coatings

Review

A Review on Graphene Based Materials and Their Antimicrobial Properties

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Abstract: Graphene-based materials are found as excellent resources and employed as efficient antimicrobial agents, and they have been receiving significant attention from scientists and researchers in this regard. By giving special attention to recent applications of graphene-based materials, the current review is dedicated to unveiling the antimicrobial properties of graphene and its hybrid composites and their preparation methods. Different factors like the number of layers, concentration, size, and shape of the antibacterial activity are thoroughly discussed. Graphene-based materials could damage the bacteria physically by directly contacting the cell membrane or wrapping the bacterial cell. It can also chemically react to bacteria through oxidative stress and charge transfer mechanisms. This review explains such mechanisms thoroughly and summarizes the antibacterial applications (wound bandages, coatings, food packaging, etc.) of graphene and its hybrid materials.

Keywords: graphene; antibacterial activity; mechanism; graphene-based materials

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1. Introduction

Frequent increase in population leads to contamination of water and air in our day-to-day life. Consequently, infectious diseases and pathogens are developed worldwide. Over the past few years, drug resistance was developed in many pathogens because of the excessive utilization of antibiotics like β-lactam, chloramphenicol, carbapenem, etc. [1,2]. Therefore, multidrug resistance of pathogens affecting humans with various infections globally could be one of the crucial problems that needed to be resolved [3]. Several antimicrobial agents like carbon nanotubes, metal nanoparticles, and metal oxide nanoparticles have been discovered to address this problem. Graphene and its hybrid materials are currently recognized as efficient antimicrobial agents and have shown a deleterious effect on plant pathogens [4–6].

Graphene (GR) is a two-dimensional structure with hexagonal carbon arrangements in a honeycomb manner in which each carbon atom undergoes sp^2 hybridization and contains one pure P_z electron. This is the reason for its exceptional electrical properties, and it is the thinnest material ever found on earth [7–9]. Graphene has grabbed much attention from researchers and scientists in several areas of nanotechnology owing to its distinctive physical, thermal, and electrical properties [10,11]. Graphene oxide is an oxidation form of graphene and having oxygen polarity groups on the planes of 2D structure, which is highly dispersible in water [5]. Graphene-based materials can hold biomolecules, particularly; graphene and graphene oxide nanomaterials could act as powerful bactericidal effects against all types of pathogenic microorganisms. This bactericidal mechanism is a complex structure and completely associated with the intrinsic properties of graphene-related materials. The parameters like the nature of the targeted microorganism, surface modification and composition, and the characteristics of the environment in which cell and graphene interaction take place are essential for efficient antibacterial activity. Current research unveiled that graphene oxide is a good candidate for antimicrobial applications, as it possesses polar oxygen groups. Thus, it can undergo oxidative stress. Several investigations have already reported that graphene, graphene oxide, and their hybrid materials could act efficiently against Gram-positive and Gram-negative bacteria [12–14]. Furthermore, graphene and graphene oxide could interact with bacterial cells either physically or chemically, responsible for their antimicrobial activity [15,16]. Physically, graphene may cause structural damage to the microorganism, capable of biologically isolating cells from their environment, ultimately leading to cell death. Furthermore, chemical interaction between graphene and microorganism releases toxic substances called reactive oxygen species (ROS). In addition, the electron transfer phenomenon could occur where electrons are progressively drained from the microbial's outer surface, which further causes ROS-independent oxidative stress leading to biological death.

Currently, plenty of research is ongoing on graphene-based materials and their composites for their enormous diversifying implications. Although comprehensive research on graphene-based composites materials has already been established [17–21], exploration of graphene products commercialization is still under process. Such composites' final performance entirely relies on the graphene dispersion and interfacial interactions associated with them. Graphene has already proved an excellent antimicrobial agent as a nano reinforcement in manufacturing hybrid composites, catalyst in catalysis, the sensor in solar cell applications, wastewater treatment, drug delivery, etc.

In literature, many reviews on graphene-based materials are reported with different perspectives [22–24]. Nevertheless, a combined analysis of preparation methods of graphene and their antimicrobial activity is infrequent. In the present review, initially, the first part dealing with preparation methods of graphene from biosources. Then, the second part highlights the antimicrobial action mode of graphene and graphene-based materials. Finally, the antibacterial applications of graphene-based materials are enumerated.

2. Preparation Methods of Graphene from Waste and Bioprecursors

The initial part of the present review emphasizes the preparation methods of graphene using waste and bio precursors. Due to the industrial revolution, waste generation and accumulation have become inevitable, so innovative approaches are required to utilize waste for valuable products. Converting this waste to use carbon materials like graphene is an appreciable approach. Graphene can be prepared by bio precursors like glucose, chitin, grape seed extract, alginate, etc. In this review, the preparation methods of graphene from different resources have been summarized, highlighting the merits and demerits. If graphene is economically produced using biomass or from waste, it can be utilized for various applications.

2.1. Significance of Preparation of Graphene from Bioresources

Inexpensive biosources have been identified from the available literature and discussed. This review intended to unveil the eco-friendly and straightforward preparation methods of graphene and augment the innovative approaches. In recent years, much research has been progressing to prepare graphene with good quality with high yield. This section will undoubtedly enable the readers to obtain valuable information over other sources and ultimately raise some challenges to the researchers to fabricate economically viable and quality graphene.

Manifold sustainable resources are essential for synthesizing graphene. This review highlights the use of various renewable bioresources such as glucose, rice husk, hemp, and disposable paper cups, and a detailed mechanism for synthesizing graphene and its applications.

2.2. Different Bioprecursors

Generally, the paper cups are made of wood, containing cellulose and hemicellulose, an extraneous material. However, a research team perceived it differently to utilize them as a starting material for preparing graphene. Hong Zhao et al. had prepared graphene using disposable paper cups as a precursor in the presence of an iron catalyst [25]. They highlighted two great uses of this approach. One is preparing graphene from waste with high yield compared to other conventional methods, and the second one is the excellent quality of the product. The preparation mechanism involves the activation treatment of paper cups with the aid of K^+ and KOH ions, and these ions are adsorbed on the structure of paper pulp. The addition of Fe catalyst results in the exchanging of ions Fe^{2+} with K^+. In the end, the Fe_3C layer could be formed when heated at higher temperatures, similar to the graphitization process. Carbon diffuses out to form multilayers of graphene over the Fe during the temperature reduction and thus resulting in the Fe/graphene composite. In the end, the etching process can be employed to eliminate Fe ions from the resultant graphene product. Besides, Pt/graphene can also be prepared [25] by exchanging Fe ions with Pt through galvanic displacement. The entire mechanism is summarized in Figure 1. This method is economically viable, quality graphene can be prepared from disposable paper cups, and it is a pioneering fabrication method that still requires optimization.

