3 Antimicrobial Perspectives for Graphene-Based Nanomaterials

Archana Ramchandra Deokar, Madhulika Sinha, Ganesh Gollavelli, and Yong-Chien Ling

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ABSTRACT

Conventional antibiotic therapies are becoming less efficient owing to the emergence of antibiotic-resistance bacterial strains. The development of novel antibacterial material to effectively inhibit or kill bacteria is crucial. During the last decade, inorganic nanoparticles and semiconductors have played an increasingly important role in combating bacterial infections. Graphene, since its discovery in 2004, has drawn tremendous attention from the scientific community as a promising nanomaterial (NM) owing to its multiple properties such as its unique mechanical stiffness, outstanding electronic transport, specific surface areas, thermal stability, conductivity, optical properties, low toxicity, and last but not the least, its excellent antibacterial property.

Here, a comprehensive view on the antibacterial properties of graphene-based NMs is summarized. In our group, efforts were made to synthesize a graphene-based photothermal agent and magnetic reduced graphene oxide functionalized with glutaraldehyde for the efficient capture and effective killing of both Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli bacteria upon near-infrared laser irradiation. We also prepared a smart magnetic graphene within 1 min by solid-state microwave reaction for drinking water purification. It can effectively kill pathogens such as E. coli, remove heavy metals, and is less toxic to zebrafish. Nevertheless, the development of graphene-based antibacterial materials toward practical applications warrants the understanding of their exact interaction mechanism. Hence, the mechanism of action for graphene-based composites toward bacteria is explored.

3.1 INTRODUCTION

Conventional antibiotic therapies have gradually become less efficient owing to the emergence of the antibiotic-resistant bacterial strains. For instance, bacterial infection caused by the New Delhi Metallo-ß-lactamase-1 type bacteria was found to be multidrug resistant, causing severe nosocomial infections worldwide (Kumarasamy et al. 2010). The rising numbers of drug-resistant diseases will eventually lead to an increasing need to develop novel and effective antimicrobial products that can combat these infections. Nanotechnology is increasingly playing an important role in combating nosocomial infections including but not limited to coating or incorporating different nanoscale metal and metal-oxide into surgical equipments (Park et al. 1998; Shah et al. 2008), medical implants (Banerjee et al. 2011), paints (Kumar et al. 2008), and textiles (Shimanovich et al. 2012).

Carbon-based nanomaterials (CNMs) have shown great promise for a number of revolutionary applications (Garg and Ling 2013; Garg et al. 2014a, b; Ke et al. 2014). CNMs such as fullerenes (C60) (Lyon et al. 2006, 2008), carbon nanotubes (CNTs) (Liu et al. 2009; Wang et al. 2013), graphene (J. C. Liu et al. 2011, S. B. Liu et al. 2011; Carpjo et al. 2012), and graphene quantum dots (GQDs) (Ristic et al. 2014) are emerging antimicrobial materials. Studies with pure microbial cultures have shown that several CNMs have significant antibacterial activity (Table 3.1). A focus area of research is to harness this property for industrial applications (Mauter and Elimelech 2008; Ahmed et al. 2012). An investigation of the potential toxicity assay of nano-C60 results has shown that in both Gram-positive and Gram-negative bacteria, nano-C60
disrupts electron transport, punctures bacterial membranes, or produces the radical-oxygen species that are toxic (Tsao et al. 2002). Carboxyfullerenes puncture the membranes of some Gram-positive bacteria but not Gram-negative bacteria. The earlier study, in which the toxicity of carboxyfullerenes toward 20 bacterial isolates was evaluated, identified the mechanism of toxicity as membrane destabilization and suggested that carboxyfullerenes could be considered as antimicrobial agents against Gram-positive and Gram-negative bacteria.

On the other hand, the antibacterial activity of CNTs has been investigated as a surrogate market in a recent report (Kang et al. 2007). The authors investigated the interaction of well-characterized, low metal content, narrowly distributed, and pristine single-walled carbon nanotubes (SWCNTs) with a model bacterium, Escherichia coli K12. The most accepted antibacterial mechanism of SWCNTs includes the direct physical contact and piercing action of aggregated SWCNTs (Kang et al. 2008). Oxidative stress from residual metal ions in CNTs might partially contribute toward the antibacterial activity. We have demonstrated that acid-functionalized SWCNTs (APSWCNTs) possess better antibacterial activity toward Gram-positive bacteria. Better activity is attributed not only to direct physical contact and piercing action, but also to molecular-scale interactions with surface functional groups of bacteria with CNTs (Deokar et al. 2013).

