drug delivery, *etc.*). Graphene-based metal nanocomposites have wide applications but this review is mainly focused on their application as potential antibacterial agents and their mechanism. Methods of synthesis of graphene-based metal nanocomposite and their antibacterial mechanism are explained as follows.

3.1. Synthesis and antibacterial mechanism of graphene-based metal nanocomposites

Some of the recently published articles include the synthesis and antibacterial effect of graphene-based metal nanoparticles such as silver and gold.[6,7,76] Fig. 4 shows the possible antibacterial mechanism of graphene-based metal nanoparticles against bacterial species.

The study by Hussain *et al.*, 2014 demonstrated that AuNPs/rGO composite prepared with an eco-friendly and nontoxic reducing agent under ultrasonication enhanced antibacterial activity against two Gram-positive bacteria (*S. aureus* and *B. subtilis*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) in comparison to GO nanosheets by leaking sugars and proteins from bacteria's cell membranes when they come into contact with AuNPs/rGO composites.[77] Recently, Turcheniuk reported that rGO–PEG–Au nanorods particles were used for photothermal ablation of bacteria, and *E. coli* inhibition was optimal at high temperatures (56–70 °C).[78] Huang *et al*., 2016 reported the synthesis of AgNPs/GO composites by a facile solution-phase synthesis method by directly reducing $AgNO_3$ on the GO matrix using Sodium Borohydride ($NaBH_4$) as a reducing agent. Moreover, the antibacterial activities of AgNPs/GO nanocomposites against Gram-negative bacterial strain (*E. coli*) and Gram-positive strain (*S. aureus*) showed good antibacterial activities.[63] Gurunathan *et al*., 2016 show preparation of GO–AgNPs with pepsin as a reducing agent and stabilizing agent, these nanoparticles have significantly higher antibacterial and anti-biofilm activities in *S. flexneri* and *S. pneumonia*.[79] Jaworski *et al*., 2018 prepared silver nanoparticles decorated on graphene oxide *via* the ultrasonic method showing excellent antimicrobial efficacy against bacteria and yeast cells as compared with Ag-NPs and GO separately.[22] Kumar P. *et al*., 2019 show the production of GO–AgNPs and rGO–AgNPs based nanocomposites by sonochemical method with improving antibacterial activities due to synergistic antibacterial mechanism.[7] Cobos *et al*., 2020 reported the *in situ* synthesis of GO–AgNPs nanohybrids by an environmentally friendly approach where excellent antimicrobial properties were observed against Gram-negative bacteria (*E. coli* and *P. aeruginosa*), Gram-positive (*S. aureus*) and the yeast (*Candida albicans*).[66] Vi *et al*., 2020 reported the synthesis of GO–Ag NPs by chemical reduction method resulted in nanomaterials showing good antibacterial activity compared to a pure sample of GO and AgNPs.[23] However, the synergetic effect of GO–Ag NPs was fully proposed by multiple mechanisms, including the physicochemical effects and ROS production. Menazea *et al*., 2020 reported synthesizing silver nanoparticles decorated onto graphene oxide sheets *via* a chemical method and studied their antibacterial activity against *E. coli*.[80] Using the microwave irradiation method, Aljaafari *et al*., 2020 prepared Au–rGO with a reducing agent (citric acid) and a binding agent (CTAB) possessing good antimicrobial and biofilm inhibition properties against foodborne pathogenic bacteria.[81] Truong *et al*., 2022 suggested that silver nanoparticles and silver nanoparticles decorated onto gra-

Table 2 Shows the published literature for the synthesis and antibacterial study of graphene-based metal/metal oxide nanocomposites