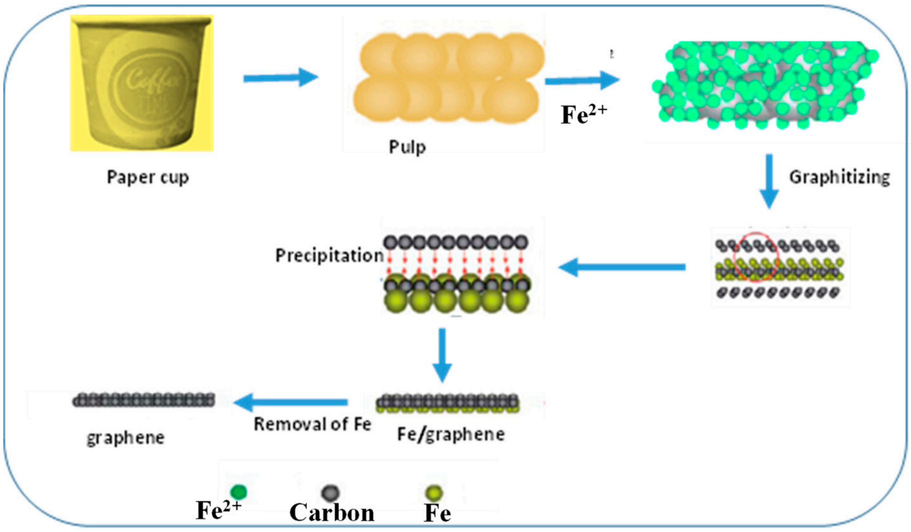

Figure 1. Formation mechanism of graphene sheets from paper cups [25].

The rice husk covers rice kernels, and rice is produced annually in huge million tons. As a consequence, rice husk is being considered a substantial agricultural waste. The potential use of rice husk has been implemented for synthesizing graphene [26–30] (see Figure 2). However, it is also utilized to manufacture silica products [31,32] because it contains more amount Si concentration. Eliminating the Si from rice husk results in a large amount of organic carbon, which is being wasted [33]. Recently, research has been conducted on deriving nanocarbon materials from the same. Research reports have revealed that a biocompatible graphene quantum dot could be prepared from rice husk with a good yield of 15%. The biocompatibility of the obtained product was confirmed using a cell viability test. Besides, silicon nanoparticles with a vast surface area can also be prepared from the rice husk as they can be utilized for comprehensive purposes. In the past, Hiroyuki et al. revealed a new synthetic method for the preparation of graphene through activation of rice husk by activating it with KOH, and substantial heating at high temperatures was conducted at 1123 K [34]. Herein, KOH helps prepare high-quality graphene containing sharp edges and can induce porosity while eliminating Si impurities. Nevertheless, the research team failed to explain the mechanism of the formation of graphene. In another report, graphene was prepared using rice husk for high-energy storage applications [35] due to the rice husk's inter-connected nanoporous structure. Therefore, quality-related issues should be thoroughly answered when utilizing rice husk to prepare graphene.

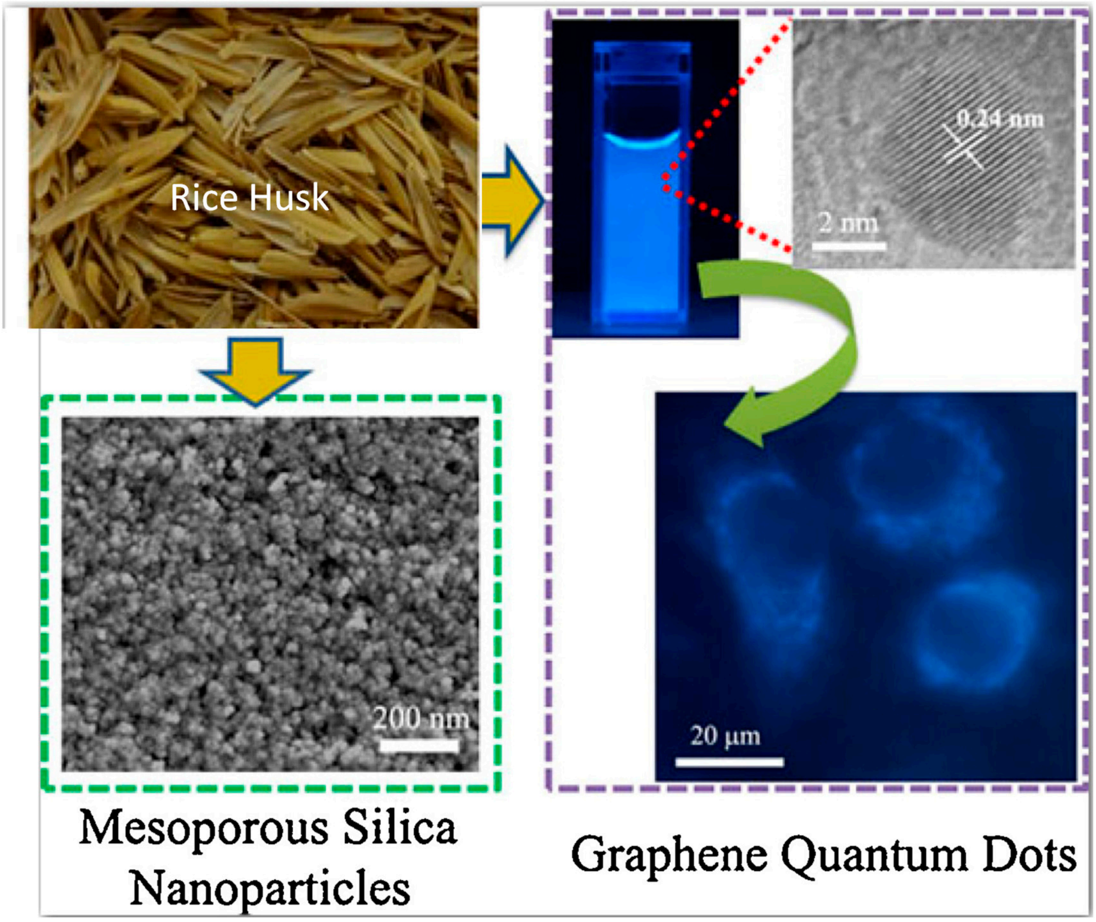

Figure 2. Utilization of rice husk for the preparation of graphene quantum dots Reprinted with permission from ref. [33] Copyright 2016 American Chemical Society.

