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Review

Recent advances and mechanism of antimicrobial efficacy of graphene-based materials: a review

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ABSTRACT

Graphene-based materials have undergone substantial investigation in recent years owing to their wide array of physicochemical characteristics. Employment of these materials in the current state, where infectious illnesses caused by microbes have severely damaged human life, has found widespread application in combating fatal infectious diseases. These materials interact with the physicochemical characteristics of the microbial cell and alter or damage them. The current review is dedicated to molecular mechanisms underlying the antimicrobial property of graphene-based materials. Various physical and chemical mechanisms leading to cell membrane stress, mechanical wrapping, photo-thermal ablation as well as oxidative stress exerting antimicrobial effect have also been thoroughly discussed. Furthermore, an overview of the interactions of these materials with membrane lipids, proteins, and nucleic acids has been provided. A thorough understanding of discussed mechanisms and interactions is essential to develop extremely effective antimicrobial nanomaterial for application as an antimicrobial agent.

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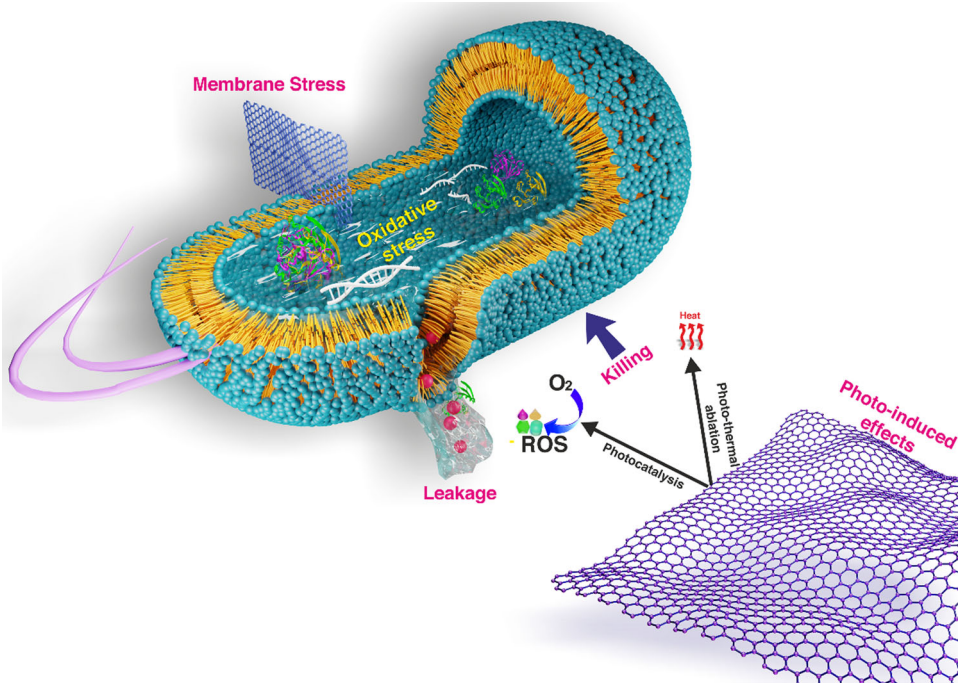
GRAPHICAL ABSTRACT



Introduction

Microbes are omnipresent; some are beneficial, while others cause severe life-threatening conditions, manifesting themselves through diseases and contamination of various products and surfaces. These disease-causing microbes are often termed pathogens and can potentially infest humans as well as plants, water, and inanimate items that are either directly or indirectly used in daily life, such as medical devices, food packing, and storage accessories [1]. The sharp rise in infectious diseases caused by bacteria forms one of the major menaces to human health and a source of suffering for millions of people worldwide [2–4]. Nosocomial infections and biofilm-forming competencies of some of the pathogenic microbes have also led to dreadful health conditions among patients and healthcare professionals [5]. Moreover, microbial contamination of potable water poses a significant quality issue globally and increases the risk to human health [6]. Several microbes cause economic losses to certain industries such as textile, water treatment, and food packaging [7]. In both industrial and medical applications, bacterial adhesion and the consequent formation of biofilm on surfaces of synthetic materials exerts severe threat to human health and causes economic losses [8, 9].

Presently, there are myriad antimicrobial agents, namely antibiotics [10], quaternary ammonium compounds, various metal/metal oxide nanoparticles, and antimicrobial peptides [11], each with its respective benefits and drawbacks. The excessive misuse of these antibiotics and undue prescription has led to the evolution of strains that are resistant to the prevailing antibiotics and thus developing antibiotic resistance among the microbes [12]. Antimicrobial resistance (AMR) occurs over time when these disease-causing microbes do not show sensitivity to the antimicrobial agent. Recently, the WHO declared AMR as one of the top 10 public health and development threats globally [13]. The WHO predicts that by 2050, up to 10 million people would die from antibiotic resistance owing to this pandemic, so there is an urgent need to find out some novel and impactful antimicrobial agents [7, 14].

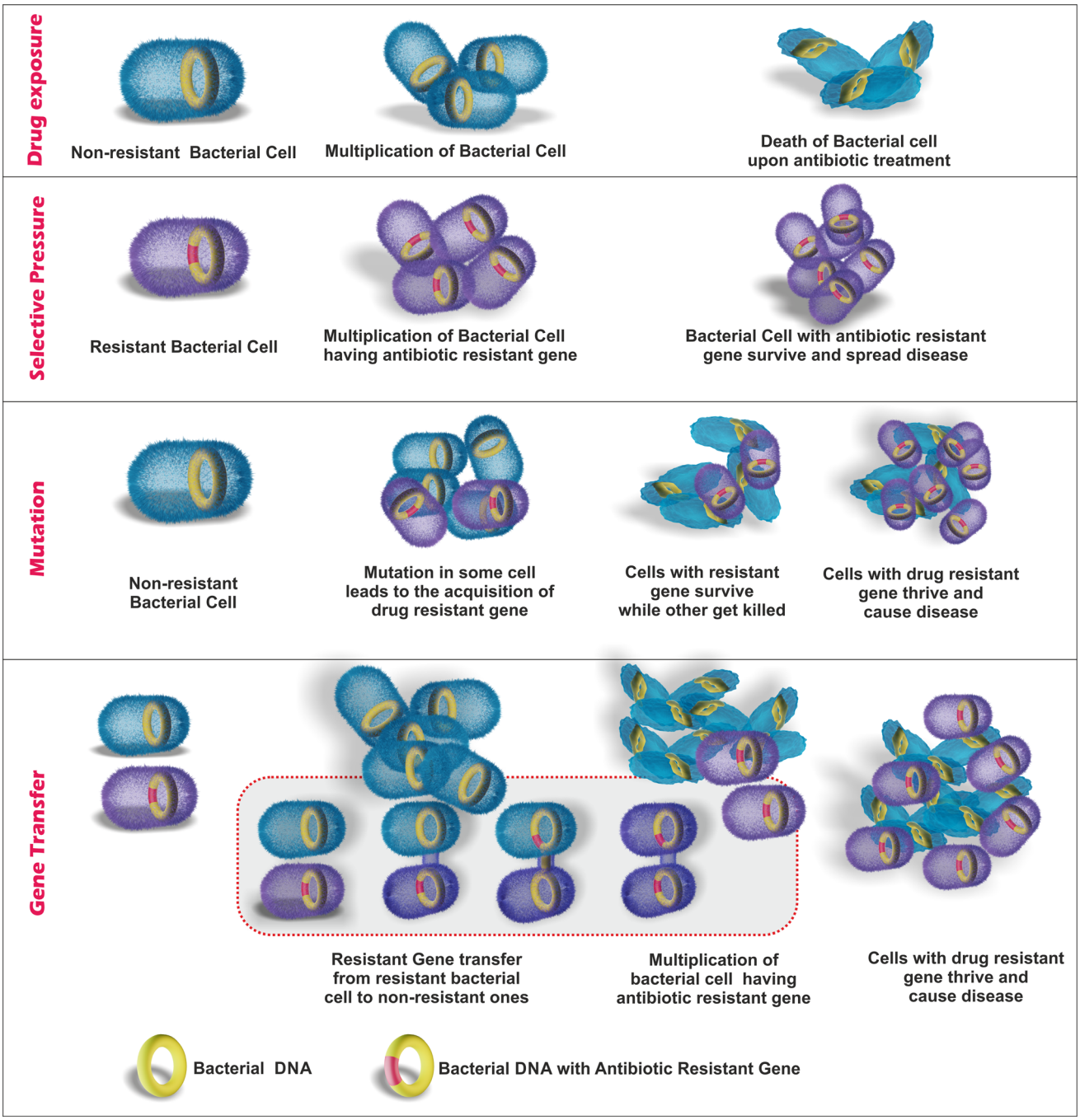


Figure 1 Mechanism of antibiotic resistance in bacterial cells.

Genetic mutation, recombination, and horizontal gene transfer by plasmids and transposons are some of the reasons for developing antibiotic resistance as illustrated in Fig. 1. Briefly, it can be explained as, non-resistant bacterial strains during antimicrobial treatment getting killed due to the effect of the antibiotic, whereas some populations of bacterial species are inherently resistant to the antibacterial agent used and thus carry the antibiotic-resistant gene (as illustrated with red colour) integrated within its genome. A serious concern arises when non-resistant bacterial strains acquire antibiotic resistance. In this case, the bacterial population which was previously susceptible to a particular antibiotic develops

resistance to that antibiotic agent by acquiring the antibiotic resistance gene in their genome. These cells with acquired antibiotic resistance genes multiply and spread the gene to its progeny cells, thereby spreading the AMR among the bacterial population [15]. Such cells form a major issue of concern as they develop resistance against the newly formed drugs used in antimicrobial therapy. Furthermore, bacterial cells may advance to resistance form by acquiring resistance to an antimicrobial agent by developing new mutations or by the acquisition of the resistance gene by horizontal gene transfer including conjugation, transformation, and transduction. Both gram-positive (G + ve) and gram-negative (G − ve) bacteria have evolved into multi-drug resistant forms and are among the principal cause of human infections worldwide [16]. Methicillin-resistant *Staphylococcus epidermidis* (MRSE) and methicillin-resistant *Staphylococcus aureus* (MRSA) are the two most clinically significant G +ve organisms' resistant to several drugs [17, 18]. Therefore, the AMR acquired by the pathogenic microbes against existing antimicrobial agents necessitated the invention of a novel class of antimicrobial agents.

Nanomaterials in the last two decades have shown tremendous potential to eliminate traditional antimicrobial agents [19, 20]. These materials are unique due to the nonpareil superlatives associated with their properties, such as their high aspect ratio, redox potential, and nano-dimensions, which differentiates them from traditional antimicrobial agents [21, 22]. One of the most studied systems is those in which two-dimensional allotropes of carbon and their derivatives such as monolayer graphene (G), graphene oxide (GO), reduced graphene oxide (rGO), and functionalized graphene oxide (-f-GO) are employed to unveil their antimicrobial potential. The 2D analogues of G provide an excellent opportunity to revamp their structures and extend the scope of their applications [23–26]. Several nano-analogues of these materials can be developed by various chemical modification methods (functionalization, doping, stacking, etc.), which include covalent as well as non-covalent transformations [27–29]. The presence of oxidative functional moieties on the surface of these nanomaterials culminates in their use as an effective antimicrobial agent [21, 30]. This has led to the conduction of vast research to evaluate mechanistic aspects of their antimicrobial action.

