

## Role of Graphene-related 2D Nanomaterials in Antimicrobial Potentials: An Overview

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### Abstract

Microbial infections profoundly impact various facets of everyday life, imposing significant economic strains on healthcare systems worldwide and resulting in death. Researchers have made considerable attempts to restrict microbial proliferation, but effective antimicrobial agents still need to be improved. A highly effective strategy for mitigating this challenge involves utilizing antimicrobial materials with chemically embedded or inherent antimicrobial properties. Recently, carbon-based nanomaterials have shown promising antibacterial results. In particular, graphene- and graphene-derived nanomaterials (GMs) demonstrate a broad range of antimicrobial activity against bacteria, fungi, and viruses. These antibacterial activities are attributed mainly to the direct physicochemical interaction between GMs and bacteria that cause deadly cellular component degradation. GMs hold a high affinity for accumulating, leading to membrane damage; similarly, after internalization, they can interact with the bacterial genome, disrupting the replicative stage. Additionally, GMs can indirectly determine bacterial death by activating the inflammatory cascade after entering the physiological environment. This mini-review delves into the potential parameters influencing antimicrobial efficacy, encompassing the number of graphene layers, concentration, size, and structural characteristics. Additionally, it explores the antimicrobial mechanisms exhibited by the graphene family against a spectrum of pathogens. Finally, it presents various antimicrobial applications underlying GMs as promising materials applicable in different fields.

**Keywords:** Graphene; Graphene oxide; Reduced graphene oxide; Nanomaterials; Antimicrobial; Mechanism

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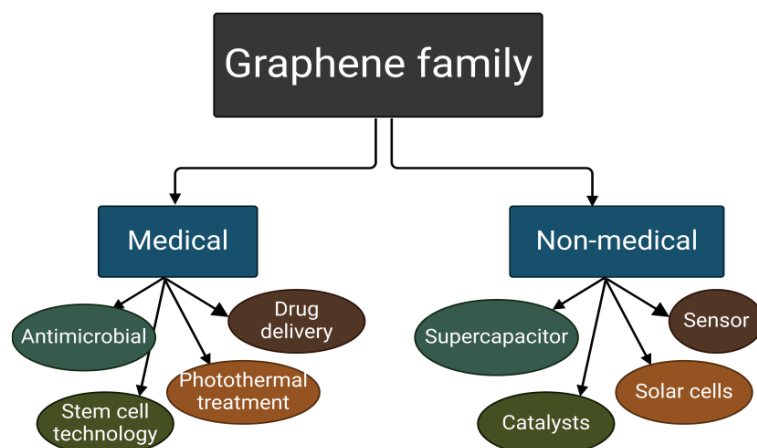
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## 1. Introduction

In the field of healthcare, it has been suggested that the development of resistance to antibiotics and other chemicals can reduce their effectiveness in treating microbial diseases [1, 2]. Microorganisms use several cellular and molecular pathways to facilitate the emergence of antibiotic resistance. Principal antibiotic resistance mechanisms include P-glycoprotein-mediated efflux of the drug, the emergence of resistance to absorption or penetration into microbial cells, endospore/biofilm formation, enzymatic microbial metabolism inactivating therapeutic agents, and mutation or inactivation of target molecules. Therefore, it is imperative to accelerate the process of searching for novel, effective agents to improve treatment effectiveness [3, 4]. The rising challenge posed by antibiotic resistance in treating infectious diseases underscores the urgent

need for alternative therapeutic agents that offer both efficacy and safety for human health [5].

Carbon-based nanomaterials have garnered significant interest from researchers across the globe in recent decades because of their affordability, simple synthesis, and compatibility with living organisms. Recently, extensive research has been devoted to investigating graphene-based nanomaterials, which are distinguished from other carbon materials by their exceptional mechanical flexibility and thermostability [6, 7]. Graphene-based nanomaterials (GBNs) exhibit potential in various domains, including stem cell technology [8], photothermal treatment [9], drug delivery [10], and biosensors [11, 12]. Furthermore, photocatalysts, microbial sensing, and biomarker sensing have also been investigated [13-15]. Figure 1 illustrates some of the mentioned uses of GBN.



**Figure 1.** Different applications of graphene-based nanomaterials.

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Furthermore, it is widely recognized that GBNs possess antimicrobial properties and are lethal to certain microorganisms. Potential pathogen inhibitors include graphene oxide as well as reduced graphene oxide. Nevertheless, the development of GBNs for antimicrobial applications is still in its early stages [16]. Graphene, among the diverse carbon allotropes, is garnering extensive research attention due to its unique properties, such as extracellular biodegradability, remarkable specific surface area, and exceptional mechanical strength [17]. Scientific literature highlights graphene-based nanomaterials for their various antibacterial agent against both Gram-positive and Gram-negative bacteria, including wrapping, membrane stress, and oxidative stress [16]. Despite the limited studies focused on graphene, *in vivo* and *in vitro* experiments unequivocally demonstrate the efficacy of graphene-loaded nanomaterials in preventing pathogenic fungal infections [18]. Furthermore, graphene and its derivatives have experienced a surge in recent years, particularly in advancing personal protective equipment to combat infectious diseases transmitted by enveloped and non-enveloped viruses, such as norovirus, coronavirus, and Ebola virus [19]. This mini-review aims to summarize recent developments in our knowledge of GBNs' potential as antimicrobials, particularly emphasizing their antiviral, antifungal, and antibacterial importance as nanotherapeutics. It starts by describing the GBN synthesis process, then outlines GBNs' antimicrobial action against bacteria, fungi, and viruses, and finally, describes different antimicrobial applications.

## 2. Graphene-based nanomaterials

Graphene (G) comprises a single layer of atomically thick, planar  $sp^2$  carbon atoms arranged as a honeycomb [20]. It possesses remarkable electronic, mechanical, and thermodynamic characteristics; its electron mobility is exceptionally high, and its surface area is enormous [21]. Although it exhibits various distinctive properties, there are some limitations for applications requiring specific characteristics [22]. A significant drawback is graphene's

propensity to aggregate, which is attributed to its hydrophobic nature, substantial surface area, and elevated surface energy, all of which contribute to its exceedingly low water dispersibility [23]. Chemical modifications were regarded as effective methods for enhancing graphene properties, forming graphene derivatives with wide-ranging applications in various domains such as sheets, platelets, ribbons, and quantum dots [24] as shown in Figure 2. Scientific literature has extensively explored graphene oxide (GO) and reduced graphene oxide (rGO) nanosheets compared to their equivalents for a number of reasons [25]. The functional groups added to GO's basal planes (hydroxyl, epoxy, carbonyl, or carboxyl) give numerous benefits due to its heterogeneous electronic configuration, including fluorescence at a wide wavelength range [25, 26] as well as water dispersibility, which is required for biomedical purposes [27]. In addition to being easily changed by chemical processes, they may be employed as fillers for polymeric or inorganic nanoscale materials [28]. GO is soluble in water and organic solvents, has a hydrophilic structure, and is readily available from Gt [29]. Functional oxygen groups on GO's surface contribute to its excellent solubility in various solvents. Specific delivery of rGO and GO to the target site can be enhanced by functionalizing them with many biomolecules, including antibodies, DNA, and RNA [30]. Moreover, their oxidation capacities and direct contact with membrane stress indicated their potent antimicrobial activity [31].

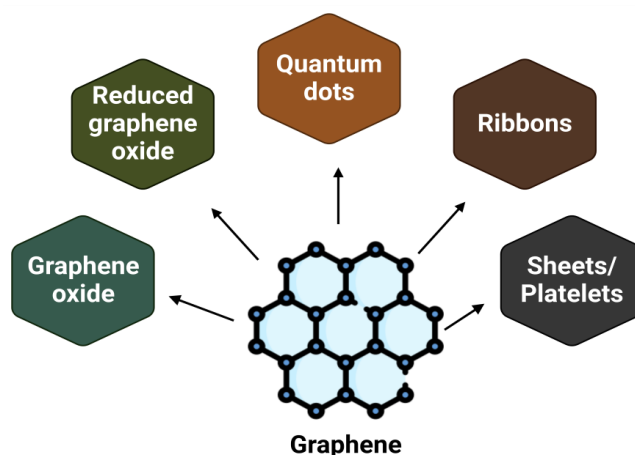
An additional graphene derivative, porous graphene (PG), possesses graphene nanosheets with a mesoporous or microporous structure and a high specific surface area. Furthermore, its excellent absorption ability promotes the flow of molecules and ions [32]. Another derivative is graphene foam, which is a three-dimensional graphene structure with a large surface area. The three-dimensional graphene's electrical and electrochemical features with variable porosity are exceptional. Although its high surface area and biocompatibility typically draw researchers' attention to the development of biosensors [33].

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Carbon dots are emerging as an important class of antimicrobial agents, in addition to graphene and its derivatives, which serve as antimicrobial agents. Despite a few side effects, graphene nanostructures are effective antimicrobial agents because of their substantial biocompatibility [34, 35].

It is also notable that 0D nanomaterials derived from graphene, such as graphene quantum dots (GQD), have been applied as antibacterial agents. Their notable attributes include superior

biocompatibility, hydrophilicity, and stability [36]. GQD nanocomposites, including silver nanoparticles, have been the subject of increased research in recent years [37]. Considering Gram-negative and Gram-positive bacteria, the antibacterial activities of silver-graphene quantum dot, AgNPs, and pure GQDs were evaluated. Compared to other materials, Ag GQDs exhibited efficacy in preventing bacterial growth [38].



