2D and Heterostructure Nanomaterial Based Strategies for Combating Drug-Resistant Bacteria

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ABSTRACT: In the last three decades, there has been a huge increase in the number of antibiotic-resistant bacterial strains, which is becoming a serious threat to public health. Since the discovery of new effective antibiotics has dramatically decreased in last ten years, there are huge initiatives to develop new antimicrobial approaches to fight drug-resistant bacterial infections. In the last decade, a new nanoparticle-based tool has emerged to combat deadly bacterial infections, which may overcome the barriers faced by antibiotic resistance. The current mini-review highlights recent reports on two-dimensional (2D) graphene oxide (GO), 2D transition metal dichalcogenides (TMD), 2D MXenes, and 2D heterostructure material-based approaches to tackle bacteria. Notably, we discuss the major design criteria which have been used to develop novel antimicrobial 2D and heterostructure materials to eliminate bacterial infections. Next, details on the various mechanisms underlying antibacterial activity for 2D and heterostructure materials such as physical/mechanical damage, lipid extraction, oxidative stress, and photothermal/photodynamic effects have been discussed. Finally, we highlight the promises, major challenges, and prospects of nanomaterial-based approaches to combat multidrug-resistant bacterial infections.

INTRODUCTION

The discovery of penicillin antibiotics by Dr. Alexander Fleming in 19281 revolutionized the medical treatment for infectious diseases. The success of penicillin encouraged scientists to discover different types of antibiotics such as cephalosporins, aminoglycosides, glycopeptides, and quinolones.2−6 It is now well documented that antibiotics kill bacteria by targeting essential survival processes.5−9 As reported by several groups, antibiotics have the ability to inhibit cell wall synthesis which interferes with the bacterial reproduction.3−9 Similarly, antibiotics have the capability to block the synthesis of vital proteins, DNA and RNA. As a result, it suspends the growth of bacteria.4−9 Unfortunately, in the last few decades bacteria have acquired resistance to antibiotics available in the market, and as a result, infections caused by drug-resistant bacteria cannot be treated easily.5−9

There are several possible mechanisms through which bacteria become drug resistant, and these are increased efflux due to overexpression of efflux pumps, enzyme inactivation, decreased cell permeability, target protection, altered target site/enzyme, etc.2,4−9 For example, it is now well documented that carbapenem-resistant Enterobacteriaceae Escherichia coli (CRE-E. coli), Gram-negative bacteria, has the capability to restrict the entry of antibiotics into the outer membrane.4−5 CRE-E. coli is capable of changing the nature of the cell wall, leading to antibiotics becoming ineffective and the bacteria becoming resistant to most available antibiotics in the market.4−9 Recent reports indicate that the “super-resistant” gene New Delhi metallo-beta-lactamase 1 (NDM-1), which exists in multidrug-resistant (MDR) bacteria, has the capability to cause enzymatic degradation of β-lactam antibiotics.4−9 As a result, these bacteria became resistant to a broad range of antibiotics.3−9 For example, Gram-positive bacteria such as Klebsiella pneumoniae have the ability to produce extended-spectrum beta-lactamases (ESBL), which allow Klebsiella pneumoniae to be resistant to virtually all beta-lactam antibiotics.4−9

In the last two decades, the development of new types of antibiotics has been declining rapidly, leaving no options to treat resistant bacteria.2,4−9 As a result, infectious disease treatment has been challenged by bacteria, which are responsible for more than a million deaths in this world every year.4−9 As per the world health organization (WHO),2 if we cannot find an...
alternative way to tackle bacteria, by 2050 drug-resistant bacteria could kill 10 million people/year which is more than the number of people dying from cancer now. All the above facts have triggered initiatives in this world for the development of novel antimicrobial compounds for targeted killing of bacteria.\textsuperscript{10–20} In the last 25 years, one of the very highly promising approaches to combating bacteria is nanomaterial-based antibacterial agents.\textsuperscript{4–35} We and other groups have demonstrated that zero-dimensional (0D) to two-dimensional (2D) nanomaterial-based approaches can be highly promising alternatives for bacteria treatment.\textsuperscript{10–35} 0D plasmonic nanoparticles such as silver nanoparticles have been demonstrated to exhibit high antibacterial activity toward drug-resistant bacteria.\textsuperscript{8–20} It is now well documented that nanoparticles combat bacteria via several different mechanisms,\textsuperscript{1–15} as shown in Figure 1.

![Figure 1. Scheme showing a possible bacteria-combating mechanism of different nanomaterials via bacterial cell damage, cell component leakage, photodynamic (PDT) killing via ROS, and photothermal (PTT) killing via disruption of genomic DNA, proteins, and cellular metabolism.](image)

Nanoparticles have the capability to physically damage the cell membrane.\textsuperscript{4–20} They can also generate reactive oxygen species (ROS) and free radicals.\textsuperscript{21–35} The above process increases the oxidative stress, which enhances the fragmentation of genomic DNA and damages the cellular structural integrity.\textsuperscript{20–35} It is now well documented that nanoparticles exhibit high membrane permeability in comparison to antibiotics.\textsuperscript{4–15} Recent reports also indicate that nanoparticle-based antimicrobial agents have good capacity to act as efflux pump inhibitors.\textsuperscript{4–20} Due to the above facts, nanomaterial-based antimicrobial agents are less prone to induce bacterial resistance in comparison to antibiotics available now in the market.\textsuperscript{20–35} As a result, scientists have great hope that nanomaterial-based antibacterial agents will provide a new way to treat antibacterial infections.\textsuperscript{4–20}

In the last two decades, many different families of nanomaterials including 0D metal nanoparticles, semiconductor nanodots, and carbon dots (one-dimensional (1D) nanorods, carbon nanotubes, 2D graphene oxide (GO), 2D transition metal dichalcogenides (TMD), and heterostructure materials) have been developed to combat bacterial infection.\textsuperscript{4–35} In the last decade, we and others have published several review articles based on 0D and 1D nanomaterial-based antibacterial agents.\textsuperscript{4–10} In this current mini-review, we have highlighted 2D-GO, TMD, MXene, and heterostructure material-based approaches to tackle bacteria.\textsuperscript{4–35} Here we have discussed the major design criteria which has been used to develop novel antimicrobial nanotherapeutics and various mechanisms of antibacterial action which have been used to eliminate drug-resistant bacterial infections. Lastly, we have highlighted the possible challenges in using 2D GO, TMD, and 2D–0D heterostructure material-based approaches in practical applications for combating bacteria.