S. no	Nano-material	Synthesis method	Conc. of material	Tested organism		Testing method	Inhibition		Modes of action	Ref.
1	Ag NP/GO	Facile solution-phase, reducing agents $NaBH_4$	2 ml	*E. coli*	*S. aureus*	Disk diffusion	10 mm	9 mm	Disrupting bacterial cell wall integrity	63
			4 ml				12 mm	11 mm		
			6 ml				13 mm	12 mm	Inhibiting cell division	
			8 ml				15 mm	14 mm		
2	GO	Modified Hummers' method	10 μg mL^{-1}	*E. coli*		Well diffusion	9.5 mm		ROS generations	23
			50 μg mL^{-1}				9.5 mm			
			100 μg mL^{-1}				11 mm			
	AgNPs	Chemical reduction method	10 μg mL^{-1}				10 mm		Penetrating the Inner cell	
			50 μg mL^{-1}				11 mm			
			100 μg mL^{-1}				13 mm			
	GO–Ag NPs	Chemical reduction method	10 μg mL^{-1}				15 mm		Physicochemical effects and ROS generations	
			50 μg mL^{-1}				16 mm			
			100 μg mL^{-1}				18 mm			
3	GO–AgNPs	Sono-chemical	10 μg mL^{-1}	*E. coli*		Plate count	100%		Ag ions can penetrate the cells and destroy the membranes	7
	rGO–AgNPs	Sono-chemical	40 μg mL^{-1}	*E. coli*		Plate count	100%			
4	GO–AgNPs	Chemical reduction method	—	*E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans*		—	—		Release of metal ions and generation of ROS	66
5	GO	Laser ablation technique		*E. coli*	*S. aureus*	Disc diffusion	6 mm	7 mm	Releasing metal ions and generating ROS	37
	AgNPs@GO						11 mm	16 mm		
	CuONPs@GO						10 mm	14 mm		
6	Au–RGO		32 μg mL^{-1}	*L. monocytogenes*		Minimum inhibitory concentration				87
			64 μg mL^{-1}	*MRSA*						
			16 μg mL^{-1}	*E. coli*						
			8 μg mL^{-1}	*S. marcescens*						
			16 μg mL^{-1}	*P. aeruginosa*						
7	rGO/Cu_2O	The biological method using cellulose acetate	20 μg mL^{-1}	*E. coli*		Broth dilution method	0.67-log		Generation of reactive oxygen species	101
			40 μg mL^{-1}				1.17-log			
			80 μg mL^{-1}				2.57-log			
66	rGO–Cu_2O	Chemical method	40 μg mL^{-1}	*E. coli*/*S. aureus*		Plate count method	70/65%		Releasing metal ions and generating ROS	37
9	GO–Fe_3O_4	Solvo thermal method	300 μg mL^{-1}	*E. coli*		Plate count method	91.5%		Releasing metal ions and generating ROS *via* contact with bacteria	87
7	GO–Fe_2O_3	Chemical method	100 μg mL^{-1}	*E. coli*		Plate count method	97%		Releasing metal ions and generating ROS	7
	GO–$MnFe_2O_4$	Chemical method	100 μg mL^{-1}	*E. coli*		Plate count method	82%			
	TiO_2–GO	Simple synthesis method	180 μg mL^{-1}	*E. coli*		Plate count method	100%		Formation Of ROS	
10	ZnO–GO	Simple and economical method	3 × 103 μg mL^{-1}	*E. coli*		Plate count method	100%		Membrane disruption due to oxidative stress	92
11	rGO–ZnO	Simple and facile one-pot chemical	500 μg mL^{-1}	*E. coli*		Plate count method	100%		Membrane disruption due to oxidative stress	93
12	GO–ZnO	Simple hydrothermal method	6.5 μg mL^{-1}	*P. aeruginosa*		Dilution method	100%		Formation of ROS due to destructing bacterial cell integrity by releasing Zn^+	85
			11.5 μg mL^{-1}	*S. aureus*			100%			
			15 μg mL^{-1}	*B. subtilis*			100%			

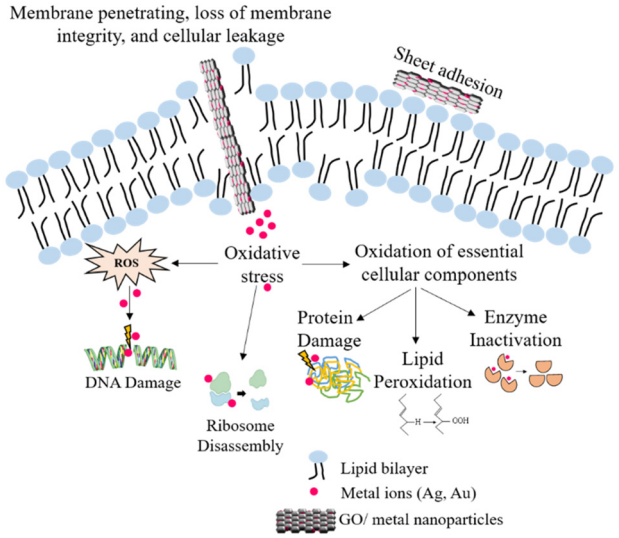

Fig. 4 Antibacterial mechanism of graphene-based metal nanocomposites.

phene oxide nanosheets are good bactericidal agents. Moreover, the order of antibacterial activity observed is GO–Ag NPrsms > GO–Ag NPs > Ag NPrsms > Ag NPs > GO against Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*).[82] Jin *et al.*, 2023 synthesis of graphene oxide on silver nanoparticles and its use in antibacterial applications synthesis. The results showed that ultraviolet irradiation could better maintain the antibacterial property of AgNPs, while GO could improve the dispersibility of AgNPs and prevent their oxidation.[83] Table 2 shows detailed parameters for recently reported metal–GO/rGO nanocomposites with mechanism of action.

4. Graphene-based metal oxide nanocomposites

Most of the metal oxides have been studied for their potential antibacterial properties, and show significant results however, their toxicity is too high to be used in biomedical applications.[73] Graphene has served as an alternative to synthesize biocompatible, stable, and cost-effective graphene-based metal oxide nanoparticles having substantial antibacterial activities.[84] Recently, graphene-based metal oxide nanocomposites have gained the attention of researchers and several studies have been reported for their synthesis of such as rGO–ZnO, GO–ZnO, rGO–Cu_2O, GO–Fe_3O_4, and rGO–TiO_2.[37,85–87] The antibacterial mechanism of these nanocomposites depends on the properties of both graphene sheets (as described earlier) and metal oxides. As mentioned earlier GO traps bacteria while the bactericidal mechanism of metal ions involves firstly, the production of oxidative stress *via* the generation of reactive oxygen species (ROS) followed by DNA, protein, lipids, and membrane damage, which finally leads to cell death. Secondly, the release of metal ions while interacting with the cell wall of bacteria. Thirdly. Leakage of internal cellular components leads to the death of bacteria. Finally, ribosome disassembly, in turn, inhibits translation further.[6,7,29] It should be noted that the bactericidal mechanism of metal and metal oxide nanocomposites is almost the same except for the fact that metal oxides do not affect ribosomal assembly as shown in Fig. 4.[88,89] Also, it has been reported that metal oxide possesses better bactericidal properties as compared with metal nanoparticles whether deposited onto GO or not.[90] This has also been suggested by Mahmoodi *et al.*, 2018 where it is stated that in the case of metal oxide NPs, the metal is already in the oxidation condition with a high ion release rate resulting in particle dissolution, while for the metal nanoparticles, the formation of an oxide layer is required first which slow down the ion release rate.[91] Metal nanoparticles (especially metal oxide nanoparticles) show great antibacterial effects are mentioned in Table 2. Various methods of synthesis of graphene-based metal oxide nanocomposite and their antibacterial mechanism against bacterial strain are explained as follows.