**Figure 2.** Different types of graphene family.

### 2.1. Synthesis of graphene and its derivatives

Many techniques have been described for synthesizing graphene-based nanomaterials. The prevailing graphene production approach involves the use of adhesive tape to partition thin Gt layers [39]. Graphene processing techniques can be broadly categorized into two distinct classes: top-down and bottom-up (Figure 3).

#### 2.1.1. Top-down techniques

**Mechanical exfoliation:** This approach allows layer formation by overcoming van der Waals binding interactions within G layers in bulk graphite (Gt) [40, 41]. The process introduced by Andre Geim and Konstantin Novoselov is the initial attempt to create G layers from bulk Gt [42]. Inspired by the scotch tape approach, 3-roll milling using an adhesive material has proven effective in synthesizing graphene layers with a thickness of 1.13-1.41 nm [42]. Results show that this approach is effective for graphene-polymer when using a suitable polymer.

**Oxidative exfoliation and reduction:** GO is primarily obtained via oxidative exfoliation. To obtain rGO, reducing oxygen functional groups could be achieved thermally, electrochemically, and chemically [43] but reducing GO using plant extracts is the most eco-friendly and alternative route to minimize the harmful effects of toxic chemicals used in reduction process [44-46]. On the other hand, chemical oxidation is the most commonly applied GQD production technique due to its simplicity and efficiency [47]. Other techniques include Hummer's method (utilizing  $\text{NaNO}_3$  and  $\text{KMnO}_4$  with  $\text{H}_2\text{SO}_4$ ), the Staudenmaier's method (using  $\text{HNO}_3$  and  $\text{KClO}_3$ ), and the Hofmann's method (involving concentrated  $\text{HNO}_3$  and  $\text{KClO}_3$ ) [48].

**Liquid-phase exfoliation:** LPE, another top-down method for graphene production, is crucial. This process disperses Gt in an appropriate liquid, exfoliates it, and then uses high-intensity ultrasound to get pure graphene [49]. This process yields

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graphene-based nanomaterials and films used in biosensors and flexible electronics.

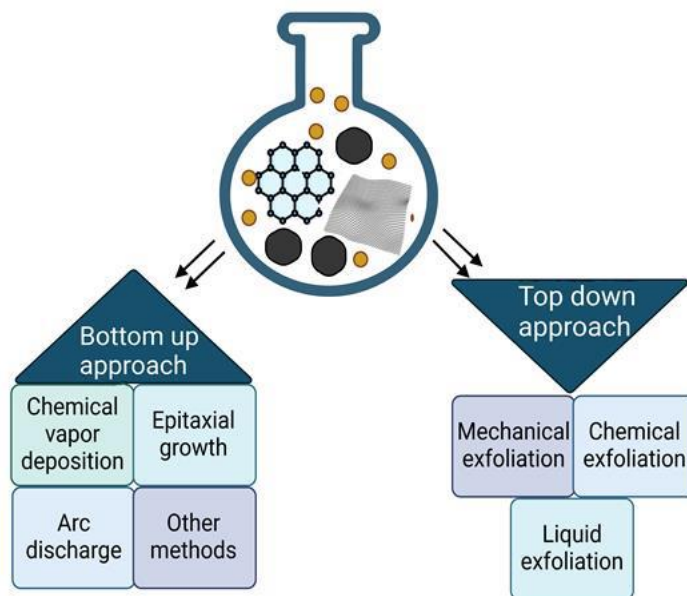
### 2.1.2. Bottom-up techniques

**Chemical vapor deposition:** This approach produces superior-quality, large-surface-area G nanosheets without defects. The metallic substrate is heated to 1000 °C to generate G sheets utilizing hydrocarbon gases as well as renewable materials [50]. Additionally, this method forms honeycomb graphene sheets by combining carbon atoms. Using liquid rather than solid metals allows for efficient, homogeneous graphene formation [51].

**Epitaxial growth:** Graphene may also be synthesized in a vacuum at 1200–1600 °C on silicon carbide (SiC). Si sublimates as well as graphene is developed by gathering carbon atoms and creating SP<sup>2</sup> at elevated temperatures [52, 53]. Sizes, prices, and

micromachining complicate this technology. It is possible to obtain graphene sheets of adjustable thickness and high-quality materials. The epitaxial graphene growing process adjusts the number of graphene layers based on temperature changes [53].

**Other bottom-up techniques:** Recently reported bottom-up approaches include arc discharge [85] and CNT unzipping. Arc discharge has been utilized to synthesize CNTs and fullerene, but now it's employed to make a few-layered graphene. CNT unzipping also produces graphene with a few layers and a single layer [54]. In bottom-up GQD production, hydrothermal, solvothermal, and ultrasound-assisted processes are used. Industry restrictions, including time and high temperatures, make these processes less preferred for large-scale production [55].



**Figure 3.** Synthesis approaches for graphene and its derivatives.

## 3. Surface modifications of graphene

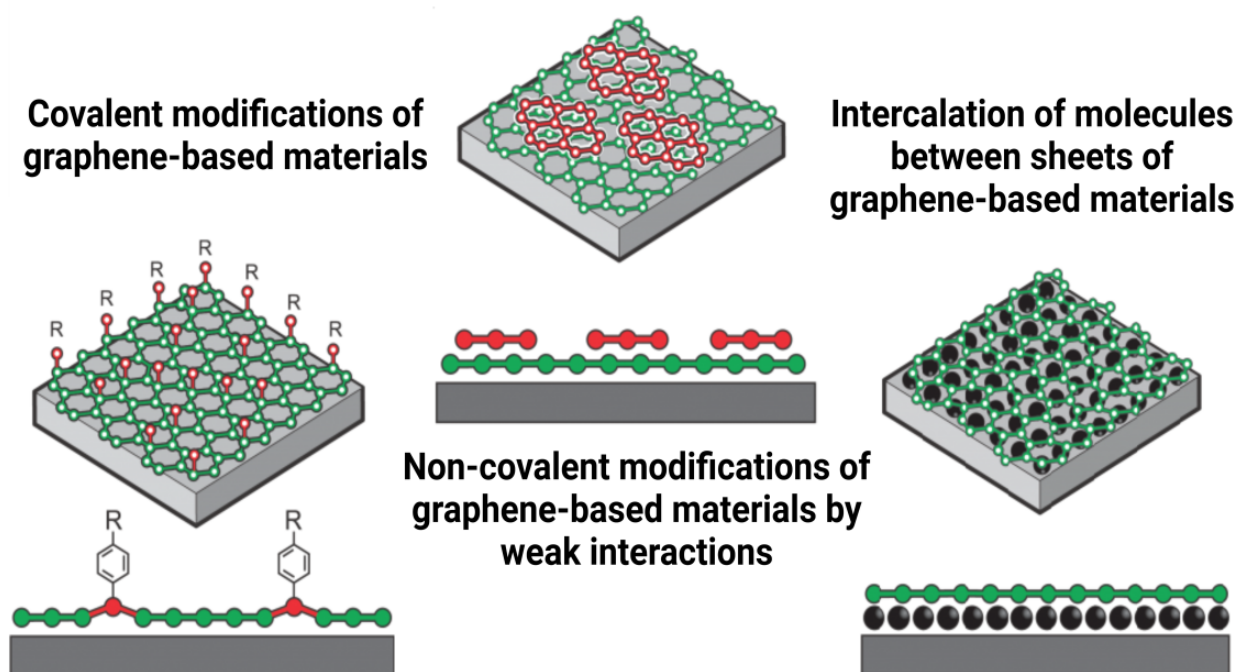
Graphene and its derivatives are widely used in various fields; however, unmodified G has limitations. In vivo, it lacks targeted, delayed, and controlled release abilities. With its charge-shielding characteristic, GO tends to agglomerate in the physical environment [56]. It also has a substantial protein adsorption capacity that macrophages quickly identify, ingest,

and consume in living cells, causing inflammation [57]. These drawbacks hinder the biomedical use of G derivatives. Increasing water solubility and stability and adding sophisticated features such as targeted, gradual, and controlled release are crucial for expanding biological applications [58]. The increased interest of scientists has led to functional alterations of G and its derivatives using metals, polymers, or composites to investigate

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their possibilities [59]. Oxygen-containing groups can be used as catalytically active centers for surface functionalization with small or macromolecules [60]. Covalent, non-covalent, and intercalation methods are capable of producing graphene-based nanomaterials [61] (Figure 4). Covalent modification involves the addition of reactive functional groups, double bonds, and polymers to the surface. The physicochemical properties of GO are enhanced by covalent modification with reactive oxygen-containing functional groups [62]. In non-covalent functionalization, electrostatic forces,  $\pi$ - $\pi$  interactions, and van

der Waals interactions generate nanomaterials of G and polymeric compounds [63]. Although it exhibits reduced stability both in vitro and in vivo, it improves functional group and structure association with G derivatives [63-65]. This enhances capabilities, including dispersion, reactive activation, and biosensor performance. Finally, the intercalation process encompasses arc discharge, ion bombardment, and annealing by heat treatment. These methods introduce an element into G, GO, or rGO, replacing diverse structural defects while preserving G's intrinsic two-dimensional structure.



**Figure 4.** Diverse approaches for the functionalization of graphene-related materials.

#### 4. Antimicrobial Activity

Graphene-related 2D nanomaterials have recently gained attention as prominent contenders in current applications, playing a crucial role in the medical and biological fields due to their ability to induce biosensing effects and establish direct interactions with different types of cells, including bacteria, fungi, viral, tumor and mammalian cells [66]. Much effort has been devoted to elucidating the broader therapeutic applications of GMs, extending beyond diagnosis and treatment to encompass microbial infections in light of their distinctive

structure and properties observed in fields such as electronics, optics, thermotics, and mechanics [67]. Despite advancements in the study of GM's antimicrobial properties, the mechanisms underlying these effects remain controversial. Nonetheless, several recent experimental findings indicate that the primary mechanisms are significantly influenced by the physicochemical properties of G-based materials [68, 69]. Table 1 summarizes the physicochemical features of multiple G materials in addition to the potential antibacterial effects, which are explored in the following subsections.