The efficient antibacterial activity of graphene-based nanomaterials (GBNs) is influenced by several physicochemical characteristics including G size, layer count, alignment, surface modification, agglomeration, dispersion of G materials, and structure of microbial species. These nanobiofactors affect the interaction of the microbial species with the GBNs and hence influence their antimicrobial activity. The size of the G material plays a crucial role in determining its antimicrobial activity [31]. Their size affects the adsorption, and dispersion, which affects interaction with the microbial species and hence the observed antimicrobial activity [32, 33]. Large-sized G has strong adsorption potential owing to greater surface energy which leads to the adsorption of the large number of microbial cells on the surface and hence more bacterial cells to be killed [34]. Furthermore, GO with reduced size displayed strong antibacterial activity in coatings [35, 36]. This was predominantly due to the higher number of defects in small-sized sheets that further leads to oxidative-stress-mediated cell death. All these nanobiofactors are certainly a related area of the present review, but the study is more focused on the mechanism and does not delve into the factors responsible for the antimicrobial effectiveness of G and based material. In this context, several mechanisms have been proposed for their antimicrobial action insinuating the role of functional moieties of G in perturbing the key cellular processes. The presence of functional groups induces extreme oxidative stress in microbes via various redox processes upon interaction. The stress induced within the cell results in the oxidation of macromolecules such as DNA, lipopolysaccharide (LPS), lipids, proteins, etc., leading to the death of the microbial cell [37]. This leads to a multisystem failure in the cell affecting its key cellular processes involving replication, transcription of DNA, and translation of mRNA into the proteins [37, 38]. Moreover, denaturation of DNA, cell membrane damage, and leakage of cytoplasmic content collectively lead to the morbidity and mortality of the pathogen.

The present review article explores the fascinating science behind the antimicrobial potential of G and its derivatives along with the mechanistic overview of their antimicrobial action. It gives a detailed account of their mechanistic aspects and focuses on the role of surface modification in regulating their activities. This review succinctly analyses significant developments in recent years and summarizes the

current state of knowledge. It creates an understanding of nanomaterials with a special focus on their architecture and the decisive role they can play in delivering the new generation of antimicrobial agents. Exclusive fundamental discussion on redox processes, membrane damage, cellular stress, mechanical wrapping, and photo-thermal ablation gives a comprehensive overview to the reader. The review concludes with a discussion of various factors that play a key role in delivering effective antimicrobial action along with key challenges in making nano-antimicrobial agents a reality.

Nanomaterials in antimicrobial resistance

The biggest threat to human health is the sharp rise of antibiotic resistance among pathogenic microbes. The current COVID-19 pandemic has obscured AMR, which has been identified as one of the potential threats to the world economy and health in recent years. The COVID-19 pandemic, which is being caused by the SARS-COV-2 (severe acute respiratory syndrome coronavirus 2) virus, is currently affecting the whole world and is the second largest global health emergency since the Spanish flu pandemic at the turn of the twentieth century. Various steps have been taken to minimalize the transmission efficiency of the disease. Amid the present pandemic, AMR to frontline medicines may be deadly to microbial infections during routine procedures such as elective surgery and C sections. According to recent research, the (COVID-19) pandemic has resulted in a larger number of individuals being brought to hospitals for intensive antimicrobial treatment that may not be effective, thereby increasing the risk of antibiotic resistance over the world [39, 40]. A large number of studies outline that AMR has been increased post-antibiotic treatment of COVID-19-positive patients. Moreover, according to the WHO report, a total of 6,893,190 lives were lost as of 6 April 2023 due to the outbreak of the pandemic and AMR is presently projected to kill about 700,000 people, annually [41, 42]. Therefore, the development of novel antimicrobials is the need of the prevailing medical status such that life-threatening effects of AMR could be minimized.

Nanomaterials have emerged as a novel tool to combat lethal bacterial diseases [43]. They are appealing options for improving treatment effectiveness against recalcitrant MDR infections due to their unique physio-chemical characteristics. Further, they can adapt diverse antimicrobial mechanisms to overcome microbial infections, each of which we will be discussing in the upcoming sections. Further, these pathways are dependent on various nanobiofactors such as size, shape, functionalization of the nanomaterial as well as size, shape, and membrane characteristics of the microbial species. They have proven to be effective against planktonic as well as biofilm infections [30, 44, 45]. Different studies have been carried out to combat antimicrobial resistance among the resistant bacterial strains of public health relevance. Li and colleagues studied the effect of GO with varying functionalities on the antibacterial potential on the antimicrobial-resistant strains of G − ve *Escherichia coli* (*E. coli*) and G +ve *Lactobacillus crispatus (L. crispatus)* [46]. They demonstrated that the hydration of GO leads to the increased density of C radicals (·C) on the surface of GO. These GOs when come in contact with the bacterial cell surface lead to the induction of lipid peroxidation in the bacterial membrane without its uptake, which further leads to cytoplasmic efflux and bacterial cell death. Further, they provided valid proof of concept by depositing hydrated GO with a large density of ·C on its surface onto the surface of glass substrates as well as silicone catheters used in medical aid and showed that it effectively killed the antibiotic-resistant strain of *E. coli*. Zheng et al. fabricated a nanocomposite consisting of lanthanum hydroxide ($La(OH)_3$) and GO, La–GO to explore its antimicrobial potential against antimicrobial-resistant strains of *E. coli*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *L. crispatus*, and *Staphylococcus aureus* (*S. aureus*) [47]. Six different nanocomposites R1to R6 with the varying mass ratios of $La(OH)_3$ to GO of 0.05, 0.1, 0.2, 0.5, 1, and 3, respectively, were evaluated for lethal effects on antibiotic-resistant bacterial cells. They showed that R2 displayed AMR-independent antibacterial effects in all tested strains. Moreover, long-term exposure of *E. coli* with $La(OH)_3$ at sub-minimum inhibitory concentration for 30 days does not induce secondary resistance in *E. coli*. Further, the displayed antimicrobial activity was due to proposed extracellular multi-target invasion which includes the association of $La(OH)_3$ with the bacterial cell membrane, dephosphorylation of membrane phospholipids, and lipid peroxidation leading to disruption of peptidoglycan layer in the bacterial cell wall. Aunkor

and coworkers examined the antibacterial activity of GO against the MDR G +ve as well as G − ve bacterial strains isolated from clinical samples and showed that it exerts concentration-dependent antibacterial activity against five G −ve bacterial strains, namely *E. coli*, *Klebsiella pneumoniae* (*K. pneumoniae*), *P. aeruginosa*, *Proteus mirabilis* (*P. mirabilis*), and *Serratia marcescens* (*S. marcescens*) and one G +ve bacterial *S. aureus* [48]. Moreover, GO-based biofilms can effectively suppress and eradicate bacterial biofilms which are frequently formed by these organisms, thus contaminating the surface of the medical equipment or implants. Therefore, G analogues find active potential significance in combatting the dreadful health conditions imposed by MDR bacterial strains.

Graphene and its derivatives as antimicrobials

GBNs include graphene oxide (GO) as well as reduced GO (rGO) and their functionalized analogues. These materials have been extensively explored for their antimicrobial activity owing to their unique physicochemical attributes. G exhibits numerous exceptional properties which make it one of the most studied wonder materials to date. Owing to these properties, G offers a fascinating material platform for the development of next-generation technologies in diverse fields, including biomedical, electronics, photonics, robotics, energy, agriculture, and so on.

Flat-monolayer of G presents a chemically inert as well as ultra-smooth surface inhibiting microbial adhesion thereby preventing microbial contamination. In addition, unique surface conjugation and the behaviour of electrons induce exceptional redox characteristics that can interfere with cellular processes [49]. However, there is a certain impediment that restricts its use in applications that call for a specific characteristic. Being a hydrophobic material, it has very low dispersibility in water and readily agglomerates due to pi-pi stacking. Chemical alterations are considered to be useful methods for modifying G's properties, yielding G derivatives that find their practicality in a variety of applications [37, 50, 51].

GO is obtained by the oxidation of G and has distorted sp^2 hybridization as well as sp^3 carbon atom [52]. The presence of oxidative functionalities such as carbonyl (C = O), carboxyl (–COOH), and epoxy (O) groups renders it with good water dispersibility and solubility [53, 54]. Thus, on the other hand, the chemically reduced form of GO (rGO) has a lesser number of oxygen-containing functional groups with a large number of electroactive sites [55]. It exhibits the properties of both pristine G as well as GO. Higher surface area and mechanical strength along with moderate dispersibility in water enhance their applicability against various pathogenic microbes. They manifest antimicrobial activity by destroying bacterial membranes, followed by lipid injuries and the release of cytoplasmic content [56]. They may also induce oxidative stress-mediated microbial killing by disrupting or destroying the vital microbial process, such as osmotic balance, respiration, energy transduction, and material transport, via oxidation owing to the presence of oxygen-containing functional groups. Larger sheets of GO and rGO cause cell entrapment, detaching the microbial cells from their external microenvironment and restricting access to nutrient and eventual growth inhibition [48]. Furthermore, there are various physicochemical characteristics including the density of functional groups, physical attributes of G material, type of microbial species, etc., that determine the antimicrobial property of GBNs [57].

Need for functional modification

G and its derived functional counterparts find extensive use in diverse fields, although unaltered G has certain limitations. It lacks targeted, delayed, and controlled release capabilities in vivo. GO, on the other hand, due to its charge-shielding property gravitates to agglomerate in the physical environment [58]. It has also been reported to possess a potent protein adsorption action which leads to quick identification, engulfment, and consumption by macrophages in living tissue, resulting in inflammation [59]. Altogether, these fallibility impedes the use of G derivatives in biomedicine. Increasing their water solubility and stability imparting them with advanced functionalities such as targeted, gradual as well as regulated release constitutes the vital strategy for improving and enhancing their biological uses.

With the rising interest among scientists, further untouched potentials of G and its derivatives were

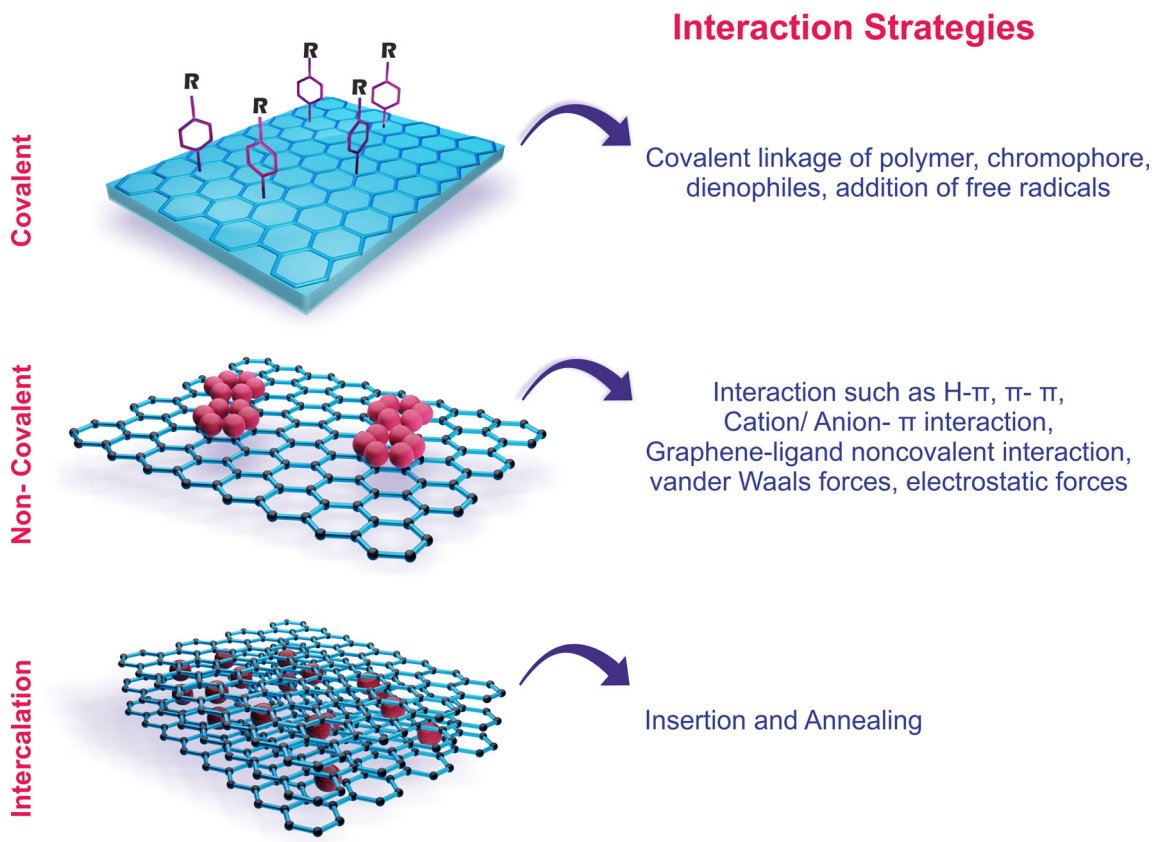


Figure 2 Different strategies for the functionalization of graphene.