4.1. Synthesis and antibacterial mechanism of graphene-based metal oxide nanocomposites

Some of the recently published articles reported the synthesis and antibacterial effect of graphene-based metal oxide nanocomposites such as ZnO, FeO_3, TiO_2, and other metal oxide nanoparticles Table 2.[6,7]

Kavitha *et al.*, 2012 reported a simple and economical preparation of zinc oxide (ZnO) decorated graphene nanosheets at low temperatures (*i.e.*, 200 °C), prepared hybrid shows good antibacterial activity against *E. coli*.[92] Recently published articles concerning metal oxide are as follows: later, Rajaura *et al.*, 2017 adapted a simple and facile one-pot chemical synthesis of zinc oxide nanoparticles (ZnO NPs) on reduced graphene oxide (rGO) and studied its antimicrobial activity by disk diffusion test where the result indicated that rGO–ZnO nanocomposites have higher antibacterial activity than rGO alone.[93] Similarly, Archana *et al.*, 2018 prepared GO–ZnO nanocomposites by a simple hydrothermal method where antimicrobial studies revealed that GO–ZnO effectively inhibits the growth in an appreciable amount. The result accredited to the antimicrobial mechanism of GO–ZnO depends on the cell membrane disruption by the release of the Zn^{2+} ions generating ROS and killing the bacterial cell.[85] Ahmadi Shadmehri *et al.*, 2019 reported a green technique to coat zinc on the surface of graphene using heat-induced oxidation where prepared graphene–ZnO shows good antibacterial properties compared to control ZnO nanoparticles and graphene.[19]

Padhi *et al.*, 2017 reported the green synthesis of Fe_3O_4/rGO nanocomposites using leaf extract of Averrhoa carambola and showed that the synthesized nanocomposite has good antimicrobial activity against *S. aureus* (MTCC-737), *B. subtilis* (MTCC-736), and *E. coli* (MTCC-443) as compared to GO and standard antibiotic (gentamycin).[94] N. Mahmoodabadi *et al.*, 2018 prepared graphene–Fe_3O_4 nanocomposites by a facile

solvothermal method and studied their antibacterial and cytotoxic properties where obtained results revealed that nanocomposites show good antibacterial activity and lower cytotoxicity compared to Fe_3O_4 NPs and GO sheets, separately.[87] Chang *et al.*, 2015 report the synthesis of Magnetic graphene oxide–TiO_2 nanocomposites, and the results of the synthesized nanocomposite indicate complete inactivation of *E. coli* within 30 min under solar irradiation.[95] A. Raja *et al.*, 2019 synthesized rGO–TiO_2 nanocomposites by a simple hydrothermal method and reported that the synthesized nanocomposites possess higher antibacterial activity against Gram-positive bacteria (*S. aureus*) than Gram-negative bacteria (*E. coli*).[96] Wanag *et al.*, 2018 reported the preparation of TiO_2/rGO using a hydrothermal method *i.e.*, under elevated pressure at 180 °C and heated at 100 °C and observed that the antimicrobial activity of hybrid nanocomposite was improved compared to unmodified TiO_2.[97] Stan *et al.*, 2018 designed antimicrobial cotton fabrics using hydrothermally synthesized nitrogen and iron co-doped TiO_2 nanoparticles decorated rGO nanocomposite and observed that the designed textile has self-cleaning characteristics.[98] Ibrahim *et al.*, 2023 GTCEnc nanocomposites containing GO–TiO_2–chitosan–escin nanostructures were prepared and characterized using diffraction, microscopy, and spectroscopy. Antimicrobial activity of GTCEnc was also investigated. Its unique nanocomposite properties and antibacterial and anticancer activity *in vitro* makes GTCEnc an effective anticancer and antibacterial agent.[99]

Another recently studied metal oxide is copper *i.e.*, CuO and Cu_2O. Z. Yang *et al.*, 2019 reported the synthesis of stable rGO–Cu_2O nanocomposites at room temperature using ascorbic acid in the presence of polyethylene glycol (PEG) and sodium hydroxide. They showed that prepared rGO–Cu_2O nanocomposites possess better antibacterial activities against *E. coli* and *S. aureus* as compared with Cu_2O.[37] Rajapaksha *et al.*, 2019 reported that GO–CuO nanocomposite suggestively inhibits the growth of both *E. coli* and S. Typhimurium bacteria better than CuONPs.[84] Similarly, Alayande *et al.*, 2020 reported that hydrothermally synthesized rGO–CuO nanocomposite films show good antibacterial activity with complete bacterial inactivation compared to CuO alone.[100] Chen *et al.*, 2019 reported a green and economical route of rGO/Cu_2O synthesis using cellulose acetate/$CuCl_2$ for highly efficient antibacterial applications.[50]

It should be noted that the bactericidal mechanism of metal and metal oxide nanocomposites is almost the same except for the fact that metal oxides do not affect ribosomal assembly as shown in Fig. 4.[88,89] Also, it has been reported that metal oxide possesses better bactericidal properties as compared with metal nanoparticles whether deposited onto GO or not.[90] This has also been suggested by Mahmoodi *et al.*, 2018 where it is stated that in the case of metal oxide NPs, the metal is already in the oxidation condition with a high ion release rate resulting in particle dissolution, while for the metal nanoparticles, the formation of an oxide layer is required first which slow down the ion release rate.[91]

5. Graphene-based multicomponent nanocomposites

Monometallic, bimetallic, and trimetallic nanocomposites have remarkable antibacterial activity. Trimetallic nanoparticles exhibited excellent antibacterial properties as compared to bimetallic or single metallic nanoparticles respectively. However, metallic nanoparticles suffer high agglomeration, and as mentioned earlier substrates like GO/rGO can help them overcome this problem. While improving their antibacterial and anticancer properties.