Glucose is a carbohydrate molecule having the structural formulae $C_6H_{12}O_6$, and it is a renewable carbon resource. Many researchers utilize graphene and its derivatives to identify glucose non-invasively due to its sensitivity to environmental changes [36]. On the other side, glucose can be starting precursor for synthesizing graphene. Recently, a report revealed the preparation of graphene from glucose with the aid of ferric chloride [37]. In this preparation method, initially, glucose was dissolved with water in the presence of $FeCl_3$. Then, the resultant mixture was heated to 80 °C, subsequent calcination at 700 °C with inert gas atmosphere results in the formation of graphene and Fe (0). In the end, Fe can be removed with the aid of HCl. Here, $FeCl_3$ plays a crucial role in forming quality graphene because it acts as a catalyst. Therefore, this method can be easily adapted for the mass production of graphene. The schematic representation of the preparation method is given in Figure 3. Xin Hao Li and his group [38] also reported the preparation of graphene utilizing glucose and formed graphene named patched graphene. In this method, dicyandiamide (DCDA) was mixed with glucose in the presence of a nitrogen atmosphere to form the layered graphitic nitride (g-C_3N_4). Complete thermolysis of the developed g-C_3N_4 results in sheet-like graphene having high crystallinity with a pure atomic structure. This method is suitable for obtaining nitrogen-doped graphene because it can regulate nitrogen content. As in the Hummer method, graphene oxide (GO) substantially aggregates into thick flakes. In this approach, entangled graphene sheets can be produced without any separation or post-purification process.

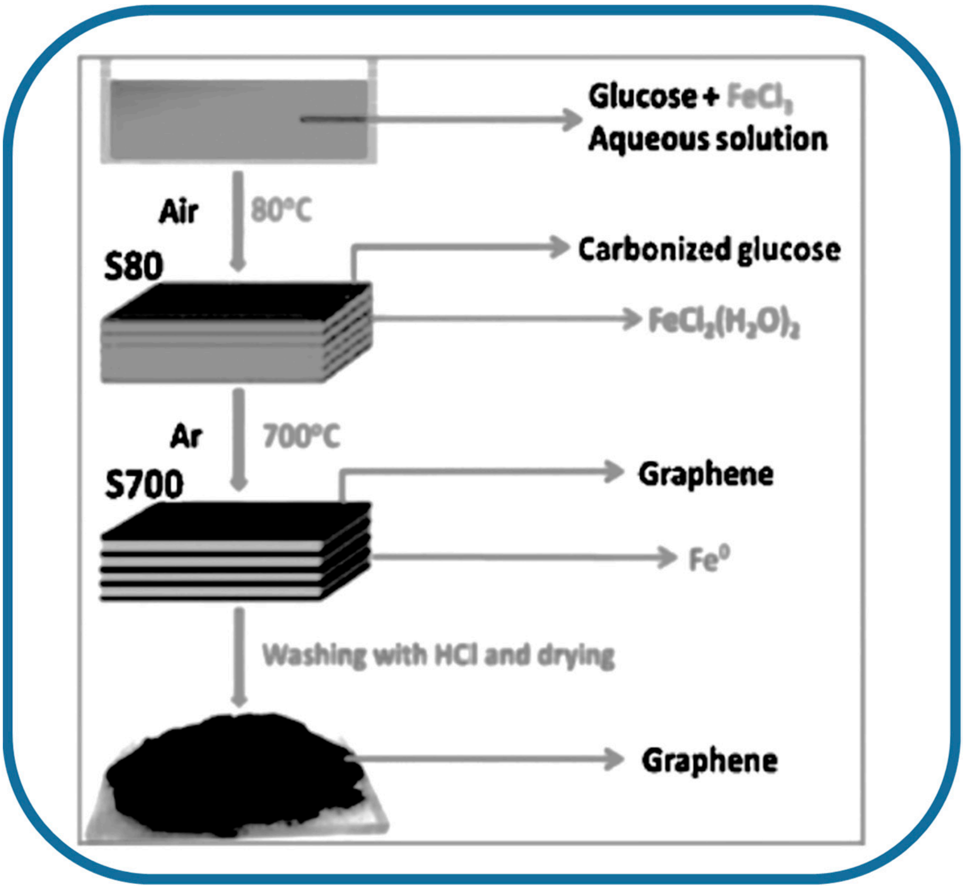

Figure 3. Preparation of graphene from glucose with the help of ferric chloride [37].

Hemp fiber is another type of bioresource that can be utilized for the preparation of graphene. It is derived from the cannabis plant known as bast. The fibrous part of this plant could be employed as clothing oil, rope, and plastics. Huanlei Wang and his coworkers synthesized nanographene from hemp fiber waste, an inexpensive starting material [39]. In another report, David Miltin revealed that Hemp bast is considered nanocomposite, which is made of hemicellulose, lignin, and crystalline cellulose [39]. The hydrothermal method was used to prepare nanocarbon material from Hemp fiber with the aid of KOH activation. Initially, the fibers were heated at 180 °C for 24 h to dissolve lignin and cellulose components. The following material was treated with KOH to prepare porous graphene (refer Figure 4). The prepared graphene can be used for energy and environmental applications.