explored by different functional modifications via metals, polymers, or other composites. Depending on the needs of certain application domains, these oxygen-containing groups or reduced doping components can be employed as catalytic active centres for surface functionalization with different compounds such as small or macromolecules [60]. It can be achieved via covalent, non-covalent, and intercalation approaches, thus forming graphene-based nanocomposites (GNCs) (Fig. 2) [27]. In the covalent modification, there is the addition of groups, namely reactive functional groups, double bonds, and polymers on their surface. It employs amides, free radicals, and other chemical processes under acidic reaction conditions to chemically react with these reactive groups on the surface, thereby constructing a covalent bond and imparting the necessary functions [61]. In GO, reactive oxygen-containing functional groups are utilized for covalent modification, thereby amplifying its physicochemical characteristics [62, 63]. Non-covalent functionalization involves electrostatic forces, π–π interaction, and van der Waals forces which are involved in the formation of nanocomposites of G with polymeric compounds [64]. It enhances the capabilities such as biosensor, dispersion, and reactive activation besides being less stable in vitro as well as in vivo which does not affect the association of the functional group and structure of the G derivatives [64–66]. Lastly, intercalation involves arc discharge, ion bombardment, annealing via heat treatment, and arc discharge to insert an element into G, GO, or rGO, subsequently resulting in the substitution of various defects in the structure and maintaining the inherent 2D structure of G.

Graphene interaction with cellular components

GBNs exert their antimicrobial effect by interacting with the cellular components including membrane lipids, proteins, and DNA/RNA. These interaction event affects the vital metabolic cell functions, thereby inhibiting bacterial growth or causing cell death. A thorough understanding of these interactions is vital to understand a deeper insight into the

antimicrobial mechanistic profile of GBNs, thus manifesting itself as being bacteriostatic or bactericidal [67]. This section presents an overview of the interactions between GBNs with proteins as well as nucleic acids. Interaction with lipid bilayer membrane has been further elaborated in the upcoming section with the mechanism counterpart.

Interaction with nucleic acids

Bacteria contain nucleic acids DNA/RNA and play an indispensable role in sustaining bacterial cells. Nucleic acids are bio-polymer consisting of nucleotides as monomeric units. These nucleotides consist of pentose sugar, a nitrogenous base, and sugar (deoxyribose/ribose). The genetic material of bacterial cells consists of double-stranded (ds) DNA along with small extra-chromosomal ds circular DNA containing the genes for antibiotic resistance. Due to the existence of oxygen- and nitrogen-containing groups in addition to the π-conjugated structure, interactions between DNA/RNA and GBNs may take place through π–π stacking, H bonding, and electrostatic adsorption [68]. Ren and coworkers showed that DNA–GO relieves DNA supercoiling and thereby induces nicks and linearization of DNA [69]. They demonstrated that GO on combining with copper ions (Cu^{2+}) causes DNA cleavage in which Cu^{2+} ions chelated by GO can interact with the bases and phosphate groups in the DNA and further result in DNA cleavage. These materials cause changes in the structural and chemical properties of the nucleic acids, leading to the death of the bacterial cell. However, not much study has been conducted to support such a phenomenon. Most of the present studies outline the free radical-induced denaturation of DNA/RNA responsible for the inactivation or killing of the microbial cell [70, 71].

Nardjess and coworkers synthesized GO nanocomposite functionalized with 2 (ferrocenylmethylamino) benzonitrile (FMB2CN) using (3-aminopropyl) triethoxysilane (APTES) as a linker molecule to study its interaction with biomolecules including DNA [72]. GO@APTES@ FMB2CN nanocomposite binds to DNA spontaneously via intercalation and demonstrated antimicrobial activity against bacteria, including *P. aeruginosa*, *E. coli*, and *S. aureus*, and fungal pathogens, namely *Candida albicans* (*C. albicans*). Aunkor et al. determined the antibacterial activity of GO against the MDR strains of *E. coli* isolated from clinical samples [48]. They analysed in vivo as well as in vitro effect of GO on DNA. For in vitro analysis, they treated the DNA extracted from *E. coli* cells with different concentrations of GO (1–0.004 μg/μL), while for in vivo analysis, *E. coli* cells were grown in the presence of varying concentrations of GO (1–0.004 μg/μL). All the extracted DNA was analysed using agarose gel electrophoresis and concluded that GO induces successful in vivo DNA destruction. In an agarose gel, a DNA band's intensity increases as DNA concentration increases, but for DNA that has been treated with GO, the band's intensity decreases as GO concentration increases (in vitro condition) [73]. In Fig. 3a, fine bands in lanes 1–3 suggest that respective concentrations exert no effect on DNA. However, reduced band intensity in lanes 4 and 5 suggests moderate degradation, and no band in lanes 6–9 indicates the successful DNA damage induced by 0.016, 0.008, and 0.004 μg/μL of GO concentration. In contrast, in Fig. 3b, DNA isolated from bacterial cells that have been exposed to GO shows specific bands from lanes 1–3, demonstrating that GO had no impact on the DNA of the treated cells. Lanes 4 and 5 highlight moderated DNA destruction, and no band in lanes 6–9 indicated complete DNA damage in vivo. Gurunathan et al. also demonstrated DNA fragmentation induced by GO, rGO, and AgNPs [74] (Fig. 3c). DNA isolated from the exposed *E. coli* cells was analysed by agarose gel electrophoresis. Specific smearing of DNA observed in the gel electrophoresis is the characteristic indication of DNA damage.

Interaction with proteins

Proteins are biological polymeric molecules consisting of amino acids as the monomeric unit linked together by peptide bonds [76]. Cellular proteins are categorized into structural and functional proteins: The former serves as a building block of the various cellular components, while the latter is involved in the regulation of cellular metabolism. GBNs interact via π–π stacking that formed between π-conjugated structures present in GBNs as well as aromatic amino acids, namely tyrosine, tryptophan, and phenylalanine. Further, Alava and coworkers using complementary G-coated quartz crystal microbalance (GQCM) and cell binding studies showed that adsorption of G on the surface of protein results in disruption in protein structure and hence affects the

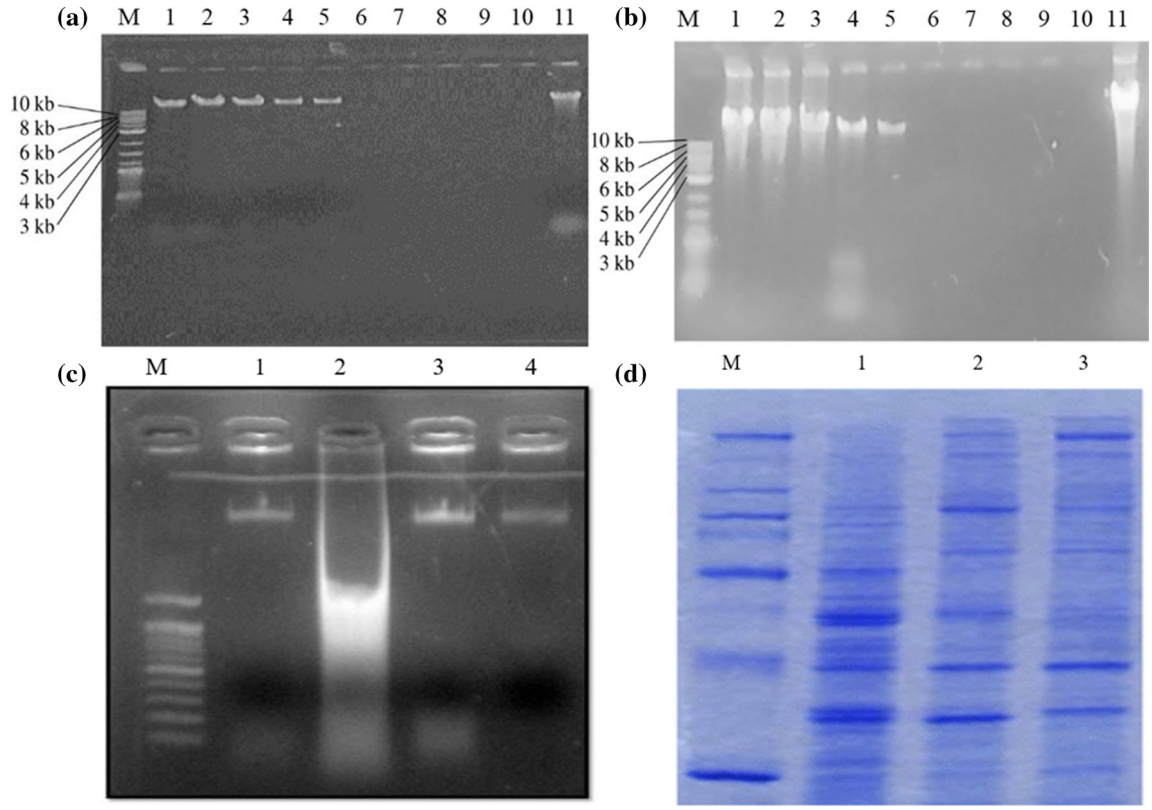


Figure 3 Gel electrophoresis results showing DNA and protein degradation in E. coli cells induced by graphene materials. (**a**) Agarose gel electrophoresis analysis of DNA extracted from cells treated with different concentrations of GO in vitro conditions. Lanes 1–9 contain DNA treated from cells treated, respectively, with 1, 0.5, 0.25, 0.13, 0.065, 0.032, 0.016, 0.008, and 0.004 μg/μL of GO. (**b**) Lanes 2–9 contain DNA from cells treated with 1, 0.5, 0.25, 0.13, 0.065, 0.032, 0.016, 0.008, and 0.004 μg/μL of GO in in vivo conditions. (**c**) DNA fragmentation in cells treated with GO, rGO, and AgNPs. DNA was isolated from treated and untreated E. coli and electrophoresed on the agarose gel. Lane M contains marker, and lanes 1, 2, 3, and 4 contain cells treated with rGO, GO, AgNP, and control, respectively. (**d**) SDS–PAGE analysis of protein extracted from treated E. coli cells. Lane 1 contains marker, lane 2 contains proteins from untreated cells, and lanes 3 and 4 contain proteins extracted from cells treated with GO and Ni/$NiFe_2O_4$ –GO, respectively. (**a**, **b**) Reproduced from ref. [48]. Copyright 2020, the Royal Society. (**c**) Adapted with permission from ref. [74]. Copyright 2012, Elsevier B.V. (**d**) Reproduced from ref. [75]. Copyright 2022, American Chemical Society.

function of the protein [77]. They have been reported to cause interference in the protein–protein interactions causing the separation of different subunits of a functional protein and hence leading to protein inactivation [67]. On the contrary, Chong et al. concluded that π–π stacking interactions between protein and GO surface lead to the strong adsorption of the protein molecule [78]. This adsorption of protein further prevents the cellular uptake as well as cell membrane adhesion of GO, thereby markedly reducing its cytotoxicity. Acharyulu and coworkers showed that the nanocomposite consisting of nickel (Ni), nickel ferrite ($NiFe_2O_4$), and GO, Ni/$NiFe_2O_4$–GO displays bactericidal activity against *E. coli* cells and leads to degradation of proteins as analysed by SDS–PAGE (sodium dodecyl sulphate–poly acryl amide gel electrophoresis) (Fig. 3d) [75].