5.1. Graphene-loaded bimetallic nanocomposites

The study Ma *et al.*, 2015 reported the synthesis of Fe_3O_4–TiO_2 nanocomposite in a solvothermal reaction and observed highly efficient antibacterial activity toward *E. coli* and *S. aureus* than pure Fe_3O_4 and TNS (titanium nanosheet).[102] Ayyaz Ahmad *et al.*, 2016 reported the *in situ* synthesis of graphene-supported Fe–Ag bimetallic nanoparticles showing excellent antibacterial activity against *B. subtilis*, *E. coli*, and *S. aureus*.[103] As reported by Zhang *et al.*, 2016 the synthesis of Pt/Ag bimetallic nanoparticles decorated on the porous reduced graphene oxide (rGO) nanosheets by facial and eco-friendly approach also achieves better antibacterial activity.[104] Perdikaki *et al.*, 2016 reported the synthesis of Ag/Cu bimetallic nanoparticles (NPs) *via in situ* grown on the surface of graphene, which was produced by chemical vapour deposition using ferrocene as a precursor and further functionalized to introduce oxygen-containing surface groups However, the bimetallic Ag/CuNP–graphene hybrids exhibit higher activity as compared to that of all other materials tested.[105] Arunima Rajan *et al.*, 2019 reported the synthesis of ZnO/Fe_3O_4/rGO nanocomposites by a simple method. However, these synthesized particles exhibited varying antibacterial activity against *S. aureus* and *E. coli*. The nanocomposite exhibits a better cidal effect on *E. coli* when compared to *S. aureus* when treated with 1 mg ml^{-1} concentration. All the nanoparticles have antibacterial properties.[106] Mallikarjuna *et al.*, 2021 performed studied the biosynthesis of Pd–Ag-decorated reduced graphene oxide (rGO) nanostructures using a green chemical approach with stevia extract for hydrogen generation under light irradiation and the time-dependent antibacterial activity of Pd/Ag/rGO NPs. Therefore, inactivation appears at 75% and 96% after 90 min and 150 min of incubation respectively, which suggests very fast action.[107]

5.2. Graphene-loaded trimetallic nanocomposites

Graphene-based trimetallic nanocomposites have been synthesized in recent years, however, used for some other applications such as DMAB dehydrocoupling catalyst[108] biomarkers detection,[109] photocatalytic remediation.[110] Zhang *et al.*, 2016 showed, that the rapid release of silver ions from Pt/Ag/rGO, enhanced antimicrobial activity which can be attributed due to galvanic action and synergistic cooperation.[104] Betul Sen *et al.*, 2018 synthesized PdRuNi@GO shows excellent performance

for the dehydrogenation of DMAB due to the synergistic effect of three metals and graphene.[108] Sharma *et al.*, 2019 described the synthesis of Fe/La/Zn@GO trimetallic nanoparticles using the micro-emulsion method which is effectively used for environmental remediation and antimicrobial studies.[110]

Recently reported graphene-based multimetallic nanocomposites along with their synthesis methods, structural arrangements, and applications are shown in Table 3.

6. Synthesis of surface-functionalized graphene-based metal/metal oxide

Surface functionalized graphene-based metal/metal oxide nanocomposites also show better antibacterial activity and less toxicity as compared to other nanomaterials. Surface functionalized graphene-based metal/metal oxide nanocomposites possess potential antibacterial activity against both types of bacterial strains and biocompatible polymers such as chitosan (Cs), polyvinyl alcohol (PVA), and polylactic acid (PLA) have superior antibacterial properties.[113] Graphene-based metal nanocomposites have poor solubility because they tend to aggregate, which limits their antibacterial effect, and this limitation is overcome by adding a polymer matrix into the graphene-based nanocomposites to form stable and biocompatible nanocomposites. Moreover, the performance of polymer-based nanocomposites has received important attention for biomedical applications, especially antibacterials.[7] Some examples of the recently published reported literature are as follows.