During the last two decades, two forms of CNMs, fullerene and CNTs, have been explored intensively (Yamago et al. 1995; Prato et al. 2008; Talyzin et al. 2011). Since the seminal work of Novoselov et al. on free-standing graphene sheets in 2004, various forms of graphene sheets have been actively explored with a wide range of technological applications such as transistors (Eda et al. 2008), solar cells (Tang et al. 2010), and sensors (Ang et al. 2008). Graphene is a flat monolayer of carbon atoms tightly packed into two-dimensional (2D) honeycomb lattice, and is the basic building block for graphitic materials of all other dimensionalities (Figure 3.1) (Geim and Novoselov 2007). It can be wrapped up into zero-dimensional fullerenes, rolled into the one-dimensional CNTs, or stacked into three-dimensional (3D) graphite. Theoretically, graphene has been studied for 60 years (Wallace 1947; Mcleure 1956; Slonczewski and Weiss 1958) and is widely used for describing the properties of various CNMs. It is one of the most fascinating carbon-based nanostructures with unique superlattices such as thinnest imaginable material, largest surface area (~2700 m$^2$/g), strongest material ever measured (theoretical limit), stiffest known material (stiffer than diamond), most stretchable crystal (up to 20% elasticity), record thermal conductivity (outperforming diamond), highest current density at room temperature ($10^6$ times than cooper), completely impermeable, intrinsic mobility (100 times more than in Si), conducts electricity in the limit of no electrons, and the lightest charge carrier (Geim and Novoselov 2007).

Recently, graphene has been explored for various biomedical applications owing to its superior biocompatibility (Chen et al. 2008; Liu et al. 2008). High surface area and versatility of surface functionalization made it a good scaffold for composite materials. However, the antibacterial activity of graphene-based materials is still at a nascent stage. Commercialization of graphene-based nanocomposites (NCs) for antibacterial applications will be of great importance owing to their low-cost mass-production and ease of preparation. Several research groups made efforts to study the antibacterial activity of graphene. The first direct evidence on graphene’s antibacterial activity was reported by Hu et al. (2010), a graphene-based antibacterial paper. The authors questioned the interactions between graphene oxide (GO) nanosheets (NSs) and bacterial and mammalian cells, and they reported the novel findings on excellent antibacterial activity and minimal cytotoxicity of GO NSs. The mechanism of interaction is attributed to either oxidative stress or the physical disruption of the cell membrane unlike the other CNMs (CNTs and fullerene) (Kang et al. 2007; Liu et al. 2009). The small diameter, high surface area, strong cell adhesion and capturing ability, and syringe-like structure of individually dispersed SWCNTs played an important role in governing CNTs antibacterial activity; whereas in case of graphene, the high-surface area, geometry of graphene-based nanomaterials (NMs), and thermal conductivity played an important role in governing its antibacterial activity. The study of the mechanism of action for graphene-based NMs is crucial in order to combat the existing antibiotic resistance.

### 3.2 GRAPHITE, GO, AND REDUCED GO

3D graphite (Gt) is made of several single-atom thick sheets of carbon atom, that is, graphene, synthesized through the

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**TABLE 3.1**

<table>
<thead>
<tr>
<th>NM</th>
<th>Starting Material</th>
<th>Synthesis Method</th>
<th>Bacterial Species/Type</th>
<th>Antibacterial Activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNTs</td>
<td>SWCNTs; Co-MCM-41 sieve</td>
<td>Centrifugation-based</td>
<td><em>E. coli</em>, <em>P. aeruginosa</em>, <em>B. subtilis</em>, and <em>S. aureus</em></td>
<td>Higher death rate if well dispersed</td>
<td>Liu et al. (2009)</td>
</tr>
<tr>
<td>GO</td>
<td>Graphite powder</td>
<td>Chemical oxidation-modified Hummers</td>
<td><em>E. coli</em></td>
<td>GO shows higher cell viability than rGO</td>
<td>J. C. Liu et al. (2011), S. B. Liu et al. (2011)</td>
</tr>
<tr>
<td>Fullerene</td>
<td>nC60+THF</td>
<td>Solvent (H$_2$O) based</td>
<td><em>E. coli</em> and <em>B. subtilis</em></td>
<td>Higher cell toxicity; no ROS generation</td>
<td>Lyon et al. (2008)</td>
</tr>
<tr>
<td>GQDs</td>
<td>Graphite rods</td>
<td>Electrochemical</td>
<td>MRSA, <em>E. coli</em></td>
<td>Effective in antibiotic resistance bac. sps.</td>
<td>Ristic et al. (2014)</td>
</tr>
</tbody>
</table>
Antimicrobial Perspectives for Graphene-Based Nanomaterials

Micromechanical exfoliation of Gt. GO is a graphene sheet with carboxylic groups at its edges and phenol, hydroxyl, and epoxide groups on its basal plane. GO can be chemically exfoliated from graphite oxide. Thermal annealing or chemical treatment can eliminate functional groups on GO to produce reduced GO (RGO) (Stankovich et al. 2007). Owing to the differences of graphene-based NMs (Gt, GtO, GO, and RGO) in electronic structure, dispersability, size, and oxidation capacity, Liu et al. (2009) made the effort to study their antibacterial properties against Gram-negative E. coli bacteria. The correlation among the antibacterial activities, glutathione (\(\gamma\)-L-glutamyl-L-cysteinyl-glycine GSH [glutathione]) oxidation, and aggregate size is summarized in Table 3.2, which can be examined from three aspects. First, the different oxidizing forms of graphite (GtO) and graphene (GO) are compared, they have similar capacities in oxidizing GSH (GtO at 21.4 ± 1.1% versus GO at 22.2 ± 0.7%); however, GO dispersion (69.3 ± 6.6%) can kill much higher fractions of E. coli than GtO (15 ± 3.7%) dispersion. GtO and GO contain almost the same number of functional groups, the only difference is their individual NS size. The results demonstrated that the aggregation of graphene NSs played an important role in governing their antibacterial activity. Materials having smaller size have higher cytotoxicity than larger ones. The results are consistent with the observation for CNTs. SWCNTs with smaller diameter possess higher antibacterial activity than larger diameter multiwall carbon nanotubes (MWCNTs) (Kang et al. 2008). Second, the authors compared the antibacterial efficiency of Gt with GtO. Although they possess almost the same particle size, Gt demonstrated higher antibacterial activity than GtO owing to their different GSH oxidizing capacity. Metallic Gt can oxidize more GSH than insulating GtO, this observation suggests that the metallicity of graphene plays an important role in its antibacterial activity. Third, the comparison between GO and RGOs