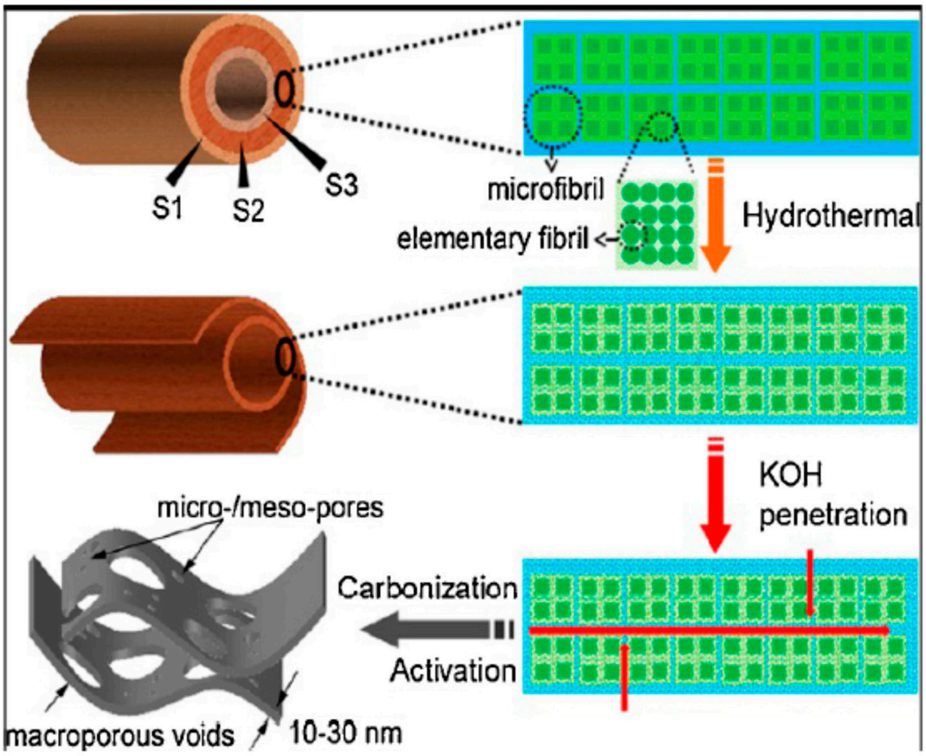

Figure 4. Preparation of graphene from Hemp fiber Reprinted with permission from ref. [39] Copyright 2013 American Chemical Society.

3. Antimicrobial Mechanism of Graphene Materials

Many research articles have summarized the antimicrobial activity of graphene and its derivatives. However, the mechanisms of antimicrobial activity of such nanomaterials are still under investigation [40,41]. Initially, graphene is invented by Andre Geim and K. Novoselov through tape peeling of two-dimensional graphene layers from graphite [42,43].

Antibacterial Activity Mechanism of Graphene-Based Materials (GBMs)

Different mechanisms of antibacterial activity of GBMs are available in the literature (see Figure 5), mainly oxidative stress through the production of reactive oxygen species [15] and the extraction of phospholipids from bacteria [44]. In the past, Zhang et al. had revealed that oxidative stress plays an important role. It entirely depends on the oxidation level of Graphene oxide (GO), which decides the cytotoxicity of GO. It was found that ROS production could be controlled in mammalian cells depending on various oxidation levels of GO [45]. They confirmed that the GO with less oxidation could enhance more ROS. These results could be further corroborated by electron spin resonance (ESR) spectrometry. It indicated that lower oxidation GO is strongly associated with indirect oxidative damage by activating H_2O_2 decomposition, which enhances the natural oxidative abilities of cells. Besides, theoretical simulations confronted that the size of oxidative groups and aromatic carbon ring of the nanographene sheet had a remarkable effect on the energy barrier of the decomposition reaction of H_2O_2.

In the past, Tu et al. had identified the effect of GO on the structural changes of *E. coli* using transmission electron microscopy (TEM) [44]. The interaction between membrane and GO is stimulated through the lipid extraction mechanism. A symbolic structure of GO can be observed inner and outer parts of *E. coli* during this process, which indicates that the GO nanosheets extract the phospholipids. The main driving force for the extraction of phospholipids is the van der Waals forces between GO sheets and membrane lipids.

After extraction, hydrophobic forces among lipids' hydrophobic tail interact with the hydrophobic part of GO (i.e., aromatic carbon ring). Besides, electrostatic interactions are developed between the hydrophilic head of lipids and unoxidized hydrophobic regions of

GO. Thus, it is speculated that there is a drastic drop in cell viability owing to the insertion of graphene and destructive lipid extraction, which imposes massive stress on the membrane. The whole process of cell viability ultimately depends on the concentration and lateral size of graphene sheets [44]. The insertion mode of action mechanism is proposed in which two-dimensional sharp graphene sheets utilize their sharp edges to destroy bacterial cells through the cell membrane and cause cell death due to leakage of intracellular material [46]. In the past, Akhavan et al. had reported that the biocidal activity of GO and rGO is because of direct contact of sharp edges with bacterial cell walls for different bacterial cells [47]. In another report, Li et al. revealed that graphene sheets penetrate inside the bacterial cell through lipid bilayer by piercing the cell wall of bacteria with its sharp edges [48].

Furthermore, the effect of incorporating various sizes of graphene sheets into the lipid layer of bacteria was unveiled by Yi et al. [49]. If the graphene is in micrometer size, it could orient near perpendicular configuration to the cell wall. In contrast, nanosize graphene could adopt a parallel position concerning the lipid bilayer of bacteria. Due to the interactions between lipids hydrocarbon tail with flat lipophilic graphene, graphene could sink between lipid tails. Consequently, graphene successfully penetrates inside the cell membrane. Nevertheless, in another report, Dallavalle had identified that graphene with smaller sizes could diffuse into the lipid membrane and orient themselves perpendicular configuration [50]. In contrast, large size graphene sheets arranging themselves across the cell membrane. Antibacterial behavior of pristine graphene and its cytotoxicity were analyzed by Pham et al. using experimental simulation and computer calculations to enhance the knowledge related to the cytotoxicity of graphene [51]. They found that graphene edges' density could significantly affect the antibacterial activity by creating an imbalance in osmotic pressure, leading to pores in bacteria's cell wall and eventually cell death. Another exciting mechanism proposed by the researchers is that the lipophilic flat surface of graphene destabilizes the 3D structure of the protein by disconnecting the protein-protein bonds on the cell membrane, causing the functional failure of bacteria [52]. As the metabolic activity of bacteria increases, the GO can be converted to graphene, resulting in a reduction reaction, which causes antibacterial activity. This effect is known as self-killing bacteria.