A mechanistic overview of the antimicrobial action

The understanding of mechanistic aspects of antimicrobial activity exerted by GBNs is essential for developing novel materials with superior antimicrobial action. The antimicrobial effect exerted by G and its derivatives functioned principally via the physical and chemical modes of action. The unique structure of G analogues with large surface area and sharp edges allows them to interact with the cellular

Table 1 Physicochemical properties of various graphene materials with their antimicrobial mechanism

Mechanism	Graphene material	Concentration	Microorganism inhibited	References
Cell membrane stress	GO	1, 0.5, 0.25, 0.13, 0.065, 0.032, 0.016, 0.008, and 0.004 μg/μL	G −ve: *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *S. marcescens*, and one G + ve: *S. aureus*	[48]
	LIG paper	–	G − ve: *E. coli* G + ve: *S. aureus*	[79]
	Gt, GtO, GO, and rGO	0.040 μg/μL	G − ve: *E. coli*	[57]
	GO, rGO	1, 2, 3, 5 μg/μL	G + ve: *S. aureus* and G − ve: *P. aeruginosa*	[80]
	GO	0.0085 μg/μL	G − ve: *P. putida* KT2440	[81]
	GO, rGO	0.02, 0.85 μg/μL	G − ve: *E. coli*	[56]
	GONWs, RGNWs	1 μg/μL	G + ve: *S. aureus* and G − ve: *E. coli*	[82]
	GO	0.1 μg/μL	G − ve: *E. coli*	[83]
	GO	0.2 μg/μL	G − ve: *E. coli*	[84]
	GO	–	G − ve: *E. coli*	[85]
	GO	0.05 μg/μL	–	[86]
	GO–NCNC	0.25, 0.125, 0.0625, 0.0313, 0.156, 0.0078 μg/μL	G + ve: *S. aureus*	[87]
	GO	0.01 μg/μL	G + ve: *S. aureus*, *E. faecalis* and G − ve: *E. coli*, *P. aeruginosa*	[88]
Mechanical wrapping	GO	50 mg/L	G − ve: *P. aeruginosa*; G + ve: *S. aureus*; Yeast: *C. albicans*	[89]
	GO	0.01 μg/μL	G + ve: *S. aureus*, *E. faecalis* and G − ve: *E. coli*, *P. aeruginosa*	[88]
	GO Langmuir–Blodgett films	–	G − ve: *E. coli*	[90]
	GO	1, 0.5, 0.25, 0.13, 0.065, 0.032, 0.016, 0.008, and 0.004 μg/μL	G − ve: *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *S. marcescens*, and one G + ve: *S. aureus*	[48]
Pore formation	G	–	G − ve: *P. aeruginosa*; G + ve: *S. aureus*	[91]
	GO	–	–	[92]
	LIG	–	Virus: PhiX174, Herpes Simplex Virus 1; G + ve: *S. aureus* and G –ve: *E. coli*	[93]
Oxidative stress	GO and rGO	0.025, 0. 05, 0.075, 0.1, 0.125, 0.15 μg/μL	G − ve: *P. aeruginosa*	[71]
	GO nanosheets	–	G − ve: *E. coli*, G + ve: *Mycobacterium smegmatis*, *S. aureus*	[94]
	GO	0.025, 0.050, 0.075, and 1 μg/μL	G − ve: *E. coli*	[95]
	GO, Carbon nanofibers	0.08 μg/μL	G + ve: MRSA, MRSE	[96]
	GBM (GO and rGO on cellulose ester membrane)	–	G − ve: *P. aeruginosa* and G + ve: *Bacillus* sp.	[97]
	GO	0.02, 0.04, 0.08, 0.16, and 0.32 μg/μL	G + ve: *S. mutans*	[98]
	GO–NCNC	0.25, 0.125, 0.0625, 0.0313, 0.156, 0.0078 μg/μL	G + ve: *S. aureus*	[87]
	Gt, GtO, GO, and rGO	0.040 μg/μL	G − ve: *E. coli*	[57]
	LIG paper	–	G − ve: *E. coli* G + ve: *S. aureus*	[79]
	GO-metal sheet	–	G − ve: *E. coli*	[99]
	$Ni/NiFe_2O_4$–GO	–	G − ve: *E. coli*	[75]
	LIG	–	G − ve: *E. coli*	[100]

Table 1 continued

Mechanism	Graphene material	Concentration	Microorganism inhibited	References
Photo-thermal ablation	GO-IO-CS	0.02 μg/μL	G + ve: *S. aureus* and G − ve: *E. coli*	[101]
	AuNR–GO	–	–	[102]

Gt: graphite; GtO: graphite oxide; GO: graphene oxide, and rGO: reduced graphene oxide; *E. coli*: *Escherichia coli*; *M. smegmatis*: *Mycobacterium smegmatis*; *S. aureus*: *Staphylococcus aureus*, MRSA: methicillin-resistant *Staphylococcus aureus*, MRSE: methicillin-resistant *Staphylococcus epidermidis*; *P. putida*: *Pseudomonas putida*; GBM: graphene-based membranes; *C. albicans*: *Candida albicans*; *S. aureus*: *Staphylococcus aureus*; *E. faecalis*: *Enterococcus faecalis*; *E. coli*: *Escherichia coli*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. mutans*: *Streptococcus mutans*; GO-IO-CS: chitosan-functionalized magnetic GO nanocomposite; AuNR: gold nanorods; NCNC: nickel colloidal nanocrystal cluster; Ni/NiFe2O4–GO: nickel/nickel ferrite graphene oxide; RGNWs: reduced graphene nanowalls; GONWs: graphene oxide nanowalls

structure. The interaction of G analogues results in either membrane stress or mechanical wrapping. In addition, their unique band structure provides an opportunity to exploit the photo-thermal effect shown by them in stimulating antimicrobial actions. On the other hand, chemical damages are induced due to the reactivity of functional moieties present on the surface. The chemical mode of action leads to a significant alteration in the atomic organization of G as well as the cell membrane of microbes and results in irreversible membrane damage. Table 1 outlines the physicochemical properties of various G materials along with the proposed antibacterial actions, which are discussed in the upcoming sections.

Physical damage

Physical damage involves mechanisms that cause damage to the physiological characteristics of the microbial cell. It involves direct physical contact of the sharp edges of the GBNs with the bacterial cell membrane which can later induce membrane stress. It also involves photo-thermal ablation and mechanical wrapping of the bacterial cell. G analogues show differential antimicrobial effect on G + ve and G − ve bacterial cells. This is principally due to the significantly different composition of the outer cell wall, which forms the first bacterial structure to be encountered by G materials. Moreover, the phospholipids' bilayer structure of the inner cell membrane interacts fascinatingly according to the hydrophobicity and hydrophilicity of the G materials and is discussed in the upcoming sections. Figure 4 shows various modes of cell membrane stress induced by GBNs.

The antibacterial action of GBNs is significantly influenced by the structure of different microbial species. G − ve and G + ve are two general classifications of bacterial cells based on the chemical makeup of the cell walls and the Gram staining response. Though, antimicrobials are classified as broad spectrum and narrow spectrum on the variety of bacterial types they inhibit. Broad spectrum is when it inhibits a wider range of bacterial types (G + ve as well as G − ve bacterial cells), while narrow spectrum is when they are effective against lesser groups of bacterial types (G + ve or G − ve bacterial cells). Field emission scanning electron microscopy (FESEM) and Transmission electron microscopy (TEM) analysis has demonstrated that difference in the outer membrane composition of these two bacterial types is linked with their difference in susceptibility towards the antimicrobial agent. The outer membrane in G − ve cells protects the bacterial cells from the destructive action of the antimicrobial agent. Lipopolysaccharides present in the outer membrane of G − ve cells develop repulsive forces through steric repulsion with the G materials leading to membrane damage. On the other hand, the presence of thick peptidoglycan, adhesins, lipoteichoic acids, and amino acids on G + ve cells helps to generate electrostatic contact, which causes G materials to physically wrap around the bacterial cell. Wang and coworkers demonstrated that the higher mortality of G + ve cells by antibacterial action of GO is due to the greater adsorption affinity of GO with teichoic acid (present on these cells) via

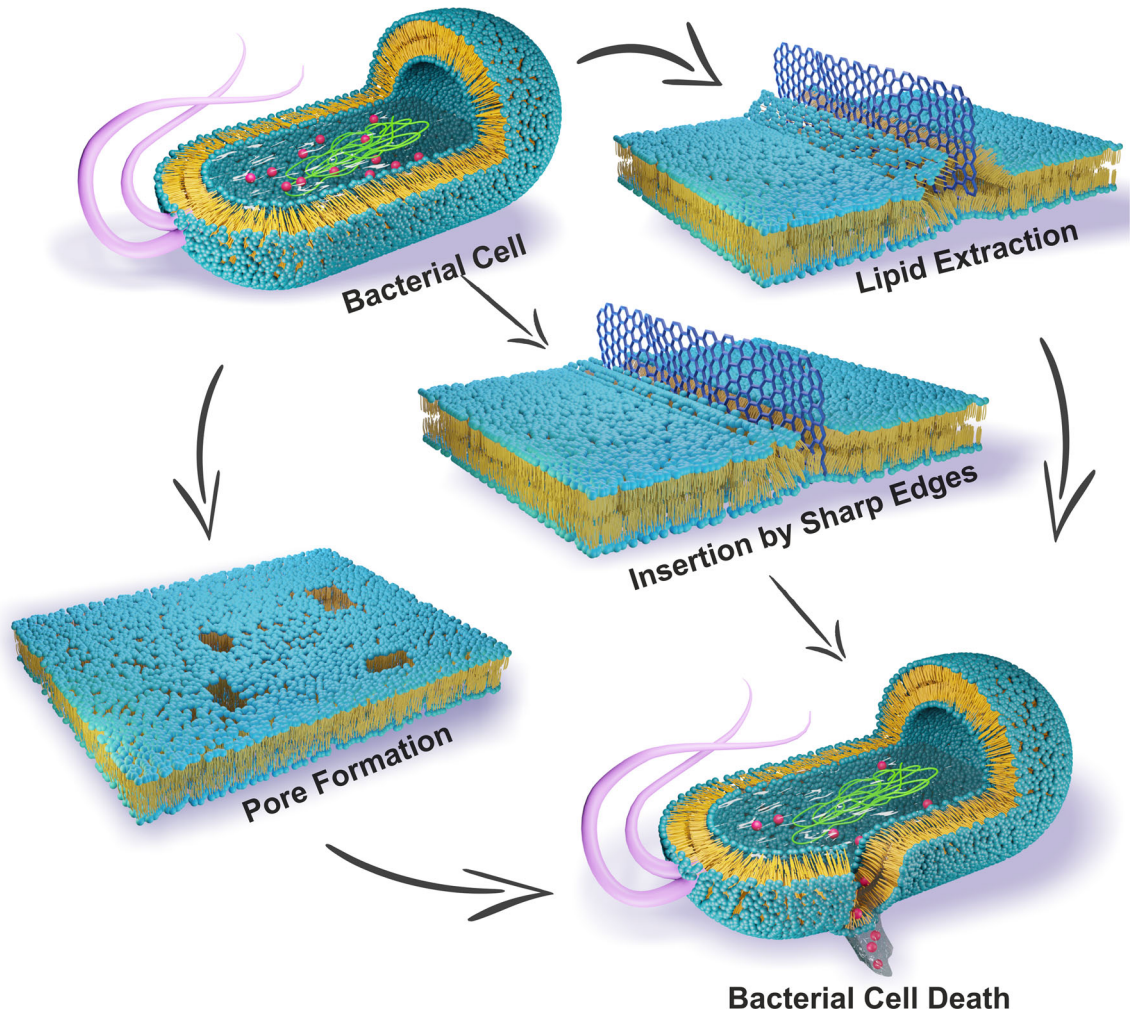


Figure 4 Different modes of membrane stress induced by graphene-based nanomaterial leading to bacterial cell death. Lipid extraction, insertion by sharp edges, and pore formation leading to cell membrane disruption, followed by loss of cellular integrity, efflux of cytoplasmic content, and finally cell death.