### Combating Bacteria Using 2D-GO- and GO-Based Heterostructures

After the first observation in 2010 by Hu et al.\textsuperscript{13} that graphene oxide (GO) and reduced graphene oxide (rGO) can be used as antibacterial materials, interest has rapidly grown to develop GO-based material for combating bacteria.\textsuperscript{11–25} GO-containing sp\textsuperscript{2} carbons and oxygen-containing functional groups such as carboxy, hydroxy, and epoxy allow high dispersion in aqueous medium.\textsuperscript{11–25} Due to the ease of synthesis in a large scale, tunable size-dependent properties, low cytotoxicity, and high biocompatibility, in the last several years our group and other groups have been developing GO-based material for biological antimicrobial agents.\textsuperscript{1–22} As reported by several groups, the antibacterial mechanism of GO involves both physical and chemical modes of action as shown in Figure 2A. We and others have shown that chemical modes of action occur via ROS generation or through direct electron transfer.\textsuperscript{11–25} On the other hand, several reported experimental data indicate that physical modes of action occur via direct contact with GO followed by penetration of the cell membrane.\textsuperscript{11–25}

Liu et al.\textsuperscript{15} compared *Escherichia coli* (*E. coli*) bacteria combating capability by GO and reduced graphene oxide (rGO) with graphite (Gt) and graphite oxide (GtO) as shown in Figures 2B–2E. Their reported data\textsuperscript{15} show low antibacterial activity for graphite and graphite oxide, which is mostly due to lower dispersion stabilities. Their reported data indicate that the antibacterial performance for GO is ∼69.3% which is much higher than that of rGO, which is ∼45.9%.\textsuperscript{15} Scanning electron microscopy (SEM) data as shown in Figures 2C–2D indicate that GO-exposed *E. coli* are almost evenly wrapped by GO layers.\textsuperscript{15} On the other hand, rGO aggregates trap *E. coli*. Liu et al.\textsuperscript{15} have argued that after wrapping the sharp edge of graphene nanosheets has the capability for significant membrane stress,\textsuperscript{15} where GO layers are acting as “cutters” to damage cell membranes. Experimental data reported by Liu et al.\textsuperscript{15} demonstrated that small-sized GO containing a high density of oxygen-containing functional groups will wrap the bacterial cells, which will induce membrane stress leading to cell death.\textsuperscript{15}

Similarly, experimental data from other groups\textsuperscript{13–24} also indicate that direct contact between Gram-positive and Gram-negative bacteria with graphene nanosheets could result in loss of bacterial membrane integrity and leakage of RNA/DNA. Zhao et al.\textsuperscript{24} have reported the possible antimicrobial mechanism associated with aerobic reduction of GO as shown in Figures 2F and 2G. Their reported data\textsuperscript{24} demonstrated that GO interacts with membrane-bound cytochrome c of *E. coli*. Zhao et al.\textsuperscript{24} have indicated that the above interaction helps for shuttling electrons from the respiratory chain to extracellular molecular oxygen. As a result, superoxide anions (O\textsuperscript{2−}) form, which in turn reduces GO.\textsuperscript{24} Zhao et al.\textsuperscript{24} showed that the reduction process interrupts the respiration chain and enhances the antimicrobial activity. From the experimental results, Zhao et al.\textsuperscript{24} concluded that GO can serve as a conductive bridge to shuttle electrons from the intracellular respiratory chain to extracellular molecular oxygen. It also promotes O\textsuperscript{2−} production, which in turn reduced GO. The above process severed oxidative damage of bacteria.\textsuperscript{24} Liu et al.\textsuperscript{20} have reported lateral size-dependent antibacterial activities of GO sheets toward *E. coli* bacteria. As shown in Figures 3A–C, their reported data indicate that larger GO sheets exhibit stronger...
antibacterial activity than smaller ones. Experimental results using atomic force microscopy (AFM) analysis indicate GO sheets interact strongly with cells as shown in Figures 3A,B.\textsuperscript{20} From AFM data, Liu et al.\textsuperscript{20} have argued that large GO sheets interact strongly with cells as shown in Figures 3A,B.\textsuperscript{20}
more easily cover cells. As a result, cells cannot proliferate once fully covered, resulting in the cell viability loss.