Weirui Xu, *et al.*, 2016 reported the self-assembly strategy to synthesize GO@CS@TiO_2 nanocomposites and observed that their antibacterial activity was concentration-dependent against *B. subtilis* and *A. niger.*[114] Bandi *et al.*, 2019 synthesized graphene/chitosan-functionalized iron oxide nanocomposites by chemical co-precipitation method and studied their antibacterial activity and anti-cancer activity which shows that the surface functionalization of iron oxide nanoparticles with chitosan enhanced the bioactivity of iron oxide nanoparticles loaded onto GO.[115] Xu, *et al.*, 2016 compared the concentration-dependent antibacterial activity of GO@CS@TiO_2 against *B. subtilis* and *A. niger*. This study suggested that the GO@CS@TiO_2 nanocomposites have a superior antibacterial effect for *B. subtilis* than for *A. niger*.[114] Liu *et al.*, 2018 demonstrated that the PVDF–2% Ag–GO composite showed excellent antibacterial activity as compared to the control to be tested. Furthermore, PVDF–1% GO–2% Ag membrane with higher Ag loading exhibits the best resistance against *E. coli* due to the release of silver ions from AgNPs, thereby enhancing the amount of cellular uptake of particle-associated Ag^+ ion. The incorporation of 1 wt% GO into PVDF/Ag further enhances their bactericidal activity, especially against *E. coli*, and the best performance is attained at 2 wt% Ag loading.[116] Suresh Bandi *et al.*, 2019 studied their antibacterial activity and anticancerous activity which shows that the surface functionalization of iron oxide nanoparticles with chitosan enhanced the bioactivity of iron oxide nanoparticles loaded onto GO.[115] Jitendra Kumar Sahoo *et al.*, 2020 reported preparing (GO–Fe_3O_4–APTES) by co-precipitation method and their antibacterial properties against Gram-negative (*E. coli*) and Gram-positive (*B. subtilis*) bacterial strains. GO/NiO/alginate nanocomposite are fabricated through the hydrothermal procedure. These surface functionalization nanocomposites have wide applications such as adsorption, degradation, chemical sensors, *etc.*[117] Bacali *et al.*, 2020 compared the antimicrobial activity of PMMA, PMMA + 1% G–AgNp, PMMA + 2% G–AgNp. Moreover, the PMMA + 2% G–AgNp samples showed better antibacterial activity against *S. aureus*, *S. mutants*, and *E. coli* than PMMA + 1% G–AgNp, PMMA.[118] Khawaja *et al.*, 2018 demonstrated a chitosan/GO/AgNPs by directly dispersing GO and AgNPs into the chitosan solution against the several bacterial strains *S. aureus*, *S. mutans*, *E. coli*, *K. pneumonia*, *P. aeruginosa*, and *S. typhi* shows significant antibacterial effect due to synergetic effects.[119] Fauzi *et al.*, 2021 reported that the PLA/GO/ZnO film with 0.2 wt% of GO–ZnO can kill 83% and 52% of *S. aureus* and *E. coli*, respectively, without light exposure. Under light exposure, the killing efficiency increases up to 99% and 98% toward *S. aureus* and *E. coli*, respectively.[113] Xiao-Jun Shen *et al.*, 2019 prepared Ag@GO/polylactic acid nanocomposite by *in situ* polymerization process and enhanced their antibacterial activity (antibacterial rate was up to 99%) by increasing the Ag@GO content due to the synergistic effect.[120]

7. Discussion

The antibacterial effect of different types of nanocomposites relies upon their chemical as well as physical aspects including size, shape, surface state, crystal structure, dispensability, attached moiety, *etc.*[121,122] Cheon *et al.* 2019 reported the antibacterial effects of nanocomposites, such as cellular uptake by cells, cellular activation, as well as intercellular distribution.[123] GO and its derivatives also have antibacterial activity against pathogens. Jiajun Qiu *et al.*, 2018 suggested that various factors play a significant role in the antibacterial activity of graphene.[124] Combining graphene and its form with metal/metal oxide shows a potential inhibitory effect due to the synergistic effect of metal ions, and graphene.[125] This mini-review provides advances in the development of metal/metal oxide nanoparticles and graphene oxide-based nanocomposites, a better understanding of the antibacterial properties of these materials, and their possibilities for commercial applications.

Several recent experimental outcomes have suggested that the characteristics of graphene-based metal/metal oxide nanocomposite (such as particle size, shape, and surface functionality) play a significant role in the mechanisms including oxidative stress, nano-knives, wrapping/trapping, cellular damage, protein damage, and so on. The antimicrobial effectiveness of graphene materials is largely determined by their lateral size, which may be modified according to the synthetic method or post-treatment.[29,75] Research has shown that size,

Table 3 List of reported literature on graphene-based multimetallic nanocomposites

S. no	Sample	Synthesis method		Structural arrangement	Antibacterial mechanism	Application	Ref.
Bi-metallic nanocomposites							
1	Ag–$CoFe_2O_4$–GO	Solvothermal reaction method	Two-step synthesis method	Mixed alloy doped with core–shell nanocomposites	Better dispersity helped it to have more contact with bacteria	Enhancing antibacterial efficiency	102
2	GO/Fe–Ag	*In situ* synthesis method	One-step synthesis method	Mixed alloy	Bimetallic-loaded graphene shows excellent antibacterial activity as compared to bare-bimetallic (because of higher ROS generation)	Enhancing antibacterial activity	103
3	Ag/CuNP–graphene	Chemical vapor deposition of further oxides using ferrocene	Two-step synthesis method	Mixed alloy	Synergistic effect of two different metals and graphene	Excellent antibacterial application	105
4	Pt/Ag@rGO	Facial and eco-friendly approach	Two-step synthesis method		Rapidly release of metal ions	Better antibacterial activity	104
5	ZnO/Fe_3O_4/rGO	Hydrothermal + coprecipitation method	Two-step synthesis method	Mixed alloy	—	Effective antibacterial activity	106
6	Pd–Ag/rGO	Green chemical method	One-step synthesis method	Mixed alloy	—	Antibacterial activity	107
7	SGO–ZnO–Ag	Hydrothermal method combined with polyol-reduction process	Two-step synthesis method	Core–shell nanoparticles	Faster as compared to mono metallic nanocomposites	Photodegradation and disinfection	111
Tri-metallic nanocomposites							
8	PdRuNi@GO	Microwave-assisted polyol method.	One-step synthesis method	Mixed alloy	—	Biocatalyst	108
9	Pd@Au@Pt/rGO	Electrodeposition	Two-step synthesis method	Mixed alloy	—	Biomarkers detection	109
10	La/Co/Ni@GO	Microwave reduction method	One-step synthesis method	Mixed alloy	Efficient charge transfers and reduced electron–hole recombination ability	Photocatalytic degradation	112
11	Fe/La/Zn@GO	Micro-emulsion method	Two-step synthesis method	Mixed alloy	—	Environmental remediation and antimicrobial studies.	110

shape, surface area, charge density, electronic state, and optical characteristics significantly evaluate the antimicrobial potency of graphene-based metal/metal oxide nanoparticles mentioned in Table 4.