Table 1. Physicochemical characteristics and antimicrobial actions of various G-based materials.

Mechanism	G-based materials	Concentrations	Microorganisms Prevented	References
Stress on cell membranes	GO	1, 0.5, 0.25, 0.13, 0.065, 0.032, 0.016, 0.008, and 0.004 µg/µL	G-: <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>P. mirabilis</i> , <i>S. marcescens</i> , and G+: <i>S. aureus</i>	[70]
	Gt, GtO, GO, and rGO	0.040 µg/µL	G-: <i>E. coli</i>	[71]
	GO, rGO	1, 2, 3, 5 µg/µL	G+: <i>S. aureus</i> and G-: <i>P. aeruginosa</i>	[72]
	GO, rGO	0.02, 0.85 µg/µL	G-: <i>E. coli</i>	[73]
	GO	0.0085 µg/µL	G-: <i>P. putida</i>	[74]
	GO	0.1 µg/µL	G-: <i>E. coli</i>	[75]
	GO	0.2 µg/µL	G-: <i>E. coli</i>	[76]
	GO	0.01 µg/µL	G+: <i>S. aureus</i> , <i>E. faecalis</i> and G-: <i>E. coli</i> , <i>P. aeruginosa</i>	[77]
Mechanical wrapping	GO	50 mg/L	G-: <i>P. aeruginosa</i> ; G+: <i>S. aureus</i> ; Fungus:	[78]
	GO	1, 0.5, 0.25, 0.13,	<i>C. albicans</i>	[70]
Oxidative stress	GO and rGO	0.065,	G-: <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>P. mirabilis</i> , <i>S. marcescens</i> , and	[79]
	GO	0.032, 0.016,	G+: <i>S. aureus</i>	[80]
	GO	0.008, and 0.004 µg/µL	G-: <i>P. aeruginosa</i>	[81]
	Gt, GtO, GO, and	0.025, 0.05,	G-: <i>E. coli</i>	[71]

#### 4.1. Factors influencing antimicrobial performance

G's physical and chemical properties significantly impact its antimicrobial activity [72]. Figure 5 highlights the factors affecting G and its derivatives' antimicrobial efficacy.

##### Bacterial type and shape:

Several studies suggest that GO and rGO can inhibit Gram-positive and Gram-negative bacteria growth; the effectiveness of the antibacterial action depends on the type, size, and shape of

the bacteria [72, 82]. Nanoparticles kill Gram-positive bacteria less effectively than Gram-negative bacteria because of their cell structure. Gram-positive bacteria cells have a 20–80 nm-thick peptidoglycan coating, preventing nanoparticle entry. Gram-negative bacterial cells possess peptidoglycan in a single layer (7–8 nm) that cannot deter nanoparticles from entering the cell [83]. GO has a greater impact on G+ bacteria (*S. aureus*) than on G- ones (*P. aeruginosa*). *P. aeruginosa* cells demonstrate susceptibility to rGO owing to their extended and curved morphology, notwithstanding the existence of a barrier within

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their membranes formed from phospholipids as well as lipopolysaccharides. *S. aureus* cells are less susceptible to rGO due to their spherical shape and reduced surface area [72]. Bacterial morphologies such as spirals, bacilli, filaments, or cocci may influence susceptibility to antibiotics [84]. Because of their sharp edges, rGO sheets were tested for antifungal activity against *A. oryzae*, *F. oxysporum*, and *A. niger* [85].

### **Oxidative stress mediation:**

Many studies on GO and rGO nanosheets have revealed distinct antibacterial properties compared with graphene. While rGO possesses a more extensive oxidation capability, smaller lateral GO layers offer better antibacterial characteristics [86, 87]. Iron oxide nanomaterials contain hydroxyl radical functional groups that promote wound healing due to oxidative stress, according to Pan et al. [88]. The antibacterial impact of the GO-loaded PMMA nanomaterial against *E. coli* was high [89]. Based on these results, GO may kill bacteria through chemical oxidative stress.

### **Graphene sheet size:**

The size of the GO sheets considerably impacts their antibacterial action. Surface coatings formed from smaller GO sheets are very antibacterial [86]. Low-size GO sheets have antibacterial action due to oxidative processes driven by higher defect density. The GO sheet area affects cell suspension bacteria growth due to cell entrapment. This method enhances antibacterial activity with sheet area [90]. For larger GO sheets, bacterial cell isolation and biocompatibility are higher [91]. Numerous researchers have attempted to determine the effect of varying the sizes of GO and rGO flakes on their cytotoxicity levels [92, 93]. However, the precise correlation between cytotoxicity and the interactions between scaffold surfaces of various GO and rGO-derived three-dimensional morphologies and their dimensions remains unknown. It is common knowledge that graphene derivative flake size influences cytotoxicity. Smaller flake sizes are highly toxic, display greater cellular uptake, and adversely impact cell performance [94].

### **Number of graphene layers:**

It influences the activity of the material as an antibacterial agent. Through molecular dynamics simulations, Wang et al. determined that bacterial lipid penetration through nanosheets of graphene comprising three layers was more difficult than through sheets containing a single layer of equivalent lateral dimensions. This finding suggests that monolayer graphene sheets have more antibacterial action [95]. Increasing the number of layers may cause graphene aggregation, thereby reducing bacterial–contact. Graphene nanosheets with fewer layers exhibit enhanced antibacterial activity [96].

### **Surface modification effect:**

The tendency of pristine graphene to aggregate may limit molecular interactions. This aggregation reduces graphene's interaction with proteins, lipids, and DNA, reducing its antibacterial properties. Aggregated graphene flakes were expected to enhance antibacterial action [97]. G and its derivatives with oxygen-containing functional groups exhibit enhanced antibacterial capabilities due to changes in surface characteristics [28]. Graphene derivatives, metal composites, and graphene-based devices embedded in polymer fibers are thought to have improved biocidal activities because of surface modifications [31].

### **Graphene time and concentration:**

Numerous studies have examined the effects of graphene-based materials' time and concentration on antibacterial activity. According to Liu et al., most bacteria are inactivated within the first hour of incubation [98]. By exposing *E. coli* cells to GO suspensions containing concentrations of 80, 40, 20, 10, and 5  $\mu\text{g/mL}$ , the bacteria's susceptibility to GO was increased. 80  $\mu\text{g/mL}$  killed approximately 90% of the microorganisms [71, 79, 99, 100]. Similarly, 80  $\mu\text{g/mL}$  rGO treatment eliminated 76.8% of bacterial cells [71]. Increased concentrations of G-based materials (25–200  $\mu\text{g/mL}$ ) were also assessed for antibacterial activity [79]. *P. aeruginosa* gradually lost viability at dosages of 75  $\mu\text{g/mL}$  for GO and 100  $\mu\text{g/mL}$  for rGO. These results indicate a threshold of 80  $\mu\text{g/mL}$  for GO (> 90% antibacterial activity).



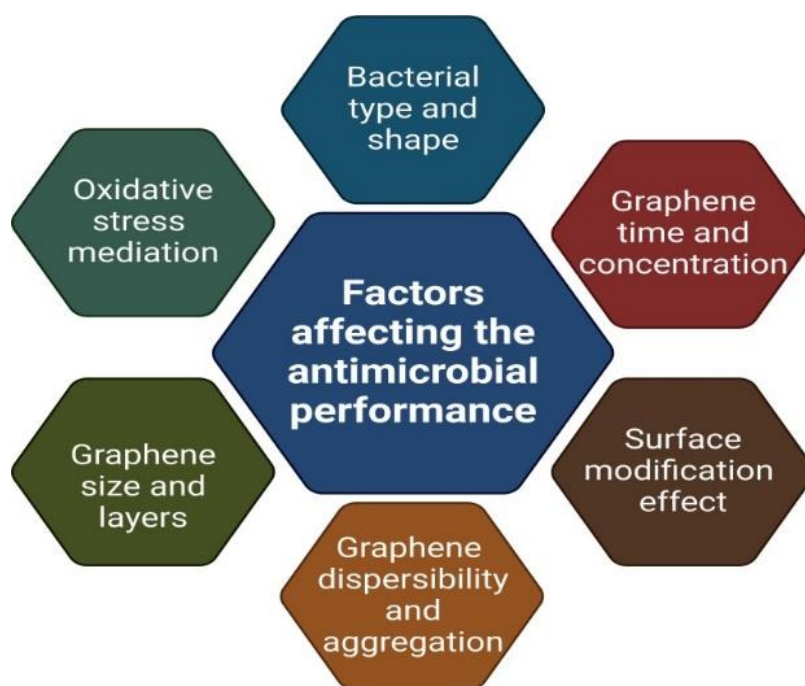
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The rGO threshold concentration is 100  $\mu\text{g/mL}$  [101]. In addition, rGO inhibits fungus growth at IC50. *A. oryzae* and *A. niger* have IC50 values of 100  $\mu\text{g/mL}$ , but *F. oxysporum* has 50  $\mu\text{g/mL}$  [102].