π–π interactions [103]. This leads to enriched expression of autolysin-based genes, leading to increased production of autolysin. These autolysins are peptidoglycan-degrading enzymes that can destroy bacterial cell walls, making them inert. Perumal and coworkers prepared G-based nanocomposites functionalized with AgNPs and vinylpyrrolidone (VPy), namely G–Ag and G–AgVPy [104]. These nanocomposites displayed a broad-spectrum antibacterial activity inhibiting G + ve as well as G − ve bacterial cells by damaging the cell membrane of these cells and eventually leading to cell death. Dan et al. fabricated GO–SiO_2 nanocomposite and studied its antimicrobial activity [105]. The synthesized nanocomposite displayed excellent antibacterial activity against *S. aureus* and *E. coli* by disrupting its cell membrane, which further results in the leakage of cellular components and eventual destruction of the bacterial cell.

Du and coworkers reinforced nickel colloidal nanocrystal cluster (NCNC) with GO to synthesize GO–NCNCs nanocomposite [87]. The fabricated nanocomposite synergistically exhibits broad-spectrum antibacterial activity against *S. aureus* and *E. coli* by the generation of ROS and membrane disruption. In their experimental procedure, they analysed the effect of GO–NCNCs, GO, and NCNCs on the viability of *S. aureus* cells by agar plate method. Live/dead fluorescence assay (Fig. 5) demonstrated the survival rate of the cells treated with respective concentrations of different treatments (GO–NCNCs, GO, NCNCs). *S. aureus* cells without any treatment

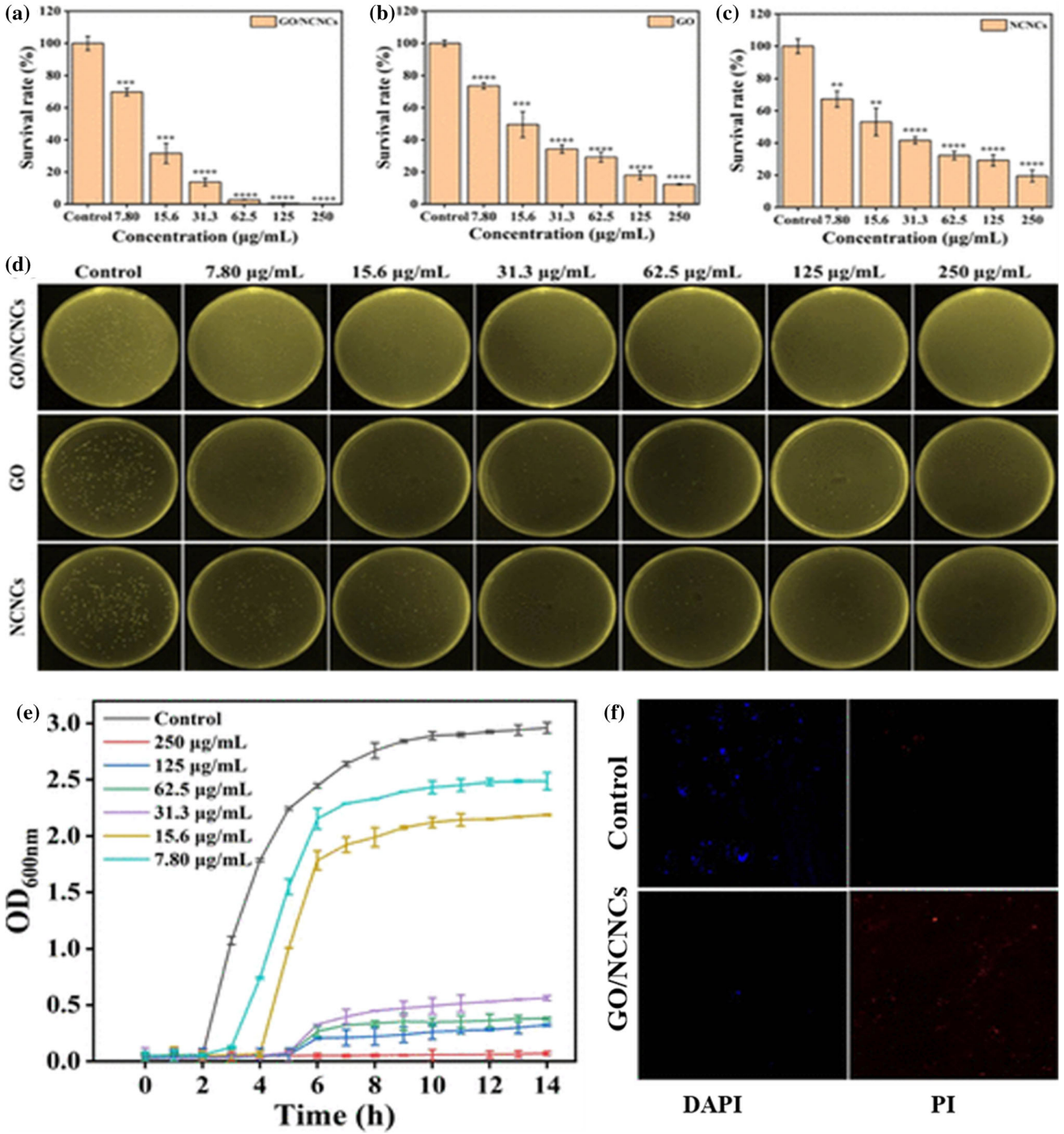


Figure 5 Antimicrobial effect of GO/NCNCs nanocomposite on the viability of *S. aureus* cells. (**a**–**c**) The dose-dependent survival rate of S. aureus cells treated with GO/NCNCs, GO and NCNCs, respectively. (**d**) Agar plate showing *S. aureus* cells in the form of colonies as appeared in plate culture of cells exposed or unexposed to different concentrations of GO/NCNCs, GO, and NCNCs (7.80–250 μg/mL). (**e**) Growth curve of *S. aureus* cultured with various concentrations of GO/NCNCs. (**f**) Fluorescence microscopy image of *S. aureus* cells without and with exposure to GO/NCNCs nanocomposite at a concentration of 125 μg/mL after staining with stain DAPI and PI. (**a**, **b**, **c**, **d**, **e**, **f**) Reproduced from ref. [87]. Copyright 2022, American Chemical Society.

served as a control group. After incubation, the survival rate was highest in the control group, while the cells treated, respectively, with GO–NCNCs, GO, and NCNCs displayed a steady dose-dependent decrease in the number of colonies observed in the agar plate (7.8–250 μg/mL), as depicted in Fig. 5d. The antibacterial rate of GO, NCNCs, and GO–NCNCs against *S. aureus* cells at a concentration of 125 μg/mL was evaluated to be 82, 70.8, and 99.5%, respectively (Fig. 5a–c). The highest antibacterial rate in GO–NCNSs demonstrates the synergistic antibacterial effect induced by GO as well as NCNCs in the developed nanocomposite GO–NCNCs against the bacterial cells. They further evaluated the antibacterial efficiency of GO–NCNCs nanocomposite by studying the growth curve and live/dead fluorescent experiment via fluorescence microscopy. *S. aureus* cells cultured in Luria–Bertani broth were incubated with GO–NCNCs in the concentration range of 7.80 to 250 μg/mL, which showed a decrease in the viable cells with the increase in the concentration of nanocomposite, as depicted in Fig. 5e. Fluorescence microscopy analysis for detecting live and dead cells displays similar results (Fig. 5f). The appearance of a large amount of red fluorescence indicates the presence of a large number of dead cells upon treatment with GO–NCNCs. These dead cells appear red after getting stained with propidium iodide (PI). Observance of less blue fluorescence points out the presence of a lesser number of live cells and appears blue due to nuclear stain DAPI (4′,6-diamidino-2-phenylindole). On the other hand, control group of cells without any treatment fluoresce blue, indicating the presence of a large number of live cells.

Cell membrane stress

Insertion/cutting mechanism The cellular membrane of the bacterial cell forms the main structure maintaining the cellular integrity as well as protecting it from exogenous damage. G materials upon interaction with cell membrane phospholipids can cause cell membrane stress by mechanisms including cutting or insertion, lipid extraction, or pore formation. The cutting/insertion mechanism was first revealed by Hu and coworkers in 2010 [56]. They demonstrated using TEM analysis that the cellular membrane of G − ve *E. coli* cells was severely damaged upon exposure to GO, rGO nanosheets, or G papers. In a different study, nanowalls of GO and rGO were found to possess higher antibacterial activity against G + ve *S. aureus* and G − ve *E. coli* cells as compared to the GO suspension and rGO nanosheets [82]. They also showed that G + ve bacterial cells were more susceptible to GBNs in comparison with the G − ve ones. This effect may probably be explained due to the presence of an outer membrane in G − ve bacteria which is lacking in G + ve cells. Further, this was confirmed by the increased RNA efflux in G + ve cells after incubation with G material as compared to G − ve cells. Various studies have come up with the agreement to this mechanism of cell membrane stress. They proved that sharp edges of the G material (G, GO, and rGO) dispersions exhibit cell membrane stress in G + ve as well as G − ve bacterial cells [57, 80]. Their sharp edges offer a blade or cutter-like effect on the membrane leading to cell membrane disruption, followed by loss of cellular integrity, efflux of cytoplasmic content, and finally cell death.

Tu and coworkers studied the atomic details using molecular dynamics simulation as well as TEM analysis on how the G material interacts with the inner and outer membranes of *E. coli* [83]. The cellular morphology of the *E. coli* cells during incubation with GO sheets for 2.5 h was analysed using TEM to predict the stages of membrane degradation. It was outlined that bacterial cells were initially transiently resistant to GO sheets at low concentrations, followed by partial damage to the cell membrane with decreased phospholipid density however with no cuts in the membrane. Finally, bacterial cells lose their membrane integrity, followed by cytoplasmic efflux and finally loss of cell viability. A molecular dynamics simulation study revealed that G nanosheets simulate a blade or knife which can cause a cutting effect in the cell membrane and direct further killing. This study clearly shows that there is the spontaneous insertion of G nanosheets into both, the inner and outer membranes of *E. coli*. Nanosheet entry follows three modes namely swing, insertional, and extraction mode. During swing mode, the G nanosheet vibrates around the atom for a small fraction of second and G edges touch the surface of the membrane several times. Then, the insertion mode follows, in which the sheet edges advance in and pierce the cell membranes due to strong van der Waals interactions with the hydrophobic interface and membrane lipids. Finally, during the extraction process, the nanosheets firmly remove the

phospholipids from the lipid bilayers on the cell surface. Due to the strong dispersion/hydrophobic interactions between lipid and G, graphene nanosheets may penetrate and remove substantial volumes of phospholipids from cell membranes [83, 92].

Further deeper insight into the insertion process was provided by analysing the physical interactions between G and lipid bilayer membranes. Profiling of the interaction energy between them demonstrates three modes, which involve a plateau of high energy representing the swing mode, followed by a sharp fall in energy corresponding to the insertion mode. Thereafter, a slow drop in energy relates to the further intensification of the interaction by repeated movement of the G sheet on the membrane, which ultimately results in the extraction of lipid molecules. Observed extraordinarily strong dispersion interactions between membrane lipids and G are due to the sp^2 atoms which can subdue the self-aggregation among the lipid molecules [83]. In addition, the insertion process is facilitated by two different mechanisms [68], firstly in which GBNs align themselves parallelly along the mid-plane of the lipid bilayer forming a sandwich-like structure with G sheet sandwiched between lipid bilayers of the membrane and secondly GBNs during insertion cut across the membrane forming a structure similar to perpendicular orientation. Further, Titov et al. stated that the G-membrane superstructure obtained by sandwich configuration of insertion is more stable than the vertical mode [106]. Initially, the G sheets orient orthogonally to the membrane, and then, attractivity between G and phospholipids causes further insertion into the membranous structure. Larger-sized G sheets with multilayers of G or having rough surfaces due to functionalization get inserted into the membrane till the end that has a perpendicular orientation to the membrane, while smaller-sized G-flakes get completely embedded into the membrane forming a sandwiched G-membrane superstructure [107]. Further sandwiched insertion mechanism was experimentally proven by Chen and colleagues in two mammalian cell lines, namely the murine macrophage cell line J774A and the murine breast cancer cell line 4T1 [108]. Moreover, Yi and Gao found that membrane splay and tension energies along with hydrophobic attraction lead to the near-perpendicular insertion mechanism of the G sheet [109]. They also showed the absence of cross-membrane penetration, thus proposing the parallel attachment of the sheet over the membrane surface due to the membrane bending and tension energies as well as a hydrophilic attraction (Fig. 6).