Besides insertion mode of action, another mechanism is explained based on direct contact of graphene basal plane with the cell membrane of bacteria, leading to the destruction of the growth of bacteria [53,54]. Recently, the direct attachment effect of CVD graphene on bacterial strains of *E. coli*, LF82, and UTI89 has been analyzed. It is concluded that these CVD graphene interfaces show no antibacterial activity due to there being no membrane damage of bacteria [40]. Furthermore, no morphological changes of bacteria were observed through SEM images. In contrast, the adherent strain of *E. coli* can quickly and easily proliferate into bio-based films.

A report revealed that the substrate electronic properties play a crucial role in destructing the Gr-coated surfaces [53]. For instance, graphene films having a large area coated on Ge and Cu restricted the bacteria growth, while graphene films coated on SiO_2 cannot inhibit the growth of bacteria. The leading cause for the antibacterial activity of Cu and Ge is that the easy transfer of electrons as graphene on the substrate can serve as electron pumping, which is responsible for oxidative stress in the membrane by pumping electrons away from the bacterial membrane. Mangadlao et al. had disclosed that sharp edges of graphene have no significant effect on its antibacterial activity. Still, the bactericidal activity is affected by the contact between GO basal planes and *E. coli* Moreover, and it was confirmed that covering or masking graphene or GO basal plane could diminish the antibacterial activity because it decreases the direct contact of GO sheet with bacteria [55].

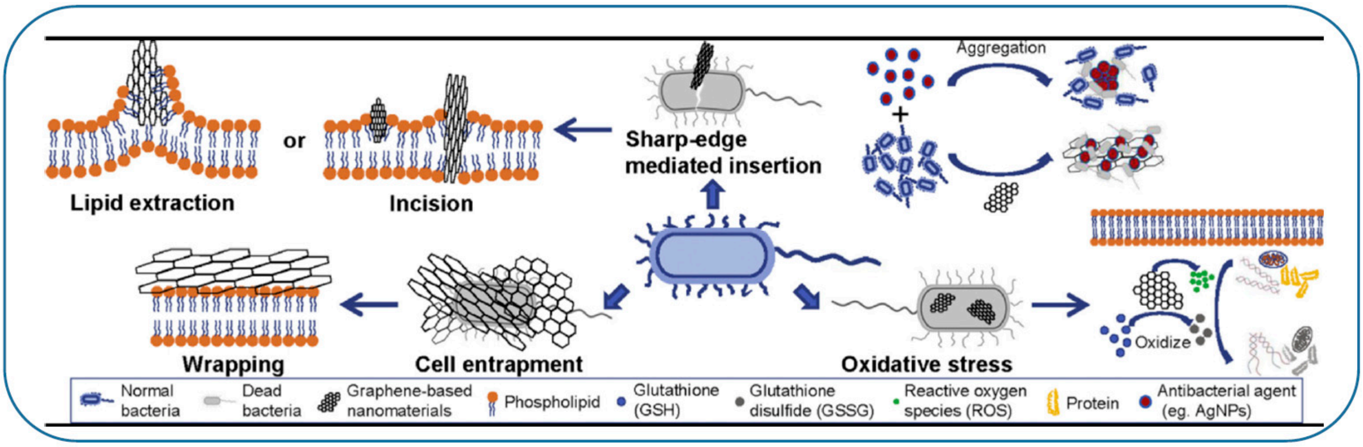

Figure 5. Schematic representation of different antimicrobial mechanisms of graphene based materials Reprinted with permission from ref. [56] Copy 2019 right from Elsevier.

4. Factors Affecting the Antibacterial Activity of Graphene and Its Derivatives

Graphene size, shape, electronic structure, and surface-related features could significantly influence antibacterial activity [12,57]. Moreover, the interaction between pathogens and nanomaterials and the conditions like medium, incubation time, and concentration significantly affect the antibacterial activity of nanomaterials [57–59].

4.1. Bacteria Shape and Type

Many reports have revealed that graphene oxide (GO) and reduced graphene oxide (rGO) can prevent gram-positive and gram-negative bacteria [60–63]. Nevertheless, size, type of bacteria, and shape significantly affect the extent of the activity. In addition, the bactericidal efficiency of nanoparticles is less for gram-positive than gram-negative due to bacteria cell structural changes [13]. Moreover, the graphene nanoparticle is difficult to enter the cell structure of gram-positive bacteria because of the thick three-dimensional layer (20–80 nm) of peptidoglycan, while gram-negative bacteria have a thin layer of 7–8 nm of the same, which is not sufficient to restrict the ingress of nanoparticle into it [64]. Therefore, *S. aureus* (Gram-positive bacteria) is more fascinated by GO than *P. aeruginosa* (Gram-negative bacteria).

On the other hand, cells of *P. aeruginosa* are more fascinated by (rGO) because of the curvature structure and elongated shape of rGO despite having a protecting layer of lipopolysaccharide and phospholipid in cell membranes. Herein, smaller surface area and spherical shape result in less susceptibility of *S. aureus* cells to rGO [65]. Besides, different shapes and morphologies of bacteria like bacillus, spiral, filament, and spherical (coccus) can also behave differently of microbes to antimicrobial agents [66].

4.2. Number of Layers (Graphene)

The number of graphene layers could also regulate the antibacterial activity of graphene-based materials [67]. In the past, Wang et al. had observed that graphene contains three layers that have more energy barrier to penetrating ability into bacterial lipid layer than single-layer graphene with the same lateral size with the help of molecular dynamics simulations [68]. This observation substantiated that single-layer graphene having more excellent antibacterial activity than multilayer. Besides, the accumulation of graphene occurs with an increasing number of layers, resulting in fewer interactions among bacteria and graphene layers. Therefore, fewer graphene layers show better antibacterial activity than more graphene layers.