### Aggregation and dispersibility of graphene:

High aggregation of graphene sheets is caused by van der Waals interactions and hydrophobicity, resulting in poor water dispersibility [103]. Chen et al., compared different graphene sheets for antibacterial effectiveness and found that dispersed GO had the highest antibacterial activity, followed by rGO, GtO, and Gt. Graphene-based materials' dispersion influences antibacterial activity [71]. Bacterial interaction is modulated by GO concentration, dispersibility, charge, and aggregate characteristics in various media; consequently, GO exhibits diverse antimicrobial properties [87]. Low GO concentrations ( $< 6.0 \mu\text{g/mL}$ ) showed robust antibacterial activity in all solutions. Non-aggregated GO mechanically disrupts bacterial

cell membranes, causing intracellular material outflow and cell death. Increasing GO concentration yielded different results. Studies on GO in deionized water showed structural stability up to 200  $\mu\text{g/mL}$  concentration. A higher GO concentration leads to enhanced antibacterial activity within these limits. The size of aggregates formed by GO in the existence of salts relies on the concentration of GO and the type of cation. GO forms floating scaffolds and aggregates in a 25–50  $\mu\text{g/mL}$  saline solution, increasing bacterial growth. Bacterial cell development is impeded when the concentration of GO exceeds 50  $\mu\text{g/mL}$ . This inhibition is achieved through the formation of sizable GO aggregates, which adhere firmly to the membranes of bacterial cells when divalent cations are present [98]. Reducing GO nanosheets results in rGO sheets that are nine times larger than GO nanosheets. Thus, larger rGO particles aggregate well, reducing their antimicrobial activity [86].



**Figure 5.** Several elements affect the antimicrobial efficacy of graphene-related 2D nanomaterials.

## 4.2. Antimicrobial mechanisms of graphene-based materials

Several studies have shown that graphene and its derivatives have antimicrobial properties, prompting discussion on their mode of action as illustrated in Table 2 [104].

**Table 2. Mechanisms of action for bacterial, fungal, and viral inhibition displayed by graphene-related 2D materials.**

Microorganisms	Action mechanism	References
<b>Bacteria</b>	<ul style="list-style-type: none"> <li>• Nanoknife effect</li> <li>• Lipid extraction</li> <li>• Pore formation within cell membrane</li> <li>• Mechanical wrapping</li> <li>• Photothermal ablation</li> <li>• ROS dependent oxidative stress</li> <li>• ROS independent oxidative stress</li> </ul>	[39, 105, 106]
<b>Fungi</b>	<ul style="list-style-type: none"> <li>• Forming spore deposits on the surface</li> <li>• Water uptake prevention</li> <li>• Inducing plasmolysis.</li> </ul>	[19, 107]
<b>Virus</b>	<ul style="list-style-type: none"> <li>• Contact mediated damage to envelope or spike protein.</li> <li>• charge effect (positively charged viral particles adsorb onto negatively charged graphene plane).</li> </ul>	[108, 109]

### Mechanism of the antibacterial activity

The antibacterial capacity of graphene (G) is attributed to various mechanisms (Figure 6) [110], including oxidative stress production of ROS [111] and phospholipid extraction from bacterial membranes [75, 87]. Zhang et al. reported that oxidative stress affects GO cytotoxicity and that ROS generation in mammalian cells changes with the degree of GO oxidation. They indicated that GO significantly increased ROS levels, with GO particles with lower oxidation degrees generating more ROS. Lower oxidation levels of GO were associated with indirect oxidative damage by facilitating the breakdown of H<sub>2</sub>O<sub>2</sub> into hydroxyl radicals, as determined by ESR spectrometry analysis. Additionally, they increased the ability of cells to oxidize directly. Based on theoretical simulations, it was shown that the energy barrier of H<sub>2</sub>O<sub>2</sub> breakdown was affected by aromatic domain sizes in nanosheets and carboxyl groups [112]. TEM showed structural alterations in *E. coli* treated with GO nanosheets, according to Tu et al. [75]. Simulated membrane-

GO interaction confirms lipid extraction. The internal and external *E. coli* membranes display a GO symbolic structure, indicating that GO nanosheets extract phospholipids. Van der Waals interactions with GO and lipids in membranes drive extraction. After extraction, the hydrophobic interaction dominates. The unoxidized portions of GO interact with the hydrophobic tails of lipids through hydrophobic interactions. Meanwhile, electrostatic interaction leads to the interaction of hydrophilic lipid heads with unoxidized hydrophobic GO regions. Therefore, damaging lipid extraction and harsh G incorporation, which adversely affects the membrane, reduces cell survival. The concentration and dimensions of G determine cell viability [75].

Another mechanism called "insertion mode of action" involves G nanosheets cutting bacterial cells through their membranes, causing cell death by intracellular material leakage [113, 114]. Akhavan and Ghaderi observed that the biocidal properties of rGO and GO are due to their rough edges directly touching the bacterial surfaces of various strains [114]. Li et al. found that the

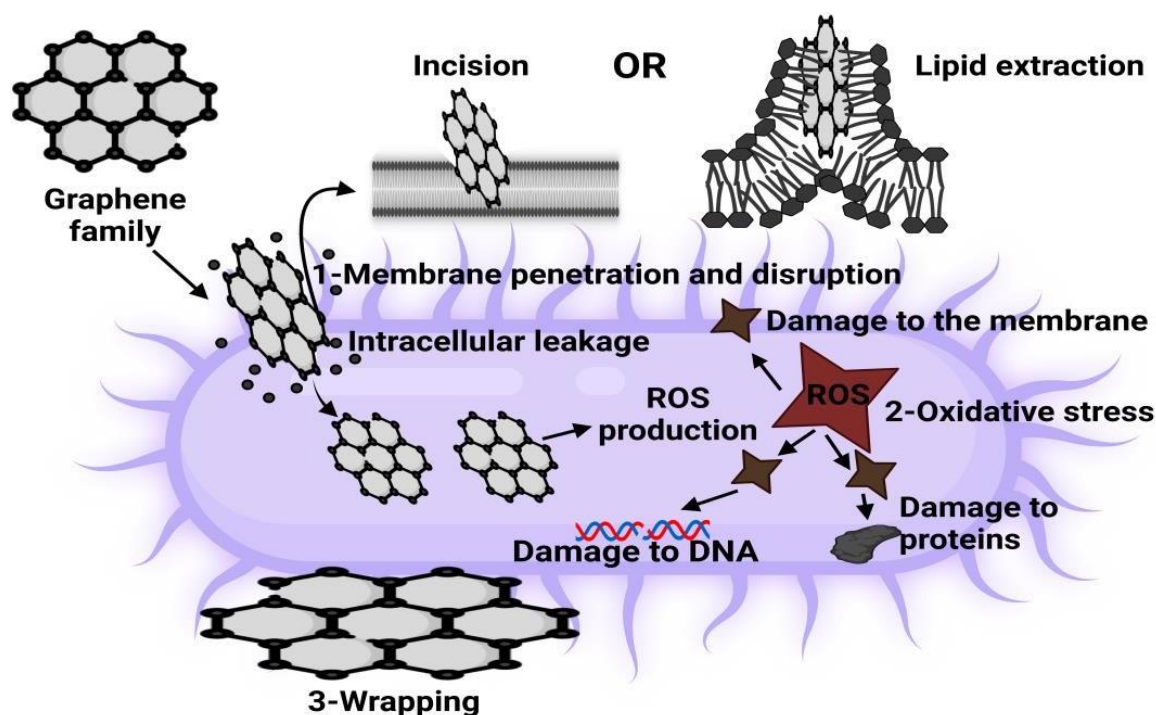
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antibacterial effect is caused by G sheets penetrating bacterial cell walls via lipid bilayers via their corners and rough edges [115].

Yi et al. observed that size affects G sheet integration into lipid bilayers. Micrometer-sized G ideally has a near-perpendicular arrangement to the cellular structures [116]. Because the hydrocarbon tail of a lipid interacts with G's flat lipophilic surface, G nanosheets sink between lipid tails and enter the cell membrane. Larger nanosheets diffuse more readily in the lipophilic region of the cell membrane by arranging themselves across it, whereas Dallavalle et al. found that smaller G sheets diffuse perpendicularly into the lipid membrane [117]. Pham et

al. studied the bactericidal properties of pure G to improve their understanding of G cytotoxicity through computer and experimental simulations [118]. They found that G-edge density causes a change in osmotic pressure, leading to cell destruction by generating holes in bacterial cells [113].

Researchers have hypothesized another mechanism for antibacterial action [119]. They revealed that G's flat surface disrupts the bonds between proteins in the cell membrane, causing functional protein dysfunction. Self-killing bacteria reduce GO to antibacterial G via glycolysis as their metabolic activity increases [120].



**Figure 6.** Schematic presentation of various antibacterial actions of graphene-related materials.

### Mechanism of action and antifungal properties of graphene-based materials

Fungi can easily spread their spores so they can infect material surfaces [121]. Fungal infection has a negative impact on human health and causes economic losses. Thus, it is necessary to have suitable materials that are resistant to fungi. An assessment was conducted on the antifungal properties of

various graphene-based materials, with a particular focus on G, GO, and rGO [122].

GO's antifungal effect is due to its sharp edges, which stress the plasma membranes of pathogenic cells. Chen et al. studied how GO interacts with fungal pathogens [123]. They found that GO suppresses cell swelling and lysing and approximately 80% of macroconidia germination. The antifungal characteristics of GO

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may be explained by the fact that GO nanosheet aggregation causes cell membrane stress, reduces cell membrane potential, and causes leaks. Because of its near-infrared photothermal treatment efficiency, GO is an effective photothermal material. Khan et al. used photothermal GO against fungi to prevent wound infection [124]. This inexpensive, non-invasive technique healed infected mouse dorsal wounds. The antifungal activity of GO was evaluated against *C. auris* as well as *S. cerevisiae*. The results indicated that GO activated by laser exhibits effective antifungal activity [124].