### Table 3.2
Correlation among Antibacterial Activities, Oxidative Stress, and Particle Size

<table>
<thead>
<tr>
<th></th>
<th>Loss of Cells (%)</th>
<th>Loss of GSH (%)</th>
<th>Particle Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GtO</td>
<td>15.0 ± 3.7</td>
<td>21.4 ± 1.1</td>
<td>6.28 ± 2.50</td>
</tr>
<tr>
<td>Gt</td>
<td>26.1 ± 4.8</td>
<td>29.9 ± 0.7</td>
<td>6.87 ± 3.12</td>
</tr>
<tr>
<td>rGO</td>
<td>45.9 ± 4.8</td>
<td>94.2 ± 1.1</td>
<td>2.75 ± 1.18</td>
</tr>
<tr>
<td>GO</td>
<td>69.3 ± 6.6</td>
<td>22.2 ± 0.7</td>
<td>0.31 ± 0.20</td>
</tr>
</tbody>
</table>

antibacterial activity depicts that smaller size GO are much more toxic than RGO toward *E. coli*. A comparative study suggests that dispersibility, particle size, and oxidative capacity of graphene-based NMs play an important role in governing their antibacterial activity. The antibacterial mechanism is likely to be a synergy of membrane and oxidative stress. Metallic Gt could be a cheapest alternative among all graphene-based NMs from the commercialization point of view. The authors suggest that physicochemical properties of graphene-based NMs such as density of functional groups, size, and conductivity can be tailored to either reducing their risks or increasing their application potential.

### TABLE 3.3

<table>
<thead>
<tr>
<th>Material</th>
<th>Synthesis Method</th>
<th>Shape</th>
<th>Size</th>
<th>Antibacterial Activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO sheets</td>
<td>Chemical oxidation-modified Hummers</td>
<td>Sheets</td>
<td>0.01–0.75 µm²</td>
<td><em>E. coli</em></td>
<td>Liu et al. (2012)</td>
</tr>
<tr>
<td>GONWs</td>
<td>Chemical exfoliation</td>
<td>Perpendicular sheets (nanowalls)</td>
<td>Single- and/or multilayer</td>
<td><em>E. coli</em> and <em>S. aureus</em></td>
<td>Akhavan et al. (2010)</td>
</tr>
<tr>
<td>Graphene NSs</td>
<td>Hydrothermal reduction of GO (in alkaline conditions)</td>
<td>NSs</td>
<td>Few layer</td>
<td><em>E. coli</em> and <em>S. typhimurium</em>, <em>E. faecalis</em> and <em>B. subtilis</em></td>
<td>Krishnamoorthy et al. (2012)</td>
</tr>
<tr>
<td>GO, rGO paper</td>
<td>Chemical oxidation-modified Hummers</td>
<td>Sheets</td>
<td>~1.5 µm, ~4.6 µm</td>
<td><em>E. coli</em></td>
<td>Hu et al. (2010)</td>
</tr>
<tr>
<td>GQDs</td>
<td>MW-assisted cleaving and reduction process</td>
<td>Spherical</td>
<td>5.1 nm</td>
<td><em>E. coli</em> and <em>S. aureus</em></td>
<td>Sun et al. (2014)</td>
</tr>
<tr>
<td>GBP–OHP</td>
<td>CVD</td>
<td>Film</td>
<td>Monolayer</td>
<td><em>H. pylori</em>, <em>E. coli</em>, and <em>S. aureus</em></td>
<td>Mannoor et al. (2012)</td>
</tr>
<tr>
<td>hRGO FET device</td>
<td>Enzymic oxidation of GO</td>
<td>Sheets</td>
<td>Monolayer</td>
<td><em>E. coli</em></td>
<td>J. Chen et al. (2014), Y. Chen et al. (2014)</td>
</tr>
</tbody>
</table>

3.3 **GRAPHENE AND ANTIBACTERIAL ACTIVITY: EFFECT OF SYNTHESIS METHOD, SIZE, AND SHAPE OF GRAPHENE**

Ever since the discovery of graphene in 2004, various synthesis methods have been developed such as exfoliation and cleavage, thermal chemical deposition, plasma enhanced chemical vapor deposition (CVD), and chemical reduction method. The physical, chemical and most importantly, antibacterial properties of graphene and/or its subtypes vary based on methods of synthesis, size, and shapes (Table 3.3). The recent achievements on the synthesis of graphene by thermal CVD have confirmed the reproducibility of good quality graphene on centimeter scale substrate and successful transfer to many other substrates including Si, glass, and polydimethylsiloxane (PDMS) (Li et al. 2009).