Wang and coworkers explained the role of thickness, oxidation state as well as surface adsorption in the insertion of G nanosheets [110]. They discovered that pristine and thin-layered G nanosheets may spontaneously enter into the bilayer and rotate to lie parallel in the centre of the membrane. Depending on the level of oxidation, the oxidized edges of the G nanosheets can breach the bilayer, with the ultimate state either parallel in the centre of the bilayer or rising erect over the bilayer. It was also seen that sheets covered with less density of lipids advance and pierce further in the bilayer with its exposed edges.

Other studies suggest that GO edges do not play a major role in its antibacterial mechanism; instead, the number of basal planes that get increased by rising the number of GO sheets plays a competent role in the mechanistic actions [90]. It was probably due to the increase in the number of bacteria being trapped in the GO sheets, leading to more bacterial cells being killed. But another study showed orientation-dependent interactions of the GBNs with bacterial membranes. They proved that GO sheets aligned parallelly to the bacterial surface have lower antibacterial activity than GBNs positioned perpendicular to the bacterial surface [84]. Mandal and coworkers explained the membrane rupture process using the X-ray reflectivity (XRR) pattern and outlined that GO flakes bring about significant structural changes in the cell membrane of *E. coli* [85] They showed that the negatively charged carboxyl group in GO gets attached to the positively charged choline of lipid heads. Furthermore, only 30% of the atoms in the produced GO are oxidized, leaving a large portion of the graphitic domain intact, which interacts hydrophobically with lipid tails to cause GO to be inserted into the membrane (GO-rich phase). This leads to antibacterial activity by rupturing the cell through attachment and penetration into the membrane.

Lipid extraction mechanism This mechanism was developed simultaneously with the insertion mechanism during simulation studies by Tu and coworkers [83]. They found that during incubation of GO nanosheets with the *E. coli* membranes (inner and outer membrane), lipid molecules get robustly

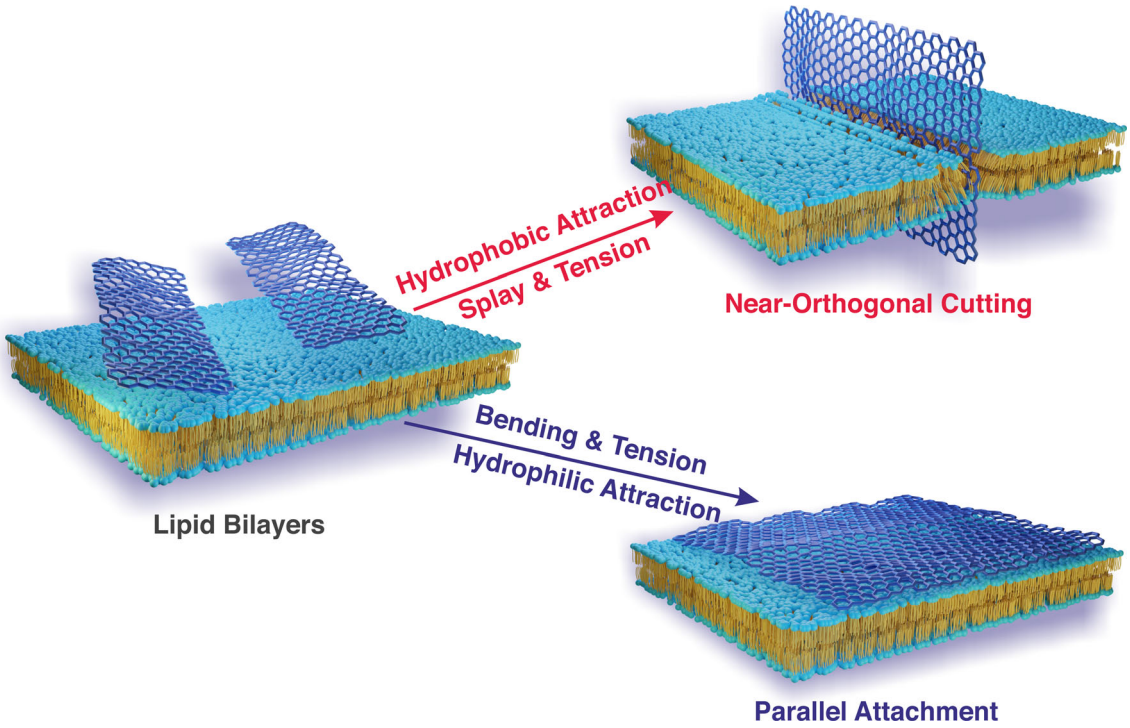


Figure 6 Hydrophilic and hydrophobic interaction between the graphene sheet and lipid membrane, favouring parallel attachment of the sheet and insertion/cutting of the membrane, respectively.

extracted from the cell membranes to both sides of the G surface. This is possible because strong dispersion interactions between GBNs and phospholipids prevailed over the hydrophobic interactions among the phospholipids. Later, Wu and coworkers using surface-enhanced infrared absorption (SEIRA) spectroscopy explained the nature of the interaction between GO and lipid molecules responsible for the extractive effect [111]. Their results proved that H-bonding, hydrophobic interactions, electrostatic attraction, and repulsion played a major role in their interactions. Further, Luan et al. described the lipid extraction event as a wetting process and proposed a wetting-based theory to explain the extraction of lipid molecules [112]. Free energy changes which occurred during the microscopic extraction of lipid accounts for the wetting of the G by the membrane lipids. Strong dispersive adhesion between G and lipid molecules plays a major role during this extraction and is dependent on G's curvature with a concave surface manifesting high lipid extraction than G's with a flat surface, while rare lipid extraction is exhibited by a convex surface. Besides, Zhang et al. using SEIRA spectroscopy, confocal laser scanning microscopy (CLSM), and electrochemical impedance spectroscopy (EIS) analysis demonstrated that the synergistic effect of weak interaction between GO and lipid membrane results in the extraction of lipids [86]. During the initial contact of GO with the membrane surface via swing motion, sharp edges facilitate its steady lodging into the membrane. This insertion is assisted by attractive forces between GO and polar head groups of the membrane. However, lipid extraction occurs only when the hydrophobic centre part of the membrane comes in contact with the corners of GO. Moreover, too strong of electrostatic attraction, as well as hydrophobic interactions, limits the lipid extraction process, thereby reducing the antibacterial capability of the GO. Therefore, under the optimum condition of attraction between GO and hydrophilic lipid head, H-bonding between the carbonyl group of lipid tail and GO may further assist its insertion into the hydrophobic alkyl chains, thus maintaining free rotation of GO and eventually causing effective lipid extraction (Fig. 7).

Pore formation Damage to the cell membrane is sometimes caused by pore formation on the bacterial cell membrane which causes osmotic imbalance and further cytoplasmic efflux and cell death [91]. Owing

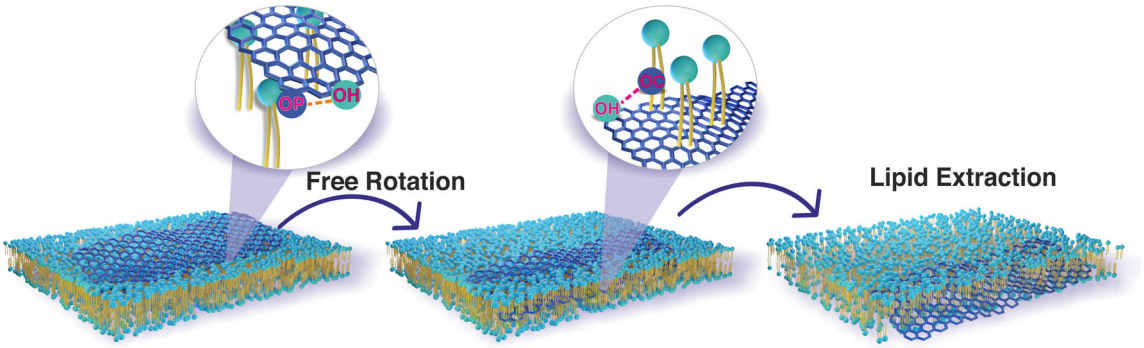


Figure 7 Lipid extraction induced by synergistic effects of weak interactions between GO and membrane lipids. Weak attractive forces between GO and the polar head group facilitated GO insertion into the membrane. Followed by H-bonding between the carbonyl group of the lipid tail and GO which further assists its insertion into the hydrophobic alkyl chains, thus maintaining free rotation of GO and eventually causing effective lipid extraction.

to its hydrophilic nature, GO tends to stay at the water-membrane interface. When it penetrates the membrane, it gets ruptured leading to localized pulling of lipids and the eventual formation of the pores [92]. Nanocomposites of GO formed by Ag nanoparticles/phosphomolybdate/rGO using a one-pot hydrothermal procedure induce pores, leakage of cell components, and eventually loss of cell membrane integrity [113]. Recently, Beikzadeh and coworkers studied the antimicrobial mechanism of LIG electrodes and proved that the charged electrodes exhibit antibacterial as well as antiviral capability [93]. They demonstrated that the hydrogen peroxide (H_2O_2) generation is not the main antibacterial mechanism and suggested that electroporation forms the principal disinfection mechanism, instead of higher capacitive electrodes. This electroporation leads to cytoplasmic efflux and eventual cell death.

Mechanical wrapping

Mechanical wrapping provides another mechanism for membrane stress-induced bacterial cell damage. G owing to its unique single-layered sp^2-bonded C-atom arranged in a thin hexagonal lattice structure leads to the formation of the barrier by surrounding itself with the bacterial cell. This causes the isolation of the cell thereby depriving it of nutrients and causing cell death. Wu et al. proved experimentally that the forces that effectively adsorb GO on lipid membranes are those that result from the balance of different interactive forces [111]. These include the electrostatic repulsion of the phosphate group, the attraction of its hydrogen bonding as well as the electrostatic and hydrophobic interaction with the choline group. Initially, it was proven that GO sheets wrap the *E. coli* cells isolating them from their microenvironment and thus affecting cell proliferation. They also outlined that smaller GO sheets were not able to isolate bacterial cells from their environment and hence displayed lesser antimicrobial capability. Later, Giulio and coworkers using optical and atomic force microscopy results demonstrated that G and its derivatives inhibit microbial growth by surrounding the microbial cell in a way that inhibits the metabolic function of the cell without affecting their membrane integrity [89]. Another research group demonstrated the role of GO and rGO on two G + ve as well as G − ve bacteria using FESEM analysis [88]. They showed that GO sheets get wrapped around cells (mechanical wrapping) preventing nutrients from entering the cell. Further, it was demonstrated that mechanical wrapping of GO sheets causes antimicrobial activity against fungal pathogens, namely *Fusarium graminearum* and *F. oxysporum*, as well as bacterial pathogens such as *Pseudomonas syringae* and *Xanthomonas campestris* pv. Undulosa [114]. This study proposes a collaborative mechanism in which GO intertwines bacterial cell and fungal spores with a wide range of aggregated GO sheets, causing local alteration of their cell membranes bringing a driving bacterial membrane potential drop and fungal spore electrolyte leakage. These incidences collectively lead to cell lysis.