At concentrations varying from 0 to 600  $\mu\text{g/mL}$ , Zhu et al. investigated the toxic properties of GO against *S. cerevisiae* [125]. They concluded that GO toxicity is dosed dependent. GO toxicity against fungi results from reduced mitochondrial transmembrane potential and increased ROS production [125]. A study by Xie et al. examined GO's antifungal effectiveness by subjecting it to *P. chrysosporium* over a period of 7 days at a concentration of 0.4  $\mu\text{g/mL}$  [126]. According to their findings, low concentration levels of GO increased cell proliferation. Low GO concentrations also acidify the culture medium. Moreover, SEM analysis revealed that at a concentration of 4  $\mu\text{g/mL}$ , GO disrupts the fiber structure of *P. chrysosporium*, resulting in dense, elongated fibers [126].

Using rGO's cytotoxicity towards fungal pathogens may produce the broad-spectrum antifungal properties of graphene-based materials. Sawangphruk et al. found that a concentration of 0–500  $\mu\text{g/mL}$  of rGO effectively inhibited pathogenic fungi [126]. For *F. oxysporum*, rGO's IC50 is 50  $\mu\text{g/mL}$ .

### Antiviral capacity of graphene-based materials

Despite the existence of attenuated and inactivated vaccines, certain viral infections continue to occur because of mutated strains. Thus, the development of effective antimicrobial drugs is critically needed. Graphene and its derivatives inhibit viruses because of their remarkable antimicrobial capabilities [127, 128]. Scientists began studying graphene derivative antiviral properties in 2012 using several approaches. Researchers found that GO derivatives, partly reduced sulfonated-GO (rGO-SO<sub>3</sub>),

can treat HSV-1 infection by promoting cellular adhesion at low doses [129]. Akhavan et al. employed G-tungsten oxide thin films under visible light to induce viral photo-inhibition [120]. GO-functioning aptamer, a type of photosensitive GO and target recognition aptamer, was developed by Hu and colleagues as a new photocatalyst [130]. When exposed to radiation, this photocatalyst suppresses viral nucleic acids and protein capsids. This reaction led to the oxidation of nucleic acid bases, particularly guanosine [130]. Furthermore, deoxyribozyme is carried intracellularly via GO, which targets HCV mRNA to block HCV gene replication [131].

The superior antiviral activity of rGO and GO compared with Gt and GtO can be attributed to their distinct monolayer structure and negative charge, which suggests the presence of a nanosheet structural association. In addition, GO mechanically destroys viruses before entering [132]. G derivatives' possible deactivation effects and mode of action on various viruses have not been investigated [127].

### 4.3. Antimicrobial activities of graphene-based nanomaterials

Antimicrobial nanomaterials of G and its derivatives are potentially effective. Graphene-attached antimicrobial nanomaterials are more stable and diffuse [133]. A variety of materials are used in the fabrication of these nanomaterials. These include metals [134], metal oxides [135], polymers [136], quaternary phosphonium salts [137], and EDTA [138].

#### Graphene-metal nanomaterials

In bacterial treatments, silver (Ag) has been used for thousands of years [139]. Ag ions can permeate bacterial cell membranes and inactivate enzymes, killing cells [140]. AgNPs destroy bacterial cell membranes. Ag ions and AgNPs destroy bacteria. However, when bare AgNPs interact with bacteria, they aggregate, resulting in a reduction in their surface area and a subsequent decline in their antibacterial activity [141]. This problem was solved by preparing G and AgNP nanomaterials using various reducing agents [142]. Using mixed additives, Das et al. [70] synthesized rGO-Ag by reducing Ag ions on GO

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sheets [143]. These nanomaterials showed improved antibacterial activity, suggesting their utility as antimicrobial agents. Maintaining AgNP dispersion in an aqueous solution is possible with GO support. A synergistic action gives the GO-Ag nanomaterial significant antibacterial properties. In the presence of GO-Ag, the decreased charge on the surface makes GO and AgNPs more likely to make contact with cells [144].

Copper (Cu), a cost-effective antibacterial agent, destroys microbes rapidly on its metallic surface. CuNPs can damage cells by oxidizing lipids and proteins, causing most bacteria to be destroyed [145]. Their aggregation, however, prevented their practical use as antibacterial agents. Increasing CuNP stability and controlling  $\text{Cu}^{2+}$  release are crucial [145]. To resolve this obstacle, Ouyang et al. developed a rGO-Cu composite by attaching CuNPs to a PLL-modified rGO surface [146]. The hybrid demonstrated significant prolonged bactericidal efficacy and water solubility, making it ideal for antimicrobial use [146]. Additionally, antibacterial agents have been formed by combining gold (Au) with GO or rGO [147]. Das et al. designed eco-friendly rGO-Au that demonstrated better bactericidal properties than GO nanosheets. As rGO nanosheets caught bacteria, AuNPs impaired their permeability, causing sugar and protein leakage and cell death [148]. The authors linked antibacterial activity to oxidative stress in antioxidant systems and membranes [148].

The damaging impact of G is attributed to its direct interaction with the bacterial membrane, in addition to its "insertion mode of action" [123, 149]. Studies have examined the attachment behavior of two *E. coli* strains with CVD gold (Au) coating, LF82 and UTI89. It is inferred that these interfaces lack antibacterial action due to the absence of membrane degradation or morphological alterations in SEM figures. Conversely, invading and adhering *E. coli* (LF82) spread rapidly in biofilms. According to Li et al. the electronic characteristics of the substrate significantly impact the damaging action of G-coated surfaces [150]. G films covered with substantial portions of Ge and Cu impeded microbial development and led to mortality, but

those on  $\text{SiO}_2$  did not. Easy electron transport is a significant antibacterial action of Cu and Ge. G on substrate junctions pumps electrons from the bacterial membrane, causing oxidative stress [150]. According to Mangadla et al., the antibacterial effectiveness of graphene is not impacted by its sharp edges. Nevertheless, bactericidal activity is affected by the interaction with *E. coli* and GO's basal plane [149]. It was discovered that 89% of *E. coli* could be killed by a GO film produced via Langmuir-Blodgett deposition, a technique that primarily restricts contact with the sharp edges of GO. Furthermore, it has been verified that hiding the basal plane of the GO diminishes its ability to inhibit bacteria as it reduces their direct contact with the GO [149].

### Graphene-metal oxide nanomaterials

Electron-hole pairs diffuse to the surface of a semiconductor metal oxide when irradiated by visible light, which is more significant than its bandgap energy. Positive holes respond to water to make hydroxyl radicals, whereas negative electrons form superoxide anion with oxygen [151]. Both types of ROS are capable of killing bacteria. Recent studies have described nanomaterials made of G and semiconductor metal oxides, resulting in improved photocatalytic properties [152]. Hybrid graphene-metal oxide semiconductor composites provide several benefits. G can greatly slow charge carrier recombination. In addition, the photocatalysts' reaction edge was shifted red from the UV to the Vis range [153]. In addition, graphene generates ROS and absorbs UV/Vis, boosting Vis light activity.

$\text{TiO}_2$  stands out as the most promising metal oxide semiconductor photocatalyst due to its chemical stability and efficiency [154]. According to Akhavan et al. G can be more effective in photo-inactivating *E. coli* under solar illumination using films made from rGO- $\text{TiO}_2$  [155]. A bare  $\text{TiO}_2$  sheet had 7.5-fold lower antibacterial activity than rGO- $\text{TiO}_2$  after 4 h of UV irradiation. rGO platelets may efficiently suppress charge carrier recombination by accepting electrons from  $\text{TiO}_2$  stimulated by UV and functioning as electron sinks [155]. Two-

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phase assembly produced gram-scale GO-TiO<sub>2</sub> nanorod materials by Sun et al. [156]. GO-TiO<sub>2</sub> nanomaterials outperform basic TiO<sub>2</sub> nanorods in photocatalytic and antibacterial activity against *E. coli* under solar-simulated light [156].

The semiconductor photocatalyst ZnO is utilized as an antimicrobial material. Some inherent flaws restrict its photocatalytic activity. Due to its broad bandgap, it can only be activated under UV irradiation, and its photocatalytic efficacy decreases with significant electron-hole recombination [157, 158]. Multiple GO-ZnO hybrids were created to overcome these constraints, resulting in improved photocatalytic and antibacterial activity due to charge separation. Wang et al. created excellent GO-ZnO materials, showing outstanding antibacterial activity and cytotoxicity of a low degree [159]. The excellent antimicrobial activities are due to GO and ZnO synergy. GO dispersed ZnONPs and encountered bacteria. Close contact enhanced Zn concentration and membrane permeability, resulting in bacterial cell death [159].

Additionally, SnO<sub>2</sub> and Fe<sub>3</sub>O<sub>4</sub> have been mixed with G for antimicrobial purposes. G-SnO<sub>2</sub> nanosheets adhere to the surface of bacteria, preventing nutrition absorption and causing cell death. G has a 3.6-fold lower cytotoxic impact than nanosheets due to synergistic effects [160]. The GO-Fe<sub>3</sub>O<sub>4</sub>-containing magnetic iron oxide nanoparticles on GO nanosheets exhibited strong antimicrobial efficacy and easy separation, thereby facilitating the disinfection of water. Their impact on *E. coli* was established through ROS generation and protein degradation [161]. GO-Fe<sub>3</sub>O<sub>4</sub> hybrid materials penetrate or adsorb into bacterial cells, resulting in intercellular content leakage and cell integrity loss.

### Graphene-polymer nanomaterials

Because of its strong interplanar contacts, G's antibacterial potential is hindered by its low solubility and processability. Incorporating G into the polymer matrix can solve this problem. Polymers with  $\pi$ -electrons can produce stable polymer-graphene dispersion.