Single-atom-thick/monolayer, *sp²* hybridized graphene is of particular interest owing to its superior mechanical, electrical, and sensing properties. Mannoor et al. (2012) printed a CVD synthesized monolayer of graphene onto water-soluble silk for wireless single bacterium detection on tooth enamel. The authors’ innovation is a fully biointerfaced sensing platform, which can be tuned to detect target analytes. They self-assembled antimicrobial peptides onto the monolayer of graphene (Figure 3.2), further they incorporated two other major functionalities of a hybrid biosensor

![FIGURE 3.2](image-url)

*FIGURE 3.2* Biotransferrable graphene wireless nanosensor. (a) Graphene is printed onto bioresorbable silk and contacts are formed containing a wireless coil. (b) Biotransfer of the nanosensing architecture onto the surface of a tooth. (c) Magnified schematic of the sensing element, illustrating wireless readout. (d) Binding of pathogenic bacteria by peptides self-assembled on the graphene nanotransducer. (Reprinted by permission from Macmillan Publishers Ltd. *Nature Communications*, Mannoor, M. S. et al. 3, 2012: 1–8, Copyright 2012.)
unit: a battery-free operation and remote wireless sensing capability. Upon reorganization and binding of specific bacterial targets by the immobilized peptides, the electrical conductivity of graphene film was modulated and wirelessly monitored using an inductively coupled radio frequency reader device. The key functionalities of the graphene/silk hybrid sensing elements are thus derived from a synergistic integration of the individual material’s properties and components. This work represents a fundamentally new paradigm in biochemical detection, and may provide an in situ, first-order monitoring and detection system for applications including point-of-care diagnostics, hospital sanitation monitoring, and food safety analysis.

The interaction of biological cells varies with respect to the surface area, number of layers, lateral dimensions, shape, and aspect ratio of graphene. Liu et al. (2012) demonstrated the effect of the lateral size of GO on Gram-negative bacterium. In particular, they prepared six different dimensions of GO by making the use of the tip sonication method. Spectroscopic analysis revealed that all six types of GO do not differ in surface chemistry nor in chemical composition. Based on time and concentration dependent experiments, the large sized GO was demonstrated to have better antibacterial activity than the smaller one. The atomic force microscopy (AFM) images depict that large sized GO sheets (Figure 3.3c,d) wraps the bacteria, thereby isolating them from growth medium which further seizes cell proliferation and leads to a huge loss in bacterial cell viability (Figure 3.3a,b).

However, the small size of GO (Figure 3.3e,f) will hold on the bacterial cell surface and cannot isolate the cells from their environment. Overall, the originated antibacterial inactivation occurs based on the size, chemical composition, and surface chemistry of GO. Moreover, the results suggested that the antimicrobial action is related to neither aggregation nor oxidation.

In another work, Akhavan and Ghaderi (2010) have studied the geometry effect of graphene nanowalls (GNWs) toward the Gram-positive Staphylococcus aureus (S. aureus) and Gram-negative E. coli, suggesting that the toxicity mechanism of CNTs includes oxidative stress, cutting off intracellular metabolic routes, and rupture of cell membrane through direct physical contact with bacteria. The authors extended this observation to graphene. The sharp edges of the graphene-based NSs with extremely high aspect ratio (the ratio of lateral size to the atomic thickness) could be proposed as the ideal nanostructure for effective direct interaction with microorganisms. The authors employed an inexpensive and versatile technique, electrophoretic deposition, for deposition of GNWs on steel substrates. Further graphene-oxide nanowalls (GONWs) were reduced to reduced graphene nanowalls (RGNWs). Figure 3.4 represents morphology of the GONWs. The single/multilayer GONWs were deposited in high density and at random orientations, but some of them were almost perpendicular to surface of the substrate. The mechanism of interaction of RGNWs was investigated by measuring the intracellular efflux of material from the damaged cell membrane of Gram-positive S. aureus.

**FIGURE 3.3** AFM amplitude and 3D images of E. coli cells after incubation with GO sheets. (a,b) E. coli incubation with deionized water for 2 h, (c,d) E. coli incubation with the 40 µg/mL GO-0 suspension for 2 h, and (e,f) E. coli after incubation with the 40 µg/mL GO-240 suspension for 2 h. The scale bars are 1 µm. (Reprinted with permission from Liu, S. B. et al., Lateral dimension-dependent antibacterial activity of graphene oxide sheets. Langmuir 28, no. 33, 2012: 12364–72. Copyright 2012 American Chemical Society.)
or Gram-negative *E. coli* bacteria. RGNWs (16% ± 3%) were found to be much more toxic than GONWs (41% ± 8%) (Figure 3.5). The higher toxicity of GNWs was attributed to more sharpening of the edges of the NWs resulting in stronger contact interaction with the cell membrane and/or better charge transfer. Owing to the high surface area and ease of functionalization, one can play a lot of chemistry around graphene either by chemical functionalization or by changing the geometry of the graphene-based NSs to make it a suitable candidate for antibacterial application. Recently, efforts were made to enhance graphene’s antibacterial property by functionalizing/modifying with metal and metal oxide nanoparticles. Such NCs possess a synergistic effect that is usually not observed in the individual material.