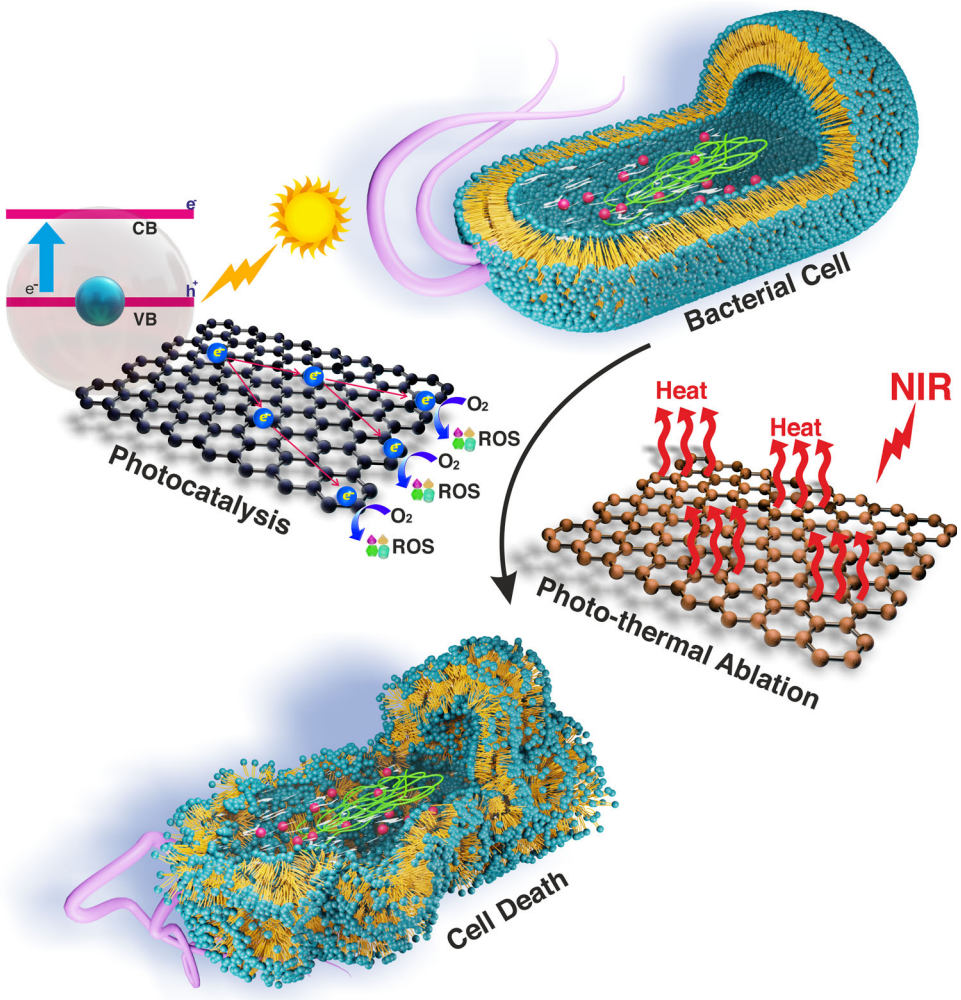


Figure 8 Photocatalysis and photo-thermal ablation mechanisms of bacterial cell death induced by GBNs. In photo-thermal ablation, NIR irradiation induces local heat and exhibits photo-thermal antibacterial activity. Photocatalytic antibacterial activity occurs by the generation of ROS when exposed to light greater than the band gap of GBNs. Generated ROS species and heat lead to the loss of bacterial cell viability.

Photo-thermal ablation

GBNs owing to their extraordinary optical properties absorb light and release it as heat. Photo-thermal ablation mechanisms utilize these light-absorbing materials combined with the pulsed laser to convert near-infrared radiations (NIR) into local heat to kill bacterial cells (Fig. 8) [115, 116]. GBNs are capable of conducting heat under near-infrared (NIR) irradiation and can be used for photothermally induced bacterial killing. For effective bacterial killing, NIR irradiation in the 700–1100 nm range is most beneficial as it can penetrate deeper into the tissues exhibiting the marked antibacterial effects [117]. It was demonstrated that magnetically reduced GO functionalized with glutaraldehyde can be used as an effective photo-thermal agent. It has a highly efficient and rapid antibacterial activity which is achieved within 10 min of exposure to NIR irradiation [118]. Moreover, G-based photo-thermal nanocomposites were used for capturing and obliterating the growth of these bacterial cells, thereby inhibiting biofilm formation. One of the chitosan-functionalized magnetic GO nanocomposites (GO-IO-CS) (IO: indium oxide; CS: chitosan) was used as a versatile therapeutic agent for inhibiting bacterial biofilms [101]. In this, positively charged functional groups on the chitosan surface readily interact and trap bacteria, while GO works as an excellent photo-thermal killer converting NIR light into local heat to increase the antibacterial activity of the prepared composite. Moreover, the super-paramagnetic characteristics of GO-IO-CS provided an efficient means to separate and aggregate the bacteria, increasing the effectiveness of photo-thermal sterilization. After 10 min of NIR irradiation, the nanocomposite is shown to successfully eradicate bacteria by damaging the lipids on the membrane leading to cell death and removal of

bacterial biofilms. Another study demonstrated the antibacterial activity by integrating GO with plasmonic noble metal gold nanorods (AuNRs) to generate a plasmonic nanohybrid with antibacterial as well as anticancer activities [102]. Here, AuNRs along with GO exhibit absorption in the NIR region. Further, photothermally antimicrobial masks were introduced during the pandemic. These masks consisting of laser-induced graphene (LIG) could reduce the germs in face masks by 81% [100]. Additionally, ten minutes of 0.75 kW/m^2 irradiation resulted in an almost 100% decrease in the bacterial load owing to the photo-thermal effect, whereas in commercial masks, around 90% of the bacterial cells were still alive after 8 h. Fan and coworkers reported that NIR-triggered thermally responsive brushes (TRB) anchored with zinc oxide (ZnO)-doped graphene nanosheets have efficient antibacterial activity [119]. Nanosheets-bacterial aggregates bring about the Zn^{2+} ion penetration into the cell membrane which triggers cell membrane disruption, hyper-thermal killing along with the release of intracellular substances, and ultimately death of the bacterial cell. This provides a rapid and safe skin wound disinfection method via a short-time photo-thermal treatment that does not damage normal skin tissues nor cause accumulative toxicities.

Chemical damage

The chemical mode of action involves free radical or oxidative stress generated by reactive oxygen species (ROS) as well as charge transfer (ROS independent) [2], both of which lead to destruction in metabolic functions and eventual cell death. Oxidative stress results in the oxidation of cellular lipids, proteins, and nucleic acids, which eventually leads to cell membrane destruction and thus inhibits microbial growth (Fig. 9) [80, 88, 120].

ROS-dependent oxidative stress

ROS-dependent oxidative stress arises when an intracellular accumulation of ROS, such as superoxide anions ($O_2\bullet^-$), hydroxyl radicals (OH•), singlet molecular oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), gets increased, which in turn leads to oxidation of vital cellular components [71]. These ROS can cause DNA damage, deactivation of proteins and lipids as well as mitochondrial inefficiency, resulting in bacterial inhibition [121]. GBNs can lead to the formation of ROS by the surface adsorption of oxygen at the

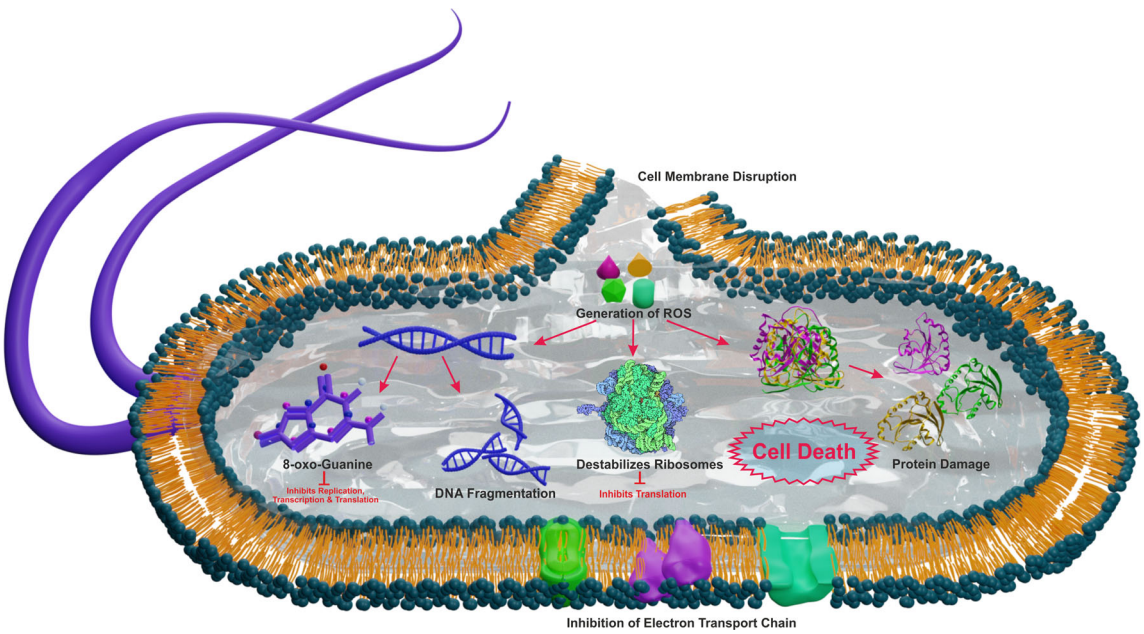


Figure 9 Oxidative-stress-induced damage to various cellular organelles leads to bacterial cell death. Produced reactive oxygen species leads to DNA damage, protein denaturation as well as ribosomal destabilization.

edges and at defect sites, which is followed by its reduction by different cellular enzymes, namely glutathione (GSH). It is a key antioxidant present in the cell and is oxidized to glutathione disulphide (GSSG) in the presence of ROS. Therefore, GSH acts as an intracellular redox-state indicator and its depletion implies an elevated ROS level as well as toxicity to the bacterial cell. Elevated ROS levels in the cells also lead to mitochondrial membrane depolarization, thereby affecting the ATP synthesis and further leading to increased ROS release, i.e. ROS induced ROS release (RIRR) [122]. These events lead to bacterial cell killing. Gurunathan and coworkers elucidated that the antibacterial activity of GO and rGO was principally due to the production of $O_2\bullet^-$, which in the experimental procedures is evaluated by using 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) [71]. XTT reacts with $O_2\bullet^-$ ion to generate water-soluble XTT formazan, which is detected spectrophotometrically at 470 mn [123, 124]. DNA destruction caused by ROS is observed in the form of different bands that are visualized when DNA from the GBNs-treated bacterial cell is analysed by agarose gel electrophoresis [71]. Lipid peroxidation is another mechanism mediated by ROS species that causes the oxidation of lipid molecules. Interaction between GBNs and lipid bilayer membrane leads to a series of radical chain reactions initiated by ROS species and causes lipid peroxidation. Lipid radicals generated by this event lead to oxidative lipid damage of the bacterial cell membrane as well as proteins and DNA [67]. Generated free radicals lead to abstraction as well as addition reaction causing the generation of carbon-centred sugar radicals or OH/H-adduct radicals of bases. Moreover, sugar moieties present in the nucleic acids produce single/double-stranded breaks in the DNA [70].