On the other hand, small GO can adhere to the bacterial surfaces, which allow weaker antibacterial activity. Due to the
Figure 4. (A) Reverse transcription polymerase chain reaction (RT-PCR) data that show *E. coli* O157:H7 killing efficiency using CNT-bridged 3D GO without the PGLa peptide, with only the PGLa peptide, and with the PGLa peptide-conjugated CNT-bridged 3D graphene oxide membrane. (B) SEM image of PGLa and glutathione-conjugated CNT-bridged graphene oxide membrane. (C) SEM image demonstrating the capture of *E. coli* O157:H7 by PGLa-conjugated CNT-bridged 3D graphene oxide-based membrane (adapted with permission from ref 12. Copyright 2015, American Chemical Society). (D) Schematic illustration shows the synergistic bacteria-killing behavior using the PDA/Ag3PO4/GO hybrid. (E) Surface morphology of the bacteria on the sample surface after culturing for 15 min in darkness versus irradiation for 15 min with 660 nm visible light. The scale bars are 1 μm. (F) Antibacterial property analysis after exposure to 660 nm visible light for 15 min. (G) H&E staining of visceral tissue slices of rats after implantation for 3 days (adapted with permission from ref 23. Copyright 2018, American Chemical Society).
penetration of the cell membrane by 2D GO, loss of membrane integrity may happen via pore formation or extraction of lipid molecules. Palmieri et al. reported how GO concentration and the surrounding media can manipulate the antimicrobial property. As shown in Figure 3D–G, their experimental data show that at low GO concentration GO cuts microorganism membranes for S. aureus and E. coli. Palmieri et al. have demonstrated that when the concentration is below 6 μg/mL GO acts as a knife that cuts bacterial membranes. On the other hand, as GO concentration increases, the GO killing efficacy increases only in water.22 Their experimental observation, as shown in Figure 3D–G, indicates that in other solutions, due to the aggregation which shields GO edges, there is no impact on microbial growth. Their reported experimental data show that when the concentration is higher than 100 μg/mL GO cluster size becomes much larger than bacteria.25 As a result, GO aggregates wrap bacteria and impede their growth. Their studies clearly show that GO versatility can be exploited for the treatments against multidrug-resistant bacteria.

Several reports in the last decade indicate that other than membrane stress mediated via wrapping oxidative stress produced by GO and rGO also can be the cause of cell death.10–24 It is now well documented that oxidative stress can interfere with bacterial metabolism.10–20 Oxidative stress induced by nanomaterials also has the capability to disrupt essential cellular functions, which can lead to cell death.14–24 Oxidative stress usually occurs via two different pathways. The first one is ROS-dependent, and the second one is the ROS-independent pathway.10–20 Liu et al. have performed in vitro GSH oxidation test experiments as shown in Figure 2E. Their reported data clearly indicate that rGOs and GT are capable of inducing superoxide anion-independent oxidative stress on bacterial cells. On the other hand, GO has the lowest GSH oxidation capacities with respect to GT, GTO, and rGO. From all the experimental data, Liu et al. have concluded that graphene-based destruction of bacteria can be attributed to both membrane damage and oxidative stress.

It is now well documented that lipid peroxidation, which is also known as ROS-mediated oxidation of lipid molecules, is a very important oxidative pathway to kill bacteria.10–24 Krishnamoorthy et al. have reported that graphene nanosheets enhanced the ultrasound-induced lipid peroxidation. Their reported data show that with respect to the control group lipid peroxidation increased by 117% and 109% after exposure to 10 and 5 μg/mL of graphene. Zucker et al. reported the disruption of phospholipid vesicles due to 2D GO and cell interactions. Their dye leakage experiment shows that the extent of membrane integrity loss was dependent on total surface area and not edge length.19 In the past decade, heterostructure building blocks using 2D GO, 1D, and 0D materials have opened unique opportunities to be studied for use as antibacterial material due to the combined advantages of the individual material.10–20 As we have discussed, although GO is able to kill bacteria, the killing efficiency is less than 50%. To improve the killing efficiency to 100%, several heterostructure-based materials have been designed recently and reported to have the capability for 100% killing of bacteria.10–20 We have reported that the PGLa peptide (Gly-Met-Ala-Ser-Lys-Ala-Gly-Ala-Ile-Ala-Gly-Lys-Ile-Ala-Lys-Val-Ala-Leu-Lys-Ala-Leu-\text{NH}_2) conjugated CNT-bridged graphene oxide membrane can be used for disinfection of pathogenic E. coli O157:H7 bacteria.

For the design of heterostructure-based antibacterial material, as shown in Figures 4A–4C, we have used 1D CNTs to physically separate 2D graphene oxide sheets from aggregation.12 To increase the percentage of killing efficiency, we have developed PGLa antimicrobial peptide which has the capability to kill E. coli O157:H7 on contact by interacting with their lipid membranes.12 As shown in Figures 4A–4C, our experimental disinfection data show that the PGLa peptide can kill roughly 70% of E. coli O157:H7 bacteria in the absence of CNT-bridged 3D graphene oxide.12 On the other hand, CNT-bridged 3D graphene can kill only 11% E. coli O157:H7 bacteria without PGLa.12 Our reported data clearly show that PGLa-attached CNT-bridged 3D graphene oxides are able to kill 100% of E. coli O157:H7 bacteria.12 We have concluded that CNT-bridged 3D graphene oxide helps to trap E. coli, and this situation allows PGLa to bind and penetrate the outer membrane of bacteria and kill E. coli O157:H7 bacteria.12

Xie et al. reported the design of hybrid polydopamine (PDA)/Ag$_2$PO$_4$/GO-based antibacterial material to achieve rapid bacteria killing and eliminate biofilms in situ. As shown in Figure 4D–4G, in their design they have explored the synergistic actions of Ag$^+$ and ROS produced by Ag$_2$PO$_4$ under irradiation of 660 nm visible light.17 For this purpose, they have tuned the bandgap of the Ag$_2$PO$_4$ NPs using GO. The observed that the synergistic killing mechanism is due to an increase in the membrane permeability by ROS, which enables Ag$^+$ to enter the bacteria more easily.23 As a result, both Ag$^+$ and ROS can destroy the DNA and proteins synergistically. Their reported experimental data indicate that the antimicrobial properties for the hybrid vary with the composition of particles.23 They have argued that due to the larger specific surface area for the heterostructure it leads to more effective release of Ag$^+$ and absorption of more photons to produce ROS.23 As shown in Figure 4G, the histological section by immunohistochemical staining of neutrophils and lymphocytes shows excellent photocatalytic antibacterial activity. Feng et al. reported a reduced graphene oxide/Au nanostar (rGO/AuNS) heterostructure based highly effective photothermal agent, which has the capability to enhance the photothermal conversion in comparison to the pure rGO nanosheets and AuNS. Their reported data show that the rGO/AuNS exhibits promising intrinsic antibacterial activity for MRSA. Very recently, we have reported the design of a polydopamine nanoparticle (PDPN) attached GO-conjugated ε-poly-L-lysine (ε-PL) antimicrobial peptide based novel heterostructure, which can be used for disinfection of drug-resistant pathogens from environmental samples. Reported data show that a PDPN-attached GO-conjugated ε-poly-L-lysine (ε-PL) based heterostructure can be used for capturing 100% bacteria. Experimental drug-resistant bacteria killing data demonstrated that the heterostructure has the capability to eradicate different drug-resistant bacteria such as β-lactamase (ESBL)-producing Klebsiella pneumoniae (KPN) and mexitilin-resistant Staphylococcus aureus (MRSA). Shoeb et al. have reported a graphene/polyindole (Gr@Pln) heterostructure-based antibacterial nanocomposite for the treatment of MRSA skin infection in BALB/c mice. Their reported experimental results show that Gr@Pln nanocomposites are highly effective in inhibiting MRSA.