Owing to the multiple drug resistance of bacterial strains, Krishnamoorthy et al. (2012) made an attempt to develop a low cost and effective graphene-based antibacterial material that could have a significant impact on environment and health care. A graphene-based antibacterial material could be a potential candidate among CNMs owing to their high thermal stability, high mechanical properties, low cost, and superior biocompatibility. The antibacterial activity of graphene-based NSs synthesized by the hydrothermal approach (under alkaline conditions using hydrazine hydrate) was evaluated based on minimum inhibitory concentration (MIC) against Gram-negative *E. coli*, *Salmonella typhimurium* and Gram-positive *Enterococcus faecalis*, *Bacillus subtilis*. The differences in cell wall structure are apparent. For example, Gram-negative bacteria possesses a thin peptidoglycan layer (~7–8 nm); whereas Gram-positive bacteria possesses a thick peptidoglycan layer (~20–80 nm) (Akhavan and Ghaderi 2010; Tyagi et al. 2013). The MIC of graphene-based NSs was compared with the commercial antibiotic kanamycin (Table 3.4). MIC values demonstrated that graphene-based NSs are much more toxic than kanamycin toward both bacteria. Moreover, the antibacterial...
TABLE 3.4
MIC of Graphene NSs and Standard Drug Kanamycin against Gram-Negative and Gram-Positive Bacterial Strains

<table>
<thead>
<tr>
<th>Bacterial Strains</th>
<th>Graphene NSs MIC (µg/mL)</th>
<th>Kanamycin MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>Gram-positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>8</td>
<td>128</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>4</td>
<td>128</td>
</tr>
</tbody>
</table>


*Results are mean ± standard deviation (n = 3).*

efficiency of graphene-based NSs toward Gram-negative is much better than Gram-positive bacteria. Similar observations were reported by Hu et al. Oxidative stress or direct physical contact of *E. coli* with graphene-based NSs resulted in the loss of cellular integrity with the disruption of the cell membrane (Stankovich et al. 2007; Akhavan and Ghaderi 2010; Hu et al. 2010). NM toxicity toward biological species not only relies on the nature of the cell wall but is also dependent on cellular enzymes and biochemical events. Toward this end, the authors made efforts to study oxidative stress that results in damage to cellular components such as DNA, lipids, and proteins. The oxidation of fatty acids leads to the generation of lipid peroxidation that stimulates a chain reaction, resulting in the disintegration of the cell membrane followed by cell death.

### 3.4 ANTIMICROBIAL PROPERTY OF GO AND GRAPHENE-METAL/POLYMER NC

The combination of two different NMs always leads to the advancement of achieving better outputs with inherent properties and also leads to a synergistic effect. Over the past several years, nanoparticulates, specifically, silver nanoparticles (AgNPs) have been extensively studied (Panacek et al. 2006; Pal et al. 2007) in the realm of antimicrobial action. Owing to their superior antimicrobial action toward a broad spectrum of pathogens, AgNPs have been used in food packing and storage materials, plastics, catheters, bandages, textiles, detergents, dental resin composites, and as a disinfection controller in water purification technologies (Benn and Westerhoff 2008). However, the problems associated with aggregation as well as releasing Ag⁺ ions somewhat limit their commercial utility. To improve the dispersibility and minimizing the Ag⁺ release, at first AgNPs were immobilized on CNTs and later on graphene and GO owing to their high surface area and ease of functionalization. The immobilization of AgNPs on graphene-based NMs not only enhances their dispersibility but also permits more prominent antimicrobial action minimizing their release into the environment. Toward this end, Ma et al. (2011) made an attempt to decorate GO with well-dispersed AgNPs in water and the as-obtained composite exhibited enhanced antibacterial activity. The antibacterial efficacy of Ag, GO, and Ag–GO was evaluated on Gram-negative *E. coli* bacteria. The results demonstrated that GO and Ag exhibited less antibacterial activity than the Ag–GO NCs. Morphological observations demonstrated substantial loss of cellular integrity of *E. coli* upon 30 min interaction with Ag–GO composite as compared with the control set. It is believed that the antibacterial action of AgNPs originates from the amount of Ag⁺ ions present in the bacterial solution, which was ~0.5 mg/L. To investigate the possibility of toxicity caused by Ag⁺, the authors measured the amount of Ag⁺ ions released from Ag–GO, which was found to be ~0.05 mg/L. This concentration was too low to induce the obtained antibacterial activity with Ag–GO. Hence, a possible antibacterial action of the Ag–GO composite is proposed by preventing nourishment of bacteria in growth medium and wrapping of the entire surface of bacteria using the Ag–GO composite. The oxygen functional groups present on the GO may form hydrogen bonds with the *E. coli* surface containing phosphates and sugars. Furthermore, the AgNPs present on the GO surface directly contact the bacteria cell wall and trigger the bacteria cell wall to rupture significantly even in a very short-time period of 5 min. Very recently, oxidation debris fragments, a by-product adsorbed on the surface of GO, have been found crucial for the nucleation and growth of AgNPs (de Faria et al. 2014). The experimental results have shown that the GO–Ag composite influences bacterial adhesion. In another interesting work, carbon nanoscrolls filled with AgNPs have been evaluated with enhanced antifungal activity (Li et al. 2013).