4.3. Graphene Sheet Size

Graphene sheet size can significantly influence antibacterial activity. GO with a smaller size show excellent antibacterial activity in surface coating based on GO [69]. During the oxidative mechanism, more significant defects created in the GO sheet causing a reduction in the size of GO had explained efficient antibacterial activity. On the other hand, the cell entrapment mechanism explained the influence of the area of GO sheet on developing bacteria on cell suspensions. According to this mechanism, greater size in GO exhibited efficient antibacterial activity. Several research works evaluated the effect of various sizes of rGO and GO on their cytotoxicity [70]. However, the exact relationship between the cytotoxicity and scaffold cell interactions of GO and rGO with different 3D structures is still not understood completely. The graphene flake size influences the cytotoxicity of graphene derivatives. Smaller size flakes are more cytotoxic, show higher cellular internalization, and can significantly affect the functionality of cells. In the past, Shi et al. synthesized few-layer rGO films by controlling the reduction of GO to a moderate level. They found that the intermediate oxidation level significantly influences the cell behavior; cell performance is greatly reduced at a high thermal reduction [71].

4.4. Concentration of Graphene-Based Materials

The concentration of graphene and its derivatives is one of the main factors that can significantly influence antibacterial activity [72]. When suspensions of GO were exposed to the cells of E. coli with different concentrations like 80, 40, 20, and 5 µg/mL, it was found that the bacterial susceptibility to GO increased with the increment of GO concentration. Besides, at the concentration of 80 µg/mL, it was identified that more than 90% of bacteria were eradicated [57,73]. Likewise, when exposed bacteria at a similar concentration of rGO at 80 µg/mL results in 76.8% mortality [57]. Moreover, antibacterial activity was evaluated at higher concentrations of graphene from 25–200 µg/mL. The mortality of *P. aeroginosa* was identified at 100 µg/mL for rGO and 75 µg/mL for GO. Based on the results, the threshold concentration of GO is 80 µg/mL, which showed more than 90% antibacterial activity. At the same time, the threshold rGO concentration for efficient activity is 100 µg/mL [40].

5. Antimicrobial Applications of Graphene and Its Composites

Many reports have revealed that graphene-based materials could be utilized as potential antimicrobial agents [74–77]. Besides, graphene-associated polymer composites or graphene reinforced polymer composites could be employed in many antimicrobial applications, including bandages, wound dressings, drug delivery, and antimicrobial films and coatings [78,79].

5.1. Graphene-Based Antimicrobial Hydrogels

Due to the inherent and distinctive properties of the 3D GO-based hydrogels, they have gained profound interest from researchers. Nevertheless, preparing such hydrogels with efficient antimicrobial ability with low cost and recyclability is complicated and challenging. Thus, in probing such hydrogels, a report disclosed the preparation of novel graphene-based hydrogels with high antimicrobial properties [80]. Another report revealed a multi-functional graphene-based hydrogel using an agarose polysaccharide that is biologically compatible and acts as a stabilizer and crosslinking agent [81], which can effectively prevent bacteria growth. Another report revealed that the commercial preservative called benzalkonium bromide was mixed with GO to obtain the dual role of the antimicrobial ability of both materials [16]. The resultant benzalkonium bromide/GO hydrogel performed efficient antimicrobial properties towards gram-positive (91%) and gram-negative (99%) bacteria. Similarly, the synergistic effect of hybrid materials of graphene hydrogel nanocomposite that is silver/graphene was examined for improved levels of antimicrobial activity. In addition, many other hybrid hydrogels like Ag/PVA/GR and Ag/GR had exhibited good responses over *E. coli* and *S. aureus* [82,83]. The following Table 1 summarizes the examples of graphene-based antimicrobial hydrogels.

Table 1. Examples of graphene-based hydrogels.

Material	Inference	Antibacterial Ability	Reference
Benzalkonium bromide/GO	Commercial preservative based benzalkonium bromide/GO hydrogel	Strong antibacterial action against gram positive (91%) and gram negative (99%)	[16]
Rose Bengal/GO/Poly vinyl alcohol (PVA)	This hydrogel can be used in photothermal therapy and photodynamic therapy	Sustainable activity against *S. aureus* and *E. coli*	[84]
Tannic acid/rGO	Plant polyphenol (tannic acid) was used for green one-step strategy is developed to fabricate three-dimensional (3D) hydrogel	99.99% activity against *S. aureus* and 58.12% against *E. coli*	[85]
Ag/rGO hydrogel	Gravity-driven 3D hydrogel for water disinfection applications	97% against *E. coli*	[86]
Electroresponsive Supramolecular GO Hydrogels	Electroresponsive hydrogel, electric field at 15 V was used to inactivate bacteria	100% against *S. aureus* and *E. coli*	[87]
GO−silver/bacterial cellulose hydrogel	Wearable Hydrogel Microfibers with sustainable antibacterial property	Sustainable activity aginst *S. aureus* and *E. coli*	[88]
Gr/PVA/Ag	Polymer based hydrogel	Good antimicrobial activity (90%)	[82]
Gr/Ag	Hybrid hydrogel	Excelnet antibacterial activity (>98%)	[83]

5.2. Packaging with Antimicrobial Ability

Flexible packaging is one of the most emerging areas in food science and technology, the addition of graphene inside polymer enhances the thermomechanical and barrier properties. Besides, graphene-based materials' antibacterial properties could be utilized for bio-based innovative packaging with antimicrobial ability. Graphene-based polylactic acid composites can be applicable in bio-applications, especially in smart food preserving applications. Many other graphene-based composites, GO/polyvinylalcohol (PVA) and LLDPE/GR, are employed for bio-based packaging [89]. A report revealed a new antimicrobial film based on GO and clove essential oil with PLA film through solution casting [90]. The resultant GO-based film is efficient in antimicrobial food packaging applications. Plasticized PLA and clove essential oil showed good bactericidal activity against *E. coli* and *S. aureus.*

To conclude, this investigation helps fight against food pathogens, and as a whole, it can be utilized in intelligent antibacterial food packaging to preserve various food products. In the same way, Wang et al. synthesized MTAC/rGO/EVOH with rGO and ethylene co vinyl alcohol multi-layer film, which defends the moisture more than 98% and has excellent antimicrobial with outstanding mechanical properties [91]. Furthermore, a report based on an rGO-ZnO hybrid with PHBV polymer [92] unveiled the efficient packaging applications since the prepared film restricted the growth of gram-negative bacteria *E. coli.* Some other examples related to smart packaging with antibacterial ability are listed in Table 2. In addition to the antibacterial activity, one should consider graphene dispersion, orientation, physicochemical interactions with other polymer substrates, and hybrid materials to develop efficient smart packaging materials.