Their experimental data with BALB/c mice, as shown in Figure 5A–5D, show that the graphene-based heterostructure has great potential to prevent bacterial infection under in vivo environments using an S. aureus-mediated skin contagion in BALB/c mice. Reported data indicate that right after the treatment using Gr@Pln nanocomposites the skin attained a typical architecture. Shoeb et al. have shown that the Gr@Pln
Combating Bacteria Using 2D-MoS\textsubscript{2} and MoS\textsubscript{2}-Based Heterostructures. Transition metal dichalcogenides (TMDs) are a unique class of 2D nanomaterials for the possible applications in drug delivery, phototherapy of cancer and bacteria, as well as its use as antibacterial wound healing agents.\textsuperscript{10,24–31} Since TMDs have bandgaps in contrast to the zero bandgap of graphene, TMD has been studied for possible photonics, optoelectronics, and other applications.\textsuperscript{10,24–31} After the first observation in 2014 by Yang et al.\textsuperscript{26} that two-dimensional (2D) chemically exfoliated MoS\textsubscript{2} can be used as antibacterial materials, the interest has rapidly grown to develop MoS\textsubscript{2}-based material for combating bacteria.\textsuperscript{10,24–31} For this purpose, MoS\textsubscript{2} nanosheets were functionalized with either hydrophobic ligands or antimicrobial peptides.\textsuperscript{10,24–31} Recently several reports indicate that the NIR-responsive MoS\textsubscript{2} nanosheets can be combined with functionalities for designing photothermal, photocatalytic, and chemical disinfection systems.\textsuperscript{10,24–31} Similarly, we and others have demonstrated that by combining with other antibacterial nanomaterials such as silver, gold, and graphene oxide one can develop highly potent antibacterial nanocomposites using MoS\textsubscript{2}-based heterostructure materials.\textsuperscript{10,24–31} Yang et al.\textsuperscript{26} have found that chemically exfoliated MoS\textsubscript{2} sheets have the capability to produce reactive oxygen species (ROS). They have suggested that antimicrobial activity for chemically exfoliated MoS\textsubscript{2} sheets is due to membrane damage and oxidation stress. Pandit et al.\textsuperscript{30} demonstrated that functionalized chemically exfoliated MoS\textsubscript{2} can be used as a highly effective antibiotic agent against Gram-positive and Gram-negative pathogens as shown in Figure 6A–6E.\textsuperscript{30} They have tested the antibacterial properties for exfoliated MoS\textsubscript{2} against MRSA and \textit{P. aeruginosa} and their corresponding biofilms.\textsuperscript{30,31} As shown in Figure 6A, in their design of positively charged MoS\textsubscript{2}, the hydrophobicity was varied by changing the alkane chain length. Figure 6D shows the position of functionalized MoS\textsubscript{2} as an antibacterial agent compared to other materials. Pandit et al.\textsuperscript{30} have reported minimum inhibitory concentration (MIC) for functionalized MoS\textsubscript{2} against MRSA and \textit{P. aeruginosa}. Their reported data indicate that for C1MoS\textsubscript{2} the MIC value is 1.88 ppm. On the other hand, the reported MIC values for C6MoS\textsubscript{2} and C8MoS\textsubscript{2} are 156 and 78 ppb, respectively.\textsuperscript{30}

Their reported high antibacterial activity using functionalized MoS\textsubscript{2} is due to the net negative charge of bacterial surfaces and the positive charge of functionalized MoS\textsubscript{2}. As a result, functionalized MoS\textsubscript{2} is highly effective to kill bacteria. As reported in Figures 6E, their experimental data indicate that functionalized materials generate less oxidative stress than exfoliated MoS\textsubscript{2}. A detailed mechanistic study reported by Pandit et al.\textsuperscript{30} indicates that ROS-independent oxidative stress generation as well as depolarization of the bacterial membrane are responsible for destroying bacteria using functionalized MoS\textsubscript{2}. Importantly, reported experimental data demonstrated that by altering the hydrophobicity of positively charged MoS\textsubscript{2} one can tune the antibacterial pathway between oxidative stress and depolarization of the bacterial membrane.
Recently, we have reported the design of the melittin antimicrobial peptide (AMP) attached MoS\textsubscript{2}-based antibacterial agent for synergistic inactivation of 100% multidrug-resistant bacteria by the combined photothermal therapy (PTT), photodynamic therapy (PDT), and antimicrobial peptide (AMP) process, as shown in Figure 7A–7C. Our reported experimental data show that using the synergistic killing mechanism of AMP-attached MoS\textsubscript{2} has the capability to kill 100% MRSA, E. coli, and KPN bacteria. In our design, the MoS\textsubscript{2}-based nanoplatform killed bacteria via an external NIR light-triggered combined photothermal and photodynamic mechanism. On the other hand, melittin antimicrobial peptide has been used to kill bacteria by pore formation as shown in Figure 7B. As shown in Figure 7C, our reported experimental data demonstrated that only 28% of multidrug-resistant bacteria (MDRB) killing is possible using a MoS\textsubscript{2}-based nanoplatform. On the other hand, only 20% of bacteria can be killed by the melittin antimicrobial peptide alone. Reported data clearly show that 100% of bacteria can be killed using a nanoplatform with NIR light. The observed synergistic killing mechanism is due to the fact that in the presence of the PEG-MoS\textsubscript{2}-AMP nanoplatform initially the melittin AMP makes pores on the surface of MDRB. Pores formed by AMP help to diffuse heat and ROS easily during PDT and PTT. Due to the above possible synergistic multimodal killing mechanism, 100% of MDRB was killed. To understand better about the membrane pore formation, we have performed a bacterial ATP leakage experiment using the ATP determination kit. Our reported data indicate a high amount of leakage of cellular ATP even at the concentration of 2.8 \( \mu \text{g/mL} \). Recently, Roy et al. have reported the design of chitosan-exfoliated MoS\textsubscript{2} nanosheets (CS-MoS\textsubscript{2}) as antibacterial agents. They have evaluated the antibacterial activity against both Gram-negative and -positive bacteria, which indicates that CS-MoS\textsubscript{2} nanosheets have the
capability for growth inhibition of both Gram-negative and Gram-positive bacteria in a concentration- and time-dependent manner, as shown in Figure 7D–7E.28