Sreeprasad et al. (2011) made efforts to synthesize multifunctional GO/RGO composite films for medical and environmental remediation application. GO/RGO-based composite films are transparent, luminescent, and antibacterial. The antibacterial activity of GO/RGO was enhanced by functionalizing with lactoferin (NLf), NLf protected gold (Au), chitosan (Ch), and combinations thereof. The addition of Ch to RGO/GO helped not only in forming stable dispersions but also in fabricating large (cm²) surface area films through a simple solvent evaporation technique. Further, photoluminescence properties were incorporated by anchoring Au@NLf onto the films. The as-formed composite showed stable luminescence in the presence of various metal ions in the solid state. The composite showed reasonable stability against pH and temperature variations as well. The as-prepared films were found transparent and the transparency could be modulated by controlling the concentration of GO/RGO with ease. The luminescent films were stable under various environmental conditions and against various metal ions demonstrating their usefulness in water purification and medical applications. The authors claimed that it could be applicable as a reverse osmosis membrane and on medical devices...
such as catheters. Figure 3.6 represents the schematic of various composites synthesized and their proposed utility. RGO/GO–NLf–Ch and RGO/GO@Ag–NLf–Ch composite material demonstrated synergistic antibacterial activity relative to that of GO/RGO (Figure 3.7). Apart from that use of bare Ch and/or AgNPs, recently, surface engineering of graphene subtypes with ethylenediamine triacetic acid (Carpio et al. 2014), zinc oxide NPs (Wang et al. 2014), Fe$_3$O$_4$ NPs (Santhosh et al. 2014), magainin I (J. Chen et al. 2014, Y. Chen et al. 2014), and magnetic Ch (Abdelhamid and Wu 2013) have resulted a wide range of NCs for antibacterial applications.

Recently, we also designed a multifunctional smart magnetic graphene (SMG) for the removal of heavy metal ions, and to disinfect disease causing bacteria utilizing a green microwave technique (Golavelli et al. 2013). Time- and concentration-dependent studies demonstrated that SMG (100 µg/mL) possesses 100% killing efficiency toward Gram-negative E. coli. The SMG exhibits low cytotoxicity toward zebrafish as an animal model. One of the possible reasons toward high antibacterial activity could be the roughness of SMG that leads to the disruption of the cell membrane of bacteria. This observation is in good agreement with Akhavan’s findings; where the sharp edges of the GONWs disrupt the bacteria cell wall (Akhavan and Ghaderi 2010). Another possibility could be the presence of hydrogen bonding between OH groups of GO and bacterial cell membrane. All aforementioned factors lead to the 100% disinfection ability of bacteria in drinking water. Furthermore, with the increased near-infrared (NIR) absorbance of SMG compared with GO, one can disinfect bacteria photothermally. Within a few minutes of interaction time, SMG (100 µg/mL) was able to disinfect ~80% of E. coli. Extending the interaction time to 1 h, a lower concentration of SMG (10 µg/mL) was able to disinfect ~95% of bacteria demonstrating its rich potential as a disinfectant in water research.

Modern life is highly dependent on polymer-based materials such as carry bags, table tops, and water pipes. Due to close association with these materials, there are several possibilities to get infected with microbial diseases. It is therefore very important to use intrinsic antimicrobial polymers to prevent pathogenic actions or to coat the polymers with antibiotics, quaternary ammonium salts, and metal NPs. The usage of organic antimicrobial agents such as antibiotics exhibit several disadvantages such as shorter life times, high decomposability, and low heat resistance (Kenawy et al. 2007). In this regime, coatings with well-known inorganic NMs such as Ag, ZnO, and TiO$_2$ have appeared quite promising. However, their relative toxicity to humans and high production costs have motivated researchers to search for potential alternatives. To this end, graphene subtypes are quite promising owing to their ease of preparation and availability. The recent in vitro and in vivo studies have demonstrated that graphene/GO is nontoxic. Graphene/GO is compatible with polymers and serve as reinforcing agents owing to their superior mechanical strengths. Santos et al. have fabricated GO with poly-N-vinyl carbazole (GO–PVK) and tested the antimicrobial
action of as-prepared GO–PVK NC film against *E. coli*. The GO–PVK NC thin film has shown more effective antibacterial activity than GO and unmodified PVK films alone (Santos et al. 2011). Very recently, a GO–PVK-modified membrane filter was effectively used for the removal of *B. subtilis* and *E. coli* (Musico et al. 2014). Despite this, it is crucial to design nontoxic composites with potent antibacterial activity. In this manner, Some et al. used poly (l-lysine) (PLL) to fabricate graphene and/or GO. PLL is a polycationic homopolymer containing naturally occurring peptide l-lysine and possesses appreciable antibacterial activity, yet promotes the growth of human cell culture. In particular, PLL was modified onto the graphene/GO surface via electrostatic and covalent interactions and further explored in designing antibacterial plastic gloves. In addition, GO–PLL and graphene–PLL composites also promote cell growth (Some et al. 2012). In view of these reports, it is quite reasonable to expect that graphene/Polymer composites are nontoxic to humans and possess rapid antimicrobial action toward certain pathogens.