Table 2. Examples of graphene-based materials for food packaging.

Material	Inference	Antibacterial Ability	Reference
Chitosan (CS)/GO composite	Green composite for good mechanical and barrier properties	Sustainable effect against *S. aureus* and *E. coli.*	[93]
CS/crosslinked GO	Thermally stable and suitable for food packaging	Against *E. coli* (90%) and gram positive B	[94]
GO with polystyrene	High mechanical strength and low water permeability	biocide effect on pathogenic bacteria	[95]
GO/PLA composite	High flexibility and lowers the oxygen permeability	Excellent antibacterial activity (>95%) against *S. aureus* and *E. coli.*	[90]
PVA/GO	Good mechanical and barrier properties	Efficient against *E. coli* (90%)	[89]
LLDPE/EVA/Gr	Excellent barrier properties	Satisfactory aginast against *S. aureus* and *E. coli.*	[89]
MTAC/rGO/EVOH	Potential food packaging	Sustainable effect on all pathogens	[91]
PHBV/rGO/ZnO	Good mechanical and barrier properties	Sustainable effect agintst *S. aureus* and *E. coli.*	[92]

5.3. Wound Dressing and Bandages

In the past, Ag-based nanomaterials were utilized to treat wounds, they were exhibited as successful wound healing materials and were clinically proven to control various infections caused by pathogens. Similarly, graphene-based materials could have potential implications in wound management (refer to Figure 6), like maintaining moisture around the wound, accelerating wound closure, and stimulating wound healing by minimizing infections without scar formation [74]. Many approaches and various graphene hybrid combinations have revealed antimicrobial properties and wound managing abilities, including graphene quantum dots and hydrogen peroxide, graphene with Ag nanoparticles, and PLA composites. The association of graphene with other bandage substrates may lead to significant benefits in antimicrobial textile composites. Previously, the fabrication of cotton fabrics together with GO was reported for a broad range of applications. It has been reported that combining graphene-based materials with Ag results in a hybrid material, which could offer efficient antibacterial activity. For example, acrylic acid and methylene bis (acrylamide) were cross-linked with GO. Ag was added to the GO (5:1) ratio and achieved significant antibacterial activity and efficient biocompatibility with good mechanical properties, which enhanced the wound healing process during the examination within two weeks [96]. Due to their antimicrobial ability, graphene-based materials could also be utilized for wound care dressings. For example, GO nanofiller merged with polyurethane (PU)-siloxane network prepared by condensation method showed efficient antibacterial activity against gram-positive, gram-negative bacteria and other fungal species [96]. Besides antimicrobial activity, the structural stability of bandages and wound dressings is very crucial for wound management. For this, graphene-based materials with high mechanical stability and easy fabrication methods have been implemented in various wound care dressing formulations for structural stability enhancement. A report identified three-fold improvement in mechanical strength of prepared GO 3D collagen made tissue scaffold [97]. Likewise, electrospinning nanofibrous membranes comprising GO with CS/PVP solutions showed improved mechanical stability [98]. The addition of GO is main responsible for improving the interactions with human fibroblast cells, which caused a more remarkable improvement in wound healing. Recently, researchers have developed the hybrid rGO material having photo thermal inhibition qualities. Moreover, it was utilized for the treatment of subcutaneous skin infections. Examples of graphene-based wound healing materials are illustrated in Table 3. The critical aspect in wound healing and management is maintaining the structural stability of wound dressing and the antimicrobial activity and adjusting the weight percentage of graphene in association with other materials/compositions for the development of efficient wound dressing materials. Moreover, key parameters to be considered are accelerating wound closure, minimizing infections, maintaining the superficial wound environment moist, and stimulating proper wound healing without any scar formation.

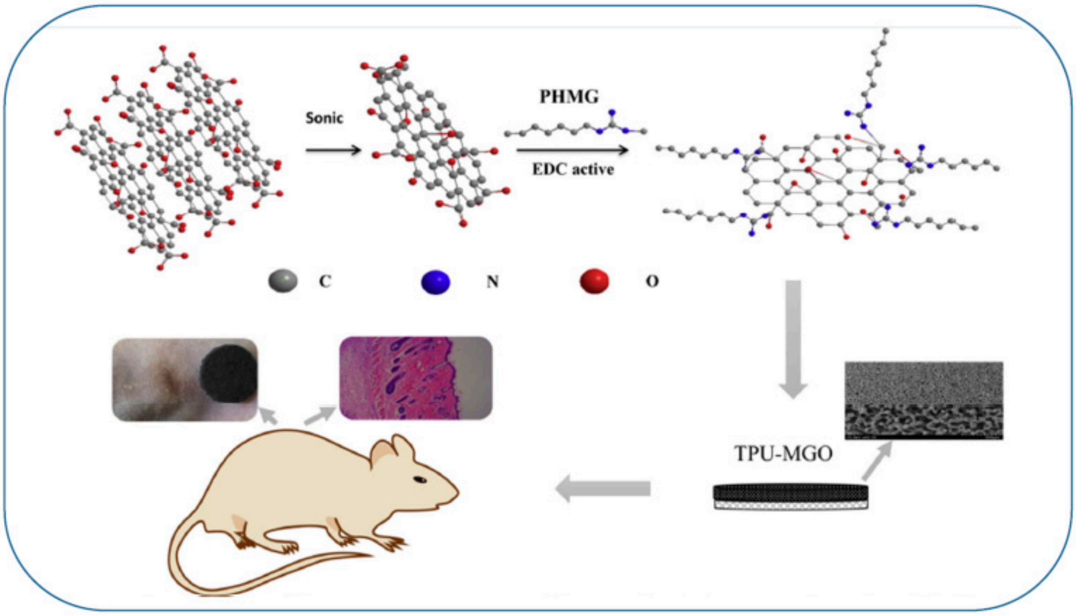

Figure 6. Wound healing ability of polyhexamethylene guanidine hydrochloride (PHMG) grafted graphene oxide (MGO) with thermoplastic polyurethane (TPU) Reprinted with permission from ref. [99] Copyright 2020 Elsevier.

Table 3. Examples of graphene-based wound dressing materials.