They have determined MIC and minimum bactericidal concentration (MBC) of the CS-MoS₂ nanosheets against both E. coli and S. aureus as shown in Figure 7E. Their reported data28 indicate that the MIC and MBC values of CS-MoS₂ nanosheets against E. coli are 30 and 60 μg/mL, respectively. Similarly, the MIC and MBC values for S. aureus are found to be 90 and 120 μg/mL, respectively.28 From their reported data28 they have concluded that due to the presence of a thick peptidoglycan layer surrounding the Gram-positive S. aureus it required a higher concentration of CS-MoS₂ for achieving antibacterial activity.28 Experimental data reported by Roy et al.28 indicated that the CS-MoS₂ nanosheets induced bacterial cell death through a combined action of membrane damage, metabolic inactivation, and oxidative stress. The detailed mechanistic study indicates that the antibacterial activity of CS-MoS₂ nanosheets happens through a multistep process, and these are membrane damage, metabolic inactivation, and oxidative stress.28 They have concluded that CS-MoS₂ nanosheets can probably be used as antibacterial coatings, wound dressings, and ultrafiltration membranes for potential biomedical and environmental applications.28

Cao et al. have reported29 the design of a poly-(dimethyldiallylammonium chloride) (PDDA)-Ag⁺-Cys-MoS₂ heterostructure and their antimicrobial properties. Their reported data as shown in Figure 8A–8D indicate that the PDDA-Ag⁺-Cys-MoS₂ exhibited enhanced broad-spectrum antibacterial activity for Gram-negative E. coli and Gram-positive S. aureus.29 On the other hand, their reported data show extremely low antibacterial ability for equivalent amounts of AgNP or AgNO₃ solution. Reported in vitro and in vivo antibacterial experiments indicate that cationic polyelectrolyte
promoted the adhesion of PDDA-Ag+-Cys-MoS2. From the experimental data, they have concluded that PDDA-Ag+-Cys-MoS2 could release large amounts of Ag+ ions at the cell walls of microorganisms. As shown in Figure 8A−8D, for in vivo experiment, Cao et al. have used the infected wound model. For this purpose, the back of the mice was slashed and injected with $1 \times 10^6$ of MRSA. In the next step, the mice were divided into seven groups, based on treatment with PBS buffer, AgNO3, AgNPs, PDDA, Cys-MoS2, Ag+-Cys-MoS2, and PDDA-Ag+-Cys-MoS2. Their experimental data, as shown in Figure 8, show that for control groups a fragmentary epidermal layer appeared on the wound after 3 day treatments. On the other hand, the intact epidermal layer emerged on the wound treated with PDDA-Ag+-Cys-MoS2 dressing for 3 days. Their reported data clearly show that PDDA-Ag+-Cys-MoS2 exhibited the best antibacterial effect and wound healing.

In this section, we have discussed 2D-MoS2 and heterostructure-based design of antibacterial strategy for killing bacteria. We have highlighted some of the proposed antibacterial mechanisms and latest developments in the practical antimicrobial applications for TMD and TMD-based heterostructures. Since 2D-MoS2-based antibacterial agent design is in the very early stages, the antibacterial mechanism has not been completely understood. Computational simulation studies are very important to analyze the interactions between 2D-TMD materials and biological components which will enable the determination of the possible mechanism. As a result, in-depth investigations are urgently needed to find out the mechanisms and different parameters influencing the antibacterial activity for TMD-based materials.

Combating Bacteria Using 2D-MXenes and Heterostructures. The emerging MXenes, a new family of multifunctional 2D materials, exhibit metallic conductivity, hydrophilic nature, and unique physiochemical performances. As a result, in the past few years, transition metal carbides, nitrides, and carbonitride-based MXenes have been introduced as novel inorganic nanosystems for biologic and biomedical applications. After the first MXene, multilayered Ti3C2, was developed in 2011, a series of 2D MXenes were designed. For the last eight years, few groups have explored possible applications in energy storage, theranostic material, chemical and biomedical sensors, etc. After the first observation in 2016 by Rasoo et al. that two-dimensional Ti3C2Tx MXenes can be used as antibacterial materials, the interest has rapidly grown to develop MXene-based material for combating bacteria. Rasoo et al. tested the antibacterial properties of Ti3C2Tx against Escherichia coli (E. coli) and Bacillus subtilis (B. subtilis). Their reported data as shown in Figures 9B−9D indicate that Ti3C2Tx exhibits a higher antibacterial efficiency toward E. coli and B. subtilis comparable with GO. As shown in Figure 9D, their experimental data on concentration-dependent antibacterial activity indicate more than 98% bacterial cell viability in the presence of 200 µg/mL of Ti3C2Tx. Antibacterial mechanic investigation data by Rasoo et al. show the damage to the cell membrane which kills bacteria.