### 3.5 GRAPHENE-BASED PHOTOTHERMAL ANTIBACTERIAL THERAPY

After the pioneering work by Yang et al. (2010), it is now widely admitted that graphene or RGO possesses inherent photothermal properties. Recently, we designed a graphene-based photothermal agent by functionalizing magnetic RGO with glutaraldehyde (MRGOA). Upon NIR irradiation, the as-prepared MRGOA composite was found to efficiently capture and kill both Gram-positive *S. aureus* and *E. coli* Gram-negative bacteria (Wu et al. 2013). The glutaraldehyde serves as an efficient capturing agent toward both bacteria, the magnetic characteristics of MRGOA allow the bacteria to be readily trapped into small volume by the external magnet. The synergistic effect increases the heating extent of MRGOA upon NIR laser irradiation and trigger the killing of the captured bacteria. The antibacterial efficiency of MRGOA was evaluated under both batch and continuous operation modes. Specifically, the batch mode was operated with a still bacteria solution, whereas the continuous mode was operated with a flowing bacteria being continuously pumped through a microfluidic chip. In contrast to MRGO and functionalized CNTs (MCNGA—single-walled CNTs [SWCNTs] functionalized with magnetic NPs and GA [glutaraldehyde]), MRGOA demonstrated better antibacterial efficiency as demonstrated in Table 3.5. The survival rate and membrane integrity assay demonstrate that 80 ppm MRGOA solution provided rapid (10 min) and effective killing up to 99% of both bacteria. The key factors toward the effective killing of bacteria include the capturing ability of GA and photothermal effects of graphene, which is capable of absorbing light and subsequently releasing the energy as a heat. Heat produced by MRGOA upon NIR laser irradiation raised the temperature to ~50°C. At this temperature the enzymes denature, inhibit the necessary intracellular reactions, damage proteins and lipids on the cell membrane, and finally lead to bacteria death. Moreover, the MRGOA cross-linked bacteria can be easily controlled by an external magnetic field and aggregated at small volumes allowing the efficient use of NIR laser irradiation to increase local heating for effective bacteria killing.

By making the use of noncovalent interactions, Wang et al. (2013) also undertook graphene-based antibacterial photothermal therapy by functionalizing nano RGO (NRGO) with anti-*S. aureus* polyclonal antibody (Ab). Here, the Ab adsorbed on...
NRGO by noncovalent interactions helps to retain the structural characteristics of Ab. The Ab-NRGO was incubated with bacteria and exposed to NIR light. Upon NIR exposure, the Ab-NRGO selectively killed *S. aureus*, however, the killing efficacy was less prominent against *E. coli* and human cells under similar experimental conditions. Recently, a variety of nanoparticles or a luminescent rare-earth complex is combined with graphene domains (Table 3.6) for optical labeling, tracking, and photothermal ablation of bacteria (Lin et al. 2014; Tian et al. 2014; Xu et al. 2014; Yang et al. 2014). These reports certainly signify the importance of graphene-based composites in photothermal antibacterial therapy and may provide new insights for future medical applications where drug-resistant pathogens in human systems can be selectively and specifically photo ablated. In line with this, the visible light-induced inactivation property of graphene composites may be used for indoor air disinfection.

**3.6 MECHANISTIC APPROACH TOWARD ANTIBACTERIAL PROPERTY OF GRAPHENE-BASED NMs**

The development of CNMs toward commercial antibacterial applications warrants the understanding of their interaction mechanism with bacterial cells. Graphene-based antibacterial materials will be of particular interest among CNMs owing to their low-cost and bulk production at laboratory scale. Oxidative stress or direct physical contact of bacterial strains with graphene-based NSs is the most acceptable mechanism of interaction that has been demonstrated (Stankovich et al. 2007; Akhavan and Ghaderi 2010). Several researchers have carried out antibacterial studies of graphene-based NMs. However, the fundamental mechanistic approach toward bacterial strains is still at the nascent stage. Moreover, some literature reports on antibacterial studies of graphene-based NMs are contrary to others. For example, some studies claim that GO is toxic toward bacteria; whereas it was also reported that GO can also enhance *E. coli* growth nonspecifically (Ruiz et al. 2011). In our group, we made an attempt to explore a novel antibacterial mechanism of SWCNTs toward both Gram-positive and Gram-negative bacteria. We have made systematic spectroscopic and microscopic studies to explore the mechanism of interaction of graphene’s sister CNTs. So far direct physical contact, piercing action, and oxidative stress from residual metal ions are the well-known killing mechanisms of CNTs toward bacteria (Kang et al. 2007, 2008). Our research team made an attempt to study the molecular-scale interactions of AFSCNTs with surface functional groups of bacteria (Deokar et al. 2013) (Figure 3.8). X-ray photoelectron spectroscopic measurements demonstrated that molecular-scale interactions might be possible in Gram-positive bacteria in addition to the aforementioned mechanisms. This might be owing to the difference in the chemical composition of the cell membranes of these two bacterial strains. The Gram-positive *S. aureus* restrains a thick peptidoglycan layer (~20–80 nm) which is composed of amino acids, teichoic acids, surface proteins, and lipoids. The peptidoglycan acts as a chelating agent and makes for the possibility of molecular-scale interactions such as hydrogen bonding and electrostatic interactions. The Gram-negative *E. coli* consists of an outer membrane (~10–15 nm), a peptidoglycan layer (~7–8 nm), and a cytoplasmic membrane (~7 nm), hence is less susceptible toward the action of AFSCNTs. Both the cytoplasmic membrane and the outer membrane contain a lipid bilayer structure. The intermediate thin peptidoglycan layer (~7–8 nm) is covered with a thick outer membrane, hence becoming more resistant toward the action of AFSCNTs. This mechanistic approach might be applicable to graphene, as SWCNTs are made of one-atom thick sheet of graphene.