7.1. Effect of the morphology of the nanocomposites

Graphene-based nanomaterials have been used as antibacterial agents due to their remarkable physicochemical properties such as large specific surface area, high electrophoretic mobility, high thermal conductivity, high mechanical strength, and biocompatibility.[126] The antibacterial potential of graphene-based metal/metal oxide nanocomposites depends on the shape and size of both deposited metal/metal oxide nanoparticles and GO/rGO sheets.[45,127] It is reported that morphologically different metal nanoparticles (such as spherical, cubical, triangular, oval, hexagonal, rod-shaped, and helical-shaped) have different antibacterial properties depending on their difference in the rate of metal ion release according to the shape and size of the metallic nanoparticle. While in the case of graphene and its derivative Zou *et al*., 2016 demonstrated that the smooth-top-side graphene film has efficient bactericidal activity against both round-shape *S. aureus* and *P. aeruginosa*, while the rough-bottom-side graphene film effectively kills rod-shaped *P. aeruginosa*. Currently, nanomaterials are attracting a lot of attention for their remarkable antibacterial properties and low cytotoxicity.[45]

The size and surface area of the nanoparticles affects their interaction with the biological system in which it is applied. The decrease in size of the nanomaterial increases their surface area exponentially relative to volume thereby making their surface more reactive. It was reported that mechanisms like cellular uptake and endocytosis of nanoparticles are affected by the particle size of nanocomposites.[128–130] On the other hand, the size of the metal nanoparticles also plays an important role in the generation of Reactive oxygen radicles, the smaller the size of the nanoparticle greater the production of ROS which leads to damaged DNA. It also has been reported that a decrease in size will increase the surface area which increases oxidation and DNA damaging ability at a much higher rate as compared to large particles with the same doses.[75,127,130–132] Therefore, the effect of size is also crucial in their antibacterial activity *i.e.*, antibacterial activity is greater for samples with a smaller size and higher oxidation ability. Moreover, lateral size is a vital element for the antimicrobial activity of graphene-derived materials. However, the antimicrobial effects are stronger in association with larger-sized GO sheets than with smaller sheets because the larger the lateral size of the graphene-derived materials, the stronger the adsorption ability, which is attributed to the higher surface energies. GMs prepared by the redox method always contain defects, regardless of the synthesis method. Meanwhile, more defects are associated with GMs with a smaller lateral size. Antimicrobial activity against *E. coli* is stronger with smaller GO nanosheets because of their greater defects. Additionally, GO-decorated with smaller metal nanoparticles demonstrated greater antibacterial activity than GO-decorated with large metal nanoparticles. The GO/metal nanocomposites have better antibacterial efficacy compared to metal nanoparticles alone.[7,75,127] Díez-Pascual *et al*., 2020 illustrated that the smaller size of nanoparticles can easily penetrate the bacterial cells and disrupt the bacterial cell by generating ROS followed by cell death.[6] Le Thanh Trinh *et al*., 2018 demonstrated that ZnO nanoparticles with a mean size of 14–26 nm was synthesized and randomly decorated on the surfaces and edges of GO sheets. Additionally, the antimicrobial properties of GO and ZnO/GO were tested against *E. coli.* The result finds that the antibacterial activity of the ZnO/GO nanocomposite was higher than that of GO against *E. coli*.[133] Considering all these factors, it can be stated that the morphology of graphene-based metal/metal oxide nanocomposites strongly determines their antibacterial properties, however, loaded concentrations of metal/metal oxide nanoparticles and/or whole nanocomposite may have differences in activity against different microorganisms.

7.2. The number of layers on graphene affects the antibacterial activity

Mohammed *et al*., 2020 suggest that if the graphene layers increase, their antimicrobial activity decreases: increased graphene layers result in a weakened "nano knife" effect, decreased dispensability, and improved aggregation tendency, leading to decreased contact between microorganisms and graphene materials. Graphene materials exhibit antimicrobial activity *i.e.*, both the surfaces and edges play a role in antimicrobial activity on the basal plane.[29,75] It may be noted that the antibacterial activity of graphene-based metal/metal oxide nanocomposite is also dependent on the number of graphene layers.

7.3. Effect of surface charge of nanocomposites

It has been reported that surface charge on nanocomposites affects their various behaviours like colloidal behaviours, selective adsorption, and transmembrane permeability.[134] Surface charge determines the colloidal property of the nanocomposites thereby influencing the organism's response. The surface charge of metal nanoparticles influences the interactions of nanoparticles with biological systems. However, positively charged nanoparticles show a higher cellular uptake than negatively and neutrally charged nanoparticles, owing to their enhanced opsonisation by plasma proteins.[122,130] Abbaszadegan *et al*., 2015 observed that the antibacterial activity of metallic nanoparticles is affected by surface charge. As a result of testing, the positively charged metallic nanoparticles have proven most effective against microorganisms, while the negatively charged and neutrally charged particles have proved the least effective.[122,135] The surfaces of GO and RGO have several functional groups such as phenolic –OH, ketone, lactone, carboxyl, quinone, and epoxy, and are negatively charged over a very wide pH range, here, edge phenolic hydroxyl and carboxyl groups make more contributions to the negative charge than the basal-plane hydroxyl and epoxy groups.[75,127,136] Moreover, due to their synergistic effect, the graphene-based metal/metal oxide nanocomposites are more