Shamsabadi et al. have reported size-dependent antibacterial properties of MXene. For this purpose, they have tested antibacterial activity for nanosheets with lateral sizes of 0.09, 0.35, 0.57, and 4.40 µm against Escherichia coli and Bacillus subtilis bacteria. Their reported data indicate that smaller nanosheets exhibit higher antibacterial activities against both...
Figure 9. (A) Schematic representation of proposed antibacterial mode-of-action of Ti$_3$C$_2$Tx MXene nanosheets (adapted with permission from ref 34. Copyright 2018, American Chemical Society). (B) SEM images of the B. subtilis treated with 50 μg/mL of Ti$_3$C$_2$Tx. (C) The cell wall stripped down after exposure to Ti$_3$C$_2$Tx nanosheets. (D) Cell viability measurements of B. subtilis treated with Ti$_3$C$_2$Tx and graphene oxide (GO) in aqueous suspension. (E) Time-dependent glutathione (0.4 mM) loss after incubation for 4 h with Ti$_3$C$_2$Tx (200 μg/mL) (adapted with permission from ref 33. Copyright 2016, American Chemical Society). (F,G) Antibacterial activity of MoS$_2$, MoS$_2$/rGO, and MoS$_2$/MXene nanomaterials against E. coli and B. subtilis bacteria. Fluorescence imaging (F) and flow cytometry (G) results of bacteria treated with 100 μg/mL of the nanomaterials for ca. 3 h in the dark. In fluorescence images, the live bacteria are green (SYTO9-stained), and the dead bacteria are red (PI-stained). 400X magnification was used (adapted with permission from 35. Copyright 2018, American Chemical Society).
The schematic representations of the proposed antibacterial mode-of-action of Ti$_3$C$_2$Tx MXene nanosheets are shown in Figure 9A. Reported antibacterial mechanism studies indicate that interactions between the sharp edges of the Ti$_3$C$_2$Tx MXene nanosheets and bacteria membrane play an important role in antibacterial properties of the nanosheets. From the experimental data, Shamsabadi et al. have concluded that MXene damages the bacterial cell wall significantly in less than 3 h by releasing DNA from the cytosol. Alimohammadi et al. have reported the comparison of antibacterial activity of vertically aligned different 2D heterostructures such as MnO$_2$/GO, MoS$_2$/rGO, and MoS$_2$/MXene. Experimental results by Alimohammadi et al. show that a vertically aligned 2D nanosheet motif exhibits higher antibacterial activity than 2D nanomaterials, as shown in Figure 9F–9G. Their reported experimental data indicate that the number of viable bacteria became 90% when B. subtilis was treated with MoS$_2$. On the other hand, the number of viable bacteria became 60% and 75%, when B. subtilis was treated with MoS$_2$/rGO and MoS$_2$/MXene, respectively.

In this section, we have discussed 2D-MXene and heterostructure-based design of antibacterial material for killing bacteria. We have highlighted very recent developments in the antimicrobial applications for 2D-MXene and 2D-MXene-based heterostructures. Since only in the past few years antibacterial properties for 2D-MXene have been realized the antibacterial mechanism is poorly understood, where further studies are very important. A systematic collaborative in vivo experimental study is highly necessary to understand the environmental impacts and the interaction between 2D-MXene with different organisms, before it can be used for society.

Although we have only highlighted 2D-GO, MoS$_2$, MXene, and heterostructure material-based antimicrobial strategies, some other 2D materials such as layered double hydroxides (LDHs), hexagonal boron nitride (BN), 2D metal oxides, and 2D kaolinite are also emerging as potential antibacterial agents.

Summary. In conclusion, in the current mini-review we have discussed recent advancements on 2D-GO, MoS$_2$, MXene, and heterostructure material-based antimicrobials, which have the capability to be used for disinfection of bacteria in vivo and ex vivo applications. Reported data from several groups have demonstrated that the tunable surface functionality of 2D-GO, MoS$_2$, MXene, and heterostructures has the ability to provide a versatile platform to combat drug-resistant bacterial infections. We have discussed how the material design can be tailored for heterostructure-based antimicrobial nanocomposites to tackle the multidrug-resistant problem. The current state of the art 2D and heterostructure material based animal model data show that the nanomaterial has strong potential to treat topical skin infections in the near future. We hope that the current mini-review will provide researchers to realize the potential of 2D multifunctional materials for combating bacteria.

Challenges. Although from the reported data we can realize that 2D-GO, MoS$_2$, MXene, and heterostructures have the capability for combating bacteria, it is still too early to apply 2D and heterostructure-based antibacterial material for real life clinical applications. Large-scale and low-cost design of 2D and heterostructure-based antibacterial material with high reproducibility is very important for practical use, which is lacking until now. The fabrication process needs to be designed in such a way that 2D and heterostructure-based antibacterial material should retain activity in complex biological media. Since for clinical applications 2D-GO, MoS$_2$, MXene, and heterostructural material-based antimicrobials can be given through either skin contact, oral, or intravenous injection, a thorough in vivo model must be evaluated to better understand their potential toxicity, clearance, and metabolism. We really need to understand the biodegradability of sheet-structured 2D materials inside the body. The central questions we need to determine is whether intravenously injected 2D-GO, MoS$_2$, MXene, and heterostructural material-based antimicrobials accumulate in the colon, lung, bone marrow, liver, spleen, and lymphatics? The pharmacological profiles of 2D and heterostructure-based antibacterial material are essential for the clinical translation, which is missing now. Interdisciplinary research evaluating these aspects needs to be performed very carefully, which will allow us to establish 2D and heterostructure nanomaterials as effective next-generation antimicrobials for bacteria.
Biographies

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Dr. Paresh Ray is a Professor in the Department of Chemistry, Jackson State University, USA. Dr. Ray’s research group is working on the interface of chemistry and biology that includes exploring 0D, 1D, 2D, and heterostructure material based new strategies for combating multidrug-resistant bacteria and imaging of cancer. Dr. Paresh Ray has published over 170 scientific publications.