Material	Inference	Antibacterial Ability	Reference
GO/cotton fabric	Excellent wound healer	Good antibacterial activity against *S. aureus* and *E. coli*	[100]
GO/CS/PVP	Increases the wound healing rate	Excellent antibacterial ability (>95%) against *S. aureus* and *E. coli*	[90]
GO/β-cylcodextrin aldehyde/PVA	biocompatible and antibacterial material for wound dressing applications	Sustainable activity against *S. aureus* and *E. coli*	[84]
GO-Polyurethane-siloxane	Good mechaincal stability with effective wound healing	Efficient against *S. aureus* and *E. coli* (>90%)	[96]
Ag/GO/acrylic acid/acrylamide	Efficient biocompatibility with promising mechanical properties	Excellent against *S. aureus* and *E. coli* (>95%)	[96]
PVA/GO-citicoline sodium lanthanum(PVA/GO-CDPC-La)	Excellent wound dressing material	Active against *S. aureus* and *E. coli* (>90%)	[101]
rGO/Vancomycin	Better wound healing effeiciency	Sustainable activity against *S. aureus* and *E. coli*	[102]
silver/reduced graphene/sodium-alginate (AGSA)	Effective wound healer	Significant activity against *S. aureus* and *E. coli* (>90%)	[103]

5.4. Antimicrobial Films and Coatings

Due to the intrinsic antimicrobial ability and advantageous properties of graphene-based materials, much research has been centralized on the applications of graphene and its derivatives based on antimicrobial films and coatings [104,105]. Graphene-related materials are found to be very applicable in antibacterial devices due to their multifunctional bio applications. The graphene-based materials used for antimicrobial films and coatings work by either triggering light or bacteria approaches via electron transfer and physical destruction. There is no specific difference between antimicrobial coating and film. Although drying treatment is required before using graphene antimicrobial coatings, graphene antimicrobial films have been utilized for manifold practical implications, and these are primarily thin and uniform. Recently, antimicrobial coating of CVD graphene merged with silver nanowires has been employed for various disinfection implications [106]. This coating was successfully applied on the ordinary plastic film of polyethylene terephthalate/polyethylene vinyl acetate, and it exhibited excellent antimicrobial against *E. coli* and *S. aureus*. Xie et al. had reported the fabrication of coating of GO-Ag hybrid collagen. It was observed that the hybrid composite displayed a quick response against *S. aureus* and *E. coli* in the presence of visible light [107]. In another report, graphene/Ag hydroxyl apatite composites were revealed as homogeneous, bioactive, and exhibited excellent corrosion stability [108]. Besides, it improved mechanical strength, reduced surface cracks, and showed perfect bactericidal activity without any side effects. Macroscopic free-standing rGO with GO-based paper was fabricated by Hu et al. and identified that the prepared product had shown a significant response against the bacterial growth (*E. coli*) with a low cytotoxic response [73]. The examples of graphene-based coatings and films from the available literature are documented in Table 4. The major challenge involved in forming graphene biofilm is that it mainly depends on the desired density and the orientation of graphene flakes on the surface [109,110]. However, few methods have been implemented to achieve the perfect orientation and density of exposed graphene sheets. Nevertheless, these methods have drawbacks and cannot be applied to coatings or surfaces of arbitrary shapes, which can be employed in all biomedical devices. Therefore, there is a need to develop scalable and straightforward methods to create arbitrary surfaces with vertically aligned graphene and hybrid materials on various biomedical devices. Moreover, such surfaces could be toxic to other cells in the surrounding environment due to the release of graphene and its derivative particles. Therefore, the toxicity of the surface should be taken care of while developing graphene-based bio surfaces and films.

Table 4. Examples of graphene-based antimicrobial films and coatings.

Material	Inference	Antibacterial Ability	Reference
Graphene with silver nanowires coated on poly ethylene vinyl acetate/poly ethylene terephthalate	Good antimicrobial coating with high disinfection capability	Excellent antibacterial activity against *C. albicans*, *S. aureus* and *E. coli*	[111]
GO/Ag/Collagen	Composite exhibited quick and effectual disinfection	Good against *S. aureus* and *E. coli*	[107]
Graphene/hydroxyapatite/Ag	Outstanding corrosion stability	Remarkable antibacterial activity without any side effects	[108]
Go/sulfonated polyanion/polyethersulfone coated on glass surface	Good coatings with high disinfection capability	Excellent antimicrobial activity against pathogens	[112]
RGO/TiO_2 film	This film is prepared through photoreduction of GO on TiO_2	Excellent activity against *E. coli*@100%	[113]
GO/Zeolitic imidazolate framework film	The composite was used as bactericidal agent to fabricate antimicrobial thin film through interfacial polymerization	Activity against *E. coli*@84.3%	[114]
Graphene and layered titanate nanosheets film	Freestanding hybrid films consisting of strongly-coupled rGO and titanate nanosheets	Activity against *E. coli*@99.98%	[115]
Graphene immobilized lysozyme/polyethersulfone mixed matrix composite	Lysozyme materials were blended into polyethersulfone (PES) casting solution to fabricate PES membrane through phase inversion method	Activity against *E. coli*@71%	[116]

6. Conclusions

In nanoscience, nanotechnology, and material science, graphene-based materials and their hybrid composites are the most promising materials due to their unique properties and versatile bio implications. In this review, the initial part is highlighted with preparation methods of graphene from various bioresources such as paper cups, rice husk, glucose, and Hemp fiber, etc. Then, the details of graphene-based materials' antibacterial mechanisms such as oxidative stress, lipid extraction, cell entrapment, incision, wrapping were discussed thoroughly, and subsequently, influencing factors (graphene sheet size, concentration, number of layers, shape, and size of bacteria) that affect the antibacterial activities are summarized. Besides, graphene-based materials' antibacterial applications (hydrogels, smart packaging, wound dressing, surface coatings, and biofilms) are described. Hence, the discussed results and provided evidence motivate the researchers to develop novel and innovative graphene-based materials and their hybrid composites for other antibacterial applications in various fields of science and technology in the coming future. To conclude, graphene and its associated hybrid nanostructures are promising materials for various potential applications in daily life. However, continuous research on graphene-based materials, mainly theoretical, is needed to develop efficient new antimicrobial materials though several research works have described the relevant achievements.

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