Table 4 Explain the physical–chemical properties influencing graphene antibacterial activity

S. no	Material name	Size, nm	Shape	Methods	Bacteria name	Conc. μg mL^{-1}	Inhibitions %	Mechanism	Ref.
	GO	0.53 μm	Wrinkles	Plate count	*P. aeruginosa*	175	100%	Damaged the cell membranes and caused cell lysis	144
	rGO	3.40 μm	Crumpled		*E. coli*	100	88%		79
	GO–AgNPs	10–40	Spheres, triangles, pentagons, ellipses, and rods	Plate count	*E. coli*/*S. aureus*	10	100%	Releasing metal ions and generating ROS *via* contact with bacteria	126
	rGO–AgNPs	—	—		*E. coli*	40	100%		145
	rGO–TiO_2	72	Spherical	Agar diffusion method	*E. coli*/*S. aureus*	N/A	N/A		96
	rGO–ZnO	14–26	—		*E. coli*	3 × 103	100%		133
	GO–ZnO	22 ± 6	Spherical	Plate count	*E. coli*	500	100%		92
	rGO–Cu_2O	25	Square		*E. coli*/*S. aureus*	40	70/65%		37
	GO–Fe_3O_4	11.64	—	Plate count	*E. coli*	300	91.5%		146
	GO–Fe_2O_3	200–250	Sphere			100	97%		120
	GO–$MnFe_2O_4$	150	Sphere			100	82%		121
	Ag NPs/GO	93	Spherical	Plate count	*S. enteritidis*	200	61	ROS production, stronger oxidative stress	6
		80			*E. coli*		89		
		80			*S. epidermidis*		76		
		80			*S. aureus*.		81		
		80			C. albicans		78		
	Ag NPs/rGO	57	Quasi-spherical		*E. coli*	40	100	ROS production, bacterial cell disruption	
		12	Spherical		*E. coli*	20	100		
	Au–rGO	50	Triangular, spherical		*S. aureus*/*B. subtilis*	250	94	Penetrate the bacterial cells and cause cell damage	
		50	Triangular, spherical		*E. coli*/*P. aeruginosa*	250	50		
		50	Triangular, spherical		*E. coli*	10	99		
	Cu_2O–rGO	30	Cubic		*E. coli*	40	70	Cellular damage including protein and lipid oxidation	
					S. aureus	40	65		
	CuO–GO	80	Ellipsoidal		*E. coli*	3	90		
					S. Typhimurium	3	99		
	TiO_2–GO	30	Spherical		*E. coli*	180	100	Generating ROS *via* oxidative stress	
	ZnO–GO	150	Triangular, spherical		*E. coli*	6.5	100		
					P. aeruginosa	6.5	100		
					S. aureus	11.5	100		

effective against Gram-negative than Gram-positive bacteria. However, the antibacterial activity of Gram-negative bacteria can easily interact with the positively charged metal nanoparticles and negatively charged graphene sheets.[63]

7.4. Effect of agglomeration and dispersion

Metal nanoparticles tend to get agglomerated and their aggregation states also influence their antibacterial activity. However, size, surface charge, composition, and other factors affect the aggregation states of nanoparticles.[122,130,132] On the other hand, graphene sheet agglomeration tends to cause a reduced surface area and shape alteration, weakens their dispersibility and adsorption capacity, which alters blade efficacy and consequently reduces their interaction with the microorganism. The properties of graphene differ in different forms, with GO dispersion showing the strongest antimicrobial activity, followed by rGO, graphite (Gt), and graphene oxide.[38,75] As a result, these findings were interpreted as different dispersion combinations of the mentioned nanomaterial, for example, proper dispersion of GO leads to thin sheets of this nanomaterial that are capable of easily wrapping bacteria, whereas when rGO is not fully exfoliated, aggregates form and its antimicrobial effect is reduced. Meanwhile, Akhavan *et al.* 2011 show that rGO results in greater bacterial inactivation than GO. This may be attributed to *E. coli* trapping and gradual wrapping of bacteria during the rGO aggregate formation. It is reported that GO/rGO sheets restrict the agglomeration of metal nanoparticles, and increase their stability as well as compatibility, similarly, the deposition of metal nanoparticles controls the aggregation of fully exfoliated graphene sheets. Hence, less agglomeration and good dispersion are important to obtain good antibacterial activity.[75,137]

7.5. Antibacterial effect of surface functionalization of graphene-based materials

The graphene family exhibits antibacterial properties, and tends to aggregate due to strong inter-plane interactions, which limit their surface area and modes of action. Surface functionalization plays a significant role in preventing agglomeration and, consequently, influencing their antimicrobial activities.[7,75] As a result, graphene has been functionalized and surface-modified using metal ions, oxides, sulfides, polymers, antibiotics, and enzymes to reduce aggregation and enhance antibacterial activity. Surface functionalized graphene-based metal/metal oxide nanocomposites possess potential antibacterial activity against both types of bacterial strains while biocompatible polymers such as chitosan (Cs), polyvinyl alcohol (PVA), and polylactic acid (PLA) have superior antibacterial properties.[7,113] Several polymer-based graphene nanocomposites have recently been proposed to enhance their antibacterial activity.