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**REFERENCES**

(1) Fleming, A. In Nobel Lectures: Physiology or Medicine; 1942—1962; Elsevier, 1964; pp 83—93.
(2) Dik, D. A.; Fisher, J. F.; Mobashery, S. Cell-Wall Recycling of the Gram-Negative Bacteria and the Nexus to Antibiotic Resistance. *Chem. Rev.* 2018, 118, 5952–5984.
(3) http://www.who.int/mediacentre/factsheets/fs194/en/ (accessed August 8, 2018).
(4) El Meouche, I.; Dunlop, M. J. Heterogeneity in Efflux Pump Expression Predisposes Antibiotic-Resistant Cells to Mutation. *Rev. Appl. Microbiol.* 2018, 39, 595–613.
(5) Anand, A.; Unnikrishnan, B.; Wei, S. C.; Chou, C. P.; Zhang, L. Z.; Huang, C. C. Graphene oxide and carbon dots as broad-spectrum antimicrobial agents—A minireview. *Nanoscale Horiz.* 2019, 4, 117–137.
(6) Li, X.; Bai, H.; Yang, Y.; Yoon, J.; Wang, S.; Zhang, X. Supramolecular ar antibacterial materials for combating antibiotic resistance. *Adv. Mater.* 2018, 31, No. 1805092.
(7) Xin, Q.; Shah, H.; Nawaz, A.; Xie, W.; Akram, M. Z.; Batool, A.; Tian, L.; Jan, S. U.; Boddula, R.; Guo, B.; Liu, Q.; Gong, J. R. Antibacterial Carbon-Based Nanomaterials. *Adv. Mater.* 2019, 31, 1804838.
(8) Ray, P. C.; Khan, S. A.; Singh, A. K.; Senapati, D.; Fan, Z. Nanomaterials for Targeted Detection and Photothermal Killing of Bacteria. *Chem. Soc. Rev.* 2012, 41, 3193–3209.
(9) Gupta, A.; Muntaz, S.; Li, C.-H.; Hussain, I.; Rotello, V. M. Combating antibacterial-resistant bacteria using nanomaterials. *Chem. Soc. Rev.* 2019, 48, 415.
(10) Begum, S.; Pramanik, A.; Gates, K.; Gao, Y.; Ray, P. C. Antimicrobial Peptide-Conjugated MoS 2-Based Nanoplatform for Multimodal Synergistic Inactivation of Bacteria. *ACS Appl. Bio Mater.* 2019, 2, 769–776.
(11) Pramanik, A.; Gates, K.; Gao, Y.; Zhang, Q.; Han, F. X.; Rightshell, C.; Sardar, S.; Ray, P. C. Composites Composed of Polydopamine Nanoparticles, Graphene Oxide, and r-Poly-L-lysine for Removal of Waterborne Contaminants and Eradication of Bacteria. *ACS Appl. Nano Mater.* 2019, 2 (6), 3339–3347.
(12) Viraka Nellore, B. P.; Kanchanapally, R.; Pedraza, F.; Sinha, S. S.; Pramanik, A.; Hamme, A. T.; Arslan, Z.; Sardar, D.; Ray, P. C. Bio-Conjugated CNT-Bridged 3D Porous Graphene Oxide Membrane for Highly Efficient Disinfection of Pathogenic Bacteria and Removal of Toxic Metals from Water. *ACS Appl. Mater. Interfaces* 2015, 7, 19210–19218.
(13) Hu, W.; Peng, C.; Luo, W.; Lv, M.; Li, X.; Li, D.; Huang, Q.; Fan, C. Graphene-Based Antibacterial Paper. *ACS Nano* 2010, 4, 4317–4323.
(14) Jia, Z.; Shi, Y.; Xiong, P.; Zhou, W.; Cheng, Y.; Zheng, Y.; Xi, T.; Wei, S. From Solution to Biointerface: Graphene Self-Assemblies of Varying Lateral Sizes and Surface Properties for Biofilm Control and Osteodifferentiation. *ACS Appl. Mater. Interfaces* 2016, 8, 17151–17165.
(15) Liu, S.; Zeng, T. H.; Hofmann, M.; Burcombe, E.; Wei, J.; Jiang, R.; Kong, J.; Chen, Y. Antibacterial Activity of Graphite, Graphite Oxide, Graphene Oxide, and Reduced Graphene Oxide: Membrane and Oxidative Stress. *ACS Nano* 2011, 5, 6971–6980.
(16) Perreault, F.; de Faria, A. F.; Nejati, S.; Elimelech, M. Antimicrobial Properties of Graphene Oxide Nanosheets: Why Size Matters. *ACS Nano* 2015, 9, 7226–7236.
(17) Krishnamoorthy, K.; Veerapandian, M.; Zhang, L.-H.; Yun, K.; Kim, S. J. Antibacterial Efficiency of Graphene Nanosheets against Pathogenic Bacteria via Lipid Peroxidation. *J. Phys. Chem. C* 2012, 116, 17280–17287.
(18) Sun, J.; Song, L.; Fan, Y.; Tian, L.; Luan, S.; Niu, S.; Ren, L.; Ming, M.; Zhao, J. Synergistic Photodynamic and Photothermal Antibacterial Nanocomposite Membrane Triggered by Single NIR Light Source. *ACS Appl. Mater. Interfaces* 2019, 11, 26581–26589.
(19) Zucker, T.; Werber, J. R.; Fishman, Z. S.; Hashmi, S. M.; Gabinet, U. R.; Lu, X.; Osuji, C. O.; Pfeiffer, L. D. Menachem Elimelech. Loss of Phospholipid Membrane Integrity Induced by Two-Dimensional Nanomaterials. *Environ. Sci. Technol. Lett.* 2017, 4, 404–409.
(20) Liu, S.; Hu, M.; Zeng, T. H.; Wu, R.; Jiang, R.; Wei, J.; Wang, L.; Kong, J.; Chen, Y. Lateral dimension-dependent antibacterial activity of graphene oxide sheets. *Langmuir* 2012, 28, 13624–13637.
(21) Shoeb, M.; Mobin, M.; Rauf, M. A.; Owais, M.; Naqvi, A. H. In Vitro and in Vivo Antimicrobial Evaluation of Graphene-Polyindole (Gr@Pn) Nanocomposite against Methicillin-Resistant Staphylococcus aureus Pathogen. *ACS Omega* 2018, 3, 9431–9440.
(22) Palmieri, V.; Bugli, F.; Lauriola, M. C.; Cacaci, M.; Torelli, R.; Ciasca, G.; Conti, C.; Sanguinetti, M.; Papi, M.; De Spriito, M. Bacteria Meet Graphene: Modulation of Graphene Oxide Nanosheet Interaction with Human Pathogens for Effective Antimicrobial Therapy. *ACS Biomater. Sci. Eng.* 2017, 3, 619–627.
(23) Xie, X.; Mao, X.; Liu, X.; Tan, L.; Cui, Z.; Yang, X.; Zhu, S.; Li, Z.; Yuan, X.; Zheng, Y.; Wai, K.; Yeung, K.; Chu, P. K.; Wu, S. Tuning the Bandgap of Photo-Sensitive Polydopamine/Ag3PO4/ Graphene Oxide Coating for Rapid, Noninvasive Disinfection of Implants. *ACS Cent. Sci.* 2018, 4, 724–738.
(24) Zhao, H.; Zhang, C.; Wang, Y.; Chen, W.; Alvarez, P. J. J. Self-Damaging Aerobic Reduction of Graphene Oxide by Escherichia coli: Role of GO-Mediated Extracellular Superoxide Formation. *Environ. Sci. Technol.* 2018, 52, 12783–12791.
(25) Feng, Y.; Chen, Q.; Yin, Q.; Pan, G.; Tu, Z.; Liu, L. Reduced Graphene Oxide Functionalized with Gold Nanostar Nanocomposites for Synergistically Killing Bacteria through Intrinsic Antimicrobial Activity and Photothermal Ablation. *ACS Appl. Bio Mater.* 2019, 2, 747–756.
(26) Yang, X.; Li, J.; Liang, T.; Ma, C.; Zhang, Y.; Chen, H.; Hanagata, N.; Su, H.; Xu, M. Antibacterial Activity of Two-Dimensional MoS2 Sheets. *Nanoscale* 2014, 6, 10126–10133.
(27) Karunakaran, S.; Pandit, S.; Basu, B.; De, M. Simultaneous Exfoliation and Functionalization of 2H-MoS2 by Thiolated Surfactants: Applications in Enhanced Antibacterial Activity. *J. Am. Chem. Soc.* 2018, 140, 12634–12644.
(28) Roy, S.; Mondal, A.; Yadav, V.; Sarkar, A.; Banerjee, R.; Sanpui, P.; Jaiswal, A. Mechanistic Insight into the Antibacterial Activity of Chitosan Exfoliated MoS2 Nanosheets: Membrane Damage, Metabolic Inactivation, and Oxidative Stress. *ACS Appl. Bio Mater.* 2019, 2, 2738–2755.