CS is a polysaccharide made of glucosamine and N-acetylglucosamine [162]. rGO-CS showed better bactericidal properties towards *E. coli* JM109 than GO [163]. Pradeep et al. formed G-based hybrids through attaching CS, CS-modified gold, and native lactoferrin to GO/rGO surfaces [164]. As a result of synergistic action, the combination of materials demonstrated maximal antibacterial activity [164]. Lee et al. developed graphene-poly-L-lysine (PLL) composites with antibacterial activity and biocompatibility by covalent bonding and electrostatic interactions between PLL and graphene derivatives [165]. In addition, adding 4-carboxy benzenediazonium salt can produce cationic PLLs that bind to carboxylic acid groups, resulting in maximum antibacterial activity [165].

### Graphene-based multicomponent nanomaterials

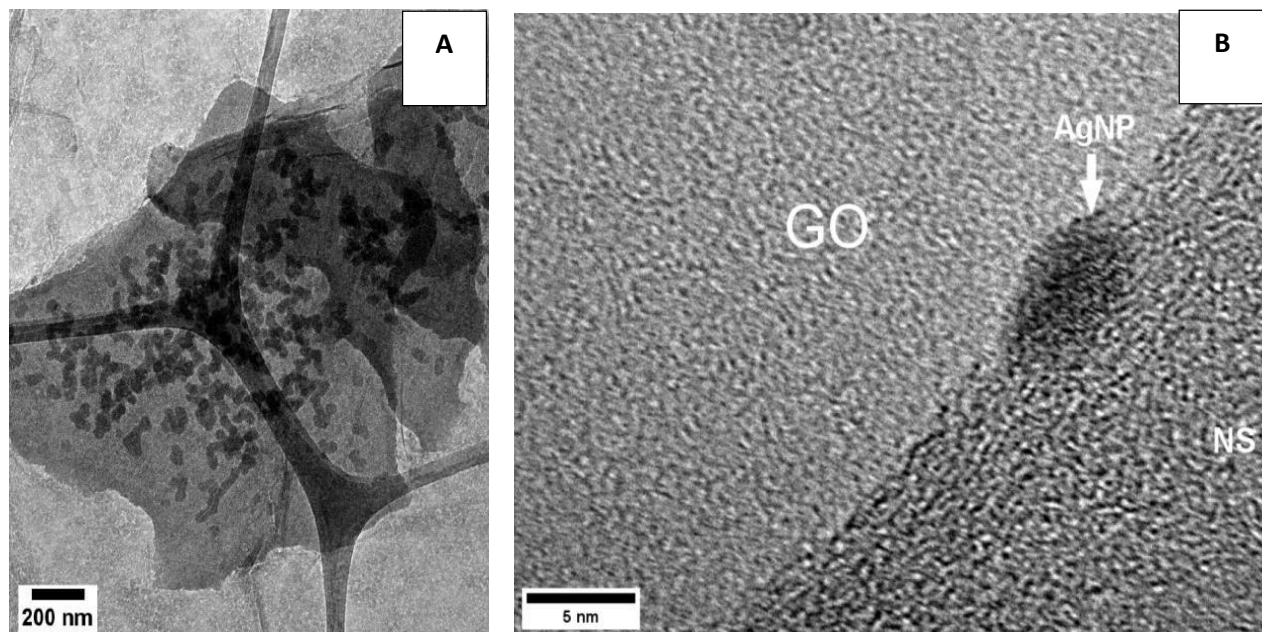
Combining several nanoparticles with G or its derivatives enhances bactericidal efficacy through synergistic action. For example, the hydrothermal synthesis of GO-Ag-TiO<sub>2</sub> nanomaterial coatings suppressed *C. jejuni*. The nanomaterials effectively prevented *C. jejuni* aggregation, proliferation, and biofilm formation [166]. Several nanomaterials with iron oxide nanoparticles have been developed. At 100  $\mu\text{g}/\text{mL}$ , magnetic GO-MnFe<sub>2</sub>O<sub>4</sub> hybrids inhibited *E. coli* by 82% after 2 h of contact [167]. GO, CoFe<sub>2</sub>O<sub>4</sub>, and Ag nanoparticle multimaterials effectively disinfect water polluted with *E. coli* and *S. aureus* at 12  $\mu\text{g}/\text{mL}$  [168]. Ultrasonication and casting were used to create a GO nanomaterial containing Ag, TiO<sub>2</sub>, and ZnO nanostructures. The nanomaterial was evaluated for antibacterial efficacy against G<sup>+</sup> and G<sup>-</sup> [169].

For example, the GO/NS/AgNPs exhibited remarkable kinetics in bacterial inactivation [170]. This composite, consisting of GO, NS, and AgNPs, demonstrated enhanced antimicrobial properties attributed to the incorporation of AgNPs onto the NS surface. The synergistic effects of these components were pivotal in elevating the material's effectiveness against bacteria. TEM images provided valuable insights into the structural characteristics of the nanomaterial. Figure 7a depicts TEM

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imagery revealing the presence of silicate nanoparticles grown on graphene oxide, highlighting the intricate morphology and the interaction between graphene oxide and nanosilica. Additionally, Figure 7b showcases high resolution TEM image

illustrating the distribution of individual silver nanoparticles on the nanosilica surface. The observed uniformity and support offered by the nanosilica to the AgNPs further underscored the material's potential in antimicrobial applications.



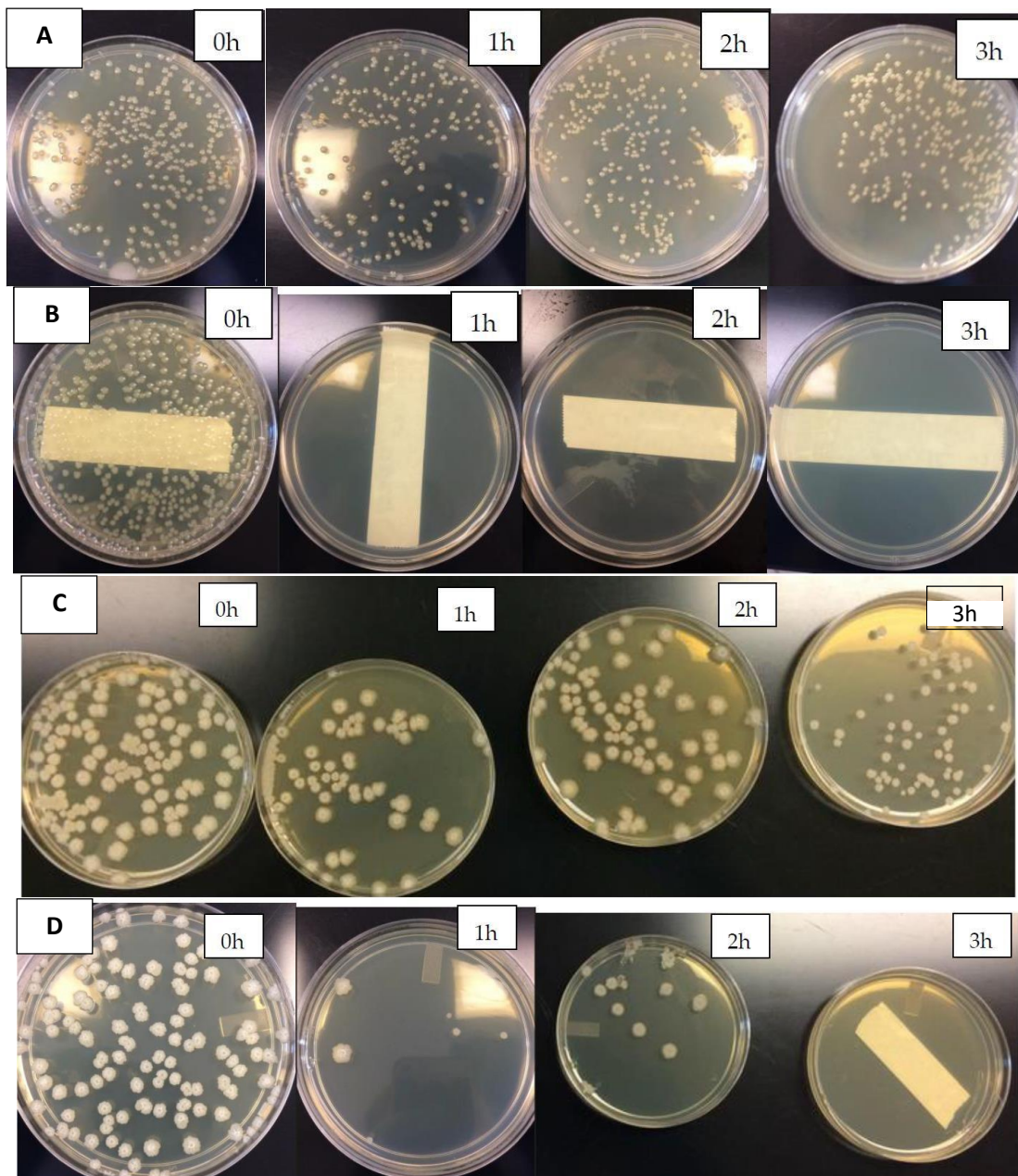
**Figure 7.** (A) TEM depicts NS grown on GO (scale bar of 200 nm); (B) High-Resolution TEM image shows single AgNP on the nanosilica (scale bar of 5 nm) [170].