**TABLE 3.5**

| Survival Rate of *S. aureus* and *E. coli* for 80 ppm MRGO, MRGOA, and MCNGA Solution after Photothermal Treatments under Batch Operation Mode |
|-----------------|-----------------|-----------------|
| **Survival Rate (%)** | **Under Dark** | **NIR Laser Irradiation** |
| **Agent** | **S. aureus** | **E. coli** | **S. aureus** | **E. coli** |
| None | 143 ± 50 | 117 ± 34 | 45 ± 14 | 39 ± 12 |
| MRGO | 147 ± 20 | 108 ± 15 | 0.4 ± 0.1 | 0.1 ± 0.1 |
| MRGOA | 110 ± 9 | 133 ± 32 | 8.7 ± 2 | 20 ± 6 |


**TABLE 3.6**

| Graphene-Based Composites for Photothermal and Photodynamic Therapy |
|----------------|----------------|----------------|----------------|----------------|----------------|
| **NM** | **Laser Wavelength (nm)** | **Power Intensity (W/cm²)** | **Irradiation Time (Min)** | **Bacterial Species/Type** | **Type of Treatment** | **References** |
| GO-IONP-Ag | 808 | 1.5 | 7 | *E. coli* and *S. aureus* | PTT | Tian et al. (2014) |
| MRGOA | 808 | 1.5 | 10 | *E. coli* and *S. aureus* | PTT | Wu et al. (2013) |
| Ab-NRGO | 808 | 1.0 | 10 | *S. aureus* | PTT | Wang et al. (2013) |
| (Au@Rubpy/GO) SERS tags | 785 | 400 mW | 10 | *E. coli* and *S. aureus* | PTT | Lin et al. (2014) |
| Eu-Van-rGO | 808 | 1.5 | 3 | DR-*E. coli*, DR-*S. aureus* | PDT | Yang et al. (2014) |
| GQD | 470 | 1.0 | 15 | *E. coli* and *S. aureus* | PTT | Ristic et al. (2014) |
Very recently, Tu et al. (2013) explored mechanistic insights for the antibacterial activity of graphene by experimental and theoretical approaches using E. coli as a model bacterium. The viable changes in the E. coli morphologies can be roughly detected at three different stages. In stage I, E. coli showed resistance toward the antibacterial action of GO, especially at low concentrations. In stage II, the E. coli cell got partially damaged and at stage III, E. coli had totally lost its cell integrity by the ‘Blade’ like action of GO. The molecular dynamics simulation studies revealed that graphene can extract huge amounts of phospholipids from the inner and outer membranes of E. coli, owing to its destructive action and strong dispersive interaction with phospholipids. According to recent reports (Hui et al. 2014), bare GO intrinsically kills both bacteria and mammalian cells, whereas masking its basal planes via noncovalent adsorption renders GO inactive against bacteria. Another mechanism for the bactericidal effect of GO shows mechanical wrapping of the pathogens and locally damaging their cell membrane resulting in cell lysis (J. Chen et al. 2014; Y. Chen et al. 2014). This is most likely owing to local perturbation of the cell membrane and decreased membrane potential in bacteria eventually leading to a leakage of electrolytes of fungal spores. Apart from this, Abdelhamid et al. (2014) demonstrated that GO effectively absorbs gramicidin (GD), which is water-insoluble, through physical interactions and improves its antibacterial activity. These emerging mechanistic approaches in conjunction with cytotoxic and/or genotoxic studies toward the antibacterial property of graphene-based NMs (Ocsoy et al. 2013; Guo and Mei 2014; Sametband et al. 2014; Sun et al. 2014) may provide new insights in designing graphene-based antibiotics in the near future.

3.7 FUTURE PERSPECTIVES

The unique physicochemical and photothermal properties, low-cost bulk production, high-surface area, and ease of functionalization in graphene have made it an attractive candidate toward the development of commercial antibacterial applications. There is still plenty of room left to explore and understand the antimicrobial mechanism of graphene-based NMs. With the ease of functionalization and high-surface area, one might play a lot of chemistry by functionalizing 2D graphene. Moreover, graphene could also be utilized for wound healing and sensing applications by tuning the size, shape, and number of layers. However, the synthesis of single-layer graphene on an industrial scale still remains a big challenge for researchers. We anticipate that owing to low cytotoxicity and superior biocompatibility of
graphene-based NMs, graphene might replace antibiotics for multi-drug resistant bacteria in the twenty-first century.

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