https://dx.doi.org/10.1021/acsomega.9b03019
ACS Omega 2020, 5, 3116–3130
(29) Cao, F.; Ju, E.; Zhang, Y.; Wang, Z.; Liu, C.; Li, W.; Huang, Y.; Dong, K.; Ren, J.; Qi, X. An efficient and benign antimicrobial depot based on silver-infused MoS2. **ACS Nano** **2017**, *11*, 4651−4659.

(30) Pandit, S.; Karunakaran, S.; Boda, S. K.; Basu, B.; De, M. High Antibacterial Activity of Functionalized Chemically Exfoliated MoS2. **ACS Appl. Mater. Interfaces** **2016**, *8*, 31567−31573.

(31) Yin, W.; Yu, J.; Lv, F. T.; Yan, L.; Zheng, L. R.; Gu, Z. J.; Zhao, Y. L. Functionalized Nano-MoS2 with Peroxidase Catalytic and Near-Infrared Photothermal Activities for Safe and Synergetic Wound Antibacterial Applications. **ACS Nano** **2016**, *10*, 11000−11011.

(32) Naguib, M.; Kurtoglu, M.; Presser, V.; Lu, J.; Niu, J.; Heon, M.; Hultman, L.; Gogotsi, Y.; Barsoum, M. W. Barsoum, Two-Dimensional Nanocrystals Produced by Exfoliation of Ti3AlC2. **Adv. Mater.** **2011**, *23*, 4248.

(33) Rasool, K.; Helal, M.; Ali, A.; Ren, C. E.; Gogotsi, Y.; Mahmoud, K. A. Antibacterial Activity of TiC2Tx MXene. **ACS Nano** **2016**, *10*, 3674−3684.

(34) Arabi Shamsabadi, A.; Sharifian Gh, M.; Anasori, B.; Soroush, M. Antimicrobial Mode-of-Action of Colloidal Ti3C2Tx MXene Nanosheets. **ACS Sustainable Chem. Eng.** **2018**, *6*, 16586−16596.

(35) Alimohammadi, F.; Sharifian Gh, M.; Attanayake, N. H.; Thenuwar, A. C.; Gogotsi, Y.; Anasori, B.; Strongin, D. R. Antimicrobial Properties of 2D MnO2 and MoS2 Nanomaterials Vertically Aligned on Graphene Materials and Ti3C2MXene. **Langmuir** **2018**, *34*, 7192.