This nanomaterial demonstrated an exceptional antibacterial efficacy, surpassing 99% against both *E. coli* and *B. subtilis*. Conducting zone of inhibition as shown in Figure 8. Moreover, the study demonstrated the remarkable efficacy of the GO/NS/AgNPs composite, achieving a substantial 7-log reduction in *E. coli* and a notable 5.2-log reduction in *B. subtilis* counts within a mere one-hour exposure period.

This impressive reduction underscores the rapid and potent antimicrobial activity of the material against both gram-negative and gram-positive bacteria. Such substantial reductions in bacterial populations within such a short timeframe highlight the potential of this composite as a rapid and effective antimicrobial agent [170].

GO/NS/AgNPs nanomaterial system epitomizes a promising frontier in antimicrobial research, harnessing the distinctive attributes of graphene oxide, nanosilica, and silver nanoparticles to accomplish potent bacterial inactivation. These findings not

only highlight the material's efficacy but also its potential for further exploration in biocompatibility studies, which are crucial for advancing its application within the medical domain. The versatility and effectiveness demonstrated by this material open avenue for diversified applications, ranging from medical devices to drug delivery systems. Moreover, its compatibility with biological systems suggests potential utilization in wound dressings, implant coatings, and other medical interventions where controlling microbial growth is paramount. As research delves deeper into understanding its mechanisms and optimizing its properties, this nanomaterial holds promise as a transformative solution in combating microbial infections and improving healthcare outcomes.



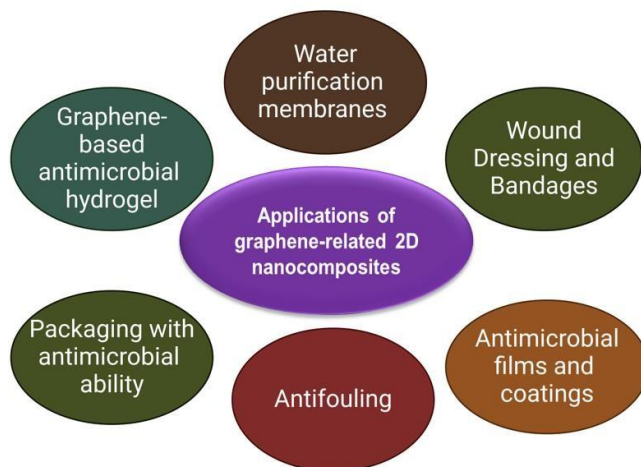
**Figure 8.** Examples of CFU plate count images of (A) The control *E. coli* samples from 0h to 3h, (B) The *E. coli*+GO/NS/AgNPs from 0h to 3h, (C) The control *B. subtilis* samples from 0h to 3h, (D) The *B. subtilis*+GO/NS/AgNPs from 0h to 3h [170].

#### 4.4. Applications of graphene-related 2D nanomaterials for antimicrobial potentials

Several studies have shown that G-related 2D nanomaterials may have antibacterial properties and can be used in several

antimicrobial applications [171]. Figure 9 highlights those potential applications that will be discussed in details.





**Figure 9.** Antimicrobial applications of graphene-related 2D nanomaterials.

### Graphene-based antimicrobial hydrogel

Researchers have shown significant interest in GO-based hydrogels due to their unique characteristics. It is challenging to develop hydrogels with effective antibacterial properties, low cost, and recyclability. The production of graphene-based hydrogels with exceptional antibacterial properties was demonstrated [172]. Another study described a G-based hydrogel employing agarose polysaccharide as a stabilizing and crosslinking agent, successfully preventing bacteria growth [173]. Another study found that benzalkonium bromide, a

commercial preservative, was combined with GO to enhance its antibacterial properties [174]. The benzalkonium bromide-GO hydrogel exhibited effective antibacterial effects on 91% G+ and 99% G- bacteria. Graphene hydrogel nanomaterial with silver was studied for its synergistic influence on antibacterial properties. Other hybrid hydrogels, such as Ag/PVA/G and Ag/G, showed promising reactions against *E. coli* as well as *S. aureus* [175, 176]. Table 3 lists some G-based antimicrobials hydrogels.

**Table 3.** A few examples of G-based hydrogels.

Material	Observation	Antimicrobial property	References
Ag/rGO hydrogel	Used in water disinfection applications	Kill 97% of <i>E. coli</i>	[177]
Tannic acid/rGO	Tannic acid used to fabricate 3D hydrogel	Effect on 99.99% of <i>S. aureus</i> and 58.12% of <i>E. coli</i>	[178]
Rose Bengal/GO/ PVA	Used in photothermal and photodynamic therapies	Active resistance to <i>S. aureus</i> and <i>E. coli</i>	[179]
GO-Ag/bacterial cellulose hydrogel	Wearable hydrogel microfibers	Active resistance to <i>S. aureus</i> and <i>E. coli</i>	[180]

### Packaging with antimicrobial ability

Flexible packaging, a growing field in food science and technology, benefits from adding G to polymers, improving their qualities. Additionally, G-based materials' antimicrobial capabilities can be applied to novel forms of packaging that possess antimicrobial properties. G-based polylactic acid materials are useful in several different usages, particularly food preservation. Several G-based materials, such as GO/PVA as

well as LLDPE/G, are used in packaging [181]. A study introduced a novel antibacterial film made from GO, essential oil of clove, and PLA film using a casting solution [182]. Therefore, the GO film is effective for antibacterial food packaging. Clove essential oil with plasticized PLA showed effective bactericidal action against *E. coli* and *S. aureus*. Table 4 outlines some G-based antimicrobials for food packaging.

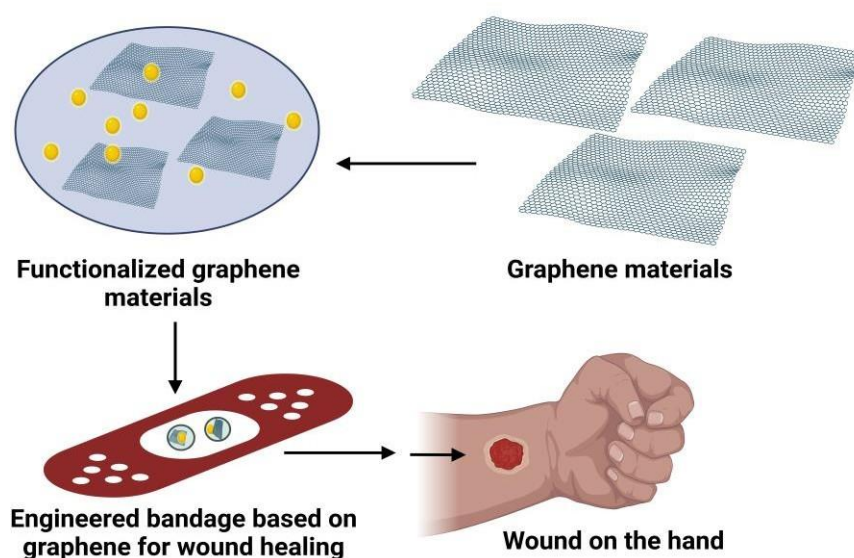
**Table 4. Some examples of G-based materials for food packaging**

Material	Observation	Antimicrobial property	References
CS/GO	Have good mechanical and barrier characteristics	Active resistance to <i>S. aureus</i> and <i>E. coli</i>	[183]
CS/crosslinked GO	Thermally stable and suitable for food packaging	Kill 90% of <i>E. coli</i>	[184]
GO with polystyrene	High mechanical strength and low water permeability	biocide effect on pathogenic bacteria	[185]
PVA/GO	Good mechanical and barrier characteristics	Kill 90% of <i>E. coli</i>	[181]

### Wound Dressing and Bandages

For many years, Ag-based nanoparticles were effective wound healing materials and clinically demonstrated to reduce pathogen-related illnesses. However, graphene-related 2D materials may improve wound treatment by preserving moisture, speeding closure, and reducing infections without scarring [186] as shown in Figure 10. Numerous graphene hybrids, such as GQDs with H<sub>2</sub>O<sub>2</sub> and G with AgNPs, in addition to PLA materials, have shown antibacterial and wound-management capabilities. Combining graphene with various bandage substrates may improve antimicrobial textile materials. Previous studies have shown the use of cotton textiles with GO in various uses. It has been demonstrated that mixing G and its derivatives and Ag can create material with effective antibacterial properties. The addition of Ag to the GO resulted in substantial antimicrobial efficacy, biocompatibility, and mechanical properties, enhancing wound healing within two weeks [187]. Wound dressings can be fabricated from graphene-based

materials. As an example, GO combined with the polyurethane-siloxane structure has shown effective antibacterial action towards G<sup>+</sup>, G<sup>-</sup>, and fungi [187]. In addition to their antibacterial action, bandages, and wound dressings must be structurally stable for effective wound treatment. G-based materials, with excellent strength in addition to easy production, are employed in wound dressings to improve their stability. A study found that GO 3D collagen tissue scaffolds had a significant increase in mechanical strength [188]. Similarly, GO-based electrospun nanofibrous membranes using CS/PVP solutions showed enhanced mechanical stability [189]. GO enhances human fibroblast cell interactions, leading to significant wound healing improvements. For effective wound healing and care, graphene weight percentages must be modified with other materials to enhance antibacterial activity. Essential factors to consider are expediting wound closure, limiting infections, keeping the area moist, and promoting rapid healing without scarring.