7.6. Antibacterial effect of graphene-based multicomponent nanocomposites

Recent achievements in the nanotechnology of metal oxides include the elaboration of nanostructured oxides consisting of two or more metallic components and the mechanism behind this study is not clear yet and still needs to be explored. In this respect, the consideration of polymetallic oxides for biological applications becomes greater since these can provide synergetic effects and unify the best physicochemical properties of their components. Some multi-metal oxide nanoparticles have shown a lesser tendency to aggregate in biological solutions and fluids, resulting in increased antibacterial activity and being highly biocompatible compared to their components. Multicomponent nanocomposites as potential antimicrobial agents owing to the beneficial synergistic effects of their components. Several graphene-based multicomponent nanocomposites have been prepared by incorporating different nanoparticles with graphene or its derivatives, which enhance antibacterial activity due to synergistic effects.[6] Along with graphene, trimetallic nanoparticles exhibited excellent antibacterial properties as compared to bimetallic or single metallic nanoparticles due to the synergistic effect of three forms of metal ions and graphene, respectively. Pascual *et al.*, 2020 reported some recent research in their study like magnetic GO–$MnFe_2O_4$ hybrids at a concentration of 100 μg mL^{-1} led to 82% inhibition on *E. coli* after only 2 h of contact. In a hybrid with polyethylenimine-wrapped Ag nanoparticles and Fe_2O_3, 100% inhibition of *E. coli* was achieved at only 0.1 μg mL^{-1}. A multicomposite of GO, $CoFe_2O_4$, and Ag nanoparticles was also developed for the disinfection of water from *E. coli* and *S. aureus*, leading to almost complete inhibition at 12 μg mL^{-1}. The study suggests that the multicomponent GO–Ag–TiO_2–ZnO nanocomposite has better antibacterial efficacy against both Gram-positive and Gram-negative bacteria, while it was found to be the most effective against Gram-negative bacteria it may be due to surface charge and increased rate of metal ion release. Based on the synergistic effect of the different components, the hybrid was found to have improved activity: Ag nanoparticles directly damaged the cell membrane, TiO_2 and ZnO generated ROS, the GO sharp edges contacted bacteria directly, and nanoparticles accumulated in the cytoplasm.[6,7,29,75]

8. Conclusion and future prospects

The development of alternative antibacterial agents is considered essential for reducing the global burden of infectious diseases. Researchers have developed novel nanomaterials, and their antibacterial activity has been tested on a variety of microorganisms. The antibacterial activity of nanomaterials varies with the type of nanoparticles, their size, shape, composition, and other factors. Recently reported graphene-based nanocomposites show excellent antibacterial properties and are biocompatible with eukaryotic cells. Metal nanoparticles (especially metal oxide nanoparticles) have great antimicrobial effects but their application in the health sector is restricted due to their toxicity at higher concentrations. There is evidence that graphene derivatives can enhance metal or metal oxide nanostructures' antibacterial activities. Owing to the synergis-

tic effects, graphene-based nanocomposites exhibit improved performance compared to nanoparticles and graphene taken separately. Nanocomposites of graphene and biomacromolecules have been found to possess low levels of cytotoxicity and exceptional biocompatibility, positioning them as valuable assets in the realms of medical diagnostics and disease management.[138] This review compares the antimicrobial efficacy of graphene-based nanocomposites by examining the effects of physicochemical properties on antimicrobial activity. Recent reports have shown utilization of graphene based nanocomposites in healthcare industry such as protective equipment (like face masks and bandages), and to prevent the development of resistance to antibiotics, water purification systems, wound healing materials, antimicrobial coatings for packaging and fabrics, surgical devices, implants (nasal, dental and other).[7,139,140] Similarly, the graphene family of nanomaterials (GFNs) can be used in other clinical practices such as tissue repair and tissue regeneration (such as bone, nerve, oral, myocardial tissues) drug delivery systems, nerve scaffolds, and recording electrodes for conditions affecting the nervous system, wound care, orthopaedic surgery, cell therapeutics, cancer immunotherapy, photo-thermal therapy and photodynamic therapy, gene/drug administration, and anti-infection.[138,141–143] These advancements underscore the bright prospects of graphene based materials in reshaping the landscape of disease treatment and clinical practices. Therefore, in order to successfully employ such nanomaterials in other advanced practical applications, in-depth studies of these nanomaterials are required.

Abbreviations

Ag	Silver
AMR	Antimicrobial resistance
ATP	Adenosine triphosphate
Au	Gold
BNPs	Bimetallic NPs
Cu	Copper
CuO	Copper oxide
DNA	Deoxyribonucleic acid
Fe	Iron
Fe_3O_4	Iron oxide
FTIR	Fourier transforms infrared spectroscopy
GO	Graphene oxide
Gt	Graphite
MDR	Multidrug resistance
Ni	Nickel
NPs	Nanoparticles
rGO	Reduced graphene oxide
RNA	Ribonucleic acid
ROS	Reactive oxygen species
Ti	Titanium
TNPs	Trimetallic NPs
Ti_2O	Titanium oxide
UV	UV-vis spectroscopy
WHO	World Health Organization
XRD	X-ray diffraction patterns
Zn	Zinc
ZnO	Zinc oxide

Ethical statement

Not applicable.

Author contributions

AS and AG planned and designed the research work. AG, HD, AS (Awantika Singh), ST, and NT Writing – original draft preparation. AS and HRK reviewing, and final editing the manuscript. HRK and AS read and approved the final manuscript.

Data availability

All the required data presented in this study is already included in the review.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose the work reports in this paper.

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