GNCs of metal ions/sulphides/oxides and nanoparticles such as cadmium sulphide (CdS), titanium dioxide (TiO2), and zinc oxide (ZnO) have been shown by multiple research groups to have antibacterial activity with the formation of ROS via photocatalytic process when exposed to light with an energy larger than their band gap. These metal oxides and sulphides act as a photocatalyst to generate ROS to kill bacterial [80, 125, 126]. Furthermore, Panda and coworkers suggest that the antibacterial action of naturally produced GO-metal films is caused by the concomitant effect of both the non-oxidative electrons transfer mechanism and the consequent formation of ROS in the exposed bacteria [99]. G-based nickel ferrite nanocomposites Ni/NiFe2O4–GO were reported to have oxidative stress-mediated antibacterial activity triggered by ROS and GHS consumption as well as protein degradation [75]. Oxidative stress induced by these nanocomposites in *E. coli* cells was proven by the exhaustion of antioxidant glutathione (GSH) in the treated cells. Upon oxidative stress in the cell, thiol bond (–SH) present in the GSH is oxidized to disulphide bond (-S–S-). Thus, quantitative estimation of thiol bonds in the GSH correlates with the level of oxidative stress. *E. coli* cells on treatment with 125 µg/mL of Ni/$NiFe_2O_4$–GO, GO and $NiFe_2O_4$ demonstrate that the highest reduction in GSH is observed in cells treated with Ni/NiFe2O4–GO, followed by $NiFe_2O_4$ and GO (Fig. 10III). However, cells without any treatment showed the least reduction in GSH. Therefore, it could be concluded that Ni/$NiFe_2O_4$ –GO nanocomposite induces oxidative stress-mediated microbial cell degradation. The further antibacterial effect induced by oxidative stress due to overexpression of ROS species in the *S. aureus* cells treated with different nanocomposites was demonstrated by fluorescence imaging of the treated cell stained with DCFH-DA (2′,7′-dichlorofluorescein diacetate) stain (Fig. 10I and II) [87, 125].

Shahraki et al. fabricated the nanocomposite consisting of rGO and carbon dots which demonstrated excellent antimicrobial activity against G + ve, *B. subtilis* and G − ve, *P. aeruginosa* [127]. It was demonstrated that following the adhesion of the material to the microbial cell wall, the formation of ROS occurs inside the cell which leads to damage to DNA and proteins, thereby impairing the metabolic functions of the bacterial cell. A similar antibacterial mechanism was demonstrated by the fabricated nanomaterial rGO–ZnO [128]. Synergistic antibacterial activity against G + ve MRSA, as well as G − ve, *S. typhimurium* and *E. coli*, was observed due to the generation of ROS under white-light illumination. Furthermore, GO-based Ag-doped ZnO nanorods showed improved broad-spectrum antibacterial activity against *S. aureus* and *E. coli* [129]. Lekshmi and coworkers proved that the flame-synthesized rGO–Ag–AgO–Ag_2O nanocomposites possessed antibacterial activity against *K. pneumoniae*, *E. coli*, and *S. aureus* which is induced by oxidative stress and photo-induced degradation [130].

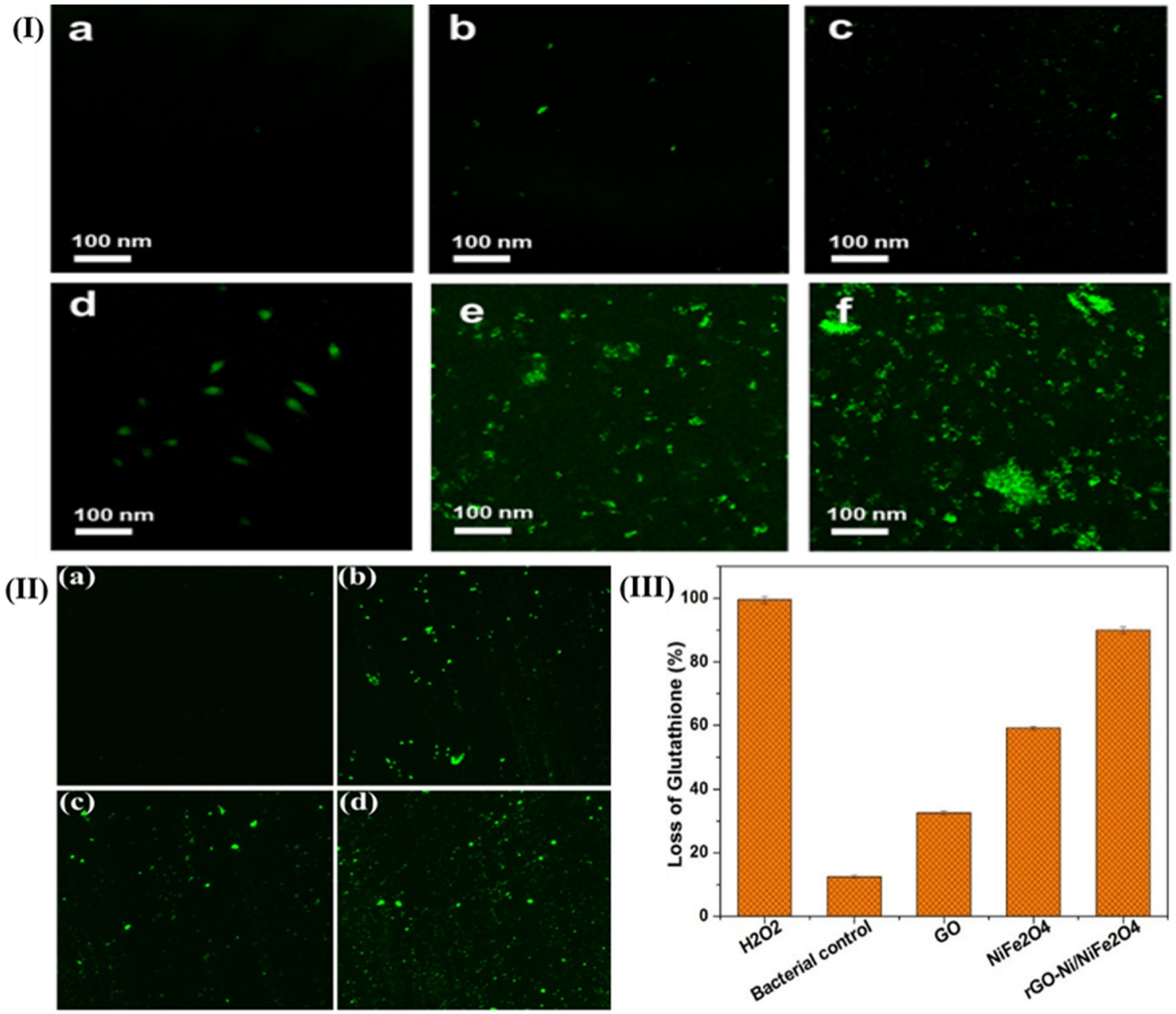


Figure 10 Determination of oxidative stress induced by graphene materials. (**I**) Fluorescence imaging for detecting ROS produced in treated microbial cells after staining with DCFH-DA stain. Results of DCFH-DA-stained *S. aureus* cells (**a**) without any treatment and treated with (**b**) GO, (**c**) AgNPs, (**d**) AgNPrsms, (**e**) GO–AgNPs, and (**f**) GO–AgNPrsms. (**II**) ROS production in S. aureus cells detected by DCFH-DA staining. Fluorescence imaging results of (**a**) untreated *S. aureus* cells, treated with (**b**) GO, (**c**) NCNCs, and (**d**) GO/NCNCs. (**III**) Level of GSH oxidation of in E. coli cells treated with GO, NiFe2O4 and rGO–Ni/NiFe2O4. (**I**) Reproduced from ref. [125]. Copyright 2023, MDPI. (**II**) Reproduced from ref. [87]. Copyright 2022, American Chemical Society. (**III**) Reproduced from ref. [75]. Copyright 2022, American Chemical Society.

ROS-independent oxidative stress

Even with the appealing concept of ROS-mediated oxidative stress, not all researchers tend to agree with it. Therefore, research continued to explore the antimicrobial oxidative stress mechanism of the G material assuming that their GMs' oxidative capability is connected to the charge transfer capability. ROS-independent oxidative stress is mediated by electron transfer from bacteria to GBNs. This electron transfer causes oxidative degradation of cellular components leading to bacterial killing. Upon sunlight simulation, electron–hole pairs are formed on the surface of G materials, which leads to the oxidation of cellular components by charge transfer [95]. These conditions lead to ROS-independent oxidative

stress causing the destruction of the bacterial antioxidant system and thereby bacterial cell death. Furthermore, it has been reported that G-metal composites may be able to remove electrons from microbial membranes more efficiently, causing the organisms to become less viable [67, 131]. Li et al. used *E. coli* and *S. aureus* cells to demonstrate the effect of antimicrobial activity of GNCs doped with three different metals, i.e. copper (Cu), germanium (Ge), and silicon dioxide (SiO_2), respectively [132]. The study findings revealed that G–Cu and G–Ge showed potent antibacterial activity because of the conductive nature of Cu and the semiconductive behaviour of Ge. But, due to the insulating behaviour, G–SiO_2 did not show effective antibacterial activity. Thus, it may be concluded that the electron transfer capability of the metal-doped G confers them with antimicrobial activity, in which GBNs act as electron acceptors leading to oxidative stress independent of ROS. Jeong and coworkers fabricated fluorine-functionalized rGO–TiO_2 nanocomposites to illustrate its antibacterial activity [133]. Irradiation with visible light leads to the prevention of charge separation between electrons and hole pair, leading to effective charge transfer. Charge transfer leads to ROS-independent oxidative stress and eventually causes bacterial cell death.

Conclusion and prospects

The present study discussed advances in G materials for antimicrobial applications. Mainly, we enthralled our discussion on the antimicrobial activity and mechanistic approaches of GBNs. These materials have aided nanotechnology and nanoscience to new horizons for their applicability as antimicrobial agents. Owing to their unique properties, these materials exhibit excellent antimicrobial activity, out of which certain captivating antibacterial as well as antifungal activities have been discussed here. Details of the mechanistic approaches such as membrane stress, photocatalysis, photo-thermal ablation, and oxidative stress have been elaborated with the fascinating research findings and impressive (eye-catching) images. Potential factors affecting these antimicrobial activities have been discussed and highlighted, which will further aid the scientific community to overcome the hurdles generated during the design of these antimicrobial agents.

Concerns about the biosafety of these materials, particularly in terms of their potential toxicity to living organisms, have induced substantial concerns. A lot of work has been carried out on the horizon of GBNs, especially related to their biosafety, and the outcomes have mostly been promising and significant. However, several issues still need to be resolved to make the GBNs more suitable in the various dimension. One of the critical concerns is the ability of these materials to accumulate inside the body over time. Studies have shown that they can accumulate in the lungs and liver, but the long-term effects of this accumulation are not yet known [134, 135]. Another hazard is the potential of these materials to harm cells and induces inflammation [136]. The degree of the damage caused to cells remains unknown; however, studies have indicated that it might lead to oxidative stress and inflammation [137]. There is also a concern that they could affect the behaviour of cells and tissues. Studies have shown that graphene oxide can affect the behaviour of cells in the brain, but the long-term effects are not yet known. Further, more research is needed to fully understand their long-term effects on living organisms.

Despite the extensive research on the mechanism of antimicrobial action of GBNs, the present research is still far from conclusive results. It remains unclear whether either GBNs exhibit ROS-mediated oxidative damage, facilitate charge transfer, or function as direct oxidant. More insight into the wrapping mechanism is needed as to how could a micrometre-graded bacterial cell be wrapped and inactivated/killed by its thousand times smaller, i.e. nanometre-sized G. Insight into the agglomeration concept of antimicrobial activity is needed as the agglomeration of the G sheet reduces the antimicrobial activity, while agglomerated rGO traps the microbial cell and enhances the antimicrobial activity. So, it is a relatively interesting and important area requiring in-depth analysis and comprehensive research which would pave the way for an improved understanding of the molecular mechanism of the antimicrobial process.

Data and code availability

Data and code availability is not applicable as no dataset was generated or analysed during the study.

Author contributions

SB and VP conceptualized the study; SB, RP, and MP reviewed the literature and wrote the original draft; SB and RP were involved in review and editing; MP and SB were involved in graphical illustration; SB, RP, and VP contributed to the final draft. All authors reviewed and approved the final version of the manuscript.

Declarations

Conflict of interest All authors declare that they have no conflicts of interest.

Ethical approval Not applicable.

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