**Figure 10.** The wound healing capability of graphene-related 2D materials.

### Antimicrobial films and coatings

Graphene-based materials' inherent antibacterial characteristics have led to extensive research on antimicrobial films and coatings [86]. Antimicrobial films and coatings made from graphene technology function by stimulating bacteria or light through electron transfer and physical destruction. Recently, CVD graphene containing silver nanowires was used as an antibacterial covering for disinfection [190]. The coating of a polyethylene terephthalate/polyethylene vinyl acetate film showed strong antibacterial properties against *E. coli* and *S. aureus*. Xie et al. developed a GO-Ag hybrid collagen coating. The hybrid material rapidly responded to *S. aureus* and *E. coli* under visible light [191]. Another study found graphene/Ag hydroxyl apatite materials to be homogenous, bioactive, and corrosion-resistant [192]. Additionally, it enhanced flexibility, decreased the appearance of cracks on the surface, and demonstrated effective antimicrobial action with no adverse impacts. Developing graphene biofilms is difficult due to the necessary thickness and arrangement of flake patterns on the surface [31, 104]. Currently, some approaches exist to obtain optimal graphene sheet orientation and density. However, these approaches have limitations and are unable to be used for

biomedical equipment coatings or surfaces easily. Scalable and easy methods are needed to manufacture surfaces with aligned G and its materials for biomedical applications [193]. Graphene and its derivative particles released from such surfaces may harm nearby cells. Therefore, consider surface toxicity while designing graphene-based biosurfaces and films [194].

For example, GO/NS/AgNPs showed extraordinarily bacterial inactivation over time [192]. The composite achieved more than 99% antibacterial efficiency against *E. coli* and *B. subtilis*. Through the zone of inhibition studies, it is highly suggested that GO/NS/AgNPs have a high potential to be applied as an effective antibacterial coating for medical equipment and other surfaces. AgNPs with an average diameter of 26 nm were functionalized on the NS surface. The material contained approximately 3% of silver nanoparticles. The silver nanoparticles on nanosilica supported over graphene oxide exhibited a 7-log reduction of *Escherichia coli* and a 5.2-log reduction of *B. subtilis* within one hour of exposure. The composite may become attractive for future biocompatibility studies to explore further applications in the medical field.

## Water purification membranes and antifouling

A membrane used for the treatment of water as well as the recycling of wastewater is susceptible to biofouling, which is the growth of biofilm on the surface of the membrane. This issue hinders long-term membrane utilization and raises costs [195]. Multiple studies have paired antimicrobial graphene-based materials with polymeric membranes to reduce biofouling [196]. These materials are suitable for various applications, including ultrafiltration, nanofiltration, desalination, wastewater treatment, and seawater metal recovery [197-199]. Cheng et al. developed graphene-based materials-composing membranes with anti-fouling properties using polysulfone covered with PDA and GO nanosheets [200]. This ultrafiltration membrane strongly inhibits *E. coli*. The additional composite elastic membrane for the treatment of water is G-rubber silicone. It inhibits microorganism adhesion to the membrane [201]. Firouzjaei et al. developed sophisticated antifouling membranes by combining GO with an Ag-based MOF in PES [196]. The composite material for forward osmosis demonstrated a synergistic interaction between GO and Ag-MOF, providing improved biofouling resistance against *E. coli*.

## 5. Conclusion and future perspectives

In recent years, the increasing bacteria multidrug resistance to traditional antibiotics has greatly hampered the development of antibacterial applications. Fortunately, various emerging 2D GBNs, which show promising potential and good opportunities in addressing antibacterial issues, have demonstrated their excellent ability to kill drug-resistant bacteria. Due to their unique features, such as a high surface- to-volume ratio, mechanical flexibility, and thermal stability, 2D GBNs are the most promising materials in nanoscience, nanotechnology, and material science. This mini-review explores recent developments and biological uses for 2D GBNs, concentrating on their antimicrobial mechanisms and characteristics. Numerous studies have demonstrated the strong antimicrobial effects of 2D GBNs on bacteria, fungi, and viruses. However,

this activity is influenced by a range of parameters linked to the target microbial species and the properties of the graphene material used. This mini-review highlights different fabrication methods for G and its derivatives. A summary of G-derived materials' antibacterial, antifungal, and antiviral mechanisms was provided after an in-depth discussion of the influencing parameters (G sheet size, concentration, number of layers, surface modification effect, bacterial morphology, and size). In addition, antimicrobial applications of G-based materials, including hydrogels, smart packaging, wound dressings, water treatment membranes with antifouling properties, surface coatings, and biofilms, are outlined. As a result of the evidence and results discussed, scientists are more likely to develop innovative G-based nanomaterials with potential antimicrobial applications in a wide range of scientific and technological fields.

Nevertheless, many aspects still require further investigation to gain a deeper understanding of the effects of G materials on microorganisms and the human body. As a potential future applicable biomaterial for substituting or reducing antibiotic usage, more evidence is needed to establish and understand G's antimicrobial activity. In addition, more evidence is required to correlate this activity with the innate immune system.

## List of Abbreviation

<b>GBN</b>	Graphene-based nanomaterials
<b>G</b>	Graphene
<b>GO</b>	Graphene oxide
<b>rGO</b>	reduced graphene oxide
<b>Gt</b>	Graphite
<b>GtO</b>	Graphite oxide
<b>PG</b>	porous graphene
<b>DNA</b>	Deoxyribonucleic acid
<b>RNA</b>	Ribonucleic acid
<b>0D</b>	zero-dimensional
<b>2D</b>	two-dimensional
<b>3D</b>	three-dimensional
<b>GQD</b>	Graphene quantum dots
<b>AgNPS</b>	Silver nanoparticles
<b>AgGQDs</b>	Silver-graphene quantum dot
<b>NaNO<sub>3</sub></b>	Sodium nitrate

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<b>KMnO<sub>4</sub></b>	Potassium permanganate
<b>H<sub>2</sub>SO<sub>4</sub></b>	Sulfuric acid
<b>HNO<sub>3</sub></b>	Nitric acid
<b>KClO<sub>3</sub></b>	Potassium chlorate
<b>LPE</b>	Liquid-phase exfoliation
<b>Si</b>	Silicon
<b>SiC</b>	Silicon carbide
<b>CNTs</b>	Carbon nanotubes
<b>GMs</b>	Graphene materials
<b>G<sup>+</sup></b>	Gram-positive
<b>G<sup>-</sup></b>	Gram-negative
<b><i>S. aureus</i></b>	<i>Staphylococcus aureus</i>
<b><i>P. aeruginosa</i></b>	<i>Pseudomonas aeruginosa</i>
<b><i>A. oryzae</i></b>	<i>Aspergillus oryzae</i>
<b><i>F. oxysporum</i></b>	<i>Fusarium oxysporum</i>
<b><i>A. niger</i></b>	<i>Aspergillus niger</i>
<b><i>E. coli</i></b>	<i>Escherichia coli</i>
<b><i>K. pneumonia</i></b>	<i>Klebsiella pneumonia</i>
<b><i>P. mirabilis</i></b>	<i>Proteus mirabilis</i>
<b><i>S. marcescens</i></b>	<i>Serratia marcescens</i>
<b><i>P. putida</i></b>	<i>Pseudomonas putida</i>
<b><i>E. faecalis</i></b>	<i>Enterococcus faecalis</i>
<b><i>S. mutans</i></b>	<i>Streptococcus mutans</i>
<b><i>C. auris</i></b>	<i>Candida auris</i>
<b><i>C. albicans</i></b>	<i>Candida albicans</i>
<b><i>S. cerevisiae</i></b>	<i>Saccharomyces cerevisiae</i>
<b><i>P. chrysosporium</i></b>	<i>Phanerochaete chrysosporium</i>
<b><i>C. jejuni</i></b>	<i>Campylobacter jejuni</i>
<b>PMMA</b>	Polymethyl methacrylate
<b>IC50</b>	Half-maximum inhibitory dose
<b>ROS</b>	Reactive oxygen species
<b>H<sub>2</sub>O<sub>2</sub></b>	Hydrogen peroxide
<b>ESR</b>	Electron spin resonance
<b>TEM</b>	Transmission electron microscopy
<b>SEM</b>	Scanning electron microscope
<b>HSV-1</b>	Herpes simplex virus type 1
<b>HCV</b>	<i>hepatitis C virus</i>
<b>mRNA</b>	messenger RiboNucleic Acid
<b>EDTA</b>	Ethylenediaminetetraacetic acid
<b>CuNPs</b>	Copper nanoparticles
<b>rGO-Cu</b>	reduced graphene oxide-encapsulated copper nanoparticles
<b>rGO-Au</b>	reduced graphene oxide-encapsulated gold nanoparticles
<b>PLL</b>	Poly-l-lysine
<b>CVD</b>	Chemical vapor deposition
<b>Ge</b>	Germanium

<b>SiO<sub>2</sub></b>	Silicon dioxide
<b>UV</b>	Ultraviolet
<b>Vis</b>	Visible
<b>TiO<sub>2</sub></b>	Titanium dioxide
<b>ZnO</b>	Zinc oxide
<b>SnO<sub>2</sub></b>	Tin dioxide
<b>Fe<sub>3</sub>O<sub>4</sub></b>	iron (II,III) oxide
<b>CS</b>	Chitosan
<b>GO-MnFe<sub>2</sub>O<sub>4</sub></b>	Graphene oxide-manganese ferrite
<b>CoFe<sub>2</sub>O</b>	Cobalt ferrite
<b>GO/NS/AgNPs</b>	Silver-silica-graphene oxide nanomaterial
<b>NS</b>	Nanosilica
<b>CFU</b>	Colony forming unit
<b>PVA</b>	Polyvinyl alcohol
<b>LLDPE</b>	Linear low density polyethylene
<b>PLA</b>	Polylactic acid
<b>PVP</b>	Polyvinylpyrrolidone
<b>PDA</b>	Polydopamine
<b>MOF</b>	Metal-organic framework
<b>PES</b>	polyethersulfone

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## Data availability

Data will be made available on request.

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